Collective behaviour

Investigating the underlying mechanisms, development, and function

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I certify that the intellectual content of this thesis is the product of my own work unless otherwise acknowledged
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CHAPTER 1

GENERAL INTRODUCTION

Animal behaviour has fascinated biologists since before the time of Darwin. Yet prior to Niko Tinbergen’s seminal paper ‘On Aims and Methods of Ethology’ in 1963, the field of animal behaviour spent many years without any “consistent public image”, with ethologists at the time differing “widely in their opinions of what their science is about” (Tinbergen 1963). Tinbergen published his paper in the hopes that he might prevent the field from “splitting up into seemingly unrelated sub-sciences”, and drew on the works of Julian Huxley to provide a framework under which all studies of animal behaviour could unify. He postulated that in order to fully understand any behavioural trait, scientists must strive to answer four questions relating to both the current and historical properties of the trait. He posed the following four questions: 1) what are the underlying mechanisms of the behaviour, 2) what is the survival value or ‘current utility’ (Bateson et al. 2013) of the behaviour, 3) How does the behaviour develop within the animal’s lifetime (i.e. what is its ontogeny) and 4) How did the behaviour evolve? By posing these four questions, Tinbergen hoped to witness the, “fusing of many sciences, all concerned with one or another aspect of behaviour, into one coherent science, for which the only correct name is “Biology of behaviour”” (Tinbergen 1963).

With this in mind, the current thesis aims to apply the framework of Tinbergen’s 4 questions to the study of collective animal behaviour, which refers specifically to the emergence of seemingly coordinated group-level behaviour through repeated interactions between individuals. Fundamentally, the basis of collective behaviour is the tendency to form groups. Aggregations of conspecifics can arise through external physical forces, such as clumped resources or converging currents, or due to mutual attraction between conspecifics. Regardless of whether animal groups arise through active or passive measures, as a strategy, living as part of a group can confer a range of benefits, from greater access to mates and
enhanced reproductive success (Robinson 1988, Cameron et al. 2009), to cooperative breeding, parental care (Balshine et al. 2001, Clutton-Brock 2002) and enhanced foraging efficiency (Pitcher et al. 1982, Rypstra 1989). It can even provide individuals with protection from aggression or predation through communal territory defence (Krebs et al. 1972, Port et al. 2011), mobbing behaviour (Hinde 1954, Krams et al. 2002), enhanced predator confusion, risk dilution and enhanced predator detection (Turner et al. 1986) (for review of group living, see Alexander (1974), Krause et al. (2002), Ward et al. (2016)).

Within these animal groups, individuals often act as a cohesive whole, resulting in the emergence of collective behaviour. One apparent example of collective behaviour is the synchronisation within moving animal groups, often seen in schools of fish or flocks of birds. In fact, the coordination between individuals within moving groups has been shown to reduce the likelihood of predators attacking (Ioannou et al. 2012), possibly through confusion effects (Landeau et al. 1986), and can benefit individuals by reducing the energetic costs of movement (Bill et al. 1976, Herskin et al. 1998). Despite the decades of research investigating collective behaviour, recent advances in tracking technology mean that more complex analyses are now possible and research can provide valuable insight into unexplored facets of collective behaviour.

This thesis aims to apply the framework of Tinbergen’s 4 questions to the study of collective behaviour and to capitalize upon these recent technological and computational advances to further our understanding of collective behaviour. Using various fish species as model systems, I address the history of grouping behaviour, investigating the development of collective movement characteristics (Chapter 2). I then detail the specific speed-mediated mechanisms producing group wide phenomena in moving animal groups, making the link between individual and group level characteristics (Chapter 3). Finally,
I investigate the adaptive value of collective behaviour in wider ecological contexts (Chapter 4 and Chapter 5).

**Development of Collective Behaviour**

Sociality, or the tendency to live in groups, is incredibly widespread throughout the animal kingdom. However, the extent to which collective behaviour is displayed or utilised varies both within and between species. This is likely due to the intricate interplay between the costs and benefits. While living in groups can improve predator detection and avoidance, enhance mate choice and foraging efficiency (Ward et al. 2016), as well as provide energetic advantages (Herskin et al. 1998, Ritz 2000, Marras et al. 2015), it comes at the cost of increased risk of infection or disease (Freeland 1976, Cote et al. 1995, Tella 2002, Godfrey et al. 2009), as well as greater competition for resources such as food (Skogland 1985) and mates (Schradin et al. 2010).

As a result, environmental or ecological conditions can influence the tendency to form groups as they determine the relative costs and benefits of grouping behaviour. Research on Trinidadian guppies has shown that the tendency to group shifts with the severity of predation (Seghers 1974). When individuals are transplanted to locations with different predation regimes, the tendency to group subsequently increases or decreases depending on whether the novel environment has higher or lower predation (Magurran et al. 1992). The physical environment can also shape social behaviour. In environments where food patches are sparse, many animals form groups due to the benefits of swarm intelligence and enhanced foraging efficiency (Snijders et al. 2018). In experiments on goldfish, *Carassius auratus*, and minnows, *Phoxinus phoxinus*, groups found food patches more quickly than solitary individuals and searching time decreased with group size (Pitcher et al. 1982). In mixed-species flocks of blue tits,
Cyanistes caeruleus, and great tits, Parus major, larger flocks were better at problem solving, leading to greater per-capita seed intake rate for larger flocks (Morand-Ferron et al. 2011).

With the benefits of foraging as part of a group well documented, it comes as no surprise that temperature (and by extension season) can alter social behaviour through its impact on the foraging environment. Yellow-eyed juncos, for instance, who must increase foraging activity at low temperatures to meet higher energy demands, form larger groups around foraging patches at colder temperatures (Caraco 1979). In coyotes, colder temperatures and snowfall during winter months reduces the availability of small rodent or insect prey, resulting in a shift from solitary or pair hunting to the formation of large hunting packs, which improves hunting success as groups are forced to target larger prey species in the winter (Bowen 1978, Gese et al. 1988). As demonstrated by these studies, plasticity in grouping behaviour is especially important for individuals living in variable environments, where the relative costs and benefits of grouping may shift on a seasonal or annual basis.

However, plasticity in grouping behaviour is important not just for individuals in seasonal environments. In fact, the conditions favouring grouping behaviour can shift on a daily (e.g. diurnal) or moment-to-moment basis. Recent research by Hansen et al. (2016) found that crimson spotted rainbowfish, Melanotaenia duboulayi, shifted inter-individual distances based on their internal nutritional state. When both fish in a pair were satiated, they swam closer together than when both fish in a pair were hungry. These shifts in grouping behaviour based on internal factors mirror shifts found in grouping behaviour based on external factors. For instance, when food cues are added to the environment, Schaerf et al. (2017) found that groups of x-ray tetras, Pristella maxillaris, increased distances to neighbours and adopted faster swimming speeds, resulting in an overall reduction in grouping behaviour. However, when predator cues were introduced into the water, these same individuals slowed down and formed
tight groups. Other work on x-ray tetras has found that individual positioning and overall information flow across groups changes based on the ratio of hungry to satiated fish within the group (Wilson et al. 2019). These immediate adjustments in grouping behaviour based on ephemeral internal or external states has been demonstrated in other species, such as banded killifish, who preferred to join the larger of two shoals after a simulated avian predator attack (Krause et al. 1994) and who change group size based on the presence of food cues or predator cues (Hoare et al. 2004).

Taken together, these studies underscore the flexibility of grouping behaviour and highlight the ways in which collective behaviour can develop or change within the lifespan of an animal. They help establish the fact that animals can assess changes in their environment and adjust behaviour accordingly. However, there is a dearth of research investigating the whether these altered group-level behaviours are a reflection of differences in the underlying mechanisms of schooling behaviour. This is especially surprising in light of recent work demonstrating how local interaction rules are not static, but in fact shift according to context (Herbert-Read et al. 2017, Schaerf et al. 2017). Herbert-Read et al. (2017) found that in addition to the long established differences in group size and cohesion between high and low predation guppies (Seghers 1974, Huizinga et al. 2009), the individual interaction rules shift based on background predation regimes. Ultimately, these studies underscore the potential insight that could be gained by extending our analysis of group behaviour beyond simple measures of neighbour distance or average group size.

Chapter 2 aims to utilise recent tracking and computational advances to determine how schooling behaviour differs between different seasons and between wild and lab-bred fish. This chapter will shed light on the extent to which individuals respond to changes in their developmental environment and how this translates into altered individual and group-level patterns. Ultimately, within the framework of
Tinbergen’s 4 questions, gaining a fuller understanding of how collective behaviour develops within an individual’s lifetime, or how it adapts to changes within the environment is crucial because it is likely to determine the current utility of the behaviour.

**Mechanisms of collective behaviour:**

To retain the benefits of living in a group, animals must also move as a group. Often, the resulting group movement is highly synchronised, with complex group-level patterns emerging, such as a torus of fish, a murmuration of starlings, a V-formation in a flock of geese or the bidirectional lane formation in termites or ants. To understand how animals achieve this coordinated collective motion, researchers have often sought to characterise the interactions occurring at the individual level and how these scale up to produce group-wide phenomena.

For many years, the behavioural mechanisms enabling such coordinated behaviour was limited to modelling approaches, such as the self-propelled particle models (Okubo 1986, Vicsek et al. 1995, Czirok et al. 2000), which created a strict set of rules by which individuals interacted with one another. Generally, these models implemented a repulsion zone immediately around the focal individual to avoid collisions and a longer-range attraction zone to facilitate group cohesion. Many of these models were able to recreate group patterns using speeds, neighbour distances and alignments consistent with real-world observations. However, these models made it difficult to draw specific conclusions about the actual behaviours adopted in natural systems because many simulations replicated observed global patterns whilst implementing vastly different rules of interaction.

This begged the question: which, if any, of these behavioural mechanisms were the ones implemented by animals in the real world? With advances in tracking software, it has only become possible in recent
years to utilise empirical research to uncover the exact mechanisms employed at the local level to produce observed global phenomena (Lukeman et al. 2010). As in the self-propelled particle models, empirical work established the existence of short-range repulsion and long-range attraction zones. However, as these forces interact, individuals began to align with neighbours at intermediate distances. Crucially, these empirical studies found that individuals adjusted distance and alignment with neighbours primarily through changes in speed (Herbert-Read et al. 2011, Katz et al. 2011).

While these studies were a crucial first step in characterising the underlying mechanisms of collective motion, subsequent research has underscored the way in which slight adjustments in local rules can change group-wide patterns. For instance, Schaerf et al. (2017) found that the way in which individuals responded to near neighbours and the impact this has on global patterns shifted with context, testing the effect of food cues and predator cues on individual and group behaviour. Similarly, Herbert-Read et al. (2017) found that slight differences in the social interaction rules adopted by guppies originating from high or low predation sites resulted in different group sizes and altered group-level cohesion. These studies underscore the importance of individual behaviour in mediating global patterns in collective behaviour.

Given that the advances facilitating these studies are relatively new, there is still a lack of empirical insight into the exact mechanisms producing the shifts between different collective states (Buhl et al. 2006, Tunstrom et al. 2013). This is surprising as moving animal collectives display a wide range of global patterns, even within the same group or species. For instance, birds can be seen mobbing predators (e.g. piñon jay (Balda et al. 1971)), aggregating loosely above fish markets (e.g. seagulls), travelling in highly ordered skeins (e.g. geese (Badgerow 1988)), or creating shape-shifting aggregations
that nevertheless maintain cohesion (e.g. European starlings (King et al. 2012))(review of avian fight formations see Heppner (1974)).

Our lack of empirical insight into the mechanisms mediating these shifting patterns is significant as it limits our ability to understand why these global patterns might exist or what their functional value is at both the individual and group level. Given that many animals aggregate but never display coordinated collective behaviour (Attanasi et al. 2014), it is interesting to investigate the mechanisms mediating the shift between collective states as a means of identifying the additional benefits individuals might derive from elevating grouping behaviour to fully synchronised collective behaviour. Research suggests that as groups coordinate collective movement, individuals benefit from enhanced foraging efficiency (Bousquet et al. 2011, Bazazi et al. 2012, Cvikel et al. 2015) and reduced vulnerability to predators (Ward et al. 2011, Ioannou et al. 2012). These benefits might be due in part to greater decision-making ability and information flow between individuals who coordinate behaviour. This is supported by recent work on flocks of starlings, *Sturnus vulgaris*, in which the authors found faster information transfer between individuals when groups were highly polarised (i.e. when individuals were highly aligned with neighbours) (Attanasi et al. 2014). This underscores the importance of investigating the mechanisms governing collective behaviour as a way of understanding the adaptive value of collective behaviour more broadly. **Chapter 3** addresses how speed, alignment and neighbour positioning all combine to govern group structure. Importantly, this chapter addresses the adaptive value of each behavioural mechanism mediating these shifting global patterns by investigating how speed and alignment impact information flow between members of the school.
Function of Collective Behaviour:

In order to stay alive, all animals must eat and avoid being eaten. This means that individuals must balance the need to forage against the need to avoid predation. Unfortunately, these two imperatives are often mutually exclusive. As animals forage, they expose themselves to greater predation risk because they inevitably spend less time on the lookout for predators (Godin et al. 1988). Conversely, as animals reduce activity levels (Ryer et al. 1998) or seek refuge from predation (Hedrick et al. 2006), they reduce the amount of time available for energy acquisition (Lima et al. 1990). Luckily, living in groups can enhance predator recognition (Mathis et al. 1996, Ferrari et al. 2007, Ward et al. 2011) and enable individuals to better assess risk and therefore allocate behaviour between these two contrasting necessities more effectively.

For instance, in deciding when and where to forage or hide, animals should integrate information from both the abiotic and biotic environment into their risk assessment. Abiotically, the level of risk an animal is exposed to when foraging or mating may be determined by the amount of protection or camouflage provided by the environment or by the level of visibility (Metcalfe 1984, Lima 1987, Cowlishaw 1997, Powolny et al. 2014). Griesser et al. (2009) found that Siberian jays reduced vigilance behaviour when living in unmanaged forests, which provide greater visual cover, compared to managed forests. However, while this reduction in risk-perception in complex environments makes sense for prey species that use hiding as a primary response to predator attacks, the opposite trend is found in species that rely on early predator detection and escape tactics (Devereux et al. 2006).

In addition to the impact of the physical or abiotic environment, the biotic or social environment can also shape an individual’s perception of risk. For instance, in larger groups, many eyes enhance the likelihood of early predator detection (Lima 1995, Ward et al. 2011). A study on free-ranging
emus, *Dromaius novaehollandiae*, found that despite decreased per-capita vigilance in larger groups, predators were detected sooner (Boland 2003). This positive impact of group size on risk perception can extend to heterospecifics within the environment as well, especially when heterospecifics are vulnerable to the same predators (Goodale et al. 2017). In mixed-species bird flocks, recordings of vocalisations of greater racket-tailed drongos, *Dicurus paradiseus*, elicited both mobbing and fleeing anti-predator behaviours in orange-billed babblers, *Turdoides rufescens*, and ashy-headed laughingthrushes, *Garrulax cinereifrons* (Goodale et al. 2014).

Despite all the evidence that risk-balancing in prey groups is shaped by both social and physical aspects of the environment, few studies have assessed how these factors interact to shape risk assessment and subsequent behavioural decisions. This is a problematic gap in the research given the likelihood that both physical and social features play a part in risk-perception. To underscore this, work on eastern chipmunks, *Tamias striatus*, has shown that vigilance behaviour increased in riskier environments, as shaped by physical aspects of the environment, and that the social environment subsequently amplified anti-predator behaviours rather than reducing them (Clermont et al. 2017). Chapter 4 sought to investigate collective behaviour within an ecological context, specifically examining how humbug damselfish in coral reef environments assess and respond to changes in risk based on both abiotic and biotic features of their environment.

Once a predator is in the vicinity of prey, this ability to assess risk may become even more important. However, less is known about how prey adjust behaviour or assess risk in environments where prey are within constant striking range of predators. In these situations, visual cues are likely to play a large role in prey behaviour. Research by Pavlov et al. (2000) found that prey living in constant proximity to potential predators maintain visual contact with predators rather than moving away. They hypothesized
that this might be a way of controlling risk by allowing prey to monitor predator behaviour. In a study by Magurran et al. (1987) minnows, *Phoxinus phoxinus*, placed in a tank with pike predators, *Esox lucius*, shifted between various anti-predator behaviours, escalating the severity of their responses as pike shifted from stationary to stalking or striking behaviour. This study demonstrated how prey scale their behavioural responses to the severity of predation threat based on predator behaviour.

Ideally, prey should assess risk by incorporating information from not just predator behaviour, but also the location of the predator’s mouth, which is the area of greatest risk often referred to as the attack cone (first described by George (1960)). Ultimately, the level of threat posed by a predator depends not only on its behaviour, but also on its proximity and alignment relative to the prey. The extent to which prey are capable of integrating all these variables into their routine behavioural decisions is unknown. While this shortcoming is due in part to the historic lack of advanced tracking capabilities, there has also been a tendency to treat risk as a fixed factor (Lima 2002). As a result, there is a dearth of research investigating how prey gauge the threat posed by a predator on a moment-to-moment basis and the extent to which they incorporate this information into their behavioural decisions. Chapter 5 investigates how prey individually and collectively responded to predation risk, as mediated by predator behaviour and angular position. Crucially, in this chapter I characterise the exact shape of the relationship between prey response variables and the relative alignment of the predator.

**CHAPTER OUTLINE**

Chapter 2 focuses on the historical properties of schooling behaviour, addressing the question of development. Specifically, this chapter will investigate the effects of season and provenance on the development of different movement characteristics. Chapter 3 uses automated tracking to investigate how speed produces the elongation seen in moving groups, detailing the exact shape of the relationship
between speed, polarization and positional composition. Importantly, this chapter investigates how individual movement characteristics influence information transfer and the potential impact speed and alignment has on group-wide functioning. **Chapter 4** investigates collective movement patterns in a wider ecological context by observing how humbug damselfish change the amount of time spent foraging rather than hiding based on social and physical aspects of their environment. In **Chapter 5**, I investigate the extent to which prey adjust their behaviour based on predator behaviour and position. Finally, in **Chapter 6**, I provide a short summary of my findings and propose potential avenues for future research.
REFERENCES


CHAPTER 2

CONSISTENCY IN SCHOOLING BEHAVIOUR BETWEEN SEASONS AND PROVENANCE

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The conditions experienced by animals shape the relative payoff of grouping behaviour. As a result, animals often shift their social tendencies as a dynamic response to their environment. However, little is known about how adjustments in the tendency to group alter the underlying mechanisms of collective behaviour. The Eastern mosquitofish, Gambusia holbrooki, is an invasive species that breeds throughout much of the year where they occur in Australia. To investigate whether the developmental or seasonal environment impacts schooling behaviour, we collected mosquitofish from 6 locations throughout New South Wales during the autumn and again during the spring. We then raised the F1 generation of spring fish in the laboratory and compared schooling behaviour between lab-bred and wild-caught fish. We quantified not only broad-scale social and asocial measures of schooling (i.e. local group structure and median nearest neighbour distances, alignment and speed), but also characterised the underlying mechanisms of schooling behaviour (i.e. the rules of interaction). We found that despite slight differences in speed and alignment, the individual interaction rules and global group properties were consistent across seasons and between wild-caught and lab-bred fish, suggesting that mosquitofish retain the ability to interact coherently within a social setting regardless of, or in spite of, differences in developmental experience.

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Introduction

Living as part of a larger social group has many associated benefits, such as increased access to mates, enhanced foraging efficiency, reduced energetic costs of movement and safety from predation, as well as some associated costs, such as greater rates of infection, increased competition for resources and greater conspicuousness to predators (Krause et al. 2002, Ward et al. 2016). While the net effect of group living is usually positive, the proximate conditions experienced by animals can strongly affect the interplay between these costs and benefits such that animals shift their social tendencies as a dynamic response to their conditions. In particular, conditions experienced during early development may play an important role in shaping patterns of behaviour.

Studies have shown that the social environment during early development can alter grouping behaviour later in life. In guppies, *Poecilia reticulata*, individuals reared with a low density of conspecifics (i.e. between 1 to 4 individuals) showed a greater tendency to shoal with conspecifics, as well as a greater ability to learn through social cues, compared to individuals reared in high density tanks (i.e. between 7 to 12 individuals) (Chapman et al. 2008). This impact of the early social environment might be particularly important for species living in seasonal environments where population densities can vary predictably between seasons, often due to factors such as recruitment to the population through breeding in spring, and mortality during harsh winter conditions (Allen 1982). Furthermore, within these seasonal environments, differences in early life conditions, such as temperature and day length, can lead to substantial physiological and behavioural differences (Seikai et al. 1986, Johnston et al. 1997, Johnston et al. 2001, Ojanguren et al. 2003, Georgakopoulou et al. 2007, Koumoundouros et al. 2009, Sfakianakis et al. 2011, Le Roy et al. 2017, Le Roy et al. 2018), although this has seldom been explored in the context of grouping behaviour.
In addition to this influence of early developmental environment on grouping behaviour, there are many examples of continued development later in life as a function of changing environmental conditions. In cooperative hunters such as coyotes, group sizes differ between populations and between seasons based on the size of their prey (Bowen 1978). In colder seasons, when the availability of rodents and insects decline, coyotes form larger groups as they shift to larger prey species, such as pronghorns (Gese et al. 1988). These larger groups increase hunting success and can be advantageous when there is enough food to share between all pack members. Similarly, empirical work on estuarine dolphins, *Sotalia guianensis*, found shifts in mean group size and cohesion based on whether groups were travelling or foraging, with the frequency of these behaviours shifting based on season (Daura-Jorge et al. 2005).

Given this apparent plasticity in grouping behaviour based on different environmental conditions, it is no surprise that studies have also found differences between wild-caught and lab-bred individuals and between experiments conducted in laboratory conditions versus natural conditions (Barnett 1958, Hayes et al. 1990, Krause et al. 2000). Work done by Irving et al. (1997) found that European minnows’ response to alarm cues differed based on whether they were in small aquaria with plastic plants and no flow or in near-natural fluvarium conditions with gravel and real weed patches. They found that the typical suite of behaviours observed in laboratory experiments were much more muted and transient when tested in near-natural conditions. Similarly, the intensity of anti-predator behaviour differed between wild-caught Atlantic salmon and their hatchery-reared offspring, with these differences being even more pronounced in the hatchery-reared F2 generation (Jackson et al. 2011).

The differences observed between laboratory and wild fish could potentially be explained by a study on first generation guppies that found a reduction in brain size when individuals were reared in laboratory rather than natural conditions (Burns et al. 2009). In fact, a study on mosquitofish found that brain size
decreased after only 6 weeks in a laboratory environment (Turschwell et al. 2016). In addition to the potential impact of these physiological changes on fish behaviour, it is likely that differences in behavioural patterns between wild and laboratory fish can result from a suite of different factors, such as smaller rearing environments, altered foraging experiences, a lack of predation experience or artificial social environments (Wright et al. 2006).

The current literature points to the fact that animals can assess changes in their environment and adapt their grouping behaviour in response. However, little is known about how these altered group-level behaviours are a reflection of differences in the underlying mechanisms of collective behaviour. Due to recent advances in tracking technology, we now have the capacity to quantify not just neighbour distances or social tendency, but to empirically characterise how individuals adjust their position or velocity as a function of the relative location and behaviour of near neighbours (a suite of responses sometimes referred to as the ‘rules of interaction’) (Lukeman et al. 2010, Herbert-Read et al. 2011, Katz et al. 2011). Recent work by Schaerf et al. (2017) and Herbert-Read et al. (2017) demonstrated that these local interaction rules are not static, but in fact shift according to context. Herbert-Read et al. (2017) found that in addition to the long established differences in group size and cohesion between high and low predation guppies (Seghers 1974, Huizinga et al. 2009), the individual interaction rules shift based on background predation regimes. Ultimately, these studies underscore the potential plasticity in the mechanisms of collective behaviour, and more research is needed to understand whether seasonal changes in the environment or differences in developmental conditions might shift schooling mechanisms. These types of studies are important as we strive to understand population-level or group-level functioning.
Here, we investigated the extent to which the Eastern mosquitofish, *Gambusia holbrooki*, a highly successful invasive fish, altered their schooling behaviour based on different seasonal environments. Mosquitofish breed throughout much of the year where they occur in Australia, hence new recruits to the population face potential differences in early life experiences according to whether they were born in winter, when populations are at their lowest densities, or in summer, when densities are at their greatest. We sought to quantify seasonal plasticity in schooling behaviour by determining not only the broad-scale social and asocial measures of schooling (i.e. local group structure and median nearest neighbour distances, alignment and speed), but also quantifying the underlying mechanisms of schooling behaviour (i.e. the rules of interaction). Furthermore, as many studies on fish are conducted within laboratory environments, often on laboratory-bred generations, we sought to understand how laboratory conditions might alter the way animals move by comparing our wild-caught fish to their laboratory-bred offspring. This was done to determine whether the difference in early life experience between lab-bred and wild-caught fish might shift the way animals move. Ultimately, by comparing fish from different seasons and from both natural and laboratory conditions, we hoped to determine the flexibility in the rules of locomotion and to uncover the effect of developmental experience on collective behaviour more broadly.
Materials and Methods

Study species and husbandry

Mosquitofish (*Gambusia holbrooki*) were collected from six sites across Sydney, all within 100km of the location where mosquitofish were originally introduced into Australia at the Royal Botanical Gardens in the 1920s (as per Seebacher et al. (2012)). Fish were collected from 2 locations within the Blue Mountains: Lake Catalina (33°42′41″S, 150°18′14″E) and Glenbrook Lagoon (33°45′37″S, 150°36′46″E), as well as from 4 locations within Sydney’s coastal plain: Homebush (33°51′51″S, 151°04′56″E), Centennial Park (33°54′00.2″S 151°14′04.5″E), Manly Dam (33°46′19″S, 151°14′45″E) and the Royal Botanical Gardens (33°51′53″A, 151°13′00″E). The purpose of using six different populations was to establish whether there was generality in our findings across populations.

Within each of these sites, fish were collected using large nets from water depths ranging from 0.1 to 0.5m. Fish were collected first during autumn, between April and May 2017, then again in spring between October and November 2017. Given that all fish were collected as mature adults, individuals in this study would have developed during the season prior to capture, meaning fish caught in autumn developed during the warmer summer months and fish caught in the spring developed during the colder winter months (see (Seebacher et al. 2012) and (Seebacher et al. 2014)). For the 3 days prior to capture, average maximum air temperatures were similar across collection sites (average ± sd: 20.7 ± 2.8°C in autumn and 22.7 ± 4°C in spring) (data was collected from weather stations throughout NSW by the Bureau of Meteorology). In the 2 weeks prior to capture, average maximum air temperatures were also similar across collection sites (21 ± 2.8°C in autumn and 22.9 ± 3.8°C). Although we did not measure water temperatures directly, the similar trends in air temperatures across sites, and the similarity in depth and water flow across locations, indicates that water temperatures would probably have been broadly similar across all locations at the time of collection.
Female individuals collected during the spring period were isolated after participating in experiments and their offspring were collected within a 1-month period. To ensure genetic diversity within our F1 generation, we used a minimum of 10 females, who are known to produce broods with mixed paternity (Zane et al. 1999), from each population. Offspring were given 3 months to reach full maturity before use in experiments. To provide offspring with social experience akin to their wild-caught counterparts, they were housed in groups of 40 within identical glass tanks containing gravel, live plants and foam sponge filters. For wild-caught fish, individuals were given 2 weeks to acclimate to laboratory conditions before use in experiments. Given that mosquitofish are a short-lived species, 3-month-old fish from the laboratory would have been similar in age to their wild-caught counterparts who were collected as mature adults from the field. Fish caught in the autumn were 22.84 mm ± 0.85 mm (mean ± s.d.), while fish caught in the spring were 21.72 ± 3.87 mm and lab-bred fish were 21.28 ± 1.28 mm (for within population and season SLs, see Table S1).

All fish were held in separate 30 litre glass stock tanks with a constant temperature of 24 ºC and a photoperiod of 12:12 hours light: dark. To standardise motivation across trials, no fish were fed within 17 hours of trials. It is important to standardise motivation because previous studies have shown that individual state within a group and amongst groups can have strong effects on mosquitofish movement and group level dynamics (Hansen et al. 2015).

**Experimental Arena:**

We used two identical square 73 x 73 cm white Perspex arenas filled to a depth of 7 cm with conditioned tap water maintained at the same temperature as the stock tanks. The depth was chosen as mosquitofish swim in a narrow horizontal plane and form shoals in shallow freshwater habitats at the
water’s surface, hence an approximation of movement based on two dimensions is appropriate. Water was aerated for 24 hours before trials in order to optimise water quality.

Before each trial, 1 litre of water from the stock tank was added to the experimental arena to provide fish with conspecific chemical cues in the water (Ward 2012). At the same time, 1 litre of water was removed to maintain water levels between trials. The arena was emptied and rinsed with water and subsequently refilled with conditioned water before each day of experiments. This process optimises water quality and provides a stable environment across trials. Lighting was provided by two LED strips with a colour temperature of 6500K, which resembles overcast daylight conditions. To isolate the arena from external disturbances, the whole experimental setup was surrounded with white poster board.

**Experimental protocol**

Given that Mosquitofish are a social species and their behaviour is shaped by their social environment (Ward, 2012), we selected experimental group sizes based on observations in the wild, which indicate that they typically travel in small groups that include both males and females (personal observation). Accordingly, we tested fish in mixed-sex groups of 4 individuals (ranging from a ratio of 1:3 to 3:1 male:female) (see Table S1 for number of trials with each sex ratio within population and season). To investigate the effect of season on schooling behaviour, we ran 12 replicates for each population collected in the Autumn, 12 replicates for each population collected in the Spring and 12 replicates for the F1 generation of each population caught during the spring (with the exception of Manly Dam, which only had enough fish for 11 replicates). In total, we conducted 215 separate trials.

Before each trial, fish were sexed and placed in small clear containers that were used to transport them to the experimental arena. These groups were gently released from the clear container into the
experimental tank and filming began after 30 minutes. Previous work with mosquitofish has established that activity levels peak after 5 minutes in the arena then gradually decrease until they stabilise around 29 minutes (Diaz 2017). Filming after 30 minutes therefore allows us to examine baseline levels of activity.

Trials were filmed from above using a Canon G1X camera positioned 1.2m directly above the arena, with a resolution of $1920 \times 1080$ pixels at 24 frames per second. The order of the trials was randomised with respect to population and sex ratio. Following completion of each trial, each fish was photographed and their body length was measured using ImageJ software (Rasband 1997-2012). Once photographed, fish were transferred into a separate holding tank to ensure no individual was used more than once.

**Data Analysis:**

Videos were converted into old format .avi files using VirtualDub (v 1.9.9), then uploaded into the semi-automated tracking software CTrax (Branson et al., 2009), which tracked the position of each individual at every frame of the video. Any errors that occurred from this automated tracking were then corrected using the Fixerrors GUI in MATLAB. This generated $(x, y)$ coordinates for each fish and their orientation (in radians) for every video frame, which was used to calculate (i) median speed (body lengths per second), (ii) median nearest-neighbour distances (NND) (BL), (iii) median angular difference in heading direction between each fish and its nearest neighbour (degrees) and (iv) mean group size. Individuals were considered to be part of a group when within 5 body lengths of at least one other individual in the group, with overall group membership determined using the algorithm described in (Hansen et al. 2015, Hansen et al. 2015). Angular differences in heading direction were only determined for fish within a group. Given that body size varied between populations, with larger fish generally observed in the colder waters of mountain habitats versus the lowland populations, we
standardised speed and nearest neighbour distances by examining them relative to body lengths using the population average (within season). Due to the fact that we did not know which trajectory corresponded to which SL, the mean SL for each trial was recorded for each individual within the trial. We used medians for our response variables because the distributions of each metric were positively skewed.

To visualize group structure and to determine the local rules of interaction, we followed methods outlined in the supplementary material of Schaerf et al. (2017). These methods produce a sequence of plots centred around a “focal individual” located at the origin travelling parallel to the positive x-axis. First, we created line graphs showing the probability of observing group members at specific x- or y-coordinates relative to the focal individual. We then created graphs showing the mean speed, mean change in speed over time and mean change in direction of motion over time of the focal individual as a function of the relative x- or y-coordinates of their neighbours. Next, we created heat plots to visualise the probability of observing groupmates at particular relative (x, y) coordinates. As with our line graphs, these density heat plots were created by centring each individual at the origin and rotating it so that its direction of travel was parallel to the positive x-axis, then recording the positions of neighbouring fish relative to the focal fish. This was repeated for each frame and for each individual in turn. Analogous methods were used to create (a) heat plots showing an individual’s mean speed given the relative position of neighbours, (b) heat plots showing an individual’s mean change in speed over time given the relative position of neighbours, (c) heat plots showing an individual’s mean change in heading direction over time when neighbouring fish were in different positions relative to the focal fish and (d) alignment heat plots showing both the mean relative directions of motion of partners at given (x, y) coordinates (illustrated with arrows) and a measure of the degree of focus of all the relative directions of motion observed for partners at given (x, y) coordinates (on a scale of 0, indicating high angular variance, to 1,
indicating perfect alignment about the mean). (The construction of the alignment plots is detailed in the supplementary material of both (Schaerf et al. 2017) and (Davis et al. 2017), and further details of the associated circular statistics can be found in (Fisher 1995)).

**Statistical Analysis**

To assess whether speed, NND, alignment and group size varied according to season or provenance, we created mixed effect models in R using the lmer package. Each behavioural measure was tested first in a mixed effect model against the categorical factor of season (i.e. autumn wild-caught vs. spring wild-caught) and then in a separate mixed effect model against provenance (i.e. spring wild-caught vs. F1 spring lab-bred). To account for the non-independence of individuals within the same group, we included standard length (which was unique for each group and repeated for each individual with the group) nested within population as a random effect. The number of males was also included as a random effect. We constrained our analysis of fish provenance to a comparison between wild-caught fish from spring and their lab-bred offspring due to the known impact of maternal effects on offspring development (Giesing et al. 2011). To meet the assumptions of normality, NND was log transformed.
Results

Basic asocial measures

Fish caught during autumn swam slower than fish caught during spring (ANOVA of mixed effect model: $F_{1,133}= 27.69$, $p<0.001$), and fish caught in the spring swam slower than their lab-bred offspring ($F_{1,132}= 12.66$, $p<0.001$) (Figure 1A).

Basic social measures

Fish caught during the autumn swam further from nearest neighbours compared to fish caught during the spring ($F_{1,78}= 4.03$, $p=0.048$), though there was no significant difference between wild-caught spring fish and lab-bred spring fish ($F_{1,90}= 0.01$, $p=0.92$) (Figure 1B). Spring fish were more aligned with nearest neighbours compared to autumn fish ($F_{1,97}= 8.28$, $p=0.005$), though less aligned compared to their lab-bred offspring ($F_{1,132}= 5.10$, $p=0.03$) (Figure 1C). Group size was not significantly different between autumn or spring fish ($F_{1,79}= 2.48$, $p=0.12$), or between spring wild-caught and lab-bred fish ($F_{1,94}= 1.25$, $p=0.27$).

Interactions between mosquitofish in moving groups

We found that for all mosquitofish, there was a greater probability of finding partner fish in front or behind rather than to either side (Figure 2A, B and C). There was also a consistent tendency to increase speeds when other groups members were further than 40mm (roughly 2 body lengths) in front of them and decrease speeds when group members were greater than 2 body lengths behind (Figure 4A and C). These results suggest that fish from both seasons and provenance use speed regulation to adjust neighbour positioning.
Figure 3C shows that average individual speed was greatest when partner fish were located along the axis of motion and when neighbours were further than 1.5 body lengths away (in front or behind). The slowest speeds occurred when neighbours were within 1 body length of the focal fish, suggesting that (in conjunction with Figure 5B and C showing a tendency to turn towards neighbours located on either side regardless of distance) mosquitofish slowed down as they turned towards lateral neighbours. This shift between moving away from neighbours within 1.5 body lengths and moving towards neighbours further than 1.5 body lengths away corresponds to the peak density of neighbours at 1.5 body lengths away along the axis of motion of focal individuals (Figure 2C).

On average, directions of motion of focal individuals and their groupmates were most closely aligned when groupmates occupied the region behind the focal individual, approximately where x < -20 mm and -20 mm < y < 20 mm, or when groupmates occupied the region in front of the focal individual, approximately where x > 20 mm and -20 mm < y < 20 mm (as illustrated by the arrows in Figure 6). Both these regions were relatively close to the axis of motion of the focal individual in terms of y-coordinates, suggesting that group members were more aligned locally when moving in single file. Observed relative directions of motion were more focussed (exhibited less variance) about the mean when groupmates were located approximately 1.5 body lengths in front or behind focal individuals (represented by the warmest colours in Figure 6). These regions of decreased variance approximately coincide to the distance at which focal individuals switched from changes in speed consistent with avoiding near neighbours (at less than 1.5 body lengths) to changes in speed consistent with attraction to farther neighbours (at distances greater than about 1.5 body lengths).

*Between autumn and spring fish*

One of the most apparent differences between mosquitofish caught during the autumn and mosquitofish caught during the spring was in their elective swimming speeds. Figure 3A and B shows that spring fish
swam faster across all relative neighbour positions and therefore maintained the same speed profile relative to neighbour positioning. As spring fish swam faster, they made greater changes to their speeds when neighbours were greater than 2 body lengths away (Figure 4A), likely a result of their greater overall speed. Similarly, spring fish increased the magnitude of changes in their direction of motion based on neighbour positioning and increased the distance over which these changes were made (Figure 4B). Average relative directions of motion were similar between autumn and spring caught fish (arrows in Figure 6). However, spring fish exhibited greater focus (and thus less variance) about these mean directions up to 5 or 6 body lengths in front or behind, whereas autumn fish had smaller regions of reduced variance about the mean direction, out to roughly 3 body lengths in front or behind (coloured portions of Figure 6).

Between spring wild-caught and lab-bred fish

We found that the mechanisms of schooling behaviour were broadly consistent across spring wild-caught and lab-bred fish. However, there was slightly greater responsiveness to partners in wild-caught groups compared to lab-bred groups, as seen by the greater magnitude changes in speed and direction of motion towards partners beyond regions where there are repulsion-like effects. For instance, despite finding greater median speeds in lab-bred fish (Figure 1A), wild-caught fish had greater mean speeds when their partners were located in front of them, beyond distances of approximately 30 mm (Figure 3C). Figure 3B shows that wild-caught fish swam faster than lab-bred fish when neighbours were within 1 body length away on the y-axis (to the left or right of focal individuals), as well as more slowly than lab-bred fish when neighbours were further than 3.5 body lengths away in the y-direction. However, along the axis of motion (Figure 3A), both lab-bred and wild-caught fish travelled at similar speeds based on neighbour positioning in front and behind. Figure 4A suggests that wild-caught fish sped up and slowed down on average with slightly greater magnitudes than lab-bred fish when their partners
were further away than 50 mm in the relative x-direction (in front or behind), and Figure 5B shows that wild-caught fish turned towards distant lateral neighbours at slightly greater turning speeds compared to lab-bred fish. The slightly greater magnitudes of changes in speed and direction observed in the wild-caught fish suggest a heightened strength of attraction to other groupmates at intermediate distances compared to lab-bred fish.

In tandem with the reduction in speed and direction of movement adjustments, lab-bred fish increased alignment with groupmates. In lab-bred fish, the region of reduced variation in heading direction was maintained as far away as 7 body lengths, whereas this region was only maintained for 5-6 body lengths in wild-caught fish (warmest colour regions in Figure 6). Alongside this increased alignment in lab-bred fish, we also see slightly greater dispersion (out to distances of approximately 75 mm in front and behind) relative to a centrally-positioned fish (Figure 2C), which may be the result of greater variation in neighbour positioning relative to a focal fish (given that NND (Figure 1B) and the standard deviation of NND did not differ between wild-caught and lab-bred fish). However, given that the heat plot in Figure 2C records not just nearest neighbour distances, but rather the relative location and distance of all neighbours, we cannot dismiss the idea that lab-bred groups might have been slightly more dispersed compared to their wild-caught counterparts.
**Figure 1:** Boxplot showing median (A) median speed (BLs⁻¹), (B) log median nearest neighbour distance (BL) and (C) median degree of difference in heading with nearest neighbour between autumn wild-caught, spring wild-caught and spring lab-bred fish (F1 Spring). The horizontal lines inside each box are the median for each species, boxes span interquartile ranges, notches represent 95% confidence intervals and whiskers extend to the minimum and maximum values of each measure.
**Figure 2:** Line graphs show the probability of observing partner fish at different $x$ (A) and $y$ (B) coordinates relative to focal fish located at the origin and travelling parallel to the positive $x$-axis (travelling from left to right in A, and out of the page towards the reader in B). Heat plots (C) show the relative frequency (estimated probability) of partner fish occupying particular $(x, y)$ coordinates relative to focal fish located at the origin and travelling parallel to the positive $x$-axis. Warmer colours denote higher relative frequencies of partner fish. Figures for autumn wild-caught, spring wild-caught and spring lab-bred fish (F1 Spring) are shown separately.
Figure 3: Line graphs show the mean speed of focal fish as a function of the relative location of partner fish on either the \(x\) (A) or \(y\) (B) axis. Focal fish are located at the origin and travelling parallel to the positive \(x\)-axis (travelling from left to right in A, and out of the page towards the reader in B). Heat plots (C) show the mean speed of focal fish (located at the origin and travelling parallel to the positive \(x\)-axis) as a function of the relative \((x, y)\) coordinates of partner fish. Warmer colours denote faster mean speeds. Figures for autumn wild-caught, spring wild-caught and spring lab-bred fish (F1 Spring) are shown separately.
Figure 4: Line graphs show mean change in speed over time of focal fish as a function of the relative $x$ (A) or $y$ (B) coordinates of partner fish. Focal fish are located at the origin and travelling parallel to the positive $x$-axis (travelling from left to right in A, and out of the page towards the reader in B). Heat plots (C) show the mean change in speed over time of focal fish (located at the origin and travelling parallel to the positive $x$-axis) as a function of the relative $(x, y)$ coordinates of partner fish. Warmer colours denote increases in speed while cooler colours denote decreases in speed. Figures for autumn wild-caught, spring wild-caught and spring lab-bred fish (F1 Spring) are shown separately.
Figure 5: Line graphs show mean change in direction of motion over time of focal fish as a function of the relative $x$ (A) and $y$ (B) coordinates of partner fish. Focal fish are located at the origin and travelling parallel to the positive $x$-axis (travelling from left to right in A, and out of the page towards the reader in B). Heat plots (C) show mean change in direction of motion over time of focal fish (located at the origin and travelling parallel to the positive $x$-axis) as a function of the relative $(x, y)$ coordinates of partner fish. Warmer colours denote turns towards the left and cooler colours denote turns towards the right. Figures for autumn wild-caught, spring wild-caught and spring lab-bred fish (F1 Spring) are shown separately.
Figure 6: Mean relative directions of motion (arrows) and associated $R$ values (a measure of the focus of all observed relative directions of motion within a particular bin used in constructing the plots, represented by the colour scale from 0 (greatest variance) to 1 (greatest focus about the mean) of partner fish based on their location relative to focal fish, located at the origin travelling parallel to the positive $x$-axis. Figures for autumn wild-caught, spring wild-caught and spring lab-bred fish (F1 Spring) are shown separately.
Discussion

Our results show that fish caught during the spring swam faster, closer together and formed denser and more polarized groups compared to fish caught during the autumn. Lab-bred offspring adopted less consistent neighbour positions and swam faster than their wild-caught counterparts, although they showed reduced responsiveness to distant neighbours. Despite these differences, we found that regardless of season or provenance, mosquitofish retained the same underlying mechanisms of schooling behaviour. Individuals generally avoided collisions by speeding up when neighbours were too close behind and slowing down when neighbours were too close in front (consistent with a zone of repulsion) and maintained cohesion by speeding up when neighbours were too far in front and slowed down when neighbours were too far behind (consistent with a zone of attraction). Overall, behaviour consistent with collision avoidance was moderated by changes in speed, whereas behaviour consistent with attraction at greater distances was moderated by both changes in speed and direction.

Across all conditions, we found that mosquitofish formed groups that were elongated on a local scale (using individuals as a point of reference) with higher densities of neighbours in front and behind compared to on either side. It could be that the lower encounter frequencies to either side of focal individuals made it difficult to detect any change in angle based on lateral neighbour crowding. Empirical work on golden shiners (*Notemigonus crysoleucas*) and x-ray tetras (*Pristella maxillaris*) found evidence that focal fish turn away from neighbours on either side when they are too close (Katz et al. 2011, Schaerf et al. 2017). However, the current study found that mosquitofish slowed down and turned towards lateral neighbours regardless of distance, meaning that changes in speed could then be used to mediate distance to neighbours. This is similar to the findings of Herbert-Read et al. (2011), who also used groups of mosquitofish. Overall, it seems like collision avoidance behaviour is moderated by
changes in speed, whereas attraction at greater distances is moderated by both changes in speed and
direction.

In addition to the similar local group formations across conditions, our results are also consistent with
the idea that individuals have 3 zones of interaction (Aoki 1982, Huth et al. 1992, Couzin et al. 2002).
Previous work has established the existence of a repulsion zone immediately surrounding each
individual, in which collision avoidance is the main concern, a zone of attraction at greater distances, in
which individuals act to avoid separation from other group members, and an orientation zone at
intermediate distances, in which individuals align their direction of movement as attraction and
repulsion forces combine. In the current study, the colder regions at the origin of our density heat plots
suggest a much lower probability of encountering neighbours within 1 body length of the focal
individual, suggestive of a repulsion zone. The tendency to speed up when neighbours are too far in
front and slow down when neighbours are too far behind seems consistent with the idea of a zone of
attraction. Finally, as repulsion shifted to attraction at intermediate distances, we saw the highest density
of neighbour encounters as well as a tendency for greater alignment. It is worth noting that alignment
can emerge without an explicit alignment rule, but rather as the result of local attraction (Strombom
2011). As mosquitofish showed a tendency to turn towards neighbours on either side regardless of
distance, we saw decreased variation in alignment with neighbours along the axis of motion, rather than
with neighbours to either side. The more elongated group formations with peaks in neighbour densities
in front and behind individuals further explains this trend in alignment along the axis of motion,
however, it runs counter to trends in other species, such as sticklebacks (Ward et al. 2017) and x-ray
tetras (Schaerf et al. 2017), who show peak neighbour densities along the $y$-axis and alignment with
individuals on all sides rather than just along the axis of motion. This may suggest that there are
differently shaped orientation zones for different species, which would be an interesting avenue for future research.

Despite these similarities, differences in the elective swimming speeds of spring, autumn and lab-bred fish led to slight differences in group-level patterns, mainly seen in the elongation of groups along the axis of motion as fish swam faster, a trend that has been found in other fish species (Kent et al. 2019). Specifically, we observed that lab-bred fish formed more elongated and polarized groups compared to spring fish, who in turn formed denser, more elongated and polarized groups compared to autumn fish. This increase in polarization with speed is inevitable given that individuals must align more closely with neighbours to avoid collisions (Lukeman 2014). In addition to speed-mediated increases in group elongation and alignment, differences in elective swimming speeds also altered interaction patterns between individuals. As spring fish swam faster, they tended to make larger adjustments in both speed and turning speed based on relative neighbour positioning. This is mirrored by previous empirical work on mosquitofish that found that individuals travelling faster made relatively larger decelerations and accelerations based on linear neighbour positioning (Herbert-Read et al. 2011). Similarly, in a study on golden shiners, Katz et al. (2011) found that speed forces (the component of acceleration parallel to an individual’s velocity vector; in the mathematical continuous time limit this quantity is the same as the change in speed over time used in our study) and turning forces (the component of acceleration perpendicular to an individual’s velocity vector; in the continuous time limit this quantity is equal to an individual’s speed multiplied by the change in direction of motion used in our study) increased as a function of a neighbour’s swimming speed. Therefore, it is unsurprising that speed increases responsiveness to neighbours given that individuals travelling faster must slow down at greater rates to avoid collisions compared to individuals travelling more slowly.
However, this trend of greater speeds correlating with increased responsiveness was not observed between wild-caught fish and their faster lab-bred offspring. Instead, lab-bred fish made smaller magnitude changes in swimming speeds across relative neighbour positions. Previous research has found that lab-bred generations show reduced cohesion (or grouping) scores compared to wild-caught fish regardless of the predation regime experienced by the source population (Seghers 1974), which is mirrored by our observation that lab-fish were more spread out or occupied a greater range of relative neighbour positions. This means that while the differences observed between seasons relates to speed, the differences observed between wild-caught and lab-bred fish may relate to lower group cohesion, or greater dispersion in lab-bred fish. Alternatively, it might be that as lab-bred fish decreased speed and angular speed regulation in response to neighbours, this resulted in greater dispersion of individuals.

Overall, we found that the mechanisms of schooling behaviour were consistent across seasons and between wild-caught and lab-bred fish. However, plasticity in elective swimming speeds between seasons lead to larger zones of interaction and greater responsiveness between spring fish compared to autumn fish. While the faster speeds adopted by lab-bred fish corresponded with an increase in alignment, lab-bred groups showed greater dispersion or variation in positioning and reduced responsiveness to neighbours. Despite these differences, the similarities are highly apparent, suggesting that mosquitofish retain the ability to interact coherently within a social setting regardless of, or in spite of, differences in developmental experience.
References


SUPPLEMENTARY INFORMATION

Table S1. Mean SL (mm), standard deviation of SL (mm), and number of trials containing 1:3 (male:female), 2:2 and 3:1 by population and season.

<table>
<thead>
<tr>
<th>Population</th>
<th>Autumn</th>
<th>Spring</th>
<th>F1 Spring</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean SL</td>
<td>s.d.</td>
<td>ratio m:f</td>
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<tr>
<td>Lake Catalina</td>
<td>22.69</td>
<td>2.76</td>
<td>3:6:3</td>
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<td>Homebush</td>
<td>23.72</td>
<td>2.69</td>
<td>5:5:2</td>
</tr>
</tbody>
</table>
CHAPTER 3

SPEED-MEDIATED PROPERTIES OF SCHOOLING

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Collectively moving animals often display a high degree of synchronisation and cohesive group-level formations, such as elongated schools of fish. These global patterns emerge as the result of localised rules of interactions. However, the exact relationship between speed, polarisation, neighbour positioning and group structure has produced conflicting results and is largely limited to modelling approaches. This hinders our ability to understand how information spreads between individuals, which may determine the collective functioning of groups. We tested how speed interacts with polarisation and positional composition to produce the elongation observed in moving groups of fish as well as how this impacts information flow between individuals. At the local level, we found that increases in speed led to increases in alignment and shifts from lateral to linear neighbour positioning. At the global level, these increases in linear neighbour positioning resulted in elongation of the group. Furthermore, mean pairwise transfer entropy increased with speed and alignment, implying an adaptive value to forming faster, more polarised and linear groups. Ultimately, this research provides vital insight into the mechanisms underlying elongation of moving animal groups and highlights the functional significance of cohesive and coordinated movement.

Author contributions: MK and AW designed the experiments, MK performed the experiments, all authors performed the analysis and edited the manuscript, which was written by MK
**Introduction**

Living in a group can improve an individual’s foraging efficiency, increase access to mates, enhance the likelihood of detecting a predator and provide benefits such as collective defence, dilution and confusion effects (Ward et al. 2016). To retain the benefits of living in a group, animals must move as a group. The resulting collective motion, exemplified by a swarm of insects, a flock of birds or a school of fish, is often characterised by a surprising degree of coordination and synchronisation.

Remarkably, these cohesive group-level patterns emerge as the result of localised rules of interactions. Generally, individuals avoid collisions by moving away from group members that are too close and maintain cohesion by moving towards group members that are too far away. At intermediate distances, these repulsion and attraction forces interact to promote orientation and alignment with neighbours (Herbert-Read et al. 2011, Katz et al. 2011). Interestingly, individuals adjust distance and alignment with neighbours primarily through changes in speed, which points to the importance of speed in structuring collective movement and group morphology more generally. In fact, the relationship between speed and collective motion in animal groups has formed the basis of recent work (e.g. Pettit et al. (2015), Jolles et al. (2017)).

As it pertains to group structure, speed tends to affect an individual’s alignment with its neighbours. Specifically, fast moving groups tend to be more polarised (Lukeman 2014). This relationship between speed and polarisation in groups is partly explained by the need to avoid collisions between fast-moving individuals, necessitating a shift from low alignment to high alignment as group speed increases (Viscido et al. 2004). More broadly, speed is key to understanding shifts between different collective states, such as the shift from loosely polarised shoals, or mills, to highly polarised schools or swarms. Tunstrom et al. (2013) assert that these different collective states can be characterised broadly using
global properties, such as polarisation and the degree of rotation, but the shifts between these states is mediated by changes in speed.

Theoretical models have made considerable progress in examining the importance of speed to both local rules of interactions and global properties of the group. For instance, Hemelrijk et al. (2008) created models of collective behaviour to demonstrate how specific group morphologies, such as oblong schools, can arise through specific interactions between individuals. They asserted that as individuals slow down to avoid colliding with individuals in front of them, individuals who had previously been neighbours move in to fill the gap, resulting in a narrowing and elongation of the school. This simple mechanistic explanation led to two hypotheses, (1) that groups with more members are denser and more elongate due to the greater frequency of collision avoidance and (2) that slower groups would become more elongate due to lower polarisation necessitating greater frequency of collision avoidance. These hypotheses have additional empirical and theoretical support (Kunz et al. 2003, Hemelrijk et al. 2010). For instance, work on saithe by Partridge et al. (1980) found greater elongation in slower schools and more circular group formations at higher speeds.

However, Breder (1959) predicted the opposite trend, with elongation occurring at faster rather than slower speeds. This also has some empirical support, such as the work done by Pitcher et al. (1983) that found school shape to be more spherical when fish were motionless compared to ellipsoid when they began stable cruising behaviour. Breder (1959) observed that the elongated body shape of fish results in individuals reducing distances to neighbours located on either side compared to neighbours in front or behind, a phenomenon that has been observed by other empirical work on fish (Olst et al. 1970, Katz et al. 2011). Breder (1959) hypothesized that this was the result of swimming movement occurring along
the horizontal plane, necessitating greater distances along the axis of motion. This would ultimately lead to more elongated schools at faster speeds as individuals require greater reaction distances.

Consequently, our understanding of the interactions between individuals and their effect on global group structure is to some extent contradictory. This hinders our ability to understand how information spreads between individuals, which is critical to our understanding of the collective functioning of groups. For instance, Attanasi et al. (2014) found faster information transfer within highly polarised groups, meaning that individuals derive an important functional benefit when they closely align with their neighbours as it allows the group to respond quickly and cohesively to real-time changes in their environment. Despite these interesting results, only a few studies have applied information theoretic measures such as transfer entropy to animal collectives or models thereof (Wang et al. 2012, Orange et al. 2015, Lord et al. 2016, Crosato et al. 2018). While these studies yielded vital insight into the process of information transfer across groups, underscoring the need to continue applying information theoretic measures to animal collectives, the current study aimed to elucidate the specific implications of speed, polarisation and positional composition on the benefits of grouping, which has not been specifically studied.

In the present experiment, we sought to test not only how speed interacts with polarisation, but how this tight relationship impacts positional composition to produce the elongated fish schools observed throughout nature. By analysing five closely related species of fish, we also determined whether these trends were qualitatively consistent across species. And finally, using a novel information theoretic approach, we characterised information flow between loosely polarised shoals travelling at slow speeds to highly polarised schools travelling at fast speeds. This research is crucial as it provides much needed empirical insight into the trends producing group-wide movement patterns and the potential fitness implications with regard to information flow.
Methods

Study animals

For this experiment, we used 5 species of rainbowfish from the family Melanotaenia (electronic supplementary material, Table S1). These species are all freshwater fish endemic to Australia and the Indo pacific. We used *M. mccullochii* from Skull Creek in Queensland (hereafter SC), *M. nigrans* from George Creek in the Northern Territory (hereafter GC), aquarium bred *M. duboulayi* (hereafter MD), *M. sp* from Burton’s Creek in the Northern Territory (hereafter BR) and *M. sp* from Bindoola Creek in Western Australia (hereafter BN). All fish were sourced from wild populations and kept for a minimum of 2 generations in an aquarium facility in the Northern Territory. Fish were transported to the aquarium facility at the University of Sydney where they were placed in 180 litre stock tanks maintained at 23°C with a 12:12 light dark cycle and fed fish flake ad libitum each evening. All fish were given a minimum of 2 weeks to acclimate to lab conditions before being used in experiments.

Experimental protocol

Trials were conducted in a white Perspex tank filled to a depth of 70 mm. We created an annulus within this tank, which promotes continuous swimming within trials. This was made using two circular white plastic inserts. The outer circle had a diameter of 1480 mm and the inner circle had a diameter of 638 mm. The tank was surrounded on all sides by white poster board to reduce the influence of external stimuli. There was no covering on the top of the experimental arena, and the arena was lit with fluorescent ceiling lights.

All trials were conducted between the hours of 8.00 and 12.00 to limit any impact that time of day may have and to standardize nutritional state as much as possible (Hansen et al. 2016). For each trial, a group of 5 fish was netted carefully out of their stock tank and placed in a small 1L holding tank. Fish were
then transported to the arena and poured gently into the annulus. To allow fish to fully acclimate to the
tank, we gave them a full hour in the tank before filming groups for 5 minutes. Video was acquired
remotely using a Panasonic HC x1000 suspended 2 m above the test tank filming at 50 fps at a
resolution of 1080 x 1920 pixels. We ran a total of 10 trials for each species.

After each trial, fish were removed from the annulus, photographed and placed in a used stock tank to
ensure no individual was used twice. These photos were then opened within ImageJ and used to
calculate standard length (SL) for each fish. The average SL for BN was 5.92 ± 0.83 cm (mean ± s.d.),
3.87 ± 0.37 cm for BR, 5.31 ± 0.59 cm for SC, 5.95 ± 0.63 cm for GC and 4.79 ± 0.54 cm for MD.

Data extraction
Videos were filmed in HD at 50 fps and analysed using the idTracker package (Perez-Escudero et al.
2014). This tracking software generated X and Y coordinates for each fish through all 15000 frames. X
and Y coordinates were then converted to mm using a ratio of known distance in mm divided by pixels.
Given the high frame rate, a moving average smoothing function spanning 10 frames (0.2 s) was used to
remove spurious fluctuations in position. These smoothed coordinates were used to calculate speed,
measured in body lengths per second using the species mean, which was done to account for size
differences between species. These measures of speed formed the basis of our analysis of speed-
mediated properties of schooling behaviour.

To understand how group morphology and polarisation relate to speed, we calculated alignment
(average deviation in angle between each fish and its nearest neighbour, NN) and proportion of the
group in front or behind each individual. These calculations were generated based on individual speed,
ranging from 0.25 BLs⁻¹ to 5.75 BLs⁻¹ in 0.5 BLs⁻¹ intervals. Fish were considered to be in front or
behind a focal individual when their \(x, y\) coordinates fell within either of the 90\(^\circ\) zones extending to the front and to the back of the focal individual (dividing lines created at 45\(^\circ\), 135\(^\circ\), 225\(^\circ\) and 315\(^\circ\) relative to the centre of mass of the focal individual). Polarisation and proportion in front or behind was averaged across each trial and then combined to produce a species average.

To investigate how speed and alignment affect information transfer within groups, we first created three speed and alignment categories. These categories were created by dividing the distribution of speed and alignment within each species into thirds. The speed cut off points between tertiles for each species were 3.45 and 4.80 BLs\(^{-1}\) for BN, 0.93 and 2.35 BLs\(^{-1}\) for BR, 1.49 and 3.27 BLs\(^{-1}\) for MD, 1.10 and 3.82 BLs\(^{-1}\) for GC and 1.05 and 2.16 BLs\(^{-1}\) for SC. The alignment cut off points between tertiles for each species were 0.76 and 0.977 for BN, 0.54 and 0.88 for BR, 0.61 and 0.91 for MD, 0.64 and 0.96 for GC and 0.62 and 0.93 for SC. Within each species’ speed and alignment tertiles, we selected the three longest continuous trajectory segments, discarding any in which a given speed or alignment was not maintained for longer than 1 s. Given that there were no trajectory segments in which fish maintained medium speeds for longer than 1 s, there were only two speed categories in the final analysis (slow and fast). For consistency, we also redacted the medium segment of alignment from the analysis. Ultimately, this left us with a total of 120 trajectory segments across all species within the lowest speed tertile, 146 trajectory segments within the highest speed tertile, 150 trajectory segments within the lowest alignment tertile and 145 trajectory segments within the highest alignment tertile.

**Calculating Transfer Entropy**

Each of these trajectory segments was used to calculate transfer entropy using methods described in (Crosato et al. 2018). Transfer entropy is a way of measuring information flow longitudinally between pairs of time series processes by quantifying how knowledge of one individual’s time series reduces the
uncertainty in predicting another individual’s time series (Schreiber 2000, Bossomaier et al. 2016). Following (Crosato et al. 2018), we calculate transfer entropy here as the average conditional mutual information about the target’s current heading update $x_n$ at time $n$ gained from the heading $y_{n-1}$ of the source at time $n-1$ (relative to the target) given a vector of the $k$ previous relative headings of the target $x_{n-1}^{(k)} = \{x_{n-1}, x_{n-2}, \ldots, x_{n-k}\}$:

$$T_{Y \rightarrow X} = \langle \log \frac{p(x_n | x_{n-1}^{k}, y_n)}{p(x_n | x_{n-1}^{k})} \rangle$$

For all trajectories within each speed and alignment category, transfer entropy was calculated as an average over samples from all relevant directed pairs of individuals within the group. Transfer entropy was calculated using the KSG estimator (Kraskov et al. 2004) with the JIDT software (Lizier 2014), using four nearest samples in the search space. We used a target embedding history length of $k=3$, which was set to minimise information from target past attributed as transfer, and a source-target delay of 100 ms (5 time steps). This process produces one measure for each trajectory segment of mean group pairwise transfer entropy across all directed pairs within the group. To test whether there was a statistically significant directed relationship between source and target, we compared our estimates of transfer entropy to surrogate distributions, which were calculated by randomizing the order of relative headings within each source trajectory segment and computing average transfer entropy for the resulting sample (techniques described in Lizier (2014)). Finally, note that whilst local or pointwise transfer entropy for each given sample for a directed pair at one specific time may be positive or negative (see (Crosato et al. 2018) for details), the average transfer entropies $T_{Y \rightarrow X}$ for each trajectory segment over these local values should in theory be non-negative. In practice, the bias correction feature of the KSG estimator can give rise to small negative average values for some trajectory segments; these should be interpreted as being consistent with the surrogate distribution.
**Statistical analysis**

Using the lme package in R (R Development Core Team 2011), we created mixed effect models to investigate the effect of speed on polarisation and positioning. Each response variable (polarisation and positioning) was separately tested against the orthogonal first- and second-order polynomials of speed (R code available in SI). This was done to investigate whether the quadratic term significantly improved the regression compared to the linear term. To analyse general trends across species, we included species as a random effect. We then used 95% confidence intervals, marginal and conditional $R^2$ values to investigate the significance of both the main effect, speed, and the random effect, species. Mixed effect models were also used to compare transfer entropy (nats) between low speeds and high speeds, as well as between low alignment and high alignment.

To visualize the relationship between speed and relative neighbour positioning, density heatplots were created for each speed tertile. This was done by anchoring each fish to the origin of the heat plot in turn and recording the relative position of neighbours when group centroids were moving slow, medium or fast, using the previously defined tertiles. For each heat plot, warmer colours denote higher encounter frequencies.
Results

Heatplots revealed a speed-mediated shift in neighbour positioning that was qualitatively consistent across all five species. Generally, there was a tendency for neighbour position to transition from the sides to the front and back as speeds increased (Figure 1).

This transition from side-by-side group positioning to linear in front-behind positioning has a quadratic relationship with the proportion of individuals in front and behind the focal individual increasing from 0.5 at slow speeds, indicating no preference for specific spatial positioning, to greater than 0.65 at faster speeds, indicating a strong preference for neighbours in front and behind. Our mixed effect model revealed a significant effect of speed$^2$ (mixed effect model: $F = 161.17$, $p < 0.001$; 95% confidence interval: -0.17 to -0.07; Figure 2). The random effect had a confidence interval of 0.03 to 0.1 and improved the fit of the model from a marginal R$^2$ value of 0.48 to a conditional R$^2$ value of 0.92 (for species-specific trendlines, see electronic supplementary material, figure S1).

Along with this speed-mediated shift in group positioning, there was also a speed-mediated increase in alignment with NN. As speeds increased, the angular deviation with NN decreased, though this plateaued at faster speeds, likely because there is a hard limit on how polarised groups can be, producing a saturating effect. Our mixed effect models revealed a significant effect of speed$^2$ (mixed effect model: $F = 553.16$, $p < 0.001$; 95% confidence interval: 47.16 to 64.31; see Figure 3). The random effect had a confidence interval of 2.80 to 10.32 and slightly improved the fit of the model from a marginal R$^2$ value of 0.87 to a conditional R$^2$ value of 0.95.

Information transfer existed between groups of fish when moving at both slow and fast speeds and low and high alignment, as determined by the transfer entropy being greater than the surrogate distribution +
Transfer entropy varied significantly between slow-moving and fast-moving fish (mixed effect model: $F = 22.12$, $p < 0.001$; 95% confidence interval: 0.007 to 0.02; Figure 4), with greater information flow occurring at fast speeds compared to slow speeds. The random effect, species, had a confidence interval of 0.001 to 0.01 and slightly improved the fit of the model from a marginal $R^2$ value of 0.19 to a conditional $R^2$ value of 0.25. Transfer entropy also varied significantly based on whether fish had low alignment or high alignment (mixed effect model: $F = 56.66$, $p < 0.001$; 95% confidence interval: 0.014 to 0.24; Figure 5). Species had a confidence interval of 0.002 to 0.01 and slightly improved the fit of the model from a marginal $R^2$ value of 0.35 to a conditional $R^2$ value of 0.41.
Figure 1: Heatplots of positional frequency when groups were moving at slow (left column), medium (middle column) and fast (right column) speeds for each of our 5 species (top to bottom: BR, GC, BN, MD, SC). Speed categories were derived from the speed distributions within each species and normalized by the maximal density value. Focal individuals are at the origin facing positive along the y-axis, with warmer colours denoting higher frequency of encounters.
Figure 2: Graph showing the mean proportion of individuals in front or behind a focal individual ± s.e. bars as a function of speed (BLs⁻¹) with a quadratic trendline. As speed increases, the proportion of individuals positioned in front or behind the focal increases sharply initially before beginning to level off.
Figure 3: Graph showing mean angle deviation with NN ± s.e. bars as a function of speed (BLs⁻¹) with a quadratic trendline. As speed increases, deviation in angle with NN decreases, meaning that overall group alignment increases.
Figure 4: Box plot of transfer entropy (Nats) when fish were travelling at slow speeds (i.e. those speeds within the lower tertile) or at fast speeds (upper tertile), measured as body lengths per second. Medians and interquartile ranges are shown. The dashed horizontal line shows the null TE + 2*s.d., indicating the threshold above which TE is significantly more than would be expected by chance.
Figure 5: Box plot of transfer entropy (Nats) when fish had low alignment (lowest tertile) or high alignment (upper tertile), measured as the degree of deviation with NN. Medians and interquartile ranges are shown. The dashed horizontal line shows the null TE $\pm 2\times$ s.d., indicating the threshold above which TE is significantly more than would be expected by chance.
Discussion

Our results underscore the importance of speed in mediating both local interactions and global patterns. At the local level, increases in speed led to increases in alignment and shifts from lateral to linear neighbour positioning. At the global level, these increases in linear neighbour positioning resulted in elongation of the group. Furthermore, mean pairwise transfer entropy increased with speed and alignment, implying an adaptive value to forming faster, more polarised and linear groups. Ultimately, this research provides vital empirical insight into the mechanisms underlying elongation of moving animal groups and highlights the functional significance of cohesive and coordinated movement.

Contrary to the theoretical work done by Hemelrijk et al. (2012), we found increasing elongation at increasing speeds. This seems to be the result of both elongated zones of interaction and a shift from favouring lateral neighbour positioning to linear neighbour positioning. In accordance with Breder (1959) and Katz et al. (2011), we found smaller distances between lateral neighbours compared to neighbours located in front or behind the focal individual. This elongation became more pronounced with speed, likely due to the need for greater reaction distances along the axis of motion. However, an important and somewhat surprising outcome of this study is the shift we found from lateral to front-back neighbour positioning at increasing speeds. We found that this shift in positional preferences had a quadratic relationship with speed. Individuals increasingly favoured leader-follower formations as speeds increased, though this preference plateaued at greater speeds, potentially signalling a stabilisation in spatial positioning at greater speeds. However, recent work by Ashraf et al. (2017) found that red nosed tetras forced to swim faster than their preferred free-swimming velocities shifted from diamond formations to phalanx formations. It is possible that the current study would have found a subsequent decrease in linear neighbour positioning had the experimental set-up not relied solely on elective group swimming speeds. Within the observed range of free-swimming speeds, our results point to an
increasingly elongated shape as groups transition from low order to high order. Importantly, this shift in neighbour positional preferences was qualitatively similar across all five species.

This consistent shift towards more linear neighbour positioning across species may imply a common benefit to forming faster, more polarised and elongated groups. While the energetic benefits of swimming in a group are well established (e.g. reduced oxygen consumption (Marras et al. 2015) and lower tail beat frequency (Herskin et al. 1998) in collectively moving fish compared to solitary fish), there may be further benefits to individuals within specific group formations. Theoretical work by Hemelrijk et al. (2015) demonstrated how individuals swimming side-by-side in a phalanx formation gained little to no energetic payoff compared to individuals swimming in a linear or diamond formation (However, see (Ashraf et al. 2017)). This may explain why we found a shift in favour of more linear formations at faster speeds as individuals would have offset the increased energy requirements of fast swimming (Beamish 1970) with greater hydrodynamic benefits of more linear formations.

In addition to the putative energetic benefits of this positional shift with speed, we found significantly greater information transfer at faster speeds and higher alignments, indicating a fitness benefit to high order groups as they increase the ability to coordinate or synchronise behaviour. Interestingly, low alignment seemed more detrimental to information flow than slow speeds given that transfer entropy between unaligned individuals was only slightly statistically larger than the null distribution. Both speed and alignment, averaged at the group level, are important, which is not surprising as speed and alignment are positively and tightly correlated. These results underscore how individuals might benefit from transitioning away from low alignment and slow speeds to faster, more aligned and linear groups.

To understand how groups move collectively and make decisions, it is first necessary to understand the
underlying interaction between individuals. This study provides an important link between individual interactions and group-level functioning, specifically showing increases in the flow of information as groups shift from low order to high order and linear movement. While previous theoretical studies have addressed the importance of density in producing oblong schools, this study has shown that at constant densities, the positional composition can shift to leader-follower formations as a function of speed. Additional research can use the present study’s findings in conjunction with school density to understand how both speed and group size might interact. Importantly, this research defines the specific relationship between speed, alignment and positional composition of groups, highlighting the importance of speed in mediating both individual behaviour and group morphology more generally.
Animal Ethics statement: This study and protocol was approved by the University of Sydney Animal Ethics Committee (Permit Number: 2015/807)

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Data Availability: Summary data and code are available at https://doi.org/10.5061/dryad.bd08ft6 or the review link https://protect-au.mimecast.com/s/7wQSCXLKZoiJ3mv5T62Yat?domain=datadryad.org. Full raw dataset available upon request.
References


**SUPPLEMENTARY INFORMATION**

**Table S1.** Species names, species abbreviations, SL (mean ± sd) and the speed and alignment cut-off points between tertiles:

<table>
<thead>
<tr>
<th>Species</th>
<th>Abbreviation</th>
<th>SL (cm) mean</th>
<th>SL (cm) sd</th>
<th>Speed cut-off lower</th>
<th>Speed cut-off upper</th>
<th>Alignment cut-off lower</th>
<th>Alignment cut-off upper</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. sp</em></td>
<td>BN</td>
<td>5.92</td>
<td>0.83</td>
<td>3.46</td>
<td>4.80</td>
<td>0.76</td>
<td>0.98</td>
</tr>
<tr>
<td><em>M. sp</em></td>
<td>BR</td>
<td>3.87</td>
<td>0.37</td>
<td>0.93</td>
<td>2.35</td>
<td>0.54</td>
<td>0.88</td>
</tr>
<tr>
<td><em>M. duboulayi</em></td>
<td>MD</td>
<td>4.79</td>
<td>0.54</td>
<td>1.49</td>
<td>3.27</td>
<td>0.61</td>
<td>0.91</td>
</tr>
<tr>
<td><em>M. nigrans</em></td>
<td>GC</td>
<td>5.95</td>
<td>0.63</td>
<td>1.98</td>
<td>3.82</td>
<td>0.64</td>
<td>0.96</td>
</tr>
<tr>
<td><em>M. mccullochii</em></td>
<td>SC</td>
<td>5.31</td>
<td>0.59</td>
<td>1.05</td>
<td>2.16</td>
<td>0.62</td>
<td>0.93</td>
</tr>
</tbody>
</table>
Effect of speed on linear positioning by species

**Figure S1.** Speed (body lengths/second) against proportion of individuals in front or behind the focal individual. BR, BN and GC had a quadratic relationship and are not significantly different. SC and MD had a linear relationship and were not significantly different. Trial averages and SE bars are shown.
CHAPTER 4

RISK BALANCING THROUGH SELECTIVE USE OF SOCIAL AND PHYSICAL INFORMATION

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To effectively balance the need to forage against the need to avoid predation, animals should utilize information from both their physical and social environments. However, most studies have considered these factors in isolation and few have investigated how animals change the use of these cues temporally. Using novel 3D modelling of the environment and 3D observations of fish movement, we investigated how local abiotic and biotic features of the environment, along with tidal patterns, impacted risk-related behaviours using humbug damselfish, *Dascyllus aruanus*, in coral reef habitats as a model system. We found that damselfish balance risk by utilizing cues from both the physical and the social environment, although the relative importance of these cues changes according to tide. At flowing tide, when food resources are typically more abundant, damselfish increased their foraging behaviour, but only when their external social environment offered protection from predation. At slack tide, when food resources are typically less abundant, damselfish were not responsive to their external social environment. Regardless of tide, damselfish living in smaller corals showed more risk-averse behaviour, emphasizing the importance of local refuge availability on risk perception. Our results underscore the flexible use of social and physical information along temporal scales and how both biotic and abiotic features influence the trade-off adopted between foraging and refuging behaviour.

Author contributions: MK designed the experiment, MK, GM, AB performed the experiments, MK, AP, WF performed the analysis and all authors edited the manuscript written by MK
Introduction

One of the greatest challenges for animals is balancing the need to forage against the need to avoid predation. Often, these imperatives are mutually exclusive, with foraging behaviour exposing an animal to increased predation risk and predator avoidance behaviour reducing the amount of time available for energy acquisition (Metcalf et al. 1984, Godin et al. 1988). However, the risk of engaging in foraging behaviour changes with different physical and social aspects of the environment, and along different temporal and spatial scales. Therefore, in deciding when and where to forage, individuals should consider the likely payoff of engaging in these behaviours against the risk of predation, a concept referred to as the risk-reward trade-off (Lima et al. 1990).

Studies have shown that animals assess risk based on both the physical and social aspects of their surroundings. For instance, animals utilize characteristics of the physical environment, such as substrate colour or habitat complexity, to determine how easily they can detect predators (Metcalf 1984), find refuge (Cowlishaw 1997) or remain camouflaged (Powolny et al. 2014). While less complex environments may increase visibility and promote early predator detection, they often contain fewer refuges and have been linked to higher prey capture rates (Longland et al. 1991). A study by Golub et al. (2005) found that habitat complexity modified risk perception in sunfish, *Lepomis gibbosus*, which increased antipredator behaviour when in more complex environments, suggesting more risk-sensitive behaviour when visibility is reduced.

The perception of risk can also shift with the social environment. In large groups, many eyes enable early predator detection (Siegfried et al. 1975, Boland 2003) and collective defense, dilution and confusion effects reduce an individual’s per-capita risk (Krause et al. 2002, Ward et al. 2016). Consequently, individuals in large groups often spend less time vigilant and more time foraging (Lima
1995, Beauchamp 2008, Creel et al. 2014) as well as show signs of reduced risk perception compared to solitary individuals (McDonald et al. 2016). Although these benefits are often limited to groups of conspecifics, these benefits can extend to mixed-species assemblages, especially when heterospecifics share predators (Goodale et al. 2017). Therefore, the behaviour of both the immediate conspecific environment and the wider heterospecific environment can act as an alarm system to alert when predators approach (Heymann et al. 2000).

Despite all the evidence that both physical and social factors shape an animal’s perception of risk, there is a lack of research that considers these factors simultaneously or whether the use of these cues shift in temporally variable environments. Recent work on eastern chipmunks, *Tamias striatus*, found that vigilance behaviour increased in riskier environments, as shaped by reduced habitat complexity and high wind conditions, which makes auditory detection of predators more difficult. Importantly, they found that this risk perception and vigilance behaviour was modified by the social environment, with neighbour density amplifying anti-predator behaviour, rather than reducing it (Clermont et al. 2017). While this study provided evidence that both physical and social aspects are important to understanding what shapes animal behaviour, less is known about how or whether animals adjust the use of each factor to temporally variable conditions.

In marine habitats, behaviour can change with the tidal patterns that drive predictable fluctuations in predator and prey abundances. For instance, humbug damselfish (*Dascyllus aruanus*) increase their foraging activity to coincide with the greatest availability of plankton during high tide (Forrester 1991) and prioritise foraging over anti-predator behaviour when the payoff for foraging is greatest (Hansen et al. 2016). This underscores the need to consider temporal scales, such as tidal cycles, when investigating the factors shaping behaviour.
The question remains as to how animals balance risk along social, physical and temporal scales. The humbug damselfish (*Dascyllus aruanus*) provides an interesting case study for this risk-reward trade-off. This species is an obligate planktivore that forms stable, restricted-entry social groups (hereafter ‘colonies’) ranging between 2 and 25 individuals, which associate closely with branching acroporan or pocilloporan coral species (Coates 1980; Forrester 1991; Sale 1971). As small reef fish, damselfish are vulnerable to predation from a wide range of predators throughout the reef environment. However, by living in social groups and by retreating into their branching coral heads when predators approach, damselfish can mitigate some of these risks (Coates 1980). Given the patchy distribution of suitable branching corals throughout the reef, the external environment can differ greatly between colonies. Importantly, as humbug damselfish rarely venture further than 1m from their home corals (Sale 1971), their behaviour is a direct reflection of their immediate environment.

Although previous work has shown an ability to respond adaptively to time of day (Burns 2016), tide and group size (Hansen et al. 2016), no study to date has investigated how the physical environment, such as the surface area of their home coral and the complexity of the surrounding environment (factors that affect the availability of refugia) affects risk perception. In complex reef environments, damselfish behaviour is likely shaped by both their local group size as well as their external social environment, which can contain both transient and resident heterospecifics and conspecifics.

This study examined the effects of 4 factors on the foraging and refuging behaviour of humbug damselfish: (1) focal coral complexity (measured as both surface area and rugosity), (2) surrounding habitat complexity, (3) local social environment (colony group size), and (4) external social environment (number of other fish in proximity to the focal colony). Given the previously described effect of tide on the behaviour of these fish, we hypothesized that a greater proportion of the colony would emerge at
high tide when food is more available, and that the proportion of the colony emerged would increase with the size of the colony and the number of ecologically similar fish in the surrounding environment. We also hypothesized that larger focal corals, and greater surrounding habitat complexity, would reduce risk-perception through decreased competition for refuge, resulting in greater emergence. Using video recording and novel 3D mapping techniques, this study aims to investigate how the risk-reward trade-off is shaped by the social and the physical environment and whether this changes with tide.
Methods:

Research was conducted within the 1st lagoon at One Tree Island Research Station on the Great Barrier Reef from October 2nd to October 8th in 2017 between the hours of 9am and 12pm. As all colonies were located within the same shallow lagoon, tidal fluctuations, plankton availability and relative risk would have been qualitatively similar across all colonies. We chose to collect data during the morning to limit any effect that time of day may have on humbug damselfish behaviour (Helfman 1986, Burns 2016). For this study, snorkelers only selected colonies that were more than 2m apart to ensure spatial independence (Sale 1971) and groups containing more than three individuals and fewer than 24 individuals. No other criteria were used to select colonies. Once suitable colonies were selected, snorkelers recorded time of day, tidal state and began filming damselfish behaviour.

Each colony was filmed once for a period of 20 minutes using two GoPro Hero 3+ cameras placed 1m away at approximately 90° to one another. Recording of damselfish from 2 angles facilitated observation of individuals in 3D space and minimised occlusion. Each camera recorded 24fps at a wide angle setting and at a resolution of 1080dpi. After the 20 minute filming period, 2 snorkelers located 5 metres to either side of the focal colony independently but simultaneously performed a fish census, including species identification and counts for 2 minutes, considering all fish within a 2m radius of the focal colony (following methods outlined in Hansen et al. (2017)). Where records differed, the average of the two estimates were taken, or the greater estimate where accounts differed by only 1 individual or species. These counts were then grouped into 3 categories: non-focal resident mid-water species, non-focal resident benthic species and non-resident mid-water species (see Table S1). These categories were created due to the expectation that damselfish would respond more strongly towards fish of similar size and ecology, specifically mid-water residents compared to benthic residents, and that the response may differ based on whether fish are local or transient within their environment. In this study, we made a
distinction between the local social environment (i.e. immediate colony group size) and the external social environment.

After this 2-minute census period, snorkelers recorded time of day and, using tidal schedules, whether the tide was flowing or slack. These tidal categories were selected because damselfish feed on zooplankton in the water column, meaning that local currents impact food availability (Russo 1977). Given that the lagoon curtails full tidal cycles, slack tides occurred when the water level fell below the perimeter of the lagoon (<1.7m above low tide sea level). Once these measures were taken, a third snorkeler mapped each focal colony and the surrounding substrate following Figueira et al. (2015). The snorkeler placed 3 calibration targets within 2m of the focal colony and took between 700-1500 photos using 3 GoPro cameras spaced evenly along a 1m pole capturing HD photos at 1fps, 1080dpi. Two of the calibration targets consisted of 2 flat disks printed with unique black and white patterns and connected by a flat metal weight. The third target had 3 metal bars of equal length connected at 90° angles. Two of the bars lay flat along the substrate and had flat disks printed with unique black and white patterns attached to the ends. The third arm sat perpendicular to the ground and had a cap with a black dot in the middle (See figure 1). Throughout the week, we collected data on a total of 55 different damselfish colonies.

**Behavioural analysis**

For each colony, both video recordings were uploaded to VirtualDub where they were synchronised by eye, placed side-by-side and exported as a single video file. After the videos had been scanned to get an accurate measure of damselfish group size, the positions of the damselfish relative to their home coral was recorded every 20 s over a 10-minute period. A damselfish was considered to have entered the coral when they were within 1 body length of the nearest edge of the coral, estimated by eye. We recorded
positions every 20 s seconds to reduce the autocorrelation between measures taken over time. All behavioural observations were carried out by the same researcher to ensure consistency. From these counts, we calculated the median proportion of the group outside the focal coral over the 10-minute observation period. Because damselfish behaviour is bimodal, with fish limited to either foraging in the water column or retreating inside when they perceive danger (Hansen et al. 2016), quantifying the proportion of the colony outside the coral head is a relevant measure of risk perception in this species.

The 10-minute behavioural observation period did not begin until all snorkelers were out of the field of view of both cameras and a 5-minute acclimation period had elapsed. This five-minute acclimation period was chosen to maximise time between turning on and turning off the camera, though damselfish resume normal behaviour after 1 minute (personal observation). On 1 occasion, the cameras stopped recording before the 10-minute period had finished so the acclimation period was reduced to 2 minutes to allow a 10-minute observation period. The proportion of fish within the coral during these 10-minutes did not differ when a full 5-minute acclimation period was given and only 7-minutes of damselfish behaviour was observed.

3D meshes
To calculate the complexity of the focal coral and surrounding habitat, we created 3D models of each damselfish colony and the local environment within 1.5m. This was done using the software package Photoscan Professional v1.3.5 (AgiSoft LLC) following methods developed by Figueira et al. (2015). We began by uploading the photos of each colony into Photoscan Professional, which aligned these at the highest settings with no pre-pair selection by identifying common features of the environment. Once the photos were aligned, the program generated a sparse point cloud in 3D space, which served to reconstruct the path along which all the photos were taken as well as the orientation of each camera.
This information was then used to populate the sparse point cloud with additional points, generating a dense point cloud, which led to the creation of a continuous 3D mesh of three-sided polygons. Agisoft then used the original images to insert colour and texture into each model. These finalized models were calibrated using each of the 3 targets, which were detected automatically by the program. The 3-pronged target was used to orient the mesh along x,y,z axes to match real-world coordinates. This process of calibration led to error estimates, which indicate the accuracy of our 3D models.

Each model was exported as an .obj file and cropped to a 3 m diameter circle centred on the focal colony using Geomagic Studio (3D Systems) (Figure 1). The surface area (m²) of these cropped models was measured, then focal corals were cut out using colour and texture to differentiate between focal coral species and external substrate. These clipped focal meshes were used to calculate focal coral surface area (hereafter focal coral size). To create a measure of only surrounding habitat complexity, we subtracted the surface area of the focal coral from the surface area of the full cropped model. Focal and non-focal habitat complexity was measured using surface area and rugosity, a measure of complexity in which real surface area is divided by geometric surface area. We also considered both focal coral size and local habitat complexity as separate measures given the high site fidelity and reliance on their focal coral rather than external environment for protection. Given previous research finding that damselfish rarely venture further than 1m from their focal coral, we measured local habitat complexity within a 1m radius of the home coral.
**Statistical Analysis**

When colonies were in very shallow water and photographed on clear days, the sun caused extensive dappling, which prevented Agisoft from aligning photos and constructing 3D models for 5 of our sites. Our final analysis therefore included 50 colonies, 25 of which were recorded at slack low tide and the remaining 25 at flowing tide. To ensure that the physical and social aspects did not significantly differ between colonies recorded at slack tide and flowing tide, we used independent student t tests on log or square-root transformed variables to meet the assumption of normality and equality of variances.

To investigate how each physical and social aspect of the environment influenced damselfish behaviour, we ran a beta regression on a model of social and physical factors against proportion of the colony outside the coral (see Table 1 for model factors). To investigate how tide might affect the importance of each social and physical factor, we also included an interaction between each factor and tide within the larger beta regression. Where there were significant interactions with tide, we ran smaller beta regressions within tide datasets to quantify the relationship between the factor and proportion of colony emerged. These smaller models were created by selecting only variables that were significant within the larger main model. Beta regressions were used to account for the bounded nature of proportion data, which were transformed away from 0 and 1 using the formula \((y * (n−1) + 0.5) / n\), where \(n\) is the sample size (Smithson et al. 2006). Our beta regressions were run using the betareg package in R (Cribari-Neto et al. 2010).

Before running the model, we tested to ensure that the assumption of multicollinearity was not violated using variance inflation factor analysis. Multicollinearity was detected between the two measures of surrounding environmental complexity, surface area (SA) and rugosity, so only surface area was included in the model. This was chosen over rugosity because surface area of the focal colony was more
important than focal coral rugosity. After running out main beta regression, we ran a general linear model of the number of non-focal mid-water residents against surrounding habitat complexity to identify whether there may have been any indirect effects on the proportion of damselfish emerged from the colony. Time of day was not included in the analysis after model weights, AIC values and delta values indicated that time of day did not improve the model. During the post-hoc analysis, we removed colony 31, filmed during flowing tide, because it had a disproportionate leverage on the results with a Cook’s distance of 0.93, which exceeds the cut-off of 0.18 set by taking 4/(n-k-1), where n is the sample size and k is the number of predictors in the model (Bollen et al. 1985).
Results

We first compared all fixed factors of interest at flowing or slack tide. There were no significant differences in humbug colony group size ($t = 1.10$, df = 48, $p = 0.05$), focal coral SA ($t = -1.15$, df = 48, $p = 0.26$), focal coral rugosity ($t = 0.67$, df = 48, $p = 0.51$), abundance of non-focal mid-water resident fish ($t = 0.13$, df = 48, $p = 0.9$), abundance of benthic resident fish ($t = 1.54$, df = 48, $p = 0.13$), abundance of mid-water non-resident fish ($t = -1.41$, df = 48, $p = 0.17$), or local habitat complexity (SA) ($t = 0.25$, df = 48, $p = 0.80$) between colonies filmed at slack tide and colonies filmed at flowing tide.

Main model

Analysis of damselfish behaviour indicated that focal coral surface area was significantly related to damselfish behaviour regardless of tide (see Table 1). As focal coral surface area increased, so too did the proportion of the colony outside the coral (Figure 2). There was no effect of focal coral rugosity on emergence behaviour, which might relate to how relevant each measure is to refuge availability. With damsels consistently occupying branching corals, the depth or intricacy of the branches may have less bearing on refuge availability than the size or surface area of the coral. The model also revealed a significant interaction between tide and the number of non-focal mid-water residents. After running beta regressions with tidal categories, we found that the external social environment did not impact damselfish emergence at slack tide (Beta regression: Estimate = -0.03, Std error = 0.02, $p = 0.07$), but had a positive relationship on the proportion of the colony emerged at flowing tide (Estimate = 3.07, Std error = 0.05, $p = 0.008$, see Figure 3).

Although local habitat complexity was not a significant predictor, there was a significant correlation between local habitat complexity and the number of non-focal mid-water resident fish, with more complex environments hosting more mid-water residents (GLM: $F_{1,47} = 11.54$, $p < 0.002$, confidence interval = 0.005, 0.018, Adjusted $R^2 = 0.2$, see Figure 4).
Table 1: Output from a beta regression examining the effect of the number of mid-water resident fish, the number of non-resident mid-water fish, the number of benthic resident fish, the surface area of the focal coral, the rugosity of the focal coral, local group size and surrounding habitat complexity on median proportion of group outside the coral depending on tide.

<table>
<thead>
<tr>
<th></th>
<th>Estimate</th>
<th>Std. Error</th>
<th>Conf Int lower</th>
<th>Conf Int upper</th>
<th>z value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>-1.075</td>
<td>1.587</td>
<td>-4.185</td>
<td>2.034</td>
<td>-0.678</td>
<td>0.498</td>
</tr>
<tr>
<td>No. mid-water resident fish</td>
<td>0.117</td>
<td>0.053</td>
<td>0.013</td>
<td>0.222</td>
<td>2.195</td>
<td>0.028</td>
</tr>
<tr>
<td>Tide</td>
<td>0.087</td>
<td>2.386</td>
<td>-4.591</td>
<td>4.764</td>
<td>0.036</td>
<td>0.971</td>
</tr>
<tr>
<td>No. mid-water non-resident fish</td>
<td>0.053</td>
<td>0.07</td>
<td>-0.084</td>
<td>0.19</td>
<td>0.762</td>
<td>0.446</td>
</tr>
<tr>
<td>No. benthic resident fish</td>
<td>0.077</td>
<td>0.06</td>
<td>-0.04</td>
<td>0.194</td>
<td>1.291</td>
<td>0.197</td>
</tr>
<tr>
<td><strong>Focal coral SA</strong></td>
<td><strong>3.04</strong></td>
<td><strong>1.344</strong></td>
<td><strong>0.407</strong></td>
<td><strong>5.674</strong></td>
<td><strong>2.262</strong></td>
<td><strong>0.024</strong></td>
</tr>
<tr>
<td>Focal coral rugosity</td>
<td>0.098</td>
<td>0.2</td>
<td>-0.295</td>
<td>0.491</td>
<td>0.488</td>
<td>0.626</td>
</tr>
<tr>
<td>Group size</td>
<td>-0.11</td>
<td>0.083</td>
<td>-0.274</td>
<td>0.053</td>
<td>-1.324</td>
<td>0.186</td>
</tr>
<tr>
<td>Surrounding environmental SA</td>
<td>-0.012</td>
<td>0.372</td>
<td>-0.741</td>
<td>0.718</td>
<td>-0.032</td>
<td>0.975</td>
</tr>
<tr>
<td><em>(No. mid-water resident fish)</em> Tide</td>
<td><em>-0.14</em></td>
<td><em>0.061</em></td>
<td><em>-0.259</em></td>
<td><em>-0.02</em></td>
<td><em>-2.292</em></td>
<td><em>0.022</em></td>
</tr>
<tr>
<td><em>(No. mid-water non-resident fish)</em> Tide</td>
<td><em>-0.057</em></td>
<td><em>0.071</em></td>
<td><em>-0.196</em></td>
<td><em>0.083</em></td>
<td><em>-0.8</em></td>
<td><em>0.424</em></td>
</tr>
<tr>
<td><em>(No. benthic resident fish)</em> Tide</td>
<td><em>-0.04</em></td>
<td><em>0.084</em></td>
<td><em>-0.205</em></td>
<td><em>0.125</em></td>
<td><em>-0.474</em></td>
<td><em>0.635</em></td>
</tr>
<tr>
<td><em>(Focal coral SA)</em> Tide</td>
<td><em>-1.102</em></td>
<td><em>1.877</em></td>
<td><em>-4.78</em></td>
<td><em>2.576</em></td>
<td><em>-0.587</em></td>
<td><em>0.557</em></td>
</tr>
<tr>
<td><em>(Focal coral rugosity)</em> Tide</td>
<td><em>-0.189</em></td>
<td><em>0.267</em></td>
<td><em>-0.713</em></td>
<td><em>0.334</em></td>
<td><em>-0.709</em></td>
<td><em>0.478</em></td>
</tr>
<tr>
<td><em>(Group size)</em> Tide</td>
<td>0.138</td>
<td>0.093</td>
<td>-0.044</td>
<td>0.32</td>
<td>1.488</td>
<td>0.137</td>
</tr>
<tr>
<td><em>(Surrounding environmental SA)</em> Tide</td>
<td>0.285</td>
<td>0.529</td>
<td>-0.752</td>
<td>1.321</td>
<td>0.538</td>
<td>0.591</td>
</tr>
</tbody>
</table>
Figure 1: 3D model of a damselfish colony. Each mesh was 3m in diameter with the focal coral at the centre. Two of the targets used to orient and calibrate the mesh can be seen in the bottom right and bottom left of the model. For the target on the right: the black dots at the centre of each black pattern are 250mm apart.
**Figure 2:** Median proportion of damselfish outside the focal coral as a function of focal coral surface area and tide (light grey triangles: slack low tide, dark grey circles: flowing tide)
Figure 3: Median proportion of damselfish outside the focal coral as a function of how many non-focal mid-water residents were counted within 2 m of focal coral when the tide was slack low (light grey triangles) or flowing (dark grey circles). The dotted trendline is shown despite the non-significant relationship between non-focal mid-water residents and proportion of damselfish emerged at slack low tide.
Figure 4: Local habitat complexity against the number of non-focal mid-water residents within 2 m of focal coral.
Discussion

Here, we show that humbug damselfish utilize cues from both the physical and the social environment, although the relative importance of the external social environment changes according to tide. Our results demonstrate an ability to prioritize foraging behaviour when flowing tides bring food past the colony and when the external social environment offers greater protection against predation. Conversely, damselfish showed no change in emergence behaviour at low tides when food resources are typically less abundant, showing a greater willingness to forgo foraging as the payoff of this behaviour decreased. Furthermore, damselfish colonies occupying smaller focal corals showed more risk-averse behaviour across both tidal categories.

This importance of focal coral size across both tidal categories speaks to the importance of local refuge availability on risk perception. Whereas many reef fish utilize structures across the reef as refuge from predation, damselfish are highly site-attached meaning that focal coral size is more important than surrounding habitat complexity in determining the availability of predator refugia. Accordingly, we found no direct impact of surrounding habitat complexity on damselfish behaviour. However, the strong correlation between surrounding habitat complexity and the abundance of non-focal mid-water residents may indicate that there is an indirect effect of habitat complexity on damselfish behaviour. Namely, that complex environments attract more heterospecific mid-water residents, whose behaviour may influence risk perception in damselfish.

This reduced risk perception may relate to the reduced per-capita risk associated with the dilution and confusion effects (Ward et al. 2016). However, it may also relate to the benefits of enhanced vigilance, with many eyes increasing the likelihood of detecting a predator (Godin et al. 1988, Ward et al. 2011), as well as access to social information within mixed-species assemblages (Goodale et al. 2017, Webster...
et al. 2017). In highly social environments, conspecifics and heterospecifics alike can act as an alarm system when shared predators approach, thereby increasing survival success. This has been found in coral reef fish, who can learn predator recognition after a single conditioning event with a heterospecific (Manassa et al. 2013), as well as monkeys, who show improved predator detection despite reductions in per capita vigilance (Wolters et al. 2003). This ready uptake of social information is likely to be an important mechanism for increasing survival in such diverse environments. However, it is interesting to note that resident benthic species had no impact on humbug damselfish behaviour or their perception of risk. This may indicate a limit to the benefits of mixed-species assemblages with information transfer existing only between species who share ecological or foraging niches or between those vulnerable to the same predators (Pollock et al. 2006, Mitchell et al. 2012).

As damselfish leave the safety of their coral, their exposure to predation increases (James et al. 2004). This may be especially pertinent at flowing tides when the abundance of predators increases (McClanahan et al. 1989). Accordingly, we found a shift in the importance of the external social environment at flowing tides with more damselfish emerging to forage as the number of mid-water residents increased. This greater emergence may also be due to the increased flow of plankton past the colonies when water is flowing (Russo 1977), indicating a greater benefit to emerging. Our results may indicate that damselfish balance the increased risk of emergence at flowing tides by emerging only as the external social environment, or number of non-focal mid-water residents, provided greater protection.

A surprising outcome of the current study was the significant effect of external social environment, but not the local social environment (i.e. group size), on damselfish emergence patterns. This may suggest that the most important interaction network is the abundance of ecologically similar fish in the vicinity
of the home coral rather than the number of colony members. Despite larger groups of conspecifics often conferring anti-predator benefits, this may be limited in damselfish due to the hierarchical structure of their colonies, which results in differential spatial distribution based on size and rank (Coates 1980). Larger and more dominant fish tend to swim higher in the water column and further from the focal coral compared to smaller and less dominant individuals (Forrester 1991). Large disparities in damselfish size may render individuals vulnerable to different suites of predators, while the wide spatial distribution may mean colony members are not always within view of each other. Potentially, damselfish have more to gain from observing ecologically similar neighbours, who may be closer in size or proximity, than taking cues from their colony mates. Ultimately, the hierarchical nature of damselfish colonies and the large size disparities between group members may limit the impact or benefit of large local colony sizes on individual behaviour.

Overall, our results underscore the flexible use of social and physical information in shaping the trade-off adopted between foraging and refuging behaviour. Specifically, we show that damselfish are risk balancers, emerging from the safety of the coral when the tide is flowing and food is more abundant, and when there are more ecologically similar neighbours in the surrounding environment, potentially offering greater protection from predation. Despite many studies highlighting individual factors impacting animal behaviour, this study provides important support for the idea that animals can integrate information from multiple sources at once. Our results highlight the multifaceted ways in which both the physical and social aspects of the environment shape risk-perception and behavioural decisions. Having established these behavioural patterns, an important avenue for future investigations would be to quantify how predation and food availability vary along circadian and tidal scales and how these in turn interact with the various abiotic and biotic features described here. This would provide valuable insight into the mechanisms driving the patterns described in the current study.
References


**SUPPLEMENTARY INFORMATION**

**Table S1.** Table showing which species were placed into each of our 3 non-focal fish categories: resident mid-water fish, resident benthic fish and non-resident mid-water fish.

<table>
<thead>
<tr>
<th>Non-focal resident fish</th>
<th>Non-resident fish</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mid-water</strong></td>
<td><strong>Benthic</strong></td>
</tr>
<tr>
<td><em>Pomacentrus chrysurus</em></td>
<td><em>Amblygobius phalaena</em></td>
</tr>
<tr>
<td><em>Pomacentrus moluccensis</em></td>
<td><em>Parapercis australis</em></td>
</tr>
<tr>
<td><em>Acanthochromis polystilus</em></td>
<td><em>Valenciennea longipinnis</em></td>
</tr>
<tr>
<td><em>Pomacentrus wadi</em></td>
<td><em>Parapercis queenslandica</em></td>
</tr>
<tr>
<td><em>Cheiilodipterus quinquelineatus</em></td>
<td><em>Ctenogobiops feroculus</em></td>
</tr>
<tr>
<td><em>Dischistodus melanotus</em></td>
<td><em>Amblyeleotris steinitzi</em></td>
</tr>
<tr>
<td><em>Dischistodus pseudochrysopoecilus</em></td>
<td><em>Cryptocentrus cinctus</em></td>
</tr>
<tr>
<td><em>Pomacentrus amboinensis</em></td>
<td><em>Ctenogobiops mitodes</em></td>
</tr>
<tr>
<td><em>Abudefduf sexfasciatus</em></td>
<td></td>
</tr>
<tr>
<td><em>Pomacentrus philippinus</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Halichoeres trimaculatus</em></td>
</tr>
<tr>
<td></td>
<td><em>Siganus lineatus</em></td>
</tr>
<tr>
<td></td>
<td><em>Thalassoma lunare</em></td>
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<tr>
<td></td>
<td><em>Scarus forsteni</em></td>
</tr>
<tr>
<td></td>
<td><em>Gerres oyena</em></td>
</tr>
<tr>
<td></td>
<td><em>Lutjanus carponotatus</em></td>
</tr>
<tr>
<td></td>
<td><em>Acanthurus auranticavus</em></td>
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<td><em>Scolopsis bilineata</em></td>
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<tr>
<td></td>
<td><em>Scolopsis bilineata</em></td>
</tr>
<tr>
<td></td>
<td><em>Hemigymnus melapterus</em></td>
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<tr>
<td></td>
<td><em>Zanclus cornutus</em></td>
</tr>
<tr>
<td></td>
<td><em>Ostracion cubicus</em></td>
</tr>
<tr>
<td></td>
<td><em>Celtinus chelonius</em></td>
</tr>
<tr>
<td></td>
<td><em>Abudefduf sexfasciatus</em></td>
</tr>
<tr>
<td></td>
<td><em>Scolopsis bilineata</em></td>
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<tr>
<td></td>
<td><em>Scolopsis bilineata</em></td>
</tr>
<tr>
<td></td>
<td><em>Hemigymnus melapterus</em></td>
</tr>
<tr>
<td></td>
<td><em>Zanclus cornutus</em></td>
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CHAPTER 5

FINE-SCALE BEHAVIOURAL ADAPTATIONS OF PREY ON A CONTINUUM OF RISK

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In the wild, prey species often live in the vicinity of predators, rendering the ability to assess risk on a moment-to-moment basis crucial to survival. Visual cues are important as they allow prey to assess predator species, size, proximity and behaviour. However, few studies have explicitly examined prey’s ability to assess risk based on predator behaviour and orientation. Using mosquitofish, Gambusia holbrooki, and their predator, jade perch, Scortum barcoo, under controlled conditions, we provide some of the first fine-scale characterisation of how prey adapt their behaviour according to their continuous assessment of risk based on both predator behaviour and angular distance to the predator’s mouth. When these predators were inactive and posed less of an immediate threat, prey were often found within the attack cone of the predator showing reductions in speed and acceleration, characteristic of predator-inspection behaviour. However, when predators became active, prey swam faster with greater acceleration and were closer together within the attack cone of predators. Most importantly, this study provides evidence that prey do not adopt a uniform response to the presence of a predator. Instead, we demonstrate that prey are capable of rapidly and dynamically updating their assessment of risk and showing fine-scale adjustments to their behaviour.

Author contributions: MK, AJWW, AJW designed the experiments, MK performed the experiments, all authors performed the analysis and edited the manuscript, which was written by MK
Introduction

The threat of predation is ubiquitous for many species. In order to survive, prey must detect and avoid predators, as well as meet daily energy requirements. A problem for prey species arises from the fact that anti-predator behaviours such as increased vigilance (Hunter et al. 1998, Jones 1998, L Quinn et al. 2006), hiding (Krause et al. 1998, Jennions et al. 2003, Hedrick et al. 2006, Hansen et al. 2016) and reduced activity rates (Skelly 1994, Wooster et al. 1995, Ryer et al. 1998) inherently decrease the amount of time and energy available for important fitness-enhancing behaviours, such as foraging, mating or territorial defence (Lima et al. 1990). However, individuals are more conspicuous (Husak et al. 2006) and less vigilant (Godin et al. 1988, Jakobsson et al. 1995, Beauchamp 2014) while engaged in these important fitness-enhancing behaviours, putting them at greater risk of predation. Due to the opportunity costs that arise from these anti-predator behaviours, prey should ideally adjust the intensity of anti-predator behaviour to the level of risk within their environment, a concept referred to as the risk sensitivity hypothesis (Helfman 1989, Brown et al. 2006). Ultimately, this ability to assess and respond appropriately to risk is an important factor determining prey survival.

To assess risk, prey must first detect the presence and location of predators in their environment. Research has shown that prey utilise chemical, visual, auditory and tactile cues to gather information on risk (Persons et al. 2001, Kusch et al. 2004, McCormick et al. 2008). Which types of cues are used in predator detection is also determined by the range and speed over which those cues are transmitted through the environment. For example, visual cues provide more immediate information about risk compared to chemical cues, which can remain within an environment after a predator has left, or auditory cues, which provide less information regarding the size or species of potential predators (Ward et al. 2010). In this way, visual cues often allow prey to assess the degree of risk with a high degree of certainty.
Visual cues can allow prey to assess risk based on predator species (Kelley et al. 2003), body width, depth or gape size (Karplus et al. 1981), body posture (Helfman 1989) and proximity (Swaisgood et al. 1999). California ground squirrels (Spermophilus beecheyi) and slimy sculpin (Cottus cognatus) increase the intensity of their anti-predator responses when confronted with large predators compared to small predators (Swaisgood et al. 1999, Chivers et al. 2001). Columbian black-tailed deer (Odocoileus hemionus columbianus) increase flight initiation distances when humans approach more directly and at faster speeds (Stankowich et al. 2005). Even at close range, some fish use visual cues to avoid the mouth of a predator during inspection behaviour given that the region in front of its mouth (sometimes referred to as the ‘attack cone’) poses the greatest threat (Magurran et al. 1990, Brown et al. 2001). In fact, prey fish use this visual information in an anti-predator behaviour called the fountain effect in which they manoeuvre away from the predator’s mouth and towards the blind spot by the tail (Hall et al. 1986).

These studies underscore the ability of prey to assess the level of risk within their environment and respond in a graded, threat-sensitive manner. Furthermore, they point to the importance of visual cues in mediating prey responses to predators. However, few studies have investigated the ability of prey to continuously assess predation-risk as a function of visual information gleaned from predator behaviour. This question is particularly relevant for prey species living in constant proximity to potential predators, a scenario that is common throughout nature. For instance, Pitcher (1980) estimated that free-ranging groups of roach, Rutilus rutilus, were seldom more than two meters away from predatory pike, Esox lucius, meaning they are constantly within striking distance of a predator. This is similar for many populations of Trinidadian guppies, Poecilia reticulata, living in high predation habitats (Seghers 1974). In these scenarios, Pavlov et al. (2000) speculate that maintaining visual contact may be more adaptive than moving away as it allows prey to monitor predator behaviour. Indeed, Magurran et al. (1987) found that minnows, Phoxinus phoxinus, swimming in the presence of pike predators, Esox lucius, shifted
between various anti-predator behaviours, escalating the severity of their response as pike shifted from stationary behaviour to stalking or striking behaviour.

The level of threat posed by a predator depends not just on its behaviour, but also on its relative proximity and orientation to the prey. Surprisingly, the extent to which prey integrate these additional variables into their risk assessment is relatively unknown (although see Handegard et al. (2012)). This apparent gap in the predator-prey research is due in part to the historic lack of advanced automated tracking software but also to the tendency to treat risk as a fixed factor (Lima 2002). As a result, little is known about how prey gauge the threat posed by a predator on a moment-to-moment basis or whether they incorporate this information into their behavioural decisions.

We sought to investigate how prey adjusted their behaviour in response to predator behaviour and orientation by allowing predator and prey to interact in controlled conditions. We hypothesized that prey would adjust their behaviour based on the predator’s activity level and based on where they were located in relation to the predator’s mouth. Specifically, we predicted that prey would increase anti-predator behaviours, reflected by increases in swimming speeds, reduced neighbour distances and increases in acceleration (Magurran et al. 1987), when they were in the attack cone in front of the predator, and when the predator was active rather than inactive. Finally, we sought to characterise for the first time the exact shape of the relationship between these response variables and the relative alignment of predator and prey.
Methods:

Collection and Husbandry:

Eastern mosquitofish, *Gambusia holbrooki*, with standard length of 22.5 ± 2.3mm (mean ± s.d.) were collected from Manly Dam, Balgowlah, Australia (33°46′35.45″S, 151°14′50.38″E) in October 2016 and transported to a temperature-controlled aquarium at the University of Sydney. All fish were housed in large stock tanks maintained at 24°C with a 12:12 light:dark cycle and fed fish flake daily. Commercially-bred jade perch, *Scortum barcoo*, with standard length of 91.5 ± 1.6mm (mean ± s.d.) were housed in individual tanks and fed a mix of pellets and live mosquitofish daily. All fish were acclimated to lab conditions for a minimum of 2 weeks before experiments began. This work was approved by the University of Sydney Animal Ethics Committee (ref 2016/1077) and was carried out in accordance with local regulations.

Experimental Apparatus and Protocol:

Experimental tanks consisted of two concentric circular arenas. The outer circular wall was opaque and tapered so that it had a diameter of 572mm at the bottom of the tank and a diameter of 692mm at the water’s surface. Tanks were filled to a depth of 70mm and kept at the same temperature as the stock tanks. The inner transparent circular arena was used to hold perch during the experiments and had a diameter of 283mm (Figure S1). A single perch (the predator) was placed in the inner enclosure the night before experiments began and given an additional hour to acclimate in the morning after the lights were turned on. No predators were fed within 24 hours of trials. After the predator’s acclimation period, mixed sex groups of 10 mosquitofish (the prey) were released into the outer annulus of the test tank. After a one-minute acclimation period, trials were filmed for 12 minutes using a Canon G1X camera filming at 1080dpi and 24fps. A total of 180 mosquitofish were used in 18 separate trials with 18 different perch predators such that all fish were tested only once.
Video Tracking and data extraction:

Videos were formatted and cropped using VirtualDub (v1.9.8) then uploaded to the manual tracking software CTrax (Branson et al. 2009). Using this automated tracking software, the $x$, $y$ coordinates of all fish (both predator and prey) were recorded at each frame over the 12-minute trials. Trajectories were then hand corrected using the Fixerrors GUI in MATLAB so that each fish had an unbroken record of its location throughout all 17,280 frames (see Figure S1).

Using a known ratio of pixels to mm, $x$, $y$ coordinates were converted to mm, then used to calculate predator and prey behaviour. Predator coordinates were used to calculate instantaneous speed and turning speed. To account for spurious fluctuations in tracked movement, coordinates were smoothed using a rolling average that spanned 5 frames (208ms). Prey coordinates were used to measure median swimming speed (mm/s), median nearest neighbour distances (mm) and median acceleration (mm/s$^2$). We calculated median swimming speeds and acceleration because both behavioural measures are highly responsive to context (Schaerf et al. 2017). Similarly, we used nearest neighbour distances as a measure of risk-perception given that prey often form more compact and cohesive groups in response to increased risk (Hager et al. 1991).

Perch behaviour was characterized by periods of activity, marked by high speeds and high turning speeds, and periods of inactivity, marked by low speeds and low turning speeds. This was determined after histograms of predators’ instantaneous speed and turning speed revealed bimodal behavioural states (Figure S2). Using these instantaneous speed and turning speed thresholds, predator behaviour was split into “active” or “inactive” states (see Supplemental methods). We then created a series of 5° bins radiating out from in front of the predator’s snout (0° to 5°) to directly behind the predator (175° to 180°). This was done so that we could calculate the behaviour of each individual prey (speed,
acceleration and nearest neighbour distance (NND)) based on predator activity state and angular distance to the predator’s mouth. We did not investigate lateralised behaviours in either the predator or prey and instead averaged prey behaviour across the predators’ left and right sides. To avoid any effect of tank geometry on prey behaviour, we analysed prey within two predator body lengths of the predator (average predator standard length: 91.5 ± 1.6 mm, therefore prey behaviour was limited to within 183mm of the predator’s centre of mass).

As perch typically stalk prey before striking, the probability of the predator striking at prey increases when they become active (Reid et al. 2010). Furthermore, previous experiments have demonstrated that prey behave differently when in front of a predator and tend to avoid the ‘attack cone’ region immediately in front of the predator’s mouth (Magurran et al. 1990, Brown et al. 2002). In light of this work, we analysed prey behaviour based on predator state and angle to the mouth. An angle of zero would indicate the prey was directly in front of the predator’s mouth, and an angle of 180 would indicate prey were at the tail of the predator.

Statistical Analysis:

Each measure of prey behaviour was tested in mixed effect models against the interaction between predator state and angle to the predator’s mouth. To fully capture the fine-scale adjustments in prey behaviour, which were often non-linear, we included orthogonal first, second, and third order polynomials to investigate whether the quadratic term significantly improved the regression compared to the linear term or the cubic term significantly improved the regression compared to the quadratic term. Orthogonal polynomials were used to reduce multicollinearity and improve model stability (Budescu 1980). While the linear fit was often significant, it failed to capture the essence of these behavioural responses (see below). When there was a significant interaction between predator state and angle, prey
behaviour was tested against angle separately based on whether predators were active or inactive. Depending on which polynomial was significant within the main model, the subsequent sub-setted model included the same degree polynomial.

Within each mixed effect model, which we created using the lme function in R (Bates et al. 2015), prey identity was nested within group and included as a random effect. This was done to account for the non-independence of individuals within the same trial. To meet the assumption of homogeneity of variance, response variables were transformed using the ordered quantile (ORQ) normalization transformation, though graphs were produced using raw data to increase interpretability.

To visualise how prey adapted their movements in response to predator activity state and location, heat plots of prey direction of movement and speed in relation to the predator’s position and orientation were created. To do this, we calculated the velocity of all prey movements that occurred in each cell of a 17 mm x 17 mm gridded array, centred with the predators positioned at (0,0) and facing along the positive y-axis. This bin size was selected because it represents the SL of the smallest mosquitofish used within any trial (17.32 mm). This was done separately for times when predators were active and inactive.
Results

Predators shifted between active and inactive activity states (Fig. S3), spending an average of 58% of each trial active and 42% inactive. Prey adjusted their median swimming speed as a function of the interaction between predator activity state and angular position relative to the predator’s mouth (Table 1). In particular, prey showed a greater range of speeds across angular positions when predators were inactive and generally moved more slowly as they approached the head or tail of the predator (Fig. 1 and see vector length (represented by the arrows) in Fig. 2). The reductions in speed when in front of a predator potentially allow individuals to update information about risk in a manner akin to predator inspection behaviour (Pitcher et al. 1986, Dugatkin et al. 1992). While prey slowed down in front of inactive predators, swimming speeds were not reduced to the same extent when in front of active predators (Fig. 1). This is likely due to the greater risk associated with occupying positions within the attack cone and can help explain the pronounced flow of prey away from the predator’s mouth and towards its tail during periods of activity in Fig. 2.

Along with these shifts in median speeds, prey also adapted their median distances to nearest neighbours as a function of the interaction between predator state and angular position relative to the predator’s mouth (Table 1). Generally, prey swam closer together in front and behind the predator, although the shape of this relationship changed with predator state. When predators were inactive, NND was lowest when prey were behind the predator whereas when predators were active, NND was lowest in front of the predator (Fig. 3). While grouping more closely is a common response to situations of heightened risk (Hamilton 1971), our results demonstrate an ability to adjust nearest neighbour distances in response not only to the presence of the predators, but also to slight changes in predator behaviour and orientation.
Prey also showed a shift in median acceleration based on predator state and angular position relative to the predator’s mouth (Table 1). Given that rapid acceleration, potentially resulting from fast start escape behaviour (Domenici et al. 1997), is an energetically taxing behaviour, prey should ideally employ this behaviour in extreme situations, such as when they find themselves in front of an active predator. Accordingly, we found a significant relationship between acceleration and angular position when predators were active with the fastest accelerations occurring directly in front of the predator’s mouth and declining as they neared the predator’s tail. When predators were inactive, there was a significant quadratic relationship with prey showing slightly greater acceleration when located to the side of the predator (Fig 4).

When the predators were inactive, prey fish tended to swim anti-clockwise around the annulus, with no discernible directional coordination with respect to the predator’s orientation (Fig. 2). However, once predators became active, prey fish fanned away from the predator’s snout and towards the predator’s tail, a pattern termed the fountain effect in the routine behavioural decisions of prey (Hall et al. 1986).
Table 1: Results from mixed effect models against each measure of prey behaviour. Individual nested within trial was included in each model as a random effect.

<table>
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<th></th>
<th>Value</th>
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<th>Conf. Int.</th>
<th>t-value</th>
<th>p-value</th>
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<td></td>
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Figure 1: Graph of median prey swimming speed (mm/s) against angle from predator’s snout when predators were inactive (green) and active (red). There was a significant cubic relationship when predators were inactive (\( \text{Angle}^3: t = 6.24, \) lower CI = 2.84, upper CI = 5.45, \( p < 0.001 \)) and significant quadratic relationship when predators were active (\( \text{Angle}^2: t = -3.82, \) lower CI = -3.73, upper CI = -1.20, \( p < 0.001 \)). Mean speeds and standard errors are shown.
**Figure 2:** Heat map of prey direction of movement and speed (as shown by the vector field (white arrows)) in relation to the predator (depicted in red at the origin of the plot). Warmer colours indicate when prey move in the same direction as the predator, cooler colours indicate when predator and prey direction of movement are opposed (measured as the cosine of the angle between the prey and predator headings).
**Figure 3:** Graph of median nearest neighbour distances (mm) against angle from predator’s snout when predators were inactive (green) and active (red). There was a significant cubic relationship between angular position and NND when predators were inactive (Angle$^3$: t = 6.09, lower CI = 3.44, upper CI =6.70, p < 0.001) and significant quadratic relationship when predators were active (Angle$^2$: t = -5.26, lower CI = -5.81, upper CI = -2.66, p < 0.001). Mean neighbour distances and standard errors are shown.
Figure 4: Graph of median acceleration (mm/s²) against angle from predator’s snout when predators were inactive (green) and active (red). There was a significant quadratic relationship between acceleration and angle when predators were inactive (Angle²: t = -2.257, lower CI = -2.979, upper CI = -0.377, p = 0.012) and active (Angle²: t = 2.00, lower CI = 0.027, upper CI = 2.583, p = 0.046). Mean acceleration and standard errors are shown.
Discussion

Here we provide evidence that prey continuously update their risk assessment and adjust their behaviour based on predator behaviour and their position relative to the predator’s mouth. When predators were inactive and posed less of an immediate threat, prey showed pronounced inspection behaviour within the attack cone of the predator with reductions in speed and acceleration. However, when predators began to move and therefore posed a greater threat, prey swam faster, closer together and increased acceleration within the attack cone of predators. Generally, during periods of reduced risk when predators were inactive, prey swam in circles around the annulus. When predators were active, prey adapted their behaviour by fanning away from the predator’s mouth and towards its tail, a manoeuvre referred to as the fountain effect (Hall et al. 1986).

In the wild, prey species often live in the vicinity of predators, rendering the ability to assess risk on a moment-to-moment basis crucial to survival. Seemingly maladaptive behaviours, such as approaching and inspecting potential sources of risk, may therefore allow prey to gain information regarding risk (Dugatkin et al. 1992). Previous work has shown that prey utilize visual cues such as eye width and gape size to assess the level of threat (Karplus et al. 1981), indicating that inspection of the most dangerous region by a predator’s head can provide vital information. In the current study, we found that when predators were inactive, prey approached regions in front of the predator’s mouth at slower speeds. While counter intuitive, this reduction in speed might decrease prey conspicuousness (Krause et al. 1995, Krupa et al. 1998) while enhancing visual acuity through reduced motion blur (Kramer et al. 2001) and increasing the likelihood of flow detection through the lateral line. These mechanisms might therefore increase the likelihood of detecting predatory attacks when in risky locations (Stewart et al. 2013, Stewart et al. 2014). Furthermore, by approaching the predator’s head rather than their side or tail, prey might signal their alertness to predators and thereby deter future attacks (Godin et al. 1995).
In accordance with the risk sensitivity hypothesis, we found that once predators were active, prey increased swimming speeds within the attack cone of the predator and swam away from its head and towards the relative safety of its tail. These increased speeds may reflect the immediate need to get out of striking distance of the predator and leave the ‘attack cone’ directly in front of its mouth (Magurran et al. 1990, Brown et al. 2001, Brown et al. 2001, Brown et al. 2002, Brown et al. 2003). In this way, prey appear to employ adaptive information gathering behaviours during times of lower risk and shift to safer, more evasive behaviours as predators posed a greater threat.

In tandem with this shift to faster swimming in front of predators, prey reduced distances between themselves and their nearest neighbours when predators were active. Grouping more closely is a common evolutionary response to predation (Krause et al. 2002, Beauchamp 2014, Ward et al. 2016, Herbert-Read et al. 2017). Indeed, theory suggests that individuals within a group can reduce risk by moving towards neighbours and by positioning themselves closer to the centre of the group, ultimately resulting in the formation of denser aggregations (Hamilton 1971). This can explain why in many systems, we see the formation of more compact groups after exposure to a predator (Foster et al. 1981, Watt et al. 1997, Viscido et al. 2002, Schaerf et al. 2017). In the current study, we found the smallest neighbour distances when prey were directly in front of an active predator, suggesting that prey were capable of gauging risk not based solely on predator presence, but based on the predator’s behavioural state and angular position. The fact that prey did not consistently form more cohesive groups in the presence of a predator implies that there may be costs associated with remaining cohesive. These costs, for example, could include increased cognitive demands associated with the coordination of this behaviour, or increased competition for resources. Ultimately, understanding how animal decision making circuits integrate multiple forms of information including the state and position of the predator,
the position of neighbours and the costs and benefits of cohesion, will provide an intriguing avenue for future research, particularly from a neurological perspective.

When predators became active, prey switched between swimming around the annulus to a manoeuvre commonly described as the ‘fountain effect’ (Hall et al. 1986), in which prey fan away from the predator’s mouth and towards the blind spot by its tail. Traditionally, observations of this behaviour describe prey rapidly accelerating out of the predator’s attack cone in response to a direct strike (Magurran et al. 1987, Christensen et al. 1993). While these flash fountain manoeuvres in direct response to predator strikes are visually apparent, it is interesting to note that the fountain pattern in this study emerged by averaging prey behaviour over the course of a trial, suggesting that these movement patterns around a predator may be occurring more passively through slight adjustments to routine behaviour. This manoeuvre may act as a way for prey to increase survival by avoiding the dangerous area in front of a predator while maintaining cohesion by reforming groups behind the threat, as reflected in the decreasing nearest neighbour distances found towards the tail of the predator. Our findings represent the first description of the fountain manoeuvre in averaged prey behaviour and ultimately serve to underscore prey’s ability to integrate information about the risk posed by different predator behaviours and different regions of the predator, lending further support to the risk sensitivity hypothesis.

By using basic routine behavioural adjustments based on predator activity and their position relative to a threat, prey fish may be able to minimise their exposure to risk through energetically efficient means. However, when prey inevitably find themselves in a dangerous situation (or position), they may need to employ more energy-consuming anti-predator responses, such as fast starts. Fast starts, or c-starts, are marked by sudden bursts of acceleration away from a threatening stimulus (Domenici et al. 1997). In the current study, we found that acceleration was greatest when prey were directly in front of an active
predator and decreased almost linearly with distance from the predator’s mouth. Previous research has shown that the ability to rapidly put distance between yourself and danger is a highly adaptive and conserved behavioural mechanism (Law et al. 1996). Evidence for the advantages of fast start behaviours have been found in research using largemouth bass and four different prey species. In that study, predators were increasingly likely to abort an attack as prey acceleration increased (Webb 1986). Similarly, the evasion success of prey corresponded to their acceleration rates (Walker et al. 2005). This means that the ability to preserve energy when risk is low and engage in the most taxing evasive behaviours only when risk is high could be important to the survival of prey species. Fittingly, prey in this study showed the greatest acceleration when in the most extreme situations, namely when they found themselves in the direct path or within striking distance of an active predator.

While previous research has expanded our understanding of how prey behaviour changes as a function of prey hunger levels (Godin et al. 1994), prey group size (Brown et al. 2006, Mathiron et al. 2015), prey provenance (Brown et al. 2009), predator diet (Brown et al. 1999, Mirza et al. 2001, Mirza et al. 2003) and predator morphology (Karplus et al. 1981, Karplus et al. 1982), much of this work has been done through the use of model predators (Karplus et al. 1982), computer animated predators (Sommer-Trembo et al. 2016), short exposure times (Ferrari et al. 2010) or the use of isolated cues, such as conspecific alarm cue (Brown et al. 2006), heterospecific alarm cue (Manassa et al. 2013) or predator odours (Brown et al. 1999, Mathiron et al. 2015). Despite the importance of these manipulative laboratory experiments, there is a dearth of empirical studies investigating the interplay between predator and prey behaviour. Many of these previous approaches have reduced predators from interactive agents to “abstract sources of risk” (Lima 2002), which prevents researchers from detecting some of the more nuanced ways in which prey can respond to the presence of a predator. We found that prey reduce risk by continuously adjusting their routine behaviour based on different information gleaned from visual cues. We found that prey respond continuously to predator activity levels and adjust
behaviour based on angular distance from the predator’s mouth, demonstrating an ability to assess risk on a moment-to-moment basis and adjust behaviour accordingly. While these insights underscore the need to investigate fine-scale interactions between predators and their prey, future research should strive to investigate these dynamics in more natural systems with free-ranging agents.
References


SUPPLEMENTARY METHODS:

Figure S1: Example of experimental setup and video tracking from predator 4 trial.
**Trajectory Analysis:**

Both predator and prey coordinates were smoothed using a moving average spanning 208ms (5 frames of video) and converted into real-world coordinates using a known reference length within the videos.

**Identifying Predator States:**

Histograms of both the predators’ instantaneous speeds (Fig. S2a) and turning speeds (Fig.S2b) were bimodal, indicating the presence of two behavioural states. Plotting predators’ instantaneous speeds versus their instantaneous turning speeds revealed more detail of these states (Fig. S2c). One state was associated with higher speeds and turning speeds (state 1), which we call the ‘active state’. The other was associated with relatively lower speeds and turning speeds (state 2), which we call the ‘inactive state’. We take the projection of a predator’s speed and turning speed along the line joining the centroids of the active and inactive state (denoted by an arrow in Fig. S2c). We define the halfway point between the two to be the boundary between these two states (as shown by the segment halfway along the arrow), and determined the occasions when each predator was in the ‘active state – state 1’ (top right region of the Fig 2c) or an ‘inactive state – state 0’ (bottom left region of Fig. S2c). Because measurements of instantaneous speed and turning speed were noisy, we attempted to identify more contiguous periods when a predator was in the active state. This was done by forcing small periods of inactivity of less than 2 seconds which are found between active periods, to also be marked as active. Results are detailed in Figure S3.
**Figure S2:** Frequency histograms of (a) log predator speed and (b) log predator turning speed. (c) Log turning speed versus log speed for all predators. We use the projection of a predator’s instantaneous speed and turning speed onto the arrow in (c) to label the predator as ‘active’ (red, top right) or ‘inactive’ (blue, bottom left) at a given point in time.
**Figure S3:** Labels of time periods of activity and inactivity for each of the 18 predator fish. The projection of a predator’s speed and turning speed along the line joining the centroids of the active and inactive state (denoted by an arrow in Fig. S1c) is shown as the noisier, faint signal on each line. The solid, binary indicator of ‘active’ vs. ‘inactive’ state for each predator is shown on top (after filtering). Each predator’s line is offset vertically from the previous one so that they are all visible.
CHAPTER 6

SUMMARY AND FUTURE DIRECTIONS

The stated aim of this thesis was to contribute to our understanding of collective behaviour by capitalising upon recent advances in tracking software and analytical techniques. Using Tinbergen’s 4 questions as a framework, this thesis investigated the mechanisms, development and function of collective behaviour. In Chapters 2 and 3, I suggest that the ability to coordinate group movement is conserved across different developmental contexts and that speed can govern certain group-level patterns in a similar way across multiple species. In chapter 3, I found that information flow increased with speed and alignment, providing support for the idea that coordinated collective movement might have an adaptive value by allowing animal groups to respond rapidly and in a synchronised manner to changes in their environment. In Chapters 4 and 5, I used different socially living species to investigate the adaptive value of collective behaviour, providing evidence that group-living species can assess and respond to risk in a nuanced manner. In a coral reef system, I discovered humbug damselfish have the ability to selectively integrate cues from their abiotic and biotic environment. In a freshwater system, I discovered that mosquitofish have the ability to alter behaviour based on slight changes in predator behaviour and position.

While this thesis has provided valuable insight into the development, mechanisms and function of collective behaviour, it did not address Tinbergen’s question regarding the evolution of collective behaviour. Generally, for a behaviour to evolve through natural selection, there must be (a) variation in behaviour within the species, (b) some selective advantages to certain behaviours and (c) the ability to
pass these differences on to future generations. Previous work has demonstrated that populations of the same species, driven by a selection pressure such as predation, can develop different grouping behaviours (Seghers 1974) that are ultimately passed on to future generations (Wright et al. 2003), thus providing an elegant example of evolution by natural selection. However, these studies have relied on basic metrics of sociality, such as average group size or neighbour distances. Less work has been done on the evolution of specific schooling mechanisms. Research has already established that plasticity in schooling mechanisms exist, both transiently (Schaerf et al. 2017) and over multiple generations (Herbert-Read et al. 2017). Furthermore, Ioannou et al. (2012) found that bluegill sunfish predators, *Lepomis macrochirus*, were less likely to attack virtual prey who formed groups and aligned with neighbours, suggesting that predation can act as a selective force on certain individual interaction rules, such as alignment or attraction. Work by Greenwood et al. (2016) also established that a specific gene was responsible for variation in “schooling ability” (i.e. positioning and alignment) between stickleback morphotypes, providing a potential mechanisms through which specific behavioural rules can be inherited. Therefore, the components necessary for evolution of specific schooling mechanisms exist, though no study has specifically studied how they shift over time or across different species. Future work could capitalise on the approach taken in Chapter 2 to compare individual interaction rules and global group properties between different species with known phylogenetic relatedness, allowing researchers to map patterns of schooling mechanisms over evolutionary time.

In chapter 3, I discussed the speed-mediated mechanisms of schooling, detailing the specific relationship between speed, alignment and positional composition. However, I limited my analysis to groups of 5 individuals, despite the fact that some of the most mesmerising examples of collective motion involves the synchronisation and coordination of hundreds of individuals. With this in mind, it would be
interesting to assess whether the relationship I found between speed, alignment and elongation in moving groups is consistent across different groups sizes. The interaction rules that were empirically derived by Katz et al. (2011) were done using groups of 2 fish. However, they also tested groups of 3, 10 and 30 and found that the interaction structure remained the same regardless of group size. Similarly, Herbert-Read et al. (2011) tested groups of 2, 4, and 8 mosquitofish and found no qualitative difference in speed or turning angle regulation between group sizes. In a field study on naturally occurring groups of sticklebacks ranging from 4 to 44 individuals, Ward et al. (2017) found that faster groups were more elongated and that larger groups tended to be faster. These results seem to provide support for the idea that the mechanisms I describe in chapter 3 might function in larger groups as well. However, research by Hemelrijk and Hildenbrandt (2008) assessed elongation as a function of both speed and density and hypothesized that there would be greater elongation at slower speeds, contrary to my findings, as well as greater elongation at greater group densities. More empirical work is needed to ascertain whether their proposed mechanisms are at play in real-world systems, or whether the mechanisms discussed in chapter 3 are consistent across different group sizes.

In chapters 4 and 5, this thesis investigated the adaptive value of collective behaviour by investigating risk-assessment in ecological and predator contexts. I began in chapter 4 by investigating the effect of the abiotic and biotic environment on emergence behaviour in humbug damselfish. While previous work has provided valuable insight into how individual environmental factors can impact animal behaviour, chapter 4 provided support for the idea that animals can integrate information from multiple sources at once. My results highlight the flexible use of social and physical information in shaping risk-perception and the balance struck between foraging and refuging behaviour. Given that I treated behaviour as bimodal, a fruitful avenue for future research would be to track behaviour in 3 dimensions and treat
distance from refuge as a more nuanced continuous measure of risk-assessment. In a study by Burns (2016), damselfish continuously reduced average speeds and distance from coral in the hour approaching sunset, which is a period of increased risk as light attenuation declines and larger crepuscular and nocturnal predators emerge (Hobson 1975). This suggests that continuous measures of speed and distance to coral are an appropriate approximation of risk-perception in damselfish. This study also found that the effect of time of day subsumed any effect of tide, suggesting that the research conducted in chapter 4 could yield interesting results were it to incorporate more nuanced measures of damselfish behaviour and investigate different times of day as well as different tidal periods.

Research on damselfish has also found a significant effect of group size on re-emergence after a threatening stimulus (Hansen et al. 2016), which runs counter to the findings in chapter 4 where only the external social environment influenced damselfish emergence behaviour. However, it is possible that the conflicting results relate to the fact that Hansen et al. (2016) used an artificial predator stimulus. This raises the interesting possibility that damselfish further shift their use of social cues when analysed in a different context, such as when they are under direct threat. Overall, an exciting opportunity exists for future research to measure behaviour continuously and to assess, as I did in chapter 4, the flexible use of social and physical features of the environment within different contexts, testing behaviour during mid day or during dusk and while undisturbed as well as after a simulated predator attack.

After investigating how different features of the environment shaped risk-perception in damselfish, I investigated how predator behaviour and angular position shaped risk-perception in mosquitofish. In chapter 5, I found that during periods of reduced risk when predators were inactive, prey swam in circles and made only slight reductions in speed and neighbour distances when located in front or behind the
predator. However, when predators became active, prey fanned away from the predator’s mouth and towards its tail while reducing distances to neighbours and increasing speeds, turning speeds and maximum acceleration within the attack cone. While much of the existing research has reduced predators to “abstract sources of risk” (Lima 2002) through their use of model predators (Karplus et al. 1982), computer animated predators (Sommer-Trembo et al. 2016), short exposure times (Ferrari et al. 2010) or isolated cues (Brown and Godin 1999, Brown et al. 2006, Manassa et al. 2013, Mathiron et al. 2015), this study has provided evidence that treating predators as interactive agents can yield important insight. That said, the mosquitofish and perch in chapter 5 could not freely interact and future research should strive to investigate predator and prey behaviour in less controlled environments. In an open field experiment, for instance, prey should ideally assess risk based on distance from predator as well as predator activity level and angular position. Furthermore, by allowing predator and prey to fully interact, research could better assess the collective behaviour of prey, comparing, for instance, how groups behave before and after an unsuccessful versus a successful attack. As demonstrated by Handegard et al. (2012) field studies of predator and prey interactions can also yield important insight into the speed at which social information can be transmitted, potentially determining the value of collective movement when under direct predation threat.

Given the two-way nature of predator-prey interactions, there is a surprising lack of research on predator behaviour as a function of prey behaviour. Research by Ioannou et al. (2012) found that predators were less likely to attack virtual prey who formed groups and aligned with neighbours. They argue that this behavioural tendency might suggest an adaptive value to coordinated collective movement in prey. Further empirical work could corroborate these patterns in fully natural systems. In fact, even within controlled experiments, such as the work done in chapter 5, it would be fascinating to investigate what
factors contributed to the shift that predators made between inactive and active states. This type of research could strive to identify which features of prey behaviour, either at the individual or group level, precipitate the shift into or out of active hunting behaviour.

Overall, there are many avenues for future research, especially in light of the recent technological and computational advances. However, given that laboratory work on fish species has been at the forefront of many recent discoveries within the field of collective behaviour, it is important that future research investigate collective behaviour in other social species and that efforts are refocused towards collecting empirical data from the field (King et al. 2018). Ultimately, collecting in-depth and long-term studies on the behaviour of animals within their natural environment is crucial towards a fuller and more realistic perspective on collective animal behaviour.
References


