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Movement Decisions and Foraging Behaviour in Shoals of Fish

The influence of internal and external stimuli

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Statement of Authorship

The research described in this thesis is the original work of the author, except where specifically acknowledged

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Chapter 1

GENERAL INTRODUCTION

Animals are frequently faced with situations where they need to make decisions, but the environments they live in are dynamic and complex, with simultaneous and partial stimuli that may make it difficult to select the best among many alternatives. Additionally, much of the information available is irrelevant and much that is relevant is uncorrelated or its importance changes according to the animal's internal state. Glimpses of a stumbling springbok will attract the attention of a hungry cheetah, but will be of less interest to one with blood still drying on its mouth from a previous meal. Animals have evolved ways of selecting and integrating important information from internal and external stimuli. This information can then be used by animals to estimate the outcomes of different options and consequently make a series of behavioural decisions that have an overall effect of improving their fitness. Deconstructing how this process works is at the heart of behavioural ecology.

Animals of the same species do not all behave the same way under equal environmental conditions. The internal state of an animal will determine many of the costs and benefits of a behavioural decision, which means that natural selection favours individuals that adjust their decisions according to their state (Houston & McNamara, 1999). Many decisions relate to the acquisition of sufficient nutrients and energy and here, energetic reserve is a decisive stimulus as it motivates an animal to seek out food which invariably leads to potential costs such as increased predation risk (Abrahams & Dill, 1989; Lima & Dill, 1990; Houston et al., 1993). This

balance between energetic requirements and predation risk determines activity levels (Sih, 1982; Dill, 1983; Lima & Dill, 1990; Houston et al., 1993; Anholt et al., 2000) and is fundamental to the formation and maintenance of social groups. The range of animals that are at least to some degree social is vast and spans the phylogenetic tree.

Within these groups individuals may attain significant benefits from foraging together: they increase the proportion of time they allocate to foraging or exploit a wider range of resources due to a reduction in predator vigilance (Magurran et al., 1985; Clark & Mangel, 1986; Elgar, 1989; Lima & Dill, 1990; Beauchamp, 2013; Beauchamp, 2014), they reduce search time locating food patches (Pitcher et al., 1982; Clark & Mangel, 1986); and reduce variation in food intake by responding to social information on food discoveries by conspecifics (Dall & Johnstone, 2002). Grouping also benefits foragers by reducing the risk of predation on individuals (Hamilton, 1971; Foster & Treherne, 1981; Magurran, 1990; Ioannou et al., 2011). However, intra-specific competition for food increases with the number of foraging conspecifics and is a major cost to grouping (Ward et al., 2006).

Whilst all animals must make decisions, the situation for animals that live in groups is special as the fitness consequences of their decisions depend on the decisions made by other individuals within the group (Giraldeau & Caraco, 2000). For many animal groups, including some that form via fission-fusion dynamics where group composition is temporary, like bird flocks, migrating ungulates or fish shoals, many of the benefits of group living, such as reduced predation risk (Ioannou et al., 2011), information transfer (Ward et al., 2008; Procaccini et al., 2011; Berdahl et al., 2013; Miller et al., 2013) and group decision-making (Biro et al., 2006; King & Cowlishaw,

2007; Ward et al., 2008; Ward et al., 2011; Ward et al., 2012) are reliant on the group remaining cohesive. Coordination between individuals is consequently vital and much recent work investigates coordinated and accurate movement by the group through information transferred by the individuals responding to and copying a change in direction by near-neighbours. How these interactions between individuals produce group-level patterns ('collective movement') is a burgeoning area of research (Romey, 1996; Couzin et al., 2002; Couzin et al., 2005; Lukeman et al., 2010; Nagy et al., 2010; Buhl et al., 2011; Herbert-Read et al., 2011; Katz et al., 2011; Herbert-Read et al., 2013; Nagy et al., 2013). How these interactions lead to group-level decisions ('collective decision-making') is less well understood but the field is growing (Pratt et al., 2002; Couzin et al., 2005; Sumpter et al., 2008; Ward et al., 2008; Sumpter & Pratt, 2009; Lihoreau et al., 2010; Couzin et al., 2011; Kao & Couzin, 2014; Kao et al., 2014).

Apart from differences in information held between individuals, groups are also composed of individuals with different and separate motivations that reflect their current physiological state and external selective pressures. These motivations are determined by long term factors such as sex, size, age, metabolic rate, immune function, parasite load and lipid reserves but are also affected by factors such as hunger, perceived predation risk, and temperature, which may change rapidly. Heterogeneity of individual state within animal groups has been under-represented in models of collective movement (but see Romey 1996; Couzin et al. 2005), despite empirical evidence bringing to light its effect on individual spacing behaviour (Krause, 1994; Romey, 1995; Krause & Ruxton, 2002). For new insights into animal collective behaviour it is necessary to relax the assumption that individuals are unchanging across time and operate under the same local interaction rules. Then grouping behaviour becomes not just a one-time static decision, but

a labile and continuously updating response determined by the interactions between internal stimuli and external environmental conditions (which includes the location of other individuals).

If one recognises the existence of heterogeneity of individual state within the same animal group it is a natural progression to consider its consequences for individual control of group decision making. Group movement may be driven by an individual, or subset of individuals within a group that are more highly motivated to move (Rands et al., 2003; Conradt et al., 2009; McClure et al., 2011; Nakayama et al., 2012a; Nakayama et al., 2012b) and considering the conflict of interests these different motivations create (Conradt & Roper, 2009; Conradt, 2012), the inclusion of these inter-individual differences into models of collective behaviour and empirical tests of their theoretical predictions are imperative for more accurate and realistic representations of animal group movement and decision-making.

Internal state, an animal's underlying physiology, is difficult to measure in behavioural studies as it usually involves invasive procedures. Nutritional state, however, is more easily manipulated and measured than other internal stimuli, and is dynamic; changing over time within an individual faster than other internal stimuli critical to foraging decisions, such as metabolic rate and lipid-levels. Nutritional state influences patterns of activity, with nutritional deficiency causing animals to increase locomotion and activity (Dethier, 1976; Abisgold & Simpson, 1987; Browne, 1993; Gill & Hart, 1994; Andersen, 1998; Asaeda et al., 2001; Riche et al., 2004; Nagata & Nagasawa, 2006; Priyadarshana et al., 2006; Colasurdo et al., 2007), however, its affect on specific movement parameters that can influence inter-individual interactions such as speed are less well developed (but see (Robinson & Pitcher, 1989b; Bazazi et al., 2011). As the

role of nutritional state has been shown to affect activity levels and locomotion it has therefore been included into random walk and diffusion models, however, these often do not take into account group behavior nor how spatial heterogeneities in the environment influence animal movement, despite the fact that many animals orientate themselves to, and move along the edges of, physical structure. Chapter 2 assesses the impact nutritional state has on the movement parameters and decisions of shoals of *Gambusia holbrooki*, especially how they interact with physical structure in their environment.

Chapter 2 highlights the importance of internal nutritional state on movement parameters and use of environmental space whilst foraging, however, its role becomes more intricate and complicated when internal state, and therefore motivations, vary between individuals within the group. Differences in internal nutritional state between members of a group is a property that emerges from individuals following the same basic rules-of-thumb; to increase their foraging effort when their energy reserves decrease and to join a conspecific whenever it forages (Rands et al., 2003). This means that in a social context, group members' physiological requirements may be temporally uncorrelated and conflict is created in their preferred choice of activity. How the group responds to these conflicts of motivations is of wide biological relevance as synchrony in activity and movement decisions may be essential in many animal groups. In these situations individuals may compromise and adopt the behaviour of other group members or the group may reach a consensus. Individuals in fission-fusion groups must continually balance their own needs with that of the group and it becomes particularly intricate in a foraging context due to competition effects and where social information is so critical. Here, if the costs of compromise or consensus are too great, groups may fission (Kerth et al., 2006; Kerth, 2010) and break up into

smaller groups that are more homogeneous in internal state. This aspect of group behaviour has been less well integrated into studies of collective movement and collective decision-making as it is often assumed that group cohesiveness is the individual's best option (Sueur et al., 2011). Chapter 3 manipulates group composition and investigates the movement decisions of individual crimson-spotted rainbow fish, *Melanotaenia duboulayi*, within three social contexts (when all fish are hungry, when all fish are satiated, and when a conflict exists between individual's motivation when half of the group are hungry and half are satiated).

Heterogeneities do not only occur within the group. One of the benefits of grouping is that social information transfer allows groups to respond collectively and accurately to environmental complexity (Berdahl et al., 2013). One aspect of environmental complexity often ignored in studies of optimal foraging is the macro-nutrient composition of food (but see Simpson et al., 2004; Geerten M. Hengeveld et al., 2009; Houston et al., 2011) which is often heterogeneously distributed amongst different food types in the environment (Simpson & Raubenheimer, 2012). Understanding and acknowledging an animal's sensory ability to recognise macro-nutrients in food is vital when developing realistic models of optimal behaviour (Simpson et al., 2004; Jordan & Ryan, 2015). Whilst individuals are known to regulate their intake through movement decisions between resources that vary in nutritional composition (Simpson & Abisgold, 1985; Despland & Simpson, 2000; Raubenheimer & Jones, 2006; Simpson & Raubenheimer, 2012), collective responses to nutritional imbalances in the environment are only beginning to be addressed (Simpson et al., 2006; Dussutour et al., 2008; Dussutour & Simpson, 2009; Simpson et al., 2010; Bazazi et al., 2011; Lihoreau et al., 2014; Lihoreau et al., 2015; Senior et al., 2015) and currently no study addresses this issue in vertebrates. Chapter 4 investigates the sensory ability

of mosquitofish to detect and regulate their intake of food on the macro-nutrient scale and how differential distributions of macro-nutrients in an environment affect individual decisions and how these scale to effect the distribution of the group within the environment.

Animals in groups do not only need to decide where and when to move in the environment. As individual fitness returns differ according to the position of individuals relative to each other, they also need decide where within a group they are spaced (Krause, 1994; Krause & Ruxton, 2002). The fitness returns of different within-group spatial positions are determined by biotic factors in the environment, predominantly predation risk (Hamilton, 1971; Rayor & Uetz, 1990; Krause, 1993a; Bumann et al., 1997; Stankowich, 2003), however, the peripheries of stationary groups (Rayor & Uetz, 1990; Romey, 1995; Rowcliffe et al., 2004) and the front of moving groups (Janson, 1990; Krause et al., 1992; Krause, 1993b) are favoured for maximising food intake. Due to the multiple selective factors affecting within-group spatial positioning the benefits and costs to individuals will vary according to their individual state. Chapter 5 investigates within-group spatial position in foraging rainbowfish and specifically utilizes a repeated measures design to isolate nutritional state from other individual differences.

The first four experimental chapters are laboratory based which allows for the collection of accurate spatio-temporal data on a small scale. This scenario necessitates the removal of many environmental variables that may be of interest to behavioural decision-making. Chapter 6 investigates colonies of humbug damselfish, *Dascyllus aruanus*, in the field incorporating the little studied environmental effect of tide on their movement-decisions in a foraging context, albeit at a coarser scale than the studies described in earlier chapters.

Within animal groups, the behaviour of conspecifics are interdependent and individuals can be viewed as a dynamic aspect of each other's external environment. Individuals that are hunting or searching for food in a group base their foraging decisions in part upon the actions of conspecifics. This allows for an increase in opportunities for individuals to acquire social information on the quality and location of food which may affect the strategic roles of individual foragers. The framework developed for investigating this relationship is called social-foraging theory, which specifically addresses the frequency dependency of foraging roles within groups considering ecological conditions in the spatial and temporal distribution of food (Giraldeau & Caraco, 2000). The vast majority of empirical tests have been conducted on birds (see (Giraldeau & Caraco, 2000; Beauchamp, 2013) for reviews) with a few investigations into social foraging theory in wild primates (Di Bitetti & Janson, 2001; Bicca-Marques & Garber, 2004; King et al., 2009). This bias in taxa is due to experimental and observational difficulties in distinguishing the tactic used by an individual, the boundaries of patches and the individual pay-offs for discrete foraging events, however, the great appeal of social foraging theory is its applicability to a vast range of animal groups. Chapter 7, therefore, investigates the effect of environmental quality on finder-joiner dynamics in three-spined sticklebacks, *Gasterosteus aculeatus*, to broaden the taxonomic scope of empirical studies in social-foraging theory and establish a model system amendable to experimental manipulation and fine-scale data collection.

Why fish?

Fish shoals are ideal systems through which to study foraging and the nature of group movement and spatial dynamics. Fishes have a long history as test subjects in this field, as they acclimate well to the laboratory where it is relatively easy to manipulate them internally and to manipulate their environmental conditions externally. Advancements in subcutaneous tagging means they can be individually identified without causing harm or adverse changes to their behaviour. In addition, due to their small size and developments in autonomous multi-agent tracking software, the position of each individual within the group can be accurately recorded over time from video. They also form complex non-kin related social groups that are known to undergo regular fission and fusion events. Perhaps the most intriguing aspect of studying these animals is their array of group level patterns and movements, which reflect the different species' varied natural history.

Four fish species have been selected for experiments in this thesis.

Mosquitofish, *Gambusia holbrooki*, (Chapters 2 and 4) are a successful invasive species to Australia and were chosen as a model system to empirically test how hunger and macro-nutrient preferences influence movement and how individuals interact with the physical environment. The reasons for this are that they are a small, facultatively shoaling species that inhabit shallow, heavily structured water bodies. They are omnivorous and have a wide range of food types. They eat zooplankton (rotiers, copepods and cladocerans), ostracods and insects (predominately chironomid larvae), with a large proportion of their diet consisting of algae and detritus.

Crimson-spotted rainbowfish, *Melanotaenia duboulayi*, (Chapter 3 and 5) are a freshwater species of fish endemic to eastern Australia. They were chosen as a system to study the impact of nutritional state on group movement dynamics as they are large enough to monitor intake rates and yet small enough to form shoals in laboratory conditions. They form small shoals of approximately 5-20+ fish, and whilst they often swim cohesively as a shoal, they undergo regular fission and fusion events, and individuals or smaller groups may break away temporarily.

Humbug damselfish, *Dascyllus aruanus,* (Chapter 6) are the only marine fish used in this thesis and the only species used in field experiments. They live in small groups of unrelated individuals ("colonies") within and around branching coral heads. They are planktivores and feed in the water column directly above and around their coral head and predation threats are alleviated through a collective response whereby the colony seeks refuge within the branches of the coral until the threat has passed. As the amount and variety of food available and therefore the feeding rate of humbug damselfish is greatest during high tide, when plankton availability is greatest, they are good natural study system to explore not only how animal groups trade-off feeding and predation threat, but also how this is affected by consistent temporal rhythms.

Three-spined sticklebacks, *Gasterosteus aculeatus*, (Chapter 7) were chosen to extend and build our understanding of finder-joiner dynamics. They were chosen as they are one of the most widely used model species in behavioural studies, are often used in foraging studies and have recently been used as a model system to explore social learning and the trade-off between using individual and social information.

Fish aggregations have long been studied in the context of foraging, nutritional state and group behaviour. Notably, the spacing and positioning of individuals reflects both internal and external stimuli and is a compromise between avoiding predation and the need to mate or find food (Keenleyside, 1955; Morgan, 1988; Robinson & Pitcher, 1989b; Robinson & Pitcher, 1989a; Krause, 1993a). Frontal positions are often better for foraging (Major, 1978; Krause et al., 1992; Krause, 1993b; Krause, 1994; DeBlois & Rose, 1996) and there is some evidence that hungry individuals emerge at the front of groups when shoaling (Krause et al., 1992; Krause, 1993b; Krause et al., 1998) where they display a stronger influence on movement direction (Huth & Wissel, 1992; Katz et al., 2011). Similarly, well-fed fish have been found to swim at the back or the middle of a moving shoal exerting their preference for group cohesion. Food deprived animals also tend to maintain a larger inter-individual distance between conspecifics (Gueron et al., 1996; Hoare et al., 2004) perhaps sacrificing safety from predation to decrease food competition (Reebs & Saulnier, 1997), whilst individuals at the front of moving groups maintain a smaller inter-individual distance, consistent with the theory that in moving groups a higher predation risk causes individuals to clump together more tightly (Bumann et al., 1997). Studying the interaction of feeding and group movement in fish shoals becomes more complex when one factors in the strong social attraction of individuals and the consequential costs and benefits of social information use (Ryer & Olla, 1991; Ryer & Olla, 1992; Webster & Laland, 2012).

It is apparent, therefore, that internal and external stimuli each affect individual position within fish shoals. A question that remains for these subjects, however, is how individual movement parameters and behavioural decisions change under varying internal and external conditions and what effect does this have on their group level movement dynamics and decision-making.

Work in this area, however, is gaining traction and fish shoals are currently a model system for integrative theoretical and experimental research into the effect of individual interactions on the emergence of collective responses. Interactions between individuals explain how collective movement evolves as a response to predation (Ioannou et al., 2012). A minority of motivated or informed fish can influence group decisions (Couzin et al., 2011), and this influence is stronger if the fish balance goal orientated motion with their tendency to be social (Ioannou et al., 2015). However, interactions with an uninformed majority can inhibit this leadership process (Couzin et al., 2011).

Fish that have consistent but individual swimming parameters (median speeds and turning speeds and speed variances) in an asocial context partly maintain these in a social context, but also conform to and adopt the speed of the group (Herbert-Read et al., 2013). Local sensing of environmental cues by individuals and subsequent interaction between these individuals shapes the collective properties of the group, allowing the group to sense complex environments more accurately than individuals (Berdahl et al., 2013).

This thesis explores the mechanisms and functions of decision-making in groups, specifically in the context of social foraging in fish shoals. While many animal groups may seem homogeneous to the naked-eye, closer inspection reveals considerable heterogeneity, as they are composed of individuals with different phenotypes and different motivations living in stochastic, complex environments. The question then, is how do individual behavioural decisions change under varying internal and external conditions and what effect does this have on group level decision-making? How do animals address conflicts of interest and competition effects whilst ensuring benefits of group living are maintained? The approach taken in this thesis has been to address these questions from many angles, using a range of freshwater and marine species and employing an array of novel experimental set-ups. Of particular importance has been the utilization of automated, multi agent tracking software, which has allowed for the description of the movement and interaction of individually identified fish at a much finer scale than in the past.

This project has direct significance to our understanding of the individual and group dynamics of social species, which is a central theme in behavioural ecology, and will inform researchers in a variety of fields from theoretical biology to sociological studies of human grouping patterns. The inclusion of internal nutritional state and external environmental factors into studies of group movement and decision-making in a foraging context is a practical way of linking the mechanistic forces behind individual behaviour to functional group-level responses. This will help expand our understanding of the evolutionary causes of group living and its ecological consequences, influencing conservation management plans and strategies to improve fisheries and aquacultural practices.

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Chapter 2

THE EFFECT OF HUNGER ON THE EXPLORATORY BEHAVIOUR OF MOSQUITOFISH, *GAMBUSIA HOLBROOKI*

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The question of how hunger affects locomotory behavior, in particular how it affects the kinematics of movement and an animal's interaction with the physical structures in its environment is of broad relevance in behavioural ecology. We experimentally manipulated the hunger levels of individual mosquitofish (*Gambusia holbrooki*) and recorded their swimming behaviour in shoals of 4 fish. We found that hungry individuals in shoals moved at greater speeds and had higher turning speeds than satiated individuals in shoals, as well as a greater variance in speed and turning speeds. We also found that hungry individuals explored more of the arena and used more of its internal space, away from the square arena's walls and displayed less wall-following behaviour than satiated individuals. A functional explanation for this change in swimming behaviour and interaction with environmental heterogeneity is discussed in the context of social foraging, as is the consequence of these results for models of search patterns and collective movement.

Keywords: Fish, Movement, Nutrition, Foraging, Exploration, Wall-following

Introduction

Animals base many of their movement decisions on their internal state, and in particular on their nutritional state. The study of nutritional deprivation on animal movement has a rich history in behavioural ecology and amongst the vertebrates fish have proven to be good models, particularly for animal group movement behaviour. Level of satiation is known to affect movement behaviour in fish, with searching activity decreasing with increasing satiation (Gill & Hart, 1994; Andersen, 1998; Riche et al., 2004; Priyadarshana et al., 2006). Furthermore, hungry fish in shoals increase their inter-individual distances compared to satiated fish (Morgan, 1988; Robinson & Pitcher, 1989b; Robinson & Pitcher, 1989a).

Our understanding of the effects of hunger on fish swimming speed is less well developed. However, Robinson and Pitcher (1989b) conducted a study in which they exposed herring *Clupea harengus* to one of three ration treatments for 13 days and showed that fish on the lowest rations swam slowest after a further 24h period of food deprivation. Fish on all three rations sped up 5 minutes after food was made available but subsequently slowed down gradually in the remaining 20 minutes of the trial. Priyadarshana *et al.* (2006) calculated the effect of satiation on swimming speeds by recording fish that had been starved for 36h as they foraged on prey and found that fish slowed down as they became satiated. Another study investigated the effect of feeding on swimming performance and metabolic response in *Silurus meridionalis* acclimated at different temperatures (Pang et al., 2010). Critical swimming speed (U_{crit}) (the highest swimming speed at which an individual fish can maintain for a set time period) and $V0_{2max}$ (maximal aerobic capacity) were higher in starved fish, however, the study was designed to investigate aerobic and anaerobic swimming performance rather than elective locomotive speeds and does

not inform about the effect of food deprivation on the speeds of socially interacting, free swimming fish. The results from Robinson and Pitcher (1989b) tells us the reaction of fish to chronic differences in ration, but arguably the more important question is how do they react to an acute changes in ration as this is a daily occurrence. Priyadarshana *et al.* (2006) comes closer to answering this question, however, the fish in this experiment were released into an arena full of prey and therefore there speed was affected by both internal (hunger) and external (prey) stimuli. Therefore, the question of how acute changes in ration affect individual swimming speed in shoals of fish remains unanswered.

The heterogeneity in physical structure of an animal's search environment is often not taken into account when discussing its movements. Random walk and diffusion models often describe animal movements in unbounded space, however, spatial heterogeneities in the landscape (for example, habitat edges) can also influence animal movement (Johnson et al., 1992; Wiens et al., 1993; Desrochers & Fortin, 2000; Morales & Ellner, 2002; Casellas et al., 2008). In addition, physical barriers affect the movement behaviour of animals (Jander & Daumer, 1974; Klotz & Reid, 1992; Klotz & Reid, 1993; Tischendorf & Wissel, 1997; Klotz et al., 2000). The tendency of animals to orient themselves via mechanical contact is known as thigmotaxis (Creed Jr & Miller, 1990; Okada & Toh, 2000; Jeanson et al., 2003) and moving along edges of physical structures is termed wall-following behaviour. Many animals tend to follow linear physical heterogeneities in the environment (Fraenkel & Gunn, 1961; Sharma et al., 2009) using them as navigational aids in the location of resources or shelter (Klotz & Reid, 1992; Klotz & Reid, 1993; Klotz et al., 2000; Collett et al., 2001; Pratt et al., 2001; Graham & Collett, 2002; Heusser & Wehner, 2002; Sharma et al., 2009). Wall-following is particularly common in laboratory

studies when animals are placed in a novel environment and has been related to anxiety in rodents (Treit & Fundytus, 1988; Simon et al., 1994) and also reduces an animal's domain of danger by decreasing the amount of open space near an individual (Schank & Alberts, 1997; Lorenzo & Lazzari, 1999) which is analogous to the manner in which animals in groups use the physical presence of conspecifics for safety (Hamilton, 1971; James et al., 2004). Therefore there is much evidence that desire for protection is a major factor in wall following behaviour.

An animal's behavioural response to hunger is an adaptive response and it should alter not only movement characteristics such as speed and how they associate with conspecifics, but also how they interact with physical structure in their environment. This study quantifies the influence of nutritional deprivation on the movement parameters of shoals of 4 mosquitofish, *Gambusia holbrooki*, in a laboratory arena.

Materials and methods

Study Species and Husbandry

Mosquitofish are an excellent model system to empirically test how hunger influences movement and how individuals interact with the physical environment as they are a small, facultatively shoaling species that inhabit shallow, heavily structured water bodies. Mosquitofish (*Gambusia holbrooki*) were collected from Lake Northam (33°53'07, 15°11'35), Sydney, Australia. They were transferred to the University of Sydney freshwater aquarium in a 50L bucket. Total transfer time was less than 30 minutes. Fish were subsequently housed in a white plastic aquarium (180

L), containing de-chlorinated, aged and aerated tap water . Gravel was provided as substrate. The fish were maintained at 22-24°C on a 12:12 hour L:D photoperiod. Fish were fed fish flake food (Nutrafin Max Tropical Fish Flakes, Rolf C. Hagen (UK) Ltd.) once per day till satiation. The fish were monitored daily for signs of stress or ill-health and were maintained consistently, as described above, for 2 months prior to the experiment.

Mosquitofish are an invasive species to Australia and are declared as 'noxious' by NSW Fisheries and the National Parks and Wildlife Service. Therefore they were not allowed to be released following capture and were humanely euthanized after the experiment using an aqueous solution of the anaesthetic MS222 at a dose rate of 250 mg/L, buffered to a pH of 7 using calcium carbonate. The fish were then placed in a freezer prior to disposal.

Experimental Arenas

All experimental trials occurred in the same arena. This was a square arena (730 × 730 × 100mm) composed of white Perspex 10mm thick filled to a depth of 60mm with de-chlorinated aged tap water. Another litre of water from an aquarium containing 100 mosquitofish was added to the arena each day before the trials commenced in an attempt to maximise the consistency of conspecific chemical cues between trials. The arena was surrounded by black plastic curtain to minimize external stimuli disturbing the fish. No substrate was used to maximize contrast of fish to the background to facilitate tracking. A triangular section of the arena measuring 100 × 100 × 141 mm was sectioned off in a corner of the arena using 5mm Perspex to create a chamber from which fish could be released at the start of the experimental trial. A second piece of Perspex

measuring (80 × 80 × 10mm) was used as a gate on the triangular section. This could be raised remotely using monofilament to release the fish into the main part of the arena. Two columnar pieces of sandstone were placed 200mm from opposite corners of the tank to create environmental enrichment and break-up the line of sight of exploring fish (Figure 1).

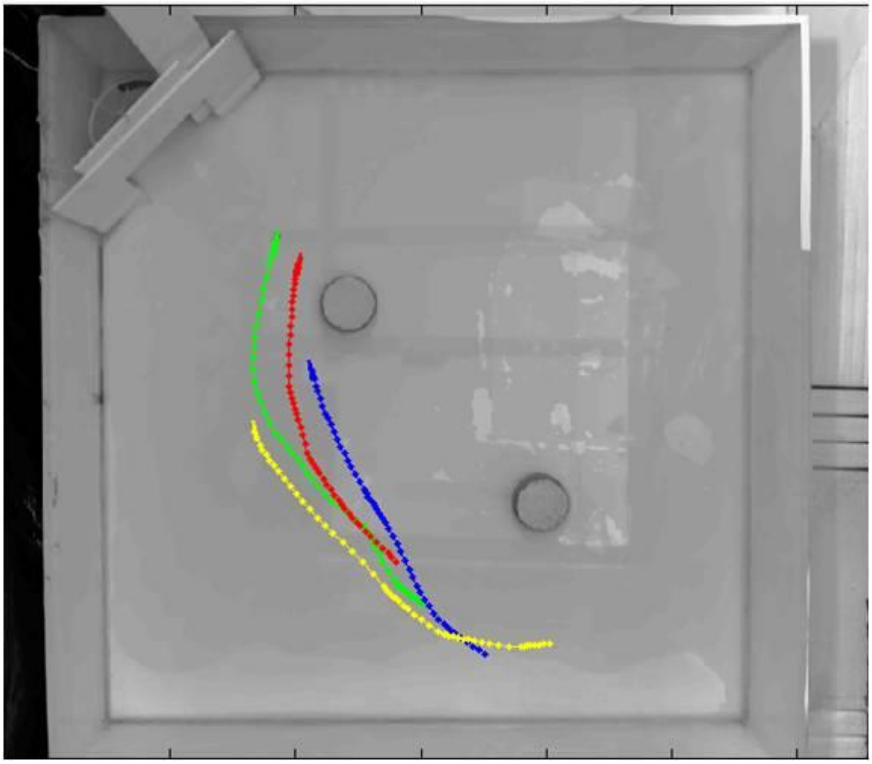

Figure 1. A screenshot from the automated tracking software program, Ctrax (Caltech ethonomics project, The Caltech Multiple Fly Tracker, Version 0.3.10, 2012) showing the individual tracks of 4 hungry mosquitofish in a 730 x 730mm white Perspex arena.

Nutritional manipulation

Thirty-two fish (30±5mm) were taken from the 180L aquarium and separated equally between 2 50L aquaria (16 fish per aquaria, in equal sex ratios). Fish in both tanks were fed fish flake food (Nutrafin Max Tropical Fish Flakes, Rolf C. Hagen (UK) Ltd.) till satiation once per day for 3 days. Before experimental trials commenced, fish in one of the aquaria were deprived of food for 24h ('hungry') while fish in the other aquaria were fed to satiation 30 min before the trial ('satiated'). This procedure was repeated with a further 32 fish (30±5mm).

Experimental trial

The experimental trials started at 09:00h. Two male and two female mosquitofish of equal size were taken from the same aquaria and placed into the triangular chamber of the experimental arena for 5 minutes, to allow the fish to get used to their surroundings. Following this, the gate was raised and the fish were left to enter the arena and swim around the arena for a further 5 minutes. All trials were filmed from directly above the arena with a web-cam (Logitech Pro 9000). Eight trials were performed a day, four replicates of the 'hungry' treatment and four replicates of the 'satiated' treatment. All trials occurred between 9:00h and 11:00h and the order was randomised. The arena was then emptied and cleaned before being employed the following day when experiments were repeated, giving 8 replicates of both treatments in total. Replicates were split between two days to ensure all fish were assayed at a similar time of the day.

Video and tracking analysis

The videos were converted from .wmv to .avs format with DirectShowSource. The .avs files were then opened with VirtualDub (v 1.9.11) where they were saved as old format .avi files after the appropriate frames were isolated and decompressed. The old format .avi file was then imported into an automated tracking software program, Ctrax (Caltech Ethonomics Project, The Caltech Multiple Fly Tracker, Version 0.3.10, 2012)(Branson et al., 2009). This program automatically tracked the position of each individual fish at every frame (15fps) of the 5 minute video. Therefore each fish had (*x, y*) coordinates (in pixels) and its orientation, θ, (in radians, relative to the positive x-axis) recorded for all 4500 frames of the video. This data was then imported into MATLAB where MATLAB FixErrors GUI was used to manually correct any errors made by the automatic tracking software, Ctrax. Each fish therefore had one consistent, unbroken track over the entire 4500 frames of the trial. Pixels were converted into mm with a conversion ratio calculated by measuring the distance between two points of known distance on the first frame of the video.

Calculations

We performed a series of calculations to examine how internal nutritional state (hungry or satiated) affected fish's speed, turning speed, nearest neighbour distances, mean neighbour distances and tendency to become isolated from the main group. We also quantified exploratory behaviour on the interior of the arena by covering the domain of the arena with a square grid and noting the number of new squares that fish passed through during each video frame. Finally, we

made use of survival analysis to examine the tendency of hungry and satiated fish to remain close to the arena's boundary. Details of all calculations are provided in the Supplementary material.

Statistical analysis

All dependent variables in the two treatments (satiated and hungry) were analysed using ANOVA's in SPSS (IBM© SPSS © Statistics, Version 20, 2011). Homogeneity of variances were checked using Levene's Test and normality was checked using Kolmogorov-Smirnov and Shapiro-Wilk Tests and by looking at histograms and Q-Q plots in SPSS. P-values were considered significant at the 0.05 level.

Results

Hungry individuals in shoals moved at significantly greater mean speeds than individuals in satiated shoals ($F_{1,14}$=12.806, p=0.003) with hungry individuals also having significantly greater variance in speed($F_{1,14}$=4.943=0.043). Hungry individuals also had significantly greater median turning speeds ($F_{1,14}$=12.688, p=0.003) (Figure 2).

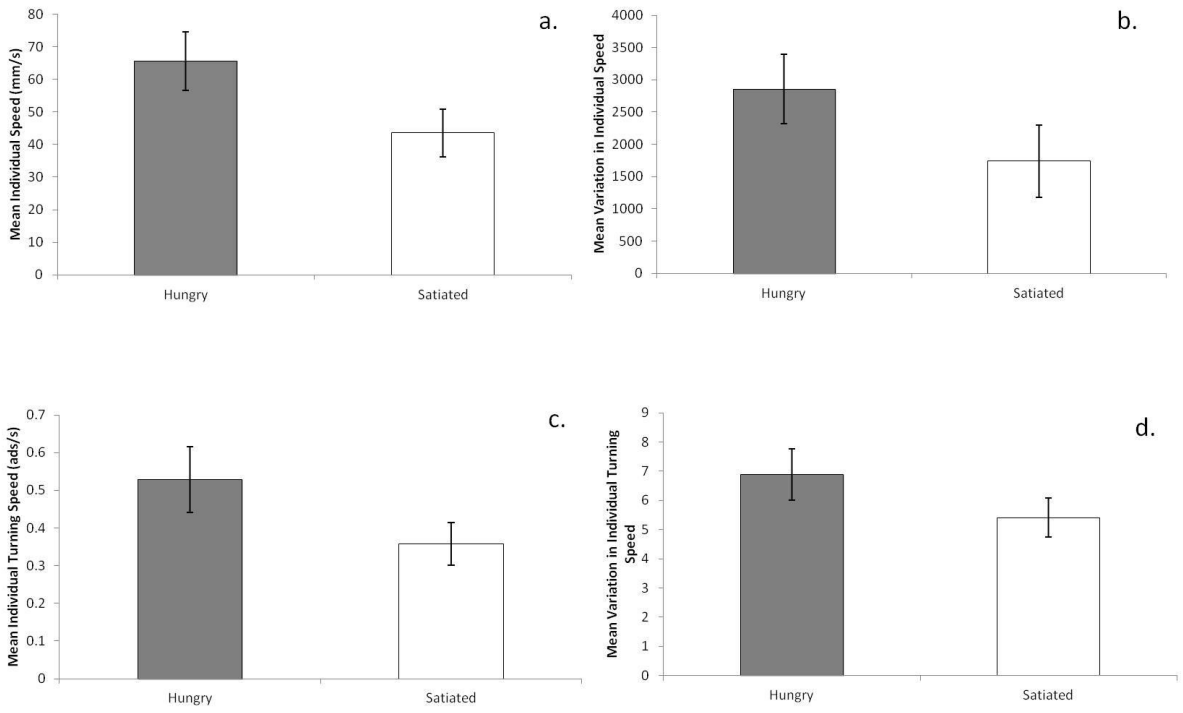

Figure 2. Significant differences between shoals of 4 hungry mosquitofish and shoals of 4 satiated mosquitofish. a.) Mean speed of individual fish (mm/s), b.) Mean variation in individual fish speeds, c.) Mean individual turning speed (rad/s), d.) Mean variation in individual turning speed.

There was no significant difference in near-neighbour distance ($F_{1,14}$=1.759, p=0.206) or mean neighbor distance ($F_{1,14}$=1.811, p=0.200) between hungry and satiated shoals, nor the number of times an individual became isolated ($F_{1,14}$=2.716, p=0.122).

Hungry individuals explored significantly more of the arena and used more of its internal space (see Table 1, Fig. 3 and 4.) for all threshold distances d_{thresh} and grid boxes of either one or two body length side lengths (see supplementary material for more details of calculations). Hungry individuals also tended to explore new grid boxes more rapidly (Figures 3 and 4).

Table 1. Table showing the mean, standard deviation and p-values of number of grid squares explored at different grid square sizes and threshold distances from the arena wall defining the area included in the analysis. Significant values are in bold.

Grid Square (body lengths)	0.5					1					2				
	Satiated		Hungry		Sig	Satiated		Hungry		Sig	Satiated		Hungry		Sig
Threshold distance (body lengths from wall)	Mean	SD	Mean	SD	P-value	Mean	SD	Mean	SD	P-value	Mean	SD	Mean	SD	P-value
0	490.3	103.0	630.4	92.0	**0.044**	262.8	51.6	318.2	36.5	0.071	105.1	17.7	118.2	9.4	0.15
1	409.3	89.6	559.7	98.2	**0.014**	227.8	44.1	290.1	41.1	**0.025**	91.4	16.2	108.8	11.2	**0.041**
2	306.7	67.4	431.5	88.6	**0.005**	174.8	35.2	230.5	36.4	**0.006**	72.8	12.4	86.6	8.3	**0.021**
3	228.1	45.7	325.0	71.1	**0.003**	131.3	24.9	173.2	33.0	**0.006**	57.2	8.8	68.7	9.7	**0.017**
4	170.5	34.4	237.1	54.6	**0.003**	97.8	18.4	128.6	25.5	**0.004**	44.4	7.0	52.3	7.1	**0.021**
5	123.3	26.9	157.4	35.4	**0.013**	70.9	15.1	86.9	16.6	**0.019**	32.8	5.4	38.2	7.0	0.055

Hungry individuals behaved significantly differently to satiated individuals in moving beyond a threshold distance from the wall region for $d_{\text{thresh}} = 30, 120 \text{ and } 150$ mm (Figure 5, panels (a), (d) and (e)). The difference in behaviour was characterised by hungry individuals having a lower probability of remaining within the threshold region than satiated individuals for a given duration t (Figure 6, panels (a), (d) and (e)). The 95% confidence regions for the parameters a and b overlapped for threshold distances of 60 mm and 90 mm (Figure 5, panels (b) and (c)). However, the general trend of observed durations and fitted survival curves at thresholds of 60 mm and 90 mm was again that hungry individuals had a lower probability of remaining within the threshold region than satiated individuals for a given duration (Figure 6, panels (b) and (c)).

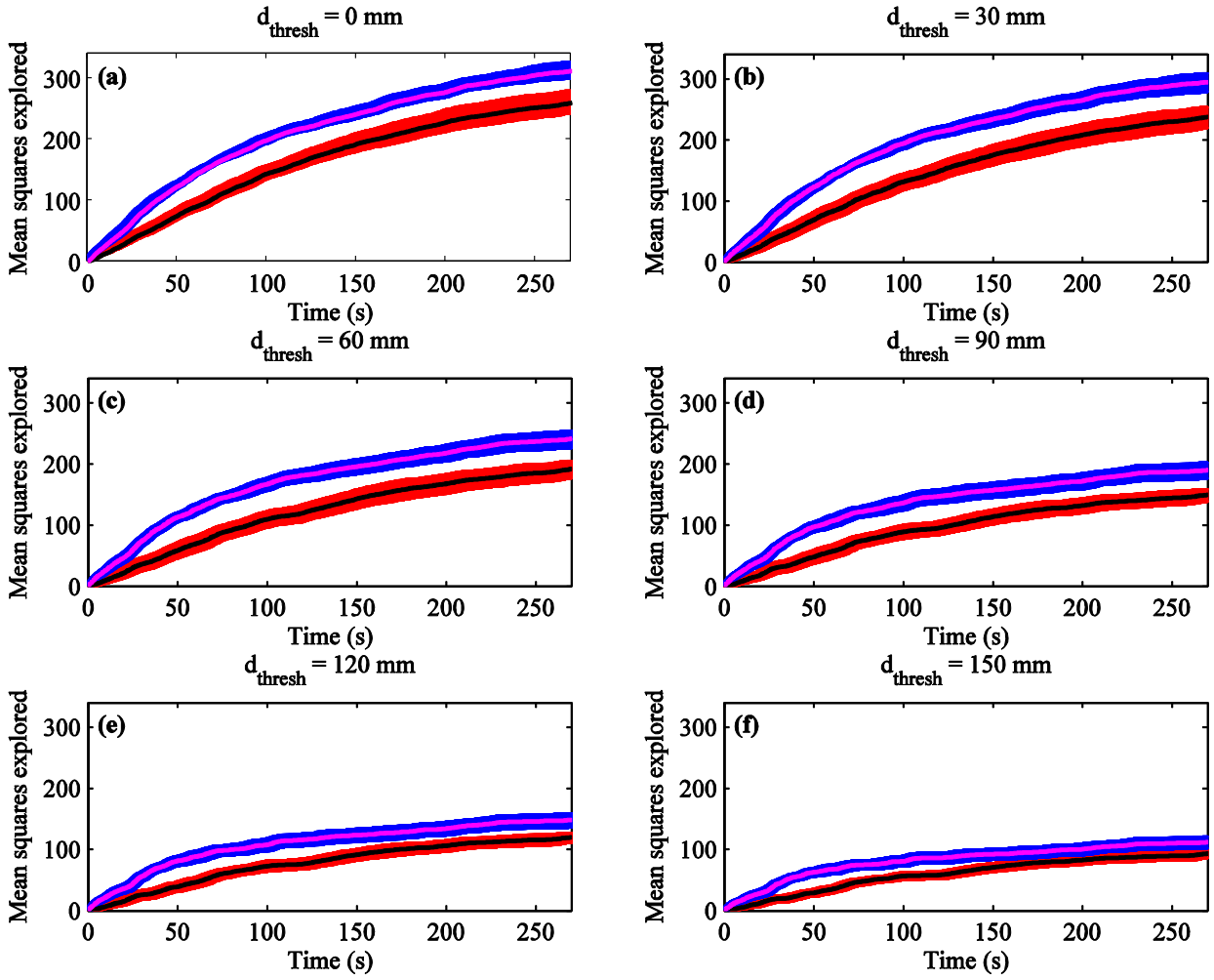

Figure 3. The rate of exploration of satiated (black line, red error bars) and hungry (magenta line, blue error bars) fish. Exploration refers to the number of new squares discovered and is therefore a representation of novel area explored, not simply distance travelled. Here, the side lengths of grid boxes were set to approximately one body length (30 mm).

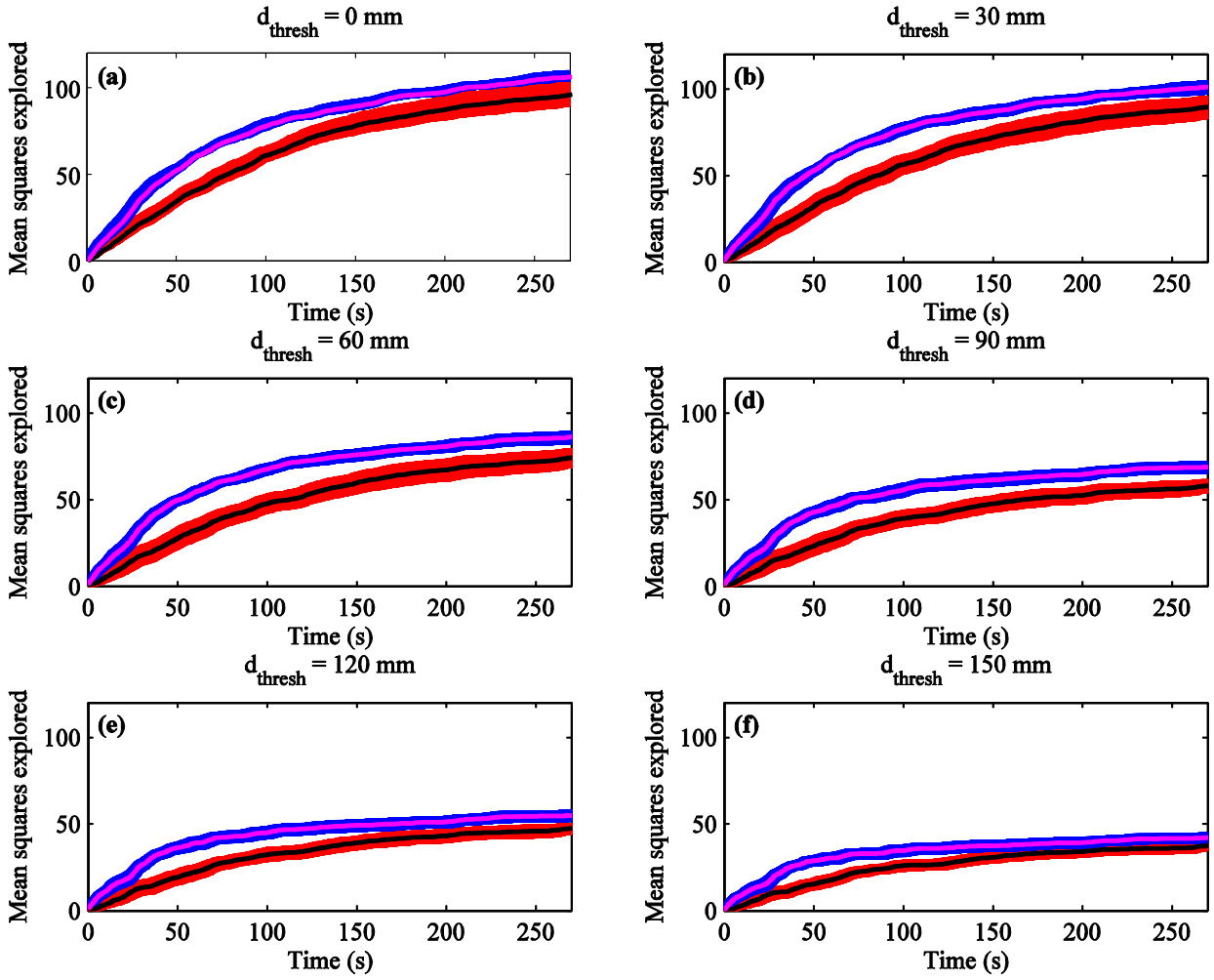

Figure 4. The rate of exploration of satiated (black line, red error bars) and hungry (magenta line, blue error bars) fish. Exploration refers to the number of new squares discovered and is therefore a representation of novel area explored, not simply distance travelled. Here, the side lengths of grid boxes were set to approximately two body lengths (60 mm).

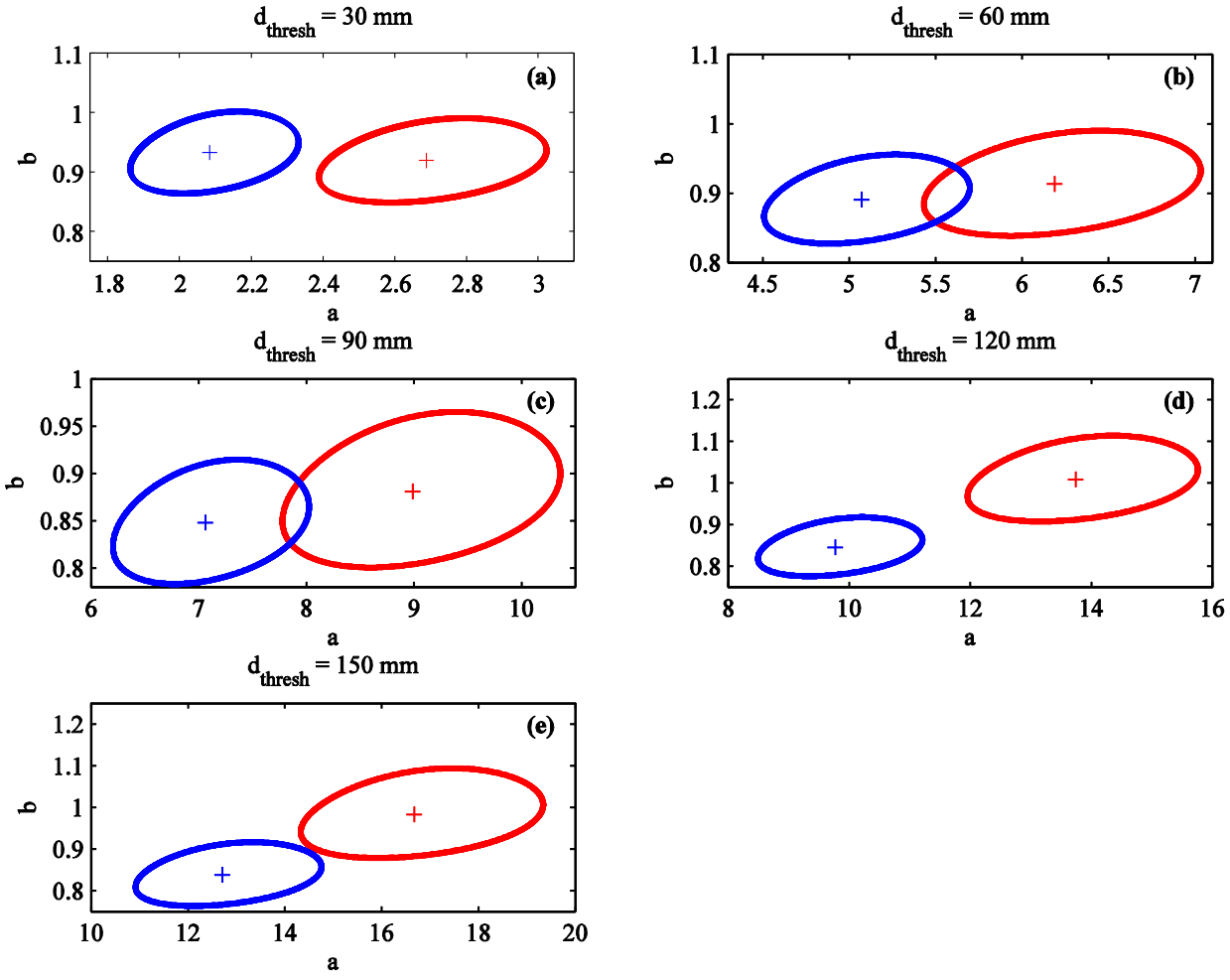

Figure 5. Bounds of the 95% confidence region for the parameters a and b of the Weibull distribution fitted to the set of durations that hungry (blue points) and satiated (red points) fish spent within a given threshold of the arena wall. Maximum likelihood estimates for a and b are plotted as plus signs (see Supplementary text for more details).

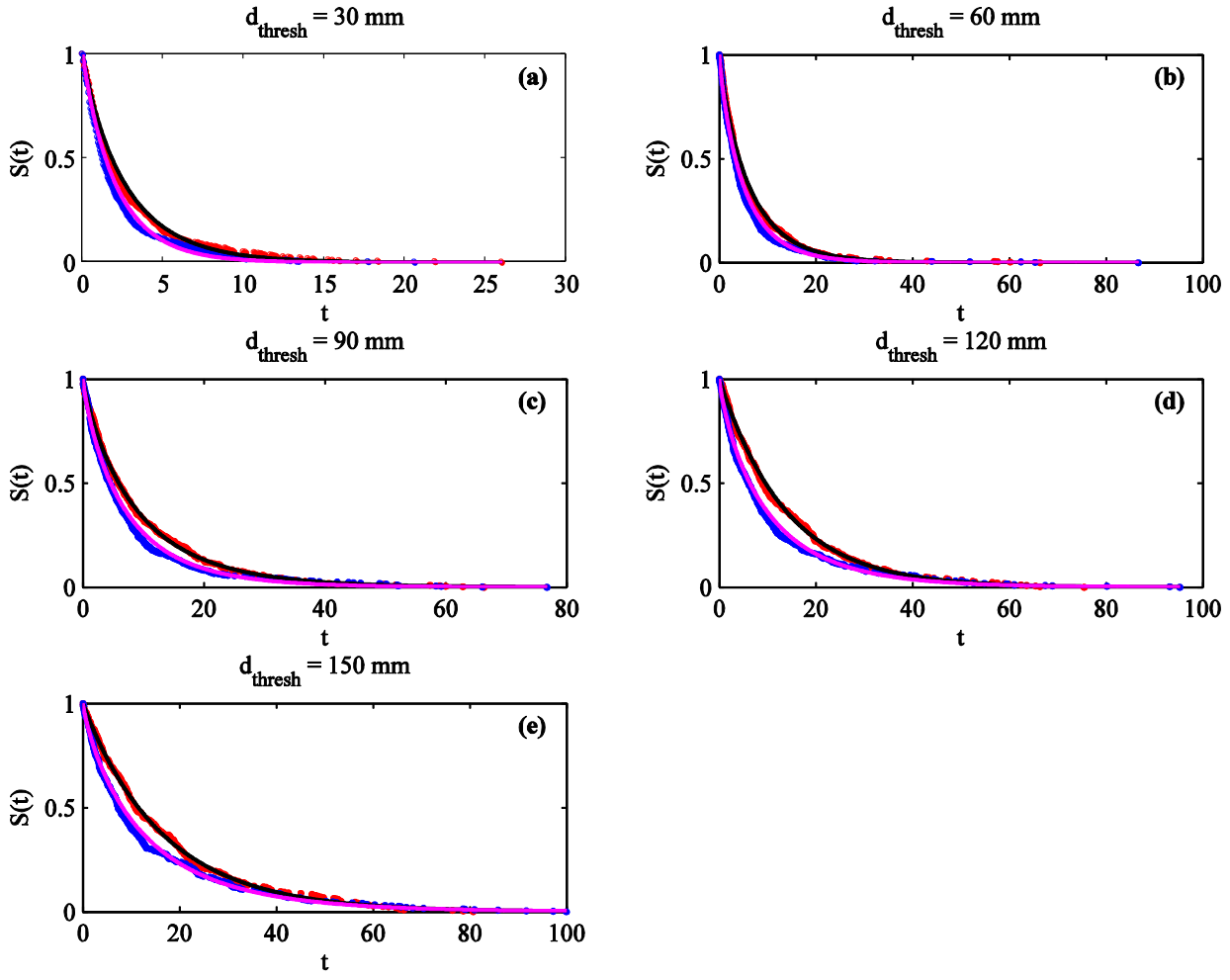

Figure 6. Observed and fitted survival functions, $S(t) = P(T > t)$, for durations spent within a given threshold of the arena wall. Each survival function represents the probability that a fish spent greater than *t* seconds within a threshold distance of the wall during a single visit. Values of $S(t)$ inferred directly from data are plotted as blue points for hungry fish and red points for satiated fish. Survival curves derived from maximum likelihood based fitting of the Weibull distribution to each set of durations are plotted as solid magenta lines for hungry fish and solid black lines for satiated fish.

Discussion

This study examined the consequences of acute changes in ration on free-swimming, freely-interacting shoals of mosquitofish and found that groups of hungry individuals had a greater mean speed and turning speeds than satiated individuals. A previous study, Robinson and Pitcher (1989b), similarly looked at the affect of starvation on swimming speed. However, it examined chronic changes in ration. Robinson and Pitcher (1989b) found that herring (*Clupea harengus*) swam most slowly on chronic low rations and explained that this was an adaptive behavioural strategy to stay within their metabolic limits. In their experiment fish entered the arena after being starved for 24h. Whilst feeding, fish initially increased their speed from their starved state, but then their speed dropped as they were fed more food. The effect of satiation caused the fish to lower their speed, but only fish that were on the highest ration eventually slowed to speeds to lower than when they were starved. In Priyadarshana *et al.* (2006), fish swam fastest when they were initially released into an arena full of plankton, after having been starved for 24h. They slowed as the trial progressed and they became satiated. Our study is not directly comparable to either experiment as food was never present in our arena, however, it is most comparable to Priyadarshana *et al.* (2006) as both studies differ from the Robinson and Pitcher (1989b) experiment in that they assess the effect of an acute shortage of food, rather than concentrating on chronic food rationing. There is evidence then that short term reductions in intake trigger an active foraging response that is characterised in part by increased swimming speeds.

Whilst studies on fish that explore the effect of hunger on speed are limited, we can discuss our findings in light of the invertebrate literature. In particular, groups of desert locusts (*Schistocerca gregaria*) increased their speed with protein deprivation (Bazazi et al., 2011). The effect of

nutritional deprivation was found to be limited for individual's tested in isolation but was found to strongly affect an individual's response to environmental stimuli, in this case, conspecifics, as they are considered a valuable source of protein (Bazazi et al., 2011). Mosquitofish assayed in isolation during pilot studies showed signs of stress wherein they either did not leave the shelter to begin exploring the arena, or froze alongside the arena walls, regardless of their treatment. A comparison between individual and group movement behaviour was therefore not possible, which is disappointing considering this would allow us to infer what aspects of group behaviour were due to the effect of hunger on individual movement and what resulted from social interaction. However, an extension of the current study beyond that of hunger into the effects of specific macronutrient deprivation (such as in Bazazi et al., 2011), is possible, and warranted, especially considering fish have a natural diet high in protein.

It could be argued that differences in movement measures between treatments may have resulted from aggressive interactions between conspecifics as hunger can increase aggression. However, we did not observe aggressive behaviour in the trials and therefore we believe that changes in fish movement behaviour are instead related to attempts to alleviate acute hunger.

The active foraging response of mosquitofish also seems to be characterized by the manner in which individuals interact with the physical structure in their environment. Both hungry and satiated fish displayed thigmotaxis and wall-following behaviour during the trials. This behaviour is not unusual for fish when placed into novel arenas (Suzuki et al., 2003). Also, it is likely that in the natural environment thigmotaxis and wall following are common behaviours. Mosquitofish follow physical structures such as sunken branches or the banks of channels (pers. obs.) and this behaviour may even have functional benefits in reducing the domain of danger or

aid in navigation, although this is yet to be tested in this species. Although all fish in these trials displayed some wall-following behaviour, importantly, hungry fish had shorter time durations of wall following behaviour (Figure 6) and, therefore, the internal nutritional state of fish is shown to affect how the shoal interacts with physical structure in the environment. Jeanson *et al.* (2003) discussed their finding that cockroaches displayed wall following behaviour by not only suggesting navigational benefits, but also that, as physical heterogeneities affect the spatial distribution of organisms, individuals that express wall-following behaviour may increase their probability of encountering conspecifics. Therefore, physical heterogeneities may be favoured areas for individuals to form aggregations (Jeanson et al., 2003; Suzuki et al., 2003). Such benefits could be extended to this study as mosquitofish are facultative shoalers and undergo shoal fission and fusion events. They are often found in heavily structured, murky water and, here, the physical heterogeneities may provide a safer location for individuals or small groups to aggregate where they will increase their likelihood of encountering other individuals and consequently form groups large enough to enter open water. This may be especially important after shoals have broken up overnight. Encountering other conspecifics whilst hungry is also important as it may increase individual's access to social information about foraging opportunities.

The behaviour of hungry groups was also characterised by faster rates of exploration and an overall increase in total area explored after 5 minutes (Table 1, Figure 3 and 4). This supports current theory that movement patterns fluctuate in response to energetic requirements (Lima & Dill, 1990; Killen et al., 2011). Hungry animals, or those stimulated by food will increase their risk-taking behaviour (in this case increasing their domain of danger by leaving the safety of the

wall) and this may involve increasing their activity levels in order to increase the scope of their foraging activity and search a wider area for food (Lima & Dill, 1990; Sogard & Olla, 1997; Killen et al., 2011).

There was a trend for near-neighbour distances, mean neighbour distances and the tendency to become isolated to be greater for hungry individuals, however, this study did not find a significant difference between the hungry and satiated treatments. Functionally, one may hypothesise that hungry individuals in shoals might show less conformity as they trade off the anti-predatory benefits of shoal cohesiveness with the need to show more individuality in their movements as they search for food and reduce competition (Reebs & Saulnier, 1997). It has been suggested that individual searching is not the most beneficial strategy for groups (Bhattacharya & Vicsek, 2013) and fish in larger shoals find food faster (Pitcher et al., 1982). However, it has been found time and time again that individuals within shoals increase the space between themselves to forage (Morgan, 1988; Robinson & Pitcher, 1989b; Robinson & Pitcher, 1989a; Romey, 1995; Gueron et al., 1996; Hoare et al., 2004). Doing so reduces competition over resources, but importantly, as long as they can still monitor the behaviour of neighbours they are still able to access social information on the location of food. The fine scale movement behaviour of foraging fish shoals deserves more attention, and it is likely that the ideal behaviour for hungry individuals is a mixture between individual searching and reaction to near-neighbours. An empirical experiment in which hunger levels of individual fish, group sizes and the quality and distribution of resources are all manipulated is warranted, particularly if the assay could not only collect accurate information on shoal cohesiveness and fission and fusion events but also measure this in respect to foraging success. In this study, it is possible that perceived threat led to

an overall decrease in inter- individual distances in fish (Krause, 1993; Tien et al., 2004; Carere et al., 2009; Bode et al., 2010) considering the light levels, high contrast background and short acclimation times in this experiment. This may have overwhelmed the subtler effect of hunger.

These findings relate to the internal nutritional state of individuals that forage when in groups and as such will rightly be viewed in context of social foraging. The allocation of tactics between individuals in producer-scrounger games is of great interest and it has been suggested that it may be based on personality (Kurvers et al., 2009), dominance (Liker & Barta, 2002), relatedness (Tóth et al., 2009) or predation risk (Mathot & Giraldeau, 2008). It was discovered that hungry sparrows scrounged more (Lendvai et al., 2004), however, considering that animals located on the edges of groups are more often producers (Barta et al., 1997; Mónus & Barta, 2008) and the result from this study and others on fish that hungry individuals are more active, take more risks (Pettersson & Brönmark, 1993) and explore more, there could well be a positive relationship between hunger levels and propensity to produce. Fish shoals would be a perfect model system for such an experiment as individual behaviour, including fine scale movement parameters, are now easily determined in large, non-kin related and socially foraging groups.

It is surprising, considering how important nutrition is to an animal's behaviour, that there is a lack of fine-scaled empirical data on the effect of nutritional stress on fish movement. This study has quantified behaviours that characterize swimming behaviour of individual mosquitofish within shoals that have undergone acute food shortage and compared these to satiated individuals within shoals. This study provides empirical data that shows hunger causes individual fish in a shoal to change their average speed, variation in speed, turning speed and exploration levels. Importantly, the fish's internal nutritional state is found to affect how they interact with the

physical heterogeneity of the environment, with hungry fish showing less wall-following behaviour and being more likely to leave the safety of the arena's walls. This data, therefore, informs us of how internal and external stimuli affect the behavioural ecology of mosquitofish and it may also be useful to parameterise more accurate models of collective movement and search pattern behaviour. It is also of commercial and animal welfare interest to aquaculture farms as it brings into consideration the placement of barriers and walls and suggests that more frequent feeding may reduce energy expenditure of fish by reducing their speeds and exploration levels.

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Supplementary Material

Speed and turning speed

Writing the coordinates of the *i*th fish in millimeters at time *t* as $\left(x_i(t), y_i(t)\right)$, we estimated the components of each fish's velocity vector, $\left(u_i(t), v_i(t)\right)$, at time *t* via the forward difference approximations:

$$u_i(t) = \frac{x_i(t+\Delta t) - x_i(t)}{\Delta t} \quad \text{and} \quad v_i(t) = \frac{y_i(t+\Delta t) - y_i(t)}{\Delta t},$$

where Δt was the constant separation between consecutive video frames (=1/15 s).

We then estimated each fish's speed at time *t* using:

$$s_i(t) = \sqrt{\left(u_i(t)\right)^2 + \left(v_i(t)\right)^2}.$$

For subsequent statistical tests we determined the mean, median and variance of each individual's time-series of speed values (denoted $\bar{s}_i(t)$, $\text{Median}\left(s_i(t)\right)$ and $\text{Var}\left(s_i(t)\right)$ respectively. At the level of each group, we then determined the means and standard deviations of $\bar{s}_i(t)$ and $\text{Median}\left(s_i(t)\right)$ across all four group members, and the mean of $\text{Var}\left(s_i(t)\right)$ (again across all four group members).

We derived each fish's turning speed from their facing directions, $\theta_i(t)$, at consecutive times, *t* and $t + \Delta t$. To do this, we first determined the *x* and *y* components of unit vectors pointing in the direction of $\theta_i(t)$ for all times *t* using:

$\hat{X}_i(t) = \cos(\theta_i(t))$ and $\hat{Y}_i(t) = \sin(\theta_i(t))$.

The magnitude of each fish's turning speed (in radians per second) at time *t* was then estimated via:

$$\alpha_i(t) = \frac{\cos^{-1}\left(\hat{X}_i(t)\hat{X}_i(t+\Delta t) + \hat{Y}_i(t)\hat{Y}_i(t+\Delta t)\right)}{\Delta t}.$$

We determined the median of each fish's turning speed time-series, denoted $\text{Median}(\alpha_i(t))$, and then determined the mean and standard deviation of $\text{Median}(\alpha_i(t))$ across all members of each group.

Neighbour distances

We determined the distances between all pairs of fish *i* and *j*, for all times *t* using the distance formula:

$$d_{i,j}(t) = \sqrt{\left(x_j(t) - x_i(t)\right)^2 + \left(y_j(t) - y_i(t)\right)^2}\ . \qquad (1)$$

For each time *t*, we determined the distance from each fish *i* to their nearest neighbour, $d_{i,nn}(t)$, and the mean distance from fish *i* to all of their neighbours, $d_{i,mn}(t)$. We then determined the means over time of both $d_{i,nn}(t)$ and $d_{i,mn}(t)$, denoted $\bar{d}_{i,nn}$ and $\bar{d}_{i,mn}$ respectively, for each fish *i*. Finally we determined the means of $\bar{d}_{i,nn}$ and $\bar{d}_{i,mn}$ across all four fish *i* in a given group.

Formation of subgroups and frequency of isolation

We developed a simple algorithm to classify fish as being part of distinct subgroups based on the distances between individual fish. In general, we identified a distinct subgroup of fish as a set of fish where no fish in the set was more than three body lengths (90 mm) from any other fish in the set at time *t*. The following analysis was applied to the final 4050 frames (4.5 minutes) of data for each trial.

For each time *t*, we started our algorithm by assigning the first fish in a given group to subgroup 1. We then identified all fish that were less than or equal to 90 mm from fish 1 using equation (1), and assigned any such fish to subgroup 1. For each newly assigned fish *i*, we then identified any other fish *j* not already assigned to a subgroup for which $d_{i,j}(t) \leq 90$ mm. If such fish were found, then they were assigned to subgroup 1, and then the process of cycling through newly assigned fish to find any other unassigned fish for which $d_{i,j}(t) \leq 90$ mm was repeated. If no fish for which $d_{i,j}(t) \leq 90$ mm were found during a cycle, then a new subgroup was started, with the first fish not already assigned to another subgroup identified as the first member of the new subgroup. The process of finding all fish less than or equal to 90 mm from the fish identified as the founding member of a new subgroup was then repeated as described above. The algorithm terminated when all fish in a group had been assigned to a subgroup for time *t*.

We identified all frames where each fish *i* was the only member of their particular subgroup. We then treated each series of consecutive time steps where a fish was the only subgroup member as a distinct instance of isolation. We tallied the instances of isolation by each fish, and then determined the mean number of instances of isolation across all group members.

Distance to the closest point on the wall

A necessary preliminary calculation for analysis of exploration of internal portions of the arena and time spent close to the boundary of the arena was the determination of the shortest distance between the arena's walls and the location of each fish for all time steps. The shortest distance between a fish located at $(x_i(t), y_i(t))$ and a single edge of the wall with corners located at (w_{x_k}, w_{y_k}) and $(w_{x_{k+1}}, w_{y_{k+1}})$ is usually given by the length of the straight line segment that is perpendicular to the wall, and that passes from the wall to $(x_i(t), y_i(t))$.

We developed a simple MATLAB script to manually extract the coordinates (in pixels) of the five corners of the arena from still images of each trial taken directly from our video footage. (We treated the additional Perspex used to section off a small part of the initially square arena as a part of the boundary, see Fig 1.) We then used the pixels to millimetres conversion ratio determined during tracking to convert the coordinates of the corners to millimetres. Next, we formed vectors of unit length that were parallel and perpendicular to each wall. The components of a unit vector parallel to a wall edge connecting corners (w_{x_k}, w_{y_k}) and $(w_{x_{k+1}}, w_{y_{k+1}})$ were:

$$\hat{w}_{x_k} = \frac{w_{x_{k+1}} - w_{x_k}}{d_{k,k+1}} \quad \text{and} \quad \hat{w}_{y_k} = \frac{w_{y_{k+1}} - w_{y_k}}{d_{k,k+1}},$$

where

$$d_{k,k+1} = \sqrt{\left(w_{x_{k+1}} - w_{x_k}\right)^2 + \left(w_{y_{k+1}} - w_{y_k}\right)^2}$$

was the distance between corners k and $k + 1$. A unit vector perpendicular to the same edge of the wall had components:

$$\hat{n}_{x_k} = \frac{-\left(w_{y_{k+1}} - w_{y_k}\right)}{d_{k,k+1}} \quad \text{and} \quad \hat{n}_{y_k} = \frac{w_{x_{k+1}} - w_{x_k}}{d_{k,k+1}}.$$

Following the above calculations, we constructed a vector from the corner of the wall with coordinates $\left(w_{x_k}, w_{y_k}\right)$ to the position of fish i at time t, $\left(x_i(t), y_i(t)\right)$. The components of this vector were:

$$a_{k,i}(t) = x_i(t) - w_{x_k} \quad \text{and} \quad b_{k,i}(t) = y_i(t) - w_{y_k}.$$

We then examined if the relative positions of the fish and the edge of the wall were such that it was possible to calculate a perpendicular distance. The necessary condition for the existence of a perpendicular distance that we employed was that the scalar component of the vector with components $\left(a_{k,i}(t), b_{k,i}(t)\right)$ in the direction of the vector from $\left(w_{x_k}, w_{y_k}\right)$ to $\left(w_{x_{k+1}}, w_{y_{k+1}}\right)$ must be greater than or equal to 0, and less than or equal to the length of the edge of the wall (that is, $0 \le a_{k,i}(t)\,\hat{w}_{x_k} + b_{k,i}(t)\,\hat{w}_{y_k} \le d_{k,k+1}$). If the necessary condition was met, then we determined the perpendicular distance from the wall to the fish via:

$$D_{k,i}(t) = \left| a_{k,i}(t)\,\hat{n}_{x_k} + b_{k,i}(t)\,\hat{n}_{y_k} \right|,$$

otherwise we did not record a distance for the given pairing of fish and wall edge.

We repeated the above calculations to determine the least distance between a given fish and each of the five edges of the arena. The minimum of these five distances was then the minimum distance between a given fish and the boundary of the arena for a given time.

Exploration of the arena

We performed a series of calculations equivalent to overlaying the domain of the arena with an $n_g \times n_g$ square grid (with grid box side lengths approximately equal to one or two body lengths) and then determining the number of new boxes in the interior of the arena that each fish passed through during each of the final 4050 frames (4.5 minutes) of each trial. The interior region was identified as being greater than a threshold distance, d_{thresh}, from the boundary of the arena. Since the selection of d_{thresh} was somewhat arbitrary, we performed calculations with $d_{\text{thresh}} = 0, 30, 60, 90, 120 \text{ or } 150 \text{ mm}$ (the body length of the mosquitofish was approximately 30 mm). The overall method that we used here was derived from a method for producing a grid based representation of a contour previously defined on a continuous domain (Schaerf & Macaskill, 2004; Schaerf & Macaskill, 2012).

We first identified all points of each fish's trajectory where the fish was greater than d_{thresh} from the boundary of the arena. We discarded all other points from each fish's trajectory, but retained the time/frame associated with the remaining points. We then shifted the coordinates of each fish so that they were guaranteed to satisfy $0 \le x_i(t) < 1400, 0 \le y_i(t) < 1400$ (mm) for all times *t* (for computational convenience). Grid boxes were identified by a pair of integers, (x_g, y_g), such that

$1 \le x_g \le n_g, 1 \le y_g \le n_g$. We scaled and re-shifted the coordinates of each fish so that we could immediately identify which grid box they occupied at time *t* via:

$$\tilde{x}_i(t) = \text{nint}\left(\left(\frac{x_i(t)}{1400}\right)(n_g - 1) + 0.5\right) \text{ and } \tilde{y}_i(t) = \text{nint}\left(\left(\frac{y_i(t)}{1400}\right)(n_g - 1) + 0.5\right),$$

where $\text{nint}(x)$ is the nearest integer to *x*. (Such a transformation guaranteed that $1 \le \tilde{x}_i(t) \le n_g, 1 \le \tilde{y}_i \le n_g$.)

Our method of recording whether or not a particular grid box had been visited by fish *i* was to construct an $n_g \times n_g$ matrix, denoted T_i, that initially had all entries set to zero. For grid box side lengths corresponding to approximately one body length we set $n_g = 46$; for grid boxes with side lengths of two body lengths we set $n_g = 23$. We then cycled through the series of transformed coordinates $(\tilde{x}_i(t), \tilde{y}_i(t))$ in increasing order; if the entry in row $\tilde{y}_i(t)$, column $\tilde{x}_i(t)$ of T_i equaled zero, we would then set the entry to 1 and record that fish *i* had explored a new square at time *t*. There was a small possibility fish might have moved a distance of more than one grid box between consecutive frames and thus grid boxes identified by integer coordinates $(\tilde{x}_i(t), \tilde{y}_i(t))$ and $(\tilde{x}_i(t + \Delta t), \tilde{y}_i(t + \Delta t))$ might not be adjacent. In the case that such a jump in position was made we identified all grid boxes that lay on the straight line segment from $(\tilde{x}_i(t), \tilde{y}_i(t))$ to $(\tilde{x}_i(t + \Delta t), \tilde{y}_i(t + \Delta t))$ using Bresenham's line algorithm) (Bresenham, 1965). Where appropriate, we set entries in T_i corresponding to the intermediate points to 1 (if T_i equaled zero at these points initially) , and added the number of these newly visited intermediate points to the tally of new grid boxes explored for time *t*. Once the number of new grid boxes explored had

been determined for all time steps, we deduced the total number of unique boxes explored up to time *t*, $c_i(t)$, for all times *t* in each time series. We then determined the mean number of unique boxes explored up to time *t* across all four fish in the trial, $\bar{c}_m(t)$ (for the *m*th trial). Finally, we determined the mean, standard deviation and standard error of $\bar{c}_m(t)$ across all 8 trials in both the hungry and satiated treatment sets for all times *t.*

Survival analysis of durations spent close to the walls

We employed survival analysis inspired by the work of Jeanson *et al.* (2003) to examine if hungry and satiated fish differed in the individual durations that they spent within a threshold distance of the arena walls (via the rate at which they moved away from the wall region, or an equivalent measure) (Jeanson et al., 2003). As with the analysis of exploration of the arena's interior, we applied our analysis to the last 4050 frames of data from each trial.

We first identified all points on each fish's trajectory that were less than or equal to a threshold distance d_{thresh} from the wall. (We performed a separate series of calculations for $d_{\text{thresh}} = 30, 60, 90, 120 \text{ or } 150 \text{ mm}$ (approximately 1 to 5 body lengths).) We identified a visit to the wall region as a set of consecutive frames where a fish was within the threshold of the wall, and hence deduced the duration of each visit in seconds using the formula ((index of last frame of visit) – (index of first frame of visit) + 1)/(frames per second). We then pooled all the durations of visits to the wall region made by all hungry fish (across all 8 trials with hungry fish) and all satiated fish (across all 8 trials with satiated fish), excluding any visits that were recorded

has having started on the first available frame or that had not concluded before the last available frame.

The survival functions, $S(t) = P(T > t)$, associated with our sets of durations represented the probability that a fish spent greater than *t* seconds within a threshold distance of the wall during a single visit. We determined observed survival functions directly from our sets of durations spent close to the wall by hungry fish and durations spent close to the wall by satiated fish for visualisation purposes. To do this, we sorted our sets of durations in ascending order and identified the unique durations in each set. For each unique duration, t_u, we counted the number of elements in the set that were greater than t_u, and hence deduced the proportion of elements in the set greater than t_u (an estimate for $S(t_u) = P(T > t_u)$). We set $S(0) = 1$, since by definition all durations spent near the wall were greater than 0 seconds.

We sought an underlying probability density function (PDF) that fit durations associated with both treatments well for a given threshold, d_{thresh} (to make a formal comparison of durations spent near the wall). By trial and error we determined that the Weibull distribution (Weibull, 1951) was a good fit for data derived from both treatment sets for all wall distance thresholds, d_{thresh}. The PDF for the Weibull distribution is:

$$f(t \mid a, b) = \frac{b}{a}\left(\frac{t}{a}\right)^{b-1} \exp\left\{-\left(\frac{t}{a}\right)^{b}\right\}; \quad t \geq 0, \qquad (2)$$

and the survival function associated with the Weibull distribution is:

$$S(t) = \int_t^{\infty} \frac{b}{a}\left(\frac{s}{a}\right)^{b-1} \exp\left\{-\left(\frac{s}{a}\right)b\right\} ds = \exp\left\{-\left(\frac{t}{a}\right)^{b}\right\},$$

where *s* is a dummy variable of integration. We estimated the scale parameter, a, and the shape parameter, b, of the Weibull distribution via MATLAB's intrinsic *fitdist* function (that makes use of maximum likelihood estimates). Goodness-of-fit of each fitted distribution was examined with a one-sample Kolmogorov-Smirnov test (via MATLAB's intrinsic *kstest* function). Table S1 reports the parameter estimates for fitted distributions for each treatment and threshold, d_{thresh}, along with statistics from the associated Kolmogorov-Smironov goodness-of-fit tests. Ultimately we wanted to know if the tendency to leave the wall region differed between hungry and satiated treatments; to determine if this was the case we compared likelihood-based 95% confidence regions for the parameters a and b for hungry and satiated treatments for each threshold, d_{thresh}. If the confidence regions did not overlap, then we treated the behaviour in leaving the threshold region as being significantly different between hungry and satiated fish and determined the relative difference in behaviour (a greater or lesser probability of staying close to the wall for longer durations) directly from plots of both our observed survival functions and fitted survival curves (derived from the Weibull distribution). Differences in behaviour could also be determined by examining the values of the maximum-likelihood estimates of a and b, which we denoted $\hat{a}$ and $\hat{b}$.

Points on the boundary of the 95% confidence region satisfied the equation:

$$l(a, b) = C, \qquad (3)$$

where $l(a,b) = -2\log\left\{L(a,b)/L(\hat{a},\hat{b})\right\}$ is the log-likelihood ratio, $L(a,b)$ is the empirical likelihood, given by

$$L(a,b) = \prod_{i=1}^{n} f(t_i \mid a,b),$$

for a set of n durations t_i (with $f(t \mid a,b)$ given by equation (2)), and C is the constant such that

$$P(\chi_\nu^2 > C) = 0.05,$$

(see, for example, (Hall & La Scala, 1990)). (χ_ν^2 is the chi-squared distribution with ν degrees of freedom.) In our case, we sought bounds on two parameters, so $\nu = 2$, and hence $C \approx 5.991$. We re-wrote equation (3) in the form:

$$g(a,b) = l(a,b) - C = 0, \qquad (4)$$

and then sought pairs of numbers (a,b) that satisfied equation (4) numerically. In practice we first chose appropriate values of a, then solved equation (4) for b for each value of a using MATLAB's intrinsic *fzero* function. We then chose appropriate values of b and solved equation (4) for a.

Table S1: Maximum likelihood estimates for parameters for the Weibull distribution and goodness-of-fit statistics from one-sample Kolmogorov-Smirnov tests under the null hypothesis that a given set of durations was drawn from the Weibull distribution with the given parameter values. d_{thresh} **is the threshold distance from the arena wall,** $\hat{a}$ **and** $\hat{b}$ **are the scale and shape parameters of the Weibull distribution respectively,** n **is the number of durations in a given set,** D_n **is the test statistic for the Kolmogorov-Smirnov test and** P **is the associated probability.**

d_{thresh}	Treatment	$\hat{a}$	$\hat{b}$	n	D_n	P
30 mm	Hungry	2.0845	0.9326	613	0.0516	0.0740
30 mm	Satiated	2.6881	0.9194	573	0.0458	0.1747
60 mm	Hungry	5.0717	0.8906	619	0.0447	0.1639
60 mm	Satiated	6.1871	0.9131	484	0.0499	0.1740
90 mm	Hungry	7.0624	0.8478	563	0.0468	0.1651
90 mm	Satiated	8.9888	0.8810	420	0.0251	0.9474
120 mm	Hungry	9.7670	0.8453	489	0.0428	0.3231
120 mm	Satiated	13.7467	1.0078	346	0.0314	0.8746
150 mm	Hungry	12.7111	0.8382	421	0.0538	0.1690
150 mm	Satiated	16.6724	0.9833	309	0.0328	0.8824

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Chapter 3

THE INFLUENCE OF NUTRITIONAL STATE ON INDIVIDUAL AND GROUP MOVEMENT BEHAVIOUR IN SHOALS OF CRIMSON-SPOTTED RAINBOWFISH (*MELANOTAENIA DUBOULAYI)*

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Groups of animals are often heterogeneously structured, and may be composed of selfish individuals responding to different internal stimuli. Group level behaviour can be determined by the slight differences in simple behavioural movement parameters structuring local interactions between conspecifics. To accurately understand individual behaviour within groups and how it affects whole group behaviour, we need to measure the responses of individuals in groups to changes in internal state and examine the outcome of these responses within the social context. Therefore we quantified the influence of nutritional state on the individual and group movement parameters of free swimming shoals of 8 rainbow fish, *Melanotaenia duboulayi.* Individual fish were experimentally manipulated to be in one of two nutritional states, hungry or satiated, and were assayed in three group compositions; all-hungry (8:0 hungry:satiated), mixed (4:4), or all-satiated (0:8). We showed that the internal nutritional state of individual fish affected basic behaviours relating to spatial positioning. The interaction between pairs of fish was dependent on the nutritional state of both fish and there was an additive effect of individual behaviour on group behaviour, which meant that group behaviour reflected the motivations of its individual members in such a way that allowed individuals to fulfil their own behavioural needs whilst still attaining the benefits of grouping.

Keywords: Shoaling behaviour, *Melanotaenia duboulayi*, Social dynamics, Nutrition, Internal state, Movement

Introduction

An individual animal's movement decisions are impacted by external stimuli from its local biotic and abiotic environment and internal stimuli, such as nutritional state. Group movement behaviour is a product of interactions between individuals that compose the group (Ward 2012). An individual tends to move towards distant neighbours, away from proximate ones, and align with neighbours at intermediate distances, copying their direction of travel and speed (Couzin et al. 2005). As individuals in a group update their position in response to that of near-neighbours, information about a potential food source or the threat of predation can transfer through the group and a consensus over the direction and timing of group movements can occur (Couzin et al. 2005; Sumpter et al. 2008; Couzin et al. 2011). Interactions between individuals within a group may be made more complex considering groups are often heterogeneously structured and composed of selfish individuals with a range of morphological and behavioural phenotypes. Individual characteristics such as sex (Piyapong et al. 2009), size (Jordan et al. 2010), colour (McRobert and Bradner, 1998), parasite load (Barber et al. 1998), personality (Sih and Watters, 2005; Cote et al. 2010a), competitive ability (Ward et al. 2006), social hierarchy (King et al. 2008; Nagy et al. 2010), metabolic rate (Biro and Stamps, 2010) and nutritional state (Bazazi et al. 2010) all may differ between conspecifics within a group and the group's movement characteristics and structural dynamics may be dependent on the strength and combination of these characteristics (Krause 1993c; Krause and Ruxton 2002; King et al. 2008; Cote et al. 2010b; Brown and Irving 2013). Such differences in characteristics between group members can create conflicts of interest; individuals seek to satisfy personal motivations whilst also retaining the benefits of animal grouping by conforming to the behaviour of other individuals (Conradt

and Roper 2009; Conradt 2012; Herbert-Read et al. 2013). Considering the ubiquity of grouping throughout the animal kingdom, the form in which these groups are structured and the manner in which they function, so that all group members retain fitness benefits despite their differing motivations, is of enduring biological significance, however, empirical manipulative studies are still lacking.

Fitness benefits of group living for many animals are dependent on an ability to achieve consensus in the timing and direction of movements. Group movement decisions often integrate the preferences of many individuals, requiring multiple movement initiators to agree on a direction (Couzin et al. 2011; Strandburg-Peshkin 2015), with the movement decision occasionally being the average of individuals' preferred directions and speeds (Hastie and Kameda 2005) as long as the difference between the preferred directions of the initiators is not large (Couzin et al. 2011; Strandburg-Peshkin 2015). In contrast to this, other work has proposed that group movement decisions may be determined by a hierarchy (Nagy et al. 2010) or dominated by a subset of group members, typically those that have greater motivation or information (Gueron et al. 1996; Reebs 2000; Robson and Traniello 2002; Rands et al. 2004; Conradt and Roper 2005, 2009; Couzin et al. 2005; Janson et al. 2005; Beekman et al. 2006; King and Cowlishaw 2007; Schultz et al. 2008; Conradt et al. 2009; Cote et al. 2010a; Johnstone and Manica 2011; Stroeymeyt et al. 2011). This process may be self-organised, with group movement decisions being determined on the basis of slight differences in simple behavioural movement parameters structuring local interactions between conspecifics (Gueron et al. 1996; Romey 1996; Couzin et al. 2005; Conradt and Roper 2009). If this idea is viewed in conjunction with the proven functional benefits that individuals attain via social conformity (Krause and

Ruxton 2002) then grouping behaviour should be considered a flexible strategy that is receptive to, on one scale, the internal motivation of each individual and on another scale, the moderating effect of multiple interactions between individuals in the group as a whole (Herbert-Read et al. 2013). This is in contrast to the idea proposed by some group behaviour models in which individuals are unchanging across time and operate under the same local behavioural rules (Hoare et al. 2004; Herbert-Read et al. 2011). To accurately understand individual behaviour within groups and how it affects whole group behaviour, we need to measure the responses of individuals in groups to changes in internal state and examine the outcome of these responses within the social context (Hoare et al. 2004; Nagy et al. 2010; Sueur et al. 2010; Berdahl et al. 2013; Jolles et al. 2013).

Nutritional intake is of central importance when defining an animal's internal state and is of benefit for experimental studies as it is easily manipulated. Fish shoals are a good vertebrate model to examine how differences in internal state affect group behaviour as nutritional deprivation is known both to cause changes to individual positioning within a shoal and to affect shoal cohesion. Also, individual nutritional state is an inadvertent cue that can be detected by conspecifics, and fish are known to associate with conspecifics based on what they have recently eaten (Krause et al. 1999; Morrell et al. 2007; Ward et al. 2011). Hungry individuals often position themselves towards the front (Krause et al. 1992) or outside of the shoal (Romey 1995) where they attain foraging benefits (Krause 1994) and can have a strong influence on group movement direction (Huth and Wissel 1992). Conversely, the middle and rear positions of moving shoals are commonly occupied by well-fed fish where they are able to minimise their domain of danger (Krause 1993a). In regards to social attraction, hungry fish may have larger

inter-individual distances (Morgan 1988; Robinson and Pitcher 1989a,b; Romey 1995; Gueron et al. 1996). There is also evidence that fish perform a calculated trade-off and spend a decreasing amount of time with the larger of two shoals as hunger increases, presumably to reduce food competition effects (Krause 1993b; Metcalfe and Thomson 1995; Reebs and Saulnier 1997) despite the increased risk of predation experienced by more isolated individuals (Bumann et al. 1997).

This experiment quantifies the influence of nutritional state on the individual and group movement parameters of free swimming shoals of 8 rainbowfish, *Melanotaenia duboulayi*. Individual fish were experimentally manipulated to be in one of two nutritional states, hungry or satiated, and are assayed in three group compositions; all-hungry (8:0 hungry:satiated), mixed (4:4), or all-satiated (0:8). All fish were tested once under each of the three group compositions. Therefore, we test whether nutritional state affects a.) individual locomotion, specifically; speed, turning speed, exploration and b.) spatial behaviour, specifically; inter-individual distance, tendency to become isoalted and position within the shoal. We then investigate how individuals relate spatially to conspecifics of the same and different nutritional state. Finally, we investigate whether nutritional effects on individual locomotory and spatial behaviour affects group level properties such as speed, turning speed, exploration, isolation, polarisation, inter-individual distance and the formation of subgroups. It is predicted that hungry individuals will swim faster than satiated individuals and explore more of the arena (Hansen et al. 2015), while also preferring to shoal in smaller sub-groups (Krause 1993b; Reebs and Saulnier 1997; Hoare et al. 2004) and closer to the front of these sub-groups (Krause et al. 1992; Krause 1994). Individuals are also expected to show evidence of assortive shoaling and increase the inter-individual

distance between hungry individuals to avoid competition (Metcalfe and Thomson 1995; Krause et al. 1999). It is predicted that group level dynamics will reflect the motivations of the individuals that compose the shoal with the mixed shoal expressing intermediate values (between the all-hungry and all-satiated shoals) for the variables assessed (Robinson and Pitcher 1989a).

Methods:

Experimental animals

Crimson-spotted rainbowfish, *Melanotaenia duboulayi*, are a freshwater species of fish endemic to eastern Australia. The fish were obtained from a commercial supplier (PISCES aquatic, Qld). They form small shoals of approximately 5-20+ fish, although individuals or smaller shoals may break away temporarily (Brown 2000). Whilst they inhabit open water, it is also common for them to aggregate around submerged logs and subsurface vegetation. Experimental fish were kept in white plastic housing aquaria (180L) in de-chlorinated aged tap water with a sponge filter at 27°C for 8 weeks in 12:12 light:dark photoperiod before the commencement of experiments. Fish used in the experiment had a body length of 60±5mm. We used juvenile fish and did not sex the fish.

Nutritional and group composition manipulation

Forty-eight fish were taken from the 180L aquarium and separated equally between six 50L holding aquaria two weeks before the experimental assay in order to reduce the effects of familiarity on shoaling preferences between trials (Frommen et al. 2007; Kydd and Brown 2009). All fish in each of the six 50L aquaria were tagged at one location on their dorsal surface with

blue or yellow visible implant elastomer (Northwest Marine Technology, Inc, Manual Elastomer Injection System, 10:1 Formulation) for individual recognition (Croft et al. 2005). There was no evidence of the fish reacting to the tags and as all fish were tagged, there was no bias between tagged and untagged fish possible. During these two weeks all fish were fed frozen bloodworms (Ocean Nutrition®) once per day till satiation (approximately 6 bloodworms per individual). Forty-eight hours before the experimental assay, a perforated plastic divider was placed in the middle of each 50L housing aquaria separating the 8 fish into two groups of 4. Fish could see and smell fish on either side of the barrier, however, it prevented fish physically crossing from either side so that the fish could be fed separately. Fish could be manipulated to be in one of two nutritional states: hungry or satiated. All 6 groups were assayed once in each of the three group composition treatments (all-hungry (8:0), mixed (4:4), and all-satiated (0:8). For the all-satiated treatment, 1 hour prior to testing all 8 fish were fed with bloodworms till satiation. For the hungry treatment all 8 fish were starved for 48h prior to testing. For the mixed treatment, 4 of the fish were starved for 48h and 4 were fed till satiation 1h prior to testing. For each group the order in which they underwent these three treatments was randomised.

Experimental Arena

The experimental arena was a square arena (1500x1500x150mm) composed of white Perspex 10mm thick filled to a depth of 60mm with de-chlorinated aged tap water at the same temperature as the holding aquaria (Fig. 1). Another litre of water from an aquarium containing 100 rainbowfish was added to the arena each day before the trials commenced in an attempt to maximise the consistency of chemical cues between trials. The arena was surrounded by opaque

white Corflute® to minimise external stimuli disturbing the fish whilst also allowing enough light for video recording. No substrate was used to maximize contrast of fish to the background to facilitate tracking. A clear plastic cylinder (100mm diameter) attached to monofilament line, extended outside of the arena and created a chamber from which fish could be released from at the start of the experimental trial. Four white ceramic square bowls (100x100x50mm) were placed upside down 400mm from the corners of the tank to create environmental enrichment and break-up the line of sight of exploring fish (Fig. 1).

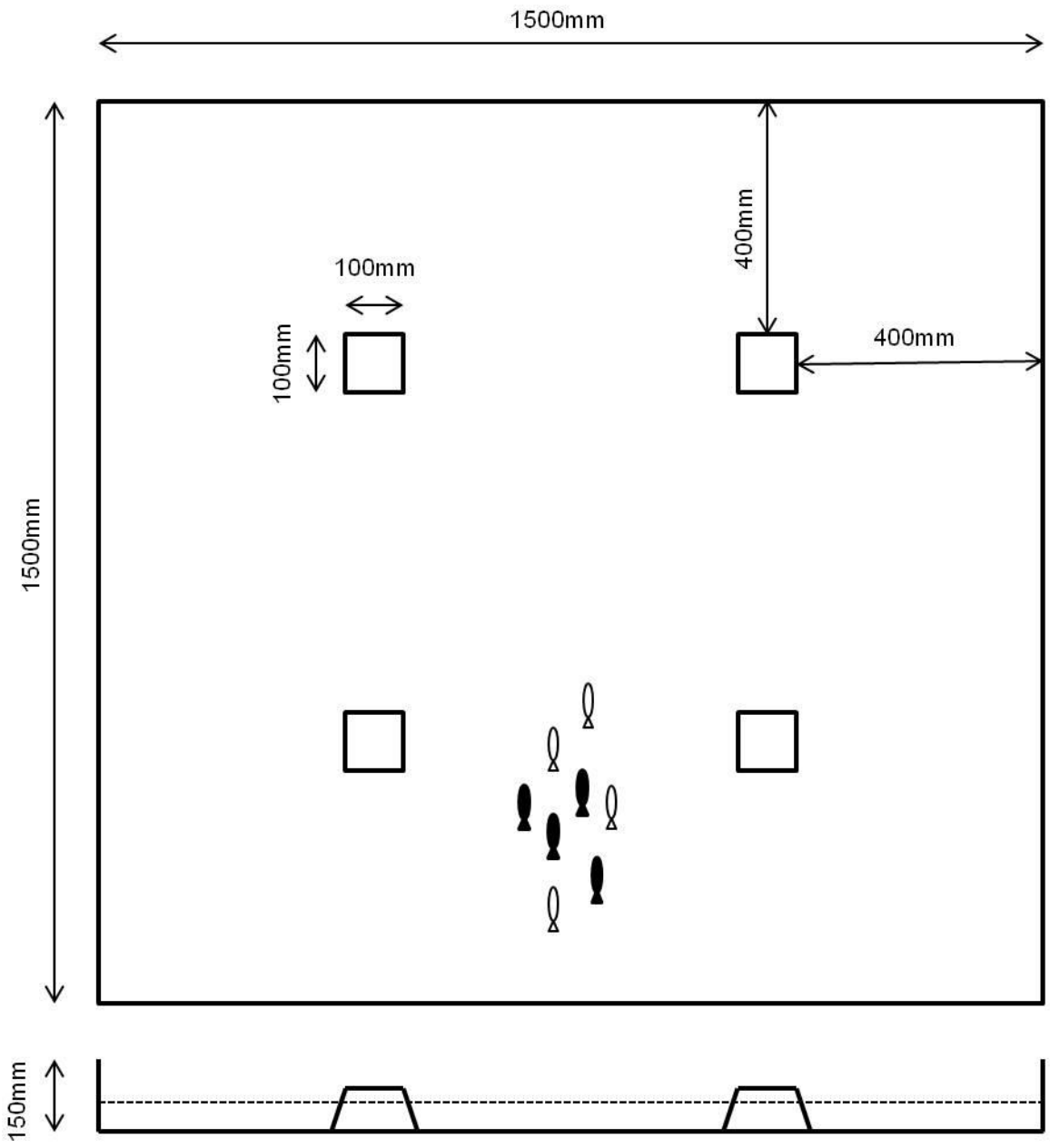

Fig. 1 Diagram of experimental arena with a 'mixed' shoal composed of 4 hungry fish ('black') and 4 satiated fish ('white'). Dotted line represents the water level (60mm).

Experimental trial

The experimental trials commenced at 10:00h and all groups were tested once every 2 days. A group of 8 fish was placed into the release chamber of the experimental arena for 10 minutes to get used to their surroundings and to rest after transfer from their holding aquarium. The chamber was subsequently raised and the fish were left to swim around the arena for 6 minutes, however, only the last 5 minutes were used for analysis. All trials were filmed from directly above the arena with a video camera.

Video and tracking analysis

Videos were recorded with a Basler® camera at 40fps and 15000Hz exposure. The footage was recorded as sequence .src files and converted to .avi with Streampix (v5, NORPIX). The .avi files were then opened with VirtualDub (v 1.10.4) where they were saved as old format .avi files after the final 12,000 frames of the video were isolated and decompressed. The old format .avi file was then imported into an automated tracking software program, Ctrax (Caltech ethonomics project, The Caltech Multiple Fly Tracker, Version 0.5.3, 2014) (Branson et al. 2009). This program automatically tracked the position of each individual fish at every frame (40fps) of the 5 minute video. Therefore each fish had (*x, y*) coordinates (in pixels) and its orientation, θ, (in radians, relative to the positive x-axis) recorded for each 12,000 frames of the video. The position of fish on the *z*-axis was not recorded as the water depth was shallow. This data was then imported into MATLAB where MATLAB FixErrors GUI was used to manually correct any errors made by the automatic tracking software, Ctrax. Each fish therefore had one consistent, unbroken track over the entire 12,000 frames of the trial. Pixels were converted into mm with a

conversion ratio calculated by measuring the distance between two points of known distance on the first frame of the video.

Calculations

We performed a series of calculations to examine how internal nutritional state (hungry or satiated) affected fish's speed, turning speed, nearest neighbour distances, mean neighbour distances, the formation of sub-groups, relative shoal position, polarisation and the tendency to become isolated from the main group. A fish was considered isolated if it was not within 3 body lengths (180mm) of any other fish. All fish part of a distinct subgroup had to be within three body lengths (180mm) of at least one other member of the subgroup. We also determined the mean over time of the distance between all pairs of fish. For the mixed groups, we noted the internal nutritional state of each fish in a pair and indentified each pair as being made up of two hungry individuals (an HH pair), a hungry individual and a satiated individual (an HS pair), or two satiated individuals (an SS pair). In the all-hungry and all-satiated group compositions half of the fish were nominally assigned as 'hungry' and 'satiated' individuals as controls. We quantified exploratory behaviour on the interior of the arena by covering the domain of the arena with a virtual square grid and noting the total number of unique squares that fish passed through during each video. Details of all calculations are provided in the Supplementary material.

Experimental design and analysis

We used R (R Core Team, 2014, R i386 3.1.2) and *lme4* to perform a linear mixed effects analysis of the relationship between the individual level dependent variables and internal

nutritional state. The dependent variables tested were; 'speed', 'turning speed', 'number of squares explored', 'near-neighbour distance', 'mean-neighbour distance', 'number of times a fish became isolated', 'number of frames spent isolated', 'relative position within a shoal' and 'personal group size'. These dependent variables were tested for homogeneity of variance with Levene's test and for normality by looking at histograms and QQ-plots. Transformations were necessary for the dependent variables 'near-neighbour distance', 'mean-neighbour distance', 'number of frames spent isolated' and 'the number of times a fish became isolated' (square root) as well as 'personal group size' (squared), to meet the assumption of normally distributed data. As a fixed effect we entered internal nutritional state into the model. We had group identity as a random effect. The linear mixed model was fit by maximum likelihood *t*-tests and used Satterthwaite approximations for degrees of freedom to approximate *p*-values. Alpha-values for rejection of statistical significance were adjusted by the Holm-Bonferroni method (Holm 1979). We also performed a principal component analysis in SPSS (IBM® SPSS® Statistics, Version 20) which supported the findings of our other analysis, the details of which can be found in the Supplementary material.

This model was run separately for each of the three group compositions as only the mixed group contained fish in both nutritional states. However, the all-hungry and all-satiated group compositions were tested as controls with nominally assigned 'hungry' and 'satiated' individuals.

To test for an effect of nutritional state on association preferences between individuals we compared mean neighbour distances between HH pairs, HS pairs and SS pairs in the three group compositions using repeated measures ANOVA in SPSS (IBM® SPSS® Statistics, Version 20).

For the group level dependent variables ('speed', 'turning speed', 'number of squares explored', 'near-neighbour distance', 'mean-neighbour distance', 'number of times fish became isolated', 'number of frames spent isolated', 'personal group size' 'number of sub-groups', 'maximum number of sub groups' and 'polarisation') we tested for differences between the three group compositions using repeated measures ANOVA in SPSS. In all repeated measures ANOVA's the assumption of sphericity was checked using Mauchly's test and if it was violated degrees of freedom were corrected using Greenhouse-Geisser estimates. Post-hoc pair-wise comparisons were investigated with Tukey's LSD.

Results

Individual locomotion

There were no significant differences in locomotion between our controls, the nominally assigned 'hungry' and 'satiated' individuals, in either the all-hungry or the all-satiated group composition treatments (Table 1). Within the mixed group composition, nutritional state similarly did not affect locomotion. There was no significant effect of nutritional state on mean speed, turning speed or the number of grid squares explored.

Table 1 LMER analysis on the effect of State (Hungry or Satiated) on individual behaviours. Significant p-values are presented in bold.

	Mixed					All-hungry					All-satiated				
	Estimate	**Standard Error**	**DF**	**t-value**	**Pr (>|t|)**	**Estimate**	**Standard Error**	**DF**	**t-value**	**Pr (>|t|)**	**Estimate**	**Standard Error**	**DF**	**t-value**	**Pr (>|t|)**
a.) Mean speed (mm/s)															
(Intercept)	115.2732	4.406				124.564	5.824				108.298	11.5436			
State	0.1303	2.3691	42	0.055	0.956	-4.132	2.994	42	-1.378	0.176	-0.9977	2.386	42	-0.418	0.678
b.) Turning speed (rad/s)															
(Intercept)	1.03513	0.08771				1.12772	0.08935				0.95988	0.13635			
State	-0.12714	0.06907	42	-1.841	0.073	-0.10892	0.06067	42	-1.795	0.08	-0.06517	0.06262	42	-1.041	0.304
c.) Number of squares explored															
(Intercept)	366.208	12.604				404.542	14.181				321.875	44.287			
State	-7.792	9.294	42	-0.838	0.407	-10.5	9.013	42	-1.165	0.251	3.417	7.34	42	0.465	0.644004
d.) NN-dist															
(Intercept)	10.214	0.4452				11.4844	0.74458				8.7717	0.2047			
State	-0.5863	0.2164	42	-2.709	**0.01**	-0.01547	0.34837	42	-0.044	0.965	0.1207	0.1713	42	0.705	0.485
e.) MN-dist															
(Intercept)	16.9553	0.9622				19.5898	1.215				13.661	0.3262			
State	-0.4382	0.1959	42	-2.236	**0.031**	0.1342	0.2585	42	0.519	0.606	0.1006	0.161	42	0.624	0.536
f.) Number of isolation events															
(Intercept)	3.41188	0.4625				4.315	0.4249				1.2607	0.2672			
State	-0.4831	0.2502	42	-1.931	0.06	-0.131	0.2307	42	-0.568	0.573	0.3311	0.2906	42	1.139	0.261
f.) Total amount of frames in isolation															
(Intercept)	33.524	4.871				47.26	6.584				10.175	1.951			
State	-8.228	2.748	42	-2.994	**0.005**	-1.388	2.703	42	-0.513	0.61	2.93	2.57	42	1.14	0.261
h.) Mean relative position															
(Intercept)	0.46945	0.01824				0.48723	0.01337				0.501059	0.015347			
State	0.05746	0.02171	48	2.647	**0.011**	0.02583	0.0189	48	1.366	0.178	-0.002292	0.021704	48	-0.106	0.916
i.) Mean shoal size															
(Intercept)	32.485	5.2699				20.9444	4.7741				53.712358	2.073262			
State	0.3947	0.7272	42	3.008	**0.004**	0.3947	0.7272	42	0.543	0.59	0.008357	0.674157	42	0.012	0.99

Individual spatial behaviour

There were no significant differences in spatial behaviour between our controls, the nominally assigned 'hungry' and 'satiated' individuals, in either the all-hungry or the all-satiated group composition treatments (Table 1). However, within the mixed group composition, nutritional state had a significant effect. Nearest-neighbour distance, mean-neighbour distance and the total number of frames in isolation were larger for hungry individuals. However, nutritional state had no significant effect on the number of times a fish became isolated. The relative position within a shoal and the mean size of the shoal occupied were both smaller for hungry individuals (Table 1).

Interactions with conspecifics

Interactions between pairs of individuals were also significantly affected by internal nutritional state ($F_{2,5}$= 11.304, p=0.003). Post Hoc tests showed that the inter-individual distances between HH pairs, HS pairs and SS pairs were all significantly different from each other, with HH pairs having the largest inter-individual distances and the inter-individual distances of HS pairs intermediate between HH pairs and SS pairs (Fig. 2).

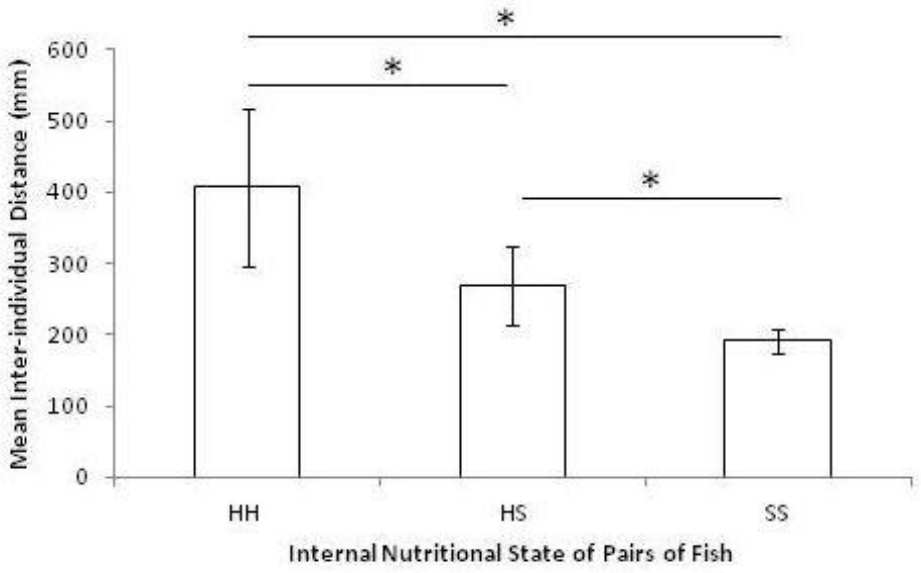

Fig. 2 Histogram of repeated measures ANOVA showing the mean inter-individual distances (mm) between pairs of individuals of known internal nutritional states. HH represents pairs including only hungry individuals, HS represents pairs including one hungry individual and one satiated individual and SS represents pairs including only satiated individuals.

Group level

Group composition treatment had a significant effect on a number of group level variables. Nearest neighbour distance ($F_{2,10}$= 7.690, p=0.009) , mean-neighbour distance ($F_{2,10}$=10.446, p=0.004), the number of isolation events ($F_{2,10}$=14.480, p=0.001), the number of frames fish spent in isolation ($F_{2,10}$= 8.946, p=0.006), the mean number of sub-groups ($F_{2,10}$=11.538, p=0.003) and the maximum number of sub groups ($F_{2,10}$=17.856, p=0.001) were all largest in the all-hungry treatment and smallest in the all-satiated treatment, with the mixed treatment intermediate to them both. The mean size of shoals occupied by each fish ($F_{2,10}$= 14.189, p=0.001) was smallest in the all-hungry treatment and largest in the all-

satiated treatment, with the mixed treatment intermediate (Fig. 3). Group composition treatment had no significant effect on mean speed ($F_{1.084,5.420}$= 0.913, p=0.389), turning speed ($F_{2,10}$= 0.725, p=0.508), number of squares explored ($F_{1.112,\ 5.61}$= 1.75, p=0.242) or polarisation ($F_{1.021,\ 5.107}$= 0.153, p=0.717).

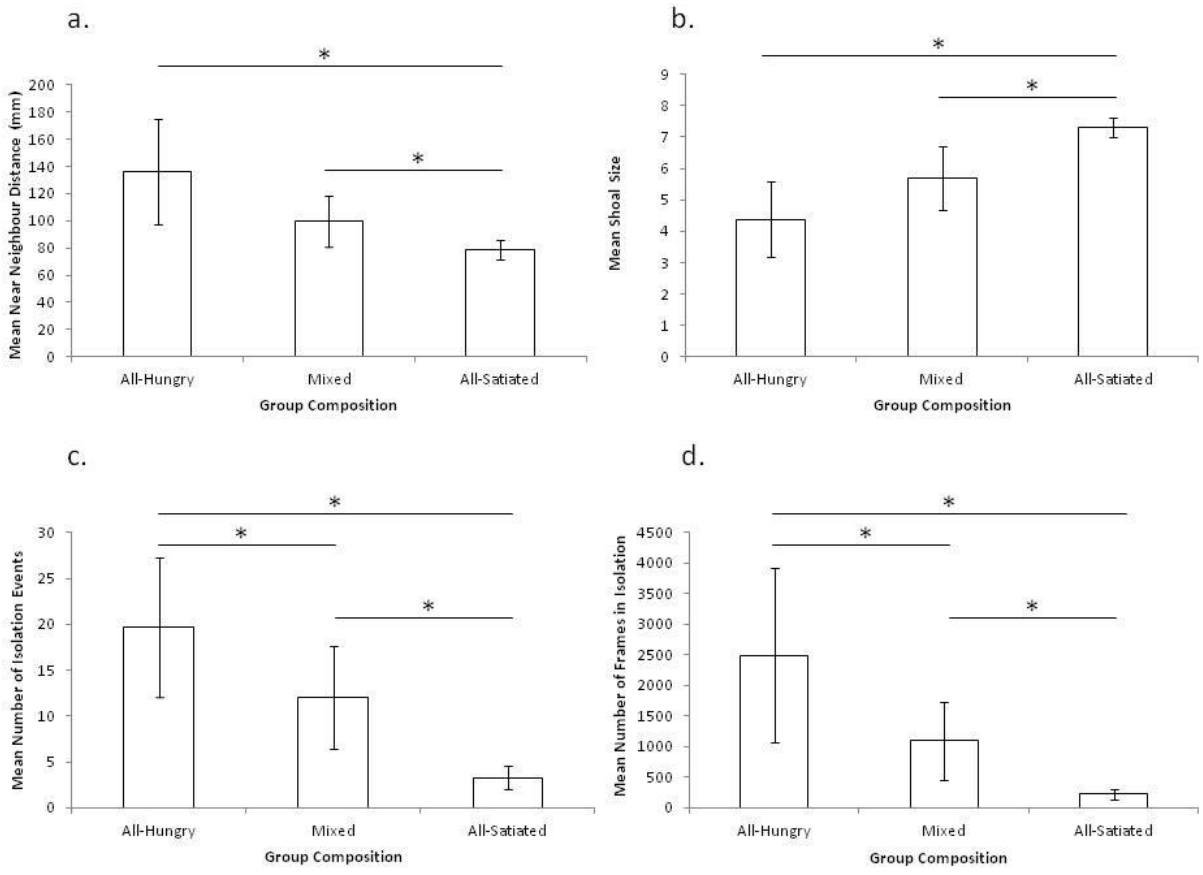

Fig. 3 Histogram of repeated measures ANOVA showing the effect of group composition (All-Hungry (8:0 hungry:satiated), Mixed (4:4) and All-Satiated (0:8)) on group level behaviours. a.) Mean Nearest Neighbour Distance (mm), b.) Mean Shoal Size, c.) Mean Number of Isolation Events, d.) Mean Number of Frames in Isolation. Asterixes* show signficant differences between treatment means.

Discussion

Individual level

Internal nutritional state affected the spatial behaviour of individual fish and these behavioural changes are reflected in their interactions with conspecifics and the overall spatial dynamics of the group. Within mixed groups, composed of both hungry and satiated individuals, there was a difference in individual spatial behaviour based on internal nutritional state. Hungry individuals had greater nearest and mean-neighbour distances, had a higher frequency of time spent in isolation, and were closer to the front of shoals than satiated individuals were. This suggests individuals were responding to internal stimuli and changing their spatial behaviour to increase foraging efficiency. Greater inter-individual distances may reduce competition (potentially increasing an individual's risk of predation (Bumann et al. 1997)), and by moving to the front of shoals fish can attain greater foraging benefits (Krause et al. 1992; Krause 1994). Field work tracking a solitary banded killifish (*Fundulus diaphanous*) of known nutritional state as it associated with a shoal of conspecifics similarly found that hungry fish spend more time in isolation (Hensor et al. 2003). Hungry individuals left the shoal more often than satiated individuals, and therefore, the study concluded that nutritional state affected the individual's decision to continue shoaling once an association was made. We assessed both the total number of frames hungry fish spent in isolation as well as the number of times they became isolated and found that only the amount of time spent in isolation was significantly different, although there was a trend for hungry fish to become isolated more times than satiated fish. This implies that hungry fish stayed isolated for longer periods of time without coming back to

within 3 body lengths of another fish. Importantly, fish would still be able to acquire social information on the location of food at this distance and, therefore, hungry individuals are likely isolating themselves to decrease food competition, whilst still remaining close enough to parasitise the food discoveries of conspecifics (Brown and Laland 2003). It is possible that their isolation is simply an emergent behaviour caused by them travelling at faster speeds and exploring more than their conspecifics (Rands et al. 2004), however, we found no evidence that internal nutritional state affected locomotory behaviour, which was surprising as this was recently found to be the case in shoals of mosquitofish (*Gambusia holbrooki*) (Hansen et al. 2015). It seems then that spatial positioning of hungry individuals is a result of a deliberate decision to isolate themselves from other individuals and it would be beneficial to investigate in what capacity an individual's zones of interaction are affected by internal nutritional state. The zones of interaction refer to the distance at which an animal is attracted to, repulsed by or aligns with a conspecific (Couzin and Krause 2003). If these are found to change with individual nutritional state, it would provide a powerful explanatory model of how different spatial arrangements of animals, in different nutritional states, are self-organised in nature.

We found that individual hungry fish occupied smaller shoals than satiated fish. This is the first empirical evidence, in this author's knowledge, of this behaviour occurring in free swimming, freely interacting shoals of fish. Two classic behavioural experiments in shoal size choice found that when given a binary choice between a large and a small shoal, both individual hungry and satiated fish preferred to join the larger shoal, but that this preference was weaker in hungry fish (Krause 1993b; Reebs and Saulnier 1997). Although highly

influential, these studies were at the time limited by their inability to track numerous fish of known identity at the same time, and hence this study builds on their findings by examining shoal size choice within a more natural environment where multiple fish of different nutritional state are able to freely interact. Hoare et al. 2004 observed that the median group size in banded killifish (*Fundulus diaphanous*), was smaller when food cues were in the water and also that solitary fish were more common. In our study (where there were no food cues added to the environment), hungry fish were often found in smaller shoals and spent more time isolated than satiated individuals. Comparing the two sets of results it is evident that external food cues and internal nutritional state can have a similar effect on individual spatial behaviour and future studies would do well look at their interaction specifically.

Interactions with conspecifics

Spatial properties of groups are not solely determined by how individuals respond to their internal state; it is also affected by how individuals react to external stimuli, including conspecifics. Theoretical and empirical studies on the spatial properties of moving animal groups examine where an individual is in relation to that of its group mates (Couzin et al. 2002, 2005; Herbert-Read et al. 2011, 2013; Strandburg-Peshkin 2015). Spatial positioning in groups should be viewed, at a minimum, as a two way interaction between individuals. Therefore, we looked at how the nutritional state of individual pairs of fish affected their inter-individual distance. Inter-individual distances between pairs of hungry fish were greater than inter-individual distances between pairs comprised of one hungry and one satiated fish. Pairs of satiated individuals were generally separated by the least distance (Fig. 2). This is evidence for assortive shoaling in relation to nutritional state, with satiated

individuals preferring to be closer to satiated individuals over hungry individuals, and hungry individuals also preferring to be close to satiated individuals rather than hungry individuals. Fish are known to select shoaling partners based on size (Croft et al. 2004; Krause et al. 2000; Ranta and Lindström 1990) as well as other phenotypic traits such as species (Krause et al. 2000), colour (McRobert and Bradner, 1998; Rodgers et al. 2010), familiarity (Griffiths and Magurran, 1997) and parasite load (Krause and Godin, 1996; Barber et al. 1998) (see Krause et al. 2000b for a review). It is relevant that hungry fish are known to prefer to shoal with smaller individuals (Hensor et al. 2003) and poorer competitors (Metcalfe and Thomson 1995) and that individuals will associate with partners based on the food type (Olsén et al. 2003; Ward et al. 2004; Morrell et al. 2007; Webster et al. 2008), quality (Ward et al. 2011), and quantity (Krause et al. 1999) they have recently eaten. The ability to sense the recent dietary intake of individuals could be from cues caused by physical changes in the fish's body shape, for example differences in stomach width and dorso-ventral extension in starved and well fed fish (Krause et al. 1999; Sumpter et al. 2008) or through the alteration of the fish's chemical cue based on nutritional state (Derby and Sorensen 2008; Giaquinto and Hara 2008; Webster et al. 2008; Ward et al. 2011). The benefits of this sensory ability may be that fish could select to associate with conspecifics that are aware of the location of food or that are good producers from whom they can scrounge from, or that fish could attain the benefits of shoaling whilst reducing the negative effects of competition by selecting to shoal with satiated fish so as to attain a higher foraging success (Krause et al. 1999). The latter is hypothesized to be what occurred in this experiment but we are not confident of the sensory mechanism that allowed for the identification of hungry and satiated individuals. Fish were fed 1 hour before the

experiment, which should have been ample time for their chemical signature to have been altered (Dosdat et al. 1996; Olsén et al. 2003). Also, fish could see how much their shoal mates ate during the nutritional manipulation phase of the experiment, and considering the fish ate till satiated, physical distortions to their body shape were likely.

Group level

Individual spatial decisions and interactions between pairs of individuals scaled up to affect group level dynamics. There was an additive effect of individual behaviour on group behaviour for all variables tested as the mixed group composition was always intermediate between the all-hungry and all-satiated group compositions (Fig. 3). Nearest neighbour distance, mean-neighbour distance and amount of time fish spent isolated were all increased as the number of hungry individuals within the group increased. The group also broke into more sub-groups when it was composed solely of hungry individuals. Changes in group sizes have been seen as an emergent feature of the interaction rules followed by individuals (Camazine et al. 2001; Couzin and Krause 2003; Rands et al. 2004). For the mixed group, it seems that the result of the conflict between hungry and satiated individuals was not an integrated group response (Stienessen and Parrish 2013) but for individuals to break up into sub-groups according to their individual needs. Satiated individuals attempted to stay in closer contact with conspecifics, however, hungry individuals isolated themselves and formed smaller sub-groups. The extent of group fission depends on the degree of the conflicting demands. Although groups broke up into sub-groups, they still were able to respond to other sub-groups. The size of the tank would have to be much greater, or the study conducted in the field, to investigate whether the conflicting demands caused by

varying nutritional levels led to true group fission events. The group level results of this experiment are very similar to the results found by Robinson and Pitcher (1989) where the swimming characteristics of mixed shoals of fish were assessed. They similarly concluded that fish behaved in mixed shoals according to their individual nutritional state and that neither the starved nor the satiated behaviour of individual fish determined overall shoal dynamics (Robinson and Pitcher 1989a).

Conclusions

The benefit of tracking freely interacting, individually identified animals is obvious: it allows for the controlled quantification of complex behaviours that are the product of interacting internal and external stimuli. It is well documented now that an individual's behaviour is dependent on the behaviour of its group members (Giraldeau and Caraco 2000; Krause and Ruxton 2002; Sumpter 2010) so it is beneficial for studies to allow individuals to interact without restrictive barriers so they can acquire information on the state of all individuals in the group and adjust their own behaviour accordingly after receiving feedback from their interactions.

Animal groups are often composed of selfish individuals with conflicting motivations and it has been suggested recently that the ability to predict group level properties of heterogeneously structured groups needs continued examination (Herbert-Read et al. 2013). This study has furthered this aim and shown that overall group properties relating to spatial dynamics reflect what is occurring at the lower levels of biological organization. Here, the internal nutritional state of individual fish affected basic behaviours relating to spatial positioning, although not locomotion. The interaction between pairs of fish was dependent

on the nutritional state of both fish and group level behaviour reflected the motivations of its individual members in such a way that allowed individuals to fulfil their own behavioural needs whilst still attaining the benefits of grouping.

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

All procedures performed were in accordance with the ethical standards of the University of Sydney.

Acknowledgments

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Supplementary Material

Calculations

Speed and turning speed

Writing the coordinates of the *i*th fish in millimeters at time *t* as $(x_i(t), y_i(t))$, we estimated the components of each fish's velocity vector, $(u_i(t), v_i(t))$, at time *t* via the forward difference approximations:

$$u_i(t) = \frac{x_i(t + \Delta t) - x_i(t)}{\Delta t} \quad \text{and} \quad v_i(t) = \frac{y_i(t + \Delta t) - y_i(t)}{\Delta t},$$

where Δt was the constant separation between consecutive video frames (=1/40 s).

We then estimated each fish's speed at time *t* using:

$$s_i(t) = \sqrt{(u_i(t))^2 + (v_i(t))^2}.$$

For subsequent statistical tests we determined the mean and variance of each individual's time-series of speed values (denoted $\bar{s}_i(t)$ and $\mathrm{Var}(s_i(t))$ respectively. At the level of each group, we then determined the means of $\bar{s}_i(t)$ and $\mathrm{Var}(s_i(t))$ across all eight group members.

We derived each fish's turning speed from their facing directions, $\theta_i(t)$, at consecutive times, *t* and $t + \Delta t$. To do this, we first determined the *x* and *y* components of unit vectors pointing in the direction of $\theta_i(t)$ for all times *t* using:

$\hat{X}_i(t) = \cos(\theta_i(t))$ and $\hat{Y}_i(t) = \sin(\theta_i(t))$.

The magnitude of each fish's turning speed (in radians per second) at time *t* was then estimated via:

$$\alpha_i(t) = \frac{\cos^{-1}\left(\hat{X}_i(t)\hat{X}_i(t+\Delta t) + \hat{Y}_i(t)\hat{Y}_i(t+\Delta t)\right)}{\Delta t}.$$

We determined the median of each fish's turning speed time-series, denoted $\text{Median}(\alpha_i(t))$, and the mean of $\text{Median}(\alpha_i(t))$ across all members of each group.

Inter-individual distances

We determined the distances between all pairs of fish *i* and *j*, for all times *t* using the distance formula:

$$d_{i,j}(t) = \sqrt{\left(x_j(t) - x_i(t)\right)^2 + \left(y_j(t) - y_i(t)\right)^2}\ . \qquad (1)$$

For each time *t*, we determined the distance from each fish *i* to their nearest neighbour, $d_{i,nn}(t)$. We then determined the mean over time of $d_{i,nn}(t)$, denoted $\bar{d}_{i,nn}$, for each fish *i*. At the group level we then calculated the mean of $\bar{d}_{i,nn}$ across all eight fish *i* in a given group.

Additionally, we determined the mean over time of the distance between all pairs of fish, *i* and *j*, $\bar{d}_{i,j}$. For mixed groups, we noted the internal nutritional state of each fish in a pair and identified each pair as being made up of two hungry individuals (an HH pair), a

hungry individual and a satiated individual (an HS pair), or two satiated individuals (an SS pair). We then separated the values of $\bar{d}_{i,j}$ into HH, HS and SS categories. In the all-hungry and all-satiated group compositions half of the fish were nominally assigned as 'hungry' and 'satiated' individuals as controls. We separated values of $\bar{d}_{i,j}$ into HH, HS, and SS categories in the all-hungry and all-satiated groups based on these nominal 'hungry' and 'satiated' assignations.

Formation of subgroups, frequency and number of frames of isolation

We used the following algorithm to classify fish as being part of distinct subgroups based on the distances between individual fish. The algorithm identified a distinct subgroup of fish as a set of fish where no fish in the set was more than three body lengths (180 mm) from any other fish in the set at time *t*.

For each time *t*, we assigned the first fish in a given group to subgroup 1. We then identified all fish that were less than or equal to 180 mm from fish 1 with the help of equation (1), and assigned any such fish to subgroup 1. For each newly assigned fish *i*, we then identified any other fish *j* not already assigned to a subgroup for which $d_{i,j}(t) \leq 180$ mm. If such fish were found, then they were assigned to subgroup 1, and then the process of cycling through newly assigned fish to find any other unassigned fish for which $d_{i,j}(t) \leq 180$ mm was repeated. If no fish for which $d_{i,j}(t) \leq 180$ mm were found during a cycle, then a new subgroup was started, with the first fish not already assigned to another

subgroup identified as the first member of the new subgroup. The process of finding all fish less than or equal to 180 mm from the fish identified as the founding member of a new subgroup was then repeated as described above. The algorithm terminated when all fish in a group had been assigned to a subgroup for time *t*. We stored the indices of all fish that appeared in each distinct subgroup for all times *t*.

We identified and tallied all frames where each fish *i* was the only member of their particular subgroup. We then treated each series of consecutive time steps where a fish was the only subgroup member as a distinct instance of isolation. We tallied the distinct instances of isolation by each fish, and then determined the mean number of instances of isolation, and the mean number of frames spent in isolation across all group members.

Relative position within each subgroup

We identified all the unique combinations of fish that appeared in each of the subgroups identified by the algorithm described above. Each combination of fish may have appeared multiple times throughout the 12,000 frames of video analysed for each trial. We identified the corresponding time steps for which the fish were grouped for each unique combination, and extracted the corresponding sections of trajectory for each subgroup member. We then determined the coordinates of the subgroup centre, $\left(c_x(t), c_y(t)\right)$, for all times that fish were grouped in a given combination (given by the mean of the *x* and *y* coordinates of all subgroup members at time *t*). We determined the components of velocity corresponding to movement of each subgroup centre using the forward difference approximations

$$c_u(t) = \frac{c_x(t+\Delta t) - c_x(t)}{\Delta t} \quad \text{and} \quad c_v(t) = \frac{c_y(t+\Delta t) - c_y(t)}{\Delta t},$$

and from these estimated the direction of motion of the group centre relative to the positive *x*-axis via $c_\theta(t) = \text{atan2}\big(c_v(t), c_u(t)\big)$. For each time step that a particular subgroup was together we then shifted the coordinates of all subgroup members so that the group centre was located at the origin, and rotated the coordinates of all group members $-c_\theta(t)$ radians about the origin such that the direction of motion of the group was now parallel to and pointing in the direction of the positive *x*-axis. We then identified the absolute position of all subgroup members based on their shifted and rotated *x*-coordinates (the fish with the greatest *x*-coordinate was identified as being at the front of the subgroup, relative to the direction of motion of the subgroup, the fish with the least *x*-coordinate was identified as being at the rear of the subgroup), and assigned each fish an integer score, $AS_i(t)$, based on their position (the first/front most fish was assigned $AS_i(t) = 1$, the second fish $AS_i(t) = 2$, and so on up to the *n*th fish in the subgroup). We also recorded the size of the subgroup for each subgroup member, $G_i(t)$. We assigned a relative group position score to each fish via:

$$RS_i(t) = \frac{AS_i(t) - 1}{G_i(t) - 1},$$

when fish were not isolated (in a subgroup of 1). Absolute and relative group position scores were only recorded for the first *n – 1* out of *n* frames for each instance that a unique combination of fish were grouped, because of the dependence of these measures on the estimated velocity of each subgroup's centre (which is only known for *n – 1* frames). We

also only recorded values of $G_i(t)$ for the first *n – 1* out of *n* frames that given subgroups had formed (leaving $G_i(t)$ undefined for the *n*th frame).

For each fish we determined the mean of their time series of relative group position scores, and the mean over time of $G_i(t)$ (denoted $\overline{G_i}$). At group level we determined the mean of $\overline{G_i}$ across all 8 fish in each group. We also determined the mean number of subgroups over time, and the maximum number of distinct subgroups observed during each trial.

Polarisation in direction of motion within each subgroup

We expressed the direction of motion of all fish for each time step with the unit vectors:

$$\hat{u}_i(t) = \frac{u_i(t)}{s_i(t)} \quad \text{and} \quad \hat{v}_i(t) = \frac{v_i(t)}{s_i(t)}.$$

We then determined the polarisation of the direction of motion of all fish in each distinct subgroup for each time step *t*. Writing $J(t)$ as the set of indices of fish in the *j*th subgroup at time *t*, the polarisation of subgroup *j* was determined by:

$$P_j(t) = \frac{\sqrt{\left(U_j(t)\right)^2 + \left(V_j(t)\right)^2}}{n_j(t)},$$

where $n_j(t)$ was the number of fish in the *j*th subgroup, $U_j(t) = \sum_{i \in J(t)} \hat{u}_i(t)$ and $V_j(t) = \sum_{i \in J(t)} \hat{v}_i(t)$. We then determined the mean polarisation across existing subgroups for each time, *t*, via:

$$P(t) = \frac{\sum_{j=1}^{n_s(t)} P_j(t)}{n_s(t)},$$

where $n_s(t)$ was the total number of subgroups in existence at time *t*. Finally, we determined the mean across time of $P(t)$ for each trial.

Exploration of arena

We performed a series of calculations equivalent to overlaying the domain of the arena with an $n_g \times n_g$ square grid (with grid box side lengths approximately equal to one body length) and then determining the total number of different grid boxes that each fish passed through during all 12,000 frames of each trial. The overall method that we used was derived from a method for producing a grid based representation of a contour previously defined on a continuous domain[65,66].

We first shifted the coordinates of each fish so that they were guaranteed to satisfy $0 \le x_i(t) < 2000, 0 \le y_i(t) < 2000$ (mm) for all times *t* (for computational convenience). Grid boxes were identified by a pair of integers, (x_g, y_g), such that $1 \le x_g \le n_g, 1 \le y_g \le n_g$. We then scaled and re-shifted the coordinates of each fish so that we could immediately identify which grid box they occupied at time *t* via:

$$\tilde{x}_i(t) = \text{nint}\left(\left(\frac{x_i(t)}{2000}\right)(n_g - 1) + 0.5\right) \text{ and } \tilde{y}_i(t) = \text{nint}\left(\left(\frac{y_i(t)}{2000}\right)(n_g - 1) + 0.5\right),$$

where $\mathrm{nint}(x)$ is the nearest integer to *x*. (Such a transformation guaranteed that $1 \le \tilde{x}_i(t) \le n_g, 1 \le \tilde{y}_i \le n_g$.) For grid box side lengths corresponding to approximately one body length we set $n_g = 34$. Using the above formulation, the grid extended beyond the domain of the arena (so it would be impossible for a fish to explore all $34 \times 34 = 1156$ squares), but the arena itself was always covered by the same number of grid squares.

We recorded whether or not fish *i* visited particular grid boxes in an $n_g \times n_g$ matrix, denoted T_i. Initially all entries of T_i were set to zero. We then cycled through the series of transformed coordinates $(\tilde{x}_i(t), \tilde{y}_i(t))$ in increasing order; if the entry in row $\tilde{y}_i(t)$, column $\tilde{x}_i(t)$ of T_i equaled zero, we would then set that same entry to 1, indicating that the corresponding grid box had been explored. There was a small possibility fish might have moved a distance of more than one grid box between consecutive frames and thus grid boxes identified by integer coordinates $(\tilde{x}_i(t), \tilde{y}_i(t))$ and $(\tilde{x}_i(t + \Delta t), \tilde{y}_i(t + \Delta t))$ might not be adjacent. In the case that such a jump in position was made we identified all grid boxes that lay on the straight line segment from $(\tilde{x}_i(t), \tilde{y}_i(t))$ to $(\tilde{x}_i(t + \Delta t), \tilde{y}_i(t + \Delta t))$ using Bresenham's line algorithm[67]. Where appropriate, we set entries in T_i corresponding to the intermediate points to 1 (if T_i equaled zero at these points initially). Once all points on a fish's trajectory had been cycled through, we tallied the total number of elements in T_i equal to 1 (that is, the total number of grid boxes that the fish passed through during the trial). We denoted the total number of squares explored by fish *i* as $n_{\mathrm{squares},i}$. We then determined the mean of $n_{\mathrm{squares},i}$ across all fish *i* in a given trial.

Principal component analysis

We performed a principal component analysis (PCA) in SPSS (IBM® SPSS® Statistics, Version 20) on the individual level dependent variables. The PCA reduced the variables into two components that explain 80% of the variance. Component 1 is mostly composed of 'spatial' variables (near-neighbour distance, mean-neighbour distance, total frames isolated, frequency of isolation events and mean shoal size (although number of squares explored could also be interpreted as another major contributor) and component 2 is composed of locomotory variables (speed, turning speed and number of squares explored). This is how variables are grouped in the main results section of the manuscript - "locomotion" and "spatial behaviour". We ran the same linear mixed model on these components as we did on the individual dependent variables and found similar results to our individual tests, PC1 was significantly affected by internal nutritional state ($t_{(1,42)}$= -2.772, p=0.008) and PC2 was not significantly affected by internal state ($t_{(1,42)}$= -1.677, p=0.101). Fig 1 shows differential clustering of individuals in hungry and satiated groups with individuals from mixed groups in between. Also the hungry and satiated individuals within the mixed groups are also spatially segregated, although less than hungry and satiated individuals from homogeneous groups.

Table 1. Principal component analysis on the individual level dependent variables showing weighting of variables and % of variance explained.

	Component	
	1	2
Speed	.425	.865
Turning Speed	.447	.738
Num Squares Explored	.485	.783
Near-neighbour distance	.908	-.290
Mean-neighbour distance	.950	-.254
Freq isolation events	.910	-.138
Total frames isolated	.945	-.242
Mean shoal size	-.933	-.237
Relative position in shoal	-.096	.259
Variance explained	**55%**	**25%**
cumulative		**80%**

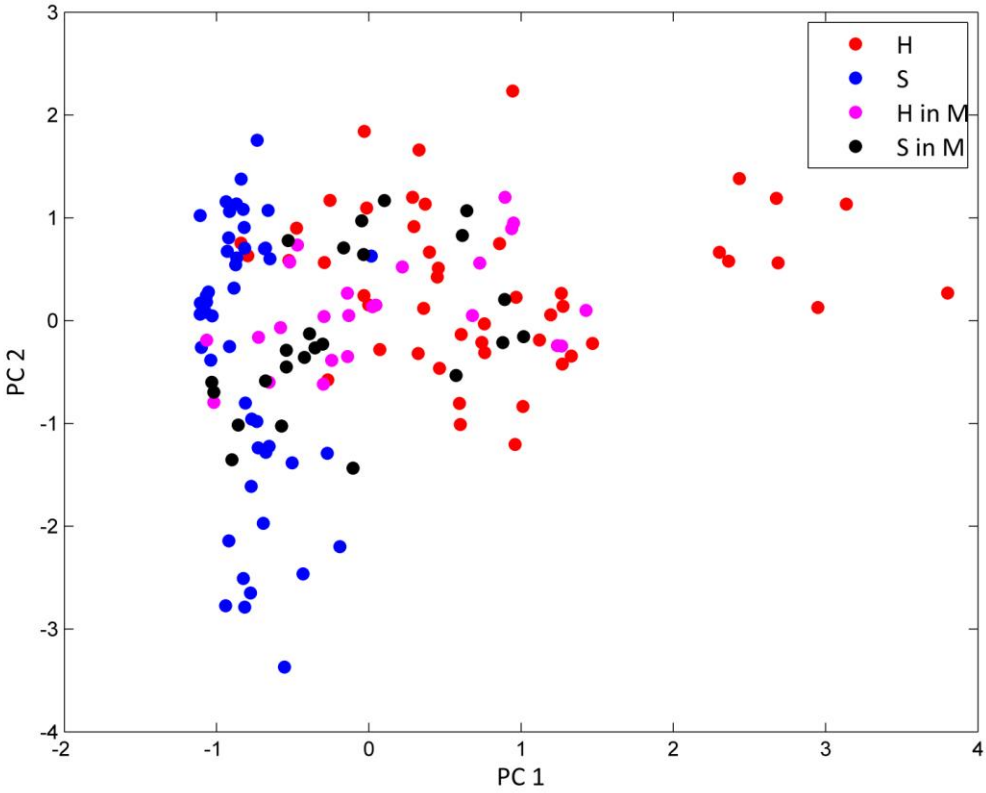

Fig 1. Clustering of hungry and satiated individuals in H (hungry groups; red dots), S (satiated groups; blue dots) and from M (mixed groups; hungry pink dots, satiated black dots).

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Chapter 4

GROUP FORAGING DECISIONS IN NUTRITIONALLY DIFFERENTIATED ENVIRONMENTS

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Foraging behaviour must be flexible enough to adapt to heterogeneities in the distribution and quality of food resources. Accurate models of optimal foraging behaviour need to acknowledge the extent to which animals can detect and regulate their intake of food based on smaller scale differences in food types. In particular, consideration of macro-nutrient distribution and how animals perceive this is limited in studies of optimal foraging, particularly in vertebrates and for animals that forage in groups. Here we track shoals of 8 mosquitofish as they forage in two environments that contain equal amounts of available energy but differ in their distribution of macro-nutrients. We provide empirical evidence that fish will distribute themselves within an environment in relation to the distribution of specific macro-nutrients, notably protein. Also, fish make foraging decisions based on the macro-nutrient composition of patches, such that their durations on patches are longer when they have a higher concentration of protein. The amount of protein does not affect the probability of a fish joining a patch, however, with low numbers of fish on the patch the probability of a fish leaving is greater per unit time step in the low protein patches than the high protein patches. Future experiments should continue to acknowledge the finer scale quality of food types when assessing optimal foraging behaviour and recognize the usefulness and appropriateness of manipulating protein concentrations when classifying patch quality in foraging experiments.

Keywords: Decision-making, Fish, Macro-nutrients, Optimal foraging, Patch duration, Protein

Introduction

It is important animals foraging in heterogeneous environments have a flexible search tactic that is attuned to the distribution of resources, and their quantity and quality, in order to focus efforts on better patches to maximize intake rate (Morgan, Brown & Thorson 1997; Thompson, Petty & Grossman 2001; Stenberg & Persson 2005). While the ability of animals to maximize their intake rate in line with optimal foraging theory is well supported by empirical evidence (Milinski 1979; Harper 1982; Godin & Keenleyside 1984; Gillis & Kramer 1987; Kennedy & Gray 1993) it is also important to recognize an animal's ability to both detect and regulate its intake of food based on smaller scale differences in quality between food types (Hengeveld *et al.* 2009; Houston, Higginson & McNamara 2011). In particular, the macro-nutrient composition can vary considerably between foods, and a single resource in nature rarely has the optimal ratio of nutrients to fulfill an animal's nutritional requirements (Simpson & Raubenheimer 2012). Individual animals compensate for nutritional imbalances in food types and regulate their own nutritional state, in part, through behavioural decisions to move between different resources that vary in nutritional composition (Simpson & Abisgold 1985; Despland & Simpson 2000; Raubenheimer & Jones 2006; Simpson & Raubenheimer 2012).

The foraging decisions animals make depend on information they receive from direct sampling of the environment and social information obtained from the location and behaviour of conspecifics (Danchin *et al.* 2004). Empirical and theoretical work has begun to explore how individual behavioural decisions, made in response to an imbalanced distribution of nutrients in the environment, may be influenced by the social context in

which these behavioural decisions are made (Simpson *et al.* 2006; Dussutour *et al.* 2008; Dussutour & Simpson 2009; Simpson *et al.* 2010; Bazazi *et al.* 2011; Lihoreau *et al.* 2014; Lihoreau *et al.* 2015; Senior *et al.* 2015). In a social foraging context, individuals obtain important benefits via group cohesion and the transfer of social information on food location and quality; however, they may also experience potential costs such as competition over resources (Giraldeau & Caraco 2000; Krause & Ruxton 2002). Therefore, foraging decisions are dependent on the decisions of conspecifics and, by extension, the nutritional requirements, or preferences, of those conspecifics.

Socially foraging individuals often form aggregations on food resources in environments with patchy food distributions, usually as a result of social enhancement, whereby the probability of joining a resource will increase with the number of conspecifics on the resource (Lihoreau, Deneubourg & Rivault 2010; Lihoreau & Rivault 2011). Aggregation is also enhanced if food patches are nutritionally imbalanced (Despland & Simpson 2000). One of the benefits of social foraging is the transfer of information about food location and quality. This socially-acquired information can in turn allow for greater per capita food intake, reduced variation in individual food intake (Caraco 1981; Clark & Mangel 1984; Ranta, Rita & Lindstrom 1993; Ruxton, Hall & Gurney 1995; Naug & Wenzel 2006) and for shortened search times (Pitcher, Magurran & Winfield 1982). However, the use of such social information can lead to increased competition, and if used indiscriminately, can promote the spread of poor quality or outdated information. As a result, individuals are often best served by adopting a mixed strategy where they acquire social information while

also individually sampling the environment to collect their own, private information (Laland, 2004; Webster & Laland 2008; Rendell *et al.* 2011). In making subsequent foraging decisions, individuals may then opt to rely on social information, which likely promotes a group foraging response characterized by group cohesion, or to adopt a more independent foraging strategy. If social cohesion of the group is too high, the group may make sub-optimal foraging decisions and aggregate on the first resource that is discovered, even if it is of poor quality (Dussutour *et al.* 2007). Lihoureau et al. (2014) related this idea to the heterogeneity of macro-nutrient distribution in the environment and hypothesized that if there is an appropriate balance of social cohesion and individuality, individuals in a group could balance their diet collectively.

We know that individual animals can make movement decisions based on the macro-nutrient composition of a diet, but empirical studies exploring the effect of sociality on these movement decisions are very limited and are heavily weighted towards invertebrate systems (Simpson & Raubenheimer 2012). Group living is widespread in the animal kingdom and investigations into the effect of macro-nutrient composition of food and its distribution in the environment on vertebrate group decision-making is important for realistic and accurate predictions of animal movement in the environment. Two processes were investigated in this experiment. Firstly, in what capacity can individuals assess the nutritional quality of a resource and how does this assessment affect the distribution of the shoal in the environment. Secondly, what affect does the ability to assess nutritional quality have on individual group-joining and leaving behaviour. This study therefore

explores the ability of fish shoals to make effective decisions in regards to resource nutritional quality in a patchy environment while balancing the factors of competition and social attraction.

Groups of eight mosquitofish (*Gambusia holbrooki*) were exposed to two environmental contexts: a 'nutritionally homogeneous environment' where all four available resource patches had the same macro-nutrient ratio, and a 'nutritionally heterogeneous environment' wherein the four patches had the same total caloric energy, but specific macro-nutrients were distributed unequally amongst them, specifically to produce two high protein patches and two low protein patches. Protein requirement is a particularly strong driver of animal behaviour (Simpson & Raubenheimer 2012) and its availability varies across patches within the environment mosquitofish inhabit. The locations of the individuals were recorded over time to assess how individuals distributed themselves in relation to the distribution of nutrients in the environment and what effect the nutritional quality of a patch had on group movement dynamics. It is hypothesized that individuals will distribute themselves in relation to the level of protein in the environment, spending more time on the high protein patches than the low protein patches in the heterogeneous environment but spending an equal amount of time on the four medium protein patches in the homogeneous environment (Houston, Higginson & McNamara 2011; Simpson & Raubenheimer 2012). It is also hypothesized that the amount of protein available on the patch will affect fish's joining and leaving behaviour, with higher protein patches having a higher probability of gaining a fish and lower probability of losing a fish than low protein patches.

Methods

Study Species and Husbandry

Mosquitofish (*Gambusia holbrooki*) were collected from Manly Dam, Sydney, Australia. They were transferred to the University of Sydney freshwater aquarium in a 50L bucket. Total transfer time was less than 60 minutes. Fish were subsequently housed in 180L aquaria, containing de-chlorinated aged tap water, a sponge filter and aerated continuously with an air stone. Gravel was provided as substrate. The fish were maintained at 26°C on a 12:12 hour L:D photoperiod under fluorescent lighting (NEC Tri-Phosphor tubes, 37 Watts, FL4055BR/37-HG). Fish were fed fish flake food (Nutrafin Max Tropical Fish Flakes, Rolf C. Hagen (UK) Ltd.) once per day till satiation. The fish were monitored daily for signs of stress or ill-health and were maintained at these conditions for 4 weeks prior to the experiment.

Mosquitofish are an invasive species to Australia and are declared as 'noxious' by NSW Fisheries and the National Parks and Wildlife Service. Therefore they were not allowed to be released following capture and thus were humanely euthanized after the experiment using an aqueous solution of the anaesthetic MS222 at a dose rate of 250 mg/L, buffered to a pH of 7 using calcium carbonate. The fish were then placed in a freezer prior to disposal.

Diet

Three diets were custom made for this experiment: a high protein diet 6:3:1 (P:C:F) and a low protein diet 2:7:1 (P:C:F) for use in the heterogeneous treatment, and a medium

protein diet 4:5:1 (P:C:F) for use in the homogeneous treatment. These ratios were chosen as they reflect intake ratios found in an array of species (Sánchez-Vázquez *et al.* 1998; Sánchez–Vázquez *et al.* 1999; Vivas *et al.* 2003; Vivas *et al.* 2006; Fortes-Silva, Martínez & Sánchez-Vázquez 2011) and they allowed for a balanced and controlled experimental design. In all diets the protein component was a combination of egg white albumin and casein, the carbohydrate source was sucrose and the fat source was cod liver oil. Protein and carbohydrate both yield approximately 17 kj/g, so all diets were considered near isocaloric (Ward, Herbert-Read & Simpson 2011). Casein has 96.3g of protein / 100g and egg white albumin has 79g of protein per 100g (see Table S1 for a full list of diet ingredients). To create the diets all dry ingredients were blended together in a food processor with 140mL of water. Agar was added to 200mL of water, micro waved till it formed a gel and then added to the dry ingredients. This mixture was blended once more and then poured into plastic Petri dishes, sealed and stored at 15°C to provide for some preservation while also preventing the build-up of condensation, which was found during pilot studies to adversely affect the texture of the diet, causing it to dissolve too rapidly in water.

Experimental Arenas

Two 5mm thick white Perspex® experimental arenas (730 x 730 x 200mm) were filled to a depth of 120mm with de-chlorinated aged tap water. The arenas were each surrounded by white Corflute® to prevent external stimuli disturbing the fish while allowing for evenly dispersed lighting for video recording. Four Perspex® squares (150 x 150 x 5mm) were

placed in the corners of the arenas, on which 4 white ceramic tiles (40 x 40 x 5mm) tiles were placed (see Fig. 1). A white wire was attached by a non-toxic silicone sealant to each of the 4 ceramic tiles. Small cubes of diet (5 x 5 x 5mm) were placed on these wires which raised the diet 60mm off the bottom of the arena. This was done as mosquitofish are mid-water and surface feeding fish. One of the arenas was allocated to be the heterogeneous environmental context and the other the homogeneous environmental context.

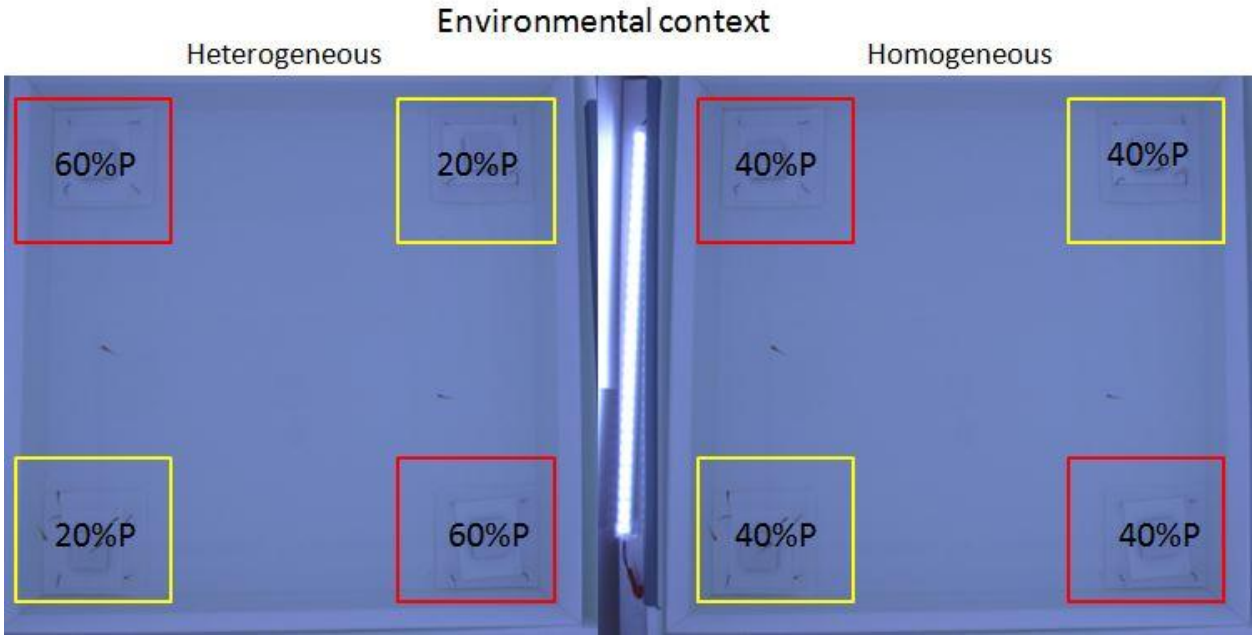

Fig. 1 Diagram of experimental arenas. There were four patches in each environmental context with 4 pieces of food on each of them. The red outlines signify the Hetero HP patches in the heterogeneous environmental context and the Hetero sp eq HP patches in the homogenous environmental context. The yellow outlines signify the Hetero LP patches in the heterogeneous environmental context and the Hetero sp eq LP patches in the homogenous environmental context. The location of these were randomized each trial. The percent protein of each patch is labeled.

Conditioning

Fish were put into the arena at 16:00h on Day1 and left for 66h to become accustomed to their environment before they were first tested at 10:00h on Day 4. At 10:00h on Day 2 and Day 3, medium protein diet was placed onto the 16 wire attachments and the fish were allowed to feed for an hour before the food was removed again. This was done to reduce the chance of food neophobia during the experimental trial.

Experimental Trial

The experimental trial commenced at 10:00h on Day 4. The fish were netted and placed into a clear plastic container that could be raised from outside of the arenas walls. The 16 pieces of diet were then placed onto the wire attachments. In the heterogeneous treatment 2 of the 4 Perspex® feeding stations contained the high protein (HP) diet and the other 2 the low protein (LP) diet. The location of these patches was randomized each trial. In the homogeneous treatment all 4 patches contained the medium protein (MP) diet (Fig. 1). Therefore, the total amount of protein and energy was the same between the 2 treatments, it was simply the distribution of the protein that was different. The arena was left undisturbed for 5 minutes before the plastic containers were raised allowing the fish to forage. The trials lasted for 15 minutes and were filmed from above at a height of 1.5m with Canon G1X Powershot video cameras. The same group was assayed under the same treatment three times, on consecutive days (Days 4, 5 and 6). Six independent replicates of each treatment were performed giving a total of 18 samples of each treatment. After the 15

minutes, although filming stopped, fish were given access to the food for a further 45 minutes to feed to satiation.

Video and tracking analysis

The videos were converted from .wmv to .avs format with DirectShowSource. The .avs files were then opened with VirtualDub (v 1.9.11) where they were saved as old format .avi files after the appropriate frames were isolated and decompressed. The old format .avi file was then imported into an automated tracking software program, Ctrax (Caltech ethonomics project, The Caltech Multiple Fly Tracker, Version 0.3.10, 2012) (Branson *et al.* 2009). This program automatically tracked the position of each individual fish at every frame (15fps) of the 5 minute video. Therefore each fish had (*x, y*) coordinates (in pixels) and its orientation, θ, (in radians, relative to the positive x-axis) recorded for each 13500 frames of the video. This data was then imported into MATLAB where MATLAB FixErrors GUI was used to manually correct any errors made by the automatic tracking software, Ctrax. Each fish therefore had one consistent, unbroken track over the entire 13500 frames of the trial. In addition, we developed a custom MATLAB script to manually identify the pixel coordinates of all four corners of each of the four Perspex® squares where the food was placed from the first frame of each video. Pixels coordinates of both the location of fish and the corners of forage patches were converted into mm with a conversion ratio calculated by measuring the distance between two points of known distance on the first frame of the video.

Patch occupancy, associated basic measures and statistical analysis

To compare between the environmental context treatments, homogeneous patches were defined by their spatial position in relation to those in the heterogeneous treatment. Therefore, the two MP patches that were spatially equivalent to the heterogeneous high protein patches (Hetero HP) were called homogeneous spatially equivalent high protein patches (Homo sp eq HP) and the two MP patches that were spatially equivalent to the heterogeneous low protein patches (Hetero LP) were called homogeneous spatially equivalent low protein patches (Homo sp eq LP).

We used the coordinates of each fish, the coordinates of the corners of each forage patch and MATLAB's intrinsic *inpolygon* function to identify the frames that each fish was either on a given forage patch (including the edge of the patch) or not on the given patch.

For each fish in a trial, we then tallied the total number of frames spent on Hetero HP patches (or the spatial equivalents of these patches in trials in the homogeneous foraging environment), HP_{total}, the total number of frames spent on Hetero LP patches (or spatial equivalent), LP_{total}, and thus the total number of frames that the fish occupied any patch, $HP_{total} + LP_{total}$. We then determined the proportion of total patch occupancy time spent on Hetero HP patches (or spatial equivalent) and the proportion of total patch occupancy time spent on Hetero LP patches (or spatial equivalent), given by $HP_{total}/(HP_{total} + LP_{total})$ and $LP_{total}/(HP_{total} + LP_{total})$ respectively. We then determined the mean (across fish in a given trial) of the proportion of total patch occupancy spent on Hetero HP patches (or spatial equivalent) and the mean of the proportion of total patch occupancy spent on Hetero LP

patches (or spatial equivalent). We also determined the mean of HP_{total}, the mean of LP_{total} and the mean of $HP_{total} + LP_{total}$ across all eight fish in each trial. (HP_{total}, LP_{total} and $HP_{total} + LP_{total}$ could all be converted to proportions of total observation time by dividing these quantities by the number of frames tracked (13500).)

We identified the number of fish on each patch as a function of time. We then determined the mean over time of the number of fish on each patch. For each trial we then determined the mean (across two patches) of the mean number of fish observed on Hetero HP patches (or spatial equivalent) and the mean (across two patches) of the mean number of fish observed on Hetero LP patches (or spatial equivalent).

For each fish we identified sets of consecutive frames of occupancy on a given patch. We identified each of these sets of frames as a distinct visit to a patch, and tallied the number of visits made to each patch by each fish. We then determined the mean (across all eight fish in a trial) number of visits to each patch, and thus the mean (across two patches) mean number of visits to Hetero HP patches and Hetero LP patches or their spatial equivalents.

We determined the duration spent on each patch during a single visit via ((the index of the last frame from a set of consecutive frames on a patch) – (the index of the first frame from the same set) + 1) × (the duration between consecutive frames (=1/15 s))). We pooled the durations of visits by all fish to each patch. Within each trial, we then further pooled all durations spent on Hetero HP patches, Hetero LP patches or their spatial equivalent. We then determined the mean and median of each set of durations of individual visits to patches with different protein content.

We used R (R Core Team, 2014, R i386 3.1.2) to run mixed model ANOVAs to explore how environmental context affected the distribution of fish. First, to assess if environmental context affected the total time fish spent on patches, we explored whether the total time fish spent on patches (dependent variable) was affected by environmental context (between subjects variable) and day (within subjects variable). We then went on to explore whether the proportion of time spent on the two hetero HP patches was significantly different to the proportion of time spent on the two homo sp eq HP patches. Further mixed model ANOVAs explored possible explanations for a difference in distribution between environmental contexts. Keeping environmental context as the between subjects variable and day as the within subjects variable we looked at mean number of fish on the patches, mean number of visits and mean and median duration of visits as dependent variables.

Survival analysis and social effects associated with patch joining and leaving

Preliminary analysis (see Results below) suggested that fish spent a greater proportion of their patch occupancy time on Hetero HP patches than on their spatial equivalents in the homogeneous foraging environment. There was no significant difference in the number of visits made to patches in the heterogeneous or homogeneous treatments, therefore the most likely factor influencing the greater proportion of patch occupancy for HP patches evident in the heterogeneous foraging environment was an adjustment to the duration of individual visits. However, there was no statistical difference in either the mean or the median

duration of patch visits. Examination of the distribution of patch visit durations for heterogeneous HP, homogeneous MP and heterogeneous LP patches (from data pooled across all trials) suggested that these durations were far from normally distributed (see supplementary Fig. S2), so a comparison of basic means or medians might not adequately illuminate any real differences. To further investigate this problem, we made use of survival analysis to examine if fish differed in the individual durations they spent on patches of different nutritional composition within each environmental context. In particular, we developed a randomisation process to examine if it was likely that durations spent on Hetero HP and Hetero LP patches differed from durations spent on patches in the homogeneous foraging environment via comparison of survival functions. (Each survival function, $S(t) = P(T > t)$, represented the probability that a fish spent a duration greater than t seconds on a patch of a particular type during a single visit.) The rationale behind developing such a randomisation process was that two patches contained high protein food, and two contained low protein food in the heterogeneous environment – we therefore thought it most appropriate to compare durations spent on Hetero HP patches and Hetero LP patches with durations spent on two sets of pairs of patches (denoted 'Set 1' and 'Set 2') selected from all the trials in the homogeneous foraging environment. The selection of which pairs of patches from the homogeneous environment to allocate to a particular set (Set 1 or Set 2) would be arbitrary, so we sought to examine a large number of such arbitrary allocations via randomisation (we performed 10000 iterations of our randomisation procedure). Survival functions were compared via log-rank tests (see for example (Kalbfleisch & Prentice 2011), and in the case that two survival functions were

found to differ we applied two measures, labelled δ_1 and δ_2, to determine which function lay mostly above or below the other (and hence which patch type fish were likely to spend a greater duration on during a single visit) (see supplementary material for more details).

We sought evidence of social influence on patch joining or leaving behaviour. To do this, we estimated the conditional probabilities of heterogeneous HP, heterogeneous LP and homogeneous MP patches gaining or loosing fish at the next, fixed, sample time step (referred to as observation), as a function of the number of fish currently on the patch and the number of fish that could possibly join the patch (identified as 'near' the patch, at a distance of 200 mm or less from the patch boundary), at the next observation time. Observation times commenced on the first video frame that was tracked and were separated by 1 s (15 frames). Details of how we estimated conditional probabilities are provided in the supplementary material.

Results

Environmental context had no significant effect on the total amount of time fish spent on the four available patches ($F_{1,10}$=1.369, p=0.82). In the heterogeneous environmental context fish spent 46% of the trial on patches, while in the homogeneous environmental context they spent 45% of the trial on patches. Day was, however, significant and the total amount of time fish spent on the four patches increased on day 5 and 6 in both in the heterogeneous environmental context and homogeneous environmental context ($F_{2,20}$=17.364, p<0.0001). There was no interaction between environmental context and day ($F_{2,20}$=1.369, p=0.277).

Environmental context did have an effect on the distribution of individuals between the 4 available patches. In the heterogeneous environmental context, fish spent 65% of their patch occupancy time on the Hetero HP patches, significantly more time than the 50% of patch occupancy time fish spent on the Homo sp eq HP patches in the homogeneous environmental context ($F_{1,10}$=33.76, p<0.001) (Fig. 2). There was a significant effect of day ($F_{2,20}$=4.861, p=0.019), with the time spent on Hetero HP patches decreasing each day and decreasing on the last day for Homo sp eq HP patches. There was no interaction between environmental context and day ($F_{2,20}$=0.212, p=0.811).

There was no significant effect of environmental context ($F_{1,10}$=1.291, p=0.282), day ($F_{2,20}$=1.059, p=0.366), nor any interaction between the two ($F_{2,20}$=0.073, p=0.929) on the mean number of visits to patches. The mean duration of an individual's singular visit to a patch was not affected by environmental context ($F_{1,10}$=0.355, p=0.564), day ($F_{2,20}$=0.811, p=0.458), nor was there an interaction between the two ($F_{2,20}$=0.837, p=0.448). Similarly, the median duration of an individual's singular visit to a patch was not affected by environmental context ($F_{1,10}$=0.506, p=0.493), day ($F_{2,20}$=2.445, p=0.112), nor was there an interaction between the two ($F_{2,20}$=0.531, p=0.596).

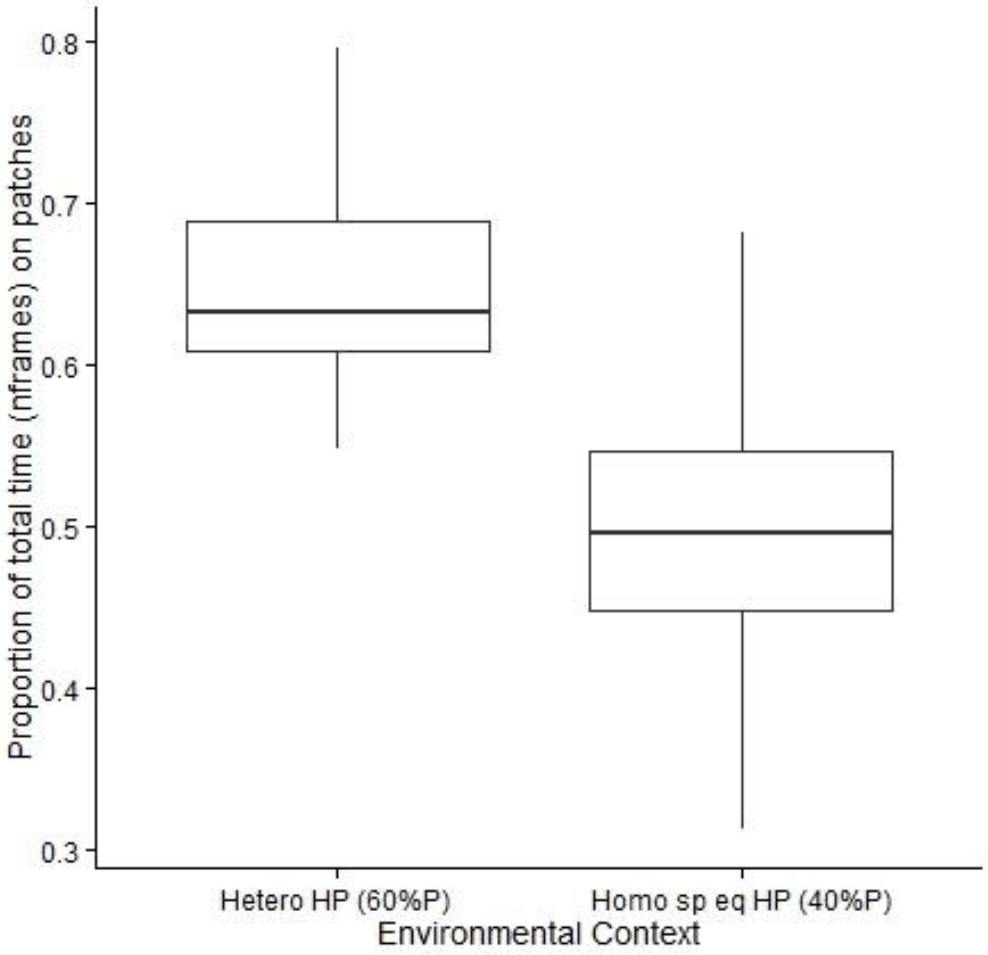

Fig. 2 Boxplots representing the mean proportion of patch occupancy spent on Hetero HP (60%P) patches and Homo sp eq HP (40%P) patches. Boxes represent first and third quartiles and whiskers extend to the highest value that is within 1.5 times the inter-quartile range.

There was, however, a significant effect of environmental context on the mean number of fish on patches, with a greater number of fish on Hetero HP patches than Homo sp eq HP patches ($F_{1,10}$=5.646, p=0.039). There was a significant effect of day ($F_{2,20}$=3.661, p=0.044), with the mean number of fish increasing from day 4 to day 5, but no significant interaction between day and environmental context ($F_{2,20}$=0.937, p=0.408).

Survival analysis

For all 10000 randomisations there was at least one survival curve that differed from the others (with $p \approx 0$ for all log-rank tests). The details of which pairs of survival curves differed varied across the set of randomisations. There were five outcomes observed, as detailed in Table 1. The consistent results across all randomisations were that the survival function for durations spent on the Hetero HP patches always differed from the survival functions for durations spent on either the randomly allocated Set 1 or Set 2 patches from the homogeneous foraging environment. According to both the δ_1 and δ_2 measures the survival function corresponding to occupancy of the Hetero HP patches always lay mostly above the survival functions associated with the Set 1 and Set 2 homogeneous patches. Therefore, mosquitofish tended to spend a greater duration on Hetero HP patches during individual visits than on any patches in the homogeneous foraging environment. In 6127 randomisations (across outcome types 2, 4 and 5) the survival function for durations spent on Hetero LP patches differed from the survival function for patches from the homogeneous environment allocated to Set 1. For 6149 randomisations (across outcome types 1, 3 and 4) there was a significant difference between Hetero LP and Set 2 survival

functions. In every instance that a difference between survival functions for the Hetero LP patches and either Set 1 or Set 2 patches from the homogeneous environment was indicated, the δ_1 and δ_2 measures both indicated that the Hetero LP survival function lay mostly beneath either the Set 1 or Set 2 survival function (or both in the case of outcome type 4). There is therefore evidence that mosquitofish tended to spend shorter durations on Hetero LP patches than on the higher protein content patches found in the homogeneous foraging environment. 1721 randomisations (outcome types 1 and 5) also suggested differences for the survival functions of sets of random patches selected from within the homogeneous foraging environment. Hetero HP and Hetero LP survival functions remained the same across all randomisations, and were identified as significantly different from one another, with the Hetero HP patch duration survival function lying mostly above the Hetero LP survival function. Figure 3 shows an example of the general pattern suggested by our analysis where the Hetero HP survival function can be seen to lie above the survival functions for Hetero LP patches as well as Homo sp eq HP and Homo sp eq LP patches.

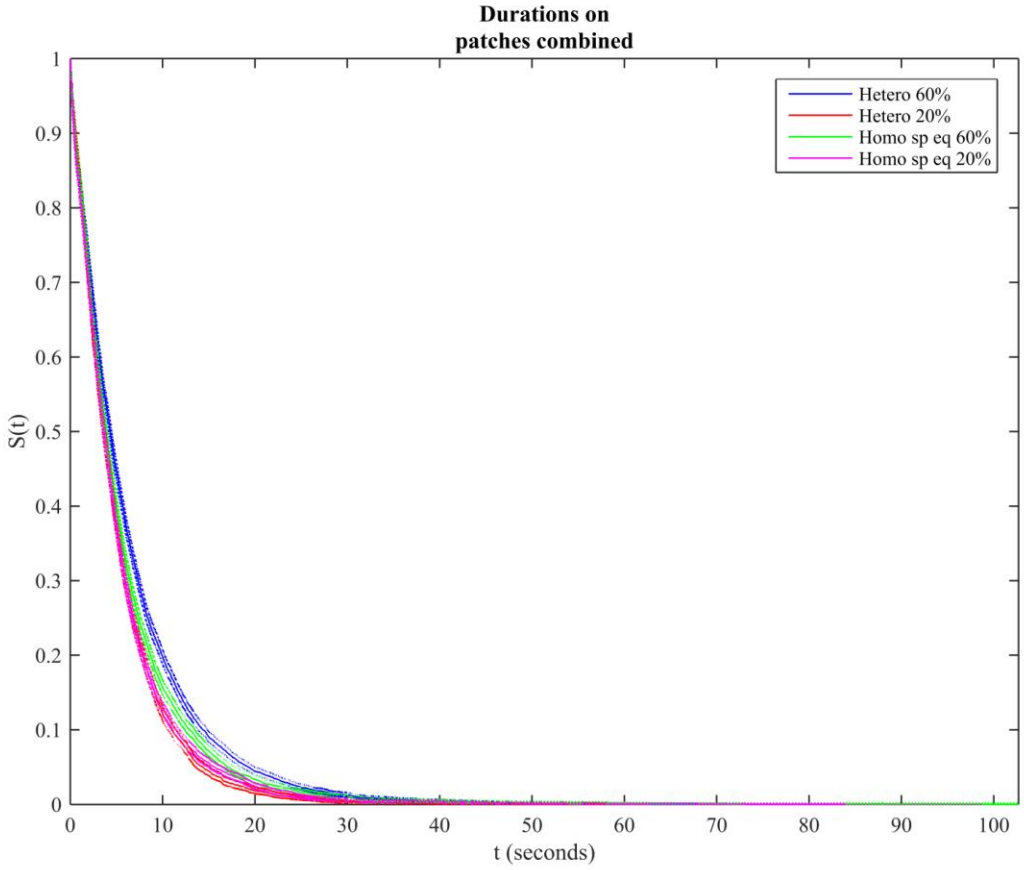

Fig. 3 Kaplan-Meier estimates of the survival functions, $S(t)$, for durations of individual visits to heterogeneous high protein forage patches (solid blue line), heterogeneous low protein forage patches (solid red line), homogeneous medium protein patches spatially equivalent to high protein patches (solid green line) and homogeneous medium protein patches spatially equivalent to low protein patches (solid magenta line). Approximate 95% intervals for each of the curves are bounded by dotted lines. At least one pair of survival curves differed ($p \approx 0$, log-rank test, *DF* = 3, test-statistic ≈ 157.77); subsequent pairwise tests suggested that all pairs of survival curves were statistically different, with the exception of the Hetero 20% (LP) survival function and the Homo sp eq 20% survival function.

Table 1: Results of pairwise comparisons of survival functions for durations spent on high protein food patches in the heterogeneous foraging environment (Hetero HP), low protein patches in the heterogeneous foraging environment (Hetero LP), food patches allocated to Set 1 from the homogeneous foraging environment via the randomisation procedure described in the supplementary text and food patches allocated to Set 2 from the homogeneous foraging environment. Each pair of survival functions was identified as being statistically different (H_1) or not (H_0) at significance level $P = 0.008\dot{3}$ via a log-rank test (subject to a Bonferroni correction that took into account all 6 possible pairwise comparisons). There were five forms of pairwise differences that appeared during the randomisation process, all of which occurred more than 500 (out of 10000) times. The frequency of each outcome is listed in the table below.

	Outcome type	1	2	3	4	5
Pair						
Hetero HP	Hetero LP	H_1	H_1	H_1	H_1	H_1
Hetero HP	Set 1	H_1	H_1	H_1	H_1	H_1
Hetero HP	Set 2	H_1	H_1	H_1	H_1	H_1
Hetero LP	Set1	H_0	H_1	H_0	H_1	H_1
Hetero LP	Set 2	H_1	H_0	H_1	H_1	H_0
Set 1	Set 2	H_1	H_0	H_0	H_0	H_1
Frequency		835	2965	3038	2276	886

Probability of joining and leaving

There is no evidence for social attraction causing aggregations on food patches. The probability of a patch gaining a fish does increase as the number of potential joiners increases (Fig. 4B-F), however, a fish is not more likely to join patch as the numbers of fish already on that patch increases (Fig. 4B). Indeed, there is a trend for the probability of a fish joining a patch to decrease as the numbers already on the patch increase (Fig. 4). The probability of a patch losing a fish increases as the number of fish on a patch increases from 1-3 (Fig. 5B-D) but then levels out as the number of fish on a patch reaches 3-5 individuals (Fig. 5D-F).

The amount of protein on the patch does not affect the probability of fish joining the patch in the heterogeneous environment (Fig. 4). However, with low numbers of fish on the patch (Figs 5B and 5C), the probability of a fish leaving is greater in the LP patches than the HP patches. This trend disappears as competition for food on the patch increases, that is, as the number of fish near and on the patch increases (Fig. 5).

Shoals spent an equal amount of time on food patches in both environmental contexts, however, the distribution of macro-nutrients in the environment had an effect on the spatial distribution of foraging mosquitofish. Fish showed a preference for the HP diet within the heterogeneous environmental context. Comparing across environmental contexts, fish spent a significantly greater time on the HP patches in the heterogeneous environment than fish did on the spatially equivalent MP patches in the homogeneous environment (Fig. 2).

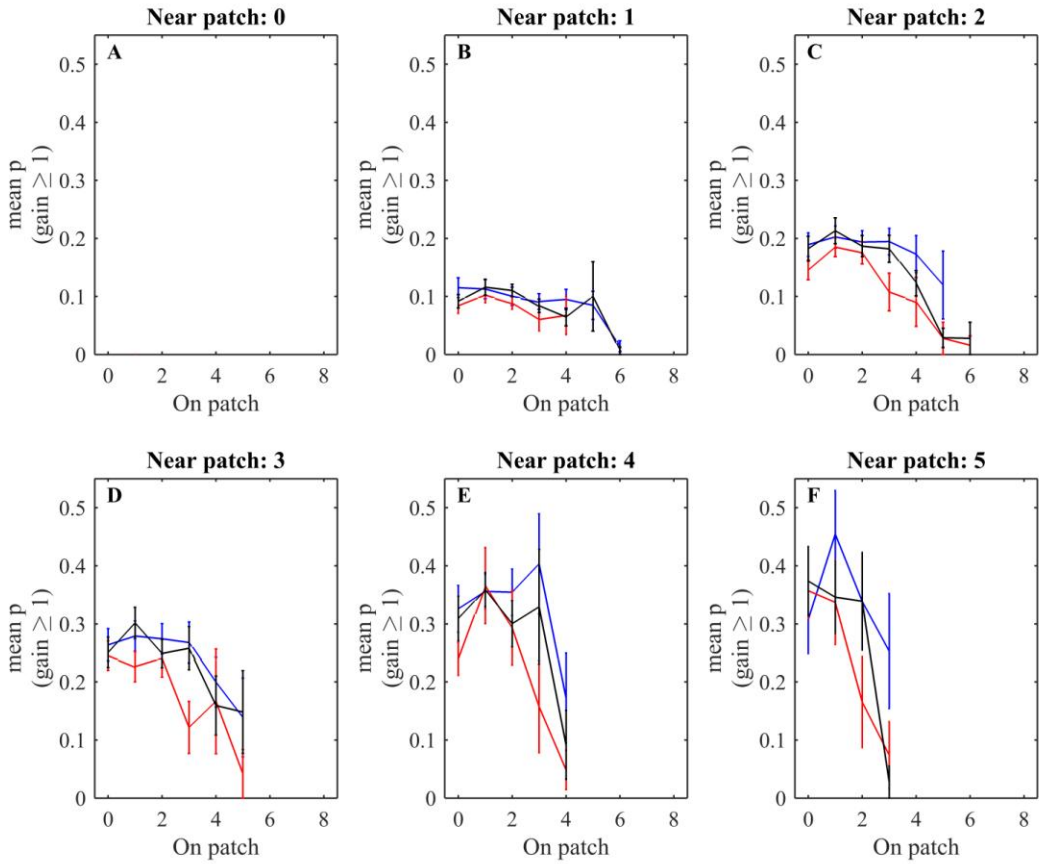

Fig. 4 Mean estimated conditional probabilities of heterogeneous HP (solid blue lines), heterogeneous LP (solid red lines) and homogeneous MP (solid black lines) patches gaining 1 or more foragers in the next time step as a function of the number of fish currently on the patch and potential joiners near (200 mm or less) the patch. Error bars are plotted ± 1 standard error above and below the estimated conditional probabilities.

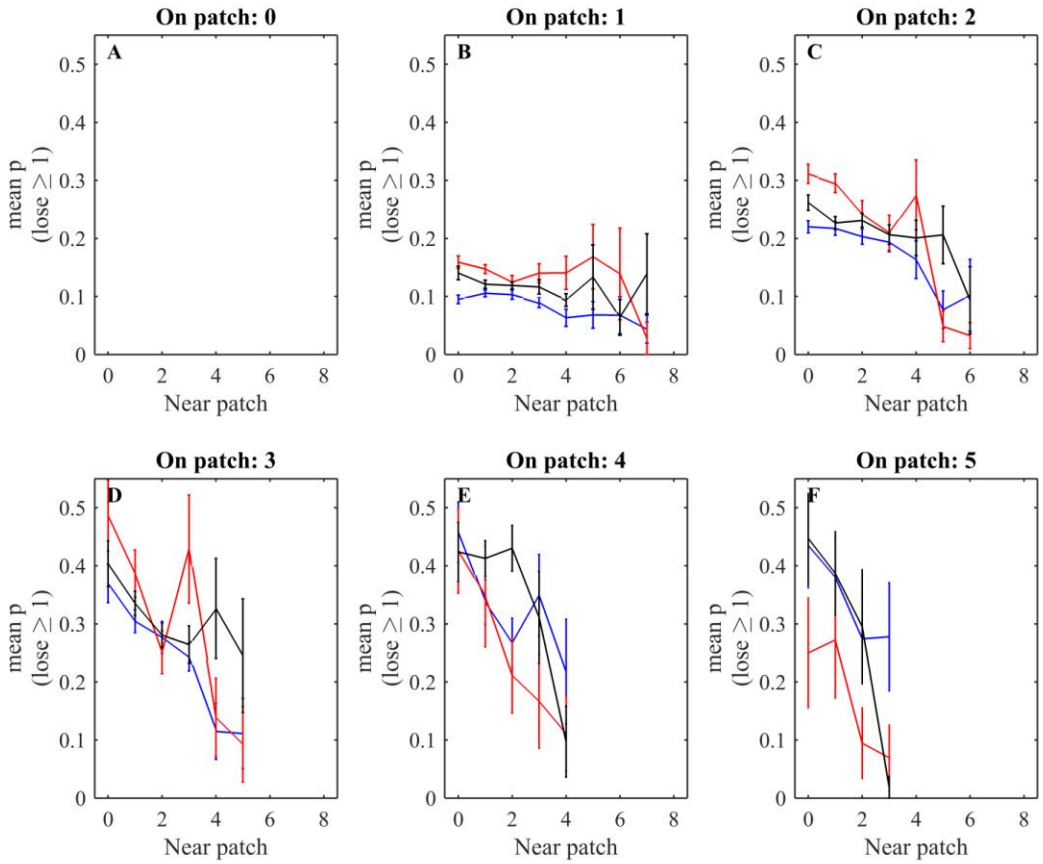

Fig. 5 Mean estimated conditional probabilities of heterogeneous HP (solid blue lines), heterogeneous LP (solid red lines) and homogeneous MP (solid black lines) patches losing 1 or more foragers in the next time step as a function of the number of fish currently on the patch and potential joiners near (200 mm or less) the patch. Error bars are plotted ± 1 standard error above and below the estimated conditional probabilities.

Discussion

This suggests that even though the total amount of energy was evenly distributed between the four patches, fish in both the heterogeneous and homogeneous environments dispersed themselves in accordance with the relative amounts of protein. Fish are known to distribute themselves around feeding stations according to their differences in productivity, fitting predictions of ideal free distribution (Milinski 1979; Pitcher, Lang & Turner 1988; Sutherland, Townsend & Patmore 1988; Abrahams 1989). Within the heterogeneous environmental context fish spent 65±0.07% of their patch occupancy time on HP diets and consequently 35±0.07% of their time on LP diets. Therefore fish did not distribute strictly according to ideal free distribution in terms of macro-nutrient distribution (if we consider protein as a replacement to total energy), as there were not three times as many fish on the HP patches (60% protein) compared to the LP patches (20% protein). However, within the homogeneous environmental context fish did spend an equal amount of time on two sets of MP patches (50±0.09%). The inclusion of macro-nutrient composition into models of optimal foraging predicted that foraging individuals should focus on nutrients that can be acquired at the fastest rate (Houston, Higginson & McNamara 2011). Certainly, fish spent more time on patches with higher protein concentration, so as long as feeding rates were similar between patches, this prediction is upheld. Fish may have also preferred high protein diets because, compared to carbohydrate, protein is often limited in availability in the environment (Simpson & Raubenheimer 2012). By focusing on consuming protein they may avoid having a shortfall of this macro-nutrient when it is unavailable. Indeed the unpredictable nature of macronutrient availability is an important part of the Houston et al.

2011 model and they suggest that considering interruptions frequently occur to a foraging individuals in the wild, for example by the arrival of conspecifics, predators or changes in abiotic conditions, an individual's foraging decision (in this case what diet to eat) should be the one that gives the greatest immediate reward (Houston, Higginson & McNamara 2011).

Fish are able to catabolise protein for energy and as such do not have a specific need for carbohydrate (Clements & Raubenheimer 2005), and this may also have played a role in fish's preference for the higher protein food patches. However, fish intake targets are often composed of some carbohydrate (Sánchez-Vázquez *et al.* 1998; Vivas *et al.* 2003; Clements & Raubenheimer 2005; Vivas *et al.* 2006; Fortes-Silva, Martínez & Sánchez-Vázquez 2011) and omnivorous animals will generally prefer to eat carbohydrate instead of dealing with physiological costs of using protein as an energy source (Clements & Raubenheimer 2005). This preference for high protein food items – at least in the short term – was an expected result, as fish are known to have intake targets that are high in protein (Clements & Raubenheimer 2005). An intake target of 60% protein is reasonably high for an omnivorous freshwater species such as the mosquitofish, nonetheless the preference for the high protein patches was clear. It is therefore important to note this preference is not a measure of mosquitofish's nutritional intake target. Firstly, the exact intake of each diet composition was not recorded (but rather a proxy of this was - the time individuals spent on a food patch) and, secondly, the observational period of the experiment only lasted 15 minutes whilst afterwards the fish were allowed to feed till

satiation unobserved. Whilst an ability to balance their macronutrient intake in this species by moving between two or more unbalanced diets could be tested in the future (likely using automatic feeders dispensing small quantifiable amounts of diets such as pellet food, as used in previous fish nutrition studies (Sánchez-Vázquez *et al.* 1998; Sánchez–Vázquez *et al.* 1999; Aranda *et al.* 2000; Vivas *et al.* 2003; Vivas *et al.* 2006; Fortes-Silva, Martínez & Sánchez-Vázquez 2011), it is important to highlight that this study looked at immediate macronutrient preference over a short time scale and does not relate to longer term macro-nutrient balancing. Pilot studies using this species found that while foraging in groups and presented simultaneously with a range of diets varying from 10-70% protein, mosquitofish preferentially fed from high protein diets (50-70%), therefore the aim of this study was to assess how this preference caused individuals in fish shoals to distribute themselves in the environment.

Time spent on patches increased each day. Considering fish had access to the food for an hour each day and were perceived to feed till satiation, we cannot explain this through cumulative hunger over the previous days of experimentation. Perhaps, despite the two days of conditioning before the experimental assay, fish still showed some sense of wariness in the environment or with the food or a combination of both. This perhaps reflects learning, either learning how to deal with the novel food item or that food is not to be found away from the patches within the arena.

Whilst the importance of protein for fish is well established (Clements & Raubenheimer 2005) and the ability of fish shoals to distribute themselves to maximize caloric intake is known (Pitcher, Lang & Turner 1988), this is the first experiment to our knowledge that shows how information acquired on the macro-nutrient make up of a food source determines how the individuals in a fish shoal distribute themselves in the environment.

The mechanisms behind this distribution pattern are also important. Preliminary examinations showed that this was not caused by the mean number of visits to the different patches. This suggests that the pattern of distribution within the environment is most likely caused by the duration of individual visits to the patches. Pooling individual durations on separate patches and comparing the means and medians of these did not explain the pattern of distribution either, however, individual durations were not normally distributed as this data was positively skewed with much of the data collected being made up of very short durations in which fish were likely simply swimming past and through the patch boundaries. This type of question is better explored through the use of survival analysis - which specifically is formulated to address duration related data. The survival analysis showed that the probability that a fish spent a greater duration on a patch was dependent on the patch's nutritional quality.

Therefore, it seems that durations on patches cause changes in the distribution of individuals in the two environments and that these durations are relative to the macronutrient composition of the available food, namely its protein concentration. When

animals are given a free choice of different food types (here macro-nutrient composition), patch resident times are theoretically determined by local information such as intake rate (Hengeveld *et al.* 2009; Houston, Higginson & McNamara 2011) and the intake rate of protein is fastest on the patches with a higher concentration of protein.

Considering, then, that there were 8 individuals within each foraging shoal and 8 available HP food items, it is interesting that fish did not just distribute themselves equally amongst these food items. Of course, there are other factors that affect the movement of individuals within groups, for example fish would have been interested in exploring new areas, reproducing and attracting mates, and responding to other social information. Our analysis of the probability of joining patches in relation to the numbers of conspecifics on the patch and near the patch did not show any evidence of social attraction causing aggregations on food patches. Logically, the probability of a patch gaining at least one fish did increase with an increase in the amount of conspecifics near the patch (Fig. 4B-F), but critically, the numbers of fish already on a patch do not appreciably raise the probability of a fish joining a patch (Fig. 4B). Here we expected to see an increase in probability due to social facilitation and perhaps even a quorum response, wherein the probability of conspecifics performing a behaviour increases after a threshold number of conspecifics performing that behaviour is reached (Ame, Rivault & Deneubourg 2004; Amé *et al.* 2006; Meunier *et al.* 2006; Ward *et al.* 2008; Sumpter & Pratt 2009; Ward, Krause & Sumpter 2012). Perhaps, extensive prior knowledge of the entire foraging environment lessened the tendencies of the subjects to rely primarily on social information. There was a trend for the probability of

a fish joining a patch to actually decrease as the numbers already on the patch increase (Fig. 4). This may be an artifact of having smaller amounts of data for these data points, although it is likely, at least in part, that as fish have prior knowledge of the environment, that they perceive it to be safe, they did not prefer social information over private information (Webster & Laland 2008). As they were aware of the type and amount food resources present elsewhere, any competition for food, even if just a minor obstruction, may simply have driven them to seek alternative food patches.

Supporting this notion is that the probability of a patch losing a fish increases as the number of fish on a patch increases from 1-3 (Fig. 5B-D) but then levels out as the number of fish on the patch reaches 3-5 individuals (Fig. 5D-F). This is likely due to their being only 4 food items available on the patch. There also seems to be a trend in the figures that a patch is less likely to lose a fish as the number of fish nearby increases, potentially suggesting guarding behaviour or that fish are simultaneously swapping places as they join and leave the patches. However, the pattern may be caused once again by the smaller amount of data for data points with these combinations of fish on and near the patch.

It was hypothesised that the amount of protein available on the patch would affect fish's joining and leaving behaviour as HP patches would have a higher probability of gaining a fish and a lower probability of losing a fish. In the heterogeneous environment, protein concentration does not affect the probability of fish joining the patch (Fig. 4). Fish may have been able to smell the protein (Ward et al. 2011) or fish may have noticed

conspecifics feeding at faster rates on the higher quality diet (Valone & Templeton 2002), or have remembered the spatial location of these more profitable patches (Milinski 1994), however, without these things being known, it is possible that fish simply did not perceive the quality of the patch unless they were themselves sampling from it. When there were low numbers of conspecifics on the patch (Figs 5B and 5C), the probability of a fish leaving was greater in the LP patches than the HP patches and this supports the result identifying longer durations on patches with higher protein concentrations. As the numbers of fish near and on the patch increases, this pattern disappears (Fig. 5) and it is likely that competition between conspecifics overrides any nutritional factors.

The mean number of individuals on patches was also different between the two environmental contexts with more individuals present on the HP patches in the heterogeneous environment than the spatially equivalent patches in the homogeneous environment. This once again may be an artifact of individuals being less likely to leave hetero HP patches, however it also suggest that fish may have been less affected by competition on the HP patches as there was surplus protein. Further experiments specifically addressing this question should be conducted. A recent theoretical exploration into the interaction of competition and nutritional decision-making has suggested that the level of competition may select for different behavioural strategies; low competition favouring a strategy of locating and consuming nutritionally balanced foods, and high competition favouring a strategy of consuming imbalanced foods in tandem with selecting complementary foods (Senior *et al.* 2015). Whilst this experiment is a good first step, an

improved system would be to use fish whose internal state has been manipulated to be deficient in certain macro-nutrients and assay their behaviour in environments where quantifiable amounts of food of known macro-nutrient quality can be introduced via feeding tubes or automatic feeders. This feeding regime, in combination with the manipulation of group size could empirically test the progressive ideas raised by Senior and co-workers in a vertebrate species.

To provide a realistic description of adaptive animal behaviour the organism's sensory capabilities must be acknowledged (Jordan & Ryan 2015). Consideration of macro-nutrient distribution and how animals perceive this is limited in studies of optimal foraging, particularly in vertebrates and for animals that forage in groups. By testing mosquitofish's perception of food quality on the macro-nutrient scale in a simple foraging experiment, we have provided empirical evidence that fish will distribute themselves within an environment in relation to the distribution of specific macro-nutrients, notably protein. Also, fish make foraging decisions based on the macronutrient composition of patches, such that their durations on patches are longer when they have a higher concentration of protein. Future experiments should continue to acknowledge the finer scale quality of food types when assessing optimal foraging behaviour (Simpson *et al.* 2004; Houston, Higginson & McNamara 2011) and recognize the usefulness and appropriateness of manipulating protein concentrations when classifying patch quality in foraging experiments.

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

All procedures performed were in accordance with the ethical standards of the University of Sydney.

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Data accessibility

For Functional Ecology papers, DYAD requires the manuscript is accepted before it will allow upload of data. A folder has been created with all of the data ready for upload and the DOI will be provided here.

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Supplementary Material

Table S1: Recipe for the three diets used in the experiment. Water: 140 mL in mixture and 200 mL in beaker for agar. Casein has 96.3g protein / 100g. Egg white has 79g protein/ 100g. Ingredients list: Egg albumin powder (MyoPure), Casein from bovine milk (SIGMA), Sucrose, Cod liver oil (Melrose Premium), Wesson salt mixture (SIGMA), Vanderzan vitamin mix for insects (SIGMA), Agar, Cellulose, Water, Methyl 4-hydroxybenzoate (SIGMA)

(g)	High Protein (60/30/10 %)	Medium Protein (40/50/10 %)	Low Protein (20/70/10 %)
Protein	19.8	13.2	6.6
Carbohydrate	9.9	16.5	23.1
Fat	3.3	3.3	3.3
Minerals	2.6	2.6	2.6
Vitamins	0.1	0.1	0.1
Agar	11	11	11
Cellulose	28	28	28
Water	340	340	340
Nipagen	0.5	0.5	0.5
TOTAL	~415.4		

Protein

	White egg powder	Casein
High Protein	12.5	10.3
Medium Protein	8.4	6.9
Low Protein	4.2	3.4

Randomisation procedure and survival analysis

We treated a duration on a patch as being right censored (that is, the duration on the patch was known to be at least that observed, but the actual duration of occupancy was unknown) if a fish occupied a patch on the first frame of a video, the last frame of a video, or both. All other durations were treated as uncensored.

We first pooled the uncensored durations spent by all fish on Hetero HP patches and the durations spent by all fish on Hetero LP patches across all 18 trials that occurred in a heterogeneous foraging environment. We also pooled all censored durations spent by all fish on the Hetero HP and Hetero LP patches.

For each iteration of the randomisation procedure we randomly selected two patches from each trial with a homogeneous foraging environment and pooled the associated durations spent on these patches into a group that we labelled 'Set 1' (with separate pools for uncensored and censored durations). Data associated with the other two patches from each trial was pooled in 'Set 2'.

For each iteration of the randomisation procedure we then constructed Kaplan-Meier estimates of the survival function, $S(t)$, for the durations spent on Hetero HP patches, Hetero LP patches, Set 1 patches and Set 2 patches (see for example [1]). Each survival function, $S(t) = P(T > t)$, represented the probability that a fish spent a duration greater than t seconds on a patch of a particular type during a single visit. The value of each survival function was determined for all observed uncensored durations of patch visits (so

that $S(t)$ was known for the same set of durations, $t \in \{t_1, t_2, \ldots, t_k\}$, for all four survival curves).

We then performed a log-rank test (again, see [1]) to determine the probability that at least one survival function differed from the others. If there was a significant difference (at significance level $\alpha = 0.05$), we then performed pairwise comparisons of all possible pairs of curves (6 pairs in total) using log-rank tests to determine which pairs differed. We applied a strict Bonferroni correction to the significance level for pairwise comparisons, so that a significant difference between a pair of curves was identified if $P < \alpha / 6 = 0.008\dot{3}$.

Finally, if a pair of survival functions differed (other than the paired survival functions for Hetero HP and Hetero LP patches), we sought to determine if one curve lay mostly above or below the other over the set of observed durations of patch occupancy. If a given survival function did lie mostly above another curve, then it was reasonable to infer that durations spent on the patches associated with the upper curve tended to be greater than those spent on patches associated with the lower curve. We used two measures to determine if one curve, $S_1(t)$, lay mostly above the other, $S_2(t)$. The first measure was the sum of the differences between the two curves over the set of uncensored durations of patch occupancy: $\delta_1 = \sum_{i=1}^{k} \left(S_1(t_i) - S_2(t_i) \right)$. If $\delta_1 > 0$ then we treated $S_1(t)$ as lying mostly above $S_2(t)$ (and hence durations of visits to the patches associated with $S_1(t)$ tended to be greater than visits to the patches associated with $S_2(t)$). If $\delta_1 < 0$ then we treated $S_2(t)$ as lying mostly above $S_1(t)$. δ_1 effectively took into account both the magnitude of the

difference between $S_1(t)$ and $S_2(t)$, and the sign of the difference between the two curves at each $t \in \{t_1, t_2, \ldots, t_k\}$. The second measure that we used was the sum of the signs of the differences between the two curves over the set of uncensored durations: $\delta_2 = \sum_{i=1}^{k} \text{sgn}(S_1(t_i) - S_2(t_i))$, with analogous interpretations of the relationship between $S_1(t)$ and $S_2(t)$ if $\delta_2 > 0$ or $\delta_2 < 0$ to those for $\delta_1 > 0$ or $\delta_1 < 0$.

We performed 10000 iterations of the above process of randomly allocating patches from each trial of the homogeneous foraging environment to Set 1 and Set 2, determination of survival functions, log-rank tests across all four survival functions, subsequent pairwise comparisons and determination of the relationship (mostly above or below) between any significantly different pairs of survival functions according to both the measures δ_1 and δ_2.

Conditional probabilities associated with patches gaining or losing fish

We estimated the conditional probabilities of heterogeneous HP, heterogeneous LP and homogeneous MP patches gaining or loosing fish at the next, fixed, sample time step (referred to as observation times hereafter), as a function of the number of fish currently on the patch and the number of fish that could possibly join the patch, at the next observation time. The ultimate goal of these calculations was to seek evidence of social influence on patch joining or leaving behaviour.

We calculated all probabilities discussed in this section from discrete video frames separated by 1 second (or 15 frames), commencing on the first video frame that was tracked.

We first needed to determine an approximate distance that fish could reasonably be observed to travel to join a patch over one second. To do this, we estimated the instantaneous velocity, and hence speed of all fish, *i*, across all trials. Writing the coordinates of fish *i* at time *t* as $\left(x_i(t), y_i(t)\right)$, we estimated the components of the fish's velocity in the *x* and *y* directions respectively using the forward difference approximations:

$$u_i(t) = \frac{x_i(t+\Delta t) - x_i(t)}{\Delta t} \quad \text{and} \quad v_i(t) = \frac{y_i(t+\Delta t) - y_i(t)}{\Delta t},$$

where $\Delta t = 1/15\,\text{s}$ was the duration between consecutive video frames. The fish's speed was then given by: $s_i(t) = \sqrt{\left(u_i(t)\right)^2 + \left(v_i(t)\right)^2}$. We pooled all observed speeds of all fish across all trials, and then constructed a histogram of the distribution of speed, illustrated in Fig. S1 A. Additionally, we determined the cumulative proportion of speed values less than or equal to the rightmost speed value on the edge of each bin associated with the histogram (Fig. S1 B). Scrutiny of the plots in Fig. S1 suggested that the mosquitofish rarely exceeded instantaneous speeds of approximately 200 mm/s. We subsequently determined that approximately 99.7% of observed speed values (3862517 out of 3874333 observations) were less than or equal to 200 mm/s. We then chose to classify fish that were not on a given forage patch (as determined during the calculation of durations spent on

patches, described above), but were 200 mm or less from the boundary of the given forage patch as a *potential joiner* for that patch at the next observation time (1 second later).

We next determined the least distance between the coordinates of each fish and the boundary of each forage patch for each observation time of each trial. Depending on the relative location of a fish and the boundary of a given forage patch, the least distance would either be a perpendicular distance from one of the edges of the boundary to the fish, or the distance between a corner of the patch and the fish's location. We first determined the perpendicular distance from each of the four edges of the boundary of a patch to a fish's location (if such a distance existed) using the calculations outlined in the Appendix of [2]. Next, writing $\left(w_{x_k}, w_{y_k}\right)$ as the coordinates of the *k*th corner of the boundary, we determined the distance from each corner to the fish, given by $d_{k,i} = \sqrt{\left(x_i(t) - w_{x_k}\right)^2 + \left(y_i(t) - w_{y_k}\right)^2}$. Ultimately we identified the distance between forage patch boundary and fish at a given observation time as the least of the four perpendicular distances from the edges of the boundary (taking into consideration if such distances existed) and the four distances to the corners of the forage patch $\left(d_{k,i}\right)$.

We defined the state of each patch for each observation time via the vector, $\mathbf{X}(t) = (I, J)$, where *I* was the number of fish on the patch at time *t* and *J* was the number of potential joiners for the same patch at time *t*. For each patch type within a given trial (heterogeneous HP, heterogeneous LP or homogeneous MP) we determined the frequency that each state was observed over the entire trial, and from this estimated the probability of each patch type obtaining each state via the relative frequency that each state was observed. (Data

relating to patch state was pooled from all four MP patches for separate trials with homogeneous foraging environments, and from both HP and both LP patches separately for separate trials with heterogeneous distributions of food.) We then determined the mean probability (across 18 trials) of observing each state for LP, MP and HP patches, along with associated standard deviations and standard errors (see Figures S3 and S4).

Next, we determined the frequency of all changes in state from $\mathbf{X}(t) = (I, J)$ at time t to $\mathbf{X}(t + \Delta t) = (I', J')$ at the next sample time $t + \Delta t$ (1 second/15 frames later) (for all $I, J, I', J' \in \{0, 1, 2, \ldots, 8\}$) for each patch type in each trial. In practice, the frequency of observed transitions for a given patch type within a trial was stored in a 9 × 9 × 9 × 9 array with ordered indices I, J, I' and J', denoted $f(I, J, I', J')$. We then converted the observed frequencies to estimates of the conditional probabilities $P\left(\mathbf{X}(t + \Delta t) = (I', J') \middle| \mathbf{X}(t) = (I, J)\right)$. To do this, we first determined the total frequency at which each starting state was observed (for each (I, J) pair); we then divided the frequencies at which each transition from state $\mathbf{X}(t) = (I, J)$ was observed by the total frequency. That is, the estimate for each conditional probability was calculated via: $P\left(\mathbf{X}(t + \Delta t) = (I', J') \middle| \mathbf{X}(t) = (I, J)\right) = \dfrac{f(I, J, I', J')}{\sum_{I'=0}^{8} \sum_{J'=0}^{8} f(I, J, I', J')}$ for each patch type within each trial. Finally, we estimated the probability that a patch of a particular type would gain one or more fish and the probability that a patch of a particular type would lose one or more fish at the next observation time conditional on the current state of the patch being $\mathbf{X}(t) = (I, J)$. For each trial, these probabilities were estimated via:

$$P\left(\text{Gain} \geq 1 \text{ fish at } t+\Delta t \middle| \mathbf{X}(t)=(I,J)\right)=\sum_{I'=I+1}^{8}\sum_{J'=0}^{8}P\left(\mathbf{X}(t+\Delta t)=(I',J') \middle| \mathbf{X}(t)=(I,J)\right) \quad \text{and}$$

$$P\left(\text{Lose} \geq 1 \text{ fish at } t+\Delta t \middle| \mathbf{X}(t)=(I,J)\right)=\sum_{I'=0}^{I-1}\sum_{J'=0}^{8}P\left(\mathbf{X}(t+\Delta t)=(I',J') \middle| \mathbf{X}(t)=(I,J)\right).$$

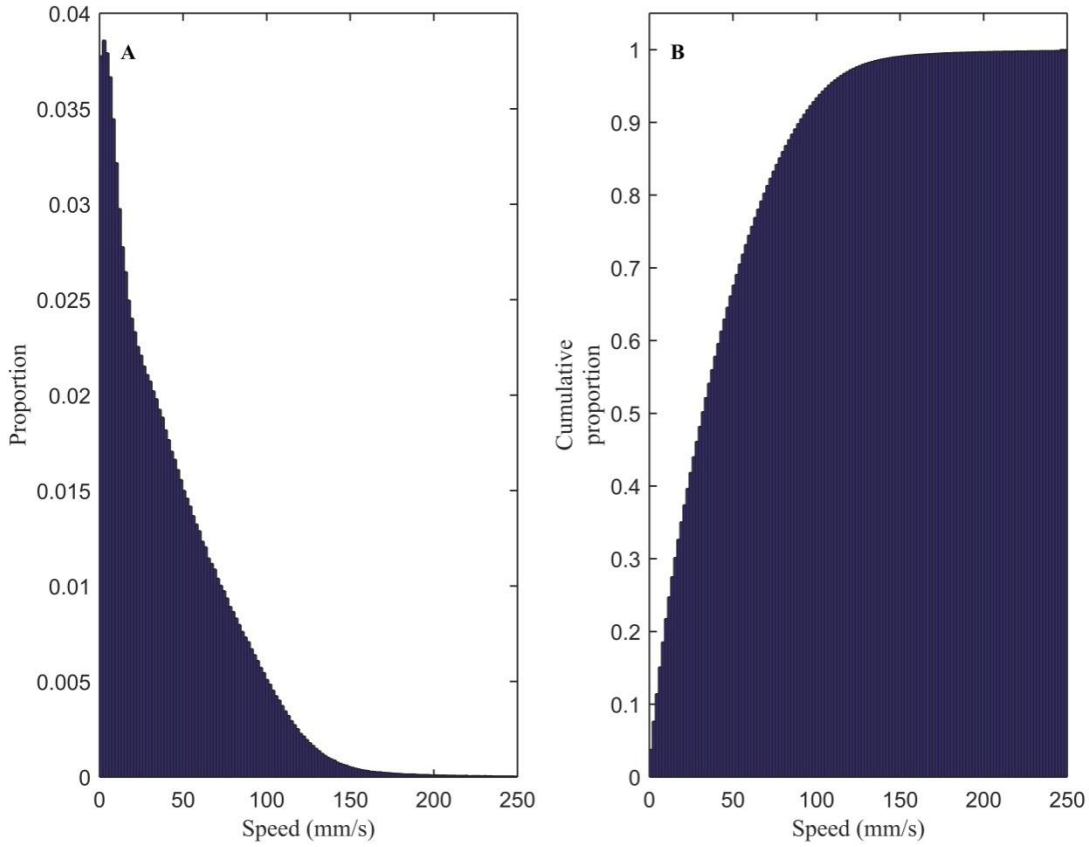

Fig S1: The proportion (A) and cumulative proportion (B) of observed speeds of mosquitofish up to 250 mm/s pooled from all 36 experimental trials.

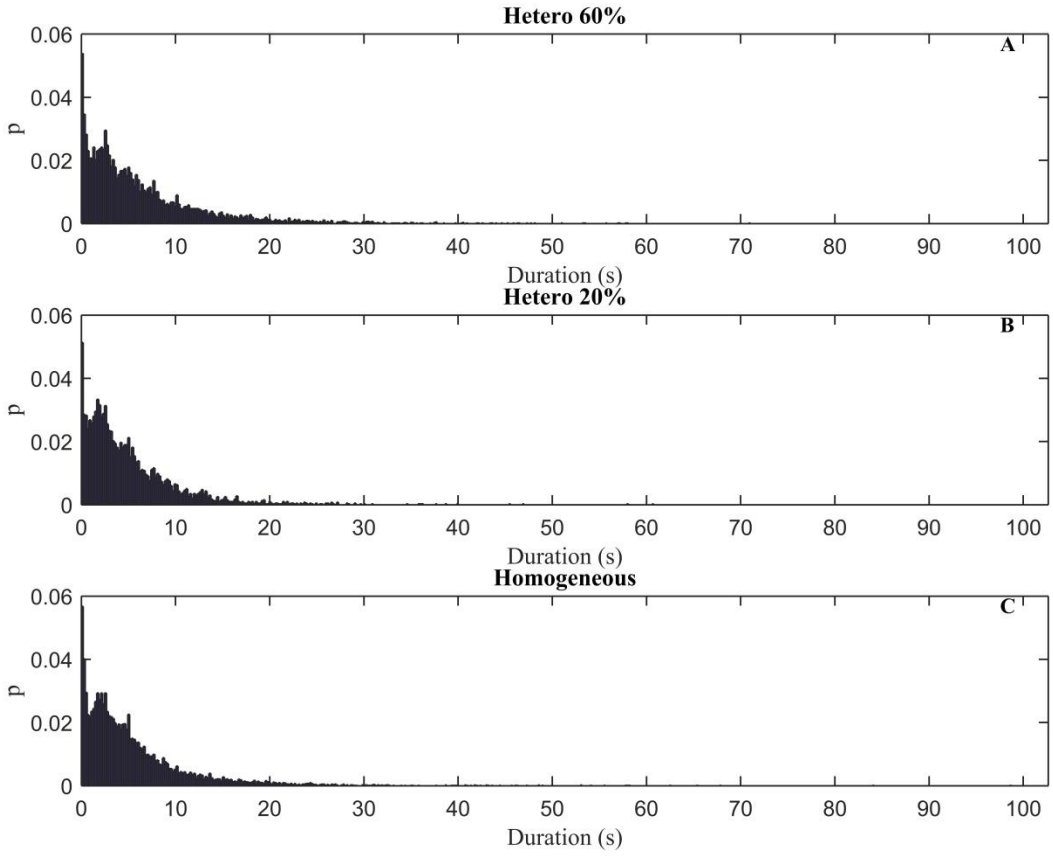

Fig S2: The distribution of observed durations of visits to heterogeneous high protein (HP/60%) patches (A), heterogeneous low protein (LP/20%) patches (B) and homogeneous medium protein (MP/40%) patches (C).

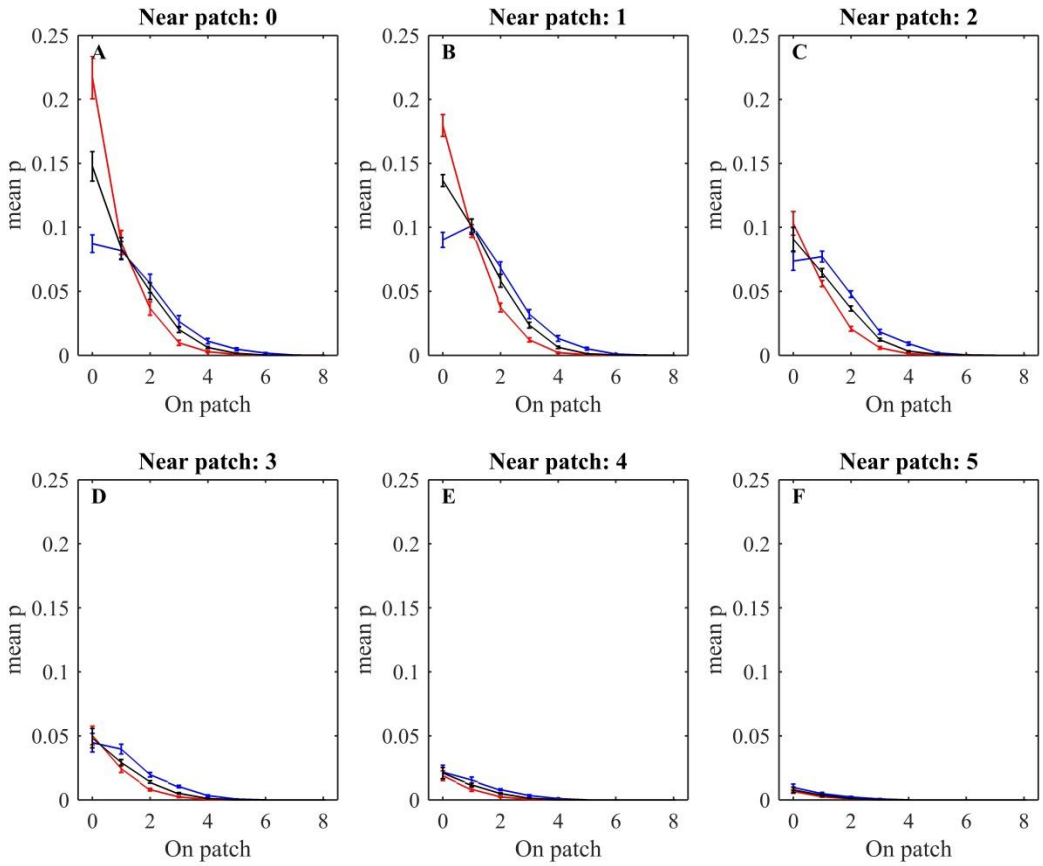

Fig S3: Estimated state probabilities for heterogeneous HP (solid blue lines), heterogeneous LP patches (solid red lines) and homogeneous MP (solid black lines) patches. Panels A—F are divided based on the number of potential joiners (from 0 to 5). Error bars are plotted ± 1 standard error above and below the mean state probabilities.

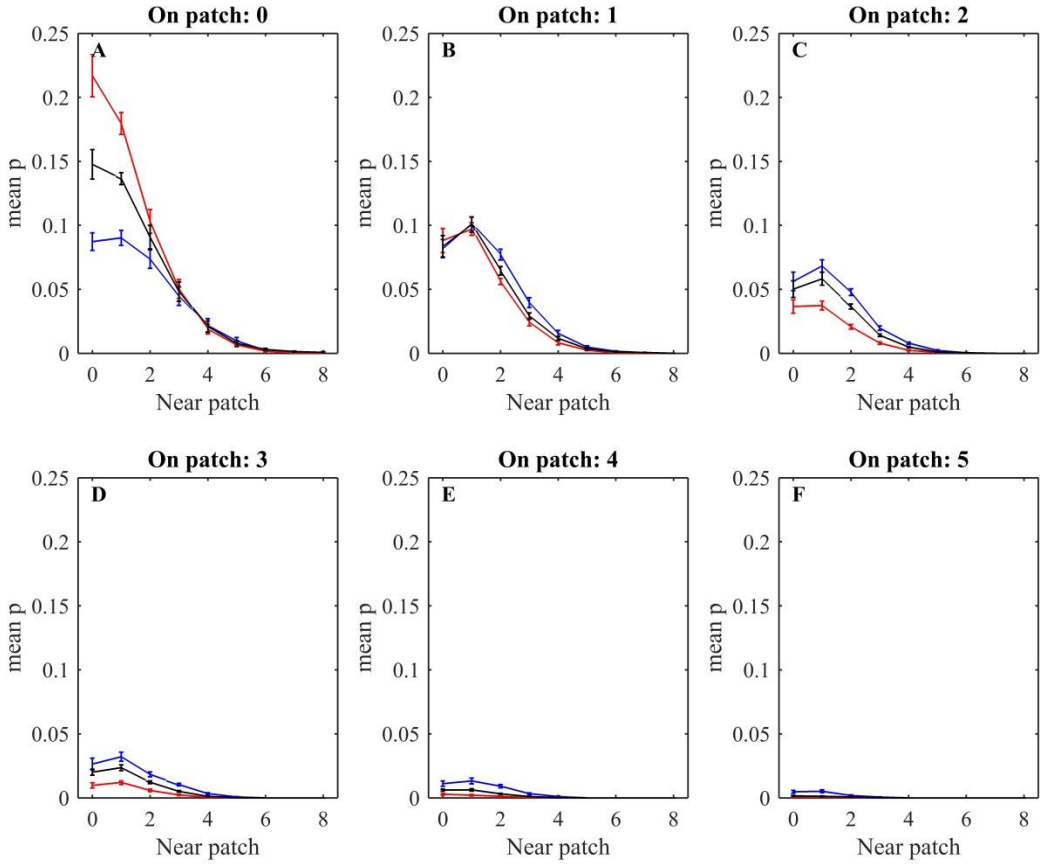

Fig S4: Estimated state probabilities for heterogeneous HP (solid blue lines), heterogeneous LP patches (solid red lines) and homogeneous MP (solid black lines) patches. Panels A—F are divided based on the number of fish on a patch (from 0 to 5). Error bars are plotted ± 1 standard error above and below the mean state probabilities.

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Chapter 5

CRIMSON-SPOTTED RAINBOWFISH (*MELANOTAENIA DUBOULAYI*) CHANGE THEIR SPATIAL POSITION ACCORDING TO NUTRITIONAL REQUIREMENT

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Decision making in moving animal groups has been shown to be disproportionately controlled by individuals at the front of groups. Therefore, an explanation of state-dependent positioning of individuals within animal groups may provide a mechanism for group movement decisions. Internal nutritional state is dynamic and can differ between members of the same group. It is also known to drive animal movement decisions. Therefore, we assayed 6 groups of 8 rainbow fish foraging in a flow tank. Half of the fish had been starved for 24h and half had been fed 1h prior. Groups were assayed again one week later but individuals were allocated to the opposite nutritional treatment. During the assay the positions of individually identified fish were recorded as were the number of food items they each ate and the position within the group they acquired them from. Food-deprived fish were more often found towards the front of the shoal; the mean weighted positional score of food-deprived fish was significantly larger than that of well-fed fish. There was no correlation between mean weighted positional scores for individuals when they were well-fed or food-deprived. There was a significant positive correlation between mean weighted positional score and number of food items acquired which displays an obvious benefit to front positions. These results suggest that positional preferences are based on nutritional state and provides a mechanism for state-dependent control of group decision-making as well as increases our understanding of what factors are important for group functioning.

Keywords: State-based behaviour, Spatial positioning, Fish shoals, Nutrition, Group behaviour

Introduction

Social animals are known to obtain clear benefits from group membership, however, the costs and benefits obtained by each individual within the group vary according to their relative spatial position in that group (Krause 1994; Krause & Ruxton 2002). The precise positional costs and benefits are determined by the biotic environment, however, it is generally recognised that animals at the front of moving groups, and at the periphery of stationary groups achieve higher rates of food intake, at the cost of greater predation risk.

A far-reaching and common example of unequal fitness returns due to spatial position is the theory of marginal predation, where the prediction is that if predators attack the closest prey, individuals towards the outside of the group are under higher risk of predation than those towards the centre of the group (Hamilton 1971; Rayor & Uetz 1990; Krause 1993a; Bumann et al. 1997; Stankowich 2003). For moving animal groups the predation threat is higher at the front edge of the group as they are the first to enter new environments and to encounter ambush predators (Bumann & Krause 1993; Bumann et al. 1997; Krause et al. 1998b). In many environments the periphery of animal groups is also where food reward is greatest, either in the quantity or quality of food items or because of reduced competition (Janson 1990a; Rayor & Uetz 1990; Forrester 1991; Black et al. 1992; Krause et al. 1992; Krause 1993c; Romey 1995; Beecham & Farnsworth 1999; Rowcliffe et al. 2004) although this depends on group size and the particular distribution of food items (Hirsch 2007). Therefore, positions within the group are often seen as a simultaneous balance between these two forces; predation risk and feeding reward (Krause 1994; Romey 1995; Beauchamp 2007, 2014; Hirsch 2007; Morrell & Romey 2008). Besides predation risk and

feeding reward, a third major factor affecting spatial positioning is energy expenditure, particularly for moving animal groups (Killen et al. 2011; Voelkl et al. 2015). Individuals at the leading edge of animal groups may be exposed to greater forces of friction than those behind in the slipstream (Bill & Herrnkind 1976; Liao et al. 2003; Svendsen et al. 2003; Portugal et al. 2014; Marras et al. 2015) and may even travel greater distances than individuals at the rear (Krause et al. 2000). Individuals may also position themselves within a group to reduce exposure to adverse environmental conditions such as harsh temperatures (Dambach & Goehlen 1999; Klok & Chown 1999; Gilbert et al. 2006).

An individual's spatial position may affect the degree to which it influences group movement and decision-making (Burns et al. 2012; Couzin et al. 2002, 2005; Leblond et al. 2006; Nagy et al. 2010). Individuals at the front of moving groups often have greater influence on the direction of movement (Huth & Wissel 1992; Bumann & Krause 1993; Katz et al. 2011) and it has been shown that a small minority of individuals can direct large groups (Reebs 2000; Swaney et al. 2001; Couzin et al. 2005; Janson et al. 2005; Beekman et al. 2006; Schultz et al. 2008; Dyer et al. 2009; Diwold et al. 2011; Stroeymeyt et al. 2011). Many groups are composed of individuals that hold varying amounts and different types of information. In these situations, individual spatial positioning within the group becomes a method in which individuals can exert control over the group. Physiological demand, personality and parasitism are all aspects that may affect motivation and the likelihood of being at the front, where individuals are able to exert disproportionate influence on group movement decisions (Ward et al. 2002, 2004, 2005; Rands et al. 2003;

Rands et al. 2008; Conradt et al. 2009; King & Cowlishaw 2009; Sueur et al. 2010a, 2013; King & Sueur 2011; McClure et al. 2011).

In many stable, restricted entry animal groups, positioning is affected by predation risk, food rewards and energetic requirements, but it is also strongly affected by dominance hierarchies and individual affiliations (Janson 1990b; Krause 1993b; Sueur et al. 2010b; Teichroeb et al. 2015). However, many animal groups are open entry systems, where group membership is temporary and group composition is therefore dynamic. In such groups, dominance relationships are thought to have less of an influence than effects such as size, metabolism and internal nutritional requirements (Krause et al. 2000). Animals that commonly form these types of groups, for example many species of fish and birds, are a preferred system for manipulative empirical experiments aimed at exploring these factors as they are not confounded by dominance hierarchies.

Within fish shoals, spatial positions are hypothesised to result from differences in size (DeBlois & Rose 1996), speed (Gueron et al. 1996), parasitism (Ward et al. 2005), predation threat (Bumann et al. 1997) and internal stimuli such as nutritional state. Hungry fish are often more spread out than satiated fish (Keenleyside 1955; Morgan 1988; Robinson & Pitcher 1989a, b) presumably to reduce competition for food, and are often found at the front of shoals (Krause et al. 1992, 1998a; Krause 1993c, roach (*Rutilus rutilus*)), where they have better access to food (Major 1978, giant trevally (*Caranx ignobilis*); Krause et al. 1992; Krause 1993c, roach; DeBlois & Rose 1996, Atlantic cod (*Gadus morhua*)). Of the studies performed under controlled conditions, Krause et al. 1992 looked at small shoals of 2 or 4 roach and found that food deprived fish (2-6 days)

were more often to be found towards the front of the shoal than well fed fish. Food deprived fish were also more often towards the front in Krause 1993c and Krause et al. 1998a. The duration of food deprivation was 3 days in Krause 1993c for fish released into larger shoals in the field and 7 days in Krause et al. 1998a for pairs of fish. Whilst these experiments are highly influential there is need to see if individual fish change positions according to their nutritional demand after shorter, more ecologically relevant, periods of food deprivation, with the same fish moving toward the front when hungry and toward the back when satiated. Also, there is a need to record how much fish eat whilst in different positions over multiple consecutive foraging opportunities to calculate the direct costs and benefits to different positions. Doing so will provide evidence on whether positional preferences are dynamic and based primarily on internal nutritional state rather than more consistent individual differences such as size, metabolic rate or behavioural syndrome.

To address this question, we conducted a repeated measures experiment on 6 groups of 8 individually identifiable rainbow fish (*Melanotaenia duboulayi*). The same individuals were assayed on two separate occasions, one week apart. On each occasion the group of 8 fish contained 4 well-fed fish (fed 1h before the experimental assay) and 4 food-deprived fish (starved for 24h). Each fish was assayed twice, once when food-deprived and once when well-fed, but always in the same group composition. This approach avoids confounding effects due to individual differences. Data was collected on their spatial position within the group and also how many food items they consumed and from what positions they attained the food. It was hypothesised that starved individuals will move

towards positions that result in them consuming the greatest proportion of food items and this is predicted to be towards the front of the shoal (Krause 1993c).

Materials and methods

Experimental animals

Crimson-spotted rainbowfish, *Melanotaenia duboulayi*, are a freshwater species of fish endemic to eastern Australia. Experimental fish were obtained from Pisces Aquatics and kept in white plastic housing aquaria (180 L) in de-chlorinated aged tap water with a sponge filter at 27°C for 10 weeks in 12:12 light:dark photoperiod before the commencement of experiments. Fish used in the experiment had a body length of 50±5mm. Forty-eight fish were taken from the 180L aquarium and separated equally between six 50L holding aquaria two weeks before the experimental assay. Fish in each of the six 50L aquaria were tagged with visible implant elastomer (Northwest Marine Technology, Inc, Manual Elastomer Injection System, 10:1 Formulation) on their dorsal surface for individual recognition. During these two weeks all fish were fed live Chironomid larvae once per day till satiation.

Experimental Arena

The experimental arena was a rectangular flow tank (3000 × 450 × 100 mm) composed of grey Perspex 5mm thick. De-chlorinated aged tap water at the same temperature as the

holding aquaria water entered one end of the flow tank through a plastic hose (30mm diameter) connected to a t-junction of PVC pipe (52mm diameter). This t-junction had 46 holes (10mm diameter) in two rows drilled into one side of it from which the water flowed out of. The water then passed through a wall of white Corflute® (100 mm) before entering the experimental arena. This was done to ensure the water flow was even across the width of the arena. This wall of white Corflute® defined the front most barrier to the experimental arena and an identical wall of white Corflute® defined the rear most barrier (Fig. 1b). Experimental fish were therefore unable to escape from either end of the experimental arena; the dimensions of the area of water accessible to fish was 1120 × 440 (× 100) mm. White plastic was attached to the base of the arena between these two barriers to allow for better contrast for fish identification. This area was also surrounded by a purpose built metal frame that was surrounded by white Corflute® to minimise external stimuli disturbing the fish whilst also allowing enough light for video recording (Fig. 1a). The water, after passing through the rear most wall of Corflute® fell through 52 holes (10mm diameter) drilled into the end and sides of the flow tank and into a 150L white plastic tub. This water was then pumped back up (Laguna PJ MAX-FLO 18000, 160W) through the hose into the t-junction and continually circulated through the tank at a constant depth of 60mm and constant flow. Evenness of the current was tested prior to the experiment using green food dye. Food could be injected into the water current by syringing 15ml of water through a small plastic hose that was filled with water, and contained a food item (Chironomid larvae). The hose was on the outside of the front most wall, which meant fish could not see food until it entered the water column and drifted towards them, whereupon the fish would make an attack on the food item. This method

also insured that the injection of the food item was not associated with the release of air bubbles or any other stimulus. A video camera (Canon AVCHDProgressive LEGRIA HFG30) and an SLR camera (Canon G1x Powershot) were attached above the experimental arena for data collection.

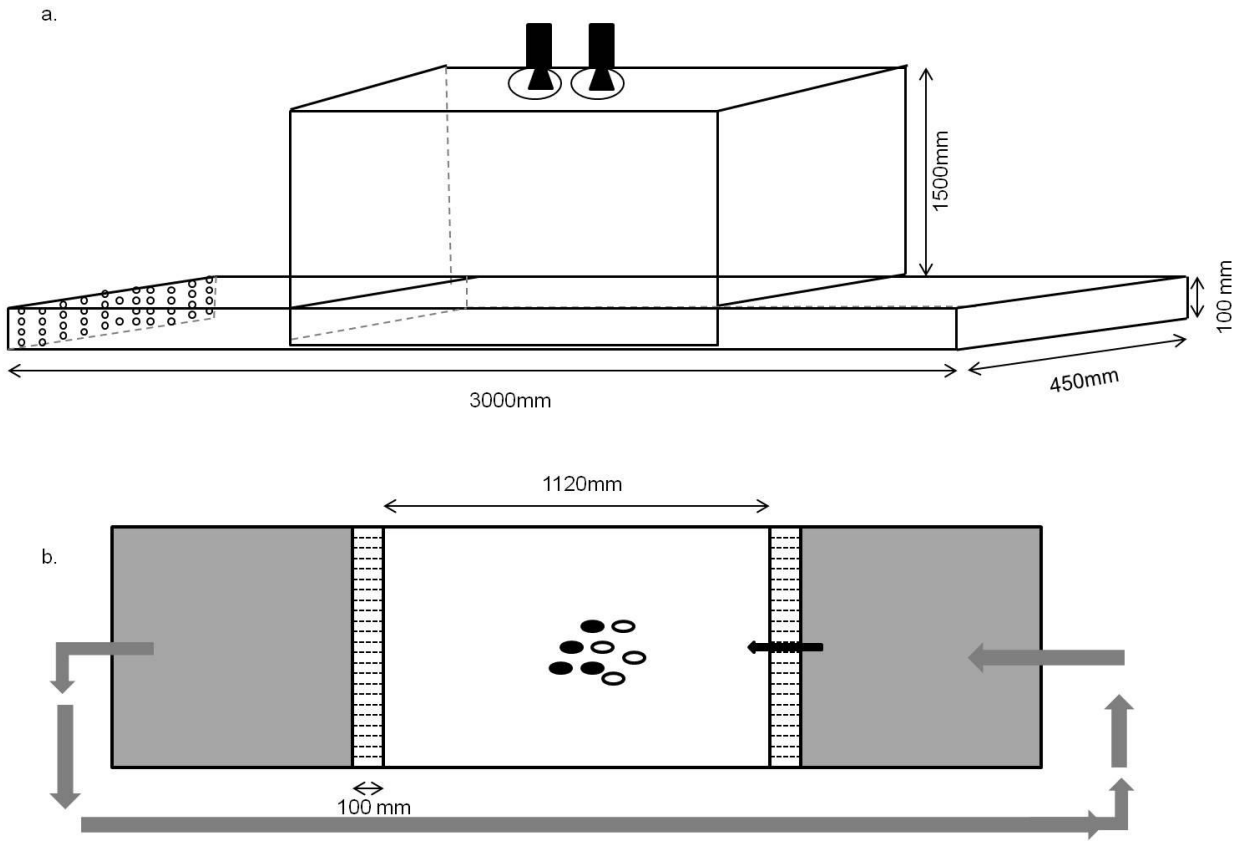

Figure 1) Diagram of the experimental arena, a rectangular flow tank showing a.) its dimensions and the positions of cameras and b.) the direction of water flow and the dimensions of the of the area of water accessible to fish defined by the Corflute® barriers.

Experimental trial

The day prior to data collection a group of 8 fish were placed in the flow tank at 17:00h and allowed to explore and get used to their surroundings overnight. A sheet of white Corflute® was placed on top of the tank to stop fish from jumping out of the arena. At 9:00h the following morning the cover was removed. At 12:00h, two glass aquaria (300 × 150 × 150mm) were placed side by side in the middle of the flow tank. The adjoining wall of these tanks was a perforated plastic divider that allowed fish to see and smell fish on either side of the barrier, however, it prevented fish physically crossing from either side so that two sub-groups of fish could be fed separately. Four fish were placed in one aquaria and 4 fish in the other. Fish could be manipulated to be in one of two nutritional states: food-deprived or well-fed. Food-deprived fish were not fed, which meant they had been starved for 24h. Well-fed fish were fed with Chironomid larvae at 13:00h via a tube that extended outside of the arena's white opaque walls till they were satiated. At 14:00h the glass aquaria were removed, releasing the fish back in to the experimental arena where they promptly formed a shoal facing into the current. After 30 minutes the video was started, and on the half minute mark of each minute a stillshot was taken remotely with the SLR over a period of 30 minutes for fish identification. This did not disturb the fish. A Chironomid larva was released from the feeding tube into the arena after 5 minutes where upon it drifted downstream toward the shoal. This was repeated 24 times, once a minute, before the film was stopped and the fish removed. The flow tank was then emptied, cleaned, and refilled before another group of fish was placed in the arena at 17:00h for assaying the next day. Each group was assayed twice, one week apart. Each individual was

assayed once when well-fed and once when food-deprived but group composition remained consistent between trials. Each group was placed into the flow tank for 24h one week before they were assayed the first time, to familiarize themselves with the experimental set-up.

Data collection and processing

It was not possible to read the individual fish identification tags from the video alone, however, by matching the video to the 24 still images we were able to determine the rank-order position of each fish in its group, relative to the end of the flow tank every thirty seconds for 24 minutes (48 positional scores per trial). The number of times that each individual spent in each position (1^{st}, 2^{nd}…8^{th}) was determined. The proportion of times an individual spent in each position was calculated and then the proportion was weighted as follows: 1^{st} x 8, 2^{nd} x 7…8^{th} x1 which meant that a higher positional score was representative of an individual that occupied frontal positions more frequently. These mean weighted positional scores were used for analysis. Also from the video, we counted the number of food items each fish consumed and the positions they attained the food from.

The 24 still images were imported to a tracking program, Image J, wherein the *x, y* coordinates in pixels of each individually identified fish was taken from the point of its snout for each of the 24 images. It was not possible to record position on the *z*-axis, however, the shallowness of the water meant that fish were on a similar *z*-plane to each other and thus ignoring the *z*-axis is not considered to be problematic. These coordinates

were then imported into MATLAB (R2014A). In addition, for each trial we recorded the coordinates of the food source in pixels (from a single image), and four reference points from the experimental arena in pixels (the top left, top right, bottom right and bottom left corners of the area accessible to the fish). We used the four reference points and the known dimensions of the area accessible to the fish (1120 mm in the *x*-direction, and 440 mm in the *y*-direction) to obtain four estimates for the number of pixels per millimetre. We used the mean of these pixels per millimetre estimates to then convert the (*x*, *y*) coordinates of each fish and the food source to millimetres for each trial. We denoted the coordinates (in mm) of each fish, *i*, in image *t* for a given trial as $\left(x_i(t), y_i(t)\right)$ and the associated coordinates of the food source as $\left(f_x(t),\ f_y(t)\right)$.

We sought to produce plots of the relative frequency that hungry and satiated individuals occupied different positions within each group. To do this, we first shifted and rotated the coordinates of all fish in all images for all trials to a standard coordinate system where a group's centroid for a given frame was located at the origin, and the food source lay on the positive *x*-axis (the line $y = 0$ for $x > 0$ in particular) as follows. For each image in each trial the group's centroid, $\left(c_x(t), c_y(t)\right)$, was given by the mean *x*- and *y*-coordinates of all group members. We then shifted the coordinates of all fish for each image according to:

$$x_{s,i}(t) = x_i(t) - c_x(t) \quad \text{and} \quad y_{s,i}(t) = y_i(t) - c_y(t).$$

We then determined the angle between the positive *x*-axis and the straight line segment passing from the group centroid to the location of the food source via $\theta(t) = \text{atan2}\left(f_y(t) - c_y(t),\ f_x(t) - c_x(t)\right)$. We rotated all shifted coordinates such that this

straight line segment would now lie on the *x*-axis (with $(c_x(t), c_y(t))$ now at the coordinate origin) using the standard transformation:

$$\begin{bmatrix} x_{r,i}(t) \\ y_{r,i}(t) \end{bmatrix} = \begin{bmatrix} \cos(-\theta(t)) & -\sin(-\theta(t)) \\ \sin(-\theta(t)) & \cos(-\theta(t)) \end{bmatrix} \begin{bmatrix} x_{s,i}(t) \\ y_{s,i}(t) \end{bmatrix}.$$

Following the shifting and rotational transformations we then separated group members into sets of hungry fish and satiated fish. We divided a portion of the domain centred on group centroids into a set of overlapping square 20 mm × 20 mm bins such that the left edges of the bins were located at $x_{l,\text{left}} = -200, -195, -190, ..., 200$ (mm), the right edges of the bins were located $x_{l,\text{right}} = -180, -175, -170, ..., 220$ (mm), the bottom edges of the bins were located at $y_{k,\text{bottom}} = -200, -195, -190, ..., 200$ (mm) and the top edges of the bins were located at $y_{k,\text{top}} = -180, -175, -170, ..., 220$ (mm). We tallied the number of times hungry fish and satiated fish were located in each bin, indexed (l, k), across all 12 trials (fish i was located in bin (l, k) in frame t if $x_{l,\text{left}} < x_{r,i}(t) \le x_{l,\text{right}}$ and $y_{k,\text{bottom}} < y_{r,i}(t) \le y_{k,\text{top}}$). We stored the tallies for hungry and satiated fish in two separate matrices. The main purpose of using overlapping bins was to smooth our plots to a certain extent; a consequence of such smoothing is that each fish likely occupied multiple bins in each image. We converted the tallies stored in the matrices for hungry and satiated fish into relative frequencies by dividing the value of each element in a given matrix by the sum of all elements in the same matrix. We then rendered the relative frequencies as a function of position relative to group centroid using MATLAB's intrinsic *surf* function. In addition to the surface plots, we constructed line graphs of the relative frequency that fish occupied

different locations examining either *x* or *y* coordinates separately. To do this, we allocated data into bin l if $x_{l,\text{left}} < x_{r,i}(t) \leq x_{l,\text{right}}$, irrespective of the fish's corresponding *y* coordinate for a plot of relative frequency as a function of *x*. Similarly we allocated data to bin k if $y_{k,\text{bottom}} < y_{r,i}(t) \leq y_{k,\text{top}}$, independent of $x_{r,i}(t)$ to generate a plot of relative frequency as a function of *y*.

To complement the analysis of weighted positional scores, we used the coordinate data imported into MATLAB to construct a bar graph to illustrate the proportion of food eaten from different positions, and the fraction of the food taken by hungry or satiated fish. For the bar graph we assigned each fish a distance rank, with the fish closest to food source assigned rank 1, up to the fish farthest from the source that was assigned rank 8. Distances were determined using the standard formula $d_i(t) = \sqrt{\left(f_x(t) - x_i(t)\right)^2 + \left(f_y(t) - y_i(t)\right)^2}$.

Statistical Analysis

We used R (R Core Team, 2014, R i386 3.1.2) and *lme4* to perform a linear mixed effects analysis of the relationship between weighted positional score and internal nutritional state. The dependent variable tested was 'mean weighted positional score'. Homogeneity of variance was tested with Levene's test and normality was assessed by looking at histograms and QQ-plots. As a fixed effect we entered internal nutritional state into the model. We had fish identity as a random effect. The linear mixed model was fit by

maximum likelihood *t*-tests and used Satterthwaite approximations for degrees of freedom to approximate *p*-values.

We performed a Pearson's correlation between individual mean weighted position score when well-fed and when food-deprived as well as between mean weighted positional score and number of food items eaten (SPSS IBM Statistics 20).

Results

The mean weighted positional score of food-deprived fish was significantly larger than that of well-fed fish ($t_{(1,96)}$=-7.086, $p<0.0001$) with food-deprived fish more frequently occupying position at the front of the shoal compared to well-fed fish (Fig. 2 and 3).

The effect of nutritional state on the weighted positional score over the first 5 minutes of the experiment, before food was made available to the shoal, similarly showed that food-deprived fish occupied positions at the front of the shoal more frequently than well-fed fish ($t_{(1,48)}$=-4.162, $p<0.0002$). However, when the two trials are analysed separately, we find that there is no effect of nutritional state on mean weighted positional score in the first trial ($t_{(1,48)}$=-1.463, $p=0.15$). In the second trial, after fish have had experience feeding in the flow tank food-deprived fish occupied positions at the front of the shoal more frequently than well-fed fish ($t_{(1,48)}$=-3.446, $p<0.002$).

There was no correlation between individuals' mean weighted positional score when it was well-fed and when it was food-deprived (r^2=0.09, $p=0.96$) which suggests individuals were not consistent in their position within the shoal between treatments.

There was a significant positive correlation between mean weighted positional score and number of food items eaten (r^2=0.47, p<0.0001). More food items were eaten by fish in the front most positions than in the rear most position, with fish in positions 1 and 2 consuming a cumulative proportion of available food items of over 60% (Fig. 4).

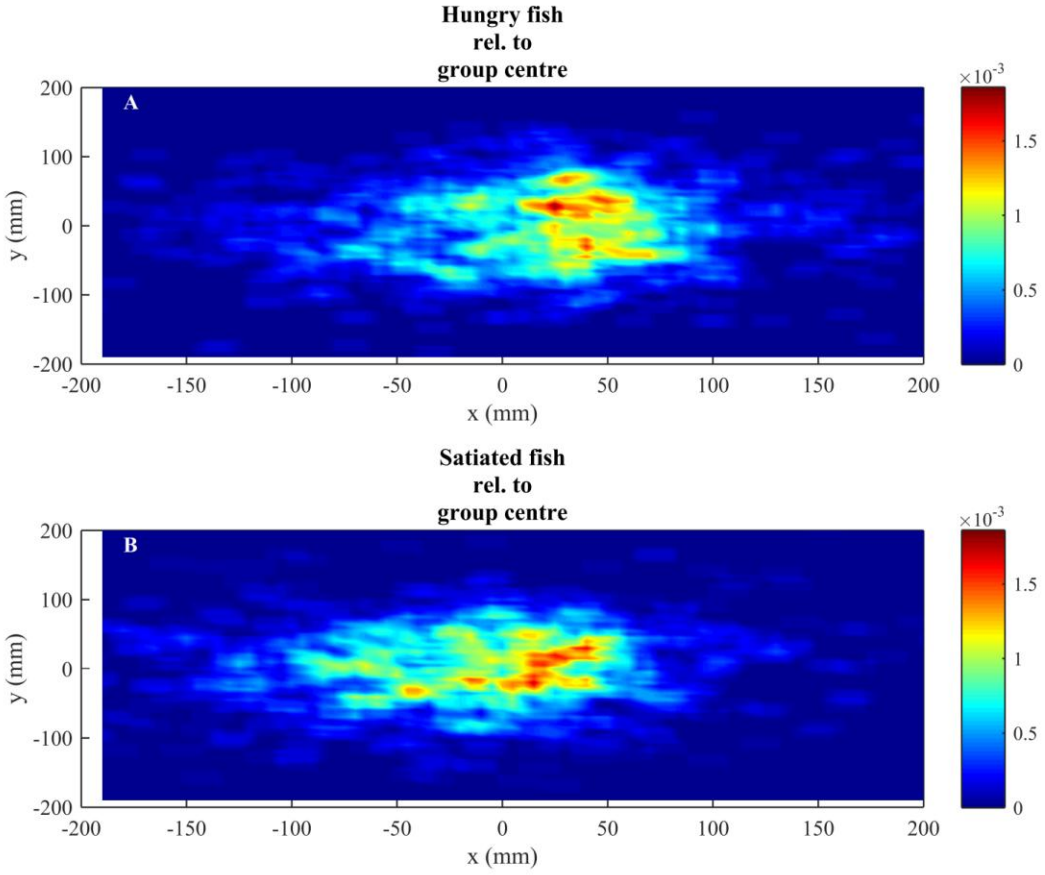

Figure 2) The relative frequency that hungry (A) and satiated (B) fish occupied different spatial locations relative to the group centroid (at (0, 0)). All coordinates were transformed such that the straight line passing from the group centroid to the food source lay on the positive *x*-axis. Both plots were smoothed through the use of overlapping square bins (see *Data collection and processing* for more details).

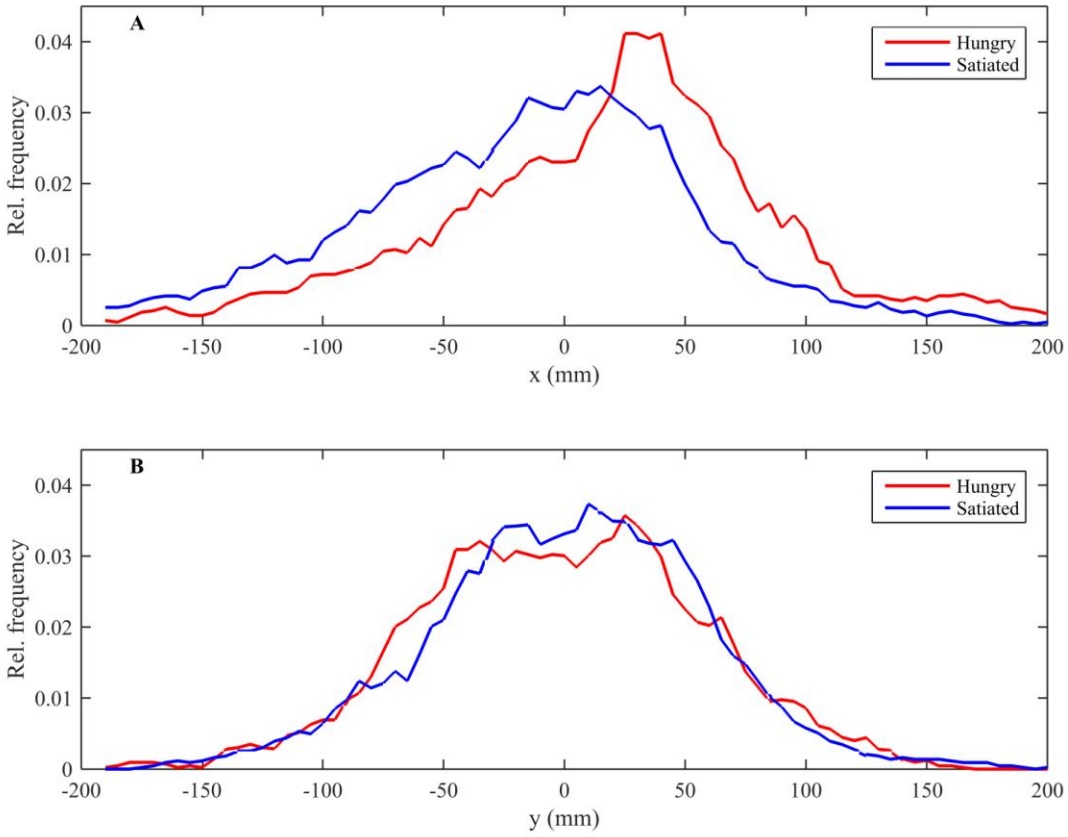

Figure 3) The relative frequency that hungry (red lines) and satiated (blue lines) fish occupied different *x*- (A) or *y*-coordinates (B) relative to the group centroid (at 0).

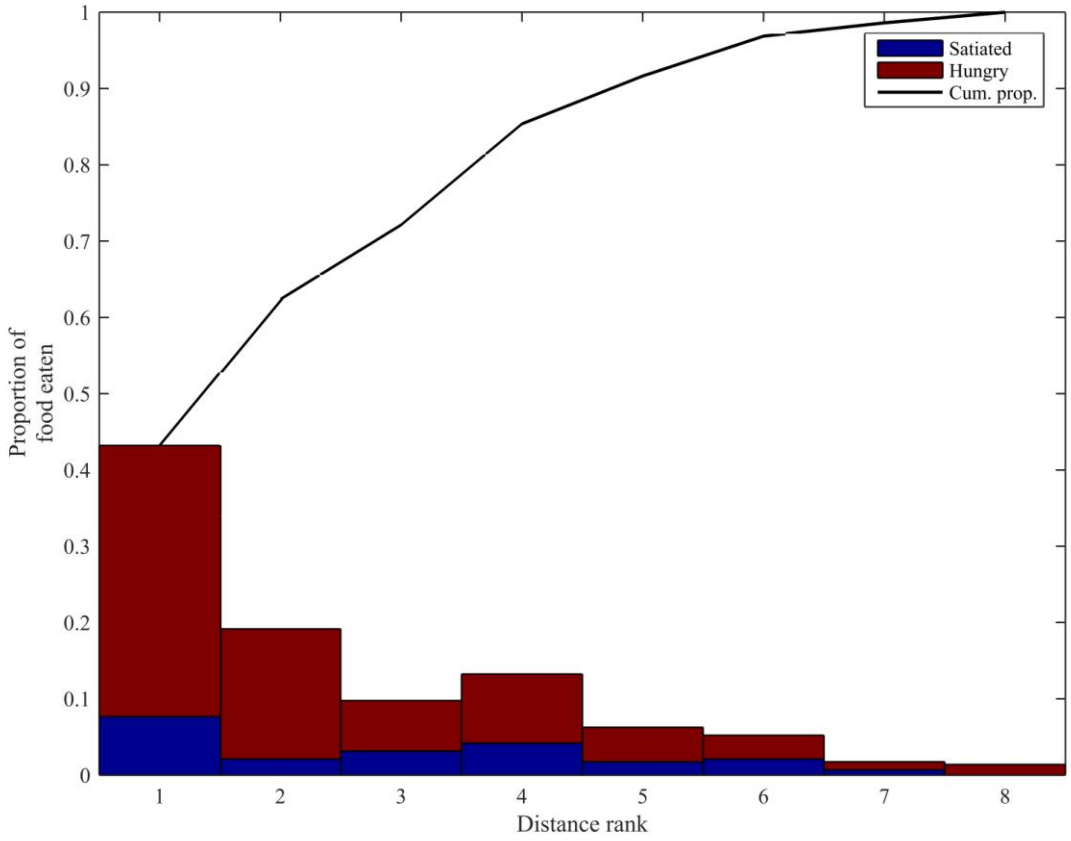

Figure 4) The proportion of available food eaten by fish with different distance ranks (determined by distance to food source: 1 = closest to food, 8 = farthest from food), divided into hungry (red) and satiated (blue) individuals. The solid black line indicates the cumulative proportion of food eaten as a function of increasing distance rank.

Discussion

As predicted food-deprived fish occupied the front most positions of the shoal more frequently than well-fed fish, evidence that positional preferences were based primarily on internal nutritional state and less so by individual differences such as size, metabolic rate or behavioural syndrome, as the same individuals were tested twice in the same group

composition, once when well-fed and once when food-deprived. Also, there was no correlation between individuals mean weighted positional score when food-deprived and when well-fed. This finding supports existing literature that proposes individuals in groups position themselves according to internal nutritional state (Krause et al. 1992, 1998a; Krause 1993c; Romey et al. 1995). For example, hungry whirligig beetles, (Coleoptera: Gyrinidae) position themselves on the periphery of the group and spatially separate themselves from near-neighbours in order to obtain the majority of food items (Romey et al. 1995).

In fish shoals, it has been suggested that food deprived individuals more often occupy the front most positions within a shoal due to a higher swimming speed or turning rate, and indeed fish do slow down as they become satiated (Priyadarshana *et al.,* 2006), however, as the shoals were tested within a flow tank the results of this experiment suggest that spatial positions may result from individuals positioning themselves in relation to shoal mates. This effect was perhaps heightened by competition for food items that entered the arena from directly in front of the shoal's facing direction. However, although the effect size was smaller, the effect of nutritional state on the weighted positional score over the first 5 minutes of the experiment in the second trial, before food was made available to the shoal, but after they had experience feeding from food drifting towards them in the first trial, showed that food-deprived fish more frequently occupied the front of the shoal. In the first 5 minutes of trial 1, when fish had no experience of food in the environment, food-deprived fish were not more frequently found at the front of the shoal, suggesting that fish may have been attempting to reduce predation risk or save energy.

Similar effects of food deprivation were found to occur in shoals of 2 roach starved for 2 or 4 days and in shoals of 4 fish starved for 4 and 6 days (Krause et al. 1992). However, Krause et al. 1992 followed a single focal fish within small shoals, whilst this experiment was able to account for the position of all 8 shoal members at each time interval, which is important considering decisions made by an individual pertaining to foraging behaviour are greatly influenced by the actions of other group members (Rands et al. 2008). Also, the time of food deprivation in Krause et al. 1992 was, at its minimum, twice as long as in the current experiment. Further experiments on roach, also had longer food deprivation periods of 3 (Krause 1993c) and 7 (Krause et al. 1998a) days. To our knowledge, 24h is the shortest food deprivation period known to have a significant effect on individual spatial positioning in fish shoals and provides strong evidence that spatial positioning based on internal nutritional state is more sensitive than previously known.

The frequency that food-deprived fish occupy frontal positions is known to increase with food deprivation (Krause et al. 1992) and fish in the wild lose the preference for frontal positions after 2 days of being allowed to forage freely (Krause 1993c). Unfortunately, the difference in the hunger levels between fish that started the trial food-deprived and fish that started the trial well-fed did not alter sufficiently to see a rotation of positions during the 30 minute trial. This was likely a combination of not introducing sufficient food for the food-deprived fish at the front to become satiated and not having a long enough trial duration for the well-fed fish at the back to become hungry. The mechanics behind the rotation of spatial positions based on dynamic changes in internal state is the next logical step for this field of research. Attempts to explore this may like to use a similar flow tank set-up as it

has proved successful in ensuring synchrony of shoal travel direction for long periods of time and allowed for accurate measurement of which individual fish ate and from what position it consumed the food item. However, future experiments should provide more food over longer trial durations and utilize automated tracking software to acquire data at a finer scale than in the current study.

Fish in the two front most positions acquired over 60% of the food (Figure 4) evidence of a clear benefit to fish that position themselves at the front of the shoal. The majority of available food being consumed by the front most individuals is similar to previous experiments calculating the number of food items eaten by individual fish within a similar sized shoal of roach (n=10, (Krause et al. 1998a). However, Krause et al. 1998a recorded the position of the fish in the shoal that ate the first and then the second of two Chironomid larvae that were placed into an arena. All fish in the shoal were hungry and the shoal only experienced a single foraging event, whilst in the current study the shoal was composed of both hungry and satiated individuals and 24 food items were drifted towards the shoal (one at a time) which meant that fish had multiple foraging opportunities and could change positions according to previous success. Therefore, the result is novel in that it records, for the first time, that over multiple consecutive foraging opportunities individual positions within fish shoals are associated with different intake rates and that hungrier fish move to the front where they receive more food. Other species have similarly showed a difference in intake rate according to spatial position, for example, individual whirligig beetles on the outer periphery consumed almost all of the food made available (Romey 1995). A proportion of food items as large as this, however, is likely due to the limited amount of

food presented to the group at any one time and the temporal dispersion of its introduction to the arena (one item, once per minute in this experiment). Higher food densities would likely lead to individuals further towards the rear or centre of the group attaining a higher proportion of the available food (O'Connell 1972; Hirsch 2007). In these environments individuals at the rear or centre could potentially acquire sufficient food to negate any benefit of moving towards the front or periphery of the group and individual spatial positioning according to internal nutritional state may not occur under such conditions.

The foraging behaviour of individual fish in shoals has already been shown to be flexible in response to changes in the distribution of food in the environment (Ryer & Olla 1992, 1995). Experiments quantifying the amount of food individuals in different shoal positions acquire under different spatial and temporal distributions of food, and how this affects the overall geometry and social dynamics of the shoal is a potentially fruitful area of future research. Individuals in the front most positions of moving shoals have a greater risk of predation (Bumann & Krause, 1993; Bumann et al. 1997; Krause et al. 1998b) and may attain further costs in increased hydrodynamic demand (Svendsen et al. 2003). It is unknown to what extent fish in this study responded to these conflicting demands. A manipulative experiment involving the addition of predator cues to the water in addition to food items as well as a calculation of individual tail-beat frequency could be a useful means of exploring the effects of conflicting demands on spatial positional choice.

The results of this paper are discussed primarily in the context of fish shoals as this is where the vast majority of empirical evidence for spatial positioning based on internal nutritional state currently exists. However, state-based positioning of individuals within

groups occurs in many other types of animal groups and it is possible that spatial positioning based on internal nutritional state plays an important role in a range of taxa (Janson 1990a; Rayor & Uetz 1990; Gueron et al. 1996; Fernandez-Juricic & Beauchamp 2008; Buhl et al. 2011; Beauchamp 2014). In many animal groups leadership or control of group travel direction is determined by select individuals often at the front of the group (Bumann and Krause 1993; Couzin et al. 2005; Leblond et al. 2006; Nagy et al. 2010) and these individuals may be those that have the greatest motivation, perhaps to seek shelter or attain nutritional balance (Rands et al. 2003, 2008; Conradt et al. 2009; King & Cowlishaw 2009; King & Sueur 2011; McClure et al. 2011; Sueur et al. 2013). This experiment provides empirical proof that internal nutritional state affects the spatial positioning of individuals within groups, providing a mechanism for state-dependent leadership, and that these positions are associated with different intake rates.

Conflict of Interest

The authors declare that they have no conflict of interest relating to this study

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Chapter 6

THE EFFECT OF TIDE ON THE EMERGENCE OF COLONIES OF HUMBUG DAMSELFISH (*DASCYLLUS ARUANUS*)

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How animals trade-off food availability and predation threats is strong determinant of animal activity and behaviour, however the majority of work on this topic has been on individual animals, despite the modulating effect the presence of conspecifics can have on both foraging and predation risk. Whilst these environmental factors (food and predation) vary spatially within habitats, they also alter temporally and in marine habitats this can be determined not only by the diel cycle but also the tidal cycle. Humbug damselfish, *Dascyllus aruanus*, live in small groups of unrelated individuals within and around branching coral heads which they collectively withdraw into to escape a predation threat. In this study we measured the proportion of individuals in the colony that were outside the coral head before and after they were scared by a fright stimulus and compared the responses at high tide and low tide. We found that a greater proportion of the shoal emerged after the fright stimulus at high tide and in larger groups than at low tide or in smaller groups. We also quantified the pattern of emergence over time and discovered that larger shoals emerge more slowly, but to a greater proportion, than smaller shoals, and a greater proportion of fish emerge at high tide, independent of shoal group size. This is the first study, to the authors' knowledge, to show that shoals of fish change their collective behavioural response to a predation threat in accordance with the tide.

Keywords: *Dascyllus aruanus*, Humbug damselfish, group decision-making, predation threat, tide

Introduction

It is widely recognised that searching for food and evading predators are two major factors influencing animal behaviour. How animals trade off these two factors is a key question in behavioural ecology (Sih 1982; Dill 1983; Lima and Dill 1990; Smith 1997). For example, an animal's selection of certain food types or foraging behaviours may change in response to the level of predation threat, and ultimately areas of higher resource profitability may be partially or completely avoided if the predation threat is perceived to be too great (Abrahams and Dill 1989; Lima and Dill 1990). Predatory threats will also affect the amount of time an animal spends in a safe place before returning to forage (Ydenberg and Dill 1986; Lima and Dill 1990). As the threat of predation exists during times when prey need to perform other activities such as feeding or finding mates, behavioural adaptations of prey should be particularly sensitive to the degree of predation risk and how it balances with current demands and opportunities (Dill 1983; Lima and Dill 1990).

In addition to the need to balance risk against reward, most animals are subject to circadian rhythms (Helfman 1986), while marine animals, particularly those that inhabit shallow waters, are also subject to circatidal rhythms (Gibson 1992). The tide can have significant effects on the appropriateness of habitats and marine animals may have behavioural patterns that are synchronized with the tidal cycle (Northcott et al. 1990; Gibson 1992). Some juvenile flat fishes time their migrations to different parts of the beach with the tide (Kuipers 1973) and activity levels of monkeyface prickleback, *Cebidichthys violaceus* (Ralston and Horn 1986), and purple marsh crab, *Sesarma reticulatum* (Palmer 1967) are also synchronised with the tide. While much work has been done on how the spatial

distribution of animals is affected by the trade-off between foraging and predation, less attention has been given to how this trade-off changes in accordance with consistent temporal rhythms (Metcalfe et al. 1999).

For animals that live in groups the behavior of conspecifics is an additional factor that interacts with other environmental stimuli to alter the trade-off between foraging and predation. The decisions of animals that live in groups are influenced by the behavior of other individuals in the group (Krause and Ruxton 2002; Ward et al. 2013) and animals need to strike a balance between conformity and individuality(Herbert-Read et al. 2013). Whilst the presence of conspecifics may decrease the risk of predation due to the many-eyes, dilution or confusion effect, food competition generally increases with the number of individuals at a food patch (Ward et al. 2006).

Humbug damselfish, *Dascyllus aruanus*, live in small groups of unrelated individuals (hereafter "colonies") within and around branching coral heads. Groups of humbug damselsfish are territorial and maintain the same group structure (Jordan et al. 2010). They are planktivores and feed in the water column directly above and around their coral head. One of the suggested explanations for the species' preference for group living is the advantage individuals receive from the increase in predator vigilance and the dilution effect (Sweatman 1985). Predation threats are reduced through a collective response in which fish seek refuge within the branches of the coral until the threat has passed. The amount and variety of food available and therefore the feeding rate of humbug damselfish is greatest during high tide, when plankton availability is greatest (Forrester 1991). This therefore creates a good natural study system to explore not only how animal groups trade-

off feeding and predation threat, but also how this is affected by consistent temporal rhythms.

Many studies have looked at habitat use and decisions of where to feed in response to predation threat (Dill 1983; Lima and Dill 1990; Sih 1982). For the territorial humbug damselfish, there is more flexibility over when to feed than there is where to feed, as the patch is restricted to the immediate area surrounding their coral head. In this system fish exist in a binary state, they are either outside of a coral refuge, in which case they are typically foraging, or they're in the coral refuge, in which case they are not. Certainly there is variance of prey distribution and type around the coral head, but for the purposes of this study the fish are considered to be either within the patch and therefore able to forage, or in hiding. Therefore, we measured the proportion of individuals in the colony that were outside the coral head before and after they were scared by a fright stimulus and compared the responses at high tide and low tide. We showed that groups of damselfish change their behavioural response to a predation threat depending on the tide.

Methods

Research was conducted at 3rd Lagoon, One Tree Island (-23° 30' 26'', 152° 5' 25''), Great Barrier Reef between March 28th and April 10th 2014. Fifty-six colonies of humbug damselfish (*Dascyllus aruanus*) were selected for this experiment, ranging in size from 3 to 24 individuals and each colony was assayed once, half were assayed during high tide and half during low tide. A trial was considered to occur at one of the two tidal categories if it

was performed within 2 hours either side of the maximum or minimum tidal amplitude. The colonies occupied *Pocillopora damicornis* and *Acropora palifera* coral heads and colonies had to be more than 5m from another colony to reduce the chance that fish would travel between colonies, which occurs when the coral heads are closely packed or continuous (Öhman et al. 1998). Care was taken to ensure the colonies used in the two treatments were spatially mixed and not clumped to reduce confounding effects of environmental variables. A precise block design was not possible, however, due to the natural distribution of colonies and because the priority was to have a similar range of group sizes between the two tidal treatments.

Fright stimulus

The fright stimulus apparatus (hereafter "apparatus") was a custom made device with an aluminum frame with a blue and white 28cm long rubber fishing lure (Williamson® Live Little Tunny Skip Jack 6951221) attached to a zip line made from monofilament line. A pulley system allowed for the user to stand 250cm from the humbug colony and shoot the model predator forwards 200cm. Care was taken to ensure the model predator approached each colony at a consistent speed of approximately 2 ms^{-1}. The apparatus was placed 50cm to the right of the colony and the model predator would reach the colony at a consistent angle and height (50 cm) from the sea floor (Figure 1). The apparatus was weighed down with two pairs of 2kg weights attached with cable ties so that it did not move in the current or when force was applied to propel the model predator towards the colony. All experiments were conducted while snorkeling at depths ranging from 160 and 330cm.

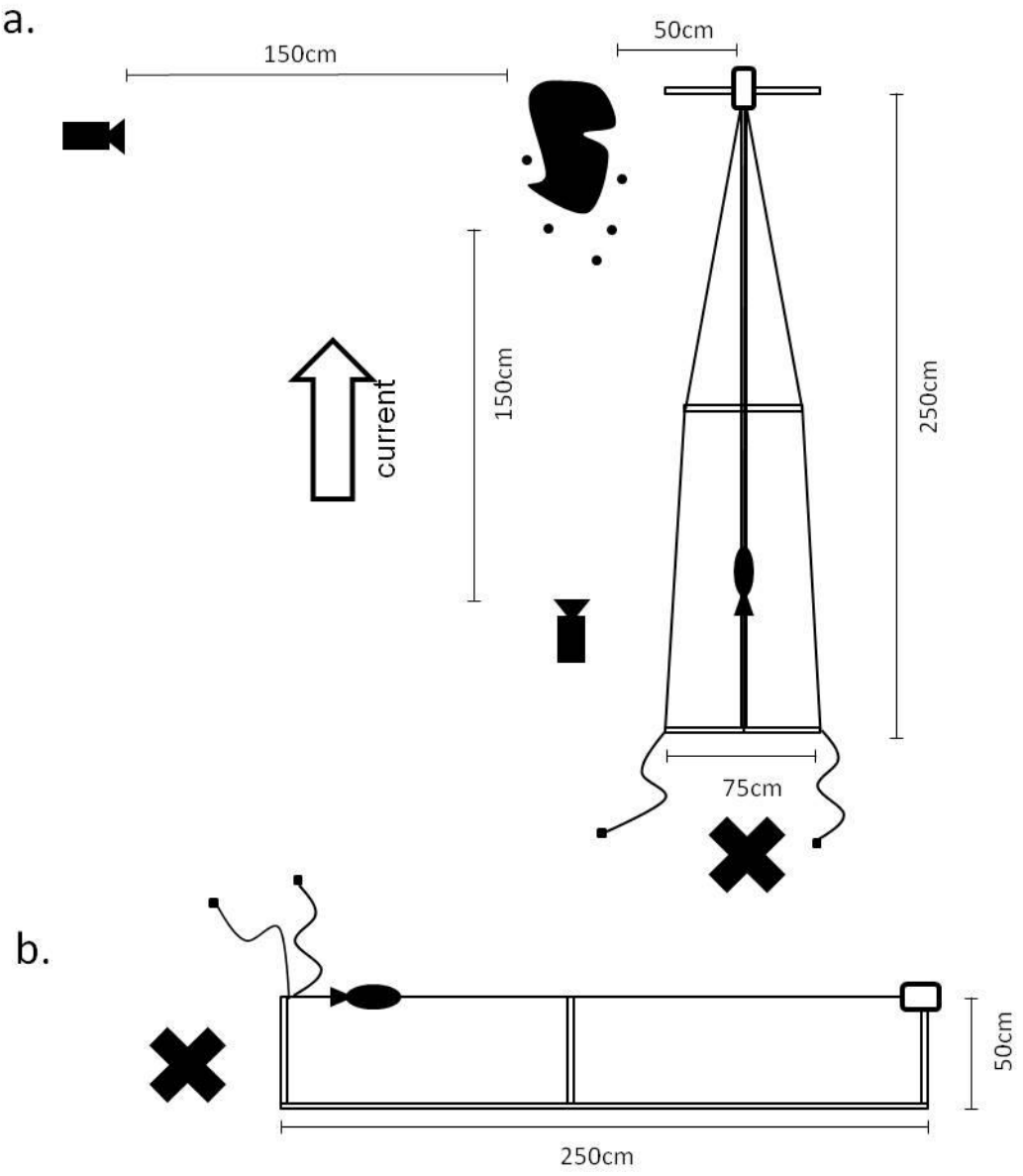

Figure 1. Diagram of experimental set-up showing placement of the fright stimulus apparatus in a.) aerial and b.) profile perspectives in relation to the direction of the current and position of the coral head (irregular black shape, dots represent fish), cameras and position of experimenter (X).

Experimental procedures

A colony was located and in preparation for the assay the apparatus was placed to the right of the coral head facing directly downstream of the current tidal flow (Figure 1). After a period of 10 min the experimenter would then place two Panasonic LUMIX underwater HD cameras 1.5m from the coral and start the film. One camera would film from the left side and one from directly in front of the colony (Figure 1). The experimenter would then stand still at the end of the apparatus for 5 min to allow the colony to resume normal foraging behaviour before pulling the fishing line and propelling the model predator towards the colony. Pilot tests confirmed that 5min was ample time for the fish to resume normal feeding behaviour. The experimenter then stayed still for the next 2 min before moving to stop the film on both cameras.

Data Collection

The videos from both cameras were converted from .wmv to .avs format with DirectShowSource. The .avs files were then opened with VirtualDub (v 1.9.11) and the video was converted from 15 frames per second to 1 frame per second. The footage 60 sec before and 60 sec after the fright stimulus was exported as a stack of 120 individual .jpeg images and opened with imageJ (Image Processing and Analysis in Java, version 1.48, 2014). Here the number of fish that were outside of the coral head were counted for each frame. A fish was considered outside of the coral head if its whole body could be seen without any coral obstructing its body. This was done for both camera angles and the largest value from either camera angle was considered as the maximum number of fish

emerged at that frame. This value was then divided by the total number of fish in the colony to give a proportion of fish emerged from the coral head every frame.

Data Analysis

Do tide and shoal size affect mean emergence?

A linear mixed effects model was used to assess whether the fright stimulus was effective, by evaluating the effect of stage (before or after the stimulus) on the proportion of fish emerged. To control for the repeated-measures nature of the data (each shoal was assessed multiple times), we included shoal identity as the random factor in the model. Throughout our analysis, proportion emerged was arcsin transformed to meet the assumptions of normality, which was assessed through visual inspection of quantile-quantile plots and plots of standardised residuals against fitted values.

We used linear mixed effects (LME) models to assess the effect of tide (high/low), group size and their interaction on the proportion of fish emerged from the coral head during the 60 seconds before and the 60 seconds after the fright stimulus. Non-significant interactions were removed following Crawley (2005) and only main effects are presented here.

Does the emergence pattern vary as a function of shoal size and tide?

Next, we assessed whether the pattern of emergence from the coral head differed depending on shoal size and tide. For each shoal, we calculated the mean and maximum proportion of the shoal that had emerged from the coral head by 5 time points after the

stimulus: 5, 10, 15, 30 and 60 seconds. We also calculated the time at which the maximum for each time category was reached. We used linear models to assess the effect of tide, shoal size and their interaction on the response variables, which were arcsin transformed to meet the assumptions of normality, assessed through visual inspection of plots as above. To account for multiple testing of similar data, p-values are adjusted following Benjamini & Hochberg's (1995) method for false discovery rate control across all 30 values (Table 1). Original and adjusted p-values are presented here.

For each colony, the number of fish emerged at each timepoint was converted to a proportion of the maximum number emerged from that colony during the trial period

Results

Do tide and shoal size affect mean emergence?

The fright stimulus was effective in causing fish to hide: there was a significant effect of stage (before/after) on the proportion emerged ($t = 58.852$, $df = 6719$, $p < 0.001$), which was lower in the minute after the stimulus than in the minute before the stimulus (Figure 2). There was no effect of either shoal size ($t = -0.625$, $df = 53$, $p = 0.535$) or tide ($t = -0.945$, $df = 53$, $p = 0.349$) on the proportion of fish emerged from the coral head before the fright stimulus, and no interaction. After the fright stimulus, however, both shoal size ($t = 4.214$, $df = 53$, $p < 0.001$) and tide ($t = -2.470$, $df = 53$, $p = 0.017$), but not their interaction, affected the proportion of fish emerged from the coral head. After the stimulus, a greater proportion emerged at high tide (Figure 2, 3) and in larger groups (Figure 3) than at low tide or in smaller groups, respectively.

Table 1. Linear models assessing the effect of tide and shoal size on a) the mean emergence, b) the maximum emergence, and c) the time to maximum emergence by 5, 10, 15, 30 and 60 seconds. P-values that remained significant after FDR control are highlighted in bold.

Time	Variable	Estimate	Std Error	t value	Model p	Adjusted p
a) Mean emergence						
5 s	Tide	-0.149	0.049	-3.052	0.004	**0.012**
	Shoal size	0.001	0.005	0.270	0.788	0.815
10 s	Tide	-0.155	0.056	-2.762	0.008	**0.016**
	Shoal size	0.012	0.006	1.954	0.056	0.076
15 s	Tide	-0.173	0.060	-2.890	0.006	**0.014**
	Shoal size	0.019	0.007	2.764	0.008	**0.017**
30 s	Tide	-0.169	0.060	-2.825	0.007	**0.015**
	Shoal size	0.025	0.007	3.792	<0.001	**0.003**
60 s	Tide	-0.134	0.053	-2.502	0.015	**0.026**
	Shoal size	0.026	0.006	4.354	<0.001	**<0.001**
b) Maximum emergence						
5 s	Tide	-0.241	0.080	-3.017	0.004	**0.012**
	Shoal size	0.007	0.009	0.824	0.414	0.496
10 s	Tide	-0.245	0.085	-2.895	0.005	**0.015**
	Shoal size	0.026	0.010	2.732	0.009	**0.016**
15 s	Tide	-0.268	0.085	-3.157	0.003	**0.010**
	Shoal size	0.030	0.010	3.182	0.002	**0.010**
30 s	Tide	-0.231	0.088	-2.622	0.011	**0.020**
	Shoal size	0.032	0.010	3.268	0.002	**0.010**
60 s	Tide	-0.124	0.102	-1.225	0.226	0.295
	Shoal size	0.0269	0.011	2.350	0.023	**0.034**
c) Time to maximum emergence						
5 s	Tide	-0.096	0.116	-0.824	0.414	0.477
	Shoal size	0.048	0.013	3.71	<0.001	**0.003**
10 s	Tide	-0.047	0.144	-0.328	0.744	0.797
	Shoal size	0.079	0.016	4.906	<0.001	**<0.001**
15 s	Tide	-0.096	0.168	-0.57	0.571	0.635
	Shoal size	0.089	0.019	4.719	<0.001	**<0.001**
30 s	Tide	0.490	0.228	2.144	0.037	0.052
	Shoal size	0.062	0.026	2.399	0.020	**0.032**
60 s	Tide	0.199	0.210	0.948	0.347	0.434
	Shoal size	0.001	0.024	0.048	0.962	0.962

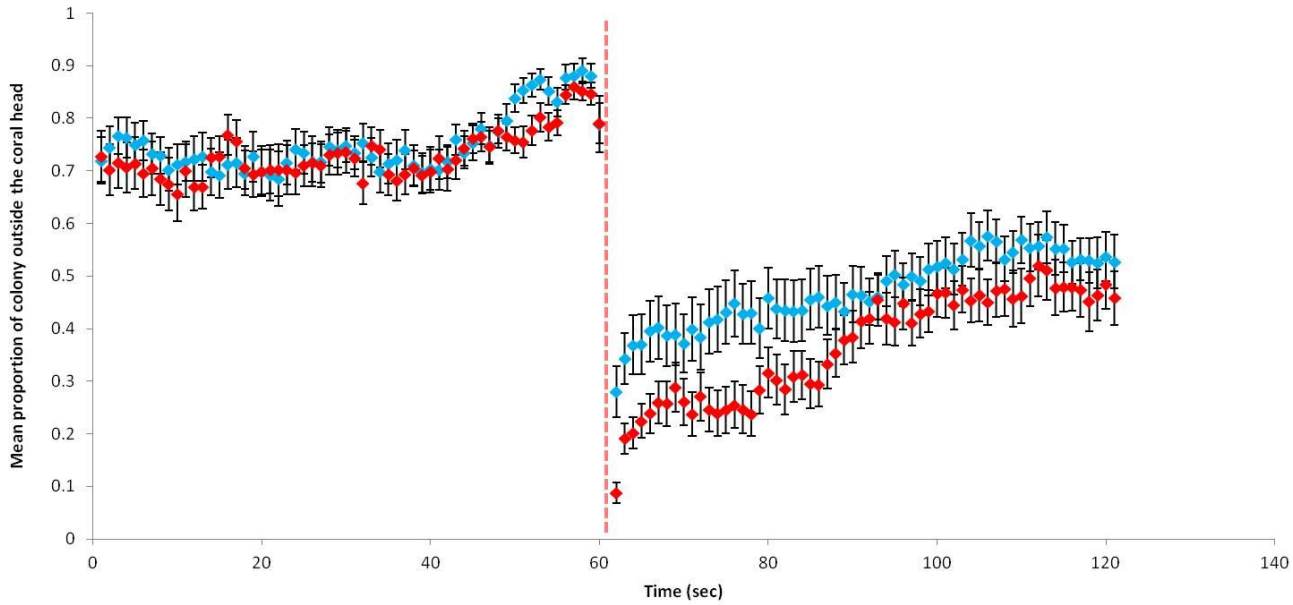

Figure 2. The mean proportion of the colony that is outside of the coral head at each frame (1 frame per sec) 60 sec before and 60 sec after the fright stimulus. The dotted red line represents the time at which the fright stimulus reached the colony. Blue markers represent the mean of colonies assayed at high tide, red markers represent the mean of the colonies assayed at low tide. Error bars are standard error of the mean.

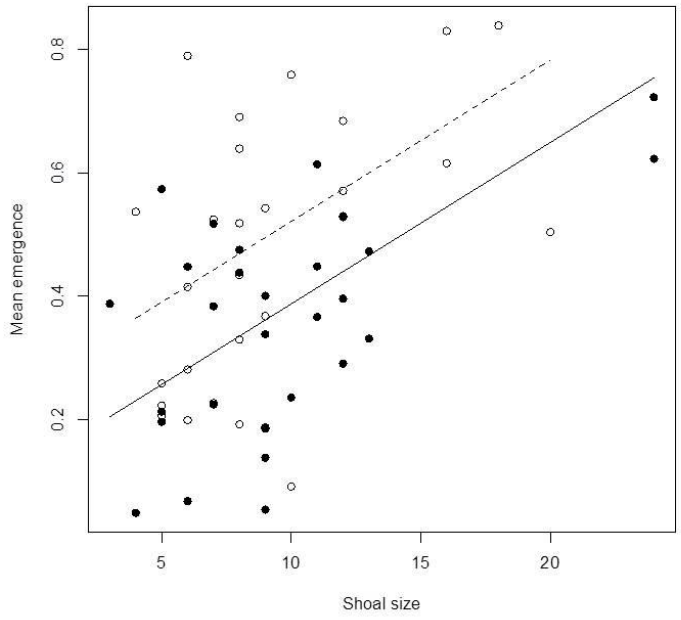

Figure 3. The mean proportion of fish emerged as a function of shoal size, at high (open circles, dashed line) and low (filled circles, solid line) tide. Fit lines are extracted from a linear model assessing the effect of shoal size and tide on the mean proportion emergence (arcsin transformed) for each shoal (tide: t = -2.502, p = 0.015, shoal size: t = 4.354, p < 0.001).

Does the emergence pattern vary as a function of shoal size and tide?

In the first 5-10 seconds after emergence, only tide had a significant effect on mean and maximum proportion emerged (Table 1a, b, Figure 4a, c), while there was no effect of shoal size. After this, both tide and shoal size (but not their interaction) determined the proportion of fish that emerged from the coral head (Table 1a, b). Shoals reached a higher mean and maximum emergence at high tide compared with low tide (Figure 4a-d), and although this was independent of group size 5 seconds after the fright stimulus (Figure 4 a, c), by 30 seconds, larger shoals had higher level of emergence than smaller shoals (Figure

4b, c). In contrast, only shoal size determined the time at which maximum emergence was reached (Table 1c), with larger shoals taking longer to reach maximum emergence than smaller shoals. Together, this suggests that larger shoals emerge more slowly, but to a greater proportion, than smaller shoals, and a greater proportion of fish emerge at high tide, independent of shoal group size.

Discussion

All shoals showed similar levels of emergence before the fright stimulus, regardless of shoal size and the state of the tide, and the fright stimulus was effective in reducing the proportion of the colony outside of the coral head in the immediate aftermath of the simulated attack (Figure 2). Both tide and shoal size affected how the fish responded to the fright stimulus with a greater proportion of the colony emerging at high tide (when food availability is highest) and in larger groups (where predation risk is likely reduced).

Humbug damselfish, like many animals who live under threat of attack, appear to act as risk balancers (Pitcher et al. 1988), emerging more quickly from their refuge when in larger groups and when there is more food. It is probable that humbug damselfish were less affected by the perceived risk of the fright stimulus at high tide as they traded off the risk of predation for the increased foraging opportunities at high tide, and indeed in this experiment fish were more polarized in the water column and seemed to be feeding more actively and at a greater rate during high tide, when we know plankton density is greatest (Forrester 1991).

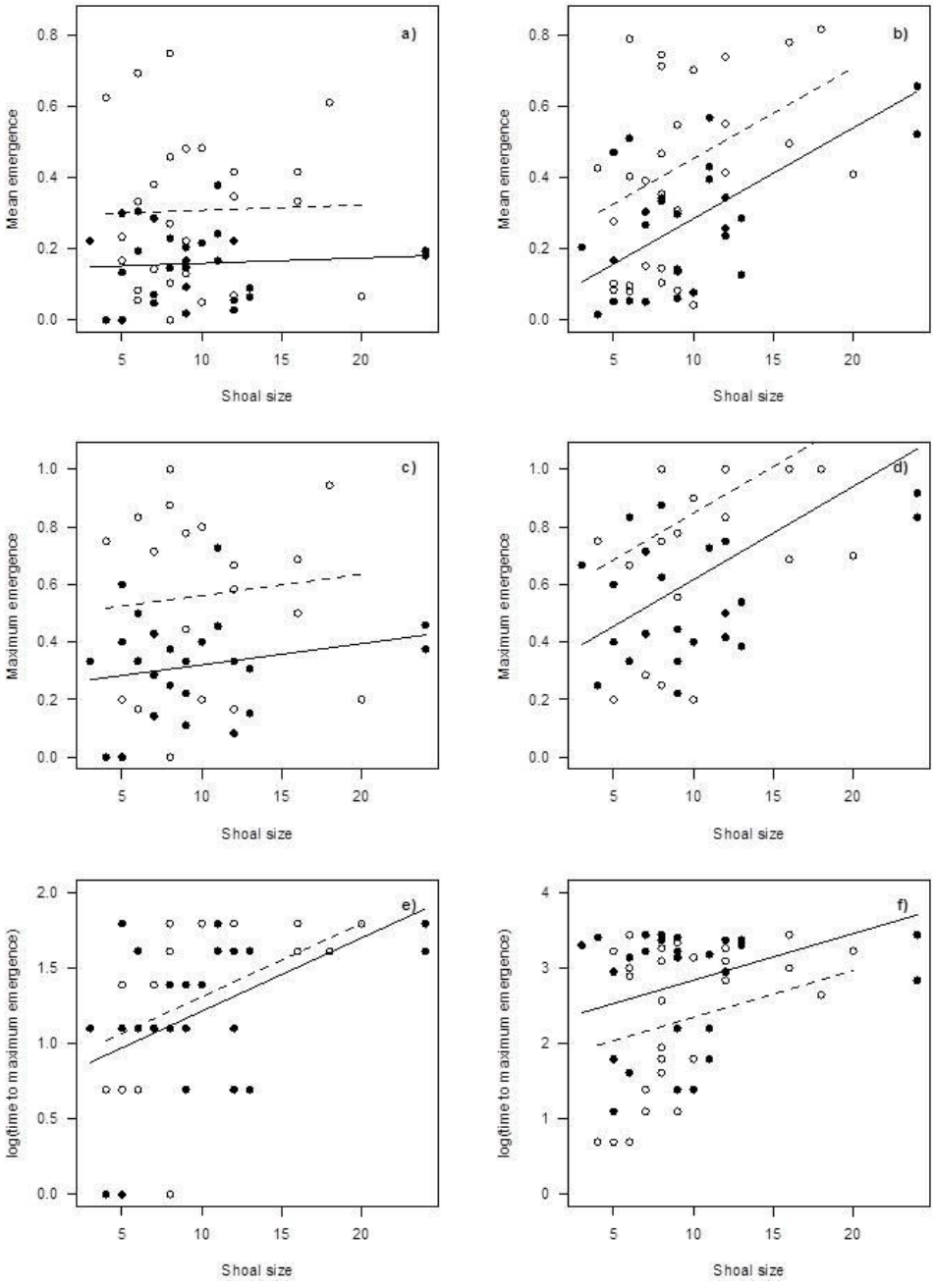

Figure 4. The mean (a, b) and maximum (c, d) proportion of fish emerged from the coral head, and the time to maximum emergence (e, f) at 5 seconds (left column) and 30 seconds (right column). Data are presented as a function of shoal size, at high (open circles, dashed line) and low (filled circles, solid line) tide.

Considering all three response variables (mean emergence, maximum emergence and time to maximum emergence) both tide and shoal size were important in determining the pattern of emergence behaviour after the fright stimulus (Table 1, Figure 4). A greater proportion of larger shoals emerged, however shoal size was not important in determining the pattern of emergence in the first 10 sec after the fright stimulus, suggesting the immediate response to the fright stimulus was similar for groups of differing size. However, after the initial 10 sec a greater proportion of group members emerged from larger colonies in comparison to smaller colonies. Distinguishing between potential causes of this pattern is problematic, it may be that each individual's assessment of its own per capita risk was lower in larger groups, or it may be because larger groups assessed the potential predation risk more accurately than smaller groups(Morgan 1988; Ward et al. 2011). Larger groups are generally more effective at collecting and integrating information and then using the information to make effective decisions than smaller groups(Couzin 2009). It is likely that a combination of increased group decision accuracy and the dilution effect contributed to the observed pattern.

Whilst shoal size was only an important factor affecting the proportion of the colony that emerged after the first 10 sec, the state of the tide was an important factor for the entire period following the fright stimulus, with a higher mean and maximum emergence occurring at high tide, independent of shoal size. Whilst there is more food in the water column for fish at high tide, there is often also an increase in predation threat, especially if high tide coincides with dawn or dusk(Munz and McFarland 1973; Helfman 1986). In this

experiment, there were certainly more predators active during high tide, predominately large schools of piscivores such as spangled emperors (*Lethrinus nebulosus*), and occasionally the damselfish made directed movements towards their coral heads as large predatory fish swam past (Pers. obs.). Despite this increased predatory threat at high tide, humbug damselfish still feed more at this time(Forrester 1991), which suggests that feeding efficiency is large enough to overcome their tendency to display risk sensitive behavior in the face of a threat.

Sixty seconds after the fright stimulus the proportion of fish outside of the coral head still had not returned to the levels before the fright stimulus, regardless of tide or shoal size. We should expect a gradual return to foraging activity levels, or perhaps even an increase to overcome the opportunities lost whilst in hiding, however, this will increase in relation to the time passed since the predation threat and depend on the severity of the threat and the likelihood of the threat returning. Juvenile Atlantic salmon, 20 min after the predation threat, only returned to 33% of their pre-predator intake rates (Metcalfe et al. 1987). Although actual intake rates were not calculated, Humbug damselfish responded surprisingly quickly to the predation threat, returning to a vulnerable position where foraging was once again possible. Perhaps it was because they face constantly high levels of predation risk threat on the coral reef and have adapted to recover from a threat quickly (particularly a false one) in order to achieve a sufficient intake of energy. Guppies from environments with high levels of predation are known to feed at greater rates and display greater tenacity after a predation threat than guppies from low predation environments.(Fraser and Gilliam 1987) Another possible explanation for the fast nature of

the damselfish's response to a predation threat is that it is driven by competition for resources. If competition is high for resources, which is probable in areas with a high predation threat, larger groups are expected to emerge faster than smaller groups. Larger shoals, however, emerged more slowly - they reached their maximum emergence later than the smaller shoals (Table 1c).

Inter-individual variation between damselfish (for example size) was not recorded in this study, however, it is known that larger individuals feed further from the coral head than smaller individuals (Forrester 1991) where they have first selection of preferred prey (Coates 1980) and this is strongly related to their linear dominance hierarchy. African cichlid fish, *Melanochromis chipokae,* further from a shelter begin their retreat to safety before fish closer to the shelter (Dill 1990) and in many bird species, the sequence of the resumption of feeding after a predation threat follows the dominance hierarchy with subordinates emerging first(Hegner 1985; Laet 1985; Hogstad 1988;) (it is suggested that subordinance in these systems may be strongly correlated with energetic need (Lima and Dill 1990)). Future research would do well to focus on individual phenotypic variability within groups, how it interacts with differences in internal state, and whether it can predict the first responder to a threat or how information is transferred throughout the group. Humbug damselfish colonies are an appropriate study system to answer these questions.

This experiment has tested how humbug damselfish colonies under natural environmental conditions respond to a predation threat. Humbug damselfish change their decision-making process after a predation threat in relation to the tide and shoal size. A greater proportion of the colony emerge after the fright stimulus at high tide and they show evidence of a social

response in larger shoals with a greater proportion of the colony emerging in larger shoals, however, contrary to expectation they did so slower than small shoals. This is the first study, to the authors' knowledge, to show that shoals of fish change their collective behavioural response to a predation threat in accordance with the tide. The humbug damselfish system has previously been used to explore the mechanisms of group movement decisions (Mann et al. 2013; Ward et al. 2013), however, this finding, that the state of the tide affects emergence behaviour, allows us to conduct new experiments to further our understanding of the effect of risk sensitivity on decision-making and information transfer - whilst simultaneously controlling for inter-group differences by performing repeated measures on the same colony at different tides.

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Chapter 7

ENVIRONMENTAL QUALITY DETERMINES FINDER-JOINER DYNAMICS IN SOCIALLY FORAGING THREE-SPINED STICKLEBACKS (*GASTEROSTEUS ACULATEUS*)

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Animals that forage in groups have access to social information concerning the quality and location of food resources available. The degree to which individuals rely on social information over their own private information depends on a myriad of ecological and social factors. In general, where resources are patchy in space and/or time, individuals that use social information and join others at previously identified food patches can reduce both search times and the variance in finding food. Here, we explore social foraging dynamics of shoals of three-spined sticklebacks (*Gasterosteus aculeatus*) and investigate when fish tend to use private information and find food themselves, or rely on social information and attend to the food discoveries of others. We show that fish's allocation to alternative foraging tactics (i.e. finding or joining) can be explained by environmental quality. In environments with high-quality food patches, fish experience a reduced finder's share and tend to adopt joining foraging tactics which result in dense co-feeding networks. In contrast, in low-quality environments, fish rely on private nformation, discovering their own food patches and rarely co-feeding. However, we found that tactic use does not result in equal foraging returns as predicted by theory, and payoffs were higher for finding in all environments we studied. Furthermore, we found no evidence that individuals consistently differed in their tendency to adopt either finder or joiner tactics and suggest that social foraging in three-spined sticklebacks represents an information sharing system. Overall, our simple experimental approach suggests that socially foraging three-spined sticklebacks show flexible behavioural rules enabling them to efficiently exploit food patches under a range of environmental conditions.

Keywords: Finder-joiner dynamics, social foraging, information sharing, social networks, three-spined sticklebacks

Introduction

Social animals can gather 'personal information' directly from environmental cues and 'social information' from the behaviour of conspecifics (Dall et al. 2005). In a foraging context, where resources are patchy in space and/or time, those individuals that use social information (i.e. attend to cues that provide information about the foraging success of conspecifics) can reduce both search times and the variance in finding food (Caraco 1981; Caraco and Giraldeau 1991; Clark and Mangel 1984; Ranta et al. 1993; Ruxton et al. 1995). However, the payoff for an individual relying upon social information decreases with an increasing number of conspecifics also using social information (Clark and Mangel 1986; Vickery et al. 1991; Barta and Giraldeau 2001; Beauchamp 2008; Kurvers et al. 2012). This is best understood by considering individuals that rely on personal information to 'find' food patches, and those relying on social information to 'join' others at food patches (Coolen et al. 2001). The more individuals choosing to join others at food patches, the greater the payoff to finding your own patch and acquiring a greater share of the resource (termed the 'finder's share') (Giraldeau and Caraco 2000).

If foraging animals can simultaneously search for and find food, while also monitoring the behavior of conspecifics for joining opportunities, then the system can be classified as an 'information sharing' system with foragers considered 'opportunists' (Clark and Mangel 1984; Vickery et al. 1991; Giraldeau and Caraco 2000). Conversely, if finding and joining are incompatible tactics, or doing both is costly, then individuals may adopt the tactic that provides the greatest expected returns; this is considered a 'producer-scrounger' system

(Barnard and Sibly 1981; Giraldeau and Caraco 2000). In producer-scrounger systems, the adoption of either tactic is frequency dependent whereby the payoffs for scrounging decrease with increasing number of individuals adopting this tactic (Caraco and Giraldea 1991). Accordingly, individuals are expected to converge to an equilibrium ratio of 'producers' and 'scroungers' in which both tactics attain the same payoff (Mottley and Giraldeau 2000).

The decisions of socially foraging animals to either (1) gather their own information and act as producers, (2) rely on others' information and act as scroungers, or (3) flexibly respond to both personal and social information and adopt either tactic are affected by a myriad of ecological and social factors. The single most important factor, however, is the quality and distribution of food resources (Giraldeau and Caraco 2000). If food resources in the environment are dispersed and low value, then the finder's share will be large and consequently, the majority of a population should independently search for food and rely on personal information. In contrast, where food resources are clumped (i.e. low density) and of high value, then this should promote the use of social information by foraging individuals. These predictions, generated by agent-based and theoretical work (Waltz 1982; Clark and Mangel 1986; Caraco and Giraldea 1991; Vickery et al. 1991; Barta and Giraldeau 2001; Beauchamp 2008, 2004; Kurvers et al. 2012) are supported by a number of empirical tests (e.g. Koops and Giraldeau 1996; Giraldeau and Livoreil 1998; Coolen et al. 2001; Beauchamp 2014, 2013).

Much recent work into social foraging theory has focused on consistent individual differences in tactic use (Beauchamp 2001; Mathot et al. 2009; Morand-Ferron et al.

2011a), and how and when intrinsic differences in dominance (Barta and Giraldeau 1998; Liker and Barta 2002; McCormack et al. 2007; King et al. 2009), metabolism (Mathot et al. 2009), exploratory tendency (Kurvers et al. 2009; Kurvers et al. 2012), sex (Pfeffer et al. 2002; King et al. 2009) and kinship (Vickery et al. 1991; Tóth et al. 2009; Mathot and Giraldeau 2010), may lead to an individual focusing on one foraging tactic over the other. Other work has looked at frequency dependent reward dynamics and how rewards from past foraging decisions will affect subsequent decisions (Giraldeau 1984; Giraldeau and Caraco 2000; Giraldeau and Dubois 2008; Katsnelson et al. 2008; Morand-Ferron and Giraldeau 2010; Morand-Ferron et al. 2011b; Dubois et al. 2012).

Using a social network approach, we are now gaining a better understanding of how individual differences in foraging behaviour can affect the structure and functioning of groups (Aplin et al. 2013). Since social information is transferred non-randomly amongst interacting individuals, this causes variation in the type and amount of information available to individuals at any one time (Croft et al. 2008; Krause et al. 2009) and an individual's position within a social network can predict foraging tactic use (Aplin et al. 2012). Specifically, individuals that are more central or well connected in a network have a higher probability of receiving and acting upon social information relating to the location of novel food patches, and thus, are better placed to adopt a joiner role (King et al. 2011). The social structuring of a group may also be associated with its spatial characteristics and individuals in the centre of groups more frequently engage in joining behaviour for the same reason (Barta et al. 1997; King et al. 2009).

Although social foraging theory is now well developed a vast majority of empirical tests have been conducted on birds in captive environments (Beauchamp 2013), with only a handful of tests on birds foraging in their natural environment (e.g. Morand-Ferron et al. 2007: *Quiscalus lugubris;* Beauchamp 2014: *Calidris pusilla*) and some investigations into social foraging theory in wild primates e.g., (King et al. 2009: *Papio ursinus*; Bicca-Marques and Garber 2004: *Saguinus sp*; Di Bitetti and Janson 2001: *Cebus apella*). The main reason for this bias in species and context is that distinguishing the tactic used by an animal, the boundaries of patches, and the individual pay-offs for discrete foraging events are experimental/observational hurdles that can prove difficult to clear. Consequently, much experimental work in laboratory settings looking at finder-joiner behaviour involves constraining individuals to one of the two tactics using specially designed apparatus (Mottley and Giraldeau 2000), or training a proportion of individuals in a foraging task so that when combined with naïve individuals only the trained individuals can express the finding foraging tactic (Ólafsdóttir et al. 2014). While this is extremely valuable and often necessary when testing predictions from producer-scrounger theory, it is less likely to represent social foraging behaviour in the wild, where animals may well perform both tactics either in consecutive foraging events or simultaneously (King et al. 2009).

Fish have a long history of being used as subjects for empirical explorations of foraging theory, particularly in relation to competition theory (reviewed by Ward et al. 2006) and ideal free distribution theory (reviewed by Milinski 1988). However, fish have rarely been used to explore finder-joiner dynamics (but see Hamilton and Dill 2003; Ólafsdóttir et al.

2014). There are considerable benefits to using fish to explore finder-joiner dynamics: (1) foraging behaviour of individual fish in shoals has been shown to be flexible in response to changes in the distribution of resources in the environment (Ryer and Olla 1992, 1995), (2) the experimental manipulation of individual state, group composition and the environment is relatively simple, and (3) they are found in a vast array of habitats and hence are morphologically and behaviourally very diverse. Finally, the experimental arenas for fish are often smaller than for other vertebrates and the entire experimental space can be filmed more accurately. Therefore, how an individual's behaviour is affected by its conspecifics at any given time can be reliably inferred. Three-spined sticklebacks (*Gasterosteus aculeatus*) are often used in foraging studies (Ranta and Juvonen 1993; Ólafsdóttir et al. 2014) and have recently been used as a model system to explore social learning and the trade-off between using individual and social information (Webster and Hart 2006; Laland et al. 2011; Webster and Laland 2012). As such they are a good choice of fish to extend and build our understanding of finder-joiner dynamics.

Here we explore the finder-joiner dynamics of socially foraging three-spined sticklebacks and ask to what degree fishes' allocation to alternative foraging strategies can be explained by environmental quality. We expected that the relative frequency of finding behaviour should decrease in environments with large and/or clumped food patches as a result of a reduced finder's share (**prediction 1**) (Giraldeau et al. 1990; Giraldeau and Livoreil 1998), resulting in larger co-feeding networks in these environments (**prediction 2**) (Krause et al. 2009; Aplin et al. 2012), and such adjustments should result in approximately equal foraging returns for the use of either tactic (**prediction 3**) (Mottley and Giraldeau 2000).

We also tested for the possibility that individuals may differ consistently in their intrinsic tendency to adopt either finder or joiner behaviour, independent of any differences in their knowledge of the environment which may reflect inter-individual differences (Morand-Ferron et al. 2011a; King et al. 2009; Kurvers et al. 2009).

Methods

Study Animals

Subjects were N=48 three-spined sticklebacks (*Gasterosteus aculeatus*), wild-caught on Swansea University campus, Wales, UK (mean ± SD = 1.12±0.26g). Subjects were kept in a holding tank (300 x 390 x 1220 mm) containing gravel substrate, plants and driftwood for 8 weeks prior to the experiment at a consistent temperature of 17°C at 8L:16D photoperiod regime. On day 1 of the experiment, 24 fish were weighed and a 6mm diameter circular plastic identification tag was placed on their first dorsal spine (Webster and Laland 2009) (Fig 1a). Fish were randomly allocated to groups of n=6 according to their identification tags (six blue, black, green, white, blue-white and yellow tags were used) resulting in four groups of 6 fish: A, B, C or D before being placed into individual 2.8L gravel-lined, aerated tanks. We used mixed sex groups of non-reproductive adults. The following day (day 2) this procedure was repeated with another 24 fish and they were randomly allocated to groups E, F, G or H. Fish remained in these individual tanks for the experimental period when not being assayed. Water was changed every two days and all fish were fed 5 defrosted bloodworms (Chironomid larvae) at 9am everyday that they were

not being assayed. Two days after being housed in individual tanks, fish were habituated to the experimental arena (see below) in their allocated groups for 60 min.

Experimental Arena and Environmental Treatments

Four identical experimental arenas were placed next to each other on the laboratory floor. The arenas were created by inserting a green plastic grid structure into a clear plastic tank (500 x 650 x 120 mm) (see (Webster and Laland 2012) for a description of a similar set-up). The plastic grid structure was made up of 100 x 100 mm squares that were 60 mm deep. We filled the grid with 30 mm of white gravel leaving 30 mm visible (Fig 1a). We filled the test arena with aged aerated water to 40 mm above the grid structure, meaning the maximum depth was 70 mm. Defrosted bloodworms could be placed onto the gravel within any grid square to create distinct foraging patches. This key feature of our experimental design meant that the head of a fish had to be within the grid square for it to be able to see the bloodworms (Webster and Laland 2012), and thus, we defined our grids as 'patches'. White card was placed between the four arenas and all four arenas were surrounded by white screen (PhotoSEL BK13CW White Screen) held up by a custom built metal frame (Fig 1b). Four photographer's lights (each with 4 x 25w 240v 6400K True Day light bulbs) lit the arenas from outside the white sheet, dispersing light evenly over the four arenas. Experiments were filmed using 2 Panasonic HDC-SD60 HD video cameras each filmed two arenas (Panasonic Corporation of North America, Seraucus, NJ, USA) mounted above the arenas (Fig 1b).

We used a 2x2 experimental design to vary the foraging environment. Factor 1 was 'patch size' and had two levels - small (2 bloodworms per patch) and large (6 bloodworms per

patch). Factor 2 was 'patch distribution' and also had two levels – clumped and dispersed. In the clumped distribution there were three clumps of three patches. The three clumps were separated by two grid squares, and the three patches within the clumps were all directly next to each other. In the dispersed treatment, all 9 patches were separated by one grid square (Fig 1c). Therefore the 4 treatments were: small and clumped (SC), small and dispersed (SD), large and clumped (LC) and large and dispersed (LD) (Fig 1c). All fish were left for 2 days in their individual tanks before they were habituated to the experimental arena in their allocated groups for 60min. A day later, each group was then assayed once in each of the 4 treatments, with a day's rest in-between assays. Trial order was controlled for each group.

Experimental Procedure

At 13:00h the day prior to the experimental assay the arenas were set-up and filled with aged aerated water. At 9:30h on the day of the experimental assay bloodworms were distributed in each of the experimental arenas according to the allocated treatment (see above). The group of fish was then placed into a plastic container within the larger arena for 10 min before being released into the arena and allowed to forage for 30 min. The fish were released from the container by pulling on a monofilament line, extending outside of the experimental arenas and surrounding screen. After 30 min the fish were returned to their individual tanks and the arenas were cleaned and set-up for the next day's assay.

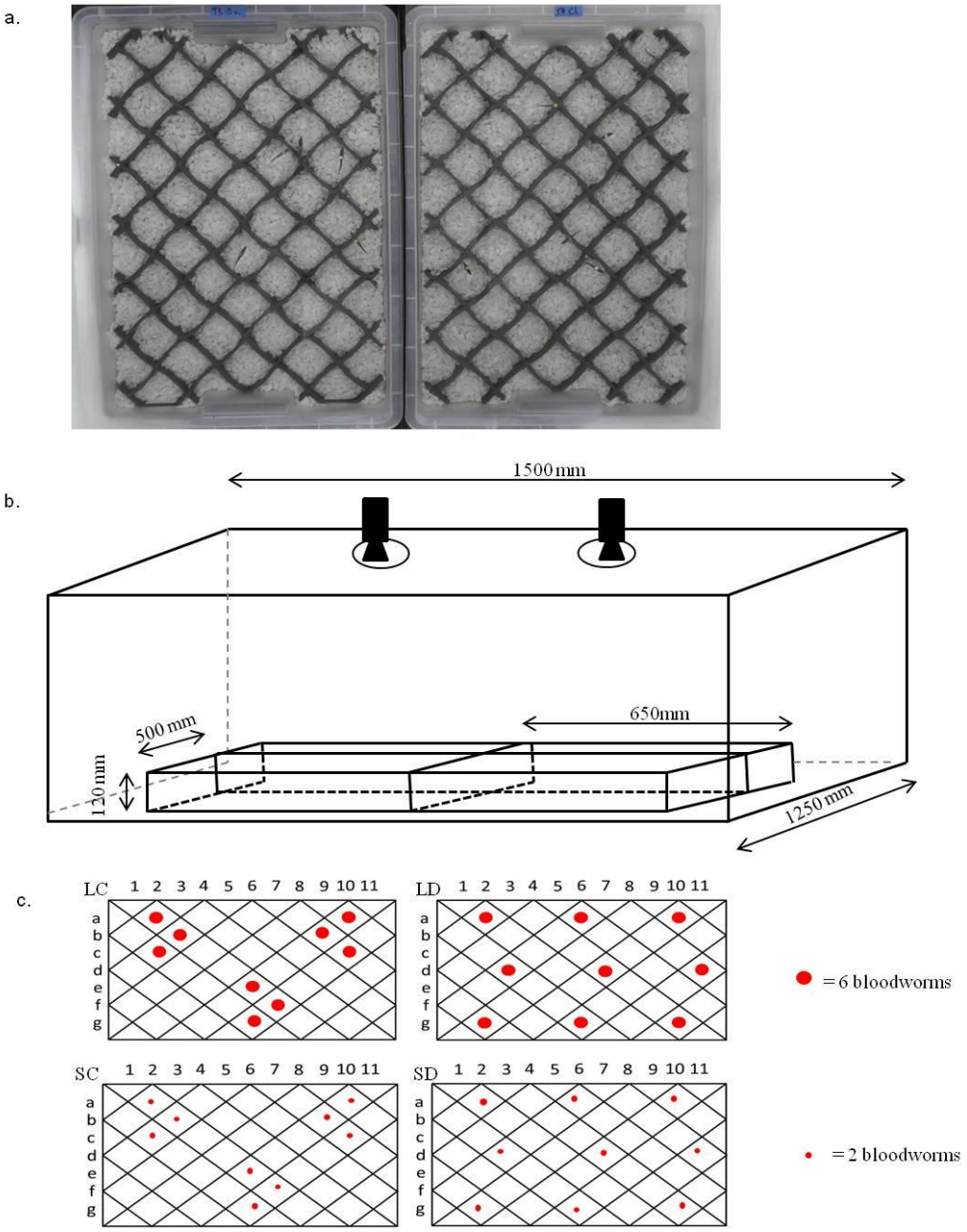

Fig 1. Experimental set-up; a.) Still-shot from experimental video of two arenas each with, individually marked fish (n=6). Fish are foraging from patches formed by the placement of green plastic grid on white gravel (see methods for details). b.) diagram of metal frame showing cameras mounted above the 4 experimental arenas. c.) the four experimental arenas showing the distribution of bloodworms in each of the 4 treatments: large and clumped (LC), large and dispersed (LD), small and clumped (SC) and small and dispersed (SD).

Data Collection

Videos were played back in VirtualDub (v 1.10.4, 1998-2012, Avery Lee) and each fish's behaviour was scored (one fish observed at a time). Every time a fish entered a patch containing bloodworm it was recorded. Following (Coolen et al. 2001), entering an unoccupied patch (by other fish) was considered "finding", whereas entering an occupied patch was considered "joining". If a fish entered an unoccupied patch and ingested at least one bloodworm, it was defined as a "finding event". If it failed to ingest the bloodworm, i.e. it pecked at it or if it subsequently spat the worm out after ingesting it (sticklebacks tend to do this as a means of manipulating the food to be able to swallow it), this was considered a "failed finding event". If a fish entered into a patch that was already occupied and ingested a worm, stole a worm out of a conspecific's mouth, or ingested a worm spat out by a conspecific, this was defined as a "joining event". If the fish entered an occupied patch but failed to ingest any bloodworm, or it attempted to steal but failed to ingest the worm, it was defined as a "failed joining event". If a conspecific had entered the patch beforehand, but the patch was unoccupied when the focal fish entered the patch and ate a bloodworm, this was still considered finding behaviour since it was not possible to know for sure whether the focal fish had attained information on the patch being previously discovered. However, if the focal fish made a directed movement towards a patch whilst a conspecific in that patch was feeding, and the focal fish subsequently ate a bloodworm from that patch then it was defined as joining behaviour (Table 1). For each foraging event recorded, we recorded: the time that the event occurred, the time passed since the focal fish's previous foraging event, the patch location where the foraging event took place, and

the identity of all other fish on the patch (where this was a joining event) as well as the identity of near-neighbours (i.e. fish within one grid square). We also recorded the number of bloodworms available at the patch before the foraging event, the event payoff (i.e. the number of bloodworms ingested by the fish), and the number of bloodworms available at the patch after the event. For an unknown reason, fish in Group H did not engage with the foraging trials (they did not eat) and so we could not use their data and are removed from all analyses.

Table 1. Definitions of behavioural tactics

Tactic	Success	Description of behaviour
Finding	Successful	-Focal fish enters an unoccupied patch and ingests ≥ 1 bloodworm
	Failed	-Focal fish enters an unoccupied patch and does not ingest a bloodworm, i.e. pecks at it or spits worm out
Joining	Successful	-Focal fish enters an occupied patch and ingests ≥ 1 bloodworm -Focal fish steals a worm out of a conspecifics mouth -Focal fish ingests a worm spat out by a conspecific
	Failed	-Focal fish enters an occupied patch and does not ingest a bloodworm, i.e. pecks at it or spits worm out -Focal fish attempts to steal a bloodworm from a conspecific but does not ingest it -Focal fish attempts to ingest a bloodworm spat out by a conspecific but does not ingest it

Statistical Analyses

To test whether the environment affected the percentage of available bloodworms eaten we fitted a linear mixed model (LMM). We fitted the percentage of bloodworms eaten as our response variable, and patch size (small, large) and patch distribution (dispersed, clumped) as fixed effects with group (A-G) and ID (1-42) fitted as random effects. This model, and

all mixed effect models described below were fitted in R (R Development Core Team, 2014, R i386 3.1.2) using *lme4* and g*lmer* packages (Bates et al. 2014) by maximum likelihood t-tests and used Satterthwaite approximations for degrees of freedom to approximate p-values.

To test prediction 1 we fitted a LMM with the percentage of an individual's events classified as "finding" as our response variable, and patch size (small, large) and patch distribution (dispersed, clumped) as fixed effects with group (A-G) and ID (1-42) fitted as random effects. We calculated the finder's share (a/F, where a = finder's advantage, the difference in amount of food items eaten when an individual finds and when it joins, and F = number of food items: (Giraldeau and Caraco 2000) across our four experimental treatments, and tested for differences across treatments using Wilcoxon signed rank test in SPSS (IBM® SPSS® Statistics, Version 20).

To test prediction 2, co-feeding networks were created based on the frequency with which fish were observed at the same foraging patch. Using these association matrices, we calculated each fish's (i) 'mean degree', which is the average of the number of individual node edges and (ii) 'network density', which is the fraction of possible node edges that are present in the network (Croft et al. 2008). These were weighted measures, the edge has a continuous value corresponding to the number of times the fish co-occurred. Networks were created using UCINET (v 6.504, 2002, Harvard, MA: Analytic Technologies). We then fitted two LMMs to explore what predicted our network measures. The first model had node degree as the response variable and patch size and distribution as fixed effects, and group and ID as random effects. The second model had network density as response

variable with patch size and distribution as fixed effects, and group as a random effect. A third model explored whether 'mean degree' predicted the proportion of feeding events that were "finding events" and was run separately for small and large patch environments as degree was correlated with patch size. We also conducted a Pearson's correlation between degree and the proportion of events that were finding events in each of the four environmental treatments.

To test prediction 3, we explored variation in individual foraging returns at the event level. We fitted a generalized linear mixed model (GLMM) with Poisson error structure. Event payoff (bloodworms consumed) was included as the response variable, and foraging decision (find, join), sex (male, female) and weight (g) were fitted as fixed effects. Group and ID were included as random effects.

We modified models to explore whether individuals varied consistently by testing the random effect ID for significance which would indicate that individuals vary consistently in their response (see e.g. Carter et al. 2012; Fürtbauer et al. 2015). In order to test whether individuals differed in their response to changes in treatment, we fitted models including fish ID as random intercept and treatment type as random slope (e.g. Carter et al. 2012; Fürtbauer et al. 2015).

Results

Descriptive statistics on the number of bloodworms eaten for each group during each treatment are summarised in Table 2 and Fig 2. In the small patch treatments, all 18

bloodworms provided were eaten, except for group C in the small-clumped distribution where they only ate 14. In the large treatments, no groups ate all the available 54 bloodworms, and on average 36±7 and 38±7 (mean ± standard deviation) bloodworms were eaten in the large-clumped and large-dispersed treatments respectively (Table 2). This meant that a larger percent of available blood worms were eaten in the environments with small patches compared to large patches (LMM: $t_{(1,124)}$= 4.823, p=0.0001) but the percent of available blood worms eaten was not affected by patch distribution (LMM: $t_{(1,124)}$= 0.755, p=0.452; Table 3a).

The frequency of finder tactic use was lower in environments with large patches (LMM: $t_{(1,121.19)}$= 3.306, p=0.001) in accordance with prediction 1, however, the distribution of resources within our small and large patch treatments had no effect (LMM: $t_{(1,121.27)}$= -1.083, p=0.28; Table 3b). Supporting and explaining these findings, the finder's share was significantly smaller in environments with large patches (Median=-0.02) compared to environments with small patches (Median=0.25) (T=1, p=0.018, r==0.89; Fig 3), however, the finder's share was not significantly different between clumped (Median=0.15) and dispersed (Median=0.16) treatments (T=10, p=0.5, r=-0.26).

Table 2. Descriptive statistics of the number of bloodworms eaten by finding (F) and joining (J) for each group in each treatment.

Number of Bloodworms Eaten												
	Small						Large					
	Clumped			Dispersed			Clumped			Dispersed		
Group	**F**	**J**	**T**	**F**	**J**	**T**	**F**	**J**	**T**	**F**	**J**	**T**
A	16	2	18	13	5	18	22	19	41	29	18	47
B	12	6	18	15	3	18	12	18	30	19	21	40
C	11	3	14	11	7	18	27	20	47	19	25	44
D	9	9	18	12	6	18	14	21	35	24	16	40
E	12	6	18	10	8	18	27	13	40	16	22	38
F	13	5	18	12	6	18	15	16	31	8	21	29
G	10	8	18	11	7	18	18	9	27	19	9	28
Mean	11.85714	5.571429	17.42857	12	6	18	19.28571	16.57143	35.85714	19.14286	18.85714	38
StDev	2.267787	2.507133	1.511858	1.632993	1.632993	0	6.156684	4.27618	7.12808	6.517376	5.209881	7.141428
Min	9	2	14	10	3	18	12	9	27	8	9	28
Max	16	9	18	15	8	18	27	21	47	29	25	47
Range	7	7	4	5	5	0	15	12	20	21	16	19

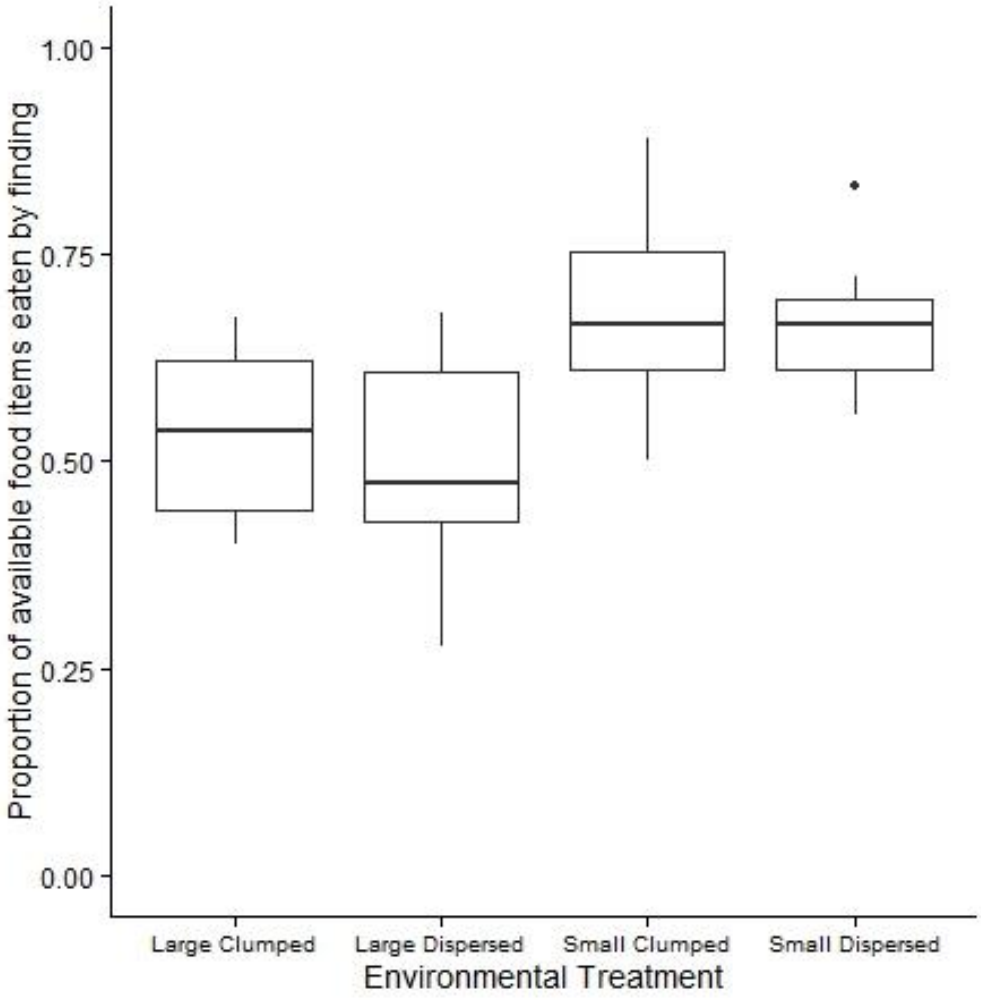

Fig 2. Boxplots representing the mean proportion of bloodworms eaten using the tactic 'finding' in the four treatments: Large and Clumped (SC), Large and Dispersed (SD), Small and Clumped (LC) and Small and Dispersed (LD). Boxes represent first and third quartiles and whiskers extend to the highest value that is within 1.5 times the inter-quartile range.

Table 3. The effect of patch size and distribution on a.) the percent of available bloodworms eaten by fish and b.) the percent of total events that were 'finding events', estimated from a linear mixed model. Group and fish identity were fitted as random effects. Significant p-values are presented in bold.

	Estimate	Standard Error	DF	t-value	Pr (>|t|)
a.) The percent of available bloodworms eaten					
(Intercept)	11.067	1.0561	122.11	10.479	
SIZE	5.0705	1.0513	124	4.823	**<0.0001**
DISTRIBUTION	0.7937	1.0513	124	0.755	0.452
b.) Percent of total events that were 'finding events'					
(Intercept)	45.488	3.568	131.67	12.747	
SIZE	12.51	3.784	121.19	3.306	**<0.002**
DISTRIBUTION	-4.096	3.783	121.27	-1.083	0.28097

Supporting prediction 2, co-feeding network measures 'mean degree' and 'network density' differed significantly across treatments, with groups having a higher 'mean degree' (LMM: $t_{(1,124)}$= 11.553, p<0.0001) and 'network density' (LMM: $t_{(1,25)}$= 6.179, p<0.0001) in their co-feeding networks when foraging in the large patch environments compared to the small patch environments. Distribution of patches within small and large-patch treatments had no significant effect on the 'mean degree' of co-feeding networks (LMM: $t_{(1,124)}$= 0.998, p=0.32), nor was 'network density' affected by the distribution of patches in the environment (LMM: $t_{(1,25)}$= 1.321, p=0.199) in line with our results above (Table 4a,b). The proportion of events that were finding events in the small patch environments was predicted by the 'mean degree' of co-feeding networks (LMM: $t_{(1,75.57)}$= -3.976, p=0.0002) so that the less connected the co-feeding networks, the higher the proportion of foraging events were finding events. Mean degree had no such effect in environments with large patches (LMM: $t_{(1,81.98)}$= -0.264, p=0.792; Table 4c,d).

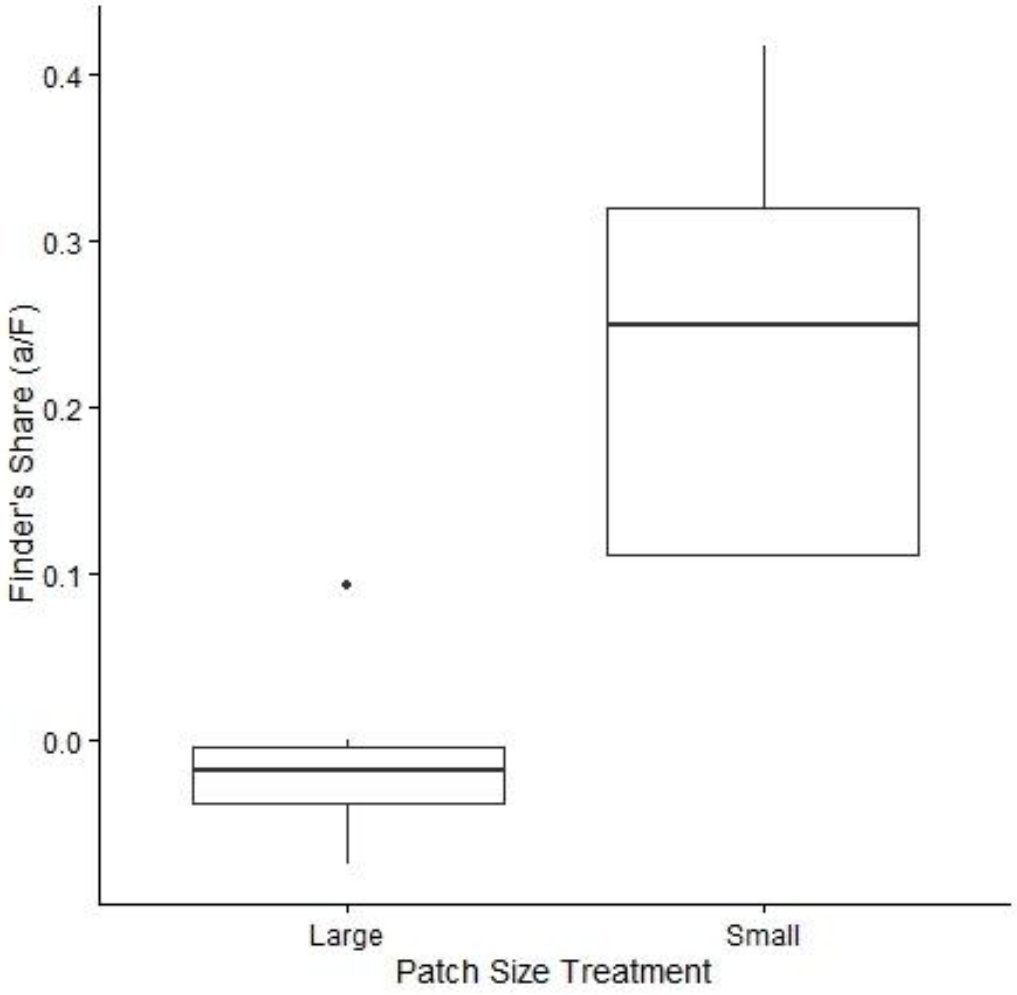

Fig 3. Boxplot of the median finder's share for the groups (n=7) in the large patch and small patch treatments. (a/F, where a = finder's advantage, the difference in amount of food items eaten when an individual finds and when it joins, and F = number of food items (Giraldeau & Caraco, 2000)). Boxes represent first and third quartiles and whiskers extend to the highest value that is within 1.5 times the inter-quartile range.

Table 4. The effect of size and distribution treatments on a.) mean degree (group and fish identity fitted as random effects) and b.) network density (group fitted as random effect), and the effect of mean degree on the proportion of events that were 'finding events' in c.) small and d.) large patch treatments estimated from linear mixed models. Significant p-values are presented in bold.

	Estimate	Standard Error	DF	t-value	Pr (>|t|)
a.) Node Degree					
(Intercept)	3.7976	0.1487	152.93	12.57	
SIZE	1.9286	0.1669	124	11.553	**<0.0001**
DISTRIBUTION	0.1667	0.1669	124	0.998	0.32
b.) Density					
(Intercept)	0.24464	0.04291	25	5.702	
SIZE	0.30614	0.04954	25	6.179	**<0.0001**
DISTRIBUTION	0.06543	0.04954	25	1.321	0.199
c.) Proportion of 'finding events' in small patch treatment					
(Intercept)	0.7817	0.06419	70.94	12.179	
DEGREE	-0.1065	0.02678	75.57	-3.976	**<0.0002**
d.) Proportion of 'finding events' in large patch treatment					
(Intercept)	0.458971	0.104244	81.52	4.403	
DEGREE	-0.00689	0.026016	81.98	-0.264	0.792

In small patch treatments, mean degree and the proportion of events that were finding events were negatively correlated (weighted $r=-0.49$, $p<0.002$ for the small clumped treatment and $r=-0.34$, $p=0.03$ for the small dispersed treatment). This pattern was not found in the large treatments (weighted $r=0.01$, $p=0.93$ for the large clumped treatment and $r=-0.09$, $p=0.55$ for the large dispersed treatment).

Contrary to our third prediction, we found unequal foraging returns for the use of either tactic, with the event payoff being greater for 'finding events' in both environments with small patches (GLMM: z = -3.549, p=0.0004; Table 5a), and large patches (GLMM: z= -2.868, p=0.004; Table 5b). In the environments with large patches, heavier individuals also had a significantly greater event payoff (GLMM: z= 1.995, p=0.046), meaning bigger fish ate more worms (Table 5b).

Significant individual differences were only found in the model exploring the effect of environmental quality on the percent of available bloodworms eaten (log likelihood = -578 to -574, X^2=8.635, p=0.013); suggesting consistent individual differences in payoffs across treatments. Including treatment as random slope revealed that individuals differed in their response to changes in treatment (log likelihood = -574 to -566, X^2=14.707, p<0.001).

Table 5. The effect of tactic; 'finding event' (F) or 'joining event' (J), sex and weight on the event payoff (number of bloodworms consumed) as estimated from a generalised linear mixed model. Separate models were run on the for the a.) small patch treatment and b.) the large patch treatment. Group and fish identity were fitted as random effects. Significant p-values are presented in bold.

	Estimate	Standard Error	z-value	Pr (>|z|)
a.) The event payoff in the small patch treatment				
(Intercept)	0.03391	0.29788	0.114	
F or J	-0.48318	0.13613	-3.549	**<0.0005**
SEX	-0.0768	0.13135	-0.585	0.558741
WEIGHT	0.10136	0.23711	0.427	0.669023
b.) The event payoff in the large patch treatment				
(Intercept)	-0.6968	0.24235	-2.875	
F or J	-0.2542	0.08862	-2.868	**<0.005**
SEX	0.08621	0.10204	0.845	0.39815
WEIGHT	0.36033	0.18062	1.995	**<0.05**

Discussion

Our investigations into the finder-joiner dynamics of socially foraging three-spined sticklebacks suggest that fish used both finding and joining behaviour to acquire resources. As predicted, the percentage of foraging events that were finding events was greater in the small patch treatment than the large patch treatment. This result fits the expectation from finder-joiner theory (Giraldeau and Livoreil 1998). In the small patch treatment, there were fewer opportunities to join, as patches were quickly depleted by the individual that arrived first. Therefore, in an environment with small patch sizes, it pays for individuals to go off and find patches rather than join those that are already discovered. Conversely, in the large patch treatment, joining still brings rewards as the patches are slower to deplete and importantly in this system there were more opportunities to steal food from conspecifics. In the small patches, bloodworms eaten by finding approximated two-thirds of the total bloodworms available, whilst in the large treatment, bloodworms eaten by finding was around half. This is reflected in the finder's share, which was significantly greater in the small patch treatment. In theory, as finder's share increases the proportion of joining events is expected to decrease (Giraldeau and Caraco 2000), and this was evident in this experiment. Although the effect of patch size on finder-joiner dynamics matched expectations, the effect of patch distribution did not (see Giraldeau and Livoreil 1998). There was no significant effect of patch distribution on the percentage of foraging events that were finding events. Although initially surprising, this is likely explained by the small difference in distance between clumped and dispersed patches. The cost of travel between patches was minimal in this experiment and future experiments exploring finder-joiner

dynamics in three-spined sticklebacks (and other small fish) should use a larger arena, where patch distribution can be manipulated to ensure the energetic cost of travel between patches is significant.

In line with our second prediction, increased joining behaviour in large patch environments resulted in more dense co-feeding networks (Krause et al. 2009; Aplin et al. 2012). The use of social network analysis to describe social foraging behaviour has developed in recent years (King et al. 2009; Aplin et al. 2014). The order of arrival at food patches has been predicted by social association and the likelihood of patch discovery has been found to be correlated with network centrality in songbirds (family Paridae) (Aplin et al. 2014). Also, less social individuals are known to be bolder individual foragers (Ward 2004; Croft et al. 2009; Kurvers et al. 2010). Therefore, we developed co-feeding networks to reflect what was happening socially on the patches in the different treatments. Patch distribution had no effect on either mean degree or network density, however, both mean degree and network density were significantly greater in the large patch treatments. The network parameters were calculated from co-feeding networks, therefore this result is a depiction of the fact that the number of conspecifics that individuals fed in close proximity with was greater when the size of the patches was greater. For the small patch treatments there was a negative correlation between degree and the proportion of events that were finding events; however this pattern did not occur within the large treatments. This provides further evidence that in the small treatment, finder's quickly depleted the available blood worms and then carried on searching, whilst in the large treatments, finder's were joined by conspecifics at the patch as the resources were not depleted as quickly.

Given that fish altered their tactic use in accordance with the quality of their environment, we expected that these adjustments should result in approximately equal foraging returns for the use of either tactic. Instead, we found that per foraging event, finding gave the larger rewards. In this experimental set-up, patches were quickly depleted by the finder and there were less bloodworms and more competition for those bloodworms when joiners arrived. Importantly, however, per patch, only half of the bloodworms in the large treatment were eaten as a result of finding behaviour (51.4% ±4.4 SE in the clumped and 49.4% ±5.9 SE in the dispersed treatments). This increased to two-thirds in the small treatments (67.9% ±5.1 SE in the clumped and 67.1% ±3.5 SE in the dispersed treatments). So, despite the unequal event payoffs in the large treatment for finding and joining behavior, joining was a more frequent behaviour and over the whole trial joining behaviour resulted in just as many blood worms being eaten as finding behaviour. Also, unequal pay-offs can arise in PS games when foragers attain different payoffs when using the same tactic. For example, dominant individuals may receive a larger reward when scrounging than more subordinate individuals (Barta and Giraldeau 1998; Stahl et al. 2001; Bugnyar and Kotrschal 2002; Liker and Barta 2002; McCormack et al. 2007; King et al. 2009; Jolles et al. 2013; Held et al. 2010). As best as we can tell dominance did not seem to play a role in our experiment, as there was no evidence of aggression between individuals, however, other individual differences may have caused unequal pay-offs for individuals using the same tactic. We found that when considering percentage of available bloodworms eaten, "fish identity" was significant and that heavier fish ate more in a single event in the large treatment. It is known that larger sticklebacks have an increased probability of successful food capture and eat at a faster rate (Gill and Hart 1996) and we

believe that, here, size determined the rate of consumption for individuals with larger individuals quickly consuming bloodworms, but often regurgitating them, providing opportunities for conspecifics to scrounge. Furthermore, our random intercept (fish identity) random slope (treatment) model suggests individual differences in plasticity where individuals differed in their ability to adjust their behaviour to the local availability and distribution of resources (Morand-Ferron et al. 2011b).

Finally, we tested for the possibility that individuals may differ consistently in their intrinsic tendency to adopt either finder or joiner behaviour, independent of any differences in their knowledge of the environment which may reflect inter-individual differences (Morand-Ferron et al. 2011a; King et al. 2009; Kurvers et al. 2009). We found that whilst we did observe variability in foraging tactic use (i.e. frequency of finding or joining) between fish and as such they can be defined as opportunists (Vickery et al. 1991), individual fish were not consistent in the frequency of their tactic use between trials (non-significant random intercept "fish identity"). As such, individual fish, in this study, cannot be categorised into finders and joiners (Giraldeau and Caraco 2000). Instead, we expect that the fish are more flexible in their foraging behaviour. Fish could only see a food item when they swam over it or whilst a conspecific was handling it. We are aware that the fish would likely be able to smell food in the arena, but we believe, considering the density of the food, fish were not able to use olfactory cues alone to precisely locate the food, and we are confident that fish never made strong directional movements towards a food item until they were within the patch itself. Seemingly then, fish in this environment could swim around monitoring other conspecifics whilst individually searching for food and

opportunistically eating food items when they became aware of them, either from an unoccupied patch or from an occupied patch. It is very difficult to establish any intent in an animal's behaviour, and to know whether individuals paid a cost in reduced searching ability by simultaneously focusing some attention on the behaviour of conspecifics. We can only record what the fish did, and as there was no consistent preference in tactic use, we believe fish acted opportunistically. It is important to note, however, that fish did not always eat a food item when they swam over it. It is not known whether this is because they did not see the food item, however, it is not because the fish ignored the food item due to satiation as often they would subsequently join and eat from a patch where conspecifics were feeding.

The results of this study can be examined in relation to earlier work on fish that have explored behavioural changes in relation to food distribution. Previous work with juvenile wall-eye Pollock (*Theragra chalcagramma*) showed that fish exposed to clumped food or dispersed food for four weeks adjusted their foraging behaviour by increasing and decreasing their use of social information respectively. Fish that were conditioned to an environment with dispersed food were then given clumped food but they continued to forage individually and did not respond to the food discoveries of conspecifics. This work suggests that fish can change foraging strategies and how they interact with conspecifics to optimally match prey distributions, however they do not do so instantly (Ryer and Olla 1995). Similarly, our study suggests that fish can alter their foraging behaviour in respect to patch size, by increasing the frequency of finding as patch size decreases and that this reflects the amount of food attained by tactic use.

Aggression was found to play a role in three-spine stickleback finder-joiner dynamics in a previous study where dominant individuals were able to use aggression to stop the joiner from using the resource (Ólafsdóttir et al. 2014). Dominant individuals, however, were those that were trained to expect food from a certain patch before foraging partners were released into the arena. Therefore, the authors differentiated between 'tolerated access' and 'opportunistic kleptoparasitism' as the joiners were only able to feed from the patch if the dominant individual allowed it or if they ate from it without the dominant fish being aware of it. There was no aggressive defense of patches in our experiment, perhaps because, although food was densely distributed, it was quickly depleted and not stable and it is known that more stable food patches make food defense more profitable and more likely (Dubois and Giraldeau 2007; Overington et al. 2008). We were, however, able to distinguish between tolerated access to patches and stealing behaviour as fish would often attempt to steal food from a conspecific's mouth or consume food that a fish had momentarily spat out, even though food was available elsewhere in the environment. This was particularly evident in the large treatment where food was rapidly consumed by a minority of individuals before being kleptoparasitized by others.

Overall, this study has confirmed the suitability of three-spine sticklebacks as a model system to further empirically test finder-joiner theory. Fishes' allocation to alternative foraging strategies can be explained by environmental quality (reduced finder's share: (Giraldeau et al. 1990; Giraldeau and Livoreil 1998), resulting in more dense co-feeding networks in these environments (Krause et al. 2009; Aplin et al. 2012), but each tactic does not result in equal foraging returns (Mottley and Giraldeau 2000), instead payoffs for

finding are greater in all the scenarios we investigated. We also found no evidence that individuals differ consistently in their intrinsic tendency to adopt either finder or joiner behaviour (Morand-Ferron et al. 2011b; King et al. 2009; Kurvers et al. 2009). Based on our set of experiments we suggest two areas where we believe considerable progress in social foraging theory can be made using this fish system. First, considering the increased use of three-spine stickleback in social learning theory (Laland et al. 2011) we suggest that future experiments explore how joining behaviour affects social learning (whether it promotes or inhibits social learning and the mechanisms behind this) (Giraldeau and Caraco 2000; Caldwell and Whiten 2003; Humle and Snowdon 2008; Ilan et al. 2013; Thornton and Malapert 2009). Second, the great advantage of the three-spined stickleback system is that it is possible to track the movements of multiple agents at the same time and therefore empirical tests of how spatial properties such as distances to patches, distances travelled, facing direction, and general shoal geometry affect tactic use and finder's advantage can be greatly advanced by collecting data at a finer scale than ever before (Giraldeau et al. 1990; Barta et al. 1997; Di Bitetti and Janson 2001; Mathot and Giraldeau 2008; Beauchamp 2013) and a promising area of future work would be to employ approximations of the fish's field of view and see if it predicts or correlates with their frequency of joining behaviour (Strandburg-Peshkin et al. 2013).

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Chapter 8

GENERAL DISCUSSION

The exploration of how environmental context and internal stimuli affect the behaviour of individuals in groups and how these scale up to affect group level properties is a central question in the field of complex systems in general and collective animal behavior in particular (Couzin et al., 2002). This thesis explores several aspects of group behaviour that are underrepresented in the literature relating to foraging in shoals of fish. In doing so, it examines how internal and external stimuli impact on individual behaviour and what the implications of these are for group behaviour. Chapters 2, 3 and 5 specifically address the effect of nutritional state on movement characteristics (2 and 3) and its effect on spatial position in the environment (2 and 3) and within the group (3 and 5). They show how nutritional state affects locomotion and spatial characteristics of individuals and the group, providing a mechanism that drives the formation of individual roles within groups and influences decision-making. Chapter 4, a nutritional ecology based experiment, showed that the macro-nutrient composition of a food patch can affect individual movement decisions and subsequently how the distribution of macro-nutrients in the environment affects the distribution of the group. In all these chapters, conspecifics were regarded as a dynamic part of each individual's environment and an integral influence on individuals' behavioural decisions. In Chapter 2, the physical structure in the environment was also shown to interact with nutritional state in affecting decisions. Chapter 6 focused primarily on external stimuli rather than internal stimuli and was the only experiment conducted in

the field. Here, fish changed their decision-making process after a predation threat in relation to external stimuli (tide and group size). The final experimental chapter, Chapter 7, explored the effect of the quality and distribution of food on the development of individual foraging tactics and in doing so will hopefully trigger further empirical explorations of social foraging theory in shoaling fish. Figure 1. provides a flow chart illustrating how nutritional state and external environmental stimuli (predation risk and food abundance) affect the movement decisions of an individual foraging in a social context. Overall, the methods adopted in this thesis have been to take a recently neglected field in foraging theory and approach it using more recent techniques in collective behaviour which emphasise the links between different levels of biological organization. In doing so, this thesis has empirically tested established foraging theory and state-based assumptions of group behaviour, whilst also integrating some often ignored yet integral external stimuli, within the collective behaviour framework. This has been done using a combination of lab and field based experiments to provide new insights into the mechanisms and functions of group living. The remainder of this thesis will discuss some areas where further research may wish to focus its efforts, highlighting the use of technological advancements to explore how individual interactions affect group behaviour in the laboratory and in the field.

There has traditionally been a heavy weighting on models in social foraging and state-based behavioural research. Whilst the theory is decades old, it has proven to be, and continues to be, central to some of the most fruitful research in behavioral ecology. This thesis has shown empirically that individuals adjust their behaviour depending on

nutritional state and that this causes changes in their interaction with near-neighbours. A coarse measure of nutritional state was adopted in this thesis, when in fact the nature of nutrition is far more complex (Simpson & Raubenheimer, 2012). This was touched upon in Chapter 4 regarding macro-nutrient distribution in the environment, and specific macro-nutrient demands of individual fish should be manipulated in foraging experiments as has been done in insect systems (Lihoreau et al., 2015), and the interaction of the divergence of macro-nutrient specific demand between individuals within a group and group size should be investigated (Senior et al., 2015). Total energetic state (ignoring the role of specific macro-nutrients) is a continuous variable, although it was dealt with categorically (hungry or satiated) in the majority of this thesis. However, the relative amounts of environmental exploration and resource exploitation should be dependent on finer degrees of satiation, with hungrier individuals exploiting a known resource more than less hungry individuals, who should spend more time exploring the environment for new resources (Katz & Naug, 2015). In a social setting this trade-off between feeding and attaining information should likely affect how individuals interact with near-neighbours due to a desire for social information. The dynamics and feedback between foraging individuals' fluctuating levels of satiation, their locomotory characteristics and their inter-individual spatial behaviour is the next step that should be taken beyond assuming internal nutritional state is stable. Further 'live' foraging experiments such as in Chapter 7, where intake is quantified over time per individual should be conducted to explore this.

The affect of satiation should be combined with other more stable inter-individual differences such as metabolism, inter-individual consistency in behaviour, and size to

explore their interactive effects on the adoption of individual roles such as leadership and collective decision-making, as inter-individual differences in state and behaviour may be important for decisions in dynamic, complex environments (Dussutour et al., 2009). Researchers may wish to use methods other than feeding to manipulate satiation and chemically alter individuals appetite (Volkoff et al., 2005) thereby isolating feeding motivation from energetic state. Environmental conditions such as; turbidity (Webster et al., 2007; Johannesen et al., 2012), salinity (Herbert-Read et al., 2010), prey distribution (Humphries et al., 2010) and predator cues (Smith & Webster, 2015), should also be considered as these will likely affect communication and inter-individual behaviour. State-based behavioural decision models (Houston et al., 1993; Rands et al., 2003) should also be extended to collective decisions, producing new hypotheses that can be empirically tested using experimental set-ups similar to the ones in this thesis, or even combined with learning experiments to investigate how individuals respond to information held by and learned from individuals of differing internal state.

How individuals base their decisions on others' state was touched upon in Chapter 3, however, far more work in this field is warranted. To do this, the sensory biology of the study organism must be a primary consideration in experimental design (Jordan & Ryan, 2015). Incorporating the true visual field of an animal is a good first step (Strandburg-Peshkin et al., 2013), however this needs to be combined with other experiments to assess what information of a conspecific's state a focal individual can sense and respond to. These experiments may wish to explore the use of interactive playback experiments, which are an important tool for understanding animal communication and its inherently

interactive nature (King, 2015). Whilst classic interactive playback experiments involve auditory cues (Schwartz, 1994), they need not be restricted to this. For example, video or computer generated images of fish of different sizes and in different positions, performing different movement behaviours could be shown to a focal individual in an arena consisting of different chemical cues (e.g. food, "Shrekstoff", or predator cues). As the focal fish responds to the image, its movements or decisions can then be used to inform the computer program how the animated fish should respond or what section of video the monitor should play and even what chemical cue is to be released into the arena (Ord et al., 2002; Van Dyk & Evans, 2008; Butkowski et al., 2011). By manipulating what signals are played back to the focal individual, experiments can collect quantifiable data on the meaning of signals and the realistic affect they have on behavioural interactions in a controlled laboratory environment (McGregor, 2000). These playback experiments will likely improve in precision as new technology such as 3D animated video stimuli (Veen et al., 2013) and robotics (Krause et al., 2011; Swain et al., 2012; Butail et al., 2013; Mitri et al., 2013) develop. The use of simulated animals and robots also has conservation potential if they can control group movement direction, restricting entry into unsafe or unfavourable zones. Of course this work necessitates species-specific experiments, and may incorporate chemosensory cues in fish, as well as auditory, visual and electrical signaling depending on the species and taxa. The combination of interactive playback experiments with subsequent tracking of the movement behaviour of social species, influenced by various environmental stimuli, would be a powerful approach to the study of the effect of communication on group decision-making.

Whilst this thesis focused on nutritional state, the influence of other internal stimuli on individual interactions and group dynamics need to be explored. Metabolic rate is of particular interest due to its holistic influence on an animal's behaviour (Norin et al., 2015). Passive assortment of individuals within groups into sub-sets with similar metabolic rates is likely to occur, possibly through differences in swimming speeds, and this has potential flow on effects into group fission events. Indeed, one of the necessary steps in the field is to expand from individual effects on the group to that of the population (Bazazi et al., 2011). This is particularly interesting on a landscape scale when groups may need to make spatial decisions over where to move for adequate resources. Ecological complexity can influence the underlying social structure of a group (Ramos-Fernández et al., 2006) and for heterogeneously structured groups faced with a decision of where in the landscape to move, if the costs of consensus outweigh those of fission, groups may potentially split into smaller sub-groups sorted by individual requirements (Conradt & Roper, 2000; Ruckstuhl & Kokko, 2002; Sueur et al., 2011). In some cases, inter-individual differences between members of a population may even lead to partial migration (Brodersen et al., 2008; Skov et al., 2010; Chapman et al., 2011; Nilsson et al., 2011; Chapman et al., 2012). An ability to predict group movement decisions from a combination of group structure and external and internal stimuli (Guttal & Couzin, 2011) would serve an important conservation tool in the future, especially if human infrastructure causes heterogeneity in the landscape or physical obstacles that impede natural movements of groups (Mammides et al., 2015). Here, like with natural events, such as seasonal differences in rainfall (Wittemyer et al., 2005), temperature (Pretzlaff et

al., 2010) or food availability (Conradt, 1998), groups may divide, causing flow on effects at a population level, perhaps altering gene flow or the spread of disease.

Linking individual behaviour to group-level behaviour and subsequently to population level effects should be a constant goal in behavioural ecology (Parrish et al., 2002; Viscido et al., 2004; Bazazi et al., 2008; Cote et al., 2010a; Cote et al., 2010b; Bazazi et al., 2011; Cote et al., 2011). It is in this area where technological advancements in GPS, uavs, satellite imagery, in situ 'smart' tags, and long-range tissue sampling, hold a wealth of potential as the accurate data they collect on individuals (spatial, physiological and behavioural) can be related to habitat structure. GPS tracked movements of social animals can been captured on a landscape scale and analysed in much the same way as small schooling fish in a laboratory arena (Strandburg-Peshkin et al., 2015) and analytical methods to screen for collective behaviours from coarse scale ecological data are being developed (Delgado et al., 2014; Langrock et al., 2014; Dalziel et al., 2015). The importance of work like this is not only to test theory in realistic environments but also to collect data subject to unpredictable environmental conditions that may further the development of theory. The attachment of sensor tags for monitoring animal movement ("biotelemetry") (Cooke et al., 2004; Wikelski et al., 2007; Payne et al., 2014) is advancing exponentially and accelerometers now allow for measurement of energy expenditure (Green et al., 2009; Halsey et al., 2009a; Halsey et al., 2009b; Gleiss et al., 2011; Halsey et al., 2011; Qasem et al., 2012), travel speed in variable environments (Bidder et al., 2012); and even behaviours such as feeding events (Yoda et al., 1999; Yoda et al., 2001; Simeone & Wilson, 2003; Halsey et al., 2009b; Halsey & White, 2010;

Kokubun et al., 2011; Iwata et al., 2012; Nathan et al., 2012; Watanabe & Takahashi, 2013; Viviant et al., 2014). The mass of data collected by the tags is becoming easier to analyse with the development of specific programs (Walker et al., 2015) that classify accelerometer data into behavioural classes automatically (Bidder et al., 2014). This fine-scale detail of behaviour can be used to assess established foraging theory relating to intake rates and movement decisions in the field and over a large spatial scale (Iwata et al., 2015). This type of information, used in combination with individual spatial positions and hence inter-individual rules of interaction, can then be used to explore collective movement decisions in the field, under realistic, natural ecological conditions (Portugal et al., 2014; Strandburg-Peshkin et al., 2015; Voelkl et al., 2015) incorporating landscape heterogeneities (Howard et al., 2015). Fish (Fréon et al., 1992; Makris et al., 2009; Handegard et al., 2012; Chapple et al., 2015) are good candidates for such work, as are birds (Wikelski et al., 2007; Kays et al., 2015; Voelkl et al., 2015), where GPS data has recently been used to explore social foraging (Evans et al., 2015). Of course larger mammals are also appropriate (Signer et al., 2010; Singh et al., 2012; Killeen et al., 2014; Langrock et al., 2014; Dalziel et al., 2015). A major issue with this type of work is the cost, however this will decrease with time and demand. Perhaps a more important issue, however, is that it does not become a descriptive science. Experimenters need to plan careful experiments that are spatially and temporally controlled and potentially include chemical manipulation of individual state. Experimenters also need to ensure they approach the systems with biologically relevant hypotheses. If this is done, an ability to link individual movement decisions, state and diet (Lesmerises et al., 2015) to accurate spatial data on habitat use would give biologists data at multiple scales and determine

causes of individual decisions and how they affect intra and inter-group spatial behaviour and thus population structure – furthering our understanding of why animals behave the way they do and how this affects their natural distribution. Hopefully this allows for appropriate, less managerial conservation efforts whilst capturing the interest of the general public along the way.

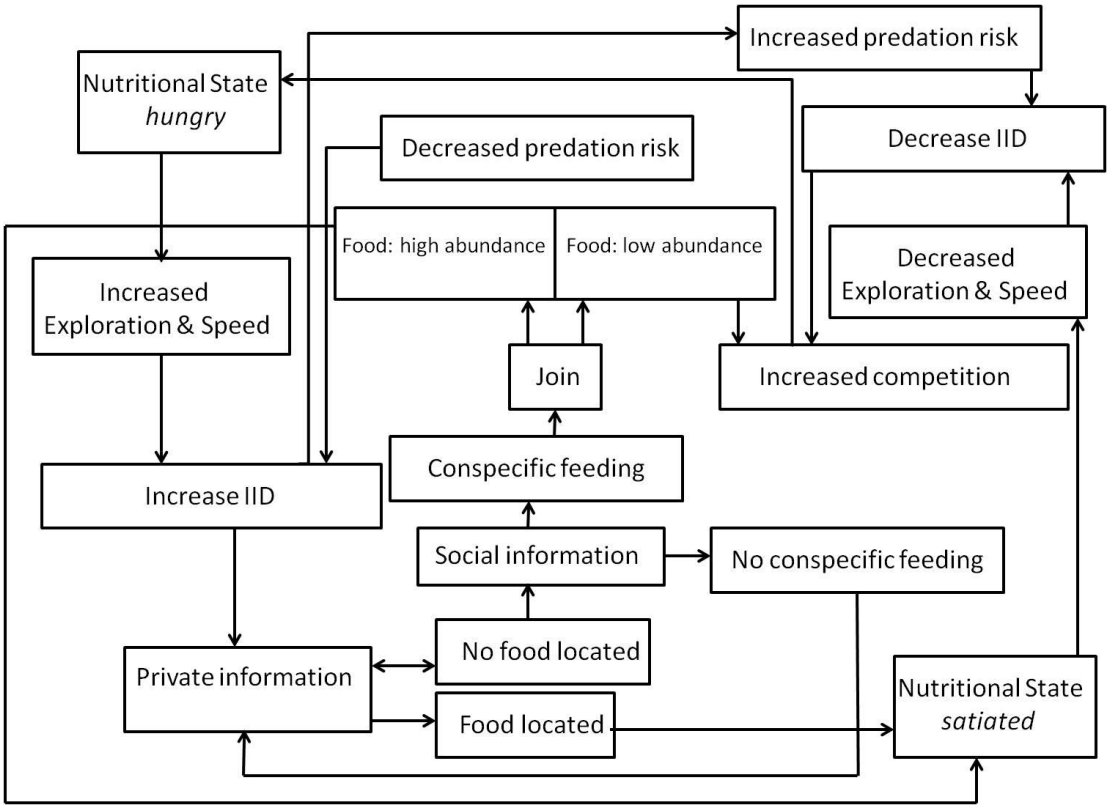

Fig. 1: Flow chart of movement decisions by an individual foraging in a social context

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