University of Hull

BEHAVIOURAL FLEXIBILITY IN RESPONSE TO ENVIRONMENTAL CHANGE

This thesis is submitted for the degree of Doctor of Philosophy

School of Biological, Biomedical and Environmental Sciences

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Candidates Declaration

I declare that the work submitted in this thesis is my own, except when otherwise stated. The work in this thesis involves collaborations with Ben Chapman (BC), Seth Norton-White (SNW), Rory Graham (RG), Katie Barnes (KB), Khia Dobbinson (KB), Lisa Bulmer (LB), Becky Mills (BM) and Rudi Schuech (RS). Lesley J Morrell (LJM) was involved with all work.

Chapter 2: SNW assisted in data collection

Chapter 3: LJM created the model

Chapter 4: RG and KB assisted in data collection

Chapter 5: KD and LB assisted in data collection

Chapter 6: BC assisted with experimental design, BM assisted in data collection, RS provided Matlab code

In addition, the work contained in this thesis has been published.

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I further declare that no part of this work has been submitted as part of any other degree. This copy has been supplied on the understanding that it is copyright material and that no quotation from the thesis may be published without proper acknowledgement.

Helen Kimbell, May 2015

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Abstract

A fundamental challenge in biology is to understand how animals can respond to the unprecedented environmental changes caused by human activities. In aquatic systems, pollution and disturbance from anthropogenic activities often impacts upon the sensory environment, and affects a number of important fitness-related behaviours. This PhD focuses on how a degraded environment impacts the behavioural responses of fish. The first section (chapters 2, 3 and 4) examines whether a degraded visual environment immediately affects the aggregation behaviour of fish as prey, and explores the implications for fish as predators. The second section (chapters 5 and 6) considers the way that previous experiences of a degraded or variable environment shape behavioural responses. Together, the results highlight the importance of considering the relationship between environmental conditions and behaviour over different time spans and during different developmental stages in order to understand how fish may respond to anthropogenic environmental change.

The first section offers new evidence on the ways that groups of fish respond to predators in turbid water, and how predators target individuals within those groups. In chapter 2, I investigate how shoals of guppies respond to a simulated aerial predator attack in increasing levels of turbidity. I find that, in turbid water, guppies form looser shoals, and alter their behaviour in response to a simulated attack by showing weaker escape manoeuvres and increasing freezing behaviour as opposed to darting escape manoeuvres. In chapter 3, I explore the 'selfish herd movement' rules that guppies use to form shoals in response to a predator attack in clear and turbid water. I find that guppies use more complex rules (moving towards a location determined by the position of multiple individuals) in clear water, resulting in large compact shoals. By comparison, guppies are unable to use these rules when forming groups in turbid water, resulting in smaller, more fragmented shoals. In chapter 4, I consider the effect of turbidity from the perspective of both predator and prey, in the context of the oddity effect. Firstly, I assess the effect of turbidity on how stickleback predators target *Daphnia* prey individuals of different sizes from within mixed groups. Secondly, I explore how turbidity influences the social choices made by sticklebacks. From the

perspective of the predator, sticklebacks selected large bodied *Daphnia* from mixed groups more than expected by chance in clear water, but not in turbid water. From the perspective of the prey, large individuals lost their preference for size-matched shoalmates in turbid water, whereas small individuals showed no social preference in either clear or turbid water. The oddity effect appears weakened in turbid water, relaxing predation pressure on large odd individuals at the expense of small individuals. Together, these three chapters consider the immediate, flexible responses of both predator and prey to short-term changes in turbidity.

The second section of the thesis explores the longer-term impacts of a degraded or variable environment on the behavioural responses of fish. In chapter 5, I investigate how adult guppies respond to different food cues (visual, olfactory, or a combination of both) with increasing levels of experience of a visually poor environment. Previous work rearing guppies under similar conditions found that individuals make a sensory switch from vision to olfaction. I find that, although guppies with more experience of a dark environment increase their foraging success in visually poor environments, they do not make a sensory switch from vision to olfaction as seen in juvenile fish. Finally, in chapter 6, I step away from the visual environment and look at how recent experience of a variable habitat combined with low or high food levels affects boldness and exploratory behaviour in guppies. While some behaviours are modified with experience of low food, for example, the time to attack a food item, I find that exploratory behaviour was not influenced by either energy state or experience of a variable environment, remaining remarkably stable over time. These final two chapters highlight the importance of investigating behavioural responses to the environment over different time spans and during different developmental stages.

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Chapter 1: General Introduction

Dealing with environmental change

Humans are having a drastic, often negative impact on the natural world. Changes to the environment caused by human activities can happen rapidly, well within the life span of an individual (Goudie 2013). Environmental stressors such as habitat loss or fragmentation, pollutants, climate change and the spread of exotic species place animals in novel situations more rapidly than they have experienced in their evolutionary past (Palumbi 2001; Sih et al. 2011; Sih 2013). The ability to respond flexibly to this kind of change is vital for the survival of an individual in the short-term and a species in the long-term (Sih et al. 2011). Behaviour is often the first mechanism employed for dealing with rapid change, as it can be altered almost instantaneously. To prevent their songs being masked by noise pollution, for example, urban birds sing more loudly (Nemeth and Brumm 2010) and alter the frequency of their song (great tits *Parus major*: Slabbekoorn and Peet 2003, song sparrow *Melospiza melodia*: Wood and Yezerinac 2006, European robins *Erithacus rubecula*: McLaughlin and Kunc 2012), while grey squirrels *Sciurus carolinensis* respond more to the visual component of a visual-auditory alarm signal from conspecifics (Partan et al. 2010). Behavioural plasticity, however, may be limited in circumstances where change becomes more permanent or dispersal away from a potential threat is limited (Schwartz et al. 2006; Thomas 2011). In these circumstances, other mechanisms may help an individual adapt to their environment, such as developmental plasticity, whereby an organism changes physiology or behaviour in response to the environment experienced during development (Tollrian 1990; Pigliucci 2001; Chapman et al. 2010b; Bell et al. 2011; Jonsson and Jonsson 2014). Adaptations such as this often depend on a critical developmental window (Dufty et al. 2002; West-Eberhard 2003), therefore the stage of life at which an animal is exposed may influence how an animal will respond.

Environmental change often impacts upon an organism's sensory environment. Disrupting senses can change the way animals communicate, find mates, forage and detect and respond to predators. For example, chemical pollution disrupts shoaling behaviour in fish (Ward et al. 2008), light pollution disrupts foraging behaviour of

moths (Macgregor et al. 2014) and noise pollution affects communication in terrestrial (Slabbekoorn and Peet 2003) and aquatic (Popper and Hastings 2009; Slabbekoorn et al. 2010) systems. Sensory channels can also interact in interesting ways, for example cuttlefish respond to increased noise by changing their colour more frequently (Kunc et al. 2014), suggesting that changes to an individual's sensory environment may have multiple effects on behaviour. In this thesis I concentrate on the visual environment, as this is often heavily impacted in aquatic environments (Richter et al. 1997; Smith et al. 2006) and may therefore have significant effects on the behaviour or aquatic organisms.

Visual environment and increasing turbidity

The visual environment varies temporally and spatially in aquatic systems due to water depth, canopy cover and water clarity. Despite this, the majority of fish have excellent vision (Guthrie and Muntz 1993) and often rely heavily on vision for a number of important behaviours, including mate choice (Endler 1991; Seehausen et al. 2008), detecting predators (Kelley and Magurran 2003a) and forming social groups (Partridge and Pitcher 1980). Human activities such as deforestation, urbanisation, increased disturbance and eutrophication have substantially increased levels of turbidity worldwide (Richter et al. 1997; Smith et al. 2006), and indeed, have caused an increasing number of areas to become permanently turbid (e.g. Lake Victoria: Hecky 1993, Baltic sea: Bonsdorff et al. 2002). Suspended particles such as algal cells or clay reduce the amount of light that can enter the water column, and increase light scattering and absorption, degrading the visual environment. Suspended particles disrupt vision in water in a comparable way to fog on land; it decreases the visibility of objects over long distances, although it has little effect over short distances (De Robertis et al. 2003). This is different from dark conditions, which degrade the visual environment more evenly (Miner and Stein 1993; Grecay and Targett 1996)

Increasing turbidity has strong implications for reproduction, growth and survival; particles can interfere directly by changing an organism's physiology (Bruton 1985), or indirectly by altering behaviour. Crucially, turbidity disrupts communication between individuals by altering the transmission of signals and the ability of an animal to receive

signals (reviewed in van der Sluijs et al. 2011). This type of disruption can interfere with sexual selection and mate choice in species that rely on visual cues to assess mate quality (Jarvenpaa and Lindstrom 2004; Engstrom-Ost and Candolin 2007; Wong et al. 2007; Sundin et al. 2010). Turbidity caused by eutrophication is thought to have led to the collapse of diversity in cichlids in Lake Victoria (East Africa), which use colour to discriminate between species (Seehausen et al. 1997; Seehausen et al. 2008). Turbidity can also affect social interactions and group dynamics, by reducing the extent to which individuals move between groups and by altering shoal choice (Fischer and Frommen 2012; Borner et al. 2015), although the extent of these impacts is not yet fully understood. Here, I consider firstly how increased turbidity interferes with grouping behaviour in the context of predator-prey interactions, and secondly how experience of a degraded visual environment affects cue use during foraging.

Predator-Prey interactions

A key driver of community structure is the complex interactions between predators and prey (Holt 1977). Predators can act directly on a community by consuming prey (Holling 1959) or indirectly, by altering prey behaviour and morphology (Lima and Dill 1990). A classic example of the influence of predation on the behaviour of organisms is diel vertical migration, whereby organisms move up towards the surface of the water at night, and return to the depths during the day to avoid predation pressure. This behaviour is observed in all oceans across a large number of taxa, and is strongly controlled by the presence of predators (Gliwicz 1986).

Predator-prey interactions are intrinsically linked to environmental conditions. In aquatic systems, for example, organisms rely on an array of cues to detect both predators and prey: olfaction, vision and the lateral line system are all important sensory modalities (Pitcher 1993). Environmental changes that impact the sensory environment can disrupt these interactions by affecting how predators detect and consume prey, and by changing prey behaviour. Chemical pollution in aquatic systems, for example, can disrupt the use of olfactory cues, which in turn interferes with a predator's ability to detect and capture prey (Kasumyan 2001) and a prey's ability to detect and respond to early warning signs of predators, such as alarm substances from

conspecifics (Scholz et al. 2000; Scott et al. 2003) or olfactory cues from predators (Olivier et al. 2006). Vision is an important cue for detecting predators or prey, as it relays the most accurate information about where an individual is in space and time. Other cues, such as olfaction, give important information about the presence, diet and even hunger level of predators (Licht 1989; Brown et al. 2001), although these cues can quickly become outdated (Giske et al. 1998; Brown 2003). From a predator's perspective, individuals can suffer from reduced capture rates when vision is disrupted, as prey become harder to locate and encounter rates between predator and prey decrease (Engstrom-Ost et al. 2006; Turesson and Bronmark 2007). This depends on the predator in question, as piscivorous fish that detect prey over long ranges are more negatively impacted than ambush predators (De Robertis et al. 2003).

These examples show how changes to the environment can alter predation pressure. This in turn can affect community structure by altering the levels of risk and survival experienced by prey. A dramatic effect of this can be seen when a predator is removed from the system; the loss of wolves from Yellowstone national park caused elk populations to explode, which subsequently caused overgrazing of Aspen (Beschta and Ripple 2009).

Focusing specifically on the impact of turbidity in aquatic environment, many studies have – perhaps surprisingly – found that prey capture rates in turbid environments remain the same as those in clear water. This suggests that predators can flexibly adjust their behaviour to overcome visual constraints (Webster et al. 2007; Johannesen et al. 2012). However, the type of individual captured in turbid water often differs from that in clear (Reid et al. 1999; Shoup and Wahl 2009; Jonsson et al. 2013). This suggests that while overall risk remains the same, individual risk changes, which could influence how prey responds and affect community structure.

Turbidity can significantly reduce the ability of prey to detect and respond to predators appropriately (Meager et al. 2006; Leahy et al. 2011; Sohel and Lindström 2015). Individuals often adjust their behaviour in turbid water, decreasing important anti-predatory responses. Organisms reduce their use of shelter and increase use of open habitat (Abrahams and Kattenfeld 1997; Lehtiniemi et al. 2005), recover more quickly from a predator attack (Gregory 1993) and continue to forage in the presence of a

predator (Lehtiniemi et al. 2005) in turbid conditions compared with clear water. It has been hypothesised that turbidity can act as a refuge for prey, affording individuals protection from predators (Gregory 1993; Engstrom-Ost et al. 2006; Engström-Öst and Mattila 2008), although this is not always the case (Abrahams and Kattenfeld 1997; Gregory and Levings 1998; Jonsson et al. 2013).

Anti-predatory responses are often costly, as they take time and energy away from other important behaviours such as foraging and mating; however, failure to respond to a predator appropriately can be lethal. Individuals need to balance the costs and risks in a given environment, and will adjust behaviour according to the level of risk (Lima and Dill 1990). For example, kangaroo rats *Dipodomys spectabilis* adjust their social behaviour with levels of moonlight, balancing the cost of missing mating opportunities, with the higher predation threat in a lighter environment (Rosenzweig 1974). If individuals are no longer able to accurately detect predators, they may decrease anti-predatory behaviours through a reduced perception in risk, even if actual levels remain unchanged. Overall, turbidity appears to reduce the distance over which prey and predators detect one another. This reduces selectivity of predators (Abrahams and Kattenfeld 1997; Reid et al. 1999; Jonsson et al. 2013) and makes anti-predatory responses less effective (Gregory 1993; Meager et al. 2006). Interactions in turbid water are thus defined by encounter rates and less by active targeting by predators and responses by prey (Abrahams and Kattenfeld 1997).

While changes in the anti-predatory behaviour of fish in turbid water have been considered extensively on an individual level, less is known about how they respond in a group. As the majority of fish spend some or most part of their lives living in groups (Shaw 1978), in part for anti-predatory benefits (Magurran 1990), it is important that we understand how changes in the environment impact this behaviour.

Grouping as an anti-predatory response

Living in groups provides multiple benefits, including improved foraging efficiency (Templeton and Giraldeau 1996), communal care of young (Gandelman et al. 1970) and reduced cost of transport (Herskin and Steffensen 1998; Weimerskirch et al. 2001). However, the main driver behind the formation and maintenance of groups is

thought to be the anti-predatory benefits (Krause and Ruxton 2002). Groups of prey show increased vigilance (Fitzgibbon 1989), communal defence (Krams and Krama 2002) and coordinated escape manoeuvres (Magurran 1990). Larger groups dilute an individual's risk of being successfully targeted (the dilution effect: Foster and Treherne 1981), and can visually confuse a predator, as they find it more difficult to lock on to and target a particular individual from within a group (the confusion effect: Landeau and Terborgh 1986). The confusion effect is enhanced when individuals within the group are morphologically and behaviourally similar. Phenotypically or behaviourally odd individuals within a group are often preferentially targeted (the oddity effect: Theodorakis 1989). The confusion and oddity effects are thought to have led to the formation of phenotypically similar groups; across taxa, animals are known to associate with individuals based on phenotypic characteristics such as body size (Svensson et al. 2000; Rodgers et al. 2011) colouration (McRobert and Bradner 1998) and species (Ward et al. 2002).

Under the threat of predation, individuals move towards one another to form large, compact groups, reducing individual risk (the selfish herd hypothesis: Hamilton 1971). As individuals will find it difficult to visually locate conspecifics in turbid conditions, the process of forming groups may be disrupted. In visually poor environments, shoals of fish form looser aggregations (Ryer and Olla 1998; Ohata et al. 2013), with fish more likely to be found as individuals rather than collectively as a shoal (Borner et al. 2015) and individuals lose their preference for associating with numerically larger shoals (Fischer and Frommen 2012). Coupled with a reduced perception of risk, this behaviour may influence how groups respond to predation, yet surprisingly little research has been conducted on this subject. In chapters 2 and 3 I expand upon the idea that turbidity affects grouping behaviour by investigating how groups form and respond to predators in increasing levels of turbidity, hypothesising that turbidity will influence the ability for individuals to detect one another and respond appropriately to predators.

In turbid water, predators appear less able to selectively target individuals (Abrahams and Kattenfeld 1997; Reid et al. 1999; Jonsson et al. 2013). It could therefore be hypothesised that the oddity and confusion effects play a less important role in turbid environments relative to their role in clear water. When choosing between groups,

animals often make choices that minimise their oddity, although this can depend on an individual's level of risk. Under the threat of predation, armoured sticklebacks *Culaea inconstans* preferentially group with non-armoured fat head minnows *Pimephales promelas* over conspecifics, as shoaling with the more vulnerable prey overrides the cost of being odd (Mathis and Chivers 2003). Many non-gape limited predators preferentially target large-bodied prey, as they represent the "better meal" (Manicom and Schwarzkopf 2011). In turn large-bodied individuals show a strong preference for size match shoals, whereas small-bodied individuals do not (Rodgers et al. 2011). I explore this idea further in Chapter 4, investigating how size selectivity is altered in predatory fish, and whether individuals take their size and level of risk into account when forming groups in turbid water.

Experience influences behaviour

Animals can adjust their behaviour in response to novel or degraded environment over very short time scales. However over longer time scales, these behavioural responses may be inadequate or unsustainable. For example, cod *Gadus morhua* increase activity when foraging in turbid conditions (Meager and Batty 2007), but this behaviour may be energetically costly over longer time periods. There are consequently alternative mechanisms by which animals can adjust their phenotype to better match their environment when experiencing altered or degraded conditions over the longer term (Stearns 1989; Tollrian 1990; Bronmark and Miner 1992; Jonsson and Jonsson 2014). Some types of plasticity may be limited to a particular life stage or age, making adjustments outside of these time periods more difficult (West-Eberhard 2003; Fischer et al. 2014). For example, stress resistance in rats can only be induced by maternal care if experienced a week after birth (Szyf et al. 2007). Changes in phenotype during early life may therefore only benefit an individual if the adult environment matches that experienced as a juvenile. Rapid changes to an animal's environment could disrupt this correlation, negating the benefits of the altered phenotype (Bateson et al. 2004; Monaghan 2008).

Evidence that organisms remain flexible throughout life is increasing (Marchinko 2003; Zhang and Meaney 2010; Ebbesson and Braithwaite 2012). Enriched environments

experienced as an adult can reverse the negative effects of an early stressful environment (Ilin and Richter-Levin 2009), and early experience of an enriched environment can be reversed by placing an individual in barren holding conditions (Näslund et al. 2012). The effects of early experience can therefore be altered by adult experience, although the extent to which this occurs is less well understood. If traits remain flexible throughout life, it could be hypothesised that adults will be able to make similar switches to those observed in juveniles. Conversely, individuals may use different mechanisms over the course of their lifespans to deal with environmental change such as behaviour modification via direct or social learning (Brown and Laland 2003). Although an individual's ability to learn is tightly linked to innate behaviour (Lima and Dill 1990), learning allows an individual to fine-tune responses in order to better match their behaviour to the environment. For example, organisms have an innate ability to respond to a generalised predator cues, but individuals can learn to recognise specific predators based on olfactory or visual cues (Chivers and Smith 1998; Brown 2003; Kelley and Magurran 2003b). In some circumstances, past experience has limited or no effect on behaviour, and the current environment alone defines how an animal behaves. For instance, Chapman et al (2009) reared guppies in high and low light conditions, but found that current lighting environment was the most important factor when looking at sexual display rates in males, with rearing environment having no effect on behaviour.

With regards to the sensory environment, organisms can compensate for the loss of one sense by increasing their reliance on an alternative sense (compensatory sensory plasticity: Rauscheker 1995). For example, blind humans increase their reliance on auditory cues (Roder et al. 1999) and juvenile guppies reared in a dark environment make a sensory switch from vision to olfaction (Chapman et al. 2010b). Such change can buffer an individual from the costs of an altered environment – the guppies in Chapman et al. (2010b) maintained foraging rates in visually poor environments – but there is scarce evidence for whether such sensory compensation can occur during later stages of life. Studies of sensory switches during later life give mixed results, as some species appear able to switch between vision and olfaction (Webster et al. 2007; Johannesen et al. 2012) while others cannot (McMahon and Holanov 1995; Fraser and Metcalfe 1997). In chapter 5, I test how recent experience (2 and 4 weeks) of a

degraded visual environment affects foraging behaviour in adult guppies, with the aim of providing evidence on whether a developmental window for sensory compensation exists in this species.

Behavioural consistency

Although behaviour is a remarkably flexible trait, animals show consistency in behaviour across time and context. Repeatability of behaviours have been termed "animal personality" (Sih et al. 2004a; Reale et al. 2007; Biro and Stamps 2008) and correlations between these traits "behavioural syndromes" or "behavioural type" (Sih et al. 2004a; Sih et al. 2004b; Bell 2007). Animal personality been observed in a diverse array of taxa (insects: Tremmel and Müller 2013, birds: Dingemanse et al. 2002, mammals: Koteja et al. 2003, fish: Bell and Sih 2007), with researchers generally focusing on 5 main axes of animal personality, thought to be important for fitness: boldness - shyness, exploration - avoidance behaviour, aggression, activity and sociability. These traits vary across individuals within a population but are consistent within individuals across time and context (Sih et al. 2004a). For example, an individual who is bold and aggressive towards rival mates will also take unnecessary risks in the face of predation (Huntingford 1976). This may appear maladaptive at first, as you would predict unlimited flexibility across situations would be favourable. This is not to imply that personality results in fixed behaviours, rather an individual's behaviour in a particular situation or context differs consistently from that of other individuals of the same population (figure 1.1a). Thus individuals can alter their behaviour, but retain rank differences (behavioural reaction norm: Dingemanse et al. 2010). Both plasticity and consistency in behaviour can be favoured in an environment, and investigating the balance between these two trade-offs is an important area of research (Briffa et al. 2008, figure 1.1). This way of considering animal behaviour differs from the traditional view that selection favours an optimal behaviour, treating individual differences as non-adaptive noise around an ideal mean, by suggesting that natural selection can favour a diversity of traits (Dingemanse and Reale 2005).

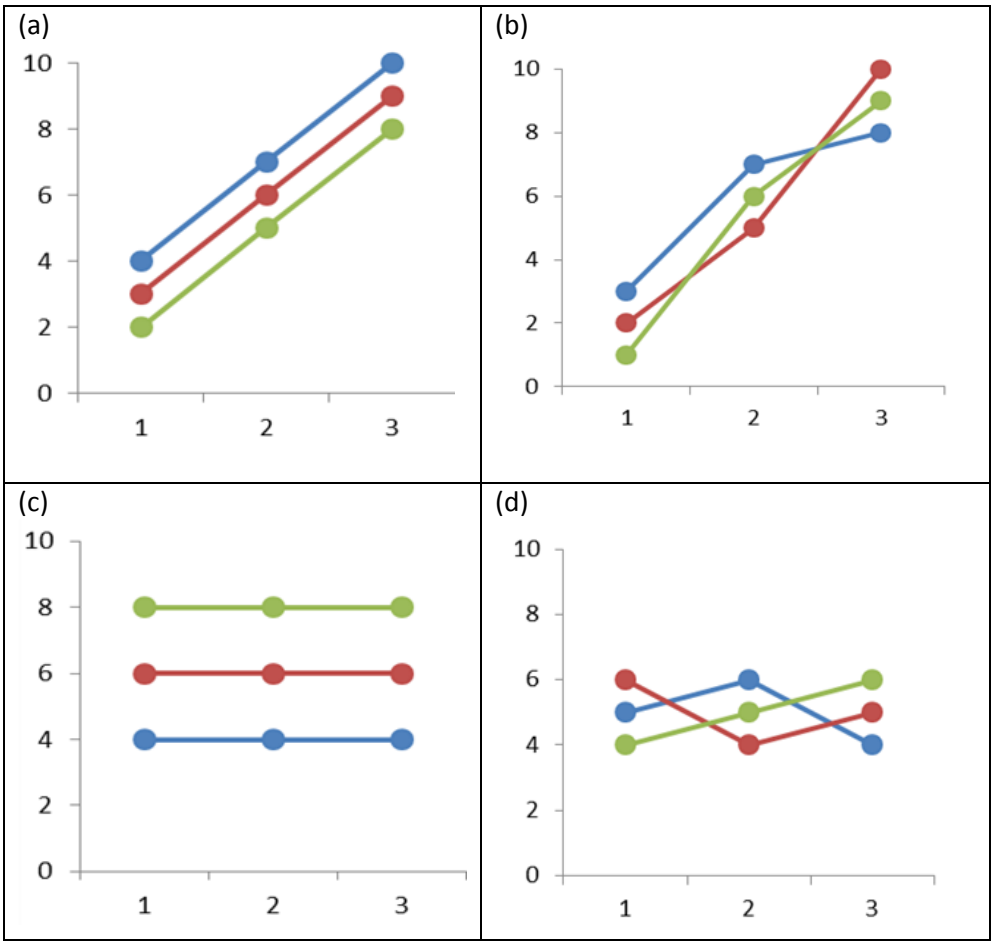

Figure 1.1: Different combinations of behavioural consistency and plasticity for 3 individuals (represented by the different colours) across 3 situations (1,2 and 3) where: (a) represents plasticity in response from each individual, but with individuals maintaining consistent relative differences (evidence for personality), (b) plasticity across situations for each individual, however consistent differences between individuals are not retained, (c) consistency in behaviour across all contexts, with no evidence of plasticity (evidence for personality), (d) no significant differences in plasticity, and no consistency in the response from individuals. (Adapted from Briffa et al. 2008)

Behaviour or personality observed in wild populations is formed by the complex interaction between genes, environment (social and physical), experience and selection pressure. At the proximate level, personality is tightly linked to an individual's internal state (Koolhaas et al. 1999; Rands et al. 2003; Dall et al. 2004; Carere et al. 2005) and experience (Braithwaite and Salvanes 2005; Dingemanse et al. 2009; Chapman et al. 2010a; Adriaenssens and Johnsson 2013; Salvanes et al. 2013), leading

to the question: how stable are personality traits in an unstable environment? An animal's state (such as its energy reserves or size) is intrinsically linked to the actions it performs. For example, when an individual's energy reserves are very low, they may take more risks in order to find food, as the benefits from obtaining food outweigh the potential risks (Krause et al. 1992; Godin and Crossman 1994; Mikheev et al. 1994). This coupled with experiential factors such as level of predation risk, environmental variability and an individual's social environment could set an individual's personality early on in life (Stamps 2003; Briffa et al. 2008). This can often be demonstrated when comparing captive bred and wild populations; captive bred salmon *Salmo salar* that experience no predation threat and high quality diets exhibit bolder and more aggressive behaviour when compared to their wild counterparts (Fleming and Einum 1997). If certain traits are set on a developmental pathway, experience later on in life could have little effect on personality traits. For example when guppies are reared on an unpredictable food supply they display bolder, more exploratory behaviours, but recent experience as an adult had no effect (Chapman et al. 2010a).

In a rapidly changing environment, understanding how individuals respond to environmental across different life stage is key to understanding how particular species will respond and survive. Comparing repeatability of behaviours between juveniles and adults of the same species is an important and relatively unexplored question (Bell et al. 2009). Is early experience more important than recent experience, or are traits altered throughout life? Evidence is growing that personality traits can be altered over short time scales; exposure to predation pressure generates a correlation between aggression and boldness in sticklebacks after just a few hours, partly through selected and plastic responses to predation (Bell and Sih 2007), and differences in bold and shy fish disappear after populations are kept in the laboratory conditions for ~ 1 month (Wilson et al. 1993). If experience of particular environments can change or erase differences in personality traits over short time scales, this could suggest that these traits are caused less by innate individual differences, rather individual differences are maintained by ecological and social forces on flexible individuals (Wilson et al. 1993). Experiments that help separate how early and adult experience shape animal personality will help us better understand the proximate causes behind consistent behaviour observed. In chapter 6, I test how recent experience of a variable

environment and energy reserves (created by using high and low feeding regimes) affects boldness and exploratory behaviours in adult guppies.

Model species

I used two species of fish to answer the questions within this thesis. The first, guppies, are small fresh water fish native to Trinidad, found in a diverse range of habitats, from high flowing rivers, to small stagnant ponds. They have been very well studied over the last 30 years, becoming an important model species in ecology and evolution. Guppies were particularly well suited to my experiments for a number of reasons. Firstly, they are highly visual organisms, responding strongly to visual cues from predators (Kelley and Magurran 2003a) and from potential mates (Long and Houde 1989; Endler 1991; Houde 1997), making them an ideal organism to study the effects of visual degradation of the environment. Secondly, guppies are a highly social species, forming loose shoals of 2 – 47 individuals in the wild (Croft et al. 2003). They are predated on by a wide variety of species including pike cichlids, crayfish and a number of bird species (Magurran 2005), and respond to predators by forming tight aggregations. Finally, they have been used in a wide range of studies considering phenotypic plasticity and early experience (Chapman et al. 2007; Chapman et al. 2008; Chapman et al. 2009; Chapman et al. 2010a; Chapman et al. 2010b), work I expand in the latter section of my thesis.

We also used 9-spine sticklebacks *Pungitius pungitius*, from a local population in Noddle Hill Nature Reserve, Bransholme, Hull (Grid Reference: 4111E, 5348N). 9-spine sticklebacks are a common species in the UK, found to inhabit a wide range of lakes, ponds and streams. Closely related 3-spined sticklebacks show strong size selectivity for large prey (Rodgers et al. 2015) and a preference for size matched conspecifics (Ranta and Lindstrom 1990; Ranta et al. 1992), making them an ideal study organism to investigate questions on size selection and oddity. Turbidity is known to affect the foraging (Webster et al. 2007; Johannesen et al. 2012) and social (Fischer and Frommen 2012) behaviours of in 3-spined sticklebacks. Sticklebacks are a small, robust and easy to house species that has become increasingly important to behavioural ecology (von Hippel 2010).

Ethical note

Sticklebacks were captured from Noddle Hill Nature Reserve, Hull, with the permission from Hull City Council. Guppies were descendants of wild-caught guppies from Trinidad. All fish were kept in aquaria approved and licenced by Home Office regulations under the Animals (Scientific Procedures) Act 1986. Experiments were approved by the ethical review committees of the School of Biological Biomedical and Environmental Sciences and the Faculty of Science and Engineering at the University of Hull (reference numbers U021 and U023).

Chapter 2: Turbidity influences individual and group level responses to predation in guppies (*Poecilia reticulata*)

Abstract

Increasing turbidity (either sedimentary or organic) from anthropogenic sources has significant negative impacts on aquatic fauna, both directly and indirectly by disrupting behaviour. In particular, anti-predator responses of individuals are reduced, which has been attributed to a reduced perception of risk. Here, we explore the effect of turbidity on shoaling behaviour, which is known to carry important anti-predator benefits, predicting that fish in turbid water should show reduced shoal cohesion (increased inter-individual distances) and reduced responses to a simulated predatory threat. We explore both the individual and shoal level responses to a predation threat at 4 different levels of turbidity. At the shoal level, we find that shoals are less cohesive in more turbid water, but that there is no effect of turbidity on shoal-level response to the predation threat. At an individual level, guppies in turbid water were more likely to freeze (rather than dart then freeze), and those that darted moved more slowly and over a shorter distance than those in clear water. Fish in turbid water also took longer to recover from a predation threat than fish in clear water. We suggest that because fish in turbid water behaved in a manner more similar to that expected from lone fish than to those in a shoal, the loss of visual contact between individuals in turbid water explains the change in behaviour, rather than a reduced perception of individual risk as is widely supposed. We suggest that turbidity could lead to a reduced collective response to predators and a loss of the protective benefits of shoaling.

Introduction

Intensified agricultural practices, urbanisation and deforestation are increasing levels of turbidity from suspended sediment and algal overgrowth in fresh water environments (Smith et al. 2006). This can have multiple negative implications for aquatic communities and is thought to be a significant contributor to declines in aquatic fauna worldwide (reviewed in Richter et al. 1997; Henley et al. 2000). At high

levels of turbidity, particles can directly affect growth and survival: sediment particles can damage gills leading to infection (Sutherland and Meyer 2007) and large algal blooms can deoxygenate water (Bruton 1985). Turbidity at lower levels acts indirectly by altering the behaviour of aquatic organisms due to the degradation of the visual environment. Turbidity can disrupt communication signals (van der Sluijs et al. 2011), impair mate choice (Engstrom-Ost and Candolin 2007; Sundin et al. 2010) and reduce the ability to detect food resources (Aksnes and Giske 1993; Utne-Palm 2002).

Crucially, turbidity can mean that individuals can no longer accurately detect predation threats (Swanbrow Becker et al. 2012), leading to changes in anti-predator behaviour and survival. In highly turbid environments, individual Atlantic cod *Gadus morhua* display poorly timed, weakened anti predatory responses (Meager et al., 2006). Other species increase behaviours often viewed as more risky (Van de Meutter et al. 2005) and decrease use of shelter in the presence of a predator (Abrahams and Kattenfeld 1997; Lehtiniemi et al. 2005). This may indicate a reduced perception of risk for some species, suggesting that turbidity acts as a shelter for prey, affording them protection from predators (Gregory and Northcote 1993; Snickars et al. 2004; Engstrom-Ost et al. 2006; Engström-Öst and Mattila 2008). In support of this theory, some visually-orientated predators can suffer from reduced capture success in turbid water (Utne 1997; Ljunggren and Sandstrom 2007), however many do not (Abrahams and Kattenfeld 1997; Gregory and Levings 1998; Reid et al. 1999; Jonsson et al. 2013). Primarily, turbidity acts to shorten the distance at which predators and prey detect each other so although prey detection by predators is impaired, those prey that are detected have less time to respond. This can make escape manoeuvres less effective (Gregory 1993; Meager et al. 2006), although shorter distances are required to move prey out of sight of predators in turbid conditions (De Robertis et al. 2003).

While changes in behaviour on an individual level have been well documented, less is known about responses to turbidity in the context of shoaling, a common and important anti-predatory tactic among many fish species (Magurran 1990; Krause and Ruxton 2002). Groups of animals detect potential threats more quickly (Magurran et al. 1985; Godin et al. 1988), perform coordinated evasive manoeuvres (Magurran and Pitcher 1987), dilute individual risk of predation (Treherne and Foster 1981; Godin 1986) and visually confuse predators, resulting in reduced targeting success (Landeau

and Terborgh 1986; Krakauer 1995; Ioannou et al. 2009). Groups become larger, tighter and more polarised when at risk from predation (Seghers 1974b; Caraco et al. 1980; Magurran and Pitcher 1987; Watt et al. 1997), enhancing these benefits. Thus, a reduced perception of risk in turbid water (Gregory and Northcote 1993; Snickars et al. 2004; Engstrom-Ost et al. 2006; Engström-Öst and Mattila 2008) could lead to reduced shoal cohesion. However, most fish rely strongly on vision for shoaling (Partridge and Pitcher 1980) meaning reduced visual distances could also disrupt this important anti-predator tactic: at low light intensities fish shoals tend to break apart (Ryer and Olla 1998; Miyazaki et al. 2000; Einfalt et al. 2012; Paciorek and McRobert 2012). Increasing turbidity is therefore expected to lead to reduced levels of shoal cohesion through either reduced perception of risk or reduced visual distances. Empirical data suggests that while moderate levels of turbidity may enhance shoaling in some species with well-developed eyes (Ohata et al. 2013), in highly turbid water, fish lose their preference for shoals composed of more individuals (Fischer and Frommen 2012) and form looser aggregations (Ohata et al. 2013).

During a predation attempt, fish in shoals perform a fast burst of motion to accelerate themselves away from the threat, leading to the flash expansion of the group (although some species remain highly cohesive during this response; Radakov, 1973). Individuals then regroup to form more cohesive shoals (Ryer and Olla, 1998). Increased cohesion reduces risk through increased predator confusion (Milinski 1977a; Krakauer 1995; Ioannou et al. 2009) and selfish herd effects, where individuals seek cover behind other shoal members (Hamilton 1971). If turbidity disrupts shoal cohesion (Fischer and Frommen 2012; Ohata et al. 2013) and reduces the perception of risk (Gregory 1993; Engström-Öst and Mattila 2008), responses to a predation event may be negatively impacted, increasing predation risk. Here, we explore how shoaling patterns of guppies *Poecilia reticulata* are influenced by increasing turbidity, and in particular, how turbidity affects both individual and shoal level responses to the visual detection of a simulated aerial predation threat. Anti-predatory behaviour is well studied in this species: guppies form loose, uncoordinated shoals (as opposed to tightly polarised schools) of 2 – 47 individuals (Croft et al. 2003), and respond to predators by using escape responses or freezing, and by increasing shoal cohesion (Magurran 2005; Fischer et al. 2015). Guppies have excellent vision (Endler 1991),

responding strongly to visual predator cues (Kelley and Magurran 2003b), making them an ideal species for this study. Our aim is to assess whether increased turbidity is likely to have negative impacts on grouping as an anti-predator response, by changing the way fish within shoals respond to a threat when they can no longer easily detect one another.

Methods

Study species and housing

All fish used in this experiment were descendants of wild-caught guppies from Trinidad. Fish were maintained in aquaria (20 x 40 x 40 cm) on a recirculating system at the University of Hull at approximately 26^{o}C (±1^{o}C) on a 12:12hr light:dark cycle and fed daily on ZM small granular feed (0.5-0.8mm; ZM Systems, Hampshire, UK). Shoals consisting of 4 guppies were created by taking female fish of similar size (all fish in a shoal measured within 0.5cm of each other) from stock tanks and moving them to separate holding tanks 20cm x 20cm x 20cm (26 shoals in total). Mean body size of individuals within shoals ranged from 1.4cm to 2.5cm. Only females were used as they form the core of guppy shoals (Croft et al. 2004) and to reduce the confounding effect of sexual behaviour on association patterns. Shoals were left in these tanks for 14 days before experiments began to allow fish to become familiar with one another (Griffiths and Magurran 1999), as familiarity can enhance anti-predator responses (Chivers et al. 1995).

Experimental design

Each shoal was exposed to 4 turbidity treatments (0, 50, 100 or 200 ± 10NTU) in a randomised order, with one week between exposures to allow for recovery. Thirty min before each trial, shoals of fish were moved to separate cylindrical holding tanks (diameter 10cm, depth 33cm) to allow the fish to acclimitise to the turbidity level. Turbidity levels were chosen as turbidity is known to reach 200NTU during rainy seasons in Trinidad (Luyten and Liley 1985), making the levels ecologically relevant. Turbidity was created using a bentonite clay-water solution and measured using an Oakton T100 portable turbidity meter. A concentrated bentonite clay solution was

created using 100g of clay suspended in 4 litres of purified water. This was filtered through fine mesh to remove larger, heavier particles that sank faster when placed in suspension, to allow for the maintenance of turbidity levels over the course of the experiment. The resulting filtrate (>1000NTU) was further diluted with water taken from the aquarium system to obtain the desired turbidity level. Turbidity was maintained in the acclimatisation tanks by pumping air into the bottom of the cylindrical tank, which re-suspended any particles that fell to the bottom.

After the acclimatisation period shoals were transferred to a white circular tank with grey sides (diameter 40cm, depth 15cm, filled to a depth of 2cm with water of the required turbidity). A monofilament fishing line ran above the centre of the tank at a 30^{o} angle, the end of which was attached to the back of the tank 10cm above the waterline. From this a model bird predator could be dropped such that it passed over the centre and came to rest against back of the tank. This approach elicits a rapid escape response in fish (Seghers 1974a; Chapman et al. 2010a), leads them to initiate aggregation (Krause and Tegeder 1994) and increase shoaling tendency (Krause et al. 1998). Thus, fish respond to the approaching aerial stimulus as if it were a predation threat, without the need for a predation event to occur. Guppies are predated on by a number of bird species in their natural habitat (Magurran 2005) and preliminary trials in clear water indicated it was effective in eliciting a behavioural response in our study fish, and thus would be appropriate for investigating the effect of turbidity on responses to a visually-detected predator. It is possible fish may respond to a mechanical stimulus caused by the model coming to rest at the back of the tank rather than the visual stimulus from flying overhead, but the majority of individuals responded when the model bird was overhead (0NTU =79%, 50NTU = 76%, 100NTU = 73%, 200NTU = 77%).

The water in the tank was kept at a depth of 2cm to allow for observation of the fish in highly turbid water and to minimise vertical movement (increasing accuracy in measuring inter-individual distances). Guppies are found in very shallow pools and streams in their natural environment, making the depth used ecologically relevant across at least some of their habitat (Luyten and Liley 1985). The shallow water also ensured that guppies were not impeded significantly in the detection of the stimulus, and that any differences in behavioural response at different turbidity levels were not

due to differences in the detection of the threat. Turbidity was maintained in the test tank by gently circulating water using an external filter. A Microsoft webcam suspended 60cm above the tank was used to record trials at 15 frames/s, and the tank was illuminated from above to ensure that a shadow passed over the tank when the predator was released.

A model bird predator was used to elicit a startle response in the guppy shoals. The model predator was an oval piece of black card 10cm long and 4cm at its widest point attached to a small circular ring at its centre through which the monofilament line was threaded. At the start of the trial, the predator was positioned at the highest point of the wire, out of sight of the tank. Fish were acclimatised in the test tank for 5 min (all fish had begun swimming normally by this point) and then their shoaling behaviour was recorded for 10 min. To initiate a startle response after the 10 min of shoaling, the model predator was released. Video recording continued through the simulated predation event and for 2 min afterwards, when trials were terminated. Water in the test tank was changed between each trial to remove any olfactory cues.

Shoal cohesion was defined as the mean inter-individual distance between individuals within a group (Miller and Gerlai 2007). As guppies form lose shoals, rather than schools, we did not measure alignment or activity synchrony, which may also component of overall cohesion. To measure shoal cohesion, videos files were converted into an image stack using VirtualDub (http://www.virtualdub.org) at 1 frame every 10 s (analysis at different frame intervals confirmed this gave an accurate representation of shoaling behaviour), which were then manually analysed in ImageJ (http://imagej.nih.gov/ij/). A scale bar drawn on the base of the tank and the tank diameter allowed accurate setting of scale for each video (pixels/cm). The XY coordinate of every fish (taken from the nose of each individual) was recorded every 10 s for 12 min (10 min before the simulated predation event, and 2 min after) and the average pairwise distance between the 4 fish calculated.

At an individual level, we observed two responses to the predator: fish would either freeze immediately (‘freeze’), or dart away from their position, using a fast burst of motion, and then freeze (‘dart then freeze’), and we recorded the number of each type of response performed by each individuals. All individuals showed one of these two

responses: no individuals darted without freezing, and no individuals remained swimming normally or showed another response. For fish that darted and then froze, we recorded their response in detail, by tracking individual movement of each of the 4 fish in the shoal for 15 consecutive frames (1 second) immediately after the predator had passed over the tank, using the plugin MtrackJ (http://www.imagescience.org/meijering/software/mtrackj/) for ImageJ. The latency to respond to the attack (defined as the number of frames taken till the fish responded by either darting or freezing), the distance moved (cm), the maximum speed reached (cm/s) and the time taken to regain normal swimming (when the fish had moved more than approximately one body length from the frozen position) were recorded for each fish in each shoal.

Individual fish were not identifiable between turbidity treatments, although shoal membership remained constant throughout the experiment. 7 fish died during the experiments and these shoals were excluded from further treatments. 2 videos were excluded as the fish were disturbed before the startle response.

Statistical analysis

Shoal cohesion was measured as the mean of the inter-individual distances between each pair of fish within the shoal at each time point for 10 min before the predator release and 2 min after. The effect of the appearance of a predator on shoal cohesion was assessed using a linear mixed effects model (LME) with time (before and after) and turbidity set as the main effects and shoal identity as a random effect to account for repeated measures and non-independence of individuals within a shoal. No interaction between time and turbidity treatment was observed, so this was removed to give the minimum adequate model (MAM; Crawley 2007). Model assumptions were checked by visual inspection of plots of residuals and were found to conform to the assumptions of normality. In order to test for differences between each of the turbidity treatments, the model was repeated using each turbidity level as the main intercept (re-levelled data).

To assess the effect of turbidity on the proportion of individuals freezing we used a generalised linear mixed effects model (GLMER) with a binomial error distribution. Turbidity was set as the main effect and shoal identity as a random factor. A GLMER

with a Poisson error distribution was used to analyse the latency to respond, with turbidity set as the main effect and shoal identity as a random factor. An additional observation-level random effect was used to account for overdispersion of the data (Harrison 2014). For fish that responded with the darting response, individual startle responses (distance moved, maximum speed) were analysed using LME models with turbidity included as a main effect and shoal as a random effect to account for both repeated measures and non-independence of individuals within a shoal. Fish that froze were excluded from this analysis as they did not move as part of their response. In order to assess whether there was a difference between each pairwise of the turbidity treatments, each model was repeated using each turbidity level as the main intercept. All analysis was carried out in R 2.15.1 (R Development Core Team 2011)

Results

Shoal Cohesion

Shoals were observed to expand and contract (decrease and increase cohesion) throughout the experiment, resulting in a large variability in shoal cohesion over time. Despite this variation, there was a significant effect of turbidity (LME: $F_{3,142} = 4.98$, $P = 0.0026$), but no effect of time (before or after predator simulation) on shoal cohesion (LME: $F_{1,142} = 0.15$, $P = 0.7$) and no interaction. The expansion of the shoals during the predator exposure was well within the normal shoaling range, with post-predator exposure shoals showing similar patterns to the pre-exposure distances.

Over the course of the experiment (before and after the aerial predation attempt), fish were found to shoal more cohesively in the lowest two levels of turbidity (0 and 50NTU) compared to the highest two level of turbidity (100 and 200NTU) (Figure 2.1: 0NTU vs 100NTU: $t = 2.7$, $P = 0.0077$, effect size = 11.15; 0NTU vs 200NTU: t = 2.79, $P = 0.006$, effect size = 11.11; 50NTU vs 100NTU: $t = 2.67$, $P = 0.0084$, effect size = 11.15; 50NTU vs 200NTU, $t = 2.77$, $P = 0.0064$, effect size = 11.12). No significant differences in shoal cohesion were observed between 0NTU and 50NTU ($t = 0.09$, $P = 0.93$, effect size = 8.93) or 100NTU and 200NTU ($t = 0.048$, $P = 0.96$, effect size = 11.11)

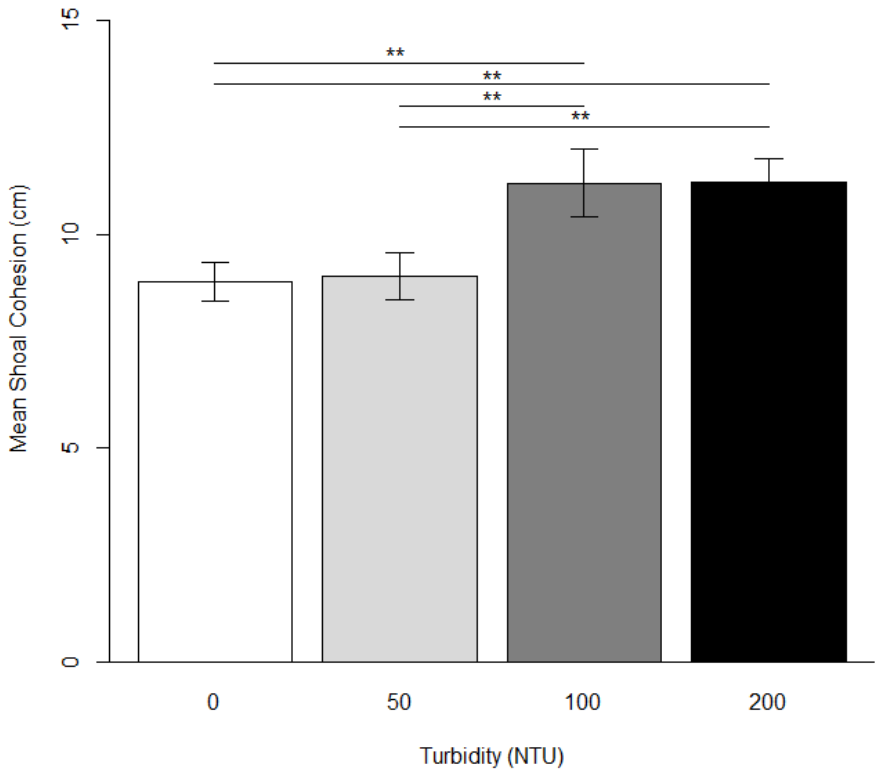

Figure 2.1: Mean shoal cohesion (cm) of fish within a shoal, measured every 10 seconds for 12 minutes in increasing levels of turbidity (±S.E.). Asterisks indicate p <0.01

Individual responses to simulated predation threat

There was a significant effect of turbidity on the proportion of fish freezing (rather than darting then freezing) immediately after the predator attack (figure 2.2a). With a higher proportion of fish found to freeze in highly turbid water compared to clear water (0NTU vs 200NTU; z = 3.05, P = 0.0023) and the 50NTU treatment (50NTU vs 200NTU, z = 2.43, P = 0.015; table 2.1).

We found no effect of turbidity treatment on the latency to respond to the predation threat (LME: $F_{3,233}$ = 1.28, P = 0.28, table 2.1), however, the strength of response differed depending on turbidity. Fish reached a higher maximum speed within the first second of movement when in clear water compared to turbid (LME: $F_{3,233}$ = 2.95, P = 0.034, figure 2.2b). Fish in clear water (0NTU) moved more quickly during the escape manoeuvre than fish in 50NTU (t = -2.60, P = 0.01) and 200NTU (t = -2.75, P = 0.0064),

but not 100NTU (t = -1.85, P = 0.065). No differences were observed between the 3 turbid treatments (table 2.1).

The total distance moved in the first second also differed between the turbidity treatments (LME: $F_{3,\ 233}$ = 4.98, P = 0.0023, figure 2.2c). Significant differences were found between clear water and highly turbid water (0NTU vs 200NTU, t= -3.74, P <0.001, table 2.1) and the lowest level of turbidity (0NTU vs 50NTU, t = -2.08, P = 0.038), with fish in the clear treatment swimming increased distances in response to the threat. A difference was also observed between the two highest turbidity treatments; 200NTU and 100NTU (t= -2.57, P = 0.010), with fish moving further in 100NTU compared to 200NTU (table 2.1).

Individuals took less time to recover from the simulated predation in clear water compared to all turbid treatments (Figure 2.2d. LME: $F_{3,327}$ = 5.01, P = 0.002). Fish recovered (began swimming normally) significantly faster in clear water compared to 200NTU (t = 3.85, P < 0.001), 100NTU (t = 2.06, P = 0.046) and 50NTU (t = 2.39, P = 0.017). No differences were observed between any of the turbid treatments (table 2.1).

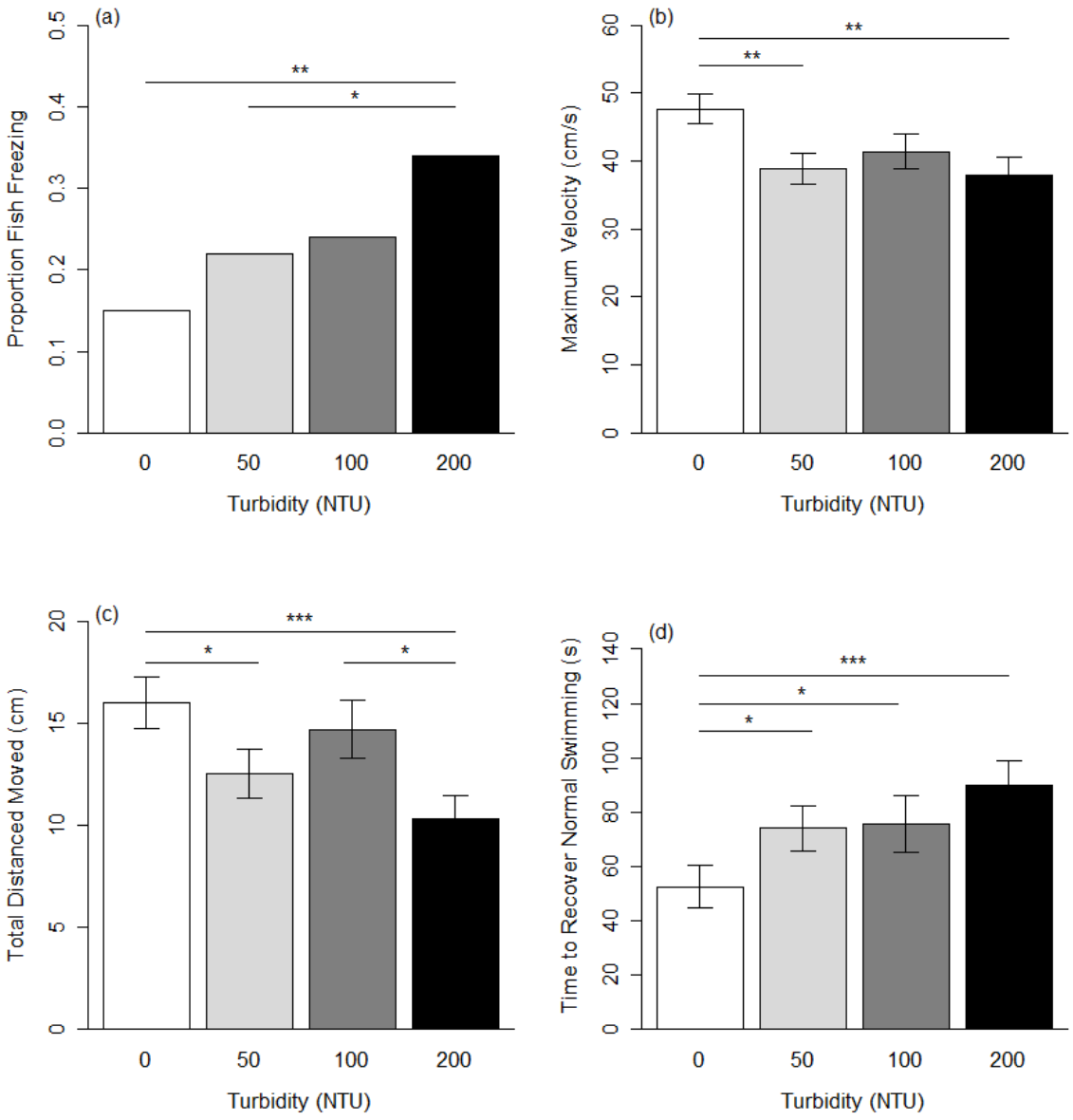

Figure 2.2: Individual level responses. a) Proportion of fish freezing in response to a simulated predator attack. b) Maximum speed (cm/s) reached in the first second of movement, c) Total distance moved (cm) within the first second of response and d) Time taken to recover normal swimming (s) after the predator simulation (±S.E.). Asterisks indicate significance: * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

Table 2.1. Pairwise comparisons from the GLMER (proportion of individuals freezing and latency to respond) and LME (maximum speed, total distance travelled and recovery time) models for the individual level responses of guppies to a simulated aerial predation threat at the 4 levels of increasing turbidity. Bold *P*-values represent significant results.

Turbidity		**D.F**	**z- or t- value**	***P*-value**	**Effect size**
Proportion of individuals freezing					
0	50	3	0.86	0.38	0.37
0	100	3	1.86	0.063	0.81
0	200	3	3.03	**0.002**	1.23
50	100	3	-1.10	0.27	0.44
50	200	3	-2.42	**0.015**	0.86
100	200	3	-1.17	0.24	0.42
Latency to respond (log seconds)					
0	50	3	-0.63	0.49	-2.22
0	100	3	-0.99	0.73	-2.16
0	200	3	-0.96	0.15	-2.28
50	100	3	1.59	0.28	-2.23
50	200	3	-0.59	0.47	-2.28
100	200	3	-1.88	0.078	-2.29
Maximum speed (cm/s)					
0	50	3, 233	-2.60	**0.01**	38.85
0	100	3, 233	-1.85	0.065	40.89
0	200	3, 233	-2.75	**0.006**	38.04
50	100	3, 233	0.61	0.54	40.88
50	200	3, 233	-1.45	0.15	38.02
100	200	3, 233	-0.83	0.41	38.08
Total distance travelled (log cm)					
0	50	3, 233	-2.43	**0.015**	-0.76
0	100	3, 233	-1.03	0.30	-0.33
0	200	3, 233	-3.77	**<0.001**	-1.21
50	100	3, 233	1.034	0.21	0.36
50	200	3, 233	-0.25	0.80	-0.4
100	200	3, 233	-2.61	**0.01**	-0.84
Recovery time (s)					
0	50	3, 327	2.23	**0.027**	72.43
0	100	3, 327	1.96	**0.05**	70.71
0	200	3, 327	3.64	**<0.001**	87.80
50	100	3, 327	-0.23	0.88	70.71
50	200	3, 327	1.46	0.14	87.80
100	200	3, 327	1.55	0.12	87.80

Discussion

Increasing levels of turbidity influenced the behaviour of guppies at both the individual and group level. Shoals were less cohesive in highly turbid water, but we found no effect of turbidity on the shoal level response to a simulated aerial predation threat. At an individual level turbidity had a strong influence on anti-predator behavioural responses. In more turbid water, individuals were more likely to freeze (rather than dart then freeze), and those that did show darting behaviour had a slower escape speed and moved a shorter distance than those in clear water, even though there was no difference in the time to initially respond to the predator. Fish in turbid water also took longer to recover from the predation threat than fish in clear water. Our finding that turbidity reduced shoal cohesion and caused individual fish to display weaker darting responses to a predator could either be explained by either a reduced perception of risk in turbid water (Gregory 1993; Miner and Stein 1996) or by constraints caused by the poor visual environment (Abrahams and Kattenfeld 1997). The finding that fish in turbid water had increased recovery times, however, contradicts the reduced perception of risk theory.

Weakened anti-predator responses in turbid water displayed by fish (Gregory 1993; Abrahams and Kattenfeld 1997; Snickars et al. 2004; Meager et al. 2006; Engström-Öst and Mattila 2008) have been attributed to a reduced perception of risk. This may be due to turbidity reducing the probability of encountering a predator (Gregory and Levings 1998) indicating that individuals are safer in turbid water, or reducing the ability of individuals to detect a predator (Meager et al. 2006) meaning the level of actual risk may remain unchanged. Studies reporting the true level of risk in turbid water show mixed results; in some cases (particularly for small juvenile fish) turbidity appears to act as a refuge, protecting prey from predators (Snickars et al. 2004; Engström-Öst and Mattila 2008), with some individuals actively seeking out turbid water (Gregory and Levings 1998). Although perception of risk may be reduced, actual risk may not: capture rates often remain the same in clear and turbid water (Reid et al. 1999; Jonsson et al. 2013) as predators compensate by using alternative cues to locate prey (Johannesen et al. 2012) or increase searching activity (Meager and Batty 2007), and as a result of inappropriate or less effective anti-predatory behaviours displayed by prey.

Our finding that fish in turbid water took longer to recover from a threat contradicts the predictions of the reduced perception of risk theory, which would predict a reduced recovery time in turbid water (Gregory 1993). Instead, the reduced visual distances in turbid water may cause fish to act as individuals rather than members of a shoal, since they are no longer able to easily detect and respond to their group-mates using vision. Fish in shoals have been found to recover more quickly than individual fish (Magurran and Pitcher 1987), supporting the suggestion that longer recovery periods in turbid environments in our experiment could indicate that the guppies' anti-predator responses are more comparable to those of lone fish. The idea that altered behaviour is due to physical constraints imposed by turbidity is further supported by our finding that, in higher turbidity, a greater proportion of individuals froze rather than darting: freezing and hiding behaviours are more often associated with lone individuals than individuals in larger groups (Magurran and Pitcher 1987; Rangeley and Kramer 1998), although Fischer et al (2015) found lone fish were more likely to dart in response to a simulated predation threat compared to fish in a shoal. In a shoal, the darting response may add to predator confusion (Ioannou et al. 2012), reducing predator attack success and decreasing risk to prey. For lone fish, however, freezing or hiding may help an individual reduce risk by avoiding detection (Magurran and Pitcher 1987). In high turbidity, the combination of reduced visual range and more dispersed shoals suggests fish are no longer able to easily detect conspecifics causing them to switch anti-predatory tactics to those more commonly associated with isolated individuals.

In contrast to previous work, we did not see an increase in shoal cohesion after the simulated attack in any of the groups (selfish herd effect: Hamilton 1971, Krause et al. 1998). This may have been because guppies tended to move to the edges of the tanks (pers. obs.), which could potentially be used or perceived as a possible refuge location by the fish, as the grey colour of the sides of the tank made individuals more cryptic against the background (Rodgers et al. 2013a). In a more open environment, we may have seen different effects. However, the shallow streams that guppies inhabit in Trinidad mean that use of refuge areas is likely to be a component of their anti-predator response. Guppies may also have received directional cues from the over-head predator stimulus and moved away from a possible attack location (Viscido et al.

2001), rather than towards their shoal-mates. An individual at risk from predation would need to balance the relative costs and benefits of protection in a group (e.g. through dilution, confusion and selfish herd effects; Krause and Ruxton 2002) against the benefits of seeking refuge as an individual. The low number of guppies in the shoal (n=4) may have also influenced behaviour within the group, although small shoals are commonly found in guppies (Croft et al. 2003). To our knowledge the interaction between group size and turbidity on anti-predatory behaviours has not been investigated, and represents an interesting avenue for future study.

In turbid water, the benefits of shoaling may be reduced as the distance at which predators can detect prey is shortened (Utne-Palm 2002), reducing the benefits of coordinated evasion and resulting in weakened shoaling preferences (Fischer and Frommen 2012) and the reduced shoal cohesion observed here and in previous studies (Ryer and Olla 1998; Ohata et al. 2013). Poor visual environments may cause predators to rely more strongly on olfactory cues (Chapman et al. 2010b), meaning that the anti-predator benefits of grouping as a means of avoiding detection (the encounter-dilution effect; Wrona and Dixon, 1991) may be reduced (Treisman 1975; Kunin 1999; Whitton et al. 2012; Wilson and Weissburg 2012).

Our study focuses on anti-predatory responses mediated by the visual detection of an over-head threat. In turbid water, however, fish may rely on alternative senses such as olfaction and the lateral line to detect and respond to both conspecifics and predators (Partridge and Pitcher 1980; Brown and Godin 1999; Stewart et al. 2013). The cues used and the responses shown will also depend on the type of predator encountered, as aquatic predators will produce different cues and may be detected at different times by members of a shoal. Spiny damselfish *Acanthochromis polyacanthus*, for example, respond more strongly to the olfactory cues of an aquatic predator in turbid water compared to clear (Leahy et al. 2011). Interestingly, the guppies in our study were able to maintain similar shoal cohesiveness and anti-predatory responses to that of clear water until relatively high levels of turbidity, suggesting individuals can use alternative cues to compensate for the poor visual environment. Olfactory cues may be important in maintaining cohesive shoals; disruption to olfactory cues through pollution interferes with shoaling behaviour (Ward et al. 2008) and alters individual responses to predators (Dixson et al. 2010), and therefore may affect group level

responses to predators. The lateral line may also compensate for the lack of vision, by providing cues to the speed and direction of other shoal members (Partridge and Pitcher 1980), although to our knowledge this remains untested in guppies, and warrants further investigation.

Our study suggests that the reduced visual distances in turbid water constrains individual responses to an aerial predation threat and may result in a reduced collective response to predators and a loss of protection gained by shoaling. This may have implications for individual survival during a predation event and for other behaviours linked to the benefits of grouping, such as anti-predator vigilance ('many eyes effect'), enhanced foraging success, transfer of information and energy conservation (reviewed in Krause and Ruxton 2002). How predators respond to the combination of changed prey behaviour and changed environmental conditions represents an interesting avenue for future study.

Chapter 3: Selfish herds of guppies follow complex rather than simple rules when information is not limited

Abstract

Under the threat of predation, animals can decrease their level of risk by moving towards other individuals to form compact groups. A significant body of theoretical work has proposed a number of movement rules, varying in complexity, which explain the process of aggregation. However, if and how animals use these rules to form compact groups is still not well understood, and how environmental factors affect the use of these rules even less so. Here, we evaluate the success of different movement rules, by comparing their movement predictions to the movement seen when shoals of guppies form under the threat of predation. We repeated the experiment in a visually poor, turbid environment to assess how the use of the movement rules changed when individuals could no longer accurately detect one another. In addition, we assessed the effect of turbidity on group formation by calculating the number of individuals each fish was associating with before and after the simulated predator attack. During a simulated predator attack, guppies in clear water were able to use complex rules that took multiple neighbours into account, forming compact groups. In turbid water, however, there was an increase in the difference between the rule predictions and fishes' movement paths, particularly for complex rules. The resulting shoals in turbid water were more fragmented, fish associated with a lower number of groupmates compared with clear water (before the threat, no difference was observed). We conclude that guppies are able to use complex rules to form dense aggregations, but that common environmental factors can limit their ability to do so.

Introduction

Aggregation by animals into groups is often considered to arise in response to the threat of predation, and the anti-predator benefits of grouping have been extensively considered (e.g. Krause and Ruxton 2002; Davies et al. 2012; Beauchamp 2014). These benefits include dilution (Foster and Treherne 1981), encounter-dilution (Turner and

Pitcher 1986; Wrona and Dixon 1991) and confusion effects (Milinski 1977a; Landeau and Terborgh 1986; Krakauer 1995; Ioannou et al. 2009), where all individuals benefit from reduced risk arising from the presence of con- or heterospecifics in close proximity (Caraco et al. 1980; Magurran and Pitcher 1987; Watt et al. 1997).

The selfish herd hypothesis (Hamilton 1971) suggests a further benefit to individuals: risk can be reduced for any particular individual in a group, but that reduction occurs at the expense of other group members, for whom risk is increased. Individual risk is defined by the 'domain of danger' (DOD), the area of space around an individual that is closer to it than to any other individual, and the selfish herd hypothesis suggests individuals should position themselves within groups to minimise the size of their own DOD (Hamilton 1971). A significant body of theoretical work has evaluated the success of various behavioural 'movement rules' in minimising DODs and creating compact groups of individuals (Morton et al. 1994; Viscido and Wethey 2002; Morrell and James 2008), either once stable aggregations have formed (Hamilton 1971; Morton et al. 1994; Viscido et al. 2001; Viscido et al. 2002; James et al. 2004) or during the process of aggregation itself (Morrell and James 2008; Morrell et al. 2011b)

In theoretical models, simple rules, where animals move towards their nearest neighbour (Hamilton 1971) tend to be outperformed by more complex rules, where the position and distance of multiple neighbours are accounted for (Viscido et al. 2002; Viscido et al. 2005; Morrell et al. 2015). These complex rules generate more compact aggregations in which a greater proportion of the group are able to reduce the size of their DOD. Simple rules can, however, result in more rapid reduction in DOD area (Morrell et al. 2011b), which is particularly important when animals have little time to respond following a predatory threat (Morrell and James 2008). Simple rules have been criticised in that they do not create the dense groups seen in nature (Morton et al. 1994; Viscido et al. 2002), while more complex rules may be cognitively too complex for animals to follow (Reluga and Viscido 2005; De Vos and O'Riain 2012).

The empirical study of selfish herd movement rules lags behind theory, with limited examples providing opposing evidence. Fur seals *Arctocephalus pusillus pusillus* moving through areas of high risk of predation from white sharks *Carcharodon carcharias*, appear to move towards their nearest neighbour rather than evaluating the

position of multiple neighbours (De Vos and O'Riain 2012). Domestic sheep move towards the centre of the group when herded by a sheep dog (King et al. 2012), while three-spined sticklebacks *Gasterosteus aculeatus* move towards an individual that can be reached more quickly rather than one which is spatially closer (Krause and Tegeder 1994), although these latter two cases did not evaluate alternative rules. Here, we investigate the selfish herd movement rules used by shoals of guppies *Poecilia reticulata* in response to a simulated predation threat in an experimental setting, comparing actual movement paths to the predictions of a simulation model. We assess the difference between the movement direction of each fish and the predicted direction if that fish were following a range of different rules, including a range of simple and complex strategies, and thus provide the first experimental comparison of multiple movement rules.

Theoretical models assume that individuals using a particular rule are able to gather all the information necessary to make an informed decision without error. In reality, errors in the evaluation of the position of neighbours may lead to movement patterns that are not consistent with optimal movement rules. As errors may be exacerbated by environmental conditions (Morrell et al. 2015), we explore the impact of increasing environmental turbidity on the selfish herd responses of our guppy shoals. In aquatic systems, increasing turbidity degrades the visual environment, shortening response distances to conspecifics (Borner et al. 2015; Kimbell and Morrell 2015), predators (Miner and Stein 1996; Meager et al. 2006) and prey (Abrahams and Kattenfeld 1997; Gregory and Levings 1998; De Robertis et al. 2003) in many species including guppies (Borner et al. 2015; Kimbell and Morrell 2015). We predict that increasing turbidity will result in either a) a switch from more complex to simpler rules as fewer shoalmates can be detected or b) increased error in evaluation of the position of shoalmates, leading to increased error in following any rule.

Methods

Study species and husbandry

All fish were descendants of wild-caught guppies from Trinidad. Fish were maintained in groups of approximately 40 in stock aquaria (200x400x400mm) on a recirculating

system at the University of Hull. Temperature was held at at ~26^{o}C on a 12:12hr light:dark cycle and fish were fed daily on ZM small granular feed (0.5-0.8mm; ZM Systems, Hampshire, UK). Experimental shoals consisting of 10 guppies (N = 12 shoals) were created by taking female fish of similar size (all fish in a shoal measured within 5mm of every other, mean size of fish in shoals varied from 15 – 29mm) from stock tanks and placing them in separate holding tanks (20 x 20 x 20cm) for 24 hours before experiments began. Only females were used as they form the core of guppy shoals (Croft et al. 2004) and to reduce the confounding effect of sexual behaviour on association patterns. Shoals were kept in these tanks for 24 hours before experiments began.

Turbid water was created using a unicellular, motile algae species *Chlamydomonas* (Phytotech lab, Kansas, USA). Algae was grown in a medium containing de-ionised water and Bold's Basal Medium Solution (Phytotech Lab, Kansas, USA) at 20^{o}c, in cylindrical culture vessels (5cm in diameter, 50cm in length) with a constant light source and airflow. Cultures were left to reach high concentrations (~400NTU) and then diluted with water from the aquarium system for experiments to reach ~20 NTU. Using this species ensures algae turbidity remains relatively stable over a period of 75 minutes (Kimbell and Morrell 2015).

Experimental Design

Experiments were carried out in a white circular shoaling tank 50cm in diameter with graduated sides, such that the water depth decreased from 5cm in a central area (20cm in diameter) to 0.5cm at the edges. This discouraged guppies from swimming around the edge of the tank or using the tank sides as a potential refuge. Shallow water restricted shoals to closer to two dimensions, and facilitated tracking of individual fish in turbid water. Trials were recorded from above using a Microsoft Lifecam suspended 40cm above the surface of the water. A monofilament fishing line was attached to two points either side of the tank out of view of the fish, and ran over the centre of the tank, passing 5cm above the camera (45cm above the water surface) at a 45 degree angle. From this a model bird predator (an oval piece of black card 10cm long and 4cm at its widest point) was dropped such that it passed over the centre of the tank, without obscuring the view of the fish.

Shoals were allowed to acclimatise in the shoaling tank for an hour. Then, at a point where the fish were dispersed across the tank (judged by eye), the model predator was released. Previous work has shown this is sufficient to elicit a clear and distinct anti-predator response in guppies (Kimbell and Morrell 2015). Each shoal was tested twice, once in clear and once in turbid water in a randomised order. After the first trial guppies were placed back into the holding tank and tested 24 hours later in the alternate water treatment. Guppies shown no acclimitisation to simulated aerial predation attempts on this timescale (Seghers 1974a; Kimbell and Morrell 2015). The water in the tank was changed after every experiment to prevent the build up of any olfactory cues. At the end of the second trial fish were measured to the nearest 0.5mm using calipers, and returned to stock tanks. As the fish were not marked, it was not possible to identify individuals within the shoal between the two treatments.

Movement rules: fish

To identify the movement pathways of individual fish, we first used VirtualDub (http://www.virtualdub.org) to convert videos into a stack of images at 15fps for each shoal. These were then manually analysed in imageJ (http://imagej.nih.gov/ij/) using the manual tracker plugin MtrackJ. Each fish was tracked by taking the XY coordinate (taken from the nose of each individual) starting from just before the simulated predator flew over the tank until they had stopped moving in response to the predator. As our interest lay in the aggregation rules used, we used only this part of the anti-predator response in our analysis. Fish typically respond to a threat using a range of responses including a C-start, darting and freezing motion: aggregation typically begins after this initial response, and so we restricted our analysis to movement occuring after this. For each individual, we used only the movement in the first 6 frames (0.4 s) after aggregation movement began, and calculate the movement speed of each individual (distance moved/time) for use in the modelling. Simultaneously, we recorded the position of every other fish in the shoal at the point at which the focal fish began aggregation, regardless of where in their own movement sequence they were. These positions were used as the start locations for the fish in modelling the predicted paths (see below). For individuals which did not initiate aggregation (remained frozen), we could not predict a path, and so these fish are

excluded from our analysis as focal fish, but are included as group mates for other fish (N = 18/120 individuals).

Movement rules: model predictions

Predicted paths were generated using the agent-based selfish herd modelling framework described in Morrell and James (2008) and Morrell et al. (2011a, b, 2015). For each shoal 10 point-like agents representing the fish were placed into a circular arena at the positions defined by the locations of the fish in the experimental trials. We assume that all individuals follow the same movement rule, and track the predicted paths of each fish over 6 timesteps. We considered 5 different movements rules (see table 3.1), following previous work on the topic: nearest neighbour (NN), 2 nearest neighbours (2NN), local crowded horizon (LCH), group centre (GC) and movement away from the final position of the simulated predator (AP).

The start of the simulation represented the time at which the focal fish started moving, and all individuals began moving simultaneously (Hamilton 1971; Morton et al. 1994; Viscido et al. 2002; Morrell and James 2008). In each timestep t ($t = 1/15^{th}$ s to match the frame rate of the video), each prey identified its target location, and moved towards that location using the speed of that individual as measured from the video. All individuals moved simultaneously and updated their target location in each timestep.

At the end of the simulation, we calculated the difference in movement direction between the start and end points of the focal fish, and the start and end points of the predicted movement path of that fish for each of the rules, giving us a movement error measured in degrees (hereafter, 'error'). The error measurement took values between 0° (representing an exact follow of the rule) and 180° (a fish moving in the opposite direction to the predictions of the movement rule; figure 3.1). We also investigated how the predicted pathway of each rule for each fish differed, and if the best-performing rule acted in combination with movement away from the predator (See supplementary material). All modelling was carried out in MATLAB R2011a.

Table 3.1: Description of the proposed movement rules for individuals aggregating under the threat of predation (adapted from Morrell and James 2008)

Rule	Description
Nearest neighbour (**NN**)[a]	Individuals moves towards closest neigbour in space
Group centre (**GC**)[b]	Individuals move towards the area in the centre of all individuals within the group
2 nearest neighours (**2NN**)[c]	Individuals moves towards the average location of 2 nearest neighbours
Local crowded horizon (**LCH**)[d]	Individuals moves towards the area with the densest concentration of conspecifics. Closer individuals have a stronger influence on direction, whereas distant individuals exert a weaker force: $f(x) = 1/1+kx$, where x is the distance from the focal individual, and k = 0.375.
Movement away from predator (**AP**)[e]	Individuals move in the opposite direction (180° angle) away from movement of predator (i.e. a potential strike location)

[a] Hamilton (1971)
[b] Viscado (2001), De Vos and O'Brien (2012)
[c] Morton et al. (1994)
[d] Viscido et al. (2000; 2002)
[e] Viscido et al. (2001)

Shoal cohesion

To evaluate overall aggregation levels, we counted the number of group-mates within 3 body lengths (Pitcher and Parrish 1993) of each fish, one frame before the simulated predator threat, and once a stable aggregation had formed. As fish were variable in size, but it was not possible to individually identify fish from the video, we used the mean body length of each shoal as our measure of distance for that shoal.

Statistics

To assess the success of each rule in explaining the movement of the fish, we compared the error measurements (difference in movement angle between the fish and the prediction) for each rule using linear mixed effects models (LME), with rule and water type as fixed effects, and shoal identity a random effect to account for the

repeated measures nature of the data. Error was square root transformed to meet the assumptions of normality. The model was then re-run on clear and turbid water separately, using rule as the fixed effect. Pairwise comparisons of rules were achieved by setting each movement rule as the main intercept (re-levelled the data) in clear and turbid water. To assess how the error for each rule changed between clear and turbid water, we compared the difference using paired Wilcoxon Signed Rank tests on each rule separately. If fish were moving randomly (i.e. not following any rule), we would predict a mean error of 90°, so we assessed whether movement was closer to each rule than to random movement using Wilcoxon Signed Rank tests. *P*-values were corrected for multiple testing using the Benjamini and Hochberg (1995) False Discovery Rate (FDR) control method.

We assessed the effect of turbidity, predation threat and their interaction on shoal cohesion using a generalised linear mixed effects model (GLMER) with a Poisson error distribution (as is appropriate for count data) and shoal identity as a random factor (to account for repeated measures). All analysis was carried out in R 3.1.2 (R Development Core Team 2011).

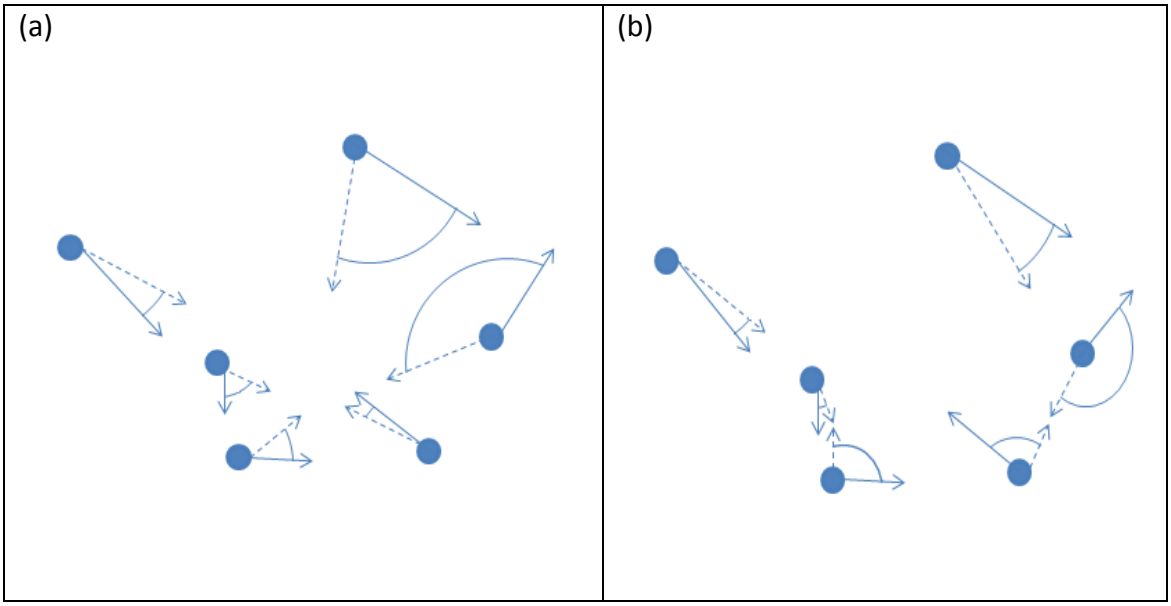

Figure 3.1: Graphical representation of the movement pathways used by fish (solid lines) compared to model simulations (dashed lines) for (a) group centre (GC) and (b) nearest neighbour (NN) movement rules. Errors are calculated in degrees from the start and end points of the actual vs simulated pathway.

Results

Movement rule

Both water clarity ($F_{1,1121}$ = 32.1, P < 0.001) and rule ($F_{1,1121}$ = 8.87, P < 0.001) had an effect on error, but there was no significant interaction. In clear water, we found a significant effect of movement rule on error rate (LME: $F_{4,571}$ = 7.74, P < 0.001; figure 2a). More complex rules, accounting for more neighbours (GC and LCH), had a lower error relative to fish movement compared to the more simple rules (NN, 2NN), and movement away from the predator (AP). In terms of their ability to predict the path of the fish, there was no significant difference between GC and LCH or between the 3 simple rules, but GC and LCH were significantly better at predicting movement paths than NN or 2NN (table 3.2). In turbid water, we saw no effect of movement rule on error rate ($F_{4,509}$ = 2.61, P = 0.3035, figure 3.2b). Pairwise comparisons suggest AP is less good at predicting movement that 2NN, GC or LCH (table 3.2). We found the more complex rules, and movement away from a predator (AP) had lower errors in clear water compared to turbid (GC: V = 3673, P = 0.002, LCH: V = 3477, P = 0.008, AP: V = 3411, P = 0.008), whereas we found no difference in the use of more simple rules between clear and turbid water (NN: V = 2895, P = 0.37, 2NN: V = 3164, P = 0.091). In clear water, all rules were better (lower error) at predicting the movement path of fish that would be expected if movement were random (P < 0.001 for all rules, table 3.2, figure 3.2). In turbid water, the more complex rules (2NN, GC, LCH) predicted movement more accurately than expected by chance while the simpler rules (AP, NN) were no better than chance at predicting movement (table 3.3).

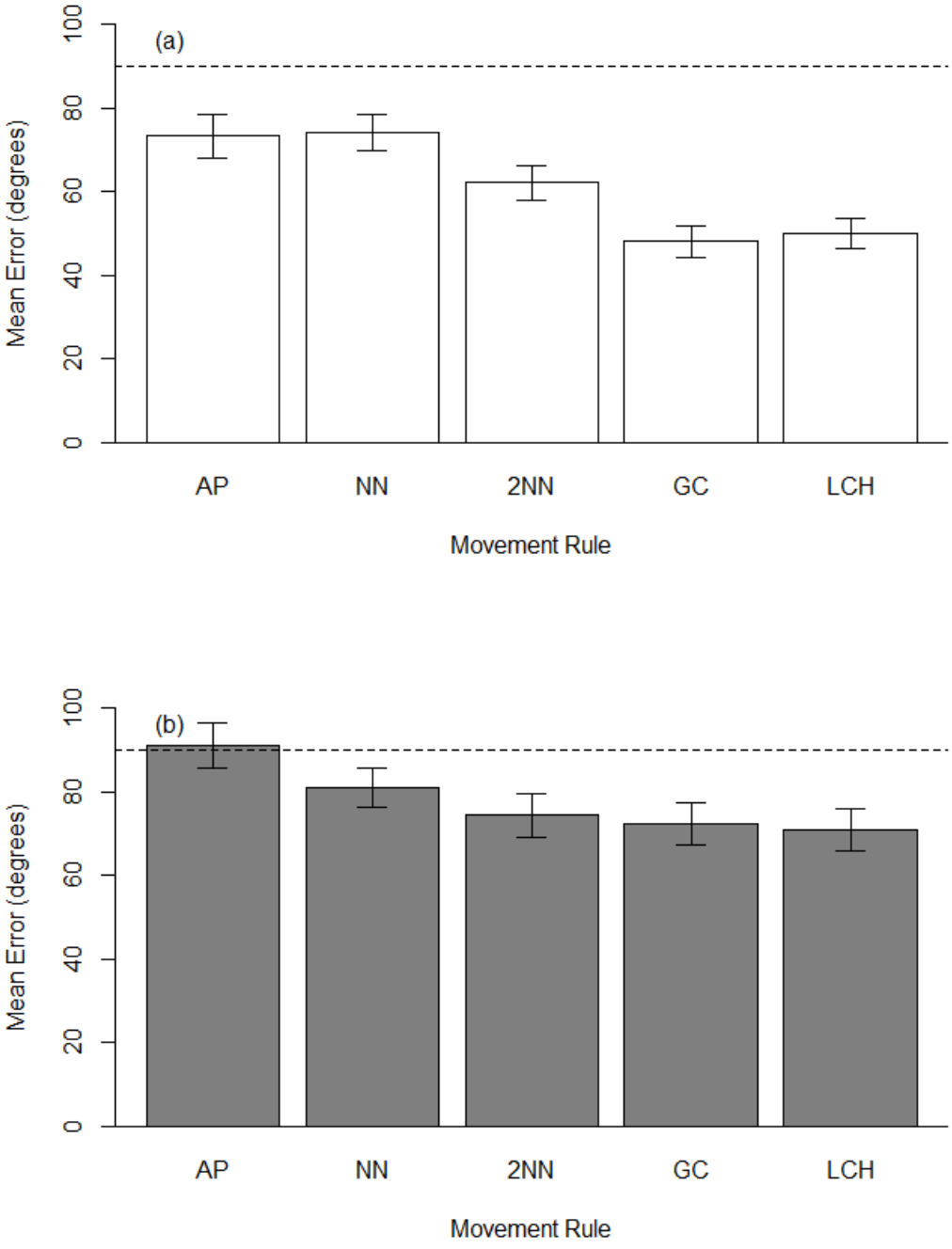

Figure 3.2. Mean error (degrees) ± S.E. between the movement path used by the fish in response to a predator attack and the 5 different movement rules (AP: away from predator, NN: nearest neighbour, 2NN: two nearest neighbours, GC: group centre, LCH: local crowded horizon) in (a) clear water and (b) turbid water. Dashed line at 90^{o} is the prediction of random movement.

Shoal cohesion

There was a significant interaction between treatment (clear and turbid) and time (before and after) on the number of nearest neighbour an individual had (GLMM: Z = 3.12, *P* = 0.0018, effect size = -0.62, figure 3.3). Number of neighbours increased after a simulated attack in both clear and turbid water, but this was more pronounced in clear water

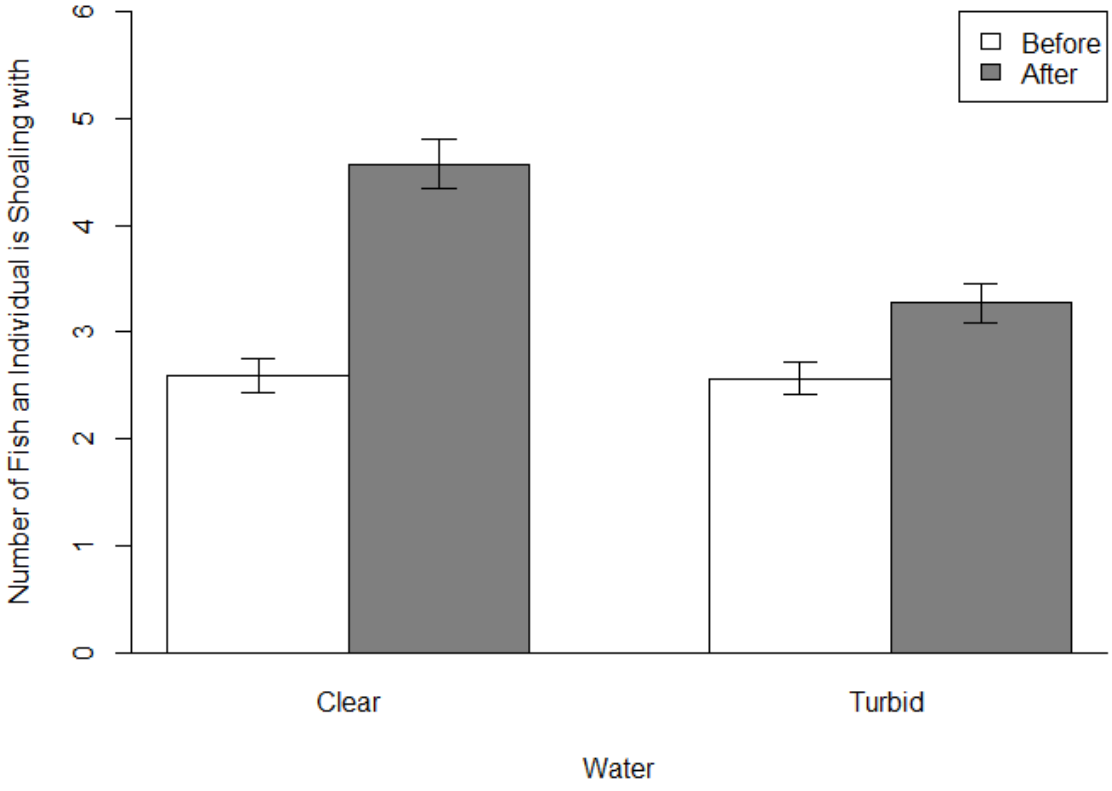

Figure 3.3: The mean (± S.E) number of fish an individual is associating with, before (open bars) and after (filled bars) a simulated predator attack in both clear and turbid water

Table 3.2: Pairwise comparisons of the ability of the 5 different movement rules to predict the movement path of the fish in clear (white) and turbid (shaded) water. Significant p-values are highlighted in bold. In all cases, N = 12 shoals of 10 fish each.

Rule	AP	NN	2NN	GC	LCH
AP		t = 0.71 *P* = 0.48	t = -1.17 *P* = 0.24	t = -3.72 ***P* < 0.001**	t = -3.3 ***P* = 0.001**
NN	t = -0.81 *P* = 0.42		t = -1.88 *P* = 0.061	t = 4.440 ***P* < 0.001**	t = 4.02 ***P* < 0.001**
2NN	**t = -2.1** ***P* = 0.04**	t = -1.28 *P* = 0.20		t = 2.55 ***P* = 0.011**	t = 2.14 ***P* = 0.033**
GC	**t = -2.5** ***P* = 0.01**	t = 1.68 *P* = 0.09	t = 0.4 *P* = 0.69		t= 0.41 *P* = 0.68
LCH	**t = -2.59** ***P* = 0.01**	t= 1.78 *P* = 0.08	t = 0.5 *P* = 0.62	t = -0.096 *P* = 0.92	

Table 3.3: Results (*P* values correct for multiple testing using FDR) from one sample Wilcoxon signed rank tests, testing if the movement used by the fish is significantly different from random (90^{o}) for each of the movement rules. N = 12 shoals each containing 10 individuals

	AP	GC	LCH	NN	2NN
Clear water	*V = 2352* ***P* = 0.004**	V = 620 ***P* <0.001**	*V = 643* ***P* <0.001**	V = 2220 ***P* <0.001**	V = 1357 ***P* <0.001**
Turbid water	V = 2795.5 P = 0.97	V = 1719 **P = 0.0019**	V = 1702 **P = 0.0016**	V = 2181 P = 0.082	V = 1875 **P = 0.0092**

Discussion

Our results demonstrate that shoaling guppies are more likely to use complex (LCH or GC) rather than simple (NN or AP) movement rules when aggregating under the threat of predation, resulting in the formation of more compact shoals, as predicted by the selfish herd hypothesis (Hamilton 1971). Our study provides the first evidence that grouping animals are able to use the position of multiple neighbours when making facultative aggregation decisions under the threat of an imminent predatory attack. Fish are able to consistently choose the numerically larger (Keenleyside 1955; Krause et al. 1998) or denser of a pair of shoals (Frommen et al. 2009) and are able to distinguish between shoal sizes of 40 and 60 individuals (Thünken et al. 2014), yet pairwise interactions are sufficient to capture spatial patterns of shoaling in groups of 30 under non-threat conditions (Katz et al. 2011). The ability of animals to use complex rules has been questioned (Morton et al. 1994; Viscido et al. 2002; Reluga and Viscido 2005), but our results suggest that guppies are cognitively capable of responding to the position of multiple group mates.

Under the degraded visual conditions associated with turbidity, we predicted that guppies would either switch from complex to simpler rules, or show a decreased ability to follow any particular rule. Our results support the second of these predictions: in turbid conditions, the difference between the predicted and actual paths of the fish increased, particularly for GC and LCH rules. This led to the formation of shoals that were more fragmented than those seen in clear water. Turbidity acts to reduce the visual information available to the individuals, and may explain why Cape fur seals move towards one or two nearest neighbours when under threat, rather than accounting for multiple group members (De Vos and O'Riain 2012).

The inability to form cohesive groups in visually poor environments could ultimately alter predation risk and survival, although this is not specifically tested here. In our study, the mean number of close neighbours did not differ between clear and turbid water before the simulated predation attack, previous work has shown that high levels of turbidity can lead to the formation of looser aggregations under non-threat conditions (Borner et al. 2015; Kimbell and Morrell 2015). This implies that already increased inter-individual distances could exacerbate the reduction in ability to respond to multiple neighbours we observed here, leading to further dispersal of prey

shoals. If aggregation is a successful predator avoidance strategy in turbid water (Johannesen et al 2014), then dispersed prey could be more at risk. This may depend on group size and the searching behaviour of predators. Further studies are needed to determine how susceptible dispersed groups of prey survive when visual cues are limited.

We found no evidence that fish were moving away from the likely location of a predatory threat (following an AP rule): error associated with movement towards conspecifics was lower than the error associated with moving away from the predator. One might expect that the direction of a predatory approach to have a significant effect on movement direction. Indeed, Viscido et al. (2001) predicted that movement paths should include movement both towards conspecifics and away from the predator, and this behaviour has been observed in fiddler crab *Uca pugilator* flocks (Viscido and Wethey 2002) and mini herds separated from droves (McLain et al. 2005) We found no evidence to support the suggestion that a combination of GC (one of the best predictors of movement) and AP resulted in a smaller error than GC alone (see Supplementary information). It is likely, therefore, that the directional information provided by the overhead stimulus was not sufficient to trigger this type of response, and our design more closely reflected the non-directional stimulus of Hamilton's (1971) 'hiding lion', where prey perceive the threat, but receive no information as to the possible direction of attack.

Although we find support for complex movement rules, we considered only a single, relatively small group size of 10 individuals (although this falls well within the normal range of shoal sizes found in the wild for this species; Croft et al. 2003). Theoretical work predicts that shoal size and density may be important in determining the best movement rule to follow, with simpler rules favoured when shoals are larger and the individuals within them are more dispersed (Morrell and James 2008). Further work is needed to investigate whether patterns of rule following differ as a function of group size both within and between species, and whether there is commonality across species in the use of different rules. Different predation strategies, for example dispersing prey before attacking, or delaying the attack until further into the centre of the group, may favour the evolution of different avoidance strategies (Demsar et al.

2015), either dynamically, as the same group faces different predators or threats, or as evolved responses across populations or species.

Supplementary Material

a) Choice of 6 frames as a reference point

For each individual, we report the error (difference between the movement direction of the fish and the predicted movement direction from the modelling) calculated 6 frames after movement began. To assess the robustness of this choice, we evaluated the error at 4, 8 and 12 frames, and found that the mean errors for each rule are very similar to those at 6 frames (table S1). Thus, our findings are robust to the timeframe we chose.

Table S1. Mean (1 SE) error (in degrees) evaluated at 4 different time points for all 5 rules, in clear (white) and turbid (shaded) water. In all cases, N = 12 shoals of 10 fish.

Time point	AP	NN	2NN	GC	LCH
4 frames	72.7 (5.2)	77.3 (4.5)	60.1 (4.4)	49.9 (3.6)	50.9 (3.7)
6 frames	73.7 (5.3)	74.6 (4.3)	61.8 (4.1)	47.8 (3.7)	50.0 (3.6)
8 frames	72.4 (5.2)	71.6 (4.5)	60.3 (4.5)	47.3 (3.8)	49.8 (3.8)
12 frames	71.2 (4.9)	72.2 (4.5)	59.4 (4.3)	48.5 (3.8)	52.8 (3.8)
4 frames	89.0 (5.4)	78.5 (4.7)	73.0 (5.2)	71.8 (5.2)	70.2 (5.2)
6 frames	92.5 (5.4)	81.8 (4.6)	75.8 (5.1)	73.7 (5.2)	72.2 (5.1)
8 frames	90.1 (5.3)	82.6 (4.7)	75.0 (4.9)	73.9 (5.1)	73.7 (4.9)
12 frames	90.6 (5.3)	86.7 (4.8)	76.9 (5.1)	71.3 (5.1)	74.4 (5.3)

b) Combined AP and GC rules

Movement away from a predator (AP) may act in combination with other movement rules (NN, 2NN, GC or LCH) to affect the direction of movement. Viscido et al (2001) predicted that movement pathways of aggregating animals would be influenced by a combination of predator direction and the location of conspecifics.

Methods

To assess this for this, we generated a rule that combined AP and GC rules at different ratios, so that the strength of the effect of the direction of the predator decreased in 10% increments from a AP:GC ratio of 100:0 (pure AP) to 0:100 (pure GC). We then compared each of these combinations to the movement pathways of individual fish using identical methology to that of the main paper.

Results

Rules including a higher level of influence from the direction of the predator (AP rule) increased the error observed, the rule was more accurate (i.e. lower error) when just GC rules (AP:GC ratio of 0:10) were compared to the movement pathway of fish, in both clear and turbid water (figure S1)

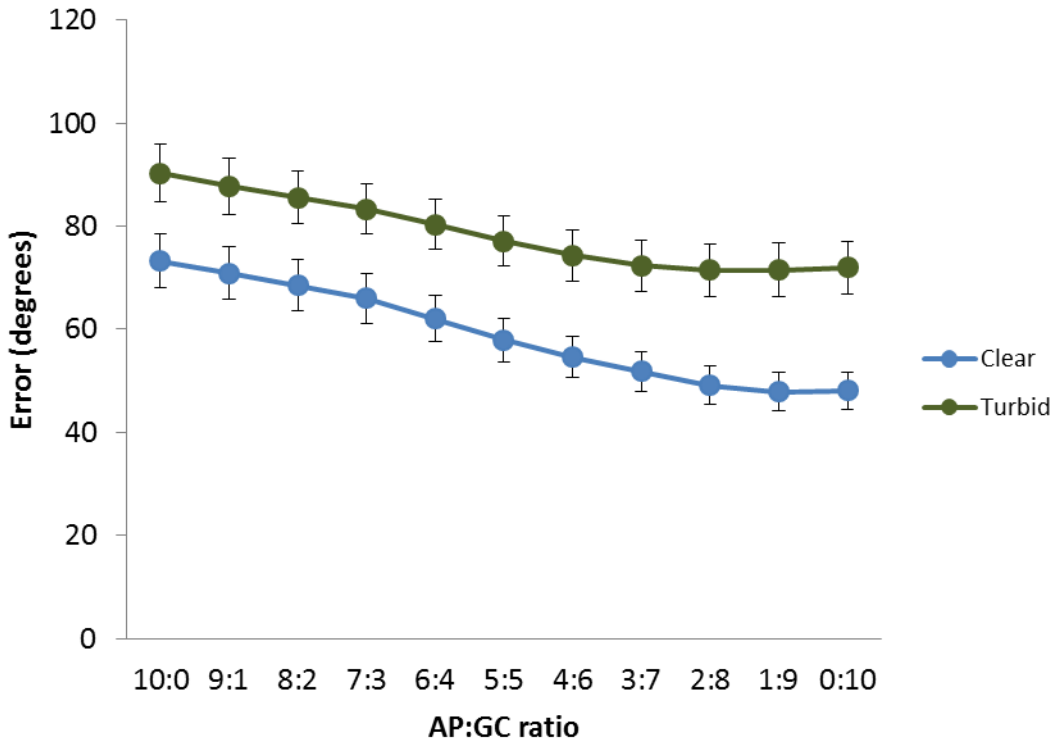

Figure S1: Mean error (± S.E.) when comparing the movement pathways of individual fish against a combined rule containing AP and GC rules at different ratios, represented here as a decreasing influence of the AP rule.

c) Rule comparisons

Some pairs of movement rules may predict similar movement paths, for example, movement following a GC rule may be similar to movement following a LCH rule as both account for multiple individuals within the small groups we evaluated. To assess the similarity of movement rule predictions, we explored the difference in predicted angle between each possible pair of rules for each individual fish. We tested whether the error was significantly different from 0° (what we would expect if the rules predicted the fish moved in the same direction) by using a one sample Wilcoxon Rank Sign test, correcting for multiple testing using the FDR method (Benjamini and Hochberg 1995)

This analysis revealed that GC and LCH made the most similar predictions (mean difference of 27.8 ± 2.5; table 1a), and that 2NN was similar to LCH (mean difference of 40.1 ± 2.9 degrees), but all pairs of rules were significantly different from one another (table S2).

Table S2: Comparison in the error (angle) ± S.E. between the different rules. A lower angle represents a similar direction. In all cases, d.f. = 2200. P-values are after correction using Benjamini & Hochberg's (1995) False Discovery Rate control method.

Rule 1	**Rule 2**	**Mean difference**	**SE**	**V**	**P**
AP	NN	92.2	3.4	24753	< 0.001
	2NN	90.5	3.5	24753	< 0.001
	GC	87.3	3.5	24753	< 0.001
	LCH	89.7	3.4	24753	< 0.001
NN	2NN	45.6	3.0	24753	< 0.001
	GC	66.8	3.2	24753	< 0.001
	LCH	55.1	3.1	24976	< 0.001
2NN	GC	59.3	3.5	24753	< 0.001
	LCH	40.1	2.9	24753	< 0.001
GC	LCH	27.8	2.5	24753	< 0.001

Chapter 4: Turbidity weakens selection for assortment in body size in groups: predator attack decisions and prey responses

Abstract

Prey animals commonly associate with similar-looking individuals to reduce predation risk, via a reduction in predator targeting accuracy (the confusion effect) and preferential targeting of distinct individuals (the oddity effect). These effects are mediated by body size, as predators often preferentially select large bodied individuals, which are at an increased risk within a group. The selection pressure to avoid oddity by associating with similar sized group-mates is therefore stronger for large individuals than small. This selection depends on the ability of both predators and prey to accurately assess body size and respond accordingly. In aquatic systems, turbidity degrades the visual environment and negatively impacts on the ability of predators to detect (and consume) prey. We assessed the effect of algal turbidity on predator-prey interactions in the context of the oddity effect from the perspective of both predator and prey. We find that sticklebacks *Pungitius pungitius* preferentially target larger *Daphnia* in mixed swarms in clear water, but not in turbid water. From the perspective of the prey, large sticklebacks preferentially associate with size-matched individuals in clear water as predicted, but not turbid water, while small individuals showed no social preference in either clear or turbid water. We suggest that a reduced ability or motivation to discriminate between prey in turbid water relaxes the predation pressure on larger prey individuals allowing greater flexibility in their shoaling decisions. Thus, turbidity may play a significant role in predator-prey interactions, altering the selection pressures on both predators and prey.

Introduction

Predator-prey interactions and anthropogenic change are two key factors influencing community structure (Holt 1977). Predation alters abundance, distribution and composition of species in a community either directly through the consumption of prey (Holling 1959), or indirectly by modifying prey behaviour (Lima and Dill 1990).

Interactions between predators and prey are highly sensitive to disturbances in the environment caused by anthropogenic activities. In aquatic environments increased turbidity, caused by de-forestation, urbanisation and eutrophication, degrades the visual environment, affecting how predators detect and target prey (De Robertis et al. 2003) and how prey responds (Gregory 1993; Meager et al. 2006). This can cause a shift in predator-prey interactions (Abrahams and Kattenfeld 1997), ultimately changing community structure through altered levels of risk and survival.

In clear water aquatic predators often show active preferences for particular prey types (Lehtiniemi et al. 2007). When there are no handling constraints, larger bodied prey are generally more profitable (optimal foraging: MacArther and Pianka 1966), and are often over-represented in the diets of planktivorous fish (Li et al. 1985; Wetterer and Bishop 1985). Thus, larger prey individuals are often at greater risk of predation (Lehtiniemi et al. 2007; Manicom and Schwarzkopf 2011). In turbid water, however, size selectivity is often impaired (Reid et al. 1999; Jonsson et al. 2013), as turbidity directly affects a predator's ability to locate and target prey. Predator reaction distances are shortened, which can lower capture success per unit of effort (Gregory and Northcote 1993; Utne 1997), or the type of prey targeted may change, while overall predation rates remain constant (Abrahams and Kattenfeld 1997; Shoup and Wahl 2009). This alters the level of risk experienced by individuals; for prey animals that aggregate, this may mean that while overall risk to the group remains constant, relative risk to individuals within the group changes.

Group formation is a common and important response to the risk of predation. In addition to reducing individual risk (the dilution effect: Foster and Treherne 1981), groups of moving prey visually confuse predators, reducing targeting accuracy (the confusion effect: Krakauer 1995, Tosh et al. 2009). This effect is enhanced in larger groups and when prey individuals are morphologically or behaviourally similar to one another (Landeau and Terborgh 1986). Predators are better able to overcome the confusion effect if a distinct or 'odd' individual is present within the group (the oddity effect: Theodorakis 1989). Predators preferentially target odd individuals as they are easier to visually isolate, making them at increased risk within a group (Milinski 1977a; Ohguchi 1978; Theodorakis 1989). Together, the confusion and oddity effects predict that individuals should preferentially group with phenotypically similar individuals, a

phenomenon well-studied in shoaling fishes (Ranta et al. 1992; McRobert and Bradner 1998; Ward and Krause 2001; Rodgers et al. 2011), but observed in other taxa including birds (Brightsmith and Villalobos 2011) and mammals (Meldrum and Ruckstuhl 2009).

In the context of the confusion and oddity effects, predator selectivity for particular prey phenotypes (e.g. large body size) means that the selection pressure to avoid oddity should be stronger for preferred phenotypes than for less preferred ones (Rodgers et al. 2015). In support of this, larger fish preferentially associate with large conspecifics, while small individuals show no such preference (Svensson et al. 2000; Rodgers et al. 2011), and larger fish are more risk averse than smaller ones when foraging (Peuhkuri 1997; Peuhkuri 1998). Changes in prey selection by predators associated with turbidity may thus alter the relative risk experienced by individuals within groups, which may have significant consequences for group formation and maintenance.

Here, we explore the effect of turbidity on predator-prey interactions in the context of the oddity effect, from the perspective of both predators and prey. Firstly, we assess predator (9-spine sticklebacks *Pungitius pungitius*) preferences for large prey *Daphnia magna* individuals in mixed groups of small and large prey, predicting that preferences for large individuals, particularly when they are odd (Rodgers et al. 2015), should be reduced in turbid water due to the previously documented reduction in size selectivity more generally. Secondly, we assess size-based association preferences of large and small sticklebacks, predicting that because predator selectivity for large individuals is reduced in turbid water, preferences for size-matched individuals in clear water should again be weakened in turbid water.

Methods

Study species and husbandry

9-spined sticklebacks were collected from Noddle Hill Nature Reserve, Hull, (Grid Reference: 4111E, 5348N) in October 2013 and housed in groups of 15-20 in 30 x 30 x 50cm aquaria (stock tanks) at the University Hull. All tanks were connected on a closed

re-circulating system, with external UV and bio-filters and a 20% weekly water change. Tanks were kept at approximately 12^{o}C on a 12:12 light:dark cycle and fish were fed daily on defrosted frozen bloodworm (chironomid larvae) and frozen *Daphnia* (purchased from Ings Lane Garden and Water Centre Ltd, Hull). Fish used were not in reproductive condition, and therefore no effort was made to sex individual fish. 30 fish were labelled as "stimulus fish" for the shoaling experiments (see below) and not used as test fish in either experiment. All other fish were used in both targeting and shoal choice experiments (see below), with at least a week between experiments.

Turbid water was created using a unicellular, motile algae *Chlamydomonas* spp (Phytotech lab, Kansas, USA). Algae was grown in a medium containing de-ionised water and Bold's Basal Medium Solution (Phytotech lab, Kansas, USA) at 20^{o}C, in cylindrical culture vessels (5cm in diameter, 50cm in length) with a constant light source and airflow. Cultures were left to reach high concentrations (~200NTU) and then diluted with water from the aquarium system for experiments to reach 12.5 NTU (±2.5NTU), which equated to approximately 30cm visual depth (measured with a Secchi disc). Turbidity was maintained in experiments using airstones.

Live *Daphnia magna* were used as prey in targeting experiments (purchased from Ings Lane Garden and Water Centre ltd, Hull). Upon arrival to the lab *Daphnia* were placed in 20 x 10 x 15cm tanks containing a small quantity of algae (*Chlamydomonas sp*, ~5NTU) for a minimum of 5 days before experiments. This provided a food source for the *Daphnia* (Ebert and Bethesda 2005) and ensured they were of a standardized colour for experiments, which otherwise may affect detection by predators (Jonsson et al. 2011). Before experiments two size classes of *Daphnia* (large: 2.5mm and small: 1.5mm) were separated from the main population into two size-matched pools (held in tanks measuring 20 x 10 x 15cm).

<u>Experiment 1: Targeting of individuals in groups</u>

To investigate how different groups were targeted, we presented sticklebacks with different combinations of large and small live *Daphnia*. 12 sticklebacks from the same stock tank were placed in a test tank (30 x 30 x 50cm) containing either clear or turbid water to a depth of 15cm and allowed to acclimatise for one hour, after which an opaque barrier was carefully placed 30 cm from one end of the tank, dividing the tank

into a smaller holding area at the back and an larger experimental area. Fish were carefully netted into the holding area and remained there for a further hour.

At the end of the experimental area, a square array of *Daphnia*, consisting of 16 water-filled $1cm^3$ transparent cubes arranged in a 4x4 grid (Rodgers et al. 2013b), was positioned externally on the end of the tank. This ensured visual, but not olfactory cues from the prey were available to the fish, and that prey individuals remained separate and could not physically interact during the experiment. Three treatments with different ratios of *Daphnia* sizes were used; 1:15 large:small (large-bodied minority), 8:8 large:small (equal ratios), and 15:1 large:small (small-bodied minority). In the two treatments with a single odd individual, the position of that individual in the grid was rotated systematically to control for any positional effects (Krause 1994). In the equal ratios treatment, large and small individuals were placed in the grid in an alternating pattern. A Microsoft LifeCam connected to a laptop was placed behind the array to record the trials, and the test tank was screened by a curtain to minimise disturbance.

At the start of each trial, a single test fish was carefully netted over the barrier from the holding area into the experimental area. This caused minimal disturbance to the fish, with the majority (211/213) of fish resuming normal swimming behaviour less than 10 seconds after being transferred to the experimental area. Fish that did not begin swimming within 2 minutes were excluded from the experiment (N=2 fish). The fish was free to view the *Daphnia* array as soon as it was netted over the barrier, and a further 10 minutes were allowed for the fish to attack an individual within the array. From the videos, we recorded the size (large or small) of the first *Daphnia* targeted, defined as the fish making a striking movement towards a particular individual within the array and making contact with the glass of the tank. Once the first attack had been made, the trial ended and the test fish was removed and returned to the stock tanks. Each fish was only used once. Fish that had not made an attack within 10 minutes of being placed over the barrier were excluded from the experiment (20/98 clear water trials, 50/119 turbid water trials). During the turbid trials, turbidity was measured using a handheld Oakton Turbidity Meter every other trial to ensure that it remained at 12.5±2.5NTU. The water in the experimental tank was changed every 12 experiments (i.e. when all the fish in one set of experiments had been used), and

Daphnia were returned to their size matched pools. In clear water we recorded N=25, N=22, N=24 successful attacks for 1:15 (large:small), 8:8 and 15:1 daphnia treatments respectively, and N=25, N=16 and N=24 successful attacks in turbid water.

Experiment 2: Shoal choice

To investigate the effect of turbidity on social decisions, we carried out a series of binary shoal choice tests (McRobert and Bradner 1998; Rodgers et al. 2011) in clear and turbid water. Three days after being introduced and acclimatised to the aquarium, 15 fish measuring between 35-40mm ("large fish") and 15 measuring between 25-30mm ("small fish") were placed in separate aquaria (on the circulating system) and labelled "stimulus fish". These fish were never used as test fish in either the targeting experiment or shoal choice experiments. These sizes were chosen as they were readily available in the population, and because three-spine sticklebacks *Gasterosteus aculeatus*, can distinguish between these size classes (Ward and Currie 2013).

The shoaling preference of each fish was assessed twice: once in clear water and once in turbid water, such that half the fish were tested in clear water first, and half in turbid water first. To allow us to identify individual fish between trials without marking, test fish were moved in groups of 12 (6 large and 6 small) to 4 identical holding tanks (40 x 20 x 20cm), each separated into 3 equal-sized compartments (each 13 x 20 x 20cm) 24 hours prior to experiments. Compartments were separated with clear perforated barriers, which allowed visual and olfactory communication between the test fish, to reduce possible stress caused by separation from conspecifics. Each fish was placed individually in a holding tank compartment, with all compartments within a holding tank containing fish of the same size (3 large or small fish per holding tank). Fish were returned to their individual compartments for 24 hours between experiments.

Shoal choice experiments were carried out in 60 x 20 x 30cm binary choice tanks. The tank was split into 3 compartments by two solid glass barriers allowing the transmission of visual but not olfactory cues, with one larger central compartment (30 x 20 x 20cm) set between two smaller compartments (15 x 20 x 20cm). The two smaller compartments contained the stimulus shoals during the experiment. Two 10cm preference zones (approximately 3 body lengths; Pitcher and Parish 1993) were

drawn up beside each stimulus compartment. Test tanks were filled to a depth of 12cm (approximately 15L) using the turbid water (see above) or clear water taken from the aquarium system and one air stone was placed in each compartment. Water was changed between each set of experiments (12 test fish, 6 large and 6 small). As no olfactory cues were exchanged between the stimulus shoals and test fish, it was not necessary to change the water between each experiment to control for the build-up of cues from the stimulus fish (which may relay information about size; Ward and Currie 2013). As the water for all experiments was taken from the aquarium system (with concentrated algae added for the turbid water experiments), cues from sticklebacks of all body sizes were present in the water.

One hour before experiments, test fish were transferred to individual 20 x 20 x 10cm tanks containing either clear or turbid water to allow for acclimatisation to test conditions. One stimulus shoal of 3 large fish and one stimulus shoal of 3 small fish, selected haphazardly from the stimulus fish tanks, were placed in the two end compartments of the binary choice tank and allowed to acclimatise for 15 minutes. After this time the focal fish was placed in the centre compartment. Observations began when the test fish resumed normal swimming behaviour (between 30 - 120 seconds). One fish was excluded from the trial as it froze for 5 minutes. During a 20 minute observation period the time spent in each preference zone (defined as a fish having more than 50% of its body within the preference zone) and the number of times it moved between preference zones (a measure of activity; Fischer and Frommen 2012, Rodgers et al. 2011) were recorded. The trial was observed from behind a curtain using a Microsoft LifeCam attached to a laptop to minimise disturbance. New stimulus shoals were taken from stimulus fish tanks after every third experiment, and the side containing the shoal of large fish was systematically alternated. To reduce the overall number of stimulus fish required, each individual was used more than once over the course of experiments, but haphazard selection of individuals from the stimulus stock tanks meant that it was unlikely the same combination of fish was selected more than once. After each trial, test fish were placed back into the holding tanks and fed defrosted frozen bloodworm. Stimulus fish were fed on completion of the day's experiments.

Statistical analysis

In the targeting experiment (experiment 1) we assessed whether sticklebacks targeted particular body sizes more than would be expected by chance using exact binomial tests. In each case, we compared the observed proportion of attacks on large *Daphnia* to expected probabilities based on random targeting. Expected proportions for the large minority, equal ratios and small minority treatments were 0.0625, 0.5 and 0.938 respectively. For example in a 1:15 ratio of large *Daphnia*: small *Daphnia*, the large *Daphnia* would be targeted 6.25% of time (1/16 x 100) if attack was random with respect to body size. 95% confidence intervals for the proportion of attacks on large *Daphnia* were calculated using the 'modified Wald' method recommended by Agresti and Coull (1998). The per capita risk to individuals was calculated as the proportion of trials in which an individual was targeted, divided by the number of size matched individuals present in the group (Rodgers et al. 2015).

For the shoal choice experiments (experiment 2), we used a generalised linear mixed effects model (GLMER) model with a binomial error distribution (as appropriate for proportion data) to assess whether the proportion of time spent shoaling with size matched individuals (shoal choice) was influenced by turbidity (clear or turbid water), test fish body size and their interaction. Fish ID was included as a random factor to account for the repeated measured design and an additional observation-level random effect was used to account for overdispersion of the data (Harrison 2014). To assess whether the shoaling preference exhibited by large and small fish in clear and turbid water differed significantly from random expectation (50% of the time with each shoal), one-sample tests were applied. Data was arcsin square root transformed to meet the assumptions of normality where possible and a one-sample t-test was used; otherwise we used a non-parametric Wilcox signed ranks test. The false discovery rate (FDR) method was applied to correct for multiple testing (Benjamini and Hochberg 1995), and we present the adjusted p-values here. We used a linear mixed effects (LME) model to assess the effect of turbidity, body size and their interaction on the total time spent shoaling with both shoals. Fish ID was included as a random effect to take into account the repeated measures design. Non-significant interactions were removed following Crawley (2007). Visual inspection of plots of residuals against fitted values and quantile-quantile plots indicated that a normal error distribution was

appropriate here (Crawley 2007). Finally, to investigate if activity (the number of times the fish switched between preference zones) was influenced by body size, turbidity and their interaction we used a GLMER model with a Poisson error distribution (as appropriate for count data) with fish ID included as a random factor.

Results

Experiment one: Targeting of individuals in groups

In clear water, large individuals were targeted significantly more than was expected by chance in the equal ratios treatment (figure 4.1a: large *Daphnia* targeted in 20/22 trials, 91%, $P < 0.001$, with random expectation 0.5) and in the large minority treatment (figure 4.1a: large individual targeted in 10/25 trials, 40%, $P < 0.001$, random expectation 0.0625). In turbid water, however, large individuals were no longer preferentially targeted at either ratio, and were chosen with a rate consistent with chance (figure 4.1a: equal ratios: large individual targeted 12/16 trials, $P = 0.08$, with a random expectation of 0.5, large minority: 4/25, $P = 0.076$, random expectation 0.0625). When large individuals made up the majority of a group (15:1) large *Daphnia* were attacked at a rate consistent with chance in both clear and turbid water (figure 1a: 100% of trials. 25/25 in clear and 21/21 in turbid water).

Per capita risk for large individuals is greatest when they form the minority in the group, and decreases as the number of large individuals increases, and in turbid water (figure 4.1b). In small individuals, per capita risk increases as their number within the group increases. Small individuals are slightly more at risk in turbid water (figure 4.1c) although they are still at lower risk overall compared to large individuals.

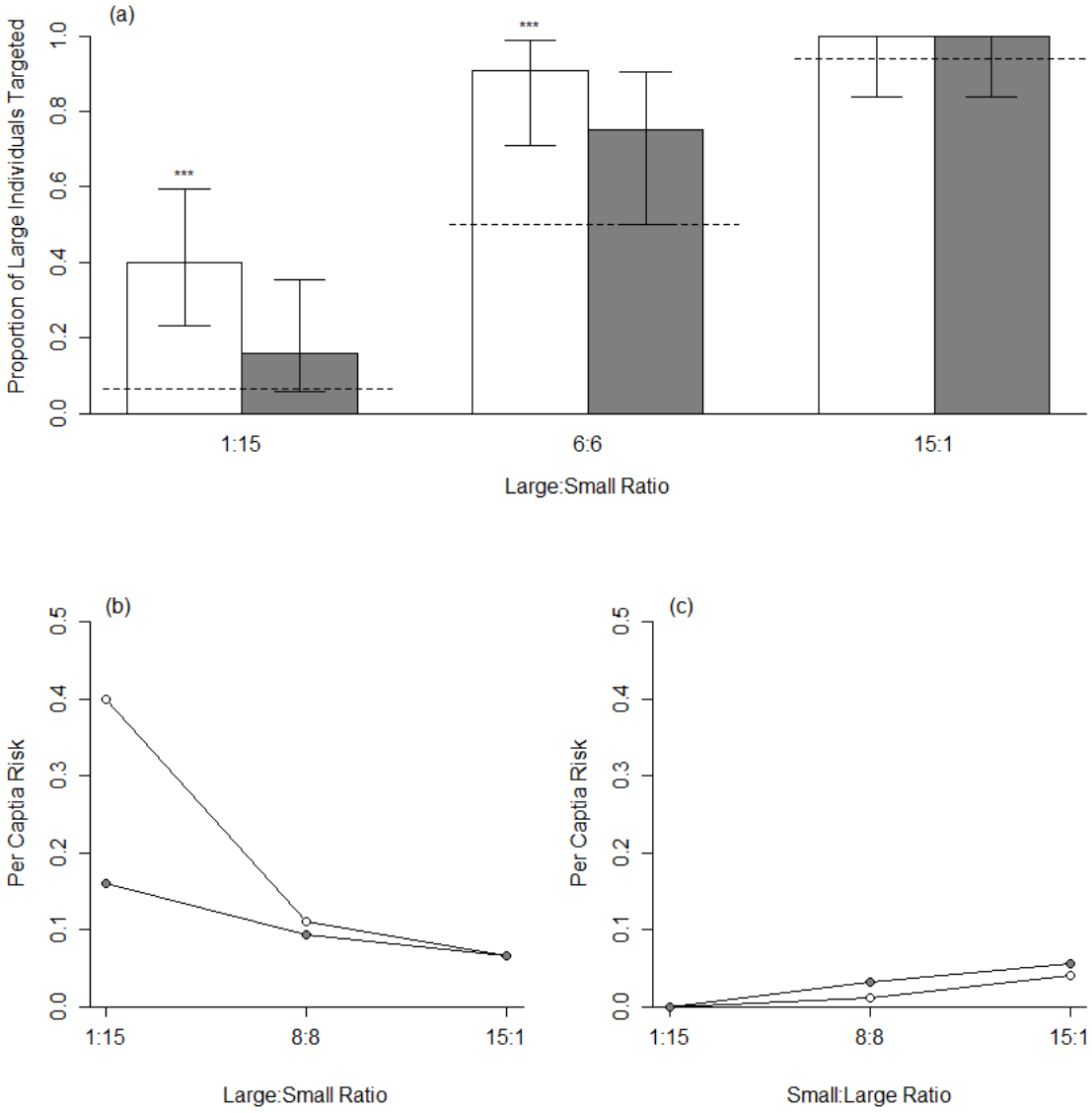

Figure 4.1: **(a)** Proportion of attacks on large individuals when large *Daphnia* were the minority (1:15), equal ratios (8:8) and majority (15:1) within the group (± 95% C.I.) in clear (light bars) and turbid (dark bars) water. Dashed lines represent the proportion expected if prey selection were random according to each prey group composition. Asterisks indicate significant differences from random expectation ($P < 0.001$). **(b)** The per capita risk for large *Daphnia* for each of the treatments (large minority, equal rations and large majority). **(c)** The per capita risk for small *Daphnia* for each of the treatments (small minority, equal rations and small majority). Open circles represent clear treatments and dark circles represent turbid treatments. Lines connecting points are for ease of visualisation.

Experiment two: Shoal choice

We found a significant interaction between water treatment and body size on the proportion of time individuals spent associating with size matched shoals (GLMER: Z = 2.22, *P* = 0.027, figure 4.2a, table 4.1a). Large individuals preferred to associate with size matched shoals in clear water (t = 3.99, adjusted *P* = 0.0024), but not in turbid water (t = 0.56, adjusted *P* = 0.36). Small individuals showed no active preference for either sized shoal in clear (V = 94, adjusted *P* = 0.64) or turbid water (V = 122, adjusted *P* = 0.64). Both large and small test fish spent significantly more time shoaling overall (total time spent shoaling) in turbid water compared to clear (LME: $F_{1,44}$= 14.52, *P* < 0.001, figure 4.2b, table 4.1b), but there was no effect of body size and no interaction (table 2). Finally, we found a significant interaction between water treatment and fish size on activity levels (GLMER: z=3.07, p = 0.002, figure 4.2c, table 4.1c). Large test fish had a higher level of activity in clear water compared to small fish, but both large and small fish reduced their activity to similar levels in turbid water. Examining the data more closely, we found that fish in turbid water were more likely to remain in one preference zone for the duration of the trial than fish in clear water (5/46, 11% clear water trials, 15/46, 33% turbid water trials).

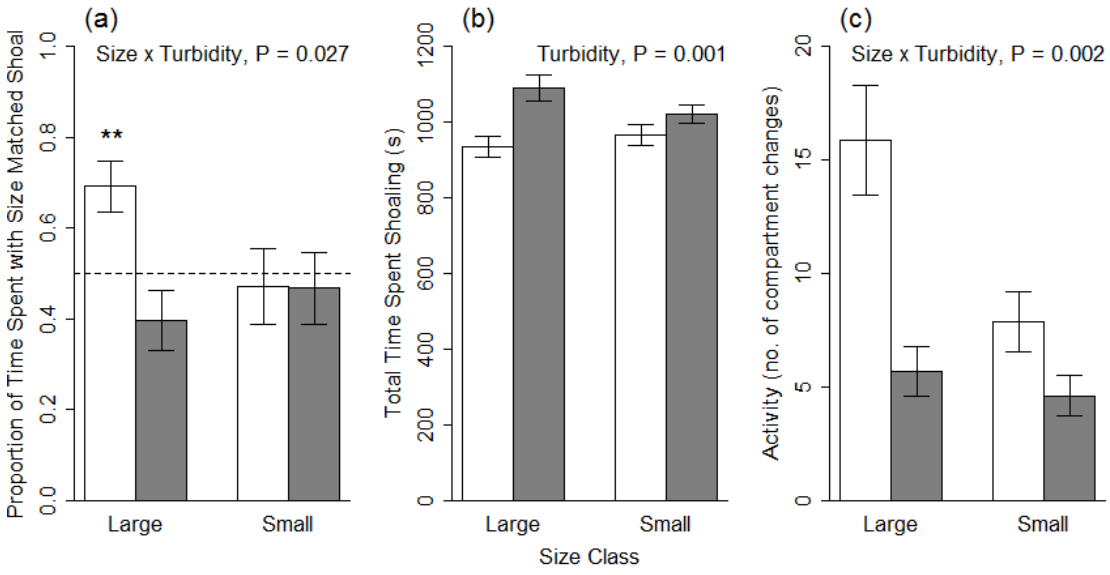

Figure 4.2. **(a)** Proportion of time spent shoaling with a size matched shoal for both large and small fish in clear (light bars) and turbid (dark bars) water (±S.E.). Dashed line represents the proportion expected if fish chose shoals by chance (0.5). **(b)** The total

time spent shoaling by large and small fish in clear and turbid water (±S.E.). **(c)** Activity (number of compartment changes) by large and small fish in clear and turbid water (±S.E.)

Table 4.1: Results of the analyses of the effects of turbidity treatment (clear or turbid), test fish body size (large or small) and their interaction on a) the proportion of time spent associating with the size matched shoal (GLMER with binomial errors), b) the total time spent shoaling (LME) and c) the number of times the test fish moved between the preference zones associated with the two shoals (activity levels; GLMER with Poisson errors). Significant p-values are highlighted in bold text.

Fixed effects	**Test statistic**	**DF**	**P**	
a) Proportion of time spent with size matched shoal				**Log odds (+S.E.)**
Turbidity	Z = -3.17		**0.002**	-4.14 (1.31)
Size	Z = -1.73		0.085	-2.34 (1.35)
Turbidity * Size	Z = 2.21		**0.027**	4.08 (1.84)
b) Total shoaling time (s)				**Effect Size**
Turbidity	F = 14.52	1,45	**<0.001**	1065.72
Size	F = 0.32	1,45	0.57	943.06
c) Activity (number of preference zone switches)				**Log odds (+S.E.)**
Turbidity	Z = -10.05		**<0.001**	-1.03 (0.10)
Size	Z = -2.79		**0.005**	-0.61 (0.22)
Turbidity * Size	Z = 3.07		**0.002**	0.49 (0.16)

Discussion

Our results suggest that turbidity weakens predator preferences for targeting odd, large-bodied individuals, and relaxes the pressure on large bodied prey to associate with similarly-sized groupmates. This suggests there is reduced selection pressure for behavioural assortment in prey, driven by confusion and oddity effects, in turbid water. Reflecting previous work (Gibson 1980; Li et al. 1985; Wetterer and Bishop

1985; Rodgers et al. 2015), we found strong predator selectivity for large prey in clear water, particularly when they were in equal ratios with small-bodied prey or were in the minority (odd) within the group. In turbid water, however, predators no longer showed this preference, a finding supported by previous work (Vinyard and Yuan 1996; Rowe et al. 2003). We suggest that turbidity therefore relaxes predation pressure on large individuals within groups, reducing the benefits of assorting by size, and resulting in a loss of the preference for associating with size-matched conspecifics seen in clear water when turbidity increases.

Large prey may be less at risk in turbid water due to visual constraints: in low-visibility conditions, predator-prey interactions occur at closer distances (Miner and Stein 1996; Meager et al. 2006), meaning predators may have reduced choice or reduced time for selection of prey from a group. Algae absorb photosynthetically active wavelengths and scatter light (Kirk 2011), reducing the contrast between objects and their background (Utne-Palm 2002), which negatively affects long-distance detection substantially more than short distance detection (De Robertis et al. 2003). For large individuals, therefore, detection distances are reduced to a greater extent than for small individuals, which may reduce size selectivity by altering encounter rates (Utne-Palm 2002; Jonsson et al. 2013). Turbidity may impact on predator confusion: if detection distances are reduced, prey swarms may appear less dense or numerically smaller, and predators therefore less susceptible to confusion effects (which are enhanced in larger and denser groups; Milinksi 1977b; Ioannou et al. 2009). The importance of oddity for successful predation would therefore also be reduced, and preferences for odd individuals in groups weakened. Further investigation is needed to explore the effects of turbidity on confusion and oddity effects.

If predators are less selective in turbid water, then prey could be expected to respond appropriately to the altered risk environment. Our results suggest that shoaling fish adjust their shoal choices in response to their immediate environment, with large fish losing their preference for size-matched shoals under turbid conditions. For large individuals, at higher risk of predation, association with size-matched individuals reduces risk by reducing oddity and enhancing confusion effects (Theodorakis 1989; Ranta et al. 1992; Rodgers et al. 2011), while for small individuals, risk is lowered through association with larger prey (Rodgers et al. 2015). If predator targeting of

large-bodied prey is reduced, and the anti-predator benefits of size-matching are reduced, we would expect to see a reduction in the strength of association preferences. Association with large-bodied individuals carries a number of costs – particularly increased competition for food (Metcalfe and Thomson 1995; Hoare 2000), which can be avoided under the relaxed selection pressures for assortment in turbid water.

Turbid water is often associated with a reduced perception of overall risk in fish (Gregory 1993; Engström-Öst and Mattila 2008), resulting in reduced anti-predator behaviour, including weakened escape responses (Gregory 1993; Meager et al. 2006), reduced use of shelter (Abrahams and Kattenfeld 1997) and decreased shoal cohesion (Kimbell and Morrell 2015) even if actual risk remains unchanged (Reid et al. 1999; Shoup and Wahl 2009). A reduction in shoaling preferences could be attributed to this effect: sticklebacks perceive that overall, rather than individual, risk is reduced and adjust their social behaviour accordingly. However, our finding that fish both increased the total time they spent in association with other shoals (figure 4.2b) and reduced their activity levels (figure 4.2c) suggests an enhanced, rather than reduced, perception of overall risk levels in turbid water for 9 spined sticklebacks. Reduced activity levels are thought to reduce encounter rates with predators and have previously been observed in shoaling fish (Fischer and Frommen 2012). By remaining with a shoal, rather than moving between shoals, individuals reduce their exposure to predators under situations where they are at increased risk through isolation (Landeau and Terborgh 1986).

As predators, fish are affected by visual constraints in turbid water, and thus the same constraint might be expected for fish as prey. Our test fish may have been unable to detect both shoals simultaneously, although the turbidity levels in our experiment (12NTU, equivalent to a secchi depth of 30cm) were chosen so that fish should be able to view both shoals simultaneously from any location within the test tank. It remains possible that distinguishing the body sizes of conspecifics is more difficult in turbid water, particularly from a distance. As a result, individuals may be unable to assess whether the shoal they were not associating with at any given time was in fact a better ‘match’ for them, phenotypically, and shoals are therefore formed via chance

encounters rather than active choice. Further work is needed to tease apart the precise mechanisms underlying the changes in shoal preference we observed.

Overall, we have shown that reduced size selectivity by predators and reduced shoal preference by prey are both consequences of increasing turbidity in aquatic environments. Together, these changes both reduce the selection pressure for prey to associate with phenotypically matched individuals, and weaken those association preferences. The confusion and oddity effects are thought to be strong drivers in the evolution of behaviours leading to the formation of phenotypically associated groups, but under turbid water we anticipate a reduction in phenotypic assortment in groups, leading to more diverse, less assorted groups. As assortativeness is associated with behaviours other than predator avoidance, such as enhanced foraging efficiency (Lindstrom and Ranta 1993; Ranta et al. 1994) and synchronisation of activity (Conradt and Roper 2000), a reduction in the pressure for assortment may increase the costs associated with other activities for animals that live in groups.

Chapter 5: Exposure to visually poor environments improves foraging performance in adult guppies under low light conditions

Abstract

Many aquatic habitats are changing rapidly as a consequence of human activities. Anthropogenic change can degrade the visual environment, through eutrophication or disturbance. Exposure to an altered visual environment during early life provides a mechanism by which animals can compensate for the effects of this change, for example, by making a sensory switch from vision to olfaction. It remains unclear if adults can make a similar sensory switch. As the visual environment changes over short timescales, it is important to understand how aquatic organisms respond at different developmental stages. We investigated how adult guppies *Poecilia reticulata* that were exposed to light or dark environments for 2 and 4 weeks responded to visual, olfactory and a combination of both food cues. We found no difference in foraging behaviour between light and dark exposed adults after 2 weeks, however after 4 weeks of exposure, dark-exposed guppies responded more strongly to food cues in the dark conditions they had experience of, regardless of the cue provided. We report an improvement in foraging in dark environments by dark-exposed adults after 4 weeks, but not via the sensory switch to olfactory cues as reported in guppies reared in dark environments from birth. Hence adults exposed to visually degraded conditions are able to forage successfully in low light conditions but the mechanism driving this compensation differs from juvenile guppies.

Introduction

Organisms live in habitats that are heterogeneous in both space and time. Individual animals are often able to respond to sudden, short-term changes in their environment by altering behaviour, an extremely plastic trait. Behavioural plasticity allows animals to adjust their behaviour rapidly to minimize negative consequences of a stressful

environment and is usually the first response to altered conditions (reviewed in Tuomainen and Candolin 2011 and Candolin and Wong 2012). However, behavioural plasticity can be limited, particularly if stressors in the environment increase, become permanent, or dispersal away from the threat is restricted (Schwartz et al. 2006; Thomas 2011). Exposure from birth provides an alternative mechanism by which animals can adapt to degraded environments, through developmental or compensatory plasticity (Rauschecker 1995; West-Eberhard 2003; Monaghan 2008). This type of plasticity is often more costly and less flexible than behavioural plasticity and can be dependent on a critical developmental window (West-Eberhard 2003).

Many habitats are changing rapidly as a result of human activities. Anthropogenic environmental change frequently disrupts an organism's sensory environment; increased noise created by roads can affect auditory communication in birds (Slabbekoorn and Peet 2003) and eutrophication in lakes degrades the visual environment, reducing foraging rates and impacting on a range of other behaviours in fish (Candolin et al. 2007; Fischer and Frommen 2012). Compensatory sensory plasticity, whereby experience of a degraded sensory environment leads to an increased capacity of an alternative sense (Rauschecker and Kniepert 1994), has been well documented in juvenile animals, including cats (Rauschecker 1995), rats (Ryugo et al. 1975), humans (Roder et al. 1999) and fish (Chapman et al. 2010b). Guppies *Poecilia reticulata* reared from birth in a low light environment, for example, make a sensory switch from vision to olfaction when detecting food cues, enabling them to maintain foraging rates (Chapman et al. 2010b). However, evidence is scarce for whether such 'sensory compensation' is a property of early ontogeny and a limited developmental window exists in which juveniles are able to make a sensory switch.

Adult fish can adjust their behaviour in response to rapid changes in the visual environment: male sticklebacks increase the frequency of mating displays and display more intense red colouration in turbid water (Candolin et al. 2007; Engstrom-Ost and Candolin 2007) and juvenile cod *Gadus morhua* increase searching activity for food sources (Meager and Batty 2007). These behaviours can compensate for the reduction in vision, but may be costly terms of time and energy spent by an individual. Adult fish may also be able to compensate for reduced visual information by increasing their reliance on an alternative sense. For example 3-spine sticklebacks *Gasterosteus*

aculeatus can maintain foraging rates by using olfactory cues after relatively short acclimatisation periods (Webster et al. 2007; Johannesen et al. 2012), suggesting that these individuals are able to cope to some extent with short term losses in vision by altering behaviour. Other species, however, appear unable to make this short-term switch (salmon *Salmo salar*: Fraser and Metcalfe 1997; largemouth bass *Micropterus salmoides*: McMahon and Holanov 2005).

While many studies investigating how adult fish respond to changes in their sensory environment consider the effects of immediate change (Webster et al. 2007; Fischer and Frommen 2012; Johannesen et al. 2012), fewer consider responses during longer-term exposure. Costly responses such as increased activity (Meager and Batty 2007) may not be sustained over longer timescales, while mechanisms such as learning (Odling-Smee and Braithwaite 2003), physiological or morphological changes (Webster et al. 2011) may allow responses to be maintained or improved. A combination of behaviour flexibility combined with sensory plasticity may buffer individuals against potential negative consequences of altered sensory environments, while maintaining the ability to deal with fluctuations in the environment.

Here we test whether adult guppies exposed to visually degraded conditions are able to compensate in a foraging task carried out under low light conditions, and test whether compensation occurs via a sensory switch from vision to olfaction. We exposed adults for varying lengths of time (2 and 4 weeks) to assess the importance of exposure duration on foraging performance and compensation. If adult guppies are able to compensate for reduced availability of visual information, we predict that dark-exposed fish will respond more strongly to olfactory food cues than light-exposed fish, improving their foraging success in low-light environments with increasing exposure time. In contrast, light-reared fish should respond most strongly to visual cues and will show reduced foraging success in low-light environments. If, however, sensory compensation is limited to a critical developmental window early in life (West-Eberhard 2003; Chapman et al. 2010b), dark-exposed fish are predicted to be unable to compensate for the degraded environment and will not switch to an increased reliance on olfactory cues.

Methods

Study species and exposure environments

All fish used in this experiment were descendants of wild-caught guppies *Poecilia reticulata* from Trinidad. Stock tanks of guppies were maintained in aquaria (20 x 40 x 40cm) at the University of Hull at ~26^{o}C on a 12:12hr light:dark cycle and fed daily on ZM fine sinking food (ZM Systems, Hampshire, UK).

135 sexually mature guppies were randomly assigned to one of two light intensity treatments: relatively high light intensity (~300 lux; "light treatment") and relatively low light intensity (~1.5 lux; "dark treatment"). Exposure tanks measured 20 x 20 x 20cm, contained an artificial plant and were held in our aquarium facility on a 12:12 hour light:dark cycle. The dark treatment was created by turning off the aquarium lights directly above the tanks (but leaving the room lights on the specified light cycle), and isolating the tanks from the main room using a thin black polycotton sheet. This ensured fish were not kept in total darkness, as some light still penetrated through the sheets. To control for the positioning of the sheet, light tanks were isolated from the room by a thin white sheet. All holding tanks (light and dark) were kept on the same circulating aquarium system with a 10% daily water change using a 50:50 mixture of purified water and filtered tap water. In order to keep the algae growth at a minimum in tanks (which may otherwise be used as a food source), UV filters were used on the system, and all tanks were carefully cleaned twice weekly and the aquarium plants changed weekly to prevent algae build up. This appeared to cause minimal disturbance to the fish.

Fish were placed into the exposure tanks at an initial density of 6 fish per tank and fed twice a day on equal quantities of crushed ZM flake food using a 5 x 2mm spatula. Fish were held in the exposure tanks for a total of 4 weeks. After 2 weeks and after 4 weeks, fish were removed from the tanks and randomly assigned to a cue treatment (visual, olfactory or both). Each fish was tested individually in light and dark test conditions, with 24 hours between trials before being returned to the tank. We carried out 21 replicates (21 groups of 6 fish) for the high light intensity treatment and 19 replicates of the low light intensity treatment.

Foraging experiment

Foraging trials used a similar methodology to that in Chapman et al (2010b). Trials took place in a rectangular plastic tank (40 x 25 x 15cm, figure 1) filled to a depth of 5cm with water taken from the aquarium system. At one end of the tank two solid, transparent cylindrical containers (diameter 7.5cm, height 10cm) also filled to a depth of 5cm were fixed to the base of the tank. They were positioned in the corners of the tank with a minimum distance of 10cm between them. The cylinders contained visual cues from food during the relevant trials, and contained no food during trials that did not involve visual cues. No olfactory cues were able to pass from the cylinders to the test tank. A 3cm preference zone was drawn around each container. Two plastic clips were placed on the outside of these containers, which held tubes to allow for olfactory cues to enter the test tank below the water line, during the relevant trials. 12cm from the opposite end of the tank a horizontal "start line" was drawn, dividing the tank into two sections (a starting section and a choice section).

The experimental tank was housed within a wooden shelter (70 x 50 x 65cm) with an open front and an opening directly above the tank where a Panasonic SDR S26 video camera was placed, so fish could be observed without disturbance. An aquarium light attached to a clamp stand was placed inside the shelter to ensure the light trial treatments received a similar light intensity as the light exposure treatment. In the dark trials this was switched off. The shelter was then covered with a black (dark trials) or white (light trials) polycotton sheet to both ensure the correct light intensity and minimise disturbance to the fish during the trials.

Visual cues were created by placing 0.2g of crushed flake food (ZM fish food) onto the surface of the water of one of the containers, using a funnel from outside the wooden shelter to minimise disturbance. Olfactory cues were created by mixing 10g of flake food in 1 litre of purified water and filtering this through a fine mesh to remove any visual cues. A control cue was made up of 1 litre of purified water with 0.2ml of yellow food dye (to match the colour of the food cues). These cues were dispensed via a peristaltic pump which released the cues through tubes connected to the cylindrical containers at a rate of 6ml per minute. An overflow pipe was placed 5cm above the base of the tank at the end of the tank opposite the cue cylinders to maintain a

constant water level. For the visual only and olfactory only treatments, only the relevant cue was added. For the both cues treatment, both visual and olfactory cues were added at the same side. The side containing the cues was randomised.

Experimental protocol

24 hours prior to experiments, all fish within a exposure tank were placed into 2 holding tanks (40 x 20 x 20cm), both separated into 3 equal-sized compartments (each 13 x 20 x 20cm) and fed. Compartments were separated with clear perforated barriers, which allowed visual and olfactory communication between the test fish, to reduce possible stress caused by separation from conspecifics. Each of the 6 fish were randomly assigned to a cue treatment (2 visual, 2 olfactory and 2 both), and tested in both light and dark trial conditions, separated by 24 hours. An hour before each trial, the fish were acclimatised to the trial lighting conditions by placing them in a separate small tank (20 x 20 x 20cm). At the start of each trial, an individual was placed in the test tank and given 2 minutes to explore the tank. After the acclimatisation period, and once the fish had returned to the start section, the food cues were added. The trial began when the fish subsequently crossed the start line (figure 5.1). Each trial lasted 5 minutes. We recorded the time spent in the preference zones of both the cue and control cylinders, from which we calculated the proportion of time spent with the cue (Chapman et al. 2010b). At the end of the 5-minute trial fish were returned to their holding tank compartment, allowing us to track individual fish between trials. When the trials for each set of fish were completed the fish were fed. They remained in the holding tanks for a further 24 hours to allow them to be re-tested under the alternative lighting environment. After the second trial, fish were released into their home tank and fed. Fish were tested after 2 weeks in their exposure tanks and again at 4 weeks. Individuals were not marked or tagged, so we were unable to track individuals between the 2 week and 4 week trials. The order the fish experienced light and a dark condition was alternated. The tank was emptied and rinsed with clean water between each experiment to remove any olfactory cues from food or the previous fish. Fish that failed to enter both control and cue zones or did not cross over the start line were removed from the analysis (2 weeks: n=79/432 and 4 weeks: 73/392).

20 fish died during the 4 week exposure period (light: 5/106, dark: 15/110), but no tanks contained fewer than 4 fish at the end of the experiment. Final sample sizes were: visual cues (2 weeks: light exposed n = 48, dark exposed n = 62. 4 weeks: light exposed n = 51, dark exposed n = 52), olfactory cues (2 weeks: light exposed n = 57, dark exposed n = 60. 4 weeks: light exposed n = 49, dark exposed n = 51), both cues (2 weeks: light exposed n = 60, dark exposed n = 66. 4 weeks: light exposed n = 60, dark exposed n = 56), with each fish tested in both light and dark conditions for one cue type at both 2 and 4 weeks.

Statistical analysis

All data were analysed using R 2.15.1 (R Development Core Team 2011). A linear mixed effect (LME) model was performed on the 2 week and 4 week trials independently. As fish were not identifiable between week 2 and week 4, it was not possible to compare between trials directly. Exposure environment (light or dark), experimental lighting (light or dark) and cue type (visual, olfactory or both) were included as main effects, with individual identify and tank identity as random factors to account for the repeated measures of the data and non-independence of individuals from the same exposure tank. Sex had no effect in our analyses and so was excluded. Non-significant 2- and 3 way-interactions were removed from the models following Crawley (2007).

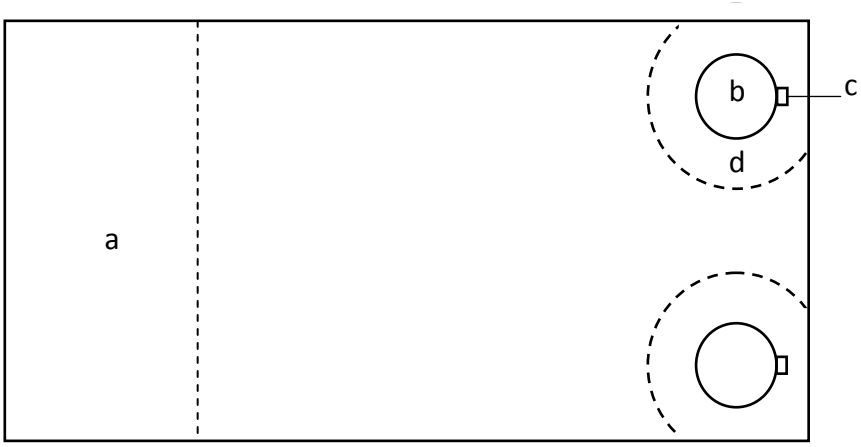

Figure 5.1: Experimental tank set up, where a) represents the starting section, where the fish must be in for a fish to start the experiment, b) 7cm solid cylindrical tube used to contain visual food cues, c) piping where the olfactory cues enter and d) 3cm preference zone

Results

After a two week exposure period, we found a significant 2-way interaction between trial lighting conditions and the food cue provided (Figure 5.2: $F_{1,139}$= 4.34, p = 0.015), but no effect of exposure environment and no other significant interactions (table 5.1). Fish spent a greater proportion of time with a cue in the light trial conditions when visual cues were present (i.e. in the visual and both treatments; figure 5.2a-c), and fish from both light and dark exposure environments responded to cues in the same way.

After 4 weeks exposure, we found a significant 2-way interaction between exposure environment and trial lighting condition (Figure 5.2: $F_{1,122}$ = 8.77, p = 0.0037, table 5.2). Fish spend a greater proportion of time with the cue in the lighting conditions they were previously exposed to irrespective of cue (visual, olfactory or both). Guppies exposed to light environments guppies spent a greater proportion of time with the food cue in light trials and dark-exposed spent a greater proportion of time with the cue under dark trial conditions (figure 5.2 d-f).

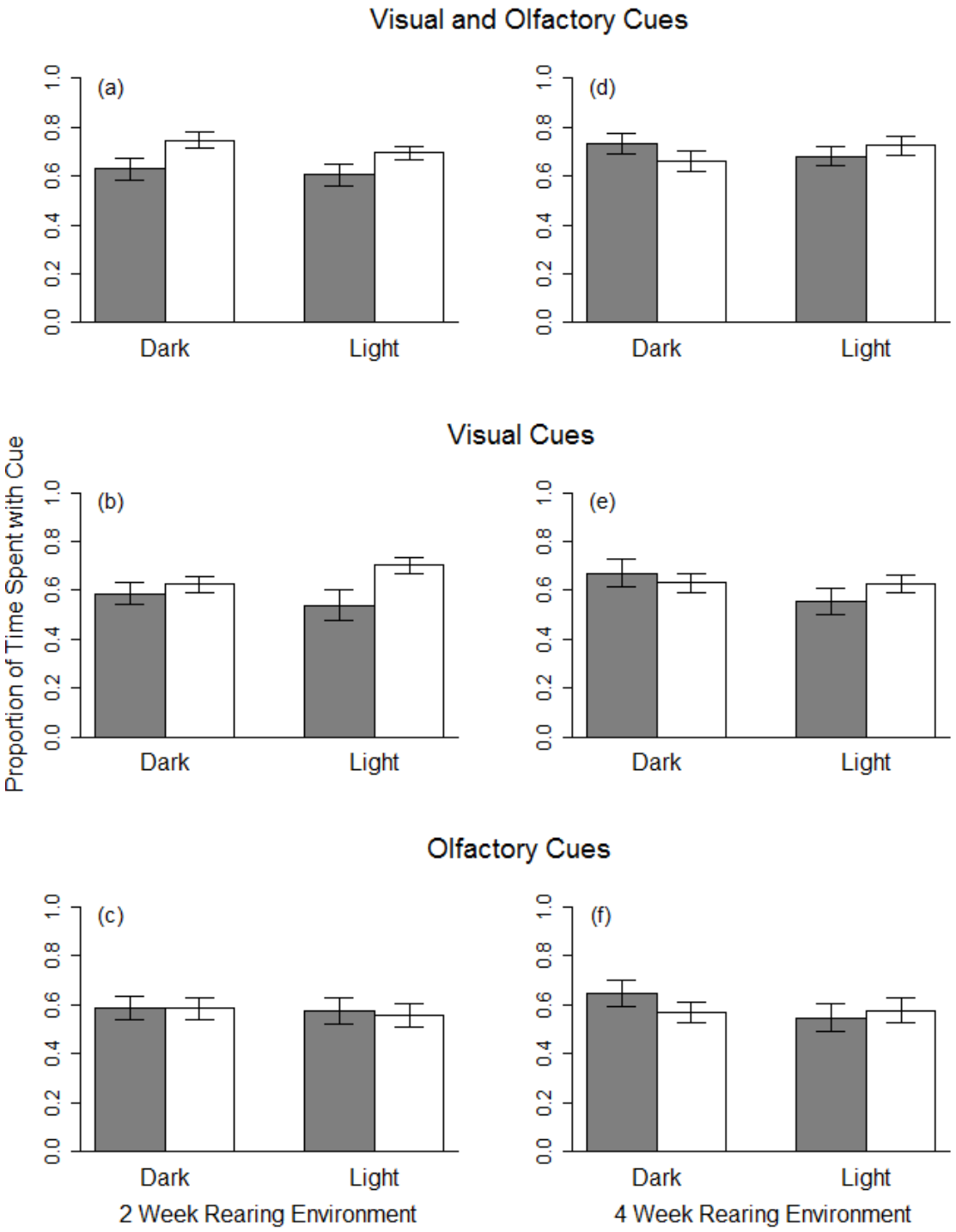

Figure 5.2. Mean proportion of time (±S.E) spent in the cue detection zone after adult fish had been exposed under light and dark conditions for 2 weeks; with **(a)** both visual and olfactory cues **(b)** visual cues and **(c)** olfactory cues, and for 4 weeks with **(d)** both visual and olfactory cues presented, **(e)** only visual cues and **(f)** only olfactory cues. Shaded bars represent dark trial conditions; unshaded bars represent light trial conditions.

Table 5.1. 2 week results: Summary of a linear mixed effect (LME) model including the 3 main effects. None-significant interactions have been removed for simplification. Emboldened p-values represent significance.

Fixed effects	**DF**	**F-value**	***P*-value**
Rearing environment	1, 38	8.96	0.0048
Trial conditions	1, 140	12.97	<0.001
Cue	2, 168	13.96	<0.001
Trial conditions* Cue	2, 140	4.32	**0.015**

Table 5.2. 4 week results: Summary of a linear mixed effect (LME) model including the 3 main effects. Non-significant interactions have been removed for simplification. Emboldened *P*-values represent significance.

Fixed effects	**DF**	**F-value**	***P*-value**
Rearing environment	1, 38	2.17	0.15
Trial conditions	1, 122	0.078	0.78
Cue	2, 153	7.67	<0.001
Rearing Environment*Trial conditions	1, 122	8.78	**0.0037**

Discussion

We found no evidence for sensory compensation in adult guppies. Dark reared fish did not develop an increased reliance on olfactory cues as previously seen in juvenile fish (Chapman et al. 2010b), suggesting a mechanism other than sensory plasticity is causing the change in behaviour. After 2 weeks, we found that only trial lighting conditions and cue type affected the proportion of time spent with the food cue, with no effect of light exposure environment. All guppies foraged more successfully in light environments when visual cues were present. This suggests that 2 weeks exposure to an altered visual environment is not sufficient time to allow guppies to adapt to a low light environment. After 4 weeks, however, guppies foraged more successfully in the environment in which they had previously been exposed, regardless of the cue type offered, suggesting that some adaptation to their environment had taken place.

More successful foraging in light conditions is unsurprising as guppies are primarily diurnal foragers (Magurran 2005) and respond strongly to visual cues across a range of behaviours (Long and Houde 1989; Endler 1991; Kelley and Magurran 2003a). A lack of short-term (after 1 hour acclimatisation to 2 week exposure) adaptation to an altered visual environment suggests that guppies cannot adjust their behaviour rapidly in response to environmental change. This contrasts with observations of sticklebacks, where foraging rates are maintained in turbid water (Webster et al 2007; Johannesen et al. 2012), but reflects the behaviour observed in other fish species (striped trumpeter *Latris lineata*: Cobcroft et al 2001, Sablefish *Anoplopoma fimbria*: De Robertis 2003). If animals are not able to respond flexibly to rapid changes in their environment, this may have detrimental effects on their foraging success. Alternatively, as short-term variability in visibility in aquatic environments is common due to water depth, turbidity or canopy cover, the benefits of changing behaviour may be outweighed by the costs of doing so (DeWitt et al. 1998), and a change in behaviour may not be observed. Male guppies reared as juveniles in dark conditions, for example, respond flexibly to current lighting environment in mating behaviour regardless of their rearing environment (Chapman et al. 2009).

Over a longer exposure period (4 weeks), however, guppies changed their behaviour, foraging more successfully in the environment to which they had been exposed regardless of the cue type available. This may be the result of learned familiarity with using different cue types, or with the environment itself. Experience of necessarily feeding in the dark may mean that dark-exposed adult guppies learn to seek out food in this environment, while light-exposed guppies, who would not normally be foraging under low light conditions, would not. Fish can learn to expect food at particular times of day (Reebs 2000), particular locations (Noda et al. 1994) or in association with particular objects (Warburton 2003) and can retain this information over a number of days (Brydges et al. 2008), although learned associations are usually built up over shorter timescales than the 4 weeks in our study. Guppies learn to associate with familiar conspecifics after 14 days (Griffiths and Magurran 1999) and learn the location of a food source in 3 trials (Lachlan et al. 1998), for example. Alternatively, guppies may become bolder in an environment with which they have become familiar, and will spend more time exploring (Martin and Reale 2008; Goldenberg et al. 2014), allowing

them to locate a food source more successfully in a test environment with which they are fully habituated with. Again, we might expect associations like this to develop more rapidly and further work is needed to tease these potential mechanisms apart.

We found no evidence of sensory plasticity, where individuals compensate for a reduction in vision by increasing reliance on olfactory cues in response to a degraded visual environment, as previously seen in juvenile guppies reared from birth (Chapman et al. 2010b). This suggests that exposure to a degraded environment during a critical developmental window (such as a transition from one life stage to the next: McCormick et al 1998) or early in life when the brain is particularly plastic (Rauschecker 1995; West-Eberhard 2003; Knudsen 2004) is required. Fishes' brains remain plastic throughout life (Ebbesson and Braithwaite 2012), and adult male guppies kept in different social conditions show different changes in brain size (Kotrschal et al. 2012) suggesting that a plastic response may be possible, but that a longer exposure period may be needed. The juvenile guppies showing evidence of sensory plasticity were reared for 72 days before testing (Chapman et al. 2010b), and 39 days was needed to see changes in brain size resulting from social conditions (Kotrschal et al. 2012).

Although adults did not demonstrate the same sensory plasticity as juveniles, we did observe some compensatory behaviour in response to a degraded visual environment. This highlights the importance of considering the impact of environmental change over different life stages and timescales. Research into the effect of environmental change on individual behaviour is usually carried out as a long-term study rearing juveniles from an early age (Cobcroft et al. 2001; Carere et al. 2005; Chapman et al. 2010b; Gray et al. 2012; Zambonino-Infante et al. 2013), or via the short-term exposure of adults to degraded conditions (Engstrom-Ost et al. 2006; Ward et al. 2008; Johannesen et al. 2012). While short-term experiments offer important insights into the immediate response of animals to a pollution event for example, longer-term studies are needed to understand the impact of more gradual or long lasting change on both adult and juvenile individuals.

Understanding how animals respond to changes to their sensory environments is critical to understanding the consequences of environmental change for individuals,

populations and communities (Candolin and Wong 2012). In the context of the visual environment, human-induced eutrophication and sedimentary turbidity is of increasing concern worldwide (Richter et al. 1997; Henley et al. 2000), with some areas, such as the Baltic Sea, becoming permanently turbid due to eutrophication (Bonsdorff et al. 2002). The ability of aquatic organisms to respond flexibly or plastically to the loss of visual information, or other senses, may differ depending on the life stage of the organism. Combined with previous work, our results suggest that extended exposure may allow adult individuals to alter their behaviour, allowing them to compensate somewhat for the detrimental effects of the change, although not to the extent to which juveniles can (Chapman et al. 2010b). The negative impact of turbidity (and other types of change) on growth and survival can occur over relatively short timescales (Berg and Northcote 1985) and raises the question of whether this compensation will be sufficient.

Chapter 6: Recent experience of a variable environment: how stable are behavioural traits in an unstable environment?

Abstract

Boldness and exploratory behaviours are often associated with how an individual will respond to rapid environmental change or novel environments. These traits are influenced by a complex interaction between an animal's genes, experience and internal state. Exposure to variable environment and restrictions in diet, for example, both increase levels of boldness and exploration. To what extent these factors influence an animal's behaviour, and over what time scales and life stages is not well understood. Here, we investigate how recent experience of a variable environment combined with a high or low food diet influence boldness and exploratory behaviours in adult guppies. We tested guppies for boldness and exploratory behaviours, by timing how long individuals took to recover from a disturbance and attack food in a novel environment, and by observing how individuals explored a novel maze. We then exposed guppies to a variable environment (created by changing the colour of the tank daily) in combination with either a high or low food diet for 2 weeks, and re-tested them. We found that diet had a significant effect on foraging and exploratory behaviour: guppies on a low food diet attacked food sooner, and those on a high food diet displayed higher activity in a novel maze, whereas experience of a variable environment had no effect on behaviour. Neither environment nor diet influenced the proportion of the maze explored, which remained stable within individuals over time. Internal state appears more important in explaining differences in behaviour than experience of a variable environment in adult guppies. These results highlight the importance of exploring personality traits over different life stages and time scales.

Introduction

Boldness and exploratory behaviours, which define how an individual responds to stressful situations, are important in determining fitness, and are good predictors as to how an animal will respond to a novel or altered environment (Wilson et al. 1993;

Wilson 1998; Sneddon 2003; Ward et al. 2004; Webster et al. 2009). Behavioural traits are often influenced by an individual's environment or state: Experience of an unpredictable environment, for example, can allow animals to alter a number of behavioural traits important to growth and survival (Moberg et al. 2011; Salvanes et al. 2013). Individuals reared in variable, enriched habitats often show increased levels of boldness and exploration in novel habitats (Braithwaite and Salvanes 2005), greater behavioural flexibility across contexts (Salvanes et al. 2013), increased learning ability (Strand et al. 2010; Salvanes et al. 2013), and forage more efficiently (Moberg et al. 2011) relative to those reared in stable environments. Environmental variability can be predictable (e.g. diurnal and annual cycles) or unpredictable, such as changes in predation risk, habitat structure and food availability, which often occur over short temporal and spatial scales. It is therefore important to understand how change affects individuals over different time scales and life stages. Although the effect of early experience of change on behaviour has been well documented, fewer studies have considered experience across different life stages, particularly how experience as an adult can alter behaviour. However, evidence is building that individuals remain remarkably flexible throughout life (Marchinko 2003; Ebbesson and Braithwaite 2012; Näslund et al. 2012).

Despite this behavioural flexibility (the ability to change behaviour in response to environmental conditions), it is now understood that individuals exhibit consistent differences across time and context, which may limit how an animal responds to environmental cues. Consistent individual differences, or "personalities" (Dall et al. 2004; Sih et al. 2004a; Sih et al. 2004b; Reale et al. 2007), have been observed in a diverse array of taxa (e.g. insects: Tremmel and Müller 2013, birds: Dingemanse et al 2002, mammals: Koteja et al 2003, fish: Bell and Sih 2007, crustaceans: Briffa et al 2008) and are influenced by both inherited genetic and environmental factors (Dingemanse et al. 2002; Drent et al. 2003; Dingemanse et al. 2009). The extent to which traits can be altered throughout life is not clear. If behaviours are set early on in life, experience as an adult may have little effect on how an animal responds to change: great tits *Parus major* remain stable in their exploratory behaviour, despite changes in season and energy state (Dingemanse et al. 2002) and guppies with experience of a temporally variable food supply early in life demonstrate bolder more

exploratory behaviour, but recent exposure as an adult has no effect (Chapman et al. 2010a). However, others have found certain traits or personality to be much more flexible; levels of boldness (defined as the propensity to take risks) can be altered of much shorter time scales (Wilson et al. 1993; Bell and Sih 2007; Frost et al. 2007).

Differences in energy state also impact behaviours often related to boldness. Individuals with lower energy states are often found to display bolder, higher risk behaviour (Milinski 1984), such as continuing to forage when predation risk is high (Dill and Fraser 1984; Gotceitas and Godin 1991; Godin and Crossman 1994), more rapid recovery from disturbances (Godin and Sproul 1988) and being more exploratory in novel environments (Mikheev et al 1994). In contrast, individuals with high energy states show more risk adverse behaviour (Godin and Crossman 1994), presumably because searching for food has a lower marginal benefit (Tremmel and Müller 2013). Responding to environmental variability can carry such energy costs: fish respond to changes in the environment by adjusting body colouration to more closely match their background over short time scales to minimise predation risk by increasing crypsis (guppies *Poecilia reticulata*: Rodgers et al 2013, rock pool gobies, *Gobius paganellus*: Stevens et al 2014). Guppies that repeatedly change colour after being exposed to systematically changing black and white backgrounds attack food items more quickly and forage for longer in a novel environment, consistent with both lower energy state and increased boldness per se (Rodgers et al 2013).

Here, we explore whether the behavioural changes observed by Rodgers et al (2013) are caused by changes in behaviour due to changes in energy state (i.e. increased boldness associated with hunger), or changes due to the variable environment (i.e. increased boldness associated with experience of a variable environment). We expose adult guppies to a colour-changing environment with both low and high food diets, and assess boldness (recovery time when placed in a novel environment and time to attack a food item) and exploratory behaviour (activity in a maze) both before and after exposure to the treatment (table 6.1). We predict that if boldness is associated with hunger, fish from low food treatments should show a greater increase in boldness-related behaviours than fish from low food treatments. If boldness is associated with environmental variability, fish from a variable environment should be bolder than those from a constant environment. If both contribute, then fish on low

food treatments in variable environments should be boldest, and those on high food diets in constant environments should be the least bold. Alternatively, if these behaviours are set early on in life, experience of variability and changes in energy state as an adult could have limited effect.

Materials and Methods

Study species and housing

All fish used in this experiment were descendants of wild-caught guppies from Trinidad. Fish were maintained in aquaria (200x400x400mm) at the University of Hull on a re-circulating system with a 10% daily water change at approximately 26^{o}C (±1^{o}C) on a 12:12hr light:dark cycle and fed daily on ZM small granular feed (0.5-0.8mm; ZM Systems, Hampshire, UK). 24 hours before the first set of experiments, groups of 4 guppies (2 male and 2 female) were removed from the stock tanks and placed into separate holding tanks (20cm x 20cm x 20cm) on the same aquarium system and fed a pinch of fine sinking food to standardise hunger levels (n = 96 individuals in total). We selected fish of different sizes in each group of 4 to allow for individual identification within each group without marking (large female; 15.2 -21.8mm, small female; 10.5 - 18.0mm, large male; 13.3 – 21mm, small male; 10.9 – 16.2mm). Fish were tested in two experiments outlined below, once before being placed into the variability and feeding treatments and once after. The experiments were performed on consecutive days, always in the same order (experiment 1 followed by experiment 2).

Experiment 1: Feeding trial

To investigate levels of boldness and motivation to feed, we investigated the time taken to recover after being placed in a novel environment, and the time taken to attack food pellets. Individual guppies from each treatment were placed in a novel tank (20 x 20 x 20) filled to the depth of 15 cm with brown card placed on the sides and base. Brown was used as it was a neutral colour that none of the fish had experienced during the treatments (see below). When guppies were initially placed in the tank they would swim to the bottom and suspend movement. We timed how long it took guppies to recover and resume swimming. After they had recovered, they were given 2

minutes to acclimatise and explore the tank. A 2.5 x 0.5mm spatula was then used to add food pellets (small pellet fish food 0.5-0.8mm) to the surface of the water and a timer started. The proportion of time (out of 5 minutes) taken for a guppy to attack the first food pellet was recorded. Experiments lasted 5 minutes; if a guppy did not attack the food, a proportion of 1 was given. The water in the tank was changed after every 4 fish to ensure olfactory cues did not build up in the water. Once the trials were completed fish were placed back into holding tanks and fed on their normal rations.

Experiment 2: Maze trial

To investigate exploratory behaviour, we placed fish in a simple maze (figure 6.1), which consisted of a brown tank (40 x 30 x 15cm) with a water depth of 5cm. 3 brown solid plastic partitions 0.5cm thick, measuring 15cm in length, were placed throughout the tank at intervals of ~13cm, creating 3 sections in the maze. A Microsoft Lifecam was suspended 50cm above the tank. The tank and camera were placed in a light cube (EZCube 51 cm light tent) illuminated from above by a daylight bulb to create even lighting over the tank to ensure accurate tracking of the fish. At the beginning of the trial fish were placed in the top right hand corner of the maze (as viewed in figure 6.1), and the trial started when the fish began swimming. The trials lasted 10 minutes, in which time the fish could explore the maze.

The videos from the trials were converted to an AVI format at 5fps using VirtualDub (http://www.virtualdub.org) and then analysed using automatic tracking software Lolitrack (http://www.loligosystems.com). The size of the tank was used to scale the videos. For each individual, we collected the XY coordinate (taken from the centre of the body) from each frame. These were used to compute: the % of time (10 minutes) spent actively swimming (defined here as movement more than 0.2cm per frame, which is approximately 1cm per second), the total distance moved (cm), the average speed (cm/s) when the fish was actively swimming, and the proportion of the maze explored. To calculate the proportion of the maze explored, we used Matlab (http://uk.mathworks.com) to create a grid section over the area of the maze. We created a 0.7cm (approximately twice the maximum width of a guppy) exploration radius around each fish. Using the XY coordinates taken from each fish at each time point, we assume the pathway between each pair of points is a straight line, thus

creating a continuous pathway for each fish through the maze. We then determined how many grid squares were covered by this pathway. The proportion of the maze explored was then calculated by dividing the number of explored grid squares by the total number of grid squares. To ensure that this value was not dependent on the size of the grid squares, we reduced the dimensions of the grid (increased the total number of squares) systematically until the calculated proportion of the maze explored stabilised (a difference of less than 1% between two grid sizes). 3 fish were excluded from analysis due to poor video quality, and 10 were removed as they froze in the maze for over 5 minutes.

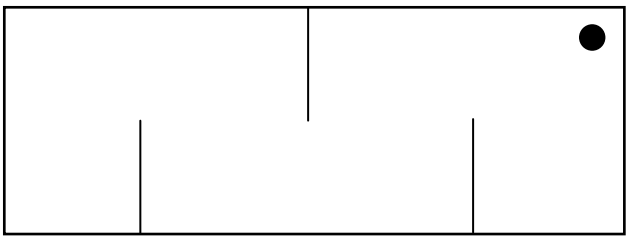

Figure 6.1: The novel maze experimental setup. Black lines indicate the barriers created, the circle represents where fish were placed in the maze to start the experiment (40 x 30 x 15cm).

Variability and feeding treatments

After experiment 2, individuals (in their groups of 4) were placed in holding tanks under one of 4 treatments for 14 days. All tanks were kept on the same recirculating system at ~26^{o} with a water depth of 15cm. The treatments were:

- Constant environment and high food (**CH**, N=24 fish)
- Constant environment and low food (**CL**, N=23 fish)
- Variable environment and high food (**VH**, N=24 fish)
- Variable environment and low food (**VL**, N=23 fish)

A variable environment was created using the protocol from Rodgers et al. (2013) by changing the background colour of the holding tanks from black to white daily, by placing either black or white card down both external sides and on the base of the tank, and by including an artificial plant of the same colour. Guppies are able to

significantly alter their colouration in response to the background within 24 hours (Rodgers et al 2013). The rear wall of all tanks was blue, and the front wall was left uncovered to allow for observation of the fish. The black and white card and plant could be changed daily with minimal disturbance to the fish. A constant environment was created by placing grey card on the outside of the tanks and a grey artificial plant in the tank, which were also changed daily to ensure each tank received the same level of disturbance. In the high food treatments, fish were fed ZM fine sinking food until satiated (approximately 0.05g) twice daily. In the low food treatments, guppies were given a 1/3 of this twice daily (Kolluru et al 2006; Grether et al 2005). Mortality during the 2 week treatment was low (2/96, 2.1%), and fish that died were not replaced.

Table 6.1: Glossary of behavioural traits observed

Behaviour	Description
Recovery time	Time taken to recover normal swimming
Time to attack food	Time taken to physically attack first food pellet
Activity	% time spent actively, defined in the tracking software as movement more than 0.2cm per frame (0.03 seconds)
Speed	Average speed (cm/s) of an individual, when actively swimming
Distance	Total distance (cm) moved over the 10 minutes
Exploration	% of the maze explored. Exploration radius of each fish is taken to be 0.7cm

Statistical analysis

We used linear mixed effect (LME) models to assess the importance of previous behaviour (behaviour recorded before the fish were put into treatment), food level (high and low) and environment (variable and constant) on the measures of boldness (time to recover and proportion of time to attack food in the feeding trials) and exploratory behaviour (average speed, total distance, activity and exploration in the maze trials). All factors and their interactions were included as main effects, with tank included as a random effect to account for non-independence of individuals within the same tank. Each model was then simplified by sequentially removing non-significant interaction terms to achieve the minimum adequate model (MAM) following Crawley

(2005). Some of the data required transformation to satisfy parametric assumptions; time to recover was log transformed and the proportion of time taken to attack a food item and the proportion of time spent active in the maze were arcsine transformed (as is appropriate for proportion data).

Sex can be important in describing behavioural differences in behaviour (Harris et al 2010; King et al 2013). To evaluate its importance on our results we ran a separate LME for each behaviour, with sex set as the main effect, and individual ID nested within tank as a random effect to account for repeated measures. We found sex did not explain any of the behaviours seen ($P > 0.05$), as a result of this (and to simplify the model), we did not include sex as a factor in our analysis (above). To test for consistency in behaviour, we calculated the intraclass correlation coefficient (Hayes and Jenkins 1997), which estimates repeatability, for all the behaviours observed for each of the 4 groups (CH, CL, VH and VL). 95% confidence intervals were calculated using the Smith method (Smith, 1956). Only individuals that had successfully completed both trials for each behaviour were included (N = 13 removed). As this method is based on an ANOVA approach, data was transformed to meet assumptions of normality (as described above).

Results

Feeding experiment

Time taken to recover in the novel feeding tank was not affected by the environment ($F_{21,1}$ 0.19, $P = 0.67$), diet ($F_{1,21} = 2.47$, $P = 0.13$) or influenced by previous behaviour ($F_{1,55} = 0.87$, $P = 0.36$, figure 6.2a, table 6.2), nor were there any significant interactions between these variables. The proportion of time (out of 5 minutes) taken to attack food was influenced by diet, with fish fed on the low food diet found to attack food faster ($F_{1,21} = 20.9$, $P < 0.001$, figure 6.2b, table 6.2). Past behaviour ($F_{1,55} = 0.71$, $P = 0.4$) and environment ($F_{1,21} = 0.02$, $P = 0.89$) had no effect on this behaviour. Neither time taken to recover or time taken to attack food was found to be repeatable across experiments (before and after being placed into treatment) (table 6.3). The water was not changed between each set of experiments (i.e. one tank of fish), which could have resulted in a build-up of olfactory food cues that may have influenced the strength of

response by individual fish. However, we would expect the influence of this to mask any effect observe, not enhance it

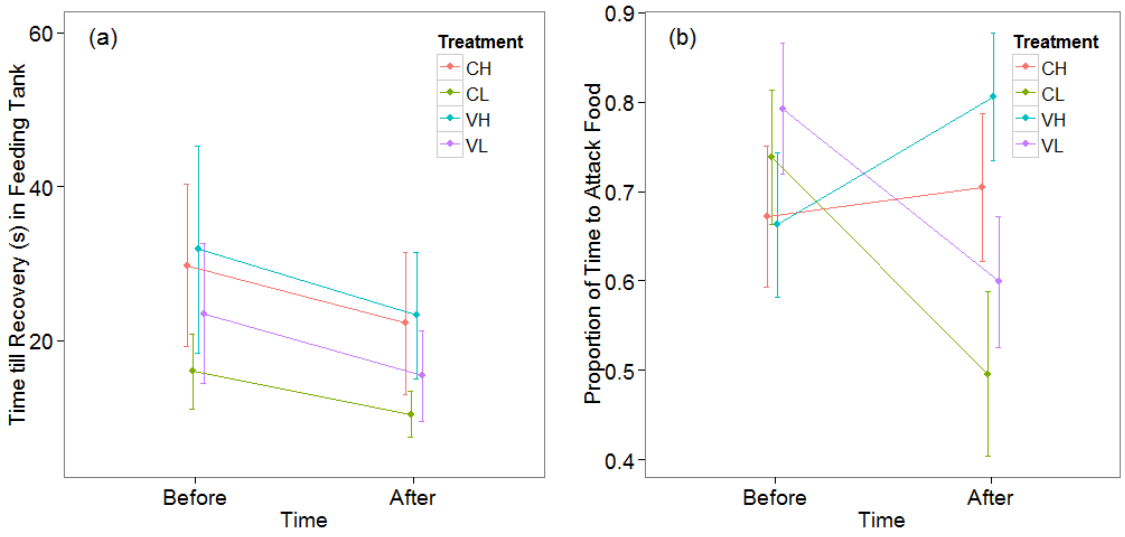

Figure 6.2: Boldness behaviours from the feeding trials before and after being placed into treatment: (a) mean time to recover when placed in the novel feeding tank and (b) time taken to attack a food item ± S.E. for each of the 4 treatments.

Maze experiment

Exploration, defined as the proportion of the maze explored, was not influenced by the environment ($F_{1,21}$ = 1.12, P = 0.33) or diet ($F_{1,21}$ = 0.034, P = 0.86), but was significantly affected by past behaviour ($F_{1,58}$ = 21.04, P < 0.001, figure 6.3a, table 6.2). Exploration remained stable across time, being found highly repeatable across experiments in all treatments (table 6.3). Activity (the proportion of time spent active), was influenced by an interaction between diet and past behaviour ($F_{1,53}$ = 4.13, P = 0.047, figure 6.3b, table 6.2). Individuals kept on the low food diet did not alter their behaviour, showing high repeatability scores (table 6.3), whereas those fed on the high food diet increased their activity. Average speed (cm/s) moved through the maze was not found to be influenced by environment ($F_{1,21}$ = 2.34, P = 0.14), diet ($F_{1,21}$ = 0.007, P = 0.94) or by past behaviour ($F_{1,56}$ = 1.71, P = 0.2, figure 6.3d, table 6.2) and was not found to be repeatable across experiments (table 1). Total distance moved (cm) was not influenced by environment ($F_{1,21}$ = 1.49, P = 0.24) or diet ($F_{1,21}$ = 0.62, P = 0.44), but was influenced by past behaviour ($F_{1,56}$ = 4.68, P = 0.035, figure 6.3c, table 6.2), although

this was not found to be repeatable across experiments in any of the treatments (table 6.3).

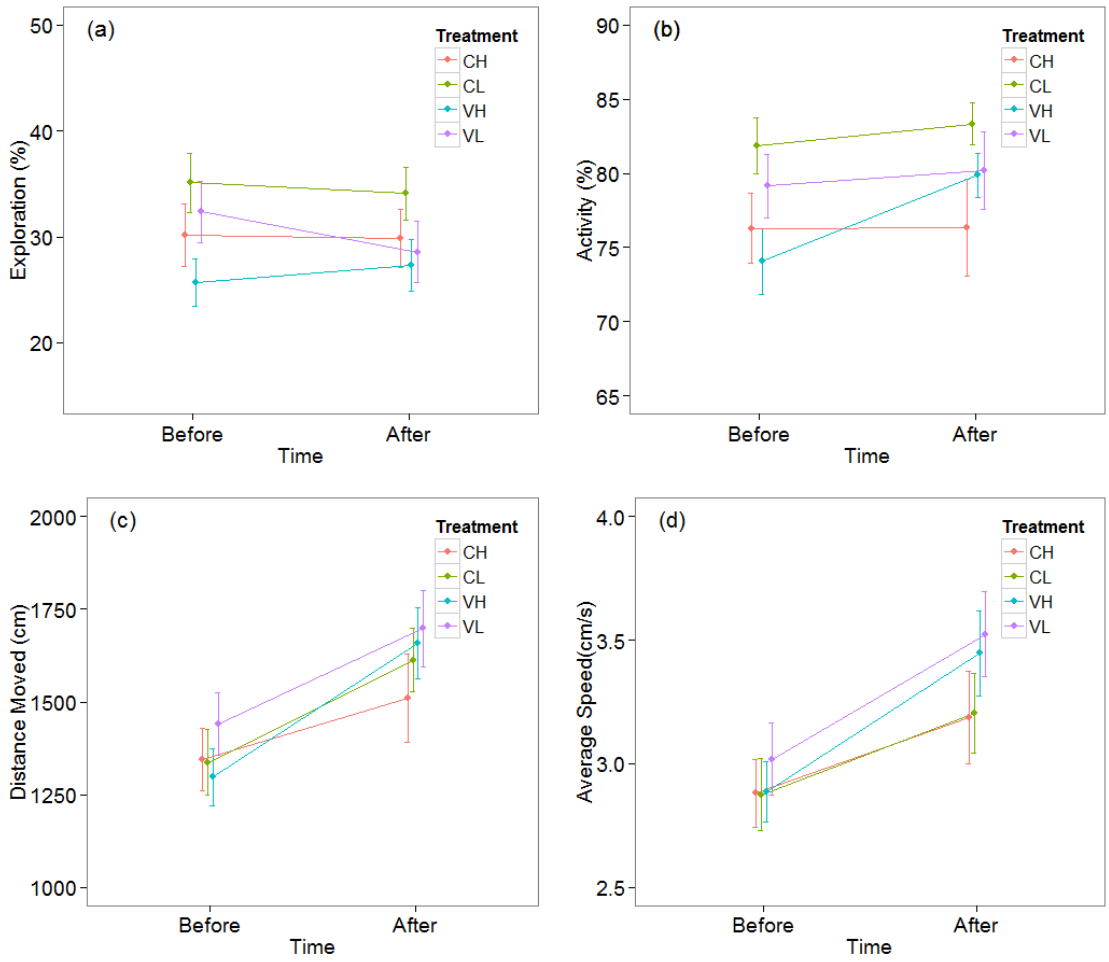

Figure 6.3: Exploratory behaviour in the maze trials before and after being placed into treatment: (a) mean % of the maze explored, (b) activity (% of time spent active), (c) total distance moved through the maze (cm) and (d) average speed (cm/s) ± S.E. for each of the 4 treatments.Table 6.2: Summary of the effect of diet and environment variability on the different behaviours observed. Ticks indicate a significant effect, crosses no effect. Interaction effects are detailed.

Behaviour	**Environment**	**Diet**	**Previous behaviour**	**Consistency (table 6.3)**
Time to recover	X	X	X	X
Time to attack	X	✓	X	X
Activity	X	Interaction effect		Low food only
Distance	X	X	✓	X
Speed	X	X	X	X
Exploration	X	X	✓	✓

Table 6.3: Results from the interclass correlation coefficient (ICC), showing repeatability between experiments (before and after) in each of the 4 treatments (CF, CV, VF, VH). Repeatable behaviours (those with 95% confidence intervals that do not cross 0) are shown in bold.

Behaviour	Repeatability score	95% Confidence intervals
Food recovery		
Constant high food	0.069	-0.41, 0.54
Constant low food	0.3	-0.091, 0.7
Variable high food	-0.035	-0.47, 0.4
Variable low food	0.02	-0.45, 0.48
Time to attack food		
Constant high food	0.058	-0.42, 0.53
Constant low food	0.13	-0.27, 0.56
Variable high food	-0.19	-0.61, 0.23
Variable low food	-0.18	-0.62, 0.26
Activity		
Constant high food	0.18	-0.28, 0.64
Constant low food	**0.31**	**-0.08, 0.7**
Variable high food	-0.11	-0.54, 0.35
Variable low food	**0.44**	**0.07, 0.8**
Speed		
Constant high food	0.17	-0.29, 0.63
Constant low food	0.058	-0.37, 0.49
Variable high food	-0.32	-0.71, 0.07
Variable low food	0.21	-0.18, 0.68
Distance		
Constant high food	0.38	-0.32, 0.61
Constant low food	0.037	-0.16, 0.65
Variable high food	-0.21	-0.66, 0.22
Variable low food	0.26	-0.77, 0.018
Exploration		
Constant high food	**0.56**	**0.24, 0.28**
Constant low food	**0.45**	**0.1, 0.79**
Variable high food	**0.48**	**0.15, 0.81**
Variable low food	**0.47**	**0.1, 0.82**

Discussion

We predicted that both environmental variability and limited food supply should increase boldness-related behaviours in comparison to constant environments and high food treatments (see table 6.1 for a summary). We found that diet had significant effects on foraging and exploratory behaviour, but there was no effect of environmental variability on any of the behavioural measures (table 6.2). Guppies in

the low food treatment began foraging more rapidly in a novel environment, but in contrast to our expectations, those fed on the high food diet were more active in a novel maze environment. We found no effect of treatment on exploratory behaviour: instead, this measure was highly consistent within individuals. The only other behaviour that showed evidence of consistency was activity, but only in individuals exposed to the low food treatments (table 6.3).

Recent experience and current environments are known to affect behavioural traits. In particular, increased hunger levels fuel risk taking and exploratory behaviour (3-spined stickleback *Gasterosteus aculeatus*: Godin and Crossman 1994, Milinski and Heller 1978, salmon *Salmo salar*: Mikheev et al. 1994), behaviours often associated with boldness. In contrast, we found that diet only affected the speed at which guppies attacked the food, not any of our other measure of boldness. This is consistent with a need to gain energy, but not necessarily through a general increase in risky behaviours. Environmental variability can also enhance risk taking: guppies experiencing unpredictable variation in the timing of food delivery behave in a bolder manner (Chapman et al. 2010a), and guppies experiencing a colour changing environment also recover more rapidly from a disturbance and attack food more readily (Rodgers et al. 2013a), a finding we were unable to replicate with our study. In contrast to our expectation that hunger would be linked to bolder behaviours, guppies from high food treatments were more active than those from low food treatments, which could perhaps be interpreted as a constraint caused by the lower energy reserves (Tremmel and Müller 2013), and reflecting a potential trade off in the allocation of energy budgets to different behaviours. This increased activity could be linked to increased motivation to seek out a shoal over finding food (Krause 1993), or increased mate searching activities (Lima and Dill 1990). For example male guppies are less attracted to food and more attracted to females with increasing satiation (Pitcher 1993).

While the majority of our behavioural measures were highly variable within and between individuals, we found that exploratory behaviour was highly consistent within individuals, and unaffected by treatment. Previous work has similarly reported exploratory behaviour to be a stable trait across time and context, not easily influenced by environment or internal state (Dingemanse et al. 2002; David et al. 2012). This may be linked to physiological mechanisms (Koolhaas et al. 1999); for

example, rainbow trout *Oncorhynchus mykiss* with reduced cortisol responses to stress tend to be bolder than those with higher responses (Øverli et al. 2005). If behaviours are linked to physiological or morphological traits, altering behaviour in response to short-term perturbations may be more complex or costly (DeWitt et al. 1998). The development of consistent behavioural traits may be linked to early experiences during prenatal or juvenile periods, where critical windows for their development may occur.

The environment experienced during development is known to shape behavioural traits such as boldness, aggression and sociability. Guppies exposed to a temporally unpredictable food supply increase exploratory and boldness behaviours (Chapman et al. 2010a), and early experience of complex habitats reduces stress responses and increases exploratory behaviour (Braithwaite and Salvanes 2005), and leads to increased brain development (Näslund et al. 2012). Early and recent experiences interact in shaping behaviour. Different behavioural traits may show different levels of flexibility, meaning some behaviours appear more sensitive to short term environmental change (Magnhagen and Staffan 2004; Frost et al. 2007; Frost et al. 2013). For example, aggressiveness is more plastic than boldness (Bell and Sih 2007). Within a trait, some individuals may be more flexible than others, and this may be linked to their overall level of that particular trait. The behaviour of bold individuals is often considered to be more stable than that of shy individuals (Carere et al. 2005; Nakayama et al. 2012) (although see Frost et al. 2013), and less aggressive mice adjust their aggressiveness in response to social context, while aggressive mice do not (Natarajan et al. 2009). This may lead to a masking of effects in studies such as ours.

Exposure to a variable environment and differences in energy state can both influence levels of boldness and exploratory behaviours in individuals (Godin and Crossman 1994; Mikheev et al. 1994; Braithwaite and Salvanes 2005; Chapman et al. 2010a). Here, we found internal state was more important than experience of a variable environment in explaining differences in behaviour of adult guppies, suggesting that short term variations in the physical environment do not have a strong influence on behaviour. This could indicate that early experience of variability is more important than recent (Chapman et al. 2010a), although further work is needed to fully understand this process. Personality traits and behavioural syndromes are more stable at certain life stages than others: Bell and Stamps (2004) found juveniles and adults

displayed consistent correlations between boldness and aggression, however these were weakened during subadulthood. Information on how animals respond over the course of their lives will provide valuable information on the flexibility of personality traits important for growth and survival.

Chapter 7: General Discussion

The aim of this thesis was to investigate how exposure to a degraded environment affected the behavioural responses of fish, and to better understand at what time scales and developmental stages these responses are effective. In the first section, I investigated how both predators and prey altered their behaviour with short-term exposure to turbidity, specifically from the perspective of the group. In the second section, I considered how previous experience of a visually degraded or variable environment influenced the behavioural responses of adult fish. In this final chapter I summarise my findings in a broader context, and discuss the importance of understanding how behavioural responses to environmental conditions vary over different time scales and developmental stages.

Turbidity influences on predator-prey interactions

In chapters 2 - 4, I explored how groups of prey formed and responded to predators, and how predators targeted individuals from within groups in a turbid environment. Turbidity is known to influence both how predators detect and target prey, and how prey responds. At the individual level, prey often show weakened, poorly timed anti-predatory responses (Gregory 1993; Meager et al. 2006), and increase behaviours viewed as more risky (Abrahams and Kattenfeld 1997; Lehtiniemi et al. 2005; Engström-Öst and Mattila 2008). It has been suggested that reduced anti-predatory responses are caused by reduced perception of risk in some species, indicating that turbidity can act as a refuge, affording prey protection from predators (Gregory 1993; Engström-Öst and Mattila 2008). In support of this, predators often suffer from reduced capture success in turbid water (Utne 1997; Ljunggren and Sandstrom 2007). However, many find that while predators lose the ability to target specific prey, overall capture success remains the same (Reid et al. 1999; Jonsson et al. 2013). In turbid water therefore, overall predation risk can remain the same, but individual risk may be altered. At the level of the group, turbidity disrupts how individuals detect and group with one another, with shoals of fish often breaking apart in turbid conditions (Ohata

et al. 2013; Borner et al. 2015); however how groups form and respond to predation in turbid environments is less understood.

In chapter 2, I investigated individual and group level responses of guppies to a simulated aerial predation attack in increasing levels of turbidity. In support of other studies, I found shoals were more dispersed in highly turbid water (Ohata et al. 2013; Borner et al. 2015), a pattern I observed both before and after the simulated predator attack. At the individual level, I found that while guppies at all levels of turbidity detected the threat at the same time, individuals in turbid water displayed higher levels of freezing behaviour, as opposed to darting, and that those that did dart moved slower and covered a shorter distance. The altered responses in our study could suggest a reduced perception of risk (Gregory 1993; Miner and Stein 1996; Engström-Öst and Mattila 2008); however guppies in turbid water also took longer to recover normal swimming, contradicting this theory. As such, I proposed that the response observed was due to constraints caused by the degraded visual environment. In turbid water, guppies are unable to accurately detect one another, and are thus responding as lone individuals, rather than collectively as a shoal, an effect that is enhanced by the decreased shoal cohesion observed at higher levels of turbidity.

Further work is needed to fully tease apart the motivation (either reduced perception or risk or visual constraints) behind the responses observed. An interesting experiment would be to compare the anti-predatory responses of lone fish with those of fish in groups in both clear and turbid water. How groups and individuals compare in response to predation has shown mixed results: some studies suggest lone prey will try to avoid detection by freezing or hiding (Magurran and Pitcher 1987; Rangeley and Kramer 1998), while others suggest that lone fish will dart in response to a simulated predation attack, whereas individuals in groups will freeze (Fischer et al. 2015). Repeating Fischer et al's (2015) experiment in turbid water would allow for assessment of whether social context and turbidity interact to influence behavioural responses to a predatory threat.

In chapter 3 I further explored how shoaling behaviour was affected by turbidity, by investigating the movement rules fish use to form cohesive groups under the threat of predation. Here, I compared the initial movement pathways guppies used to form

groups against 5 model simulations of movement rules, varying in complexity. According to the selfish herd hypothesis (Hamilton 1971) individuals can reduce their domain of danger (DOD), the area of space around an individual that is closer to it than to any other individual, by moving towards their nearest neighbour. Increasingly complex rules that include the position of multiple neighbours have since been proposed, with the aim of finding a "realistic" movement rule that generates patterns of aggregation similar to those seen in nature (Morton et al. 1994; Viscido et al. 2002; Morrell and James 2008). Simple rules, such as nearest neighbour (where individuals move towards the spatially closest group mate), have been criticised for failing to result in one cohesive group (Hamilton 1971), while complex rules accounting for multiple neighbourism, which generate dense cohesive groups, have been criticised for being too cognitively difficult for animals to follow. I provided evidence that the movement behaviour of guppies more closely matches the predictions of complex rules, which resulted in the formation of compact groups. Turbidity interfered with this behaviour, presumably by reducing visual information about the location of group members. In turbid water, guppies showed a greater error compared to the predicted movement pathways, particularly for the most complex rules, which resulted in more dispersed, fragmented shoals.

In contrast to the results of chapter 2, in chapter 3, guppy shoals had similar shoal cohesion in clear and turbid water before the simulated predator attack. Guppies appear able to maintain shoal cohesion until relatively high levels of turbidity, a result supported by chapter 2 and other researcher (Borner et al. 2015). In chapter 3 however, I found a significant difference in cohesion after the threat, whereas in chapter 2, where I found cohesion stayed the same before and after a threat. This could highlight the importance of considering shoal size; in chapter 2 I used shoals of 4 fish, whereas in chapter 3 I used shoals of 10 fish. Smaller shoals of fish respond differently to larger: Shoals of 10 minnows *Phoxinus phoxinus* are more likely to abandon shoaling and hide in response to predation, whereas shoals of 20 or 50 will remain as a group (Magurran and Pitcher 1987). Future work could explore how differences in shoal size affects grouping behaviour in turbid water. Theoretical models predict that size and density are also important in determining the rules to follow when grouping (Morrell and James 2008). For example complex rules are useful in

small compact populations, such as ours, but simpler rules are more successful in large, low density populations (Morrell and James 2008).

In chapter 4, I explored how turbidity affected predator-prey interactions in the context of the confusion and oddity effects. Moving groups of animals can visually confuse predators (Krakauer 1995), an effect which is enhanced when individuals within the group are morphologically or behaviourally similar. Phenotypically distinct, or "odd" individuals allow a predator to overcome the confusion effect, and are often preferentially targeted (Landeau and Terborgh 1986). The confusion and oddity effects together put pressure on individuals to assort by phenotype (Ranta et al. 1992; McRobert and Bradner 1998; Ward and Krause 2001). However, the level of risk experienced by an individual can affect the level of pressure to associate with phenotypically matched individuals. In clear water, predators often chose larger bodied prey, as they are generally more profitable (Li et al. 1985; Wetterer and Bishop 1985), meaning large individuals have a higher level of risk within a group. The oddity and confusion effect are therefore mediated by body size, with smaller individuals being less at risk in a mixed-sized group, even if they are odd (Rodgers et al. 2015). Turbidity disrupts a predator's ability to preferentially select prey by size (Abrahams and Kattenfeld 1997; Reid et al. 1999; Jonsson et al. 2013), which may influence levels of risk and survival, and may have implications for the structuring of animal groups in relation to phenotypic characteristics.

In chapter 4, I explored how sticklebacks (as predators) targeted individual *Daphnia magna* of different sizes from within a group, and how sticklebacks (as prey) chose to form groups based on body size. In line with previous work, I found preferential targeting of large bodied prey in clear water, particularly when they were in equal ratios with small-bodied prey, or the odd individual within the group (Rodgers et al. 2015). This preference, however, was lost in turbid water, leading to a relaxed predation pressure on large individuals within groups, but an increase in risk to small-bodied individuals. This difference in risk has the potential to change the pressure to assort by phenotype: indeed, I found large bodied sticklebacks lost their preference for shoaling with size-matched conspecifics in turbid water, while small bodied sticklebacks showed no preference in either clear or turbid water. Behavioural assortment in prey, driven by confusion and oddity effects appeared weakened in

turbid water. My results suggest that relaxed pressure on large individuals reduces the benefits of assorting by size, which could impact community structure through altered levels of risk and survival.

The change in shoaling behaviour of the sticklebacks observed in chapter 4 could be attributed to a reduced perception of risk or to visual constraints in turbid water (Gregory 1993; Engström-Öst and Mattila 2008). If sticklebacks perceive a reduced predation threat they may alter their behaviour accordingly, as the costs associated with grouping with large individuals, such as increased competition, may no longer outweigh the anti-predatory benefits of minimising oddity. However in chapter 2, I suggested that visual constraints, rather than reduced perception of risk, better explained the reduced anti-predatory responses observed. In support of previous work (Fischer and Frommen 2012), I found sticklebacks reduced their activity when choosing between shoals in turbid water relative to clear water. In turbid water individuals switched between shoals less, occasionally remaining with one shoal for the duration of the experiment. This behaviour suggests an enhanced, rather than reduced perception of risk: by remaining with a shoal, rather than moving between shoals, individuals reduce their exposure to predators under situations where they are at increased risk through isolation (Landeau and Terborgh 1986). I therefore suggested that test fish may have been unable to distinguish between two simultaneous shoals simultaneously, with shoals being formed via chance encounters, rather than active choice.

Chapters 2-4 suggest that turbidity could have a range of effects on predator confusion and the role of phenotypic oddity in structuring animal groups. In chapter 4 I found the oddity effect was weakened in turbid water, and in chapters 2 and 3 that turbidity disrupts shoal formation and cohesion, potentially reducing the benefits associated with predator confusion as shoals become more dispersed. Turbidity disrupts a predator's ability to detect and locate prey (Utne 1997; Ljunggren and Sandstrom 2007), and could reduce the ability of a predator to detect all individuals within a group, consequently reducing its susceptibility to the confusion effect, which is enhanced with increasing group size and density (Krakauer 1995). My research suggests that some of the benefits associated with group living may be reduced in turbid water. However, some benefits of grouping may remain: research has suggested

grouping is a beneficial strategy to prey, as it reduces the likelihood of being encountered by a predator, particularly if that predator is using olfaction (Johannesen et al. 2014). Grouping provides a range of other benefits: The encounter-dilution effect suggests that predators are less likely to encounter groups of prey than the size of the group would predict (Wrona and Dixon 1991). This effect is likely to be maintained or even enhanced in turbid water, as the detection of prey is generally hampered (Utne 1997; Granqvist and Mattila 2004).

If aggregation is a beneficial strategy in turbid conditions (Johannesen et al. 2014), then dispersed groups observed may be detrimental to survival. My research focused primarily on the use of visual cues, however many predators rely on tactile or olfactory cues to locate and target prey. Predators relying on olfactory cues, for example, may not be impacted by increasing levels of turbidity in the same way as visual predators. Tactile predators, however can suffer from confusion effects (Jeschke and Tollrian 2007), as they lack the high spatial resolution thought necessary to single and target individuals from within a group. If exposure to a degraded environment means predators switch to alternative cues more susceptible to confusions effects, how predators respond to groups may be changed in other ways. Further work teasing apart how different cues influence group detection and targeting for predators using different sensory modalities is needed.

In chapters 2, 3 and 4 I considered short term, flexible behaviour of individuals when faced with the loss of the vital visual sense. However, an animal's response may be altered with the length of time it is exposed to a degraded environment, and its stage of life. For example guppies are unable to respond to short term changes in their visual environment by relying on olfactory cues, however long term experience over ontogeny allows them to make a sensory switch from vision (Chapman et al. 2010b). In chapters 5 and 6 I focus on the effect recent experience has on adult behaviour.

Recent experience and behavioural responses

In chapters 5 and 6 I explore the effect of longer-term exposure to degraded or variable environments on fish behaviour. In chapter 5 I exposed adult guppies to a visually poor low light environment for 2 and 4 weeks, and assessed their ability to

locate food using visual or olfactory food cues or a combination of both. Previous work has shown guppies experiencing a dark environment during development make a sensory switch from vision to olfaction, thus enabling them to successfully locate food sources in visually poor environments (Chapman et al. 2010b). I found no evidence for similar sensory compensation in adult guppies. After 2 weeks I saw no change in behaviour, with both light and dark exposed guppies responding more strongly to visual cues in a light environment. After 4 weeks exposure, however, guppies foraged more successfully in the environment they had experience of, regardless of the cue provided. This suggests that although guppies are able to adapt to their environment with prolonged exposure, the mechanism behind the change differs from that of juveniles.

Intriguingly, my research suggests that individuals respond to an altered environment differently over the course of their life span. This could suggest the existence of a critical period in an individual's life where sensory plasticity can develop. One weakness with directly comparing this study to those conducted on juveniles is the difference in the length of exposure time. Chapman et al (2010b) reared guppies for 72 days, whereas I exposed adult guppies for a maximum of 28 days. More research is needed to fully understand how animals alter the use of senses throughout life, and to fully understand the flexibility of this behaviour. Some species appear able to flexible use alternative cues flexibly when vision is limited (Webster et al. 2007; Johannesen et al. 2012) whereas others do not (McMahon and Holanov 1995; Fraser and Metcalfe 1997). One possibility would be to rear juvenile guppies in light and dark conditions, and then switch them into an alternate environment at maturity. By looking at the flexibility of response within an individual, we could better understand the timing in the expression of sensory plasticity, and better understand the importance or recent vs early experience. This has important implications when considering the rate of change of aquatic environments, particularly in terms of turbidity (Richter et al. 1997; Henley et al. 2000), raising the question of whether the types of behavioural compensation observed in juveniles and adults will be enough to buffer individuals against the negative impacts of turbidity.

Finally, in chapter 6, I move away from the visual environment, to consider how recent exposure to a variable environment together with a high or low feeding regime

influenced boldness behaviour in adult guppies. Personality traits, such as boldness and exploratory behaviour, are formed by a complex interaction between an individual's genes (Dingemanse et al. 2002), internal state (Koolhaas et al. 1999) and experience (Braithwaite and Salvanes 2005). Early experience of a variable environment can increase boldness and exploratory behaviours (Braithwaite and Salvanes 2005; Chapman et al. 2010a), as can low energy states (Godin and Crossman 1994; Mikheev et al. 1994). However, the two do not always act independently, as responding to a variable environment can come at an energetic cost: guppies exposed to an environment that varied in daily in colour attacked food items more quickly and foraged for longer in novel environment (Rodgers et al. 2013a). Here, I explored whether changes in guppy behaviour were due to a variable environment, or the energetic costs, by exposing adult guppies to a variable environment (daily colour change) in combination with a high or low food diet for two weeks. I investigated their latency to recover and attack food in a novel environment, and their propensity to explore a novel maze environment both before and after being placed into treatment.

Diet had a significant effect on foraging and exploratory behaviour, whereas exposure to a variable environment had no effect. Guppies in the low food treatment attacked food more quickly; consistent with both increased boldness and increased hunger, but this did not translate into any other measures of boldness. Those receiving the higher food diet showed an increase in activity compared to those on the low food diet, potentially reflecting an increase in other behaviours such as searching for mates, or shoal mates (Lima and Dill 1990; Pitcher 1993). Exploratory behaviour (the proportion of the maze explored) was highly repeatable, suggesting consistency in behaviour despite changes to energy state and environment, at least over a time scale of a few weeks.

I found that recent experience had little effect on an individual's behaviour. A number of studies have considered development of personality across different life stages: Bell and Stamps (2004) assessed behavioural traits in sticklebacks multiple times at different stages in their development, finding that correlations between aggression and boldness were repeatable at juvenile and adulthood, but not subadulthood. Kandra et al. (2012) found that as Siberian dwarf hamsters *Phodopus sungorus* aged, correlations between boldness and activity changed from negative to positive.

Juveniles and adults may differ in their levels of repeatability, depending on selection pressures. For example Bell et al. (2009) hypothesised that if traits expressed early in life are under strong selection pressure, we would see an increase in repeatability with age. How important early experience is in comparison to recent experience within the same species is a fascinating question that requires greater attention.

In this experiment I chose to concentrate on levels of boldness, perhaps the most well studied personality trait, as is it particularly important in predicting how an individual will respond to change. Bold individuals out compete shy (Ward et al. 2004; Webster et al. 2009), are more willing to consume novel food sources (Wilson 1998) and will more readily explore a novel environment (Budaev 1997), although there are higher mortality risks associated with bold behaviour (Bell and Sih 2007; reviewed in Smith and Blumstein 2008). Levels of boldness are also correlated to other traits, such as aggression (Bell and Stamps 2004) and sociability (Budaev 1997). By narrowing my search down to boldness and exploratory behaviours, I could have missed other changes in behaviour, such as changes in shoaling behaviour (Chapman et al. 2010a). Understanding how flexible animals are across different traits and throughout life may provide us with a greater understanding of how particular species will respond to environmental change.

Conclusion

In conclusion, short term exposure to turbidity severely impacts the ability of fish to form groups and respond to predators, and reduces selective foraging by predators. I find that individuals can vary their behavioural responses to a degraded or variable environment with increasing exposure time; however these appear to differ from the responses of juveniles. Together, these results highlight the importance of considering behavioural responses over different time spans and during different developmental stages when trying to understand how individuals will respond to anthropogenic environmental change.

References

Abrahams, M. & Kattenfeld, M. 1997. The role of turbidity as a constraint on predator-prey interactions in aquatic environments. *Behavioral Ecology and Sociobiology,* 40**,** 169-174.

Adriaenssens, B. & Johnsson, J. I. 2013. Natural selection, plasticity and the emergence of a behavioural syndrome in the wild. *Ecol Lett,* 16**,** 47-55.

Agresti, A. & Coull, B. A. 1998. Approximate is better than "exact" for interval estimation of binomial proportions. *The American Statistician,* 52**,** 119-126.

Aksnes, D. L. & Giske, J. 1993. A theoretical-model of aquatic visual feeding. *Ecological Modelling,* 67**,** 233-250.

Bateson, P., Barker, D., Clutton-Brock, T., Deb, D., D'udine, B., Foley, R. A., Gluckman, P., Godfrey, K., Kirkwood, T., Lahr, M. M., Mcnamara, J., Metcalfe, N. B., Monaghan, P., Spencer, H. G. & Sultan, S. E. 2004. Developmental plasticity and human health. *Nature,* 430**,** 419-421.

Beauchamp, G. 2014. *Social predation. How group living benefits predators and prey,* London, Academic Press.

Bell, A. M. 2007. Future directions in behavioural syndromes research. *Proc Biol Sci,* 274**,** 755-61.

Bell, A. M., Dingemanse, N. J., Hankison, S. J., Langenhof, M. B. W. & Rollins, K. 2011. Early exposure to nonlethal predation risk by size-selective predators increases somatic growth and decreases size at adulthood in threespined sticklebacks. *Journal of Evolutionary Biology,* 24**,** 943-953.

Bell, A. M., Hankison, S. J. & Laskowski, K. L. 2009. The repeatability of behaviour: A meta-analysis. *Animal Behaviour,* 77**,** 771-783.

Bell, A. M. & Sih, A. 2007. Exposure to predation generates personality in threespined sticklebacks (gasterosteus aculeatus). *Ecol Lett,* 10**,** 828-34.

Bell, A. M. & Stamps, J. A. 2004. Development of behavioural differences between individuals and populations of sticklebacks, gasterosteus aculeatus. *Animal Behaviour,* 68**,** 1339-1348.

Benjamini, Y. & Hochberg, Y. 1995. Controlling the false discovery rate - a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society Series B-Methodological,* 57**,** 289-300.

Berg, L. & Northcote, T. G. 1985. Changes in territorial, gill-flaring, and feeding-behavior in juvenile coho salmon (oncorhynchus-kisutch) following short-term pulses of suspended sediment. *Canadian Journal of Fisheries and Aquatic Sciences,* 42**,** 1410-1417.

Beschta, R. L. & Ripple, W. J. 2009. Large predators and trophic cascades in terrestrial ecosystems of the western united states. *Biological Conservation,* 142**,** 2401-2414.

Biro, P. A. & Stamps, J. A. 2008. Are animal personality traits linked to life-history productivity? *Trends in Ecology & Evolution,* 23**,** 361-368.

Bonsdorff, E., Ronnberg, C. & Aarnio, K. 2002. Some ecological properties in relation to eutrophication in the baltic sea. *Hydrobiologia,* 475**,** 371-377.

Borner, K. K., Krause, S., Mehner, T., Uusi-Heikkila, S., Ramnarine, I. W. & Krause, J. 2015. Turbidity affects social dynamics in trinidadian guppies. *Behavioral Ecology and Sociobiology,* 69**,** 645-651.

Braithwaite, V. A. & Salvanes, A. G. 2005. Environmental variability in the early rearing environment generates behaviourally flexible cod: Implications for rehabilitating wild populations. *Proc Biol Sci,* 272**,** 1107-13.

Briffa, M., Rundle, S. D. & Fryer, A. 2008. Comparing the strength of behavioural plasticity and consistency across situations: Animal personalities in the hermit crab pagurus bernhardus. *Proc Biol Sci,* 275**,** 1305-11.

Brightsmith, D. J. & Villalobos, E. M. 2011. Parrot behavior at a peruvian clay lick. *Wilson Journal of Ornithology,* 123**,** 595-602.

Bronmark, C. & Miner, J. G. 1992. Predator-induced phenotypical change in body morphology in crucian carp. *Science,* 258**,** 1348-1350.

Brown, C. & Laland, K. N. 2003. Social learning in fishes: A review. *Fish and Fisheries,* 4**,** 280-288.

Brown, G. E. 2003. Learning about danger: Chemical alarm cues and local risk assessment in prey fishes. *Fish and Fisheries,* 4**,** 227-234.

Brown, G. E. & Godin, J. G. J. 1999. Who dares, learns: Chemical inspection behaviour and acquired predator recognition in a characin fish. *Animal Behaviour,* 57**,** 475-481.

Brown, G. E., Golub, J. L. & Plata, D. L. 2001. Attack cone avoidance during predator inspection visits by wild finescale dace (phoxinus neogaeus): The effects of predator diet. *Journal of Chemical Ecology,* 27**,** 1657-1666.

Bruton, M. N. 1985. The effects of suspensoids on fish. *Hydrobiologia,* 125**,** 221-241.

Brydges, N. M., Heathcote, R. J. P. & Braithwaite, V. A. 2008. Habitat stability and predation pressure influence learning and memory in populations of three-spined sticklebacks. *Animal Behaviour,* 75**,** 935-942.

Budaev, S. V. 1997. Alternative styles in the european wrasse, symphodus ocellatus: Boldness-related schooling tendency. *Environmental Biology of Fishes,* 49**,** 71-78.

Candolin, U., Salesto, T. & Evers, M. 2007. Changed environmental conditions weaken sexual selection in sticklebacks. *Journal of Evolutionary Biology,* 20**,** 233-239.

Candolin, U. & Wong, B. B. M. 2012. *Behavioural responses to a changing world. Mechanisms & consequences,* Oxford, Oxford University Press.

Caraco, T., Martindale, S. & Pulliam, H. R. 1980. Avian flocking in the presence of a predator. *Nature,* 285**,** 400-401.

Carere, C., Drent, P. J., Privitera, L., Koolhaas, J. M. & Groothuis, T. G. G. 2005. Personalities in great tits, parus major: Stability and consistency. *Animal Behaviour,* 70**,** 795-805.

Chapman, B. B., Morrell, L. J., Benton, T. G. & Krause, J. 2007. Early interactions with adults mediate the development of predator defenses in guppies. *Behavioral Ecology,* 19**,** 87-93.

Chapman, B. B., Morrell, L. J. & Krause, J. 2009. Plasticity in male courtship behaviour as a function of light intensity in guppies. *Behavioral Ecology and Sociobiology,* 63**,** 1757-1763.

Chapman, B. B., Morrell, L. J. & Krause, J. 2010a. Unpredictability in food supply during early life influences boldness in fish. *Behavioral Ecology,* 21**,** 501-506.

Chapman, B. B., Morrell, L. J., Tosh, C. R. & Krause, J. 2010b. Behavioural consequences of sensory plasticity in guppies. *Proceedings of the Royal Society B-Biological Sciences,* 277**,** 1395-1401.

Chapman, B. B., Ward, A. J. W. & Krause, J. 2008. Schooling and learning: Early social environment predicts social learning ability in the guppy, poecilia reticulata. *Animal Behaviour,* 76**,** 923-929.

Chivers, D. P., Brown, G. E. & Smith, R. J. F. 1995. Familiarity and shoal cohesion in fathead minnows (*pimpehales promelas*) - implications for antipredator behavior. *Canadian Journal of Zoology-Revue Canadienne De Zoologie,* 73**,** 955-960.

Chivers, D. P. & Smith, R. J. F. 1998. Chemical alarm signalling in aquatic predator-prey systems: A review and prospectus. *Ecoscience,* 5**,** 338-352.

Cobcroft, J. M., Pankhurst, P. M., Hart, P. R. & Battaglene, S. C. 2001. The effects of light intensity and algae-induced turbidity on feeding behaviour of larval striped trumpeter. *Journal of Fish Biology,* 59**,** 1181-1197.

Conradt, L. & Roper, T. J. 2000. Activity synchrony and social cohesion: A fission-fusion model. *Proceedings of the Royal Society B-Biological Sciences,* 267**,** 2213-2218.
Crawley, M. 2007. *The r book,* Chichester, John Wiley & Sons, Ltd.
Croft, D. P., Arrowsmith, B. J., Bielby, J., Skinner, K., White, E., Couzin, I. D., Magurran, A. E., Ramnarine, I. & Krause, J. 2003. Mechanisms underlying shoal composition in the trinidadian guppy, poecilia reticulata. *Oikos,* 100**,** 429-438.
Croft, D. P., Krause, J. & James, R. 2004. Social networks in the guppy (poecilia reticulata). *Proceedings of the Royal Society B-Biological Sciences,* 271**,** S516-S519.
Dall, S. R. X., Houston, A. I. & Mcnamara, J. M. 2004. The behavioural ecology of personality: Consistent individual differences from an adaptive perspective. *Ecology Letters,* 7**,** 734-739.
David, M., Auclair, Y. & Cézilly, F. 2012. Assessing short-and long-term repeatability and stability of personality in captive zebra finches using longitudinal data. *Ethology,* 118**,** 932-942.
Davies, N. B., Krebs, K. R. & West, S. W. 2012. *An introduction to behavioural ecology,* Chichester, Wiley-Blackwell.
De Robertis, A., Ryer, C. H., Veloza, A. & Brodeur, R. D. 2003. Differential effects of turbidity on prey consumption of piscivorous and planktivorous fish. *Canadian Journal of Fisheries and Aquatic Sciences,* 60**,** 1517-1526.
De Vos, A. & O'riain, M. J. 2012. Movement in a selfish seal herd: Do seals follow simple or complex movement rules? *Behavioral Ecology,* 24**,** 190-197.
Demsar, J., Hemelrijk, C., K., Hildrenbrandt, H. & Bajec, I., L. 2015. Simulating predator attacks on schools. Evolving composite tactics. *Ecological Modelling,* 304**,** 22-33.
Dewitt, T. J., Sih, A. & Wilson, D. S. 1998. Costs and limits of phenotypic plasticity. *Trends in Ecology & Evolution,* 13**,** 77-81.
Dill, L. M. & Fraser, A. H. 1984. Risk of predation and the feeding behavior of juvenile coho salmon (oncorhynchus kisutch). *Behavioral Ecology and Sociobiology,* 16**,** 65-71.
Dingemanse, N., Both, C., Drent, P. J., Van Oers, K. & Van Noordwijk, A. 2002. Repeatability and heritability of exploratory behaviour in great tits from the wild. *Animal Behaviour,* 64**,** 929-938.
Dingemanse, N. J., Kazem, A. J., Reale, D. & Wright, J. 2010. Behavioural reaction norms: Animal personality meets individual plasticity. *Trends Ecol Evol,* 25**,** 81-9.
Dingemanse, N. J. & Reale, D. 2005. Natural selection and animal personality. *Behaviour,* 142**,** 1159-1184.
Dingemanse, N. J., Van Der Plas, F., Wright, J., Reale, D., Schrama, M., Roff, D. A., Van Der Zee, E. & Barber, I. 2009. Individual experience and evolutionary history of predation affect expression of heritable variation in fish personality and morphology. *Proc Biol Sci,* 276**,** 1285-93.
Dixson, D. L., Munday, P. L. & Jones, G. P. 2010. Ocean acidification disrupts the innate ability of fish to detect predator olfactory cues. *Ecology Letters,* 13**,** 68-75.
Drent, P. J., Van Oers, K. & Van Noordwijk, A. J. 2003. Realized heritability of personalities in the great tit (parus major). *Proceedings of the Royal Society B-Biological Sciences,* 270**,** 45-51.
Dufty, A. M., Clobert, J. & Moller, A. P. 2002. Hormones, developmental plasticity and adaptation. *Trends in Ecology & Evolution,* 17**,** 190-196.
Ebbesson, L. O. E. & Braithwaite, V. A. 2012. Environmental effects on fish neural plasticity and cognition. *Journal of Fish Biology,* 81**,** 2151-2174.
Ebert, D. & Bethesda 2005. *Ecology, epidemiology, and evolution of parasitism in daphnia*, National Library of Medicine (US), National Center for Biotechnology.
Einfalt, L. M., Grace, E. J. & Wahl, D. H. 2012. Effects of simulated light intensity, habitat complexity and forage type on predator-prey interactions in walleye sander vitreus. *Ecology of Freshwater Fish,* 21**,** 560-569.

Endler, J. A. 1991. Variation in the appearance of guppy color patterns to guppies and their predators under different visual conditions. *Vision Research,* 31**,** 587-608.
Engstrom-Ost, J. & Candolin, U. 2007. Human-induced water turbidity alters selection on sexual displays in sticklebacks. *Behavioral Ecology,* 18**,** 393-398.
Engstrom-Ost, J., Karjalainen, M. & Viitasalo, M. 2006. Feeding and refuge use by small fish in the presence of cyanobacteria blooms. *Environmental Biology of Fishes,* 76**,** 109-117.
Engström-Öst, J. & Mattila, J. 2008. Foraging, growth and habitat choice in turbid water: An experimental study with fish larvae in the baltic sea. *Marine Ecology Progress Series,* 359**,** 275-281.
Fischer, B., Van Doorn, G. S., Dieckmann, U. & Taborsky, B. 2014. The evolution of age-dependent plasticity. *American Naturalist,* 183**,** 108-125.
Fischer, E., Schwartz, A., Hoke, K. & Soares, D. 2015. Social context modulates predator evasion strategy in guppies. *Ethology*.
Fischer, S. & Frommen, J. G. 2012. Eutrophication alters social preferences in three-spined sticklebacks (gasterosteus aculeatus). *Behavioral Ecology and Sociobiology,* 67**,** 293-299.
Fleming, I. A. & Einum, S. 1997. Experimental tests of genetic divergence of farmed from wild atlantic salmon due to domestication. *Ices Journal of Marine Science,* 54**,** 1051-1063.
Foster, W. A. & Treherne, J. E. 1981. Evidence for the dilution effect in the selfish herd from fish predation on a marine insect. *Nature,* 293**,** 466-467.
Fraser, N. H. C. & Metcalfe, N. B. 1997. The costs of becoming nocturnal: Feeding efficiency in relation to light intensity in juvenile atlantic salmon. *Functional Ecology,* 11**,** 385-391.
Frommen, J. G., Hiermes, M. & Bakker, T. C. M. 2009. Disentangling the effects of group size and density on shoaling decisions of three-spined sticklebacks (gasterosteus aculeatus). *Behavioral Ecology and Sociobiology,* 63**,** 1141-1148.
Frost, A. J., Thomson, J. S., Smith, C., Burton, H. C., Davis, B., Watts, P. C. & Sneddon, L. U. 2013. Environmental change alters personality in the rainbow trout, oncorhynchus mykiss. *Animal Behaviour,* 85**,** 1199-1207.
Frost, A. J., Winrow-Giffen, A., Ashley, P. J. & Sneddon, L. U. 2007. Plasticity in animal personality traits: Does prior experience alter the degree of boldness? *Proc Biol Sci,* 274**,** 333-9.
Gandelman, R., Paschke, R. E., Zarrow, M. X. & Denenberg, V. H. 1970. Care of young under communal conditions in the mouse mus-musculus. *Developmental Psychobiology,* 3**,** 245-250.
Gibson, R. M. 1980. Optimal prey-size selection by 3-spined sticklebacks (gasterosteus-aculeatus) - a test of the apparent-size hypothesis. *Zeitschrift Fur Tierpsychologie-Journal of Comparative Ethology,* 52**,** 291-307.
Giske, J., Huse, G. & Fiksen, O. 1998. Modelling spatial dynamics of fish. *Reviews in Fish Biology and Fisheries,* 8**,** 57-91.
Gliwicz, M. Z. 1986. Predation and the evolution of vertical migration in zooplankton. *Nature,* 320**,** 746-748.
Godin, J.-G. J. & Crossman, S. L. 1994. Hunger-dependent predator inspection and foraging behaviours in the threespine stickleback (gasterosteus aculeatus) under predation risk. *Behavioral Ecology and Sociobiology,* 34**,** 359-366.
Godin, J.-G. J. & Sproul, C. D. 1988. Risk taking in parasitized sticklebacks under threat of predation: Effects of energetic need and food availability. *Canadian Journal of Zoology,* 66**,** 2360-2367.
Godin, J. 1986. Risk of predation and foraging behavior in shoaling banded killifish fundulus-diaphanus. *Canadian Journal of Zoology,* 64**,** 1675-1678.
Godin, J., Classon, L. J. & Abrahams, M. V. 1988. Group vigilance and shoal size in a small characin fish. *Behaviour,* 104**,** 29-40.

Goldenberg, S. U., Borcherding, J. & Heynen, M. 2014. Balancing the response to predation—the effects of shoal size, predation risk and habituation on behaviour of juvenile perch. *Behavioral Ecology and Sociobiology,* 68**,** 989-998.

Gotceitas, V. & Godin, J.-G. J. 1991. Foraging under the risk of predation in juvenile atlantic salmon (salmo salar l.): Effects of social status and hunger. *Behavioral Ecology and Sociobiology,* 29**,** 255-261.

Goudie, A. S. 2013. *The human impact on the natural environment: Past, present, and future*, John Wiley & Sons.

Granqvist, M. & Mattila, J. 2004. The effects of turbidity and light intensity on the consumption of mysids by juvenile perch (perca fluviatilis l.). *Biology of the baltic sea.* Springer.

Gray, S. M., Mcdonnell, L. H., Cinquemani, F. G. & Chapman, L. J. 2012. As clear as mud: Turbidity induces behavioral changes in the african cichlid pseudocrenilabrus multicolor. *Current Zoology,* 58**,** 146-157.

Grecay, P. A. & Targett, T. E. 1996. Effects of turbidity, light level and prey concentration on feeding of juvenile weakfish cynoscion regalis. *Marine ecology progress series. Oldendorf,* 131**,** 11-16.

Gregory, R. S. 1993. Effect of turbidity on the predator avoidance-behavior of juvenile chinook salmon (*oncorhynchus tshwytshca*). *Canadian Journal of Fisheries and Aquatic Sciences,* 50**,** 241-246.

Gregory, R. S. & Levings, C. D. 1998. Turbidity reduces predation on migrating juvenile pacific salmon. *Transactions of the American Fisheries Society,* 127**,** 275-285.

Gregory, R. S. & Northcote, T. G. 1993. Surface, planktonic, and benthic foraging by juvenile chinook salmon (*oncorhynchus tshwytscha*) in turbid laboratory conditions. *Canadian Journal of Fisheries and Aquatic Sciences,* 50**,** 233-240.

Griffiths, S. W. & Magurran, A. E. 1999. Schooling decisions in guppies (poecilia reticulata) are based on familiarity rather than kin recognition by phenotype matching. *Behavioral Ecology and Sociobiology,* 45**,** 437-443.

Guthrie, D. M. & Muntz, W. R. A. 1993. *Role of vision in fish behaviour*, Chapman and Hall Ltd., 2-6 Boundary Row, London SE1 8HN, England; Chapman and Hall, Inc., 29 West 35th Street, New York, New York, USA.

Hamilton, W. D. 1971. Geometry for selfish herd. *Journal of Theoretical Biology,* 31**,** 295-&.

Harrison, X. A. 2014. Using observation-level random effects to model overdispersion in count data in ecology and evolution. *PeerJ,* 2**,** e616.

Hayes, J. P. & Jenkins, S. H. 1997. Individual variation in mammals. *Journal of Mammalogy,* 78**,** 274-293.

Hecky, R. E. 1993. The eutrophication of lake victoria. *In:* Sladeckova, A. (ed.) *International association of theoretical and applied limnology - proceedings, vol 25, pt 1.* Stuttgart: E Schweizerbart'sche Verlagsbuchhandlung.

Henley, W. F., Patterson, M. A., Neves, R. J. & Lemly, A. D. 2000. Effects of sedimentation and turbidity on lotic food webs: A concise review for natural resource managers. *Reviews in Fisheries Science,* 8**,** 125-139.

Herskin, J. & Steffensen, J. F. 1998. Energy savings in sea bass swimming in a school: Measurements of tail beat frequency and oxygen consumption at different swimming speeds. *Journal of Fish Biology,* 53**,** 366-376.

Hoare, D. 2000. Body size and shoaling in fish. *Journal of Fish Biology,* 57**,** 1351-1366.

Holling, C. S. 1959. The components of predation as revealed by a study of small-mammal predation of the european pine sawfly. *The Canadian Entomologist,* 69**,** 293-320.

Holt, R. D. 1977. Predation, apparent competition, and the structure of prey communities. *Theoretical population biology,* 12**,** 197-229.

Houde, A. E. 1997. *Sex, color, and mate choice in guppies,* Princeton, New Jersey, Princeton University Press.

Huntingford, F. A. 1976. Relationship between anti-predator behavior and aggression among conspecifics in 3-spined stickleback, gasterosteus-aculeatus. *Animal Behaviour,* 24**,** 245-260.

Ilin, Y. & Richter-Levin, G. 2009. Enriched environment experience overcomes learning deficits and depressive-like behavior induced by juvenile stress. *Plos One,* 4**,** 12.

Ioannou, C. C., Guttal, V. & Couzin, I. D. 2012. Predatory fish select for coordinated collective motion in virtual prey. *Science,* 337**,** 1212-1215.

Ioannou, C. C., Morrell, L. J., Ruxton, G. D. & Krause, J. 2009. The effect of prey density on predators: Conspicuousness and attack success are sensitive to spatial scale. *American Naturalist,* 173**,** 499-506.

James, R., Bennett, P. G. & Krause, J. 2004. Geometry for mutualistic and selfish herds: The limited domain of danger. *J Theor Biol,* 228**,** 107-13.

Jarvenpaa, M. & Lindstrom, K. 2004. Water turbidity by algal blooms causes mating system breakdown in a shallow-water fish, the sand goby pomatoschistus minutus. *Proceedings of the Royal Society B-Biological Sciences,* 271**,** 2361-2365.

Johannesen, A., Dunn, A. M. & Morrell, L. J. 2012. Olfactory cue use by three-spined sticklebacks foraging in turbid water: Prey detection or prey location? *Animal Behaviour,* 84**,** 151-158.

Johannesen, A., Dunn, A. M. & Morrell, L. J. 2014. Prey aggregation is an effective olfactory predator avoidance strategy. *PeerJ,* 2**,** e408.

Jonsson, B. & Jonsson, N. 2014. Early environment influences later performance in fishes. *Journal of Fish Biology,* 85**,** 151-188.

Jonsson, M., Hylander, S., Ranaker, L., Nilsson, P. A. & Bronmark, C. 2011. Foraging success of juvenile pike esox lucius depends on visual conditions and prey pigmentation. *Journal of Fish Biology,* 79**,** 290-297.

Jonsson, M., Ranaker, L., Nilsson, P. A. & Bronmark, C. 2013. Foraging efficiency and prey selectivity in a visual predator: Differential effects of turbid and humic water. *Canadian Journal of Fisheries and Aquatic Sciences,* 70**,** 1685-1690.

Kanda, L. L., Louon, L. & Straley, K. 2012. Stability in activity and boldness across time and context in captive siberian dwarf hamsters. *Ethology,* 118**,** 518-533.

Kasumyan, A. O. 2001. Effect of the cheical pollution agents on feeding behavior and sensibility of fishes to nutritonal stimuli. *Voprosy Ikhtiologii,* 41**,** 82-95.

Katz, Y., Tunstrom, K., Ioannou, C. C., Huepe, C. & Couzin, I. D. 2011. Inferring the structure and dynamics of interactions in schooling fish. *Proceedings of the National Academy of Sciences of the United States of America,* 108**,** 18720-18725.

Keenleyside, M. H. A. 1955. Some aspects of the schooling behaviour of fish. *Behaviour* 8**,** 183 - 248.

Kelley, J. L. & Magurran, A. E. 2003a. Effects of relaxed predation pressure on visual predator recognition in the guppy. *Behavioral Ecology and Sociobiology,* 54**,** 225-232.

Kelley, J. L. & Magurran, A. E. 2003b. Learned predator recognition and antipredator responses in fishes. *Fish and Fisheries,* 4**,** 216-226.

Kimbell, H. & Morrell, L. J. 2015. Turbidity alters anti-predatory behaviour in guppy (poecilia reticulata) shoals. *Animal Behaviour,* 103**,** 179-185.

King, A. J., Wilson, A. M., Wilshin, S. D., Lowe, J., Haddadi, H., Hailes, S. & Morton, A. J. 2012. Selfish-herd behaviour of sheep under threat. *Curr Biol,* 22**,** R561-2.

Kirk, J. T. O. 2011. *Light and photosynthesis in aquatic ecosystems*, Cambridge University Press.

Knudsen, E. I. 2004. Sensitive periods in the development of the brain and behavior. *Journal of Cognitive Neuroscience,* 16**,** 1412-1425.

Koolhaas, J. M., Korte, S. M., De Boer, S. F., Van Der Vegt, B. J., Van Reenen, C. G., Hopster, H., De Jong, I. C., Ruis, M. a. W. & Blokhuis, H. J. 1999. Coping styles in animals: Current status in behavior and stress-physiology. *Neuroscience and Biobehavioral Reviews,* 23**,** 925-935.

Koteja, P., Carter, P. A., Swallow, J. G. & Garland, T. 2003. Food wasting by house mice: Variation among individuals, families, and genetic lines. *Physiology & Behavior,* 80**,** 375-383.

Kotrschal, A., Rogell, B., Maklakov, A. A. & Kolm, N. 2012. Sex-specific plasticity in brain morphology depends on social environment of the guppy, poecilia reticulata. *Behavioral Ecology and Sociobiology,* 66**,** 1485-1492.

Krakauer, D. C. 1995. Groups confuse predators by exploiting perceptual bottlenecks - a connectionist model of the confusion effect. *Behavioral Ecology and Sociobiology,* 36**,** 421-429.

Krams, I. & Krama, T. 2002. Interspecific reciprocity explains mobbing behaviour of the breeding chaffinches, fringilla coelebs. *Proceedings of the Royal Society B-Biological Sciences,* 269**,** 2345-2350.

Krause, J. 1993. The influence of hunger on shoal size choice by 3-spined sticklebacks, gasterosteus-aculeatus. *Journal of Fish Biology,* 43**,** 775-780.

Krause, J. 1994. Differential fitness returns in relation to spatial position in groups. *Biological Reviews of the Cambridge Philosophical Society,* 69**,** 187-206.

Krause, J., Bumann, D. & Todt, D. 1992. Relationship between the position preference and nutritional state of individuals in schools of juvenile roach (rutilus-rutilus). *Behavioral Ecology and Sociobiology,* 30**,** 177-180.

Krause, J., Godin, J. & Rubenstein, D. 1998. Group choice as a function of group size differences and assessment time in fish: The influence of species vulnerability to predation. *Ethology,* 104**,** 68-74.

Krause, J. & Ruxton, G. 2002. *Living in groups,* Oxford, Oxford University Press.

Krause, J. & Tegeder, R. W. 1994. The mechanism of aggregation behavior in fish shoals - individuals minimize approach time to neighbors. *Animal Behaviour,* 48**,** 353-359.

Kunc, H. P., Lyons, G. N., Sigwart, J. D., Mclaughlin, K. E. & Houghton, J. D. 2014. Anthropogenic noise affects behavior across sensory modalities. *Am Nat,* 184**,** E93-100.

Kunin, W. E. 1999. Patterns of herbivore incidence on experimental arrays and field populations of ragwort, senecio jacobaea. *Oikos,* 84**,** 515-525.

Lachlan, R. F., Crooks, L. & Laland, K. N. 1998. Who follows whom? Shoaling preferences and social learning of foraging information in guppies. *Animal Behaviour,* 56**,** 181-190.

Landeau, L. & Terborgh, J. 1986. Oddity and the confusion effect in predation. *Animal Behaviour,* 34**,** 1372-1380.

Leahy, S. M., Mccormick, M. I., Mitchell, M. D. & Ferrari, M. C. 2011. To fear or to feed: The effects of turbidity on perception of risk by a marine fish. *Biol Lett,* 7**,** 811-3.

Lehtiniemi, M., Engstrom-Ost, J. & Viitasalo, M. 2005. Turbidity decreases anti-predator behaviour in pike larvae, esox lucius. *Environmental Biology of Fishes,* 73**,** 1-8.

Lehtiniemi, M., Hakala, T., Saesmaa, S. & Viitasalo, M. 2007. Prey selection by the larvae of three species of littoral fishes on natural zooplankton assemblages. *Aquatic Ecology,* 41**,** 85-94.

Li, K. T., Wetterer, J. K. & Hairston, N. G. J. 1985. Fish size visual resolution and prey selectivity. *Ecology (Washington D C),* 66**,** 1729-1735.

Licht, T. 1989. Discriminating between hungry and satiated predators - the response of guppies (poecilia-reticulata) from high and low predation sites). *Ethology,* 82**,** 238-243.

Lima, S. L. & Dill, L. M. 1990. Behavioral decisions made under the risk of predation - a review and prospectus. *Canadian Journal of Zoology-Revue Canadienne De Zoologie,* 68**,** 619-640.

Lindstrom, K. & Ranta, E. 1993. Foraging group-structure among individuals differing in competitive ability. *Annales Zoologici Fennici,* 30**,** 225-232.

Ljunggren, L. & Sandstrom, A. 2007. Influence of visual conditions on foraging and growth of juvenile fishes with dissimilar sensory physiology. *Journal of Fish Biology,* 70**,** 1319-1334.

Long, K. D. & Houde, A. E. 1989. Orange spots as a visual cue for female mate choice in the guppy (poecilia-reticulata). *Ethology,* 82**,** 316-324.
Luyten, P. H. & Liley, N. R. 1985. Geographic variation in the sexual behaviour of the guppy, *poecilia reticulata*. *Behaviour* 95**,** 164-179.
Macarther, R. H. & Pianka, E. R. 1966. On optimal use of a patchy environment. *Am Nat***,** 603-609.
Macgregor, C. J., Pocock, M. J. O., Fox, R. & Evans, D. M. 2014. Pollination by nocturnal lepidoptera, and the effects of light pollution: A review. *Ecological Entomology,* 40**,** 187-198.
Magnhagen, C. & Staffan, F. 2004. Is boldness affected by group composition in young-of-the-year perch (perca fluviatilis)? *Behavioral Ecology and Sociobiology,* 57**,** 295-303.
Magurran, A. E. 1990. The adaptive significance of schooling as an antipredator defense in fish. *Annales Zoologici Fennici,* 27**,** 51-66.
Magurran, A. E. 2005. *Evolutionary ecology of the trinidadian guppy,* Oxford, Oxford University Press.
Magurran, A. E., Oulton, W. J. & Pitcher, T. J. 1985. Vigilant behavior and shoal size in minnows. *Zeitschrift Fur Tierpsychologie-Journal of Comparative Ethology,* 67**,** 167-178.
Magurran, A. E. & Pitcher, T. J. 1987. Provenance, shoal size and the sociobiology of predator-evasion behavior in minnow shoals. *Proceedings of the Royal Society Series B-Biological Sciences,* 229**,** 439-465.
Manicom, C. & Schwarzkopf, L. 2011. Diet and prey selection of sympatric tropical skinks. *Austral Ecology,* 36**,** 485-496.
Marchinko, K. B. 2003. Dramatic phenotypic plasticity in barnacle legs (balanus glandula darwin): Magnitude, age dependence, and speed of response. *Evolution,* 57**,** 1281-1290.
Martin, J. G. A. & Reale, D. 2008. Temperament, risk assessment and habituation to novelty in eastern chipmunks, tamias striatus. *Animal Behaviour,* 75**,** 309-318.
Mathis, A. & Chivers, D. P. 2003. Overriding the oddity effect in mixed-species aggregations: Group choice by armored and nonarmored prey. *Behavioral Ecology,* 14**,** 334-339.
Mccormick, S. D., Hansen, L. P., Quinn, T. P. & Saunders, R. L. 1998. Movement, migration, and smolting of atlantic salmon (salmo salar). *Canadian Journal of Fisheries and Aquatic Sciences,* 55**,** 77-92.
Mclain, D. K., Pratt, A. E. & Kirschstein, K. 2005. Predator-driven fragmentation of fiddler crab droves into selfish miniherds of biased composition. *Journal of Experimental Marine Biology and Ecology,* 315**,** 1-15.
Mclaughlin, K. E. & Kunc, H. P. 2013. Experimentally increased noise levels change spatial and singing behaviour. *Biol Lett,* 9**,** 20120771.
Mcmahon, T. E. & Holanov, S. H. 1995. Foraging success of largemouth bass at different light intensities - implications for time and depth of feeding. *Journal of Fish Biology,* 46**,** 759-767.
Mcrobert, S. P. & Bradner, J. 1998. The influence of body coloration on shoaling preferences in fish. *Animal Behaviour,* 56**,** 611-615.
Meager, J. J. & Batty, R. S. 2007. Effects of turbidity on the spontaneous and prey-searching activity of juvenile atlantic cod (gadus morhua). *Philos Trans R Soc Lond B Biol Sci,* 362**,** 2123-30.
Meager, J. J., Domenici, P., Shingles, A. & Utne-Palm, A. C. 2006. Escape responses in juvenile atlantic cod gadus morhua l.: The effects of turbidity and predator speed. *J Exp Biol,* 209**,** 4174-84.
Meldrum, G. E. & Ruckstuhl, K. E. 2009. Mixed-sex group formation by bighorn sheep in winter: Trading costs of synchrony for benefits of group living. *Animal Behaviour,* 77**,** 919-929.
Metcalfe, N. B. & Thomson, B. C. 1995. Fish recognize and prefer to shoal with poor competitors. *Proceedings of the Royal Society B-Biological Sciences,* 259**,** 207-210.

Mikheev, V., Metcalfe, N., Huntingford, F. & Thorpe, J. 1994. Size-related differences in behaviour and spatial distribution of juvenile atlantic salmon in a novel environment. *Journal of fish biology,* 45**,** 379-386.

Milinski, M. 1977a. Do all members of a swarm suffer same predation. *Zeitschrift Fur Tierpsychologie-Journal of Comparative Ethology,* 45**,** 373-388.

Milinski, M. 1977b. Experiments on selection by predators against spatial oddity of their prey. *Zeitschrift Fur Tierpsychologie-Journal of Comparative Ethology,* 43**,** 311-325.

Milinski, M. 1984. A predator's costs of overcoming the confusion-effect of swarming prey. *Animal Behaviour,* 32**,** 1157-1162.

Milinski, M. & Heller, R. 1978. Influence of a predator on optimal foraging behavior of sticklebacks (gasterosteus-aculeatus l). *Nature,* 275**,** 642-644.

Miller, N. & Gerlai, R. 2007. Quantification of shoaling behaviour in zebrafish (danio rerio). *Behavioural Brain Research,* 184**,** 157-166.

Miner, J. G. & Stein, R. A. 1993. Interactive influence of turbidity and light on larval bluegill (lepomis macrochirus) foraging. *Canadian Journal of Fisheries and Aquatic Sciences,* 50**,** 781-788.

Miner, J. G. & Stein, R. A. 1996. Detection of predators and habitat choice by small bluegills: Effects of turbidity and alternative prey. *Transactions of the American Fisheries Society,* 125**,** 97-103.

Miyazaki, T., Shiozawa, S., Kogane, T., Masuda, R., Maruyama, K. & Tsukamoto, K. 2000. Developmental changes of the light intensity threshold for school formation in the striped jack pseudocaranx dentex. *Marine Ecology Progress Series,* 192**,** 267-275.

Moberg, O., Braithwaite, V. A., Jensen, K. H. & Salvanes, A. G. V. 2011. Effects of habitat enrichment and food availability on the foraging behaviour of juvenile atlantic cod (gadus morhua l). *Environmental Biology of Fishes,* 91**,** 449-457.

Monaghan, P. 2008. Early growth conditions, phenotypic development and environmental change. *Philosophical Transactions of the Royal Society B-Biological Sciences,* 363**,** 1635-1645.

Morrell, L. J., Greenwood, L. & Ruxton, G. D. 2015. Consequences of variation in predator attack for the evolution of the selfish herd. *Evolutionary Ecology,* 29**,** 107-121.

Morrell, L. J. & James, R. 2008. Mechanisms for aggregation in animals: Rule success depends on ecological variables. *Behavioral Ecology,* 19**,** 193-201.

Morrell, L. J., Ruxton, G. D. & James, R. 2011a. Spatial positioning in the selfish herd. *Behavioral Ecology,* 22**,** 16-22.

Morrell, L. J., Ruxton, G. D. & James, R. 2011b. The temporal selfish herd: Predation risk while aggregations form. *Proc Biol Sci,* 278**,** 605-12.

Morton, T. L., Haefner, J. W., Nugala, V., Decino, R. D. & Mendes, L. 1994. The selfish herd revisited: Do simple movement rules reduce relative predation risk? *Journal of Theoretical Biology***,** 73-79.

Nakayama, S., Johnstone, R. A. & Manica, A. 2012. Temperament and hunger interact to determine the emergence of leaders in pairs of foraging fish. *PLoS One,* 7**,** e43747.

Näslund, J., Aarestrup, K., Thomassen, Søren t., Johnsson, Jörgen i. & Post, J. 2012. Early enrichment effects on brain development in hatchery-reared atlantic salmon (salmo salar): No evidence for a critical period. *Canadian Journal of Fisheries and Aquatic Sciences,* 69**,** 1481-1490.

Natarajan, D., De Vries, H., Saaltink, D.-J., De Boer, S. F. & Koolhaas, J. M. 2009. Delineation of violence from functional aggression in mice: An ethological approach. *Behavior genetics,* 39**,** 73-90.

Nemeth, E. & Brumm, H. 2010. Birds and anthropogenic noise: Are urban songs adaptive? *Am Nat,* 176**,** 465-75.

Noda, M., Gushima, K. & Kakuda, S. 1994. Local prey search based on spatial memory and expectation in the planktivorous reef fish, chromis-chrysurus (pomacentridae). *Animal Behaviour,* 47**,** 1413-1422.

Odling-Smee, L. & Braithwaite, V. A. 2003. The role of learning in fish orientation. *Fish and Fisheries,* 4**,** 235-246.

Ohata, R., Masuda, R., Takahashi, K. & Yamashita, Y. 2013. Moderate turbidity enhances schooling behaviour in fish larvae in coastal waters. *ICES Journal of Marine Science,* 71**,** 925-929.

Ohguchi, O. 1978. Experiments on selection against color oddity of water fleas by 3-spined sticklebacks. *Zeitschrift Fur Tierpsychologie-Journal of Comparative Ethology,* 47**,** 254-267.

Olivier, A., Leduc, H. C., Roh, E., Harvey, M. C. & Brown, G. E. 2006. Impaired detection of chemical alarm cues by juvenile wild atlantic salmon (salmo salar) in a weakly acidic environment. *Canadian Journal of Fisheries and Aquatic Sciences,* 63**,** 2356-2363.

Øverli, Ø., Winberg, S. & Pottinger, T. G. 2005. Behavioral and neuroendocrine correlates of selection for stress responsiveness in rainbow trout—a review. *Integrative and Comparative Biology,* 45**,** 463-474.

Paciorek, T. & Mcrobert, S. 2012. Daily variation in the shoaling behavior of zebrafish danio rerio. *Current Zoology,* 58**,** 129-137.

Palumbi, S. R. 2001. Evolution - humans as the world's greatest evolutionary force. *Science,* 293**,** 1786-1790.

Partan, S. R., Fulmer, A. G., Gounard, M. a. M. & Redmond, J. E. 2010. Multimodal alarm behavior in urban and rural gray squirrels studied by means of observation and a mechanical robot. *Current Zoology,* 56**,** 313-326.

Partridge, B. L. & Pitcher, T. J. 1980. The sensory basis of fish schools - relative roles of lateral line and vision. *Journal of Comparative Physiology,* 135**,** 315-325.

Peuhkuri, N. 1997. Size-assortative shoaling in fish: The effect of oddity on foraging behaviour. *Animal Behaviour,* 54**,** 271-278.

Peuhkuri, N. 1998. Shoal composition, body size and foraging in sticklebacks. *Behavioral Ecology and Sociobiology,* 43**,** 333-337.

Pigliucci, M. 2001. *Syntheses in ecology and evolution. Phenotypic plasticity: Beyond nature and nuture*, Johns Hopkins University Press, 2715 N. Charles St., Baltimore, MD, 212184319, USA; Johns Hopkins University Press, London, UK.

Pitcher, T. J. 1993. *The behaviour of teleost fishes,* London, Chapman & Hall.

Pitcher, T. J. & Parrish, J. K. 1993. *Functions of shoaling behaviour in teleosts*, Chapman and Hall Ltd., 2-6 Boundary Row, London SE1 8HN, England; Chapman and Hall, Inc., 29 West 35th Street, New York, New York, USA.

Popper, A. N. & Hastings, M. C. 2009. The effects of anthropogenic sources of sound on fishes. *J Fish Biol,* 75**,** 455-89.

Radakov, D. V. 1973. Schooling in the ecology of fish. *Haisted Press, New York*.

Rands, S. A., Cowlishaw, G., Pettifor, R. A., Rowcliffe, J. M. & Johnstone, R. A. 2003. Spontaneous emergence of leaders and followers in foraging pairs. *Nature,* 423**,** 432-434.

Rangeley, R. W. & Kramer, D. L. 1998. Density-dependent antipredator tactics and habitat selection in juvenile pollock. *Ecology,* 79**,** 943-952.

Ranta, E. & Lindstrom, K. 1990. Assortative schooling in 3-spined sticklebacks. *Annales Zoologici Fennici,* 27**,** 67-75.

Ranta, E., Lindstrom, K. & Peuhkuri, N. 1992. Size matters when 3-spined sticklebacks go to school. *Animal Behaviour,* 43**,** 160-162.

Ranta, E., Peuhkuri, N. & Laurila, A. 1994. A theoretical exploration of antipredatory and foraging factors promoting phenotype-assorted fish schools. *Ecoscience,* 1**,** 99-106.

Rauschecker, J. P. 1995. Compensatory plasticity and sensory substitution in the cerebral-cortex. *Trends in Neurosciences,* 18**,** 36-43.

Rauschecker, J. P. & Kniepert, U. 1994. Auditory localization behavior in visually deprived cats. *European Journal of Neuroscience,* 6**,** 149-160.

Reale, D., Reader, S. M., Sol, D., Mcdougall, P. T. & Dingemanse, N. J. 2007. Integrating animal temperament within ecology and evolution. *Biol Rev Camb Philos Soc,* 82**,** 291-318.
Reebs, S. G. 2000. Can a minority of informed leaders determine the foraging movements of a fish shoal? *Animal Behaviour,* 59**,** 403-409.
Reid, S. M., Fox, M. G. & Whillans, T. H. 1999. Influence of turbidity on piscivory in largemouth bass (micropterus salmoides). *Canadian Journal of Fisheries and Aquatic Sciences,* 56**,** 1362-1369.
Reluga, T. C. & Viscido, S. 2005. Simulated evolution of selfish herd behavior. *J Theor Biol,* 234**,** 213-25.
Richter, B. D., Braun, D. P., Mendelson, M. A. & Master, L. L. 1997. Threats to imperiled freshwater fauna. *Conservation Biology,* 11**,** 1081-1093.
Roder, B., Teder-Salejarvi, W., Sterr, A., Rosler, F., Hillyard, S. A. & Neville, H. J. 1999. Improved auditory spatial tuning in blind humans. *Nature,* 400**,** 162-166.
Rodgers, G. M., Downing, B. & Morrell, L. J. 2015. Prey body size mediates the predation risk associated with being "odd". *Behavioral Ecology,* 26**,** 242-246.
Rodgers, G. M., Gladman, N. W., Corless, H. F. & Morrell, L. J. 2013a. Costs of colour change in fish: Food intake and behavioural decisions. *Journal of Experimental Biology,* 216**,** 2760-2767.
Rodgers, G. M., Kimbell, H. & Morrell, L. J. 2013b. Mixed-phenotype grouping: The interaction between oddity and crypsis. *Oecologia,* 172**,** 59-68.
Rodgers, G. M., Ward, J. R., Askwith, B. & Morrell, L. J. 2011. Balancing the dilution and oddity effects: Decisions depend on body size. *PLoS One,* 6**,** e14819.
Rosenzweig, M. L. 1974. On the optimal aboveground activity of bannertail kangaroo rats. *Journal of Mammalogy,* 55**,** 193-199.
Rowe, D. K., Dean, T. L., Williams, E. & Smith, J. P. 2003. Effects of turbidity on the ability of juvenile rainbow trout, oncorhynchus mykiss, to feed on limnetic and benthic prey in laboratory tanks. *New Zealand Journal of Marine and Freshwater Research,* 37**,** 45-52.
Ryer, C. H. & Olla, B. L. 1998. Effect of light on juvenile walleye pollock shoaling and their interaction with predators. *Marine Ecology Progress Series,* 167**,** 215-226.
Ryugo, D. K., Ryugo, R., Globus, A. & Killackey, H. P. 1975. Increased spine density in auditory-cortex following visual or somatic deafferentation. *Brain Research,* 90**,** 143-146.
Salvanes, A. G. V., Moberg, O., Ebbesson, L. O. E., Nilsen, T. O., Jensen, K. H. & Braithwaite, V. A. 2013. Environmental enrichment promotes neural plasticity and cognitive ability in fish. *Proceedings of the Royal Society B-Biological Sciences,* 280.
Scholz, N. L., Truelove, N. K., French, B. L., Berejikian, B. A., Quinn, T. P., Casillas, E. & Collier, T. K. 2000. Diazinon disrupts antipredator and homing behaviors in chinook salmon (oncorhynchus tshawytscha). *Canadian Journal of Fisheries and Aquatic Sciences,* 57**,** 1911-1918.
Schwartz, M. W., Iverson, L. R., Prasad, A. M., Matthews, S. N. & O'connor, R. J. 2006. Predicting extinctions as a result of climate change. *Ecology,* 87**,** 1611-1615.
Scott, G. R., Sloman, K. A., Rouleau, C. & Wood, C. M. 2003. Cadmium disrupts behavioural and physiological responses to alarm substance in juvenile rainbow trout (oncorhynchus mykiss). *Journal of Experimental Biology,* 206**,** 1779-1790.
Seehausen, O., Terai, Y., Magalhaes, I. S., Carleton, K. L., Mrosso, H. D., Miyagi, R., Van Der Sluijs, I., Schneider, M. V., Maan, M. E., Tachida, H., Imai, H. & Okada, N. 2008. Speciation through sensory drive in cichlid fish. *Nature,* 455**,** 620-6.
Seehausen, O., Vanalphen, J. J. M. & Witte, F. 1997. Cichlid fish diversity threatened by eutrophication that curbs sexual selection. *Science,* 277**,** 1808-1811.
Seghers, B. H. 1974a. Geographic variation in responses of guppies (poecilia-reticulata) to aerial predators. *Oecologia,* 14**,** 93-98.
Seghers, B. H. 1974b. Schooling behavior in guppy (poecilia-reticulata) - evolutionary response to predation. *Evolution,* 28**,** 486-489.
Shaw, E. 1978. Schooling fishes. *American Scientist,* 66**,** 166-175.

Shoup, D. E. & Wahl, D. H. 2009. The effects of turbidity on prey selection by piscivorous largemouth bass. *Transactions of the American Fisheries Society,* 138**,** 1018-1027.
Sih, A. 2013. Understanding variation in behavioural responses to human-induced rapid environmental change: A conceptual overview. *Animal Behaviour,* 85**,** 1077-1088.
Sih, A., Bell, A. & Johnson, J. C. 2004a. Behavioral syndromes: An ecological and evolutionary overview. *Trends Ecol Evol,* 19**,** 372-8.
Sih, A., Bell, A., Johnson, J. C. & Ziemba, R., E. 2004b. Behavioural syndromes. An integrative overview. *The Quarterly review of biology,* 79**,** 242-277.
Sih, A., Ferrari, M. C. O. & Harris, D. J. 2011. Evolution and behavioural responses to human-induced rapid environmental change. *Evolutionary Applications,* 4**,** 367-387.
Slabbekoorn, H., Bouton, N., Van Opzeeland, I., Coers, A., Ten Cate, C. & Popper, A. N. 2010. A noisy spring: The impact of globally rising underwater sound levels on fish. *Trends Ecol Evol,* 25**,** 419-27.
Slabbekoorn, H. & Peet, M. 2003. Ecology: Birds sing at a higher pitch in urban noise - great tits hit the high notes to ensure that their mating calls are heard above the city's din. *Nature,* 424**,** 267-267.
Smith, B. R. & Blumstein, D. T. 2008. Fitness consequences of personality: A meta-analysis. *Behavioral Ecology,* 19**,** 448-455.
Smith, V. H., Joye, S. B. & Howarth, R. W. 2006. Eutrophication of freshwater and marine ecosystems. *Limnology and Oceanography,* 51**,** 351-355.
Sneddon, L. U. 2003. The bold and the shy: Individual differences in rainbow trout. *Journal of Fish Biology,* 62**,** 971-975.
Snickars, M., Sandstrom, A. & Mattila, J. 2004. Antipredator behaviour of 0+ year perca fluviatilis: Effect of vegetation density and turbidity. *Journal of Fish Biology,* 65**,** 1604-1613.
Sohel, S. & Lindström, K. 2015. Algal turbidity reduces risk assessment ability of the three-spined stickleback. *Ethology,* 121**,** 548-555.
Stamps, J. 2003. Behavioural processes affecting development: Tinbergen's fourth question comes of age. *Animal Behaviour,* 66**,** 1-13.
Stearns, S. C. 1989. The evolutionary significance of phenotypic plasticity - phenotypic sources of variation among organisms can be described by developmental switches and reaction norms. *Bioscience,* 39**,** 436-445.
Stewart, W. J., Cardenas, G. S. & Mchenry, M. J. 2013. Zebrafish larvae evade predators by sensing water flow. *Journal of Experimental Biology,* 216**,** 388-398.
Strand, D. A., Utne-Palm, A. C., Jakobsen, P. J., Braithwaite, V. A., Jensen, K. H. & Salvanes, A. G. V. 2010. Enrichment promotes learning in fish. *Marine Ecology Progress Series,* 412**,** 273-282.
Sundin, J., Berglund, A. & Rosenqvist, G. 2010. Turbidity hampers mate choice in a pipefish. *Ethology,* 116**,** 713-721.
Sutherland, A. B. & Meyer, J. L. 2007. Effects of increased suspended sediment on growth rate and gill condition of two southern appalachian minnows. *Environmental Biology of Fishes,* 80**,** 389-403.
Svensson, P. A., Barber, I. & Forsgren, E. 2000. Shoaling behaviour of the two-spotted goby. *Journal of Fish Biology,* 56**,** 1477-1487.
Swanbrow Becker, L. J., Gabor, C. R. & Koenig, W. 2012. Effects of turbidity and visual vs. Chemical cues on anti-predator response in the endangered fountain darter (etheostoma fonticola). *Ethology,* 118**,** 994-1000.
Szyf, M., Weaver, I. & Meaney, M. 2007. Maternal care, the epigenome and phenotypic differences in behavior. *Reproductive Toxicology,* 24**,** 9-19.
Templeton, J. J. & Giraldeau, L. A. 1996. Vicarious sampling: The use of personal and public information by starlings foraging in a simple patchy environment. *Behavioral Ecology and Sociobiology,* 38**,** 105-114.

Theodorakis, C. W. 1989. Size segregation and the effects of oddity on predation risk in minnow schools. *Animal Behaviour,* 38**,** 496-502.
Thomas, C. D. 2011. Translocation of species, climate change, and the end of trying to recreate past ecological communities. *Trends in Ecology & Evolution,* 26**,** 216-221.
Thünken, T., Eigster, M. & Frommen, J. G. 2014. Context-dependent group size preferences in large shoals of three-spined sticklebacks. *Animal Behaviour,* 90**,** 205-210.
Tollrian, R. 1990. Predator-induced helmet formation in daphnia-cucullata (sars). *Archiv Fur Hydrobiologie,* 119**,** 191-196.
Tosh, C., Krause, J. & Ruxton, G. 2009. Basic features, conjunctive searches, and the confusion effect in predator-prey interactions. *Behavioral Ecology and Sociobiology,* 63**,** 473-475.
Treherne, J. E. & Foster, W. A. 1981. Group transmission of predator avoidance-behavior in a marine insect - the trafalgar effect. *Animal Behaviour,* 29**,** 911-917.
Treisman, M. 1975. Predation and evolution of gregariousness .2. Economic model for predator-prey interaction. *Animal Behaviour,* 23**,** 801-825.
Tremmel, M. & Müller, C. 2013. Insect personality depends on environmental conditions. *Behavioral Ecology,* 24**,** 386-392.
Tuomainen, U. & Candolin, U. 2011. Behavioural responses to human-induced environmental change. *Biological Reviews,* 86**,** 640-657.
Turesson, H. & Bronmark, C. 2007. Predator-prey encounter rates in freshwater piscivores: Effects of prey density and water transparency. *Oecologia,* 153**,** 281-290.
Turner, G. F. & Pitcher, T. J. 1986. Attack abatement - a model for group protection by combined avoidance and dilution. *American Naturalist,* 128**,** 228-240.
Utne-Palm, A. C. 2002. Visual feeding of fish in a turbid environment: Physical and behavioural aspects. *Marine and Freshwater Behaviour and Physiology,* 35**,** 111-128.
Utne, A. C. W. 1997. The effect of turbidity and illumination on the reaction distance and search time of the marine planktivore gobiusculus flavescens. *Journal of Fish Biology,* 50**,** 926-938.
Van De Meutter, F., De Meester, L. & Stoks, R. 2005. Water turbidity affects predator-prey interactions in a fish-damselfly system. *Oecologia,* 144**,** 327-36.
Van Der Sluijs, I., Gray, S. M., Amorim, M. C. P., Barber, I., Candolin, U., Hendry, A. P., Krahe, R., Maan, M. E., Utne-Palm, A. C., Wagner, H. J. & Wong, B. B. M. 2011. Communication in troubled waters: Responses of fish communication systems to changing environments. *Evolutionary Ecology,* 25**,** 623-640.
Vinyard, G. L. & Yuan, A. C. 1996. Effects of turbidity on feeding rates of lahontan cutthroat trout (oncorhynchus clarki henshawi) and lahontan redside shiner (richardsonius egregius). *Great Basin Naturalist,* 56**,** 157-161.
Viscido, S., Miller, M. & Wethey, D. S. 2002. The dilemma of the selfish herd. The search for a realistic movement rule. *Journal of Theoretical Biology***,** 183-194.
Viscido, S. V., Miller, M. & Wethey, D. S. 2001. The response of a selfish herd to an attack from outside the group perimeter. *Journal of Theoretical Biology,* 208**,** 315-328.
Viscido, S. V., Parrish, J. K. & Grünbaum, D. 2005. The effect of population size and number of influential neighbors on the emergent properties of fish schools. *Ecological Modelling,* 183**,** 347-363.
Viscido, S. V. & Wethey, D. S. 2002. Quantitative analysis of fiddler crab flock movement: Evidence for 'selfish herd' behaviour. *Animal Behaviour,* 63**,** 735-741.
Von Hippel, F. A. 2010. *Tinbergen's legacy in behaviour: Sixty years of landmark stickleback papers,* Leiden, Netherlands, Koninklijke Brill.
Warburton, K. 2003. Learning of foraging skills by fish. *Fish and Fisheries,* 4**,** 203-215.
Ward, A. J., Duff, A. J., Horsfall, J. S. & Currie, S. 2008. Scents and scents-ability: Pollution disrupts chemical social recognition and shoaling in fish. *Proc Biol Sci,* 275**,** 101-5.
Ward, A. J. W., Axford, S. & Krause, J. 2002. Mixed-species shoaling in fish: The sensory mechanisms and costs of shoal choice. *Behavioral Ecology and Sociobiology,* 52**,** 182-187.

Ward, A. J. W. & Currie, S. 2013. Shoaling fish can size-assort by chemical cues alone. *Behavioral Ecology and Sociobiology,* 67**,** 667-673.
Ward, A. J. W. & Krause, J. 2001. Body length assortative shoaling in the european minnow, phoxinus phoxinus. *Animal Behaviour,* 62**,** 617-621.
Ward, A. J. W., Thomas, P., Hart, P. J. B. & Krause, J. 2004. Correlates of boldness in three-spined sticklebacks (gasterosteus aculeatus). *Behavioral Ecology and Sociobiology,* 55**,** 561-568.
Watt, P. J., Nottingham, S. F. & Young, S. 1997. Toad tadpole aggregation behaviour: Evidence for a predator avoidance function. *Animal Behaviour,* 54**,** 865-872.
Webster, M., Ward, A. & Hart, P. 2009. Individual boldness affects interspecific interactions in sticklebacks. *Behavioral Ecology and Sociobiology,* 63**,** 511-520.
Webster, M. M., Atton, N., Hart, P. J. B. & Ward, A. J. W. 2011. Habitat-specific morphological variation among threespine sticklebacks (gasterosteus aculeatus) within a drainage basin. *Plos One,* 6**,** 10.
Webster, M. M., Atton, N., Ward, A. J. W. & Hart, P. J. B. 2007. Turbidity and foraging rate in threespine sticklebacks: The importance of visual and chemical prey cues. *Behaviour,* 144**,** 1347-1360.
Weimerskirch, H., Martin, J., Clerquin, Y., Alexandre, P. & Jiraskova, S. 2001. Energy saving in flight formation - pelicans flying in a 'v' can glide for extended periods using the other birds' air streams. *Nature,* 413**,** 697-698.
West-Eberhard, M. J. 2003. *Developmental plasticity and evolution,* New York, Oxford University Press.
Wetterer, J. K. & Bishop, C. J. 1985. Planktivore prey selection - the reactive field volume model vs the apparent size model. *Ecology,* 66**,** 457-464.
Whitton, T. A., Jenkins, S. R., Richardson, C. A. & Hiddink, J. G. 2012. Aggregated prey and predation rates: Juvenile shore crabs (carcinus maenas) foraging on post-larval cockles (cerastoderma edule). *Journal of Experimental Marine Biology and Ecology,* 432**,** 29-36.
Wilson, D. S. 1998. Adaptive individual differences within single populations. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences,* 353**,** 199-205.
Wilson, D. S., Coleman, K., Clark, A. B. & Biederman, L. 1993. Shy bold continuum in pumpkinseed sunfish (lepomis-gibbosus) - an ecological study of a psychological trait. *Journal of Comparative Psychology,* 107**,** 250-260.
Wilson, M. L. & Weissburg, M. J. 2012. Temporal and spatial sampling strategies maintain tracking success of whelks to prey patches of differing distributions. *Animal Behaviour,* 84**,** 1323-1330.
Wong, B. B. M., Candolin, U. & Lindstrom, K. 2007. Environmental deterioration compromises socially enforced signals of male quality in three-spined sticklebacks. *American Naturalist,* 170**,** 184-189.
Wood, W. E. & Yezerinac, S. M. 2006. Song sparrow (melospiza melodia) song varies with urban noise. *Auk,* 123**,** 650-659.
Wrona, F. J. & Dixon, R. W. J. 1991. Group-size and predation risk - a field analysis of encounter and dilution effects. *American Naturalist,* 137**,** 186-201.
Zambonino-Infante, J. L., Claireaux, G., Ernande, B., Jolivet, A., Quazuguel, P., Severe, A., Huelvan, C. & Mazurais, D. 2013. Hypoxia tolerance of common sole juveniles depends on dietary regime and temperature at the larval stage: Evidence for environmental conditioning. *Proceedings of the Royal Society B-Biological Sciences,* 280**,** 9.
Zhang, T. Y. & Meaney, M. J. 2010. Epigenetics and the environmental regulation of the genome and its function. *Annual review of psychology.* Palo Alto: Annual Reviews.