University of Southampton Research Repository

University of Southampton

Faculty of Engineering and Physical Sciences

School of Engineering

Fish Behavioural Response to Static Visual Cues

by

James Miles

ORCID ID: 0000-0001-6448-6343

Thesis for the degree of Doctor of Philosophy

September 2021

University of Southampton

Abstract

Faculty of Engineering and Physical Sciences

School of Engineering

Doctor of Philosophy

Fish Behavioural Response to Static Visual Cues

by
James Miles

Fish have been shown to associate with visual cues, whereas flying animals tend to avoid areas of visual complexity, but the reasons for this difference in reaction remain unclear. For fish, visual cues provide reliable information on their position in space and help them align with hydrodynamic flow. Vision is also vital to maintain cohesion in schools and for communication. This thesis aims to improve fundamental knowledge regarding how European freshwater fish behave in relation to static abiotic visual cues (patterns on surfaces) by answering two main research questions; how (1) flowing water, and (2) group size, affects the response of fish to visual cues?

Affiliation with visual cues was greater in flowing than static water. It is hypothesised that the higher level of association with visual cues in flow provides fish with reliable reference points, which may improve swimming performance (the 'Station Holding Hypothesis'). However, the lack of correlation between association with visual cues and flow velocity and the short duration of visits to the visual cues for both common minnow (*Phoxinus phoxinus*) and brown trout (*Salmo trutta*) implies that controlling swimming is not a primary reason for the observed behaviour. The association with visual cues continued under static water suggesting that visual cues may provide other benefits such as physical refuge from predators or an opportunity for crypsis.

Both individuals and groups of minnow exhibited a positive affiliation for the visual cues, travelling at a slower speed and spending more time closer to the striped walls. Individual fish tended to show a stronger association with visual cues when they came across them. However, larger groups responded to the presence of stripes more quickly due to enhanced detection efficiency and information transfer between group members. The greater association with the striped walls exhibited by individuals may reflect a greater dependence on environmental information or anti-predator behaviour (the 'Predator Refuge Hypothesis') when separate from a group.

Groups of minnow aligned more side-by-side when associating with visual cues in flowing water compared with a control in which visual cues were absent. However, the sub-structure (distance to nearest neighbour and polarisation) of groups was unaffected. Although the whole group responded by associating with visual cues, members continued to maintain cohesiveness and align with one another, presumably to benefit from energetically advantageous formations, suggesting that the equivalent benefits could not be gained by aligning with environmental visual cues.

The findings of this thesis enhance fundamental understanding of fish behavioural response to stimuli and demonstrate the importance of visual cues in dictating behaviour. This research may give rise to some useful applications, for example in fish guidance technology and improving animal welfare in aquaculture.

Keywords: *Optic flow, vision, rheotaxis, stimulus, minnow, trout.*

Table of Contents

Table of Tables

Table of Figures

Research Thesis: Declaration of Authorship

Name: James Miles

Title: *Fish Behavioural Response to Static Visual Cues*

I declare that this thesis and the work presented in it is my own and has been generated by me as the result of my own original research.

I confirm that:

1. This work was done wholly or mainly while in candidature for a research degree at this University;
2. Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
3. Where I have consulted the published work of others, this is always clearly attributed;
4. Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;
5. I have acknowledged all main sources of help;
6. Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;
7. Parts of this work have been published as:

Miles, J., Vowles, A.S. & Kemp, P. (2021). The response of common minnow (*Phoxinus phoxinus*) to visual cues under flowing and static water conditions. *Animal Behaviour*. https://doi.org/10.1016/j.anbehav.2021.07.004.

The accompanying data for this thesis can be found at https://doi.org/10.5258/SOTON/D2160

Signature: .... Date:..........................

Acknowledgements

Firstly, I would like to thank my two supervisors Prof. Paul Kemp and Dr Andrew Vowles for their guidance, knowledge and expertise throughout this research and assistance with getting these chapters ready for submission for journal publication.

Secondly, thank you to Dr Tory Tsuzaki for his guidance and assistance with experimental planning towards the beginning of my PhD and the rest of the members of the International Centre for Ecohydraulics Research (ICER) for their contributions of time and expertise when attaining fish in the field, covering experiments and valuable feedback in seminars.

While completing this thesis I have received help and support from a number of other close friends both in Southampton and elsewhere – thank you Jack for your analysis ideas! Also, thank you to Georgia for being an excellent proof-reader, for bouncing ideas off and spending countless hours covering my experiments. Finally, thanks to my parents and sister for their continued support over the last 30 years and without whom I would not be undertaking this PhD.

Thank you to Test Valley Trout Ltd. for their keen interest in the research being undertaken in addition to their assistance and provision of Brown Trout used in Chapter 5.

Funding for this research was provided by the Natural Environment Research Council (NERC) and the Engineering and Physical Sciences Research Council (EPSRC) under the United Kingdom Research and Innovation (UKRI) department under grant agreement number 1786331.

Definitions and Abbreviations

Organisms referred to throughout this thesis.

Families

COMMON NAME	LATIN NAME
Sturgeon	*Acipenseridae*
Locust/Grasshopper	*Acrididae*
Eel	*Anguillidae*
Honeybee (Genus)	*Apis*
Bumblebee (Genus)	*Bombus*
Pigeon	*Columbidae*
Carp	*Cyprinidae*
Catfish	*Pimelodidae*
Salmon	*Salmonidae*
Owl (Order)	*Strigiformes*
Hummingbird	*Trochilidae*

Species

COMMON NAME	LATIN NAME
Climbing perch	*Anabas testudineus*
European eel	*Anguilla anguilla*
Western honeybee	*Apis mellifera*
Mexican tetra	*Astyanax mexicanus*
Anna’s hummingbird	*Calypte anna*
Central stoneroller	*Campostoma anomalum*
Goldfish	Carassius auratus
Dwarf pufferfish	*Carinotetraodon travancoricus*
Three-lined cory	*Corydoras trilineatus*
Zebrafish	*Danio rerio*
Giant danio	*Devario aequipinnatus*
Northern pike	*Esox Lucius*
Elephantnose fish	*Gnathonemus petersii*
Red-nose tetra	*Hemigrammus bleheri*
Humans	*Homo sapiens*
Budgerigar	*Melopsittacus undulatus*
House fly	*Musca domestica*
Golden shiners	*Notemigonus crysoleucas*
Medaka / Japanese rice fish	*Oryzias latipes*
European perch	Perca fluviatilis
Harbour seal	*Phoca vitulina*
Common minnow	*Phoxinus phoxinus*
Atlantic salmon	*Salmo salar*
Brown trout	*Salmo trutta*
Common cuttlefish	*Sepia officinalis*

Acronyms

ANOVA	Analysis of variance
BL	Body length
GLMM	Generalised linear-mixed model
ICER	International Centre for Ecohydraulics Research
LM	Linear model
LMM	Linear-mixed model
R.P.M	Revolutions per minute
TS	Topic search
WOS	Web of Science

General Terms

Abiotic	Non-living physical or chemical features of the environment.
Acclimation	The physiological adjustment of an organism to new or changing environmental conditions.
Anthropogenic	Consequence of human activity.
Behavioural guidance system	A method to deter fish from or attract fish to certain locations – usually harmful to the fish (e.g. hydropower intakes) by using a stimulus that fish detect and respond to.
Benthic	Living on or in reference to the substrate/lowest level of a body of water.
Binocular vision	The ability to focus on an object using both eyes to aid depth of field perception and gauge distance to objects.
Biotic	Living or biological aspects of the environment.
Conservation	The protection of resources – e.g. preventing species extinctions.

Ecosystem functions / services	Natural processes that provide value to humanity through goods and services.
Fish pass / fishway	A structure designed to allow fish to pass upstream of an anthropogenic barrier to movement (e.g. dam or weir). Commonly a series of stepped pools that water flows down.
Fitness	The reproductive success of an individual. Quantified by the contribution of genes an individual makes to the next generation, relative to the contribution made by others in its population.
Gaze convergence	The inward rotation of both eyes toward each other to focus on an object and maintain single binocular vision.
Habitat connectivity	The degree to which the landscape facilitates the movement of species between suitable habitat patches.
Habitat fragmentation	The subdivision of habitat into smaller and more isolated patches. Can be caused by both natural processes and anthropogenic activity.
Habitat	The natural environment of an organism, which is an area that provides the resources (e.g. food, space) necessary for its existence.
Habituation	A reduction in the magnitude of a response to a stimulus after repeat encounter or exposure to it.
Hydraulics	The study of the mechanical properties of liquids.
Hydrodynamics	Subdiscipline of hydraulics that describes the flow of fluids.
Innate	In this thesis meaning - a natural behaviour that does not have to be learnt.
Lotic	Flowing water environments.
Migration	The movement of an organism from one area to another.
Mitigation	An action intended to reduce the adverse impact of an anthropogenic disturbance.

Definitions and Abbreviations

Motion parallax	Objects closer to an observer appear to move at a faster rate than those further away.
Optokinetic response	Tracking moving objects with the eye using a combination of a slow-phase and fast-phase eye movements. It is seen when objects move out of the field of vision and the eye then moves to focus on a new object or returns to its original position.
Overspills	A section of a dam or weir designed to release excess water at high flows.
Parr	A juvenile life-stage of anadromous salmonids resident in freshwater before transformation into smolts.
Rheotaxis	The orientation of fish to water currents.
School	A shoal of fish swimming in the same direction in a coordinated manner.
Screens	A physical barrier used to trap or deflect debris or living organisms from entering a particular area (e.g. hydropower turbine intake).
Shoal	A group of fish with some cohesion and social interaction between individuals.
Smolt	A juvenile life-stage of anadromous salmonids between parr and adult. They generally undergo downstream migration into saline environments.
Swimming capability	A measure of the efficiency or ability of fish to swim in flow. Can be quantified in many ways - for example, burst, sustained, critical and prolonged swim speeds are each measured differently. Capabilities differ between species because of varying swimming modes, methods of propulsion, and drag.
Top-down control	A system in which the apex (or top) predator controls the structure of the ecosystem.

Chapter 1 Introduction

Freshwater comprises approximately 0.01% of the total volume of water on earth (Stiassny, 1996; Lynch *et al.*, 2016), yet it provides habitat for approximately one third of all vertebrate species (Dudgeon *et al.*, 2006). Fish constitute the majority of such species, with roughly 40% of all fish utilising freshwater ecosystems (Helfman *et al.*, 2009; Lynch *et al.*, 2016). Fish perform important ecosystem functions including regulating food web dynamics, carbon flux and sediment processes. For example, northern pike (*Esox Lucius*), a large predatory fish, can have top-down control on the fish community composition (He and Kitchell, 1990); in lakes, fish supress zooplankton, indirectly increasing the flux of carbon between the atmosphere and the lake (Schindler, 1997); and in lotic environments benthic feeders, such as the stoneroller (*Campostoma anomalum*), re-suspend silt and organic matter into the current while foraging (Holmlund and Hammer, 1999).

Freshwater is arguably the most important resource to humankind (Dudgeon *et al.*, 2006). Ponds, lakes, streams, rivers and wetlands provide an extensive variety of goods and services to society, including agriculture, power production, industry, recreation and tourism (Lin *et al.*, 2021). Fisheries also provide livelihoods and a considerable source of protein for hundreds of millions of people in regions where employment and alternative sources of protein are scarce (Limburg and Waldman, 2009; McIntyre, Liermann and Revenga, 2016). Estimating the global economic, cultural, and social value of freshwater ecosystem services is non-trivial, and difficult to evaluate. However, Costanza *et al.* (1997) estimated that wetlands, lakes and rivers, which constitute 1.1% of the world’s surface area, provide ecosystem services worth over $4 trillion per year, equivalent to 20% of global ecosystem services at this time. The central role of fish in aquatic ecosystems makes them good indicators of ecosystem change (Lynch *et al.*, 2016). Ecosystems with high species richness exhibit increased resilience (Dowing and Leibold, 2010), emphasising the importance of conserving diverse freshwater fish communities (Lynch *et al.*, 2016).

Humans have been dramatically altering aquatic ecosystems by over-exploitation of fisheries, construction of barriers, extraction of water for irrigation and cooling systems, introduction of non-native species and the disposal of waste products into waterways for thousands of years (Limburg and Waldman, 2009). The human dependence on freshwater and the services it provides has resulted in every major city across the world being situated on or in proximity to a river (Silva, Serdoura and Pinto, 2006). The disproportionately large concentration of freshwater biodiversity covering just 1% of the earth’s surface means that anthropogenic disturbances to these ecosystems can have dramatic impacts. These effects are further exacerbated by the inherent connectivity of river systems, whereby a disturbance in one location can have detrimental effects throughout a catchment (Ward and Stanford, 1995). A major threat to biodiversity is habitat fragmentation caused by weirs and dams, which interrupt the natural flow of the river and prevent fish from reaching important habitats such as spawning grounds (Nilsson *et al.*, 2005). The hydropower industry generates more electricity than any other renewable source and is a major cause of large barriers in rivers (IEA, 2020). The industry continues to grow at roughly 3% per year generating roughly 4,333 Terawatt-hour (TWh) globally in 2019 (IEA, 2020). Their impact on fish varies hugely depending on the size and type of structure, how frequently they occur within a catchment, environmental conditions and the swimming capability and timing of fish migration. Ineffective movement of fish past a barrier can disrupt lifecycles, isolate populations and cause the accumulation of fish in reservoirs (Larinier & Travade, 2002), leaving them vulnerable to predation (Jepsen, Aarestrup, Økland, & Rasmussen, 1998; Jepsen, Pedersen, & Thorstad, 2000). High fish mortality occurs at hydropower and water intake facilities where fish pass over hazardous overspills, become impinged by screens, collide with turbine blades or experience high sheer stresses (Elder, Woodley, Weiland, & Strecker, 2016; Fu *et al.*, 2016; Larinier, 2008). Non-native species are also a significant threat to freshwater ecosystems. It is estimated that there are 134 invasive freshwater species in Great Britain (Gherardi *et al.*, 2008; Keller *et al.*, 2011), bringing threats of disease, competition for habitat and prey, ultimately causing a decline in the number of native species and in extreme cases triggering ecosystem shifts (Rahel, 2002).

The current decline in freshwater biodiversity is greater than any other major ecosystem on earth (Abell, 2002; Dudgeon *et al.*, 2006; Revenga *et al.*, 2010; Strayer and Dudgeon, 2010), with 65% of inland habitat classified as moderately or highly threatened by anthropogenic stressors (Vörösmarty *et al.*, 2010), and most wild fisheries near maximum sustainable exploitation levels (FAO, 2018). Climate change is predicted to alter precipitation and run-off patterns threatening to dramatically alter freshwater ecosystems (Woodward, Perkins and Brown, 2010). Habitat loss and climate change have caused European eel (*Anguilla anguilla*) populations to decline, recently leading to a ‘critically endangered’ reclassification (Birdlife International, 2012; Jacoby *et al.*, 2015), and although sturgeons (*Acipenseridae*) have succeeded for millions of years, their sensitivity to over-fishing and habitat degradation has endangered or eradicated many populations around the world (Birstein, Bemis and Waldman, 1997). As a result, it will become increasingly important to monitor the freshwater environment and mitigate for human disturbances.

The connectedness of river systems means that terrestrial conservation strategies are not appropriate for freshwater habitat unless borders are set around a full catchment (Naiman and

Latterell, 2005). Legislation is in place to protect, restore and manage watersheds such as the European Water Framework Directive (2000/60/EC) (EU, 2000), which focuses on the free movement of species throughout their natural range (Hering *et al.*, 2010). In addition, there is general legislation in place to protect priority species and maintain fishing productivity, for example the EU habitats directive (1992), the Salmon and Freshwater Fisheries Act (1975) (England and Wales) and the Endangered Species Act (1993). However, the complexity of socio-political boundaries often leads to the ownership, governance and management of a single catchment being divided (Dallimer and Strange, 2015).

Environmental conditions and species composition within freshwater ecosystems are dynamic, motivating fish to undertake migrations to improve feeding opportunities, escape predators and find appropriate spawning habitat. Salmon, which return from the sea to their native rivers in huge numbers to spawn, are the most renowned migratory species. However, it is now clear that a great number of species undergo extensive migrations (Baras and Lucas, 2001; Hutchings, 2002). For example, Amazonian catfish migrate the full extent of the Amazon, spawning at the headwaters in the Andes (Barthem and Goulding, 1997). The eggs and larvae then passively drift downstream to the estuary where juveniles begin a progressive life-long upstream migration covering thousands of kilometres (Barthem and Goulding, 1997). Many species also make short distance, lateral and vertical migrations to forage on floodplains or use deep water as a refuge, which can result in a substantial movement of biomass and nutrients from one region to another (Fausch *et al.*, 2002).

Other than actively transporting fish or removing barriers, fish passes are the only widespread mitigation measure to improve the permeability of barriers to fish movement in rivers. If used correctly, fish passes should allow species to use their complete natural habitat range, while excluding them from unfavourable habitat (e.g. large reservoirs). Fish passes can also lead to the unwanted dispersal of non-native species. However, in most scenarios, fish passes aim to be 100% effective for native species, allowing them to transcend a barrier with no additional energy expenditure or delay. Nevertheless, in practice, they generally perform poorly and are species specific, favouring stronger swimmers and economically important species such as salmonids (Stuart and Mallen-Cooper, 1999; Bunt, Castro-Santos and Haro, 2012). Fish use a variety of sensory cues (e.g. hydraulic) to navigate and find a safe route upstream. The main challenge when designing a fish pass is to achieve sufficient flow velocity at the entrance to attract fish, but appropriate velocities within the pass to allow fish to ascend successfully (Bunt, 2001). However, there is no legislation in place, other than broad guidelines, to regulate fish pass design or determine its location on a structure, both of which greatly affect the passage efficiency (Larinier, 1990; Brackley, 2016). Another priority is to reduce mortality from hydropower turbines and water intakes systems. Physical barriers to movement, such as screens, are often ineffective because they accumulate debris, reduce the efficiency of the turbines, and are unsuitable for deflecting the full range of species and life stages (Larinier *et al.*, 2004). As a result, behavioural guidance systems are a growing area of research that aim to deter fish from unfavourable areas or guide fish towards safer passages. To date, the use of bubble screens, strobe lighting, electric fields and acoustic deterrents have been implemented with limited success (Welton, Beaumont and Clarke, 2002; Noatch and Suski, 2012; Miehls, Johnson and Hrodey, 2017). Inherently, a comprehensive understanding of fish behaviour and swimming capability is central to designing effective fish passes and guidance systems (Clay, 2017).

Detailed knowledge of the swimming capabilities of many species are known from studies using swimming chambers. This knowledge is needed when designing fish passes with appropriate water velocities for target species. However, understanding the fundamental behavioural response of fish to stimuli is essential to truly understand how a fish will react to environmental and artificial cues (Enders, Gessel and Williams, 2009; Rice, Lancaster and Kemp, 2010). Fish response to sound, light and many different hydrodynamic stimuli has been tested for a variety of key species in controlled experiments (Ali, 1961; Taylor, Pegg and Chick, 2005; Russon, Kemp and Calles, 2010; Vowles and Kemp, 2012; Vowles *et al.*, 2014), as well as reactions and behaviours to river infrastructure such as culverts (Kemp and Williams, 2008) and weirs (Kemp *et al.*, 2006). This knowledge can then be used to help design more effective mitigation measures for human disturbances.

There is a huge variation in morphology and behaviour among fish. Despite this, all fish have the same two locomotory problems; i) propulsion and ii) manoeuvring and stabilising their position. A series of neuromasts in the head of a fish create a lateral line that can detect movement and flow of water around them (Baker and Montgomery, 1999). Since fish are often competing with flow, an innate behaviour called rheotaxis has evolved whereby fish orientate themselves with the direction of a current. This can either be positive, where they face the flow, or negative, where they avoid the flow. Generally, this behaviour is used by fish to hold the position in flowing water relative to a stationary background. Theoretically without a frame of reference, fish should be unable to discern their own movement within a current. For example, in a current a fish's swim speed may not match the speed over the ground and when drifting passively with the current almost no movement will be detected by the lateral line. Hence, it is widely believed that vision is used by fish to establish position and dictate movement (Arnold, 1974). For example, Dijkgraaf (1963), found that even when sensory feedback from the lateral line was blocked, swimming behaviour was unaffected. However, it is also known that rheotaxis is a complex and dynamic process that depends on a range of sensory cues, and changes in flow velocity or turbulence can vastly influence the reliance on each sense (Bak-Coleman, Paley & Coombs, 2013).

At the present time much of the fundamental research into fish behaviour is driven by the necessity to understand how fish respond to stimuli, such as sound or hydrodynamic flow, and apply the findings to solve real world problems. Fish guidance technology is one such field of research that requires detailed knowledge about baseline behavioural responses to stimuli to manipulate fish behaviour and develop mitigation strategies that reduce harmful impacts of river infrastructure and improve the efficiency of fish passes. Although the phototactic reaction to light (Bulkowski and Meade, 1983; Marchesan *et al.*, 2005) and the use of strobe lighting as a deterrent have been investigated for some species (Richards, Chipps and Brown, 2007; Noatch and Suski, 2012; Fjeldstad, Alfredsen and Boissy, 2014), visual cues have not been widely considered and this holds true for the wider literature on fish behaviour. Given that many species of fish have large, highly evolved eyes and they depend on vision as a source of sensory information to aid navigation and other fundamental behaviours such as rheotaxis, schooling, predator detection and feeding, the potential to influence fish movement using passive abiotic visual cues may become a valuable tool in the management and conservation of fish worldwide. Given the lack of literature focusing on fish behavioural response to visual cues and their potential to be considered as a possible guidance mechanism (illustrated in Section 2.2), there is a greater need for fundamental knowledge about how and why fish respond to visual cues.

How visual cues shape the movement and navigation of certain birds, insects and mammals has been studied; yet there is limited published research about the same adaptation in fish.

1.1 How animals navigate using sight

To move safely through their environment, organisms continuously monitor their surroundings while controlling their heading and speed to avoid collisions. Animals that are regularly in contact with a surface can use this to provide information about distance travelled and their speed of movement (Wolf, 2011). However, within a dynamic environment, such as air and water, wind and flow impede the ability for an animal to gain reliable information about its speed of motion relative to nearby objects. Gibson (1952) theorised that an animal should be able to extract all of the information required for safe locomotion by monitoring the way in which its surroundings transformed as the animal itself moved. This concept is termed ‘optic flow’, defined as;

> ‘The pattern of apparent motion of objects, surfaces, and edges in a visual scene caused by the relative motion between an observer and a scene’ (Kim & Park, 2017)

In the 1970s, David Marr described vision as the process of creating a three-dimensional description of the world from a two-dimensional visual array on the retina (Marr and Nishihara, 1978). Although not exclusive across the animal kingdom, sight is the predominant primary sense used to form a three-dimensional perception of the world and information on self-motion and perspective is essential for efficient visual processing (Kern *et al.*, 2005; Zeil, Boeddeker and Hemmi, 2008). For example, when an animal moves in response to a visual scene it alters the scene, creating a feedback loop. Consequently, coordination between vision and behaviour has shaped the design of the visual processing mechanisms and the underlying neural physiology (Zeil, Boeddeker and Hemmi, 2008).

An object can move in two fundamental ways. Movement in a straight line from one point in space to another is termed ‘translational motion’, whereas an object turning about its axis is termed ‘rotational motion’. Generally, motion is a combination of the two and each provides an animal with different information about their environment. When rotating, the visual scene moves in one direction so that any two points on the image move at the same angular speed, irrespective of distance to an object (Figure 1.1). Whereas when moving in a straight line, the image diverges from the focal point at the front, flows past the animal and converges directly behind. Translational movement results in motion parallax, where any two points of the image move at a velocity proportional to the speed of movement and inversely proportional to the distance to the object (Srinivasan, 2011a) (Figure 1.1). Consequently, nearby objects have higher image motion than those further away. This motion parallax is central to separating the two components of optic flow and provides more information about the surrounding environment (Rieger and Lawton, 1985; Warren, 1998; Duffy, 2000; Sunkara, DeAngelis and Angelaki, 2016).

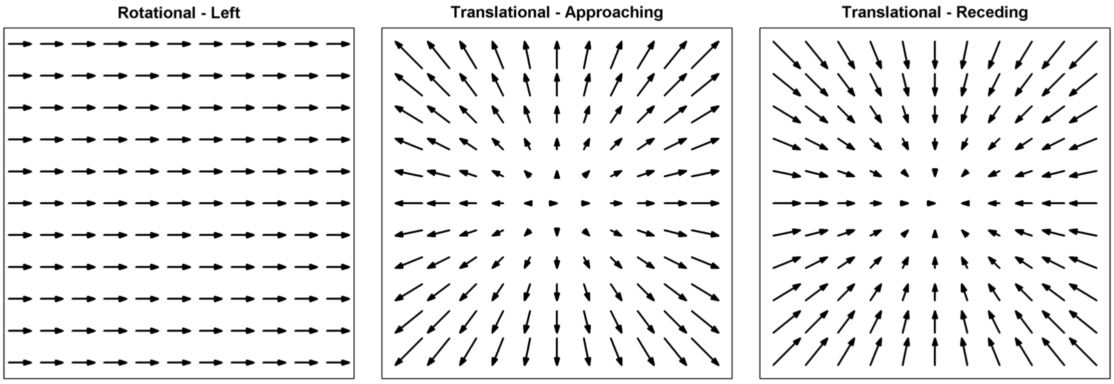

Figure 1.1. Diagram representing image motion during translational (centre & right) and rotational (left) optic flow. The length of the arrow represents the speed of image motion and the arrow dictates the direction. Created by author.

Image processing is not instantaneous. For example, during rapid movement of a camera or an animal's eye, the image changes throughout processing resulting in an inability to resolve details, called motion blur. The animal kingdom has evolved mechanisms to reduce the effect of motion blur and improve the ability to process visual information. When actively exploring an image, vertebrates perform saccadic eye or head movements, during which visual processing is blocked by the brain, resulting in a series of stabilised images with no noticeable gap in perception to the observer (Cassin *et al.*, 1990; Rucci and Poletti, 2015; Mostofi, Boi and Rucci, 2016). Furthermore, mechanisms to improve visual processing by minimising movement around the three rotational axes have shaped animal behaviour. For example, birds on a moving perch use neck, hip and leg muscles to keep the head and eyes stationary in space (Katzir *et al.*, 2001; Theunissen and Troje, 2017). Similarly, even in wind, hovering insects have an amazing ability to maintain a stable position (Kelber and Zeil, 1997). These movements compensate for the perceived motion of a visual scene, stabilising the image on the retina. This behaviour is termed the optomotor response and is commonly seen in insects, hummingbirds and fish (Rock and Smith, 1986). The motor circuit controlling these behaviours is generally regulated by closed-loop neural feedback (Buckley & Toyoizumi, 2018). This complex association between vision and movement means that little is known about the role of the primary visual cortex (Nelken, 2004; Carandini *et al.*, 2005) because much of what we know about neurobiology has been investigated under controlled conditions where visual mechanisms have been detached from behaviour. Hence, to fully appreciate how the nervous system computes behaviourally relevant information from a visual scene, it is important to conduct studies on freely behaving animals.

Using vision to navigate is common across the animal kingdom and optic flow has been studied across a broad range of species, from house flies (*Musca domestica*) to humans (*Homo sapiens*) and even in harbour seals (*Phoca vitulina*) (Geurten *et al.*, 2017) and the common cuttlefish (*Sepia officinalis*) (Helmer *et al.*, 2017). Those that contend with navigation in a dynamic fluid, such as air or water, with no physical contact with their surroundings are of particular interest because wind and hydrodynamic flow can provide a false impression of movement, hindering navigation. There are a variety of theories about how animals perceive and process the relative motion of objects to modify behaviour. Some of the leading theories and proven experimental methods for visual processing mechanisms and resulting navigational behaviour are described in the following section, with comparisons made between three relevant taxa; insects, birds and fish.

1.1.1 Insects

Given their small size and comparatively simple neural mechanisms and eye physiology, insects have an amazing ability to navigate, avoid obstacles and coordinate behaviour. As a result, there has been a clear bias towards studying their underlying mechanisms for visual perception and behaviour (Bhagavatula, Claudianos, Ibbotson, & Srinivasan, 2011; Galizia, Eisenhardt, & Giurfa, 2011; Zeil *et al.*, 2008). Insects have immobile eyes with fixed-focus optics (Srinivasan *et al.*, 1998). Therefore, distance estimation cannot be inferred from gaze convergence or by the refractive power necessary to focus on an object (Srinivasan *et al.*, 1998). Furthermore, in contrast to larger animals, the closeness of insect eyes means that binocular vision can only be used at extremely close range (Horridge, 1987; Srinivasan *et al.*, 1998). Although there is some disagreement about the underlying neural and behavioural mechanisms, it is believed that insects rely heavily on optic flow to view the world in three-dimensions and coordinate movement and navigation (Srinivasan, 2011a). It can explain insect behaviour when negotiating narrow gaps and controlling speed, flight path, altitude and landing manoeuvres. Studies have shown that a wide range of insects control their flight path by steering away from regions of strong optic flow and towards regions of weak optic flow, thus innately balancing the rate of image motion across both eyes (Hassenstein and Reichardt, 1956; Borst, 2009). Srinivasan *et al.* (1996), demonstrated this behaviour in honeybees (*Apis mellifera*) flying through a tunnel. When the walls were equally textured, bees flew directly down the centre of the tunnel (Figure 1.2). However, when one wall was made to move against the motion of the bee, the bee flew down the path where both eyes experienced equal image velocities, moving away from the strong optic flow (Srinivasan *et al.*, 1996).

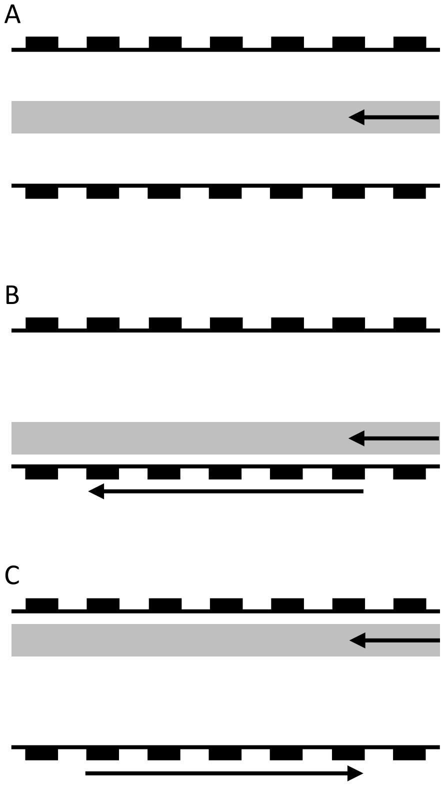

Figure 1.2. Flightpaths of honeybees through a patterned tunnel. Bees balanced the speed of image motion experienced by both eyes. Shaded areas are flight paths of bees. The arrows represent the direction of bee flight and the movement of the vertical lines on the tunnel walls. A) static walls, B) left wall with flight, and C) left wall opposite to flight direction [recreated from Srinivasan (1992)].

Insects regulate their flight speed by maintaining a constant image velocity across the eye. Studies on fruit flies (Fry *et al.*, 2009), bumblebees (Dyhr and Higgins, 2010) and honeybees (Srinivasan *et al.*, 1996) have all showed comparatively faster flight speeds when patterns on the two walls of a corridor were moved in the same direction as the insect. This mechanism causes insects to reduce their speed in cluttered environments or narrow passages, whereas in wide-open spaces such as fields they increase it (Barron and Srinivasan, 2006; Baird, Kornfeldt and Dacke, 2010). It has also been proposed that insects use image motion in the front part of the visual field to anticipate potential changes in flight speed (Baird, Kornfeldt and Dacke, 2010). Bees also regulate altitude by maintaining a constant speed of ground motion (Baird *et al.*, 2006). As expected, moving the floor in the same direction as the bees' flight caused them to lower their altitude and vice versa (Portelli, Ruffier and Franceschini, 2010). Bees tend to favour flight speeds and ground speeds with a similar rate of image motion (Baird *et al.*, 2006; Portelli, Ruffier and Franceschini, 2010), suggesting that the same mechanisms regulate flight path, speed and altitude. For example, in wide tunnels or open areas, bees have been shown to use one wall to control flight path, maintaining a constant distance from it (Serres *et al.*, 2008).

When approaching a surface at a constant speed, the angular speed of the surface edge increases exponentially. As honeybees approach a surface they use an elegant strategy maintaining the angular speed of the surface image on the eye, which in turn regulates their speed, causing them to decelerate as they approach the landing surface (Chahl, Srinivasan and Zhang, 2004). Assessing the rate of image expansion is also used as a strategy to help avoid collisions with nearby objects by estimating time to impact (Tammero and Dickinson, 2002).

Stationary locusts sway their head from side to side to observe motion parallax and estimate the distance to an object. In a series of well-designed experiments, a target was oscillated from side to side in synchrony with the peering movements of the insect (Sobel, 1990). When the target was oscillated in phase with the movement of the head, thereby decreasing the speed and amplitude of the object's image on the retina, the locust consistently overestimated the range to the target (Sobel, 1990). Similarly, wasps estimate distances to stationary objects by pivoting around them or producing a zig-zag flight, creating an image motion where distant objects move in the same direction as pivoting and those in the near-field against it (Voss and Zeil, 1998; Zeil, Boeddeker and Hemmi, 2008). While our understanding of simple insect navigation behaviours is fairly robust, the navigation behaviours of more complex vertebrates remain a challenge to study (Franz and Mallot, 2000).

1.1.2 Birds

Birds have large regions of their brains dedicated to visual processing (Frost, 2010; Wylie, Gutierrez-Ibanez and Iwaniuk, 2015) and a handful of behavioural studies demonstrated that birds use visual cues to navigate and control flight (Bhagavatula *et al.*, 2011). However, behaviours and neural mechanisms tend to be less stereotyped and there is a greater divergence of strategies compared with insects. Much of the literature is dedicated to optic flow but the results are relevant to understanding how animals respond to visual cues in general. When subjected to the same test as Srinivasan *et al.* (1996) performed on insects, budgerigars (*Melopsittacus undulatus*) used the same centring strategy, balancing the rate of image motion from vertical black and white lines on the walls (Figure 1.3; Bhagavatula *et al.*, 2011).

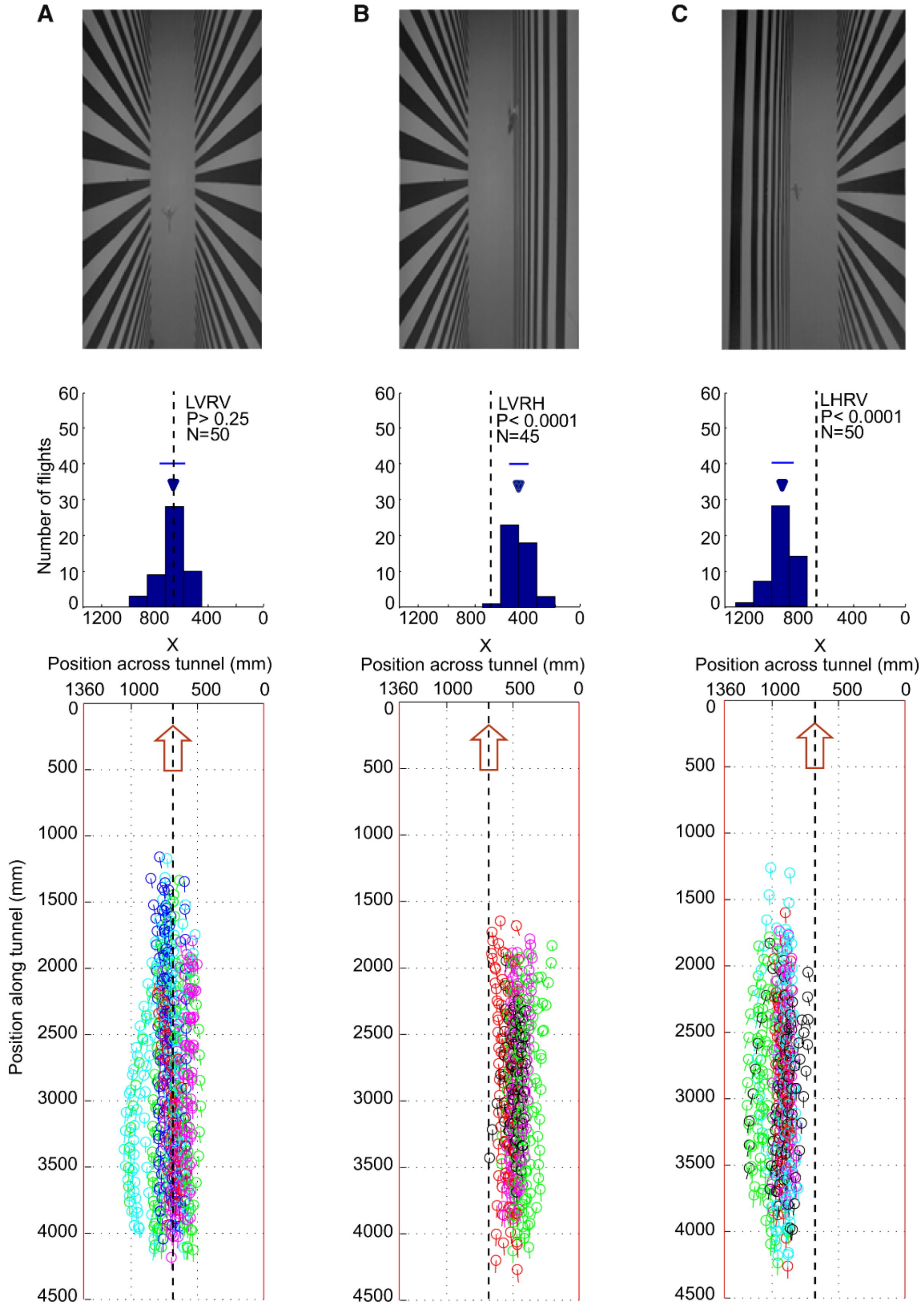

Figure 1.3. Flightpaths of budgerigars through a patterned tunnel with histogram showing the distribution of trajectories. Circles in lower plots represent the position of the bird's head and the lines the body orientations. Three treatments are presented: (A) Both walls presenting strong optic flow cues (vertical stripes). (B) Left wall presenting strong optic flow and right weak (horizontal stripes) and (C) Right wall presenting strong optic flow and left weak. Reprinted from Bhagavatula *et al.* (2011) with permission from Elsevier.

Conversely, hummingbirds (*Calypte anna*) ignored the patterns on the walls and continued to move down the midline of the tunnel, except when lines were at a specific spatial frequency (Dakin, Fellows and Altshuler, 2016). A possible explanation is visual fusion during image processing (when information is combined from multiple images) resulting in a loss of resolution at certain flight speeds (Dakin, Fellows and Altshuler, 2016). A series of other tests revealed that the hummingbirds' overall strategy was not to balance pattern velocity (such as seen in budgerigars) but to balance the rate of visual expansion from the focal point (Dakin, Fellows and Altshuler, 2016). It is believed that a hummingbird's capability to hover and move in multiple directions causes them to react to optic flow differently, relying on visual expansion rather than image motion to navigate during translational flight (Altshuler and Srinivasan, 2018). Interestingly, all species across the animal kingdom studied to date have enhanced gain in front to back visual motion, except hummingbirds, which show equal response to motion in all six major directions (Goller and Altshuler, 2014).

Budgerigars alter their flight speeds depending on visual cues. When image motion cues were generated in the same direction as flight, budgerigars increased their flight speed proportionately to the speed of optic flow (Schiffner and Srinivasan, 2015, 2016). However, a decrease in flight speed was not observed when optic flow was reversed, suggesting that birds do not solely rely on optic flow to control their flight speed (Schiffner and Srinivasan, 2015, 2016). Perhaps more interestingly, Schiffner and Srinivasan (2016) found that when budgerigars flew through a narrowing tunnel where the width decreased linearly from one end to the other, birds flew at two distinct speeds switching from a high speed (10 ms^{-1}) in the wider section to a lower speed (4-5 ms^{-1}) in the narrower section (Schiffner and Srinivasan, 2016). Likewise, when flying through an aperture, the speed of approach was irrespective of width (Vo, Schiffner and Srinivasan, 2016), again supporting the findings of Schiffner and Srinivasan (2016) that birds prefer two distinct speeds. The difference observed between birds and insects, which continuously adjust their speed, is thought to optimise energy expenditure and improve the ability to estimate the distance to objects using vision (Altshuler and Srinivasan, 2018). These findings do not provide conclusive evidence that optic flow is used by birds to estimate the width of the tunnel and regulate speed but the findings do indicate that visual cues do play a considerable role in flight control.

When horizontal lines were moved up or down on the walls of a tunnel, hummingbirds showed an optomotor response by altering their flight elevation accordingly (Dakin, Fellows and Altshuler, 2016). However, this has not yet been tested in other bird species. Observations of common swifts (*Apus apus*) in the wild found that they were able to adapt their heading to account for wind drift using visual cues at lower altitudes but failed to compensate at higher altitudes (Hedenström and Åkesson, 2017), where birds were unable to distinguish between objects on the ground. When landing, insects and birds have been shown to aim for high contrast visual features to guide them (Bhagavatula, Claudianos, Ibbotson, & Srinivasan, 2009; Lehrer, Srinivasan, & Zhang, 1990; Lehrer & Srinivasan, 1993). For example, bees and birds tended to aim for the edge of a coloured disk, even though a food source was placed at the centre (Bhagavatula *et al.*, 2009; Lehrer *et al.*, 1990).

Vision is also key to flight in flocks. The two main formations adopted are cluster formations, such as those seen in flocks of starlings, which are generally characterised by synchronised changes in direction, or line formations, for instance the V-formations of flying ducks. Computer models have been able to visually mimic organised flight using simple rules, generating hypotheses about the mechanisms that drive them (Bajec and Heppner, 2009; Ling *et al.*, 2019). In V-formations, the angle between birds means that geese are able to see all other geese in the formation despite having a considerable blind spot behind them and poor binocular vision in front (Heppner, 1985). Similarly, vision is the most obvious medium by which to transfer information in bird flocks to produce coordinated behaviour (Bajec and Heppner, 2009).

1.1.3 Fish

Fish eyes closely resemble those of birds and mammals and their physiology is well understood. In nearly all species, eyes are laterally located which limits the ability to use binocular vision. However, it is not known whether fish make use of motion parallax to estimate distances to objects or perceive the depth of field. Numerous studies have shown evidence or made use of the optomotor response of fish (Rock & Smith, 1986; Shaw, 1965), where fish move to stabilise their visual field on the eye. However, when travelling along a straight path, fish show a different response to visual cues compared with both insects and birds (Scholtyssek *et al.*, 2014). When zebrafish (*Danio rerio*) were exposed to visual cues that provided strong optic flow on one wall and weak optic flow on the other, fish showed preference for the stronger optic flow (Scholtyssek *et al.*, 2014) (Figure 1.4). This contradicts the behaviour of insects and birds, which balance the rate of image motion between their two eyes, flying closer to visual cues that provide weak optic flow (Srinivasan, 2011b). Scholtyssek *et al.* (2014), proposed that this response was to ensure the strongest possible visual signal in a medium where visibility can be limited and because the consequence of a collision with an object in water are less severe than when flying. In the same study, Scholtyssek *et al.* (2014) found that zebrafish did not regulate their swim speeds in response to changes in the strength of visual feedback, thus they did not maintain a constant rate of optic flow in the lateral visual field (Figure 1.4). Yet, in flow where hydrodynamic perception of speed is inhibited, vision is the main sense available for estimating swim speed. As a result, there are many unanswered questions regarding how fish regulate their speeds, navigate, and avoid colliding with obstacles in flowing water.

Studies investigating the role of vision in rheotaxis have been done by disabling vision or the lateral line. For example, Elder and Coombs (Elder and Coombs, 2015) found that Red-nose tetra (*Astyanax mexicanus*) were able to perform rheotaxis when either their lateral line or vision was blocked. It has also been shown that when giant danios (*Devario aequipinnatus*) are deprived of either sight or lateral-line senses, they exhibit subtle alterations in behaviour (Bak-Coleman *et al.*, 2013; Bak-Coleman and Coombs, 2014). Without vision the directionality of heading was reduced and fish exhibited sweeping behaviours to sense flow using the lateral-line. These results indicate that if deprived of one sense, fish use others to compensate, illustrating that navigation is a complex multisensory behaviour (Bak-Coleman *et al.*, 2013; Bak-Coleman and Coombs, 2014). Hence, stereotyped behaviours such as rheotaxis and the optomotor response are likely to be directly influenced by multiple stimuli and neural pathways.

Vision is essential in the collective behaviour of fish (Pita *et al.*, 2015). The eyes are located laterally so that fish can maintain visual contact with neighbours and schooling species tend to display marks that aid in visual information transfer between members such as horizontal stripes down the body or marks on the fins (Bone and Moore, 2008). In addition, it has been shown that without the use of vision schools become less cohesive (Partridge and Pitcher, 1980). The importance of vision in the coordination of group movement has been demonstrated but the effect of external environmental visual cues on the behaviour of groups of fish has been largely overlooked.

Fish reactions to visual cues can change throughout their lifetime. By using the optomotor response, Bak-Coleman *et al.* (2015), found that the reaction of zebrafish to visual cues changed throughout ontogenesis. They found that larval zebrafish swam in the direction of optic flow, whereas adults seemed to rely solely on non-visual cues, ignoring optic flow and showing no preference for orientating upstream (Bak-Coleman, Smith and Coombs, 2015). Therefore, it was concluded that the optomotor response may not be the dominant influence on rheotaxis. Veselov *et al.* (1998) tested the optomotor response in Atlantic salmon (*Salmo salar*) parr and smolts. They found that their response changed considerably throughout maturation from parr, which exhibited strong positive rheotactic behaviour, to smolts, which showed a weaker rheotaxis and optomotor response (Veselov *et al.*, 1998). This means that smolts are less likely to move synchronously with a visual scene to stabilise the image on the eye, thus, in a river, displacing them downstream with the flow. The transformation in behaviour is thought to promote downstream migration and pelagic shoaling behaviour (Veselov *et al.*, 1998).

The use of the optomotor response has been demonstrated in fish but why they show the opposite reaction to insects and birds when swimming through a tunnel lined with optic flow cues remains unclear. A summary of the finding of this review are presented in Table 1.1 as a comparison of visual guidance strategies between insects, birds and fish. The subsequent literature review quantifies the current literature concerning fish behavioural response to abiotic visual cues to display research trends and biases, and to identify knowledge gaps for the continuation of this research.

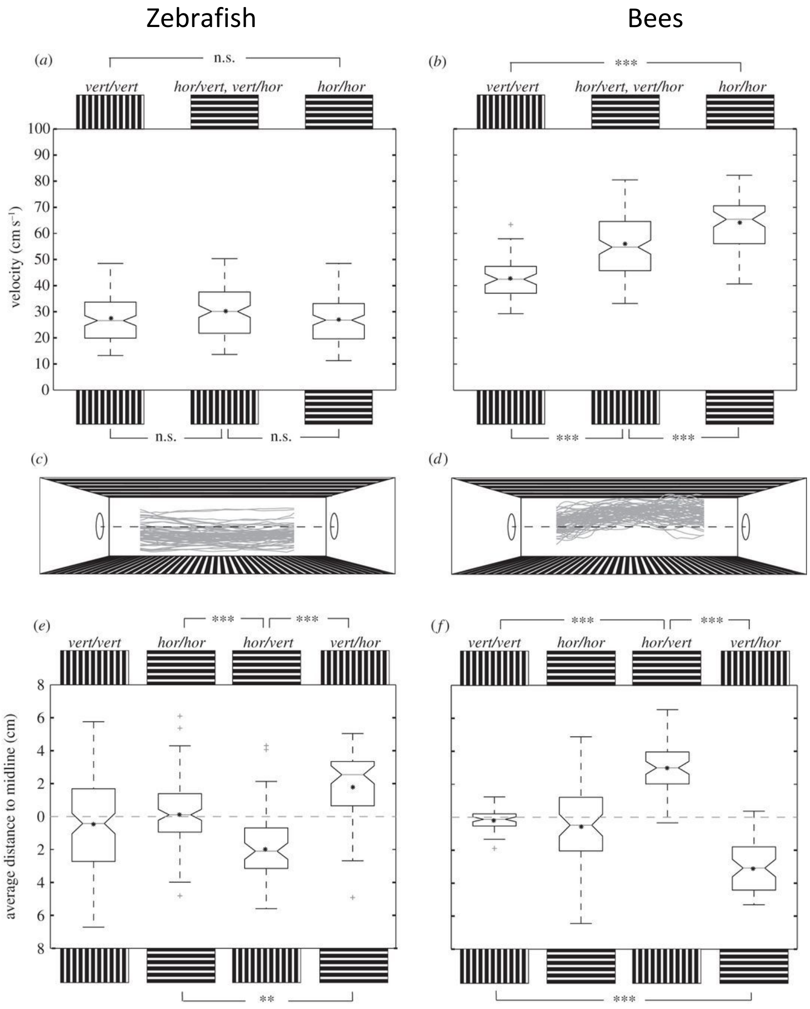

Figure 1.4. Paths of zebrafish (left) and bumblebees (right) through a patterned tunnel under four optic flow treatments. (a) and (b) represent the velocity of fish and bees through the tunnel, respectively. Grey lines in (c) and (d) represent individual paths and (e) and (f) indicate the mean distance from the tunnel midline with confidence intervals for each treatment group. Patterns top and bottom of each graph represent the relevant pattern for each wall. Asterisks represent the degree of significance between treatments. Modified from: Scholtyssek *et al.*, (2014) with permission from The Royal Society.

Table 1.1. Summary comparison of visual guidance strategies in insects, birds and fish.

	Insects	**Birds**	**Fish**
Optomotor response	Balance the rate of optic flow across both eyes[1] Steer away from strong and move towards weak optic flow (centring strategy)[1]	Budgerigars balance the rate of optic flow across both eyes[3] Hummingbirds balance the rate of visual expansion[4]	Attracted towards strong optic flow/visual cues[2] Optomotor response found in many species but varies in intensity Often rely more on other senses (e.g. mechanosensory)
Speed control	Maintain a constant image velocity across each eye[7] • Fly faster when exposed to a slow image velocity • Fly slower when exposed to a fast image velocity	Budgerigars increase flight speed if optic flow cues match the direction of flight but do not slow down if optic flow opposes flight direction[5] Two distinct speeds - one for cluttered environments and one for open environments[5]	Zebrafish do not seem to alter their speeds depending on their exposure to optic flow[2]
Height control	Maintain a constant ground image velocity • Fly lower if slow ground image velocity • Fly higher if fast ground image velocity	Use optic flow cues at low altitude, unable to do so at high altitude[6] Hummingbirds adjust their altitude with dynamic optic flow (horizontal stripes) on tunnel walls[4]	-
Landing	Maintain the angular speed of the surface image on the eye[8] Aim for high contrast visual features	Utilise rate of object expansion Aim for high contrast visual features	-
Range estimation	Make use of motion parallax Move head from side to side Employ zig-zag flight[9]	Have binocular vision Make use of motion parallax Move head from side to side	-

[1] *Srinivasen et al., 1992*
[2] *Schotyssek et al., 2014*
[3] *Bhagavatula et al., 2011*
[4] Dakin et al., 2014
[5] *Schiffner and Srinivasan, 2016*
[6] *Hedenström and Åkesson, 2017*
[7] *Srinivasan et al., 1996*
[8] Chahl et al., 2004
[9] *Voss and Zeil, 1998*

1.2 Preliminary research aims and objectives

The following aims and objectives were initially set to identify gaps in scientific knowledge for the development of further research in this field, before adapting a set of finalised aims and objectives stated at the end of the following Chapter.

The preliminary aim of this thesis is to:

1) improve fundamental knowledge of the behavioural response of European freshwater fish to static abiotic visual cues.

To meet this aim, the following objectives were initially established to be met in the subsequent chapter (Chapter 2):

I. Review existing literature on how organisms across the animal kingdom use visual cues to navigate and compare with current knowledge on fish responses to visual cues.
II. Perform a quantitative review of the literature regarding the behavioural response of fish to abiotic visual cues to highlight research trends and biases, and to identify knowledge gaps for the continuation of this research.

Chapter 2 Quantitative Literature Review: Fish response to visual cues

2.1 Introduction

Following on from the comparative review of how insects, birds and fish respond to visual cues, two quantitative reviews were carried out. Initially a literature search for papers relating to fish guidance technology was undertaken to determine the amount of research targeting the use of visual cues as a possible guidance mechanism (Section 2.2). This was then followed by a more extensive review of articles on the broader theme of fish and visual cues (Section 2.3). A systematic analysis of the published literature was undertaken to provide an overview of published literature in the topic area to determine past and recent trends, to align future research and methodologies to gaps in knowledge and to further define the objectives of this thesis. This approach is robust because it follows a replicable methodology and attempts to avoid unintended human bias. However, there are some biases documented in the literature regarding internet based search methods, for instance search engines provide very different results and some favour natural sciences and engineering over arts (Bar-Ilan, 2008; Mongeon and Paul-Hus, 2016).

2.2 Visual cues as a guidance mechanism

Fish guidance technologies rely on a fundamental understanding of fish responses to specific stimuli to mitigate against human disturbances and ultimately conserve species and ecosystems. Therefore, research on freshwater fish behaviour is often driven by the necessity for robust knowledge of fish responses to stimuli. A literature search for papers on fish guidance covering six major senses was undertaken to determine the extent to which literature targeted each form of sensory guidance. The search found a total of 153 papers on Web of Science (WOS) and 347 papers on Scopus relating to fish guidance (Table 2.1). Sub-searches within these results were conducted using the strings in Table 2.1 to reveal the proportion of articles relating to each of the six categories of fish senses. Duplicates between WOS and Scopus were not filtered out.

The results reveal that by far the most common field of research in fish guidance was hydraulic cues. This is unsurprising because fish responses to flow regimes and turbulence are fundamental to fish behaviour and have been studied for over a century (Liao, 2007). However, of the 500 studies only 32 (6.4% of the total) had terms relating to vision as keywords, the fewest of any category (Table 2.1). These results demonstrate that the use of visual cues as a guidance mechanism has been largely disregarded in the field of fish guidance without much investigation, highlighting an opportunity for further research.

Table 2.1. Search results for fish guidance research and sub-search for each sense stimuli (Subcategories) in Web of Science (WOS) and Scopus. Searches were carried out on 11/06/2021.

Topic	**Search string**	**WOS**	**Scopus**	**%**
Fish guidance	(‘fish guid*’ OR ‘fish barrier’ OR ‘fish deterrent’ OR ‘fish screen’)	153	347	
Subcategories				
Hydraulics	AND (flow OR hydro* OR hydraulic)	73	194	53.4
Electricity	AND (electr*)	18	61	15.8
Sound	AND (acoustic OR sound OR noise OR aud*)	21	43	12.8
Vision	**AND (vis* OR optic* OR sight)**	**14**	**18**	**6.4**
Physical	AND (physical)	12	27	7.8
Chemical	AND (chemo* OR chemical OR smell OR olfaction)	16	20	7.2

2.3 Fish behavioural response to visual cues

To summarise the literature regarding fish responses to visual cues and to ensure that this thesis contains original research a major quantitative literature review was conducted. A number of metrics were investigated to collate information about the types of studies being carried out with the aim of highlighting opportunities for further work.

Specifically, this quantitative review aims to meet the following objectives:

i) Identify broad trends within the literature of fish and visual cues
ii) Create a subset of highly relevant literature from the general search; specifically, experimental studies on fish behavioural response to abiotic visual cues.
iii) From the subset of literature:
    a. Identify trends, methodologies employed, and the type of visual cues that have been used in behavioural studies
    b. Collate information about species and group size studied
    c. Identify gaps and biases in the literature as the foundation for the development of future research

2.3.1 Literature search methodology

A search of peer-reviewed literature was conducted on Web of Science and Scopus on 18 June, 2018. A topic search (TS) was conducted using the following string of search terms on both platforms to identify publications that refer to *'optic flow' or 'visual cue' or optomotor* and *fish* within the title, abstract, author keywords and keywords (Table 2.2). Only articles written in English and published between 1960 and 2018 were retained. Although the syntax varies between search engines, searches were functionally identical. The findings of this literature review provided information on which to base the thesis aims and objectives. On 18 June 2021 a qualitive review of literature published since the first analysis in 2018 was undertaken. The abstracts and keywords were assessed to confirm that there were not any obvious dissimilarities in research trends or experimental methods compared with the initial review.

Table 2.2. Search strings used to on Web of Science (WOS) and Scopus to find relevant literature on fish and visual cues.

Search engine	Search string	Hits
WOS	(TS=((‘optic* flow’ OR ‘visual cue’ OR optomotor) AND (*fish))) AND LANGUAGE: (English) AND DOCUMENT TYPES: (Article)	241
Scopus	TITLE-ABS-KEY ((’optic* flow’ OR ‘visual cue’ OR optomotor) AND (*fish)) AND (LIMIT-TO (DOCTYPE , ’ar’)) AND (LIMIT-TO (LANGUAGE , ’English’))	721

An inventory of publications was produced including columns for ‘year of publication’, ‘authors’, ‘title’, ‘topic’ and ‘species studied’. Eight broad topics were developed to categorise each article at the authors discretion. This was done so that specific objectives could be met. Titles and abstracts were screened and categorised by topic or topics if they covered multiple themes. Due to the diversity of species studied, fish were grouped by Order. A subset of highly relevant articles was retained for more detailed analysis. As described in Objective [ii] articles were considered highly relevant if they specifically carried out an experiment to investigate a behavioural response of fish to a static or moving abiotic visual cue. To further group the literature, experimental methodology was recorded as either ‘field’, ‘flume’, ‘tank’, ‘tank (optomotor)’, ‘tank (maze)’ or ‘tank (choice)’. Whether the research was conducted on ‘individuals’ or ‘groups’ of fish was documented. Information about the visual cues was also collected using ten broad categories described in Table

2.4. If any required information was absent from the title or abstract the methodology was referred to.

2.3.2 Results and discussion

Broad trends in the literature

The initial literature search combining results from WOS and Scopus returned a total of 782 unique peer reviewed publications. After reviews and irrelevant papers were discarded 435 articles focusing on fish species and visual cues remained. The earliest study, undertaken using optomotor apparatus in 1965, found that the speed and direction of groups of fish matched the revolutions per minute (R.P.M) of the rotating optomotor drum displaying vertical black and white stripes (Shaw and Tucker, 1965). Until 1990 there was a slow increase in publications in this area (Figure 2.1). However, since then there has been almost a fourfold increase in the rate of publication, from 51 in the 1990's to more than 191 since 2010. Indeed, over half of all research in this area has been published since 2008 (52.4%) (Figure 2.1). Similar increases in the rate of publication have been seen across most disciplines, therefore Figure 2.2 provides additional information about how the rate of publications within the eight research topics have changed over time.

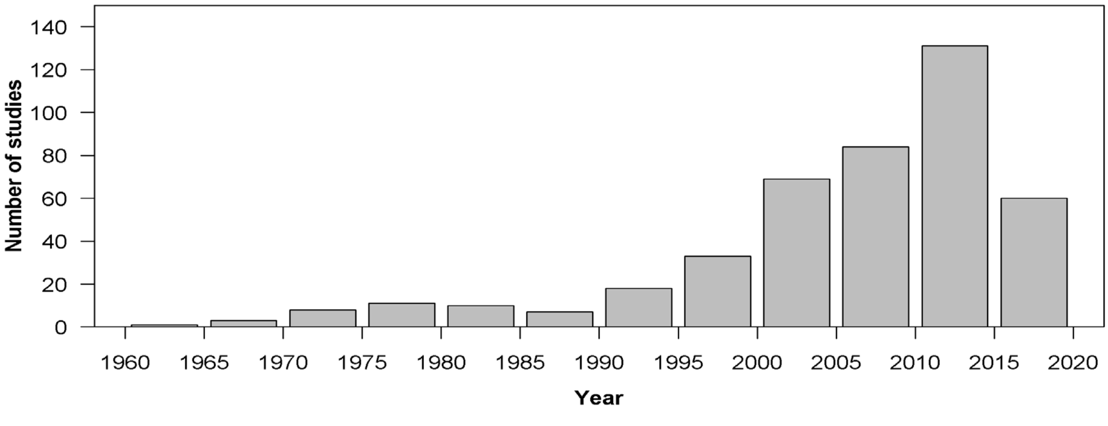

Figure 2.1. Number of peer reviewed research articles on topic: 'fish' and 'visual cues' by publication date. Data collated from WOS and Scopus on fish and visual cues to create an inventory of 435 research articles published between 1960 and 2018.

Publications covered a range of subject areas but were categorised into eight broad topics. The most studied topic was ecology/behaviour, making up 40% of all articles. This was closely followed by papers focusing on optometry and physiology with 103 and 95 papers, respectively. The following table briefly discusses the trends identified within each topic (Table 2.3).

The publication rate for all topics increased after 1990 but particularly for ecology/behavioural studies and more recently for learning studies (Figure 2.2). Comparatively modest increases in many of the other topics including social interactions, reproduction, optometry, health and anthropogenic impacts, have been seen since 2000. The increased awareness of the importance of conservation has probably driven the growth in studies in ecology/behaviour but there have been comparatively few studies focusing on man-made impacts on how fish use or respond to visual cues.

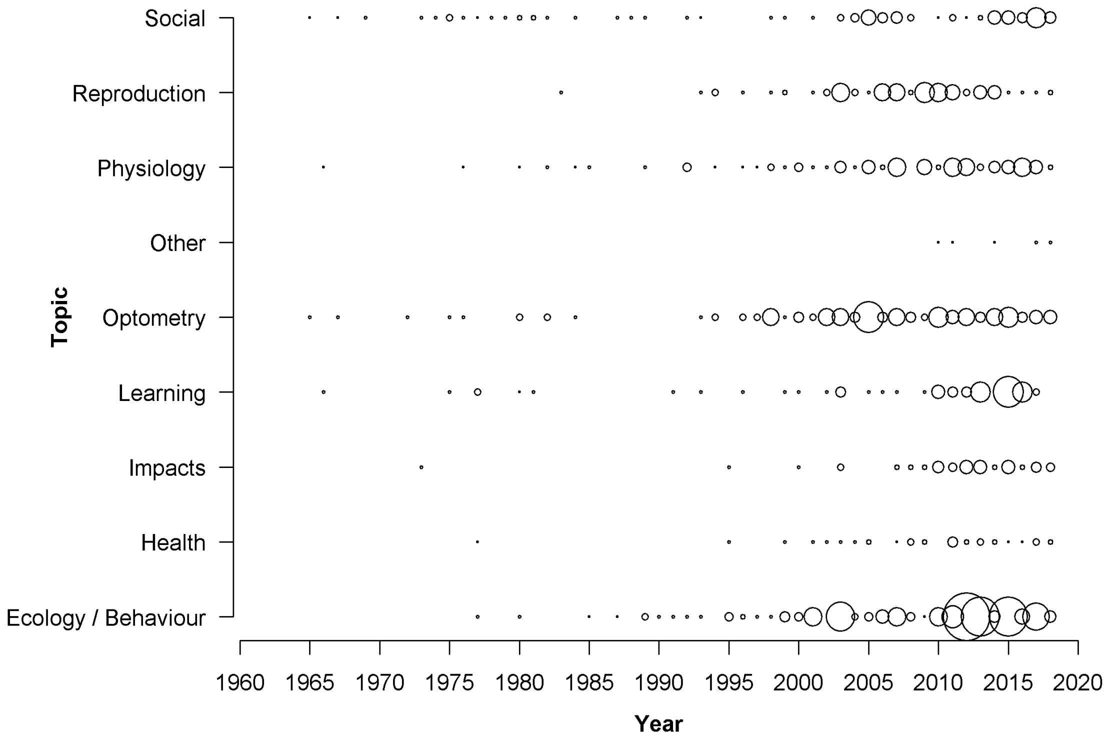

Figure 2.2. Publication year against topic for each paper from the full uncensored dataset consisting of 435 articles. Size of each point is proportional to the number of papers published on that topic in the given year. Articles may appear in more than one topic.

The majority of papers within the preliminary search tended to focus on response of fish to biotic visual cues (Table 2.4), for example, mate choice during sexual selection and conspecific or predator-prey recognition. Behavioural responses under light and dark conditions were also common. Patterns were used in roughly a fifth of all studies but almost all of these were within optomotor apparatus.

Table 2.3. Description of topics and trends noticed during the full literature search of 435 articles.

Topic	Count	Description / trends
Ecology / Behaviour	155	Many ecology papers studied predator-prey interactions, focusing on biotic visual cues, particularly how the response to chemical or visual cues interact when searching for prey or evading predators. Sensory perception studies were used to investigate which senses were most important for navigation. This was often achieved by inhibiting certain senses (e.g. visual vs chemical vs electro vs lateral line).
Optometry	103	All aspects of fish sight were studied. Specifically; motion detection, visual acuity, visual sensitivity and spectral sensitivity. The aims of many papers were to better understand visual processing and neural physiology.
Physiology	95	Physiological studies tended to examine either the structure of the eye, the neural networks associated with processing visual information or the innate link between vision and complex behaviours. Ablation of certain neurons or brain regions was a common method to discover which regions are most important to fish vision and behaviour.
Social	72	Social interactions and schooling behaviour - Biotic visual cues (e.g. conspecifics) constituted most of the literature on this topic. Abiotic visual cues were studied to a lesser extent, for example, the optomotor response to elicit shoaling or schooling behaviour.
Reproduction/ Development	66	How senses/vision develops during ontogenesis. Many studies focused primarily on fish larval stages. A large field of research focussed on how and where fish larvae choose to settle on coral reefs using detection of conspecifics and visual cues alone. How fish use biotic visual cues and displays during sexual selection constituted much of the remaining research.
Learning	52	Operant conditioning experiments were used to investigate whether visual cues could be associated with food rewards. Several experiments also studied the extent to which groups of fish are capable of social learning.
Anthropogenic impacts	45	Research focused on many topics. Most notably how changing environmental conditions affect the use of vision, such as increasing turbidity and climate change. A couple of studies were attempting to develop mitigation for anthropogenic structures.
Health	37	Toxicology and the effect of hormones or chemicals on zebrafish behaviour was commonly used to help understand neural mechanisms. Optomotor apparatus was the most common methodology to improve both human medical research and to assess impacts of environmental chemical pollution on fish behaviour.

Table 2.4. Types of visual cue used in experiments and the proportion of studies using each technique.

Visual cue	Description	No. studies	% of studies
Biotic	Any living visual cue (e.g. conspecifics, predators and prey)	253	58.2
Pattern	Any regular repeating visual cue – (almost always black and white stripes)	90	20.7
Light	Can refer to spectral sensitivities (colour), light intensity or light and dark	60	13.8
Landmarks	Novel or natural objects within a spatial learning task	23	5.3
Association	Operant conditioning or a trained response to a coloured target, a novel or natural object or a predator/conspecific cue.	10	2.3
Colour	Coloured targets, panels or light	9	2.1
Habitat	General habitat complexity	9	2.1
Fishing gear	Pelagic trawling nets	7	1.6
Novel objects	For association, detection or discrimination tasks	4	0.9
Bubbles	Walls of rising air bubbles	1	0.2

2.3.2.1 Subset of relevant literature

A total of 98 studies that carried out experiments to investigate a behavioural response of fish to a static or moving abiotic visual cues were retained for more detailed analysis. Studies whose aim was not primarily to improve knowledge of animal behaviour (e.g. toxicology, health science or understanding neural physiology) were removed but those assessing the visual capability of fish by using a behavioural response to a visual cue (e.g. the optomotor response) were retained. Spatial learning and fish schooling studies were retained if abiotic visual cues were used. The following graph and table display the distribution of topics within the subset (Figure 2.3; Table 2.5). A total of ten research papers were retained across the categories of health, impacts, and reproduction (Figure 2.3).

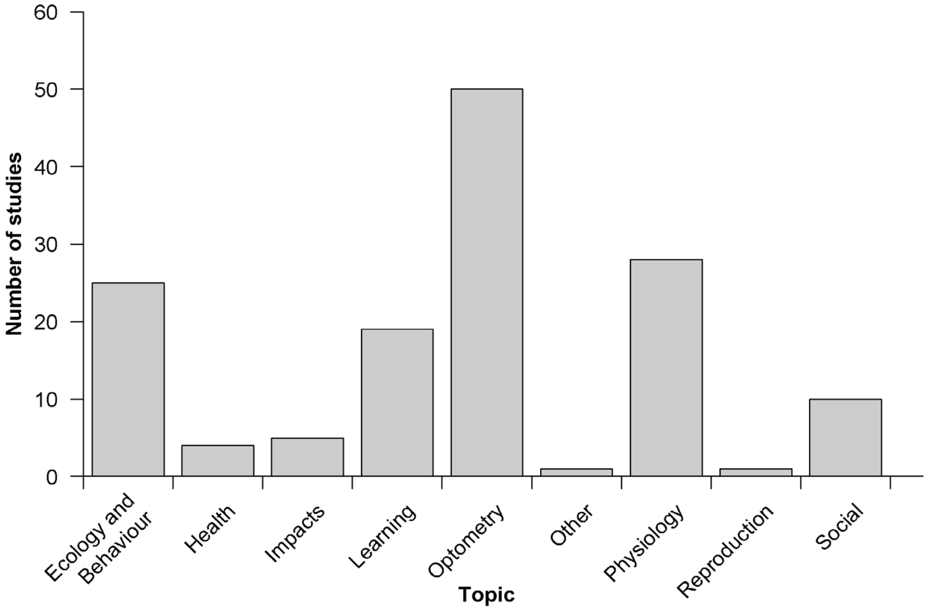

Figure 2.3. Topics of articles within the subset of highly relevant literature (98 articles in total) – some studies covered more than one topic.

Topics covered

Within the subset of literature relating to the topic of ecology, 90% of studies relating to predation were excluded because they focused on biotic, rather than abiotic visual cues. Studies focusing on habitat use and visual navigation were retained and within these spatial mapping and prey response to predator cues under different habitat complexities were overarching themes. For example, Rilov *et al.*, (2007) found that males courted less frequently and explored to a lesser extent if their visual field was cluttered. Similarly, Sheenaja and Thomas (2011) investigated spatial learning capabilities of climbing perch (*Anabas testudineus*), and found that fish collected from flowing environments were less successful at navigating using visual landmarks than those taken from a pond. It was hypothesised that flowing water provides a less stable habitat with unreliable visual landmarks.

Table 2.5. Summary of the number of papers in the full literature search and subset of highly relevant literature by topic.

Topic	Articles				Proportion of articles retained
	No. in Full	% Full	No. in Subset	% Subset	
Ecology / Behaviour	155	35.6	25	25.5	0.16
Health	37	8.5	4	4.1	0.11
Impacts	45	10.3	5	5.1	0.11
Learning	52	11.7	18	18.4	0.37
Optometry	103	23.7	50	51.0	0.49
Other	6	1.4	1	1.0	0.17
Physiology	95	21.8	28	28.6	0.29
Reproduction	66	15.2	1	1.0	0.02
Social	72	16.6	10	10.2	0.14

Within the topic of optometry, physiological studies investigating toxicology and chemical pollution were discarded but those relating to behavioural responses to visual cues and the optomotor reaction were retained. The large number of studies using optomotor apparatus increased the relative proportion of papers under this topic from 21.8% to 28.6%.

Methodologies

Unsurprisingly, across all topics, experimental tanks were by far the most frequent methodology, with 87.7% of the retained papers carrying out experiments within static tanks (Table 2.6). Fifteen spatial learning experiments used variations of T-mazes or additional apparatus to provide the fish with a choice between cues to help understand fish cognition. For example, when comparing the spatial learning abilities of sand-dwelling gobies to those that reside in rock-pools it was found that rock-pool gobies favoured landmarks to navigate to a goal, whereas sand-dwelling species relied almost entirely on turn direction (White and Brown, 2015). Flumes, defined as experiments in which fish were subjected to flowing water, were used in 12 studies. However, in all these experiments the response to visual cues was secondary to understanding fish rheotactic response to hydrodynamic flow. Additionally, specific visual cues including patterns or novel objects were only added to the experiment in four of the 12 studies, with the remaining studies simply testing a response between light and dark conditions.

Table 2.6. Percentage of shortlisted papers using each methodology. Some papers used more than one methodology.

Methodology	% of papers
Field	5.1
Flume	12.2
Lab	7.1
Modelling	1
Tank	35.7
- Optomotor	36.7
- Maze	9.2
- Choice	6.1

Species and group size

Cypriniformes were by far the most studied order of fish with 21 of the 92 highly relevant studies using zebrafish as the subject species. Most studies focused on freshwater species. However, there were several studies on coral reef fish and marine demersal fish species such as gadoids. A large proportion of the full literature search revealed that the larval stages of development were studied because they possess simple neural mechanisms and exhibit less complex behaviour compared with adult fish. However, within the subset of highly relevant literature 83% of the studies used adult fish with only 11% and 6% using juvenile and larvae stages, respectively.

Articles investigating the response of groups of fish to visual cues were underrepresented with 80 of the retained studies focusing solely on individual fish. Taking measurements from individual fish is logical when measuring a species visual ability or a conditioned response. However, considering social interactions between fish are common and almost all fish species school at some stage in their lives, group responses are arguably more relevant and ecologically important. Those studies that did focus on group responses found vision and visual cues play an important role in schooling behaviour. For example, Shaw (1965) and Shaw and Tucker (1965) first discovered that groups of fish continued to swim in the same direction and matched the rotational speed of a visual stimulus even after the stimulus was removed. Later research has showed that the optomotor response can provoke coordinated movements between two conspecific species, the medaka (*Oryzias latipes*) and dwarf pufferfish (*Carinotetraodon travancoricus*) (Imada *et al.*, 2010).

Metrics used to quantify behaviour

Fish behaviour is relatively complex and challenging to interpret because uncontrolled variables can impact how a fish responds to a treatment and individual fish display unique behavioural traits. As a result, there are countless metrics that can be used to quantify and assess how an experimental treatment affects behaviour. Within the subset of highly relevant literature two metrics stood out. Providing fish with a choice was the most frequently used metric to quantify behaviour. This was generally achieved using a choice maze. The second most common set of metrics measured the strength of the optomotor response as either alignment (polarisation), orientation or R.P.M relative to the moving stimulus. Analysing fine scale behaviour by tracking the movements of fish from video footage has been employed for decades and provided detailed information on fine scale fish behaviour from startle responses (Pantoni *et al.*, 2020) to modelling the collective movements of shoals (Delcourt *et al.*, 2013). However, this method of quantifying behaviour was uncommon overall.

2.4 Biases in the literature and opportunities for future work

To date, the majority of research on animal response to visual cues has been in relation to optic flow and has attempted to develop knowledge of neural mechanisms, either to improve fundamental understanding of animal behaviour or to develop elegant models and technology for automated robot navigation using lightweight sensors and minimal computer processing. A disproportionate amount of research has been dedicated to insects due to their comparatively simple neural mechanisms and behaviours, leaving species with more complex, less stereotyped, behaviour, including birds, bats and fish, yet to be studied in any depth. Although gaps remain in our fundamental knowledge of fish behavioural response to visual cues there are many potential applications for this research.

Thus far, no research has attempted to apply current knowledge of visual cues to manipulate fish behaviour to mitigate anthropogenic disturbances, benefit conservation efforts or improve the fishing and aquaculture industry. Some of the only examples of applied research in the literature attempted to reduce bycatch and improve fish catches in pelagic trawl nets where fish were affected by the optomotor response (Hemmings, 1973; Jones, Summerbell, & O’Neill, 2008; Kim, 2015). Behavioural deterrents and fish guidance technology make up a large part of applied research, which attempts to manipulate fish movement by using sensory stimuli from flow (Coutant, 2001), light (Vowles and Kemp, 2012; Fjeldstad, Alfredsen and Boissy, 2014), acoustics (Maes *et al.*, 2004), electricity (Noatch and Suski, 2012) and combinations of stimuli such as bubbles and sound (Welton, Beaumont and Clarke, 2002). Yet, despite considerable literature on fish vision and its role in rheotaxis, passive visual cues have been largely disregarded as a behavioural guidance strategy. There are a number of potential applications for manipulating fish behaviour using visual cues, for example; improving distribution and avoiding overcrowding in aquaculture, directing and guiding fish movement in rivers; reducing delay at barriers and helping to avoid mortalities at pump intake systems and from collisions with hydropower turbines (Enders, Gessel and Williams, 2009). The literature review established that vision is central to fish navigation, rheotaxis, schooling behaviour, conspecific recognition and sexual selection. However, the review also demonstrated that fundamental understanding of fish behavioural responses to visual cues is necessary before this knowledge can be applied elsewhere.

Until now, fish vision and neural physiology has generally been assessed using zebrafish in highly controlled conditions where vision has been detached from behaviour. For example, assessing the optokinetic response of the eye on tethered or immobilised fish, making experiments on freely behaving fish to assess responses to visual cues relatively uncommon. Nevertheless, there are several relevant studies (Bak-Coleman *et al.*, 2015; Scholtyssek *et al.*, 2014) that quantify the response of freely behaving zebrafish to visual cues (Scholtyssek *et al.*, 2014; Bak-Coleman, Smith and Coombs, 2015). Exotic species, including zebrafish, elephantnose fish (*Gnathonemus petersii*), three-lined cory (*Corydoras trilineatus*) and Red-nose tetra have been chosen specifically because they have well-known neural physiology, use electric cues to navigate or have poor eyesight. This focus on exotic fish or species that have either enhanced or inferior sensory ability has left a knowledge gap in the fundamental behavioural responses of common European freshwater fish to optic flow and visual cues.

Given that most freshwater fish live in or encounter lotic environments, there is an underrepresentation of studies specifically looking at fish behavioural response to abiotic visual cues within flowing water. Both mechanosensory cues and vision are central to fish rheotaxis, which has been studied extensively, for example, fish can compensate and perform rheotaxis with either the lateral line senses or vision blocked but fail to orientate to the flow if both senses are inhibited (Suli *et al.*, 2012). However, just three of the seven highly-relevant studies that focused on fish rheotaxis used a pattern as a visual cue. The other four studies demonstrated that vision plays an important role in rheotaxis simply by comparing rheotaxis with and without vision (or light vs dark). Although the optomotor response in flow has briefly been studied for zebrafish and zebrafish larvae (Bak-Coleman, Smith and Coombs, 2015), migratory fish, such as salmonids and the European eel, will exhibit different responses because their life histories require timely navigation within a flowing environment. It would be informative to compare the responses between species with very different swimming capabilities, body morphologies or life history strategies to ascertain whether any responses are ubiquitous or vary between species.

Fish can be highly sociable, performing coordinated elaborate behaviours and schooling in large numbers (Katz *et al.*, 2011; Keenleyside, 1955). Although it is clear that visual cues and optic flow play a role in schooling behaviour, there has been little research focusing on how groups of fish might respond differently to visual cues compared with individual fish. This is especially important to consider in sociable fish, such as minnow or salmonid smolts because individuals will not necessarily exhibit their natural behaviours when tested alone. All eleven studies carried out in flowing water used individual fish as a subject, exemplifying the clear gap in our knowledge concerning how collective behaviour effects fish response to visual cues in flowing water.

2.5 Thesis aims and objectives

As stated in section 1.2, this thesis aims to:

> Improve fundamental knowledge of the behavioural response of European freshwater fish to static abiotic visual cues.

To meet this aim, two primary objectives, highlighted by the literature review, were assessed to understand how visual cues shape fish behaviour in:

1) flowing water
2) groups

Four secondary objectives were developed to further explore related behaviours to objectives 1 and 2:

3) Enhance understanding of the role of visual cues in rheotaxis
4) Compare the response of species with different behavioural ecologies to visual cues
5) Determine whether fish habituate to the sight of visual cues
6) Determine whether the presence of visual cues alters the structure/formation of fish groups in flowing water

A schematic of how these objectives contribute to the thesis is shown in Figure 2.4.

2.6 Thesis overview

Several experiments were conducted to meet the thesis aim and objectives (Figure 2.4). The species used and general methodologies employed in the experiments and analysis are described in Chapter 3. The four results chapters (4-7) are presented as stand-alone bodies of research which introduce and report the methodology and results of each experiment before discussing the findings. Firstly, Chapter 4 investigated the response of common minnow to visual cues to broadly assess the primary objectives of this thesis. To achieve this, the response of common minnow to visual cues was quantified in static and flowing water, and between individuals and groups. The findings of this chapter then form the basis for the three following results chapters that go on to investigate the primary objectives in more depth by addressing the secondary objectives. As such, the primary objectives are addressed across multiple chapters and some chapters address multiple objectives. Chapters 5 and 7 primarily focus on the behaviour of fish in flowing water, Chapter 6 focuses on collective behaviour in static water and Chapter 7 collective behaviour in flowing water. The final chapter (Chapter 8) discusses the four results chapters in relation to the overall thesis aim and adds context to the results. It also suggests some avenues for future work and potential applications for this research.

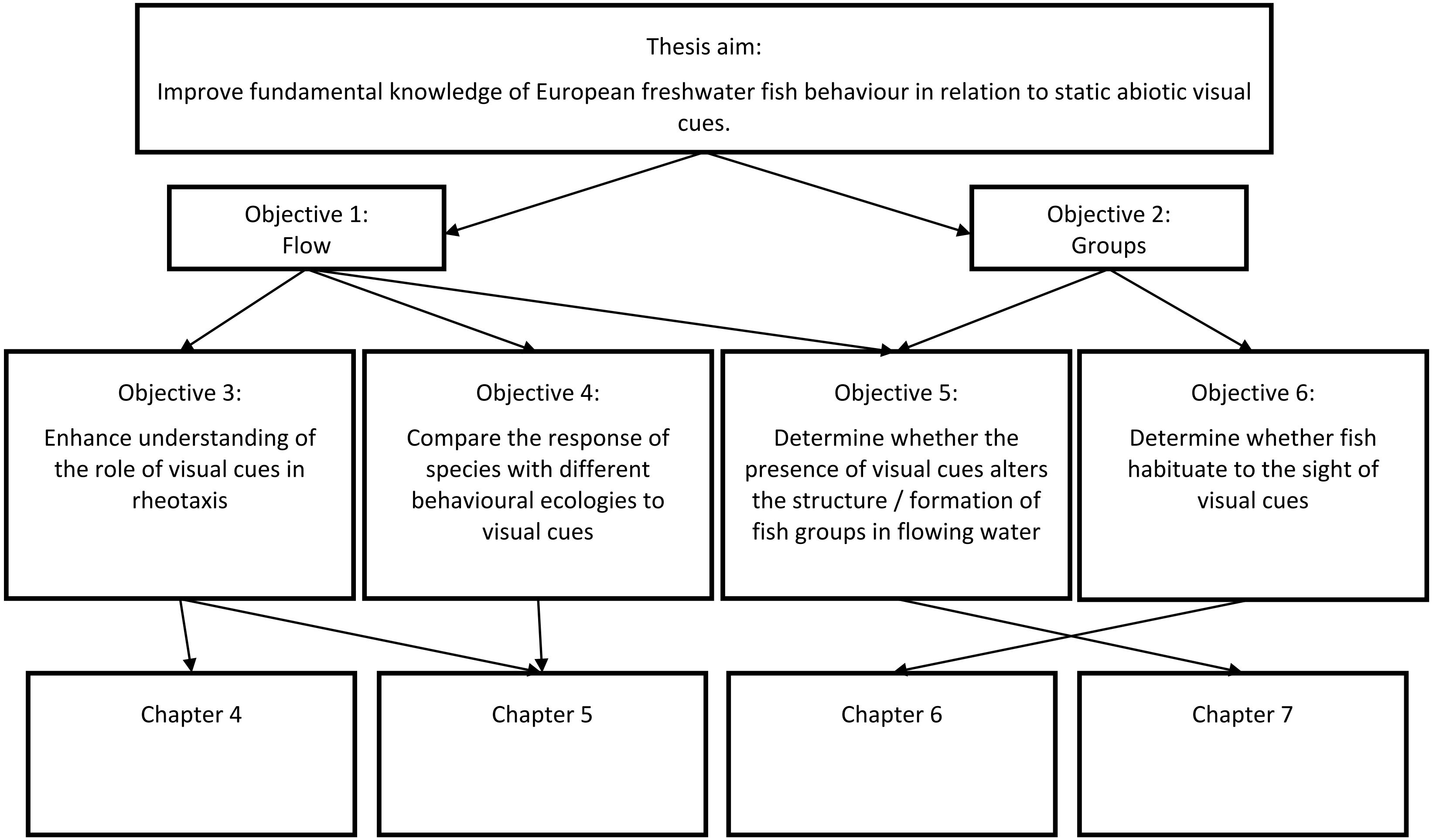

Figure 2.4. Schematic of thesis aims and objectives corresponding to each of the results chapters.

Chapter 3 Approach and research methodology

The following chapter introduces the methodologies, species and equipment used to meet the aims and objectives of this thesis. Detailed methodologies, specific to each experiment, can be found in the relevant chapters (Chapters 4 - 7).

3.1 Experimental facilities

Compared with insects, fish have enhanced vision and a more complex neural physiology. Consequently, behavioural responses to visual cues are likely to be less stereotyped than those observed in insects. Furthermore, the fundamental nature of this research and knowing that fish respond strongly to hydrodynamic cues means that experiments need to be carefully designed and executed under highly controlled conditions to isolate the response of fish to visual cues and minimise any behaviours that might occur as a response to other stimuli.

Research conducted as part of this thesis was carried out at the International Centre for Ecohydraulics Research (ICER, University of Southampton, UK) using experimental tanks and open channel flume facilities. This approach has been widely adopted to gain fundamental information on freshwater and migratory fish because it allows high-resolution behavioural data to be collected whilst controlling for experimental conditions to regulate confounding variables, such as temperature and light.

Two recirculatory flumes were used at the ICER facility. The experiment in Chapter 4 was conducted in a 16 m rectangular, glass sided, steel-based flume driven by three centrifugal pumps that could be used in combination or individually (Figure 3.1). Only the smallest pump was necessary for the required flow rates. A weir at the downstream end of the flume could be adjusted to maintain a constant water depth for all trials. No structures needed to be inserted into the rectangular channel, however, white PVC was placed on the flume base and attached to the flume walls using magnetic strips so that the visual cue treatments could be efficiently randomised between trials. The white PVC minimised reference points to which the fish had access within the channel and a hide erected around the outside of the flume using white blackout material ensured that visual cues and light sources from the surrounding vicinity did not disturb the fish.

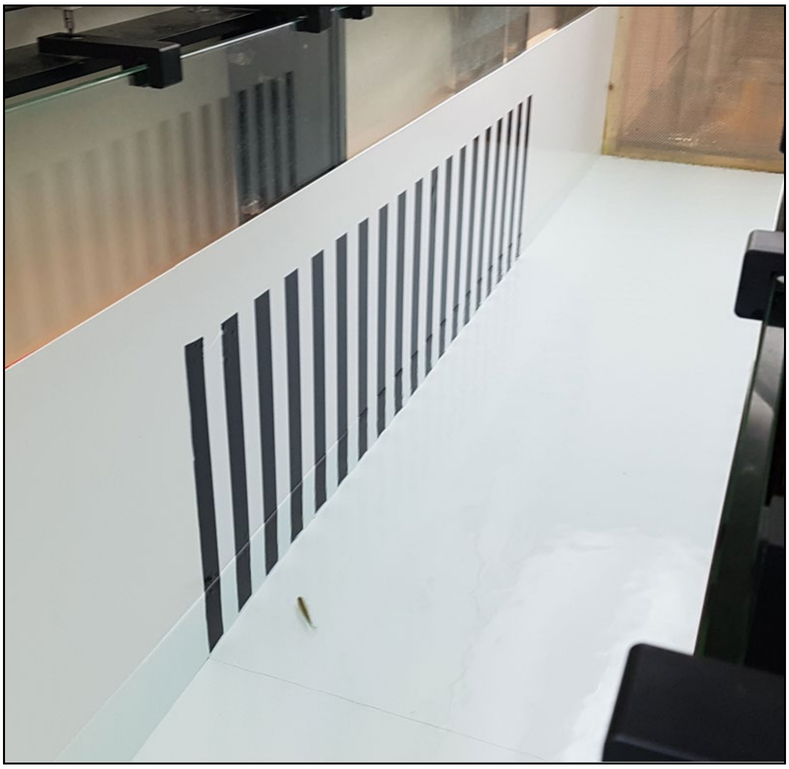

Figure 3.1. Photograph of experiment from Chapter 4 being performed in the ICER 16m rectangular flume facility, Southampton, UK.

Experiments in Chapter 5 and Chapter 7 were conducted in a smaller 12 m open-channel recirculating flume (Figure 3.2). For these experiments, a length of 1 m was necessary that was isolated from the rest of the flume using white flow straighteners to minimise any behaviour that could be caused by hydraulic cues. The rest of the experimental setup was similar to that described in Chapter 4 using white PVC to avoid visual reference points and a hide to minimise disturbances to the fish.

An experimental tank was used in Chapter 7 when the experiment was carried out in static water. Water from the fish holding facility was used in trials to avoid any disturbance to the fish when being transferred between the two tanks. Two computer monitors (iiyama ProLite B2283HS; Diagonal: 54.7 cm; resolution: 1920 x 1080 @75Hz) were required to display visual cues at each end of the experimental tank.

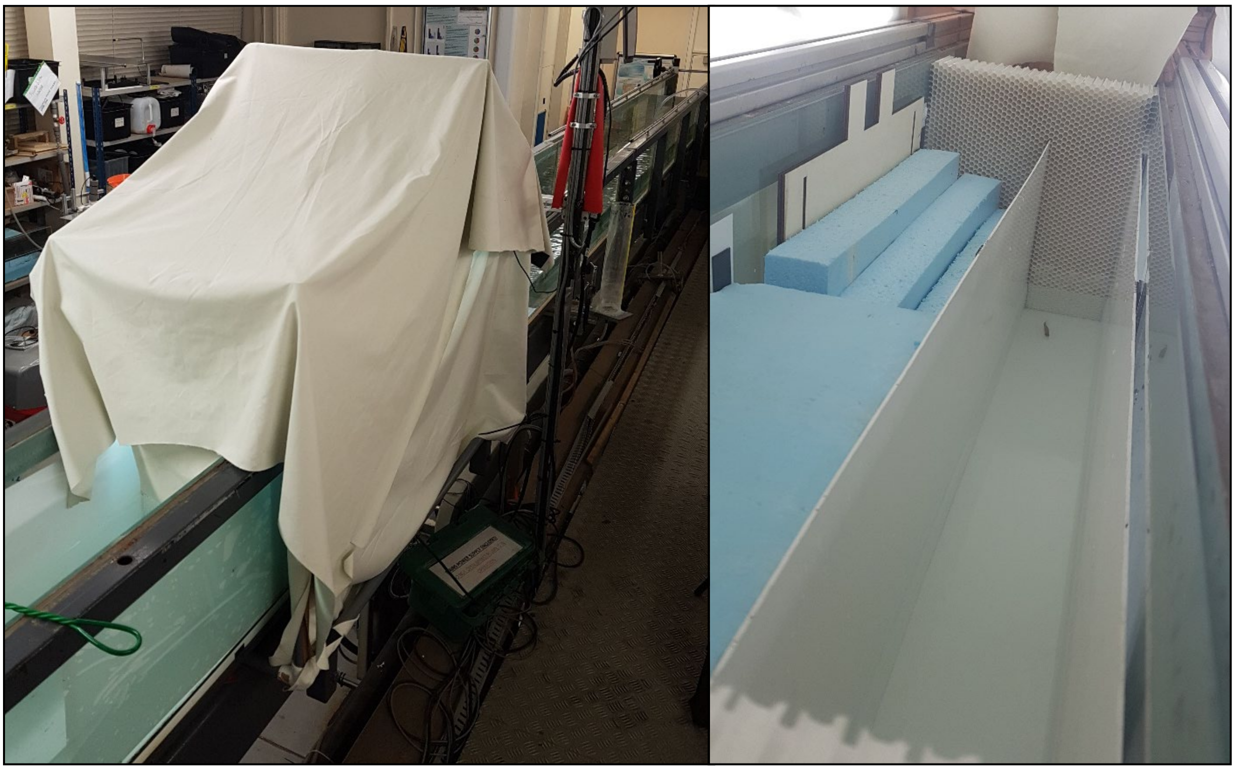

Figure 3.2. Photograph of the experimental set-up in the 12m open-channel recirculating plint flume used for experiments in Chapter 5 and Chapter 7. Experimental flume and hide (left). Experimental channel used (within the hide) in Chapter 5 with minnow (right).

3.2 Fish species

Species were chosen carefully based on previous knowledge, body form, life-history, eye-sight capability and availability. Below is a short introduction to the two species used throughout this thesis.

3.2.1 Common minnow

The common minnow is a non-migratory freshwater cyprinid species found across Europe in streams, rivers and less commonly in lakes (Frost, 1943). They are a highly social species often forming large shoals, which makes them an ideal species for studies looking at group behaviour because there is a reasonable basis of literature on them and they tend to shoal naturally in an experimental setting (Magurran, 1986). They have a characteristic dark stripe down the side and males have red patches around the fins and abdomen during the summer (Figure 3.3). Common minnow generally live for between 2-5 years, are of low conservation concern (IUCN, 2021), and are abundant across the UK. Minnow were fed fish flake food daily whilst held in aquaria.

Figure 3.3. Example of minnow displaying schooling behaviour in the aquaria used to house minnow during experiments for Chapter 5 and Chapter 7.

There is very little information on the visual capabilities of common minnow, however they have large eyes relative to their body size and are known to use vision to maintain cohesion when schooling (de Bie, Manes and Kemp, 2020). There is considerable literature regarding their visual capabilities of zebrafish, a cyprinid and of a similar size to minnow. Therefore, when designing experiments minnow visual capability was assumed to be roughly comparable to zebrafish.

3.2.2 Brown trout

Brown trout (*Salmo trutta*) is a Salmonid species native to Europe which has been widely introduced to freshwater habitats globally (Valiente *et al.*, 2010). They can have two life-history strategies; resident freshwater and anadromous sea-run fish, both of which may develop within the same population (Nevoux *et al.*, 2019). In the UK, brown trout are of high commercial importance due to sport fishing and are therefore bred in fish farming facilities throughout the country. In the wild they breed on gravel riverbeds between January and March and can live up to roughly 20 years. Wild brown trout generally feed on invertebrates, larvae and small fish. When held for the experiment brown trout were fed on the same food pellets that they were accustomed to feed at the trout farm facility.

The economic and ecological importance of brown trout has led to a vast body of research on them, including from a fish passage perspective. They are adapted to high flowing environments and feed using station-holding behaviour and consequently they are mainly a solitary, territorial species. Their eyes are located laterally, which provides them with a large visual field and they have evolved elliptical eyes that provide them with a large region of the visual field that is capable of binocular vision (Randall, 2014). There is also considerable evidence that trout are able see polarised light (Hawryshyn, 1992). Brown trout populations are under threat from the spreading of salmon lice from fish farming, invasive species, poor water quality, and barriers to movement in rivers and coastal areas (Nevoux *et al.*, 2019).

3.3 Visual cues

The systematic literature review (Section Chapter 2) provided information about which abiotic visual cues have been used in previous research. Vertical black and white stripes were by far the most utilised option for strong visual feedback and horizontal black and white stripes for weak visual feedback. A few studies also incorporated other abiotic visual cue patterns.

The optimum visual acuity and resolution are found in the literature for several species. The most researched species with robust measurements of visual capability in this field was zebrafish, a closely related species and of a similar size to common minnow. The stripe width for each experiment was determined with experimental set-up and subject species visual acuity and resolution in mind. In general, it was assured that the spatial resolution of the stripes (as seen from the centre of the channel) was within the optimum range of the visual acuity of the test species. The consistent response of fish to the visual cues displayed in each experiment provided confidence that appropriate visual cues were being presented. Vertical black and white stripes were used throughout the experiments in this thesis to ensure consistency. Fish response to different visual cue patterns, spatial frequencies or spectral frequencies was not within the scope of this thesis. Further studies may wish to investigate which visual cues provide the strongest response.

Visual cues were displayed on the walls of the flume or tank so that overhead video could track the position of the fish relative to a white background (Figure 3.1). Visual cues were minimised within the rest of the experimental area by lining the walls and floor with white PVC plastic sheeting. In Chapter 6 visual cues were displayed on a computer monitor because the visual cues had to periodically change between a control and visual cue treatment within the same trial. There is considerable evidence that fish do respond to images displayed on monitors because the flicker fusion frequencies of cyprinid vision [e.g. for zebrafish (Makhankov, 2005)] are lower than the computer monitor refresh rate of 75Hz.

3.4 Quantifying fish behaviour

3.4.1 Videography

Videography was employed to enable the movements of fish to be recorded and post processed using video tracking software. Custom video tracking code in MATLAB 2018a (The MathWorks, 2018) enabled high resolution data to be obtained, something that is difficult to do in the field and in real time. Tracking on a frame-by-frame basis provided the raw data from which behaviour was analysed using the following methodology in MATLAB displayed in Figure 3.4.

Initially a median image, created from 30 images spaced evenly throughout the recording of the trial, was used to create an image in which the fish had been 'removed' (Figure 3.4.B). This was then compared with the current frame (Figure 3.4.A) by detecting differences in contrast to isolate the position of the silhouette of the fish (Figure 3.4.C). The position of each fish was quantified as the centroid of the pixels making up the fish's silhouette. In addition, the heading of the fish was quantified by placing a centreline through the fish's silhouette and measuring the deviation from the horizontal (Figure 3.4.D). The orientation did not account for heading as the code was unable to differentiate between the head and tail of fish. In Chapter 5, when assessing the use of visual cues during rheotaxis, the position of the fish's eye was deemed more appropriate for the analysis, therefore the coordinates were taken from the most upstream position of the fish's silhouette as an approximate position of the eye. However, this was only used if the fish was in flowing water when the alignment of the fish was highly directional. If multiple fish were in each frame (Chapter 4 and Chapter 6) then the centroid and heading of each fish was obtained. From this the group centroid was calculated and the standard deviation of fish headings provided a measure of the degree of alignment of individual members of the group (*group polarisation*). The raw data on fish positions and alignments from each video frame allowed the behaviour to be quantified using a variety of metrics from the recordings as described in the relevant chapters.

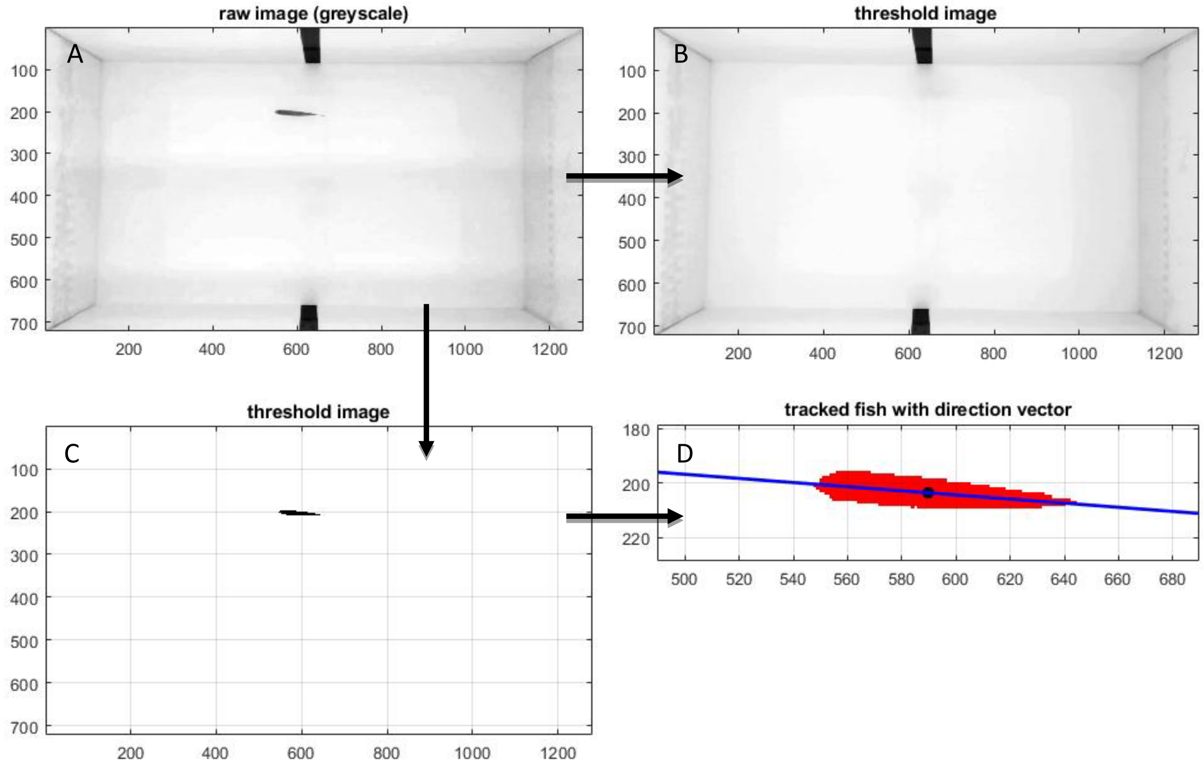

Figure 3.4. Example images from the fish tracking methodology. A – Raw image representing one frame within the video recording. B – Median image calculated over 30 frames throughout the recording to remove the fish. C – Threshold image calculated by differences in contrast between the median image (B) and current frame (A). D – subset of C detailing the centroid and alignment of the fish.

The accuracy of fish locations due to image resolution was considered negligible. In addition, the depth of the fish in the water column was not considered when measuring swim speed and the location of fish. This resulted in a very small inaccuracy of the fish's location at the perimeters of the image/tank of approximately 2mm in chapters 5, 6 and 7 and up to 5mm in Chapter 4 due to the larger flume width and water depth. These errors were considered negligible due to the size of the flumes used in comparison to the error and the variation in animal behaviour observed.

3.5 Hydraulic measurements

Hydraulic measurements were obtained for all experiments that were undertaken in flowing water. Hydraulic measurements in Chapter 4 were collected using a Valeport electromagnetic velocity meter (Model 801, Valeport Totnes, UK), which measures unidirectional flow. Therefore, flow velocity was only measured parallel to the channel walls.

For experiments in Chapter 5 and Chapter 7 measurements were taken at a higher resolution using an Acoustic Doppler Velocimeter (ADV) because the experimental area was considerably smaller than in Chapter 4. ADVs allow an accurate 3-D velocity reading in a precise location by emitting a pair of acoustic pulses and measuring the changes in frequency of the returning signal (Voulgaris and Trowbridge, 1998). As measurements are made in three planes a number of hydraulic parameters can be calculated including mean streamwise velocity, velocity index and turbulent kinetic energy (Johnson and Cowen, 2017). When using an ADV, readings with a signal-to-noise ratio (SNR) > 20 dB and a correlation > 80% were used to estimate flow velocity.

3.6 Animal welfare / Ethics

Ethics for all experimental chapters was reviewed by the Animal Welfare and Ethics Review Board and approval granted by the University of Southampton Ethics and Research Governance Committee. Permission was obtained from the Environment Agency (England) to extract common minnow from their natural habitat and to return them to the source river shortly after completion of the trials. Brown trout were sourced from a fishery and returned at the end of the experiment. Experiments were carried out in compliance with Home Office regulations and regulated procedures were not necessary for this thesis. Fish were held in holding tanks with appropriate aeration and filtration systems. Feeding and water quality testing was performed daily and regular water changes (20 – 50%) ensured high water quality was maintained (ammonia < $0.25 mgL^{-1}$, nitrite < $0.25 mgL^{-1}$, and nitrate < $50 mgL^{-1}$) within the holding facilities throughout all experiments. Individuals were allowed adequate time to acclimate to any new environment, handled with care, with handling time kept to a minimum. There was no evidence of an adverse reaction, stress or fatigue from exposure to the visual cues or flowing water treatments during all experiments. Flow velocity was within the sustained swimming speed of equivalent size minnow as measured in an open channel flume for all experiments (Holthe *et al.*, 2009).

Chapter 4 The response of common minnow (*Phoxinus phoxinus*) to visual cues under flowing and static water conditions

Summary

While fixed visual cues provide reliable reference points for navigation in static water, it remains unclear whether fish energetically benefit from their presence in flowing water. Furthermore, benefits of visual feedback from fixed sources may reduce for group members that can gain additional information from others. Using an open-channel flume, this experimental chapter investigated how fish response to stationary visual stimuli is influenced by flow and group membership under two treatments: vertical black and white stripes placed on (1) both side-walls of the channel, (2) one side-wall only; compared with a control where both walls were uniform white. Fish response was compared under flowing and static water, and between individuals and groups of five. Fish exhibited a positive affiliation for the visual cues; travelling at a slower speed and spending more time closer to the striped walls. Fish spent more time at the sides of the flume under flowing conditions, presumably utilising the lower velocities at the wall boundary to reduce energy expenditure. When only one wall presented visual cues, individual fish spent a median of 97% of time associated with it under flowing water, suggesting some energetic benefit in lotic conditions. This may result from a greater ability to maintain station or control position relative to a reference point, and/or the use of visual stimuli as a proxy indicator of physical structure that may provide drag-reducing refuge. A lesser association with the striped wall under static water suggests that visual cues provide other non-hydrodynamic benefits, such as physical refuge from predators or opportunities for crypsis. Conversely, less association with the striped walls exhibited by shoals may reflect a greater dependence on information provided by conspecifics, or increased security associated with being part of a group. This chapter indicates that fixed visual cues are likely to provide several benefits that vary depending on flow and group membership. These are investigated further in Chapters 5, 6 and 7.

4.1 Introduction

Moving animals continuously monitor their surroundings while controlling their heading and speed to avoid collisions. Within dynamic environments such as air, wind can impede an animal's ability to gain reliable information on its spatial position. Consequently, successful navigation of airborne animals, such as birds and insects, is highly dependent on visual cues (Zeil, Boeddeker and Hemmi, 2008; Schiffner and Srinivasan, 2015; Hedenström and Åkesson, 2017). The apparent motion of these stimuli relative to the movements of the observing animal is known as optic flow. During flight, honeybees and budgerigars control their path, speed and altitude by maintaining a constant rate of optic flow on the retina (Bhagavatula *et al.*, 2011; Linander, Baird and Dacke, 2017). This enables them to move away from areas they perceive to be 'cluttered', reducing the risk of them colliding with physical objects (Lecoeur *et al.*, 2019).

Although fish encounter similar challenges to insects and birds, they display distinctly different responses to optic flow. For example, when individual zebrafish are presented with one vertically and one horizontally striped wall on opposite sides of a tunnel under static water conditions, they react by swimming closer to the vertical stripes that provide stronger visual feedback because they are oriented perpendicular to the direction of swimming (Scholtyssek *et al.*, 2014). This is in contrast to bees, which steer away from vertical stripes when presented with the same choice (Scholtyssek *et al.*, 2014). It is suggested that the zebrafish may move towards stronger visual stimuli because they provide useful reference points in an environment that has the potential to be turbid (Scholtyssek *et al.*, 2014).

The majority of studies focusing on fish response to visual cues have been conducted under static water conditions. However, water currents are a fundamental property of lotic aquatic environments that can be used to enhance navigation (Milner, Solomon and Smith, 2012; Vowles *et al.*, 2014), while fish suffer the risk of being displaced downstream if they cannot fix on a stationary point of reference (Arnold, 1974; Engelmann, Hanke and Bleckmann, 2002). How fish navigate or maintain position in flowing water has been a long-standing question (Arnold, 1974), and likely depends on the availability of visual, tactile and/or hydrodynamic information. Fish can compensate and perform rheotaxis with either the lateral line or vision blocked, but fail to orient to the flow if both are inhibited (Suli *et al.*, 2012), indicating that rheotaxis is a multi-sensory process involving both vision and the lateral line system (Bak-Coleman *et al.*, 2013; Kulpa, Bak-Coleman and Coombs, 2015; Olive *et al.*, 2016). Although fish move closer to visual cues in static water (Scholtyssek *et al.*, 2014); the role of vision in rheotaxis suggests energetic benefits may be gained from access to visual cues for navigation and station holding in flowing water.

Vision plays a central role in the collective behaviour of fish and is critical to the formation and maintenance of shoals and schools (Partridge and Pitcher, 1980). For example, shoaling is elicited when fish are exposed to a moving visual cue (Shaw and Tucker, 1965; Pitcher, 1986), and for members of a shoal that have been blinded in one eye, their ability to maintain position within the group is dependent on other fish being present on their intact, rather than sightless, side (Shaw, 1962). In flowing water, group members benefit from adopting formations that reduce the energetic cost of locomotion (Hemelrijk *et al.*, 2015; Ashraf *et al.*, 2016), such as by swimming side-by-side (de Bie, Manes and Kemp, (2020), for minnow) and synchronising frequency of tail beats at higher velocities (Ashraf *et al.* (2017) for red-nose tetra, *Hemigrammus bleheri*). The transfer of visual information between members of a group allows multiple individuals to benefit from a discovery made by a few. For example, goldfish (*Carassius auratus*) and minnow detect food patches more efficiently when in a larger group (Pitcher, Magurran and Winfield, 1982), and forage at a faster rate and for longer compared with smaller groups that need to remain more vigilant of predators (Magurran and Pitcher, 1983; Morgan, 1988). Given the visual information transfer between conspecifics when navigating in groups, the necessity for members to maintain visual contact with the surrounding environment may be lower than for isolated individuals, and thus stationary visual cues may be of lesser importance.

The experimental approach used in this chapter determined the fine-scale fish response to: (1) visual cues (vertical stripes) and how this is influenced by (2) flow and (3) group size. It was predicted that: (i) fish will associate with visual cues; (ii) that this will be stronger in flowing than static water because they will gain energetic benefits (reduced risk of displacement and greater ability to control speed of movement) from the utilisation of fixed points of reference; and (iii) members of groups will have a lower affinity for visual cues than isolated individuals because information on relative position can be gained from others.

4.2 Methods

To compare the response of individuals and groups of common minnow to visual cues in flowing and static water an experiment was conducted using an open channel flume under two treatments: [T1] vertical black and white stripes placed on one side-wall only, while the other was white (randomly alternated between trials to control for lateral bias of flume hydraulics and/or fish behaviour); [T2] vertical black and white stripes placed on both side-walls of the flume, and [C] a control with uniform white walls. The minnow was selected as the model because it is relatively common and of low conservation concern, easily captured and maintained, and tends to display natural behaviours, including the formation of cohesive shoals, under laboratory conditions.

4.2.1 Experimental setup

Experiments were conducted at ICER (University of Southampton, UK), using a rectangular open-channel recirculating flume (16 m long x 0.6 m wide x 0.8 m deep), between 24 September and 25 October 2018. White laminate PVC sheeting was secured to the inner walls and base of the channel to reduce visual points of reference and to increase contrast between the fish and the background so that automated video processing could be enhanced. To reduce hydrodynamic complexity, a flow straightener was installed upstream of a 4.8 m long experimental section that was isolated from the remainder of the channel by mesh partitions. A blackout hide was erected over the experimental section to prevent disturbance to the fish, and LED strip lighting (Brillihood - LED-Batten - 4 FT - 36 W, 2950 lumen, frequency peaks: 450 nm & 550-600 nm) with reflectors and diffusers installed to enhance uniformity of illumination.

A 1 m long acclimatisation zone was created at the most downstream end of the experimental area using a retractable screen. Upstream of this was an approach zone (1.48 m long), devoid of visual cues, followed by the treatment zone of an equivalent length along which visual cues were displayed during trials (Figure 4.1). A further zone in which the test visual cues were absent extended 0.84 m upstream of the treatment zone. Two overhead Logitech webcams (HD Pro Webcam C920; 30 frames/second, Resolution = 1080p) were installed 1.3 m directly above the centre of the approach and treatment zones (Figure 4.1).

Vertical black and white stripes created strong optic flow on the walls of the treatment zone during the trials (Figure 4.1). A stripe width of 4 cm was selected to provide a spatial frequency (0.066 cycles/degree), as seen from the centre of the flume, that is within the optimum range for the visual acuity of zebrafish, the closest related species to minnow for which robust measurements of visual resolution exist (Tappeiner *et al.*, 2012). The stripe width chosen was comparable to that presented during previous optic flow studies using zebrafish (Scholtyssek *et al.*, 2014; Bak-Coleman, Smith and Coombs, 2015).

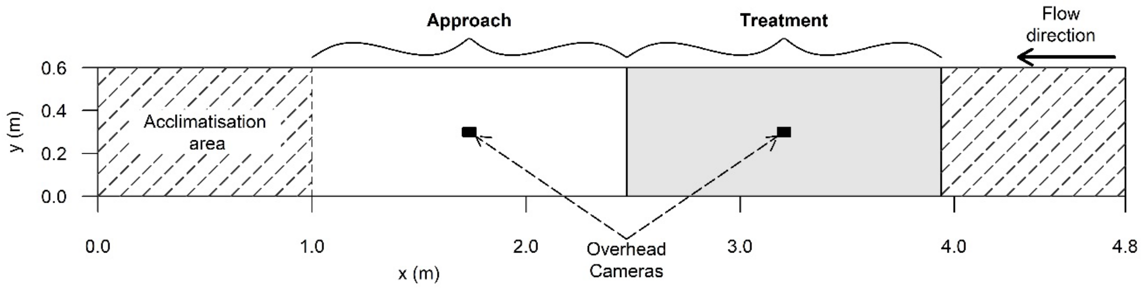

Figure 4.1. Plan of the experimental channel used to assess the response of individual and groups (5 fish) of common minnow to visual cues under static and flowing water. Fish were released from the acclimatisation area and flow was from right to left (arrow). Fish behaviour was recorded by overhead cameras positioned above the centre of the approach and treatment zones. Visual cues were displayed on the flume walls within the treatment zone during treatments (T1 and T2) only.

4.2.2 Flow regimes

Unidirectional flow velocity was measured over a period of 10 seconds at 40 locations throughout the experimental area using a Valeport electromagnetic velocity meter (Model 801, Valeport Totnes, UK). Mean [± SD] velocities were 0.34 [± 0.26] cm s^{-1} and 17.4 [± 2.56] cm s^{-1} for the 'static' and 'flowing water' regimes, respectively. Velocity during the 'flowing water' treatment was slightly lower than the published sustained swimming speed of common minnow of comparable size measured in an open channel (Holthe *et al.*, 2009). Water depth was maintained at 17 cm for both regimes.

4.2.3 Fish maintenance

Common minnow were collected from the River Itchen Navigation, St. Catherine's Hill, Winchester, UK, (lat: 51.049783 long: -1.311416), using a 5 m seine net, and transported in aerated river water to holding tanks at the ICER facility. No more than 350 minnow were collected on three separate occasions between 19 September and 18 October 2018. They were held for a maximum of 16 days before return to a location upstream of the fishing site.

The fish were maintained in two 1200 L holding tanks and fed daily. Water quality was monitored and regular water changes (20 – 50%) ensured a high standard was maintained (ammonia < 0.25 mg L^{-1}, nitrite < 0.25 mg L^{-1}, and nitrate < 50 mg L^{-1}). Mean ± SD holding tank temperature was 16.4 ± 0.5 °C. Lighting was set to match the natural photoperiod throughout the study.

4.2.4 Experimental Protocol

Fish were acclimatised in a perforated container located at the downstream end of the flume for at least 30 minutes prior to the start of trials. An individual or group of five fish were selected at random and moved to the acclimatisation zone at the downstream end of the channel for a further 10 minutes to encourage exploratory behaviour and, in the case of groups, the formation of a shoal. At the start of each trial the retractable screen was lifted, enabling fish access to the experimental area for 30 minutes, after which they were caught, weighed (g) and measured (fork length, mm). Fish length (mean ± SD = 47.6 ± 5.63 mm, N = 580; ANOVA (Analysis of variance): $F_{11,196} = 0.51$, $P = 0.90$) and mass (mean ± SD = 1.15 ± 0.40 g, N = 580; ANOVA: $F_{11,196} = 0.58$, $P = 0.84$) did not differ between treatments. Mean ± SD flume water temperature was 16.7 ± 0.6 °C and did not differ between treatments (ANOVA: $F_{11,196} = 1.02$, $P = 0.43$). To minimise the effect of confounding variables, treatments were randomised throughout the experimental period, and there was no bias in the start time of treatments (earliest: 07:59; latest: 18:00). Fish were used in one trial only and at least 14 repeats were conducted for each condition tested (Table 4.1).

4.2.5 Behavioural and statistical analysis

Video data was post-processed using custom-written tracking software (The MathWorks, 2018). The spatial coordinates of fish centroids were recorded every third of a second by identifying changes in contrast between fish silhouettes and the background. Where multiple fish were present within a single frame the coordinates of each fish were recorded, and the centroid of the group calculated by minimising the sum squared distances between each coordinate.

As fish volitionally explored the experimental area during each 30-minute trial, multiple paths through the approach and treatment zones were recorded in both the upstream and downstream directions. Consequently, data from the approach and treatment zone were analysed independently. The positions of fish as they passed through the approach and treatment zones were used to determine their response to visual cues through the following three metrics: (1) *Visual cue association* - quantified as the time spent in the treatment zone expressed as a proportion of total time spent in the approach and treatment zones, (2) *Ground speed* - calculated as quotient of distance and time between consecutive frames, and (3) *Distance to closest wall* - quantified as the distance to the closest wall from the individual fish or group centroid. The closest member of the group to a wall in each frame was also analysed but as the results did not differ between the two metrics, and therefore the group centroid was chosen for the final analysis. Only passes in the upstream direction and in the mid 90 cm of each zone were used to calculate *Ground speed* and *Distance to closest wall* because the behaviour in the approach zone may have been influenced by the presence of stripes in the upstream treatment zone, and flow influenced the ground speed of fish swimming downstream. Due to the group splitting between zones and times where fish did not form cohesive shoals, frames containing fewer than three fish were excluded to ensure that data was representative of group behaviour. This resulted in 22% of group data being removed from the analysis.

A factorial experimental design was used to quantify the influence of flow regime (static versus flowing water) and group membership (individuals versus group of five) on fish response to visual cues (T1, T2, and C), resulting in a total of 12 conditions. Statistical analysis was performed using R software (Version 3.4.1 R Development Core Team, 2017). Each metric was analysed separately with visual cue, flow regime and group membership as explanatory variables. Interactions between explanatory variables were also tested. Mean *Ground speed* and *Distance to closest wall* were calculated for each pass and then averaged by trial to avoid pseudo-replication due to disproportionately more data obtained from longer passes or trials that contained a greater number of passes. Shapiro-Wilk and Levene's tests were performed to check for normality and homogeneity of variance, respectively. Where the error distribution of the response variable deviated from normality, an appropriate transformation was used to normalise the data or error distribution assumed within the model. Model fit was assessed using the DHARMa package (Hartig, 2020). Chi-square and F statistics were calculated using the car package (Fox and Weisberg, 2014). Post-hoc tests were conducted using the phia package (De Rosario-Martinez, 2015). As mean water temperature and fish size did not differ between treatments they were not included as covariates.

Proportion data was arcsine square-root transformed prior to use in the analysis of *Visual cue association* using a two-way Anova. A linear mixed model (LMM) (Lme4 package; Bates *et al.*, 2015) was used to compare the *Ground speed* between the approach and treatment zones with a random effect variable of *Trial ID* to account for data from the same fish in both zones during a trial. The response variable was $\log_{10}$ transformed to normalize the model residuals. *Ground speed* and *Distance to closest wall* were $\log_{10}$ and square-root transformed, respectively, prior to analysing the effect of explanatory variables using Anova.

4.3 Results

4.3.1 Response to visual cues

Both flow and the presence of visual cues influenced the proportion of time fish spent in the treatment zone (ANOVA: $F_{1,203} = 9.7$, $P < 0.001$). Fish spent a greater proportion of time in the treatment zone when either one (mean ± SE = 0.67 ± 0.05; ANOVA: $F_{1,203} = 49.6$, $P < 0.001$) or two walls (mean ± SE = 0.69 ± 0.07; ANOVA: $F_{1,203} = 64.4$, $P < 0.001$) displayed visual cues compared with the control (mean ± SE = 0.48 ± 0.05; Figure 4.2), but no difference was observed between the one and two striped wall visual treatments (ANOVA: $F_{1,203} = 1.36$, $P = 0.25$).

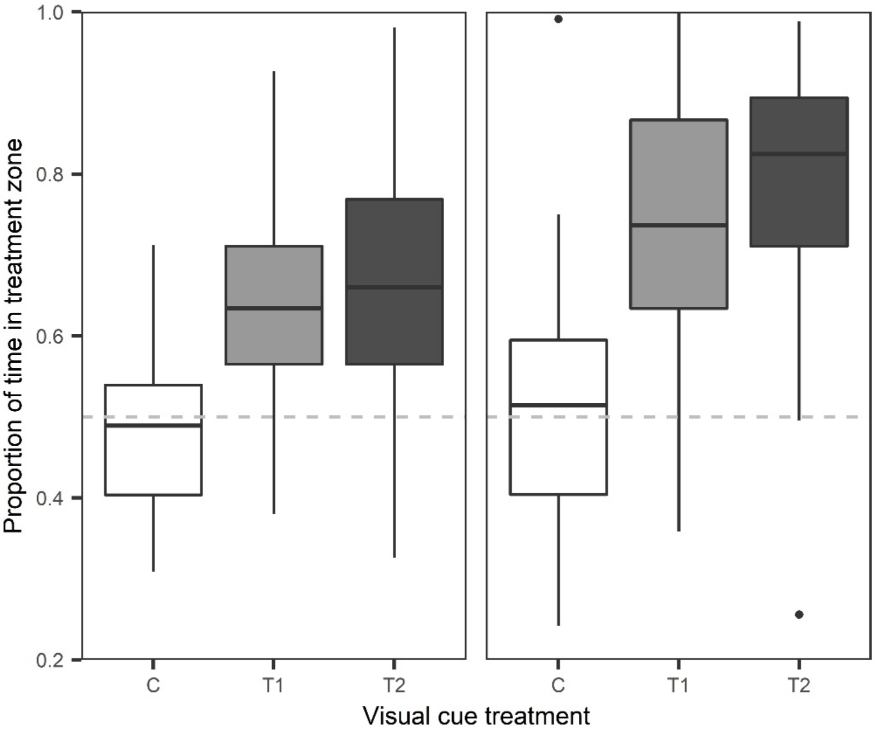

Figure 4.2. Proportion of time minnow spent within the treatment zone compared with the approach zone under the three visual cue treatments in static (left) and flowing (right) water. Boxes show the lower quartile, median and upper quartile and the whisker displays the range of data within 1.5 x the IQR. The points represent outliers (data beyond these limits).

Fish tended to swim in close proximity to the flume walls, independent of visual cue treatment, resulting in a bimodal lateral distribution for the control and two striped wall conditions (Figure 4.3). However, when only one wall displayed visual cues the lateral distribution was skewed as fish tended to associate with the striped wall (Figure 4.3; bold text in Table 4.1). When visual cues were present fish swam roughly twice as close to the channel walls (One wall: median [IQR] = 6.3 [6.8] cm, ANOVA: $F_{1,188}$ = 16.1, $P < 0.001$; Two walls: median [IQR] = 6.2 [8.5] cm, $F_{1,188}$ = 14.0, $P < 0.001$) compared with the control (median [IQR] = 12.2 [10.8] cm; Figure 4.4). There was no difference in the *Distance to closest wall* between the one and two wall visual cue treatments (ANOVA: $F_{1,188}$ = 0.02, P = 0.88). In the absence of visual cues (control) there was no difference in the median *Ground speed* between approach and treatment zones (LMM: $F_{1,201}$ = 0.30, P = 0.59). However, considering the treatment zone in isolation, all three explanatory variables influenced *Ground speed* (ANOVA: $F_{11,188}$ = 5.51, $P < 0.001$), including an interaction between group membership and visual cue treatment (ANOVA: $F_{2,188}$ = 5.9, P = 0.003, Figure 4.5). There was no difference in ground speed between groups and individuals when visual cues (either one or two) were displayed. However, during the control individuals generally swam faster than groups (ANOVA: $F_{11,188}$ = 9.98, $P < 0.001$).

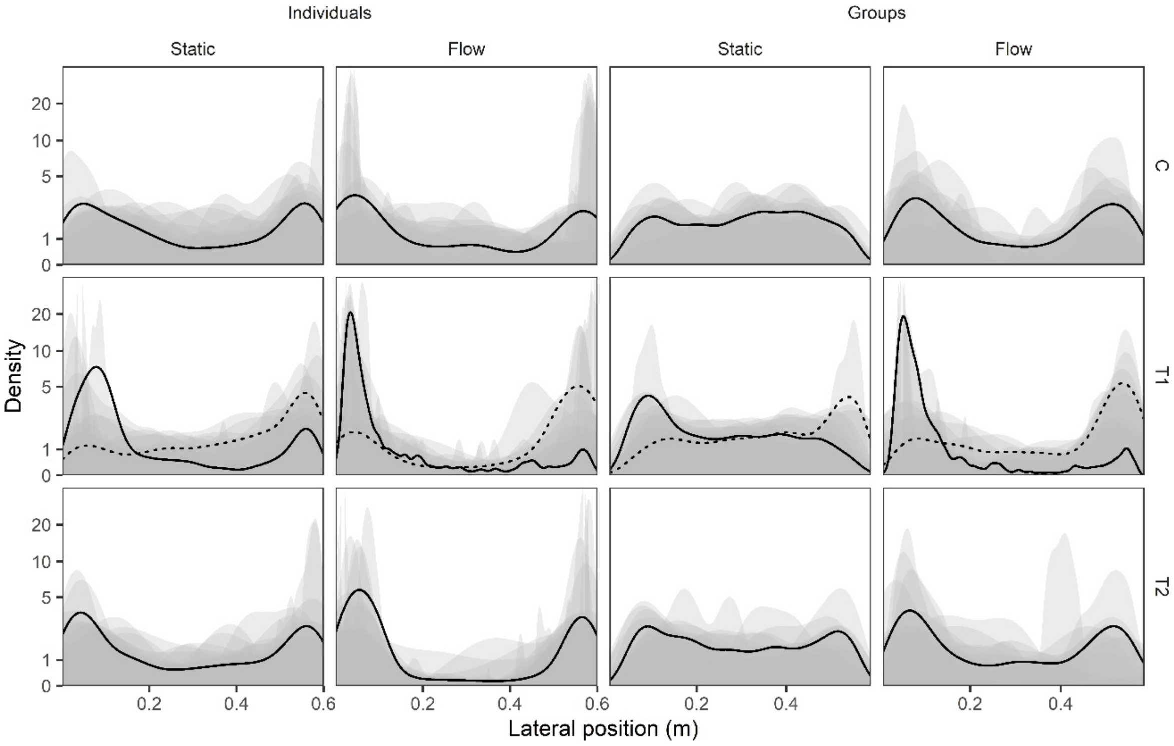

Figure 4.3. Probability density function plot for lateral distribution of fish in the treatment zone for all 12 treatment groups. Contribution of data varied between fish. Line type varies for the different visual cue treatments. In the middle row the solid line represents trials when the visual cues were on the left side of the flume and the dashed line, the right. The area under the curve is equal to one – to calculate the probability of a fish being in a region combine the density with the range on the x-axis to calculate area under the curve.

Table 4.1. Proportion of time associated with striped walls. Median proportion of time associated with the one striped wall (T1, rows in bold) and the right side of the flume in the two striped wall treatment (T2) and control (C) in the treatment zone. IQR = interquartile range.

Visual cue treatment	Flow regime	Group size	Proportion by stripe / right	IQR	Sample size (N)
C	Static	1	0.456	0.252	19
T1			**0.738**	**0.274**	**20**
T2			0.558	0.218	20
C	Flow	1	0.325	0.660	20
T1			**0.970**	**0.298**	**21**
T2			0.475	0.599	21
C	Static	5	0.408	0.255	14
T1			**0.668**	**0.160**	**16**
T2			0.547	0.333	14
C	Flow	5	0.612	0.734	14
T1			**0.906**	**0.267**	**15**
T2			0.636	0.450	15

4.3.2 Influence of flow on response to visual cues

Association with visual cues was greatest in flowing water (ANOVA: $F_{1,203} = 14.8$, $P = 0.01$), with fish spending on average 16.8% more time in the treatment zone (mean ± SE = 0.74 ± 0.07) compared with when they were in static water (mean ± SE = 0.63 ± 0.06, Figure 4.2). Fish also associated more closely with the striped wall (Figure 4.3; Table 4.1) and swam closer to the flume walls in flowing water (median [IQR] = 5.5 [5.7] cm) compared with static water (median [IQR] = 12.4 [11.2] cm) for all visual cue treatments including the control (ANOVA: $F_{1,188} = 76.4$, $P < 0.001$).

The *Ground speed* of minnow was slightly slower in flow (median [IQR] = 14.7 [10.9] cms^{-1}) than static water (median [IQR] = 18.9 [11.1] cms^{-1}; ANOVA: $F_{1,188} = 4.98$, $P = 0.026$; Figure 4.5).

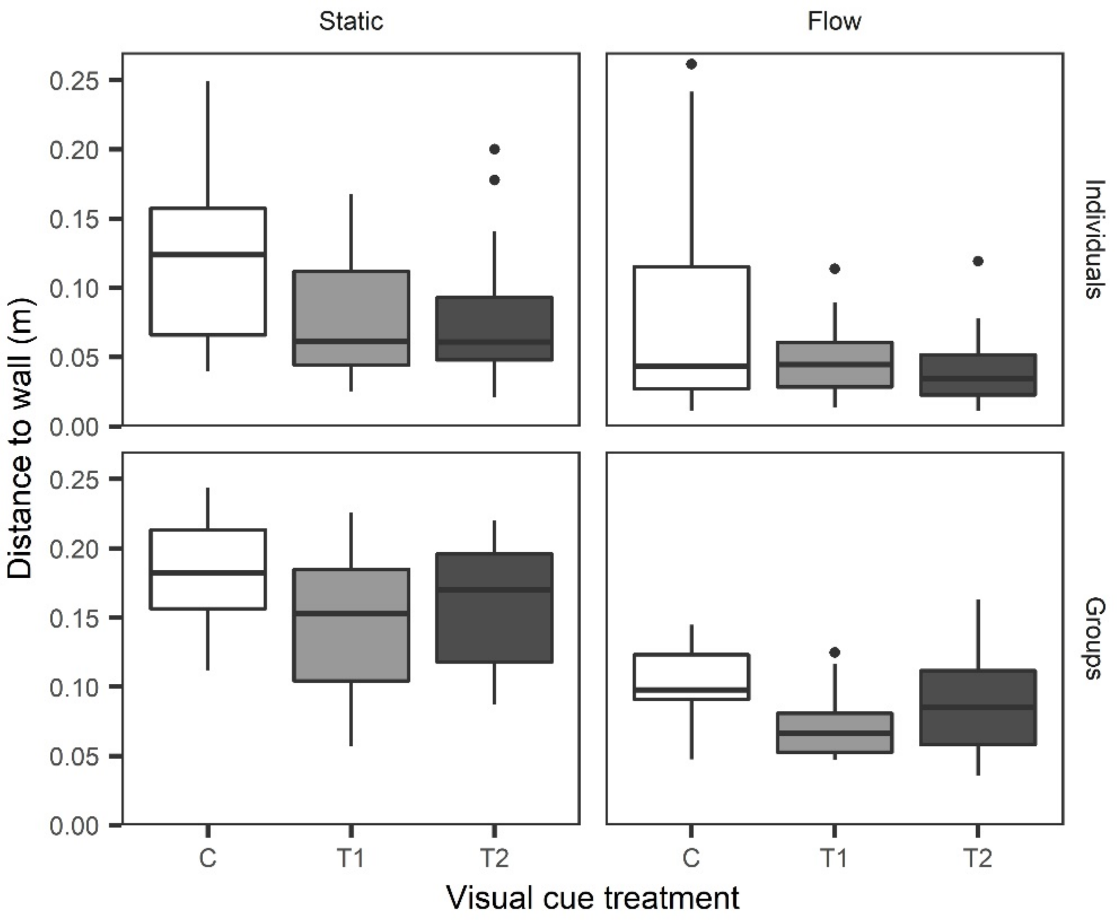

Figure 4.4. The median distance to closest wall for each treatment. Static water (left), Flowing water (right), individuals (upper) and groups (lower). Boxes show the lower quartile, median and upper quartile and the whisker displays the range of data within 1.5 x the IQR. The points represent outliers (data beyond these limits).

4.3.3 Influence of group membership on response to visual cues.

Group size did not influence the proportion of time spent in the treatment zone (ANOVA: $F_{1,203}$ = 1.6, $P = 0.21$), but individual fish associated with the striped wall to a greater extent (Figure 4.3; Table 4.1) and swam closer to the flume walls (median [IQR] = 5.5 [7.0] cm) compared with groups (median [IQR] = 12.2 [8.6] cm, ANOVA: $F_{1,188}$ = 69.1, $P < 0.001$; Figure 4.4).

Groups of fish did not vary their *Ground speed* across any of the visual cue treatments (ANOVA: $F_{2,188}$ = 2.04, $P = 0.13$). However, individual fish swam slower when more visual cues were displayed. Specifically, individuals swam slower when one (ANOVA: $F_{1,188}$ = 15.9, $P < 0.001$) or two walls (ANOVA: $F_{1,188}$ = 45.3, P = 0.001) displayed visual cues compared with the control and when two walls displayed visual cues compared with just one (ANOVA: $F_{1,188}$ = 8.17, P = 0.02; Figure 4.5).

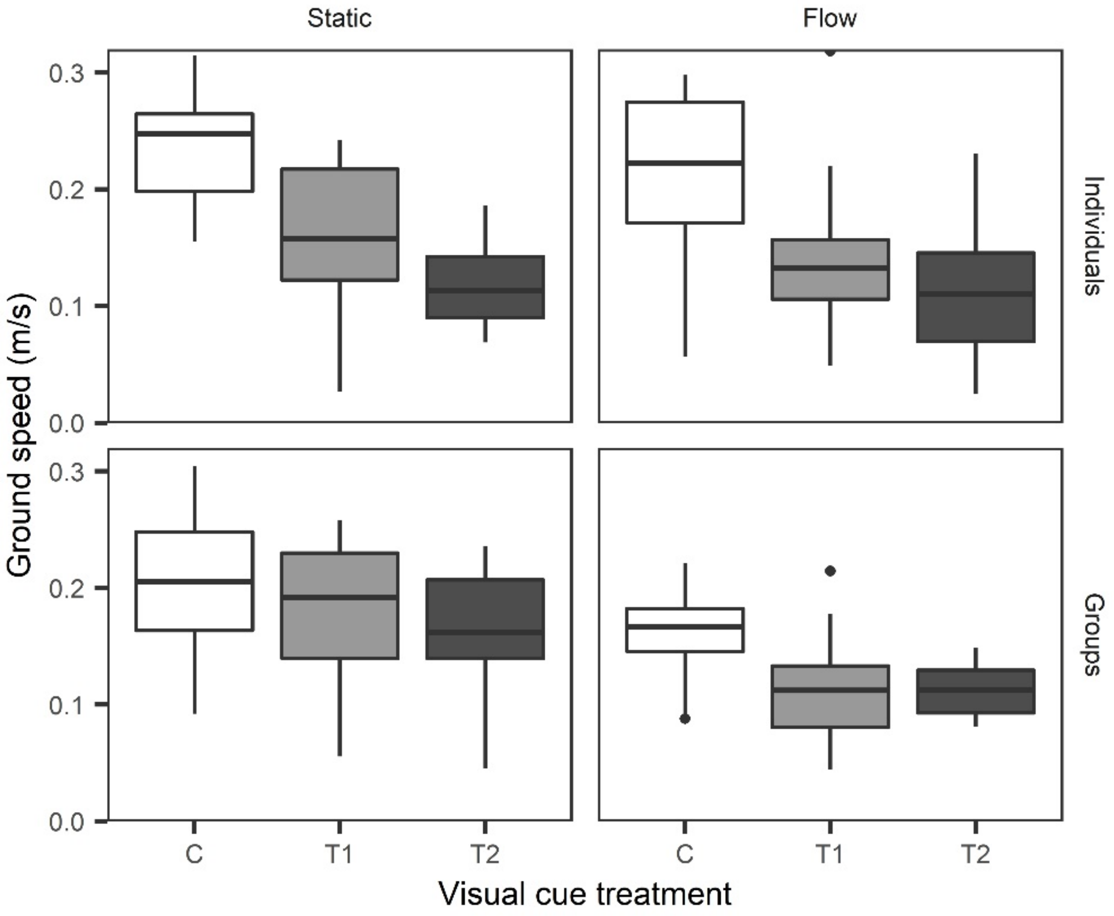

Figure 4.5. Median ground speed stratified by visual cue treatment for each condition. Static water (left), Flowing water (right), individuals (upper) and groups (lower). Boxes show the lower quartile, median and upper quartile and the whisker displays the range of data within 1.5 x the IQR. The points represent outliers (data beyond these limits).

4.4 Discussion

This chapter quantified the response of minnow to visual cues and investigated how that was influenced by flowing water and group membership. In-line with previous work with zebrafish (Scholtyssek *et al.*, 2014), minnow swam in proximity to visual cues in static water, but as predicted their association became stronger in flowing water. In addition, individual fish showed a stronger affinity to the stripes compared with groups of five. The stronger association in flowing water indicates that fish may accrue energetic benefits by using visual reference points to control their swimming. In groups, information transfer between conspecifics may reduce the necessity for fixed reference points, and/or individual fish may be more inclined to seek refuge alongside the visual cues because the advantages gained from being a member of a group are absent.

Consistent with the first prediction, minnow tended to show an innate association with visual cues irrespective of flow and group membership. Fish reduced their *Ground speed* and chose to spend more time alongside the visual cues. In addition, fish associated with the striped side of the channel when offered a choice. This supports the findings of previous studies that found fish to favour the side of the tank displaying the stronger visual feedback (Scholtyssek *et al.*, 2014). This contrasts insects and birds that balance bilateral optic flow. If fish were balancing optic flow across both eyes they would move away from the striped wall if offered a choice or move directly down the centre of the flume when both walls displayed the same cues. Although fish exhibited a general attraction to visual cues, abiotic (flow) and biotic (presence of conspecifics) cues influenced the magnitude of affiliation.

In agreement with the second prediction, minnow clearly associated with visual cues to a greater extent in flowing compared with static water. It was expected that fish would utilise the lower velocities at the channel boundaries to reduce their energetic costs in flowing water (Watson *et al.*, 2018), but the clear preference for the single striped wall when the opposite was uniform indicates that there are likely benefits associated with visual cues. In nature, holding position in a current may provide multiple ecological advantages, including the lessening of energetic costs of locomotion, improved access to food, and reduced probability of detection by a predator through motion camouflage (Johansen, Akanyeti and Liao, 2020). Fish living in a unidirectional flowing environment have adapted behaviours such as rheotaxis and position holding, to avoid being displaced downstream. Vision has been demonstrated to play an important role in rheotaxis (Arnold, 1974), and therefore the stripes may have provided fixed reference points to help fish hold position (the 'Station Holding Hypothesis'). However, fish associate with visual cues even in static water, suggesting other benefits in addition to reduced energetic expenditure. The visual complexity associated with a striped, rather than uniform, landscape may indicate structural complexity and thus attract fish to areas where they might seek shelter from unfavourable hydrodynamic conditions or predators (Orpwood *et al.*, 2008; Ioannou and Krause, 2009). Alternatively, a complex striped background may itself bestow benefits in terms of reduced predator detection if the outline of the fish is disrupted, independent of whether physical structure is present (Scharf, Manderson and Fabrizio, 2006; Kjernsmo and Merilaita, 2012). It should also be noted that, the minnow may perceive the predation risk to be greater in flowing water, and therefore, that they use the visual cue to a greater extent as a refuge in flowing water.

Groups of minnow were less inclined to spend time close to the stripes and tended to show more exploratory behaviour compared with individuals, supporting the third prediction. Shoaling plays an important role in lowering predation risk, and so individuals may seek alternative strategies by using cover (Pitcher and Parrish, 1993). For example, Orpwood *et al.* (2008) found that minnow tended to school in areas of low habitat complexity but sought refuge and remained still in more complex habitats. Attraction of individuals to visual cues may, therefore, reflect a sheltering or refuge seeking response with the stripes offering a form of visual camouflage (the 'Predator Refuge Hypothesis'). Considering minnow are a social species, when conspecifics were absent individuals may have experienced higher stress levels and this could provide an alternative explanation for the increased association. The importance of vision on the collective behaviour of fish has been demonstrated (Partridge and Pitcher, 1980; Pita *et al.*, 2015; Lemasson *et al.*, 2018), with subtle forms of information transfer between schooling fish, usually for resource or predator detection (Magurran and Higham, 1988; Brabazon, Cui and O'Neill, 2015). Additionally, groups of fish adopt side-by-side formations to enhance visual information transfer to benefit from energy efficient formations in flow (de Bie, Manes and Kemp, 2020). Given that fish gain information on their relative position from conspecifics, a reduced onus on group members to maintain visual contact with environmental cues is logical and would explain the looser association of groups with the visual cues in this study. Additionally, this behaviour could be driven by a cognitive constraint whereby individuals are unable to monitor both environmental factors as well as other group members simultaneously (Ioannou et al., 2017). Consequently, it is possible that group members infer information about environmental features from the behaviour of conspecifics and use this information to better maintain ideal distances from environmental features. Alternatively, the energetic benefits groups gain by adopting energy efficient formations (Ashraf *et al.*, 2016) may outweigh those gained by controlling position using fixed visual cues.

4.4.1 Conclusions

Based on the observations in this chapter, the benefits of association with the visual cues provided by striped walls are likely to be greatest when isolated minnow inhabit flowing water. This is likely because fish in flowing water use visual cues as reference points to control position for energetic benefit. Furthermore, the vertical stripes may resemble macrophytes in the minnow's natural habitat and the observed association may reflect the seeking of physical refuge from flow or predators. For groups, information transfer between conspecifics may explain their reduced need to use visual cues, either to save energy by fixing on a known location, or as an indicator of refuge, e.g. from a predator or adverse flow. There is a necessity for both the 'Station

Holding Hypothesis' and 'Predator Refuge Hypothesis' to be further explored throughout this thesis to determine what is driving the observed association with visual cues.

A fundamental understanding of how fish react to environmental stimuli may help inform environmental engineers and fisheries managers, particularly in relation to fish guidance. In this respect, further research to investigate whether fish habituate to the presence of visual stimuli, or whether such signals can be used to elicit a consistent response over a range of environmental conditions and for multiple species, remains an avenue of interest.

Chapter 5 The influence of flow velocity on the response of rheophilic fish to visual cues

Summary

The strong association with visual cues exhibited by rheophilic fish in flowing water may reduce the energetic costs of maintaining position due to the provision of spatial points of reference. If this ‘Station Holding Hypothesis’ is true, a positive relationship between the association with visual cues and flow velocity is expected. This hypothesis was tested experimentally by quantifying the response of common minnow and brown trout to visual cues under three flow velocities. In contradiction to the prediction, there was no evidence that the association with strong visual cues was positively related to flow velocity when fish were presented with vertical black stripes in an open channel flume, although interspecific variation in response was observed. The time that fish spent associated with the zone in which visual cues were presented during the treatment was compared with a control measuring the time that fish spent associated with the zone in which visual cues were absent. The association with visual cues was relatively weak in trout that spent only 50% more time in the central zone during the treatment than during the control (when visual cues were absent), compared with the minnow that spent 650% more time associated with the central zone when visual cues were present. Trout tended to be more exploratory and made short visits to the area where visual cues were present, whereas minnow associated with the cues for longer. The strong association with visual cues independent of flow velocity exhibited by minnow and the weak association across all velocities shown by trout suggests that this behaviour is unlikely to be a strategy to minimise the energetic cost of swimming in flowing water. Minnow may use the visual cues as a proxy indicator of physical structure that provides alternative benefits, such as refuge from predators. Trout may employ alternative cues (e.g. mechanosensory) to seek more energetically favourable regions of the experimental area, reducing the importance of stationary visual stimuli.

5.1 Introduction

Within flowing fluvial environments fish face the risk of being displaced by currents. Consequently, most species have adapted rheotactic behaviours to orient and control their position relative to the flow direction (Arnold, 1974). This allows fish to perform directional migrations or hold station to intercept prey or odours within the current (Veselov *et al.*, 1998; Smith *et al.*, 2005). Mechanosensory and visual cues are used by fish to control position relative to their surroundings (Suli *et al.*, 2012). As fish are able to perform rheotaxis with either vision or their lateral line inhibited, the relative role of each is still debated (Montgomery, Baker and Carton, 1997; Bak-Coleman, Smith and Coombs, 2015). The lateral line enables fish to determine directionality of flow and orientate accordingly, but without a fixed point of reference it is unclear how fish maintain position or detect their relative motion within a flow (Arnold, 1974). Therefore, it is accepted that vision plays some role in station holding behaviour.

Fish control their speed and movement within dynamic flowing environments by stabilising their field of view using an innate optomotor response (Protasov & Altakov, 1961; Shaw, 1965), possibly as a position stabilising reflex to aid navigation and maintain cohesiveness in shoals (Shaw & Sachs, 1967; Veselov *et al.*, 1998). In addition to the optomotor response, fish tend to associate with strong visual reference points when navigating through both still (Scholtyssek *et al.*, 2014; Chapter 4) and flowing water (Chapter 4). When offered a choice between a striped and plain white wall fish almost always chose to associate with the wall displaying the strong visual cues (Chapter 4). There are currently two explanations for this behaviour. The first hypothesis suggests that fish may associate with visual cues because they act as a proxy for physical structure in which to seek shelter from predators (the 'Predator Refuge Hypothesis'). The second hypothesis suggests that static visual cues provide a reliable point of reference from which to control position and swimming speed and potentially reduce energetic costs of readjusting position (the 'Station Holding Hypothesis'), as the observed association is stronger in flowing water (Chapter 4). Further work is needed to test these hypotheses.

Fish are known to associate with physical structure in flowing water to reduce energy expenditure. For example, fish perform specialised behaviours, such as entraining, bow riding (Kerr, Manes and Kemp, 2016), and Kármán gaiting (Liao, 2007), to reduce energetic costs of maintaining position in complex flows. These behaviours take advantage of predictable hydrodynamic features, such as vortices produced by physical objects (e.g. cylinders or boulders), and vision is likely to play an important role when associating with fixed structures. For some rheophilic fishes, efficient station maintenance is critical to their behavioural ecology as it allows them to minimise energy expenditure, for example when intercepting passing invertebrate drift (Vivancos and Closs, 2019). Association with visual cues might provide a means to help fish do this, either because it indicates the presence of physical structure and associated benefits, or because it provides information on spatial position. However, it is unknown, whether the presence of visual cues alone is sufficient to enhance swimming performance associated with station maintenance in the absence of the beneficial hydrodynamic characteristics produced by stationary physical objects within the flow.

In addition to ascertaining whether static visual cues provide fish with energetic benefits in flowing water, resulting in a positive relationship between association with visual cues and flow velocity, a secondary question is how universal such a relationship might be. Stream-dwelling fish exhibit a diverse array of behavioural strategies linked to their ecology and life-history characteristics. For example, direct visual cues from external abiotic sources may be more important to solitary species than those that live in groups that are able to obtain information from their conspecifics in addition to that provided by the surrounding environment. This is exemplified by both red-nose tetra and common minnow that use visual information shared between conspecifics in flowing water to adopt group formations that optimise energy expenditure (Ashraf *et al.*, 2017; de Bie *et al.*, 2020). Indeed, pairs of minnow enhance visual two-way information transfer by adopting a side-by-side formation when in flow compared with a tandem formation (follow-the-leader) when in still water in which information transfer is one-way only (de Bie, Manes and Kemp, 2020). In this chapter a reductionist approach was adopted by using only a single fish in each trial to control for the confounding effect of visual information transfer between group members, and by using two common stream-dwelling species, the common minnow and brown trout, with different life-history characteristics, to quantify interspecific variation.

This experimental chapter aimed to explore: (1) the relationship between association with visual cues and flow velocity, a proxy for energy expenditure; and (2) interspecific variation in the response exhibited. Two separate experiments were conducted at three flow velocities to test the following predictions: (i) fish will associate with visual cues; (ii) the relationship between association with visual cues and flow velocity will be positive (supporting the 'Station Holding Hypothesis'); and (iii) interspecific variation in response will be observed, with typically group living minnow being less likely to form strong associations with visual cues than trout, which are likely to utilise visual cues to hold station in the water column and against the flow to feed.

5.2 Method

Experiments were designed to test fish response when visual cues were present and absent (control) across three flow velocities (low, mid and high; Table 5.1), creating six treatment groups. Both minnow and trout were tested across all six treatments, but flow velocities were adjusted between species to account for differences in their swimming capabilities. Between 10 and 21 replicates were conducted for each treatment (Table 5.1), resulting in a total of 109 trials using individual minnow and 76 trials using trout.

5.2.1 Experimental setup

Two experiments were conducted at the ICER (University of Southampton, UK) within a rectangular, open-channel recirculating flume (12.0 m x 0.3 m x 0.4 m) between 1 and 24 March 2021 (minnow) and 13 and 20 January 2020 (trout). An experimental area of length 1.0 m and width 0.12 m (minnow) and length 1.0 m and width 0.3 m (trout) was separated from the rest of the flume using flow straighteners, which also minimised turbulence. Flume width differed between experiments to accommodate difference is body size between the two species. Within the experimental area white laminate PVC sheeting was secured to the flume's base and walls to minimise visual reference points for the fish and maximise contrast between the fish and the background to improve video processing. Any reference points outside the flume were blocked using a blackout hide, within which indirect, diffused LED strip lights (Brillihood - LED-Batten-4FT-36 W, 2950 lumen, frequency peaks: 450 nm & 550-600 nm) provided illumination.

Visual cues were provided by vertical black and white stripes down the centre of each wall. Three 20 mm wide black stripes were used for minnow and a single 35 mm black stripe was used for trout. Stripe width was different between experiments to accommodate the difference in flume width and guaranteed that the cues fell within the optimum visual acuity range of each species (measured from centre of flume: minnow = 0.03 cycles/degree; trout = 0.04 cycles/degree) (Nakano *et al.*, 2006; Rubin, 2012). Three stripes were used for minnow to improve visual cue detection in the narrower channel. For the control trials, both walls were devoid of any visual cues. A Logitech webcam (HD Pro Webcam C920; 30 fames/second, Resolution = 1080p) positioned 90 cm above the centre of the experimental area captured fish movement and behaviour.

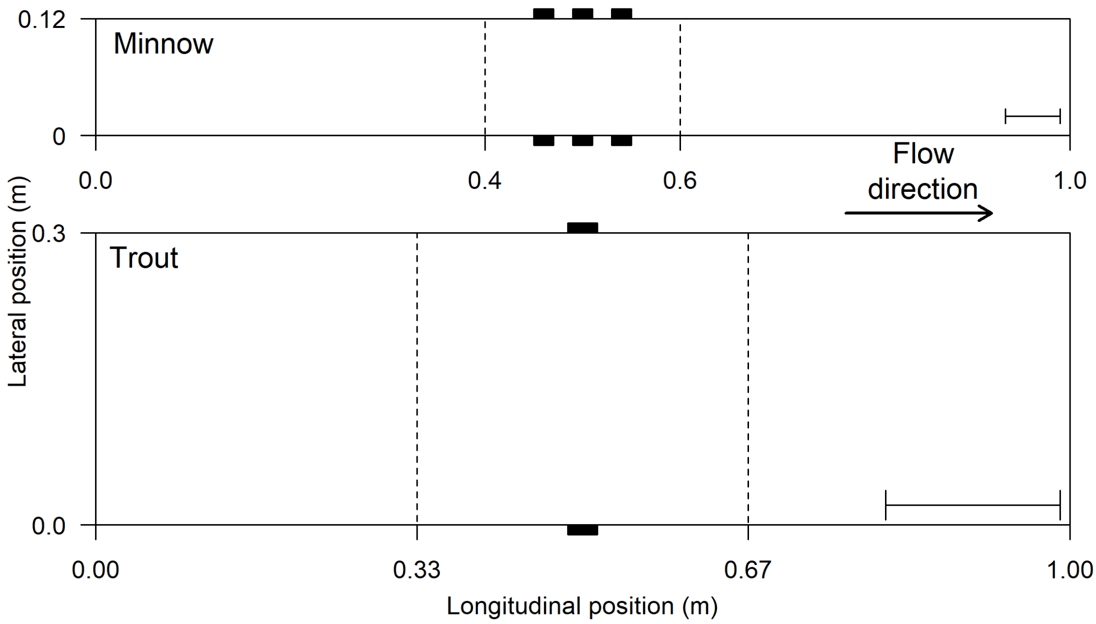

Figure 5.1. Plan view of the experimental set-up for minnow (top) and trout (bottom). The experimental area was isolated from the rest of the flume using flow straighteners (upstream and downstream). Dotted lines display the central zone and small black boxes at the sides of the flume the position of the visual cues (vertical stripes). Flow was from left to right. The scale bar in the bottom left of each box represents the mean fish fork length for that species.

5.2.2 Flow velocities

Both experiments were conducted at three flow velocities (low, mid and high; Table 5.1). For minnow, the velocities were approximately 1.5, 2.5, and 3.5 body lengths per second (BL s^{-1}) and within the sustained swimming speed measured in an open channel (Holthe *et al.*, 2009). For trout, the velocities were approximately 1, 2 and 3 BL s^{-1} and also within their sustained swimming speed (Clough *et al.*, 2004). Velocity was measured at five locations front to back of the experimental area at 50% water depth using a Nortek Vectrino+ 16 MHz Acoustic Doppler Velocimeter (ADV) at a sampling frequency of 50 Hz for 30 seconds in each location. As the experimental area was wider for trout than that of minnow, three lateral measurements were also taken, totalling 15 locations. Only measurements with a signal-to-noise ratio (SNR) > 20 dB and a correlation > 80% were used to estimate flow velocity. Water depth was maintained at 10 cm (minnow) and 12.0 cm (trout) for all velocity regimes.

Table 5.1. Summary of mean flow velocities (± SD) and sample sizes for each treatment group.

Species	Velocity	Velocity (cms^{-1})	SD	Sample size (N)	
				Control	Treatment
Common minnow (*Phoxinus phoxinus*)	Low	9.0	± 1.2	16	21
	Mid	15.0	± 1.7	15	20
	High	19.3	± 1.5	16	21
Brown trout (*Salmo trutta*)	Low	17.5	± 0.2	10	14
	Mid	33.3	± 0.3	10	15
	High	45.4	± 0.2	10	14

5.2.3 Capture and maintenance of experimental fish

Common minnow (n = 109; mean fork length = 54 ± 6 mm; mean weight = 2.07 ± 0.74 g) were collected from the River Itchen (Riverside Park, Southampton, UK, lat: 50°56'05.2'N lon: 1°22'23.9'W) on 25 February and 5 March 2021 using a 5 m seine net. They were transported to holding tanks at the University of Southampton in 50 L containers of aerated river water. Minnow were acclimated in a 200 L perforated, aerated container within the flume sump as the water temperature could be controlled to closely match that of the river (11 °C). Water temperature was slowly increased over three days to 15 °C, which was ambient for the laboratory. Prior to use in the trials, minnow were moved to four 120 L holding tanks (mean temperature = 15.8 ± 0.6 °C; maximum stocking density = 0.59 kg m^{-3}) for a minimum of 72 hours. Brown trout (n = 76; mean fork length = 179 ± 9.9 mm; mean weight = 76.9 ± 11.4 g) were collected from a nearby trout farm and transported to the University of Southampton in a 250 L aerated tank on 10 January 2020. Fish were held in a 1200 L temperature-controlled holding tank (mean temperature = 10.9 ± 0.7 °C; maximum stocking density = 5.84 kg m^{-3}) for 72 hours prior to use in trials. Feeding and water quality testing was performed daily for both species and regular water changes (50%) were performed to ensure high water quality was maintained (ammonia < 0.25 mg L^{-1}, nitrite < 0.25 mg L^{-1}, and nitrate < 50 mg L^{-1}). Lighting regimes matched the natural photoperiod throughout the experimental period for both minnow and trout.

5.2.4 Experimental Protocol

Prior to trials commencing, minnow and trout were placed in flume water for 15 minutes and overnight, respectively, to adjust to the water temperature. Fish were released into the experimental area and allowed to volitionally explore. Recording began as soon as the fish were placed into the experimental area. After 30 minutes had elapsed, the fish were removed from the flume before being measured (fork length, mm) and weighed (g). Fish length (ANOVA: Minnow: $F_{3,104} = 0.76$, $P = 0.51$; Trout: $F_{3,72} = 0.31$, $P = 0.82$) and weight (ANOVA: Minnow: $F_{3,104} = 0.62$, $P = 0.61$; Trout: $F_{3,73} = 0.71$, $P = 0.55$) did not differ between treatments. Treatments were pseudo-randomised to minimise any potential effects from confounding variables. The mean ± SD flume water temperature did not differ between treatments throughout the experimental period (ANOVA: Minnow: 15.0 ± 0.6°C; $F_{3,105} = 0.94$, $P = 0.43$; Trout: 12.6 ± 0.31°C; $F_{3,73} = 0.74$, $P = 0.48$).

5.2.5 Behavioural metrics

Video data was processed in Matlab (The MathWorks, 2018) using custom automated fish-tracking software that recorded the coordinates of the fish's head every third of a second for the entire 30-minute trial by measuring changes in contrast between the fish and the background. The position of the fish was taken from the upstream end of the fish's silhouette, which was deemed to be the approximate location of the eye and therefore the most appropriate measure of position relative to the visual cues for this chapter. The first five minutes of each trial were excluded from analysis to provide time to acclimate to the flow and exclude any erratic behaviour associated with introduction to a new environment. No startle responses or escape behaviour were observed after this period. Using the coordinates recorded, three metrics were calculated to analyse the behaviour of fish relative to the central (test) zone between treatments, A*ssociation, Number of visits,* and *Visit duration* (Table 5.2). Fish located in the central zone were deemed to be associating with visual cues under the treatments (Figure 5.1). The same metrics were used for both species.

Table 5.2. Definitions of the behavioural metrics devised to statistically analyse fish behaviour during each trial.

Behavioural metric	**Definition**	**Transformation**	
		Minnow	**Trout**
Association	Percentage of trial time spent within the central zone (%)	Square-root	Natural log
Number of visits	Number of visits to the central zone from either the front or the back of the experimental area	Square-root	Square-root
Visit duration	Time spent in the central zone on any one occasion (s)	Natural log	Natural log

5.2.6 Statistical analysis

Linear models were constructed in R (R Core Team, 2020) and used to assess all metrics. Shapiro-Wilk and Levene's tests were performed to check for normality and homogeneity of variance, respectively. Where appropriate natural log or square-root transformations were performed to normalise the response variables (Table 5.2). The behavioural metrics were used as response variables with visual cue and flow velocity as explanatory variables. Interactions between explanatory variables were assessed for both species. Chi-square and F statistics were calculated using the car package (Fox and Weisberg, 2014), and post-hoc tests using the phia package for analysis of interaction terms (De Rosario-Martinez, 2015).

5.3 Results

5.3.1 Association with the central zone

The only explanatory variable to affect the proportion of time minnow spent in the central zone was visual cue treatment (ANOVA: $F_{5,101} = 16.0$, $P < 0.001$). On average, minnow spent six times longer in the central zone when visual cues were present (median [IQR]: 28.5 [31.7] % of trial time) compared with the control (when visual cues were absent) (4.3 [6.7] %; ANOVA: $F_{1,101} = 72.4$, $P < 0.001$; Figure 5.2a). The proportion of time associating with visual cues was not related to flow velocity (ANOVA: $F_{2,101} = 1.33$, $P = 0.27$).

For trout, the proportion of time spent in the central zone was greater in the presence of visual cues (ANOVA: $F_{(1, 67)} = 7.7$, $P = 0.007$). Trout also spent a greater proportion of time in the central zone at higher flow velocities (ANOVA: $F_{(2, 67)} = 11.7$, $P < 0.001$). However, no interaction between visual cue and flow velocity was detected in the full model (ANOVA: $F_{2,67} = 1.07$, $P = 0.35$). Therefore, flow velocity did not influence the proportion of time trout spent associating with visual cues. Overall, trout spent (median [IQR]) 20.5 [15]% of time in the central zone when visual cues were present compared with 14.0 [11.3]% in the control, and 23.9 [11.6]% at the highest flow velocity compared with 11.5 [6.7]% at the low velocity (Figure 5.2b). Association was greater at the high flow velocity compared with mid (ANOVA: $F_{1,67} = 6.9$, $P = 0.02$) and low flow velocity (ANOVA: $F_{1,67} = 22.6$, $P < 0.001$), and association at the mid flow velocity was greater than the low flow velocity (ANOVA: $F_{1,67} = 4.7$, $P = 0.04$).

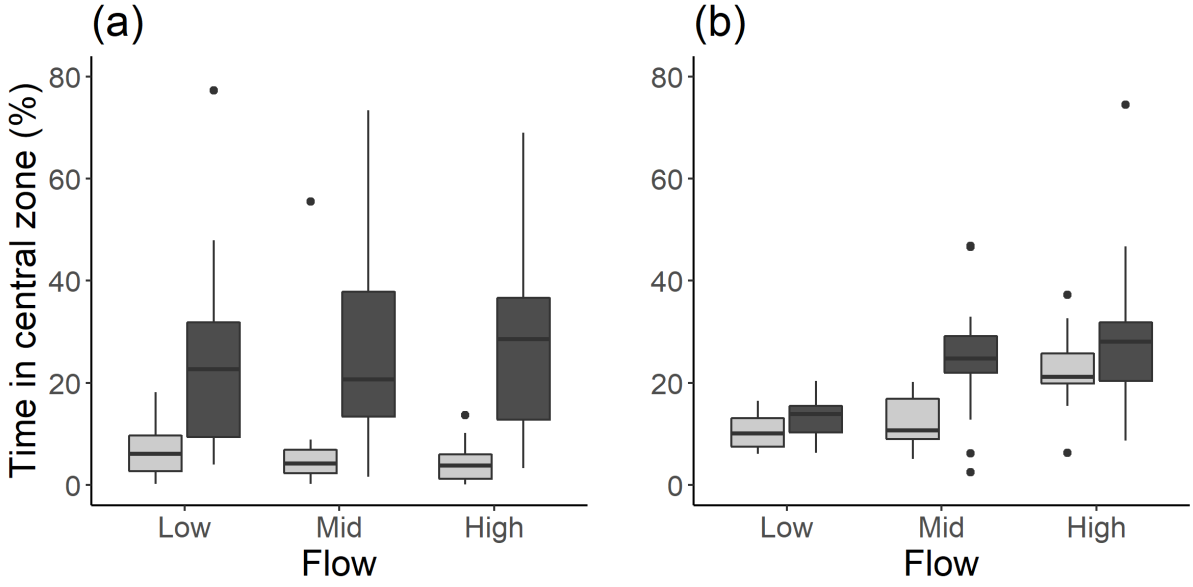

Figure 5.2. The percentage of time spent in the central zone of a flume with (dark grey) and without visual cues (light grey – the control) under three flow velocities for (a) common minnow and (b) brown trout. Boxes show the lower quartile, median and upper quartile and the whisker displays the range of data within 1.5 x the IQR. The points represent outliers (data beyond these limits).

5.3.2 Number of visits to the central zone

For minnow, neither the presence of visual cues (ANOVA: $F_{1,101} = 1.32$, $P = 0.25$) nor flow velocity (ANOVA: $F_{2,101} = 0.15$; $P = 0.86$) affected the number of visits to the central zone (Figure 3a).

For trout, velocity influenced the number of visits to the central zone (ANOVA: $F_{2,69}$ = 27.5, P < 0.001) but the presence of visual cues did not (ANOVA: $F_{1,67}$ = 2.33, P = 0.13). There was no interaction between fixed factors (ANOVA: $F_{2,67}$ = 0.03, P = 0.97). Overall trout made over twice as many visits to the central zone at the highest (median [IQR] = 114 [57.2]) compared with the lowest velocity (median [IQR] = 54.5 [30.2]; Figure 5.3b). Post hoc tests indicated a greater number of visits to the central zone at the high compared with the mid (ANOVA: $F_{1,67}$ = 25.9, P < 0.001) and low flow velocities (ANOVA: $F_{1,67}$ = 50.1, P < 0.001) and at the mid compared with the low flow velocity (ANOVA: $F_{1,67}$ = 4.2, P = 0.045).

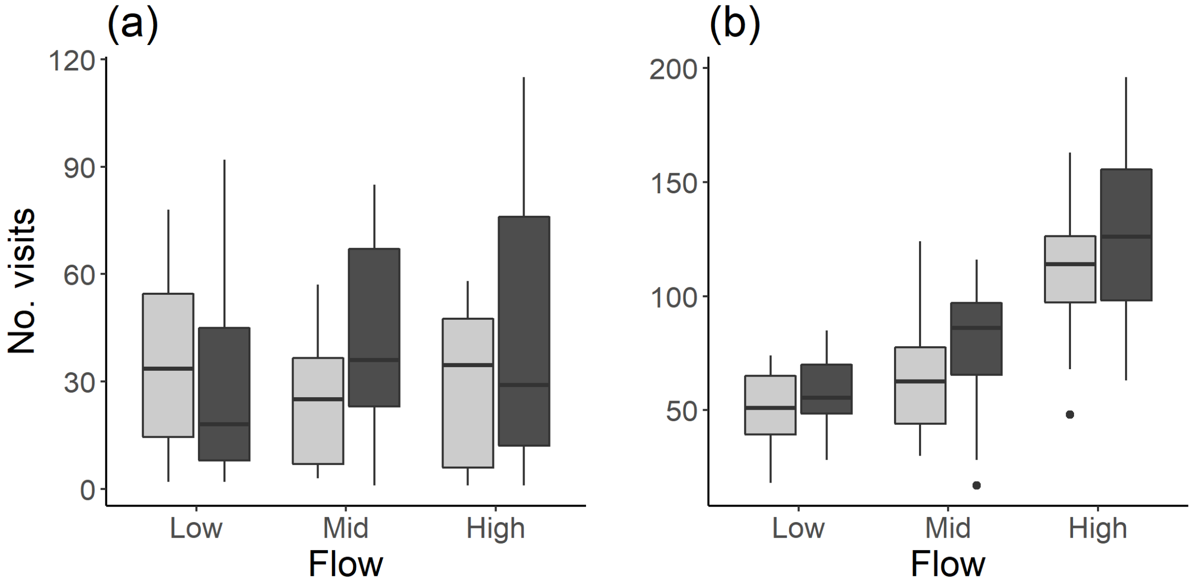

Figure 5.3. The number of visits to the central zone for the control (light grey) and visual cue treatment (dark grey) at the three flow velocities for (a) common minnow and (b) brown trout. Note the difference in y-axis scale. Boxes show the lower quartile, median and upper quartile and the whisker displays the range of data within 1.5 x the IQR. The points represent outliers (data beyond these limits).

5.3.3 Duration of visits to central zone

For minnow, the median [IQR] duration of each visit to the central zone was approximately three times longer when visual cues were present (6.74 [7.12] s) compared with the control (2.10 [1.11] s; ANOVA: $F_{(1,89)}$ = 107.3, P < 0.001). There was no difference in duration of visits across the three flow velocities (ANOVA: $F_{2,89}$ = 0.77, P = 0.46; Figure 4a) nor was there an interaction between explanatory variables (ANOVA: $F_{2,89}$ = 0.81, P = 0.45).

For trout, the duration of visits to the central zone did not differ with visual cue (ANOVA: $F_{1,66}$ = 2.72, P = 0.10) or flow velocity (ANOVA: $F_{2,66}$ = 2.61, P = 0.08;

Figure **5.4**b).

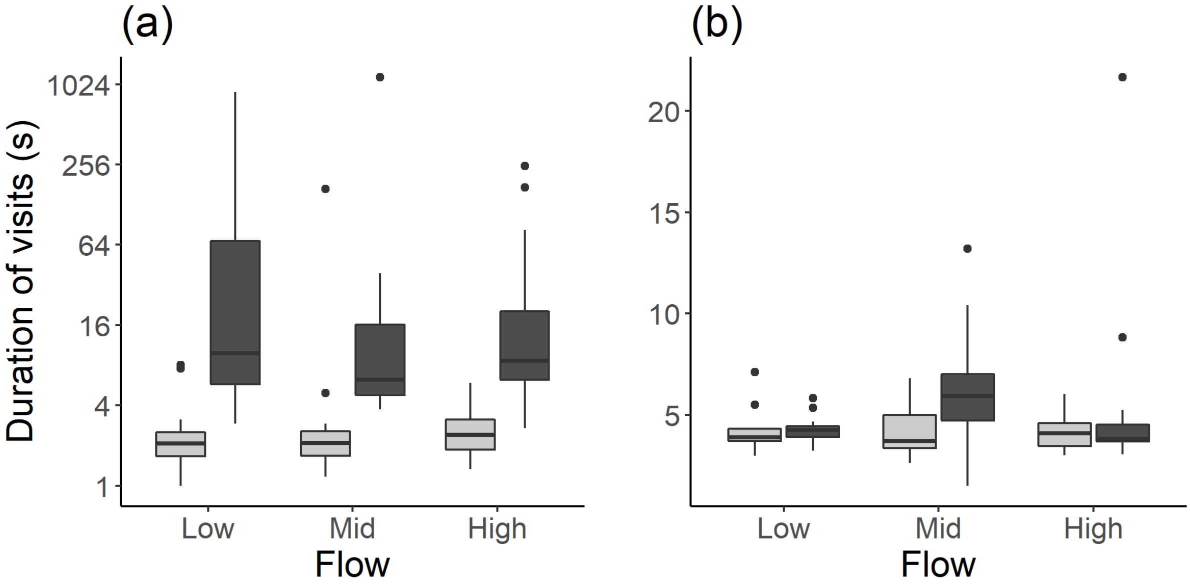

Figure 5.4. Median duration of visits to the central zone for the control (light grey) and visual cue treatment (dark grey) at the three flow velocities for (a) common minnow and (b) brown trout. Note the difference in y-axis scale - the scale for minnow has been log transformed. Boxes show the lower quartile, median and upper quartile and the whisker displays the range of data within 1.5 x the IQR. The points represent outliers (data beyond these limits).

5.4 Discussion

This chapter tested a 'Station Holding Hypothesis' which suggests that the strong association with static visual cues observed by fish in flowing water is driven by energetic benefits gained through an enhanced ability to maintain position due to the provision of spatial points of reference. Based on this hypothesis, it was predicted that fish will (a) associate with static visual cues and (b) associate more strongly at higher flow velocities. Interspecific differences in the influence of flow velocity on response to visual cues was tested using two common stream-dwelling species. In support of the first prediction, both common minnow and brown trout associated with the static visual cues, although the nature of response varied by species. Minnow, a shoaling fish, showed a strong and consistent association with visual cues, but this was independent of flow velocity, contradicting the second prediction. By comparison, trout, a species well adapted to holding position in flowing water (Bleckmann *et al.*, 2012), spent more time in the central zone at higher velocities but this was irrespective of the presence of visual cues. Overall, there was weak association with visual cues for trout overall, with a tendency at high velocity to exhibit exploratory behaviour rather than to hold station. The relatively low association with visual cues (trout) and the lack of a relationship with flow velocity (minnow & trout) contradicts the 'Station Holding Hypothesis' and suggests that responses were unlikely to reflect the use of visual reference points to control position, predominantly for energetic benefit.

This chapter documents the association with visual cues by fish in flowing water in support of previous observations (Scholtyssek *et al.*, 2014; Chapter 4). Minnow spent considerably more time in the central zone of a flume when visual cues were present compared with when visual cues were absent at all three incremental flow velocities. However, the lack of a positive relationship between association with visual cues and flow velocity contradicts the second prediction. Minnow are a highly social species and are often found in large shoals (Magurran, 1986). Isolated individuals, such as those used in this study, may perceive high levels of vulnerability to threats and experience elevated stress levels when not part of a group (Short *et al.*, 2020). Rather than benefitting energetically from the use of static visual cues through station holding, the observed association may instead be driven by refuge seeking if the vertical stripes are used as proxy indicators of physical structure or an opportunity for crypsis. The 'Predator Refuge Hypothesis' may explain the consistent association over all three flow velocities in this experiment, and the previously observed strong association with visual cues in static water (Chapter 4) when minimising energy expenditure while holding position is irrelevant.

Compared with minnow, trout exhibited a comparatively weak association for visual cues compared with minnow across all three flow velocities. A positive relationship between association with the central zone and flow velocity was observed but this occurred independently of the presence of visual cues. As such, trout spent more time in the central zone when visual cues were present but did not increase their level of association compared with the control at higher, more energetically costly flow velocities opposing the second prediction. Although a greater proportion of time was spent in the central zone at the highest velocity, this is likely to be because of elevated exploratory behaviour as exemplified by the large number of short duration visits to the central zone, rather than station holding alongside the visual cues. If trout were actively using the visual cues as a reference point, they would be expected to spend more time adjacent to the stripes on each visit. Instead, they explored the whole experimental area more at higher flow velocities. As for minnow, the results obtained for trout provide no evidence in support of the 'Station Holding Hypothesis'.

It was predicted that trout would associate with visual cues in flowing water in a manner consistent with that expected based on the ‘Station Holding Hypothesis’. This is because they rely on station holding as a drift feeding strategy and as a form of motion camouflage by using background matching (Donnelly and Whoriskey, 1991). For group-living minnow, which have adapted to gain information on their spatial position from other group members, it was predicted that they would utilise the stripes to a lesser degree. Nevertheless, neither species showed a positive relationship between association with visual cues and flow velocity and minnow showed an overall higher level of association compared with trout. This difference in behavioural response between minnow and trout to static visual cues contradicts the third prediction and likely reflects differences in their behavioural ecology. Minnow are social and can be found living in large groups enabling individuals within the group to benefit from reduced predatory risk and access to social information, which improves predator and resource detection, thus allowing members to allocate more time for activities that improve individual fitness (Krause *et al.*, 2002; Ward and Webster, 2016). Trout are adept at station holding, which is a multisensory process (Arnold, 1974). Therefore, fish may adjust to primarily use the sense that provides the most robust information about the flow field and their environment at specific velocities. In the case of trout, visual cues may not have been necessary because other cues (i.e. hydrodynamic) may provide the fish with enough information to control swimming at faster flows. It is well known that rheophilic fish seek predictable hydrodynamic structures, such as Kàrmàn vortices, to reduce locomotory costs (Przybilla *et al.*, 2010). As it was not possible in this experiment to stop turbulence from increasing at higher flows; fish may have received alternative information about the flow field or found different areas of the experimental zone with favourable hydrodynamics thus reducing their need for visual reference points. It has been observed that Mexican tetra (*Astyanax mexicanus*) performed rheotaxis better in turbulent conditions at low flows (Elder and Coombs, 2015): therefore, at the lowest flow trout may have located to more turbulent areas close to the flow straighteners or rested against the physical structure at the back of the experimental area. The importance of vision in the rheotactic response to flow is still debated (Arnold, 1974; Bak-Coleman, Smith and Coombs, 2015). Although the results presented here provide no further evidence for the necessity of the optomotor response or the importance of vision in eliciting rheotaxis, it was found that both minnow and trout associated with visual cues, providing further evidence that a behavioural response to visual cues is ubiquitous between species.

It should be noted that although both minnow and trout spent more time alongside the visual cues compared with the control; the overall percentage of time spent associating with cues was low (generally < 30%). This suggests that both species show interest in the visual cues but do not predominantly use them as a reference point either because: they do not provide adequate benefit; the flow velocities or experimental conditions did not encourage it, or the fish are not adapted to realise any benefit. Additionally, it was found that some fish tended to associate with visual cues far more than others and there is evidence that individual personalities can affect considerably their use of a test environment (Kareklas, Elwood and Holland, 2017). For instance, individual green sturgeon (*Acipenser medirostris*) varied in their response to visual cues during rheotaxis: most relied on mechanosensory stimuli, but some relied on visual cues (Johnston *et al.*, 2017). Furthermore, the chosen velocities used were selected based on previous literature with some trial and error. For both species the velocities used were evenly spread at approximately 1, 2 and 3 BLs^{-1}, but the range may not have been enough to detect a difference in behaviour relative to velocity and could be one explanation for the same observed response by minnow across velocity treatments. In contrast to the trout that were obtained from a hatchery, the minnow were captured from a natural river setting and therefore had more familiarity with flowing water. Studies on sticklebacks have found that fish from rivers are better than those from ponds at finding prey patches using flow and those from ponds are more likely to use visual cues (Braithwaite and Girvan, 2003). Therefore, the source of the fish may have impacted the extent to which the visual cues influenced their interspecific behaviour. As no direct measurement of energetic expenditure was made, it would be interesting to use a respirometer to assess whether the association with visual cues translates into an energetic benefit. Finally, there were subtle differences in the experimental designs between the two species (i.e. trout had one large stripe and minnow had three narrow stripes) which may account for some of the differences detected in the observed behaviour.

5.4.1 Conclusions

This chapter has demonstrated that both minnow and trout associate with visual cues in flowing water, but the association does not increase at higher more energetically demanding flow velocities. Minnow showed a greater degree of association compared with trout across all flow velocities but neither species consistently use the visual cues to help control swimming in flowing water. This suggests that their association was not related to a reduction in energy expenditure. Considering that trout are strong swimmers, which are adept at holding position in flowing water, while minnow are more social, the differences seen in their responses may reflect differences in behavioural ecology, vulnerability to predation and/or swimming capability. Finally, as station holding is a multisensory process, the role of vision may only account for part of the results observed in this chapter. Decoupling the interaction between vision and the mechanosensory cues (e.g. from the lateral line) remains challenging, and further work needs to be conducted to understand fully fish association with visual cues.

Chapter 6 The role of collective behaviour in fish response to visual cues

Summary

The benefits of collective behaviour are bestowed on members of groups, which outperform smaller aggregations in cognitive tasks. Vision determines the nature and magnitude of benefit accrued by facilitating the transfer of information between group members. In fish, association with stationary visual cues appears important, possibly for multiple reasons, which may be context dependent. Visual cues may provide a point of reference under flowing water conditions (the 'Station Holding Hypothesis') against which to track changes in position. Alternatively, visual cues may either provide a proxy indicator of physical structure in which prey may seek shelter from predators or offer an opportunity for crypsis (the 'Predator Refuge Hypothesis'). How affinity for visual cues is influenced by group membership remains unclear. Under standing water conditions, this chapter investigated the influence of: (1) group size (isolated individual, groups of five, and 20 common minnow); and (2) number of exposures, on the response to visual cues created by vertical black and white stripes. The stripes were displayed either at (a) one end of an experimental tank, while the other was uniform white, or (b) both ends. Reponses were compared with those observed under a control condition in which stripes were absent. Visual cues were pseudo-randomly presented in 15-minute treatment periods over six-hours. Three predictions were made: first, due to a more efficient flow of information, larger groups would respond more rapidly (*Rate of response*) to initial presentation of the visual cues; Secondly, assuming the 'Predator Refuge Hypothesis' is the primary explanation for an affinity for visual cues in standing water, and recognising that larger groups experience greater benefits of group membership in relation to reduced predatory risk, there would be a stronger association (*Strength of association* and *Final association*) with stripes for individuals or smaller groups. Thirdly, it was also predicted that the association with visual cues exhibited by larger groups would diminish more over time compared with smaller groups that are generally more risk averse. As expected, larger groups exhibited a faster *Rate of response* when visual cues were presented, and individual fish showed a greater *Strength of association* compared with the largest group size once they found visual cues. However, the enhanced visual cue detection and information transfer resulted in a more frequent *Final association* by larger groups compared with both smaller groups and individuals. Contrary to the final prediction, association and *Rate of response* of fish to visual cues did not decrease over time for any group size, suggesting that the response may reflect an innate

‘rule of thumb’ or that the periods of the experiment were insufficient to observe an effect of time.

6.1 Introduction

Group-living has evolved across the animal kingdom because individual members benefit from increased fitness. Although animals aggregate for a multitude of reasons, such as to reduce the probability of predation (Morrell, Ruxton and James, 2011) or find mates (Krause *et al.*, 2002), more efficient transfer of information is likely one of the most important advantages (Ward & Webster, 2016). Larger groups often perform better at cognitive tasks, such as finding food and identifying predators, than those that are smaller (Ioannou, 2017), possibly because they have a higher probability of detecting threats or changes in their environment (MacGregor, Herbert-Read and Ioannou, 2020). Social information transfer can alert unaware group members to the presence of a predator (Handegard *et al.*, 2012; van der Marel, López-Darias and Waterman, 2019) and improve overall probability of detection, allowing individuals to allocate more time to foraging rather than to being vigilant (Beauchamp, 2019). Furthermore, larger groups tend to find food more rapidly, encouraging food-sharing and enabling all members to profit from a discovery by a single member (Pitcher, Magurran and Winfield, 1982). The spread of information through a group is often governed by simple interactions between neighbours that can produce seemingly complex collective behaviours (e.g. flocking birds, schooling fish, swarming bees) without members having direct knowledge of the source.

Vision is typically the primary sense used to organise group behaviour in moving fish (Couzin *et al.*, 2007). Those within groups often align with neighbours, for example as an antipredator defence when schooling (Faucher *et al.*, 2010), or to enhance hydrodynamic performance (Ashraf *et al.*, 2017) and improve the speed and strength of visual information transfer and collective movement (de Bie, Manes and Kemp, 2020). However, the role of external visual stimuli in governing these behaviours is often overlooked (Odling-Smee, Simpson and Braithwaite, 2011; Schaerf, Dillingham and Ward, 2017). In particular, space use in relation to non-threatening visual cues has rarely been investigated, despite their importance in navigation (Odling-Smee, Simpson and Braithwaite, 2011; Sovrano, Baratti and Lee, 2020), seeking refuge from predators (Valdimarsson and Metcalfe, 1998), and rheotactic response (Bak-Coleman *et al.*, 2013).

Fish have been observed to associate with stationary cues when moving under flowing (Chapter 4 and Chapter 5 for common minnow) and static water (Sholtyssek *et al.*, 2014 for Zebrafish). This association may be explained if the visual cues provide a point of reference from which to control position and speed of movement in flowing water (the ‘Station Holding Hypothesis’, Chapter 4).

This reasoning fails, however, when considering a similar affinity is exhibited in standing water, as observed for individual minnow that had a stronger association with visual cues than small (5 fish) groups (Chapter 4). This behaviour may reflect the use of stationary visual stimuli, such as vertical black stripes, as a proxy indicator of the presence of physical structure, for example in which to seek shelter from a predator, and/or because a more complex background may reduce the risk of detection and predation by disrupting the body outline (the ‘Predator Refuge Hypothesis’). A stronger preference exhibited by isolated individuals, which are unable to benefit from the antipredator benefits of group membership, may indicate a higher perceived value for such proxy shelter (Podolsrky *et al.*, 1995; Chapter 4). Although such explanations are logical, they remain largely speculative with little supporting evidence. Further insight is needed into the mechanisms of how group size affects the use of, or response to, stationary visual cues in static water, where the value of such cues to navigation and control of position is unlikely to be as important as when in a lotic environment.

There is clear evidence that social context affects the behaviour of fish. In larger groups, where predation risk or the cost of exploiting resources is generally less severe than that for small groups and isolated individuals, members tend to recover more rapidly from a disturbance or discover resources (Webster, Ward and Hart, 2007). Conversely, smaller groups, for which the predation risk is higher, exhibit reduced exploratory behaviour and increased group cohesion (Magurran and Pitcher, 1983; Herbert-Read *et al.*, 2017). In essence, larger groups are expected to respond to changes in their environment more quickly, while smaller groups are driven to seek shelter and associate with visual cues more strongly. Considering this, the overall association with visual cues may be a trade-off between the rate of discovery and the strength of association thereafter.

While social context, such as group membership and the size of the aggregation, plays an important role in determining the behaviour exhibited, it remains unclear how it influences the nature and magnitude of the response over time. Habituation is a form of non-associative learning in which an animal decreases its responsiveness to repeated stimuli (Rankin *et al.*, 2009). For example, European perch (*Perca fluviatilis*) react strongly on first encountering a predator in an experimental setting, but exhibit a diminished response over time due to habituation (Goldenberg, Borcherding and Heynen, 2014). This demonstrates that the magnitude of behaviour, risk-taking in this case, can be time dependent, and investigations should quantify how response varies after repeated exposures to develop a fuller understanding of an animal behavioural ecology (Goldenberg, Borcherding and Heynen, 2014). It is unclear whether fish response to static visual cues remains stable over time or diminishes with repeated exposure, and what influence group membership and size has on this process. Focusing on the ‘Predator Refuge Hypothesis’, multiple contradictory hypotheses may be generated. For example, an association with a proxy indicator of refugia might be innate, i.e. individual fish follow a 'rule of thumb', and the response occurs undiminished over time, independent of group size. Alternatively, a waning in the association with visual cues over time might reflect learning that vertical stripes bestow no benefits of physical structure in which fish can hide, although visual complexity of the background may impede prey detection (Merilaita, 2003; Dimitrova and Merilaita, 2010), for example, if it disrupts the body outline. If habituation does occur, it may be expected for this to be greater and more noticeable in larger groups because members are less risk averse and more likely to recover rapidly from disturbances (Webster, Ward and Hart, 2007).

To further understand the role of: (1) collective behaviour and (2) the effect of repeated exposures on the response of fish to visual cues, individual and groups (five or 20) of minnow were pseudo-randomly exposed to a series of vertical black and white stripes displayed at the ends of a tank over six hours. Focusing on testing the 'Predator Refuge Hypothesis', it was predicted that, compared with individuals and small groups, larger groups would: (Hypothesis [H]1) detect the visual cues more rapidly (*Rate of response*); but (H2) exhibit a weaker association due to the higher security associated with group membership. It was also predicted that larger groups would exhibit: (H3) a diminishing association over time compared with the more risk averse smaller groups and individuals. Association was quantified as *Strength of association* (the proportion of time spent next to the stripes after encountering them) and *Final association* (whether fish were associating with visual cues at the end of a treatment period). The change in association over time was measured across the entire trial for all three metrics (*Rate of response, Strength of association* and *Final association*).

6.2 Method

6.2.1 Model species and husbandry

The common minnow was selected as the model due to their widespread distribution, ease of collection and maintenance in the laboratory, and tendency to form well defined shoals. Minnow (N = 336; length: 53.8 mm [SD ± 5.7 mm]; mass ± SD: 1.81 g ± 0.60 g) were caught in the River Itchen (Riverside Park, Southampton, UK, lat: 50°56'05.2'N lon: 1°22'23.9'W) on three occasions during October and November 2019 and transported in an 80 L tank containing aerated river water to the ICER facility at the University of Southampton (UK). They were maintained in a 1200 L holding tank at a mean ± SD temperature of 15.7 °C ± 1.08 °C with a photoperiod of 12:12 H generated using overhead fluorescent strip lighting on an automatic timer. Feeding and water quality checks were carried out daily and water changes (20 - 50%) performed when necessary to ensure high standards were maintained (ammonia < 0.20 mg L^{-1}, nitrite < 0.20 mg L^{-1}, and nitrate < 50 mg L^{-1}).

6.2.2 Experimental setup

Experiments were conducted using a rectangular tank (1.5 m long, 0.5 m wide, 0.6 m deep, Figure 6.1). The base and inner side-walls of the tank were covered with white laminate PVC sheeting to minimise visual cues and improve contrast between the fish and the background for automated video processing. A blackout hide erected around the tank prevented visual disturbance from external sources and indirect LED strip lighting either side of the tank (Brillihood - LED-Batten-4FT-36W, 2950 lumen, frequency peaks: 450nm & 550-600nm) provided uniform illumination during trials. A Logitech webcam (HD Pro Webcam C920; 30 fames/second, Resolution = 1080p) positioned 1.2 m above the centre of the experimental area recorded the movement and behaviour of fish during the trials.

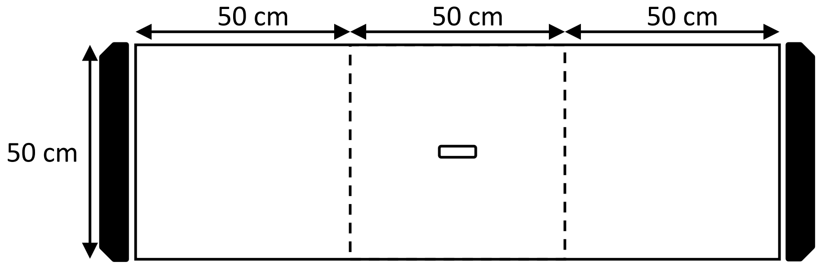

Figure 6.1. Plan view of experimental setup. Black blocks at either end of an experimental tank represent the positions of the computer monitors used to assess fish response to visual cues and the clear rectangle in the centre shows the position of an overhead camera. The dashed lines represent the three equal sized zones used in the analysis.

Visual cues in the form of vertical black and white stripes (40 mm wide) were displayed on two monitors (iiyama ProLite B2283HS) covering each end of the tank. The width of stripes matched those used in previous chapters in which the same species showed a clear response to visual cues (Chapter 4). There was no evidence that the monitor refresh rate of 60 Hz effected fish behaviour and previous studies have found that flicker fusion frequencies measured in other cyprinids (e.g. zebrafish, Makhankov, 2005) are lower than this.

6.2.3 Experimental Design and Protocol

Fish were allowed one hour to acclimate to the experimental tank prior to the start of each six-hour trial. A randomised complete block experimental design was used in which fish in each trial were repeatedly exposed to two visual cue treatments and a control and their response recorded over a total of 24 periods. Each trial comprised six blocks, each of which consisted of an initial 15-minute pre-treatment control period during which both screens presented a uniform white background, followed by three successive and pseudo-randomly selected 15-minute treatment periods during which three combinations of visual cues were presented as vertical black and white stripes displayed on the screens in a pseudo-randomised order (six blocks of one control and three treatment periods = 24 periods, Figure 6.2). In each block, all three possible combinations of stripes (both [B], left [L], right [R] monitors, Figure 6.2) were presented without replacement after the initial pre-treatment control period to ensure the design was balanced and fish in each trial received all unique transitions exactly twice (e.g. from B to L, Figure 6.2). Accordingly, there were two visual cue treatments: Treatment 1 (T1) consisted of a single-striped end (L or R monitor while the opposite remained uniform white); and Treatment 2 (T2) presented visual cues on both monitors (B). These were compared with a control (C) during which both monitors displayed a uniform white background. The order of combinations was pseudo-randomised for each trial.

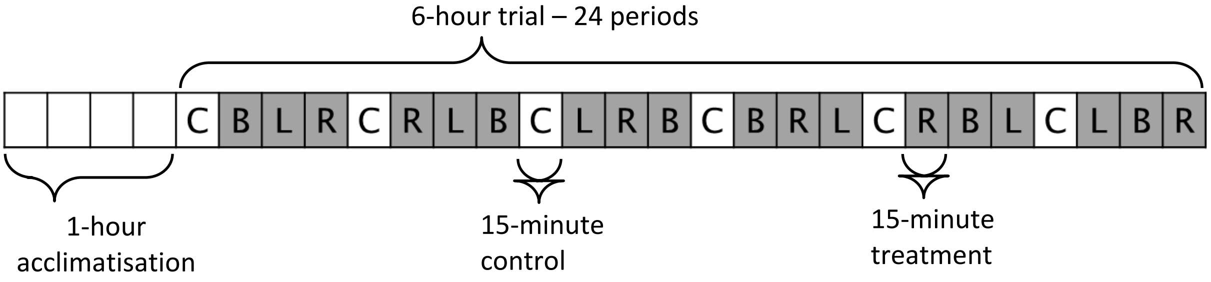

Figure 6.2. Example sequence of visual cue exposures used to assess the response of common minnow when alone or in groups of five or 20. After the acclimation period, each trial consisted of six blocks of four x 15-minute periods that included a pre-treatment control (clear boxes - C) followed by three randomly selected (without replacement) treatment periods (grey boxes) in a randomised complete block design. The three visual cue combinations comprised two treatments: T1 consisted of a single-striped end (L or R); and T2 presented visual cues on both monitors (B).

Trials were performed between 21 October and 12 December 2019 and group size treatment (individual fish or groups of five or 20) was selected in a pseudo-random fashion. The fish were selected randomly without replacement from the holding tank and used in one trial only. A total of 42 trials (individual fish: n = 15; five fish: n = 15; twenty fish: n = 12) were conducted between 08:00 and 18:00 with a single trial conducted per day. At the end of each trial, the fish were measured and weighed before being returned to a separate holding tank. Water was transferred from the holding tank to the experimental tank and 50% water changes conducted between trials. Mean ± SD water temperature in the experimental tank was 16.8 ± 0.76 °C and did not differ between group treatments ($P = 0.71$).

6.2.4 Data processing and analysis

Fish behaviour was quantified using data extracted from video footage using a customised fish tracking algorithm in Matlab (The MathWorks, 2018) that detected fish based on differences in contrast between a mean frame and each subsequent frame. The centroid of each individual within a group and the centroid of the shoal was calculated each second during the trials. From these coordinates the following metrics were quantified to assess the hypotheses: (1) *Rate of response* (H1) – the time taken for at least 50% of the group to enter the striped end of the tank in T1, or in the case of the control and T2 the opposite third of the tank to which they were located at the start of the treatment period. Periods in which fish did not actively move to associate with the visual cues because they were already located at end of the tank that displayed the stripes were removed from further analysis (Table 6.1). Similarly, treatment periods during which fish did not enter the opposite third of the tank for the entire duration of the period were also removed. This ensured that only fish that actively moved to associate with the stripes were assessed making the analysis equitable. (2) Association (H2) was measured in two ways: (a) *Strength of association,* the proportion of time spent at the striped end of the tank during a treatment period after at least 50% of the group had entered the third of the tank displaying visual cues for the first time (i.e. responded to the visual cues); and (b) *Final Association*, the third of the tank the majority of fish were located throughout the final five minutes of a treatment period. *Final association* was defined as positive when the majority (> 50%) of the group occupied positions in the striped third of the tank for the median of the final 5 minutes of a treatment period. For the control and T2; each treatment period was allocated a randomly assigned ‘striped’ end so that they could be statistically compared with T1. The final 5 minutes was chosen to allow time for fish to detect and respond to the change in visual cues displayed within the tank. *Strength of association* was measured from the first instance that the majority of fish entered the third of the tank displaying visual cues when only one-end displayed them, otherwise (for the control and T2) it was measured from the first instance that the majority of fish entered the opposite third of the tank from which they began the treatment period. Metrics were calculated for each 15-minute treatment period within trials. To assess the change over time (H3), any change in the *Rate of response*, *Strength of association* and *Final association* was measured over the entire 6-hour trial (i.e. across the 24 periods).

6.2.5 Statistical analysis

Statistical analysis was performed in R using linear mixed-effects models (LMM) and generalised linear mixed-effects models (GLMM) in the lme4 package (Bates *et al.*, 2015; R Core Team, 2020). GLMMs were used for *Final association* because the dependent variable was binary (associating with visual cues or not) and involved repeated exposures of the treatment to the same individuals over the course of each trial. Each of the three metrics were assigned as response variables in separate models with visual cue treatment, group size and treatment period as explanatory variables. An initial analysis found no significant difference between T2 and the control for each metric. Therefore, data was pooled for T2 and the control for further statistical comparison with T1. Trial number was included as a random factor in all models to control for non-independence between treatment periods. Interactions were considered and all explanatory variables included in the final model. A measure of the change in response over time (H3) was included in each of the models as an interaction between group size and treatment period. Model fit was assessed using the DHARMa package (Hartig, 2020), and post-hoc comparisons and interactions were assessed using the phia package (De Rosario-Martinez, 2015). The *Rate of response* and *Strength of association* metrics were log and arcsine square-root transformed, respectively, to meet normality assumptions before performing a LMM. The *Final association* metric was analysed using a binomial GLMM.

6.3 Results

6.3.1 Rate of response to visual cues

Groups size was the only explanatory variable to affect the *Rate of response* to a change in visual cue. The *Rate of response* did not differ between the visual cue treatments or control for all group sizes (LMM; individuals: χ^2_1= 3.5, P = 0.18; five: χ^2_1= 1.3, P = 0.52; twenty: χ^2_1= 0.39, P = 0.53). However, as predicted (H1) the largest groups (20 fish) moved more rapidly to the opposite end of the tank (T2 and C) or the visual cues (T1) (median [IQR]: 28.5 [60.0] s) than groups of five fish (median [IQR]: 65.0 [136.0] s; LMM: X^2_1= 7.0, P = 0.016) or individuals (median [IQR]: 96.0 [300.0] s; LMM: χ^2_1 = 23.6, P < 0.001; Figure 6.3), with groups of five fish groups responding faster than individual fish (LMM: χ^2_1= 5.6, P = 0.018).

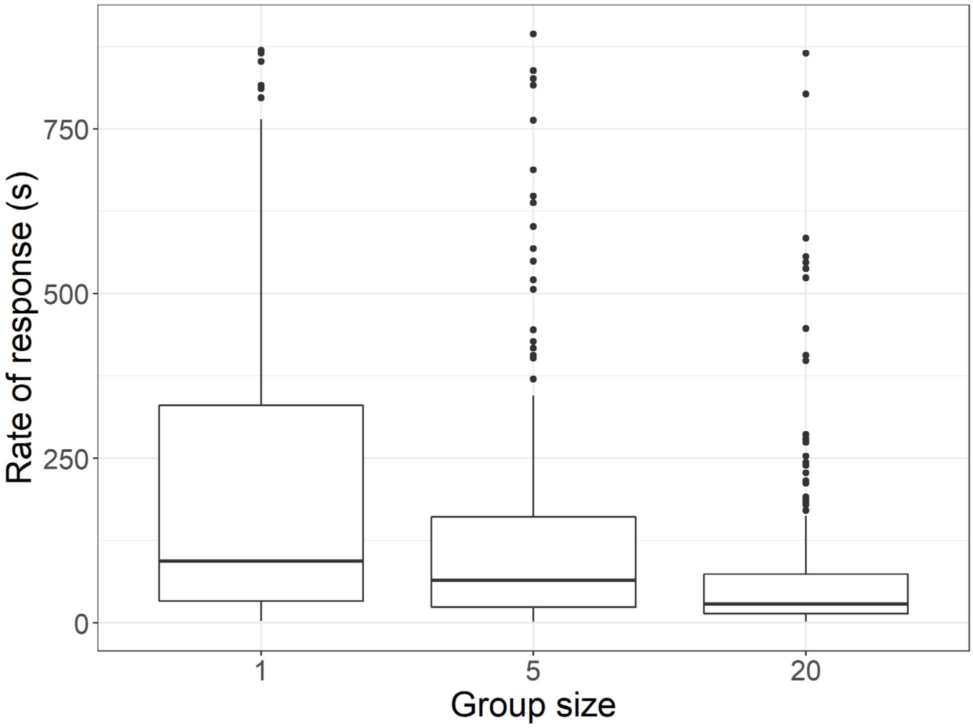

Figure 6.3. Median time for at least 50% of the group to move into: the opposite third of the tank (T2 and C); or towards visual cues (T1) for all three group sizes (*Rate of response*). Data for all visual cue treatments has been combined. Boxes show the lower quartile, median and upper quartile and the whisker displays the range of data within 1.5 x the IQR. The points represent outliers (data beyond these limits).

Across all trials, groups of twenty fish failed to enter the opposite third of the tank to which they started on only two occasions, compared with 106 occasions for individual fish (Table 6.1). Individual minnow were least likely to explore the opposite end of the tank from which they started when one screen only displayed visual cues (T1), whereas groups of five were least likely when both screens displayed visual cues (T2). Both individuals and groups of five were most likely to explore the opposite end of the tank from which they started during the control (Table 6.1).

Table 6.1. Percentage (%) of exposures during which fish did not enter the opposite third of the tank from which they started for each visual cue treatment and group size.

Group size	Control	T1	T2
1	30.2	39.8	35.5
5	10.8	13.5	20.5
20	3.3	0.0	0.0

6.3.2 Strength of association

When considering all three group sizes (one, five and 20 fish), fish that entered the third of the tank that displayed visual cues in T1 showed a strong association, spending a median [IQR] of 72.1 [38.1] % of the remainder of the treatment period in that section compared with 49.2 [50.2] % when both screens displayed visual cues (T2) and 45.0 [41.6] % during the control. There was no difference in association between the control and T2 (LMM: χ^2_1= 2.01, P = 0.15; Figure 6.4).

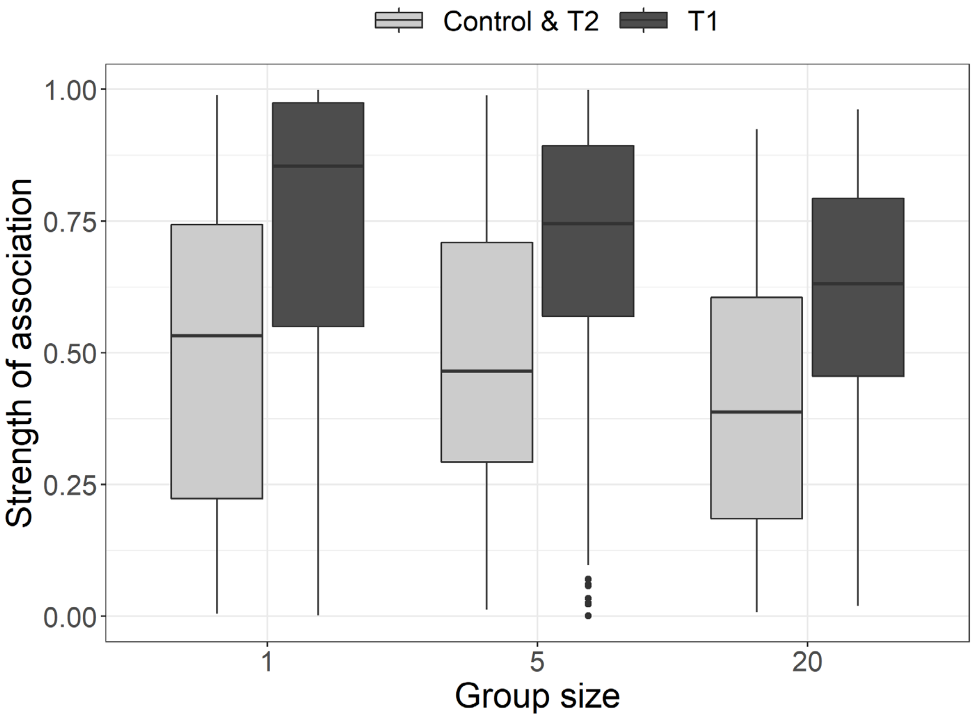

Figure 6.4. The proportion of time at least 50% of the group spent associating with the visual cues once they had entered the third of the tank that displayed them (T1; dark grey), or in the opposite third to which they were initially located during the control & T2 (light grey). The light grey boxes contain data from exposures during which both screens displayed visual cues (T2) and when both were uniform white (control). Boxes show the lower quartile, median and upper quartile and the whisker displays the range of data within 1.5 x the IQR. The points represent outliers (data beyond these limits).

In support of the second prediction (H2), the largest groups (20 fish) exhibited a weaker association with visual cues or randomly assigned side than individuals during both the control (LMM: $\chi^2_1 = 12.3$, $P = 0.002$) and T1 (LMM: $\chi^2_1 = 13.2$, $P = 0.002$). However, no difference in association was detected between individuals and groups of five fish, or between groups of five and twenty, during the control or T1 (LMM: one-five: $\chi^2_1 = 2.37$, $P = 0.12$; five-twenty: $\chi^2_1 = 3.27$, $P = 0.07$). All group sizes associated more strongly with visual cues in T1 compared with T2 and the control (LMM: one fish: $\chi^2_1 = 32.7$, $P < 0.001$, five fish: $\chi^2_1 = 43.6$, $P < 0.001$; twenty fish: $\chi^2_1 = 45.1$, $P < 0.001$, Figure 6.4).

6.3.3 Final association

During T1, groups of 20 (GLMM: $\chi^2_1 = 35.6$, $P < 0.001$) and five (GLMM: $\chi^2_1 = 17.3$, $P < 0.001$) fish spent the final five minutes of each treatment period associated with the striped end more often than the randomly assigned end in the control and T2. This was not observed in individuals (GLMM: $\chi^2_1 = 3.4$, $P = 0.07$; Figure 6.5).

Final association did not differ with group size in the control (GLMM: $\chi^2_1 = 0.06$, $P = 0.79$). However, during T1 groups of 20 fish were more likely to associate with visual cues at the end of a treatment period, with this being the case in 83.3% of occasions compared with 75.6% for five fish and 58.0% for individuals (Figure 6.5). Overall, groups of 20 and five fish occupied the striped end of the tank more often than individuals (GLMM: twenty: $\chi^2_1 = 20.0$, $P < 0.001$; five: $\chi^2_1 = 7.9$, $P = 0.024$), contradicting the second prediction (H2). *Final association* did not differ between groups of 20 and five fish (GLMM: $\chi^2_1 = 3.89$, $P = 0.19$).

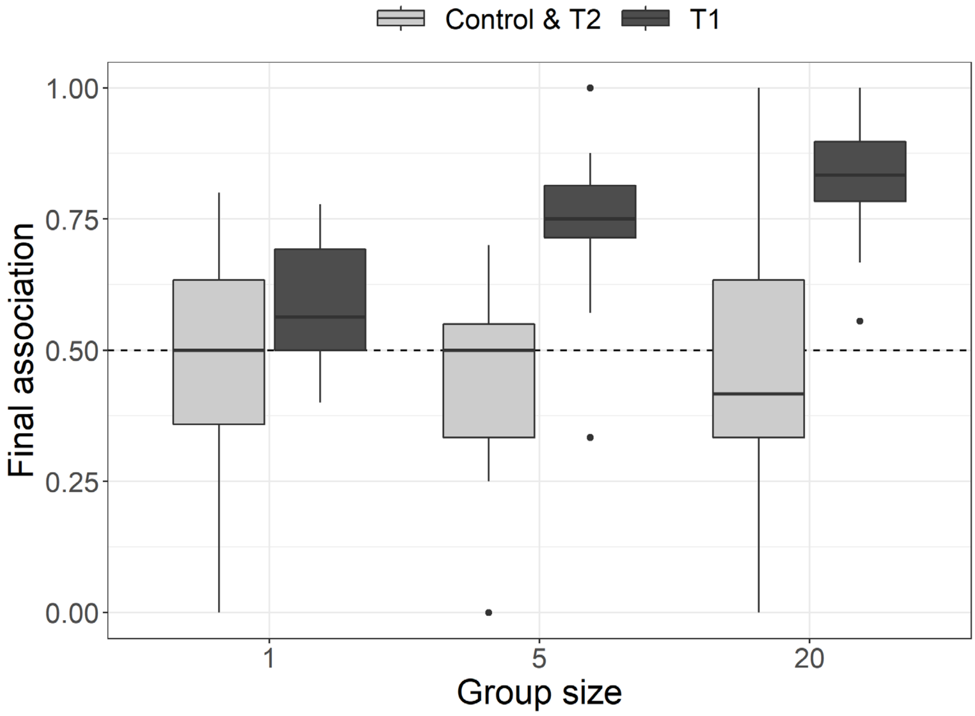

Figure 6.5. The proportion of exposures during which at least 50% of the group were at the (1) striped end of the tank (T1; dark grey) or (2) randomly assigned end (Control & T2; light grey) at the end of the treatment period. Boxes show the lower quartile, median and upper quartile and the whisker displays the range of data within 1.5 x the IQR. The points represent outliers (data beyond these limits).

6.3.4 Change in response to visual cues over time

Change in fish response to visual cues over time was measured for all three metrics across group size. In contradiction to the final prediction (H3), the *Rate of response* (LMM: $F_{1,37} = 0.66$, $P = 0.41$), *Strength of association* (LMM: $F_{1,38} = 0.07$, $P = 0.78$) and *Final association* (GLMM: $F_{1,38} = 0.01$, $P = 0.92$) of fish to visual cues did not decrease during the trials for any of the group sizes.

6.4 Discussion

The primary aim of this chapter was to determine how the response of fish to static visual cues is influenced by group size (individuals or shoals of five or 20 members) in the context of a 'Predator Refuge Hypothesis' that predicts that fish will associate with a black and white striped background in standing water because the stripes may provide a proxy indicator of physical structure in which to shelter from predators and/or because a more complex background disrupts the outline of the fish, both of which are likely to reduce predation risk. As expected, when presented with visual cues, the larger shoals of minnow associated with the striped backgrounds faster than the smaller groups and individuals, suggesting that a greater availability of social information provided by others led to a more rapid *Rate of response*. However, despite frequently being associated with the visual cues at the end of each treatment period, on average such association was weaker for the larger groups, presumably because the anti-predator benefits of group membership partially off-set the value of a complex background that is of greater importance to the smaller groups and solitary fish. Finally, and in contradiction to the final hypothesis, there was no evidence of habituation to the visual cues over the time-scales of the experiment, as association did not diminish over time for any of the group sizes tested. Overall, the results indicate that membership of larger groups result in a more rapid *Rate of response*, likely as a result of more efficient information transfer between conspecifics, and frequent *Final association* with the visual cues at the end of each treatment period, while the *Strength of association* with visual cues is higher for individuals than for shoals, presumably because alternative anti-predator strategies are required in the absence of opportunities to join a group.

In support of the first hypothesis, the largest group reacted the most rapidly to the presentation of black and white stripes at the ends of the tank. On average, groups of 20 fish responded to the visual cues more than twice as quickly as shoals of five fish, and in approximately one-third of the time taken by individuals. In the absence of flowing water and a predatory threat, the largest groups formed loose shoals in which members tended to be haphazardly oriented, thus enhancing the collective panoramic visual field [although potentially increasing individual occlusion by neighbours depending on position within the group, Davidson *et al.* (2021)], and increasing the probability of detecting, monitoring and transferring information related to changes in their environment when compared with smaller groups and individuals (MacGregor, Herbert-Read and Ioannou, 2020). A more rapid response by the largest groups was also observed when both ends of the tank displayed the stripes and under the control condition in which visual cues were absent, indicating that larger shoals tended to be more exploratory. This elevated exploratory behaviour exhibited may reflect greater safety in numbers experienced by larger groups (Mathiron, Crane and Ferrari, 2015) and the potential to maintain shoal integrity despite occasional dispersal of a proportion of individuals (Hoare *et al.*, 2004), perhaps to access environmental information over greater spatial scales to be later shared with the wider group. In contrast, individual fish are likely to exhibit heightened antipredator responses and less exploratory behaviours than group members (Magurran, Oulton and Pitcher, 1985; Beauchamp, 2019). In this study, compared with groups a large proportion of individuals failed to associate with the stripes or enter the opposite third of the tank to that in which they were located when the treatment period commenced, and particularly when visual cues were displayed at their starting end. This may have been because individual fish did not have access to the social information afforded to group members and may have been less motivated to seek alternative refuge even though the visual cues with which they were associating periodically disappeared.

The results supported the second hypothesis that the association with visual cues would be weaker (i.e. lower *Strength of association*) for the largest group. Despite being more exploratory and responding more rapidly to the visual cues, groups of 20 fish had a lower *Strength of association* than individuals because those that did initially associate with the visual cues tended to continue to do so. The result agrees with those obtained in Chapter 4, which also observed individual minnow associating more strongly with visual cues than groups of five in both static and flowing water, perhaps because they were more inclined to seek the visual/ cryptic refuge provided by vertical black and white stripes in an otherwise homogeneous environment. The observation that under static water conditions all three group sizes associated with visual cues after their initial encounter, and the observation that the *Strength of association* was highest for individual fish unable to benefit from the antipredator advantages of group membership, provides evidence in support of the ‘Predator Refuge Hypothesis’. Interestingly, the larger groups (of 20 fish) exhibited more frequent *Final association* (at the end of each treatment period) with visual cues than individuals, illustrating the importance of the complex background despite their exploratory tendencies. Larger groups of fish have been shown to make better cognitive decisions through more efficient social information transfer (Ward *et al.*, 2011), and in this experiment the larger groups were better able to respond rapidly to the display of visual cues and benefitted more frequently from any anti-predator benefits bestowed than the smaller groups and individuals.

The final prediction was that larger groups would display a decreased association with visual cues over time compared with smaller groups and individuals that are more likely to remain risk averse. In fact, no temporal changes in fish response relative to the metrics measured were observed, at least not over the time scales of this study, in any of the three group sizes. Fish did not alter their *association* with, or *Rate of response* to, the display of visual cues, suggesting that the reaction is an innate behaviour and not governed by habituation. The responses appear unlikely to be driven by inquisitiveness, but rather by an instinctive drive to associate with structures or complex backgrounds, which in turn may provide hydrodynamic refuge (in lotic environments) or reduce predatory risk (Everett and Ruiz, 1993).

6.4.1 Conclusions

As predicted, larger groups tended to react faster and more frequently to the presence of visual cues; although once a striped background had been encountered smaller groups tended to associate more strongly. The results primarily reflect the increased efficiency with which larger groups initially associated with the visual cues, because of their increased exploratory behaviour. Interestingly, over the course of the experiment there was no evidence of habituation to the complex background, suggesting that association may be an innate behavioural response. Given how reliably fish groups associate with visual cues, several applications may emerge; such as facilitating management of fish within aquaculture systems, or the development of fish guidance technology by offering a passive and inexpensive attractant in a field that typically employs repellents. Further work is needed to quantify the nature and magnitude of responses to visual cues for a range of species to validate the findings of experimental studies in field settings.

Chapter 7 Do environmental visual cues alter the structure of fish shoals in flowing water?

Summary

Fish use vision to align with members of a shoal and enable efficient information transfer, to coordinate group movement and to reduce energetic expenditure. In flowing water, groups of fish have also been shown to associate with environmental visual cues and it has been hypothesised that this helps control swimming (the ‘Station Holding Hypothesis’), as a means of reducing energetic expenditure. However, it remains unclear whether the presence of environmental visual cues reduces the necessity for shoaling fish to align with other group members. This chapter compared the formations adopted by groups of five and two fish (European minnow) in flowing water in the presence and absence of visual cues (vertical black stripes on opposite walls of an open channel flume). It was hypothesised that when visual cues were present: (i) group members would align more laterally (shoal structure), spanning the channel to gain information about their position from their environment, and (ii) group sub-structure (group polarisation and distance to the nearest neighbour) would be more variable as members rely to a lesser extent on others for information about their spatial position. With access to visual cues both groups of five and pairs aligned more side-by-side in comparison with the control. Pairs of fish consistently associated (defined as the proportion of time alongside visual cues) with visual cues. This suggests that the presence of environmental visual cues does alter the shoal structure. However, group sub-structure (polarisation or distance to their nearest neighbour) remains unaffected. Although the whole group associated with visual cues, fish continued to align with other group members presumably to benefit from formations that provided a reduction in energetic cost (e.g. because of altered hydrodynamics) which could not be gained by aligning with the environmental visual cues. Further studies could directly measure energetic expenditure of fish shoals in the presence and absence of environmental visual cues to better quantify the magnitude of any energetic benefits, which, based on the small effect size presented in this chapter, are predicted to be minimal.

7.1 Introduction

Vision is often the primary sense used by vertebrates to view and perceive their environment allowing them to navigate, interact with conspecifics, detect predators, and find food and potential mates. Vision is also of fundamental importance for the collective behaviour of animals, which can be beneficial because larger groups have a higher probability of detecting changes in their environment (Lima, 1995; MacGregor, Herbert-Read and Ioannou, 2020; Chapter 6). Consequently, when in a group, all members can benefit from discoveries made by the minority thus reducing the onus on individual members to remain vigilant of predators and to find food patches. This allows them to invest more time in other activities that increase their fitness such as feeding or finding a mate (Krausz, 2013). However, there are costs to collective living such as disease, competition for resources and stress, which can make collective decisions and maintaining group cohesion challenging (Ford and Swearer, 2013; Tindale and Winget, 2019).

Fish are known to be one of the most social vertebrate groups with some species forming aggregations in their tens of millions (Makris *et al.*, 2006). Studies have shown that vision plays an essential role in the coordination of group behaviour in fish (Partridge and Pitcher, 1980). Numerous attempts have been made to replicate the mechanics of group movement using simple rules that generally govern interactions between neighbouring members (Gautrais *et al.*, 2009; Katz *et al.*, 2011; Crosato *et al.*, 2017). For example, visual interaction networks have been created for groups of golden shiners (*Notemigonus crysoleucas*) to further understand the propagation of behaviour across the group (Strandburg-Peshkin *et al.*, 2013). In addition to coordinating movement, Weihs (1973) first demonstrated that groups of fish can optimise their energy expenditure by adopting formations that allow individuals to exploit hydrodynamic structures produced by conspecifics. The two main mechanisms, which work by reducing the relative flow velocity in the direction of fish movement are the channelling effect and the vortex hypothesis. The channelling effect is created when two individuals swim side-by-side reducing the relative flow velocity between them (Wang and Wu, 2011), and the vortex hypothesis allows individuals to benefit from the vortices produced in the wake of individuals in front of them (Weihs, 1973). The optimal formation to take advantage of the vortex hypothesis is a diamond, but the channelling effect works well with rectangular formations when individuals are side by side (Weihs, 1973; Daghooghi and Borazjani, 2015). Additionally, several studies have demonstrated that shoal structure is influenced by flow velocity. At high flow velocities (> 15 ms^{-1} for red-nose tetra and > 11 cms^{-1} for common minnow) fish swim in a side-by-side formation taking advantage of the channelling effect and synchronising their tail-beat frequencies to gain an energetic advantage (Ashraf *et al.*, 2017; de Bie, Manes and Kemp, 2020). Whereas in still or slow-flowing water groups tend to show reduced cohesion and polarisation (Ashraf *et al.*, 2017; Shelton *et al.*, 2020). It was hypothesised that a side-by-side formation facilitates visual information transfer, helping to maintain alignment and tail beat synchronicity in fast flowing water (de Bie, Manes and Kemp, 2020). The importance of collective behaviour in faster flowing water can be seen in wild zebrafish, which choose to form larger, more cohesive shoals compared with when in still and slow-flowing environments (Shelton *et al.*, 2020). These results highlight the potential importance of collective behaviour in environments where flow acts as an energetic constraint and fish are forced to adopt hydrodynamic formations (Shelton *et al.*, 2020).

In addition to biotic cues, association with abiotic environmental visual cues has been theorised to bestow benefits for fish (Chapter 4). Association tends to be stronger in flowing water compared with static water suggesting that access to visual cues may be particularly advantageous in flow (Chapter 4). It was hypothesised in Chapter 4 that robust visual reference points provide energetic benefit because of improved control of self-motion (the ‘Station Holding Hypothesis’). In shoals, individual fish often indirectly acquire environmental information by responding to the movements of neighbouring fish (Ioannou *et al.*, 2011). This reduced reliance on external cues is likely to drive the weaker association with visual cues shown by groups in comparison to individuals, as members gain information about their position from conspecifics (Chapter 4). The extent to which the structure of a group is compromised by the presence of visual cues may be correlated to the importance of visual cues to fish groups. For example, the cohesion of the group might decrease because group members rely to a greater extent on environmental visual cues and to a lesser extent on conspecifics to orientate themselves in flow. However, the impact of environmental visual cues on the structure of fish shoals remains largely unexplored.

Given that fish use vision to align with neighbours to form more energetically advantageous formations in the absence of visual cues (de Bie, Manes and Kemp, 2020) yet show a strong association with them when they are present, this chapter aimed to determine how environmental visual cues alter the formation of fish shoals. Initially, the formation of five fish was assessed at one flow velocity to determine if environmental visual cues alter the spatial arrangement of small groups. Both the overall shoal structure (longitudinal and lateral cohesion) and the shoal sub-structure (distance to nearest neighbour and group polarisation) were quantified. It was predicted that: (1) Groups would align more laterally in the presence of environmental visual cues as members would be more inclined to span the channel to gain information about their position. (2) Group polarisation and distance to the nearest neighbour would be more variable because individuals rely on their neighbours to a lesser extent for information about their spatial position. This was followed by a second experiment using two fish (the simplest subunit of a shoal) at two flow velocities to more clearly assess fine scale interactions that occur between fish without the complexity of additional group members. It also assessed the relationship across flow velocities. The same predictions as made for shoals of five fish were made for groups of two. In addition, it was predicted that: (3) Fish would spend more time shoaling when visual cues were present because visual cues would provide a common reference point around which to associate. (4) Fish would align more laterally at higher flows and when visual cues were present.

7.2 Method

7.2.1 Study Subjects and Husbandry

The common minnow (N = 220; mean ± SD fork length: 54.7 ± 0.41 mm; mean ± SD weight: 1.83 ± 0.36 g) was used as a model organism because they form cohesive shoals and previous chapters have demonstrated a tendency to associate with environmental visual cues in flowing water (Chapter 4 and Chapter 5). Experiments were carried out on two separate occasions. Initially an experiment was conducted on five fish at one flow velocity. This was then followed by a study using two fish at two flow velocities. For both experiments minnow were captured from the River Itchen (Riverside Park, Southampton, UK, lat: 50°56'05.2’N lon: 1°22'23.9’W) using a seine net and transported in an 80 L aerated container of river water to the University of Southampton. A maximum of 120 fish at any one time were housed across four 100 L glass aquariums (mean ± SD temperature: Two fish: 15.8 ± 0.6 °C; Five fish: 21.2 ± 0.41 °C), for a maximum of 14 days. Fish were kept for at least 72 hours prior to trials commencing, visually isolated from fish in other aquaria, fed daily on commercial flake food and exposed to the natural photoperiod.

Fish were used in one trial only and at least 15 repeats were used for each treatment group. There was no difference in the fork length (ANOVA: Five fish: $F_{1, 38} = 0.57$, $P = 0.46$; Two fish: $F_{2, 57} = 0.50$, $P = 0.62$) and mass (ANOVA: Five fish: $F_{1, 38} = 0.01$, $P = 0.93$; Two fish: $F_{2, 57} = 0.15$, $P = 0.86$) of fish between treatment groups (Table 7.1). Likewise, there was no difference in flume temperature between treatments (ANOVA: Five fish: $F_{1, 38} = 1.9$, $P = 0.17$; Two fish: $F_{2, 57} = 0.14$, $P = 0.87$).

Table 7.1. Flow velocities, sample sizes and fish measurements for each treatment group. Measurements are presented as mean ± standard deviation (SD).

No. Fish	**Treatment**			**Sample size (N)**	**Fork length (mm) (Mean ± SD)**	**Mass (g) (Mean ± SD)**
	Flow	**Velocity ± SD (cms^{-1})**	**Visual cue**			
Five	High	16.0 ± 1.9	No	20	54.2 ± 2.1	1.68 ± 0.23
			Yes	20	53.6 ± 3.1	1.69 ± 0.29
Two	Low	9.0 ± 1.2	No	15	54.0 ± 4.9	1.88 ± 0.52
			Yes	15	54.7 ± 4.0	1.95 ± 0.38
	High	15.0 ± 1.7	No	15	55.1 ± 4.6	2.04 ± 0.51
			Yes	15	55.5 ± 3.2	1.89 ± 0.36

7.2.2 Experimental setup and protocol

Experiments were conducted at the University of Southampton - ICER - within a rectangular, open-channel recirculating flume (12m x 0.3m x 0.39m) between 9 September and 24 October 2019 (Five fish) and 2 and 18 March 2021 (Two fish). Experimental set-ups differed between the two experiments (Figure 7.1). For five fish an experimental arena of 0.5 m x 0.3 m (length x width) was used and for two fish an area of 1 m x 0.12 m was used with the channel narrowed using high density engineered foam. In both cases an experimental arena was isolated from the rest of the flume using two uniform white flow straighteners to reduce any environmental cues the fish could gain from hydrodynamic turbulence (Figure 7.1). White laminate PVC sheeting was secured to the base and side-walls of the channel to improve video processing by maximising the contrast between the fish and the background. The PVC minimised the number of environmental visual reference points within the flume and a blackout hide reduced reference points from outside the flume. Within the hide, LED strip lights (Brillihood - LED-Batten-4FT-36W, 2950 lumen, frequency peaks: 450nm & 550-600nm) provided indirect, defused illumination. Flow velocity was maintained at 16.0 cms^{-1} for groups of five (approximately 3 BLs^{-1}), and water depth at 8.5 cm for both treatments. When using groups of two fish, water depth was maintained at 10 cm and flow velocity at 9.0 cms^{-1} and 15 cms^{-1} for the low and high flow treatment, respectively.

Visual reference points were provided by a single 20 mm wide vertical black stripe (five fish) or three 15 mm vertical black stripes (two fish) midway along the length of the experimental arena (**Figure 7.1**). Three stripes were used with pairs of fish to enhance the probability of visual cue detection in a narrower channel. The stripe widths as seen from the centre of the experimental arena fell within the optimum visual acuity of zebrafish (0.26 cycles/°), a small cyprinid species with a robust measure of visual acuity (Rubin, 2012). During the control treatment the walls were uniform white. The behaviour of fish was recorded throughout the 30-minute trial using a Logitech webcam (HD Pro Webcam C920; 30 fames/second, Resolution = 1080p) positioned 90 cm above the centre of the experimental arena.

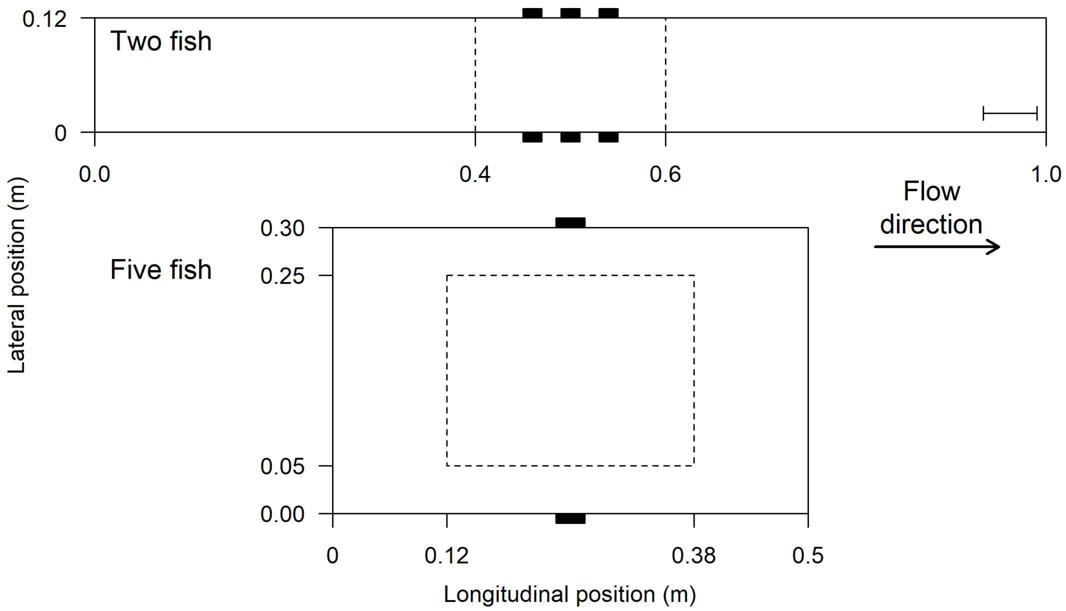

Figure 7.1. Plan view of experimental arena. Black rectangles at the midpoint of each wall represent the position of visual cues. Only frames during which the group centroid was within the dashed rectangles (central zone) were used when analysing group structure. Scale in bottom-right of the upper diagram represents the mean fork-length of individuals.

Prior to trials, five or two fish were randomly selected from a holding tank and isolated in flume water for 15 minutes to acclimate. The group were then released into the experimental arena and recording commenced immediately. Fish were left for 30 minutes to explore the experimental area before being weighed and measured.

7.2.3 Data processing and analysis

Video data was processed using automated fish tracking software in Matlab (The MathWorks, 2018). The coordinates of each fish's centroid and angle of the midline relative to the flume was calculated from a binary image of the fish's silhouette, obtained by subtracting the image background for each frame. To avoid incorporating group formations that were affected by the boundaries of the experimental arena or those unlikely to be within visual range of the cues only frames during which the centroid of the group was within the central zone of the flume were analysed (Figure 7.1). Similarly, dispersed groups whose cohesion was greater than 10 cm and those groups moving faster than 10 cms^{-1} were removed from the analysis. This was done to avoid analysing the behaviour of groups where members were at opposite ends of the experimental area and therefore were not behaving in a collective manner. During the first five minutes of each trial fish were deemed to be acclimating to the flow conditions and therefore they were not used in the analysis. After this, groups no longer showed erratic or escape behaviour. There were times when fish overlapped (i.e. swam over one another) and as a result they were measured as one fish. However, the spatial arrangement of the group could still be inferred from these frames so they were retained for analysis.

The fish coordinates and midlines were used to quantify the group formation within each frame using four metrics: Shoal structure was measured using 1) *Lateral cohesion* – standard deviation of lateral coordinates (spread of the group perpendicular to flow), and 2) *Longitudinal cohesion* – standard deviation of longitudinal coordinates (spread of the group in the direction of flow). The shoal sub-structure (i.e. relationship between group members) was quantified as 3) *Group polarisation* – standard deviation of the individual midline angles for each frame, and 4) *Distance to closest neighbour* – the average distance from each member of the group to their respective nearest member. To quantify the collective behaviour of two fish the same metrics were used. In addition, the *Association* – the percentage of time within the central zone of the experimental arena, and *Time shoaling* – the percentage of time within 1 body length (BL) of one another when in the central zone of the channel were quantified for two fish. One BL was used as a threshold because cohesive shoals usually have a nearest neighbour distance of < 1 BL (Ashraf *et al.*, 2017) and the threshold distance needed to be considerably lower than the width of the channel.

For density plots of five fish the relative position of each fish from the group centroid was plotted (Figure 7.2). For two fish the downstream fish was used as a reference fish and the absolute lateral distance (x-axis; $|x|$) was used so fish appeared to always be on the upper-right side of the reference fish (i.e. there were no negative x coordinates) (Figure 7.5).

7.2.4 Statistical analysis

Statistical analysis was performed in R software using the lme4 package (Bates *et al.*, 2015; R Core Team, 2020). An ANOVA was used when assessing *Association* and *Time shoaling*. When data for individual frames were being statistically analysed (*lateral cohesion, longitudinal cohesion, distance to nearest neighbour* and *group polarisation*) linear mixed-models (LMM) were performed with Trial ID added as a random variable to account for across-group variability or any un-measured factor between trials. Visual cue treatment was used as an explanatory variable along with flow when analysing the behaviour of two fish. Transformations were necessary to meet the model assumptions. For five fish log transformations were required for all four metrics. For two fish, *Association* and *Time shoaling* were arcsine square-root transformed, *Lateral* and *Longitudinal cohesion* were square-root transformed and *Group polarisation* was log transformed before models were performed. Model fit and residual diagnosis was achieved using the DHARMa package (Hartig, 2020), and Post-hoc tests to assess interaction terms was done using the phia package (De Rosario-Martinez, 2015).

7.3 Results

7.3.1 Five fish

Groups tended to swim in diamond formation and were slightly more longitudinally aligned than laterally, spanning a median [IQR] of 2.58 [1.44] cm laterally across the flume compared with 3.53 [2.14] cm longitudinally (Figure 7.2). The presence of visual cues did not alter group longitudinal cohesion (LMM: $F_{1,39} = 0.21$, $P = 0.65$), but fish tended to be more laterally aligned when visual cues were present compared with the control (LMM: $F_{1,39} = 9.71$, $P = 0.004$; Table 7.2; Figure 7.2).

In addition, individuals did not seem to alter their interaction or association with other group members. Group polarisation (LMM: $F_{1,39} = 1.87$, $P = 0.18$) and distance to nearest neighbour (LMM: $F_{1,39} = 0.02$, $P = 0.89$) did not differ between visual cue treatments.

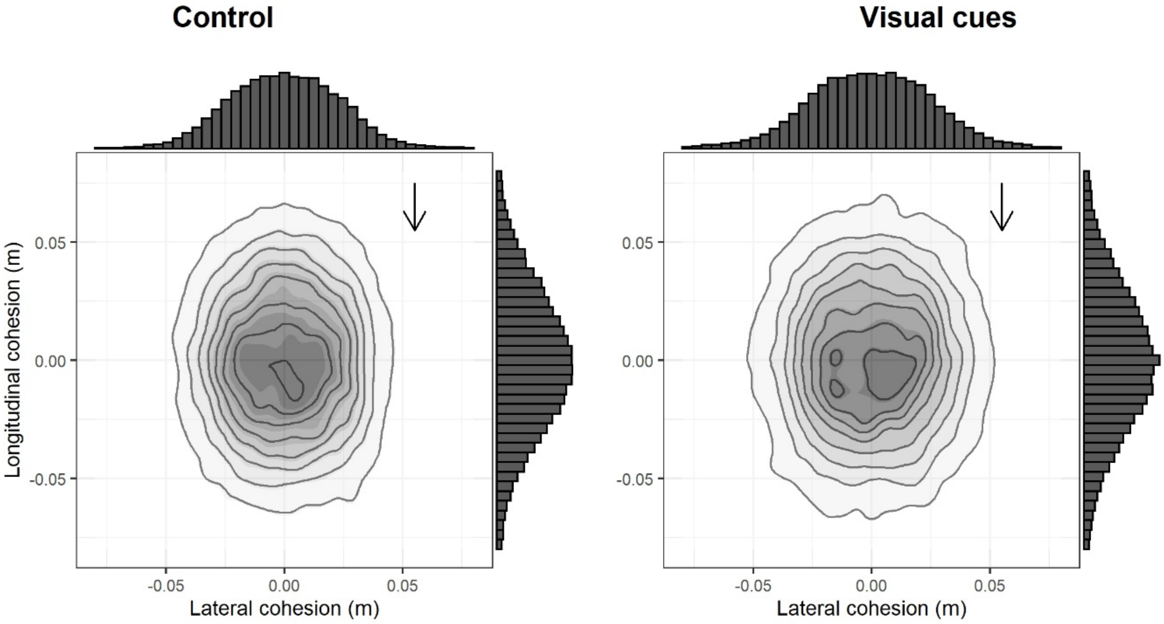

Figure 7.2. Density plot of fish positions relative to the group centroid using kernel density estimation during the control (left) and visual cue (right) treatments. Histograms display the longitudinal and lateral distribution of fish coordinates. Direction of flow from top to bottom.

Table 7.2. Summary statistics for each metric for groups of five fish by treatment. Interquartile range (IQR). Level of significance represented by *.

Five fish	**Control**		**Visual Cues**		
	Median	**IQR**	**Median**	**IQR**	
Lateral cohesion (cm)	2.47	± 1.26	2.71	± 1.65	**
Longitudinal cohesion (cm)	3.49	± 2.09	3.57	± 2.16	
Group polarisation (degrees)	7.68	± 6.53	8.03	± 6.74	
Distance to nearest neighbour (cm)	3.29	± 1.26	3.39	± 1.24	

7.3.2 Two fish

Fish associated with environmental visual cues across both flow regimes (ANOVA: $F_{1,54} = 95.4$, $P < 0.001$; Figure 7.3). However, there was no difference in the association between the low and high flow condition (ANOVA: $F_{1,54} = 0.66$, $P = 0.41$). No interaction was detected between visual cue treatment and flow condition (ANOVA: $F_{1,54} = 0.02$, $P = 0.88$).

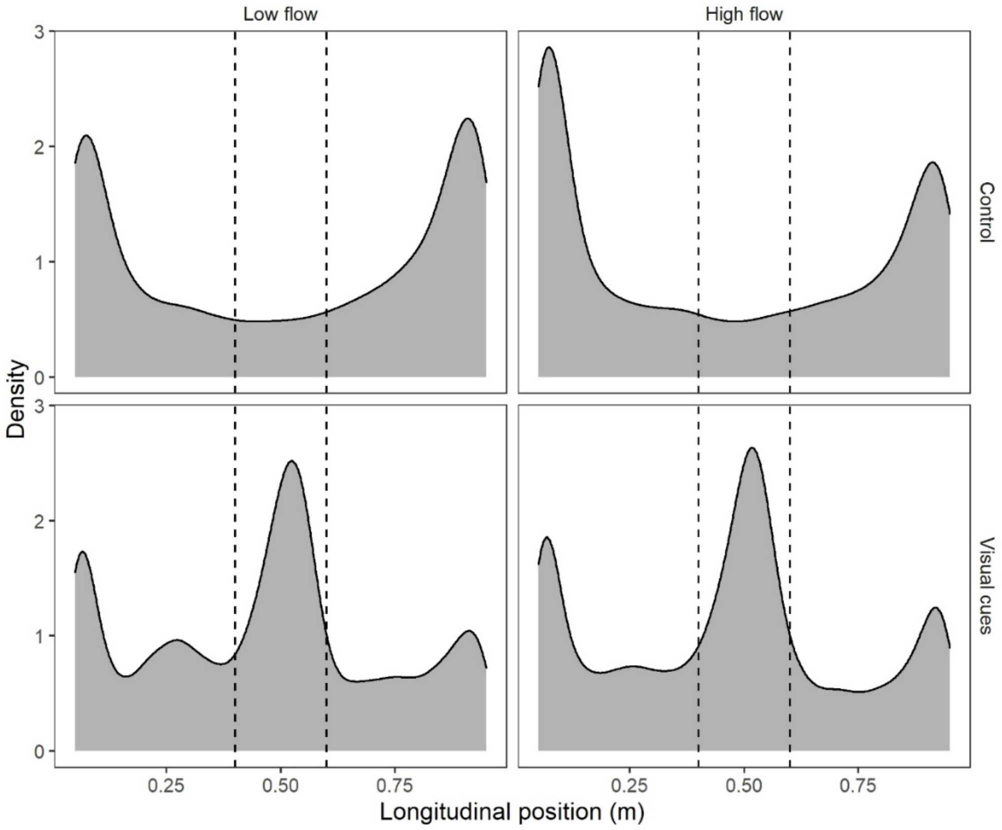

Figure 7.3. Probability density function of fish coordinates longitudinally along the channel for each treatment group. Dashed vertical lines represent the area in which fish were assumed to be associating with visual cues.

When within the central zone of the experimental area fish shoaled for a median [IQR] of 77.3 [14.7] % of the time during the control compared with 92.8 [9.4] % when visual cues were present (ANOVA: $F_{1,53} = 34.6$, $P < 0.001$; Figure 7.4). Flow condition did not affect the time shoaling (ANOVA: $F_{1,53} = 2.45$, $P = 0.12$) nor was there an interaction between flow condition and visual cue treatment (ANOVA: $F_{1,53} = 0.17$, $P = 0.69$).

There was a subtle difference in the formation that pairs of fish adopted between the four treatment groups (Figure 7.5). During the control the longitudinal cohesion (median [IQR] = 3.4 [3.8] cm) was less compared with when visual cues were present (median [IQR] = 2.57 [3.6] cm; LMM: $F_{1,57} = 12.4$, $P < 0.001$). Flow did not affect the longitudinal cohesion (LMM: $F_{1,57} = 3.25$, P = 0.08) and visual cue treatment (LMM: $F_{1,57} = 1.58$, $P = 0.22$) and flow (LMM: $F_{1,57} = 3.50$, $P = 0.56$) had no effect on the lateral cohesion of pairs of fish. Pairs of fish tended to be more aligned at higher flows (LMM: $F_{1,57} = 24.8$, $P < 0.001$) and when visual cues were present (Table 7.3; Figure 7.5; LMM: $F_{1,57} = 10.3$, $P = 0.002$).

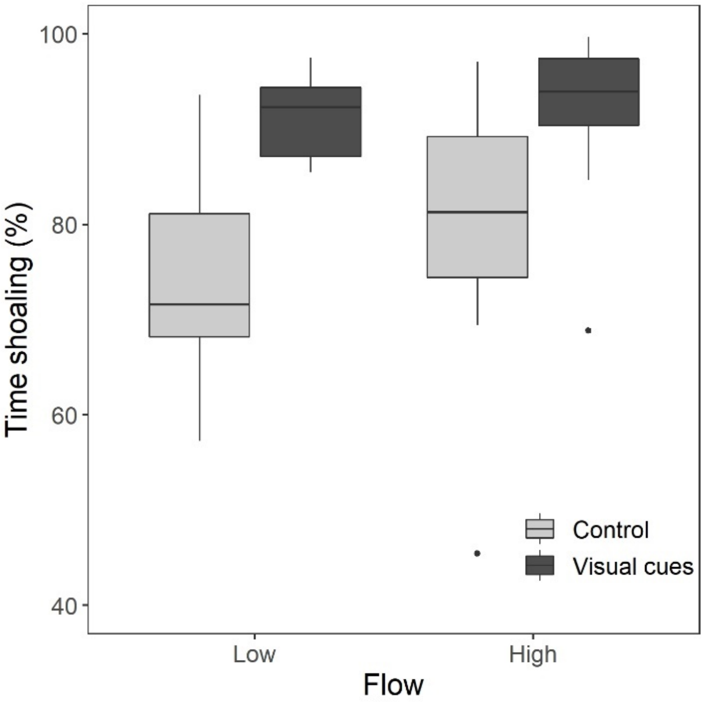

Figure 7.4. Percentage of time shoaling (within 1 BL) when in the central zone of the channel (i.e. associating with the visual cues) for control (light grey) and treatment (dark grey) groups across the two flow conditions. Boxes show the lower quartile, median and upper quartile and the whisker displays the range of data within 1.5 x the IQR. The points represent outliers (data beyond these limits).

Table 7.3. Summary statistics for each metric for groups of two fish by treatment. Level of significance represented by *.

Two fish	**Control**		**Visual Cues**		
	Low	**High**	**Low**	**High**	
Lateral cohesion (cm)	1.71 [1.89]	1.92 [1.57]	1.71 [1.63]	1.61 [1.28]	
Longitudinal cohesion (cm)	3.69 [3.82]	3.23 [3.83]	2.53 [3.94]	2.60 [3.29]	***
Group polarisation (degrees)	7.75 [14.3]	5.50 [9.39]	6.00 [10.7]	4.55 [5.56]	**
Distance to nearest neighbour (cm)	4.55 [3.24]	4.07 [2.96]	3.75 [3.40]	3.62 [2.64]	

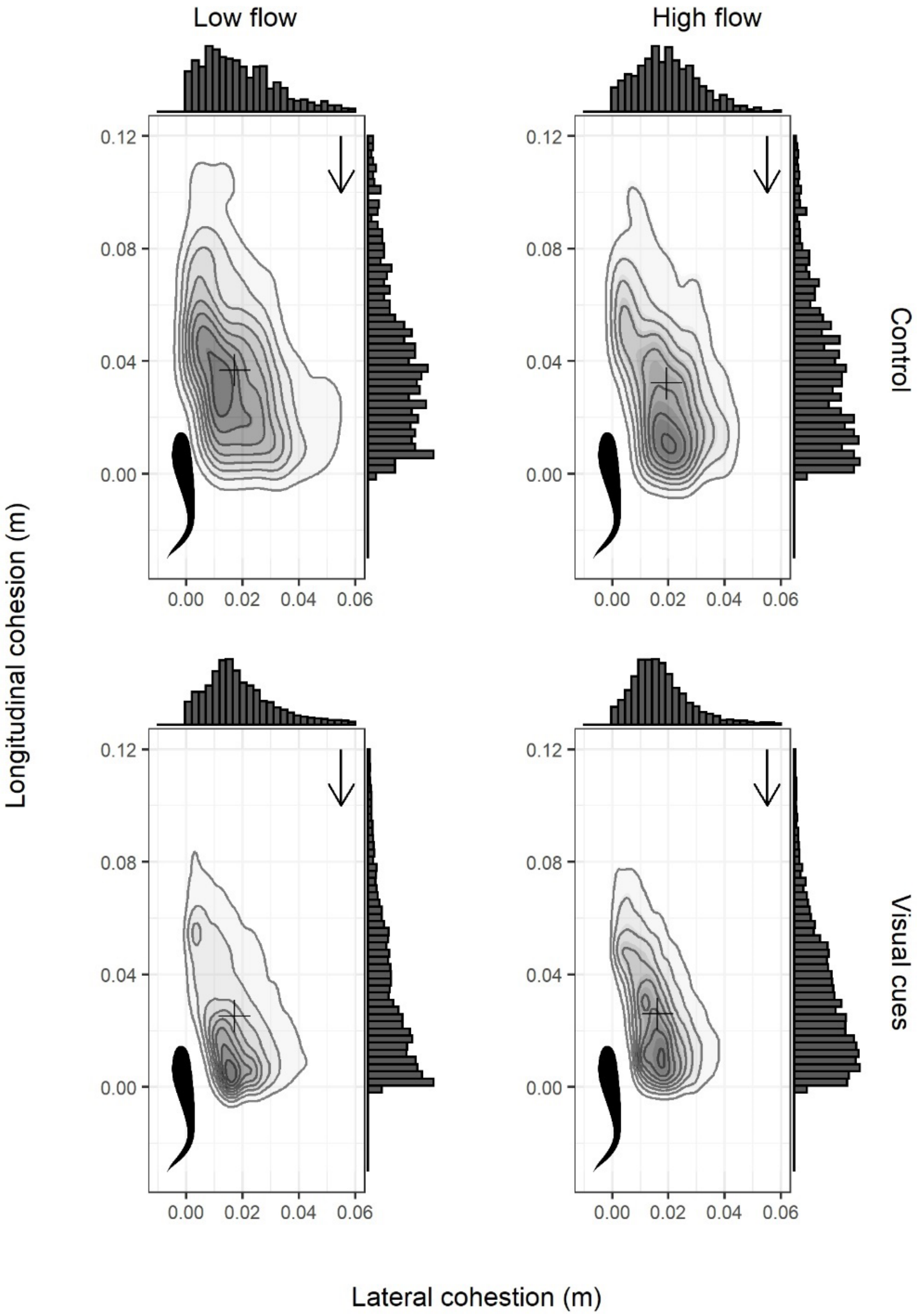

Figure 7.5. Relative position of fish under the four treatment conditions using kernel density estimation – Control (A, B), Visual cues (C, D) and low (A, C) and high (B, D) flow velocity. Density plots were created using data from when fish were within 10cm of each other in the central zone. Black cross represents the median location of fish. Direction of flow from top to bottom.

7.4 Discussion

This study aimed to determine the effect of environmental visual cues on the formation of fish shoals in flowing water by quantifying the structure of groups of five fish and pairs of fish in the presence and absence of visual cues. There were subtle differences in the formation of minnow between treatments. Both groups of five and pairs tended to align more side by side (laterally) when visual cues were present. However, the differences in formations were slight and provided no strong evidence that members of a group favour information from environmental visual cues over the those obtained by maintaining group cohesion.

The overall shoal structure and sub-structure of groups of five minnow was analysed to determine the effect of visual cues on group formation. The first hypothesis stated that the lateral group cohesion would decrease in the presence of visual cues as members would be more inclined to align laterally across the channel to gain information about their position from the environmental visual cues presented on the channel walls. For example, group members would be spread across the channel with some associating with the stripe on the right wall and other members, the left. This was observed as five fish aligned more laterally when visual cues were present providing support for the first hypothesis. When analysing the sub-structure of the group, no difference was detected in the polarisation of individuals within the group or distance to closest neighbour between the control and treatment, providing evidence against hypothesis two. Fish adopt energy efficient formations in flowing water and when schooling by controlling their distance to neighbours to take advantage of the hydrodynamic structures produced when swimming (Weihs, 1973). There are a small number of formations that have theoretically been shown to provide energetic benefit including diamond, rectangle and side-by-side formations which make use of the vortex hypothesis and channelling effect (Ashraf *et al.*, 2017; Daghooghi & Borazjani, 2015; Weihs, 1973). The formations adopted in this experiment were fluid but generally a diamond formation was formed with slightly greater longitudinal than lateral cohesion, similar to those described by Weihs (1973). In addition, the consistent distance to nearest neighbour and polarisation of group members supports studies demonstrating that maintaining a constant distance between neighbours and synchronising tail-beat-frequencies can reduce energy expenditure (Ashraf *et al.*, 2016; Ashraf *et al.*, 2017). The lack of change in the sub-structure of the group between treatments provides strong evidence that fish do not compromise on group structure even though the group as a whole reacts to the presence of visual cues. Considering that one of the advantages of collective behaviour is to reduce the energetic cost of locomotion (Marras *et al.*, 2015) it is likely that the group members gained more benefit from being part of the group in an energetically advantageous formation than could be gained by compromising on group cohesion with members relying on external visual reference points to help control position.

Two fish showed a clear association with visual cues, in line with previous chapters (see Chapter 4) and shoaled more frequently when visual cues were present. During the control, when environmental visual cues were absent, two fish adopted a follow the leader formation, demonstrated by the decreased longitudinal cohesion, whereas when visual cues were present fish tended to align more side-by-side. This could be because when visual cues are present both fish are actively aligning with visual cues as a focal point either to control swimming or as a refuge seeking response as described in Chapter 4. Other studies have found that flow velocity influences the structure of fish groups. For example, two fish have been shown to align side-by-side in flowing water but align more longitudinally in static water (de Bie, Manes and Kemp, 2020). In this study, fish did not align more side-by-side or increase association at higher velocities contradicting the results of de Bie, Manes and Kemp (2020), but presenting an identical result to Chapter 5, in which individual fish did not alter their association with visual cues across flow velocities. Considering that the association did not increase at the high flow velocity and the results of the experiment on groups of five fish are inconsistent, it seems unlikely that the observed behaviour was induced by individuals seeking visual cues to help control their position in flow. The fish may have been searching for sanctuary by the visual cues, although further work need to be done to more conclusively support the ‘Predator Refuge Hypothesis’.

When associating with visual cues, both groups of five and pairs of fish showed a similar result by aligning more side-by-side. For groups of five fish this was demonstrated by a decrease in lateral cohesion, whereas for pairs it was demonstrated by an increase in their longitudinal cohesion in the presence of visual cues. Neither group size altered their distances to nearest neighbours, but pairs aligned more when visual cues were present. As both experiments produced similar results it is fairly clear that groups of fish choose to associate with visual cues which can alter the overall structure of the group, but they do not compromise on the group sub-structure. The reasons for this are unknown but it is likely that group members maintain a constant distance to neighbours for energetic benefit.

The flow velocity had no effect on any of the results presented here, but this may be because the range of flow velocities (9.0 – 16.0 cms^{-1}) was not great enough to elicit a substantial difference in behaviour. In addition, the length of the experimental arena was increased when assessing two-fish so that association behaviour could be defined more clearly. As a result, the visual cues caused the fish to hold position for longer in the central area and when they did this they were aligned laterally. In contrast, when visual cues were absent, minnow were less likely to hold position in the middle and swam through without delay, resulting in them being more longitudinally spaced. Further studies could test whether the exploratory behaviour or total distance travelled decreases when the full length of both channel walls is covered in visual cues verses a control.

7.4.1 Conclusions

Although association with the visual cues was evident, especially for the pairs of fish, this study provided no strong evidence that environmental visual cues alter the sub-structure of fish shoals. However, there were slight differences detected in the overall shoal structure as groups and pairs tended to align more side-by-side in the presence of visual cues. Thus, it seems that groups of fish move to associate with visual cues but continue to rely on other group members. It might be the case that groups can passively gain information from their environment without physically changing the way they interact with other group members. As such, further studies that quantify energy expenditure using a swim chamber would more conclusively determine whether groups gain any energetic advantage from the presence of environmental visual cues.

Chapter 8 Discussion

Knowledge of the fundamental behavioural responses of living things to stimuli is essential to understanding the world around us and can give rise to some unexpected applications. The research presented in this thesis was undertaken in an experimental setting to improve fundamental knowledge of the behavioural response of European freshwater fish to static abiotic visual cues. It did this by addressing six objectives (two primary and four secondary objectives; section 2.5 and Table 8.1, page 31). The primary objectives were to understand how visual cues shape the behaviour of fish in (i) flowing water and (ii) groups. These primary objectives were addressed within multiple experimental chapters (4-7) whereas the secondary objectives were each addressed within a single chapter. First this chapter will review how the programme of research transpired before discussing the findings of this thesis in relation to the primary objectives, drawing on the outcomes of the secondary objectives throughout. In addition, potential limitations of these studies and avenues of further interest will be discussed before finally placing this research within a broader context and suggesting some potential applications.

8.1.1 Topic rationale

An initial literature review (Section 1.1) recognised differences in the response of fish (Scholtyssek *et al.*, 2014), insects (Mauss and Borst, 2020) and birds (Schiffner and Srinivasan, 2015) to optic flow, providing an interesting basis from which to investigate the topic. It was clear that fish showed the opposite reaction to visual cues compared with flying animals (Scholtyssek *et al.*, 2014). Scholtyssek *et al.* (2014) hypothesised that fish may favour strong visual cues as a strategy to control swimming, or because they live in a medium in which vision is often limited. However, the reason for this difference and whether this response was universal among fish species remained largely unexplored. The quantitative literature review in Section Chapter 2 aimed to identify gaps and biases in the literature by exploring the methodologies and species used in previous studies which focused on fish behavioural responses to abiotic visual cues in experimental studies. The review revealed that previous research placed considerable focus on the optomotor response and on fish response to moving visual cues, but there was very little documented research regarding fish behaviour around stationary visual cues. In addition, this research was performed on individual trained fish in static water (Scholtyssek *et al.*, 2014), leaving many unanswered questions about fish response under different circumstances such as in flowing water or in groups, despite their ecological importance. Assessing fish behaviour in relation to these two themes became primary objectives (objectives 1 and 2) of this thesis.

8.1.2 Primary Objectives

The primary objectives were first investigated in Chapter 4 by assessing the response of common minnow to visual cues under flowing and static water conditions (objective 1), and in groups and when alone (objective 2). The results clearly demonstrated that common minnow associate with visual cues in static water, supporting the results from previous studies, which investigated whether zebrafish navigate using optic flow (Scholtyssek *et al.*, 2014). The results provide more evidence that fish do not rely on optic flow in the same way as insects and birds but instead suggests that fish gain benefits by being alongside reliable reference points. In addition, minnow associated more strongly in flowing water in which navigation and stabilising position is more challenging. Considering the increased associationwith visual cues in flow, it was hypothesised that fish may make use of passive visual cues to control position and perhaps gain some energetic benefit from doing so (the 'Station Holding Hypothesis'). Additionally, groups of five minnow showed a weaker association than individuals, resulting in a second hypothesis: the 'Predator Refuge Hypothesis'. This is the hypothesis that individual fish were more vulnerable to predation when away from a group and therefore sought refuge alongside visual cues that resemble structure in the fish's natural environment. These two hypotheses, presented in Chapter 4, were further investigated for the remainder of the thesis to understand what is driving this association with visual cues by exploring the secondary objectives 3-6.

Primary objective 1 – Influence of flowing water

The response of fish to visual cues in flowing water (objective 1) was primarily investigated in Chapter 5 and Chapter 7 on individuals and groups, respectively. The multi-sensory nature of rheotaxis and the relative role of vision and the lateral-line in the behaviour remains one of the largely unanswered questions in sensory ecology dating back to Lyon (1904). Chapter 4 revealed a stronger association of both individuals and groups to visual cues when in flowing compared with when in static water. This led to the development of a 'Station Holding Hypothesis', which was investigated in more detail in Chapter 5. It did this by addressing objectives 3 and 4 to enhance understanding of the role of visual cues in rheotaxis and to compare the responses of individual fish from two different species to visual cues. The common minnow and brown trout are two distinct species with contrasting behavioural ecologies (Section 3.2), yet the results presented in Chapter 5 demonstrated that neither species displayed a clear bias to associate with visual cues to a greater extent at higher, more energy-demanding flow velocities. In addition, the overall association was generally lower than 30% and the remaining time was spent away from the stripes either at the front or back of the experimental area where the hydrodynamic conditions were potentially more favourable. Therefore, the inconsistency by which fish held position alongside the visual cues suggests that aligning with visual cues to control speed and direction of movement for energetic benefit (the ‘Station Holding Hypothesis’) is not the primary reason for the observed relationship. This does not mean that fish do not use visual cues as a reference point, but the inconsistency of the findings suggest that fish do not need to actively associate with strong visual stimuli to control swimming. In addition, the consistent association with visual cues in static water shown in Chapter 4 and Chapter 6 provide evidence that the advantages of association with visual cues may differ in static water and the stronger association in flow likely demonstrates that there is some additional benefit to the association in flow. Studies elsewhere have shown that mechanosensory cues (from the lateral-line) alone can mediate rheotaxis (Suli *et al.*, 2012; Kulpa, Bak-Coleman and Coombs, 2015) and the relative role of different sensory cues has recently been reviewed and reiterates the sensory complexity of the process given that fish can compensate and perform rheotaxis after the loss of one or more senses (Coombs, Bak-Coleman and Montgomery, 2020).

Primary objective 2 – Influence of group behaviour

Primary objective 2 explored how collective behaviour alters fish response to visual cues. Initially, the results of Chapter 4 found that individual fish show a stronger association with visual cues compared with groups of five. This led to the development of the ‘Predator Refuge Hypothesis’ which suggested that visual cues represent physical structure in which fish may seek shelter from predators or reduce the risk of detection from predators by residing against a dark background. The effect of group size on the response of fish to visual cues was further investigated in Chapter 6 by assessing the rate of response and strength of association of one, five and 20 fish to visual cues. It was found that larger groups of fish respond more quickly to a change in the visual environment compared with individuals, and that larger groups associated with visual cues more frequently throughout the experiment. This contradicts the results presented in Chapter 4, where individuals showed a higher association. This result is likely to be a consequence of differences between the experimental designs and the behaviour of the fish when encountering the presented visual cues. In Chapter 4 the experimental design required the fish to actively explore and swim past the visual cues which were at the upstream end of the experimental arena. In this scenario, fish behaviour was only assessed when fish were within the treatment zone, so only the behaviour of fish that were in close proximity to the stripes (within detection distance) was quantified. Those that did not encounter them (e.g. timid fish) were not included in the analysis. In contrast, Chapter 6 took place in a confined tank where fish were already occupying the space in which visual cues were displayed. In this scenario individual fish could reside at one end of the tank and not know if there were visual cues present at the other, all the time having their behaviour quantified relative to that of larger groups. As such, the results of Chapter 4 represent the greater desire for individuals in comparison to groups to seek refuge alongside visual cues when they encounter them. In contradiction, Chapter 6 represents the increased efficiency by which groups may detect changes in their environment compared with individuals. This general feature of collective behaviour has been shown elsewhere in the literature. For example, in social foraging, group members can benefit when other members locate food sources (Beauchamp, 2013) or detect predators (Beauchamp, 2019). In this case, groups of fish discovered the visual cues more rapidly than individuals and therefore had more time remaining to associate with the stripes. However, this does not indicate that individuals were less inclined to associate with the visual cues as individuals showed a strong association once the cues were discovered. Importantly, the results also indicate that groups of twenty fish still show a strong preference to associate with visual cues which could have implications in an applied setting, for instance, in fish guidance. Lastly, the stronger association shown by a predominately shoaling species, which are more vulnerable to predation in their natural habitat than brown trout, may indicate that the 'Predator Refuge Hypothesis' has the potential to hold true.

Many species across the animal kingdom use motion camouflage (Bian, Elgar and Peters, 2016) and background matching (Donnelly and Whoriskey, 1991; Kjernsmo and Merilaita, 2012) as a form of crypsis to avoid being detected by predators or prey. If fish were using the visual cues in the experiments as a form of refuge seeking, one would expect the fish to remain relatively motionless when alongside the cues to avoid being detected. However, fish rarely maintained a steady position alongside the visual cues for more than a few seconds in flowing water providing some doubt that the association was an active anti-predator response. Instead, the frequent, fleeting visits to the central area where the stripes were displayed in Chapter 5 suggests the association may be an act of curiosity considering that the stripes provide a novel visual stimulus. If the association was born out of curiosity, then one would expect the response to diminish over time as fish lose interest. Nevertheless, Chapter 6 clearly demonstrated that the association did not diminish over time, as fish continued to associate with visual cues to the same extent after 6 hours. This provides strong evidence that the response is not purely curiosity but may be an innate behaviour and provide some essential evolutionary advantage. Interestingly, a study focusing on the response of fish groups to visually noisy environments found that moving light bands across the tank did deter fish from the visually noisy environments but did not cause fish to seek refuge (Attwell *et al.,* 2020).

Objective 6 – Influence on group structure

The final objective was to determine how the structure of groups changes around visual cues (Objective 6). To meet this objective two experiments were carried out in Chapter 7 using two group sizes of common minnow to quantify how the formation of fish differed with and without access to robust visual cues. Numerous studies have found that fish align more laterally in fast flowing water for energetic benefit (Ashraf *et al.*, 2016; de Bie, Manes and Kemp, 2020). As hypothesised, when visual cues were on the side-walls of the channel, groups of five fish spread out across the channel. Similarly, pairs of fish also aligned more laterally (i.e. side by side) when visual cues were present. This suggests that visual cues provide a common reference point around which to gather. However, there was no detected difference in the group sub-structure (distance to nearest neighbour, group polarisation) suggesting that although the group may associate with visual cues the relationship with other group members is unchanged. The consistent relationship with other group members both when alongside stripes and during the control may be evidence that visual cues are not important for controlling position and agrees with the outcomes from the objectives 1 and 3, which provide little evidence to support the ‘Station Holding Hypothesis’.

Nonetheless, the overall responses in all four chapters suggest that the behaviour around visual cues may be highly context dependent. When fish were alone in a large channel such as exemplified in Chapter 4, or when in a large group in static water such as in Chapter 6, they clearly showed a preference for stripes, and the results presented in this thesis suggest this is most likely driven by a refuge seeking response. When in a smaller area in flowing water, the use of stripes as a reference point was possibly dependent on whether fish found a suitable hydrodynamic refuge elsewhere within the experimental area.

Table 8.1. Summary of thesis hypotheses and inference for the observed behaviour.

Chapter	**Objective**	**Hypothesis/Prediction**	**Finding**	**Inference**
4	Already Known	Fish will associate with visual cues	Yes	
	1	Association will be stronger in flowing than static water because fish will gain energetic benefits (reduced risk of displacement and greater ability to control speed of movement) from the utilisation of fixed points of reference	Yes	Fish gain benefits by being alongside reliable reference points in flowing water. Potentially for energetic benefit
	2	Members of groups will have a lower affinity for visual cues than isolated individuals because information on relative position can be gained from others	Yes	Groups have less need to rely on environmental reference points as they can align with other group members. Individuals may be more risk averse and use visual cues as a refuge
5	Already Known	Fish will associate with visual cues	Yes	
	3	The association will be positively related to flow velocity	No – Association with visual cues was the same across flow velocities	Aligning with visual cues to control speed and direction of movement for energetic benefits is not the primary reason for the observed relationship
	4	The response will be stronger in trout compared with minnow as they naturally hold station and drift feed	No – stronger association for minnow	Considering that minnow are a social species and were used individually the increased association of minnow suggests the response may be refuge seeking

Chapter	**Objective**	**Hypothesis/Prediction**	**Finding**	**Inference**
6	2	Larger groups would associate with visual cues more rapidly when exposed to them because of enhanced detection	Yes	Larger groups were able to detect the visual cues more quickly
	2	Larger groups would exhibit a weaker association	No – Similar strength of association across all group sizes but larger groups associated more frequently	Larger groups more frequently associated with stripes reflecting the increased efficiency by which groups detect changes in their environment compared with individuals
	5	Larger groups would be more likely to experience a diminished association over time because smaller groups and individuals will be more risk averse	No - no difference in response was detected over time	The association is unlikely to be one of curiosity. More likely it is an innate behaviour
7	6	Groups of five would align more laterally in the presence of visual cues	Yes	Groups spanned the channel – either out of curiosity or to gain information about their position from environmental visual cues
	6	Group polarisation and distance to the nearest neighbour would be more variable when associating with visual cues, as members relied to a lesser extent on their neighbours for information about their spatial position	No difference in the internal structure of groups was detected	Visual cues do not provide enough of a benefit or distraction to disrupt the cohesion and sub-structure of groups
	2	Fish would spend more time shoaling when visual cues were present	Yes	The presence of visual cues probably provided a common reference point around which to associate

Chapter	**Objective**	**Hypothesis/Prediction**	**Finding**	**Inference**
	6	Fish would align more laterally at higher flows (de Bie, Manes and Kemp, 2020) and when visual cues were present	No/Yes	No difference in lateral alignment was detected between the two flow speeds but two fish aligned more side-by-side when visual cues were present.

8.2 Contributions to existing knowledge

This thesis has made several original contributions to existing knowledge and improved fundamental understanding of fish responses to visual cues:

The review of fish responses to visual cues in Section Chapter 2 outlined the absence of literature focusing on responses to static visual cues. Only one experimental study had investigated how fish use optic flow when navigating through a channel providing some interesting results that contrasted the behaviour seen in insects and birds (Scholtyssek *et al.*, 2014). However, this study had some obvious limitations, for example it was undertaken using trained fish and only took place in static water. Chapter 4 built on this experiment in an open channel flume by quantifying the behaviour of freely behaving fish in flowing water and in groups to determine whether the results hold true under different conditions. The results of this work have been published in Animal Behaviour: Miles, J., Vowles, A.S. & Kemp, P. (2021). The response of common minnow (*Phoxinus phoxinus*) to visual cues under flowing and static water conditions. Animal Behaviour. https://doi.org/10.1016/j.anbehav.2021.07.004.

Further chapters built on Chapter 4 in an attempt to decipher why the association with visual cues was occurring. The two main avenues of interest arose from the discovery that minnow tended to show a stronger association in flowing water and when alone. Chapter 5 went someway to enhance current knowledge of the role of visual cues in rheotaxis (Objective 3). It illustrated that visual cues may only play a small role, considering the infrequent associations with visual cues at higher flow velocities. This work is in preparation for journal submission as Miles *et al.* The influence of flow velocity on the response of rheophilic fish to visual cues.

How this affinity for visual cues is influenced by collective behaviour was assessed in Chapter 6. The results of this chapter demonstrated that larger groups of minnow respond to changes in their visual environment faster than smaller groups, tying in well with the wider literature on collective behavioural responses to environmental stimuli. It also established that large groups of fish frequently chose to associate with visual cues albeit a species that is relatively vulnerable to predation, again signifying that seeking refuge from predators is a likely driver for this behaviour. This chapter will be submitted for publication as Miles *et al.* The role of collective behaviour in fish response to visual cues.

Finally, Chapter 7 established that the structure of fish groups remains relatively unaffected by the presence of visual cues and suggests that their influence on the interactions between group members is minimal even though the group as a whole may react to visual cues.

The results of this thesis highlight the complexities of fish behaviour and provide a good platform from which to further investigate the subject. There is considerable scope to investigate the Predator Refuge Hypothesis further in experimental studies to further understand the association with visual cues. In addition, there may be opportunities to exploit this behavioural response to solve ecological problems. Opportunities for further work and potential applications for this research are discussed below.

8.3 Further studies

There are several unanswered questions regarding why fish associate with and respond to visual cues. Considering the small effect size of responses in flowing water, the results suggest that the primary drive to associate with visual cues is unlikely to be a mechanism to help control movement (the ‘Station Holding Hypothesis’). The more likely motive for visual cue association is refuge seeking (the ‘Predator Refuge Hypothesis’), considering that the vertical black and white stripes used in this study may resemble macrophytes in the fish’s natural environment. In addition, given the homogeneity of the test environments the dark stripes would have provided a surface where the fish had the closest background match. Since there is evidence that refuge seeking is affected by individual traits such as boldness (Webster, Ward and Hart, 2007) further work measuring the effect of individual boldness on the strength of association with visual cues may help conclude that the response is driven by these mechanisms. Similarly, a positive correlation between association and individual level of predation risk would more conclusively support the ‘Predator Refuge Hypothesis’.

It is likely that the motivation to associate with visual cues has multiple drivers and is not triggered solely by refuge seeking. Given that Chapter 5 was inconclusive regarding whether visual cues may provide some aid to controlling position in flowing water, future studies using a swim chamber that quantifies energy expenditure may go a long way to answering this question. Finally, considering the strong behavioural response of minnow and trout to visual cues in an experimental setting, it would be interesting to trial the response of other species in a more natural setting with extra visual distractions. Further work in either a laboratory setting or in a natural river will provide useful information about whether the response of fish to visual cues is strong enough to be applicable elsewhere.

8.4 Potential applications

Fish continue to provide essential nutrition to human populations globally, as exemplified by the growth of the aquaculture industry by 131% since 2000 (FAO, 2020). Despite this - and partly as a result of this, fish populations continue to face numerous man-made ecological challenges from overfishing, invasive species, pollution, climate change, alongside habitat fragmentation; modification; and destruction. With such a fast-growing aquaculture industry animal welfare standards can be difficult to maintain (Ashley, 2007). The association with, and lack of habituation to, visual cues may provide multiple non-invasive methods to combat some of these issues; for example, applying visual cues to aquaculture tanks with large stock densities may aid the dispersion and/or distribution of fish and in turn, reduce stress and disease. In addition, recent research has drawn attention to the use of fish behavioural responses to stimuli for improved welfare and growth in aquaculture (Macaulay *et al.*, 2020). Visual cues may provide one such stimuli within aquaculture systems that may stimulate complex cognitive behaviours in fish by encouraging social learning and in turn improve fish welfare.

Habitat modification, especially barriers to movement, can cause considerable ecological harm in freshwater habitats (Rahel and McLaughlin, 2018). For fish, barriers can affect many species at all life stages and there is currently no satisfactory solution to aid the efficient navigation of species through waterways and past barriers without major disadvantages. Many of the current solutions aim to deter fish from harmful infrastructure, for example, strobe lighting (Mussen and Cech, 2019) or acoustic deterrents (Putland and Mensinger, 2019) have had varying success at managing fish populations or preventing passage through hydropower turbines. As such, the demonstrated consistency by which fish react to, and associate with, static visual cues exemplified in this work may provide promising applications in real world settings, especially considering that static visual cues can provide a non-invasive, low-cost solution to attract fish to an area which many other forms of fish guidance do not achieve. Further work testing guidance using visual cues in controlled settings, before assessing the effectiveness in natural settings would be the next logical steps to understanding whether there is merit in using visual cues to improve fish passage across multiple species of fish and life stages.

8.5 Conclusion

The aim of this thesis was to further understand the response of fish to static abiotic visual cues. To meet this aim, two primary avenues of interest were assessed: 1) how flowing water effects the response of fish to visual cues, and 2) how this response varies with different group sizes. Association with visual cues was ubiquitous across all experiments. Individual fish and those in flowing water generally showed the highest association, but there was limited evidence that this association was used to aid control over self-motion for minnow and trout in flowing water (the 'Station Holding Hypothesis'). This was particularly evident by the inconsistent association at higher flow velocities and because fish did not alter their group structure when near visual cues. Given the higher association of individuals (in both static and flowing water) observed in Chapter 4, it appears that one of the primary drivers may be a refuge seeking anti-predator response (the 'Predator Refuge Hypothesis'). Interestingly fish did not habituate to the sight of visual cues over time suggesting that the response to visual cues is innate, potentially giving rise to some valuable applications. This work has demonstrated that there are multiple drivers motivating the behavioural response of fish to visual cues and further research is necessary to understand the complex behaviours in more detail.

List of References

Abell, R. (2002) ‘Conservation Biology for the Biodiversity Crisis: A freshwater follow-up’, *Conservation Biology*, 16(5), pp. 1435–1437. doi: 10.1046/j.1523-1739.2002.01532.x.

Ali, M. A. (1961) ‘Histophysiological Studies on the Juvenile Atlantic Salmon (Salmo Salar) Retina: Ii. Responses To Light Intensities, Wavelengths, Temperatures, and Continuous Light or Dark’, *Canadian Journal of Zoology*, 39(4), pp. 511–526. doi: 10.1139/z61-055.

Altshuler, D. L. and Srinivasan, M. V. (2018) ‘Comparison of visually guided flight in insects and birds’, *Frontiers in Neuroscience*, 12(MAR), p. 157. doi: 10.3389/fnins.2018.00157.

Arnheim, R. and Gibson, J. J. (1952) ‘The Perception of the Visual World’, *The Journal of Aesthetics and Art Criticism*, 11(2), p. 172. doi: 10.2307/426044.

Arnold, G. P. (1974) ‘Rheotropism in fishes’, *Biological Reviews of the Cambridge Philosophical Society*, 49(4), pp. 515–576. doi: 10.1111/j.1469-185X.1974.tb01173.x.

Ashley, P. J. (2007) ‘Fish welfare: Current issues in aquaculture’, *Applied Animal Behaviour Science*, 104(3–4), pp. 199–235. doi: 10.1016/j.applanim.2006.09.001.

Ashraf, I. *et al.* (2016) ‘Synchronization and collective swimming patterns in fish (Hemigrammus bleheri)’, *Journal of the Royal Society Interface*, 13(123), p. 20160734. doi: 10.1098/rsif.2016.0734.

Ashraf, I. *et al.* (2017) ‘Simple phalanx pattern leads to energy saving in cohesive fish schooling’, *Proceedings of the National Academy of Sciences of the United States of America*, 114(36), pp. 9599–9604. doi: 10.1073/pnas.1706503114.

Attwell, J.R., Ioannou, C.C., Reid, C.R. and Herbert-Read, J.E. (2021). Fish avoid visually noisy environments where prey targeting is reduced. *The American Naturalist, 198(3),* pp.421-432.

Baird, E. *et al.* (2006) ‘Visual control of flight speed and height in the honeybee’, in *Lecture Notes in Computer Science (including subseries Lecture Notes in Artificial Intelligence and Lecture Notes in Bioinformatics)*, pp. 40–51. doi: 10.1007/11840541_4.

Baird, E., Kornfeldt, T. and Dacke, M. (2010) ‘Minimum viewing angle for visually guided ground speed control in bumblebees’, *Journal of Experimental Biology*, 213(10), pp. 1625–1632. doi: 10.1242/jeb.038802.

Bajec, I. L. and Heppner, F. H. (2009) ‘Organized flight in birds’, *Animal Behaviour*, 78(4), pp. 777–

789. doi: 10.1016/j.anbehav.2009.07.007.

Bak-Coleman, J. *et al.* (2013) ‘The spatiotemporal dynamics of rheotactic behavior depends on flow speed and available sensory information’, *Journal of Experimental Biology*, 216(21), pp. 4011–4024. doi: 10.1242/jeb.090480.

Bak-Coleman, J. and Coombs, S. (2014) ‘Sedentary behavior as a factor in determining lateral line contributions to rheotaxis’, *Journal of Experimental Biology*, 217(13), pp. 2338–2347.

Bak-Coleman, J., Smith, D. and Coombs, S. (2015) ‘Going with, then against the flow: Evidence against the optomotor hypothesis of fish rheotaxis’, *Animal Behaviour*, 107, pp. 7–17. doi: 10.1016/j.anbehav.2015.06.007.

Baker, C. F. and Montgomery, J. C. (1999) ‘The sensory basis of rheotaxis in the blind Mexican cave fish, Astyanax fasciatus’, *Journal of Comparative Physiology - A Sensory, Neural, and Behavioral Physiology*, 184(5), pp. 519–527. doi: 10.1007/s003590050351.

Bar-Ilan, J. (2008) ‘Which h-index? - A comparison of WoS, Scopus and Google Scholar’, *Scientometrics*, 74(2), pp. 257–271. doi: 10.1007/s11192-008-0216-y.

Baras, E. and Lucas, M. C. (2001) ‘Impacts of man’s modifications of river hydrology on the migration of freshwater fishes: A mechanistic perspective’, *Ecohydrology and Hydrobiology*, 1(3), pp. 291–304.

Barron, A. and Srinivasan, M. V. (2006) ‘Visual regulation of ground speed and headwind compensation in freely flying honey bees (Apis mellifera L.)’, *Journal of Experimental Biology*, 209(5), pp. 978–984. doi: 10.1242/jeb.02085.

Barthem, R. and Goulding, M. (1997) *The catfish connection: ecology, migration, and conservation of Amazon predators*. Columbia University Press.

Bates, D. *et al.* (2015) ‘Fitting linear mixed-effects models using lme4’, *Journal of Statistical Software*, 67(1), pp. 1–48. doi: 10.18637/jss.v067.i01.

Beauchamp, G. (2013) *Social Predation: How Group Living Benefits Predators and Prey*, *Social Predation: How Group Living Benefits Predators and Prey*. Elsevier. doi: 10.1016/C2012-0-03532-6.

Beauchamp, G. (2019) ‘On how risk and group size interact to influence vigilance’, *Biological Reviews*, 94(6), pp. 1918–1934. doi: 10.1111/brv.12540.

Bhagavatula, P. *et al.* (2009) ‘Edge detection in landing budgerigars (Melopsittacus undulatus)’, *PLoS ONE*, 4(10), p. e7301. doi: 10.1371/journal.pone.0007301.

Bhagavatula, P. S. *et al.* (2011) ‘Optic flow cues guide flight in birds’, *Current Biology*, 21(21), pp. 1794–1799. doi: 10.1016/j.cub.2011.09.009.

Bian, X., Elgar, M. A. and Peters, R. A. (2016) ‘The swaying behavior of Extatosoma tiaratum: motion camouflage in a stick insect?’, *Behavioral Ecology*, 27(1), pp. 83–92.

de Bie, J., Manes, C. and Kemp, P. S. (2020) ‘Collective behaviour of fish in the presence and absence of flow’, *Animal Behaviour*, 167, pp. 151–159. doi: 10.1016/j.anbehav.2020.07.003.

Birdlife International (2012) *The IUCN Red List of Threatened Species 2012*, *Chaunoproctus ferreorostris*. Available at: http://www.iucnredlist.org/details/168639/0.

Birstein, V. J., Bemis, W. E. and Waldman, J. R. (1997) ‘The threatened status of acipenseriform species: a summary’, in *Sturgeon biodiversity and conservation*. Springer, pp. 427–435.

Bleckmann, H. *et al.* (2012) ‘Station holding of trout: Behavior, physiology and hydrodynamics’, in *Notes on Numerical Fluid Mechanics and Multidisciplinary Design*. Springer, pp. 161–177. doi: 10.1007/978-3-642-28302-4_10.

Bone, Q. and Moore, R. (2008) *Biology of fishes*. Taylor \& Francis.

Borst, A. (2009) ‘Drosophila’s View on Insect Vision’, *Current Biology*, 19(1), pp. R36--R47. doi: 10.1016/j.cub.2008.11.001.

Brabazon, A., Cui, W. and O’Neill, M. (2015) ‘Information propagation in a social network: The case of a fish schooling algorithm’, in *Intelligent Systems Reference Library*. Springer, pp. 27–51. doi: 10.1007/978-3-319-15916-4_2.

Brackley, R. (2016) *Interactions Between Migrating Salmonids and Low-Head Hydropower Schemes*. University of Glasgow.

Braithwaite, V. A. and Girvan, J. R. (2003) ‘Use of water flow direction to provide spatial information in a small-scale orientation task’, *Journal of Fish Biology*, 63(SUPPL. A), pp. 74–83. doi: 10.1111/j.1095-8649.2003.00218.x.

Bulkowski, L. and Meade, J. W. (1983) ‘Changes in Phototaxis during Early Development of Walleye’, *Transactions of the American Fisheries Society*, 112(3), pp. 445–447. doi: 10.1577/1548-8659(1983)112<445:cipded>2.0.co;2.

Bunt, C. M. (2001) ‘Fishway entrance modifications enhance fish attraction’, *Fisheries Management and Ecology*, 8(2), pp. 95–105. doi: 10.1046/j.1365-2400.2001.00238.x.

Bunt, C. M., Castro-Santos, T. and Haro, A. (2012) ‘Performance of fish passage structures at upstream barriers to migration’, *River Research and Applications*, 28(4), pp. 457–478. doi: 10.1002/rra.1565.

Carandini, M. *et al.* (2005) ‘Do we know what the early visual system does?’, *Journal of Neuroscience*, 25(46), pp. 10577–10597. doi: 10.1523/JNEUROSCI.3726-05.2005.

Cassin, B. *et al.* (1990) ‘Dictionary of eye terminology, Triad Pub’, *Co., Gainesville Florida*.

Chahl, J. S., Srinivasan, M. V. and Zhang, S. W. (2004) ‘Landing strategies in honeybees and applications to uninhabited airborne vehicles’, *International Journal of Robotics Research*, 23(2), pp. 101–110. doi: 10.1177/0278364904041320.

Clay, C. H. (2017) *Design of fishways and other fish facilities*. CRC Press.

Clough, S. *et al.* (2004) ‘Swimming speeds in fish: phase 2’.

Coombs, S., Bak-Coleman, J. and Montgomery, J. (2020) ‘Rheotaxis revisited: A multi-behavioral and multisensory perspective on how fish orient to flow’, *Journal of Experimental Biology*, 223(23). doi: 10.1242/jeb.223008.

Costanza, R. and Folke, C. (1997) ‘Valuing ecosystem services with efficiency, fairness and sustainability as goals’, *Natures Services: Societal Dependence on Natural Ecosystems*, pp. 49–70.

Coutant, C. C. (2001) ‘Turbulent attraction flows for guiding juvenile salmonids at dams’, in *American Fisheries Society Symposium*, pp. 57–77.

Couzin, I. D. *et al.* (2007) ‘Social Organization and Information Transfer in Schooling Fishes’, *Fish Cognition and Behavior*, 2, pp. 166–185. doi: 10.1002/9780470996058.ch9.

Crosato, E. *et al.* (2017) ‘Informative and misinformative interactions in a school of fish’, *arXiv*, 12(4), pp. 283–305.

Daghooghi, M. and Borazjani, I. (2015) ‘The hydrodynamic advantages of synchronized swimming in a rectangular pattern’, *Bioinspiration and Biomimetics*, 10(5), p. 56018. doi: 10.1088/1748-3190/10/5/056018.

Dakin, R., Fellows, T. K. and Altshuler, D. L. (2016) ‘Visual guidance of forward flight in hummingbirds reveals control based on image features instead of pattern velocity’, *Proceedings of the National Academy of Sciences of the United States of America*, 113(31), pp. 8849–8854. doi: 10.1073/pnas.1603221113.

Dallimer, M. and Strange, N. (2015) ‘Why socio-political borders and boundaries matter in conservation’, *Trends in Ecology and Evolution*, 30(3), pp. 132–139. doi: 10.1016/j.tree.2014.12.004.

Davidson, J. D. *et al.* (2021) ‘Collective detection based on visual information in animal groups’, *Journal of the Royal Society Interface*, 18(180). doi: 10.1098/rsif.2021.0142.

Delcourt, J. *et al.* (2013) ‘Video multitracking of fish behaviour: a synthesis and future perspectives’, *Fish and Fisheries*, 14(2), pp. 186–204.

Dijkgraaf, S. (1963) ‘The functioning and significance of the lateral-line organs’, *Biological Reviews*, 38(1), pp. 51–105.

Dimitrova, M. and Merilaita, S. (2010) ‘Prey concealment: Visual background complexity and prey contrast distribution’, *Behavioral Ecology*, 21(1), pp. 176–181. doi: 10.1093/beheco/arp174.

Donnelly, W. A. and Whoriskey, F. G. (1991) ‘ Background-Color Acclimation of Brook Trout for Crypsis Reduces Risk of Predation by Hooded Mergansers Lophodytes cucullatus ’, *North American Journal of Fisheries Management*, 11(2), pp. 206–211. doi: 10.1577/1548-8675(1991)011<0206:bcaobt>2.3.co;2.

Dowing, A. L. and Leibold, M. A. (2010) ‘Species richness facilitates ecosystem resilience in aquatic food webs’, *Freshwater Biology*, 55(10), pp. 2123–2137.

Dudgeon, D. *et al.* (2006) ‘Freshwater biodiversity: Importance, threats, status and conservation challenges’, *Biological Reviews of the Cambridge Philosophical Society*, 81(2), pp. 163–182. doi: 10.1017/S1464793105006950.

Duffy, C. J. (2000) *Neuronal Processing of Optic Flow*, *International Review of Neurobiology*. Academic Press. Available at: http://www.sciencedirect.com/science/article/pii/S0074774208607436.

Dyhr, J. R. and Higgins, C. M. (2010) ‘The spatial frequency tuning of optic-flow-dependent behaviors in the bumblebee Bombus impatiens’, *Journal of Experimental Biology*, 213(10), pp. 1643–1650. doi: 10.1242/jeb.041426.

EEC (1992) ‘“Habitatdirektivet”. Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora. Svensk versjon på http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:1992L0043:20070101:SV:PDF’, *Official Journal of the European Union*, 206, pp. 7–50.

Elder, J. and Coombs, S. (2015) ‘The influence of turbulence on the sensory basis of rheotaxis’, *Journal of comparative physiology A*, 201(7), pp. 667–680.

Elder, T. *et al.* (2016) ‘Factors influencing the survival of outmigrating juvenile salmonids through multiple dam passages: an individual-based approach’, *Ecology and Evolution*, 6(16), pp. 5881–5892. doi: 10.1002/ece3.2326.

Enders, E. C., Gessel, M. H. and Williams, J. G. (2009) ‘Development of successful fish passage structures for downstream migrants requires knowledge of their behavioural response to accelerating flow’, *Canadian Journal of Fisheries and Aquatic Sciences*, 66(12), pp. 2109–2117. doi: 10.1139/F09-141.

Engelmann, J., Hanke, W. and Bleckmann, H. (2002) ‘Lateral line reception in still- and running water’, *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology*, 188(7), pp. 513–526. doi: 10.1007/s00359-002-0326-6.

EU (2000) ‘Council Directive of 23 October 2000 establishing a framework for Community action in the field of water policy’, *Directive number 2000/60/EC*, 2000/60/EC, p. Brussels. Available at: http://jncc.defra.gov.uk/page-1375 %0D.

Everett, R. A. and Ruiz, G. M. (1993) ‘Coarse woody debris as a refuge from predation in aquatic communities - An experimental test’, *Oecologia*, 93(4), pp. 475–486. doi: 10.1007/BF00328954.

FAO (2018) *The State of World Fisheries and Aquaculture 2018 - Meeting the sustainable development goals.* Rome.

FAO (2020) *World Food and Agriculture - Statistical Yearbook 2020*, *World Food and Agriculture - Statistical Yearbook 2020*. Rome. doi: 10.4060/cb1329en.

Faucher, K. *et al.* (2010) ‘Fish lateral system is required for accurate control of shoaling behaviour’, *Animal Behaviour*, 79(3), pp. 679–687. doi: 10.1016/j.anbehav.2009.12.020.

Fausch, K. D. *et al.* (2002) ‘Landscapes to riverscapes: Bridging the gap between research and conservation of stream fishes’, *BioScience*, 52(6), pp. 483–498. doi: 10.1641/0006-3568(2002)052[0483:LTRBTG]2.0.CO;2.

Fjeldstad, H. P., Alfredsen, K. and Boissy, T. (2014) ‘Optimising Atlantic salmon smolt survival by use of hydropower simulation modelling in a regulated river’, *Fisheries Management and Ecology*, 21(1), pp. 22–31. doi: 10.1111/fme.12044.

Ford, J. R. and Swearer, S. E. (2013) ‘Two’s company, three’s a crowd: Food and shelter limitation outweigh the benefits of group living in a shoaling fish', *Ecology*, 94(5), pp. 1069–1077. doi: 10.1890/12-1891.1.

Fox, J. and Weisberg, S. (2014) *An R Companion to Applied Regression: Appendices*. Second, *Robust Regression in R*. Second. Thousand Oaks {CA}: Sage. Available at: http://socserv.socsci.mcmaster.ca/jfox/Books/Companion/appendix.html.

Franz, M. O. and Mallot, H. A. (2000) 'Biomimetic robot navigation', *Robotics and Autonomous Systems*, 30(1), pp. 133–153. doi: 10.1016/S0921-8890(99)00069-X.

Frost, B. J. (2010) 'A taxonomy of different forms of visual motion detection and their underlying neural mechanisms', *Brain, Behavior and Evolution*, 75(3), pp. 218–235. doi: 10.1159/000314284.

Frost, W. E. (1943) 'The natural history of the minnow, Phoxinus phoxinus', *The Journal of Animal Ecology*, pp. 139–162.

Fry, S. N. *et al.* (2009) 'Visual control of flight speed in Drosophila melanogaster', *Journal of Experimental Biology*, 212(8), pp. 1120–1130.

Fu, T. *et al.* (2016) 'Assessing hydraulic conditions through Francis turbines using an autonomous sensor device', *Renewable Energy*, 99, pp. 1244–1252. doi: 10.1016/j.renene.2016.08.029.

Gautrais, J. *et al.* (2009) 'Analyzing fish movement as a persistent turning walker', *Journal of mathematical biology*, 58(3), pp. 429–445.

Geurten, B. R. H. *et al.* (2017) 'Saccadic movement strategy in a semiaquatic species - The harbour seal (Phoca vitulina)', *Journal of Experimental Biology*, 220(8), pp. 1503–1508. doi: 10.1242/jeb.150763.

Gherardi, F. *et al.* (2008) 'Alien Invertebrates and Fish in European Inland Waters', in *Handbook of Alien Species in Europe*. Springer, pp. 81–92. doi: 10.1007/978-1-4020-8280-1_6.

Goldenberg, S. U., Borcherding, J. and 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(6), pp. 989–998. doi: 10.1007/s00265-014-1711-1.

Goller, B. and Altshuler, D. L. (2014) 'Hummingbirds control hovering flight by stabilizing visual motion', *Proceedings of the National Academy of Sciences of the United States of America*, 111(51), pp. 18375–18380. doi: 10.1073/pnas.1415975111.

Handegard, N. O. *et al.* (2012) 'The dynamics of coordinated group hunting and collective information transfer among schooling prey', *Current Biology*, 22(13), pp. 1213–1217. doi:

10.1016/j.cub.2012.04.050.

Hartig, F. (2020) ‘DHARMa: residual diagnostics for hierarchical (Multi-Level/Mixed) regression models. The Comprehensive R Archive Network (CRAN), R package version 0.3. 2.0’. Available at: https://cran.r-project.org/web/packages/DHARMa/vignettes/DHARMa.html (accessed Jul 4, 2020).

Hassenstein, V. B. and Reichardt, W. (1956) ‘System theoretical analysis of time, sequence and sign analysis of the motion perception of the snout-beetle Chlorophanus’, *Z Naturforsch B*, 11(9–10), pp. 513–524.

Hawryshyn, C. W. (1992) ‘Polarization vision in fish’, *American Scientist*, 80(2), pp. 164–175.

He, X. and Kitchell, J. F. (1990) ‘Direct and Indirect Effects of Predation on a Fish Community: A Whole-Lake Experiment’, *Transactions of the American Fisheries Society*, 119(5), pp. 825–835. doi: 10.1577/1548-8659(1990)119<0825:daieop>2.3.co;2.

Hedenström, A. and Åkesson, S. (2017) ‘Adaptive airspeed adjustment and compensation for wind drift in the common swift: differences between day and night’, *Animal Behaviour*, 127, pp. 117–123. doi: 10.1016/j.anbehav.2017.03.010.

Helfman, G. S. *et al.* (2009) *The diversity of fishes: biology, evolution, and ecology*, *Atlantic*. John Wiley & Sons. doi: 10.1007/978-1-4615-2664-3_1.

Helmer, D. *et al.* (2017) ‘Saccadic movement strategy in common cuttlefish (Sepia officinalis)’, *Frontiers in Physiology*, 7(JAN), p. 660. doi: 10.3389/fphys.2016.00660.

Hemelrijk, C. K. *et al.* (2015) ‘The increased efficiency of fish swimming in a school’, *Fish and Fisheries*, 16(3), pp. 511–521. doi: 10.1111/faf.12072.

Hemmings, C. C. (1973) ‘Direct observation of the behaviour of fish in relation to fishing gear’, *Helgoländer Wissenschaftliche Meeresuntersuchungen*, 24(1–4), pp. 348–360. doi: 10.1007/BF01609525.

Heppner, F. H. (1985) ‘Visual Angle and Formation Flight in Canada Geese (Branta canadensis)’, *The Auk*, 102(1), pp. 195–198. doi: 10.2307/4086847.

Herbert-Read, J. E. *et al.* (2017) ‘How predation shapes the social interaction rules of shoaling fish’, *Proceedings of the Royal Society B: Biological Sciences*, 284(1861), p. 20171126. doi: 10.1098/rspb.2017.1126.

Hering, D. *et al.* (2010) ‘The European Water Framework Directive at the age of 10: A critical review of the achievements with recommendations for the future', *Science of the Total Environment*, 408(19), pp. 4007–4019. doi: 10.1016/j.scitotenv.2010.05.031.

Hoare, D. J. *et al.* (2004) 'Context-dependent group size choice in fish', *Animal Behaviour*, 67(1), pp. 155–164. doi: 10.1016/j.anbehav.2003.04.004.

Holmlund, C. M. and Hammer, M. (1999) 'Ecosystem services generated by fish populations', *Ecological Economics*, 29(2), pp. 253–268. doi: 10.1016/S0921-8009(99)00015-4.

Holthe, E. *et al.* (2009) 'Swimming performance of the European minnow', *Boreal Environment Research*, 14(2), pp. 272–278.

Horridge, G. A. (1987) 'The evolution of visual processing and the construction of seeing systems', *Proceedings of the Royal Society of London - Biological Sciences*, 230(1260), pp. 279–292. doi: 10.1098/rspb.1987.0020.

Hutchings, J. A. (2002) *Migration of Freshwater Fishes*, *Copeia*. John Wiley & Sons. doi: 10.1643/0045-8511(2002)002[0878:]2.0.co;2.

IEA (2020) *Hydropower*. Paris. Available at: https://www.iea.org/reports/hydropower.

Imada, H. *et al.* (2010) 'Coordinated and cohesive movement of two small conspecific fish induced by eliciting a simultaneous optomotor response', *PLoS ONE*, 5(6), p. e11248. doi: 10.1371/journal.pone.0011248.

Ioannou, C. C. *et al.* (2011) 'Social organisation and information transfer in schooling fish', *Fish cognition and behavior*, 2, pp. 217–239.

Ioannou, C. C. (2017) 'Swarm intelligence in fish? The difficulty in demonstrating distributed and self-organised collective intelligence in (some) animal groups', *Behavioural Processes*, 141, pp. 141–151. doi: 10.1016/j.beproc.2016.10.005.

Ioannou, C. C. and Krause, J. (2009) 'Interactions between background matching and motion during visual detection can explain why cryptic animals keep still', *Biology Letters*, 5(2), pp. 191–193. doi: 10.1098/rsbl.2008.0758.

Irvin, W. R. (1993) 'Endangered species act [8]', *Science*, 259(5094), p. 443. doi: 10.1126/science.259.5094.443.

IUCN (2021) *The IUCN Red List of Threatened Species.*, *Version 2021-1*. Available at: https://www.iucnredlist.org.

Jacoby, D. M. P. *et al.* (2015) ‘Synergistic patterns of threat and the challenges facing global anguillid eel conservation’, *Global Ecology and Conservation*, 4, pp. 321–333. doi: 10.1016/j.gecco.2015.07.009.

Jepsen, N. *et al.* (1998) ‘Survival of radio-tagged Atlantic salmon (Salmo salar L.) and trout (Salmo trutta L.) smolts passing a reservoir during seaward migration’, *Hydrobiologia*, 371/372(August), pp. 347–353. doi: 10.1007/978-94-011-5090-3.

Jepsen, N., Pedersen, S. and Thorstad, E. (2000) ‘Behavioural interactions between prey (trout smolts) and predators (pike and pikeperch) in an impounded river’, *River Research and Applications*, 16(2), pp. 189–198. doi: 10.1002/(sici)1099-1646(200003/04)16:2<189::aid-rrr570>3.0.co;2-n.

Johansen, J. L., Akanyeti, O. and Liao, J. C. (2020) ‘Oxygen consumption of drift-feeding rainbow trout: The energetic tradeoff between locomotion and feeding in flow’, *Journal of Experimental Biology*, 223(12). doi: 10.1242/jeb.220962.

Johnson, E. D. and Cowen, E. A. (2017) ‘Remote determination of the velocity index and mean streamwise velocity profiles’, *Water Resources Research*, 53(9), pp. 7521–7535. doi: 10.1002/2017WR020504.

Johnston, M. E. *et al.* (2017) ‘Experimental evaluation of the use of vision and barbels as references for rheotaxis in green sturgeon’, *Journal of Experimental Marine Biology and Ecology*, 496, pp. 9–15. doi: 10.1016/j.jembe.2017.04.002.

Jones, E. G., Summerbell, K. and O’Neill, F. (2008) ‘The influence of towing speed and fish density on the behaviour of haddock in a trawl cod-end’, *Fisheries Research*, 94(2), pp. 166–174. doi: 10.1016/j.fishres.2008.06.010.

Kareklas, K., Elwood, R. W. and Holland, R. A. (2017) ‘Personality effects on spatial learning: Comparisons between visual conditions in a weakly electric fish’, *Ethology*, 123(8), pp. 551–559. doi: 10.1111/eth.12629.

Katz, Y. *et al.* (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(46), pp. 18720–18725. doi: 10.1073/pnas.1107583108.

Katzir, G. *et al.* (2001) ‘Head stabilization in herons’, *Journal of Comparative Physiology - A Sensory, Neural, and Behavioral Physiology*, 187(6), pp. 423–432. doi: 10.1007/s003590100210.

Keenleyside, M.H. (1955). Some aspects of the schooling behaviour of fish. *Behaviour*, pp.183-

248.

Kelber, A. and Zeil, J. (1997) ‘Tetragonisca guard bees interpret expanding and contracting patterns as unintended displacement in space’, *Journal of Comparative Physiology - A Sensory, Neural, and Behavioral Physiology*, 181(3), pp. 257–265. doi: 10.1007/s003590050112.

Keller, R. P. *et al.* (2011) ‘Invasive species in Europe: Ecology, status, and policy’, *Environmental Sciences Europe*, 23(1), p. 23. doi: 10.1186/2190-4715-23-23.

Kemp, P. S. *et al.* (2006) ‘The behaviour of Pacific salmonid smolts during passage over two experimental weirs under light and dark conditions’, *River Research and Applications*, 22(4), pp. 429–440. doi: 10.1002/rra.913.

Kemp, P. S. and Williams, J. G. (2008) ‘Response of migrating chinook salmon (ONCORHYNCHUS TSHAWYTSCHA) smolts to in-stream structure associated with culverts’, *River Research and Applications*, 24(5), pp. 571–579.

Kern, R. *et al.* (2005) ‘Function of a fly motion-sensitive neuron matches eye movements during free flight’, *PLoS Biology*, 3(6), pp. 1130–1138. doi: 10.1371/journal.pbio.0030171.

Kerr, J. R., Manes, C. and Kemp, P. S. (2016) ‘Assessing hydrodynamic space use of brown trout, Salmo trutta, in a complex flow environment: A return to first principles’, *Journal of Experimental Biology*, 219(21), pp. 3480–3491. doi: 10.1242/jeb.134775.

Kim, J. B. and Park, C. (2017) ‘A study on VR sickness prediction of HMD contents using machine learning technique’, in *Communications in Computer and Information Science*, pp. 38–41. doi: 10.1007/978-3-319-58753-0_6.

Kim, Y. (2015) ‘Escape response of juvenile fish in a shaking codend determined from tank experiments’, *Fisheries Research*, 161, pp. 273–279. doi: 10.1016/j.fishres.2014.08.012.

Kjernsmo, K. and Merilaita, S. (2012) ‘Background choice as an anti-predator strategy: The roles of background matching and visual complexity in the habitat choice of the least killifish’, *Proceedings of the Royal Society B: Biological Sciences*, 279(1745), pp. 4192–4198. doi: 10.1098/rspb.2012.1547.

Krause, J. *et al.* (2002) *Living in groups*. Oxford University Press.

Krausz, R. R. (2013) *Living in Groups*, *Transactional Analysis Journal*. Oxford University Press. doi: 10.1177/0362153713519414.

Kulpa, M., Bak-Coleman, J. and Coombs, S. (2015) ‘The lateral line is necessary for blind cavefish rheotaxis in non-uniform flow', *Journal of Experimental Biology*, 218(10), pp. 1603–1612. doi: 10.1242/jeb.119537.

Larinier, M. (1990) 'Experience of fish passage in France: fish pass design criteria and downstream migration problems.', in *Proceedings of the international symposium on fishways, Gifu Japan*, pp. 65–74.

Larinier, M. (2008) 'Fish passage experience at small-scale hydro-electric power plants in France', *Hydrobiologia*, 609(1), pp. 97–108. doi: 10.1007/s10750-008-9398-9.

Larinier, M. and Marmulla, G. (2004) 'Fish passes: types, principles and geographical distribution - An overview', in *Proceedings of the Second International Symposium on the Management of Large Rivers for Fisheries Volume II.*, p. 22pp.

Larinier, M. and Travade, F. (2002) 'Chapter 13 Downstream Migration : Problems and Facilities', *Rivers*, (1), pp. 181–205. doi: 10.1051/kmae/2002102.

Lecoeur, J. *et al.* (2019) 'The role of optic flow pooling in insect flight control in cluttered environments', *Scientific Reports*, 9(1), pp. 1–13. doi: 10.1038/s41598-019-44187-2.

Lehrer, M., Srinivasan, M. V. and Zhang, S. W. (1990) 'Visual edge detection in the honeybee and its chromatic properties', *Proceedings of the Royal Society B: Biological Sciences*, 238(1293), pp. 321–330. doi: 10.1098/rspb.1990.0002.

Lehrer, M. and Srinivasan, M. V (1993) 'Object detection by honeybees: Why do they land on edges?', *Journal of Comparative Physiology A*, 173(1), pp. 23–32.

Lemasson, B. *et al.* (2018) 'Motion cues tune social influence in shoaling fish', *Scientific Reports*, 8(1), p. 9785. doi: 10.1038/s41598-018-27807-1.

Liao, J. C. (2007) 'A review of fish swimming mechanics and behaviour in altered flows', *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, 362(1487), pp. 1973–1993. doi: 10.1098/rstb.2007.2082.

Lima, S. L. (1995) 'Back to the basics of anti-predatory vigilance: the group-size effect', *Animal Behaviour*, 49(1), pp. 11–20.

Limburg, Karin E and Waldman, J. R. (2009) 'Dramatic declines in North Atlantic diadromous fishes', *BioScience*, 59(11), pp. 955–965. doi: 10.1525/bio.2009.59.11.7.

Limburg, Karin E. and Waldman, J. R. (2009) 'Dramatic Declines in North Atlantic Diadromous Fishes', *BioScience*, 59(11), pp. 955–965. doi: 10.1525/bio.2009.59.11.7.

Lin, J. *et al.* (2021) ‘Changes in supply and demand mediate the effects of land-use change on freshwater ecosystem services flows’, *Science of the Total Environment*, 763, p. 143012. doi: 10.1016/j.scitotenv.2020.143012.

Linander, N., Baird, E. and Dacke, M. (2017) ‘How bumblebees use lateral and ventral optic flow cues for position control in environments of different proximity’, *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology*, 203(5), pp. 343–351. doi: 10.1007/s00359-017-1173-9.

Ling, H. *et al.* (2019) ‘Collective turns in jackdaw flocks: Kinematics and information transfer’, *Journal of the Royal Society Interface*, 16(159), p. 20190450. doi: 10.1098/rsif.2019.0450.

Lynch, A. J. *et al.* (2016) ‘The social, economic, and environmental importance of inland fish and fisheries’, *Environmental Reviews*, 24(2), pp. 115–121. doi: 10.1139/er-2015-0064.

Lyon, E. P. (1904) ‘On rheotropism. I.Rheotropism in fishes’, *American Journal of Physiology-Legacy Content*, 12(2), pp. 149–161.

Macaulay, G. *et al.* (2020) ‘Challenges and benefits of applying fish behaviour to improve production and welfare in industrial aquaculture’, *Reviews in Aquaculture*. doi: 10.1111/raq.12505.

MacGregor, H. E. A., Herbert-Read, J. E. and Ioannou, C. C. (2020) ‘Information can explain the dynamics of group order in animal collective behaviour’, *Nature Communications*, 11(1), pp. 1–8. doi: 10.1038/s41467-020-16578-x.

Maes, J. *et al.* (2004) ‘Field evaluation of a sound system to reduce estuarine fish intake rates at a power plant cooling water inlet’, *Journal of Fish Biology*, 64(4), pp. 938–946. doi: 10.1111/j.1095-8649.2004.00360.x.

Magurran, A. E. (1986) ‘The development of shoaling behaviour in the European minnow, Phoxinus phoxinus’, *Journal of Fish Biology*, 29, pp. 159–169.

Magurran, A. E. and Higham, A. (1988) ‘Information Transfer across Fish Shoals under Predator Threat’, *Ethology*, 78(2), pp. 153–158. doi: 10.1111/j.1439-0310.1988.tb00226.x.

Magurran, A. E., Oulton, W. J. and Pitcher, T. J. (1985) ‘Vigilant Behaviour and Shoal Size in Minnows’, *Zeitschrift für Tierpsychologie*, 67(1–4), pp. 167–178. doi: 10.1111/j.1439-0310.1985.tb01386.x.

Magurran, A. E. and Pitcher, T. J. (1983) ‘Foraging, timidity and shoal size in minnows and goldfish', *Behavioral Ecology and Sociobiology*, 12(2), pp. 147–152. doi: 10.1007/BF00343206.

Makhankov, Y. (2005) *Assessment of outer retinal function in genetically modified zebrafish larvae by electroretinography (ECG).*, *Ph.D Dissertation*. ETH Zurich. Available at: http://e-collection.library.ethz.ch/eserv/eth:28238/eth- 28238-02.pdf/.

Makris, N. C. *et al.* (2006) 'Fish population and behavior revealed by instantaneous continental shelf-scale imaging', *Science*, 311(5761), pp. 660–663.

Marchesan, M. *et al.* (2005) 'Behavioural effects of artificial light on fish species of commercial interest', *Fisheries Research*, 73(1–2), pp. 171–185. doi: 10.1016/j.fishres.2004.12.009.

van der Marel, A., López-Darias, M. and Waterman, J. M. (2019) 'Group-enhanced predator detection and quality of vigilance in a social ground squirrel', *Animal Behaviour*, 151, pp. 43–52. doi: 10.1016/j.anbehav.2019.02.017.

Marr, D. and Nishihara, H. K. (1978) 'Representation and recognition of the spatial organization of three-dimensional shapes.', *Proceedings of the Royal Society of London. Series B, Containing papers of a Biological character. Royal Society (Great Britain)*, 200(1140), pp. 269–294. doi: 10.1098/rspb.1978.0020.

Marras, S. *et al.* (2015) 'Fish swimming in schools save energy regardless of their spatial position', *Behavioral Ecology and Sociobiology*, 69(2), pp. 19–226. doi: 10.1007/s00265-014-1834-4.

Mathiron, A. G. E., Crane, A. L. and Ferrari, M. C. O. (2015) 'Individual vs. social learning of predator information in fish: does group size affect learning efficacy?', *Behavioral Ecology and Sociobiology*, 69(6), pp. 939–949. doi: 10.1007/s00265-015-1905-1.

Mauss, A. S. and Borst, A. (2020) 'Optic flow-based course control in insects', *Current Opinion in Neurobiology*, 60, pp. 21–27. doi: 10.1016/j.conb.2019.10.007.

Merilaita, S. (2003) 'Visual background complexity facilitates the evolution of camouflage', *Evolution*, 57(6), pp. 1248–1254. doi: 10.1111/j.0014-3820.2003.tb00333.x.

Miehls, S. M., Johnson, N. S. and Hrodey, P. J. (2017) 'Test of a Nonphysical Barrier Consisting of Light, Sound, and Bubble Screen to Block Upstream Movement of Sea Lampreys in an Experimental Raceway', *North American Journal of Fisheries Management*, 37(3), pp. 660–666.

Miles, J., Vowles, A. S. and Kemp, P. S. (2021) 'The response of common minnows, Phoxinus phoxinus, to visual cues under flowing and static water conditions', *Animal Behaviour*, 179, pp. 289–296.

Milner, N. J., Solomon, D. J. and Smith, G. W. (2012) ‘The role of river flow in the migration of adult Atlantic salmon, Salmo salar, through estuaries and rivers’, *Fisheries Management and Ecology*, 19(6), pp. 537–547. doi: 10.1111/fme.12011.

Mongeon, P. and Paul-Hus, A. (2016) ‘The journal coverage of Web of Science and Scopus: a comparative analysis’, *Scientometrics*, 106(1), pp. 213–228. doi: 10.1007/s11192-015-1765-5.

Montgomery, J. C., Baker, C. F. and Carton, A. G. (1997) ‘The lateral line can mediate rheotaxis in fish’, *Nature*, 389(6654), pp. 960–963. doi: 10.1038/40135.

Morgan, M. J. (1988) ‘The effect of hunger, shoal size and the presence of a predator on shoal cohesiveness in bluntnose minnows, Pimephales notatus Rafinesque’, *Journal of Fish Biology*, 32(6), pp. 963–971. doi: 10.1111/j.1095-8649.1988.tb05439.x.

Morrell, L. J., Ruxton, G. D. and James, R. (2011) ‘Spatial positioning in the selfish herd’, *Behavioral Ecology*, 22(1), pp. 16–22. doi: 10.1093/beheco/arq157.

Mostofi, N., Boi, M. and Rucci, M. (2016) ‘Are the visual transients from microsaccades helpful? Measuring the influences of small saccades on contrast sensitivity’, *Vision Research*, 118, pp. 60–69. doi: 10.1016/j.visres.2015.01.003.

Mussen, T. D. and Cech, J. J. (2019) ‘Assessing the use of vibrations and strobe lights at fish screens as enhanced deterrents for two estuarine fishes’, *Journal of Fish Biology*, 95(1), pp. 238–246. doi: 10.1111/jfb.13776.

Naiman, R. J. and Latterell, J. J. (2005) ‘Principles for linking fish habitat to fisheries management and conservation’, *Journal of Fish Biology*, 67(SUPPL. B), pp. 166–185. doi: 10.1111/j.0022-1112.2005.00921.x.

Nakano, N. *et al.* (2006) ‘Color vision, spectral sensitivity, accommodation, and visual acuity in juvenile masu salmon Oncorhynchus masou masou’, *Fisheries Science*, 72(2), pp. 239–249. doi: 10.1111/j.1444-2906.2006.01144.x.

Nelken, I. (2004) ‘Processing of complex stimuli and natural scenes in the auditory cortex’, *Current Opinion in Neurobiology*, 14(4), pp. 474–480. doi: 10.1016/j.conb.2004.06.005.

Nevoux, M. *et al.* (2019) ‘Environmental influences on life history strategies in partially anadromous brown trout (Salmo trutta, Salmonidae)’, *Fish and Fisheries*, 20(6), pp. 1051–1082.

Nilsson, C. *et al.* (2005) ‘Fragmentation and Flow Regulation of the World’s Large River Systems’, *Science*, 308(5720), pp. 405–408.

Noatch, M. R. and Suski, C. D. (2012) 'Non-physical barriers to deter fish movements', *Environmental Reviews*, 20(1), pp. 71–82.

Odling-Smee, L., Simpson, S. D. and Braithwaite, V. A. (2011) 'The Role of Learning in Fish Orientation', *Fish Cognition and Behavior*, 4(3), pp. 166–185. doi: 10.1002/9781444342536.ch8.

Olive, R. *et al.* (2016) 'Rheotaxis of larval zebrafish: Behavioral study of a multi-sensory process', *Frontiers in Systems Neuroscience*, 10(FEB), p. 14. doi: 10.3389/fnsys.2016.00014.

Orpwood, J. E. *et al.* (2008) 'Minnows and the selfish herd: effects of predation risk on shoaling behaviour are dependent on habitat complexity', *Animal Behaviour*, 76(1), pp. 143–152. doi: 10.1016/j.anbehav.2008.01.016.

Pantoni, M. M. *et al.* (2020) 'Quantifying the acoustic startle response in mice using standard digital video', *Frontiers in Behavioral Neuroscience*, 14, p. 83.

Partridge, B. L. and Pitcher, T. J. (1980) 'The sensory basis of fish schools: Relative roles of lateral line and vision', *Journal of Comparative Physiology □ A*, 135(4), pp. 315–325. doi: 10.1007/BF00657647.

Peter B McIntyre, Liermann, C. A. R. and Revenga, C. (2016) 'Linking freshwater fishery management to global food security and biodiversity conservation', *Proceedings of the National Academy of Sciences*, 113(45), pp. 12880–12885.

Pita, D. *et al.* (2015) 'Vision in two cyprinid fish: Implications for collective behavior', *PeerJ*, 2015(8), p. e1113. doi: 10.7717/peerj.1113.

Pitcher, T. J. (1986) 'Functions of shoaling behaviour in teleosts', in *The behaviour of teleost fishes*. Springer, pp. 294–337.

Pitcher, T. J., Magurran, A. E. and Winfield, I. J. (1982) 'Fish in larger shoals find food faster', *Behavioral Ecology and Sociobiology*, 10(2), pp. 149–151. doi: 10.1007/BF00300175.

Pitcher, T. J. and Parrish, J. K. (1993) 'Functions of shoaling behaviour in teleosts', in *Behaviour of Teleost Fishes*. Springer, pp. 363–439. doi: 10.1007/978-94-011-1578-0_12.

Podolsrky, D., Uiblein, F. and Winkler, H. (1995) 'Visual habitat choice in cyprinid fishes: an experimental approach', *Ecology of Freshwater Fish*, 4(4), pp. 160–167. doi: 10.1111/j.1600-0633.1995.tb00028.x.

Portelli, G., Ruffier, F. and Franceschini, N. (2010) 'Honeybees change their height to restore their optic flow', *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral*

Physiology, 196(4), pp. 307–313. doi: 10.1007/s00359-010-0510-z.

Protasov, V. R. and Altakov, U. P. (1961) ‘The possibility of using the optomotor reaction for controlling the movement of fishes’, *Plynoe. Khoz*, 2, pp. 29–32.

Przybilla, A. *et al.* (2010) ‘Entraining in trout: A behavioural and hydrodynamic analysis’, *Journal of Experimental Biology*, 213(17), pp. 2976–2986. doi: 10.1242/jeb.041632.

Putland, R. L. and Mensinger, A. F. (2019) ‘Acoustic deterrents to manage fish populations’, *Reviews in Fish Biology and Fisheries*, pp. 1–19.

R Core Team (2020) ‘R: A Language and Environment for Statistical Computing’. Vienna, Austria. Available at: https://www.r-project.org/.

Rahel, F. J. (2002) ‘Homogenization of Freshwater Faunas’, *Annual Review of Ecology and Systematics*, 33(1), pp. 291–315. doi: 10.1146/annurev.ecolsys.33.010802.150429.

Rahel, F. J. and McLaughlin, R. L. (2018) ‘Selective fragmentation and the management of fish movement across anthropogenic barriers’, *Ecological Applications*, 28(8), pp. 2066–2081. doi: 10.1002/eap.1795.

Randall, J. (2014) *Trout Sense: A Fly Fisher’s Guide to What Trout See, Hear, \& Smell*. Stackpole Books.

Rankin, C. H. *et al.* (2009) ‘Habituation revisited: An updated and revised description of the behavioral characteristics of habituation’, *Neurobiology of Learning and Memory*, 92(2), pp. 135–138. doi: 10.1016/j.nlm.2008.09.012.

Revenga, C. *et al.* (2010) ‘Prospects for monitoring freshwater ecosystems towards the 2010 targets Rapid response Email alerting service Prospects for monitoring freshwater ecosystems towards the 2010 targets’, *Society*, 360(1454), pp. 397–413. doi: 10.1098/rstb.2004.1595.

Rice, S. P., Lancaster, J. and Kemp, P. (2010) ‘Experimentation at the interface of fluvial geomorphology, stream ecology and hydraulic engineering and the development of an effective, interdisciplinary river science’, *Earth Surface Processes and Landforms*, 35(1), pp. 64–77. doi: 10.1002/esp.1838.

Richards, N. S., Chipps, S. R. and Brown, M. L. (2007) ‘Stress Response and Avoidance Behavior of Fishes as Influenced by High-Frequency Strobe Lights’, *North American Journal of Fisheries Management*, 27(4), pp. 1310–1315. doi: 10.1577/m06-239.1.

Rieger, J. H. and Lawton, D. T. (1985) ‘Processing differential image motion’, *Journal of the Optical*

Society of America A, 2(2), p. 354. doi: 10.1364/josaa.2.000354.

Rilov, G. *et al.* (2007) ‘Complex habitats may not always benefit prey: Linking visual field with reef fish behavior and distribution’, *Marine Ecology Progress Series*, 329, pp. 225–238. doi: 10.3354/meps329225.

Rock, I. and Smith, D. (1986) ‘The optomotor response and induced motion of the self’, *Perception*, 15(4), pp. 497–502. doi: 10.1068/p150497.

De Rosario-Martinez, H. (2015) ‘phia: Post-Hoc Interaction Analysis’. Available at: https://cran.r-project.org/package=phia.

Rubin, G. S. (2012) ‘Visual Acuity and Contrast Sensitivity’, *Retina Fifth Edition*, 1(1), pp. 300–306. doi: 10.1016/B978-1-4557-0737-9.00011-4.

Rucci, M. and Poletti, M. (2015) ‘Control and Functions of Fixational Eye Movements’, *Annual review of vision science*, 1, pp. 499–518. doi: 10.1146/annurev-vision-082114-035742.

Russon, I. J., Kemp, P. S. and Calles, O. (2010) ‘Response of downstream migrating adult European eels (Anguilla anguilla) to bar racks under experimental conditions’, *Ecology of Freshwater Fish*, 19(2), pp. 197–205. doi: 10.1111/j.1600-0633.2009.00404.x.

SAFFA (1975) ‘Salmon and Freshwater Fisheries Act’.

Schaerf, T. M., Dillingham, P. W. and Ward, A. J. W. (2017) ‘The effects of external cues on individual and collective behavior of shoaling fish’, *Science Advances*, 3(6), p. e1603201. doi: 10.1126/sciadv.1603201.

Scharf, F. S., Manderson, J. P. and Fabrizio, M. C. (2006) ‘The effects of seafloor habitat complexity on survival of juvenile fishes: Species-specific interactions with structural refuge’, *Journal of Experimental Marine Biology and Ecology*, 335(2), pp. 167–176. doi: 10.1016/j.jembe.2006.03.018.

Schiffner, I. and Srinivasan, M. V. (2015) ‘Direct evidence for vision-based control of flight speed in budgerigars’, *Scientific Reports*, 5, p. 10992. doi: 10.1038/srep10992.

Schiffner, I. and Srinivasan, M. V. (2016) ‘Budgerigar flight in a varying environment: Flight at distinct speeds?’, *Biology Letters*, 12(6), p. 20160221. doi: 10.1098/rsbl.2016.0221.

Schindler, D. W. (1997) ‘Widespread effects of climatic warming on freshwater ecosystems in North America’, *Hydrological Processes*, 11(8), pp. 1043–1067.

Scholtyssek, C. *et al.* (2014) 'Control of self-motion in dynamic fluids: Fish do it differently from bees', *Biology Letters*, 10(5), p. 20140279. doi: 10.1098/rsbl.2014.0279.

Serres, J. R. *et al.* (2008) 'A bee in the corridor: Centering and wall-following', *Naturwissenschaften*, 95(12), pp. 1181–1187. doi: 10.1007/s00114-008-0440-6.

Shaw, E. (1962) 'The Schooling of Fishes', *Scientific American*, 206(6), pp. 128–141. doi: 10.1038/scientificamerican0662-128.

Shaw, E. (1965) 'The optomotor response and the schooling of fish', *Spec. Publs int. Commn NW. Atlant. Fish*, 6, pp. 753–755.

Shaw, E. and Sachs, B. (1967) 'Development of the Optomotor Response in the Schooling Fish, Menidia Menidia', *Journal of Comparative and Physiological Psychology*, 63(3), pp. 385–388. doi: 10.1037/h0024636.

Shaw, E. and Tucker, A. (1965) 'The optomotor reaction of schooling carangid fishes', *Animal Behaviour*, 13(2–3), pp. 330--IN7. doi: 10.1016/0003-3472(65)90052-7.

Sheenaja, K. K. and Thomas, K. J. (2011) 'Influence of habitat complexity on route learning among different populations of climbing perch (Anabas testudineus Bloch, 1792)', *Marine and freshwater behaviour and physiology*, 44(6), pp. 349–358.

Shelton, D. S. *et al.* (2020) 'Collective behavior in wild zebrafish', *Zebrafish*, 17(4), pp. 243–252. doi: 10.1089/zeb.2019.1851.

Short, M. *et al.* (2020) 'Influence of acoustics on the collective behaviour of a shoaling freshwater fish', *Freshwater Biology*, 65(12), pp. 2186–2195. doi: 10.1111/fwb.13612.

Silva, J. B., Serdoura, F. and Pinto, P. (2006) 'Urban Rivers as Factors of Urban (Dis)integration', in *42nd ISOCARP Congress*, pp. 14–18.

Smith, B. B. *et al.* (2005) 'Current-flow and odour stimulate rheotaxis and attraction in common carp', *South Australian Research and Development Institute (Aquatic Science), SARDI Publication Number RD04/0064--3, Adelaide*.

Sobel, E. C. (1990) 'The locust's use of motion parallax to measure distance', *Journal of Comparative Physiology A*, 167(5), pp. 579–588. doi: 10.1007/BF00192653.

Sovrano, V. A., Baratti, G. and Lee, S. A. (2020) 'The role of learning and environmental geometry in landmark-based spatial reorientation of fish (Xenotoca eiseni)', *PloS one*, 15(3), p. e0229608.

Srinivasan, M. V. *et al.* (1996) 'Honeybee navigation en route to the goal: Visual flight control and odometry', *Journal of Experimental Biology*, 199(1), pp. 237–244. doi: 10.1242/jeb.199.1.237.

Srinivasan, M. V. *et al.* (1999) 'Robot navigation inspired by principles of insect vision', in *Robotics and Autonomous Systems*, pp. 203–216. doi: 10.1016/S0921-8890(98)00069-4.

Srinivasan, M. V. (2011a) 'Honeybees as a model for the study of visually guided flight, navigation, and biologically inspired robotics', *Physiological Reviews*, 91(2), pp. 413–460. doi: 10.1152/physrev.00005.2010.

Srinivasan, M. V. (2011b) 'Visual control of navigation in insects and its relevance for robotics', *Current Opinion in Neurobiology*, 21(4), pp. 535–543. doi: 10.1016/j.conb.2011.05.020.

Srinivasan, M. V (1992) 'How bees exploit optic flow: behavioural experiments and neural models', *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, 337(1281), pp. 253–259. doi: 10.1098/rstb.1992.0103.

Stiassny, M. L. J. (1996) 'An Overview of Freshwater Biodiversity: With Some Lessons from African Fishes', *Fisheries*, 21(9), pp. 7–13. doi: 10.1577/1548-8446(1996)021<0007:aoofb>2.0.co;2.

Strandburg-Peshkin, A. *et al.* (2013) 'Visual sensory networks and effective information transfer in animal groups', *Current Biology*, 23(17), pp. R709--R711. doi: 10.1016/j.cub.2013.07.059.

Strayer, D. L. and Dudgeon, D. (2010) 'Freshwater biodiversity conservation: Recent progress and future challenges', *Journal of the North American Benthological Society*, 29(1), pp. 344–358. doi: 10.1899/08-171.1.

Stuart, I. G. and Mallen-Cooper, M. (1999) 'An assessment of the effectiveness of a vertical-slot fishway for non-salmonid fish at a tidal barrier on a large tropical/subtropical river', *River Research and Applications*, 15(6), pp. 575–590. doi: 10.1002/(sici)1099-1646(199911/12)15:6<575::aid-rrr562>3.0.co;2-q.

Suli, A. *et al.* (2012) 'Rheotaxis in larval zebrafish is mediated by lateral line mechanosensory hair cells', *PLoS ONE*, 7(2), p. e29727. doi: 10.1371/journal.pone.0029727.

Sunkara, A., DeAngelis, G. C. and Angelaki, D. E. (2016) 'Joint representation of translational and rotational components of optic flow in parietal cortex', *Proceedings of the National Academy of Sciences of the United States of America*, 113(18), pp. 5077–5082. doi: 10.1073/pnas.1604818113.

Tammero, L. F. and Dickinson, M. H. (2002) 'Collision-avoidance and landing responses are mediated by separate pathways in the fruit fly, Drosophila melanogaster', *Journal of Experimental*

Biology, 205(18), pp. 2785–2798.

Tappeiner, C. *et al.* (2012) ‘Visual acuity and contrast sensitivity of adult zebrafish’, *Frontiers in zoology*, 9(1), p. 10.

Taylor, R. M., Pegg, M. A. and Chick, J. H. (2005) ‘Response of bighead carp to a bioacoustic behavioural fish guidance system’, *Fisheries Management and Ecology*, 12(4), pp. 283–286. doi: 10.1111/j.1365-2400.2005.00446.x.

The MathWorks, I. (2018) ‘MATLAB and Statistics Toolbox Release’.

Theunissen, L. M. and Troje, N. F. (2017) ‘Head Stabilization in the Pigeon: Role of Vision to Correct for Translational and Rotational Disturbances’, *Frontiers in neuroscience*, 11, p. 551.

Tindale, R. S. and Winget, J. R. (2019) ‘Group decision-making’, in *Oxford Research Encyclopedia of Psychology*.

Valdimarsson, S. K. and Metcalfe, N. B. (1998) ‘Shelter selection in juvenile Atlantic salmon, or why do salmon seek shelter in winter?’, *Journal of Fish Biology*, 52(1), pp. 42–49.

Valiente, A. G. *et al.* (2010) ‘Not all lineages are equally invasive: genetic origin and life-history in Atlantic salmon and brown trout acclimated to the Southern Hemisphere’, *Biological Invasions*, 12(10), pp. 3485–3495.

Version 3.4.1 R Development Core Team (2017) ‘R: A language and environment for statistical computing. R Foundation for Statistical Computing’. Vienna, Austria. ISBN 3-900051-07-0. Available at: http://www.r-project.org.

Veselov, A. E. *et al.* (1998) ‘Ontogenesis of rheotactic and optomotor responses of juvenile Atlantic salmon’, *Aquaculture*, 168(1–4), pp. 17–26.

Vivancos, A. and Closs, G. (2019) ‘Using fine-scale spatial analysis to study behavioural strategies prevalence in wild groups of drift-feeding fish’, *Canadian Journal of Fisheries and Aquatic Sciences*, 76(10), pp. 1854–1861. doi: 10.1139/cjfas-2018-0221.

Vo, H. D., Schiffner, I. and Srinivasan, M. V. (2016) ‘Anticipatory Manoeuvres in Bird Flight’, *Scientific Reports*, 6, p. 27591. doi: 10.1038/srep27591.

Vörösmarty, C. J. *et al.* (2010) ‘Global threats to human water security and river biodiversity’, *Nature*, 467(7315), pp. 555–561. doi: 10.1038/nature09440.

Voss, R. and Zeil, J. (1998) ‘Active vision in insects: An analysis of object-directed zig-zag flights in wasps (Odynerus spinipes, Eumenidae)', *Journal of Comparative Physiology - A Sensory, Neural, and Behavioral Physiology*, 182(3), pp. 377–387. doi: 10.1007/s003590050187.

Voulgaris, G. and Trowbridge, J. H. (1998) 'Evaluation of the Acoustic Doppler Velocimeter (ADV) for Turbulence Measurements*', *Journal of Atmospheric and Oceanic Technology*, 15(1), pp. 272–289. doi: 10.1175/1520-0426(1998)015<0272:EOTADV>2.0.CO;2.

Vowles, A. S. *et al.* (2014) 'Effects of avoidance behaviour on downstream fish passage through areas of accelerating flow when light and dark', *Animal Behaviour*, 92, pp. 101–109. doi: 10.1016/j.anbehav.2014.03.006.

Vowles, A. S. and Kemp, P. S. (2012) 'Effects of light on the behaviour of brown trout (Salmo trutta) encountering accelerating flow: Application to downstream fish passage', *Ecological Engineering*, 47, pp. 247–253. doi: 10.1016/j.ecoleng.2012.06.021.

Wang, L. and Wu, C. (2011) 'Energy saving mechanism of "channeling effect" in fish school swimming', *Lixue Xuebao/Chinese Journal of Theoretical and Applied Mechanics*, 43(1), pp. 18–23.

Ward, A. J. W. *et al.* (2011) 'Fast and accurate decisions through collective vigilance in fish shoals', *Proceedings of the National Academy of Sciences of the United States of America*, 108(6), pp. 2312–2315. doi: 10.1073/pnas.1007102108.

Ward, A. and Webster, M. (2016) 'Sociality: the behaviour of group-living animals'.

Ward, J. V. and Stanford, J. A. (1995) 'The serial discontinuity concept: Extending the model to floodplain rivers', *Regulated Rivers: Research & Management*, 10(2–4), pp. 159–168. doi: 10.1002/rrr.3450100211.

Warren, W. H. (1998) 'Visually Controlled Locomotion: 40 Years Later', *Ecological Psychology*, 10(3–4), pp. 177–219. doi: 10.1080/10407413.1998.9652682.

Watson, J. R. *et al.* (2018) 'Utilising the boundary layer to help restore the connectivity of fish habitats and populations', *bioRxiv*, 122, pp. 286–294. doi: 10.1101/332338.

Webster, M. M., Ward, A. J. W. and Hart, P. J. B. (2007) 'Boldness is influenced by social context in threespine sticklebacks (Gasterosteus aculeatus)', *Behaviour*, 144(3), p. 351.

Weihs, D. (1973) 'Hydromechanics of fish schooling', *Nature*, 241(5387), p. 290.

Welton, J. S., Beaumont, W. R. C. and Clarke, R. T. (2002) 'The efficacy of air, sound and acoustic bubble screens in deflecting Atlantic salmon, Salmo salar L., smolts in the River Frome, UK', *Fisheries Management and Ecology*, 9(1), pp. 11–18. doi: 10.1046/j.1365-2400.2002.00252.x.

Welton, J S, Beaumont, W. R. C. and Clarke, R. T. (2002) ‘The efficacy of air, sound and acoustic bubble screens in deflecting Atlantic salmon, Salmo salar L., smolts in the River Frome, UK’, *Fisheries Management and Ecology*, 9(1), pp. 11–18. doi: 10.1046/j.1365-2400.2002.00252.x.

White, G. E. and Brown, C. (2015) ‘Cue choice and spatial learning ability are affected by habitat complexity in intertidal gobies’, *Behavioral Ecology*, 26(1), pp. 178–184. doi: 10.1093/beheco/aru178.

Wolf, H. (2011). Odometry and insect navigation. *Journal of Experimental Biology*, *214*(10), pp.1629-1641.

Woodward, G., Perkins, D. M. and Brown, L. E. (2010) ‘Climate change and freshwater ecosystems: Impacts across multiple levels of organization’, *Philosophical Transactions of the Royal Society B: Biological Sciences*, 365(1549), pp. 2093–2106. doi: 10.1098/rstb.2010.0055.

Wylie, D. R., Gutierrez-Ibanez, C. and Iwaniuk, A. N. (2015) ‘Integrating brain, behaviour and phylogeny to understand the evolution of sensory systems in birds’, *Frontiers in Neuroscience*, 9(JUL), p. 281. doi: 10.3389/fnins.2015.00281.

York, N. *et al.* (2011) *Honeybee neurobiology and behavior – a tribute for Randolf Menzel*, *Darwin*. Springer Science & Business Media. Available at: http://books.google.de/books?id=5by7JmlPQQ4C.

Zeil, J., Boeddeker, N. and Hemmi, J. M. (2008) ‘Vision and the organization of behaviour’, *Current Biology*, 18(8), pp. R320--R323. doi: 10.1016/j.cub.2008.02.017.