Predator-Prey Interactions in Aquatic Environments

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Submitted in accordance with the requirements for the degree of

Doctor of Philosophy

The University of Leeds

School of Biology

March 2013

The candidate confirms that the work submitted is her own, except where work which has formed part of jointly authored publications has been included. The contribution of the candidate and the other authors to this work has been explicitly indicated below. The candidate confirms that appropriate credit has been given within the thesis where reference has been made to the work of others.

The work in this thesis involves collaborations with Leo Murphy (LM), Charlotte Leviston (CL), Hugin Kárason Mortensen (HKM), Sarah Pickering (SP), Emily Costello (EC), and Ben Whittaker (BW). Their contributions to each chapter are highlighted below. Lesley J Morrell and Alison M Dunn were involved in all the work.

Chapter two: LM assisted in data collection

Chapter three: CL and HKM assisted in data collection

Chapter four: SP assisted in experimental design and data collection

Chapter six: EC and BW assisted in experimental design and data collection

Chapter two contains work from a jointly authored publication:

Johannesen, A., Dunn, A. M., & Morrell, L. J. (2012). Olfactory cue use by three-spined sticklebacks foraging in turbid water: prey detection or prey location? Animal Behaviour, 84(1), 151–158. doi:10.1016/j.anbehav.2012.04.024

Author contributions are as follows: AJ designed the experiments, gathered and analysed data and wrote the paper. AMD and LJM provided useful feedback on experimental design, data analysis and initial drafts.

Acknowledgements

A special thank you goes to my supervisor, now at the University of Hull, Lesley Morrell for patiently answering all my emails asking what at times have been inane and boring questions. I would also like to thank her for putting up with my incredibly poor writing and getting me through this with her calming and optimistic attitude. Thank you to my local supervisor Alison Dunn and the entire lab group here in Leeds expertly run by Alison, which includes (in alphabetical order) Lucy Anderson, Katie Arundell, Mandy Bunke, Gwendolen Rodgers (Lesley's student), Paula Rosewarne and Nigel Taylor. Without people like you, I doubt I could ever get through this ordeal. You have shown me that we all struggle with this and that there are many different ways to complete a PhD, none better than the other.

Much of my data has been collected with the help of undergraduate and Master's students, whose help was acknowledged on the previous page. I would also like to thank the people looking after my fish at the aquariums at the University of Leeds; Scott Fawcett and Neil Crossley. People who have helped me with fieldwork on the Faroe Islands; Biofar (Faroese Marine Biological Research Station) and all their staff for advice and field equipment, academics at Havstovan (Faroe Marine Research Institute) and Náttúruvísindadeildin at Fróðskaparsetur Føroya (Faculty of Natural and Health Sciences, The University of the Faroe Islands). A special thank you goes to Fiskaaling (Aquaculture Research Station of the Faroes) for providing facilities, study animals and especially Regin Arge for providing useful feedback on experiment design for chapter 6 of this thesis.

My deepest gratitude to the Faroese Research Council (Granskingarráðið) for funding and support throughout my PhD.

I would like to thank my mum for bringing me up to strive for the best that I can do and to believe in my ability to succeed. This thank you is not limited to my mum, as I have been surrounded by wonderful people throughout my upbringing who have all supported me and believed in me.

In terms of my development into a person who is interested in science, Gerald Durrell deserves a mention for awakening my passion for animal behaviour and the natural world. Unfortunately, I was never lucky enough to meet him, but I'll always have Korfu. I would also like to thank my mathematics teachers in school and college and my statistics teachers in university, especially Mark Rosbotham, for being patient with me and explaining until I understood how the maths worked. I could never learn by rote, so understanding things properly was always very important to me. That probably made me an annoying student.

Finally, I have to thank my partner in life and soul mate - even though there is no such thing as a soul let alone a soul mate. Thank you so much, Heini Reinert. I don't just want to thank you for the beautiful drawing of a stickleback that you made for me. I want to thank you for reminding me, when the stress was driving me crazy, that failing this wouldn't be disastrous. For reminding me, that everything would be ok and for believing in me when I didn't believe in myself. Finally, thank you for putting up with my extreme mood swings and distracting me with endless episodes of anime on the internet.

Abstract

In the first half of this thesis, I have focused on predator ability to locate prey using olfaction and how prey aggregation and turbulence affect prey detection. In chapter 2 I investigate the ability of three spined sticklebacks to compensate for loss of visual cues by using olfaction and find that they can use olfactory cues but that these most likely help the fish detect prey rather than locate prey. In chapter 3 I explore the effect of prey aggregation as an anti-predator strategy when avoiding an olfactory predator and find that aggregated prey survive longer than do dispersed prey. In order to further investigate why this may be, I carried out an experiment using *Gammarus pulex* as the predator where I recorded search time as a function of prey group size. I found that similarly to detection distance, search time relates to the square root of the number of prey. Finally, I investigate the effect that turbulence in flowing water may have on prey group detection using three spined sticklebacks in a y-maze. I find that risk of detection increases with prey group size but that turbulence lowers this risk. This may mean that there are thresholds below which size prey groups can benefit from turbulence as a 'sensory refuge' thus avoiding predators.

In the second part of my thesis I focus on the interactions between a cleaner fish and a parasite in an aquaculture setting focusing on whether said fish is useful as a cleaner in industry. I carry out experiments to investigate the use of lumpfish as salmon cleaners in terms of cleaning efficiency and behaviour. I find that while some lumpfish do clean salmon, the required circumstances are still unknown and that further work including selective breeding, personality and effects of tanks is necessary.

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Chapter 1: General Introduction

In this thesis I consider two separate, but related areas of research. In chapters 2-5 I investigate the behaviour of predators foraging using olfactory cues, and how prey aggregation and water turbulence affect risk of detection and prey survival. I do this using two model predators; three spined sticklebacks (*Gasterosteus aculeatus*) and *Gammarus pulex*. As prey, I use bloodworm (chironomid larvae) either frozen and defrosted (usually) or live. In chapter 6 I take an applied approach to the study of predator-prey interactions. This applied chapter investigates whether lumpfish (*Cyclopterus lumpus*) are predators of salmon lice (*Lepeophtheirus salmonis*) and whether they could function as cleaner fish in the salmon farming industry. These two areas relate by investigating foraging behaviour and how predators locate prey in the two separate contexts of behavioural ecology and applied animal behaviour. In this introduction I shall introduce predator-prey interactions from the perspective of ecology first and then move towards applied work on particularly cleaner fish. Finally I describe the study species and provide a short overview of each chapter.

1.1 Predator-prey interactions

A major mechanism regulating organism abundance, behaviour and phenotypic traits is the interaction between predators and their prey (Rosenzweig and MacArthur 1963). Predators and prey are involved in continuous 'arms races' of constant adaptation and modification in both predators and prey to enhance fitness (Dawkins and Krebs 1979). When the environment changes, for example due to climate change or habitat fragmentation, interactions between species are affected in a number of ways including a decrease in

pollinators affecting plant fertilisation or a shift in parasitoid-host relationships (reviewed in Tylianakis et al. 2008). The relationship between predators and their prey can also be affected through a change in predator numbers due to destruction of habitat, potentially leading to lower predation pressure on prey as seen in coleopterans preying on bark beetles in patchy red pine habitats (Ryall and Fahrig 2005).

Predators can affect prey populations in a number of different ways. Firstly, predators may reduce prey numbers through consumption of prey (Brockelman 1969). Even in this situation, the relationship may not be simple. There are, for instance, systems in which populations oscillate due to complex interactions between predator and prey numbers, such as the lynx-snowshoe hare cycle (Krebs et al. 1995). In addition to direct consumption, the presence of a predator may affect prey behaviour or morphology directly (Peckarsky et al. 2008), this can lead to population changes on other trophic levels in what is known as “trait mediated indirect interactions” or TMIIs (Utsumi et al. 2010). For example, increased anti-predator vigilance decreases the time available for foraging across taxonomic groups (Holmes 1984). Exposure to cues of predation can also induce phenotypically plastic responses in morphology: *Daphnia* make costly changes to their exoskeleton to avoid predators (Riessen 2012). In other organisms phenotypic changes happen as a response to long-term selective pressure, such as the evolution of less conspicuous colouration in guppies as a response to predation (Millar et al. 2006).

Behavioural changes due to a change in the ecosystem, such as a drop in food availability can affect survival: bivalves such as *Macoma balthica* burrow deeper into sand as a response to predator cues (Griffiths and Richardson 2006), which results in lower food intake (de Goeij and Luttikhuizen 1998) thereby lowering their reproductive fitness. However, when

food supply is short, *M. balthica* choose to stay near the surface to feed. There they become heavily predated upon, resulting in a drop in *M. balthica* population with inadequate food supply being the indirect cause (van Gils et al. 2009). TMIIs can also cause top-down trophic cascades where the presence of a predator affects the behaviour of a prey species in such a way as to cause changes in population sizes at lower trophic levels. For example in New Jersey (USA) the invasive green crab (*Carcinus maenas*) preys on algae grazers such as *Littorina littorea*. When *L. littorea* are exposed to green crab risk cues their grazing is suppressed, which in turn affects the fucoid algal communities grazed on by *L. littorea* (Trussell et al. 2002).

1.2 Detection of prey

Predators use a range of senses to detect prey and use a range of methods to locate prey (Obrist et al. 1993, Nakata 2010, Gracheva et al. 2010). Senses include those with which we are familiar, such as vision, olfaction (smell) and hearing. Hearing, for example, is used either to detect sounds in the environment such as owls detecting prey from rustling undergrowth (Takahashi et al. 2003) or by emitting sounds and using the echoes to navigate to prey as used by bats (Obrist et al. 1993, Jones and Holderied 2007). Other senses, perhaps more exotic to humans, include tactile signals such as those used by spiders to detect movement in a web (Nakata 2010) or electrical signals like those employed by some species of shark and crayfish (Kajiura and Holland 2002, Patullo and Macmillan 2010).

Perhaps the most well-studied sensory modality is vision (Hairston et al. 1982, Spaethe et al. 2001, Ioannou et al. 2011), reflecting our own reliance on visual cues. There is a wide selection of literature exploring how vision is used in a range of foraging situations and how different animals use vision to locate food. Examples range from the use of infrared or

ultraviolet light in snakes and bees (Spaethe et al. 2001, Gracheva et al. 2010) to long distance spotting of prey achieved by, for example, falcons (Tucker et al. 2000). Visual sensitivity may be enhanced where visual cues are less readily available, such as the improved night vision observed in some nocturnal predators (e.g. many cats and deep sea fishes; reviewed in Warrant, 2004).

1.3 Olfactory prey detection

Many predators are able to use olfaction to locate prey. Some aquatic predators use olfactory cues and can follow olfactory plumes over long distances. Cod (*Gadus Morhua*), for example, can detect mackerel (*Scomber* sp.) bait from several hundred metres away and locate the bait using rheotaxis, swimming against the direction of flow up the odour plume (Løkkeborg 1998). Evidence suggests that crustaceans and molluscs are able to navigate up stream towards a food source following odour plumes using a combination of rheotaxis (moving against flow direction to follow the plume) and chemotaxis (orienting towards a chemical stimulus regardless of flow) (Webster and Weissburg 2001, Weissburg and Dusenbery 2002, Ferner and Weissburg 2005). In turbulent flow (flow that deviates from straight, laminar flow due to obstacles or rough surfaces, causing differences in flow speed and direction within the water column), some fast moving predators such as crabs and crayfish are slower and less successful at locating prey but slow moving predators such as gastropods are able to use time averaging sampling methods that allow them to stay within a turbulent odour plum and move in a straight line towards prey (Ferner and Weissburg 2005). This would suggest that turbulence may offer a ‘sensory refuge’ (an area where prey are concealed from predators) to prey hiding from a fast moving predator and that prey may choose to aggregate in turbulent areas.

While many predators use mainly vision or olfaction to detect prey, many other species use a combination of senses to detect prey. Some predators, for examples owls, have evolved to function in poor lighting conditions during nocturnal hunting and have both strong hearing and night vision to rely on to detect prey (Takahashi et al. 2003). There are also more short term situations such as fluxes in turbidity (turbidity refers to a reductions of water clarity, usually caused by suspended particles in the water. This causes fog-like visual conditions where light is scattered reducing long distance visibility while not necessarily affecting short distance visibility unlike low light conditions where visibility at short distances is equally affected), where a predator will need to employ senses other than vision. Turbidity in water lowers visual range of predators (Grecay and Targett 1996, Utne 1997, Mazur and Beauchamp 2003, Sweka and Hartman 2003) and the type and colour of suspended particles affect the visibility of prey in different ways (Utne-Palm 1999, Liljendahl-Nurminen et al. 2008). However, little is known about the ability of visual foragers to compensate for a lack of visual cues using olfaction. One study on sticklebacks finds that sticklebacks are able to locate prey in turbid water, but if olfactory cues are concealed with the addition of extra olfactory cue mixed into the water, this lowers prey finding performance (Webster et al. 2007a). This indicates that perhaps sticklebacks are able to use olfaction to compensate for the loss of visual cues available, but whether they do so by navigating an odour plume or whether the olfactory cues have another effect is not well understood.

1.4 Aggregation as an anti-predator defence

Visual predators have driven a range of evolved responses in prey, including the evolution of cryptic colouration (Howlett and Majerus 1987). Another well-known predator-

avoidance strategy is that of living in groups (Krause and Ruxton 2002). Aggregation results many trade-offs related to costs such as balancing competition for resources (Schülke 2003) and increased risk of parasitism and disease (Rifkin et al. 2012) with benefits such as communal rearing of young (Gandelman et al. 1970) or improved foraging efficiency (Packer and Ruttan 1988). Anti-predator benefits include the dilution of individual risk (Foster and Treherne 1981), the confusion effect (reduced targeting efficiency by predators faced with multiple targets; Ruxton et al. 2007), increased overall levels of vigilance (with a reduced individual commitment; Roberts 1996) and communal defence (mobbing; Krams et al. 2010). However, aggregation comes at a cost of increased conspicuousness to predators: A group of animals is usually more easily detected than a single individual due to the increased area the group occupies (Ioannou and Krause 2008). Therefore, there is a trade-off between avoiding detection and gaining safety in numbers. This means that for an animal to benefit from aggregating, the risk to each individual animal must be smaller when it is in a group than when it is alone.

If surviving as part of a group is dependent on the relative risk to an individual, it must mean that a group has to offer benefits outweighing the increased risk of detection that comes with larger group sizes. This could happen through dilution of risk, where only one or a few of the group are eaten once discovered (Foster and Treherne 1981). A flock of birds may be safer simply because the predator relies on stealth and thus is exposed as soon as the attack happens and all but (perhaps) one unlucky bird escape (see for example how finches respond to predator attacks; Lima and Bednekoff 2011). Immobile prey may have to rely on being in a large enough group to satiate the predator before it gets to them. In this kind of situation, aggregation is only beneficial if detection does not increase proportionally with group size. In other words, if a group grows to twice the size, then it must be less than twice as easily

detected (Taylor 1976b, 1979, Turner and Pitcher 1986). In visual terms at least, it is fairly well established that detection of groups increases asymptotically with group size. Several empirical studies combined with mathematical models find that this is the case for sticklebacks (Ioannou and Krause 2008, Ioannou et al. 2011), great tits (*Parus major*) (Riipi et al. 2001) and humans (Jackson et al. 2005). In an old model by Vine (1973), it is predicted that detection radius is related to N^0.45 (as well as some constants relating to predator visual acuity and prey size), which leads to diminishing returns in terms of detection distance as prey group sizes increase.

If a predator has unlimited time in which to search for prey, a group would eventually be discovered regardless of the rate of increase in detection with group size, so in order for the dilution effect to be really useful to prey, encounter-dilution or predator avoidance (where few large groups are far apart making searching costly) must be combined with dilution of risk (attack abatement; Turner and Pitcher 1986). When this happens, situations may occur where fewer predators survive: in a turbid lake, the combination of prey aggregation and predator ability to only consume one prey per encounter increased the effective distance to prey, which was exacerbated by low visibility resulting in fewer available prey and thus lower predator numbers (Turesson and Brönmark 2007).

There has been very little empirical investigation on how prey group size may affect prey detection when predators are using olfactory, rather than visual, cues (but see work on biting flies; (Hargrove and Vale 1978, Eiras and Jepson 2009, Takken 2011). However, one study on whelk (*Busycon carica*) tracking ability finds that when prey are placed in line with flow direction (effectively aggregating the prey to create one odour plume for all prey) whelks find prey faster and move in a straighter line towards prey (Wilson and Weissburg 2012). Cain

(1985) proposes a model in which the benefits from aggregation depend on sensory acuity of the predator as well as prey density. In his model, which is based on insect predators and plants (i.e. immobile prey with no option of escape) aggregation in prey becomes beneficial as prey density grows. Additionally, if the predator has high sensory acuity or a large detection radius then aggregation becomes beneficial at lower densities and if the predator is not good at detecting prey, aggregation starts becoming beneficial at higher prey densities. According to theoretical predictions by Treisman (1975), probability of detection should increase linearly with prey when the predator employs olfaction to locate prey, and according to Bossert and Wilson (1963) detection distance to prey should increase linearly with prey number. Predictions based on this would be that prey avoiding an olfactory predator would not benefit from attack abatement by aggregating. However, a recent empirical study on moth antennal responses to patches of sex pheromones indicates that detection distance increases proportionally only with the square root of the number of individuals (Andersson et al. 2013). In this case, aggregation may well be a beneficial anti-predator strategy when avoiding an olfactory predator. However, at present, while there is evidence that predators navigate more easily to prey when they are aggregated (Wilson and Weissburg 2012), whether aggregation improves prey survival when being predated upon by an olfactory predator is unknown.

1.5 Industry application of knowledge about foraging behaviour – aquaculture

While a thorough understanding of predator-prey interactions and foraging behaviour in aquatic organisms may be useful in for example a conservation context, there may be other applications too. One such application is biological control of parasites within the aquaculture industry. Within the field of predator-prey interactions, this thesis focuses on finding food, so a natural step into applied animal behaviour studies is to investigate whether and how a

potential cleaner fish finds parasite prey on other fish. The methods employed in the applied investigation in chapter 6 are different to those in the previous chapters, as are the aims and objectives. However, as I shall argue next, the investigation of cleaner fish foraging behaviour is an important step towards improved animal welfare and sustainable farming and therefore a worthy subject of applied research related to the work carried out in the previous chapters.

1.6 Cleaner fish in the aquaculture industry

The aquaculture industry is a rapidly growing component of the fisheries industry. In 2010, it accounted for 40% of all seafood produced globally (see figure 1.1; FAO 2012). Because of the rapid growth in industrial aquaculture, the industry, in particular the fish farming industry suffers from problems ranging from pollution to fish diseases (Ashley 2007, FAO 2012).

Problems stem from lacking sustainability of feed sources (FAO 2012), localised pollution due to high stocking densities (Mente et al. 2006), health risks caused by easy transmission of diseases and parasites between so many animals in close proximity (Conte 2004, Ashley 2007), and implications for local wild ecosystems (McGinnity et al. 2003, Mente et al. 2006). Diadromous fish (fish that spend part of their lives in fresh water and part of their lives in salt water, such as salmon and some species of trout) culture makes up 6% of all aquaculture in the world. Of that, salmonids account for almost half of the production and approximately 99% of commercial Atlantic salmon (*Salmo salar*) is cultured (FAO 2012). For salmon farmers, one of the major problems are salmon lice (*Lepeophtheirus salmonis*) as these cause injury and pain as they feed on fish scales, causing welfare problems as well as financial losses (Ashley 2007, Gjerde and Saltkjelvik 2009, Costello 2009, Taylor 2011). On-going

research into treatment primarily focuses on the chemical removal of salmon lice (Tully and McFadden 2000, Fallang et al. 2004). While some of these treatments are currently effective (Burridge et al. 2010), some of the effective treatments (Avermectins, Pyrethoids and Chitin synthesis inhibitors) contain substances harmful to other crustacea such as prawns and lobsters (Burridge et al. 2010). Additionally, louse resistance to the treatments is growing, and some treatments (such as hydrogen peroxide) can be harmful to the salmon as well as the lice, and so cause welfare problems and financial loss for salmon farmers (Kiemer and Black 1997, Treasurer et al. 2000, Burridge et al. 2010).

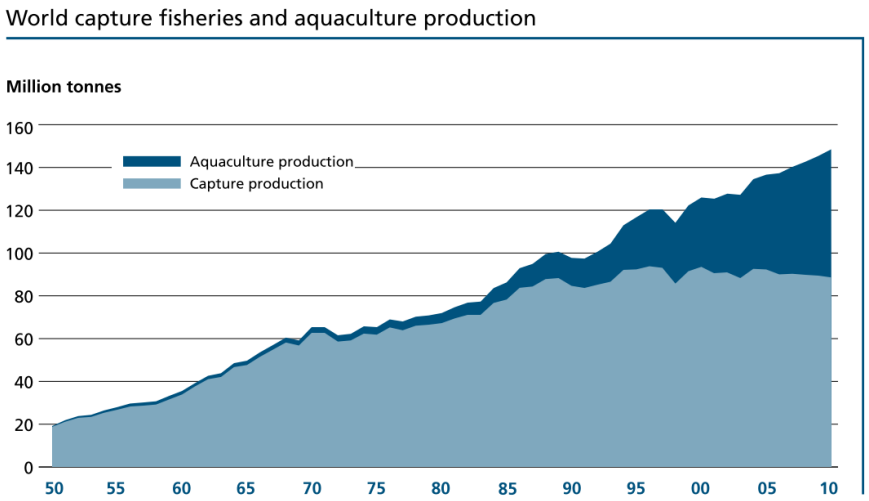

Figure 1.1. Amount of fish and other marine and aquatic species landed from aquaculture and capture fisheries from 1950 to 2010. The figure is from (FAO 2012).

One potential solution to the problem of fish lice is the use of 'cleaner' fish such as goldsinny, rock cook, and corkwing wrasse (*Ctenolabrus rupestris*, *Centrolabrus exoletus* and *Crenolabrus melops*, respectively), which remove lice directly, without harming the salmon (Treasurer 2002). Cleaner fish could potentially slow or reverse the growth of louse populations, reducing the frequency of chemical treatments, and therefore reducing the build-up of resistance to those treatments (Treasurer 1994, Deady et al. 1995). In the salmon industry, the wrasse species mentioned above are most commonly used as cleaners. However,

not all locations (such as the Faroe Islands) have native wrasse, so alternative options need to be explored to avoid the introduction of alien species. Some evidence suggests that lumpfish (*Cyclopterus lumpus*) may be used as an alternative cleaning species in areas where they, but not wrasse, are native (Schaer and Vestvik 2012). In the second part of this thesis, I investigate the potential of lumpfish as cleaner fish for the Salmon industry in the Faroe Islands, and assess the factors that might act to promote cleaning behaviour.

1.7 Model species

*1.7.1 Three-spined sticklebacks (*Gasterosteus aculeatus*)*

Three-spined sticklebacks are small fish (adults reach approximately 7cm total body length) found in a range of habitats in the Northern hemisphere, surviving and breeding in fresh, brackish and sea water, with tolerance of a wide range of temperatures (Wootton 1976). They are opportunistic feeders that consume benthic as well as littoral prey such as insect larvae, copepods and arthropods (Ward et al. 2004a, Rafferty and Boughman 2006). Sticklebacks are mainly visual foragers (Wootton 1984), though they do use olfactory cues for a range of purposes such as mate choice and shoaling decisions (Webster, Goldsmith, et al. 2007; Heuschele et al. 2009). Habitats include both clear and turbid water and they can coincide with sediment dwelling chironomid larvae, which will be used as prey in my experiments (de Haas et al. 2006). Their primarily visual foraging mode, ability to use olfactory cues, and position as a model species in behavioural ecology (Huntingford and Ruiz-Gomez 2009) makes them an ideal model species for the investigation of how predators find visually concealed prey organisms.

1.7.2 Gammarus pulex

Gammarus pulex (henceforth referred to as *Gammarus*) are freshwater amphipods sometimes referred to as fresh water shrimp in the aquarist community. They are usually found in streams where they feed on decomposing vegetation, though they are opportunistic feeders who will consume small invertebrates including smaller *Gammarus* (Moore 1975, Willoughby and Sutcliffe 1976, McGrath et al. 2007). They are small (adults ranging from 1-2cm in length Cussans, 1904) and have a life span of one to two years (Sutcliffe et al. 1981).They have compound eyes, but they predominately use chemical cues to detect food (Åbjörnsson et al. 2000, Lange et al. 2005). Their small size and reliance on olfaction makes them a suitable olfactory forager for use in my experiments on how prey group size affects detection.

1.7.3 Bloodworm (Chironomid larvae)

Chironomids (family: Chironomidae) are also known as non-biting midges. Their larvae are an important part of the diet of fresh water fish such as sticklebacks and trout (Hunt and Jones 1972, Wootton 1976). Chironomid larvae are found in a wide variety of aquatic environments, both fresh and brackish water (Armitage et al. 1995) and they are sometimes used as an indicator species in climate research (Larocque et al. 2001). Some species of chironomids, which have red larvae (bloodworm) are exploited commercially as pet food. The chironomid larvae used in experiments for this thesis are commercially available bloodworm sold as fish food.

*1.7.4 Lumpfish (*Cyclopterus lumpus*)*

Lumpfish are rotund, sedentary fin fish reaching approximately 50cm in length as adults (Davenport 1985). Especially while young, they use a modified pectoral fin sucker to attach to smooth surfaces to rest (Brown 1986). They spend pre-adult stages of their life in open seas and come back to coastal waters to breed, with males showing red mating colouration and exhibiting egg tending and guarding behaviour (Goulet et al. 1986, Mitamura et al. 2012). They are opportunistic feeders, consuming mainly invertebrates found in and around seaweed as juveniles (Brown 1986, Williams and Brown 1991, Killen et al. 2007) while their open sea diet is less well known. Lumpfish are used industrially mainly as a source of caviar substitute though their flesh is also eaten in Scandinavia (Monfort 2002). There is some indication that lumpfish will consume salmon lice (Schaer and Vestvik 2012) but the conditions that enable and promote this, and how and why they do so is unknown.

*1.7.5 Salmon lice (*Lepeophtheirus salmonis*)*

L. salmonis are copepod parasites of salmon belonging to the family Caligidae. They have 10 life stages of which the first three are free swimming (Pike and Wadsworth 1999). Salmon lice consume the mucus, skin and blood of their host (Ross et al. 2000) and are able to move between hosts at pre-adult and adult life stages (Stephenson 2012). In addition to causing welfare problems, heavy infestations of salmon lice are a financial burden on aquaculturists (Johnson et al. 2004, Costello 2009)

*1.7.6 Salmon (*Salmo salar*)*

Atlantic salmon are anadromous fin fish native to the North Atlantic Ocean. The fry spend one to three years in fresh water streams and lakes before migrating to the ocean where they stay for one to five years before returning to fresh water to spawn (reviewed in: (Klemetsen et al. 2003). While in the ocean, the salmon are vulnerable to infection with salmon lice (*Lepeophtheirus salmonis*). Salmon is a popular food item and the demand is largely being met by the aquaculture industry (FAO 2012). This industry is not without drawbacks as both escaped domestic salmon as well was salmon lice breeding in salmon farms are thought to have a negative impact on wild populations of salmon (Gross 1998, McGinnity et al. 2003, Krkosek et al. 2013, Skilbrei et al. 2013).

1.8 Outline of thesis chapters

In chapter 2, I assess whether sticklebacks are able to compensate for the loss of visual cues by using olfaction when searching for food. Evidence suggests that in some species, increased reliance on olfaction can compensate for reduced availability of visual cues (Webster et al. 2007a, Chapman et al. 2010). I investigate this using two approaches; 1) a binary choice experiment to test whether sticklebacks are able to detect the presence of prey and narrow their search down to a particular area using olfaction alone and 2) a foraging experiment to test whether foraging ability is hindered by confusing olfactory cues.

In chapter 3 I investigate how aggregation affects survival in prey. Prey were hidden from view using either refuges or turbid water to investigate whether aggregation confers a survival advantage against an olfactory predator (as it does for prey avoiding visual predators; Ioannou et al., 2011).

In chapter 4 I investigated in more detail how the size of a group (contrasted with the aggregated versus dispersed set up of chapter 3) affects olfactory detection using an olfactory specialist, *Gammarus*. Two experiments are used to test two different ideas: the first assesses how prey group size affects ability to track prey along a concentration gradient, and the second is an assessment of the foraging effort of a predator once prey has been tracked and located.

In chapter 5, I investigate whether sticklebacks are able to successfully locate an odour source in flowing water, and how cue concentration (group size), affects success rate, finding that the predators are increasingly successful at locating groups as the size of the group increases. Previous work has suggested that turbulence can help conceal prey from predators (Ferner and Weissburg 2005), as prey can exploit 'sensory refuges' where predators are less able to detect them (Smee and Weissburg 2006).

In chapter 6, I take an applied approach to mechanisms of locating prey and investigate the foraging behaviour of lumpfish (*Cyclopterus lumpus*) in the context of their potential use as cleaner fish in the salmon farming industry. In order to investigate this, I carry out a series of experiments using farmed salmon infected with salmon lice as prey for lumpfish.

1.9 Ethical Note

All sticklebacks captured for the purpose of gathering data for this thesis were kept at home office licensed facilities, though no work carried out required a license. All experiments were carried out in agreement with UK laws and regulations and were discussed with licensed technicians at the central biological facilities at the University of Leeds. Sticklebacks were released once experiments were finished in accordance with home office regulations and in agreement with DEFRA.

The applied work was carried out on the Faroe Islands, where legislation differs from the in the UK and local laws and regulations were adhered to at all times. All lumpfish used in experiments were caught for breeding purposes and every effort was made to ensure high welfare standards throughout all experiments. Farmed salmon were used in these experiments and were supplied by Fiskaaling's own breeding facilities. Infecting salmon with lice is not a licenced procedure on the Faroe Islands, but in order to minimise stress, infection levels were kept to a minimum and salmon were de-loused as soon as possible after the conclusion of trials. Experimental procedures as well as husbandry practices were carried out in accordance with advice from the resident vet and animal technicians. While lumpfish were used as breeding stock after experiments were finished, salmon were humanely euthanized by qualified staff as they could not be relocated for biosecurity reasons.

Chapter 2: Olfactory cue use by three-spined sticklebacks foraging in turbid water: prey detection or prey location?

Abstract

Finding prey items, when senses are limited to olfaction, is composed of two distinct stages; the detection of prey and the location of prey. While specialist olfactory foragers are able to locate prey using olfactory cues alone, this may not be the case for foragers who rely primarily on vision. Visual predators in aquatic systems may be faced with poor visual conditions such as natural or human-induced turbidity. The ability of visual predators to compensate for poor visual conditions by using other senses is not well understood although it is widely accepted that primarily visual fish can detect and use olfactory cues for a range of purposes. I investigated the ability of the three spined stickleback (*Gasterosteus aculeatus*)to a) detect the presence of prey and b) to precisely locate prey, using olfaction, in clear and turbid (two levels) water. When provided with only a visual cue, or only an olfactory cue, sticklebacks showed a similar ability to detect prey, but a combination of those cues improved their performance. In open-arena foraging trials, a dispersed olfactory cue added to the water (masking cues from the prey) in the arena improved foraging success, contrary to my expectations, while activity levels and swimming speed did not change as a result of olfactory cue availability. I suggest that olfaction functions to allow visual predators to detect rather than locate prey, and that olfactory cues also have an appetitive effect, enhancing motivation to forage.

2.1. Introduction

Predators use a range of senses to find prey including vision, olfaction and the detection of electric fields (Goerlitz et al. 2008, Nakata 2010, Patullo and Macmillan 2010, Gracheva et al. 2010). For predators using visual cues to forage, detecting and locating a prey item occur simultaneously. For predators using olfactory cues, however, the detection of a cue may convey very little information about the location of a prey item (Conover 2007). In such systems, finding a prey item (or mate, or other resource) using olfaction can be considered as two discrete steps: detection, where an individual is alerted to the presence of food in the vicinity; and location, where detected item is found. The step from detection to location when using olfaction may depend on factors such as wind or flow speed and turbulence, the strength of the cue, and the sensitivity of chemoreception by the individual (Conover 2007, Carthey et al. 2011). For example in mice (*Mus domesticus*), cue patchiness is an important factor determining foraging success (Carthey et al. 2011) and plume tracking insects need both an olfactory cue and wind direction in order to successfully navigate to the source of the cue (Cardé and Willis 2008).

In aquatic systems, many fish predators rely primarily on vision, yet visual cues can be highly limited, as water is often turbid or too deep to allow light to penetrate (Davies-Colley and Smith 2001, Utne-Palm 2002). Fish also use olfaction in a range of behaviours, including mate choice (cichlids (*Pseudotropheus emmiltos)*; Plenderleith et al. 2005, sticklebacks; Rafferty and Boughman 2006, Heuschele et al. 2009), as a social cue (sticklebacks; Ward et al. 2004a, 2005, perch (*Perca fluviatilis)*; Behrmann-Godel et al. 2005), to detect predators (rainbow trout (*Oncorhynchus mykiss)*; Brown et al. 2011, minnows (*Pimephales promelas)*; Ferrari et al. 2010) and to detect prey (cod (*Gadus morhua)*; Løkkeborg 1998). Thus, changes to the visual (e.g. through turbidity; Utne 1997, Quesenberry et al. 2007) or olfactory (e.g.

through altered pH; Moore 1994, Heuschele and Candolin 2007) environment can negatively impact on the ability of fish to detect and locate prey items.

Turbid conditions can be caused by natural events, such as algal blooms due to seasonal shifts in temperature and light availability; and from anthropogenic activities such as excess fertiliser from agriculture reaching waterways, or erosion caused by deforestation or construction (Richter et al. 1997, Henley et al. 2000, Donohue and Molinos 2009). Highly turbid water is known to be detrimental to a visual forager: in high-production lakes lowered encounter rates between predators and prey lead to fewer large fish predators in comparison to low-production lakes (Turesson and Brönmark 2007). Across a range of fish species, reaction distance to prey decreases with increasing turbidity (Utne 1997, Sweka and Hartman 2003, Pekcan-Hekim and Lappalainen 2006, Quesenberry et al. 2007) and increased turbidity decreases foraging success (Gregory and Northcote 1993, Sweka and Hartman 2003, Granqvist and Mattila 2004).

However, in some cases, high turbidity has little impact on foraging success (Miner and Stein 1993, Grecay and Targett 1996, Granqvist and Mattila 2004, Quesenberry et al. 2007). This may be related to the size of the predator and its prey (Utne-Palm 2002): A small predator feeding on plankton will often find itself close to prey, so reaction distances can be short without negatively affecting the predator. In contrast, larger predators that eat sparser prey are more likely to be negatively affected by turbidity (Turesson and Brönmark 2007). While some predators are not adversely affected by turbidity because of their size and prey density, others may be able to compensate for the loss of available visual cues with changes in behaviour (Andersen et al. 2008) or through developmental plasticity, making use of other senses such as olfaction (Chapman et al. 2010).

Here, I investigate whether three-spined sticklebacks (*Gasterosteus aculeatus*) can use olfaction to compensate for a reduction in the availability of visual foraging information due to increased turbidity. The three-spined stickleback is a visual predator occupying a wide range of habitats including very turbid water (Wootton 1976, Utne-Palm 2002, Engström-Öst and Candolin 2006, Webster et al. 2007b). Sticklebacks are known to use olfaction across a range of behaviours: they compensate for poor visual conditions by using olfactory cues in mate choice, allowing them to accurately assess male quality (Reusch et al. 2001), and base shoaling preferences on habitat-derived olfactory cues (Ward et al. 2004a, 2005). Webster et al. (2007) demonstrated that an excess of olfactory prey cue homogenously mixed in with the foraging water resulted in decreased foraging success in sticklebacks compared to those foraging in water containing only olfactory prey cue released by the prey items present. The masking or concealing effect that the excess olfactory prey cue had indicates a key role for olfaction in foraging in this species. Thus, as primarily visual foragers, but with a well-documented sense of smell, sticklebacks are an ideal model system in which to test the hypothesis that olfaction allows individuals to compensate for the reduced availability of visual cues in turbid water.

Here, I use two complementary approaches to investigate the use of visual and olfactory cues in stickleback foraging, in the context of both prey detection and prey location. In the first 'prey detection' experiment I test the hypotheses that a) sticklebacks can use olfaction to detect prey and b) reliance on olfactory cues to detect prey increases with increasing turbidity. In the second, 'foraging success' experiment, I test the hypotheses that a) increasing turbidity reduces the ability of fish to locate prey items and b) this effect is increased when olfactory prey cues are masked by the addition of excess prey cue to the water

(thus providing no information about the location of prey items). Together, these experiments allow us to test the general hypothesis that sticklebacks compensate for poor visual conditions by using olfactory cues to detect and locate dispersed prey.

2.2. Methods

2.2.1 Study Species and Housing

Two hundred and fifty three-spined sticklebacks (*Gasterosteus aculeatus*) 45-55mm long were caught using small (single or two person) seine nets from brackish water ponds near Saltfleet, Lincolnshire, UK (53° 25' 59.55"N, 0° 10' 49.41"E). Fish were placed in commercial fish transportation bags at maximum density of five fish per litre. Each bag was filled with 25% water from the source water body, and 75% air (total bag volume of 20 litres), and bags were packed into plastic boxes. Fish were returned by car to the laboratory in Leeds, and no fish died during transportation. At my facilities, the fish were kept in groups of between 50 and 150 fish in fresh water holding tanks (60x90x45cm) on a 10:14 hour light/dark cycle at a temperature of 16 ± 1 °C and pH was 6.5-7.0. The holding tanks were enriched with gravel substrate and artificial plants. They were fed defrosted frozen bloodworm (chironomid larvae) once daily. The fish were maintained in the laboratory for 18 months after which they were released again where caught in agreement with the Home Office and DEFRA. To control for any potential confounding effect of social background, fish from each holding tank were evenly distributed between treatments. The prey species used in my experiments were live bloodworm sourced from a local pet shop (Experiment 1: prey detection) and frozen bloodworm sourced from a commercial fish food provider (Experiment 2: foraging success).

2.2.2 Experiment 1: Prey detection

To investigate whether sticklebacks could use olfactory cues to detect prey, I used a binary choice design (similar to that of Chapman et al., 2010). Fish were presented with two containers, one containing prey and one without prey. I used three cue-availability treatments (olfactory, visual and combined cues), each repeated in three turbidity environments (clear, medium and high; see below for details) with 25 trials in each group (a total of 225 trials). Some trials (N = 47) were excluded due to the fish not entering a selection zone (see below), giving a total sample size of 178. A web-cam positioned above the arena and connected to a laptop next to the experimental arena was used to monitor the fish during acclimatisation and record the trials.

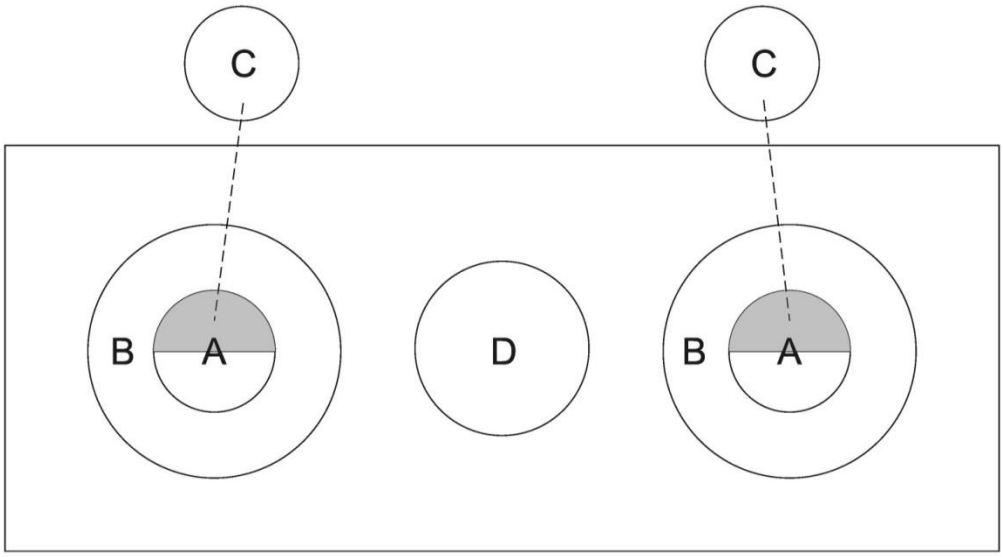

Figure 2.1. Binary choice arena measuring 54 x 34 cm. (A) indicates the cue containers, one half opaque (shaded) and one half transparent (unshaded). The containers were perforated for the olfactory and combined treatment, but intact for the visual treatment. (B) indicates the selection zones of 5cm and (C) indicates the containers holding the cue drip positioned at the side of the arena with tubing (dashed lines). (D) indicates the opaque cylinder for acclimatisation.

The choice arena (54 x 34cm, filled to a depth of 5cm; figure 2.1) contained two prey containers, positioned at opposite ends, 10 cm from the tank wall, and an opaque shelter

positioned in the tank centre. Around each prey container I marked a 5cm wide 'selection zone'. Each prey container was constructed from a 100ml plastic beaker divided vertically into two equal sections, one transparent and one opaque (see figure 2.1a for positioning of the containers). Live bloodworm prey placed into the transparent section provided visual cues to the predator (in the visual only and combined cue treatments), while prey placed in the opaque section (in the olfactory only treatment) did not. Live prey were used as movement is an important visual cue (Utne-Palm 2002). For treatments where an olfactory cue was available (the olfactory and combined cue treatments), the containers were perforated with 1mm holes spread at 1cm intervals across the entire surface of the container. For the visual only treatment, the container remained unperforated. In each trial, one container held prey while the other did not. The side containing the prey was randomised between trials to control for any potential side bias.

To facilitate the transmission of olfactory cues from the container in to the surrounding water (for the olfactory only and combined treatments), an additional olfactory cue was dripped via airline tubing into the container containing prey at a rate of one drop per 10 seconds amounting to approximately 5ml of drip per trial dripping into approximately 9 litres of water in the arena. A control drip of water was added to the container without prey. I performed a series of pilot trials using water dyed with food colouring to visualise patterns of cue dispersal, prior to the start of experimental trials. These pilot trials indicated that over the course of 30 minutes, the cue would disperse to create a cylindrical odour plume approximately 2 cm wide around the container with a sharp concentration gradient. These pilots indicated no visually detectable current caused by the olfactory cue drip. To control for the presence of the tubing, it was left in place for the visual only treatments, but no cue was added.

The olfactory cue was generated from the water in which the live bloodworm were stored. The bloodworm were supplied in small plastic bags containing approximately 150ml of water, and I housed the bloodworm in this water in the laboratory for up to two days after purchase (bloodworm survived for no more than three days in the laboratory). Thus, the water used for the olfactory cue used was generated by housing bloodworm in water for three to five days. In order to achieve the required volume of olfactory cue, the water used to house the bloodworm was diluted immediately before use one part water, one part bloodworm housing water. As the cue water had a slight pink tinge, a small amount of red food colouring was added to the control water. Pilot trials indicated that there was no effect of the food colouring on fish response to the water.

In addition to randomly assigning the side containing the prey cue, I also carried out cue treatments in a random order. Trials were recorded on video and analysed blind to cue treatment and the side containing the cue. A separate spreadsheet held information on cue treatment and on which container held prey items for each trial. Although much was done to ensure randomisation, all clear water trials were carried out before the turbid trials. The initial experiment in clear water was designed to test whether sticklebacks could detect the olfactory cue in my experimental set up. This pilot indicated that detection of the prey when olfactory cues were available was similar to detection when both cues were available (ANOVA: $F_{1,59}$ = 1.45, P = 0.24), and so these results were incorporated into the full experiment. Within the clear water trials, cue treatment was randomised and videos analysed blind, as for the main experiment.

Turbidity was created by dissolving industrial clay (Commercial Clay Ltd) in conditioned water (Abrahams and Kattenfeld 1997, Ferrari et al. 2010). High turbidity (488.69 ± 5.46 NTU) was created from 1g of clay per litre of water and medium turbidity using 0.5g/L (296.51 ± 4.77 NTU). Turbidity dropped to 437.05 ± 7.96 NTU and 250.63 ± 5.10 NTU respectively over a period of 15 minutes (five minutes acclimatisation plus 10 minutes trial time). Turbidity differed significantly between high turbidity and medium turbidity treatments (ANOVA: $F_{1,112}$ = 682.9, $P < 0.001$). The clay did not alter the pH of the water used in my trials. Clear water treatments contained no clay (~0.1 NTU). The fish showed no symptoms of ill health during or following experiments. It is likely that the turbidity levels chosen for these experiments were higher than is usually seen in the wild, but as the trials ran in small volumes of water, high turbidity was necessary to prevent the fish from seeing prey at short distances. At the turbidities I used, the secchi disk distance (indicative of the distance the fish would be able to see through the water) was approximately 3cm for high turbidity and 10 cm for medium turbidity.

Fish were starved for 24 hours prior to trials in order to standardise motivation to feed. Individual fish were placed in the shelter and left for five minutes to acclimatise, in order to minimise decrease in turbidity and in line with other studies (Engström-Öst and Candolin 2006, Quesenberry et al. 2007, Webster et al. 2007a). After the acclimatisation period, the video recording was started and the fish was released into the arena by raising the shelter above water level using a remote pulley system. Each trial lasted 10 minutes, after which the fish was caught and measured, and the trial number assigned to the video. The arena was emptied of water and refilled for each subsequent trial to remove olfactory cues from previous trials. Total time spent in each selection zone was recorded from the video.

2.2.3 Analysis

Statistical analysis was carried out in R version 2.13.0 (R Core Team 2013) using a generalised linear model (glm) with quasibinomial errors to analyse the proportion of time spent in the selection zone with the container holding prey as opposed to the empty container selection zone. The model was run with interactions first and when an interaction was found between turbidity and treatment, *post hoc* glms in each turbidity level were run with a Bonferroni correction for multiple tests in order to test for main effects of cue availability.

2.2.4 Experiment 2: Foraging success

As detecting prey in a binary choice test does not necessarily equate to the ability to locate prey, I carried out a second experiment, in which predators located and consumed prey in an open arena, again under three differing turbidity levels (as above).

Foraging success trials were carried out in a 100x100cm arena with a water depth of 5cm (figure 2.2). A 10x10cm floating polystyrene shelter was positioned in the centre of the arena, held in place by lengths of white sewing thread attached to the centre of two opposite sides of the arena. Eight bloodworms were placed at evenly spaced predetermined spots (25cm from the arena wall and 25cm from the nearest neighbouring prey) surrounding the shelter. Defrosted frozen bloodworms were used as prey to prevent excessive movement away from these locations during the trial. A high definition webcam (Logitech Webcam Pro 9000), suspended above the arena was used to remotely monitor and record trials.

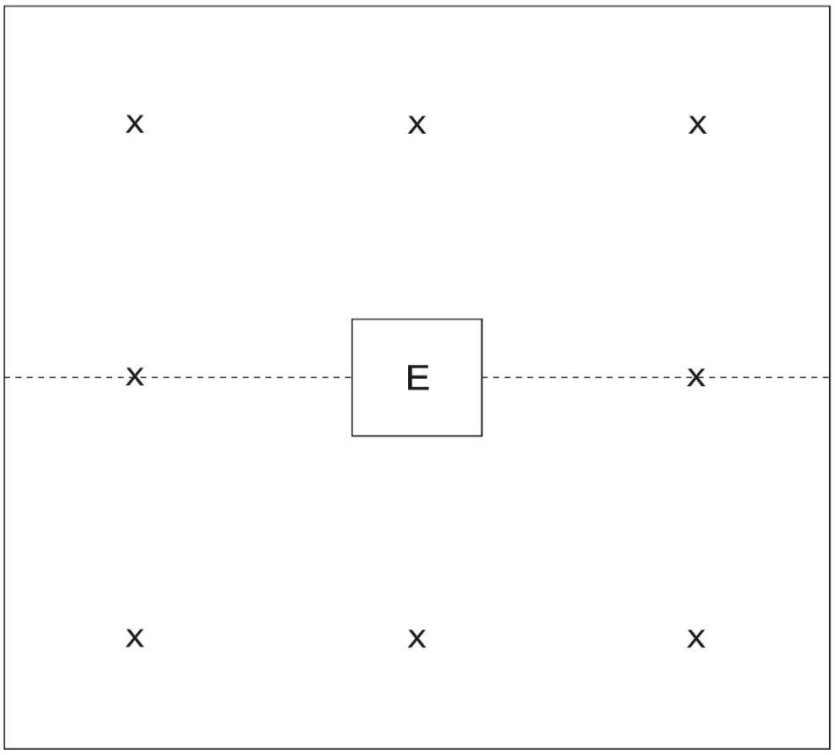

Figure 2.2. Foraging arena measuring 100x100 cm. (E) is floating shelter at centre of arena held in place with sewing thread (dashed lines). (X) mark the predetermined spots where prey were placed prior to trials. The distance between each prey and to either shelter or arena edge was approximately 25 cm.

I used two cue availability treatments: “visual and olfactory” and “visual only”. The visual and olfactory treatment allowed the stickleback to use both senses (although visual cue availability was dependent on turbidity levels – I make no assumptions about the effect of clay on the olfactory cue available). In the visual only treatment, I prevented the use of olfactory cues to locate prey by adding additional olfactory cue to the water used to fill the arena, ensuring that the cue was well-mixed with the water before the arena was filled. The added olfactory cue was created following the methodology in Webster *et al* (2007) from the filtered extract of macerated frozen bloodworm (1g of bloodworm per 20 litres of water final concentration). The added olfactory cue was intended to override any olfactory cue emanating from the prey items, thus preventing the fish from using this cue to locate the prey. Fish were fed then starved for 24 hours preceding trials to standardise motivation to feed. Each cue

availability treatment was carried out in three different turbidity treatments, as above. On each day of experimentation, I carried out two to three trials at each turbidity level. Within a day, turbidity levels were grouped (for logistical reasons), but between days, the order in which different turbidity levels were trialled was randomised.

Individual fish were released under the shelter, where they would hide. Any fish that did not hide under the shelter or did not emerge from the shelter within 15 minutes were removed and excluded from the experiment (N = 92 fish). The 15 minute emergence limit was imposed in order to avoid overlap in turbidity treatments due to settling of clay over time. I recorded the time taken for the fish to emerge, defined as the time at which the full extent of its body was free of the shelter. Mean time until emergence from the shelter did not differ significantly between cue or turbidity treatment groups (Cox Proportional Hazards survival model, likelihood ratio $test_3$ = 3.38, P = 0.34). Turbidity was measured (for the majority of trials) before the fish was released and after the trial was complete. Turbidity decreased over time from 646.38 ± 12.74 (mean ± SE) NTU to 460 ± 20.69 (high turbidity, N = 18 & 26 respectively), and from 391.15 ± 9.35 NTU, to 286.83 ± 9.1 NTU (medium turbidity, N = 29 in both cases) over a maximum of 35 minutes (maximum time permitted in the shelter plus maximum foraging time). Thus, despite decreases in turbidity over time, turbidity in the medium and high turbidity treatments differed significantly ($F_{1,53}$=63.06, P<0.0001). Once the fish had emerged, I started video recording and the fish was allowed to forage until all prey were eaten or for 20 minutes, at which point the trial was terminated. Fish were measured to the nearest mm (total body length) at the end of each trial.

Data were manually extracted from videos using Etholog (2.2.5) and Windows Media Player. The time spent engaged in each of the four behaviours outlined in Table 2.1 was

recorded. In addition, I recorded the time taken to emerge from the shelter (see above) and the time of consumption of each individual prey.

Table 2.1. Behaviours recorded in the foraging trials.

Behaviour	Description
Swimming	Moving around in the arena including saltatory and steady movement, but not along the edges of the arena.
Hiding	The fish is under the shelter and invisible to the observer
Edge	Continuous swimming along the edge of the arena
Inactive	Time spent immobile for at least 5 seconds in one bout

2.2.5 Analysis

All analysis was carried out in R (R Core Team 2013). Cox Proportional Hazards Survival Models (Therneau and Lumley 2011) and Mixed Effects Cox Models (Therneau 2011) were used to analyse my three response variables: the total time until emergence from shelter, the total time until first prey was eaten and the total time until each prey was eaten, as a function of turbidity and cue availability treatment.

In a subsequent analysis, I focused only on the time when the fish was actively swimming in the arena, excluding time when the fish was hiding, inactive or swimming around the edges of the arena. This measure best represents active search for prey, as all other behaviours were counterproductive to locating the bloodworm. Swimming time analyses were also carried out using Mixed Effects Cox Models, but using swimming time instead of total time until consumption of each prey. Both time until consumption of first and all prey were analysed.

A Mixed Effects GLM using the R package lme4 (Bates et al. 2011) with binomial errors was used to test for difference in number of prey eaten. Size of fish as a random factor (to account for the fact that smaller fish might eat fewer prey) and an observation level random variable was included to account for over dispersion (Bates et al. 2011). No interaction between cue and environment was found, so this was removed and the minimum adequate model (MAM) is presented.

Each behaviour recorded represented a proportion of the total time budget recorded, so the measurements were not independent, with the increase of time spent on one behaviour necessarily causing the decrease in one or more of the others. As this type of data may cause spurious correlations, it is best treated like a composition - that is - each variable should be treated as a proportion either dependent on the whole or of the other variables (Aitchison 1982). Therefore, the compositions package in R (Boogaart et al. 2011a) was used to transform the data (using the isometric log ration transform in the package) into a composition suitable for linear analysis (Boogaart and Tolosana-Delgado 2006, Boogaart 2008, Boogaart et al. 2011b), and using a MANOVA to test for differences in time budgets. Individual behaviours were analysed using generalised linear models with quasibinomial errors. Swimming speeds were analysed using a linear model with two factors (turbidity and cue availability) after log transforming the data in order to meet the assumptions of a linear model.

2.3. Results

2.3.1 Experiment 1: Prey detection

There was a significant interaction between the effect of turbidity and cue on time spent with the prey container (ANOVA: $F_{4,169}$ = 2.455, P = 0.048). High turbidity affected time

spent with the prey container when a visual cue only was available. Single factor analysis on treatments at separate turbidity levels, using a Bonferroni correction for multiple tests, revealed that fish in the visual only and olfactory only treatments spent significantly less time with the correct cup compared to when both cues were available, when turbidity levels were high (Binomial GLM: Olfactory only: t_{60} = -2.467, P = 0.0166 Visual only: t_{60} = -4.233, P = 0.0001; figure 2.3). There was no significant difference between treatments in clear water and medium turbidity (ANOVA: $F_{2,59}$ = 1.45, P = 0.24 and $F_{2,52}$ = 2.22, P = 0.12 respectively).

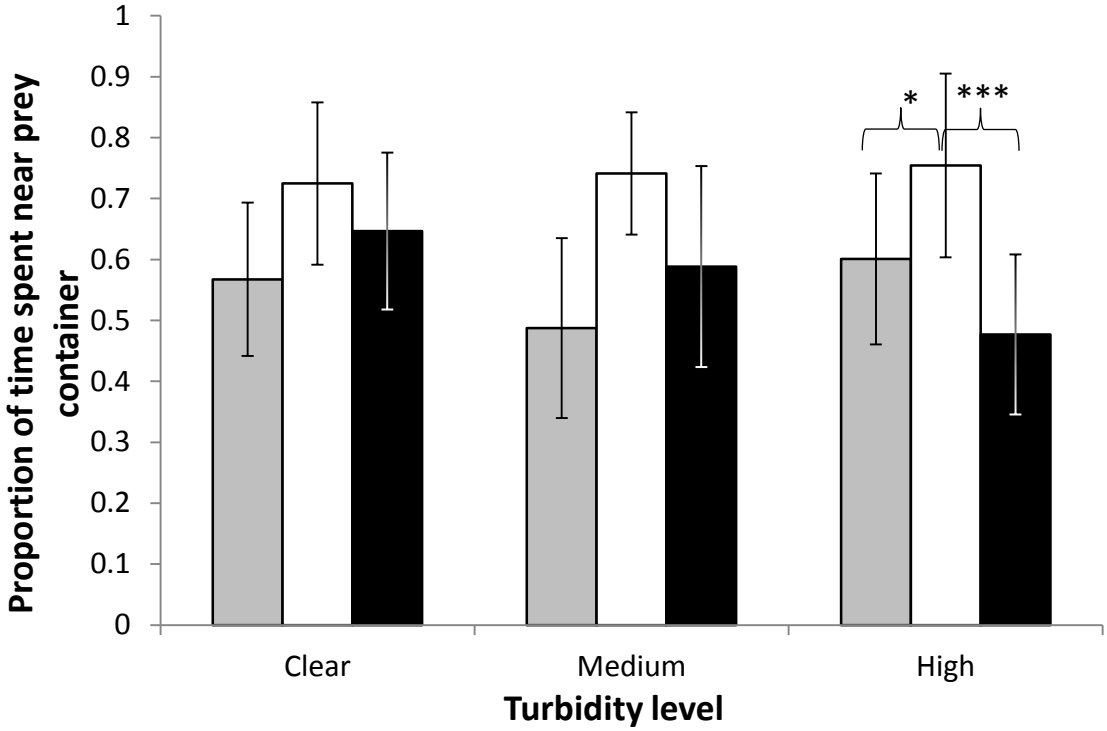

Figure 2.3. Mean proportion of time spent with the prey container with error bars of two standard errors. Grey is olfactory cue only, white is combined cues and black is visual cue only. * P<0.05, *** P<0.001.

2.3.2 Experiment 2: Foraging success

More prey were eaten in treatments with an added olfactory cue compared to treatments without an added olfactory cue (Mixed Effects GLM, z = 1.976, N = 90, P = 0.048) and fewer prey were eaten at high turbidity (z = -4.053, N = 90, P < 0.001) but not medium turbidity (z = -0.898, N= 90, P = 0.369) compared to clear water. There was no significant

interaction between cue treatment and turbidity level on the number of prey eaten. Comparison of the z values indicates a greater effect of turbidity than presence/absence of olfactory cue.

There was no significant difference in the total time until the first prey was eaten between clear water and high turbidity (z = -0.658, N = 90, P = 0.51) or between added cue and no added cue (z = 1.165, N = 90, P = 0.24), in a Cox Proportional Hazards model (figure 2.4a). There was, however, a significant difference between clear and medium turbidity, with medium turbidity leading to a decrease in the time taken until the capture of the first prey (z = 2.95, N = 90, P = 0.003). When looking at swimming time only to the first prey being eaten (figure 2.4b), high turbidity leads to a significant increase in the time taken until the first prey is eaten, compared to clear water (z = -3.219, N = 90, P = 0.0013). The other treatment combinations do not differ significantly from clear water with no added cue (medium turbidity: z = 1.369, N = 90, P = 0.17 and added cue: z = -0.109, N = 90, P = 0.91).

Preys survived longer (total time) in medium and highly turbid water than in clear water and with an added olfactory cue they were eaten sooner than with no added cue (Mixed Effects Cox model, cue: z = 2.86, N = 90, P = 0.0042, turbidity: medium: z = -2.24, N = 90, P = 0.025, high: z = -7.36, N = 90, P < 0.0001; figure 2.4c), but there was no interaction between turbidity and cue availability. Repeating this analysis using active swimming time only revealed a significant interaction between turbidity and cue availability on the survival of prey (z = 3.27, N = 90, P = 0.0011, figure 2.4d). The interaction effect suggests that at high turbidity, the addition of the olfactory cue increases the ‘hazard’ (the risk to the prey of being eaten). Post hoc tests (with Bonferroni correction for multiple tests) revealed that added cue significantly shortened the lives of prey in clear water (z = 2.66, N = 30, P = 0.0078) but no effect was found

at medium turbidity (z = -0.4, N = 30, P = 0.69). Both with and without added cue, increasing turbidity increased the time until prey were eaten (no added cue: medium: z = -5.68, N = 45, P < 0.001, high: z = -12.98, N = 45, P < 0.001; added cue: medium: z = -5.80, N = 45, P < 0.001, high: z = -9.73, N = 45, P < 0.001).

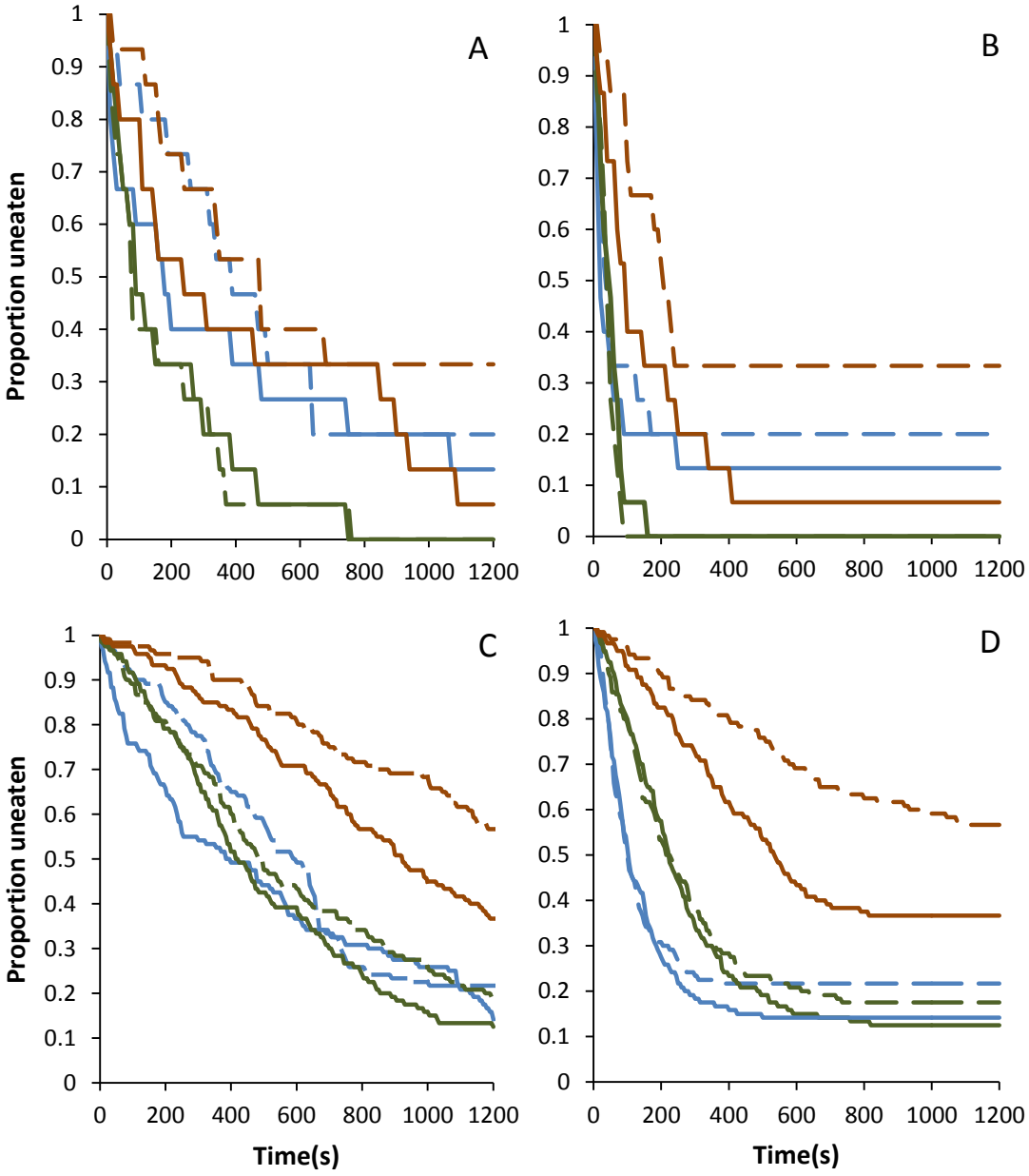

Figure 2.4. Survival curves for total time to first prey (A), swimming time to first prey (B), total time for all prey (C) and swimming time to all prey (D). Lines are: solid line = added olfactory cue; dashes = no added cue; blue lines = clear water; green lines = medium turbid water; brown lines = highly turbid water.

I found no significant interaction effect between olfactory cue and turbidity level on time budgets (MANOVA following transformation using compositions $F_{6,166}$ = 1.34, P = 0.242). There was a highly significant main effect of turbidity ($F_{6,170}$ = 4.84, P < 0.001) but no effect of olfactory cue treatment ($F_{3,84}$ = 1.49, P = 0.224) on behaviour. The above analysis looks at the effect on activity budget as a whole, and when looking at individual behaviours, fish spent a significantly larger proportion of time actively swimming in medium and high turbidity than in clear water (Quasibinomial GLM, t_{89} = 3.45, P < 0.001 and t_{89} = 3.80, P < 0.001 respectively; figure 2.5a). In time spent hiding, there was no significant interaction between added olfactory cue and turbidity ($F_{2,84}$ = 2.09, P = 0.13). After removing the interaction term, the fish spent significantly less time in hiding in both medium and high turbidity than they did in clear water (t_{86} = -5.28, P < 0.0001 and t_{86} = -5.17, P < 0.0001, figure 2.5b).

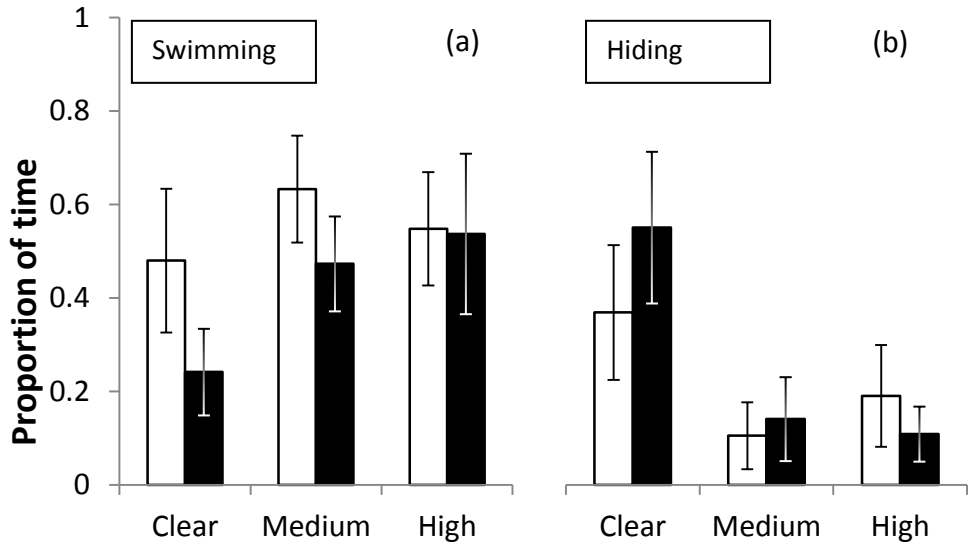

Figure 2.5. The proportion of time spent swimming (a) and hiding (b) in the six treatment groups. Black bars are no added cue, and white bars are added olfactory cue.

Swimming speeds did not differ between added cue and no added cue ($F_{1,59}$ = 00.832, P = 0.365), but speeds were higher at medium and high turbidity than in clear water (medium: t_{58} = 2.908, P = 0.005, high: t_{58} = 2.990, P = 0.004).

2.4 Discussion

My results suggest that olfaction plays an important role in foraging, particularly in turbid waters. At high turbidity, prey detection was enhanced by the presence of both visual and olfactory cues compared to one cue type alone. Surprisingly, I found that when foraging in highly turbid waters, the addition of a masking olfactory cue did not hamper the location of prey items, instead increasing predation risk on the bloodworm. Based on the results of a previous study (Webster et al. 2007a) I predicted that flooding the arena with olfactory cues from bloodworm would conceal the location of the prey to a predator using olfaction to find them. Instead, foraging success was increased with the addition of this olfactory cue in my study. I suggest that the added olfactory cue may have had an appetitive effect on the fish, stimulating them to actively search for or consume prey. I found no difference, however, in the time spent actively foraging, or swimming speed between the two olfactory cue treatments.

In line with previous studies, overall foraging success was decreased in highly turbid waters (Pekcan-Hekim and Lappalainen 2006, Nurminen et al. 2010b, 2010a). However, my prey detection results in particular suggest that the detrimental effect of turbidity may be mediated by the use of olfactory cues from prey for foraging sticklebacks. Previous work has found that when juvenile guppies (*Poecilia reticulata*) are reared in an environment where visual cues are limited, individuals increase reliance on olfactory cues in foraging, to the extent that their overall foraging success is not negatively impacted by reduced visual information (Chapman et al. 2010). Such an increased reliance on olfaction provides a way for individuals to compensate for potentially detrimental effects of environmental change on foraging success and survival, although the mechanisms underlying this are not yet known (Chapman et al.

2010). Sticklebacks are often found naturally in highly turbid water, so early experience of this environment may allow for enhanced use of olfactory cues.

While my study finds a negative effect of high turbidity on the ability of sticklebacks to detect and locate prey, other studies have found that a moderate level of turbidity can have a positive effect on foraging success (Gregory and Northcote 1993) as well as reaction distance (Utne 1997, Utne-Palm 1999). While an increase in reaction distance can be explained by how prey will sometimes stand out more against a turbid background than clear water (Utne-Palm 2002), this cannot explain why juvenile chinook salmon (*Oncorhynchus tshawytscha*) have higher foraging success when foraging for benthic or surface prey. These prey do not have a turbid water background, so would not be more easily detected for this reason. Turbidity causes a decreased anti-predator response in fathead minnows (*Pimephales promelas*) and chinook salmon (Gregory 1993, Abrahams and Kattenfeld 1997) and it may well be that improved foraging at moderate turbidity is at least partly due to change in foraging behaviour caused by a decreased perception of risk.

The contrast between my results and those of Webster *et al* (2007) is interesting, and may be explained by the configuration of the prey in the different experiments. In Webster *et al*'s (2007) experiment, prey items (sections of bloodworm) were partially concealed within a darker coloured substrate, while the prey in my experiment were in high contrast to the flat white background of the test arena. High turbidity reduces the long-range availability of visual cues (Berg and Northcote 1985, Mazur and Beauchamp 2003, Quesenberry et al. 2007), but once close to the prey, the short-range availability of cues will be affected by small-scale habitat structure: prey concealed within the substrate are less likely to be located than those clearly visible, when using visual cues alone. I suggest that for my fish, the appetitive effect of

the added olfactory cue, combined with the availability of short-range visual cues, allowed for increased consumption of prey.

It is possible that the clay used to create turbidity in my experiments may have affected the availability of olfactory cues, which I did not control for in my trials. However, if the clay had a strong negative effect on the availability or perception of olfactory cues, I would expect to see a decrease in the availability/use of olfactory cues in the medium and high turbidity treatments, and a reduced effect of the added olfactory cue in the foraging experiment in turbid water treatments, and this is not reflected in my results. Therefore, while the clay used in my trials may have had some effect on olfactory cue, the primary effect seems to be in limiting visual cues.

Olfaction is known to play a key role in a number of other behaviours in sticklebacks specifically, and in other fish species. Olfactory cues are an important component of social decision-making (Ward et al. 2004a, 2005) and mate choice (Rafferty and Boughman 2006, Heuschele and Candolin 2007, Heuschele et al. 2009). In sticklebacks, increased algal turbidity leads to an increased reliance on olfactory cues in mate choice in comparison to clear water, where visual cues are of primary importance, with knock-on implications for mate selection and the direction of sexual selection (Heuschele et al. 2009). Roach (*Rutilus rutilus*), when exposed to olfactory predator cues from either pike (*Esox lucius*) or perch (*Perca fluviatilis*), are able to successfully identify the predator species and take suitable species dependent evasive action (Martin et al. 2010). Together with previous studies, my results suggest that sticklebacks are able to flexibly rely on olfactory cues, although this may not always compensate for the reduction in visual cue availability caused by turbidity.

My results suggest that in sticklebacks, olfactory cues are used primarily for prey detection, with vision used for final prey location. Where there is no water movement, pervasive olfactory cues alert the fish to the presence of prey in the immediate environment. Highly localised cues may be of less use, as they remain undetected until the predator is very close to the cue source, where vision may successfully be used to locate prey. Where wind or water flow disperses cues, olfactory predators may use anemo- (moving up-wind) or rheotaxis (upstream movement) in addition to chemotaxis to locate prey (Zimmer-Faust et al. 1995), utilising information provided by moving air or water to follow an odour plume to its source, but this information may be disrupted by turbulence (Weissburg et al. 2002). How and whether primarily visual foragers like sticklebacks utilise flow to track odour plumes is unknown (however, see Løkkeborg 1998, Cripps et al. 2011).

Chapter 3: Prey aggregation is an effective olfactory predator avoidance strategy

Abstract

Prey aggregation is a well-known predator avoidance strategy. For immobile prey, the effectiveness of aggregation depends on the inability of the predator to consume all prey once discovered or the inability of the predator to discover single large groups as easily as several small groups. While the benefits of aggregation against visual predators are well-known, the benefits to prey when predators use other sensory modes are less well understood. We investigated the potential benefits of prey aggregation as a predator avoidance strategy when visual cues are not available, using a fish predator and chironomid larvae as prey. Prey aggregation increased the time until detection by predators, but once discovered, aggregated prey suffered high mortality. In the field, however, survival was not affected as strongly by initial discovery. This indicates that aggregation is an effective anti-predator behaviour for prey avoiding olfactory predators.

3.1 Introduction

Predator-prey interactions are one of the major factors influencing patterns of species diversity and abundance in ecosystems (Chesson and Kuang 2008). Predators influence prey abundance and distribution through both consumption and non-consumptive effects (Preisser et al. 2007) such as predator avoidance behaviours, which may limit prey access to resources (Griffiths and Richardson 2006). Aggregation into groups is a common response to the risk of predation (Krause and Ruxton 2002). Individuals benefit from the dilution effect if a predator is unable to consume all prey in a group (Foster and Treherne 1981) and from encounter dilution, where aggregated prey are encountered less often assuming population size is kept constant (Wrona and Dixon 1991). Together, this leads to a situation where fewer predators survive because cost of finding a prey group is high, and more prey survive as predators only consume few prey per encounter (Turner and Pitcher 1986, Turesson and Brönmark 2007).

Prey detection is likely to be dependent on a predator's sensory acuity and modality (Cain 1985). Theory predicts that as a group of prey grows, the ability of a visual predator to detect the group will increase at a slower rate (that is, a group of *N* individuals should less than *N* times more detectable than a single individual; Brock and Riffenburgh 1960, Treisman 1975, Turner and Pitcher 1986). This is supported by empirical evidence for visual predators; Riipi et al (2001) found a non-proportional relationship between detectability and prey group size in great tits (*Parus major*) searching for aposematic prey, a finding reflected by humans seeking computer-generated prey (Jackson et al. 2005) and sticklebacks (*Gasterosteus aculeatus*) attacking *Daphnia* swarms (Ioannou et al. 2011).

Whether encounter-dilution effects operate when predators use other sensory modalities is unclear. Close neighbours are likely to produce odour plumes that interact, increasing both the area of the odour plume and the amount of stimulant (Monismith et al. 1990). Treisman (1975) suggests that a group of *N* individuals should be detectable by an olfactory predator at a distance *N* times as great as that for a single prey, resulting in an area in which the group can be detected N^2 times as large as for a single prey (or a volume N^3 times as large). If this is the case, encounter-dilution would not take place, and grouping would not be favoured unless the predator is highly sensitive to olfactory cues and does not preferentially target large groups over small ones (Cain 1985). Recent empirical data indicates that whelks (*Busycon carica*) move more directly and quickly towards clam (*Mercenaria mercenaria*) prey patches when the prey items were positioned in line with water flow (aggregating the odour cues produced by the clams) compared to when they were positioned perpendicular to the flow (Wilson and Weissburg 2012). In a study of moth antennal responses to patches of sex pheromones (Andersson et al. 2013) found that detection distance increased proportionally to the square root of the number of odour sources, a relationship supported by meta-analysis of trap catches in relation to attractant release rate across a range of insect species (Andersson et al. 2013).

To our knowledge, no study has directly contrasted visual and olfactory prey detection on grouped and aggregated prey by the same predator species. Predators may use both vision and olfaction in detecting prey, increasing reliance on olfaction under poor visual conditions (Chapman et al. 2010). We predict that the benefits of aggregation as an anti-predator defence will be reduced or eliminated when predators hunt using olfaction rather than vision. To test this prediction, we investigate the ability of sticklebacks (*Gasterosteus aculeatus*) to detect and consume dispersed and aggregated prey (bloodworm) when visual cues are and are not

available. Sticklebacks are often found in waters that are highly variable in turbidity (Wootton 1976) and employ olfaction to detect prey in turbid water to compensate for the loss of visual cues (Johannesen et al. 2012). As a measure of detection, we monitor the survival of prey (bloodworm) over time when dispersed and aggregated, and in clear (visual and olfactory cues available) and turbid (no visual cues available) water. Additionally, we test the effect of three levels of aggregation in the field in order to include more naturally sized foraging settings and multiple predators.

3.2 Methods

3.2.1. Laboratory experiment: the effects of aggregation and turbidity (cue availability) on prey detection

3.2.1.1 Study species and housing

Three spined sticklebacks were caught by netting from small waterbodies in Saltfleet, Lincolnshire (53°25'59.55" N, 0°10'49.41" E) in November 2010 and 2011. On both occasions, 250 fish were caught and were transported in commercial fish bags to the aquarium facilities at the University of Leeds. Fish were housed in groups of approximately 50 in grey plastic tubs (60x90x45cm) with gravel substrate and artificial plants for environmental enrichment, at 14±2°C and on a 14:10 hour light:dark cycle. Fish were fed *ad libitum* on defrosted frozen bloodworm (chironomids) once daily. Our prey species were frozen (and defrosted) bloodworm from a commercial fish food supplier. Each group of fish was released one year after capture at the location where caught (in agreement with the Home Office and DEFRA).

3.2.1.2 Procedure

Our experimental procedure followed that in Johannesen et al. (2012) and is briefly summarised here. We investigated two levels of prey aggregation (aggregated and dispersed) and two levels of water clarity (clear and turbid) in a crossed design, giving four treatments (clear-aggregated, clear-dispersed, turbid-aggregated and turbid-dispersed). In each trial, eight designated locations in a foraging arena (100x100 cm, depth 5cm, with a 10 x 10 cm central floating polystyrene shelter) were allocated either one prey each (dispersed prey) or eight prey in one location (aggregated prey) allocated at random. Each location was a distance of 25cm from the nearest neighbours and 25cm from the arena wall. Turbid water was created by the suspension of commercial clay (low temperature white clay from Commercial Clay Ltd.) in conditioned water at 0.5g/l. Water was changed between trials to remove olfactory cues from previous fish or prey, and fish were starved for 24 hours before testing to standardise motivation to feed.

Trials were video recorded from above. In each trial a single fish was released under the floating shelter to acclimatise and time to emerge (be fully free of the shelter) was recorded. Fish that did not hide under the shelter on release did not participate in the experiment and were returned to the holding tank. Fish that did not emerge within 15 minutes of release were excluded from the experiment. Turbidity in the arena decreased over time, from 391.15 ± 9.35 NTU before fish were released to 286.83 ± 9.1 NTU after 35 minutes (measured before fish were captured after the trial). Therefore, fish were given a maximum of 35 minutes in the foraging arena, consisting of up to 15 minutes before emergence, plus 20 minutes foraging time. Fish were measured (+/- 1mm total body length) using callipers after each trial. Environment (turbid/clear) did not affect time to emergence (Negative Binomial

GLM, z=-1.63, df=61, P=0.1). This suggests that our manipulation of visual cues did not influence motivation to hunt for prey and/or perceived predation risk of the fish.

Data on foraging behaviour and time of prey capture were manually extracted from videos using Etholog (2.25) and Windows Media Player. Sticklebacks vary considerably in boldness (Ward et al. 2004b, Frost et al. 2007, Harcourt et al. 2010), leading to variation in time spent hiding (and therefore not foraging). Thus, to standardise search time for all fish, we recorded prey capture as a function of time spent actively swimming.

3.2.4 Field experiment: Predators searching for aggregated and dispersed prey using olfactory cues

Our laboratory experiment necessarily constrained the search area available for each predator, increasing the likelihood of chance encounter. In ponds and lakes, search volume or area is much greater, and there may additionally be multiple predators (individuals or species) in the environment, affecting how many prey may be consumed and increasing the likelihood of local or stimulus enhancement (where the activity of an individual draws the attention of an observer towards a location or object; Spence 1937, Thorpe 1956), or social learning (Brown and Laland 2003). To test the real-world validity of some of our findings, we also carried out a field experiment to assess the survival of visually hidden prey at different levels of aggregation.

Fieldwork was carried out on the Faroe Islands, where there is a low diversity of aquatic species, making natural systems much simpler than those in warmer climates (Malmquist 2002, Brodersen 2011). The largest predators in a typical rock pool above the tidal line are *Gammarus duebeni* (Roberts 1995) and sometimes three spined sticklebacks (*Gasterosteus aculeatus*). These ponds also contain a range of invertebrate prey species,

including midge larvae. Ponds (N=11) were 5-50 m^2 in size, contained sticklebacks and did not directly connect to any other pond in the study.

3.2.4.1 Procedure

We created "feeding stations" to conceal visual, but not olfactory, cues from prey. Each feeding station consisted of a weighted transparent cylindrical plastic "skeleton" (12cm diameter, 8cm height) covered in two layers of fine-mesh material (nylon tights, 40 denier) with two entrance holes (2x2cm) positioned at opposite sides of the station (figure 3.1). In each pond, we placed six stations close to the edge (to allow access by the experimenter), approximately 1m apart. Stations were added two to four days prior to the first observation day to counter any effects of neophilia or neophobia (Frost et al. 2007, Archard and Braithwaite 2011). To reduce disturbance, feeding stations were left in the ponds for the duration of the trials.

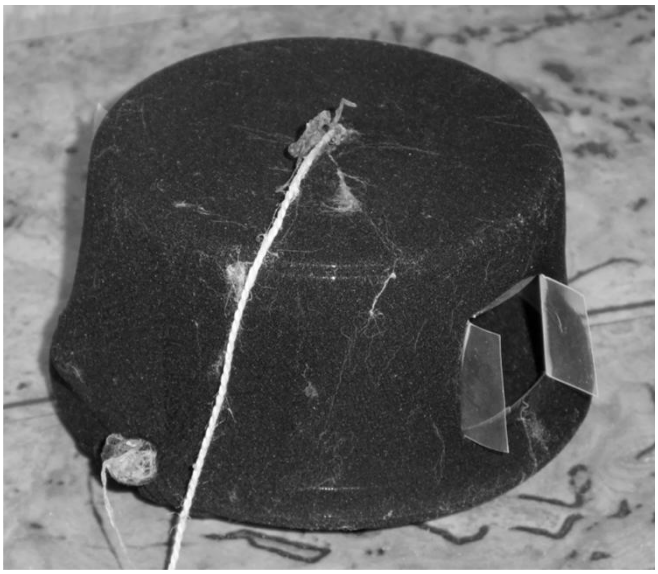

Figure 3.1. "Feeding station" after use in field trials. Cotton thread attached at the top assisted in positioning and retrieval of stations and to the right is an entrance hole with "doors" intact to ensure opening was not blocked by straying material. A similar opening is found on the opposite side of the station.

In each pond, we investigated three levels of prey aggregation (aggregated; 30 prey in one of the six feeding stations, semi-dispersed; 10 prey in each of three of the six stations, and dispersed prey; five prey in each of the six stations). Aggregated prey were allocated to a feeding station at random and semi-dispersed prey were allocated to alternating feeding stations (starting point chosen at random). The order in which the treatments were placed in each pond was systematically rotated ensuring each possible trial sequence was included at least once and no more than twice. To minimise any possible effects of learning and reduce disturbance, a minimum of four days was left between each trial within a pond. Prey used in these trials were frozen bloodworm sourced from a local pet shop. The bloodworm were defrosted and the refrozen in tap water ice cubes in the prey groups sizes above for ease of handling in the field.

On the day of each trial, the ice cubes containing prey were positioned in their allocated feeding stations. Plain ice cubes (containing no prey) were placed in all other stations to control for the presence of the observer at each station and any cues from the tap water that may have been used by potential predators. After 10, 20, 30, 40, 50, 70 and 90 minutes, the observer returned to the pool and counted the number of uneaten prey in each station. Stations containing no prey were also checked to control for the presence of the observer and the disturbance caused by removing and replacing the feeding station. The timer was stopped when the observer returned to the pool, and restarted when counting was complete, so that the time while disturbed by researcher was not included in the time available to the fish to forage in the stations.

3.2.6 Analysis

All data analysis was carried out in R v 2.13.0 (R Core Team 2013). For the laboratory data, prey within a trial were not independent of one another. To account for this, we created multiple events (each predator could encounter multiple prey 'events') models using the Andersen-Gill version of Cox Proportional Hazards models in the package 'survival' (Therneau and Grambsch 2000, Therneau and Lumley 2011). By incorporating 'trial' as a clustering factor in the model, each prey encountered was an event for each individual stickleback.

Our initial model of the laboratory data did not meet the necessary assumption of proportional hazards (Chi-squared=85.6, P<0.001; Therneau and Grambsch 2000). When this assumption is violated, it is an indication that the survival curves are not the same shape and do not follow similar hazards distributions (i.e. the risk to a prey individual in one treatment is not a simple multiplication of the risk in another treatment, for any given time point). This is especially problematic when survival curves cross (as they do in our case; figure 3.2) (Therneau and Grambsch 2000). In order to remedy this, we split our data set in two ("initial prey discovery" and "subsequent survival of prey") and analysed these separately (figure 3.3). The assumption of proportional hazards was met in the case of initial prey discovery (Chi-squared=3.27, P=0.351). In the case of subsequent prey discovery, the assumption of proportional hazards was not met (Chi-squared=176.4, P<0.001). However, survival curves did not cross (figure 3.3b), so although predictions based on this model should be treated with caution (Therneau and Grambsch 2000), it does give an indication of whether the survival of prey differed between treatments.

The data from field trials were interval censored, meaning the exact time of each prey being eaten was not known. Times were defined as the start and stop time of the interval in

which prey were eaten, and we fitted a non-parametric maximum likelihood estimate (NPMLE) of the survival distribution (Turnbull 1976). Hypothesis testing was performed using a non-parametric weighted k-sample logrank test with Sun's scores, using the packages 'interval' and 'icens' developed for analysing interval censored data (Fay and Shaw 2010, Gentleman and Vandal 2011).

3.3 Results

3.3.1 Laboratory experiment – does turbidity affect best aggregation strategy?

The survival curve for aggregated prey in turbid water showed a very different pattern to the survival curve for other treatment groups (figure 3.2). As the assumption proportional hazards was not met (Chi-squared=85.6, P<0.001; see above), this suggests that overall patterns of survival differ significantly as a function of treatment grouping.

Aggregation is beneficial in increasing the time to initial detection in both clear and turbid water, but has a greater effect in turbid water; there was a significant interaction between water clarity and level of aggregation (CoxPH; z=2.24, n=61, P=0.025) on the time until the first prey was discovered (figure 3.3a). For dispersed prey, initial discovery happens sooner in turbid water than clear water while for aggregated prey it happens sooner in clear water (figure 3.3a).

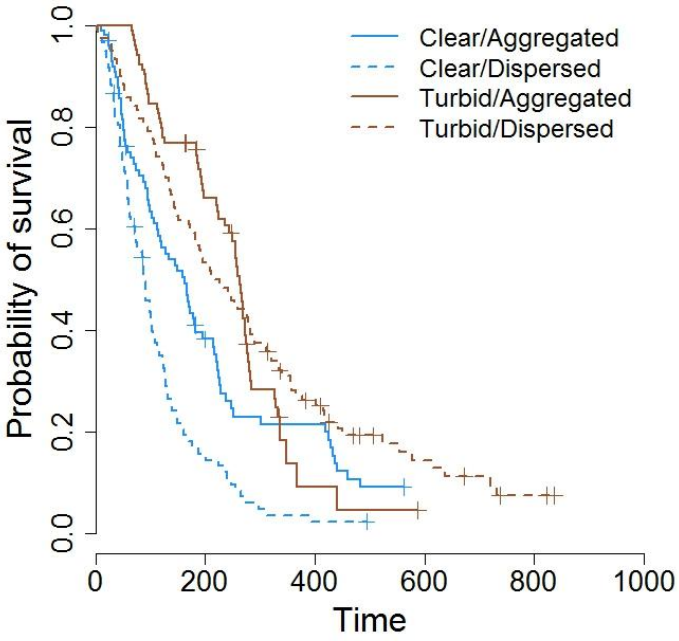

Figure 3.2. Kaplan-Meier survival curves for the four groups of prey. Crosses signify censored events where the observations for a particular trial ended before all prey were eaten. The curve for aggregated prey in turbid water shows a different pattern to the curves for the other three treatments.

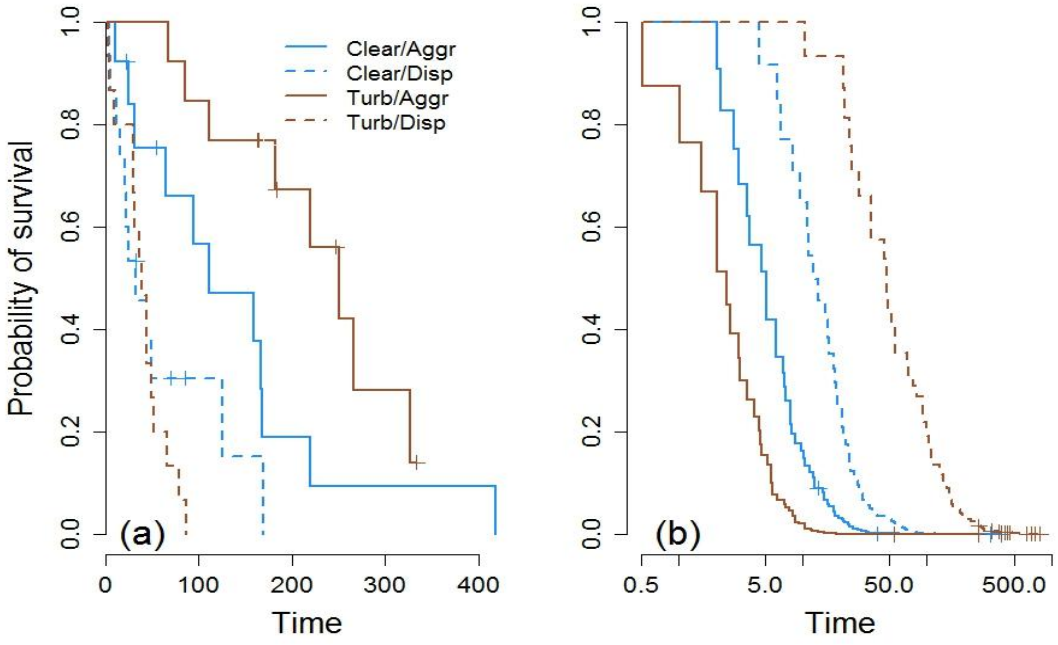

Figure 3.3. Kaplan-Meier curves for time to discovery of first (a) and subsequent (b) prey. The solid lines represent aggregated prey and dashed lines dispersed prey. Brown represents turbid water and blue represents clear water. Crosses signify censoring events. In (b), the time axis was logged to improve clarity.

For time to consume subsequent prey, there was also a significant interaction between the water clarity and level of aggregation (CoxPH, z=-3.173, n=302, P=0.002). Survival is highest for dispersed prey in turbid water, while aggregated prey survive for longer in clear water than in turbid water (figure 3.3b). Therefore, after the discovery of the first prey, aggregation appears to be beneficial in clear water (aggregated prey survive longer in clear water than in turbid water), but not in turbid water (where dispersed prey have higher survival).

3.3.2 Field experiment: do prey in a more natural setting benefit from aggregating?

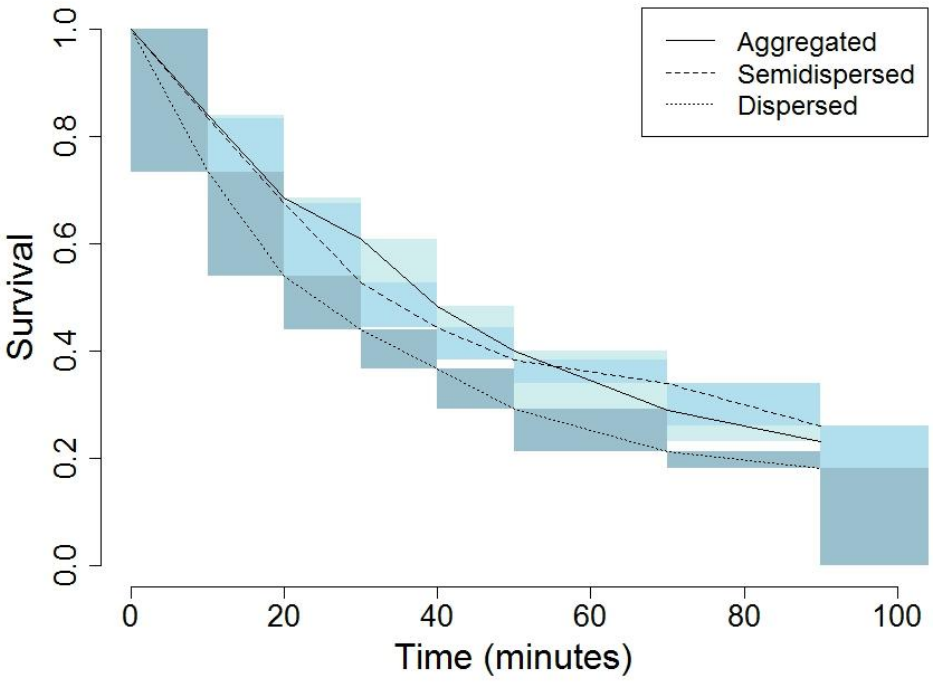

Figure 3.4. Interval censored survival curves for the field data. Possible stepwise changes in survival lie within the shaded area for each curve. Aggregated: solid line, light blue shading, semi-dispersed: dashed line, medium blue shading, dispersed: dotted line, dark blue shading.

The three levels of aggregation differed significantly in survival (Asymptotic Logrank k-sample test with Sun's scores, Chi-squared=13.16, P=0.001) with dispersed prey being

discovered and consumed the most quickly (Suns' score statistics: dispersed: 42.17, aggregated: -19.11, semi-dispersed: -23.06).

3.4 Discussion

Aggregation as a predator avoidance strategy is effective both for visually conspicuous and concealed prey. Aggregated prey, with and without visual cues available to the predator, had improved survival over dispersed prey in terms of initial detection. However, once an aggregation has been detected in the lab, the prey did not survive for very long. This likely occurred because predators were able to find and consume all the prey in an aggregation after having discovered the first prey, and the dead prey could not take any evasive action in response to the proximity of the predator. In the natural pond setting, overall survival of aggregated and semi-dispersed prey was higher than that of dispersed prey.

This suggests that aggregation should be an adaptive strategy for species living in water of varying turbidity or in habitats with structural refuges hiding them from view as well as where the predator of immediate concern does not use visual cues. In fact, aggregation as an anti-predator strategy when the predator does not use visual cues is seen in a number of species such as the sediment dwelling *Chironomus riparius* larvae, who aggregate in response to predator presence (Rasmussen and Downing 1988) and stream dwelling caddis flies (*Rhyacophila vao*) that avoid predation by the planarian predator *Polycelis coronata* by communally pupating on the same stone (Wrona and Dixon 1991). Additionally, studies such as Taylor's (1977) study on southern grasshopper mice, which found that buried aggregated prey were found less easily than dispersed prey, seem to confirm our findings that aggregation is adaptive against predators using sensory modalities other than vision.

There is evidence in our results to suggest that the protection provided by aggregating depends partly on the availability of visual cues as well as the perception of risk by the predator. Once discovered, aggregated prey did not survive for long, but those in clear water survived for longer than those in turbid water. We suggest that despite data on time to emergence, a perceived risk involved in foraging in clear open water (Abrahams and Kattenfeld 1997) decreased foraging effort and allowed aggregated prey to survive longer in clear water than in turbid water.

In the field, aggregated prey did not experience the accelerated death rate once discovered as they did in the laboratory. There is some indication that benefits to prey depend on size or number of predators (Brock and Riffenburgh 1960) and sticklebacks are able to learn from visual foraging cues from conspecifics (Webster and Laland 2012), resulting in increased discovery if one stickleback in the group starts consuming prey. However darkness or turbid water should reduce the likelihood of this happening, as initial discovery by one predator would not be observed visually by other predators. Lateral line detection of the movement of conspecifics (Coombs 1999) is likely to be too short-range to be relevant in this context, however the importance of noises generate by foraging might warrant further exploration. In our experiment, prey as well as any predator feeding on them, were concealed in feeding stations, which may have prevented visual social cues from being transmitted to other sticklebacks in the area. Prey groups were also much larger, which likely prevented individual sticklebacks from consuming all prey. Together, this may have limited the rapid consumption of prey seen in the laboratory.

The benefits of aggregation are likely to depend on the sensory acuity of the predator with predators unable to detect prey approaching random search efficiency (Cain 1985). However, a predator able to detect the presence of prey and perhaps even an indication of the number of prey should perform better than random by increased search effort, especially if that effort can be focused in the general area surrounding prey. Sticklebacks use both visual and olfactory cues in foraging, and when visual cues are not available, the presence of olfactory cues increases foraging efficiency (Johannesen et al. 2012). Therefore, strong cue concentrations around aggregated prey could increase search effort, potentially countering the benefit prey derive from aggregating. Similarly, theory on the relationship between olfactory cues and detection of prey groups predicts that grouping should not be favoured as detection radius increases with group size (Treisman 1975). In our study, however, it is clear that aggregation is beneficial to prey, at least at the predator-prey ratios tested here. There is some evidence to suggest that olfactory detection radius increases with group size (Andersson et al. 2013), but it is still not clear how increased detection affects aggregated prey in different systems such as one where only one prey item is captured and the rest escape and how predator sensory acuity interacts with prey group sizes.

Aggregations are ubiquitous and part of many important life functions. Understanding detectability and survival of aggregated prey will help us understand the adaptive mechanisms driving distributions of prey organisms and how these interact with predators. Our study provides insight into some adaptive reasons to aggregate in a system that is different from the usual visual predator system. Many natural predators rely on visual cues but the consequences of low to no availability of visual cues have been relatively neglected by scientists, likely because of the dominant importance of vision to humans. We demonstrate that aggregations are beneficial to prey avoiding non-specialist olfactory foragers. However, a better

understanding of the relationship between group size, predator sensory acuity and detectability is needed as well as empirical investigation of systems where all but the first prey escape after detection. Understanding the relative consequences of vision versus olfaction by freshwater aquatic predators must be given increased urgency by our need to predict system responses to climate change. Water light levels will be influenced by changed rainfall patterns through a number of mechanisms: changes in typical cloud cover, changes in river flow patterns (and thus sediment levels in the water column) and water levels, increased algal growth through runoff of nitrate fertilisers from agricultural land. Since predation is a fundamental interaction structuring communities, changes in the relative importance of vision and olfaction in prey detection could have far reaching implications ecologically. Our work provides a small step towards improved ability to predict these effects.

Chapter 4: Detectability of prey as a function of prey aggregation

Abstract

Aggregation is a common predator-avoidance strategy for many prey species. For prey that are avoiding olfactory predators, theoretical work makes contrasting predictions. Some models predict that the distance at which predators can detect prey should increase linearly with prey group size, thus making aggregation detrimental to prey, while other work predicts that prey groups should benefit from aggregation. Empirical data (Andersson et al. 2013) suggests that the distance at which predators can detect prey increases linearly with the square root of the number of prey, when air movement transports odour cues towards predators. However, the ability to detect prey does not translate directly to the ability to locate that prey, and thus the relationship between search time and number of prey number in a group may differ from the relationship between group size and detection distance. In this chapter, I investigate how group size influences the time taken to locate prey by *Gammarus pulex* searching for bloodworm. I find that prey are discovered more quickly when in larger groups and there is a linear relationship between the square root of search time and square root of prey group size. However, prey groups are more likely to be discovered at intermediate group sizes. Large prey groups, while benefitting from attack abatement may suffer detrimental trait mediated effects as predators may choose to increase search efforts in areas with high prey density while not necessarily finding prey quickly.

4.1 Introduction

Aggregation is a common predator avoidance strategy in a range of animals and even plants (Elgar 1989, Jakobsen et al. 1994, Lima 1995, Kunin 1999, Morrell et al. 2011). Benefits of being in a group include communal effort put into vigilance (Brown 1999), mobbing (Krams et al. 2010) and confusion of predators (Ruxton et al. 2007). However, not all prey species are sufficiently mobile to allow them to utilise these active defence measures (e.g. *Mytilus edulis;* Reimer and Tedengren 1997, *Senecio jacobaea*; Kunin 1999 and *Dreissena polymorpha*; Kobak et al. 2010), and even if active avoidance of predation is possible, the benefits could be off-set by increased conspicuousness (Taylor 1976).

For prey that are unable to mount an active or even passive defence (such as a thicker shell; Trussell 1996) against predators, there are two main avenues which could be exploited: avoiding detection through crypsis or the use of a refuge, and the dilution of risk through aggregating with many other prey. Crypsis is a common defence and many animals successfully avoid predators in this way (Howlett and Majerus 1987). However, crypsis can be costly (Dunham and Tierney 1983) and for some species, aggregation may be a better strategy. If a predator is unable to consume all prey in a group, then some individuals will survive an attack, known as the dilution effect (Foster and Treherne 1981). Additionally, if prey density remains constant, aggregated prey will be less easily encountered by chance resulting in 'encounter-dilution' (Turesson and Brönmark 2007). Together, the dilution and encounter-dilution effects combine to reduce individual risk through 'attack abatement' (Turner and Pitcher 1986).

When a predator detects an olfactory cue, very little information other than the presence of prey is immediately conveyed. Thus, for a predator to make use of an olfactory

cue to locate food, it must do at least one of three things: track the cue in a flowing environment such as wind (anemotaxis) or water current (rheotaxis), be able to detect and follow a cue concentration difference (chemotaxis) or increase search effort in the area where the cue is detected (Guevara-Fiore et al. 2010). Previous work has demonstrated that many aquatic predators are able to track odour plumes upstream towards prey, including whelks (Ferner and Weissburg 2005), crabs (Finelli et al. 2011) and fish (Løkkeborg 1998). Finally, some fish make use of odour cues in a way that indicates greater foraging effort in the presence of prey cue (chapter 2; Johannesen et al. 2012).

The selective advantage of aggregation is subject to debate: theory predicts an advantage of encounter-dilution must outweigh costs of increased conspicuousness of groups (e.g. see theoretical models by Taylor 1976b, 1976a, 1979, Cain 1985). Recently, Ioannou et al. (2011) have shown both theoretically and empirically that when predators use vision to detect prey, the increased conspicuousness of groups is offset by the decrease in encounter rate as groups increase size in a finite population, providing good evidence that aggregation is beneficial as a means of diluting risk through attack abatement. However, whether this holds true when predators hunt using olfaction is less well established. Recent studies have suggested that the detection range in a flowing environment increases relative to the square root of the number of prey in a group (Andersson et al. 2013), which would indicate that grouping should be beneficial. However the relationship between group size and the effect on predator search behaviour and ability to track the odour plume to its source (locate prey) is unknown, yet this is a fundamental component of predation success.

In this chapter, I will use an olfactory forager *Gammarus pulex* (henceforth referred to as *Gammarus*) to investigate how prey group size affects search effort and the time taken to

detect the prey in a still water environment (where water flow provides no clue as to prey location). Although *Gammarus* are known primarily as shredders and detritivores (Åbjörnsson et al. 2000), they are opportunistic feeders (Sutcliffe et al. 1981) that predate on a range of invertebrates (Fielding et al. 2003). They are known to use olfactory cues in a range of situations including mating behaviour (Dunn et al. 2008), predator avoidance (Åbjörnsson et al. 2000) and foraging (Lange et al. 2005). I predict that higher concentrations of olfactory cue (from larger groups) should increase *Gammarus* efforts to access or locate prey once olfactory cue is detected. Additionally though results from chapter 2 indicate that olfactory cues in still water may not provide good information on directionality allowing predators to locate prey, *Gammarus* are primarily olfactory predators, so may be able to better use olfactory cues in this manner, so I predict a decrease in search time with an increase in prey group size.

4.2 Methods

4.2.1 Study species and housing

Three hundred *Gammarus pulex* were collected from leaf litter in a stream in Goldenacre Park, Leeds using sieves (2mm mesh size) and transported by road to the University of Leeds in two four litre cool boxes. In the laboratory, *Gammarus* were approximately equally divided between three clear plastic tanks (20x30x20 cm) filled with conditioned, fresh tap water. All tanks were enriched with leaf litter substrate (obtained from Goldenacre Park during animal collection) to provide shelter and food resources, and were supplied with an air stone to oxygenate the water. Tanks were held in a temperature controlled room at 17°C with a 16:8 h light:dark cycle. *Gammarus* will feed on leaf litter, but in addition to the leaf litter substrate, they were also fed defrosted frozen bloodworm two to

three times per week to prevent cannibalism and to familiarise them with the prey used in the experiments.

4.2.2 Experiment 1 – speed of prey location as a function of prey group size.

The aim of this experiment was to investigate the probability that *Gammarus* find prey and the time taken to find it, as a function of prey group size. An arena measuring 10x15cm was filled with 5cm depth of conditioned water. Pilot observations indicated that *Gammarus* behaviour was more similar to that in the home tank (i.e. more natural) when some home tank substrate was added to the arena, so approximately 1ml of substrate from the home tank was spread across the base of the tank to minimise stress and maximise natural behaviour. A shelter consisting of a piece of fabric (3cm by 3cm piece of 40 denier black tights) weighted with gravel was added to the centre of the tank. Two opaque barriers 2.5cm high and 3cm wide were positioned across two opposite corners of the tank (leaving a triangle measuring approximately 2cm by 2cm by 3cm behind each barrier). The barriers prevented accidental access to the prey by the *Gammarus*, which usually remain on the base of the tank unless prey is detected. After five minutes acclimatisation in the tank by an individual *Gammarus*, prey were added to one corner and at the same time, 1cm strands of red cotton thread were added to the other corner to control for the use of any visual cues by the foraging predator. A set weight of bloodworm was added to the prey corner, as prey were variable in size and differently sized prey would produce different quantities of olfactory cues. Eight treatments were considered (weight ± SD): 0.005 ± 0.001g (approximately one prey, N=28), 0.012 ± 0.001g (three prey, N=27), 0.019 ± 0.001g (five prey, N=24), 0.024 ± 0.001g (seven prey, N=28), 0.035 ± 0.002g (10 prey, N=20), 0.057 ± 0.001g (15 prey, N=28), 0.085 ± 0.001g (20 prey, N=26), 0.118 ± 0.001g (30 prey, N=21). An equal number of cotton threads were added to the control corner. *Gammarus* were given 10 minutes to forage and the time taken to locate prey was

noted. Location was defined as a *Gammarus* grabbing hold of prey. This involved the *Gammarus* swimming upwards away from the bottom of the arena to cross the barrier and then swimming down to grab the prey, thus making it an event unlikely to happen by chance. *Gammarus* were tested for interest in prey (motivation to search) at the end of each trial by placing three to four bloodworm near the *Gammarus*. Any *Gammarus* that did not take prey offered to them in this way were excluded from analysis (n=2).

4.2.3 Activity levels

In order to determine any effect that olfactory cue might have on activity levels and whether this may affect the time taken to locate prey, activity of individual *Gammarus* was measured from the trial videos. To measure activity, the number of times an individual crossed a line dividing the tank diagonally from the prey corner to the opposing corner was recorded. The crossing rate (crossings per second) could be adequately measured in two minutes of observation time, so crossings were recorded for two minutes. In order to ensure that olfactory cue had dispersed into the tank from the prey corner at the onset of observation, the start time was two minutes after prey were added. In some trials, *Gammarus* located prey within four minutes, leaving less than two minutes of observation time. Those trials were excluded from activity analysis. I was able to extract a minimum of 10 trials from all prey group sizes, so for prey group sizes where there were more than 10 suitable trials, those used for analysis were chosen at random.

4.2.4 Experiment 2 – preference for prey containers as a function of prey group size

In this experiment, the strength of preference for olfactory cues from prey groups of different sizes was measured, using association time with an inaccessible prey group as a

measure of preference strength. The arena used was identical to that used in experiment 1, except that there were no barriers in the corners. Four prey containers were placed in the arena, one in each corner. The containers were constructed from the inverted bulb ends of disposable plastic pipettes with holes cut into them (0.5x0.5cm holes 0.5cm apart) and then covered with a layer of black fabric (40 denier tights). Each container was weighed down with gravel and in each trial there were three "dummy containers" and one "prey container". Dummy containers were "baited" with 1 cm strands of red cotton thread to mimic bloodworm prey without providing olfactory cues. The number of strands was appropriate to the prey group size being tested, thus controlling for visual cues of the prey. As in experiment 1, prey containers were baited with a set weight of bloodworm (based on initial weighing of the corresponding number of prey). Six treatments were considered: 0.006 ± 0.001g (approximately one prey, N=16), 0.018 ± 0.001g (five prey, N=16), 0.035 ± 0.001g (10 prey, N=16), 0.084 ± 0.002g (20 prey, N=12), 0.117 ± 0.002g (30 prey, N=11), 0.153 ± 0.002g (40 prey, N=12).

A dummy container was placed in each corner of the tank, then a single *Gammarus* was placed in the arena and left to acclimatise for five minutes. At the end of the acclimatisation period, one dummy container was removed and replaced with a prey container and all other containers were lifted from and returned to the water. The order in which containers were removed and replaced was randomised to control for the effect of disturbing containers. Trials were recorded from overhead using a webcam positioned above the arena. Each *Gammarus* was allowed 10 minutes to search for prey and the time spent with dummy containers as well as the prey container was recorded. After each trial, three to four bloodworm were added to the arena close to the position of the *Gammarus* to assess motivation to feed. Any individuals that did not take bloodworm prey were excluded from

analysis (n=1). Finally, the arena was carefully rinsed and prey containers rinsed and covered in new fabric to remove all olfactory cues from previous trials.

4.2.5 Analysis

All analysis was carried out in R version 2.15.2 (R Core Team 2013). In experiment 1, following Andersson et al. (2012), I predicted a relationship between the search time and the square root of the number of prey. To normalise residuals, search time was also square root transformed. Proportion of prey detected was analysed using a Chi-squared test for association. Activity was analysed using a linear model of number of crossings as a function of prey number. In experiment 2, preference for prey container was analysed using time (in seconds) with prey container and with the other containers in a mixed effects GLM with binomial errors. The fixed effect factor was number of prey and as data were over dispersed, a trial level random effect was added to the model.

4.3 Results

4.3.1 Experiment 1

Gammarus were most likely to locate the prey at intermediate group sizes (Chi-square=15.9, DF=7, P=0.03; figure 4.1). Where prey were located, there was a significant effect of group size on time to location (linear regression; $F_{1,91}$=4.82, P=0.031; figure 4. 2) with drop in time to location being related to the square root of the number of prey. There was no significant relationship found between activity levels and number of prey (Linear model; $F_{1,78}$=2.18, P=0.14).

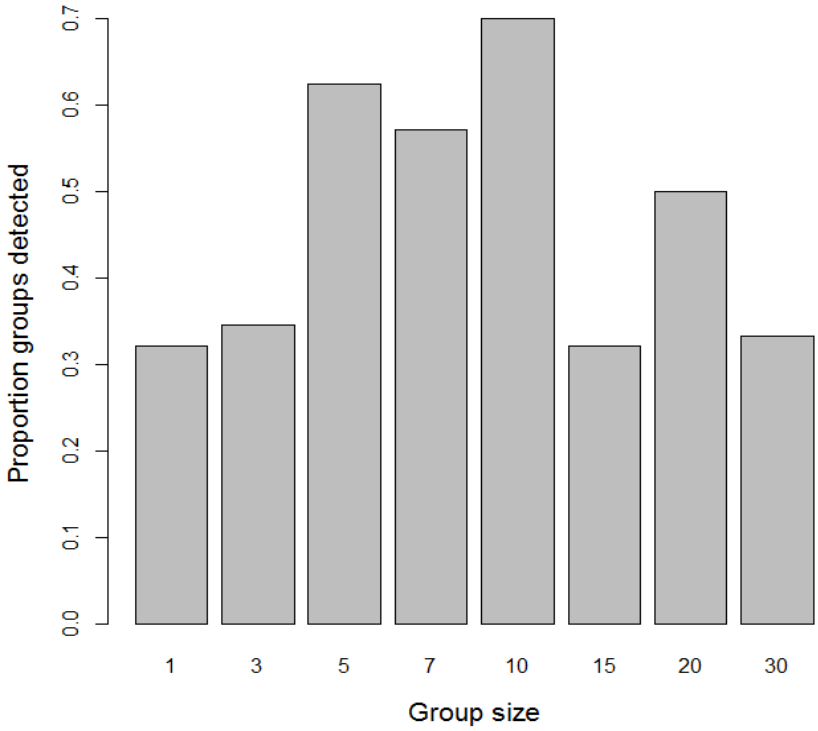

Figure 4.1. Proportion of prey groups located as a function of group size. More prey groups were located by the predator at intermediate group sizes.

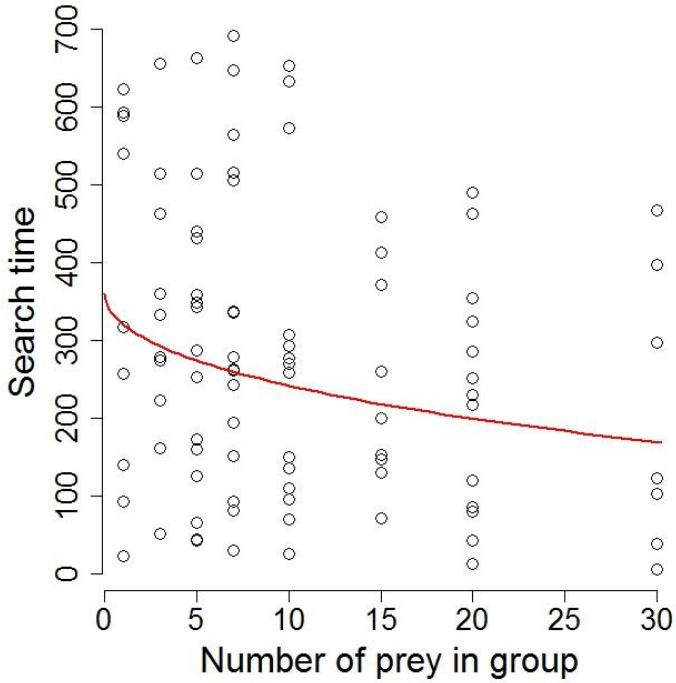

Figure 4.2. Time taken to locate prey as a function of the number of prey in the group. The red line is the prediction from the statistical analysis relating the square root of search time to the square root of the number of prey.

4.3.2 Experiment 2

Number of prey had a significant effect on the proportion of time spent with the appropriate container (ANOVA comparing two binomial mixed effects GLMs, one with and one without number of prey as factor; Chi-squared=23.21, df=5, P<0.001; table 4.1). From the summary (table 4.1) and figure 4.3 it is clear that there is a threshold where group sizes 10 and up attract *Gammarus* for a larger proportion of the time than group sizes one and five.

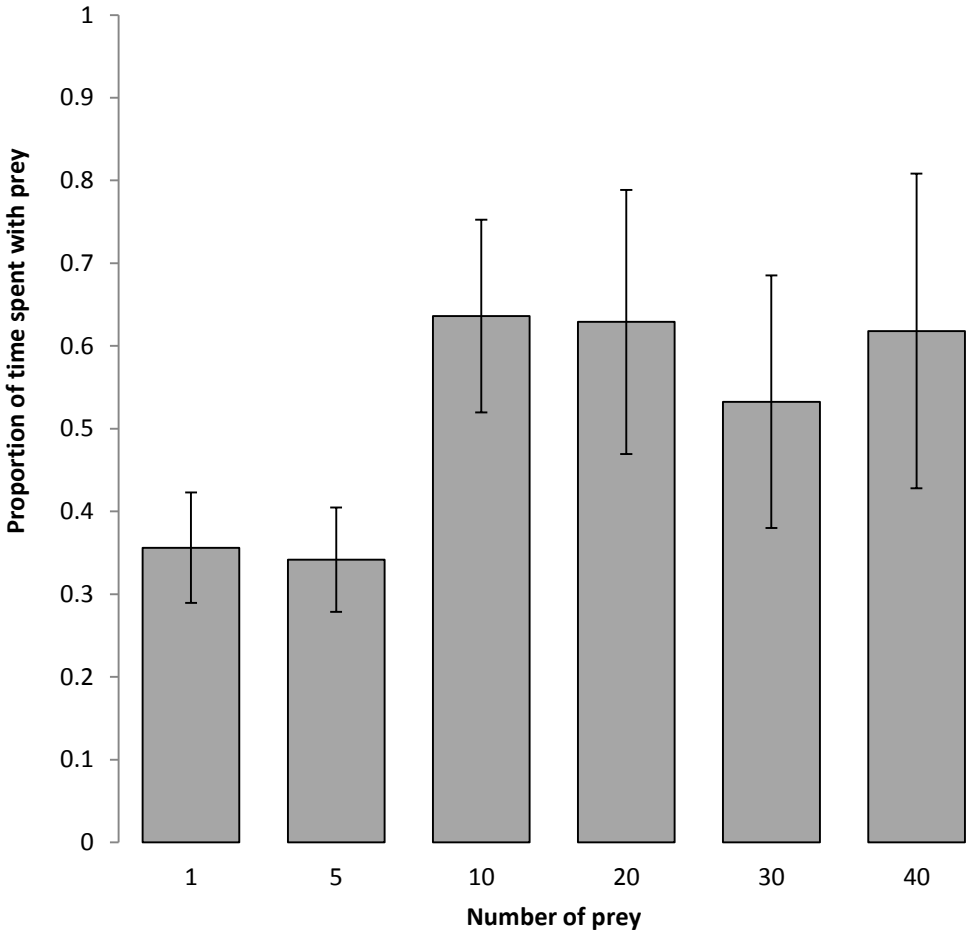

Figure 4.3. Proportion of time spent with the prey container as a function of the number of prey in container. Error bars are two standard errors.

Table 4.1. Summary statistics of proportion of time spent with prey containers at prey group sizes 1 and 5 compared with group sizes 10, 20, 30, and 40 (Mixed effects GLM with binomial errors). * Denote significant difference from intercept group size at 0.05 probability. No differences were found in comparisons between group sizes 10, 20, 30, and 40.

Intercept group size	Group size	z	P
1	5	-0.21	0.833
	10	3.24	0.001*
	20	3.37	0.001*
	30	1.9	0.057
	40	2.97	0.003*
5	10	3.45	0.001*
	20	3.56	0.000*
	30	2.09	0.037*
	40	3.16	0.002*

4.4 Discussion

My results suggest that aggregation is a beneficial strategy for prey. Search time decreases asymptotically with prey group size and probability of being found by the predator decreases when group size reaches a threshold. I found a negative relationship between the square root of the number of prey and the square root of search time. This corresponds well with previous work on olfactory prey detection distance, which also relates to the square root of the number of prey in the group (Andersson et al. 2013). Interestingly, the proportion of prey groups detected did not show a linear relationship with the number of prey in the group, but rather seemed to reach a maximum at an intermediate group size. In experiment 2, I found that there is a threshold group size (five prey individuals) below which *Gammarus* show little preference for the prey container, and above which there is a stronger preference. This suggests that while preference increases with group size, it does not do so linearly, a similar pattern found for the detection of groups using visual cues (Jackson et al. 2005, Ioannou et al. 2011). The difference in patterns between the search time and the proportion of prey found cannot be related to search effort, as unexpectedly I found no evidence that prey cue affected

search effort (Guevara-Fiore et al. 2010). This may indicate that prey location is not a simple matter of detection distance in olfactory terms and that the mechanisms of searching for prey once detected are important in understanding how prey group size affects location risk to prey.

For olfactory predators, the distance at which they can detect prey groups may not translate directly into risk of attack on those groups. Firstly, detecting prey and locating prey are two separate processes for olfactory predators (unlike for visual predators), as I discuss in chapter 2 (Johannesen et al. 2012). If a predator locates and attacks prey that it detects at a distance related to the square root of the number of prey, the prey will benefit from attack abatement (Turner and Pitcher 1986) as the group size will grow faster than the relative detection distance, reflecting the pattern observed for visual predator-prey systems (Ioannou et al 2011). However, it is possible that if prey are detected by an olfactory predator then the predator may increase search effort in that area (see also chapter 2 in this thesis). As the presence of a predator affects prey behaviour (Trussell et al. 2003), olfactory prey detection has two potential effects on prey; consumption and behavioural. If prey are difficult to locate with a long search time post detection, the non-consumptive effect of prolonged predator presence could potentially have a greater negative effect on prey than the consumptive effect.

The pattern seen in the probability of prey being located by *Gammarus* could be explained by olfactory cue flooding of the arena due to the large number of prey, which has been shown in sticklebacks to lower foraging efficiency (Webster et al. 2007a). However, in my experiment, search time continued decreasing beyond the point where proportion of prey located dropped, which seems to contradict this explanation. Additionally, in chapter 2, an excess prey cue improved foraging success both in terms of search time and proportion of prey

located by sticklebacks. Neither sticklebacks in chapter 2 nor *Gammarus* in this chapter increased activity levels in response to an increase in olfactory cues. However, the best explanation for the results seen in chapter 2 was that the high concentration of olfactory cue had an appetitive effect on sticklebacks. Perhaps this is not the case in *Gammarus* or perhaps the larger group sizes in this experiment did not cause an olfactory cue flooding.

My results suggest that while search time decreases with increase in prey group size, the proportion of prey located only increases until an 'optimal' group size is reached beyond which proportion of prey group located decreases. The implications are twofold. Firstly, aggregated prey avoiding an olfactory predator benefit from attack abatement as their risk of discovery does not increase linearly with prey group size (Turner and Pitcher 1986, Andersson et al. 2013). Secondly, aggregated prey at large group sizes may suffer a negative trait mediated effect (Rasmussen and Downing 1988, Griffiths and Richardson 2006) due to prolonged presence of an olfactory predator attracted to olfactory cues (male guppies increase search effort in response to female olfactory cues; Guevara-Fiore et al., 2010). If this is the case, visually concealed prey should aggregate in smaller groups so as to not attract predator attention for very long. However, our results did not indicate any increased foraging effort by *Gammarus* as prey group sizes increased. The ideal strategy for the prey most likely depends on predator foraging strategy. Further study on olfactory predator behaviour may reveal to what extent predators focus search efforts based on localised olfactory cues and to what extent this may affect undiscovered prey behaviour and survival.

Chapter 5: Turbulence lowers risk of detection of aggregated prey

Abstract

Prey aggregation increases risk of detection by olfactory predators in flowing water. However, turbulence is known to lower detection risk as well as prolong time taken to locate prey. Here I investigate how turbulence affects risk of detection in groups of prey (bloodworm) avoiding non-specialist olfactory predators (three spined sticklebacks). A y-maze with flowing water was used and a significant preference for arms with olfactory prey cue was taken to mean that prey were detectable. I found that while increasing prey group sizes did increase the risk of detection by the predator, creating turbulence downstream from prey cue input lowered the risk of detection for both intermediate and large groups. In the intermediate group size, turbulence lowered preference for the prey arm of the y-maze to that of chance whereas preference was still significantly larger than chance for the prey arm when prey groups were large. This indicates that there may be a threshold group size below which prey can aggregate and remain undetected using turbulence as a 'sensory refuge'.

5.1 Introduction

Olfactory cues provide a useful means for predators to detect prey in a range of environments and situations; examples include mice (*Mus musculus*) finding buried peanuts and blue crabs (*Callinectes sapidus*) navigating upstream through an odour plume (Zimmer-Faust et al. 1995, Carthey et al. 2011). For many animals, locating prey, home range or mates involves navigating odour plumes in air or water (DeBose and Nevitt 2008). There are among other things a study showing "sniffing" behaviour in flounders (Pleuronectidae) as a response to prey odour cues (Nevitt 1991) and another indicating that cod (*Gadus Morhua*) are able to track odour plumes to prey (Løkkeborg 1998). In the laboratory, much work has been carried out on the ability of slow moving olfactory predators such as whelks (*Busycon carica*) to detect and locate prey using olfactory cues (Webster and Weissburg 2001, Ferner and Weissburg 2005).

Attack abatement is a lowering of predation risk that may occur when prey aggregate, and is the combination of 1) dilution of risk to prey when discovered by a predator due to their large number preventing the predator from consuming all of the prey and 2) low prey encounter rates due to greater distances between aggregated prey groups. For attack abatement to occur, prey crypsis must not decrease enough with group size to make prey as easily encountered in a group as when dispersed (Turner and Pitcher 1986). Recent evidence suggests a direct relationship between prey group size and the relative distance at which predators may be able to detect prey using olfactory cues (Andersson et al. 2013). Grouping is often favoured as part of a predator-avoidance strategy for prey faced with visual predators (Riipi et al. 2001), but aggregation may be counter-productive if increasing group size makes prey increasingly easier for predators to find using olfaction (Kunin 1999). However, if

detection distance increases with the square root of the number of prey (Andersson et al. 2013), aggregation is favoured providing potential predators are unable to consume all prey once discovered. In knobbed whelks (*Busycon carica*), there is good evidence that both prey cue concentration (which is related to group size), and distribution are important in a flowing environment (Wilson and Weissburg 2012). At low prey densities, prey were more easily found if they were positioned in line with flow. This difference was not apparent at high prey densities.

Prey group size may affect the ability of prey to avoid predators, and prey must balance the increasing conspicuousness of larger groups against the benefits of aggregation such as attack abatement or mobbing (Krause and Ruxton 2002). However, environmental factors such as turbulence or flow speed may affect the extent to which aggregated prey are exposed as a result of their group size, as the movement of water may break up the odour plume (Webster and Weissburg 2001). Some studies suggest that turbulence may provide 'sensory refuges' (where predators are unable to locate prey) or at least make tracking of odour plumes much more difficult (Webster and Weissburg 2001, Ferner and Weissburg 2005). If turbulence can effectively lower predator success, prey would do well to aggregate in those places that offer the protection of turbulence. However, aggregation in such refuges will increase the concentration of odour emitting from them, which may negate the benefits of using the refuge.

Here I investigate the effectiveness of aggregating in turbulent and non-turbulent environments as an anti-predator strategy. My prey are chironomid larvae and their predator is the three spined stickleback (*Gasterosteus aculeatus*). Sticklebacks are able to detect prey using olfactory cues (Johannesen et al. 2012) and are also known to use olfactory cues for

mate choice (Heuschele and Candolin 2007). They may be found in both flowing and still environments and may therefore be able to track an odour plume to the source. However, as they are known to not rely strongly on olfaction to locate prey, they may not be able to track a plume in turbulent water. If this is the case, aggregated prey otherwise easily detected may find refuge from non-specialist olfactory predators in turbulent areas. We predict that while large groups of prey are detected more easily than small groups, turbulence provides an effective refuge, favouring aggregation in turbulent areas over dispersal in laminar flow.

5.2 Methods

5.2.1 Experimental species, transportation and housing

Three spined sticklebacks (4-5cm full body length) were caught in a pond in Saltfleet, Lincolnshire in November, 2011 (53° 25' 59.55"N, 0° 10' 49.41"E) by netting from land and transported to our facilities in Leeds by car (three hour journey). Two hundred fish were packed in five fish bags (10 litres each) of 30-50 fish in each and packed in a plastic box for transportation. No fish died during transportation. Fish were housed in grey fibreglass tanks (0.5x0.5x1.0m) with gravel substrate, plastic plants, rocks and plant pots for enrichment and two air driven mechanical filters with an activated carbon layer. Light regime was 10/14 light/dark, the temperature was held at 14 ± 2 °C and fish were fed daily on defrosted frozen bloodworm. Fish were kept for six months to one year for experimentation prior to release where caught in agreement with the Home Office and Defra.

5.2.2 Procedure

Trials were carried out in a flow-through Y maze (Ward et al. 2011; figure 5.1). The stem of the maze measured 40cm by 33cm and each arm measured 20cm by 20cm, with a water depth of 9cm throughout. Conditioned water was pumped from a header tank into the maze entered the maze over a horizontal barrier in both arms of the Y, and passed through a collimator to reduce turbulence. Water left the flume through three mesh-covered exit holes evenly spaced across the base of the stem of the Y, and was not re-circulated. The stem of the Y also contained a 'release zone' (40cm by 20cm), with a removable barrier, where fish were placed at the start of the trial. Flow in the maze was measured at approximately 0.03 m/s. Trials were observed from behind a screen via a webcam connected to a laptop to reduce disturbance to the fish.

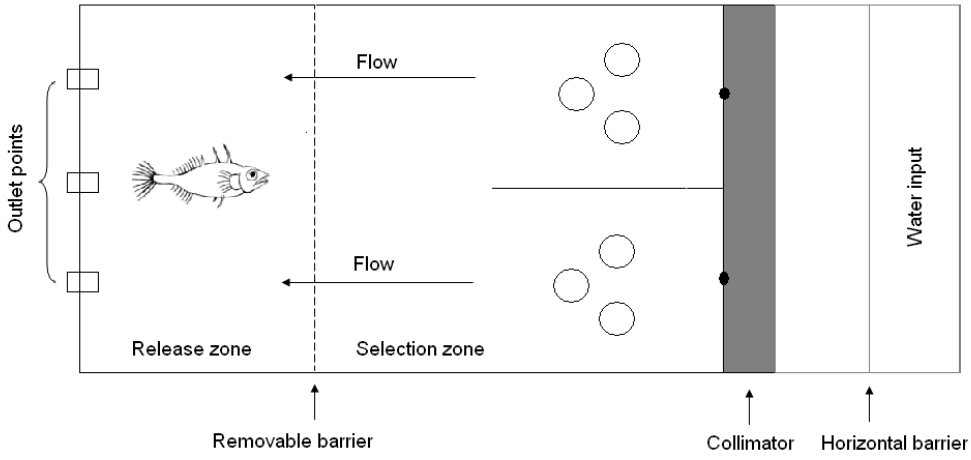

Figure 5.1. Layout of Y-maze measuring 75cm x 40cm. Water flowed over a horizontal barrier before entering the Y-maze to ensure even flow on both sides. Cue input points are marked by a black dot. Large open circles represent the cylinders added to the tank in the turbulence treatments.

We created olfactory cues by macerating 20g frozen bloodworm, which was then filtered through a Whatman filter with 200ml of water using a Buchner funnel and diluted to predetermined concentrations (pilot trial: equivalent to 5g of bloodworm per litre, main

experiment: low: 5g/l medium: 10g/l and high: 20g/l). As aggregated prey produce higher concentrations of olfactory cues (Moir and Weissburg 2009), increasing cue concentration is equivalent to increasing group size (Andersson et al. 2013). Cues were delivered to the maze using either separating funnels (pilot trial) or two peristaltic pumps (main trial). Cue strengths were chosen on the basis of a pilot trial indicating that a concentration of 5g/ml delivered at 20ml/minute was sufficient to allow significantly more fish than random to choose the cue side of the maze (two tailed exact binomial test: N = 15/20, p = 0.041). As the peristaltic pumps delivered a slower maximum flow rate than that used in the pilot trial (10ml/minute), concentrations for the main experiment were determined such that the 'medium' concentration selected delivered cue at a similar rate to that in the pilot experiment. In each trial, an olfactory cue entered at one arm of the maze, and a conditioned water control entered at the other at the same rate. Cue side was allocated at random in order to control for side preference. After the trial, the maze was emptied and refilled with conditioned water to remove olfactory cues from the previous trial.

At the start of the trial, the tank was filled with conditioned water and a single stickleback was placed into the release zone and allowed to acclimatise for a minimum of five minutes or until it resumed normal behaviour (start – stop swimming at moderate speed). Any fish that had not resumed normal behaviour within 15 minutes were excluded (N=23 across all trials). Following acclimatisation, the pump in the header tank was switched on. After two minutes (allowing for stabilisation of flow), the peristaltic pumps delivering the cue and control water were turned on. The behaviour of the test fish was then monitored. After a minimum of two minutes, or once the fish had visited both sides of the stem of the Y, the barrier was raised using a pulley system and the fish was allowed up to five minutes to reach the top of one arm of the Y, making a choice. Fish that did not visit both sides of the stem of the Y within five

minutes (N = 8 fish) or did not make a choice (N = 6) were excluded from the experiment. Final sample sizes were: low: N = 16, medium: N = 16, high: N = 16). Time to acclimatise (visit both sides of the maze after cues were introduced), time to choose (following release) and which choice was made (cue or control) were recorded.

Following completion of the initial experiment, we investigated the effect of adding turbulence to the water on the behavioural measures. Three cylinders were added to each arm of the Y maze to create downstream turbulence (see figure 5.1). Visualisation of the flow using food dye indicated that odour plumes were split and dispersed to a greater extent when the cylinders were present compared to when they were absent. We used an identical protocol in the turbulence experiments, and investigated two cue concentrations: medium and high (low was not used as the concentration experiment indicated that this concentration was not preferred by the fish over the control, see results). Eight fish were excluded from this experiment, giving final sample sizes of N = 17 for medium cue concentration and N = 17 for high cue concentration.

5.2.3 Analysis

Data were analysed using R v 2.13.0 (R Core Team 2013). Time to acclimatise and time to choose were analysed using a Cox proportional hazards survival model (survival package in R; Therneau and Lumley 2011) and choice of side was analysed using binomial exact tests (proportion of fish choosing the cue side over the control side against a random expectation of 0.5).

5.3 Results

In the pilot trial, fish selected the cue arm of the Y maze significantly more often than the control arm (N = 15/20 fish, P (success) = 0.75, p = 0.041). In the main experiment, fish tested in the turbulent water condition took less time to acclimatise than those in the 'no added turbulence' condition (coxph: Chi-squared = 25.81, df = 1, P<0.001), but there was no effect of cue concentration or turbulence on time to choose once acclimatised (coxph: Chi-squared = 6.22, df = 5, P = 0.29). This suggests that turbulence may lower perceived risk.

In the 'no added turbulence' condition, fish selected the cue arm over the control arm at medium (N = 13/16, P (success) = 0.8125, p = 0.021) and high (N = 15/16, P (success) = 0.938, p < 0.001) cue concentrations, but not at the low cue concentration (N = 11/16, P (success) = 0.688, p = 0.21). There was a difference between detection rate at high cue concentration and low cue concentration (comparison with P (success) = 0.688; N = 15/16, p = 0.03), but neither differed from medium cue concentration. When turbulence was added, fish preferentially selected the cue arm at high (N = 14/17, P (success) = 0.824, p = 0.013) but not medium (N = 10/17, P (success) = 0.588, p = 0.629) cue concentrations.

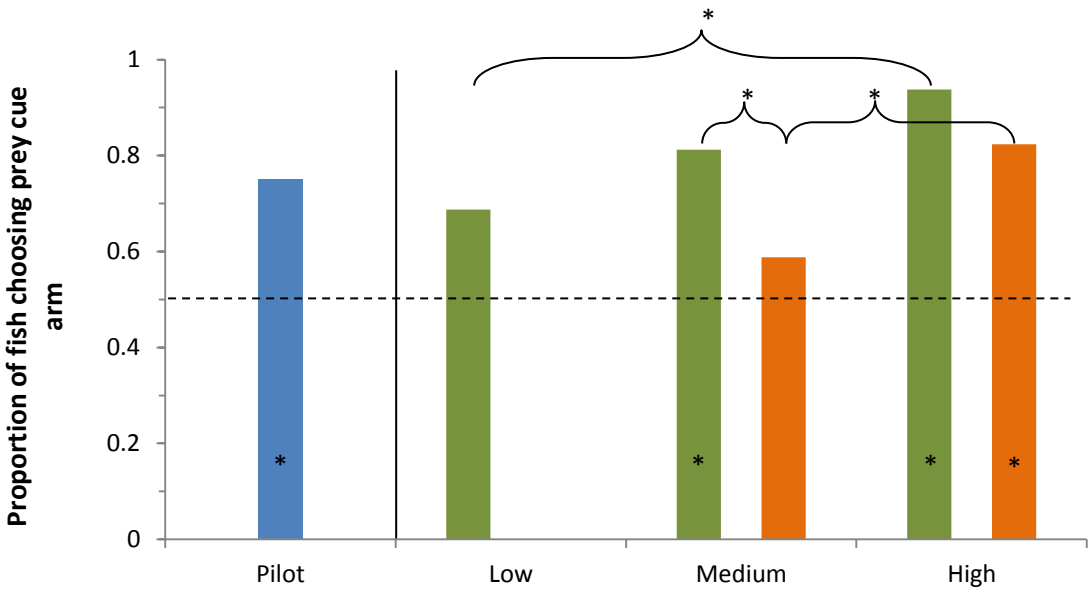

Figure 5.2. Proportion of fish choosing prey side in y-maze. * signify significant differences from random choice of side. Green bars are low turbulence treatments and orange bars are added turbulence treatments. The dashed line indicates random choice of side in the y-maze. The solid line separates the pilot trials (blue bar, no added turbulence) from the main experiment.

At medium cue concentration, proportion of prey detected was significantly reduced in turbulent water (comparison with P (success) = 0.8125; N = 10/17, P = 0.027). There was no difference at high cue concentrations (comparison with P (success) = 0.938; N = 14/17, P = 0.086) and detection ratio in turbulent water was significantly smaller at medium cue concentration than high cue concentration (comparison with P (success) = 0.824; N = 10/17, p = 0.02).

5.4 Discussion

Our results suggest that while aggregation increases the detection of prey by predators in a flowing water environment, there may be benefits to aggregating in turbulent environments. Fish chose the prey cue arm significantly more often than expected by random

encounter at both medium and high cue concentrations in non-turbulent flow. The addition of turbulence reduced predator success at medium, but not high, cue concentrations. This suggests that there may be a threshold effect favouring small to medium aggregations in areas of turbulence, but that large aggregations would not be favoured.

Theory suggests that aggregations should become increasingly detectable to olfactory predators, but the pattern of this increase is not clear (Treisman 1975, Kiltie 1980, Kunin 1999). Recent work finds that for insects, the distance at which an odour source can be detected is proportional to the square root of the number of individual sources (Andersson et al. 2013). In still water, aggregation is advantageous against stickleback predators (Johannesen et al, in prep, chapter 3), and detection of prey by *Gammarus* increases with group size, but at a rate that is less than linear (Johannesen et al, in prep, chapter 4), reflecting well-established patterns observed for visual predators locating prey (Ioannou et al. 2011).

While specialist olfactory predators are generally able to track odour plumes effectively in laminar flow (Vickers 2000), previous evidence suggests that tracking plumes is difficult in turbulent water (Webster and Weissburg 2001, Weissburg and Dusenbery 2002, Ferner and Weissburg 2005). Additionally, there may be areas of flow in which the ability of prey to detect their predators exceeds that of predators to detect their prey (termed ‘sensory refuges’; Weissburg and Zimmer-Faust 1993). However, if prey aggregate in sensory refuges, the combined chemical signal from multiple individuals (Monismith et al. 1990, Wilson and Weissburg 2012) may increase their detectability to predators, negating the benefits of using such refuges. Our results support this hypothesis.

Aggregation as an anti-predator strategy is beneficial to prey animals in many ways, including communal defence, dilution of individual risk, and confusion of predators (Krause and Ruxton 2002). Thus, while groups may be more detectable than individuals, aggregation may still be favoured if these factors outweigh the costs of increased detection rates by predators. For example, if predators are able to only take a single prey item from an aggregation (while the rest escape), the benefits offered by dilution of individual risk may outweigh the increased detection of groups, providing groups of N are not more than N times as detectable as a single individual (Kiltie 1980, Turner and Pitcher 1986).

The study of anti-predator aggregation has primarily focused on predators that use vision to detect their prey, while the effect of olfactory predators is less well known. Our work suggests that group size interacts with environmental parameters, and that the evolution of grouping in response to predation may be highly dependent on the flow environment. Turbulence affects different predators in different ways, dependent on the sensory capabilities and sampling strategies of the predators (Ferner and Weissburg 2005). How these differences affect detection distance, the interaction between detection and odour concentration in turbulent environments, and the implications for the evolution of grouping, are unknown.

Chapter 6: The use of lumpfish as cleaner fish on salmon farms

Abstract

Salmon lice cause serious financial and welfare problems on salmon farms. Chemical de-lousing treatments are widely used in the industry, but lice build up resistance to treatments and some treatments are potentially harmful to other species, especially other crustaceans. Three different species of wrasse have been used in Atlantic salmon farms as salmon cleaners with positive results in some cases. On the Faroe Islands, there are no suitable native wrasses and since importing new species is not advisable, an alternative species could be beneficial as a substitute or in addition to chemical louse treatments. I investigate the cleaning behaviour and efficiency of lumpfish (*Cyclopterus lumpus*) in a series of de-lousing experiments and a cleaning behaviour experiment. I find that lumpfish are not reliable cleaners and in no case did lumpfish lower louse population as much as a commercial chemical treatment. However, in behavioural trials, lumpfish were seen to clean and exhibit interest in salmon/lice. Because of the severity of problems caused by salmon lice, this warrants further investigation.

6.1 Introduction

Fish are an increasingly important food resource for human populations, with demand increasing with or faster than human population growth (FAO 2012). However, reliance on over-exploited wild stocks is high and poses great risk to fish and invertebrate populations world-wide (FAO 2012). The aquaculture industry is rapidly expanding in an attempt to counter the problem of limited wild stocks. In 2011, approximately 41% of all world fisheries production came from aquaculture, increasing from 34% only five years earlier. In that time, total wild catches have not changed (90-90.4 million tonnes) while the increase in aquaculture production accounts for all of the change in share of production (47.3-63.6 million tonnes) (FAO 2012).

For the Atlantic salmon (*Salmo salar*) farming industry, salmon lice (*Lepeophtheirus salmonis*) are a major issue. Rae (2002) estimated that in Scotland, the industry spends £20-30m a year on louse treatments and lice are growing resistant to chemical treatments almost as quickly as they are developed (Treasurer et al. 2000, Sevatdal and Horsberg 2003, Fallang et al. 2004). In addition to financial costs of drugs to fight louse infestations, salmon lice pose a severe welfare problem to salmon by removing scales, causing sores that may get infected and lead to osmoregulatory failure (Johnson et al. 2004). Fish welfare is a subject that has been long ignored, but in recent years it has become more frequently discussed and it is now widely accepted as an issue that needs to be addressed (Huntingford et al. 2006, Ashley 2007, Barber 2007), so giving careful consideration of the possible solutions to the welfare problems caused by salmon lice is increasingly important.

One proposed solution to this problem is the use of cleaner fish to remove lice from salmon. Fish of the family Labridae are well-known as cleaner fish, particularly on coral reefs (Bshary and Schäffer 2002). This approach has been tried with Goldsinny wrasse (*Ctenolabrus rupestris*) in, for example, Scotland and Ireland with some indication of successful reduction in louse numbers (Treasurer 1994, 2002, Deady et al. 1995). However, not all regions have species of wrasse suitable for use in this way, so an exploration of other species, both fresh-water and marine is necessary in order to provide comprehensive biological control of parasites in aquaculture.

The Faroe Islands has an extensive aquaculture industry, particularly salmon farming, contributing significantly to the local economy with approximately 12% of fish landed on the Faroe Islands or by Faroese vessels in 2011 being farmed fish (Hagstova-Føroya 2012). However, there are no native wrasse species that could be used as cleaner fish. In this region, two possible alternative cleaner fish have been suggested: thicklip grey mullet (*Chelon labrosus*) and lumpfish (*Cyclopterus lumpus*). There is some evidence from the Norwegian aquaculture industry that lumpfish may clean salmon (Willumsen 2001, Schaer and Vestvik 2012), but their cleaning behaviour and efficacy is not known. Here, I investigate the potential of lumpfish as cleaners in the salmon industry. I investigate the propensity to clean and cleaning efficiency of wild caught and captive reared lumpfish cleaning both adult and juvenile salmon.

6.2 Methods

6.2.1 Study species

Lumpfish (*Cyclopterus lumpus)* are found in the North Atlantic with a range spanning from Russia to Canada. As juveniles, lumpfish are sedentary and opportunistic feeders spending most of their time attached to smooth surfaces inshore or in free floating seaweed (Ingólfsson and Kristjánsson 2002). They leave for open waters at later life stages before returning to shore to breed at two years of age (Davenport 1985, Mitamura et al. 2012).

For this work, lumpfish from four different sources were used in order to investigate the effect of rearing background and age/size of lumpfish on any cleaning behaviour displayed. I collected juveniles (approximately 3-5cm long lumpfish estimated to be about one to three months old) in two bouts, once in the summer of 2011 and once in the early spring 2012. This was done by collecting seaweed attached to ropes hanging off buoys and docks and gently shaking it over a tray to encourage the fish attached to the seaweed to let go. Fish were collected from the tray and transferred to transport containers (glass jars, 8x12cm, no more than five fish per jar) before being taken by car (maximum one hour journey) to the aquaculture research laboratories in Nesvík on the Faroe Islands (62° 13' 2.70" N , 7° 1' 7.26" W). The facilities comprised a captive breeding section housing Atlantic cod and lumpfish, a rearing section housing lumpfish, and outdoor facilities including cylindrical tanks and flow through 'ponds' for plankton production. The two cohorts of juvenile lumpfish were reared in Nesvík for nine and three months respectively and were used as the captive reared lumpfish in our trials (captive reared sub-adult and captive reared juvenile respectively; table 6.1). I also used juvenile lumpfish caught in the same manner one to two weeks before trials in the summer of 2012 (wild-caught juveniles, approximately 6cm at time of capture). Finally, I

sourced lumpfish from the summer 2011 cohort from a commercial fishing vessel using purse seine nets to catch mackerel (wild caught sub-adults). Sizes can be found in table 6.1.

Lumpfish were stocked in light grey or green cylindrical fibreglass tanks (1.5m in diameter and 0.5m deep) with continually flowing seawater, were held on a 10:14h day/night cycle and fed a locally produced salmon feed. Food was provided using a 24 hour automated feeding system during rearing and then switched to hand feeding *ad libitum* two times per day two to three weeks before trials began. To maintain water quality and to minimise risk of contaminating the local area with salmon lice or diseases, incoming seawater (pumped in from the shore) was filtered and treated with UV light before reaching the tanks and similarly treated before exiting the facility. All tanks were on a flow through system, and water only reached one tank before leaving the facility. Tanks did not initially contain any enrichment, but one month prior to experiments PVC tubes and artificial seaweed were added to the tanks.

Salmon were supplied in two sizes (adult and yearling) by Fiskaaling's Skopun hatchery (Table 6.1). Adult salmon were housed in green cylindrical tanks measuring 3m x 1.1m (diameter x depth) with constant water flow and covered with a lid to prevent avian predation. Tanks were outside, so were lit with natural light throughout trials (light entered tanks through a mesh covered open section of the lids). Fish were fed *ad libitum* once per day on salmon feed.

Table 6.1. Fish categories and sizes

Fish group	Mean total body length ± standard deviation (cm)
Captive reared sub-adult lumpfish (CSL)	18.3 ± 2.8
Captive reared juvenile lumpfish (CJL)	6.0 ± 1.0
Wild caught sub-adult lumpfish (WSL)	21.1 ± 2.1
Wild caught juvenile lumpfish (WJL)	6.6 ± 1.9
Adult salmon (AS)	54.9 ± 5.1
Yearling salmon (YS)	20.3 ± 1.3

Salmon lice were collected from six different salmon farms throughout the experimental period. Lice were collected from salmon farms on a weekly basis as they do not survive for long without a host. Our louse collections coincided with biweekly louse counts carried out by Fiskaaling as part of health authorities' routine for monitoring of louse load on salmon farms. Salmon were netted from salmon cages, anaesthetised using Finquel, lice were counted and removed, and salmon were returned to the oxygen rich cages after recovering to near consciousness in an oxygenated tank. Lice were kept in lidded buckets containing sea water and transported to Nesvík, where the water was oxygenated until they were used to infect salmon. As infection with lice has welfare implications, salmon were only infected immediately before trials, and all lice were removed either during trials as part of the experimental procedure or after each week long trial.

6.2.2 Procedure

6.2.3 Experiment 1. Cleaning efficiency

I assessed the cleaning efficiency of lumpfish in a three-part experiment. For each experiment, experimental details are summarised in Table 6.2:

1) Experiment 1 compared the effectiveness of captive reared sub-adult lumpfish (CSL), captive reared juvenile lumpfish (CJL), wild caught sub-adult lumpfish (WSL) and Salmosan (a commonly used anti-louse treatment) in louse removal against a control group of adult salmon with no treatment present. The aim was to determine firstly whether lumpfish can lower louse infestation compared to a no treatment control and a chemical treatment, and secondly whether age/rearing conditions of the lumpfish have an effect on cleaning efficiency.

2) As salmon cages are made from net on a metal frame, experiment 2 investigated the presence/absence of lumpfish (CSL) crossed with presence/absence of netting to assess a) the effect that netting may have on louse infestation as salmon may be able to dislodge lice by rubbing on the net and b) the effect on lumpfish ability to clean as a net prevents the lumpfish from resting using their sucker.
3) In experiment 3, I investigated the effect of a seaweed shelter and previous experience of young lumpfish on louse removal from juvenile salmon. Literature suggests that lumpfish use seaweed as shelter (Ingólfsson and Kristjánsson 2002), but juvenile salmon are also known to use shelters when available (Gries and Juanes 1998).

In each experiment, salmon were anaesthetised using Finquel, measured (to the nearest cm) and transferred to a tank with fresh oxygenated seawater containing lice. After one minute, salmon were removed from the lousing tank and the number of lice that had attached was noted before the salmon (still unconscious) was transferred to the experimental tank with fast flowing oxygenated water in order to aid the salmon in regaining consciousness. Salmon usually regained consciousness within two minutes and showed no sign of drowsiness after 10 minutes. Once all salmon had been infected (louse load was an average of 4.6 ± 2.3 SD lice per fish), lumpfish were measured and assigned to the experimental tanks (see table 6.2 for tank dimensions for each part of the experiment). Trials ran for a set number of days (table 6.2) during which salmon were fed ad lib one to two times per day (lumpfish were not fed, but were observed taking salmon feed on occasion). At the end of each trial, lumpfish and then salmon were caught, salmon were anaesthetised and any remaining lice were removed and counted.

Table 6.2. Overview of treatments in the three cleaning experiments carried out here.

Experiment	Treatment groups	Days per trial	Tank diameter and depth (m)	Number of Lumpfish:Salmon
1	AS in trials with: CSL (N = 11 trials) CJL (N = 9 trials) WSL N = 11 trials) Salmosan louse treatment (N = 8 trials) Control (N = 10 trials)	7	1.5 x 1.1	2:3
2	AS in trials with: CSL: With net covering (N=5 trials) and without net (N=5 trials) No lumpfish: With net covering (N=3 trials) and without net (N=3 trials)	7	2.5 x 1.5	4:6
3	YS in trials with: CJL: No shelter (N=8 trials) and with shelter (N=7 trials) WJL (N=3 trials) and no lumpfish (N=8 trials)	5	0.7 x 0.75	2:4

6.2.4 Analysis

Differences in louse infestation between treatments were analysed using a Generalised Linear Mixed effects model with binomial error structure using number of lice left after the trials as 'failures' and the numeric drop in lice counts as 'successes' and an observation level random effect to account for over dispersion. Lice counts were pooled per tank per trial as lice are known to be able to move from salmon to salmon (Pike and Wadsworth 1999) making individual counts per salmon unreliable. All analysis was carried out in R 2.15.3 (R Core Team 2013).

6.2.5 Experiment 2. Cleaning behaviour

To assess cleaning behaviour and shelter use, I carried out observations of juvenile lumpfish behaviour when in the presence of salmon. These were the same lumpfish as those used in part three of the cleaning efficiency experiments, which allowed us to make a direct comparison between cleaning behaviour of fish and the cleaning efficiency seen in the trials where those fish were used. However, for logistical reasons, only 12 fish completed both behaviour trials and efficiency trials, though 15 fish took part in behaviour trials in total. Each trial ran for two consecutive days in light grey cylindrical tanks (0.7m radius and 0.75m deep) and five infected salmon (with two to six lice each) were used in each trial. Cleaning behaviour was observed under two treatments; with no shelter and with a shelter for lumpfish and/or salmon to use. In half of the trials, the shelter was available on the first day and in the other half it was available on the second. Lumpfish were randomly assigned to trials with shelter on the first or second day. Three lumpfish (one focal fish and two companions) were starved for 24 hours to standardise motivation to feed and left in an open top mesh enclosure (cylindrical, 15cm diameter and 30cm long, 3mm mesh) inside the experimental tank overnight to allow them to acclimatise to the environment and the presence of salmon. The following day, at the start of the trial, the lumpfish were released by lowering the enclosure, allowing lumpfish to swim out through the top and the enclosure was removed. In order to measure activity levels, behaviour (sitting or swimming) as well as position in the water column (upper or lower half) were recorded on release and every five minutes subsequently for three hours (33 observations per fish). Finally, any instances of the lumpfish approaching or nipping at a salmon/louse were noted together with the duration of any such interactions. The number of nips at a salmon was used as a measure of cleaning activity, which I then related to overall activity levels (proportion of times noted as swimming), previous experience with salmon

(including cleaning trials and previous day in a behavioural trial, measured in days) and tank use (proportion of times noted as in upper or lower half of tank).

6.2.6 Analysis

GLMs with quasi-poisson error distributions were used to investigate the effect of treatment (shelter availability) and day of trial on the number of nips made at salmon. To investigate the effect of treatment and day of trial on activity levels and tank use, GLMs with quasi-binomial error distributions were used with the response variables being times noted as swimming/sitting and times in the upper/lower half of the water column respectively. GLMs with Poisson error distributions were used to estimate the effect of activity levels on propensity to clean. Activity was determined as the proportion of time spent swimming during behavioural trials (calculated from the five minute interval scan samples) and propensity to clean was the number of times a lumpfish nipped at salmon. An additional analysis used data from behavioural trials in combination with the small salmon cleaning efficiency data (part 3) to investigate whether the known propensity to clean, previous experience with louse infested salmon and activity levels had an effect on cleaning efficiency. In order to test for this, we used 12 fish from behavioural trials, where the cleaning rate was known from the cleaning trials and used the behavioural traits and experience as predictors in a binomial GLMer with treatment group as a random factor.

6.3 Results

6.3.1 Cleaning efficiency

In experiment 1, the number of lice declined in all five treatments (including control). However, no differences were found in the proportional drop in louse population on the salmon between CSL, CJL, WSL and the control (Difference from Control (N=49): CJL; z=-1.706, P=0.09, CSL; z=-0.912, P=0.36, WSL; z=-1.18, P=0.24). The only effective treatment was Salmosan with a drop of 95% of lice compared to the 48% drop in the control treatment (figure 6.1a, difference from Control; z=5.95, P<0.001). In experiment 2, there was some indication that there may be an interaction between the presence of net coating and lumpfish with a net coating having a positive effect on louse removal when no lumpfish were present and no or a negative effect when lumpfish were present (see figure 6.1b), but this was not significant (z=-1.891, N=16, P=0.06). However, after removing the interaction term, I did find a significant effect of treatment with the presence of lumpfish causing a 42% drop in lice compared to a 16% drop in the control treatment (z=3.19, N=16, P=0.001; figure 6.1b). In experiment 3 I found no differences between the control and any of the treatment groups (Difference from Control (N=26): CJL; z=-1.28, P=0.2, CJL+shelter; z=0.01, P=0.99, WJL; z=1.26, P=0.21; figure 6.1c). Interestingly, there was a significant difference between controls in experiments 1 and 2 (quasi-binomial GLM: t=2.39, N=25, P=0.03) with the control treatment in experiment 3 not differing from either of the other two (quasi-binomial GLM (N=25): Exp 1; t=1.34, P=0.2, Exp 2; t=-0.75, P=0.46).

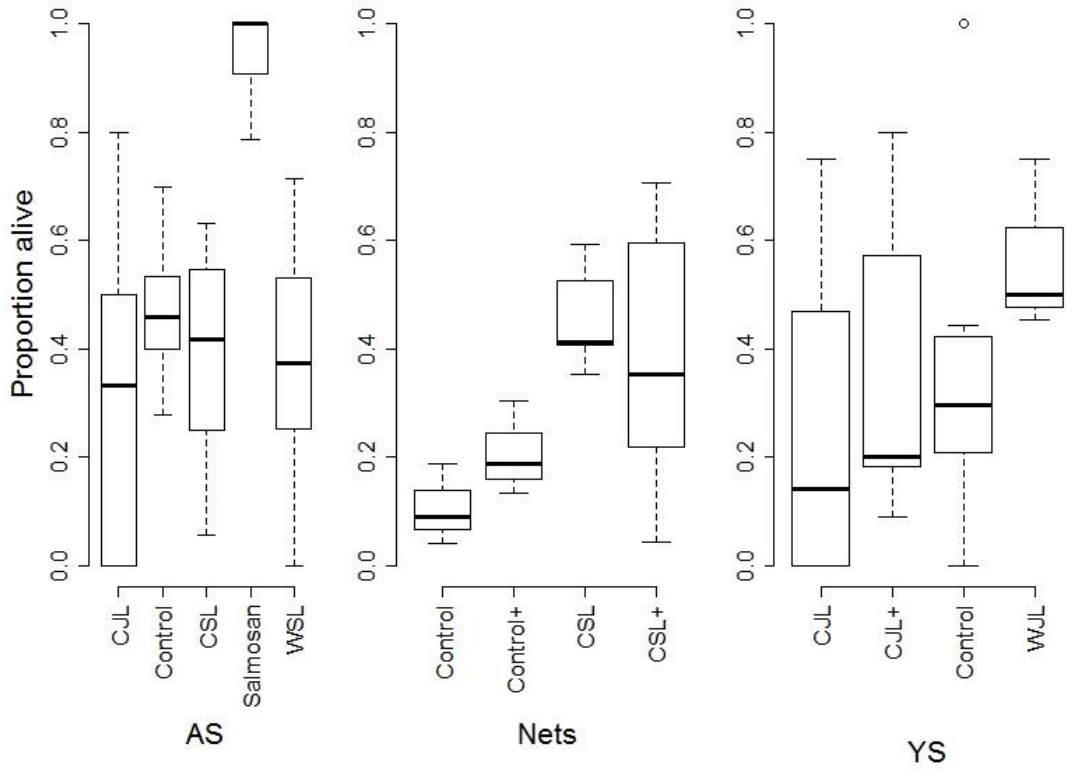

Figure 6.1. Change in louse populations in experiments testing for cleaning efficiency for a) experiment 1, captive reared juvenile (CJL) and sub-adult (CSL) lumpfish, wild caught sub-adult lumpfish (WSL) and Salmosan adult salmon (AS), b) experiment 2 with net lining in tanks (+ signifies added net lining) and captive reared sub-adult lumpfish (CSL), and c) experiment 3 with yearling salmon (YS) and juvenile captive reared (CJL) and wild caught (WJL) lumpfish (+ signifies added shelter). In experiment 1, only Salmosan caused a significantly different decrease in lice from the control. In experiment 2, there was an effect of captive reared sub-adult lumpfish (CSL) and in experiment 3, there were no significant differences between Control and treatments.

6.3.2 Cleaning behaviour

Lumpfish spent 82.5% of their time swimming and 68% of their time in the upper half of the water column on average. Generally, fish that were swimming a lot did not spend much time in the lower half of the water column (see figure 6.2) but the reverse was not necessarily the case.

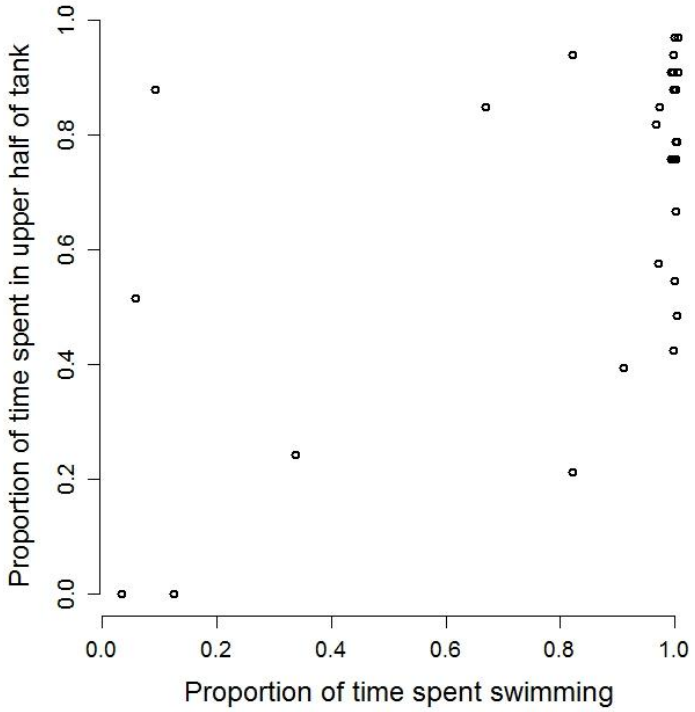

Figure 6.2. Relationship between the proportion of time spent in the upper half of the tank and proportion of time spent swimming. Most fish spent most of their time swimming in the upper half of the tank.

Cleaning behaviour was not very frequent with only five out of 15 fish nipping at salmon and with seven fish approaching salmon. The maximum number of nips seen was 18 in a three hour session with the average for fish nipping was six nips per session. There was no significant effect of availability of shelter or day of trial on the number of nips (GLM with quasipoisson errors: Shelter; $F_{1,28}$=0.11, P=0.74, Day; $F_{1,28}$=0.07, P=0.8), on activity levels (GLM with quasibinomial errors: Shelter; $LRT_{1,28}$=56.66, P=0.14, Day; $LRT_{1,28}$=0.91, P=0.85) or on time spent at the top or bottom of tank (GLM with quasibinomial errors: Shelter; $LRT_{1,28}$= 0.64, P=0.82, Day; $LRT_{1,28}$=0.05, P=0.95).

There were statistically significant relationships between activity levels and propensity to clean (GLM with quasipoisson errors: $F_{1,27}$=4.24, P=0.049; figure 6.3b) and between previous

exposure to lice infested salmon and propensity to clean (GLM with quasipoisson errors; $F_{1,27}$=11.55, P=0.002; figure 6.3a).

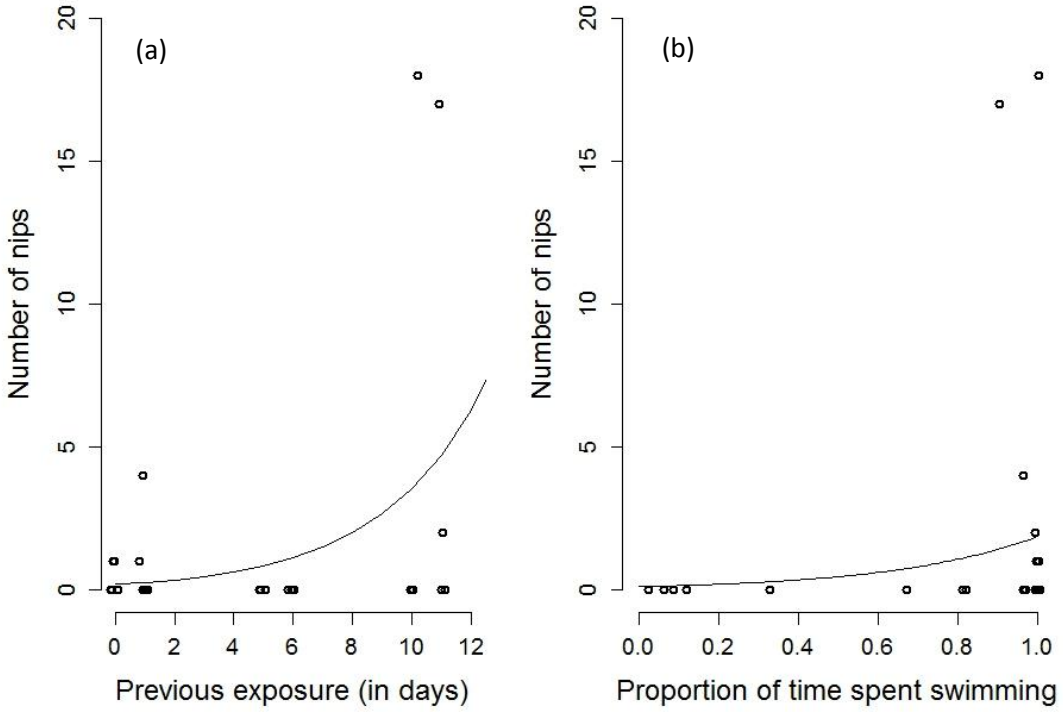

Figure 6.3. The number of times a lumpfish nipped at salmon or lice as functions of a) previous exposure to infested salmon in days and b) lumpfish activity level calculated from the proportion of times noted as swimming. The lines represent individual covariate model fits.

Observed activity levels and number of nips by individual lumpfish from behavioural experiments was not related to their cleaning efficiency in the cleaning experiments and neither was previous experience with salmon (Binomial GLMM of cleaning data (N=24) with cleaning trial treatments (shelter/no shelter/WJL) as random factor: Activity; z=-1.74, 0.08, Experience; z=1.29, P=0.20, Nips; z=1.71, P=0.09).

6.4 Discussion

My results suggest that using lumpfish as cleaner fish may not be immediately effective. In two out of three cleaning efficiency experiments, I found no evidence for louse removal by juvenile lumpfish. Louse numbers on adult salmon declined by approximately 40% over the course of experiment 1, which investigated the effect of size and rearing background of lumpfish on cleaning efficiency. However, there was no difference in louse decline between the control treatment and the lumpfish treatments in this experiment. A similar result was observed with small salmon (experiment 3). However, louse numbers showed a greater decline in the lumpfish (40%) compared to control treatments (10-20%) in the 2nd experiment where the cages were lined with netting.

In the trials using a net lining for the tanks (experiment 2) I found that the presence of lumpfish significantly lowered louse populations, but netting had no effect. Lumpfish use smooth surfaces to attach to while resting (Killen et al. 2007), so it was hypothesised that cleaning efficiency would be affected in tanks devoid of such surfaces. However, in this experiment, this was not the case. In general fish farming practice, salmon cages are devoid of smooth surfaces barring a few plastic pipes that could conceivably be large enough for smaller lumpfish (smaller than 20cm in length) to attach to (Muir and Scott 2000). Future investigation should investigate whether there is an effect of available resting space on cleaning efficiency of lumpfish, but lumpfish welfare should also be taken into consideration as it is clear that when presented with the option of attaching to a smooth surface, they do take advantage of that.

There was a significant difference in drop in louse populations between control treatments in the two large salmon experiments (AS, experiment 1 and Nets, experiment 2). The reason for this difference is unclear. There may be environmental causes; tanks used in experiment 2 were much larger than those in experiment 1 and the number of salmon and lumpfish present in the trials were also different. If tank size or stocking density can significantly affect the number of lice surviving for one week in control treatments (when comparing experiment 1 and 2), then future experiments considering the effectiveness of a louse treatment ought to take this into consideration. Ideally, treatments should be tested on site under usual farming conditions to estimate their real effect. A smaller number of fish were used in each trial in experiment 1 than in experiment 2, which may have changed the social dynamics resulting in differences in behaviour of salmon, lumpfish or both and consequently cleaning efficiency. Replication was unfortunately low, particularly in experiment 2. Future work should systematically alter the social and physical environments to ascertain the factors that maximise louse drops.

In the behavioural trials I found some indication that cleaning propensity is affected by activity level and previous experience. Only the most active individuals showed any propensity to clean and among those individuals, the number of days of previous experience was positively related to the number of nips at salmon (this may have been either taking a louse or aggressive behaviour towards the salmon itself). In lumpfish, activity is associated with hunger as satiated lumpfish will usually attach to a surface rather than actively forage for food (Killen et al. 2007). This suggests that those lumpfish that were most motivated to feed were also the most likely to exhibit cleaning behaviours. If this is the case, then perhaps the effects seen were not based on inherent behavioural traits but on more transient hunger levels (Krause et al. 1998, Biro and Booth 2009). However, not all active fish approached salmon, so my results

are inconclusive and perhaps a more detailed measure of individual behavioural traits, such as boldness (Ward et al. 2004b, Harcourt et al. 2010, Archard and Braithwaite 2011) are needed to investigate why some lumpfish approached salmon and some did not. Future work could focus on comparing cleaning efficiency of 'bold' and 'shy' individuals (or other aspects of behavioural syndromes and personality; Bell, 2007) to determine how much cleaning behaviours are dependent on behavioural traits.

Because I found such great variation in inclination to clean in my behavioural trials, with most fish, even those that were active, showing no interest in salmon, I suggest it may be useful to selectively breed 'cleaning' lumpfish. While cleaning may not be part of a behavioural syndrome, there may be genetic basis for propensity to clean. However, one problem encountered in my study was that of individuals showing interest in cleaning salmon also being aggressive towards salmon (personal observations, unfortunately not adequately recorded as part of sampling). If future experiments reveal aggressive tendencies in cleaners, this is a serious welfare concern, which may outweigh the benefits of lowering louse populations. Additionally, selectively breeding cleaners may also result in more aggressive individuals causing welfare problems for salmon, as well as potential welfare problems for lumpfish caused by increased aggression (Saxby et al. 2010). However, thorough research into aggressive behaviours, propensity to clean and other potential behavioural and welfare concerns in addition to husbandry measures such as provision of shelters, could prevent serious problems from arising (Brown 1986, Deady et al. 1995, Gries and Juanes 1998).

Because of the financial and welfare implications of salmon louse infections (Ashley 2007, Costello 2009), good management practices and louse treatments are necessary. As chemical treatments are often only useful for a time until lice build up resistance (Treasurer et

al. 2000, Fallang et al. 2004), other ways to manage infestations can be useful alternatives or additions to chemical treatments. Cleaner fish do alleviate the problem to some extent (Deady et al. 1995, Treasurer 2002), so finding suitable salmon cleaning species across the areas where salmon are farmed should be given priority. Though my results are inconclusive, there is some potential in lumpfish that ought to be investigated further. Even low level louse infestations can cause health problems including affecting osmoregulation in salmon and 0.05 adult or pre-adult lice per square centimetre of salmon is considered the point at which lice begin to have a long term effect (Ross et al. 2000, Stien et al. 2013) with an expected upper limit of 0.12 lice per square centimetre of fish (this is approximately 10 pre-adult or adult lice on a small post-smolt salmon, 15g) where the infestation becomes lethal (Finstad et al. 2000, Stien et al. 2013). The Faroese health authorities have regulations in place requiring chemical de-lousing if infestations reach more than two adult lice per salmon or 10 pre-adult lice. If cleaner fish can reduce salmon louse loads by 40%, this can keep louse loads low enough to make chemical treatments unnecessary in otherwise borderline salmon cages, which could slow down build-up of resistance as well as lessening potential harm to other crustaceans in the ecosystem.

Chapter 7: General Discussion

In this thesis I investigated two aspects of the broad field of predator-prey interactions. As visual predator-prey systems have been well studied, my primary focus was on the relationship between prey aggregation and the success of a predator hunting using olfaction. In chapter 2 I explored the ability of sticklebacks to compensate for loss of visual cues by using olfactory cues instead. Chapters 3 to 5 explored how prey aggregation affects detection by olfactory predators. Chapter 3 focused on a non-specialist forager, the stickleback and prey survival at different aggregation levels and chapter 4 explored prey detection by *Gammarus* when searching for bloodworm in a range of group sizes. In chapter 5 I investigated how prey detection by sticklebacks is affected by group sizes and turbulence in a flowing environment. Finally, in chapter 6 I studied predator-prey interactions from a more applied perspective, and focused on the industrial use of a potential cleaner fish in reducing louse loads in salmon farming.

Olfactory prey detection

In chapters 2-5, I investigate the ability of predators to detect and locate prey, and consider this in the context of risk to prey. Predator sensory modality has important implications for predator-prey interactions, and for the detection and location of prey. When a visual predator sees a prey item, the prey has been both detected and located at the same time. However, when a predator smells prey, or detects prey using sound, those prey have not necessarily been located even though they have been detected. The risk to prey from being located by a predator differs to that of being detected. If an olfactory predator detects prey in

an area, it may be able to track the odour plume to its source, or it may intensify foraging efforts in the area, but may not find prey. However, the presence of a predator will affect prey through trait mediated effects as well such as a decrease in time spent foraging (Trussell et al. 2003) or morphological changes (in sticklebacks; Frommen et al., 2010, *Daphnia*; Riessen, 2012). If prey are located by the predator, their risk is that of imminent death if they cannot employ defensive mechanisms at that stage or if they are not in a group large enough to benefit from dilution of risk (Foster and Treherne 1981). In this section of the thesis, I address questions of both prey detection and prey location, and give insight into the risks to prey associated with aggregating when predators hunt using olfaction.

It is fairly well established that temporary loss of visual cues negatively affects the ability of primarily visual predators to locate prey (Grecay and Targett 1996, Utne 1997, Zingel and Paaver 2010, Vollset and Bailey 2011). In chapter 2, I found that sticklebacks can partially compensate for a reduction in the availability of visual cues and that the presence of strong olfactory cues in the water act to improve foraging success. When an excess of olfactory cue was added to the water, stickleback foraging success was increased, contrary to my expectation that the excess cue would mask any location cues and reduce the ability of predators to locate the prey. Instead, I suggest that sticklebacks don't use olfactory cues to locate prey but rather to detect the presence of prey in an area and then intensify their search effort. My results differed from a previous study where an added olfactory cue decreased foraging efficiency (Webster et al. 2007a), which could potentially be explained by a difference in the experimental design. Prey in my study were exposed on a white flat surface making them visually detectable within a very short distance in the high turbidity treatment and those in the study by Webster et al. (2007) were on a gravel substrate where close-range olfactory cues may have been more useful than they were in my study.

If perceived high prey density improves foraging success, this could have a detrimental effect on aggregated prey as they could conceivably cause a localised high density olfactory prey cue. This could act either through the mechanisms suggested in chapter 2, or through an increased ability of predators to track odour plumes arising from larger groups. In chapter 3 I investigated how prey survival or stickleback foraging success is affected by prey aggregation. I found aggregated prey were not as easily detected as dispersed prey, with aggregated prey surviving longer than dispersed prey in a field experiment and being discovered later than dispersed prey in a laboratory experiment. This reflects findings for prey avoiding visual predators, where encounter-dilution attack abatement effects acts to reduce individual risk (Jackson et al. 2005, Ioannou et al. 2011). Thus, aggregating, despite potentially alerting the predator to the presence of prey, is beneficial to prey avoiding an olfactory forager. However, in my laboratory experiment (chapter 3), the prey were rapidly consumed by the predator upon discovery to the extent that their survival decreased to below that of dispersed prey, as the dead prey could not take any evasive action. This would imply that in order to fully avoid being consumed once an aggregation is discovered, prey should aggregate in groups larger than that which the predator can consume, and thus individuals could benefit through dilution effects (Wrona and Dixon 1991). If prey groups are large and prey density remains constant, distances between prey groups combined with the inability of the predator to consume all prey in a group, will lead to attack abatement (Turner and Pitcher 1986).

From chapter 3, I concluded that aggregating is beneficial to prey when predators hunt using olfactory cues. My results from chapters 4 and 5, however, indicate that larger groups are more easily detected than small group, reflecting again the patterns that are observed in visual predator-prey systems. The benefits of aggregating in response to an olfactory predator

could therefore also be a balance between the increased detectability of groups and encounter-dilution effects (Taylor 1976a, 1979). In chapter 4 I found that time taken for an olfactory predator to detect a prey group decreased with increasing size of the group. In chapter 5, larger groups were also more easily detected by a primarily visual forager than smaller groups, when the ability of a predator to locate an odour source was tested in flowing water. So how is risk of detection and location of prey affected when prey aggregate?

In chapter 4 I found that while increasing levels of prey aggregation decreases the time until a primarily olfactory predator discovers the group, increasing group size does not increase the proportion of prey groups that are discovered. That is, the proportion of prey discovered decreased after an 'optimal' (for the predator) group size. Prey group size did not affect foraging activity or effort at reaching prey, so the two relationships of a) time to location and b) proportion of prey location with prey group size give conflicting answers to the question of how prey group size affects location. This contrasts with my findings using sticklebacks, where a strong olfactory cue in the water improved foraging success both in terms of shorter search time and proportion of prey located. I suggest that in *Gammarus* the lower proportion of prey detected at large group sizes can be explained by a flooding effect preventing the predator from locating prey as easily (Webster et al. 2007a). As *Gammarus* primarily use olfaction rather than vision (Åbjörnsson et al. 2000), they may respond differently to an excess of olfactory prey cue in comparison to the sticklebacks in chapter 2, who improved their foraging success under excess prey cue conditions. However, if prey were harder for *Gammarus* to find when the groups were large, this does not explain why larger prey groups were found more quickly, especially as activity levels did not differ as a function of group size and so cannot explain the increased time to locate the prey. A recent meta-analysis of empirical olfactory detection studies has suggested that there is a clear relationship between

the square root of the number of prey and distance at which they can be detected (Andersson et al. 2013). I did not investigate detection distance, but the search time data suggests a similar pattern: I found a linear decrease in the square root of search time with an increase in the square root of the number of prey. This may suggest that the square root of the number of prey is a good predictor for effects on detection and location, though time and distance must be treated differently, and further work is necessary to elucidate the relationship between group size and predation risk when predators hunt using olfactory cues.

In flowing water with a stickleback predator (chapter 5), larger prey groups are more likely to be found by predators, reflecting the findings of a recent study on knobbed whelks that found that aggregated prey were more easily located than dispersed prey (Wilson and Weissburg 2012). This would suggest that prey are at increased risk if they aggregate, but no studies to date have investigated prey survival in flowing water as a function of increasing group size. Chapter 5 also investigated the effect of turbulence on the ability of predators to locate prey groups of different sizes. Previous work has suggested that turbulence makes odour plume tracking more difficult, particularly for fast moving predators such as crabs (whereas slow moving predators such as whelks can still successfully locate prey; Ferner and Weissburg 2005), and that 'sensory refuges' may exist, where turbulent water movement allows prey to hide from olfactory predators (Weissburg and Dusenbery 2002). In chapter 5, I suggest that turbulence may increase a threshold group size that prey can aggregate in while staying below the detection limit: turbulence allowed medium sized, but not large, groups to effectively 'hide' from the predator. This suggests that turbulence can provide a 'sensory refuge', allowing prey to aggregate and thus benefit from dilution of risk if discovered (Foster and Treherne 1981). If this is the case, field studies should reveal larger aggregations of prey upstream of turbulent water than in equivalent positions upstream of laminar water. There is

still much to discover on this subject such as how the intensity of turbulence or even shape or type of turbulence (Vickers 2000) affects ability of predators to detect prey and whether there are distance restrictions or prey group sizes above which prey will always be detected, thus limiting the size of aggregations.

In chapters 2-5, I stress the importance of considering the difference between detection and location when investigating olfactory predation. Visual predation is often investigated in terms of detection distance (Sweka and Hartman 2003, Quesenberry et al. 2007) or visual angle (Ioannou and Krause 2008), though search time is often used as a measure of detection (Ioannou et al. 2009). However, when investigating visual detection no distinction between detection and location is necessary due to the nature of vision.

When investigating olfactory cue detection as a function of prey group size, detection distance seems the most likely measure to be used (Treisman 1975, Kunin 1999, Andersson et al. 2013). This may be because location of prey can happen either once the olfactory predator makes contact with prey after tracking an odour plume (Ferner and Weissburg 2005) or because the predator switches to other senses once the prey is within range of those senses, be it close range vision in turbid water (Utne-Palm 1999), detection of vibrations in water or substrate (Coombs 1999) or echolocation (Thies et al. 1998). Because of this potential switch in sensory modality once the predator is near its prey, determining whether prey were located using olfaction or other senses and when the predator changed its sensory modality can be difficult. This complicates measures of olfactory foraging relating to location rather than detection. However, because detection does not mean location when the predator is an olfactory predator, the non-consumptive effects on prey (Preisser et al. 2005) related to the time taken by the predator to search for prey should be considered.

Avoiding olfactory predators may be as much a question of diminishing negative trait mediated effects (Flynn and Smee 2010, Riessen 2012) as it is about avoiding consumptive effects. This could potentially mean that despite increased survival through attack abatement (Turner and Pitcher 1986), prey that are easily detected due to their large group size, suffer detrimental effects due to a larger presence of predators in their general area. Perhaps chiefly for these reasons, turbulence in flowing environments is a useful way for prey to benefit from attack abatement as well as diminishing the presence of searching predators in the area (chapter 5).

Cleaner fish in the aquaculture industry

In chapter 6 I investigated the potential use of lumpfish as cleaner fish for Faroese salmon farms, where salmon lice present a significant economic and welfare problem. I found that lumpfish are highly variable in their inclination to clean salmon: while some individuals actively removed lice from salmon, the majority showed little interest in the salmon or the lice. In one of three experiments, I found that lumpfish caused a significant reduction in louse load relative to the control, but this finding was not reflected in the other two experiments, where lumpfish appeared to have little effect on louse loads. These results indicate that while there is potential for lumpfish to clean salmon, further work is needed to establish the conditions under which cleaning behaviour is promoted.

As salmon lice are an on-going problem in the salmon farming industry (Ashley 2007), finding alternative or complementary methods of reducing louse populations, and maintaining low levels of infestation, on salmon farms has been and still is of great importance. A range of

chemical treatments are available (Rae 2002, Pike and Wadsworth 1999), but resistance builds up to most of these, and thus additional control measures are necessary. Because wrasse have been shown to be effective cleaners with over five million wrasse being stocked annually in Norway (Treasurer 2002), the use of cleaner fish is a feasible option on the Faroe Islands, though wrasse are not available. However, because of the inconclusive results of my study, more investigation is needed.

Future research should focus on three aspects of lumpfish cleaning:

1) Selective breeding of cleaning lumpfish to reach a high proportion of cleaners in the population. This should be done carefully, however as there are potential welfare implications to consider. There may be a link between cleaning propensity and aggression (chapter 6), which could lead to selection for aggressive individuals. As farmed salmon and therefore also lumpfish used as cleaners are kept at high densities in large numbers, aggression could cause serious welfare problems for both lumpfish and salmon. Even putting aggression aside, selective breeding should always be done carefully to avoid unwanted health or welfare problems.

2) Further investigation of why lumpfish differed in interest in salmon. While an attempt was made at standardising hunger levels, lumpfish may have differed in body condition, though we found no effect of size. Interest in cleaning showed a weak link with activity levels and an investigation of whether there are any reliable personality traits linked with cleaning propensity and whether aggression is one of those traits ought to be investigated.

3) Finally, reasons for the difference in control drop in louse population are unknown, but as they were quite large, future investigation into the cleaning propensity of

lumpfish ought to take into account tank size and stocking density. Preferably, trials ought to run on salmon farms, which is where cleaning efficiency is needed.

Future work

There have been recent advances in knowledge regarding olfactory foraging and prey aggregation. My work has consistently found that aggregation is beneficial to prey avoiding an olfactory predator and the relationship between detection distance and prey group size is fairly well established (Andersson et al. 2013), indicating that as detection distance increases asymptotically with prey group size, aggregation will lead to attack abatement providing not all prey are consumed once discovered (Turner and Pitcher 1986).

However, while detection distance works well as a predictor of risk in a visual framework (Taylor 1979, Quesenberry et al. 2007), this is not necessarily the case in an olfactory framework (Ferner and Weissburg 2005, Robinson et al. 2011). While we may be able to use prey group size to predict the distance at which an olfactory predator can detect prey (Andersson et al. 2013), this does not necessarily provide enough information on the risk to prey in terms of attack abatement as the relationship between prey detection and prey location is not properly quantified. Specifically, the relative risk posed by olfactory specialists (e.g. knobbed whelks; Ferner and Weissburg 2005) and opportunists (e.g. sticklebacks - chapter 2; Johannesen et al. 2012), social predators able to use olfactory social foraging cues (Colasurdo and Despland 2005) and the effect of flow and turbulence (Koehl 2006) is not well understood. If predator as well as prey aggregation (Fryxell et al. 2007) is added to the system, predictions based on a traditional framework, even when modified to account for a difference

in sensory mode by the predator, do not hold. Also, the ability of the predator to modify its behaviour based on prey behaviour changes relative benefits of aggregation (Nachman 2006).

Substantial changes to the way we think of olfactory prey detection and aggregation could go some way to predict consumptive effects of predators, but would not adequately consider non-consumptive effects of predators (Trussell et al. 2003), which may be more important for prey avoiding olfactory predators than their visual counterparts. Prey avoiding an olfactory predator that has a long search time from detection, potentially not finding the prey at all may be more at risk from the detrimental effects of prolonged predator presence, such as reduced foraging, breeding or making costly morphological changes (Preisser et al. 2005, Dunn et al. 2008, Frommen et al. 2010) than of actual consumptive effects. A theoretical framework which includes search time as defined by the time from detection to location and risk of location after detection is needed to fully understand how olfactory prey detection affects consumptive risk to prey and would be useful for predictions regarding non-consumptive effects as well.

Anthropogenic effects on ecosystems such as ocean acidification (Dixson et al. 2010) and algal blooms (Engström-Öst et al. 2009) alter the way in which predators and prey are able to detect each other. Knowing the consequences of for example a shift towards greater reliance on olfactory cues as a response to fluxes in turbidity or the effect of weaker olfactory cues due to changes in pH is necessary in order to predict effects both in terms of density mediated interactions as well as trait mediated interactions (be it direct or indirect).

Conclusions

Because of the disconnect in olfactory foraging between prey detection and prey location, current models and risk to aggregated prey should take into consideration search times and probability of location given detection. Even then, non-consumptive effects are likely to be of great importance in systems with olfactory predators, perhaps especially non-specialists for whom tracking odour plumes is potentially more challenging. Work should focus on the effect of predator search time once prey have been detected on prey behaviour and fitness and how environmental factors interact with predator foraging behaviour in their effects on prey.

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