FORAGING BEHAVIOUR OF SHOALING FISHES: INFORMATION
GATHERING AND PREY COMPETITION

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by

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ABSTRACT

Group foraging is associated with a number of costs and benefits, and these must be balanced by individuals if they are to gain an adaptive advantage. In this thesis I investigated aspects of the group foraging behaviour of shoaling fishes.

Firstly I investigated the use of asocial and social information use; the potential for inexpensively acquiring information about prey resources is one advantage of social foraging. Threespine sticklebacks (*Gasterosteus aculeatus*) assimilated and used private information about prey distribution between different subhabitats when foraging alone. They also used a number of different social information cues, and when these conflicted with their private information, they based their foraging decisions upon the former, suggesting that there are costs associated with non-conformist foraging behavior.

Secondly I investigated prey competition, a major cost associated with social foraging. I found that increasing group stability, and by inference familiarity, led to a decrease in the rate of kleptoparasitic prey competition within shoals when they were foraging for dispersed prey. When prey were concentrated however there was no effect of group stability upon prey competition level. Prey competition was less intense between familiar individuals that were embedded in unfamiliar shoals than it was between these and their unfamiliar shoal mates.

Finally I investigated the role of individual behavioural variation in relation to social information use and prey competition. Boldness across a number of contexts was seen to correlate with individual competitive ability, predicting the outcomes of both inter- and intra-specific prey competition interactions. Interestingly, the use of public information, a risk-averse strategy consistent with the shy behavioural phenotype, was not seen to be related to individual boldness.

The broader significance of the findings of this thesis are considered in the context of previous research, and directions for future work are identified and discussed.
DECLARATION

Some of the experiments contained in this thesis were carried out in collaboration with others. Jo Goldsmith assisted with the collection of data in Chapter 3. Ashley Ward assisted with the collection of data in Chapters 3, 6 and 7. Paul Hart supervised the production of this thesis and was involved in all of the experiments. Work arising from this thesis has been published, or submitted for publication in a number of journals. Details of published and submitted works arising from this thesis are given below.

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Chapter 1

Introduction
1.1 Living in groups

Group living is common in nature, with many species from a range of taxa displaying grouping behaviour during at least some stages of their lives (Krause & Ruxton 2002). Animals form groups for a number of reasons and are subject to a range of benefits and costs when doing so.

Two of the primary potential benefits derived from group living are reduced predation risk and enhanced foraging success, and these are discussed below. Other benefits potentially derived from group living that are not discussed further include increased mating opportunities (Westbeat et al. 2000), the maintenance of homeostasis (Andrews & Belknap 1986; Clark & Faeth 1998) and the reduction of energy expenditure when traveling (Herskin & Steffensen 1988; Fish 1991; Weimerskirch et al. 2001).

Individuals within groups potentially bear lower per capita predation risk costs than do solitary individuals for a number of reasons. Firstly, larger groups are more likely to detect approaching predators sooner, the so-called many eyes effect (Roberts 1996; Krause & Ruxton 2002). This is due to the likelihood that within a larger group many individuals will be looking out for threats at any given moment. Coupled with the potential for greater rates of social transmission of information, this allows individual group members to decrease their own investment in predator detection without increasing the risk of missing an incoming threat (Krause & Ruxton 2002). During attacks by predators, group members benefit from attack dilution effects, whereby the risk to any one individual decreases as group size increases (Wrona & Dixon 1991). This effect can be enhanced further through active behavioural strategies such as predator swamping behaviour, whereby group members synchronise behaviours such as flight responses or emergence from refuges (Sweeney & Vannote 1982), predator confusion, where group behaviour reduces the capacity of the predator to select and follow a target individual (Fels et al. 1995), and selfish herd
behaviour, where individuals actively attempt to reduce their own likelihood of capture relative to that of their group mates (Hamilton 1971).

The enhancement of foraging returns is another key benefit afforded to many group living animals. Grouping may facilitate the exploitation of prey resources that are otherwise unattainable to the individual. For example, pack hunters may be able to subdue large or dangerous prey that they could not capture if they were to hunt alone (Creel & Creel 1995).

Many of the benefits relating to group foraging however are associated with the acquisition of social information. Larger groups of foragers can cover more ground, or search a given unit of habitat more rapidly than can an individual. As such the prey encounter rate of a larger group is likely to be greater, and many or all group members can exploit this by pursuing scrounging strategies, using social information cues to detect prey finds by others that they themselves can then attempt to exploit. Social information refers to cues produced either actively or passively by other foragers that provide information about a resource. Different types of social information are recognised, and these are categorised by the amount of information that they potentially provide the receiver. Local enhancement is a form of social information by which the receiver detects and is drawn to other foragers that are already exploiting a resource. Local enhancement cues may provide no information to the receiver about either how to exploit the resource, or whether it is of low or high quality. In the context of foraging, local enhancement cues may be used by a forager as a proxy for the location of a prey patch, since groups of feeding animals are often more conspicuous than the prey that they are exploiting.

Public information is a more specialised form of social information, in that it specifically conveys to the receiver usable information about the quality of a resource (Valone 1989; Valone & Templeton 2002). Group members observing the foraging performance of others feeding at multiple prey patches may use information such as prey capture rates in order to detect more profitable patches, allowing them to maximise their own potential prey capture
rate without the need to expend energy and incur risk of predation by sampling each patch themselves.

Group members also benefit from facilitation effects. In the context of foraging, social facilitation describes a situation whereby the presence of others directly or indirectly allows a forager to increase its own foraging efficiency. This process does not involve social learning, since the forager utilises skills or information that it already possesses. Rather, it frees the individual from other constraints, allowing it to allocate more effort towards foraging (Galef 1988; Day et al. 2000). For example, foraging within a larger group allows an individual to reduce investment in predator vigilance, and to spend more effort searching for and consuming prey. As such, this is tied to the many eyes effect, described above.

Group foragers are also subject to a range of costs. There are predation risk costs associated with group living, since though grouped prey benefit from a lower per capita chance of capture during an attack, evidence suggests that being in a group may initially attract predators (Krause & Ruxton 2002). This may be because predators can more easily detect groups than they can individuals, because they exploit confusion that may occur between fleeing prey that are reacting to social information rather than the predator itself, or because the predators preferentially attack larger groups in the hope of capturing multiple prey (Krause & Ruxton 2002; Botham et al. 2005; 2006).

Another key cost associated with group living is competition for resources. Competition can take several forms, either indirect or direct. Exploitation is a form of indirect competition: in the context of foraging, individuals that are feeding upon the same patch cause it to become depleted, preventing subsequently arriving group members from using it. This type of competition does not assume that competitors must encounter one another, or occupy the same space at the same time. Scramble competition occurs when one forager detects its competitors and attempts to reach, capture and process a prey item before they do.
Finally, contest competition occurs when two or more foragers directly and usually aggressively compete to obtain a prey item (Ward et al. 2006).

The balance between the costs and benefits of group living can vary over time as a function of prevailing environmental and social conditions, and the changing requirements and objectives of the individual. Grouping behaviour is therefore subject to a variety of trade-offs that must be assessed, updated and balanced if the individual is to gain an adaptive advantage from group living.

1.2 Shoaling in fishes

Group living is very common in fishes and over half of all known species are thought to form shoals during some stage of their development (Shaw 1978). Many fish shoals possess a fission-fusion social structure: group structure is dynamic and the shoals themselves are ephemeral entities. Individual shoals may split into small shoals, whilst separate shoals meet to form larger ones. Shoals may pass through one another, exchanging individuals as they do so, whilst other individuals may leave their shoals in search of new ones. Shoals break up and reform, and individuals come and go both passively and actively, and the rate at which they do so is determined by such factors as inter-shoal encounter rates, and local environmental, predation, foraging, and sexual pressures. In some systems, shoals may meet and exchange individuals as frequently as every few minutes or hours (Hoare et al. 2000; Croft et al. 2003). Nonetheless, shoal composition in fish shoals is far from random. Group membership has adaptive implications, relating to for example, levels of intraspecific competition (Peuhkuri 1997), and oddity effects (Theodorakis 1989). As a consequence of this, and despite the potential instability of shoal structure, fish shoals in nature tend to be highly sorted by general factors such as species, body size, parasite load, and other phenotypic factors (Krause et al. 1996; Hoare et al. 2000, Ward & Krause 2001). Even once these criteria are satisfied, individuals make further fine-scale assessments based upon more
subtle criteria such as patterns of familiarity (Griffiths 2003; Ward and Hart 2003; Griffiths and Ward 2006), and resource use (Olsen et al. 2003; Ward et al. 2004; 2005).

1.3 The scope of this thesis

The interplay between the benefits of social foraging and the costs of prey competition form the broader focus of this thesis. Specifically I examine the ways in which individuals use social information to make foraging decisions, and the ways in which competition is mediated. Emphasis is placed upon the group, for example by looking at patterns of overall competition levels, and by examining the effects of resource use patterns upon group membership decisions and shoal cohesion. Emphasis is also placed upon the individual, by considering the role of variation in behavioural phenotypes in relation to information use and the outcomes of prey competition. Specific predictions relating to information use and competition are made and tested on a chapter by chapter basis and the findings of this thesis are discussed in the context of the current theory concerning social foraging and adaptive behavioural strategies.

1.4 Study species

Small shoaling fish are very amenable for studies of social behaviour; shoals can be readily accommodated the laboratory, where their environment can easily be manipulated. This provides the researcher with a degree of flexibility and control when designing experiments that can be difficult to achieve when working with some other vertebrates. The threespine stickleback (Gasterosteus aculeatus) was used throughout this thesis because it is a well established as a study organism with much known about its behavioural ecology, evolution and genetics (Bell & Foster 1994). A number of factors contribute to its tractability as model species for such research; these include its wide distribution, and adaptability to laboratory conditions. The species complex is found throughout the temperate and sub-Arctic Northern
Hemisphere, occurring in rivers and lakes as well as in estuarine and coastal marine habitats. Studies of the adaptive responses of different populations of this species to the great variety of selective pressures operating upon them across their distribution have furthered our insight into evolutionary processes in vertebrates (Bell & Foster 1994; Shapiro et al. 2004). In addition to this research on extant populations, studies allying ecological assays with paleontological techniques have allowed us to make inferences about the evolutionary dynamics of extinct populations (Purnell et al. 2006), adding a temporal dimension to our understanding of the ecology of the species. Three-spine stickleback acclimated to laboratory conditions readily display a broad repertoire of complex behaviours, and behavioural ecologists have exploited this in order to controlled, ecologically relevant research in a variety of areas (Bell & Foster 1994).

In Chapter 7 of this thesis I also used the closely related ninespine stickleback (*Pungitius pungitius*) in order to study interspecific interactions. The two species frequently occur in sympathy and often actively aggregate with one another (Hart 2003). Mixed species aggregations commonly occur in the wild, and the study of fine-scale behavioural interactions between co-occurring species affords both natural realism and greater scope to our understanding of them.
Chapter 2

Foraging and information use: private versus social information
2.1 INTRODUCTION

Fine-scale habitat structure can be unstable and subject to rearrangement through space and time, affecting the ability of foraging predators to detect prey. In order to optimise prey capture rates under such conditions, and under the added constraints of competition from conspecifics and the risk of predation, the generalist forager should benefit from a flexible repertoire of behaviours allowing it to assimilate and process information from multiple sources. Such an individual potentially has the option of basing its foraging decisions upon private information, such as learned responses and prior experience arising from asocial interactions with the environment, or social information, that is, cues generated by other foragers pertaining to the location, means of access to, or quality of a prey resource.

The process of discrimination between private and social information by social animals is the subject of much ongoing research (Valone and Templeton 2002; Laland 2004). The common consensus is that private information is of greater value because it is more reliable, and prevents the formation of informational cascades, whereby erroneous information is received and accrued, and sub-optimal behavioural responses are elicited (Giraldeau et al. 2002; Kendal et al. In Press). Collecting private information can be costly to the forager however, in terms of time and energy expenditure and potentially heightened predation risk. For these reasons the use of social information is reasoned to be likely widespread in nature (Danchin et al. 2004; Dall et al. 2005).

Previous studies have determined that various fish species are capable of assimilating and recalling private information, using spatial and temporal cues to aid orientation and prey patch location (Milinski 1994; Odling-Smee and Braithwaite, 2003). The capacity to recall and make use of such cues varies within and between both species and populations as a function of ecological conditions, in particular the temporal stability of the habitat (Mackney and Hughes 1995; Girvan and Braithwaite 1998; Hughes and Blight 1999) suggesting that the mechanism of recollection and utilisation of previous experience in fish is plastic and
adaptive. In nature foragers must select not only between patches of known spatial location, but also between areas of unexplored subhabitat, structurally differing components of the greater forage plain. Examples of subhabitats in aquatic habitats may include deposits of different substrate materials, areas of different flow velocity and patches of different vegetation type. Differing subhabitats are known to harbour different assemblages of prey species (Taniguchi et al. 2003; Boyero and Bosch. 2004; Taniguchi and Tokeshi. 2004), facilitating the patchy distribution of prey assumed by many models of optimal foraging (Charnov. 1976; Stephens and Krebs. 1986). Through the discrimination of different subhabitat types, a foraging fish should be able to assess the potential of previously unsearched areas of subhabitat, providing that it has previously sampled an area of similar type, and is able to recall its foraging success there. Evidence from field and laboratory studies has identified subhabitat preferences in species such as silver hake (Merluccius bilinearis. Auster et al. 1997; 2003) and threespine stickleback (Webster and Hart. 2004) that may be experience-based strategies, serving to actively enhance encounter rates with prey.

In addition to privately acquired information, shoaling fish also base foraging decisions on information obtained through the observation of the feeding performances of others (Laland & Williams 1997; Lachlan et al. 1998; Coolen et al. 2003; Kendal et al. 2004; van Bergen et al. 2004). Such information may include local enhancement cues, which specifically relate to prey patch location, or public information cues, which infer prey patch quality (Coolen et al. 2003). Such cues may be current, in that they are available as the recipient is actively discriminating between patches, or they may be prior, in that they are provided before, but not during, the period when the recipient selects a foraging site.

Given this, a foraging fish may potentially have several conflicting sources of information on which to base foraging decisions. In this study I aimed to determine whether threespine sticklebacks were capable of developing foraging preferences for subhabitats
based upon their previous foraging successes there. I predicted that they use their own previous experience when selecting between subhabitats, even when conflicting social information cues were provided.

2.2 METHODS

2.2.1 Collection and Housing of Experimental Fish

Approximately 500 sub-adult threespine stickleback measuring 15-20 mm total length were collected using dip nets in September 2004 from Stonton Brook, Leicestershire, UK. They were distributed between 12 holding tanks (40 x 25 x 28 cm deep, water depth 25 cm, bare Perspex substrate, water temperature 11°C; photoperiod 12:12 h) and fed daily to satiation on frozen Chironomid larvae. They were held under these conditions for approximately four months prior to the beginning of the study.

2.2.2 Subhabitat conditioning procedure

Fish were conditioned to display subhabitat foraging preferences prior to experimental trials. Conditioning was carried out in visually and chemically isolated tanks (40 x 25 x 28 cm deep, water depth 25 cm), each of which was divided into two equally sized sections of two types of substrate material over which fish foraged for benthic prey items. One substrate comprised fine sand (grain size ≤ 1 mm) and the other comprised assorted angular sandstone gravels, where particles had a longest axis length range of 5–20 mm. These two substrate types represented different subhabitat units, similar to those that river dwelling fish may encounter in nature. Each tank held four fish, size matched to one another to within <1mm total length. Over the course of a conditioning period prey, 5mm sections of thawed chironomid larvae were provided in one subhabitat only. These were provided in excess once per day, and were distributed evenly across the surface of the subhabitat using a 1cm³
syringe. The duration of the conditioning period, and the subhabitat to which fish were conditioned varied between experiments, as described below.

2.2.3 Experimental tank and procedure

Subhabitat preference binary choice trials were conducted in an experimental tank (54 x 25 x 30 cm deep; water depth 25 cm), covered at the sides with opaque plastic to eliminate outside disturbance. The tank contained two subhabitat types of equal area, containing the same substrate materials that the fish had experienced during the conditioning phase of the experiments. The left-right positioning of these substrate materials within the tank was randomised for each trial in order to eliminate any tank end bias. Prey items were randomly distributed across each subhabitat; prey densities varied between experiments as stipulated in the experimental descriptions below. Sections of Chironomid larvae 5mm in length were used as prey in all trials.

Fish were deprived of food for 18 hours prior to testing in order to increase foraging motivation. Before the start of each trial the test fish was held for three minutes in a 25 cm tall mesh holding tower (mesh size 2 mm) in the centre of the binary choice tank, such that it could view both substrate types. The tower was raised and removed, releasing the fish to explore the tank and the trial began when the fish located and consumed the first prey item. With this approach I could be certain that the fish has made the transition from any stress related behaviour to the foraging behaviour that I was seeking to quantify. Each trial lasted 3 min and I recorded the amount of time spent directly over, but less than 10 cm above each substrate and the number of feeding strikes made into each subhabitat type. Multiple consecutive strikes made at a single prey item were deemed to constitute a single handling process and were recorded as such.

2.2.4 Control experiment: pre-existing subhabitat preferences
A previous study revealed pre-existing subhabitat preferences in threespine stickleback (Webster and Hart 2004). I reasoned that any pre-existing preferences should not persist over the four-month period during which fish were held in the laboratory prior to the beginning of the experimental series. To confirm this assumption I conducted a control experiment in which fish that had not experienced either substrate type were given a binary choice using the tank and protocol described above. One randomly selected fish per holding tank \((n= 12)\) was tested for subhabitat preference at an experimental prey density ratio of 1: 1 \((0.008 \text{ prey items per cm}^2 \text{ on each substrate type})\).

### 2.2.5 Experiment 1: The rise of subhabitat foraging preferences

The purpose of this experiment was to determine whether subhabitat foraging preferences arose in response to experience of successful foraging on a given subhabitat type. Twenty-four groups of four fish were established in their own subhabitat conditioning tanks as described above. Initially I provided prey on the sand subhabitat only. Every second day for 32 days one fish was randomly selected from each group and tested for subhabitat preference using the procedure and binary choice tank described above. After 18 days I randomly selected 12 of the 24 groups and switched prey provision from the sand to the gravel subhabitat type. Binary choice testing continued as previously; this made it possible to observe the rate at which a preference arose for the sand subhabitat containing prey, and at what rate it decayed and was replaced when only the gravel subhabitat held prey. In the remaining 12 groups I continued to provide prey on the sand subhabitat only; these groups served as controls, for comparison with those groups in which prey provision was switched. Preferences were examined at only one experimental prey density ratio, 1: 1 \((0.008 \text{ items per cm}^2 \text{ in each subhabitat in the experimental tank})\).
2.2.6 Experiment 2: Recent experience versus conflicting real-time assessment

In this experiment I aimed to determine whether test fish that had experienced predictable and regular prey distribution over the course of a conditioning phase would use previous experience alone to select between subhabitats or whether they would use what I termed real-time assessment. By the definition used in this study, real-time assessment would involve visiting both subhabitat types and allocating proportionally more foraging effort to that which held more prey, following the assumptions of the ideal free distribution (Fretwell & Lucas 1970; Milinski & Parker 1991). I achieved this by presenting test fish with binary choice tests in which prey density between subhabitats varied from what they had previously experienced, such that using previous experience alone would not provide optimal foraging returns.

Twenty-four groups of four fish were established in their own subhabitat conditioning tanks as described above. In the first 12 of these tanks prey was provided on only the sand subhabitat, and in the second 12 only on the gravel subhabitat. Fish were held under these conditions for a period of 14 days.

Following this conditioning period I tested subhabitat preferences of fish conditioned on the sand and gravel subhabitat under four different prey density ratios: 1) 1:1, sand: gravel (0.008 items per cm$^2$ in each subhabitat). 2) 3:1 (0.024 items per cm$^2$ in the sand versus 0.008 items per cm$^2$ in the gravel subhabitat). 3) 1:3 (0.008 items per cm$^2$ on the sand versus 0.024 items per cm$^2$ in the gravel subhabitat). 4) 0:0, a control with no prey present in either subhabitat. Trials were conducted using the experimental tank and procedure described above. One fish from each conditioning tank was tested at each prey density ratio, giving 12 trials per treatment and over the course of the experiment no fish was tested more than once.
2.2.7 Experiment 3: Recent experience versus conflicting local enhancement and public information

In this experiment I aimed to determine whether test fish persisted in the use of recent experience when contradictory local enhancement, and current and prior public information cues were available from stimulus conspecifics. Thirty-six groups of four fish were established in their own subhabitat conditioning tanks as described above. Twelve groups were used for each of three parts of experiment 3. In all groups fish were conditioned to preferentially forage on the sand substrate type for 14 days prior to testing. Additionally, I set up 12 identically sized tanks of four fish each in which the substrate was bare Perspex. These were size-matched to the experimental fish, but had had no experience of either substrate type during the six-month period for which they had been held in the laboratory. These were also held for 14 days.

2.2.8 Local enhancement

The experimental test tank described above was modified by the addition of two stimulus chambers. These were 25 cm tall, 10 cm diameter clear acrylic cylinders with four 2mm diameter perforations per cm² of vertical surface and a clear unperforated acrylic base covered with the corresponding substrate material. Each chamber was placed centrally within one of subhabitat types, and contained a group of stimulus fish, size matched to one another and to the focal fish. These were randomly drawn from laboratory stock tanks housing fish that were not otherwise used in this study. Test fish had had no prior experience of these fish, and therefore were unfamiliar with them. No stimulus fish was used more than once.

I conducted four sets of trials; eight fish in the sand subhabitat versus three in the gravel subhabitat, suggesting better foraging in the sand subhabitat (Coolen et al. 2003), and in agreement with previous experience; three fish in the sand subhabitat versus eight in the
gravel subhabitat, in contradiction to previous experience; and two control experiments. In the first control, three fish were present in each subhabitat and the focal fish was conditioned to prefer the sand subhabitat as in the previous two trial sets. In the second control three fish were also present in each subhabitat, however the focal fish were derived from the Perspex substrate tanks, and had no pre-existing subhabitat preference. Test fish were held in the holding tower as described in the experimental tank procedure above for three minutes, during which time they could observe both stimulus shoals. Prey density was equal on both substrate types outside the stimulus chambers with 0.008 prey items per cm$^2$; no prey was provided within the stimulus chambers. Trials progressed and data was collected as described above.

2.2.9 Current public information

The experimental test tank was set up with the stimulus chambers as described above for the local enhancement trials. In this set of trials, prey was provided within the stimulus chambers as well as outside, allowing the stimulus fish to feed, and the focal fish to monitor them. Prey items were introduced into the bottom of each stimulus chamber from a syringe, via a 10mm diameter opaque acrylic tube after the three-minute settling period of the focal fish, and immediately before it was released. The acrylic tube prevented the focal fish from seeing the delivery of the prey into the stimulus chamber. The substrate within the stimulus chamber was set lower than the surrounding substrate so that the focal fish was unable to see the prey within the chamber after its delivery. I conducted four sets of trials. Stimulus shoals each comprised three size matched fish on either substrate in each trial set. In the first trial set the stimulus chamber in the sand subhabitat was provided with 30 prey items, compared to 10 in the stimulus chamber in the gravel subhabitat, suggesting better foraging in the sand subhabitat, in agreement with previous experience. In the second trial set the number of prey items delivered to each stimulus chamber was reversed, suggesting better foraging in the
gravel subhabitat, in contradiction to previous experience. In the third trial series, 10 prey items were delivered to each stimulus chamber. In the final trial series, 10 prey items were also provided to each, however the focal fish were derived from the Perspex substrate tanks, and had no pre-existing subhabitat preference. Stimulus fish readily consumed prey items in all trials, though in no trial were all prey items within a stimulus chamber seen to be consumed before the trial ended. Prey density was equal on both substrate types outside the stimulus chambers, with 0.008 prey items per cm$^2$.

2.2.10 Prior public information

The procedure for assessing the influence of prior public information upon the foraging decisions of the focal fish was identical to that used in the current public information trial series, with the exception that the foraging cues provided by the stimulus fish were provided before the focal fish was allowed to forage. The focal fish was held in the holding tower for a three-minute period as previously. This was followed by the introduction of prey items to the stimulus chambers, also as previously. The focal fish, still within the holding tower, was allowed to observe the stimulus shoals feeding for three minutes, after which the stimulus shoals were removed. The focal fish was given a further one-minute settling period, and was then released from the holding tower, allowing the trial to progress as described above. There were four trial sets as described in the current public information section, and the prey density was equal on both substrate types outside the stimulus chambers, with 0.008 prey items per cm$^2$, also as above.

2.2.11 Statistical Analysis

In Part 1, for within treatment analyses, both time allocation and foraging rate data were analysed using Friedman tests with equal groups paired comparison post-hoc analyses (Langley 1979). To make comparisons between treatments I used Wilcoxon-signed rank
tests. In the control experiment, and in parts 2 and 3 I analysed time allocation and foraging rate data as follows: the proportion of time allocated to each subhabitat was converted to a proportion of the total trial time and the proportion of time allocated to the gravel subhabitat was then subtracted from that allocated to the sand subhabitat. These values were compared with a null expected value of zero using Wilcoxon Signed Rank Tests. All proportional data were arcsine transformed before analyses were performed.

2.3 RESULTS

2.3.1 Control experiment: pre-existing subhabitat preferences

The amount of foraging effort directed by fish to either experimental subhabitat was not statistically different, either in terms of time allocation (Wilcoxon signed ranks test: n= 12, Z = -0.31, P= 0.91), or foraging rate (n= 12, Z = -0.70 P= 0.48).

2.3.2 Experiment 1: The rise of subhabitat foraging preferences

The pattern of proportional time allocation to the subhabitats in the experimental treatment was seen to vary significantly over the duration of the study (Friedman test: $X^2_{(1.15)} = 137.90$, P<0.001). Post-hoc analysis revealed that the allocation of time to the sand subhabitat became significantly different from the first day of testing on day 14 (P= 0.05). Prey provision was switched to the gravel subhabitat on day 18; a post-hoc test showed that fish were allocating significantly more time to this subhabitat compared to day 18 by day 24 (P= 0.01). The proportional time allocation the control treatment, in which prey provision was not switched, also varied significantly over the course of the study (Friedman test: $X^2_{(1.15)} = 137.90$, P<0.001). A significant preference for the sand subhabitat arose by day 16 (Post-hoc analysis, P= 0.01), but there was no subsequent significant switch in subhabitat preferences as seen in the experimental treatment (Figure 2.1).
This pattern was also seen in the direction of foraging effort over time in the experimental treatment (Friedman test: $X^2_{(1, 15)} = 75.37, P = 0.001$). In this treatment foraging effort allocation to the sand subhabitat increased from the beginning of the study, becoming significantly different from the first day of testing by day 14 (Post-hoc analysis: $P = 0.05$). Following the switching of prey provision to the gravel subhabitat fish began allocation more foraging effort there, showing a significant preference for that subhabitat by day 26 compared to day 18 (Post-hoc analysis: $P = 0.001$). In the control treatment there was an initial increase over time in the allocation of foraging effort to the sand subhabitat ($X^2_{(1, 15)} = 26.19, P = 0.03$), which became significantly different from that seen on the first day of testing by day 16 (Post-hoc analysis: $P = 0.05$). As with time allocation, there was no subsequent switch in subhabitat preference in the control treatment (Figure 2.2).
Figure 2.1. Changes over the course of the experiment in the proportional time allocation (mean +/- Standard Error) by fish to either subhabitat type in a treatment where prey was provided in the sand subhabitat only over the duration of the study (a), and a treatment where prey provision was switched to the gravel subhabitat after 18 days (b). A positive value indicates preference for the sand subhabitat, and a negative value indicates preference for the gravel subhabitat.
Figure 2.2. Changes over the course of the experiment in the allocation of foraging effort (median +/- Quartiles) by fish to either subhabitat type in a treatment where prey was provided in the sand subhabitat only over the duration of the study (a), and a treatment where prey provision was switched to the gravel subhabitat after 18 days (b). Positive and negative values indicate greater foraging rates in the sand and gravel subhabitats respectively.
2.3.3 Experiment 2: Recent experience versus conflicting real-time assessment

Test fish provided with previous experience of finding prey in either gravel or sand subhabitats displayed significant foraging preferences for that subhabitat in trials. This was reflected both in the allocation of time and foraging rate.

2.3.4 Prey provided on the gravel subhabitat

Time allocation to the gravel subhabitat was significantly greater in trials where prey density was equal, and where it was greater in either the gravel or the sand subhabitat (Wilcoxon signed ranks test: n= 12, Z = 2.52, P= 0.012; n= 12, Z = -2.00, P= 0.045; n= 12, Z = -2.38, P= 0.017 respectively). This preference was also reflected in the foraging rates (n=12, Z= -2.50, P= 0.01; n=12, Z= -2.74, P= 0.006; and n=12, Z= -2.25, P= 0.02 respectively). In trials where no prey was present, no time allocation preferences were seen (n= 12, Z = -1.13, P= 0.25) (Figure 2.3).

2.3.5 Prey provided on the sand subhabitat

Time allocation to the sand subhabitat was seen to be significantly greater when prey density was equal in both subhabitats, when it was greater in the sand subhabitat, and also when it was greater in the gravel subhabitat (Wilcoxon signed ranks test: n= 12, Z = -2.12, P= 0.034; n= 12, Z = -2.85, P= 0.004; n= 12, Z = -2.21, P= 0.027 respectively). This was also true of the foraging rate (n=12, Z= -2.56, P= 0.009; n=12, Z= -2.93, P= 0.007; and n=12, Z= -2.28, P= 0.01 respectively). However, in trials where no prey was present in either subhabitat no time allocation preferences were seen (n= 12, Z = -0.19, P= 0.84) (Figure 2.4).
Figure 2.3. The difference in time allocation (a) and foraging rate (b) of fish conditioned to preferentially forage in the gravel subhabitat. The black bar indicates the median, the box shows the inter-quartile range and the error bars indicate 95% confidence intervals. A positive score indicates a preference for the sand subhabitat and a negative score indicates a preference for the gravel subhabitat.
Figure 2.4. The difference in time allocation (a) and foraging rate (b) of fish conditioned to preferentially forage in the sand subhabitat. The black bar indicates the median, the box shows the inter-quartile range and the error bars indicate 95% confidence intervals. A positive score indicates a preference for the sand subhabitat and a negative score indicates a preference for the gravel subhabitat.
2.3.6 Experiment 3: Recent experience versus conflicting local enhancement and public information

2.3.7 Local enhancement

Fish were seen to display time allocation preferences and greater foraging rates in the sand subhabitat when it held the larger stimulus shoal (Wilcoxon signed ranks test: time allocation: n= 12, Z = -3.21, P= 0.001; foraging rate: n=12 Z= -3.02, P= 0.002). When the stimulus shoal was larger on the gravel subhabitat they displayed a greater time allocation (n= 12, Z = -2.71, P= 0.005), but no difference in foraging rate (n= 12, Z= -1.16, P= 0.10). In trials where the stimulus shoals were identical in size no preference for either subhabitat was seen, either where test fish had been conditioned to prefer sand subhabitat (time allocation: n= 12, Z = -1.02, P= 0.30; foraging rate: n=12, Z= -0.05, P= 0.95) or where they had no previous experience of either (time allocation: n= 12, Z = -1.14, P= 0.15; foraging rate: n=12, Z= -0.36, P= 0.75) (Figure 2.5).

2.3.8 Current public information

When current public information cues were available test fish were seen to direct greater time allocation and foraging rate to the subhabitat indicated to be of higher quality. This was true when greater quality was inferred both of the sand subhabitat (time allocation: n= 12, Z = -3.00, P= 0.003; foraging rate: n=12, Z= -2.03, P= 0.02) and the gravel subhabitat (time allocation: n= 12, Z = -3.10, P= 0.002; foraging rate: n=12, Z= -2.69, P= 0.007). However, when both subhabitats were indicated to be of equal quality, no preference existed (time allocation: n= 12, Z = -0.27, P= 0.78; foraging rate: n=12, Z= -0.25, P= 0.79). Likewise, no preference existed in fish that had no previous experience of either subhabitat when both subhabitats were indicated to be of equal quality (time allocation: n= 12, Z = -0.54, P= 0.58; foraging rate: n=12, Z= -0.17, P= 0.86) (Figure 2.6).
2.3.9 Prior public information

Test fish apparently made no use of prior public information, eliciting a preference for the sand subhabitat to which they were preconditioned in trials where the sand subhabitat was previously indicated to be of higher quality (time allocation: n=12, Z = -3.00, P= 0.003; foraging rate: n=12, Z= -2.53, P= 0.01), where the gravel subhabitat was previously indicated to be of higher quality (time allocation: n=12, Z = -2.98, P= 0.003; foraging rate: n=12, Z= -2.95, P= 0.003), and when both patches were indicated to be of equal quality (time allocation: n=12, Z = -3.06, P= 0.002; foraging rate: n=12, Z= -2.84, P= 0.004). In trials where test fish had no previous experience of either subhabitat, when both subhabitats were previously indicated to be of equal quality, no preference was seen to exist (time allocation: n=12, Z = -0.70, P= 0.48; foraging rate: n=12, Z= -1.08, P= 0.28) (Figure 2.7).
Figure 2.5. The difference in time allocation (a) and foraging rate (b) of fish when local enhancement cues were provided. The x-axis labels indicate the subhabitat of inferred higher quality. Focal fish had been preconditioned to preferentially forage in the sand subhabitat, except in the control trials in which focal fish had no preconditioned subhabitat preference. The black bar indicates the median, the box shows the inter-quartile range and the error bars indicate 95% confidence intervals. A positive score indicates a preference for the sand subhabitat and a negative score indicates a preference for the gravel subhabitat.
Figure 2.6. The difference in time allocation (a) and foraging rate (b) of fish when current public information cues were provided. The x-axis labels indicate the subhabitat of inferred higher quality. Focal fish had been preconditioned to preferentially forage in the sand subhabitat, except in the control trials in which focal fish had no preconditioned subhabitat preference. The black bar indicates the median, the box shows the inter-quartile range and the error bars indicate 95% confidence intervals. A positive score indicates a preference for the sand subhabitat and a negative score indicates a preference for the gravel subhabitat.
Figure 2.7. The difference in time allocation (a) and foraging rate (b) of fish when prior public information cues were provided. The x-axis labels indicate the subhabitat of inferred higher quality. Focal fish had been preconditioned to preferentially forage in the sand subhabitat, except in the control trials in which focal fish had no preconditioned subhabitat preference. The black bar indicates the median, the box shows the inter-quartile range and the error bars indicate 95% confidence intervals. A positive score indicates a preference for the sand subhabitat and a negative score indicates a preference for the gravel subhabitat.
2.4 DISCUSSION

Individual test fish acquired foraging preferences for subhabitats in which prey had previously been consistently presented. These arose gradually and were maintained in experimental trials irrespective of the actual prey densities in either subhabitat. When local enhancement and current public information cues were provided, they were used by individuals in place of previous experience. Fish did not use prior public information however, and individuals tested in the absence of stimulus shoals that they had just observed feeding reverted to foraging preferences consistent with their previous experience. A control experiment revealed that fish had no pre-existing foraging preferences for either experimental subhabitat type.

It was seen that once established, preferences were strong, with fish selecting the subhabitat in which they had previous experience of finding prey irrespective of its actual prey density relative to the other subhabitat. Particle size differed between subhabitats and this probably influenced the availability of visual and chemical cues pertaining to the location of prey items since the larger inter-particle voids in the gravel subhabitat could have obscured prey. Despite this, test fish conditioned find prey in either subhabitat displayed foraging preferences there subsequently. Such a behavioural strategy appears to be maladaptive, and is contrary to optimal foraging theory (Charnov 1976; Stephens and Krebs 1986), which predicts that foragers should sample patches frequently, amassing and updating accurate information on each. Interestingly, earlier studies reveal that other species, including walleye pollock (*Theragra chalcogramma* Ryer and Olla 1995), Atlantic cod (*Gadus morhua* Steingrund and Fernö 1997), and turbot (*Scophthalmus maximus* Ellis et al. 2002) continue to exploit prey types or to persist in the use of foraging tactics that have recently yielded optimal returns, even when doing so becomes apparently sub-optimal. It is possible that if prey yield is consistently predictable in a certain subhabitat, it may become less costly to forage there than to expend effort and incur risk in sampling areas that have
held fewer prey on earlier occasions. In this study, following the change in the provision of prey to the gravel subhabitat, preference for the sand subhabitat was seen to persist initially before steadily degrading. This may be an example of reversal learning (Frank et al. 1972; Levin & Vergara 1987), generally considered to be costly since individuals must learn to recognise and handle new resources.

Additionally I found that fish did not display any significant subhabitat preference when no prey was provided. These findings suggest that positive reinforcement through the presence of prey is required for subhabitat preferences to persist. This could serve to readjust foraging preferences, countering maladaptive behaviours in which fish persistently exploit habitat or prey types that no longer provide optimal returns. The rate at which individual foraging preferences arise and decay may be linked to the temporal stability of the habitat in which the fish lives, something that has previously been shown to affect spatial memory capacity and retention (Mackney and Hughes 1995; Warburton 2003).

Individuals made use of local enhancement and current public information cues irrespective of their previous experience, foraging in the subhabitat of inferred higher quality, or partitioning effort equally between subhabitats when public information suggested no difference existed between them. When local enhancement cues were provided, fish always preferred to forage in the subhabitat that held the largest shoal. This is contrary to the dictates of ideal free distribution theory, but similar to the findings of Gotceitas & Colgan (1991) who documented a similar preference for association with numerically larger groups, suggesting that foraging fish may use the numbers of conspecifics at prey patches as a proxy to infer patch quality. Social foraging facilitates greater prey detection rates (Pitcher et al. 1982; Pitcher & Magurran 1983; Lachlan et al. 1997; Day et al. 2000), and the potential returns from foraging as a group member in an unknown subhabitat may outweigh those from foraging alone or in a smaller group, even in a previously preferred area. Whilst the numbers of foragers present may be a basic measure of patch prey yield, their feeding
rates provide more subtle information on patch quality (Coolen et al. 2003). If this information is available to an individual during the period in which it is selecting between patches, then it represents a more recent and reliable source than any private information coming from previous experience, and should therefore be given more weighting. As in this study, Coolen et al. (2003) found that threespine stickleback did not however use prior public information. They went on to show that the closely related ninespine stickleback did so, drawing upon cues from con- and heterospecifics alike. This interspecific difference is likely explained by the differences in habitat use and behaviour that separate and define their respective niches (Godin and Clark 1997; Hart 2003). The ninespine stickleback has less robust bodily armour than that of the threespine stickleback making it more vulnerable to predation, and it has been suggested that it therefore relies to a greater extent upon public information, which can be collected from cover (Coolen et al. 2003).

The adaptive benefits of using previous experience to enhance future foraging successes are clear, since the use of recalled information relating habitat structure to prey yield allows the potential to forage in a non-random manner, even in unexplored areas. Nonetheless caution must be exercised when considering the utility and prevalence of these behaviours in nature. This study provides strong evidence of the use of private information by individuals, but suggests that in the more naturally realistic situation of social foraging, public information use and social conformity predominate. Private information such as recent experience may be used by foragers in situations where public information is limited or lacking completely, where risks associated with social non-conformity, such as enhanced predation risk are low, or where potential pay-offs from innovative behaviour are high. Previous studies have shown that species including ninespine stickleback (Coolen et al. 2003; van Bergen et al. 2004), and guppies (*Poecilia reticulata* Kendal et al. 2004) discriminate between private and social information, selecting the most reliable source, and foregoing social conformity only when the potential costs of doing so are low. This is
consistent with the social release hypothesis (Brown & Laland 2002), which suggests that whilst social conformity prevails, in the absence of demonstrators, naïve individuals have the opportunity to innovate. Related to this, hungry fish may trade off the benefits of shoaling to improve their potential share of any prey that is found (Krause, 1993). Internal state, a relative constant in this study, could therefore also be a factor in determining which information source is acted upon. In order to support these assertions further research is required.
Chapter 3

Habitat specific chemical cues and shoaling preferences
3.1 INTRODUCTION

Animals that forage in groups can use cues generated by other foragers in order to gain information about prey patch location and quality. In Chapter 2 I found that threespine sticklebacks preferentially used local enhancement and public information cues over their own learned private information when foraging, reasoning that such social conformity might allow them to reduce costs associated with predation risk. In this chapter I investigated the use of another type of social information, one that can be derived from the assimilation and self-referent matching of habitat specific chemical cues.

Though group living is common in nature, group composition is often unstable and highly dynamic, changing through fission and fusion processes that operate under a variety of dynamic environmental, predation, foraging, and sexual pressures (Raman 1997; Hoare et al. 2000; Croft et al. 2003). Consequently, individuals may have to make frequent decisions about which groups to join, and which individuals to associate with. Group membership has adaptive implications, relating to for example, levels of intraspecific competition (Peuhkuri 1997), and oddity effects (Theodorakis 1989). As a consequence of this, social groups in nature tend to be highly sorted by general factors such as species, phenotype and parasite load (Krause et al. 1996; Hoare et al. 2000), whilst individuals within these groups make fine-scale assessments based upon further, more subtle criteria.

In shoaling fishes, recognition of individual conspecifics has been posited as an important mechanism facilitating fine-scale shoal assortment, and numerous laboratory studies have revealed that fish of many species preferentially associate with individuals with which they have recently and repeatedly interacted (reviewed by Griffiths 2003; Ward and Hart 2003; Griffiths and Ward 2006). This effect is termed familiarity, and familiar shoals have been shown to engage in lower levels of agonistic prey competition (Højesjø et al. 1998; Utne-Palm and Hart 2000; Seppä et al. 2001; Chapters 4 and 5 in this thesis), to forage more
efficiency (Swaney et al. 2001; Ward and Hart 2005), to transfer information and innovative
behaviors more rapidly amongst members (Laland and Williams 1997; Lachlan et al. 1998).
and to benefit from lower predation risk through greater shoal cohesion (Chivers et al. 1995).
Evidence for familiarity as an important shoal structuring mechanism in nature remains
equivocal however. Shoal fidelity and stable shoaling preferences have been reported in
some systems (see Klimley and Holloway 1999; Griffiths and Magurran 1997a; Ward et al.
2002), whilst others detected no evidence of persistent association patterns (see Helfman
1984; Hoare et al. 2000; Godin et al. 2003; Magurran and Queiroz 2003). This may be
attributed to the fact that whilst these shoaling preferences are seen to arise over several days
or weeks in the laboratory (Griffiths and Magurran 1997b; Croft et al. 2004a), the
composition of free-ranging shoals often changes within seconds to hours in nature (Hoare et
al. 2000; Croft et al. 2003). Furthermore, the cognitive demands of individual recognition
limit the number of individual identities that can be learned (Griffiths and Magurran 1997a).

Recent research has identified a more parsimonious mechanism of recognition, one based
upon self referent matching of recent prey and habitat use that may be used over familiar
(Salvelinus alpinus) preferentially associated with conspecifics that had been fed upon the
same type of artificial feed. Ward et al (2004; 2005) found that threespine stickleback
shoaled to a greater extent with individuals that had not only consumed the same prey as
themselves, but also those that had spent time in the same habitat. Unlike preferences based
upon learned recognition, these association preferences were evident after a single day, and
did not assume previous interaction between individuals. These preferences were based upon
olfactory rather than visual cues, ones that could even be assimilated from closely related
heterospecifics, and most significantly, fish were seen to preferentially shoal with unfamiliar
conspecifics with a similar recent habitat use history over familiar fish that differed in this
respect.
The mechanism of self-referent habitat use matching potentially represents a general means of recognition, one that might allow foraging fish to match patterns of resource use. This study aimed, firstly to determine how quickly these preferences arose and decayed, and secondly to determine the effects of habitat use history upon shoal cohesion. I predicted that fish should develop preferences based upon habitat specific cues more rapidly that the period of 24 hours described by Ward et al. (2004; 2005), since a mechanism that is slow to detect and process habitat use patterns is likely to yield erroneous information leading to maladaptive behavioural responses. I also predicted that shoal cohesion should be greater in amongst groups that share a common recent habitat use history, allowing for greater potential for the detection of social information.

3.2 METHODS

3.2.1 Fish collection and housing

Several hundred juvenile threespine stickleback measuring 15 to 20 mm standard length were collected from Stonton Brook, Leicestershire, UK in July and August 2005. They were divided into groups of approximately 50, and each was placed into a chemically and visually isolated holding tank. (40 by 25 by 25 cm, water depth 20 cm, 1 cm deep fine sand substrate, flow through rate 0.1 L / min). The water temperature and light: dark regimes were held at 10°C and 12: 12 hours respectively for the duration of the experimental period. They were fed frozen Chironomid larvae once per day. Fish were held under these conditions for eight weeks prior to the beginning of the study.

3.2.2 Part 1. How long do association preferences based upon habitat chemical cues take to break down and build up?
Previous studies have shown that fish preferentially associate with others with similar habitat experience to themselves (Ward et al. 2004; 2005). Here I investigated the short-term breakdown and build up of these preferences by quantifying the changing association preferences of focal fish that had been moved between habitat treatments in a laboratory study.

### 3.2.3 Habitat conditioning treatments

I used two habitat treatments, which I termed treatments A and B. Treatment A consisted of regular freshwater obtained from the recirculating laboratory supply, and simulated areas of habitat free from decaying organic matter. Treatment B simulated a habitat unit with high concentrations of tannins, characteristic of areas where decaying vegetation matter accumulates, such as beneath riparian vegetation, along wind-exposed littoral zones of still-water habitats, or in slow-flowing depositional habitats in stream channels. I replicated these habitat conditions by using 0.5 ml / L of a purpose designed solution (Blackwater Extract, Tetra GmbH. Herrenteich 78, 49324 Melle, Germany). Both of these habitat types occur along the channel of Stonton brook, giving the experimental design ecological relevance.

I performed four habitat time exposure experiments. Within each experiment I performed 12 trials where the focal fish had been conditioned to habitat treatment A, and 12 where it had been conditioned to habitat treatment B, giving a total of 24 trials per experiment. Focal fish were conditioned individually to their respective habitat treatment in visually and chemically isolated 12 L aquaria. These were not connected to the recirculating flow through system of the laboratory, and were set in 10°C water baths in order to maintain constant temperatures. Stimulus fish were also held under these conditions, separate from the focal fish, at a density of 6 fish per tank. Fish were held in habitat conditioning tanks for a period of 48 hours before trials began during which time they were not fed.

In the first habitat exposure experiment focal fish were exposed to habitat treatment A or B and then given a binary choice test between shoaling with stimulus fish from habitat
treatment A or B using the apparatus and procedure described below. In the second habitat exposure experiment, the focal fish were exposed to either habitat treatment A or B for 48 hours as above, but were then switched to the opposite habitat and held there for 30 minutes before being given the binary choice association test. That is, focal fish conditioned for 48 hours to treatment A, were then exposed to treatment B for 30 minutes before testing, and vice versa. In the third experiment focal fish were exposed to the opposite habitat for 120 minutes before being tested, and in the fourth and final experiment for 240 minutes.

Within each trial I used fish that had previously been housed together during the eight-week period prior to the beginning of the study. I did this in order to control for familiarity-based preferences (Griffiths 2003; Ward and Hart 2003; Griffiths and Ward 2006). I could not achieve this by simply segregating fish in the laboratory, since I had no data on their previous patterns of interaction in the field. Instead I ensured that whilst patterns of familiarity were potentially high between individuals within trials they were also homogenous.

### 3.2.4 Experimental tank

I tested association preferences using a standard binary choice experiment in an experimental tank (39cm by 17cm by 18cm deep, water depth 15 cm). At each end of the tank along its longest axis was an 8cm wide stimulus chamber, separated from the central section of the tank by screens of colorless perforated plastic (perforation diameter 0.1 cm, 5±1 perforations/cm²). This allowed the exchange of both visual and chemical cues. A 2 cm deep substrate of 0.5 cm aquarium gravel was provided in the central section of the tank and in the two stimulus chambers. Three 10 cm lengths of artificial plastic vegetation were floated on the surface in each of the stimulus chambers. These served to keep the stimulus fish from becoming stressed by providing overhead cover. On the outside of the glass I marked two association zones, indicated by vertical black lines, 2 cm from each of the
stimulus chambers. This distance falls well within the range of inter-individual distances seen in free-ranging shoals (Pitcher and Parrish 1993). The experimental tank contained freshwater obtained from the recirculating laboratory supply. Water from the recirculating laboratory supply was pumped into the centre of each of the two stimulus chambers at a rate of 20 cm$^3$ per minute, and allowed to drain out of an overflow outlet located at the waterline at the centre of the rear wall of the tank. This served to carry chemical cues from stimulus fish from either compartment into the central section of the tank where the focal fish was housed. Two test tanks were set up, and used alternately between trials.

3.2.5 Experimental procedure

Within each trial, the focal and stimulus fish were size matched by standard length to within <1 mm of each other. Three stimulus fish from habitat treatment A were added to one stimulus chamber, and three from habitat treatment B were added to the other. Each group of three stimulus fish was taken from within the same conditioning tank. Their positions were randomised between trials in order to control for tank-end bias. These were allowed to settle for 2 minutes. A single focal fish was then selected and was placed within a 7cm by 7cm by 22cm tall holding unit constructed from the same perforated material as the stimulus compartment screens. The holding unit was situated in the centre of the test tank. The focal fish was held with the holding unit for 1 minute, during which time it could assimilate visual and chemical cues from the stimulus shoals. The holding unit was then removed and the focal fish released, beginning the trial. The trial duration was 2 minutes and I recorded the first shoaling choice of the focal fish and the total amount of time it spent shoaling with either stimulus shoal. Focal fish from habitat treatments A and B were tested alternately.

Following each trial I added new stimulus shoals to the second test tank. While these were settling for 2 minutes I changed the water in the first test tank to prevent habitat cues from accumulating between trials. When adding stimulus shoals and focal fish I took care
not to add water or debris from the treatment tanks. This ensured that any habitat cues generated came from the stimulus fish only.

3.2.6 Statistical analyses

I investigated the first choice of the focal fish by comparing the number of trials in which they first shoaled with fish from the same habitat treatment as themselves against the number of trials in which they first shoaled with fish from the other habitat treatment, using a binary test with a null expected distribution of 50%. I compared shoaling preferences by subtracting the proportion of time spent by the test fish shoaling with fish from the same habitat, from the amount of time it spent shoaling with fish from the other habitat, and compared these values to a null expected value of zero using Wilcoxon signed rank tests. I did this for each of the four habitat exposure time experiments. Finally, I compared these values between the four habitat exposure time experiments using a one-way ANOVA. All time data were converted into proportions of total trial time and arcsine transformed before analyses were carried out.

3.2.7 Part 2. Do habitat specific chemical cues influence shoal cohesion?

I sought to determine whether heterogeneity in recent habitat experience affected shoal cohesion. I ran an experiment with four treatments: one mixed shoal treatment, where each fish in the shoal had been conditioned within a different habitat, and three comparison treatments, in which all the fish in each shoal had been conditioned to the same habitat. Shoals contained four fish size matched to one another to within <1 mm and I conducted 20 trials per treatment.

3.2.8 Mixed habitat experiment
I used four habitat conditions, one freshwater, using water taken from the recirculating flow through system of the laboratory, one of low and one of high concentration tannin conditions (0.25 ml / L and 0.75 ml / L respectively of blackwater extract) and one with saline water (specific gravity 1.012, using Instant Ocean synthetic sea salt, Aquarium Systems, Sarrebourg Cedex, France). Three-spine sticklebacks live in both freshwater and marine habitats, and are commonly found in tidal estuaries, so the latter saline treatment is ecologically relevant.

Fish were conditioned to their respective habitat treatment individually in visually and chemically isolated 12 L aquaria. These were not connected to the recirculating flow through system of the laboratory, and were set in 10°C water baths in order to maintain constant temperatures. Fish were held under these conditions for 48 hours before being tested, using the procedure described below.

3.2.9 Comparison treatments

These were carried out as above except that within each treatment all fish were conditioned to the same habitat conditions. I ran one freshwater set, in which all of the 12 L conditioning aquaria contained water obtained from the recirculating laboratory supply, and one saline set (specific gravity 1.012). I only ran one tannin set, using a concentration of 0.5ml / L blackwater extract, intermediate between the two used in the mixed habitat treatment described above. As above, fish were conditioned individually and I performed 20 trials per treatment.

3.2.10 Experimental tank and procedure

I used an experimental tank (60 cm by 45 cm by 15 cm deep, water depth of 8 cm) surrounded on all sides by 60 cm tall non-transparent screening. A remote controlled 3.2
A mega pixel digital camera attached to a tripod was placed centrally above the tank at a height of 80 cm.

Experimental shoals of four fish each were formed. Fish had been conditioned separately for the previous 48 hours, but I formed the shoals from fish that had been housed together in the laboratory immediately before this in order to control for familiarity effects, as described in part 1. In the mixed habitat experience treatment I selected one fish from each of the four habitat conditions to form a shoal of heterogeneous recent habitat experience. In the three comparison treatments all fish had been held under the same habitat conditions.

The members of the experimental shoal were placed together in the centre of the experimental tank, where they were allowed to settle for 5 minutes. As before care was taken to ensure that tank water or debris were not transferred to the test tank. Following this I took one digital photograph of the shoal every minute for a further 5 minutes, giving a total of 5 images per shoal. I used the digital measuring program TPSdig32 (Rohlf 2005) to analyze the images. For each image I measured the nearest neighbour distance for each individual. Measurements were made of the smallest distance between the bodies of the nearest neighbours. I also measured the standard length of each individual, with all of these measurements made in pixels. Each nearest neighbour distance was then divided by the mean standard length of the shoal, giving a measurement of inter-individual spacing that was standardised into body lengths. I made measurements in two dimensions, since the 8 cm water depth restricted the potential for shoals to form in three dimensions. For each experimental shoal I calculated the mean nearest neighbour distance based upon the five images taken during the trial, giving a total of 20 measurements each for the mixed habitat treatment, and for each of the three comparison treatments.

3.2.11 Statistical analysis
I compared nearest neighbour distances for each of the four treatments using a one-way ANOVA and Bonferroni post-hoc analyses.

3.3 RESULTS

3.3.1 Part 1. How long do association preferences based upon habitat chemical cues take to break down and build up?

When test fish were taken from their respective habitat treatment tank and tested immediately, they showed a significant preference for shoaling with stimulus fish from the same habitat treatment as themselves, both in terms of their first choice (binomial test, null expected distribution 50%: \( p<0.001 \)) and time spent shoaling with either stimulus shoal (Wilcoxon signed rank test: \( n=24, Z=-2.996, p=0.003 \)). When fish were transferred to the opposite habitat treatment for either 30 minutes or 120 minutes before being tested they showed no preference for either stimulus shoal, either in terms of their first choice (binomial test, null expected distribution 50%: \( p=1 \); and \( p=0.307, 30 \) minutes and \( p=0.003 \), 120 minutes respectively), or in terms of time spent shoaling (Wilcoxon signed rank test: \( n=24, Z=-1.443, p=0.148 \); and \( n=24, Z=-0.543, p=0.587, 30 \) minutes and 120 minutes respectively). When fish were transferred to the opposite habitat treatment for 240 minutes before being tested they showed an association preference for fish from the new habitat. This was not seen in terms of first choice (binomial test, null expected distribution 50%: \( p=0.063 \)), but it was clear in terms of time allocation (Wilcoxon signed rank test: \( n=24, Z=-2.557, p=0.010 \)).

Finally, the amount of time that the test fish spent shoaling with stimulus fish from the same habitat to which it was originally conditioned, was lower in treatments where the test fish had spent more time in the opposite habitat treatment (one-way ANOVA: \( F_{(3, 92)}= 9.419, p<0.001 \). Table 3.1. Bonferroni post-hoc, 0 minutes in opposite habitat versus 120 minutes in...
opposite habitat \( P < 0.001 \); 0 minutes in opposite habitat versus 240 minutes in opposite habitat \( P < 0.001 \). Figure 3.1).

**Figure 3.1.** Association preferences (proportion of trial time shoaling with fish from the same habitat minus proportion of trial time shoaling with fish from the different habitat +/- S.E.).

**Table 3.1.** The results of a one-way ANOVA comparing the proportion of time that focal fish from four treatment groups spent shoaling with stimulus groups that had been exposed to the same habitat as themselves. Fish from the four treatment groups had been exposed to an alternate habitat treatment for 0, 30, 120 and 240 minutes respectively (\( n = 24 \) fish per treatment group).

<table>
<thead>
<tr>
<th></th>
<th>Sum of Squares</th>
<th>Degrees of freedom</th>
<th>Mean Square</th>
<th>( F )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Between groups</strong></td>
<td>9196.60</td>
<td>3</td>
<td>9196.60</td>
<td>9.41</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Within groups</strong></td>
<td>3106.80</td>
<td>92</td>
<td>33.65</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>1228.64</td>
<td>95</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
3.3.2 Part 2. Do habitat chemical cues affect shoal cohesion?

Shoal cohesion was lower in the treatment where shoals were composed of fish from different habitat types compared to treatments where shoals were composed of fish from the same habitat type (one-way ANOVA: $F_{(3, 76)} = 16.578$, $P<0.001$. Table 3.2. Bonferroni post-hoc, $P<0.001$ in all cases. Figure 3.2).

![Figure 3.2](image)

Figure 3.2. Shoals composed of fish from the same habitat were more cohesive than shoals composed of fish from different habitats (mean nearest neighbor distance +/- S.E.).
Table 3.2. The results of a one-way ANOVA comparing mean nearest neighbour distances within shoals of fish in which all constituent group members had been housed under the same or different habitat treatments (n=20 shoals per treatment).

<table>
<thead>
<tr>
<th></th>
<th>Sum of Squares</th>
<th>Degrees of freedom</th>
<th>Mean Square</th>
<th>F</th>
<th>P</th>
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<td>Between groups</td>
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<td>7410.69</td>
<td>16.57</td>
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<tr>
<td>Within groups</td>
<td>3535.78</td>
<td>76</td>
<td>33.65</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>829.00</td>
<td>79</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3.4 DISCUSSION

This study shows that the association preferences of threespine sticklebacks for conspecifics that have recently occupied the same habitat as themselves alter on a timescale of hours to reflect changes in the choosing individual’s own habitat use. Furthermore, it was seen that individuals from the same habitat form more cohesive shoals than do those with differing habitat use history.

Associating with others that are using the same resources likely infers both benefits and costs. Social foragers benefit from higher rates of prey detection than do solitary foragers. and by shoaling with others that are exploiting similar units of habitat, a forager may benefit by assimilating and acting upon social cues pertaining to the location and means of access to specific resources that a solitary individual may not otherwise detect. Viewed in this context, association preferences mediated by habitat-use specific cues may represent a form of social or informational parasitism. Whether such habitat cues allow receivers to indirectly gauge resource quality, that is, whether they constitute a form of public information (Valone and Templeton 2002) is unknown, and is worthy of further investigation. A likely cost of actively grouping with others that are exploiting similar resources is increased competition. This may be manifest in various forms, ranging from exploitative competition, whereby the
presence of additional individuals causes the resource to be depleted more rapidly. To contest competition, where individuals actively and aggressively compete over a resource (Ward et al. 2006). These, and other costs and benefits must be balanced against each other by individuals when making decisions on whether to join a group, and may in nature be subject to trade-offs dictated by the intensity of prevailing local environmental and social pressures.

This mechanism of recognition is potentially widespread in nature since many habitats comprise multiple, distinct subunits, and individuals within populations vary their use of these through space and time. These patterns of variation may be discrete, as in the case of trophic polymorphism (Bolnick et al. 2003), or they maybe be periodic, reflecting patterns of short-term use of certain resources by ecological generalists. Trophic polymorphism is commonly manifest in fishes as division between benthic and pelagic resource specialization (e.g. Robinson and Wilson 1994; Mittelbach et al. 1999; Proulx and Magnan 2004).

Ecomorphs specializing upon resources in one habitat type are thought to trade away foraging efficiency in others (Svanbäck and Eklöv 2003; 2004), however little is know about how individuals move between these habitat units, and at what rates they encounter and interact with other habitat specialists. It is conceivable that encounters between habitat specialists may occur regularly, particularly along the frontiers and transitional zones between different habitat types. Here, the ability to recognize and discriminate between resource-use specific cues could allow individuals to assess potential shoal mates, enabling them to ally patterns of resource use, and to avoid associating with groups that are exploiting different prey, something that may put them at a competitive disadvantage. Even at the within-habitat scale, generalist foragers often specialise upon resources within foraging sessions, for example by adopting search images when hunting for cryptic or concealed prey (Reid and Shettleworth 1992; Morgan and Brown 1996). Such behavior can lead to individuals disproportionately concentrating foraging effort upon certain prey types (Quevedo and Olsson 2006), or subunits of the habitat (Webster and Hart 2004; 2006;
Chapter 2, this thesis), even though resource use may vary between foraging sessions over the course of a day. Here it was seen that the ability to detect differences between the habitat use of conspecifics after just four hours, a much shorter time period than the 24 hours previously reported by Ward et al. (2005). The ability to detect short-term changes in the nature of the habitat specific cues accrued and released by different groups of foragers allows individuals to recognize and discriminate between potential shoal mates, and to accurately match their own resource use patterns accordingly.

It was also seen that shoals composed of individuals with shared recent habitat history were more cohesive than those from different habitat types, with the observed inter-individual distances falling well within those determined by Pitcher and Parish (1993) to constitute active shoaling, even in the mixed habitat cue treatment. Assuming that a function of shoaling with groups engaged in similar resource use patterns is to increase foraging efficiency via informational parasitism, then such foragers may form more cohesive groups in order to better observe and assimilate social information cues. Such cues are likely to be visual in mode, revealing the successful discovery or capture of prey, and allowing the observer to join the finder in exploiting the patch, or to engage in kleptoparasitic behavior. Maintaining close proximity to others may be especially necessary in structurally complex habitats where fields of vision are otherwise restricted. Where individuals differ in resource use they may form looser aggregations because differences in individual search image use render social information less valuable, or because heterogeneity in prey detection and handling efficiency puts some individuals at a foraging disadvantage, increasing the costs of competition.

Associating with others of shared habitat use history may infer a second advantage, in that it may reduce predation risk by decreasing olfactory oddity. Many predators use prey odor cues when hunting, and prey species may evolve behavioral counter measures in order to minimize their risk of detection or capture by predators that use such cues (e.g. Roberts et
al. 2001; Reneerkens et al. 2005; Pastro and Banks 2006). In this context, grouping with other fish that are producing similar habitat specific chemical cues may seem counterintuitive, since doing so potentially presents a larger or more concentrated stimulus to searching predators. However it may be that by doing so, a group of fish present a common olfactory profile, something that may reduce per capita predation risk at close quarters through the reduction of the oddity effect. They may further off-set this disadvantage by forming more cohesive shoals as observed in part 2 of this study.

That habitat derived cues influence social interactions between fish is a relatively new finding, and there exists the possibility that some of the behaviours previously ascribed to the recognition of familiar individuals may be actually facilitated by this more general means of recognition. Certainly familiar recognition is used by fish in numerous behavioural contexts, such predator inspection (Milinski, 1990), and the studies detailed in following chapters of this thesis investigate its role in competitive interactions. Nonetheless, much further research is needed in order to identify and separate the relative roles of these two mechanisms in influencing social interactions between fish.
Chapter 4

Social foraging and prey competition a):

Effects of group stability and prey distribution
4.1 INTRODUCTION

Group living is widespread in nature, and animals may actively aggregate for a variety of reasons. Individuals that forage together bear lower per capita vigilance and predation risk costs and stand to gain from potentially higher prey detection rates compared to those foraging alone. A detriment of social foraging is the increased potential for competition with conspecifics for prey once it is discovered, since animals are often compelled to compete amongst themselves in order to maximize their share of it. Competition can be costly in terms of time and energy expenditure or risk of injury or predation, and selection should favour the adoption of behaviors that most efficiently minimize the intensity or duration of conflict whilst simultaneously preserving the benefits of sociality (Giraldeau and Caraco 2000).

These costs can be reduced if individuals are able to discriminate between their group mates in a competitive context on the basis of recent associations and interactions and moderate their own behavior accordingly when interacting with others of higher or lower competitive standing. This allows disputes to be settled without the need for intense or prolonged agonistic conflict (Barnard and Burke 1979). Research has revealed that species in many taxa posses the capacity to recognise familiar individuals and to change certain behaviors when interacting with them (Barnard and Burke 1979; De Vries 1998; Dugatkin and Earley 2004), and a great deal of work on familiarity has been carried out on shoaling fish.

This work (reviewed by Griffiths 2003; Ward and Hart 2003; Griffiths and Ward 2006) has shown that individuals in many species prefer to associate with familiars over non-familiars. It has revealed that in some species familiar groups forage more efficiently than groups composed of unfamiliars (Swaney et al. 2001), that they are more cohesive and may therefore be less susceptible to predation (Chivers 1995), and that information diffuses more
rapidly between members (Laland and Williams 1997; Lachlan et al. 1998). Familiarity is also considered to be an important factor in reducing aggression within groups of foraging fish (Seppä et al. 2001), however despite the large literature on familiarity effects in general, relatively few studies have specifically examined the role of familiarity in relation to prey competition.

One study, a detailed analysis of the effects of individual recognition upon social behavior in sea trout (*Salmo trutta*) by Höjesjö et al. (1998) revealed that food intake and growth were higher, and that dominance hierarchies were more stable in familiar than in unfamiliar groups. Similarly, Seppä et al. (2001) showed greater growth rates and lower mortality in familiar groups of Arctic char (*Salvelinus alpinus*). Utne-Palm and Hart (2000) studied prey resource share and aggression within pairs of threespine sticklebacks as a function of time spent together and showed that in longer established pairs the disparity in prey share and levels of foraging related aggression were lower.

This study aimed to identify temporal changes in kleptoparasitic prey competition as familiarity developed within groups of initially unfamiliar fish. Kleptoparasitism, in this study, took the form of contest competition, with aggressive interaction between the captor and challenger for prey items. Contest competition is distinct from both scramble and exploitation competition (Ward et al. 2006), although both of these can also be used alongside kleptoparasitic foraging strategies. I selected contest competition for the focus of this study, as it is costly to the kleptoparasite in terms of lost foraging time and potentially heightened predation risk through lower vigilance and greater conspicuousness. costs that are balanced against the benefit of usurping another’s foraging effort. Using threespine stickleback I created shoals of five fish and monitored foraging rates and related competitive behaviors in each individual over a four-week period.

I looked at two different prey distributions, concentrated and dispersed, determined by the spatio-temporal presentation of prey. The concentrated distribution treatment considered
prey that was presented simultaneously in a spatially focused patch. This is ecologically relevant since foraging effort is often mediated by fine-scale habitat structure (Webster and Hart 2004; 2006. Chapter 2 in this thesis) and many models of optimum foraging assume such patchy prey distribution. (Charnov 1976; Stephens and Krebs 1986). The dispersed distribution treatment considered prey that was presented sequentially and in differing locations. In nature drifting prey can assume an unpredictable spatio-temporal distribution, and is known to form a substantial proportion of the diet of many stream dwelling fishes (Flecker, 1992) such as those used in our study.

I predicted that levels of kleptoparasitism would be greater when prey was spatially and temporally dispersed compared to when it was concentrated, since the value of competing for a given item is greater given that no other prey are present at that moment. I also predicted that levels of kleptoparasitism, and the disparity in prey share between individuals should fall over time, and that individuals should make adaptive decisions about which shoal mates they associate with based upon their relative competitive abilities.

4.2 METHODS

4.2.1 Fish Collection and Housing

Two hundred sub-adult threespine stickleback measuring 25 to 30 mm standard length were collected from a 350 m long reach of Stonton Brook, Leicestershire, UK in September 2004 using dip nets. They were transported by road for 40 minutes to the laboratory, where they were divided equally between 16 chemically and visually isolated holding tanks (40 by 25 by 25 cm, water depth 20 cm) with a 1 cm deep fine sand substrate. Fish were fed frozen Chironomid larvae once per day. The water temperature and light: dark regimes were held at 11°C and 12: 12 hours respectively over the duration of the study. Fish were held under these conditions for six weeks.
4.2.2 Experimental Groups

Thirty-six experimental groups comprising five fish each were created, with individuals within each experimental group drawn from different holding tanks. Twelve groups were used in concentrated prey trials, 12 in dispersed prey trials, and 12 were used in a control experiment designed to control for prey delivery rate predictability in the dispersed prey trials. In each group fish were size matched to within <1 mm standard length and every individual was given an identification tag (described below). Experimental groups were each housed within their own chemically and visually isolated tanks (22 x 15 x 15 cm, 1 cm fine sand substrate).

Fish were tested in foraging trials on every fourth day over a 28 day period as described below. On the days in which the fish were not tested they were fed frozen Chironomid larvae once per day. These were provided in excess and were distributed evenly within the tank. This ensured that all fish were able to feed to satiation on the days that they were not tested, since I had predicted that differences in foraging ability would result in different levels of prey intake during trials. If prey were limited over the course of the whole study (rather than only on the days of testing) then one might expect to see differences in nutritional status between individuals leading to differences in foraging and competitive motivation that could affect our results. This method of feeding was used in both the concentrated and dispersed prey trials. Providing excess prey and conducting tests at four day intervals thus ensured that hunger levels were standardized between trials.

4.2.3 Tagging Procedure

In order to be able to recognise individuals within experimental groups I gave each fish an identification tag. I used fluorescing Visible Implant Elastomer (VIE) tags, a purpose-
designed product manufactured by Northwest Marine Technology Inc. Fish were first cooled in 6°C water for several minutes. This was performed in place of anaesthesia, which can cause high levels of stress and mortality in such small fish (personal observation). A tag measuring approximately 3 by 0.4 mm was implanted into the dorsal surface of each fish using a 0.4 mm diameter needle. Tags were positioned in front of, along side, or behind the first dorsal spine and yellow or green tags were used, producing a unique mark for each group member. Immediately after tagging, fish were transferred to aerated 11°C water to recover. No fish died following this procedure. The tissues of the dorsal musculature were sufficiently transparent for the tags to be visible when fish were viewed side on and all fish were seen to retain their tags over the duration of the study.

4.2.4 Competition for dispersed prey: experimental arena

Trials took place in an experimental tank (40 by 25 by 25 cm, water depth 20 cm, 1 cm deep fine sand substrate), the sides and rear of which were non-transparent, to minimise outside disturbance. Observations were made via a vision slit in an opaque screen to remove observer effects. One side of the tank contained a row of five equally spaced 5 mm holes at the waterline. These served as prey introduction points, through which prey items could be introduced over the course of a trial via a 5 cm³ syringe. Twenty-five 5 mm long sections of Chironomid larvae were used as prey. These were introduced sequentially, as described below. Fish of the size used in this study have been seen to consume up to ten 5mm Chironomid sections within five minutes (Webster, unpublished data), so competition for prey should be expected to persist even when an individual has consumed its expected share of five prey items.
4.2.5 Competition for dispersed prey: experimental procedure

Food was withheld for 18 hours before the trial began. Experimental groups were transferred from their respective tank to the holding unit, where they were allowed to acclimatize for a settling period of 5 minutes before the holding unit was removed and the trial began. Individual prey items were added through the prey introduction points, and 10 seconds were allowed to elapse between the ingestion of one prey item before the introduction of the next. Prey was introduced through a different point each time, in a pre-determined random order. I recorded the number of prey items consumed by each individual, and the number of contested prey items. Tests were repeated every fourth day for 28 days following experimental group formation.

4.2.6 Competition for dispersed prey: control for prey delivery rate predictability

A fall in the contest rate for prey over time in the dispersed prey experimental series could be attributed either to changes in behaviour as a function of the time the shoal had spent together, or to learning of the prey delivery rate by test fish. That is, if over time fish were to learn that multiple prey would be presented over the course of the trial, then the value of competing for singular items may be diminished. In order to separate these effects I conducted a control experiment in which 12 groups of five fish were housed under the same conditions as those in the dispersed prey competition experiment, but were not tested until day 28, the final day of testing. If the contest rate in these groups was not significantly lower than that seen on day 4, the first day of testing in the dispersed prey competition groups, I would be unable to rule out experience of the prey delivery rate as a causal factor of any fall in the contest rate for prey over time in the distributed prey competition experiment. I used the same apparatus and procedure as described above, with fish in this control experiment being deprived of food for 18 hours as they were in the dispersed prey competition experiments every fourth day, the exception being that testing was only conducted on day
28. I recorded the number of prey items eaten by each individual and the number of contests that occurred.

4.2.7 Competition for concentrated prey: experimental arena

Trials took place in an experimental tank (40 by 25 by 25 cm. water depth 20 cm. 1 cm deep fine sand substrate) with non-transparent side and rear walls. Observations were made via a vision slit in an opaque screen. Twenty-five 5 mm long sections of dead Chironomid larvae were distributed equally within a 12 cm square. 1 cm tall colourless Perspex dish set into the substrate. The dish also contained substrate material and was situated centrally on the bottom at one end of the tank. Prey items were immobile and were placed upon the surface of the substrate in the dish. At the opposite end of the observation tank, 21 cm from the prey patch, a holding unit was situated. This consisted of a 7 cm square, 22 cm tall tower constructed from perforated colourless Perspex. This was used to hold experimental groups prior to testing, allowing them to acclimate, and facilitated visual and chemical assessment of the experimental arena.

4.2.8 Competition for concentrated prey: experimental procedure

Test fish were deprived of food for 18 hours prior to the commencement of trials in order to increase foraging motivation. Experimental groups were transferred from their respective tank to the holding unit, where they were allowed to acclimatize for a settling period of 5 minutes. Following the settling period, the holding tower was removed and the fish released into the arena. Fish were allowed to explore the arena and the trial began when a fish detected and engaged the first prey item. Trials ran for 5 minutes, or until all prey had been consumed, which ever occurred first, and I recorded the number of prey items eaten by each individual and the number of contests that occurred. Trials were repeated every fourth day for 28 days following experimental group formation.
4.2.9 Kleptoparasitic prey contests

Kleptoparasitic prey contests occurred when an individual attempted to obtain a prey item from the jaws of the fish that had originally captured it. This behaviour had two components, the pursuit of the initial captor by the challenger, followed by the seizure of the prey by the challenger, resulting in either the retention and ingestion of the prey by the captor or it being yielded to the challenger. The prey items used in this study were of sufficient size to require a period of handling before ingestion, thus providing the opportunity for kleptoparasitism to occur.

4.2.10 Relative competitive ability and association preference

At the end of the experimental period, 3 days after the final foraging trial and 31 days post group formation, I sought to determine whether individuals could identify shoal mates with respect to their relative competitive ability, and whether they displayed association preferences based upon this. Within each experimental group, for both prey distribution treatments, I determined the mean proportional prey share consumed by each individual over the whole study period. Based on prey share rankings I found that within each group two individuals consumed substantially more prey than did the other three group members on average (see results section). I randomly selected one of these three fish as a focal fish in each group. This individual was then presented with a standard binary choice between shoaling with either the two fish that obtained higher prey shares than it did or the two individuals with which it shared a similar amount of prey over the course of the study (Metcalfe and Thomson 1995).

Binary choice experiments were conducted in a 39cm by 17cm by 18cm deep, water depth 15 cm binary choice arena with a 1cm deep fine sand substrate. At either end of the arena was a 6 cm wide stimulus compartment in which the stimulus fish were housed. The focal fish was held in a 7cm by 7cm by 22 cm tall holding tower prior to the commencement
of the trial. The walls of the holding tower and the stimulus compartments were constructed from colourless perforated Perspex, allowing visual and chemical exchanges to occur. I conducted trials in the absence of prey as I sought to determine whether the focal individual displayed an associated preference based upon condition independent individual recognition, rather than through the use of overt cues from the stimulus fish such as prey handling ability or aggressive behavior. Focal fish were hunger motivated through 18 hours of food deprivation prior to each trial. After a 5 minute settling period, the tower was raised, the focal fish released, and the trial commenced. Each trial ran for 3 minutes, and I recorded the proportion of time the focal fish spent within 5 cm of either stimulus group compartment.

4.2.11 Statistical Analyses

I used the Friedman test, a non-parametric repeated measures analysis, and performed post-hoc analyses using equal groups paired comparisons as described by Langley (1979) to compare differences in the coefficient of variance of prey share, and contest rates for prey between days of testing within the two prey distribution treatments. I calculated the coefficient of variance of prey share for each group on each day, comparing these between days, as described above. If the coefficient of variance were to increase over days of testing this would indicate that prey share was becoming less equal within groups. Conversely, if it decreased, this would imply prey share was becoming more equal. The mean prey share of each individual in each group over the whole study period was determined. I calculated the coefficient of variance of mean prey share for each group and compared this to a null expected value of zero using Wilcoxon signed rank tests. A value of zero would be obtained if prey share were equal within groups, since the standard deviation would also be zero. Next I determined the daily contest rate by dividing the number of contested prey items within each group by the total number consumed on each day of testing. I compared differences in overall contest rates for prey within days of testing between prey distribution treatments, and
between the dispersed prey treatment and the control experiment using Mann-Whitney U tests. Finally I calculated the shoaling preferences of lower ranked fish for better or similar-ranked individuals by converting the amount of time allocated to each stimulus shoal to a proportion of the total time spent shoaling, subtracting one from the other and comparing this to a null expected value of zero using Wilcoxon signed rank tests.

4.3 RESULTS

4.3.1 Prey resource share

4.3.2 Dispersed Prey

The mean share of prey consumed by each individual over the duration of the study was not equal (Wilcoxon signed rank test comparing the coefficient of variance of mean prey share with a null expected value of zero: $Z = -3.59, p = 0.001$ Figure 4.1a). A Friedman test of the coefficient of variance of prey share between days of testing revealed no changes in prey share within groups over time $X^2_{(1, 6)} = 4.22, n= 12, P=0.64$.

4.3.3 Concentrated Prey

Individual prey share was unequal, both for mean prey share (Wilcoxon signed rank test: $Z = -3.06, p=0.002$ Figure 4.1b) and prey share patterns over time (Friedman test: $X^2_{(1, 6)} = 6.07, n = 12, P=0.41$).

4.3.4 Contest rate for prey

4.3.5 Dispersed Prey

The prey contest rate decreased significantly over the 28 day study period (Friedman test: $X^2_{(1, 6)} = 14.70, n= 12, P=0.02$, Figure 4.2), with post-hoc analyses revealing that it was
significantly lower on day 28 than on days 4, 8 and 12 (equal groups paired comparisons: P= 0.01; P= 0.05; P= 0.05 respectively. Figure 4.2).

4.3.6 Control for learnt prey predictability

The contest rate seen on day 28 in the control experiment was significantly lower than that seen on day 4 in the dispersed prey competition experiment (Mann-Whitney U test: Z_{(4,11)}=-3.37, P<0.001), but not significantly different to that seen on day 28 in the dispersed prey competition experiment (Mann-Whitney U test: Z_{(4,11)}=-0.07, P=0.46).

4.3.7 Concentrated Prey

The prey contest rate did not differ significantly between days of testing in trials where fish foraged for concentrated prey items (Friedman test: X^2_{(6,11)}=5.24, n= 12, P=0.51. Figure 4.2).

4.3.8 Differences in contest rate between dispersed and concentrated prey trials

The contest rate on day 4 was significantly higher in the dispersed prey trials than in the concentrated prey trials (Mann-Whitney U test: Z_{(4,11)}=-2.28, P=0.022. Thereafter contest rate was not seen to differ between treatments (Mann-Whitney U tests: Day 8: Z_{(4,11)}=-1.45, P=0.16; Day 12: Z_{(4,11)}=1.04, P=0.29; Day 16: Z_{(4,11)}=-0.57, P=0.56; Day 20: Z_{(4,11)}=-1.26, P=0.17; Day 24: Z_{(4,11)}=-0.75, P=0.45; Z_{(1,11)}=-1.08, P=0.13 days 8 to 28 respectively. Figure 4.2).
Figure 4.1. Differences in mean proportional prey share (+/- S.E.) between highest and lowest prey consumers in dispersed prey (a) and concentrated prey (b) trials over the duration of the study period.
Figure 4.2. The proportion of consumed prey items that were contested by two or more individuals prior to ingestion (+/- Standard error) was seen to significantly decrease over time in dispersed prey trials (solid line) but not in concentrated prey trials (broken line). A control experiment in the drift foraging trials revealed this to be a function of the time that fish spent together rather than a response to the experimental procedure.

4.3.9 Relative competitive ability and association preference

4.3.10 Dispersed Prey

I tested whether focal individuals given a binary choice between associating with shoal mates that had previously and consistently consumed a greater share of prey over the course of the trials, and with shoal mates from which it had not differed in prey intake, and failed to detect any shoaling preference (Wilcoxon signed rank test: \( Z = -0.78, n = 12, P = 0.43 \)).

4.3.11 Concentrated Prey

Focal individuals given a binary choice between associating with shoal mates that had previously and consistently consumed a greater share of prey over the course of the trials.
and with shoal mates from which it had not differed in prey intake showed no preference for either (Wilcoxon signed rank test: \( Z = -1.06, n = 12, P = 0.28 \). Figure 4.3).

![Graph showing prey distribution: Dispersed and Concentrated.]

**Figure 4.3.** The mean proportion of time +/- standard errors that an intermediately ranked focal fish spent shoaling with two shoal mates that had consumed proportionately more (grey bars) or less (white bars) prey than it had.

### 4.4 DISCUSSION

The rate of kleptoparasitic prey contests in dispersed prey trials declined over the course of the study, but this was not the case in the concentrated prey trials where the contest rate remained low and stable over time. A control experiment revealed the fall in contest rates in the dispersed prey trials to be a function of the time that fish spent together, rather than a response to exposure to the experimental protocol. Individual prey intake within groups was not equal, and disparity in prey share did not change over the course of the study, even in dispersed prey trials where the contest rate was seen to decrease.
Prey contest rates were initially greater when prey was spatially and temporally dispersed compared to when it was concentrated. This is unsurprising, since the benefits of kleptoparasitism and aggressive prey competition are predicted to increase within groups of foragers as rates of prey detection fall (Amat and Obeso 1991; Sirot 2000; Broom and Ruxton 2003). Furthermore, though foragers often obtain prey both by searching for it themselves, and by observing other foragers in order to steal their captures (Ha and Ha 2003), producer-scrounger models assume that they cannot engage in both types of behavior simultaneously (e.g. Barnard and Sibly 1981; Vickery et al. 1991, and empirical work by Mottley and Giraleau 2000, but see Smith et al. 2002). Under such circumstances, where conventional foraging and kleptoparasitism are simultaneously incompatible, the overall prey detection rate of a group of a given size will fall as the proportion of speculative kleptoparasites increases (Coolen 2002). Consequently the prey returns of kleptoparasites will also diminish. As such, the contest rate for prey should remain relatively low and relatively constant when food resources are spatially and temporally concentrated, though this is likely to be affected by prey patch depletion, something I did not examine in this study. Conversely, when prey is spatially and temporally dispersed, prey detection rates at a given point in time will be lower, increasing the relative value of competing for singular prey items. Additionally, it is conceivable that foragers rely on social information to a greater extent than when prey distribution is more predictable, and may therefore switch more frequently between searching the prey patch and observing conspecifics, so increasing the opportunity to detect and kleptoparasitise their prey captures (Rafacz and Templeton 2003). Again, this is something that I did not explicitly test in this study, and further research in this area could be useful.

The stable contest rate level seen in the concentrated prey trials, and attained over time in the dispersed prey trials may represent a food return-predation risk trade-off baseline. The fall over time in the dispersed prey trials may be attributed to the development of familiarity
between individuals, and previous studies have demonstrated lower levels of aggressive interaction within groups that are composed of familiar individuals (Højesjø et al. 1998; Seppä et al. 2001; Utne-Palm and Hart 2000). Competition is costly, using time that could otherwise be invested engaging in other behaviours, and has been shown to increase the risk of predation to the individuals taking part, both when competing for prey (Jakobsson et al. 1995; Slotow and Paxinos 1997), and, for example, mating opportunities (Candolin 1997; Kelly and Godin 2001). Familiarity promotes greater rates of foraging and social learning (Laland and Williams 1997; Lachlan et al. 1998; Swaney et al. 2001), and as familiarity develops within a group, this may outweigh the benefits to be gained from kleptoparasitising others, leading to a decrease in aggressive interaction.

The development of familiarity is not the only possible explanation for the observed changes in competition levels in the dispersed prey experiment however. It has recently been shown that fish can recognise others using self referent matching of chemical cues pertaining to recent prey and habitat use (Ward et al. 2004b; 2005; chapter 3 in this thesis). In addition to this, hierarchies can develop and persist through winner and loser effects, which allow individuals to assess their chances of winning or losing contests based respectively upon their past victories or losses (Barnard and Burk 1979; Hollis et al. 1995). Neither of these mechanisms assumes the capacity of a group member to recognise conspecifics individually. Interestingly, in this study there was no evidence of individual recognition through shoaling preference of intermediately ranked prey consumers, though this may represent insufficient statistical power arising from a sample size of 12 replicates. Metcalfe and Thomson (1995) found that intermediately ranked European minnows (Phoxinus phoxinus) displayed a preference for shoaling with poorer competitors, presumably because it afforded the choosing individual a potentially greater prey share.

In addition to the contest rate differences seen between the two experimental prey distribution modes, another finding of this study was significant disparity in prey share.
Inequality in prey share can be explained by factors such as variation in foraging experience, individual physiological state, and predation risk versus prey return trade-offs. For example, individuals consuming more of a prey resource may simply be consistently better foragers, perhaps because they are consistently faster swimmers who therefore prevail in scramble competition, because they are more vigilant for or more efficient at handling prey, or because they are driven to forage at a greater rate due to higher metabolic demands (McCarthy 2001). Related to this, they may have greater innate tendencies to take risks when foraging. Some individuals are known to consistently engage in such behavior to a greater extent than others across a range of different contexts (Bell and Stamps, 2004; Bell, 2005; Ward et al., 2004a). Further to this, more danger prone, or bold individuals were seen by Ward et al. (2004a) to be better foraging competitors than their danger averse, or shy opponents. The bold-shy axis phenomenon could account for the prevalence of both of these behavioral phenotypes in nature, since the seemingly disadvantaged shy individuals are thought to trade off a greater share of a contested resource in favour of lower mortality through reduced predation risk (Huntingford, 1976).

In this chapter I considered the effects of increasing group stability upon patterns of prey share and competition. I build up the findings of this study in the following chapters, where I further investigate the roles of familiarity (Chapter 5) and bold-shy behaviour (Chapters 6 and 7) in the context of prey competition.
Social foraging and prey competition b):

Familiarity and group composition
5.1 INTRODUCTION

In Chapter 4 I considered the role of familiarity in mediating prey competition within shoals of foraging fish; in this chapter I examined interplay between familiarity and competition under more naturally realistic conditions, in which levels of familiarity within shoals were unequal. Numerous laboratory studies have revealed that in a variety of fish species, individuals preferentially associate with familiar over unfamiliar conspecifics (reviewed by Griffiths 2003). In nature most free-ranging fish shoals are open in structure with their composition changing subject to fission-fusion processes. They tend to be highly sorted by factors including species, phenotype and parasite load (Krause et al. 1996; Hoare et al. 2000), but evidence for familiarity as an important shoal structuring mechanism remains equivocal. Klimley & Holloway (1999) described a high level of shoal fidelity in pelagic yellowfin tuna (*Thunnus albacares*), whilst Griffiths & Magurran (1997a) and Ward et al. (2002) described stable association preferences between individuals in channel-bound populations of guppies and threespine stickleback respectively. Conversely, studies of species including yellow perch (*Perca flavescens*, Helfman 1984), banded killifish (*Fundulus diaphanous*, Hoare et al. 2000), guppies (Godin et al. 2003) and red-bellied piranha (*Pygocentrus nattereri*, Magurran & Queiroz 2003) reported no evidence for shoal fidelity or association preferences for familiars.

This apparent difference may be attributed to the fact that whilst these shoaling preferences are seen to arise over several days or weeks in the laboratory (e.g. Griffiths & Magurran 1997b; Croft et al. 2004a), the composition of free-ranging shoals often changes over a period of seconds to hours in nature, as individuals leave and join groups under the influence of temporally dynamic predation, foraging or sexual pressures (Hoare et al. 2000; Croft et al. 2003a). Despite this instability, research in a range of social vertebrate species suggests that even where overall group fidelity is low, small groups of individuals may still
persistently associate with one another, giving rise to social association networks (Croft et al. 2004b; Cross et al. 2004; 2005; Lusseau 2004; Lusseau & Newman 2005; Muller & Thalmann 2000; Vonhof et al. 2004). These sub-groups of individuals may still benefit from the advantages of associating with familiar conspecifics, even when the composition of the larger group is constantly changing around them.

This study aimed to investigate whether pairs of familiar fish within an otherwise unfamiliar shoal could benefit from the effects of reduced prey competition and associated agonistic behaviours previously seen in studies where all individuals were equally familiar to one another. I created experimental shoals, embedding familiar pairs of threespine sticklebacks into groups of unfamiliar conspecifics. I predicted that during foraging trials, members of familiar pairs would contest prey with each other to a lesser extent and consume a greater share of the prey resource than their unfamiliar conspecifics.

5.2 METHODS

5.2.1 Fish Collection and Housing

One hundred and twenty sub-adult threespine stickleback measuring 27 to 30 mm standard length were collected from a 500 m stretch of Stonton Brook, Leicestershire, UK in March 2005. There they were distributed equally between 12 chemically and visually isolated holding tanks, (40 by 25 by 25 cm, water depth 20 cm, 1 cm deep fine sand substrate, flow through rate approximately 0.1 l min). The water temperature and light: dark regimes were held at 11°C and 12: 12 hours respectively over the duration of the study. Two days after capture these fish were given an identification tag using the procedure described in Chapter 4 of this thesis. The fish were held in these tanks for a further 30 days prior to the beginning of experiments. They were fed frozen Chironomid larvae once per day.
5.2.2 Experimental groups

Fish were deprived of food for 24 hours in order to generate feeding motivation. I then created 20 experimental groups of six fish per group. Five pairs of fish were taken from each of the first four holding tanks and placed into their own chemically and visually isolated holding tank. These pairs of fish had been housed together for 32 days previously; I termed these individuals familiars. To each familiar pair I added four fish from the remaining eight holding tanks. Within each group, no two of these were drawn from the same holding tank, and these fish had previously been held separately both from each other, and from the familiar pair. I termed these individuals unfamiliars. Thus I created 20 experimental groups of six individuals, two of whom were familiar to each other, but unfamiliar to the other four members, who were also unfamiliar to one another. Each experimental group was held together for one hour before the trial was conducted.

5.2.3 Experimental procedure

Trials took place in an experimental tank (40 x 25 x 25 cm, water depth 20 cm. 1 cm deep fine sand substrate), the sides and rear of which were covered with non-transparent screening, to minimise outside disturbance. Observations were made via a vision slit in an opaque screen to remove observer effects. One side of the tank contained a row of five equally spaced 5 mm holes at the waterline. These served as prey introduction points, through which prey items could be introduced over the course of a trial via a 5 cm$^3$ syringe in 2 cm$^3$ water. Sections of Chironomid larvae 5mm long were used as prey. These were introduced sequentially, as described below.

Prior to the commencement of a trial the experimental group was placed in the experimental tank and allowed to settle for 5 minutes. Individual prey items were introduced through via the prey introduction points, and 10 seconds was allowed to elapse between the
ingestion of one prey item before the introduction of the next. Prey items were introduced through a different point each time, in a predetermined random order. A total of 18 prey were offered; fish of the size used in this study have been seen to consume up to 10 5mm Chironomid sections within five minutes (Personal observation), so competition for prey should be expected to persist even when an individual has consumed its expected share of three prey items. I recorded the following: the number of prey items consumed by familiar and unfamiliar fish, the number of contested prey items, and the identity, i.e. familiar pair member or unfamiliar shoal mate, of the individual which first engaged the prey, of that which contested it, and of the individual which ultimately consumed it.

5.2.4 Statistical Analyses
I determined the mean proportional prey share of familiar and unfamiliar individuals within each group and compared these data using a Wilcoxon signed rank test. All proportional data were arcsine transformed before analyses were performed. I calculated the mean rate of prey contests per prey item per individual. I produced three classifications of interactions: 1) Prey contests that occurred between members of familiar pairs; for each member of the familiar pair there was one other familiar individual with which it could interact. 2) Prey contests that occurred between members of familiar pairs and unfamiliar fish, where the familiar pair member initiated the contest: for each member of the familiar pair there were four unfamiliar individuals with which it could interact. 3) Prey contests that occurred between unfamiliar fish, where the contest was not initiated by a member of a familiar pair; for each unfamiliar fish there were five unfamiliar individuals with which it could interact, since the members of the familiar pair were unfamiliar to the other four fish. Contest rates within each interaction were standardised by dividing the number of contests within each classification by number of competitors that an individual could have interacted with. I compared the standardised proportion of contests that occurred within each classification per group using a Friedman
test. and performed post-hoc analyses using equal groups paired comparisons as described by Langley (1979). Finally I looked at contests that occurred between familiar and unfamiliar individuals. I compared the proportion of these contests that were initiated by familiar and unfamiliar individuals. I then compared the proportion of contest won by familiar and unfamiliar both when they had initiated or received the contest challenge. Again I used Wilcoxon signed rank tests to perform these analyses.

5.3 RESULTS

5.3.1 Prey Share

The mean proportional prey shares of unfamiliar individuals and members of familiar pairs did not differ significantly from each other (Wilcoxon signed rank test: $n= 20, Z= -0.67, P= 0.49$. Figure 5.1).

![Proportional Prey Share](image)

**Figure 5.1.** Mean proportional prey share (+/- S.E.) of members of familiar pairs and unfamiliar fish.
5.3.2 Contest Rate

The mean number of prey contests per trial (+/- S.E.) was 6.95 (+/- 0.94, n= 20). Within experimental groups the contest rate for prey items between members of familiar pairs was significantly lower than seen between unfamiliar individuals and for members of familiar pairs versus unfamiliar individuals (Friedman test: $X^2_{(2;20)}= 19.45$, P<0.001, Figure 5.2). In order to control for effects of satiation that may have caused fish to stop competing for prey and biased our results, I compared the number of contests that occurred for the first nine prey items against the number occurring for the second nine, finding no differences (Wilcoxon signed rank test: n= 20, Z= -0.16, P= 0.86).

![Figure 5.2](image)

Figure 5.2. The mean (+/- S.E.) proportion of prey contests per individual per shoal between familiar and unfamiliar individuals.

5.3.3 Contest Victories

In prey contests between familiar and unfamiliar individuals each were equally likely to engage in kleptoparasitism (Wilcoxon signed rank test: n= 20, Z= -0.50, P= 0.61), and both were equally likely to be victorious, both when they initiated (Wilcoxon signed rank test: n=...
20. $Z = -0.24, P = 0.81$) or received (Wilcoxon signed rank test: $n = 20, Z = -0.18, P = 0.85$) the contest challenge (Figure 5.3).

![Proportion of contests](image)

**Figure 5.3.** Mean proportion of contests initiated and contest victories (+/- S.E.) by members of familiar pairs (white bars) and unfamiliar fish (grey bars).

5.4 DISCUSSION

Members of familiar pairs engaged in fewer kleptoparasitic prey contests with each other than they did with unfamiliars, and than did unfamiliars with each other. Both familiar pair members and unfamiliar individuals were not seen to differ in their likelihood of initiating contests however, and this is interesting as it suggests that the lower contest rate between pair members was due to a non-random allocation of competitive effort by these individuals rather than a general tendency to engage in fewer contests *per se*.

Familiar and unfamiliar fish were not seen to differ in terms of their prey resource share. We consider it unlikely that this is due to sample size effects ($n = 20$ experimental groups). Though it is known that individual fish that are otherwise phenotypically similar can differ
consistently in their competitive ability (Metcalfe & Thomson 1995; Ward et al. 2004a; Chapters 4, 6 and 7 in this thesis), the fish used in this study were selected randomly, and variation in competitive ability should have been spread equally between treatments. Here it was seen that variation in prey share was relatively low (Figure 1). These results suggest that discriminating between familiar and unfamiliar individuals in the context of prey competition may instead bring indirect benefits. Prey competition is costly, using up time that could otherwise be spent engaging in other behaviours, and increasing the risk of predation through reducing the vigilance, or increasing the conspicuousness of competing individuals (Jakobsson et al. 1995; Slotow & Paxinos 1997). Given this, a strategy that reduces prey contest rates whilst preserving the benefits of group foraging is likely to be of adaptive value.

The mechanism facilitating recognition of familiars upon which this effect depends remains unclear, and may vary according to species, habitat stability, population density and other factors. A system of learned recognition may operate, whereby individuals recognize, or can quickly gauge, the hierarchical standing of their opponent allowing them to concede contests with known superior competitors, in which they are more likely to lose out on the contested resource anyway, or rapidly escalate contests with subordinates, where they are more likely to win, without the need for protracted interaction (Barnard & Burk 1979). This may account for the differences in contest rates observed in this study between familiars and between familiars and non-familiars: when faced with an unfamiliar but phenotypically similar competitor, the outcome of a contest may not be so readily predictable and the value to both parties of pursuing a prey contest is therefore potentially greater. This however is a fairly complex mechanism, placing substantial cognitive demands upon the individual, and with several associated costs. Firstly, prolonged interaction over a substantial period of time may be required for identities to be learned (Griffiths 2003) and secondly there may be an upper limit on the number of individual identities than can be learned and recalled (Griffiths...
In nature these demands may be irreconcilable with the temporally dynamic structure of free-ranging shoals, and the potentially very high number of conspecifics that an individual may associate with on a day-to-day basis (Ward et al. 2002). Winner and loser effects, allowing individuals to assess their chances of winning or losing contests based respectively upon their past victories or losses (Hollis et al. 1995) liberates them from the cognitive constraints of learning separate identities, but this mechanism alone cannot explain how familiars are differentiated from non-familiars. A general assortive mechanism, recently shown to operate in shoaling fishes (Ward et al. 2004b; 2005; Chapter 3 in this thesis) is one of self-referent matching of chemical cues pertaining to recent prey and habitat use. Again, this mechanism does not assume the capacity for individual recognition. It represents an inexpensive means of assessing group composition and stability, one that can be easily over-ridden, and useful future work could consider the roles of these different mechanisms in free-ranging populations, and the conditions under which each or either is used.

In groups where animals leave and join at different rates over time, the ability to discriminate between familiars and unfamiliars brings another benefit, one analogous to heterogeneous advantage. This is a phenomenon much studied in a kin-selective context, whereby individual fitness is higher in groups where genetic diversity is greater (Griffiths & Armstrong 2001; Greenberg et al. 2002). In this case however we consider diversity in levels of familiarity. In fishes, familiar groups have been shown to forage and disseminate foraging-related information amongst themselves more efficiently than groups of unfamiliar individuals (Laland & Williams 1997; Lachlan et al. 1998). Furthermore, unfamiliar outsiders have been shown to exploit this effect, by identifying and preferentially associating with familiar groups in order to enhance their own foraging rates through local enhancement and social facilitation (Ward & Hart 2005). By discriminating between their longer established, and more foraging-efficient group members and any relative newcomers that
may be usurping the successes of the group, and allocating competitive effort accordingly. Individuals may better balance the costs of prey competition with conspecifics exploiting a common resource with the benefits of kleptoparasitising the efforts of others.
Chapter 6

Bold / shy behavioural variation: prey competition and social information use
6.1 INTRODUCTION

The bold-shy axis describes the degree to which animals balance fundamental trade-offs between returns and risk when undertaking such tasks as foraging, inspecting predators and competing for resources (Wilson et al. 1994; Wilson 1998; Sih et al. 2004a). Studies have revealed that in numerous species, individuals that behave boldly in one context, also behave boldly in other, separate contexts and that within a given population, the distribution of individuals along the bold / shy axis can be substantial (Verbeek et al. 1996; Dugatkin & Alfieri 2003; Bell & Stamps 2004; Ward et al. 2004; Bell 2005; Quinn & Cresswell 2005). On the other hand, behaving in a consistently bold or shy manner in all situations is potentially maladaptive, since it may prevent animals from producing optimal responses to different stimuli. Accordingly, there is also evidence to suggest that some animals modify their responses, behaving boldly only when it is adaptive to do so (Coleman & Wilson 1998; Reale et al. 2000; Lopez et al. 2005).

Little is known of how social context relates to the boldness of individuals however. Aspects of sociality such a social facilitation and the use of social information clearly affect the way individuals exploit resources, interact with their environment, and the extent to which they take risks when doing so. This is important since many previous studies of boldness have focused upon the bold / shy responses of single animals only, often restoring the social context only to look at aggressive or competitive interactions. The impact of many of the day-to-day costs such as predation risk and resource sampling can be less severe for animals that live in groups than it would be if they were living alone. This is because in groups there are likely to be at any given time certain members that are engaged in sampling resources, or watching for predators, and who actively or passively transmit information derived from these activities throughout the group. To the individual group member this can reduce the required minimum investment of time and energy into meeting these costs.
allowing them to spend more time engaged in other often non-compatible activities, such as searching for and consuming prey (Galef 1988; Baird et al. 1991; Day et al. 2001; Krause & Ruxton 2002). Related to this, social animals can assimilate and use public information about their surroundings. Public information is a specialized form of social information, one that specifically conveys to the receiver information about the quality of a resource (Valone 1989; Valone & Templeton 2002). If we are to further our understanding of the role of boldness in determining the behaviour of members of free-ranging populations we need to know how such behaviour is expressed under naturally realistic conditions when these cues are available.

In this study we examined the influence of social context upon the expression of bold/shy behavioural responses by individual threespine sticklebacks (*Gasterosteus aculeatus*). We measured the behaviour of focal fish in two separate contexts, activity levels in novel surroundings and foraging when under simulated predation risk, predicting that individuals that are more active should also resume foraging more rapidly following a simulated attack from a predator. Previous studies have shown that these behaviours can be correlated in this way in this species (Bell and Stamps 2004). Using these and further assays we performed a series of three experiments designed to determine the relationship between social context and individual bold/shy behavioural tendencies. The first of these experiments considered prey competition and group size. Prey competition is costly, and it can potentially increase the risk of predation of those individuals taking part (Jakobsson et al., 1995; Slotow & Paxinos, 1997). For this reason we predicted that the prey share of a focal fish would be correlated with its behaviour in other contexts when the size group of competitors was low, in accordance with previous findings (Ward et al. 2004), but not when the group size was larger. We reasoned that in numerically larger groups where the per capita predation risk was lower it would be adaptive to behave boldly by competing more vigorously and that an individual’s prey share would no longer correlate with its behaviour in other contexts. In the
second experiment we tested the prediction that an individual’s behaviour would be correlated between the two behavioural contexts, activity in novel surroundings and foraging behaviour under simulated predation risk when it was tested alone in each but not when it was tested within a group of conspecifics in one or other of these contexts. Again we reasoned that social facilitation through the presence of shoal mates should allow individual group members to behave more boldly than they would if they were alone. In our third experiment, we considered the use of public information by threespine sticklebacks. Previous research has revealed that threespine sticklebacks can obtain public information about prey patch quality by observing the foraging success of attendant conspecifics, and that they use this information to discriminate between patches, preferentially foraging at patches where prey yield is higher (Webster & Hart 2006a). Doing so allows them to maximise their foraging efficiency and potentially reduce predation risk, by removing the need to sample multiple patches themselves. Given this, we predicted that individuals that were less active and which took longer to resume foraging following a failed attack by a simulated predator would use social information to a greater extent.

6.2 METHODS

6.2.1 Fish Collection and Housing
Threespine sticklebacks (30 to 35 mm standard length) were collected from the Great Eau river, Lincolnshire, UK in June 2006. They were housed in the laboratory in groups of 20 with each group assigned to its own holding tank (40 by 25 by 25 cm, water depth 20 cm, sand substrate, and artificial vegetation for cover). The water temperature and light: dark regimes were held at 10°C and 12: 12 hours. Fish were fed frozen chironomid larvae once per day, unless otherwise stated below. They were held under these conditions for three
months before the experiments began. Over the course of the study no fish was used more than once.

6.2.2 Part 1. Does boldness predict prey share in group foraging trials?

The aim of Part 1 was to determine a) whether the competitive ability of an individual, determined by its share of a finite prey resource, was related to its behaviour in other contexts and b) whether this relationship was affected by the size of the group in which it was competing. Fourteen groups of two and 14 groups of six fish were established with each housed in its own chemically and visually isolated tank (40 by 25 by 25 cm, water depth 20 cm, sand substrate). All fish were size matched to within <1 mm standard length. One focal fish was randomly selected from each group. This fish was identified by the pigmentation patterns along its flanks and a digital photograph of each focal fish was taken whilst it was still within its tank, along with a written record describing its markings, to ensure that each focal fish could be quickly and accurately identified. Fish were held under these conditions for 24 hours before experiments began. Each group / focal fish was subjected to three experiments according to the following schedule:

Day 1: the experimental groups were established
Day 2: prey share competition trial 1
Day 4: prey share competition trial 2
Day 6: prey share competition trial 3
Day 8: focal fish activity level test
Day 10: latency to resume foraging under simulated predation risk test

These assays are described below.

6.2.3 Statistical analysis for part 1
We calculated the mean proportional prey share of the focal fish, and its movement rate as a proportion of the total trial time. These data were not normally distributed therefore non-parametric statistics were used. We performed Spearman Rank correlations to investigate relationships between individual activity levels and latency to resume foraging, activity levels and prey share, and latency to resume foraging, and prey share. We did this for each group size treatment, performing a total of six correlations.

6.2.4 Part 2. Is individual boldness affected by social facilitation?

Individual animals can benefit from reduced predator vigilance costs by being a member of a group, potentially allowing them to engage in certain other behaviours to a greater extent than they could if they were alone (Krause & Ruxton 2002). In this part of the study we sought to determine a) if focal fish would be more active and resume foraging more rapidly under predation risk when they were in groups compared to others tested alone, and b) whether behavioural responses were correlated within individuals when they were tested alone in one context but not in the other.

Thirty-six focal threespine sticklebacks were used, with each housed in its own chemically and visually isolated tank (40 by 25 by 25 cm, water depth 20 cm, with a 1 cm deep fine sand substrate). In addition to the focal fish, each tank contained two companion fish. These individuals were included to minimise stress in the focal fish, and were not used in any of the experiments. As described above, focal fish were identified by their flank pigmentation patterns. Three experimental groups of 12 focal fish each were established, and tested according to the following schedule:

Day 1: focal and companion fish assigned to holding tanks
Day 3: activity level test
Day 5: latency to resume foraging under simulated predation risk test
The first experimental group was used in a control treatment. Individuals were tested alone in the activity level test on day 3, and then alone again in the foraging under simulated predation risk test on day 5 according to the assays detailed below. Individuals from the second experimental group were tested in a modified version of the activity level test on day 3, described below. They were then tested alone on day 5 in the foraging under simulated predation risk test, also described below. Finally, individuals from the third experimental group were tested alone in the activity level test on day 3. They were then tested in a modified version of the simulated predation risk test on day 5, described below.

6.2.5 Statistical analysis for part 2

Data were not normally distributed and we used non parametric statistics for the following analyses. We compared firstly the movement rates and secondly the latency to resume foraging of the focal fish from the three experimental groups using Kruskal-Wallis test with Langley post-hoc analyses Langley (1979). We then performed Spearman Rank correlations to investigate relationships between individual activity levels and latency to resume foraging within each of these groups performing a total of three correlations.

6.2.6 Part 3. Is boldness correlated with the use of public information?

Foraging animals can gauge prey patch quality using public information, by monitoring the foraging success of other patch users, and previous research has shown that threespine stickleback are capable of doing so (Webster & Hart 2006a). The aim of this part of the study was to determine whether the use of public information by individuals was related to their behaviour in other contexts. Thirty focal fish were used and each was housed in its own chemically and visually isolated tank (40 by 25 by 25 cm, water depth 20 cm, with a 1 cm deep fine sand substrate). In addition to the focal fish, each tank contained two companion
fish as described in Part 2. Experiments were performed according to the following schedule:

Day 1: focal and companion fish assigned to holding tanks
Day 3: activity level test
Day 5: latency to resume foraging under simulated predation risk test
Day 7: use of public information test

These assays are described below.

6.2.7 Statistical analysis for part 3

We sought firstly to determine whether the focal fish had used public information. We subtracted the proportion of trial time spent by the focal fish shoaling with the stimulus group housed at the poor quality feeder from that spent shoaling with the stimulus group housed at the high quality feeder. We compared these values to a null value of zero using a Wilcoxon signed rank test. We then used three Spearman Rank correlations to look for correlations between these values and individual activity levels and latency to resume foraging.

6.2.8 Experimental behaviour assays

These behavioural assays were used to measure intra-individual behavioural correlations across different contexts. They were selected because they have previously been used in studies of boldness in this species. Previous studies have revealed that behavioural tendencies in these contexts are relatively stable within individuals (Ward et al. 2004; Bell 2005) and for this reason, with the exception of the prey competition test, each was only performed once.

6.2.9 Prey competition test (Part 1)
Fish were tested within their own holding tank so as to minimise stress. Each group was provided with single, sequentially delivered 3 mm long sections of chironomid larvae, a natural prey type that fish had been fed during their time in captivity. Prey items suspended in 2 ml of tank water were added via a 5 ml syringe through one of five holes, spaced 2 cm apart at the water line of each aquarium. The order in which each hole was used was randomly pre-selected to prevent fish from predicting and monopolising the feeding position and pre-selected to avoid experimenter bias. In the trials where the group contained two fish a total of six prey items were introduced, and in the trials where the group size consisted of six fish a total of 10 prey items were added. The ratio of prey items to group members was therefore lower in the trials where the group size was six. This approach was adopted in order to minimise the chance that superior competitors might consume many prey items and become satiated before the experimental trial was completed. Prey were delivered sequentially, with a 30 s period between the ingestion of one item by a fish and the introduction of the next. In each trial the prey share of the focal fish was recorded. Prey competition took the form of both scramble competition, whereby multiple foragers were able to detect prey items directly and sought to be the first to reach, handle and consume them, and also contest competition, whereby individuals actively and aggressively contested ownership of the resource (Ward et al. 2006; Webster & Hart 2006b). This test was performed three times according to the above schedule, and was used to calculate the mean proportional prey intake of the focal fish. Immediately after testing excess chironomid larvae were added to the tank, so that all fish could feed until satiated. This served to standardise hunger levels between trials. Fish were fed again the following day, after which all uneaten prey were removed, and the fish were deprived of food for 24 hours until the next feeding trial. It is unlikely that familiarity could have had any influence upon individual prey share over such a short timeframe and previous research has shown that although levels of
agonistic competition for prey items may decrease within groups of this species over a 28 day period, levels of individual prey share remain stable (Webster & Hart 2006b).

6.2.10 Activity level test (Parts 1-3)

When animals enter new surroundings they face a trade-off between increasing their activity in order to gather information about their new environment, something that may also increase their likelihood of being detected by a predator, or remaining inactive, at the cost of a potentially reduced prey intake. In this test we recorded the activity rates of focal fish in a previously unexplored test tank. Testing took place in a tank measuring 60 by 30 by 30 cm, with a water depth of 27 cm and a 2 cm deep layer of 5 mm gravel. The tank was otherwise unfurnished, and was covered externally with black screening on the sides and rear. The focal fish was introduced to the centre of the tank within a 7 by 7 cm, 27 cm tall holding unit. This was constructed of clear, perforated plastic, allowing the fish to assimilate visual and chemical cues from the test tank. The fish was held in the unit and allowed to settle for 5 minutes, before this was removed, releasing the fish, and beginning the trial. The trial ran for 5 minutes and point sampling (Lehner 1996) was used at 15 s intervals (giving n= 20 measurements) to record whether the fish was active or whether it was stationary on the substrate. Observations were made via a slit in a black screen in order to remove observer effects. Following each trial the fish was returned to its respective tank and the water in the test tank was replaced.

6.2.11 Modified version of the activity level test (Part 2)

In Part 2 we used a modified version of this test, in that four additional fish were present to the test tank. These were size matched to within <1 mm standard length of the focal fish. They were obtained from a different stock tank to the focal fish and were therefore unfamiliar with it. They were added five minutes before the focal fish was added, and were
allowed to move around the tank whilst the focal fish was acclimatised for five further within the holding tower. Following the release of the focal fish its movement rate was determined using point sampling, as above. Following the completion of a trial the water and four additional fish were replaced.

6.2.12 Latency to resume foraging under simulated predation risk test (Parts 1-3)

Foraging animals are compelled to cease feeding and seek refuge or take evasive action when predators attack. Foragers face a trade-off regarding when to resume feeding following a failed attack: too soon and the predator may still be close by, too late and their rake of prey intake decreases. In this test we measured the latency of focal fish to resume foraging following a simulated attack from a fish-eating bird.

We used a test tank dived into three chambers. At one end of the tank were two 15 by 12 cm chambers situated side by side, leaving a larger 30 by 48 cm chamber in the remainder of the tank. The left-hand chamber held the focal fish, whilst the right-hand chamber received the simulated predator attack. The walls of the two smaller chambers that faced into the larger chamber were made from one-way glass, aligned so that the focal fish within the left-hand chamber could see into the larger chamber. All of the other walls were constructed from clear, regular glass and the focal fish was therefore able to see both into the right-hand chamber as well as into the larger chamber. All of the chambers contained a 2 cm layer of 5 mm gravel and were filled with water to a depth of 27 cm. The larger section was otherwise empty except in the modified version of this test used in Part 2 and described further below. Three 10 cm strands of artificial vegetation were floated on the surface of the left-hand chamber in order to minimise stress to the focal fish.

Ten 3 mm long sections of chironomid larvae were placed across the bottom of the left-hand chamber and 5 minutes later the focal fish was added. A 100 g weight was suspended 20 cm above the container on the right. The focal fish was added to the container on the left
and allowed to begin to feed. After the focal fish had consumed one prey item the weight was released and allowed to drop into the right-hand container. This caused a disturbance designed to simulate a failed attack from a fish-eating bird, and was sufficient to induce a fright response in all of the focal fish. The latency of the focal fish to resume foraging was recorded, and taken as a second measure of boldness. Following each trial the water and prey were replaced in the container on the left, and 5 minutes were allowed to elapse before the next trial was carried out (versions of this assay have been used successfully in a number of previous studies (Bell and Stamps 2004; Ward et al. 2004; Bell 2005).

**6.2.13 Modified version of the foraging under simulated predation risk test (Part 2)**

In Part 2 we used a modified version of this test, in that four additional fish were present to the larger chamber of the test tank. The focal fish, held in the left-hand chamber was able to see these addition fish. The additional fish were unable to see either the focal fish or the disturbance caused by the falling weight because of the one way glass. They were also unable to see the reaction of the focal fish to the falling weight, preventing them too from displaying any fright responses that might in turn have affected the behaviour of the focal fish. As above, the focal fish was allowed to begin to feed before the weight was dropped, eliciting a fright response, and we recorded its latency to resume feeding.

**6.2.14 Use of public information test (Part 3)**

In this test we quantified the use of public information of focal fish by measured to proportion of time that they spent shoaling with each of two stimulus shoals, one feeding from a high yield feeder and one from a low yield feeder. A binary choice arena was established in an experimental tank (39cm by 17cm by 18cm deep, water depth 15 cm). At each end of the tank along its longest axis was an 8cm wide stimulus chamber, separated from the central section of the tank by screens of colourless perforated plastic (perforation
diameter 0.1 cm, 5±1 perforations/cm²). This allowed the exchange of both visual and chemical cues. A 2 cm deep substrate of 0.5 cm gravel was provided in the central section of the tank and in the two stimulus chambers. Three 10 cm lengths of artificial plastic vegetation were floated on the surface in each of the stimulus chambers. These served to keep the stimulus fish from becoming stressed by providing overhead cover. On the outside of the glass two association zones were marked by vertical black lines, 2 cm from each of the stimulus chambers. This distance falls well within the range of inter-individual distances seen in free-ranging fish shoals (Pitcher & Parrish 1993). In the centre of each stimulus chamber was a vertically positioned 2 cm diameter, 30 cm long white plastic tube. Each tube was cut diagonally across the last 3 cm of its length at a 45° angle, and positioned so that this end rested on the substrate, with the longest side of the tube facing into the test tank towards the focal fish. These served as prey delivery tubes. Positioning them in this way prevented the focal fish from seeing the uneaten prey as it was delivered to the chamber. Instead the focal fish could gauge only patch quality by observing the prey capture success of either stimulus shoal.

Three stimulus fish were added to each stimulus chamber. These were size matched to within <1 mm of each other and to the focal fish. Stimulus fish were drawn from different stock tanks to the focal fish, and were therefore unfamiliar to it. The focal fish was introduced to the centre of the tank within a 7 by 7 cm, 27 cm tall holding unit. This was constructed of clear, perforated plastic, allowing the fish to assimilate visual and chemical cues from the test tank. The focal and stimulus fish were then allowed to settle for five minutes. After this period prey was added to the stimulus chambers. One chamber was randomly assigned to be a high quality prey patch and the other a low quality prey patch. A single prey item was added every 10 seconds to the high quality prey patch, whilst the low quality patch received a prey item every 30 seconds. Prey consisted of a 3 mm long section of chironomid larvae. These were delivered via the prey delivery tube in 1 ml of prey
conditioned water, obtained by crushing 1.5 g (wet mass) of frozen Chironomid larvae in 10 ml of tank water. In order to control for prey odour effects at the intervals when no prey was being delivered to the low quality patch. 1 ml of prey condition water alone was added at the same time as the high quality patch was receiving a prey item. These prey items were added whilst the focal fish was still being held in the holding unit. Prey cues were provided in this way for 2 minutes before the removal of the holding unit and the release of the focal fish, which marked the beginning the trial. The trial ran for 5 further minutes, during which time prey and / or prey odour cue provision to the two stimulus chambers continued. In all trials the stimulus fish were seen to seize the prey as soon as it emerged from the delivery tube. The amount of time that the focal fish spent within the 2 cm association zone in front of the high or low quality prey patch group was recorded and used as an indicator of its use of public information (Coolen et al. 2003, 2005; van Bergen et al. 2004; Webster & Hart 2006a).

6.3 RESULTS

6.3.1 Part 1. Does boldness predict prey share in group foraging trials?

Individuals that were more active also tended to resume foraging sooner under predation risk. Spearman Rank correlations revealed that individual activity levels were negatively correlated with latency to resume foraging in both group size treatments (group size two: n= 14, r= -0.72, P= 0.004; group six: n= 14, r= -0.62, P= 0.014). Furthermore, movement rate was positively correlated with prey share (group size two: n= 14, r= 0.69, P= 0.007; group six: n= 14, r= 0.60, P= 0.027), whilst latency to resume foraging was negatively correlated with prey share (group size two: n= 14, r= -0.55, P= 0.040; group six: n= 14, r= -0.76, P= 0.001, Figure 6.1).
Figure 6.1. Scatterplots showing correlations between activity levels, latency to resume foraging following a simulated attack by a predator and proportional prey share when foraging in a group. Plot a) shows that individuals that were more active also resumed foraging more rapidly following an attack. Plot b) shows that...
individuals that were more active also consumed more prey in group competition trials. Plot c) shows that individuals that resumed foraging more rapidly following an attack also consumed more prey in group competition trials. Focal fish that were tested in prey competition trials of group size two are shown by white points and a grey line, and focal fish that were subsequently tested in prey competition trials of group size six are shown by black points and a black line. Each data point represents one individual fish.

6.3.2 Part 2. Is individual boldness affected by social facilitation?

Focal fish in experimental group 2 where conspecifics were present in the activity level test were more active than those in groups 1 and 3, where the focal fish were tested alone (Kruskal-Wallis test: H = 10.84; degrees of freedom = 2, P = 0.004, Langley post-hoc: P < 0.001 in both cases). Similarly, focal fish in experimental group 3 where conspecifics were present in the latency to resume foraging test tended to resume feeding more rapidly than did those in groups 1 and 2 (H = 12.41; degrees of freedom = 2, P = 0.003, Langley post-hoc: P < 0.001 in both cases).

Individual activity levels were negatively correlated with latency to resume foraging in the first experimental group where individuals were tested alone in both tests (Spearman Rank correlation: n = 12, r = -0.60, P = 0.038). We saw no correlations in the second (n = 12, r = 0.10, P = 0.73) or third experimental groups (n = 12, r = 0.21, P = 0.35) where conspecifics were present in the activity level or latency to resume foraging test respectively (Figure 6.2).
Figure 6.2. Boxplots showing the proportion of trial time spent moving (a) and the latency to resume foraging following a simulated predator attack of fish in three treatment groups (b). In each case the plot shows the median values, inter-quartile range and the 95% confidence intervals. Fish in group 1 were tested alone in both the activity level and the latency to resume foraging tests. Fish in group 2 were tested in the presence of conspecifics in the activity level test and alone in the latency to resume foraging test. Fish in group 3 were tested alone in the activity level test and in the presence of conspecifics in the latency to resume foraging test. Please note that in the plot for group 2 on graph a) the values representing the median, upper quartile and upper confidence interval were identical.
6.3.3 Part 3. Is boldness correlated with the use of public information?

A Wilcoxon signed rank test revealed that focal fish spent significantly more time with the stimulus group housed next to the rich feeder (n= 30, Z= 2.50, P=0.012) suggesting that they were using public information. As in Parts 1 and 2, individual activity levels were negatively correlated with latency to resume foraging (Spearman Rank correlation: n= 12, r= -0.81, P<0.001). There were no relationships however between of public information and either activity levels or the latency to resume foraging (n= 30, r= -0.04, P= 0.83 and n= 30, r= 0.05, P= 0.79 respectively. Figure 6.3).
Figure 6.3. Scatterplot a) shows that individuals that were more active also resumed foraging more rapidly following an attack. Scatterplots b) and c) reveal no correlation between the use of public information and
either the latency to resume foraging following an attack or activity levels. Public information use was inferred from the net proportion of time that the focal fish spent shoaling with a stimulus group that was feeding from a rich prey patch in a binary choice test. Each data point represents one individual fish.

6.4 DISCUSSION

In each of our three experiments we saw that individual fish that were more active were also more likely to resume foraging sooner following a simulated predator attack. This robust result is consistent with previous findings in the threespine stickleback and in other species (Bell & Stamps 2004; Ward et al. 2004; Bell 2005).

In Part 1 we saw that individual prey share was positively correlated with activity levels, and negatively correlated with the latency to resume foraging, both when the group size numbered two, and also in larger groups consisting of six individuals. This finding is at odds with our initial prediction that boldness should become uncoupled from prey share in larger groups because of social facilitation effects. Previous research has revealed that individual bold / shy behaviour directly influences prey share in social foraging situations, and that this can have long term effects upon growth rates (Ward et al. 2004). A simple explanation for the persistent correlations between prey share and the two response variables between the group size treatments is that the difference in group sizes, two versus six, was insufficient to bring about a change in the behaviour of the focal fish. In the second part of this study however, we saw that similar differences in group size (one versus five individuals) were sufficient to produce differences in the behaviour of the focal fish, suggesting that this may not be the case. Another potential explanation relates to winner and loser effects, which allow individuals to assess their chances of winning or losing contests based respectively upon their past record of victories or losses (Barnard & Burk 1979; Hollis et al. 1995). Work by Hollis et al. (1995) on aggressive interactions between blue gouramis (Trichogaster trichopterus) revealed that previously successful fish went on to win more contests whilst unsuccessful fish persisted in losing contests. It is conceivable that winner and loser effects
could be associated with boldness since bolder individuals frequently out-compete shyer ones in prey contests (Ward et al. 2004; Webster et al. submitted). Further research in this area would be useful. Finally, engaging in competition has also been shown to increase the risk of predation to participants (Jakobsson et al. 1995; Slotow & Paxinos 1997), for example by increasing their conspicuousness to, and by reducing their capacity to detect nearby predators. In this context competition could be viewed as a trade-off between potentially receiving a greater share of a contested resource versus exposure to greater risk of predation. The avoidance of such risk, at the cost of reduced prey share, is consistent with the shy behavioural disposition. The results of this part of our study may go some way towards explaining how significant disparity in prey share can persist within stable groups of animals (e.g. Metcalfe & Thomson 1995; Webster & Hart 2006b).

In Part 2 of our study we found significant effects group size upon boldness. Here we saw that the correlation between individual activity levels and latency to resume foraging, readily apparent in the control treatment when focal fish were tested alone in both contexts, was absent when additional conspecifics were present in either one of the two tests. The links between larger group size and greater social facilitation effects are broadly understood and the theory is well supported by numerous empirical studies (reviewed in Krause & Ruxton 2002). In any given situation, individuals may make assessments of the levels of risk that they are prepared to incur. Per capita risk is lower when in a larger group and this potentially allows individuals in such groups to engage in risky behaviours to a greater extent before unacceptable thresholds of risk are reached. Accordingly, in our study we saw that focal fish were more active, and resumed foraging more rapidly in the presence of conspecifics, relative to others that were tested alone. Few studies have made the link between boldness and social facilitation effects however, and indeed many previous studies have focused on the bold / shy responses of single animals, often restoring the social context only to look at aggressive or competitive interactions. One study to consider the social aspect in relation to
boldness, that of Magnhagen & Staffan (2005), showed that shy Eurasian perch (*Perca fluviatilis*) behaved more boldly when they were embedded within shoals consisting only of other shy individuals, compared to when they were initially held in shoals containing a mixture of bold and shy conspecifics. Their finding suggests that there may be an influence not only of the presence of other individuals upon the behaviour of a given animal, but also a more subtle effect of group composition.

Part 3 of our study revealed that although test fish were using public information, there was no correlation between boldness and public information use, in disagreement with our initial prediction. Research by Coolen et al. (2003) found that ninespine sticklebacks (*Pungitius pungitius*) used public information to a greater extent than did sympatric threespine sticklebacks. Godin & Clark (1997) reported that ninespine formed more cohesive shoals and inspected predators to a lesser extent than did threespine from the same population. Of these two closely related species, threespines generally possesses longer dorsal and pelvic spines and more robust bodily armour than do ninespines, and this is seen as an adaptive countermeasure to predation risk. It has been suggested that the less armoured and therefore more vulnerable ninespine stickleback should minimise predation risk through the adoption of shy-type behavioural strategies, such as greater reliance upon social information, whilst the armoured threespine stickleback is better equipped to gather and use private information. Interestingly, research by Webster & Hart (2006a) revealed that threespine sticklebacks gathered from a different population both to that studied by Coolen et al. (2003), and to that used in this study, also used public information. Further research by these authors (Webster et al. Submitted) has shown that threespine and ninespine sticklebacks from a sympatric population did not differ in their mean behavioural responses in a range of contexts, despite obvious differences in armour morphology. These results suggest that intra- and interspecific patterns of social information use vary between populations under the influence of as yet undetermined pressures, most likely including
predation risk and environmental instability. Clearly, further research in these areas is required.

Two hypotheses, the constraint and the adaptation hypotheses, have been developed to explain the presence and prevalence of bold / shy behavioural variation within and between populations. The constraint hypothesis argues that certain behavioural responses are controlled by the same underlying hormones or genes and are therefore correlated with one another. As a consequence, individuals should exhibit similar levels of boldness across different contexts, since the uncoupling of behaviours would require substantial mutation or evolution of the underlying genetic or endocrinal architecture. The adaptation hypothesis states that behaviours should become correlated only when it is adaptive for them to do so, whilst associations between behaviours that lead to a decrease in fitness should be selected against (Cheverud 1996; Bell & Stamps 2004; Bell 2005). As such, correlation between behaviours should vary between different populations, under the influence of the prevailing selective pressures acting upon each. These two theories oppose one another, since the former assumes the restriction of selection, whilst the latter is a consequence of it. The results of our study demonstrate that at least some bold / shy behavioural responses are context specific, in that individuals that behave shyly when alone in one situation may act more boldly when in the presence of conspecifics, a more naturally realistic situation for social species. This is consistent with the predictions of the adaptation hypothesis and contrary to those of the constraint hypothesis. Related to this, if social context influences boldness then making inferences about the bold / shy behaviour of social animals in nature using data derived from tests of single individuals may be misleading. Context specificity as a determining influence upon bold / shy behaviours and behavioural correlations has previously been demonstrated in species from a range of taxonomic groups including mammals (Reale et al. 2000), reptiles (Lopez et al. 2005), fish (Coleman & Wilson 1998) and cephalopods (Sinn & Moltschaniwskyj 2005). Furthermore, individual variation in
boldness has been seen to be correlated with body size (Brown et al. 2005; Lopez et al. 2005). morphology and health (Lopez et al. 2005) suggesting that physiology may play a role. As of yet neither of the constraint or the adaptation hypothesis has been rigorously tested. Future work should aim to definitively test these hypotheses by targeting multiple populations of a given study species that together are subject to a range of quantifiable selection pressures. By accounting for rates of mutation and genetic drift, such a study could confirm or refute either of these hypotheses, and would give us greater insight into how bold / shy axes come to exist, and into those selective agents that determine how the members of a population are distributed along it.
Chapter 7

Bold / shy behavioural variation and interspecific interactions
Within populations of animals, individuals can vary in the degree to which they engage in danger-prone behaviors, something that governs the way that they balance fundamental trade-offs between returns and risk when foraging, collecting information and competing for resources (Wilson et al. 1994; Wilson 1998; Sih et al. 2004). This form of behavioral variation has been referred to as the bold-shy axis (Ward et al. 2004), where individuals that accept greater degrees of risk are termed bold, and those which avoid or minimize risk are termed shy. Within a given population the distribution of individuals along this continuum can be substantial. Studies of bold-shy behavior in species from a variety of taxa have revealed that boldness tendencies can be correlated between behaviors, such that, for example, individuals that are more active in exploring a novel habitat may also act more boldly when under potential or perceived predation threat (Dugatkin and Alfieri 2003; Bell and Stamps 2004; Bell 2005; Quinn and Cresswell 2005), or show a greater capacity for learning (Sneddon 2003) relative to their less active conspecifics. Furthermore, boldness under certain contexts has been shown to correlate positively with ability when competing for resources (Verbeek et al. 1996; Ward et al. 2004). It is thought that the benefits of being bold, such as greater resource access and growth rates, are offset by the cost of higher exposure to mortality through predation, allowing the seemingly disadvantaged shy individuals to persist (Wilson et al. 1994; Wilson 1998).

Whilst research in this area has provided insight into the fine scale dynamics of intrapopulation behavioral ecology, less is known about how bold-shy behavioral variation affects interactions between species. In a series of laboratory studies we investigated the role of boldness in interspecific interactions between two ecologically similar species of fish, the threespine (*Gasterosteus aculeatus*) and the ninespine (*Pungitius pungitius*) stickleback. Both species are generalist predators of pelagic and benthic invertebrates and frequently...
occur sympatrically. They are thought to minimize competitive interactions through different microhabitat preferences, with the ninespine stickleback foraging to a greater extent amongst vegetation, and the better-armored threespine stickleback feeding more in open water. Threespine sticklebacks are generally better armored than are ninespine sticklebacks, possessing longer spines and more robust lateral plates along their flanks. These defenses are considered to be an adaptive response to the greater risk of predation incurred by occupying open habitats where cover from predators is less readily available (Godin and Clark 1997; Copp and Kovac 2003; Hart 2003). These habitat preferences are not definitive however, and in nature these species often shoal together, with interactions between them likely to be common where they co-occur.

In Part 1 of this study we compared the microhabitat preferences of the two species when a) in still or flowing water, and b) in the presence of conspecific and heterospecific local enhancement cues. Based upon previous research (Copp and Kovac 2003; Hart 2003) we predicted that ninespine sticklebacks should show a stronger preference for vegetated habitats that threespine sticklebacks under still water conditions. Since holding position in flowing water places energy demands upon fish, and because vegetated habitats provide shelter from flowing water, we further predicted that both species would show a greater preference for the vegetated habitat under flowing water conditions compared to when no flow was provided. Finally we predicted that habitat choice would be influenced by social cues. Social cues are used readily by both of these species (Coolen et al. 2003; Webster and Hart 2006), and we predicted that focal fish given the choice between open or vegetated habitat would preferentially occupy whichever one contained a shoal of conspecifics, irrespective of any innate habitat preference. In Parts 2 and 3 we performed a number of experimental assays that quantified individual behavioural variation across a number of related contexts. These included habitat preferences, activity levels, latency to leave refuge and approach prey, and the ability to compete for prey. Looking for correlations between
related contexts provided two advantages. Firstly, it allowed us to measure behaviors that directly affect habitat use overlap and resource competition, the focus of this study. Secondly, it provided a measure of the consistency of behavioural tendencies within individuals. In Part 2 we looked at the effect of individual behavioural variation upon microhabitat choice. We predicted that we would see both species specific differences in habitat preferences (Copp and Kovac 2003; Hart 2003) and also an effect of individual variation in behaviour. Specifically, we predicted that individuals that were more active, or which approached prey stimuli more readily would also spend less time sheltering in the vegetated habitat. In Part 3 we investigated prey competition between heterospecific pairs, predicting that individual competitive ability would be correlated with activity levels, habitat preferences and readiness to approach a prey patch. We made this assertion based upon previous research that has revealed relationships between an individual’s competitive ability and its tendency to incur risk in other situations in single-species interactions (Ward et al. 2004).

7.2 METHODS

7.2.1 Fish Collection and Housing

We collected threespine and ninespine sticklebacks from Melton Brook, Leicestershire, UK in September 2005. They were divided into conspecific groups of 20 and each group was assigned to its own chemically and visually isolated holding tank (40 by 25 by 25 cm, water depth 20 cm, with a 1 cm deep fine sand substrate, and artificial vegetation for cover). The water temperature and light: dark regimes were held at 10°C and 12: 12 hours respectively over the duration of the study. Maintaining this temperature and photoperiod was sufficient to inhibit reproductive behavior and prevented the fish from developing nuptial coloring.
Ward et al. (2004) reported that non-reproductive male and female adult threespine sticklebacks did not differing their boldness or behavior. Fish were fed both live *Daphnia* spp. and frozen Chironomid larvae once per day, unless otherwise stated below. They were held under these conditions for three months before the experiments began.

### 7.2.2 Part 1. Use of open and vegetated habitat: the effects of flow rate and social cues

a) Habitat use under still and flowing water conditions

We compared the habitat use of threespine and ninespine sticklebacks in groups of six fish under still and flowing water conditions. To do this, we used an aquarium measuring $3 \times 0.8 \times 0.25$ m ($1 \times w \times h$) filled to a depth of 12 cm. Within this tank, we used mesh screens to create an experimental arena measuring $1.2 \times 0.8$ m. We fixed a patch of artificial plants along the longest side of this arena to create a complex habitat zone to mimic a weeded area in the fishes' natural environment. In total, we used 30 plastic plants, set in a regular lattice pattern, creating a zone measuring $0.7 \times 0.21$ m. We used two treatments: in the first we varied the species composition of the experimental group and in the second we varied the flow within the experimental arena. We used three different group compositions, either six threespine sticklebacks, six ninespine sticklebacks or a mixed group composed of three of each species. We used two flow rates, 0 and $2.3 \pm 0.1$ cm/s. This gave a total of six experimental groups. Water was pumped into the experimental area via a series of baffles in order to homogenize flow strength and minimize turbulence across the width of the tank. Having passed through the experimental area the water was and recirculated to the first pump at the upstream end of the tank. For each experimental replicate, we added a group of six fish and allowed them to acclimatize. After the fish had been in the arena for 30 minutes we took a single photograph using a Nikon digital camera positioned 1.4 m above the arena.
The photographer was concealed from the fish using a cloth barrier to avoid scaring the fish. From this image we measured the distance of each fish to the artificial weed patch and took an average for the group. In the mixed groups we averaged the two species separately. Where a fish was already in the weed patch, it was given a score of 0. No fish was used more than once throughout the experiment. We ran 20 replicates for each of the 6 experimental permutations, and no fish was used more than once.

b) The effect of social cues upon habitat choice

We sought to determine whether preferences for vegetated or open habitat, thought to differ between our study species (Copp and Kovac 2003; Hart 2003) were expressed independent of, or under the influence of conspecific social cues. We used binary choice experiments to determine the habitat preferences of each species when either a shoal of conspecifics or a shoal of heterospecifics was present in each habitat. We used a 60 by 30 by 30 cm tank, water depth 27 cm with a 2 cm fine gravel substrate. The tank was divided into two 30 by 30 cm habitat zones. One contained artificial vegetation constructed from 5 mm thick strands of green twine, arranged in 16 clumps of 10 strands each, running to the water surface and weighted at the bottom. The other habitat zone was left open. In the centre of each habitat zone we placed a 7 by 7 cm by 27 cm tall holding unit. These were used to hold stimulus shoals of fish, as described below. Half way in between these, in the centre of the tank we placed a third holding unit of the same size. This was used to hold the focal fish prior to the beginning of each trial. Holding units were made from clear perforated plastic, allowing the exchange of visual and chemical cues, and focal fish in the central holding unit had an unrestricted view of both stimulus shoals. We ran a total of four sets of trials, with eight replicates each:
1) Threespine stickleback focal fish with a conspecific stimulus shoal in the open habitat unit and a heterospecific stimulus shoal in the vegetated habitat zone.

2) Threespine stickleback focal fish with a heterospecific stimulus shoal in the open habitat unit and a conspecific stimulus shoal in the vegetated habitat zone.

3) Ninespine stickleback focal fish with a conspecific stimulus shoal in the open habitat unit and a heterospecific stimulus shoal in the vegetated habitat zone.

4) Ninespine stickleback focal fish with a heterospecific stimulus shoal in the open habitat unit and a conspecific stimulus shoal in the vegetated habitat zone.

Stimulus shoals contained three individuals, one with three- and one with ninespine sticklebacks. All stimulus and focal fish were size-matched to within <2 mm standard length within trials. All fish had been housed separately from each other previously, and were therefore unfamiliar to one another. Stimulus shoals were placed in their respective holding units and allowed to settle for 5 minutes. The focal fish was then placed in the central holding unit and allowed to settle for a further 2 minutes. After this time the central holding unit was raised, releasing the focal fish and beginning the trial. The trial ran for three minutes and we recorded the amount of time spent by the focal fish in each habitat unit. Observations were made via a vision slit in a black screen in order to remove observer effects. No focal or stimulus fish was used more than once.

7.2.3 Statistical analysis for Part 1

a) Habitat use under still and flowing water conditions

We calculated the mean distance of the test fish from the vegetation patch in each trial. In the mixed species trials we calculated a mean value for each species. In the single species we
used a two-way ANOVA to look for species specific differences, the effects of flowing
versus still water conditions, and any interaction between these treatments upon the mean
distance of fish from the vegetation patch. In the mixed species trials we subtracted the mean
distance from the vegetation of the threespines from that of the ninespines and compared
these to a null expected value of zero using Wilcoxon signed ranks tests, performing two
analyses, one for the mixed and one for the flowing water treatment.

b) The effect of social cues upon habitat choice

We converted the amount of time spent by focal fish in each habitat unit into a proportion of
the total trial time. We subtracted the proportion of time spent in the habitat unit that
contained the heterospecific stimulus shoal from that spent in the habitat unit that contained
the conspecific stimulus shoal, and compared them to a null expected value of 0 using
Wilcoxon signed rank tests. We performed these analyses for each of the four sets of trials.
Data were not normally distributed, precluding the use of parametric statistics.

7.2.4 Part 2. Behavioral correlates of habitat use

The aim of this experiment was to determine whether preferences for vegetated areas of
habitat were due to species specific differences, as predicted by previous research (Copp and
Kovac 2003; Hart 2003) or whether they were predicted by individual variations in
behavioural tendencies. We quantified two such behavioural tendencies: activity in a novel
environment, and the latency to cross a barrier and approach a prey patch. We used 34
threespine and 35 ninespine stickleback, measuring between 31 and 50 mm standard length.
For each fish we recorded the blotted mass to the nearest 0.001 g, and then placed it in its
own separate chemically and visually isolated holding tank measuring 30 by 20 by 20 cm,
water depth 17 cm with a 1 cm deep fine sand substrate. Artificial vegetation was provided for cover to minimize stress.

For each individual we collected a range of behavioral and body size data according to the following experimental schedule:

Day 1: Fish assigned to its own holding tank
Day 3: Activity level test
Day 5: Habitat preference test
Day 9: Latency to approach a prey patch test

Fish were fed one hour after being placed in their holding tanks on day 1, and one hour after being tested on days 3, 5 and 9. Fish were also fed on day 7. They were otherwise not fed during the experimental period. Following the end of the testing schedule on day 9 fish were transferred to new tanks, where they were retained as laboratory stock. Details of each of the tests performed are given below. No fish was used more than once. All testing took place in tanks measuring 60 by 30 by 30 cm, with a water depth of 27 cm and a 2 cm deep layer of 5 mm gravel. These tanks were covered externally with black screening on the sides and rear to minimize outside disturbance. All observations were made via a slit in a black screen in order to remove observer effects.

Activity level test

Fish were tested in an open environment; aside from a 2 cm deep layer of 5 mm gravel the tank used in this treatment was unfurnished. Prey odor cues were provided to stimulate the fish to forage. The fish therefore faced a trade-off between increasing activity to search for prey, something that might increase their likelihood of being detected by a predator, or remaining inactive, at the cost of a potentially reduced prey intake. We obtained a prey odor solution by crushing 1.5 g (wet mass) of frozen Chironomid larvae in 10 ml of tank water.
This was filtered through fine gauze to remove solid debris and was added to the tank and allowed to disperse for two minutes before the introduction of the fish. We used prey odor cues rather than actual prey because this stimulates the fish to forage whilst preventing them from becoming satiated and ceasing foraging behavior before the trial ends. Fish had been fed Chironomid larvae since being captured, so this odor was not novel to them. The focal fish was introduced to the centre of the tank within a 7 by 7 cm, 27 cm tall holding unit. This was constructed of clear, perforated plastic, allowing the fish to assimilate visual and chemical cues from the test tank. The fish was held in the unit and allowed to settle for 5 minutes, before this was removed, releasing the fish, and beginning the trial. The trial ran for 5 minutes and we used point sampling (Lehner 1996) at 15 s intervals (giving n= 20 measurements) to record whether the fish was swimming or stationary, and whether the fish was more than 5 cm from the substrate, since remaining low in the water column can constitute a form of refuging behavior. Following each trial the fish was returned to its respective home tank and the water in the test tank was replaced and fresh prey odor cues were added.

Habitat preference test

In a variation on the experiments conducted in Part 1, we performed a habitat preference test in order to test the putative species-specific habitat preferences described by Copp and Kovac (2003) and Hart (2003). This experiment provided the data for the dependant variable in Part 2 of the study. One half of the tank contained artificial vegetation constructed from 5 mm thick strands of green twine. These were arranged in 16 clumps of 10 strands each, running to the water surface and weighted at the bottom. They were spaced 5 cm apart in a grid within one 30 by 30 cm half of the tank. The other half of the tank was open, containing no structure apart from the gravel substrate. The tank was covered externally with black
screening on the sides and rear. The fish was introduced to the centre of the tank within a 7 by 7 cm, 27 cm tall holding unit. This was constructed of clear, perforated plastic, allowing the fish to assimilate visual and chemical cues from the test tank. The fish was held in the unit for 5 minutes, before this was removed, releasing the fish, and beginning the trial. The trial ran for 5 minutes and we used point sampling (Lehner 1996) at 15 s intervals (giving n=20 measurements) to record whether the fish was in the vegetated or the open half of the tank. Following each trial the fish was returned to its respective tank and the water in the test tank was replaced.

Latency to approach a prey patch

Visual predators are more likely to detect prey when they pass over substrates or backgrounds against which they are conspicuous (Merilaita et al. 1999), and there is evidence to suggest that many species of fish mediate such predation risk by actively avoiding structurally simple or non-cryptic substrates (e.g. Gotceitas and Brown 1993; Houtman and Dill 1994). In this study we measured the latency of focal fish to move from a refuge over a white, open area to reach a prey patch that lay beyond it. The test tank was divided into three sections along its longest axis. Those at either end were 15 cm wide. One contained a prey patch in the form of a centrally placed 5 cm diameter 30 cm tall clear plastic cylinder containing approximately 200 live *Daphnia* spp. The other end section contained artificial vegetation constructed from 5 mm thick strands of green twine, arranged in 5 clumps of 10 strands each, running to the water surface and weighted at the bottom. Three were placed 5 cm apart, and 5 cm from the end of the tank, and the remaining two were placed 5 cm in front of these, and 10 cm apart from each other. This section formed a refuge. The central section between the prey patch and the refuge contained a 30 by 30 cm white plastic sheet laid flat upon the substrate. Fish were highly conspicuous against this
background, something that would in nature increase their risk of being detected by a predator. Fish had to cross this central zone in order to reach the prey patch. We placed a 7 by 7 cm, 27 cm tall holding unit in the refuge section, between the two forward-most clumps of vegetation. This was constructed of clear, perforated plastic, allowing the fish to assimilate visual and chemical cues from the test tank. The fish was held in the unit for 5 minutes, before this was removed, releasing the fish, and beginning the trial. We recorded the time taken for the fish to leave the refuge, cross the central section and attempt to strike the *Daphnia* within the prey patch cylinder. We imposed a cut-off time of 10 minutes on each trial: fish that had not completed the trial in this time were recorded as having taken 10 minutes to do so.

### 7.2.5 Statistical analysis for Part 2

We used a general linear model to identify predictors of habitat use. All proportional data were found to be normally distributed following arcsine transformation. Habitat use (the proportion of time spent in the vegetated habitat in the habitat preference test described above) was designated as the dependant variable. Species was assigned as a fixed factor, and the proportion of time spent <5cm from the substrate and the proportional movement rate of each individual, both quantified in the activity level test, and the latency of each individual to approach the prey patch were included as covariates. In the latency to approach the prey patch test, eight of the 34 threespines and seven of the 35 ninespines failed to leave cover and approach the prey patch. For the purposes of analysis there were recorded as having taken 10 minutes to do so, the cut off time that we imposed upon the trial duration. Latency to approach the prey patch was converted to a proportion of the total trial time (10 minutes) before analyses. Body mass was also included as a covariate, and data describing this variable were seen to be normally distributed. Finally, we performed two Spearman rank
correlations, one for each species, comparing the activity levels of individuals with their latency to approach the prey patch.

7.2.6 Part 3. Interspecific prey competition: behavioral correlates of individual variation in competitive ability

In the final part of this study we looked at short-term competitive interactions between individuals of the two species. We looked to see if they differed in their ability to compete for a common prey species, and aimed to identify behavioral covariates of competitive ability that might influence competition outcomes.

We formed 14 pairs of one threespine and one ninespine stickleback, size matched to <5 mm standard length. Each pair was housed in a chemically and visually isolated tank measuring (30 by 20 by 20 cm, water depth 17 cm with a 1 cm deep fine sand substrate). Pairs of fish were always housed together, except during trials where fish were tested alone. For each pair we ran three prey competition trials, and collected a range of behavioral data according to the following experimental schedule:

Day 1: Fish weighed to the nearest 0.001 g and assigned in pairs to their holding tank

Day 3: Prey competition trial 1

Day 5: Prey competition trial 2

Day 7: Prey competition trial 3

Day 9: Activity level test

Day 11: Individual habitat preference test

Day 15: Latency to approach a prey patch test

Fish were fed one hour before being placed in their holding tanks on day 1, and within one hour of being tested on every second day. Fish were also fed on day 13. They were otherwise
not fed during the experimental period. Following the end of the testing schedule on day 15, fish were transferred to new stock tanks. No fish was used more than once. Details of each of the tests performed are given below.

Prey competition trials

Fish were tested within their own holding tank so as to minimize stress. Fish were provided with single, sequentially delivered *Daphnia* spp., a common prey type of both species. Prey suspended in 2 ml of tank water were added via a 5 ml syringe through one of five holes, spaced 2 cm apart at the water line of each aquarium. The order in which each hole was used was randomized to prevent fish from monopolizing or predicting optimum feeding positions. We introduced a total of six prey items, with a 30 s period between the ingestion of one item and the introduction of the next. We recorded the species in each pair that consumed the prey item in each instance. This test was performed three times according to the above schedule, and was used to calculate the mean prey intake per partner per pair. Immediately after testing we provided excess *Daphnia* to the tank, so that both fish could feed until satiated. Uneaten prey were removed after 30 minutes. This served to standardize hunger levels between trials.

Other behavioral trials

The members of each pair were tested individually in the activity level test, the habitat preference test, and the latency to approach a prey patch test as described above for Part 2.

7.2.7 Statistical analysis for Part 3
Within each pair we subtracted the mean proportional prey share of the ninespine stickleback from that of the threespine stickleback. This gave a value where a positive score corresponded to a greater prey share for the threespine stickleback and a negative score indicated a greater prey share for the ninespine stickleback. We also did this for body mass, the proportional movement rate of each pair member, the proportion of time spent <5cm from the substrate (both quantified in the activity level test), and the latency of each pair member to approach the prey patch, which was converted to a proportion of the total trial time (imposed at 10 minutes). In this latter test, two of the 14 threespines and one of the 14 ninespines failed to leave cover and approach the prey patch. For the purposes of analysis there were recorded as having taken ten minutes to do so. Because data were not normally distributed we used non-parametric statistics throughout. We used Spearman rank correlations to compare each of these variables with the observed prey share difference values. In order to compare competitive ability for prey between species we compared the difference in prey share between species from each trial against a null expected value of 0 using a Wilcoxon signed rank test.

7.3 RESULTS

7.3.1 Part 1. Use of open and vegetated habitat: the effects of flow rate and social cues

a) Habitat use under still and flowing water conditions

We saw no differences between species, nor any effect of flow treatment or any interaction between these, upon the use of vegetated habitat by fish in this experiment (see the results of a two-way ANOVA presented in Table 7.1). We also saw no differences in vegetation use
between species in mixed species groups (no flow: Wilcoxon signed rank test: \( n=20, Z=-0.80, P=0.42 \); flowing water: \( n=20, Z=-0.91, P=0.36 \), Figure 7.1).

**Table 7.1.** A two-way ANOVA revealed that there were no species specific differences, no effect of experimental flow regime (still water or flowing water), nor any interaction effect between these upon the mean distances that threespine and ninespine sticklebacks ventured from an experimental vegetation patch.

<table>
<thead>
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<th>Source</th>
<th>Degrees of Freedom</th>
<th>F</th>
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<tr>
<td>Total</td>
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</table>
Figure 7.1. Threespine and ninespine sticklebacks were not seen to differ in the mean distance that they maintained from the vegetation patch, either under still- or flowing water (2.3 cm / s) treatment regimes. Filled bars represent threespine sticklebacks and open bars represent ninespine sticklebacks.

b) The effect of social cues upon habitat choice

Focal fish were seen to always prefer the habitat in which the conspecific shoal was located (Wilcoxon signed rank test: threespine sticklebacks, open habitat, n=8, Z= -2.60, P= 0.010; vegetated habitat, n=8, Z= -2.53, P= 0.011; ninespine sticklebacks, open habitat, n=8, Z= -1.82, P= 0.017; vegetated habitat, n=8, Z= -2.05, P= 0.012; Figure 7.2).
Figure 7.2. Fish spent more time in the habitat that contained a conspecific shoal than the one containing a heterospecific shoal in a binary choice study. Filled bars represent threespine sticklebacks and open bars represent ninespine sticklebacks. The horizontal line represents the null expected time allocation of 50%.

7.3.2 Part 2. Behavioral correlates of habitat use

A general linear model revealed both inter- and intraspecific predictors of habitat use. Specifically, ninespine sticklebacks were seen to spend more time in the vegetated habitat than did threespine sticklebacks, whilst fish of both species that spent more time moving in the activity level tests, also spent more time in the open habitat (Table 7.2). Spearman Rank correlations revealed that individuals that spent more time moving in the activity level test tended to approach the prey patch sooner in the latency to approach the prey patch test
(threespine sticklebacks: $r = -0.42$, $n=34$, $P=0.03$; ninespine sticklebacks: $r = -0.33$, $n=35$, $P=0.04$; Figure 7.3.).

Table 7.2. A General Linear Model revealed that both movement rate and species predicted vegetated habitat use, accounting for a total of 49% of the observed variation between them.

**Dependent variable: Proportion of trial time spent in the vegetated habitat**

<table>
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<th>Source</th>
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</tr>
</thead>
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</tr>
<tr>
<td>Proportion of trial time &lt;5 cm from substrate</td>
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<tr>
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<td>0.55</td>
</tr>
<tr>
<td>Mass</td>
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<td>0.42</td>
</tr>
<tr>
<td>Error</td>
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<tr>
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</tr>
<tr>
<td>Corrected Total</td>
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</tr>
</tbody>
</table>

$R^2 = 0.49$

Figure 7.3.
There was no species level difference between threespine and ninespine sticklebacks in their competitive ability for *Daphnia* prey (Wilcoxon signed rank test: n=14, Z= -0.85, P= 0.39).

At the individual level, Spearman Rank correlations revealed no relationship between differences in prey share and differences between the mass of the two competitors (r= -0.11, n= 14, P= 0.34), differences between the time spent by either competitor in the vegetated habitat in the habitat preferences tests (r= -0.21, n= 14, P= 0.46), or differences between the
time spent <5 cm from the substrate by either competitor in the activity level test ($r=0.38$, $n=14$, $P=0.17$). We did however find significant relationships between differences in prey share and differences in activity levels ($r=0.49$, $n=14$, $P=0.04$), with individuals that spent more time moving in the activity level test commanding a great share of prey in during the competition trials. We also saw that individuals that left the refuge to move towards the prey patch sooner in the latency to approach the prey patch test also tended to consume a greater prey share ($r=0.52$, $n=14$, $P=0.03$, Figure 7.4).
Figure 7.4. Differences in prey share within pairs of competing threespine and ninespine sticklebacks were correlated with differences in a) the latency to leave cover and approach a prey patch, and b) the proportion of time that they spent moving. Each data point represents one heterospecific pair, with a positive value indicating that the threespine stickleback scored higher, and a negative score indicating that the ninespine stickleback scored higher. Values are proportional.
This study revealed that individual level variation in behavior can have significant effects upon interspecific interactions. We saw that three behaviors, habitat use, movement rate and latency to approach prey were related to one another. We also saw that movement and latency to approach prey were correlated with competitive ability. Spending more time moving, more time in the open, and taking less time to leave shelter and approach prey is consistent with the 'bold' type behavioral phenotype described previously in sticklebacks (Bell and Stamps 2004; Ward et al. 2004; Bell 2005) as well as in other species (Wilson et al. 1994; Wilson 1998; Sih et al. 2004).

Parts 1 and 2 of our study considered habitat use. In Part 1 a) we saw no differences in the distance that either species ventured from the cover provided by experimental vegetation patches, and this was not affected by flow regime. In Part 2 however, we did observe species specific differences in habitat use patterns, specifically that ninespine sticklebacks tended to remain in the vegetated habitat to a greater extent than threespine sticklebacks. This latter finding supports previous research (Godin and Clark 1997; Copp and Kovac 2003; Hart 2003), and is in accordance with our initial predictions. The apparent discrepancy between Part 1 a) and Part 2 may be explained by social facilitation effects. In Part 1 a) fish were tested in groups, while in Part 2 they were tested alone. Being within a group generally decreases per capita predation risk, allowing animals to invest less effort in vigilance and, in the case of ninespine sticklebacks, potentially reducing their reliance upon cover to mediate predation risk. Social conformity, the adoption by individuals of the behavior of the majority of the group may also have influenced cover use in Part 1 a). This was seen to be the case in Part 1 b) where we observed that fish of both species preferentially shoaled in either open or vegetated habitats when stimulus shoals of conspecifics were present there.
Part 2 of our study revealed the importance of individual variation in behaviour in determining habitat use. For both species, the proportion of time spent in the vegetation patch was negatively correlated with their movement rate, quantified in a separate activity level test. Utilizing the shelter of a vegetated habitat may constitute an anti-predatory response, whilst reducing swimming activity is also a common reaction to perceived risk (Lehtiniemi 2005). Movement rate was also correlated with the latency to leave cover and approach prey, indicating that behavioral tendencies pertaining to different aspects of habitat use are correlated within individuals.

In Part 3 we found no evidence of interspecific differences in prey competitive ability. Rather, we found that individual behavioral variation was again a better predictor of prey competition outcomes. Competitors that were more active, or that left shelter and approached prey stimuli more rapidly were more likely to consume a greater proportion of the contested resource. This finding confirmed our initial prediction, and supports and builds upon the findings of a previous study (Ward et al. 2004) which revealed that bold threespine sticklebacks out competed shy ones in intraspecific prey competition.

Taken together, the findings of our study suggest that whilst species specific differences in preferences for open and vegetated areas of habitat do exist, individual habitat choice is also strongly influenced by social attraction to conspecifics, and by individual behavioural variation. Furthermore it would seem that variation at the individual level, rather than at the species level can be more important in resolving foraging contests. All of these factors can potentially influence patterns of habitat use overlap and resource competition between species, determining both the frequency with which it occurs and the outcome of the resulting interactions. Detailed comparisons between ecologically similar species therefore, cannot necessarily be made based upon the species specific mean scores determined for given behavioral or ecological traits. Rather, if high resolution analyses of interspecific or community interactions are to be completed accurately, appreciation of the nature and
prevalence of behavioral and ecological diversity at the within-species level of scale is required.

Overlaps in resource use occur to varying extents between species of ecological generalists, whose broad niches encompass a range of resources. Competition between such species is often offset by character displacement in one or more of the competing species (Schluter and McPhail 1992; Schluter 2000). The extent to which resource use overlap and resulting character displacement occurs and the form that they take, is likely to be affected by environmental pressures operating at the population level. In this study we considered interactions between sympatric populations from a single site, however for other populations, where different environmental conditions prevail, the extent of niche overlap may differ, in turn affecting the potential for intraspecific individual variation to influence interactions within and between species. Such environmental pressures may include habitat structure, and the diversity of predator and prey assemblages. These in turn affect niche differentiation and the predation pressure to which each species is subject. When two competing species differ in their vulnerability to predation, then species specific differences in boldness and associated behaviors might be expected. Threespine sticklebacks are more robustly armored than are ninespine sticklebacks, generally possessing longer spines and more substantial lateral plates. Godin and Clark (1997), studying sympatric threespine and ninespine sticklebacks, showed that the threespines shoaled less cohesively in the presence of predatory fish, and inspected these predators to a greater extent than did the ninespines. Related to this, Coolen et al. (2003) found that these species differed in their use of public information, with ninespine sticklebacks using such information more than threespines. They suggested that doing so allowed ninespines to reduce the need to sample foraging sites firsthand, a strategy that potentially decreases their exposure to predators. Where predation pressure is intense, we might therefore expect to see greater segregation between ecologically similar species, and therefore less scope for ecological interaction.
Intrapopulation variation in bold-shy behavior has already been shown to be a widespread phenomenon in the animal kingdom, one that influences population level interactions and promotes differential fitness. In this study we have shown that bold-shy behavioral variation can also affect interactions between species, and that it potentially plays a role in determining community level interactions. Further work could build upon these findings by examining the magnitude of this variation in multiple populations of ecologically similar sympatric species that are subject to a range of selective pressures. Such an approach could further our understanding of the role of environmental heterogeneity in driving both intra- and interpopulation variation in boldness, and also give us a clearer appreciation of how it can influence interactions between species.
Chapter 8

Summary and further work
In this chapter the main findings of the experiments presented in the previous chapters of this thesis are drawn together. The specific findings of each study in relation to previous research and existing theory have already been discussed in detail in each of Chapters 2 to 7. Here the main findings are summarised and future research directions that build upon these contained within this thesis are suggested.

8.1 Summary

8.1.1 Using social information

In Chapter 2, threespine sticklebacks were seen to be capable of using private information when foraging, relating to habitat substructure to their experience of foraging success. They were seen to be able to discriminate between different types of subhabitat in a foraging context. This finding builds upon previous research that has shown that fish use search images when foraging (Hughes & Croy 1993) and that they can recall the relative profitability of multiple, spatially fixed prey patches (Milinski 1994). Basing their foraging preferences upon habitat characteristics potentially allows fish to obtain a crude estimate of prey yield in patches of habitat that they have never previously exploited, provided that they are equipped with and can recall previous experience of foraging in similar subhabitats.

Threespine stickleback were also seen to make use of a variety of social information cues when foraging. These included simple local enhancement as well as more subtle forms of public information. Interestingly, when these social cues were in conflict with their private information, test fish made use of social information cues. This suggests that social conformity is prioritised over individual experience, perhaps because the costs of conformity are lower than the potential risks associated with deviation from the behaviour of the majority. The trade-offs between innovation and conformity in relation to information use are discussed further below.
Chapter 3 considered the role of recent habitat use in relation to shoaling preferences and shoal structuring patterns. Fish were seen to be able to track changes in patterns of habitat use, using unknown habitat specific chemical cues, with a relatively high degree of temporal resolution. It is suggested that fish should use resource derived cues, a form of olfactory social information, as a means of matching resource use patterns in order to obtain social information that could enhance their foraging efficiency. This is a relatively new finding and avenues for further research to test this assertion are suggested below.

8.1.2 Competing for prey

Resource competition is thought to be a major cost of group living. In Chapter 4 it was seen that levels of prey competition were affected by prey distribution. When prey was dispersed both through space and time, levels of aggressive competition were initially high, compared to when prey distribution was patchy. This was interpreted as being due to the potential payoffs from competing being higher when prey provision was uncertain and unpredictable compared to when multiple prey were simultaneously available. An additional variable was seen to be group stability. It was seen that the initially high prey competition levels when prey were dispersed decreased over time in sequential trials as a function of group stability. The interpretation of this finding is difficult. Previously, similar findings have been explained by familiarity (e.g. Utne-Palm & Hart 2000), however when group composition is stable, with no individuals leaving nor others joining the potentially beneficial effects of discriminating between familiars and non-familiars is lost. When all individuals are familiar there can be no effects of heterogeneous advantage; the potential benefits to be gained from competing with members of an all familiar group should be the same as when all members of the group are unfamiliar. Interestingly, an experiment detailed in Chapter 4 does indeed suggest a role of familiar recognition in mediating prey contests. Here it was seen the competition levels were lower between members of embedded familiar pairs than they were
between non-familiar group members. This implies that some form of recognition is taking place, and further research is required to better determine the role of familiarity in relation to prey competition.

Another finding of Chapter 3 was that prey shares differed substantially between individuals. This was not affected by familiarity and was seen under both modes of prey distribution. Experiments conducted in Chapters 6 and 7 demonstrated that individual variation in bold/shy behaviour strongly predicted prey share and prey contest outcomes, both in interactions between threespine sticklebacks and also in interspecific contests between threespine and ninespine sticklebacks.

8.2 Further work

8.2.1 Trade-offs between using private and social information

In the experiments detailed in Chapter 2 of this thesis, fish that were equipped with asocially acquired private information were seen to defer to certain social information cues when the two were in conflict. This was interpreted as an adaptive response, since behaving differently from the majority of the group, for example by visiting a different prey patch, potentially increases the exposure of that individual to predation risk. This finding suggests that the potential costs of nonconformity are greater than the potential costs of using social information (such costs may include eliciting maladaptive behaviours in response to inaccurate social information). These data were acquired from fish obtained from a single population. It is possible that the costs and benefits of nonconformity may vary between populations as a function of local selective pressures such as predation risk, prey distribution and competitive intensity. Fish may respond to this by displaying a greater tendency towards using private information over conflicting social information, if the costs associated with doing so are lower.
Related to this, the use of socially versus privately acquired information should be linked to local rates of environmental change. When environmental variables such as prey type and distribution and habitat structural complexity are relatively stable over time individuals should display conformist behaviours, relying to a greater extent upon socially transmitted information, in order to preserve and maximise group living benefits such as predator defense. Conversely, when environmental variables are unstable and subject to change over time individuals stand to gain from using innovative behavioural strategies. This is because social information may become outdated more rapidly, leading to the development of informational cascades. By gathering and acting upon private information an individual stands to gain from potentially greater foraging efficiency (Boyd & Richerson 1985).

Further work should therefore be directed towards quantifying differences in the degrees of social conformity between populations of species where the costs of conformity differ. Predation risk is one factor that likely affects these costs, and the well established study system of high and low predation pressure populations of Trinidadian guppies represents an ideal focus for this research. Such work, closely tied to current theory on innovation versus imitation (Boyd & Richerson 1985), could greatly increase our understanding of the constraints that act upon adaptive information use by social animals.

8.2.2 Social information in a producer-scrounger relationship

Most existing research on the use of social information has focused upon the receiver of the information, for example by examining the trade off they face between using inexpensive, but potentially outdated social information versus accurate private information that may be costly to collect. Less research effort has been directed towards the individuals that are producing the information, and the costs and benefits that they are subject to. Information producers and receivers may be viewed as producers and scroungers, with the receiving individuals scrounging information that is being produced by those individuals that are
exploiting the resource. The benefit to the receiver of this information has been well
documented, but the costs faced by the producers of the information, and the means by
which they potentially reduce them warrant further investigation.

Costs to information producers of attracting receivers probably primarily centre around
resource competition. Attracting further individuals to a prey patch should lead to the
resource being depleted more rapidly and may lead to direct and aggressive competition, and
the further costs that this can bring. It should therefore be adaptive for individuals that have
found a new resource to attempt to conceal their discovery from other foragers by reducing
the amount of information that they transmit. How they might do this is unclear. They may
attempt to handle and consume prey more rapidly, perhaps by investing less effort into other
behaviours such as predator vigilance. They may carry prey off to be consumed away from
the patch, or they may even monitor the degree to which they are being observed. only
feeding when potential information receivers are distracted or engaged in other behaviours.

Conversely, there may be other occasions when it would be better for an individual to
attract other foragers to a prey patch that it has discovered. When predation risk is high for
example, having other foragers close by may reduce the extent to which the discoverer of the
prey patch needs to engage in predator vigilance, a social facilitation effect, allowing it to
increase its own prey intake despite the greater number of potential competitors in
attendance.

Research effort should therefore focus upon answering questions such as: (1) Do
individual foragers actively attempt to control the intensity and the nature of the information
cues that they are producing? (2) What behavioural mechanisms do they employ to do this?
(3) Is there evidence to suggest that some individuals are primarily information generators
(i.e. producers) whilst others are primarily information receivers (i.e. scroungers)? Or do
individuals act as both with equal frequency? In this thesis I found no evidence that public
information use was linked to bold / shy behaviour (Chapter 6), whilst Coolen et al. (2003)
documented a difference that can potential be explained by the differential predation risk levels that either species is subject to. Finally, (4). Under what circumstances does the cost of generating social information vary, and is the information producer able to control the way in which it transmits information to others to reflect these changing circumstances, in order to gain an adaptive advantage?

8.2.3 Self referent matching of prey and habitat derived cues

The self-referent matching of prey and habitat derived chemical cues as a means of shoal assortment is a recently recognized phenomenon (Olsen et al. 2003; Ward et al. 2004; 2005; In Review: Chapter 3 of this thesis). As such there are numerous questions to be answered, and scope for much further research. Firstly we must determine how the mechanism operates: which chemical compounds are involved, and how are they sequestered from the prey or habitat that the fish is using? How are they transmitted by the fish, via the mucous coating of the epidermis, or through the excretion of waste perhaps? And is their release a passive or active mechanism? Finally, how are they detected by the receiving individual, and by what (if any) cognitive process does self-referent recognition take place?

As important a question as how the mechanism of self-referent matching operates in why it is used. In Chapter 3, two non-exclusive hypotheses were introduced, one based upon information use, and the other based upon the reduction of olfactory oddity.

The information use hypothesis proposes that individuals should group with others that have been exploiting the same resources as itself in order to reduce sampling costs by obtaining social information cues from them. Future work could test this hypothesis by comparing rates of social transmission of information between resource use matched and mismatched individuals, and by comparing rates of learning from demonstrators by naïve individuals that share or differ in their resource use patterns.
The olfactory oddity hypothesis predicts that self-referent resource use matching is a counter measure to predation risk imposed by predators that use olfaction or chemotaxis to detect prey. Such predators may include the predatory aquatic larvae of some beetles, as well as predatory fishes. Though vision is the primary sensory mode of many predatory fish species, olfaction is also important, and may be relied upon to a greater extent in structurally complex or highly turbid environments. Research exploring this hypothesis could take two starting points. Firstly, the role of direct predator choice could be examined; predators could be exposed to multiple odour trails from prey fish and given the choice of approaching or attempting to prey upon individuals treated to have a matched odour (i.e. that of the majority of the stimulus group) or an odd odour. A second experimental series could consider the behavioural responses of olfactory oddity in the prey fish, by asking whether olfactorily odd individuals display any risk adverse behaviours that might offset the putative costs of oddity.

Self-referent resource matching is potentially adaptive and may be a key mechanism involved in social organisation in nature. It has already been demonstrated that this mechanism operates in natural systems (Ward et al. 2006), and it is know that self-referent matching overrides preferences based upon individual recognition in some species (Ward et al. 2004; 2005). It is a less costly mechanism of recognition that is familiarity, since it does not assume prolonged prior interaction between individuals, nor does it require the capacity to learn and recall individual identities. Furthermore, it has the potential to explain some of the behaviours and benefits that have previously been ascribed to familiar recognition.

8.2.4 Familiarity as a mechanism of recognition and assortment in nature
Familiarity, the ability of an individual to recognize others with whom it has recently interacted, has been widely documented in shoaling fishes (Griffiths 2003; Ward and Hart 2003; Griffiths and Ward 2006). Some of the putative benefits gained by shoaling with familiar individuals are discussed in Chapter 3 of this thesis, and experiments detailed in
Chapters 4 and 5 suggest that familiarity can reduce the intensity of prey competition in certain contexts. Despite the substantial body of evidence suggesting that familiar recognition exerts substantial evidence on shoal choice behaviour in the laboratory, the important of familiarity as a shoal structuring mechanism in nature remains equivocal. A possible explanation for this relates to the costs associated with the acquisition of familiarity, and learning the identities of others. Firstly, it takes time to acquire familiarity based preferences, often several days or weeks of persistent interaction (Griffiths and Magurran 1997b; Croft et al. 2004a). Secondly, the cognitive demands of individual recognition limit the number of individual identities that can be learned (Griffiths and Magurran 1997a). In nature the fission-fusion structure of the shoals of many species may limit the amount of time for which fish can interact with one another. Shoals are known to exchange individuals as often as every few minutes or hours (e.g. Croft et al. 2003; Hoare et al. 2003), and this instability might be enough to prevent familiarity from developing. Furthermore, the number of individuals any one fish might encounter and interact with is potentially massive, far exceeding the number of individual identities that it could learn or recall (e.g. Griffiths and Magurran 1997a). In the laboratory setting both of these confounding variables are controlled for; the density of individuals is held constant whilst shoal composition is enforced within the usually relatively small holding tank.

Research effort should therefore be focused upon a direct assessment of the role of familiarity in mediating shoal structure in free ranging shoals. Such an approach could examine patterns of shoal fidelity by individuals, perhaps by taking advantage of recent advances in PIT (Passive Integrated Transponder) tag technology (Skov et al. 2005) and social network theory (Watts & Strogatz 1998). Comparisons could be drawn between naturally formed shoals and artificial shoals that have been conditioned to be familiar in the laboratory. Much of the laboratory evidence for familiarity is derived from binary choice tests in which focal fish are given the choice between shoaling with one or another treatment.
groups in an otherwise socially sterile environment. By conducting more complex shoal preference experiments on free-ranging shoals or upon captive shoals in a mesocosm setting we should be able to better determine whether the role of familiarity as a determining factor in shoal structuring has been overstated.

8.2.5 Bold / shy behavioural phenotypes

Bold / shy behavioural correlations have been documented both within (e.g. Chapters 6 and 7 of this thesis) and also between populations (Bell 2005). It is not presently clear how certain behaviours come to be correlated with one another, and two hypotheses, the constraint and the adaptation hypotheses, have been posited (Bell 2005). The constraint hypothesis dictates that certain behavioral responses vary in concert because they are controlled by the same endocrine mechanisms, or are associated with the same assemblages of genes. As a consequence, changes in the form of one behavior should be accompanied by corresponding changes in the other correlated behaviors, since the uncoupling of behaviors would require substantial mutation or evolution of the underlying genetic or endocrinial architecture. The adaptation hypothesis states that behaviors should become correlated only when it is of adaptive value for them to do so, whilst associations between behaviors that lead to a decrease fitness should be selected against (Cheverud 1996). As such, correlation between behaviors should vary between populations, under the influence of the prevailing selective pressures acting upon each. These two theories oppose one another, since the former assumes the restriction of selection, whilst the latter is a consequence of it. Many of the empirical studies from which our current understanding of behavioral syndromes is derived have focused upon single populations of their chosen study species, meaning that neither of these hypotheses has been rigorously tested. Exceptions to this pattern include works by Palmer and Dingle (1986) and Dingle et al. (1988), and Bell and Stamps (2004) and Bell (2005) who observed correlated behaviors in one but not in another of two
populations of their respective insect and fish study species. Another study, by Riechert and Hedrick (1993) detected similar patterns of correlation between behaviors in two spider populations. Finding that behavioral correlations differ between two populations does not necessarily rule out the constraint hypothesis, since the possibility of mutation or genetic drift must be controlled for.

There is substantial evidence from single population studies that the expression of boldness is flexible and context dependent, something that the constraint hypothesis should militate against. Behaving in a consistently bold or shy manner is potentially costly, preventing individuals from eliciting optimal responses to different threats or stimuli. Given this, flexibility between contexts in bold/shy responses likely brings adaptive benefits, and previous research provides support for this (Coleman & Wilson 1998; Reale et al. 2000; Lopez et al. 2005). Interestingly, research by Lopez et al. (2005) revealed behaviours within a single population of Iberian rock lizards (Lacerta monticola) that could be grouped into two independent bold-shy continua, suggesting a more complex situation whereby different behaviours may be arranged into multiple syndromes.

It is conceivable that either mechanisms operate in different species, and there is need for research that explicitly tests the constraint and adaptation hypotheses by targeting behavioral syndromes within multiple populations that together are subject to a range of selective pressures. Such a study should account for rates of mutation and genetic drift between populations, and should ultimately aim to identify those genes that are involved in behavioural syndromes.

8.2.6 Interspecific interactions

The findings detailed in chapter 7 suggest that intraspecific individual variation in behaviour can have significant determining effects upon interspecific interactions between ecologically similar species. Over time, competition between ecological generalist species is generally
reduced by character displacement in one or more of the competing species (Schluter & McPhail 1992; Schluter 2000). The extent to which resource use overlap occurs, and the magnitude and nature of any associated character displacement is likely to be affected by population level differences in habitat conditions. In Chapter 7 I considered interactions between sympatric populations from a single site, however for other populations, where different environmental conditions prevail, the extent of niche overlap may differ, in turn affecting the potential for sources of intraspecific individual variation, such as bold-shy tendencies to influence interactions within and between species. Such environmental variables include habitat structure, the abundance of predators and prey assemblages, which may produce greater niche differentiation, or different exposure to predation pressure between species. When two competing species differ in their vulnerability to predation, then species specific differences in boldness and associated behaviours might be expected. In the context of stickleback interactions, the threespine stickleback is more robustly armoured than the ninespine stickleback, generally possessing longer spines and more substantial lateral plates. Godin & Clark (1997), studying sympatric threespines and ninespines, showed that the threespines shoaled less cohesively in the presence of predatory fish, and inspected these predators to a greater extent than did the ninespines. Related to this, Coolen et al. (2003) found that these species differed in their use of public information, with ninespine sticklebacks using such information more than threespines. They suggested that doing so allowed ninespines to reduce the need to sample foraging sites firsthand, a strategy that potentially decreases their exposure to predators. Where predation pressure is severe, we might therefore expect to see greater segregation between species that are similar in their ecology but that differ in their vulnerability to predation, something that could reduce potential for ecological interaction. The findings of chapter 6 could be built upon by a program of research that targets bold / shy behavioural traits within multiple sympatric populations that are subject to a range of environmental variables, in order to determine how
differing susceptibility to specific selection pressures affects ecological differentiation between potentially competing species.

8.3 Conclusions

A foraging fish can draw information from a variety of sources, both asocially and through the assimilation of social cues generated by those with which it is shoaling. This flexibility affords individuals the capacity to pursue adaptive foraging strategies in changing and uncertain environments. In this experimental series it was seen that fish adhered to patterns of social conformity when their private information conflicted with that of the group majority, suggesting that the benefits of social foraging outweigh the costs of resource competition. Finally, it was seen that individual behavioural variation strongly affected interactions that took place between individuals, highlighting the significance of fine-scale variation in relation to group level dynamics.
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