The Importance of Predator Behaviour on Risk to Prey

Christos C. Ioannou

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The candidate confirms that the work submitted is his/her own and that appropriate credit has been given where reference has been made to the work of others.

Abstract

Both predator and prey have evolved to maximise reproductive success by balancing food intake with risk. There has been a bias in predator-prey studies, where prey behaviour has been examined in detail, yet predators are assumed to follow simplistic rules. I use three-spined sticklebacks predating upon invertebrate prey to test a range of ways in which prey risk was hypothesised to be affected by predator behaviour.

The relationship between encounter rate and prey density has been recently shown not to be directly proportional, and theoretical arguments have been made that predator search behaviour can explain this trend. I test these arguments, and show acceleration of a predator's search path can in fact lead to the observed less-than-directly proportional relationship between prey density and encounter rate.

The perceptual constraints of predators can have major impacts on prey risk. Once encountered, an attack was more likely when prey were encountered late in a search, probably due to a decrease in anti-predator vigilance as the fish became more habituated to the arena. In a subsequent study, larger groups of prey were more quickly found, as were larger numbers of groups. This led to the conclusion that the field of attention is a subset of the total visual field, and this is also supported by denser prey being more conspicuous.

Although the predator responded to increased prey group size and density with a reduced time to detect and attack prey, attacks on such groups were less successful due to the confusion effect. Interestingly, I show the effect of prey density to be sensitive to spatial scale, where a large-scale measure of density affected conspicuousness, and a small-scale measure affected attack success. This was explained by a reduction in the total number of prey in the visual field as a group of prey is approached and attacked.

In the final chapter, I turn my attention to differences in temperament within a predator population, and how this affects prey risk. As expected, bolder fish represented a greater risk to the prey. However, as larger fish tended to be more bold, suggesting boldness was driven by their perception of predation risk, a within-community behaviourally-mediated trophic cascade may occur, where the risk to prey is driven by their predators' own perceived risk of predation. This shows that optimal foraging decisions under the threat of predation, as well as perceptual constraints, can mediate the effect of predator behaviour on prey risk.

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Declaration

The work contained in this thesis has involved collaborations with Graeme D. Ruxton (GDR), Colin R. Tosh (CRT), Leigh Neville (LN), Lesley J. Morrell (LJM) and Matt Payne (MP). Their contributions are indicated below. Jens Krause was involved in all the studies.

Chapter II: GDR assisted in guiding my experimental design and statistical analysis.

Chapter IV: CRT helped design the experimental setup and LN assisted in the data collection and video analysis.

Chapter V: LJM and GDR provided advice on the statistical analysis and experimental design.

Chapter VI: MP assisted in the data collection.

In addition, all work contained within this theses has been published or submitted for publication, in each case the co-authors have provided input into the ideas discussed:

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Ioannou C.C., Ruxton G.D., Krause J. Search rate, attack probability and the relationship between prey density and prey encounter rate. BEHAVIORAL ECOLOGY 19: 842 – 846, doi:10.1093/beheco/arn038.

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Chapter I - General Introduction

The relationship between predators and their prey has fascinated ecologists since the inception of the subject. Almost all animal species are subject to the risk of predation at some point in their lives, and even dominant top predators must often survive vulnerable juvenile stages. Behavioural adaptations to minimise risk have attracted a vast research effort, as have the costs of these adaptations and their mechanisms. For example, living in groups is well documented to reduce predation risk with an associated cost (Krause and Ruxton 2002), and the proximate mechanism of forming and maintaining groups can be explained by collective animal behaviour (Ballerini et al. 2008). Predation has direct effects on prey through mortality, but also has indirect effects via decisions on when, where and how prey choose to forage (Beckerman et al. 1997) and reproduce (Creel et al. 2007).

Compared to our good understanding of anti-predation behaviour, much less is known about how predator behaviour influences predator-prey relationships (Lima 2002). Whilst prey are selected to minimise being encountered and detected, predators maximise these probabilities. Similarly, selection to detect predators early and escape efficiently is mirrored by co-evolution in predators to minimise being detected and prey escaping. These selection pressures are not isolated, and must be balanced with the ability to forage (for prey) and avoid predation risk themselves (for predators). Thus, although predator and prey seem diametrically opposed, they are both subject to balancing food intake with risk (Lima 1998), hence maximising the universal evolutionary currency of reproductive success. Understanding predators in such a way avoids considering them as unresponsive sources of risk (Lima 2002), which may be suitable for non-biological risk, such as earthquakes, but not for predators. In this thesis, I explore a number of aspects of predator behaviour which have an impact on prey risk, behaviour and evolution.

Optimal foraging predators (and the effect of risk)

Illustrating the point that predators are under multiple selective pressures are examples where predators evolve behaviours that decrease, rather than increase, the consumption rate of prey and hence their predation risk. In avoiding their own risk of predation, predators will often forage less (Lima 1998); for example, salamanders increase refuge use as a response to predatory fish, reducing risk to their isopod prey (Huang and Sih 1991). Refuge use by predators can also lead to greater spatial predictability, and hence prey can change patterns in space use to avoid encounters with spatially predictable predators. Male guppies in high predation rivers, for example, avoid deeper water where attacks by fish predators are more common (Croft et al. 2006) and it is believed these predators occupy deeper water as a refuge to avoid attacks from aerial predators. Generally, greater activity levels will increase encounter rates with both predators and prey (Anholt and Werner 1995), so predators will trade-off these two factors based on the degree of risk and availability of food to determine the optimal degree of activity (Anholt et al. 2000).

Optimal foraging decisions made by predators increase the net intake of prey, so will, by definition, have impacts on prey risk (Stephens and Krebs 1986). The ideal free distribution (Fretwell and Lucas 1970) predicts multiple predators should distribute between two or more patches in proportion to prey profitability in each patch. As profitability will often be positively related to prey density, prey risk may be more equal between two patches than expected from differences in prey abundance alone. Optimal foraging can also affect the preference for different prey types (Sih and Christensen 2001). Nutritional deficits can lead to switching to an alternative prey type after an extended period of feeding on a prey lacking in a required nutrient (Kear 1962). Selectivity for prey types can also change with prey availability (type III functional response; Holling 1965); belted kingfishers (*Megaceryle alcyon*), for instance, attack their preferred prey (large fish) disproportionately more than the alternative (crayfish), but only when the prey were more available (in this case, when the habitat was less structured; Kelly 1996).

Whether a particular predator behaviour affects prey risk, and whether it increases or decreases risk, will often depend on prey variables such as behaviour, morphology, population density and habitat. In the kingfisher example, the risk for crayfish decreases in open habitats as the birds specialise on large fish. A number of predators conduct area concentrated search after encountering prey (carrion crows, Tinbergen et al. 1967; sticklebacks, Thomas 1974), improving the subsequent rate of encountering cryptic, aggregated prey. However, this depends on the prey being aggregated, and area concentrated search will reduce encounter rates with prey if they are evenly distributed (Hill et al. 2002).

Flexible predators, flexible prey

Dependence on prey context can lead to phenotypically plastic behaviour by predators in response. For generalist predators, the success of a hunting strategy will depend on the prey type, and this has led to the evolution of flexible hunting strategies with a single predator using different methods of attack and capture specific to the prey type encountered. Great grey shrikes (*Lanius excubitor*) use different strategies to capture insects, mice and birds, with each method presumably adjusted to the prey's ability to escape and the risk of injury they pose (Curio 1976 and references therein). Attack behaviour has also been shown to be flexible depending on whether the prey is positioned against a substrate or in the water column (Nyberg 1971), and with the distance of the prey to a refuge (Sparrowe 1972).

Prey can also respond adaptively to predator behaviour. Of particular interest is predator inspection, as it involves the assessment of a predator's motivational state (and hence potential risk) followed by an appropriate behavioural response by the prey (Botham et al. 2006). To return to the ideal free distribution example, prey can often also move between the patches, with each patch providing food but also the risk of predation based on the number of predators present. In this three-level trophic system, a dynamic game arises as prey switch between patches to balance risk and intake, as do predators to maximise prey consumption (Iwasa 1982; Hugie and Dill 1994). This interaction between predator and prey behaviour can lead to a 'behavioural response race' (Sih 1984). In this scenario, predators will track prey distribution when predators are relatively more mobile (leading to the original ideal free distribution), prey will avoid predators when they are relatively more mobile, but there may be no correlation between predator and prey distributions when they are equally mobile and responsive to each other's movements (Sih 1984).

Absolute or relative risk?

The importance of a predator behaviour may affect risk to prey absolutely, i.e. the risk alters for all prey in the habitat, or may affect risk relative to other prey in the population. A well-researched example of frequency rather than density dependence is apostatic selection, i.e. the disproportionately greater consumption of common prey phenotypes (Allen et al. 1998). To improve the probability of detecting prey, predators form 'search images' based on the characteristics of prey already encountered, which are more likely to be the common phenotype (Tinbergen 1960). Although the risk for prey increases overall, this will not increase risk for both phenotypes equally; the common phenotype will suffer a disproportionately large increase in risk, while the risk for the rare phenotype will often decrease as they do not match the search image and are not detected as easily. Hence, the effect of search images is greater for prey types relative to one another than the effect it has on the prey population as a whole. This frequency-dependent predation can maintain polymorphism in prey populations (Bond and Kamil 2002) and is underpinned by the development of search images in predators to maximise intake.

Predator constraints

Thus far I have used examples to illustrate predator behaviours that arise from trade-offs between incompatible behaviours, i.e. whether and where to forage or take refuge, whether to be selective between prey types, or whether to specialise and change search path after encountering prey. In this sense, these can be seen as behavioural 'decisions' made by the predator to maximise intake at minimum risk. However, predator behaviour relevant to prey risk often arises from constraints in morphology (which also arise from trade-offs, but at developmental, physiological and evolutionary levels). A widespread example is the use of crypsis to avoid detection by predators (Ruxton et al. 2004; Caro 2005). An inability to detect cryptic prey arises from a constraint in sensory ability (Dukas 2002), rather than as a behavioural decision not to detect cryptic prey. Perception of the ultraviolet part of the spectrum in birds, which makes some 'cryptic' prey (to mammal predators) conspicuous (Cuthill et al. 2000), demonstrates the importance of considering the predator and their biological constraints. Although most research has focused on visual crypsis, a growing number of studies have demonstrated effective crypsis in other sensory modalities, such as olfaction (Akino et al. 2004) and acoustics (Belwood & Morris 1987).

Putting the pieces together

This discussion shows the wide range of ways in which predator behaviour can affect prey risk. Figure 1.1 presents a schematic of how these factors are interrelated. Of particular interest is the feedback from prey behaviour and other prey variables to the predator strategy, illustrating that neither the anti-predation behaviours of prey nor the behaviour of predators can be viewed in isolation (Lima 2002). Predator behaviours that affect prey risk can be categorised in a number of ways: behaviours that affect risk absolutely or relative to other prey, behaviours that are driven by optimal decision-making versus those based on predator constraints, and those that are affected by prey context. However, multiple factors are likely be at work in any predator-prey interaction at any one time. For example, the rate of encounter between predator and prey will depend on the search strategy predators use, but this interacts with whether prey are distributed evenly, randomly or aggregated in the landscape (Travis and Palmer 2005). More torturous search paths take longer to encounter prey, with this effect being magnified when prey are aggregated. Similarly, the time taken to capture, consume and digest prey (handling time) creates an upper limit on predation rate, leading to the type II functional response to prey density described by Holling (1959). Although handling time is based on a constraint of the predator, it will also vary with prey type and behaviour.

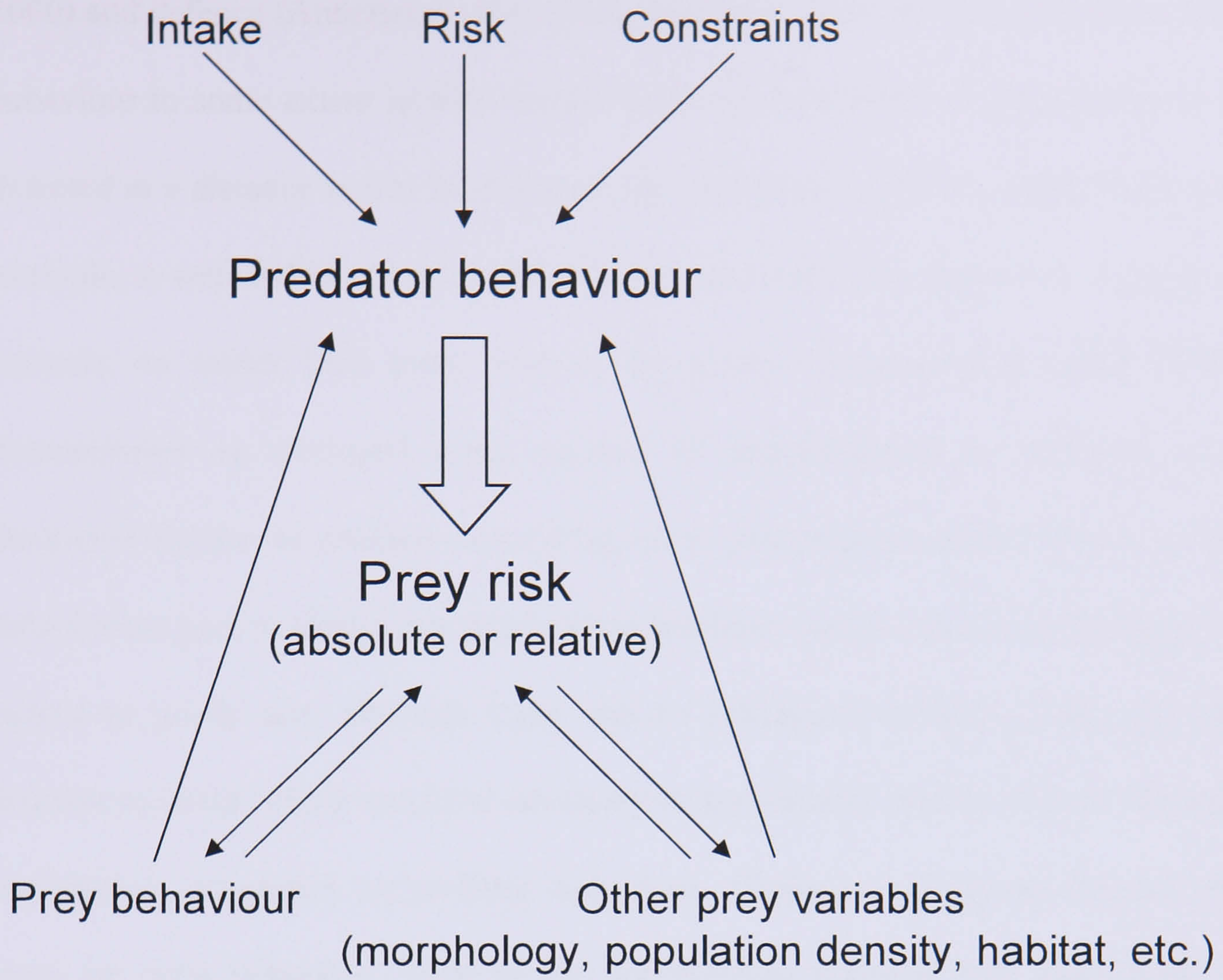


Figure 1.1. The factors directly influencing the relationship between predator behaviour and prey risk (for clarity, direct interactions between these factors are not shown). Note that the intake for the predator is equal to the absolute risk posed to prey.

Predator behaviour and living in groups

Predator behaviour has a particular importance in the evolution of group living as an anti-predatory response. A number of anti-predatory functions of group living have been identified (reviewed by Krause and Ruxton 2002), including group vigilance, group defence, predatory confusion and attack abatement. Group vigilance (Treves

2000) and defence (Andersson 1976; Andersson and Wiklund 1978) rely on predator behaviour to some extent as they depend on the susceptibility of the predator to be detected at a distance and to be overcome by coordinated defence, respectively. For example, a well hidden sit-and-wait predator may never be spotted by a group of animals, no matter how many eyes in the group. Cresswell and Quinn (2004) demonstrated an increased attack success of sparrowhawks on redshanks with decreased distance to predator-concealing cover, presumably because there was less time for the prey to detect the approaching predator. Attack success was negatively related to group size, although there was no interaction between group size and distance to cover, which would be necessary to demonstrate that the success of group vigilance is dependent on predator behaviour. Vigilance and group defence rely more on prey behaviour, such as the coordination between prey individuals in reacting to an attack, and most research has concentrated on this area (Krause and Ruxton 2002).

In contrast, the confusion effect is highly dependent on predator behaviour. It arises as a perceptual constraint of the predator: multiple targets in larger groups overload the information processing capability of the predator, and so attacks on the prey become less successful. Unlike vigilance and group defence, coordinated movement between prey individuals in the group is not necessary (Ruxton et al. 2007). In fact, the current theoretical framework for understanding the confusion effect, neural network models (Krakauer 1995; Tosh et al. 2006), uses prey of different group sizes and arrangements as the visual input, with no interaction between prey or even any prey movement.

The effect of confusion can alter risk to prey both absolutely and relatively. A number of studies demonstrate a reduction in attack rate and success with increased group size (reviewed by Jeschke and Tollrian 2007) which will reduce the rate of predation absolutely. However, this decrease in intake for the predator can result in a shift to focus attacks on smaller groups or spatially and phenotypically odd individuals (Milinksi 1977a; 1977b; Landeau and Terborgh 1986), increasing their relative risk. Under the perceptual constraint which creates the confusion effect, predators can make an optimal behavioural decision to switch to less-confusing prey. Group vigilance and defence may also have a similar effect, and there is some evidence sparrowhawks will attack the more vulnerable flock out of a pair (based on their group size and distance to cover) more often than expected by chance (Cresswell and Quinn 2004). Thus, although predator behaviour may not be instrumental in directly reducing risk for large groups through vigilance and defence, it may have an indirect effect as the predator shifts attacks to more vulnerable groups.

The advantage prey groups gain from attack abatement also arises from predator behaviour. As Turner and Pitcher (1986) showed, the effect relies on two components working simultaneously: avoidance, where larger groups are attacked less than proportionally to their size, and risk dilution, where only a constant number of prey can be consumed per encounter. Avoidance is often explained by the relationship between prey group size and detection rate being less than directly proportional (Vine 1973; Triesman 1978; Riipi et al. 2001), which arises as a perceptual constraint of the predator. Risk dilution also depends on predator traits, as handling times will place a limit on the number of prey consumed (Holling 1959). However, the importance of handling time will be relative to the ability of prey to respond to the initial attack by relocating away from the predator and so ending the encounter. In fact, this restriction on the number of prey consumed per encounter may also explain the avoidance effect. Attacking larger groups may provide little benefit for the predator relative to smaller groups if handling time restricts the rate of prey consumption (so the reward will not be proportional to group size; Holling 1959). Empirical studies have shown support for avoidance and risk dilution, together leading to a reduction in per capita risk for larger groups via attack abatement (Wrona and Dixon 1991; Jensen and Larsson 2002). However, the importance of predator behaviour has not been examined in detail, for example by manipulating prey conspicuousness or handling times.

Importance of predator behaviour within groups

Predator behaviour also has implications for the spatial positioning of individuals within groups. Hamilton's selfish herd (1971) assumed predators appear anywhere in the habitat and target the nearest prey. Thus, to minimise being attacked, prey should minimise the area around themselves closer to them than any other prey, i.e. their domain of danger. The compaction of prey groups in response to a predation threat is often used as support for the selfish herd theory (Magurran and Pitcher 1987; Viscido and Wethey 2002), and exemplifies a direct response by prey to a predator behaviour (attacking prey with large domains of danger). Numerous studies have shown greater risk on the periphery of groups as predicted by Hamilton (Krause 1994: Krause and Ruxton 2002). However, few studies have quantified actual domains of danger and shown them to be directly proportional to the risk of mortality. Without demonstrating this, the increased risk on the periphery could be due to predators attacking prey from outside the group where they encounter the prey first (Stankowich 2003), which would show risk was greater on the periphery and lesser in the centre than expected from the domains of danger.

James et al. (2004) considered the domain of danger being limited in size, outside of which the predator would not attack the prey. This could occur from limitations on the distance a successful attack can be launched, or the range over which prey can be perceived. By considering the predator, James et al. (2004) removed an unrealistic prediction of selfish herd models that peripheral prey would have infinitely large domains of danger. A number of rules have been proposed for how prey should move once a predator is detected to minimise domain of danger (Morrell and James 2008). The effectiveness of these different rules have been shown to be dependent on both predator behaviour and prey context (Morrell and James 2008). In addition to prey population size and density, the time it takes a predator to attack has an influence on the ability of different movement rules to reduce domain of danger.

For mobile groups, leading individuals have been shown to be more at risk than central or other peripheral individuals (Krause et al. 1998), with reduced inter-individual distances in the front of groups (Bumann et al. 1997). Bumann et al. (1997) demonstrated this gradient of risk from the front to the back of groups is greatest when the predator is stationary, as encounters only occur at the front of groups. Clearly, how predators encounter and select prey from groups affects the relative risk of different spatial positions, as well as the rules prey should use to minimise their personal risk. Further experimental work is needed to support or refute these ideas, testing them directly with detailed spatial information on prey positions, and connecting particular predatory behaviours to adaptive movement responses by prey.

The predator

Throughout these experiments I use the three-spined stickleback (*Gasterosteus aculeatus* L.), a common freshwater and estuarine teleost fish, as a model predator. A number of studies on stickleback behaviour exist (Wootton 1992), and extensive studies specific to the feeding behaviour of three-spined stickleback have been made (Tugendhat 1960; Beukema 1968). Their small size and ease of habituation in the laboratory make them ideal study organisms, and being generalist predators on invertebrate prey (avoiding ethical issues) make them particularly well-suited to investigating predator-prey relationships. For all laboratory studies, the fish were caught from the estuary of the Great Eau river, U.K. (grid reference TF 456935). They were kept in a large glass stock tank (210x50x50cm, water depth 35cm) or grey fiberglass tanks (85 x 55cm, water depth 50cm) at 15-16^{o}c on a 13:11 day:night light cycle for at least three months before testing, being fed defrosted bloodworm each day. The fish were not sexed, as they were not in reproductive condition and therefore there were no phenotypic differences between males and females.

Chapter Overview

Rather than predators being 'black boxes' of risk (Lima 2002), the effect of predator behaviour on prey risk is multi-facetted and can have subtle, but important, implications for prey ecology and evolution. In this thesis, I consider a range of contemporary issues in behavioural ecology that may be resolved by focusing on the behaviour of predators. I begin by considering the effect of prey density on the rate of encountering prey. Recent experimental work has demonstrated that encounter rate increases with density, but less than directly proportionally (Mols et al. 2004). In response, Ruxton (2005) and Travis and Palmer (2005) proposed three possible mechanisms for this, two of which depend on the search behaviour of the predator. In chapter II, I present an experimental test of these ideas by recording search behaviour by the fish in an effectively one-dimensional circular arena.

In chapter III I consider the next stage in predation, the detection of prey. I test how prey group size affects conspicuousness, both in the laboratory and the field. The effect of the number of groups is also tested, as is the proposed mechanism behind the detection of larger groups (visual angle; Triesman 1978). Chapter IV deals with the confusion effect, and explicitly tests predictions made by neural network models (Tosh et al. 2006) which seek to give a conceptual explanation of the confusion effect. I also examine whether the number of individuals, their density, or the area they occupy causes the confusion effect. Following from this distinction between group size and density, in chapter V it is investigated how prey density effects conspicuousness to a predator and attack success, and whether this is sensitive to the spatial scale at which density is measured.

In chapters III to V, the experiments are designed to isolate the effect of the predator's perceptual constraints on detecting and accurately attacking prey that live in groups. A different aspect of predator behaviour is examined in chapter VI, where I test whether an element of predator temperament, in this case boldness, has effects on the risk to prey. Finally, in chapter VII, I discuss the importance of this work for predator and prey relationships, and potential further work.

Chapter II - Search rate, attack probability and the relationship between prey density and prey encounter rate

Abstract

Although numerous influential models in ecology assume a directly proportional relationship between prey density and prey encounter rate, a recent test of this assumption found that the actual relationship was non-linear (rising slower than proportionately). Three-spined sticklebacks were used to test two recently-proposed mechanisms based on predator search behavior that may induce this non-linearity: the effect of increasing search rate over the course of the search, and the effect of spatial correlation in areas searched. To test these, I carried out experiments in which fish explored a novel apparatus of 16 cells (15x16cm) arranged in a ring, with each cell connected to the two adjacent cells by small openings. Hence, the arena was effectively one-dimensional, simplifying the recording of search paths and removing edge effects. The relationship between prey density and encounter rate was found to be less than directly proportional, confirming the findings of a previous study using great tits (*Parus major*) searching for caterpillars. In the majority of trials the search rate accelerated as more of the arena was searched. Moreover, the greater this acceleration, the less prey density and encounter rate were directly proportional. There was no evidence found, however, that spatial autocorrelation had any effect. I also found an interesting effect of prey density on the likelihood that an encounter

with a prey would lead to an attack. Once prey were encountered, the probability of attack increased with the number of cells already visited, leading to a higher probability of attacking first-encountered prey at lower prey densities.

Introduction

An encounter between two agents is the first step in any interaction between them, whether they are predator and prey, parasite and host, competitors, or randomly moving molecules. In numerous models using encounters between predator and prey, a central assumption is that the encounter rate of a predator with potential prey is directly proportional to prey density (Mols et al. 2004), an assumption forming the basis of many classical models in ecology (for example, the Lotka-Volterra predator-prey model; Lotka 1925). This assumption initially seems reasonable, and follows from analogy with physicists' models of collisions between randomly moving gas particles (e.g. Denny and Gaines 2000). However, a recent experimental study using great tits (*Parus major*) searching for winter moth caterpillars (*Operophtera brumata* L.) explicitly tested this assumption for the first time, and found that the time to first encounter did not decline with increasing density as steeply as would be expected from a directly proportional relationship between prey density and encounter rate (Mols et al. 2004).

Two untested hypotheses have recently been presented to explain this result. Firstly, when prey are aggregated, increasing density should not proportionally reduce the average distance to the nearest prey and thus the time to first encounter prey (Travis and Palmer 2005). Ruxton (2005) proposed an alternative, but not mutually exclusive, mechanism. If predators increase their search rate whilst exploring a habitat, prey at high densities will be encountered when search rate is relatively low; in contrast, low density prey will be encountered after a longer period of time, when search rate has accelerated to higher levels. Thus, prey at low densities will be encountered sooner than would be expected on the basis of the time taken to encounter high-density prey. Following Ruxton (2005), I define search rate as the area searched per unit time, which can be derived from the time spent in each patch searching for food.

Another property of predator searching, the degree of spatial correlation in the search, also has an effect on the relationship between prey density and encounter rate (Travis and Palmer 2005). Increasing spatial correlation (i.e. increasing overlap) of the search path increases the time to encounter prey at a low density to a greater extent than at higher densities. Such overlap might occur if prey are stationary and predators do not remember or mark areas that they have previously searched. However, this mechanism predicts the opposite trend to that found by Mols et al. (2004), a trend where encounter rate increases with prey density faster than would be expected from a directly proportional relationship.

In addition to the predicted effect on the relationship between prey density and encounter rate, search behavior is well established as having an important role in other aspects of predator-prey interactions. Gendron and Staddon (1983) proposed a trade-off between search rate and detection of prey, where the probability that prey is detected increases with the time spent searching an area. As animals have a limited attention (Dukas 2002), they must balance the rate at which they search (quantity) with how well the area is searched (quality). Hence, although high density prey may be encountered more slowly than expected from the time taken to encounter prey at low density, a greater proportion of encounters may lead to detection as more time is spent in each area. The increase in search rate Ruxton (2005) proposed to explain the observed relationship between prey density and encounter rate may thus be accompanied by a decrease in the detection rate of encountered prey (Gendron and Staddon 1983).

In this study, I examine exploratory behavior of three-spined sticklebacks in an unfamiliar habitat, testing whether the time taken to encounter prey (*Daphnia magna*) is inversely proportional to prey density. Detailed recording of search paths then allowed us to determine which search behaviors (change in search rate and overlap of the search path) affect this relationship, as proposed by the models of Travis and Palmer (2005) and Ruxton (2005). Further, given that increasing search rate has been predicted to negatively affect the probability that encountered prey are actually detected (Gendron and Staddon 1983), I examined whether prey density and the area already searched affected the probability prey was attacked once the first encounter had taken place.

Method

Exploratory behavior of sticklebacks (mean length±s.d = 46.0±4.54mm) in a novel environment was monitored in an arena of discrete compartments in which they could search for prey (figure 2.1). The arena consisted of 16 numbered cells (each approximately 15x16cm, water depth = 11cm) arranged in a circle (diameter 82cm), with each cell connected to the two cells adjacent by a small opening (3 width x 7cm high). As the fish could move between cells only in one plane (clockwise or anti-clockwise), the arena was effectively one dimensional, simplifying the recording and analysis of the movement pattern. In addition, the arena was finite but lacked any boundary, reducing bias between the cells and removing edge effects.

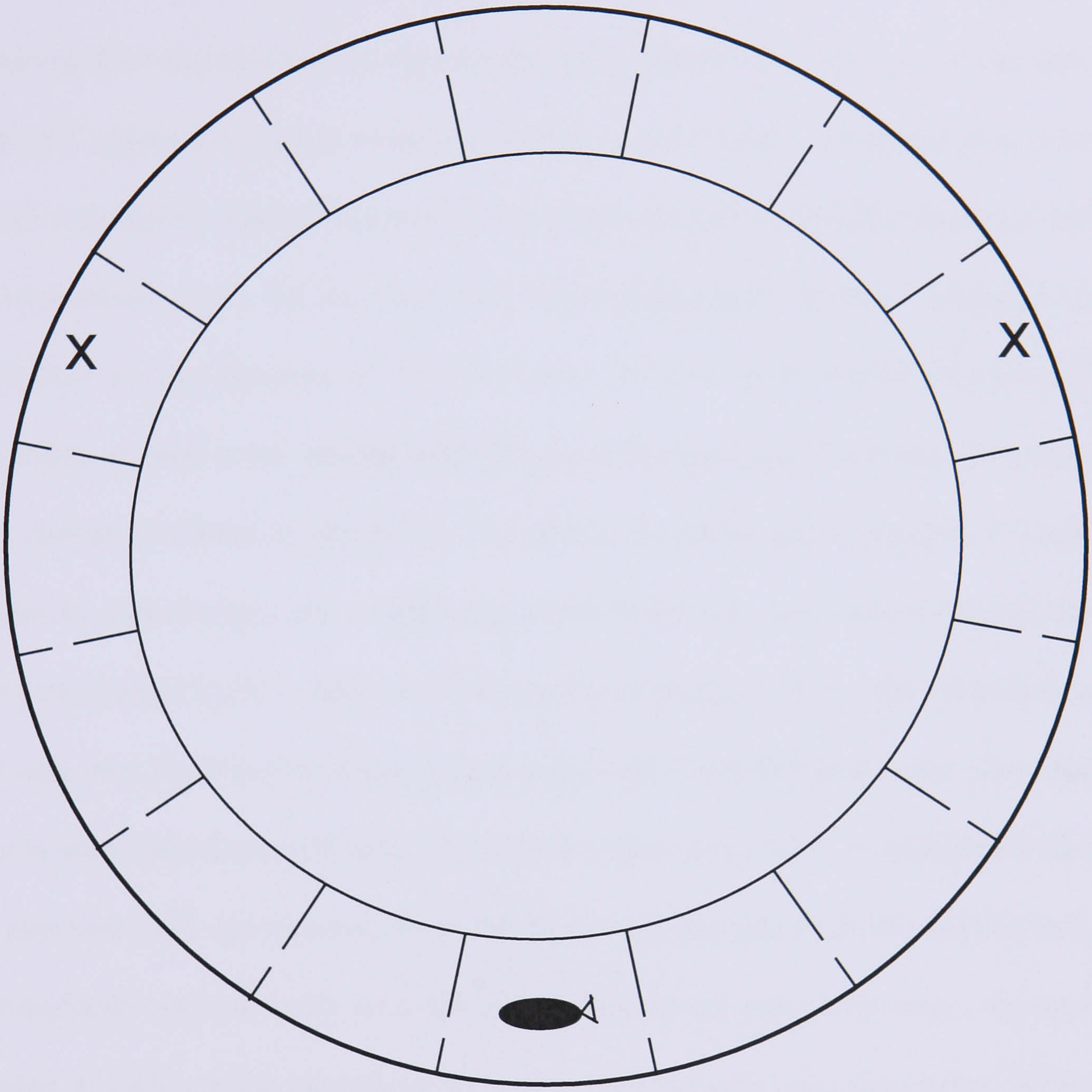


Figure 2.1. The experimental arena used to assess the relationship between search behavior and the rate of encountering and attacking prey (not to scale). Each cell is approximately 15x16cm, connected to the two cells adjacent by 3x7cm openings. The diameter of the arena is 82cm. The fish can move in only one dimension (clockwise and/or anti-clockwise); as the arena is continuous, there is no boundary,

so edge effects are minimized. X's mark the position of prey at a density of 0.11 prey per cell, i.e. 1 in 9 cells contain prey (not including the starting cell).

In the first experiment, a test tube (length = 7.5cm, internal diameter = 1cm, water depth = 6cm) was placed on the outer wall of each cell. Two of these tubes contained the same number of *Daphnia* prey (both tubes with either 1 or 10 per tube, with prey group size varied randomly between trials). Tubes containing prey were placed a randomly selected distance of 1 to 7 cells in both directions (clockwise and anti-clockwise) from the starting cell. Encountering prey at these distances is equivalent to prey densities of 1, 0.33, 0.20, 0.14, 0.11, 0.09 or 0.08 prey per cell (assuming no prey in the starting cell). This is made more clear if one considered the prey density presented in figure 2.1. Our design is a designed to simulate a whole habitat by considering only a small segment between two prey, hence, beyond the two prey marked by X's, there would be another 8 empty cells in either direction, a cell with prey, followed by 8 empty prey and so on. From this it becomes clear that prey is encountered every 9 cells, i.e. a density per cell of 0.11. A stickleback (fed the previous day) was removed from the stock tank and placed in the starting cell. Starting cells were randomly selected. As the fish moved around the arena, the total number of cells visited (including the cell containing prey), the time taken before entering a cell with prey and whether an attack was made once prey were encountered were recorded. The trial ended once a cell containing prey was left, regardless of whether an attack was made, as stickleback behavior is known to change after encountering prey (Thomas 1974). Thirty trials were carried out at each group size, with each fish being used only once.

Both prey density and the time taken to encounter prey were log10 transformed. Linear regression was used to estimate the relationship between log10 (prey density) and log10 (time to encounter), as in Mols et al. (2004). The gradient of the regression line in log-log space is equivalent to the exponent of the relationship before log10 transforming. Values equal to -1 indicate an inversely proportional relationship between density and time to encounter, more negative values indicate that the time to encounter decreases more rapidly with density than proportionally, and less negative values that time to encounter decreases less rapidly. In addition, the probability that prey was attacked once encountered was analyzed using logistic regression, with prey group size and the number of sites visited as explanatory variables. The effect of density on the proportion of first encounters that resulted in attacks was analyzed using a binomial generalized linear model.

The second experiment repeated the procedure from the first, except detailed exploratory behavior by the fish was monitored by recording the time spent in each cell and the identity of the cell. In addition, as only the search pattern was of interest and to achieve an extended search path, no prey were present. For any prey distribution considered post hoc, the recorded search path allows straightforward calculation of predicted time to first encounter prey as if such a prey distribution had actually been present. Trials ended when the fish had visited all cells (56% of trials), when they had visited more than 30 cells without visiting all cells (42% of trials), or when they spent more than 20 minutes exploring the arena without fulfilling either one of these requirements (2% of trials). 50 trials were carried out in total, with each fish being used only once.

Using the time spent in each cell, the time taken to reach 1, 2, 3, 4, 5, 6, 7 and 8 cells from the starting cell (in either clockwise or anti-clockwise directions) was calculated for each of the 50 search paths. This is equivalent to the time taken to encounter prey at these distances from the starting cell as in the first experiment, but avoided changes in search behavior upon encountering prey (Thomas 1974). The exponent of the relationship between hypothetical prey density (derived using the distances from the starting cell) and time to first encounter was determined for each path, using linear regression in log-log space, as in the first experiment. A linear model was then applied with this exponent as the dependent variable, and spatial correlation and the change in search rate of each path as covariates. The degree of spatial autocorrelation was calculated as the proportion of cells visited where the fish changed direction (and exits to the same cell that it entered from). The exponent of the relationship between the number of cells visited and the time spent in each cell (again calculated from linear regression in log-log space) was used as a single value to describe the change in search rate over a trial. Negative values indicate an increase in search rate over the course of a trial, zero represents no change, and positive values a decrease in search rate.

All statistical analyses were carried out in R version 2.4.1 or SPSS version 14.

Results

Increased prey density significantly reduced the time taken to encounter prey (figure 2.2; linear regression, t_{58}=-3.28, P=0.002). However, the gradient of the relationship was -0.55 ± 0.17 (± 1 S.E.), significantly greater (i.e. less negative) than the -1 expected for an inversely proportional relationship between density and time to first encounter (t_{58}=2.67, P<0.01). Thus, although my experiment predicts an increasing encounter rate with increasing prey density, like Mols et al. (2004) but unlike classical theory, the increase is slower than linear.

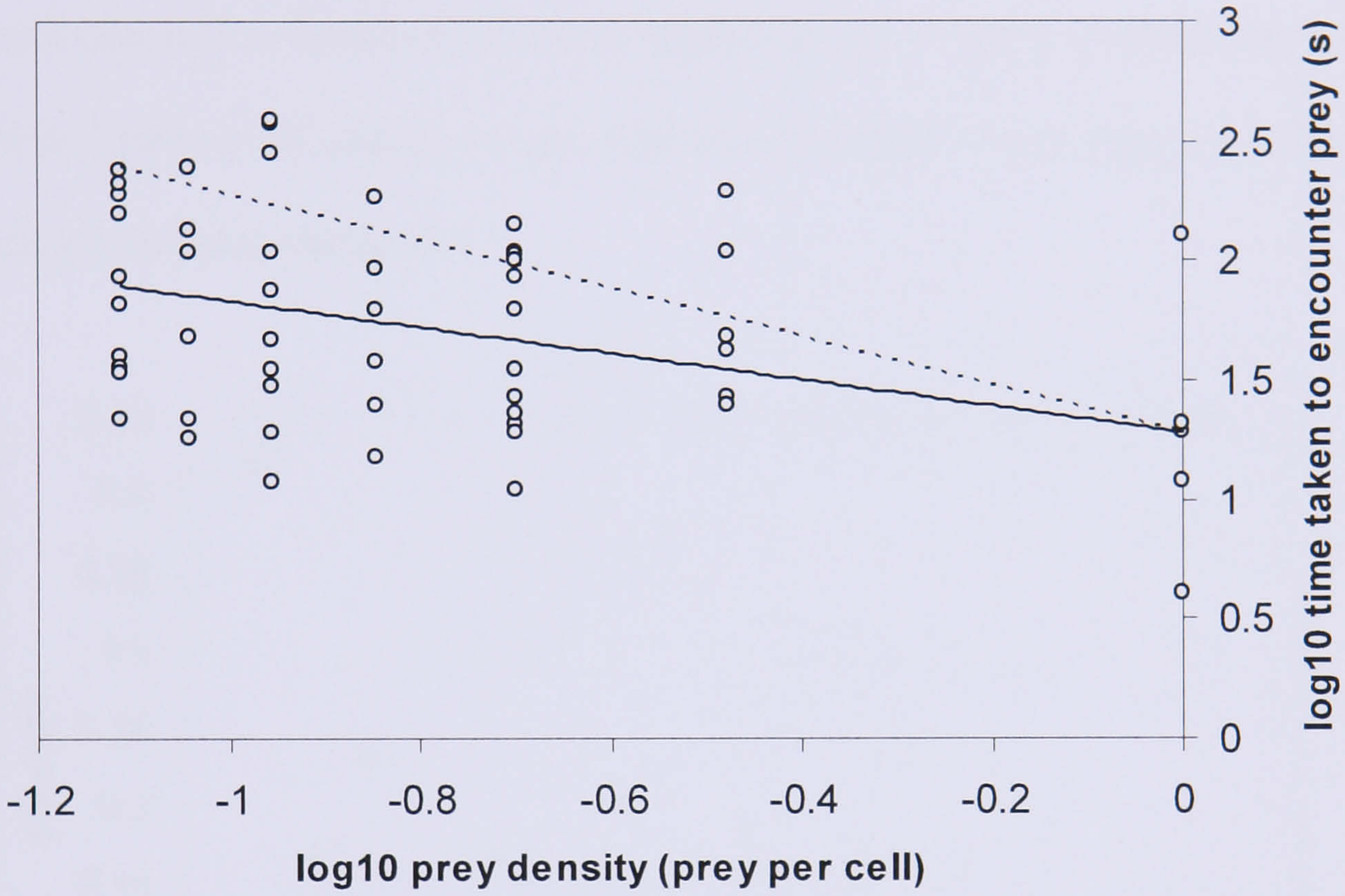


Figure 2.2. The observed relationship between prey density and the time taken to first encounter prey. The dashed line is the expected relationship if encounter rate is proportional to prey density (i.e. a gradient of -1 between density and first encounter in log-log space), scaled to meet the y-axis at the same point as the solid line fitted to the data. The gradient of the line fitted to the experimental data is significantly less than -1 (i.e. is significantly different to the dashed line).

Of first encounters with prey, 23.3% resulted in attacks. The probability of attack was not significantly affected by prey group size (Logistic regression, Wald=0.55, P=0.46), with 6 attacks out of 30 encounters at group size 1, and 8 attacks out of 30 encounters at group size 10. In contrast, the probability of attack increased with the total number of cells visited before encountering prey (Wald=4.32, P<0.05). The fitted model predicted an attack in less than 10% of encounters after visiting 2 cells, compared to over 65% after 25 cells were visited. This led to a greater proportion of first encounters at lower prey densities resulting in an attack (figure 2.3; Generalized Linear Model, d.f.=5, P<0.05), although the result should be treated with caution as zero attacks at a density of one prey per cell may have disproportionate influence.

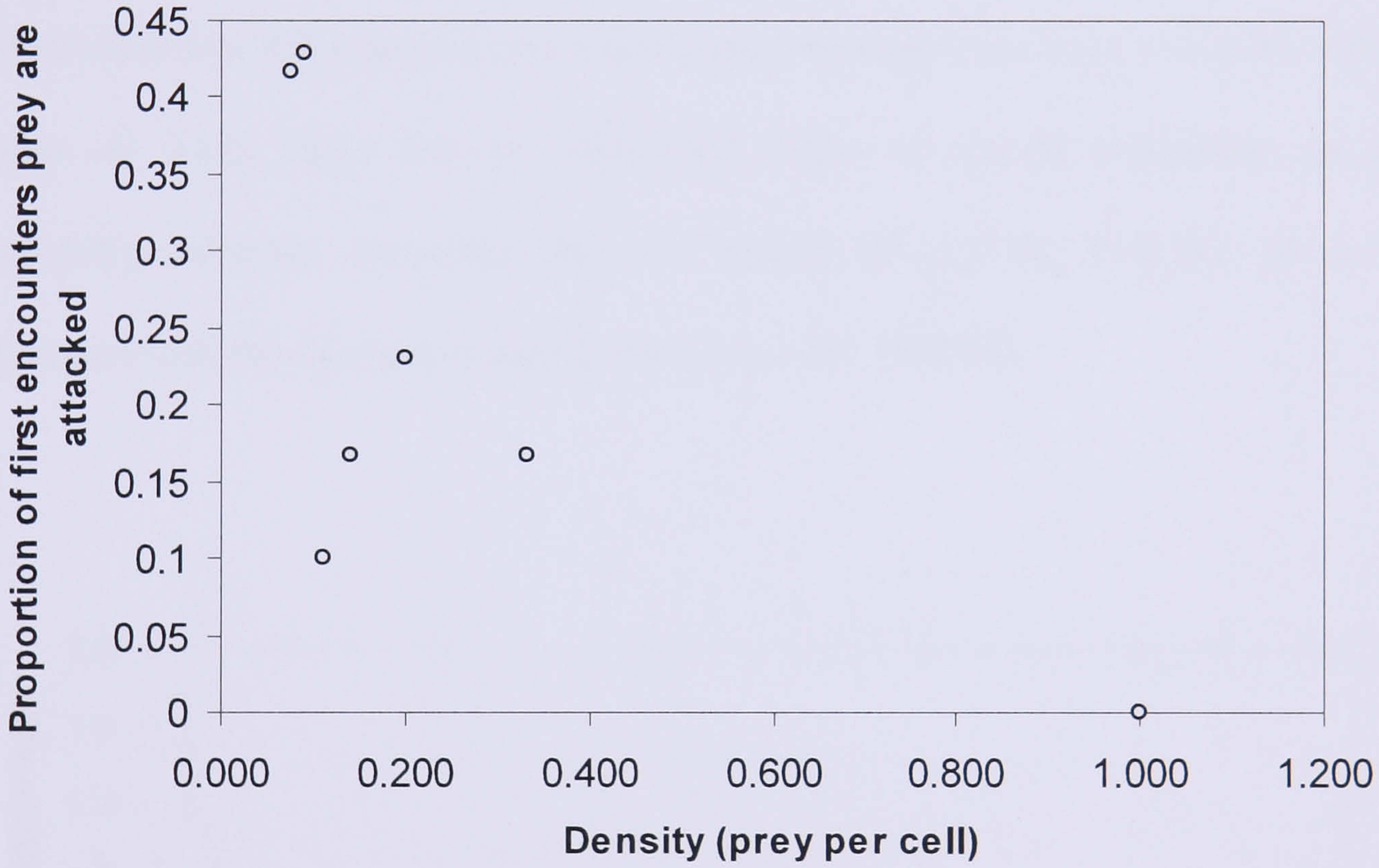
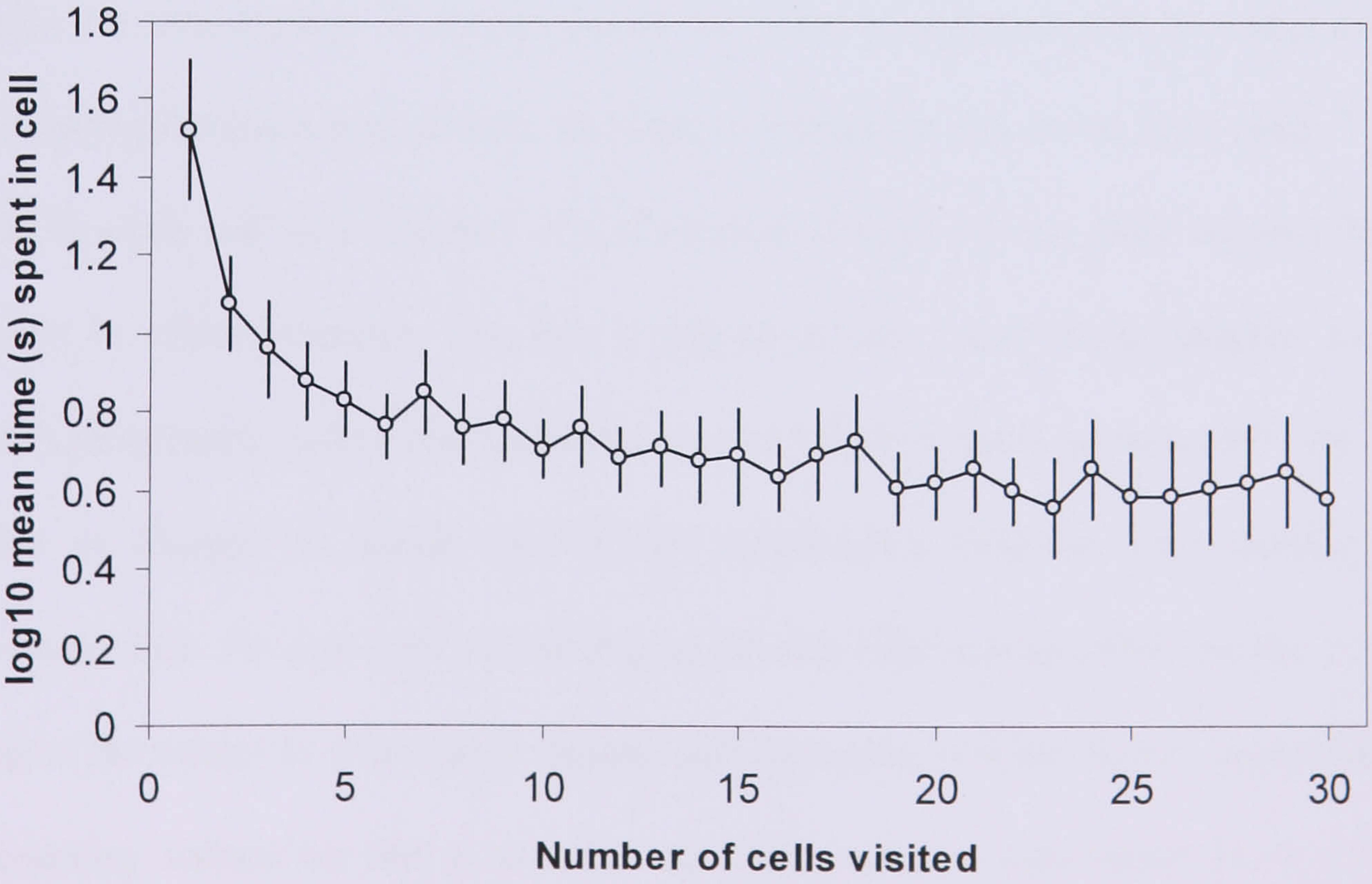


Figure 2.3. The proportion of first encounters where prey are attacked as a function of prey density. As prey density increases, the probability that the prey first encountered are attacked decreases.

As the trials progressed, the search rate by the sticklebacks increased (figure 2.4a). Specifically, the mean exponent of the relationship between the number of cells visited and the time spent in each cell was significantly less than 0 (One sample t-test, t_{49}=12.9, P<0.0005). The increase in search rate had a significant effect on the relationship between prey density and the time to first encounter (figure 2.4b; General Linear Model, $F_{1,46}$=29.6, P<0.0001). The greater the increase in search rate, the less steeply encounter rate increased with increasing density. If there had been no change in search rate over a trial (i.e. the exponent equaled zero), there would be a directly proportional effect of density on encounter rate (i.e. the exponent between density and time to first encounter = -1; figure 4b). Although there was wide variation in the degree of spatial correlation between trials (the proportion of cells visited where the fish changed direction ranged between trials from 0 to 0.47, with a median of 0.13), there was no significant effect of spatial correlation on the relationship between encounter rate and density ($F_{1,46}$=0.02, P=0.88), or as an interaction with the increase in search rate ($F_{1,46}$=0.9, P=0.34).

(a).



(b).

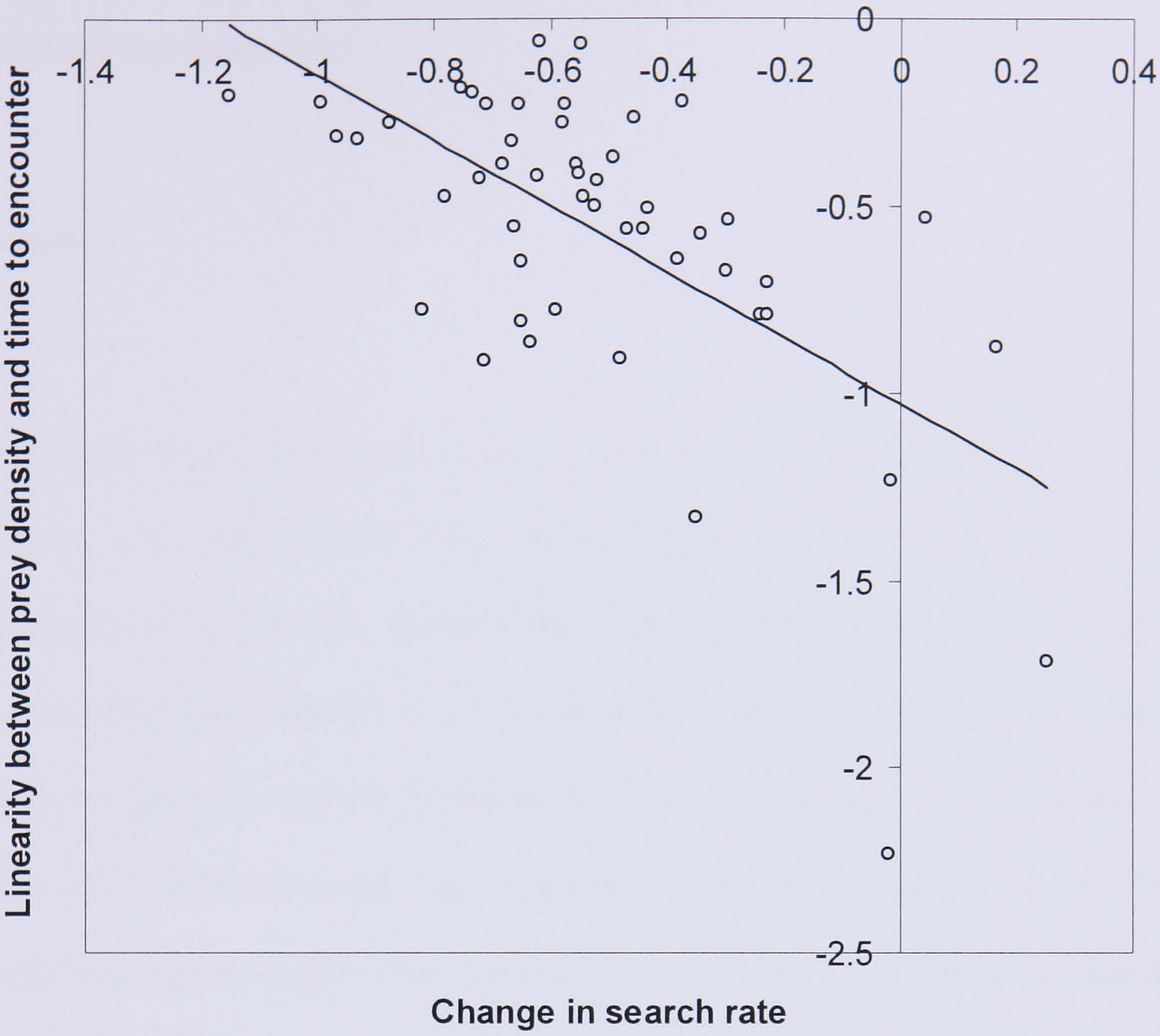


Figure 2.4. The change in search rate as the arena is explored, and its effect on the relationship between prey density and time to encounter. (a). Mean time (with 2 s.e.) spent in each cell as a function of the number of cells visited, with no prey being present to affect behavior. The fish is placed in cell 1 and timing begins. As the search progresses, search rate increases (i.e. less time is spent in each cell). (b). The effect of change in search rate on the relationship between prey density and encounter rate. As expected, increasing search rate (decreasing values on the x axis) reduces the extent to which prey density and encounter rate are directly proportional (increasing values on the y axis; -1 on the y axis is equivalent to a directly proportional relationship between density and encounter rate). The graph shows this

effect is not cancelled by the autocorrelation of the search, the other factor affecting the time taken to reach the hypothetical prey. Instead, this factor causes the variance around the regression line.

Discussion

If a predator increases its search rate during a search, the encounter rate with prey increases with prey density slower than expected from a directly proportional relationship, as predicted by Ruxton (2005). The greater this increase in search rate, the less effect prey density has on encounter rate. As it takes longer to encounter low-density prey, during which time the rate of search increases, encountering prey at low densities occurs sooner than expected from the time taken to encounter high-density prey (which occurs when search rate is low). This could explain the result of Mols et al. (2004) where this relationship between density and encounter was first observed, but the predator's search pattern was not recorded. Although my setup was highly simplified to minimize edge effects and enable detailed recording of search patterns, it is not dissimilar from the littoral zone where sticklebacks are most often found; the littoral zone consists of small, discrete volumes of space partitioned from one another by macrophytes that may harbor prey or predators.

For search rate to have an effect on the relationship between density and encounter rate, the scale at which the search accelerates must be appropriate to the scale over which prey density varies. The few fish that did not alter search rate during the trial may have accelerated searching over a larger spatial scale, but would experience a directly proportional relationship between density and encounter rate at the scale of the experiment. Conversely, if search rate increases and approaches a maximum very quickly, before the first prey is encountered at high densities, there will be little change in search rate when prey are encountered at lower densities. For example, Shipley et al. (1996) demonstrated smaller mammalian herbivores that accelerate and decelerate rapidly show a more inversely proportional relationship between encounter rate and distance between plants compared to larger herbivores, where the range of distances between plants was the same for all species.

An improvement in searching ability with practice and/or a decrease in perceived predation risk (i.e. familiarity with the arena) over the trials could also cause an increase in search rate over time (Ruxton 2005). A way to distinguish between these two possibilities would be to measure reaction times to a simulated predation event; a more rapid response would indicate a greater degree of vigilance, and if this is negatively related to search rate, it would support the idea of declining perceived risk as the search progresses. Both of these factors depend on predators being initially unfamiliar with their environment, as they were in my experiment. Repeating the experiment with fish habituated to the arena, and with a mechanism of introducing the fish into the arena with less disturbance would test whether the result can be generalised to predators familiar with their habitat. Some uncertainty, however, is always expected in the risks associated with an environment, leading to an acceleration of searches over time. It is expected that temporal and spatial heterogeneity in the predator's environment will have a positive effect on the increase in search rate, providing the scale of heterogeneity is matched to the scale of movement of the predator. Collecting field data on the search patterns of predators from various habitats would be especially illuminating in this regard, helping to determine the generality of the results presented here.

Variation in detection probability once an encounter has taken place was not a component of the models of Ruxton (2005) and Travis and Palmer (2005), although this factor might be expected to also vary with search rate and the distribution of prey. For cryptic prey, it has been shown that increasing search rate can decrease the probability of detecting prey per encounter (Gendron and Staddon 1984). However, my results suggest that, perhaps due to the decrease in anti-predatory vigilance by the fish as the search progresses, the increase in search rate is accompanied by an increase in the probability of detection. This would occur if scanning the environment for prey and being vigilant for predators are to some extent mutually exclusive, and declining investment in anti-predatory vigilance allows increased investment in prey detection. Gendron and Staddon's (1983, 1984) model and lab study lacked vigilance as a variable component of attention, and instead proposed a trade-off between the area searched (i.e. search rate) and detecting prey (positively related to the time spent searching in an area). Essentially, they assumed that the less time a forager spends in a certain area (i.e. the higher the search rate) the less chance any cryptic prey in that area will have of being detected. This is an entirely reasonable mechanism, but here I argue that it may be compensated for or even dominated by the effect of reduced anti-predator vigilance allowing both an increase in the rate at which local habitat is encountered and an increase in attention devoted to scanning the currently-experienced local habitat for prey.

An alternative explanation for the lack of attacks at high densities is that prey were ignored after being detected. Distinguishing whether prey were not detected or that they were detected but ignored could not be done with my experimental design. Both are possible: being unable to detect the prey depends on constraints to limited attention (Dukas 2002), while detecting prey but ignoring them can be explained by optimal foraging decisions under the (potential) risk of predation (Lima 1998). Encountering prey early in a search is used as a cue for its abundance in the general habitat, so early encounters may be devalued relative to the potential predation risk involved in foraging activity (Krause and Godin 1996). There is some evidence of dietary conservatism where novel prey are ignored (Marples et al. 1998), although this seems unlikely in this experiment as a familiar, natural prey of the stickleback was used; in addition, prey novelty cannot explain why prey were attacked more often at low densities. Based on absolute and relative encounter rates, most studies have concentrated on the decision of which prey type to attack (e.g., Holling 1965; Kelly 1996; Allen et al. 1998), rather than the decision of whether to attack or not. Ignoring prey at high encounter rates may have a logical basis, although I am unaware of any study that explicitly demonstrates prey encountered early in a search are ignored after being detected.

It has been established that the relationship between prey density and encounter rate can be non-linear in both the empirical studies that test this assumption, and evidence is now presented supporting one of the proposed mechanisms for this trend. I can only speculate on what impact this non-linearity has for ecological models that have so far been built on the assumption of a directly proportional relationship. At spatial and temporal scales greater than those used in this study, predator search rate is also likely to increase with hunger, and hence respond to low encounter rates with prey (Wieser 1991). Thus, predator populations should be less adversely affected by low prey densities than originally thought, and, equally, the impact of predation on low-density prey populations will be greater than expected from its impact at high densities. It would be of particular interest to include the relationship between density and encounter rate as a function of environmental heterogeneity (via the increase in search rate), given that heterogeneity has additional effects on ecological processes other than that on predator searching behavior (for example, Cronin and Reeve 2005). Additionally, the results apply beyond trophic interactions to any system with actively searching agents, such as pollinators searching for inflorescences and mate searching.

Unlike the explanation proposed by Travis and Palmer (2005), based on prey distribution, the mechanism of increasing search rate is dependent on predator behavior, and highlights the importance of considering predators as agents under selection rather than unresponsive sources of risk (Lima 2002). Moreover, my results demonstrate caution should be exercised with untested assumptions, especially when these assumptions form the basis of classical models underpinning our understanding of ecology (for example, Lotka 1925; Holling 1959). The assumption that encounter rate is directly proportional to density may be met with gas particles showing Brownian motion (Denny and Gaines 2000), but can fail in predator-prey systems when predator search behavior is sufficiently complex that ideal gas particles provide a poor and misleading analogue.

After prey are encountered, what other factors affect the probability prey are detected? In the following chapter, I explore how the size and number of groups effects detection rate, and provide a simple experimental test of the proposed mechanism for why larger groups are more conspicuous: the greater visual angle they subtend (Vine 1973).

Chapter III - Searching for prey: the effect of group size and number

Abstract

If larger groups are discovered (and thus attacked) by predators more often, the benefits of group living can be reduced or even cancelled. Thus, the relationship between group size and detection rate is critical in assessing risk to prey individuals. Three-spined sticklebacks predating *Daphnia magna* were used to assess this relationship, with the time taken to approach prey as a proxy for detection rate. In a field experiment, a group of 100 prey was approached and attacked more rapidly than a group of 2. In addition, the time to approach prey was found to be inversely proportional to group size (over a range of 2 to 50 *Daphnia*) under laboratory conditions. Multiple groups of the same size (2 or 30) were then presented to determine the effect of group number on approach time. The time to approach the prey was significantly reduced compared to when these group sizes were presented alone. This effect of group number suggests the asymptote observed in the relationship between group size and approach time was limited by the encounter rate with the prey. There was no significant interaction between group size and number, suggesting prey encounter (number of groups) and prey detection (group size) acted independently in reducing approach time. In a final experiment, it was demonstrated that the visual angle produced by the prey group could account for the greater detection of larger groups.

Introduction

If predators detect and attack larger groups more often, the anti-predator benefits of group living may be reduced to the point where individuals in larger groups are more at risk than solitary individuals, or other non-predation benefits are lost (Vine 1973: Turner and Pitcher 1986). Generally, detection rate increases with group size, although this effect saturates (Vine 1973; Triesman 1978). For example, attack rate on bird flocks has been observed to increase with group size (e.g. Lindström 1989; Cresswell 1994). However, this trend is not universal: Treherne and Foster (1982) observed attack rate on a marine insect to be independent of group size, and FitzGibbon (1990) observed cheetahs preferentially attacking smaller groups, probably reflecting their greater attack success with smaller groups. An issue with these studies is that they rely on correlational field observations with no experimental manipulation of group size; thus, a third variable may be influencing predator attacks and group size, such as time of day (Krause and Ruxton 2002).

Overcoming these limitations, the experimental work of Riipi et al. (2001) and Jackson et al. (2005) both demonstrated a positive relationship between group size and detection rate, and again this effect saturated at relatively low group sizes. However, both used artificial prey items: almonds in Riipi et al. (2001) and computer generated 'prey' in Jackson et al. (2005). Thus, although this approach reveals the constraints on predator searching ability, and can be favourable as the novel nature of the stimulus minimises the effect of past experience, its relevance to prey risk itself is questionable. Utne (1997) demonstrated an increase in detection rate with prey group size by the goby, using their natural prey *Calanus finmarchicus.*

With only two group sizes (1 and 10), however, any saturating effect of group size on detection rate could not be examined in this case.

The mechanisms behind these trends have been modelled using the idea that larger groups produce a greater visual angle on the retina of a predator, and are thus more likely to be detected (Vine 1973; Triesman 1978). However, this concept has yet to be explicitly tested in a predator-prey system, especially controlling for other effects of large group size. such as the increased activity of individuals in larger groups (Krause and Godin 1995; Grand and Dill 1999).

How these relationships between group size and detection rate hold when multiple groups are presented has not previously been examined. Assuming the predator has a limited visual field that sweeps out as it moves, and is independent of prey distribution, increasing the number of prey groups in a fixed area should decrease the time to detection (Vine 1971; Travis and Palmer 2005). However, this argument is one of encounter, rather than detection, as it depends on the number of groups entering the visual field, and not on their ability to be detected. Although an encounter is necessary, detection of the prey must then also occur. and this is likely to increase with increasing prey group size. Encounter rate is determined solely by spatial position of prey, whereas detection rate is dependent on how conspicuous the prey are. Interestingly, in a prey population of finite size, group formation decreases encounter rate, while increasing the probability of detecting of encountered groups. To minimise detection, prey must therefore balance both the risks of being encountered and being detected by predators. although previous studies have examined either encounter or detection rates, and rarely compared the two factors.

In this study I use a single predator-prey system (the three-spined stickleback and *Daphnia magna*) to attempt to quantify the relationship between group size and (visual) detection rate and how this relationship alters when multiple groups are presented. In addition, a field manipulation study is presented to determine whether larger groups are detected sooner in a field setting without the confounding factors of other field studies. Finally, I investigate whether an increased visual angle can account for the increased detection rate of larger groups by varying visual angle through the distance to the group, thus controlling for other effects of larger group sizes.

Method

Field experiment

Trials were carried out at Balmaha pond (NS 42 91), near Loch Lomond, Scotland, on the 11th and 12th June, 2006. Sticklebacks were the only fish observed in the littoral zone. *Daphnia* were also present in the pond (Colin Adams, pers. comm.), although none were observed in the littoral on test days.

To determine the effect of prey group size on detection rate by predators in the field, the following procedure was carried out. Fourteen sites were selected in the littoral zone of the pond, at least 1.3m apart from one another. To enable recognition of sites between days, photographs were taken of each site and labelled with a marker on the nearest part of bank. This allowed accurate repeated placing of the stimulus in the same location. The stimulus consisted of either 2 or 100 *Daphnia* (mean length±s.d = 2.1±0.2mm), placed in a sealed glass vial (length = 12cm, internal diameter = 1.3cm). These vials were then gently placed on the benthos at each site, using fishing line attached to a translucent rod. Occasionally fish approached the vials within 5 seconds, suggesting this method caused minimal disturbance to the fish. The time it took for a stickleback to approach the vial, defined as orientation to the vial within one body length, was recorded, as was the time taken to make the first attack. This method assumes the time to detect prey is negatively related to the detection rate of the stimulus, and has been used in previous studies (e.g. Gendron and Staddon 1984). However, it is conceivable predators may also delay the time to approach a detected stimulus, such as when a choice is to be made between two similar stimuli (Tegeder and Krause 1995). Trials were limited to ten minutes. Both group sizes were presented at each site, separated by one day. The order the treatments were presented in a site was randomly determined, as was the order the sites were tested per day, under the condition that two adjacent sides were not tested consecutively.

Lab experiments

Effect of group size on time to detect prey

The trends from the field experiment were examined in more detail in a laboratory setting using a greater range of prey group sizes. Fifty fish (40.3±5.18mm) were placed in a fibreglass tank which was adapted as shown in figure 3.1, with a water depth of 13 cm. The tank was covered with a white sheet to reduce disturbance and produce a diffuse, even lighting in the tank. Lighting was provided by diffuse fluorescent ceiling lights.

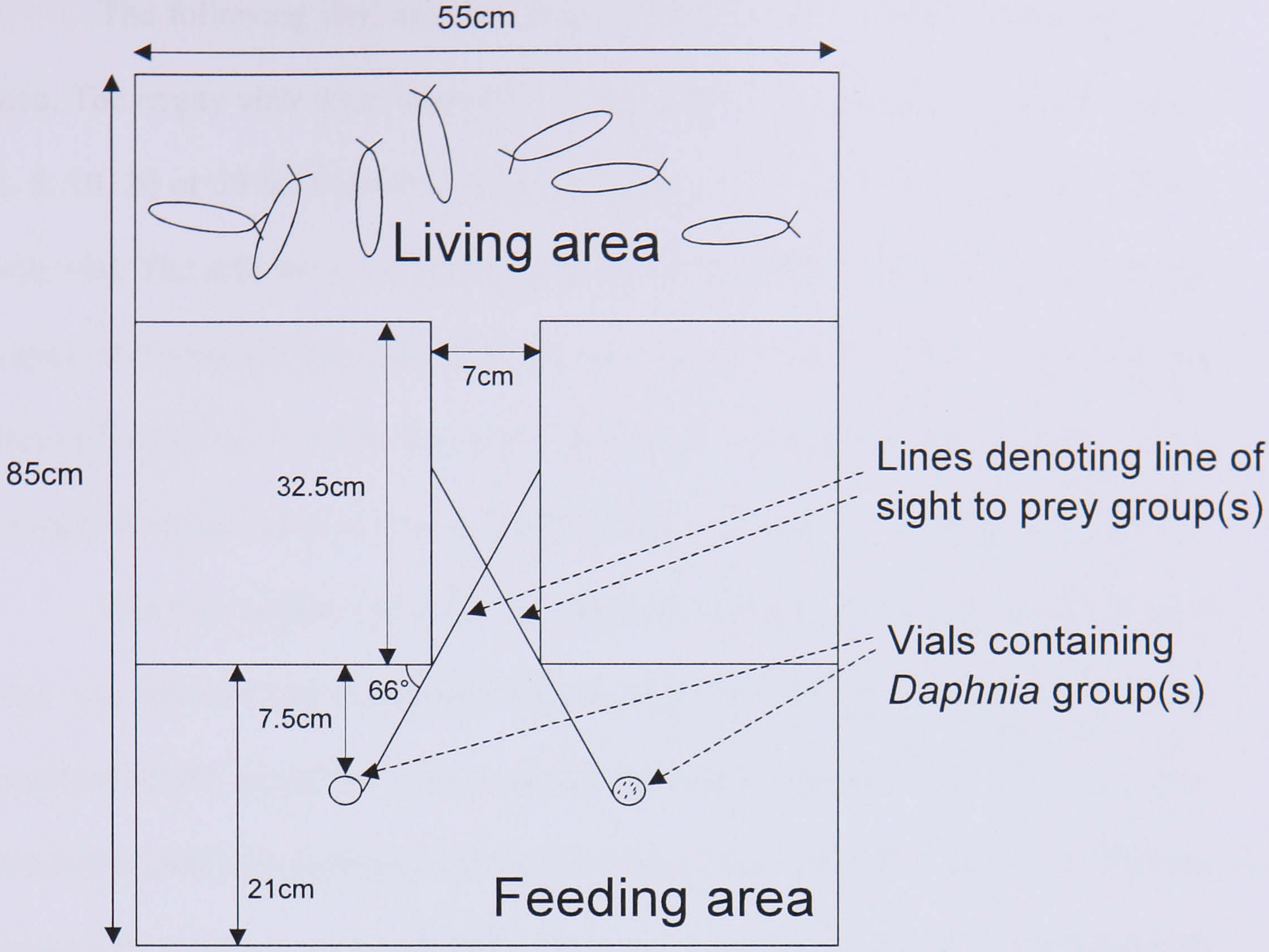


Figure 3.1. The experimental setup used to determine the effect of group size and the number of groups on detection rate in the laboratory (not to scale). Approach time was measured as the time taken from crossing the line of sight to orientating to the prey group within one body length.

Fish were fed live *Daphnia* and defrosted bloodworm in the feeding area of the tank at 17.00 daily, and the fish were then left overnight to habituate. A gate at the feeding end of the channel was left open to allow exploration of the whole tank, with inverted glass vials (height = 5.3cm, internal diameter = 2.3cm, height of visible area = 3.9cm), containing only water and identical to those used in the trials, placed on the gravel also to allow habituation. Sand was placed in the cap of the vials to allow sinking, and to stop the *Daphnia* being hidden during the trials (figure 3.1).

The following day, any fish in the feeding area were moved into the living area. The empty vials were replaced with one containing a group of *Daphnia* (either 2, 5, 10, 30 or 50 individuals of mean length±s.d = 2.1±0.2mm) and another water-only vial. The activity in the feeding side of the tank was observed remotely using a camcorder mounted under the white sheet. The time taken for a fish to approach the *Daphnia* (defined as in the field experiment) after crossing the line indicating a line of sight to the prey was recorded, as well as the time taken to make the first attack.

Once the attack was made, the gate was closed and the fish in the feeding area was removed and not reused. On occasion, the gate was lowered if a fish that approached the group, but did not attack, indicated leaving the feeding area. This method avoided the problems associated with pseudo-replication. If fish entered the feeding area without approaching the *Daphnia* group, the gate was not lowered and the fish not removed. As we assume no detection had taken place (as defined by an approach), there should have been little influence of the visit on subsequent visits by the same fish.

All testing took place between 10.00 and 17.00, with removed fish being replaced from the stock tanks at 17.00. The side with the *Daphnia* group was randomised for each trial. The order of group sizes presented was randomised using a complete random block, i.e. each group size was tested every five trials, but within those five trials, the order of testing was random. This assisted in reducing variability due to time of day, changes in boldness, the effect of reducing the population size, and changes in population composition between days. Fish were returned to the source site after completion of the experiments.

Effect of multiple groups

Using the same procedure as above, detection rates of two further treatments (2 vs. 2 and 30 vs. 30 prey) were tested to determine the effect of multiple groups. In these experiments both vials contained prey. These treatments were determined from the results of the above experiment. As in the previous experiment, approach and attack time for the first attack on a group were recorded.

The role of visual angle

In the final experiment, the mechanisms behind the effect of group size on detection rate were explored. To isolate the effect of visual angle, group size was kept constant, and the distance from the starting point of the fish to the prey group was varied instead. The apparatus in figure 3.1 was adapted to increase the feeding area to 43x55cm and the channel modified to a length and width of 7x5cm. Additional variables were identical to those in the previous lab experiments.

The procedure was identical to that in the other lab experiments, except that the *Daphnia* stimulus consisted of a glass vial containing 10 *Daphnia* (1.8±0.3mm), placed at an angle of 40° to the channel at one of five distances (7, 13, 19, 25 or 31cm from the nearest point of the channel opening). Only one side of the feeding area was used to present prey, to minimise variation in lighting between the two sides of the tank. Activity of fish in the feeding area was recorded using a digital camcorder, and the path coordinates were manually extracted using Image J (version 1.34).

As the time required to reach the group was not constant as in the previous experiments, the time taken to approach was not an appropriate indicator of detection rate. Instead, the path characteristics of the fish were analysed to infer detection of prey, as in Confer and Blades (1975), Utne (1997) and Utne-Palm (1999). Although these authors used reaction distance, defined as the distance from the pre-attack pause to the prey, sticklebacks do not demonstrate such clear pause-travel search as the fish used in these studies (*Lepomis gibbosus* and *Gobiusculus flavescens*), and so reaction distance was not suitable. As an alternative, path straightness was calculated from straight-line distance to the prey divided by the observed distance travelled (i.e. the path taken relative to the minimum path). This measure has similar properties to reaction distance as it assumes the fish take straighter paths to prey when they are detected. It also has the advantage of taking into account the whole path of the fish, rather than only the position the fish decides to attack the prey. Average speed was also calculated from the observed path length / time taken (s). Although the prey group was placed at five different distances from the entrance, variation in where the fish crossed the line of sight meant that the distance the fish had to travel was more accurately measured as the point the fish crossed the line to the prey.

Data analysis

Data were $\log_{10}$ transformed where data did not meeting parametric assumptions of normality and homogeneity of variances. Residuals were normally distributed in the paired t-tests. The effect of visual angle on path straightness was analysed using a

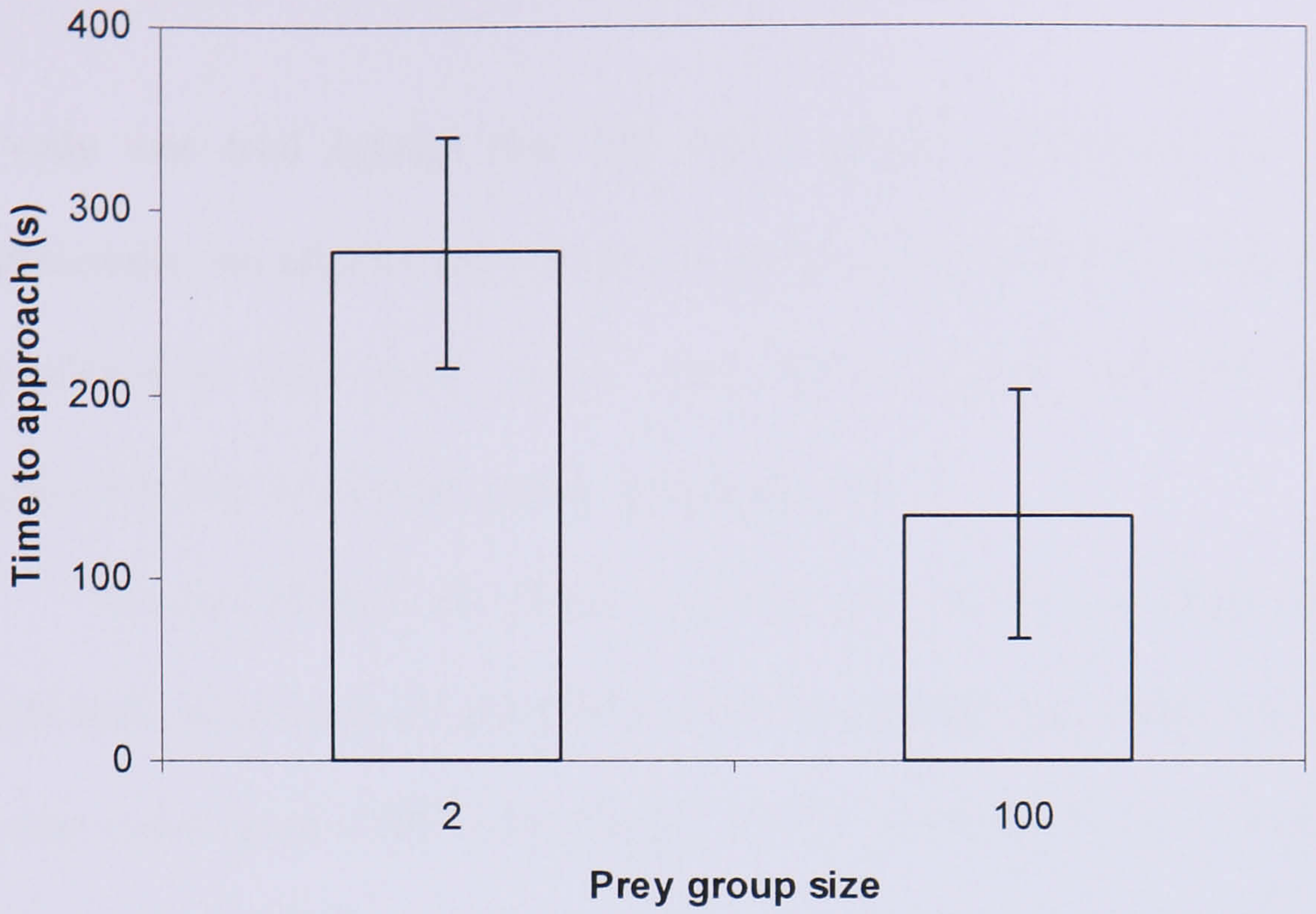
Spearman's rank correlation, as residuals were not normally distributed even after transformation. All statistical tests were carried out using SPSS version 14.

Results

Field experiment

There was considerable variation in approach times to the prey groups, varying from 3 seconds to over ten minutes. Nevertheless, *Daphnia* groups of 100 were approached significantly sooner than groups of 2 (figure 3.2a; paired t-test: $t_{13}=2.166$, $P<0.05$) and were also attacked sooner (figure 3.2b; paired t-test: $t_{13}=2.185$, $P<0.05$).

(a).



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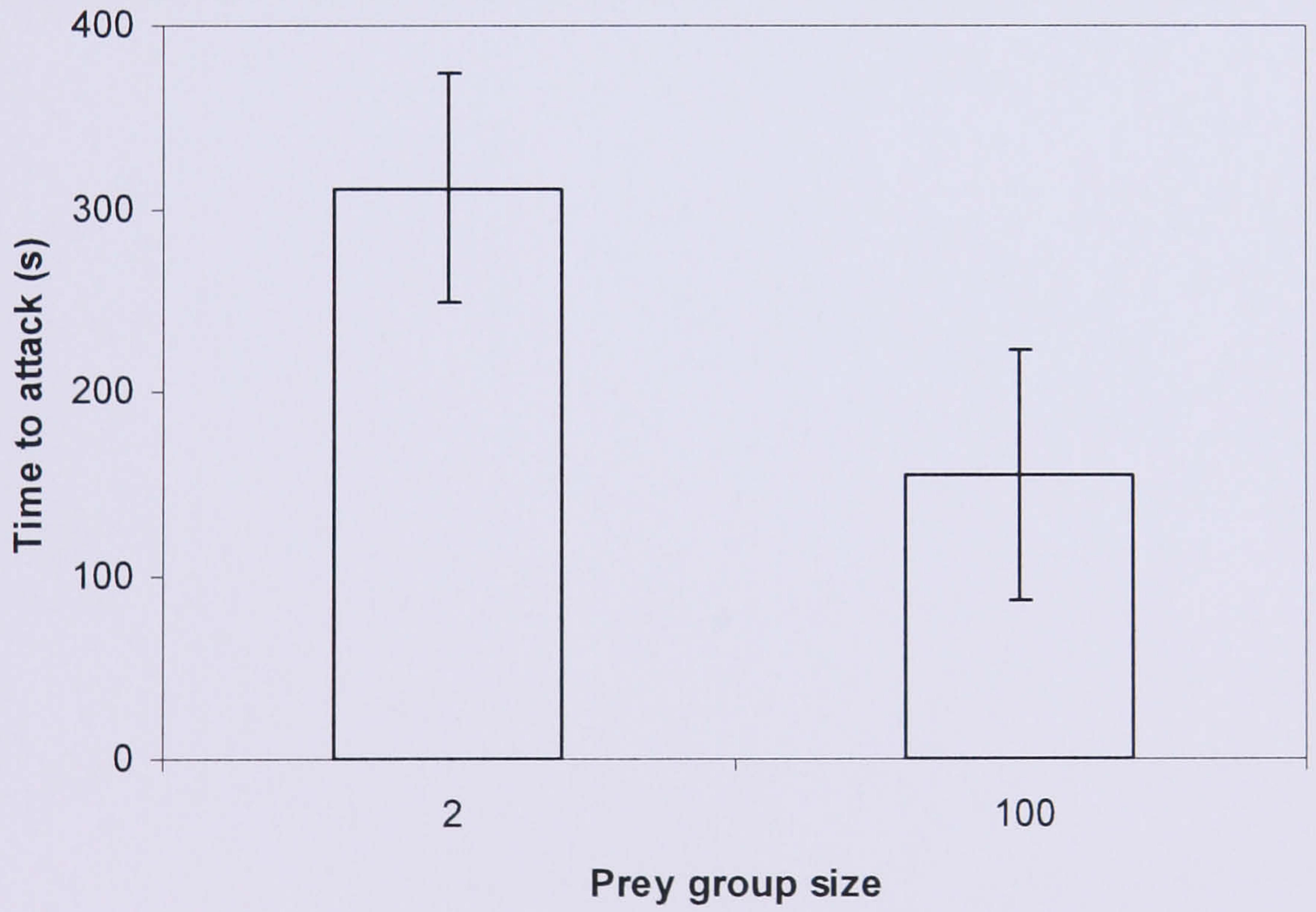


Figure 3.2. The effect of group size on mean approach (a) and attack (b) time (seconds) of sticklebacks predating *Daphnia* in the field. Error bars are one standard error.

Effect of group size

In only one trial (group size 50) was a group approached but not attacked. Additionally, no attacks were made on empty vials, while all *Daphnia* groups were approached at least once. These observations suggest the fish recognised the *Daphnia* as prey in all trials and at all group sizes.

To test whether the effect of group size on time to detect prey saturates, linear and inverse curves were fitted to the $\log_{10}$ transformed times to approach. The inverse curve gave a better fit (figure 3.3: $F_{1,50}=16.45$, $r^2=0.25$, $P<0.0005$) than a linear regression ($F_{1,50}=3.45$, $r^2 = 0.07$, $P = 0.069$). This suggests the effect of group size on detection rate saturates, which would be even more pronounced if the time to approach had not been transformed to meet parametric assumptions.

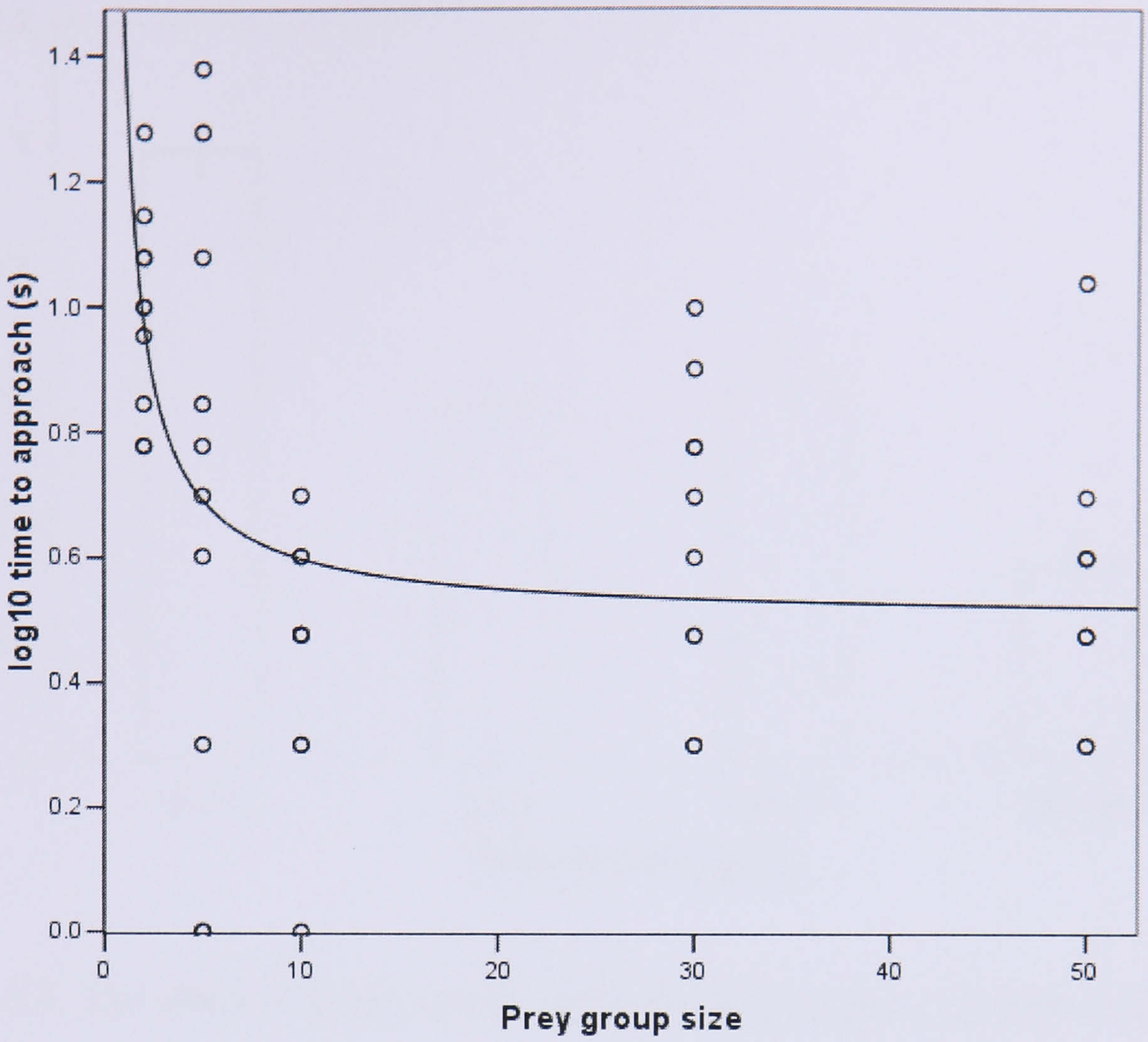


Figure 3.3. The relationship between group size and detection rate in the laboratory, as measured as log10 time to approach (s). An inverse relationship fits the data ($P<0.0005$) closer than a linear one ($P=0.069$).

Multiple groups

As in the single group study, multiple groups of 30 were approached significantly sooner than multiple groups of 2. Comparison to single groups of the same size revealed multiple groups were approached sooner, although there was no significant interaction between the number of groups (single or multiple) and group size (figure 3.4; two-way ANOVA, group size: $F_{1,40}=21.48$, $P<0.0005$, number of groups: $F_{1,40}=30.50$, $P<0.0005$, group size*no. groups: $F_{1,40}=0.74$, $P=0.40$).

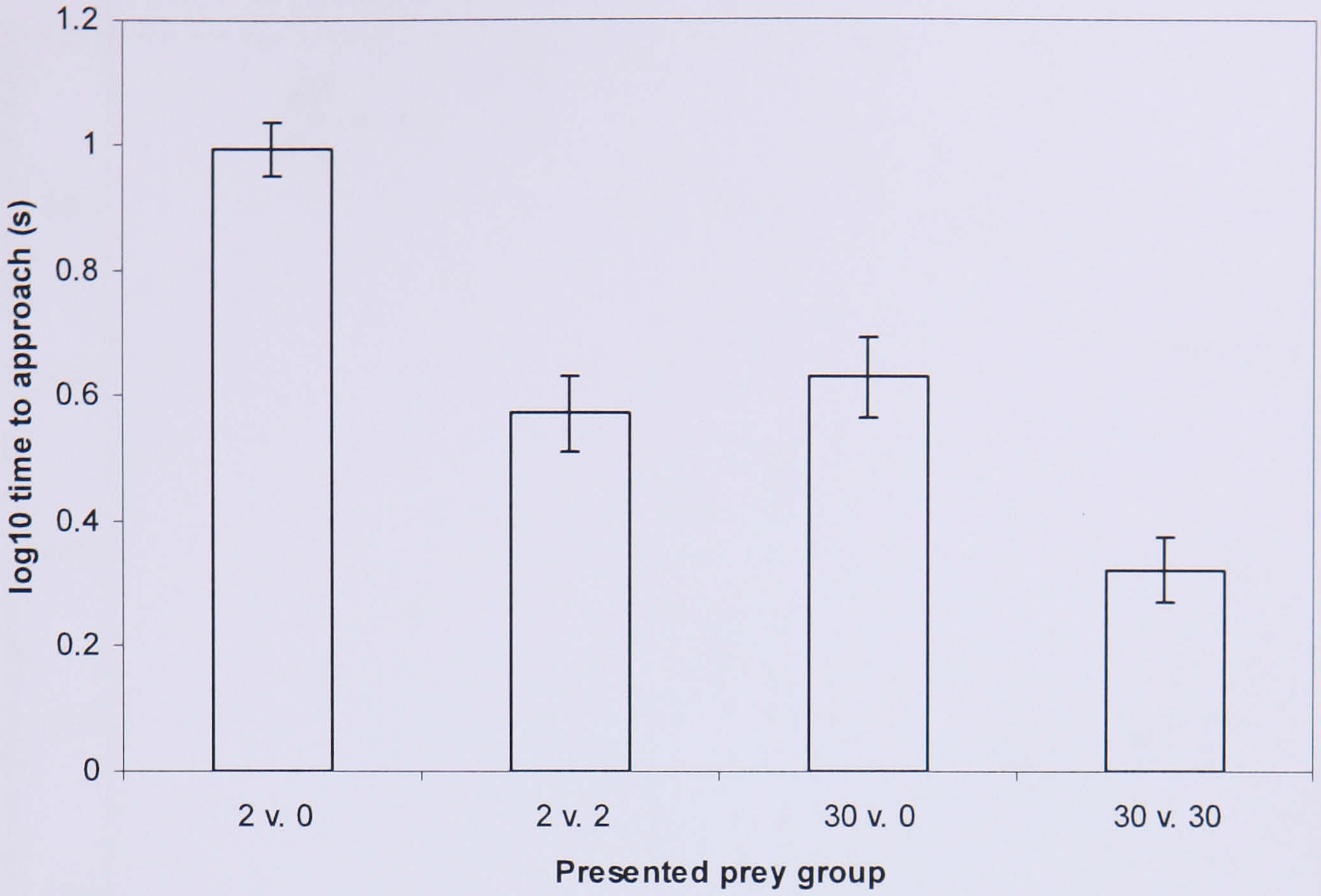


Figure 3.4. The effect of group size (2 or 30) and the number of groups (1 or 2) on mean approach time (s). Error bars are one standard error. N =10 in each treatment.

Visual angle

As the distance from the predator's starting position to the prey group increased, the path the predator took became longer relative to the straight-line distance (figure 3.5; Spearman's rank, r_s=-0.496, n=32, P<0.005), indicating distance had a significant effect on visual detection of the prey group. However, there was no evidence that distance had an effect on the average swimming speed of the fish (Spearman's rank, r_s=-0.015, n=32, P=0.936).

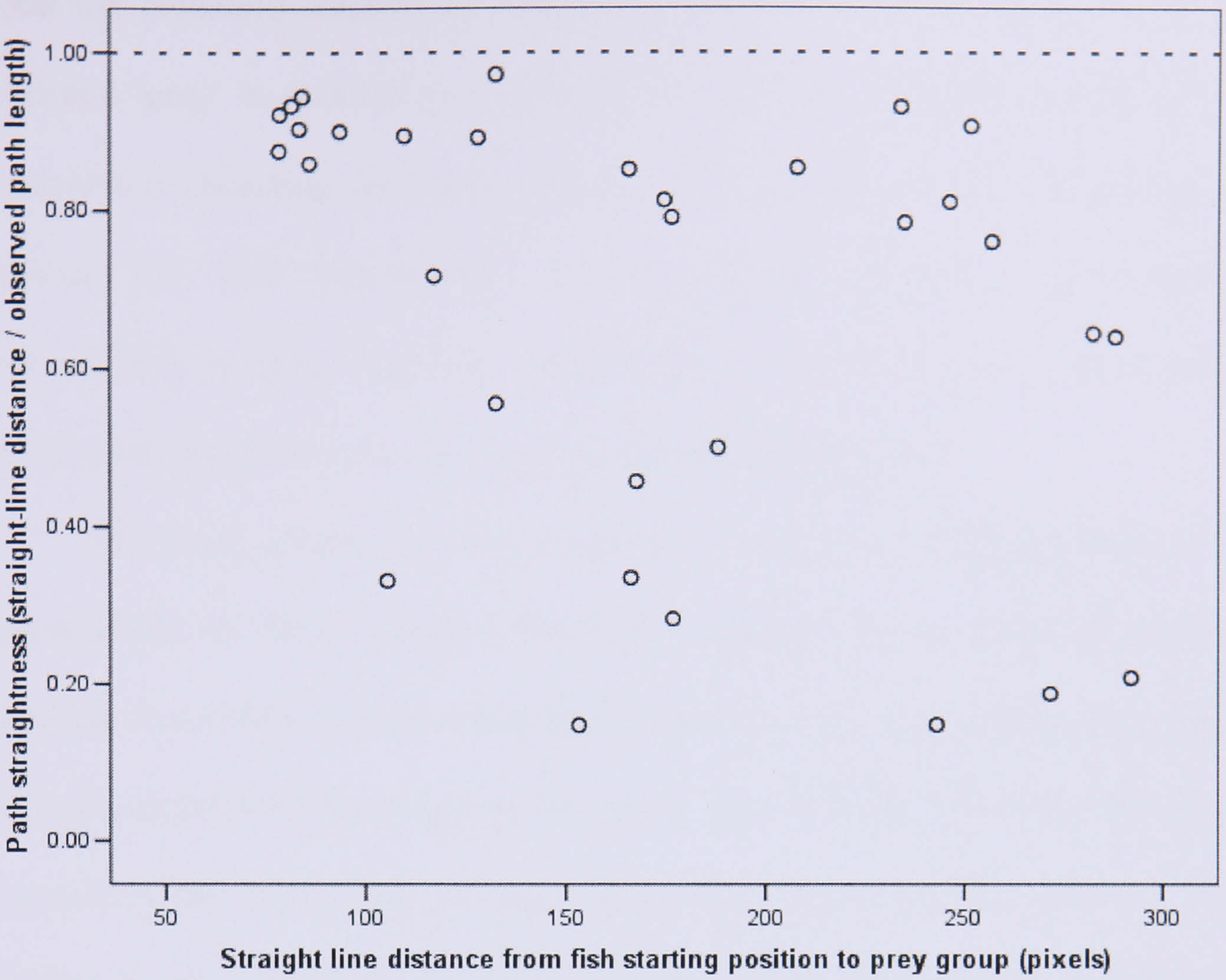


Figure 3.5. The effect of visual angle (as manipulated by varying the distance from the fish to a group of 10 *Daphnia*) on the path taken by the fish to find the prey group. Path straightness is calculated as the straight-line distance (from the fish's starting position to the prey) divided by the observed path length. The dashed line represents a perfectly straight path.

Discussion

The results from this study demonstrate that larger prey group sizes are detected more rapidly, although this effect saturated within a range of small group sizes of 2 to 10 individuals. This is in agreement with the experimental work of Riipi et al. (2001) and Jackson et al. (2005), where a similar saturating effect of group size on detection rate was observed. Although in their experiments artificial prey were used (great tits predating almonds in Riipi et al. (2001), humans predating computer generated 'prey' in Jackson et al. (2005)), this agreement suggests the perceptual constraints in detecting prey are universal across (at least) vertebrate predators. In addition, the field manipulation study confirmed descriptive field studies, predominantly in raptors attacking bird flocks (e.g. Creswell 1994), where larger groups were approached and attacked sooner than smaller groups.

The results demonstrate that the more rapid detection of larger groups can be accounted for by the increased visual angle produced by the larger group size. Although in the single group experiment I could not rule out an effect of increased per capita activity with increased group size (Grand and Dill 1999), this effect was removed in the experiment varying visual angle using only the distance from predator to prey. However, in this experiment, light attenuation with increased distance could not be ruled out, which was not an issue in the single group experiment. Thus, the observed effects could be due to different mechanisms in each of the experiments, and although I suspect there was some effect of these mechanisms, a single (and hence more parsimonious) mechanism, visual angle, can account for both of the observed trends. In addition, the experiments of Riipi et al. (2001) and Jackson et al. (2005) used prey unresponsive to group size, and achieved similar results.

The importance of visual angle in detection of prey groups has formed the mechanistic basis for the limited amount of theoretical work on group detection rates (Vine 1971; Treisman 1978). The relative contribution of visual angle to detection rate is unclear, however. For example, increased per capita activity in larger groups (Grand and Dill 1999) has been shown to increase detection rate (Krause and Godin 1995), and the effect of decreasing inter-individual distances between group members on detection rate remains unexamined. In addition, the study did not reveal whether the total visual angle is produced by the area occupied by the boundaries of the group or by the sum of the area of each group member. Actual quantification of visual angles subtended by groups of prey, and how these effect detection rates, is required to explore these issues further.

Although I have concentrated on a mechanistic explanation, based on the perceptual limitations of predators when searching for prey, a faster response to increased group size could represent the greater reward of increased prey group size (Lima 1998). Similarly, increasing distance to prey would increase travel costs, explaining why the fish took relatively longer routes to approach prey further away. The observed relationship between group size and detection rate will be sensitive to both perceptual constraints and optimal foraging decisions, and the relative contribution of each will be sensitive to the details of the predator-prey system involved. For example, when prey are difficult to capture in large groups, due to the confusion effect or increased vigilance and group defence with larger groups (Krause and Ruxton 2002), attacks on small groups may be more likely as larger groups will be detected but ignored (e.g. FitzGibbon 1990). In this study, however, competition for food was observed to be intense in both the stock tanks and the living area of the test tank, so it was expected fish would respond with an approach as soon as prey was detected, rather than delaying to gather more information about the environment. Moreover, the reduction in approach time observed with multiple groups would not be expected from foraging theory, as the fish had to choose a group to approach (in fact, time to approach would be expected to be delayed if a choice was to be made between two stimuli, especially if the two stimuli are similar; Tegeder and Krause 1995).

The saturation observed in the relationship between group size and the time to approach in the single group experiment could be due to a number of factors, such as a minimum information processing time or travel time to the prey. However, time to approach decreased further when multiple, rather than single, groups of 30 were presented to the predator; this suggests detection time was not limited by information processing or travel time, but rather by a factor sensitive to the number of groups, i.e. encounter rate. Increasing group number reduced the time needed to scan the feeding area (increasing encounter rate), while increasing group size increased the probability of detection once inside the field of attention. This explanation depends on the portion of the visual field attended to being relatively small (Dukas 2002), so that both groups were not perceived simultaneously. If both groups were perceived simultaneously, the time to approach a group of 30 would be equal whether presented alone or with another group of 30, as expected from the relationship between group size and approach time. Separation of encounter and detection is thus supported by a lack of an interaction between group size and number.

These results raise interesting questions as to the nature of the relationship between encounter and detection effects in terms of the risk experienced by prey. For example, in a finite prey population, prey aggregation and the formation of groups decreases encounter rate (Travis and Palmer 2005), but increases detection rate as the groups formed are larger (Turner and Pitcher 1986). My results suggest, however, that although larger groups are detected at a greater rate, the lower encounter rate of aggregated prey leads to a net reduction in risk (i.e. detection of a group from 2 vs. 2 was similar to a single group of 30). This issue deserves further theoretical and empirical attention. In addition, the saturation in detection rate with group sizes greater than 10 suggests larger groups will be favoured.

In the next chapter I examine what happens after the group is detected. In this post-detection phase, the rate of attacks and their success can depend strongly on group size, so that even though larger groups are more conspicuous, individuals may still be safer in larger groups.

Chapter IV - The confusion effect: from neural networks to reduced predation risk

Abstract

The confusion effect is often cited as an anti-predatory benefit of group living, and has been demonstrated by numerous studies across a range of taxa. However, there have been relatively few studies examining the mechanism(s) behind the effect, and no experimental test of its supposed theoretical basis (information degradation in neural networks) using a natural predator-prey pairing. In agreement with other studies, I demonstrate that attack success of the three-spined stickleback is reduced by an increase in *Daphnia magna* group size. Neural network models attempt to explain this trend with multiple prey inducing poor neural mapping of target prey, thus leading to an increase in the spatial error of each attack. I explicitly tested this prediction, and demonstrate that the decrease in attack success by sticklebacks does correspond to an increase in spatial targeting error with larger prey group size. Finally, I show that the number of targets, rather than the density or area occupied by the group, has the greatest effect on reducing the rate of attack. These results are discussed in the context of the information-processing constraints of predators, the ultimate cause of the confusion effect.

Introduction

The confusion effect (Miller 1922) is one of several mechanisms that reduce predation risk in group-living prey; others include group defence, increased vigilance and attack abatement (reviewed by Krause and Ruxton 2002). As predators have to process spatial information of multiple targets when prey aggregate, the accuracy of this processing declines with multiple prey, causing the confusion effect (Krakauer 1995). Thus, aggregation by prey exploits the information-processing constraint of predators. It is this neurological constraint that distinguishes the confusion effect from other anti-predatory grouping mechanisms.

The effect operates at two stages of the predation cycle (Lima and Dill 1990). Firstly, there may be a reduction in attack rate (e.g. Milinski 1977a) as costs of overcoming confusion are too high, for example due to a decreased attention to their own predators (as in the simulated aerial attacks on feeding sticklebacks by Milinski 1984). If an attack is launched, the probability of success can also be affected by the confusion effect (Krause and Ruxton 2002), i.e. the ratio of attacks to kills is increased (e.g. cephalopods predating fish: Neill and Cullen 1974; raptors predating redshanks: Creswell 1994). The inverse of this attack-to-kill ratio is attack success (i.e. the number of kills per attack), which will be used throughout this paper (as used by Tosh et al. 2006). Interestingly, attack rate should increase with prey density as encounter rate increases (the functional response, discussed in terms of the confusion effect by Jeschke and Tollrian 2005). In addition, a randomly striking predator will increase attack success as density increases, as the probability of striking a space containing a prey increases with density.

The experimental literature on the confusion effect greatly outnumbers theoretical work. A possible explanation is that the underlying mechanism is believed to be neurological, which is problematic to model, especially without the use of computationally intensive techniques. Only three published studies attempt to model this effect: the neural network models of Krakauer (1995), Tosh et al. (2006) and Tosh and Ruxton (2006). Neural network models aim to represent the neurological processing of visual information by animals, from input at the retina to the representation of this input onto a neural topographic 'map'. The neural network approach has proved successful in simulating an observed confusion effect of humans predating computer-generated prey (Tosh et al. 2006). Possibly due to the relative novelty of this theoretical approach, however, no study has yet attempted to bridge the gap between theoretical and experimental work using a natural predator-prey system.

Neural network models of the confusion effect make a key, untested prediction for the behaviour of a predator attacking prey groups: accuracy is reduced due to the poor neural mapping of targeted prey, induced by the large number of potential targets. Thus, it is expected that the greater the degree of confusion, the greater the spatial targeting error of each attack. This mechanism is believed to account for the lower attack success observed in many experimental studies, although there is no empirical evidence that poor neural mapping leads directly to an increase in spatial targeting error (Tosh et al. 2006), and alternative mechanisms may also be in operation. For example, tracking of a potential prey may be interrupted by another prey overlapping the target item, even though the original strike would have been successful (Neill and Cullen 1974; Ohguchi 1981). Overlap may result in an alternative individual being captured, although this is less likely if prey are relatively fast moving and a period of tracking the target is required before the strike can be launched. This issue can be overcome by presenting prey in a two dimensional plane, isolating the effect of large prey numbers on neural mapping, although this approach has not been used outside of studies using humans (Tosh et al. 2006).

Neural network models focus primarily on the effect of prey number, although there is some theoretical (Krakauer 1995, but see Tosh et al. 2006) and experimental (Milinski 1977b) evidence that increasing density of groups can increase the confusion effect as well. Prey groups from a range of taxa demonstrate compaction on detecting a predator (for example, Magurran and Pitcher 1987), although this may also be accounted for by a selfish herd effect (Hamilton 1971). The question of whether the density, size (number) or area occupied by a group cause the confusion effect is often overlooked as prey are often presented in a constant area or volume and prey number manipulated. As density is a derived variable (density = number / area or volume), it is difficult to distinguish which variable (density, number or area) is having the effect, as only one variable can be controlled between treatments. Milinski (1977b) demonstrated that increasing density of the group increased preference for strays, although such a density effect has yet to be shown when a single group (without strays) is presented.

Three experiments were conducted to attempt to bridge the gap between predictions of neural network models and experimental work demonstrating risk reduction in prey. I tested whether there is a decrease in attack success associated with increased prey group size, thus reducing risk to aggregated prey. I then examined whether this decrease in success could be accounted for by a larger targeting error from the target individual as predicted by neural network models. In the final experiment, I test whether density, area and/or number have effects on predator confusion, by measuring the number of attacks per time unit. By investigating the commonly used stickleback-*Daphnia* system (e.g. Ohguchi 1981), the results presented are directly comparable to those of other studies, and are discussed in relation to neural network models and how these can be developed further.

Method

Experiment 1 – Attack success

An opaque, white, cylindrical container (diameter 25cm, height 25cm, water depth 4cm) was filled with 1 litre of water, with either 20 or 500 *Daphnia magna* (mean length±s.d. = 2.1±0.2mm) allowed to habituate for 10 minutes. The upper *Daphnia* density was within those found under natural conditions (e.g. Jensen and Larsson 2002). A three-spined stickleback (5.6±0.52cm), fed the day previously, was gently added to the container from individual holding (held in tanks 45x11x13cm for at least 48hrs). Larger fish were used in this experiment compared to experiments 2 and 3 to facilitate detailed observation of feeding behaviour. Lighting was provided by diffuse ceiling fluorescent tubes. The feeding behaviour of each fish was filmed for 30 min, using a camcorder mounted 1m above the tank, recording the number of attacks and whether each failed, the prey was rejected, or resulted in consumption (kill). Trials were aborted if no kills were made within 30 minutes. Each fish was tested at both *Daphnia* group sizes, separated by a week. Out of twenty fish tested, only two fish did not consume at least one *Daphnia* in either treatment, and a further five did not consume in one of the two treatments. Thus, the sample size for the repeated measures tests was reduced to 13. Individual fish and treatments were tested in a random order.

To maintain prey number, *Daphnia* were replaced immediately after each consumption using two pipettes protruding into the container by 1cm at the water surface. The pipettes were placed opposite to each other; the pipette further from the fish was used to replace the prey to minimise disturbance, and this method did not appear to affect feeding behaviour. A video monitor was used to indirectly observe consumption. Additionally, two holes in the sides of the container, equidistant from the pipettes and also at the water surface, allowed overflow (due to water added with the *Daphnia*) to maintain water volume. A similar apparatus was used by Heller and Milinski (1979).

Experiment 2 – Targeting error and group size

I then examined whether any effect of prey group size on attack success corresponded with an effect on the accuracy of each attack, as measured by the spatial error from the target. Forty sticklebacks (35mm±5mm) were placed in a tank (46 x 31 x 31cm, water depth 29cm) the evening before testing and then left to habituate overnight. The tank was split into two areas: a living area (31 x 31 x 31cm) and a feeding area (15 x 31 x 31cm), separated by a white Perspex wall. The two areas were connected by a 10 x 10cm square door cut into the horizontal centre of the wall, so that the top of the door was flush with the water surface. An additional piece of Perspex (19 x 15cm) was suspended 2cm behind the door to block any line of sight from the living area into the feeding area. This also provided a white background to facilitate recording of *Daphnia* positions.

The next day, all fish were moved into the living area. and either 5 or 20 *Daphnia* (2.7±0.17mm) were placed in a 10cm x 10cm glass chamber with an internal width of 4mm (thus restricting overlap between *Daphnia* but allowing free swimming movements). The chamber was mounted in the feeding area on the tank wall opposite the door. After a number of minutes, individual fish would enter the feeding area from the living area, and attack the *Daphnia* prey. The attack of the fish was remotely viewed and recorded using a camcorder facing the feeding area. Once an attack was made, the fish was removed. and the *Daphnia* individuals changed. *Daphnia* were presented in a random order at either group size 5 or 20.

Frame by frame analysis was used to determine the error of each strike. A strike was defined as the first snout touch on the chamber wall, a reliable indication of which was given by an overhead mirror viewing the fish's approach to the chamber. Of each frame at the moment of the strike, the coordinates of the stickleback's mouth and the centre of each *Daphnia* were extracted manually using ImageJ (version 1.34s). This allowed calculation of the strike's targeting error (the distance (pixels) from the strike to the nearest *Daphnia*). 21 trials were carried out with a *Daphnia* group size of 5 and 20 trials with a group size of 20.

Experiment 3 – Prey number, area or density?

Finally. I tested whether prey number, area or density (or a combination of these) cause the confusion effect. *Daphnia* were presented in a chamber of nine adjacent plastic cuvettes (each with internal dimensions of 1cm x 1cm) attached to a tank (19 x 10 x 25, water depth=22) wall facing a camcorder. Water depth in these cuvettes was kept constant at 1.5cm. including cuvettes without *Daphnia*.

For each treatment, *Daphnia* (2.1±0.2mm) were placed in the seven middle cuvettes in the following arrangements (figure 4.3):

A: Large area, low density, low number: 5,5,5,5,5,5,5.

B: Small area, high density, low number: 0,0,0,35,0,0,0.

C: Large area, density and number: 35,35,35,35,35,35,35.

Thus, each variable (area, number and density) was increased by a factor of seven between high and low treatments. As density is derived from number / area, two variables must vary between treatments, and only one can be controlled. Thus, a minimum of three treatments must be used to distinguish which variables are having an effect on predator confusion. By expanding the area occupied by the *Daphnia* group horizontally, the problem highlighted by Milinski (1977b) are avoided. In that experiment, area was varied using water depth within a single test tube, although this affected *Daphnia* distribution within the tube, possibly confounding the result.

After 10 minutes habituation of the *Daphnia*, an opaque cover was placed between the chamber and the inside of the tank. A stickleback was transferred from individual holding (held in tanks 45x11x13cm for at least 48hrs) to the tank, and after 5 minutes further habituation, the opaque cover was gently removed. After the initial approach of the fish to the cuvettes (an approach was defined as being within 1cm), the number of strikes made was recorded for five minutes. The trial was aborted if the fish did not approach within 10 min. Three out of twenty fish did not approach in any of the trials. Each fish was tested at all three arrangements, one arrangement per day over three days. Individual fish and treatments were tested in a random order.

Data analysis

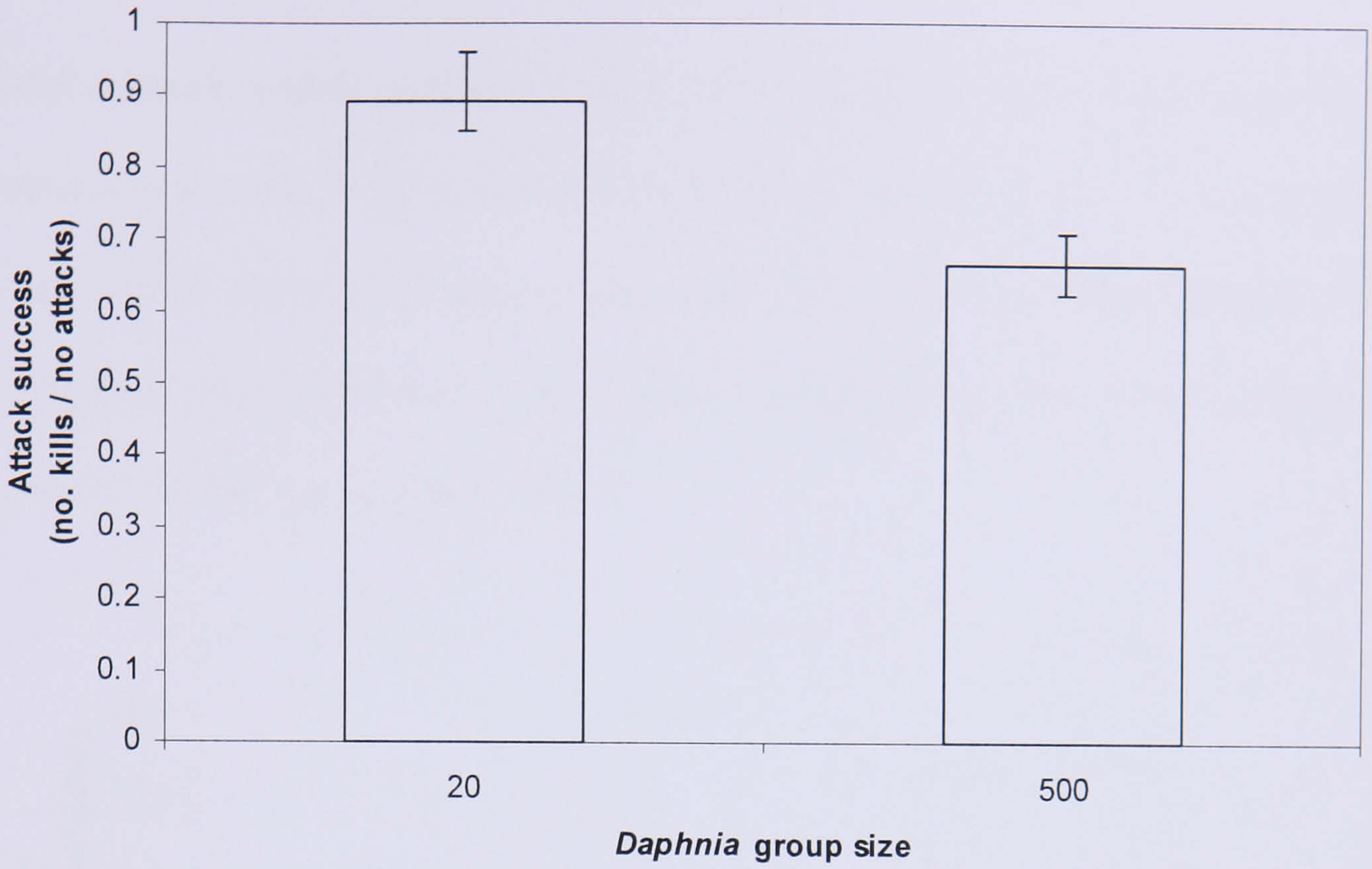
Where data did not meet assumptions of normality and homogeneity of variance, data was log10 transformed where possible, or non-parametric statistics were used. As repeated measures designs were used in experiments 1 and 3, Wilcoxon Signed ranks tests were used to compare *Daphnia* treatments. All statistics were carried out in SPSS version 11.

Results

Experiment 1 – Attack success

To determine the effect of aggregation on the confusion effect, three-spined sticklebacks were added to groups of *Daphnia* of 20 or 500 individuals, and foraging success recorded. The median number of attacks was 28 when attacking a group of 20 prey versus 34 when attacking a group of 500 (although this difference was non-significant: Z=-0.157, n=13, P=0.910). The median number of kills was greater on the smaller group size, being 25 (group of 20) versus 15 (group of 500) attacks. Although this effect was also not significant (Z=-0.315, n=13, P=0.774), together attack success (no. kills / no. attacks) was more successful at the lower, compared to the higher, *Daphnia* group size (figure 4.1a; Wilcoxon Signed ranks test, Z=-2.824, n=13, P=0.002). Median attack success (no. kills / no. attacks) when attacking prey at group size 500 was 67%, compared to 86% at the smaller group size of 20. There was a difference in the number of failed attempts (figure 4.1b; Z=-2.49, n=13, P=0.01).

(a).



(b).

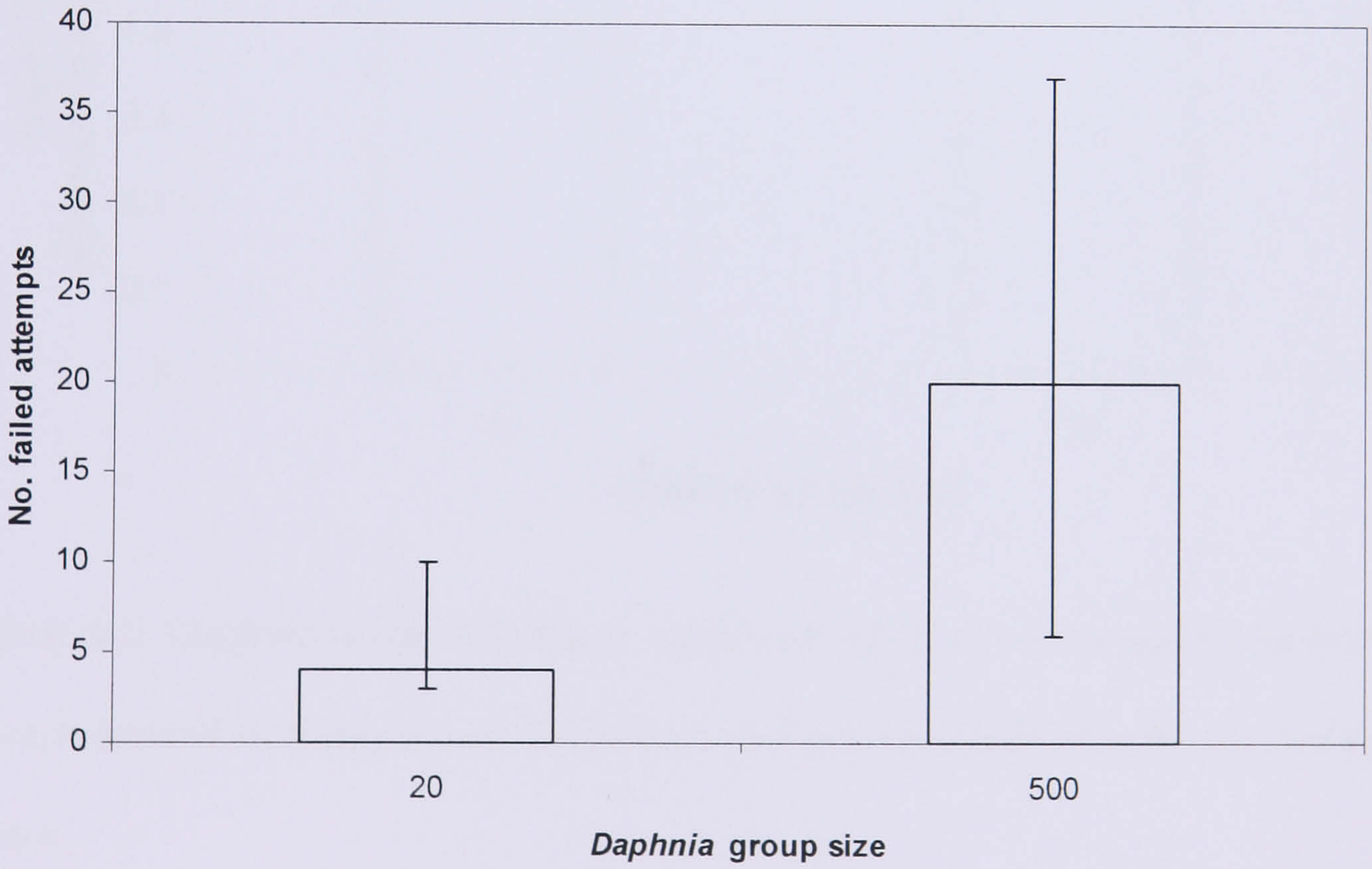


Figure 4.1. *Daphnia* group size has a significant effect on: (a) attack success (no. kills / no. attacks) and (b) number of failed attempts by three-spined sticklebacks. Medians are given with inter-quartile range.

Experiment 2 – Targeting error and group size

Neural network models predict that the mechanism for the above decrease in attack success is a decrease in the accuracy of each attack. Targeting error (measured as the distance of the strike to the nearest prey individual) was significantly greater when 20 *Daphnia* were presented to sticklebacks compared to when 5 were presented (figure 4.2; T-test, t_{39}=-2.036, $P<0.05$).

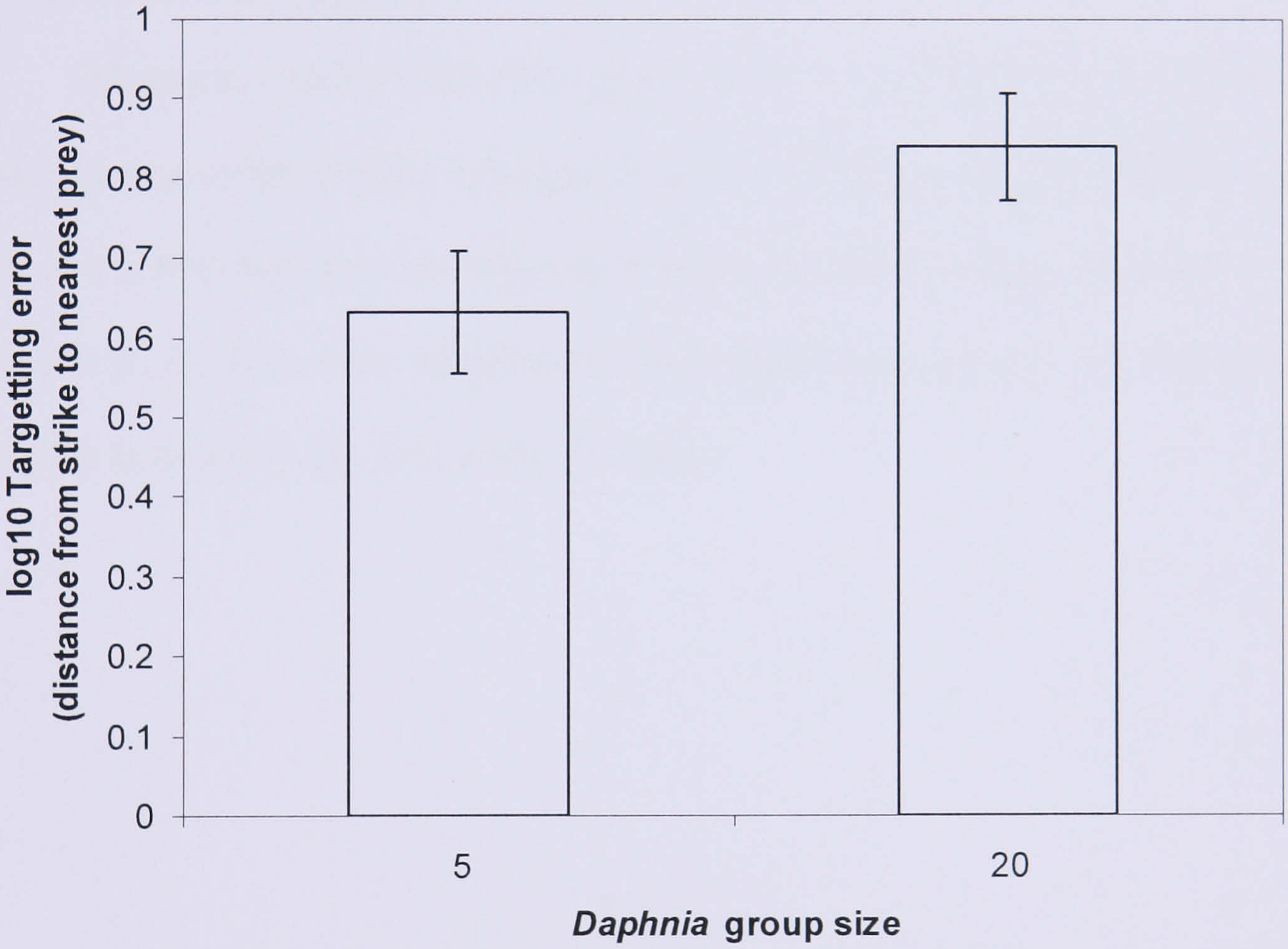


Figure 4.2. *Daphnia* group size has a significant effect on mean spatial targeting error, measured as distance from the strike to the nearest *Daphnia* (with one standard error).

Experiment 3 – Prey number, area or density?

In the former two experiments, it has been unclear whether the decreased foraging ability (measured as attack success and targeting error) has been due to an increase in the number of targets, or an increase in their density. This is because the area/volume of the group has been kept constant. To address this issue, the final experiment examined how attack rate was affected by these three variables (number, area and/or density) separately.

When prey number was held constant (i.e. A versus B), there was no effect of density or area on the number of attacks (figure 4.3; Wilcoxon Signed ranks test, $Z=-0.79$, $n=12$, $P=0.46$). However, when the number of potential targets increased (A to C and B to C), there were significantly fewer attacks (A versus C: $Z=-1.99$, $n=14$, $P=0.043$; B versus C: $Z=-2.22$, $n=10$, $P=0.039$).

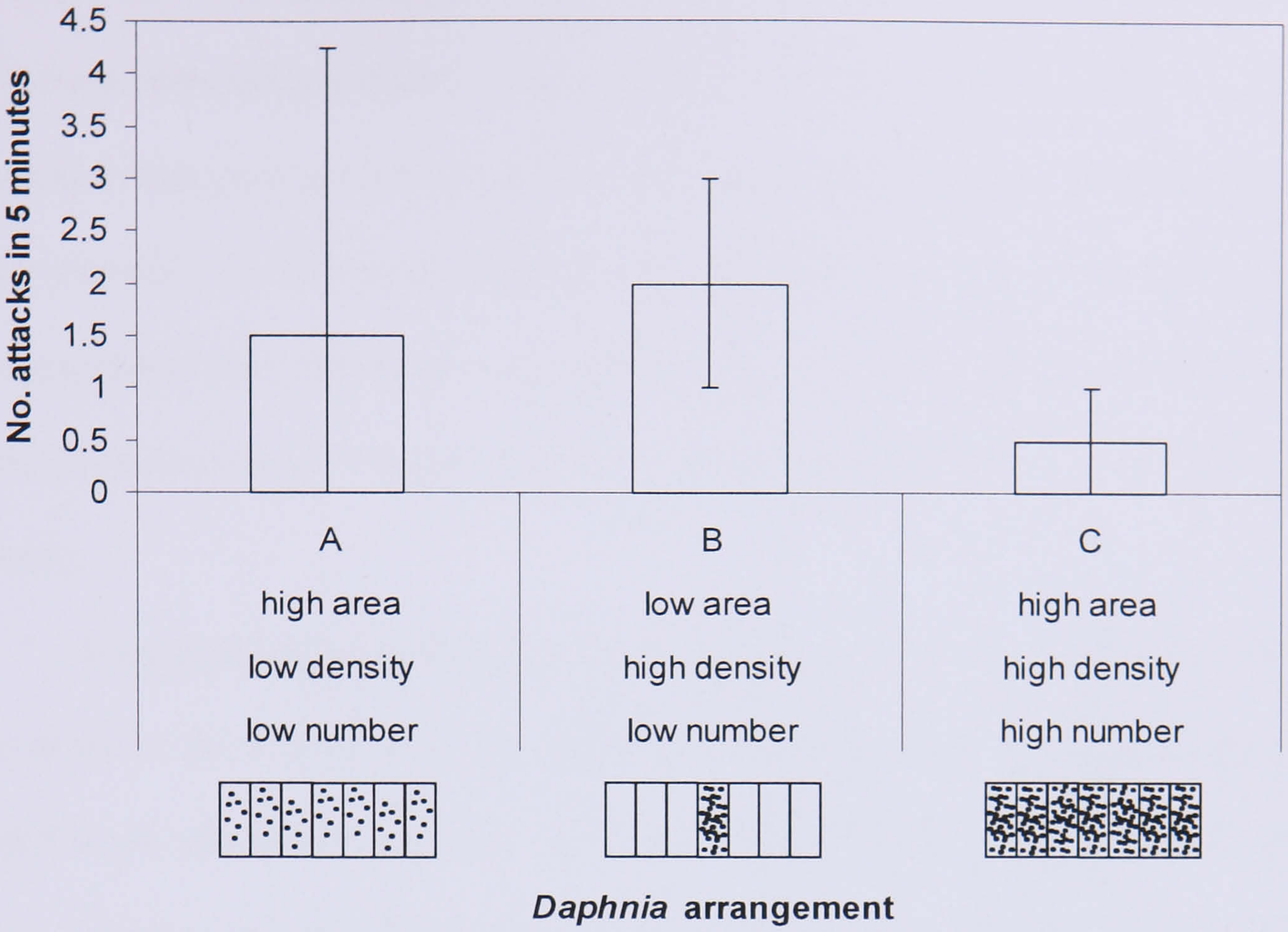


Figure 4.3. Attack rate on three *Daphnia* group arrangements, with arrangements represented below the x-axis. Medians are given with inter-quartile range. Treatments are: A: low number, density and high area; B: low number, high density and low area; C: high number, density and area.

Discussion

Increased *Daphnia* group size was associated with reduced success of attacks by sticklebacks. Overlap between individuals is a commonly cited possible mechanism for this trend (Neill and Cullen 1974; Ohguchi 1981), although I have demonstrated explicitly that targeting error (the spatial error from the nearest prey to the strike) increased when large group sizes are presented in a two-dimensional plane, thus excluding overlap. This result can be explained by the poor neural mapping of targeted prey as predicted from the neural network models of Krakauer

(1995), Tosh et al. (2006) and Tosh and Ruxton (2006), and is the first verification of these models using a natural predator-prey system. My study lends support to the prediction that poor neural mapping of prey position (induced by numerous prey) leads directly to an increased targeting error of attacks, and hence to a fitness benefit for aggregated prey. However, a quantitative (rather than qualitative) test of neural network models is still lacking, and is in part due to the abstract nature of these models.

By employing a continuous measure of targeting error, i.e. the distance from the strike to the nearest prey, the predictions of neural network models could be tested more specifically than has been possible previously. Detail would be lost using a binary success/failure measurement based on a critical distance from the nearest prey, such as the results presented by Tosh et al. (2006) using human predators. Although success/failure is relevant to prey risk, as demonstrated by experiment 1, targeting error (a continuous variable) is more useful in understanding the mechanism(s) causing the confusion effect. Hence, this is the first study to suggest that poor neural mapping due to multiple targets leads to increased error in targeting prey. It was assumed the nearest individual to a strike was the intended target, although neural network models and the experimental test using humans (Tosh et al. 2006) pre-assigned the target to be attacked. As this is not possible using non-human predators, it is likely my results are a conservative estimate of the confusion effect, as it was more likely a non-target individual was nearer to the strike than the intended target at the higher density. If a single prey was assigned to be the target *a priori*, the problem that an increase in density would lead a random predator improving targeting accuracy would not arise.

Together with the other literature on the subject, this study illustrates that the confusion effect is an umbrella term, with multiple, non-mutually exclusive causes and consequences, even within a particular predator-prey system. Experiment 1 demonstrates the confusion effect manifests itself in a reduction of attack success, experiment 2 in an increase in spatial targeting error, and experiment 3 in a decrease in attack rate. Consequences of confusion in the stickleback also include the focus of attacks on less dense parts of the group (Milinski 1977a), preference for spatially and phenotypically odd individuals (Milinski 1977b; Ohguchi 1981), greater latency before attacking (Milinski 1979), and reduced attack duration (Milinski 1979). Which of these factors are relevant to predation events in natural systems is dependent on the predator-prey system under examination (Ruxton et al. 2007), and also on environmental factors, such as the proximity of refuges for prey to escape capture.

How these multiple causes and consequences interact is an interesting area for future research into the confusion effect. I speculate in figure 4.4 how poor neural mapping may account for increased latency before the first attack, reduced attack rate, and increased preference for strays as remediation behaviour to counteract low attack success. For example, the model of Tosh et al. (2006) found that the accuracy of neural mapping increased when stray individuals were targeted. Whether attack success increases with attack latency, reduction of attack rate and the targeting of stray individuals needs to be tested under experimental conditions, thereby giving an adaptive explanation to these phenomena from the perspective of predator behaviour. There is some suggestion from humans predating computer generated prey that while prey group size had no effect on missing the assigned target, there was an increase in the time taken to make a successful attack (Ruxton et al. 2007). However, my results suggest that amelioration mechanisms are far from completely compensating, as decreased attack success and targeting accuracy was still observed with increasing prey group size.

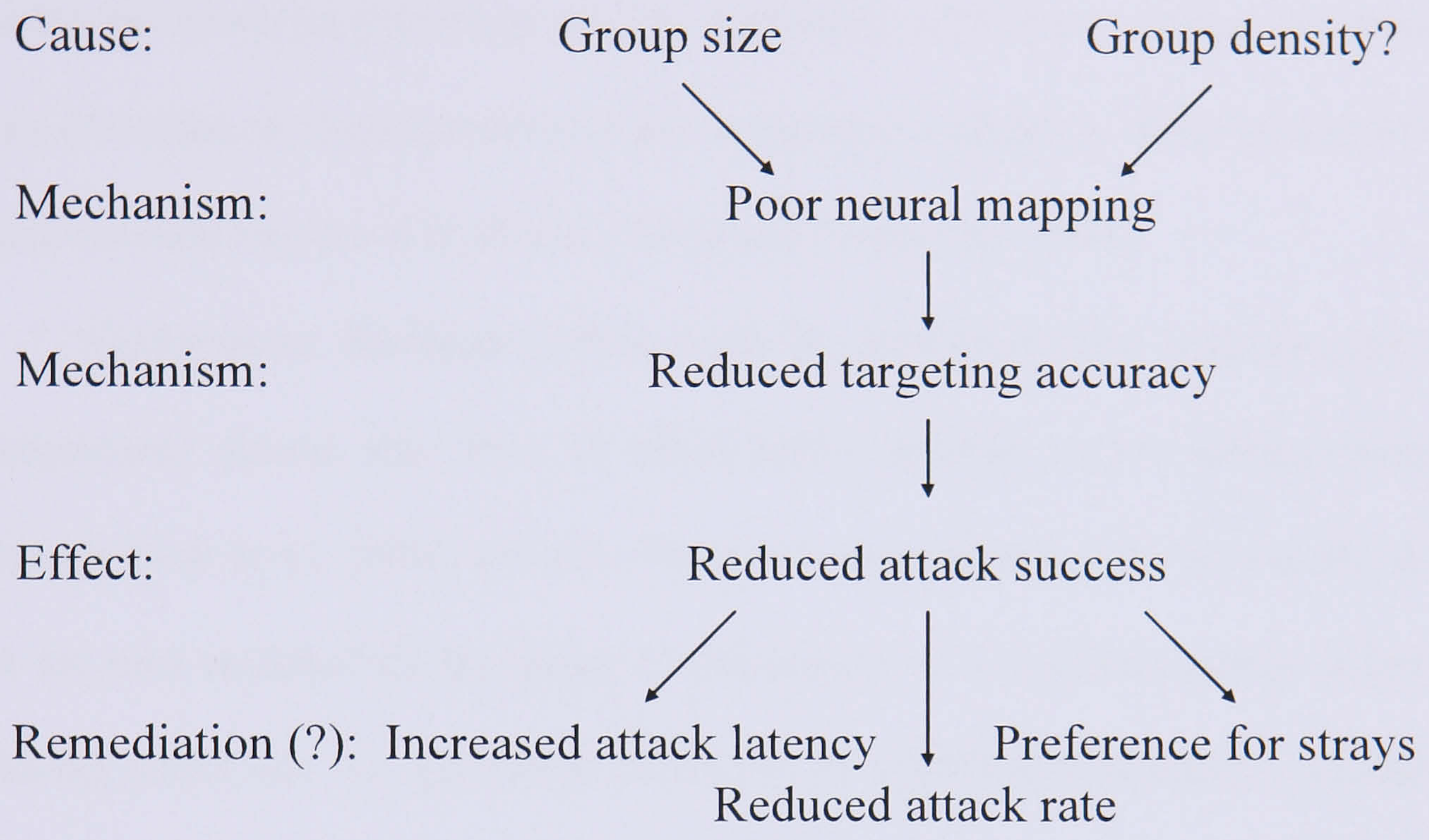


Figure 4.4. Hypothesised causes and consequences of the confusion effect. According to neural network models, numerous targets (within the visual range of the predator) cause poor neural mapping of the target individual, which leads to a decrease in attack success. It is hypothesised that predators may attempt to ameliorate this effect by taking longer to attack prey, and focusing attacks on less dense parts of the swarm and/or strays.

The density of a prey group is a variable derived from the total number of prey per unit area or volume; thus it is not clear which factor is predominantly responsible for the confusion effect (i.e. density, number or area; Milinski 1977b). My three-treatment design in experiment 3 demonstrates that increasing the number of prey causes a confusion effect compared to a prey group with the same area but low density, and also a group with the same density but small area. The experimental design could not rule out the observed effect being caused by an increase in both density (from treatment A) and area (from treatment B), rather than an increase in number *per se*. However, the design adopted was necessary given the problem of independent manipulation of the three variables, and the interpretation presented remains the most parsimonious one. In agreement with these results, the primary cause of confusion is predicted to be due to numerous targets as demonstrated by the neural network models of Krakauer (1995) and Tosh et al. (2006).

Furthermore, Krakauer (1995) made the prediction that increasing density (compaction) should also have an effect under specific neural wiring schemes, although Tosh et al. (2006) did not. There was no evidence from the current study that the area occupied by the group or the density of individuals had an effect on reducing attack rate. In agreement, Ruxton et al. (2007) demonstrated with human subjects that prey compaction had no effect on the probability of prey escape, nor on the time taken to make successful attacks. These findings suggest that to maximise the confusion effect, prey should aim to increase group size, and that the compaction demonstrated by many species (e.g. Magurran and Pitcher 1987) may be due to a selfish herd effect (Hamilton 1971). Clearly, however, some compaction is required for all individuals in a group to come within the visual field of the predator.

Milinski (1977b) also examined this aspect of the confusion effect using sticklebacks, and demonstrated increasing density to have an effect on increasing confusion, with no effect of prey number and area when density was held constant. However, the methodology differed in that the stickleback's preference for strays was measured, while the attack rate on a single group was measured in the current study. It is also reasonable to assume that both density and number may have an effect on the same measure of confusion, but at different scales. For example, if the visual field of a stickleback is already filled by a *Daphnia* swarm, additional prey on the periphery, i.e. outside the field, would not be expected to increase the confusion effect. In contrast, additional prey items to a swarm within the visual field (increasing the volume it occupies, but not its density) may increase confusion, as was found in this study. The effects of number, area and density on the degree of predator confusion are almost certainly non-linear (for example, Tosh et al. 2006), thus the scale at which the effect is examined is critical in its demonstration. This suggests further potential work for neural network models in examining the confusion effect, by considering prey groups exceeding the visual field of the predator.

In agreement with previous work, three-spined sticklebacks seem to suffer from a confusion effect when presented with multiple prey, and this is associated with an increase in targeting error as predicted by neural network models. The reduction in risk for *Daphnia* with increased aggregation has been shown repeatedly, from the perspective of predator behaviour (Milinski 1977a; 1977b) and also changes in *Daphnia* behaviour when presented with predator cues (Jensen and Larsson 2002; Young et al. 1994). Thus, *Daphnia* aggregation does appear to have adaptive value in reducing predation risk from fish predators.

Although the density of prey within a group may have little effect on the rate of attacks, it may have an effect on the detection of the group. I explore this in the next chapter, and also ask whether attack accuracy can be affected by the density of prey within a group. I examine whether these density effects are sensitive to spatial scale, which may help explain why there was no effect of density on the confusion effect in this chapter.

Chapter V - The effect of prey density on predators: conspicuousness and attack success are sensitive to spatial scale

Abstract

In contrast to the numerous studies that have examined the response of predators to prey group size, little is known about how prey density affects prey detection and the accuracy of attacks. I demonstrate that increasing the density of *Daphnia magna* swarms increases conspicuousness to a natural predator, the three-spined stickleback. Individuals in denser parts of groups were more conspicuous, as the fish attacked denser prey than would be expected if they attacked the nearest prey upon entering the feeding chamber. The spatial error of attacks also increased with the local density around the target; hence, different stages of predation (searching for, versus successfully attacking, prey) seem to select for opposing responses to prey density. However, whereas the effect of density on target selection only occurred using a global measure of density (average inter-individual distance), the effect on attack error was only significant using a local measure of density (domain of danger). This effect of spatial scale reflects the reduction in the number of prey in the visual field of the predator as an attack progresses. This provides a perceptual basis for the importance of spatial scale in density-dependent processes, via the spatial scale effect on predator-induced mortality.

Introduction

The spatial distribution of individuals relative to one another is a major selective force on both plants and animals, and has led to a wide range of morphological and behavioural strategies to facilitate dispersal from, or aggregation towards, conspecifics (e.g. Strong 1988). Predation risk in particular has been shown to be an important factor in driving spatial relationships between animals, affecting both the formation of groups and the pattern of spacing within groups (Krause and Ruxton 2002). Although group size (the number of individuals in a group) has attracted the vast majority of theoretical and empirical attention when considering the effect of spatial distribution on predator behaviour (Krause and Ruxton 2002), prey density (the spatial proximity of individuals to one another) may also affect prey detection, target selection and attack success. As population density, not group size, has held prominence in our understanding of ecological processes from the classical models of Lotka-Volterra (Lotka 1925) onwards, how predation responds to prey density at a behavioural level is essential to explaining why predator-induced mortality varies with density.

Little is known about how the density of prey affects conspicuousness to predators (Jackson et al. 2005). The greater visual angle produced by larger group sizes has formed the mechanistic basis for theoretical work on group detection rates (Vine 1973; Treisman 1978), with an implicit assumption of these models being that spacing between individuals remains constant, so that the dimensions of the group are directly related to group size. In the majority of animal groups this is not the case. Inter-individual distances vary with spatial position in a group (Bumann et al. 1997), and groups also are highly dynamic; for instance, compaction is often observed as a response to heightened predation risk (Magurran and Pitcher 1987) and individual positional preferences may alter over time (Morrell & Romey 2008). Although spacing in groups is clearly important to prey, how this affects detection of the group remains largely untested. Using humans 'predating' static, computer generated prey, Jackson et al. (2005) demonstrated a positive effect of group size on the detection of cryptic prey, but no density effect. Moreover, to my knowledge, no theoretical model directly considers an effect of density. This is likely to be due to the difficulty of modelling visual processing beyond a simple visual angle approach, and visual angle may be shown to be an inadequate model if group compaction can increase detection rate.

The selfish herd model (Hamilton 1971) is often cited to explain the formation and compaction of animal groups. The key assumption is that the predator can appear anywhere in the environment and targets the nearest prey; thus, the area around an individual nearer to it than to any other individual is proportional to its relative risk of predation (the "domain of danger"). To minimise this area, individuals move toward one another, forming groups (Hamilton 1971). Targeting the nearest prey assumes the predator either always detects the nearest prey first, or already has knowledge of prey locations and attacks the nearest. Even though attacking the nearest prey may be energetically efficient, and may be more likely to result in a successful attack since the prey has less time to react (as demonstrated by Cresswell and Quinn 2004), limitations of knowledge on prey positions may stop this occurring. When predators are constrained in this way, factors other than proximity may affect which prey is targeted. Although it is known that preference for particular phenotypes can violate the assumption of targeting the nearest individual (Stankowich 2003), it has not been considered that if greater densities of prey within groups are more conspicuous, individuals with smaller domains of danger, because they are in a compact part of the group, may actually be at greater risk than spatially isolated individuals. There is some evidence that denser parts of groups are more conspicuous, as three-spined sticklebacks initially attacked dense parts of *Daphnia* swarms more frequently when prey were cryptic (Milinski 1977b), although the density of attacked individuals was not quantified.

Compared to the effect on conspicuousness, more attention has been given to the effect of prey density on the post-detection phase of predation. The confusion effect has been shown to increase with the density of prey, independently of group size, leading to a preference for spatially isolated individuals (Milinski 1977b). However, other experiments have failed to observe an effect, both with fish using attack rate (Ioannou et al. 2008; see chapter IV) and with humans measuring the time taken to make an attack and attack success (Ruxton et al. 2007). This disagreement in the literature may be due to density being measured at different spatial scales, in addition to differences in the response variable used to assess the confusion effect (see chapter IV).

There are a number of theories, therefore, that make predictions about the relationship between the density of prey and predation risk. Whereas denser prey may be more conspicuous to predators, isolated individuals may be encountered first (due to selfish herd effects) and/or be attacked with more success (due to the confusion effect). Hence, the optimal response of a predator to prey density, and the optimal density for prey, is unclear and may vary with the stage of predation. A similar issue occurs with the group size of prey: although larger group sizes may be more conspicuous to a predator (Riipi et al. 2001; chapter III), the success of attacks may decline due to the confusion effect (Cresswell 1994).

I conducted three experiments to examine the effect of prey distribution on multiple stages of the predation cycle (Lima and Dill 1990): detection of prey, targeting of an individual and attack success. I used a three-treatment design to determine whether the number of *Daphnia*, the area occupied by the group, and/or the density of individuals has the effect on detection rate normally associated with increased group size. To control for increased per capita activity in larger groups (Grand and Dill 1999), which can increase conspicuousness (Krause and Godin 1995), I examined whether the distance between isolated, individual prey affected detection rate, where there was no possibility of chemical or tactile interaction between individuals. I then investigated whether any effect of prey density on detection rate also applies to the targeting of individuals from within a group, and whether this is affected by group size. The relative importance of prey distribution on conspicuousness versus a selfish herd effect was assessed, measuring prey density at both local (domain of danger) and global (average inter-individual distance) spatial scales. These measures of density were then used to determine the effect of prey density on the spatial error of attacks, which is negatively related to attack success.

Method

Group size, prey density or area of the group?

Thirty-five three-spined sticklebacks (mean standard length±s.d. = 42±4.9mm) were placed in a tank (44.5 x 24.5cm, water depth 13cm) the evening before testing, fed defrosted bloodworm at 17.00 and then left to habituate overnight. The tank was split into two areas: a living area (30 x 24.5cm) and a feeding area (14.5 x 24.5cm), separated by a white Perspex wall. A 5 x 2cm (width x height) rectangular door was cut into the bottom of the horizontal centre of the wall, with a Perspex gate allowing the door to be closed remotely. The gate was left open to allow exploration of the whole tank. Seven test tubes (length = 7.5cm, internal diameter = 1cm, water depth = 5cm), containing only water and identical to those later used in the trials, were arranged in a horizontal row in the feeding area on the wall opposite the door. The water levels of the test tubes and the tank were identical. Both of the lateral tank walls were made of one-way glass to allow observations with minimum disturbance to the fish. This could have possibly reflected the prey in the lateral walls, but the reflected images would be much further from the predator than the real prey, and the fish never indicated behaviourally that they detected the reflected image. The tank was covered with a white sheet to reduce disturbance and produce a diffuse, even lighting in the tank. Lighting was provided by an overhead fluorescent light.

The following day, any fish in the feeding area were moved into the living area and the gate was lowered. The seven empty test tubes were replaced with one of three *Daphnia* (2.7±0.17mm) treatments visually represented in figure 5.1. Each of the seven tubes contained either zero, one or five *Daphnia*, in the following arrangements:

A: 0,1,1,1,1,1,0 (low number and low density with large area)

B: 0,0,0,5,0,0,0 (low number, high density with small area)

C: 0,5,5,5,5,5,0 (high number and high density with large area)

Thus, each factor (number, density, and area) varied by a factor of five between high and low treatments. As density is derived from number divided by area, two variables must vary between treatments, and only one can be controlled. A minimum of three treatments must be used to distinguish which variables are having an effect on the conspicuousness of the group.

At the start of the trial, the gate was opened. By allowing the fish to swim into the feeding area when ready and without disturbance, in addition to being habituated overnight in the tank, the fish readily attacked the prey once detected. The time taken to approach the *Daphnia* (defined as an orientated approach to prey within one body length) after crossing the threshold of the door was recorded using a stopwatch. Only approaches followed by attacks were analysed: this occurred in the vast majority of cases (>90%). The number of visits by fish to the feeding area before an approach was also recorded. More than one visit was made in 38 out of the 84 trials, although the number of visits was not affected by treatment (see results). Trials were only included in the analysis if the fish that approached and attacked the prey did so without other fish in the feeding area. Once the attack was made, the gate was closed and the fish removed, avoiding problems associated with pseudoreplication. Although this removal may have disturbed the remaining fish in the living area, they quickly resumed normal swimming behaviour, and the disturbance would extend the time taken for the fish in the next trial to enter the feeding area, rather than the time taken to approach the prey. Note that sticklebacks do not release Schreckstoff upon being attacked by a predator (unlike minnows, for example; Krause 1993).

The order in which treatments were presented was randomised using a complete random block, i.e. three consecutive trials constituted a block, with each of the three treatments appearing in each block in a random order. This assisted in spreading equally between treatments any variability due to time of day, changes in boldness, and the effect of reducing the remaining fish population size. 28 trials were carried out for each treatment, using different *Daphnia* individuals in each trial.

To determine whether the results of the above experiment were due directly to the physical properties of the group, rather than indirectly through increased activity per individual when *Daphnia* were placed in the same test tube, I manipulated density without manipulating *Daphnia* numbers per tube. The procedure above was repeated using the same apparatus, although the stimulus consisted of 8 test tubes with *Daphnia* arranged either as 0,1,1,0,0,0,0,0 or 0,1,0,0,0,0,1,0 (figure 5.2). The prey were placed off the centre of the test tube row in the high density treatment to maintain the approximate prey position of the prey (for example, prey positioned centrally may be more conspicuous to the fish). Thus, the two *Daphnia* were either placed clumped (high density) or separated (low density), but there was no opportunity for tactile or chemical interaction between the *Daphnia* in either treatment. In the high density treatment, the side of the group was alternated between trials. As the number of *Daphnia* in the two treatments was constant, a repeated-measures design was employed whereby the same two *Daphnia* were used in both treatments and then replaced for the next pair of treatments. This assisted in controlling for inter-*Daphnia* variability in activity and appearance. Thirteen such pairs of treatments were carried out, and in 7 of these 26 trials fish visited the feeding area before the fish that approached and attacked the prey. The order of treatments was randomised within each replicate.

A Generalized Linear Model (GLM) was used with a negative binomial error distribution to determine significant differences between the three prey arrangements in the time to approach the prey. A negative binomial distribution was suitable as most approaches were very brief, but some lasted much longer. A paired t-test was used in the second experiment to compare the time to approach clumped versus separated pairs, as the same two *Daphnia* were used in pairs of high and low treatments. Residuals were normally distributed in this case.

Distribution and the targeting of prey

In the previous chapter (chapter IV), an effect of prey group size on targeting error (the distance from a strike to the nearest prey) was demonstrated (experiment 2). Here, the video footage and data from this previous study have been reanalysed, to examine whether prey distribution within groups affects selection of a target from the group. Additionally, the effect of prey density on targeting error was examined. Details of the procedure can be found in chapter IV, and I only detail the analysis here.

From data obtained in chapter IV (coordinates of each *Daphnia* in every group at the moment they were first attacked), two measures of density were calculated for every individual in each prey group: the domain of danger (DOD) and the average inter-individual distance (IID). The DOD is defined as the area around an individual that contains all points closer to that individual than to any other (Hamilton 1971); it thus considers only nearest neighbours and is hence a 'local' measurement of density. It was approximated by generating 980 uniform random coordinates within the limits of the glass chamber for each group, then counting the number of these points closer to an individual than to any other. The domain of danger was quantified for each individual in each group. In contrast, the average inter-individual distance is global measure of density, as it considers all group members. For each prey individual in each group, this was calculated as the average distance from an individual to all others in the group.

The nearest individual to the strike was assumed to be the target prey (chapter IV). To control for differences in prey distributions between trials, the density of the target prey was compared to the median density of all individuals in that group (i.e. relative density of target = density of target / median density of group). Values of 1 indicate the predators targeted individuals of median density, values <1 they target individuals close to others, and >1 they target isolated individuals (these values correspond to 0, <0 and >0, respectively, when the data is log10 transformed). The method was repeated using the still image when the fish first entered the feeding area (when it was naïve to the presence of prey), again measuring the position of the snout and all *Daphnia* prey. Relative density measures for the nearest individual to the fish's snout were calculated as above, for both the DOD and IID.

Quantifying the relative density of the prey nearest upon entry allowed a comparison with the density of the prey targeted, and hence reveal any tendency by the predator to attack denser, or less dense, prey. Hence, mixed models were used with trial identity as a random factor, and prey group size and phase of predation (nearest prey upon entry or targeted prey) as fixed factors. Both the relative DOD and IID of the prey were analysed using this method, and log10 transformed before analysis. Two of the videos were damaged at the point the fish entered the feeding area, i.e. before they were analysed, so the sample size for the group size of 20 was reduced to 18 trials in this analysis.

To determine the effect of prey density on the subsequent success of an attack following prey detection, the distance from the strike to the nearest prey was calculated. Log10 targeting error was used as a response variable in a linear model with prey group size and density (DOD) as explanatory variables, then repeated using IID as the measure of density. All statistical analyses were carried out in SPSS version 13 or R version 2.4.1.

Results

Group size, prey density or area of the group?

Overall, there was a significant difference between treatments in the time taken for the fish to approach the prey groups (figure 5.1; GLM, $LRT_{2,81}=21.86$, $P<0.0001$). There was no significant difference in approach time between the two treatments where density was held constant but number and area varied (C versus B; $z=0.64$, $P=0.52$). However, there was a significant increase in the time taken to approach when the group was less dense, both relative to a dense group occupying a smaller area (A versus B; $z=-3.56$., $P<0.0005$), and to a more numerous group occupying the same area (A versus C; $z=-4.18$, $P<0.0001$). Thus, density had the greatest effect on approach time, with approach time declining with increasing density of the group rather the total number of individuals in the group or the spatial extent of the group. Although increased per individual activity in larger groups (Grand and Dill 1999) could explain this density effect, clumped isolated individual prey (i.e. prey at high density but unable to interact) were approached significantly sooner than separated, isolated individual prey (figure 5.2; paired T-test, $t_{12}=3.2$, $P<0.01$).

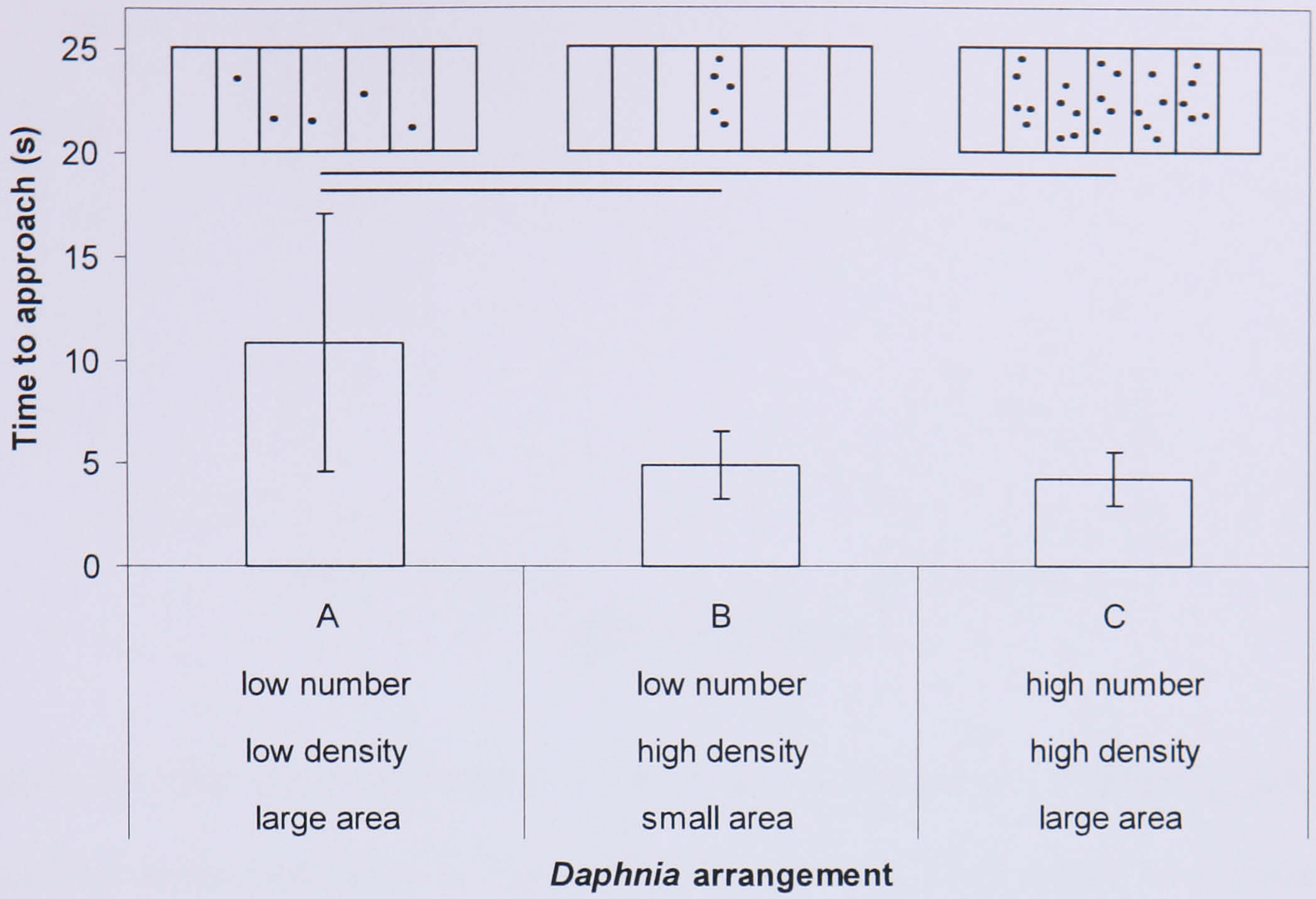


Figure 5.1. The effect of prey distribution on detection rate, measured as the time taken to approach the prey. As increases in prey group size are associated with an increase in the area occupied by the group and/or an increase in the density of individuals, it is problematic to isolate which is having an effect on increasing the conspicuousness of the group. When density increased, by either compacting the group (A to B) or adding more individuals (A to C), there was a significant increase in the detection rate of the group (indicated by horizontal lines above the bars: $P<0.05$). Means are presented with two standard errors.

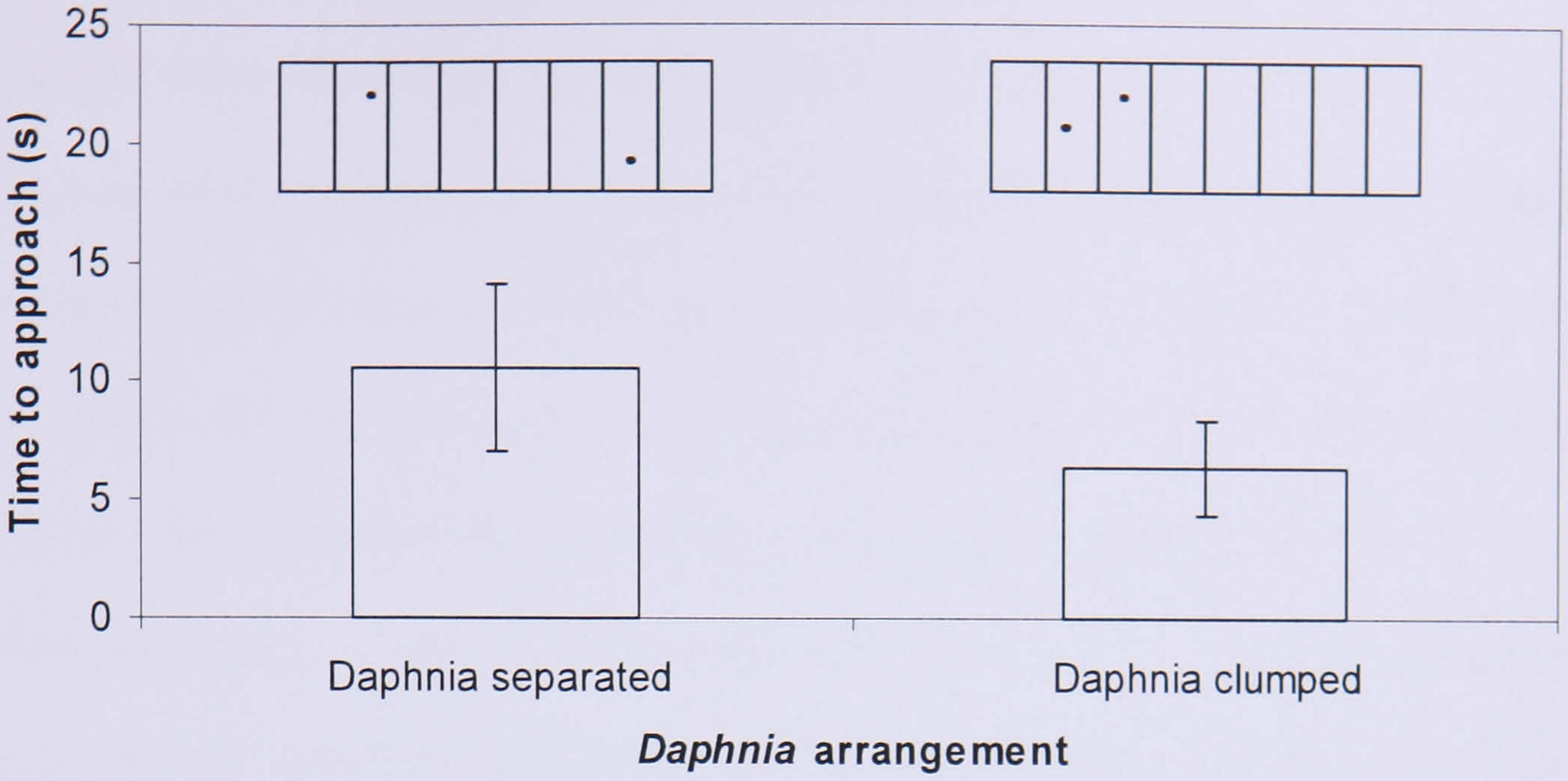


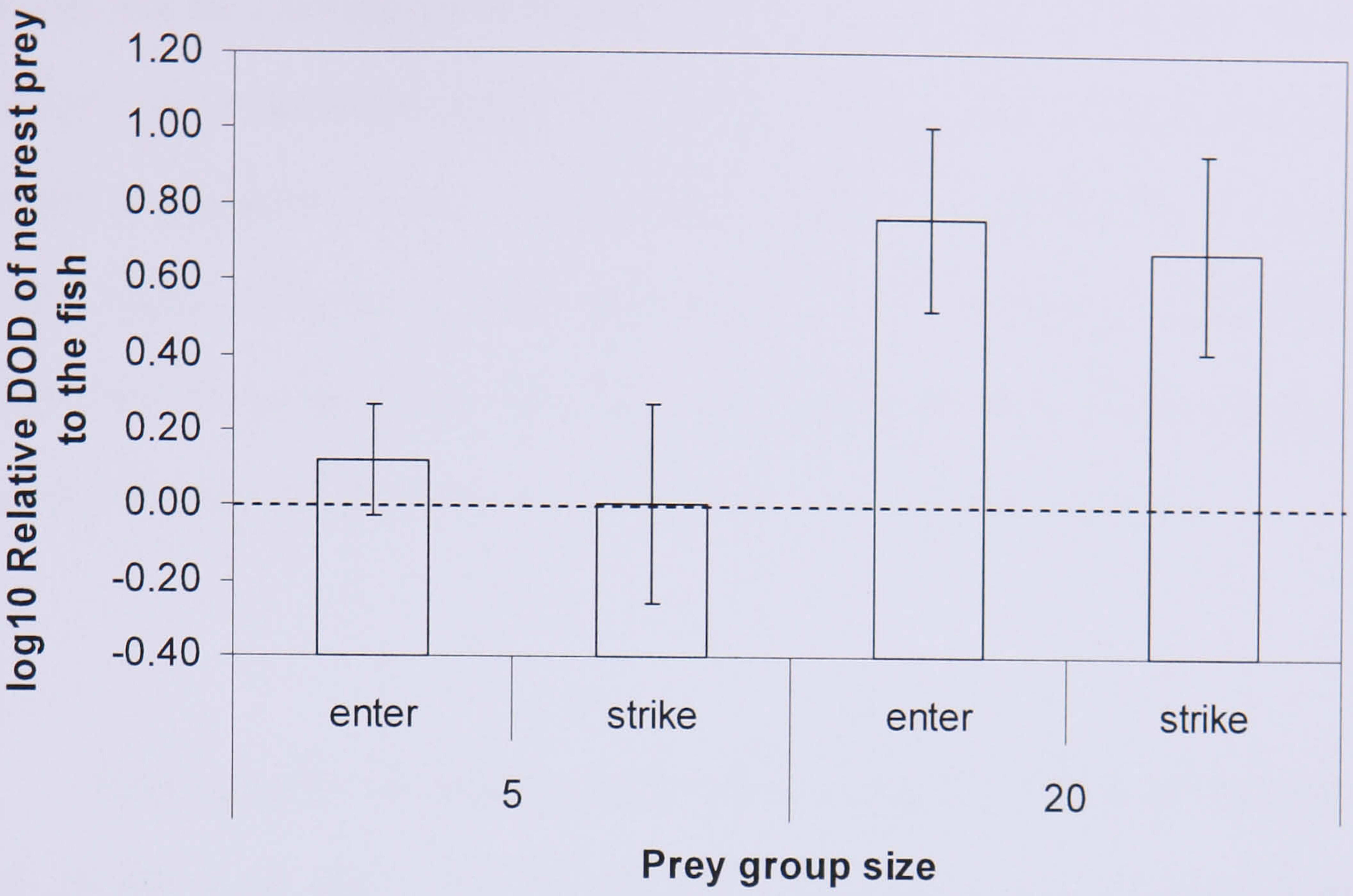
Figure 5.2. The effect of distance between two isolated prey on detection rate, measured as the time taken to approach the prey. When the two prey were closer together, they became significantly more conspicuous to the predator. Means are shown with two standard errors.

The number of visits to the feeding area before the prey were approached was not significantly affected by treatment in either the experiment comparing group size, density and area (GLM with poisson distributed errors, $LRT_{2,81}$=2.52, P=0.28) or the experiment varying the proximity between isolated prey (Wilcoxon Signed Ranks test, Z=-0.43, n=13, P=0.78).

Distribution and the targeting of prey

I then examined whether individuals in more or less compact regions in a group are more likely to be attacked. Two measures of density were used, one at a local spatial scale (the domain of danger, DOD) and one at a global scale (the average inter-individual distance, IID). Although the two relative measures (relative density of target = density of target prey / median density of group) were positively correlated when all data were pooled (Spearman's rank, $r_s=0.50$, n=78, $P<0.001$), there was enough variation to give quantitatively different results when examining the effect of relative density. When using a local measure of density (the DOD), the prey nearest the fish in a group of 20 was in a significantly less dense position relative to the group than the nearest prey to the fish in a group of 5 (figure 5.3A; Mixed model, $F_{1,37}=27.04$, $P<0.0001$). However, there was no significant change in relative density from the nearest prey upon entering the feeding area to the prey that was targeted, i.e. local density had no effect on targeting prey ($F_{1,38}=0.96$, $P=0.33$). In contrast, the fish targeted prey in significantly denser parts of the group than the individual they were nearest to at the moment of entering the feeding area (figure 5.3B; Mixed model, $F_{1,38}=11.69$, $P<0.005$) when using a global measure of density (the average IID). There was no significant effect of group size using this measure of density ($F_{1,37}=3.33$, $P=0.08$). No interactions were significant at either spatial scale ($P>0.05$ in both cases), and the random factor, trial identity, had no effect either at a local scale (Likelihood ratio=1.23, $P=0.26$) nor at a global scale (Likelihood ratio=1.63, $P=0.20$).

(a).



(b).

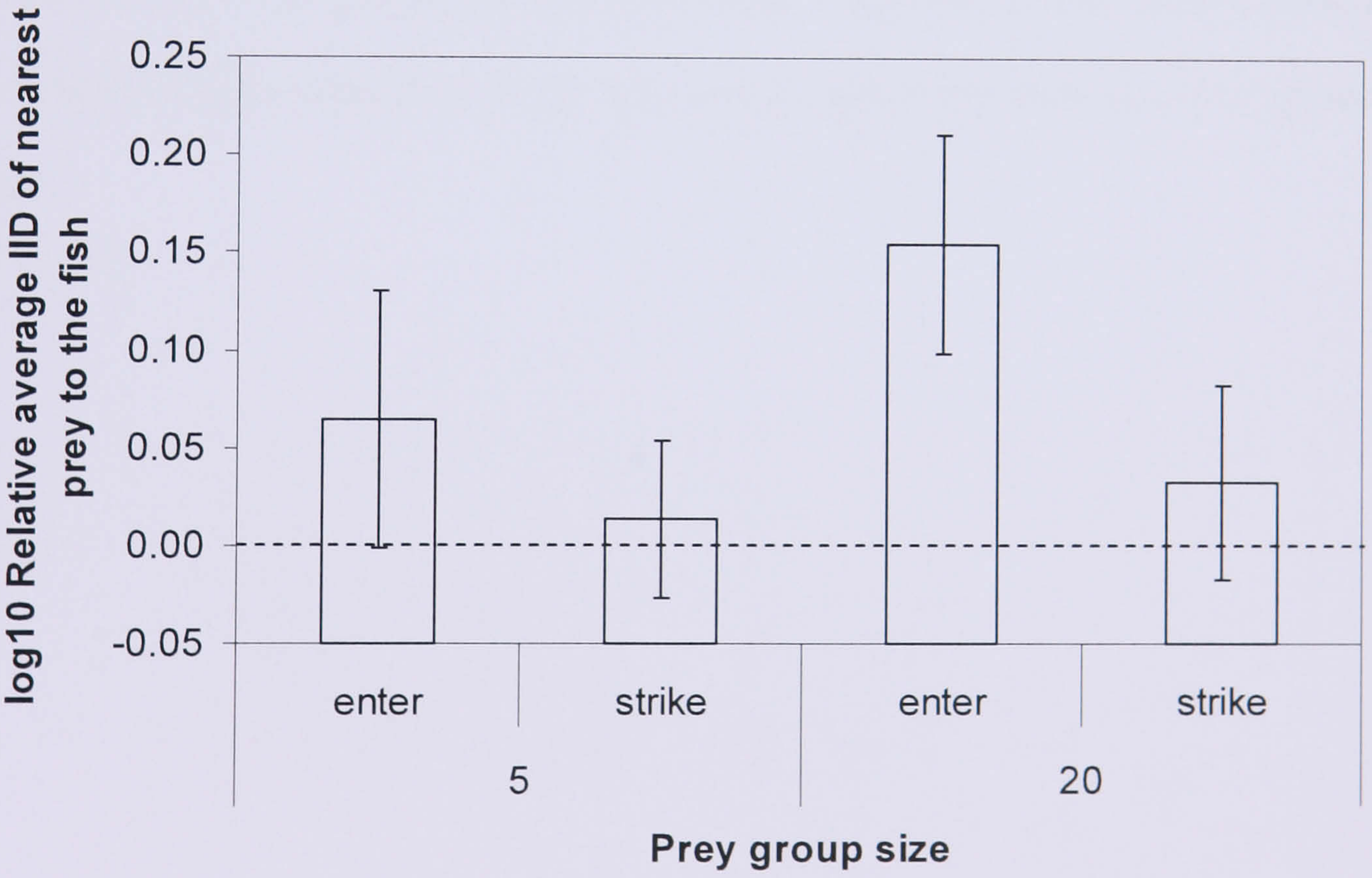


Figure 5.3. The density (mean +/- 2SE) in the vicinity of the prey individual nearest to the fish, relative to the median density of the group. 'Enter' refers to the density of the prey nearest to the fish upon entering the feeding area, and 'strike' the relative

density of the individual actually targeted. After log10 transformation, values <0 indicate that the individual is more dense than the group median, 0 indicates equal density to the group median (dotted line), and >0 to prey less dense than the median. Density was quantified at two spatial scales: a local scale (the domain of danger (DOD); figure 5.3A) and a global scale (average inter-individual distance (IID); figure 5.3B). When the IID was used there was a significant shift from the density of the nearest individual upon entry to the targeting of a more dense individual.

Targeting error significantly reduced as the domain of danger of the target prey became larger (figure 5.4; $F_{1,39}=5.49$, $P<0.05$). The average inter-individual distance of the target, however, had no effect on the spatial error of attacks ($F_{1,39}=0.61$, $P=0.44$). Hence, prey at low densities were targeted with more accuracy compared to higher density prey, but only when measuring density at a local spatial scale.

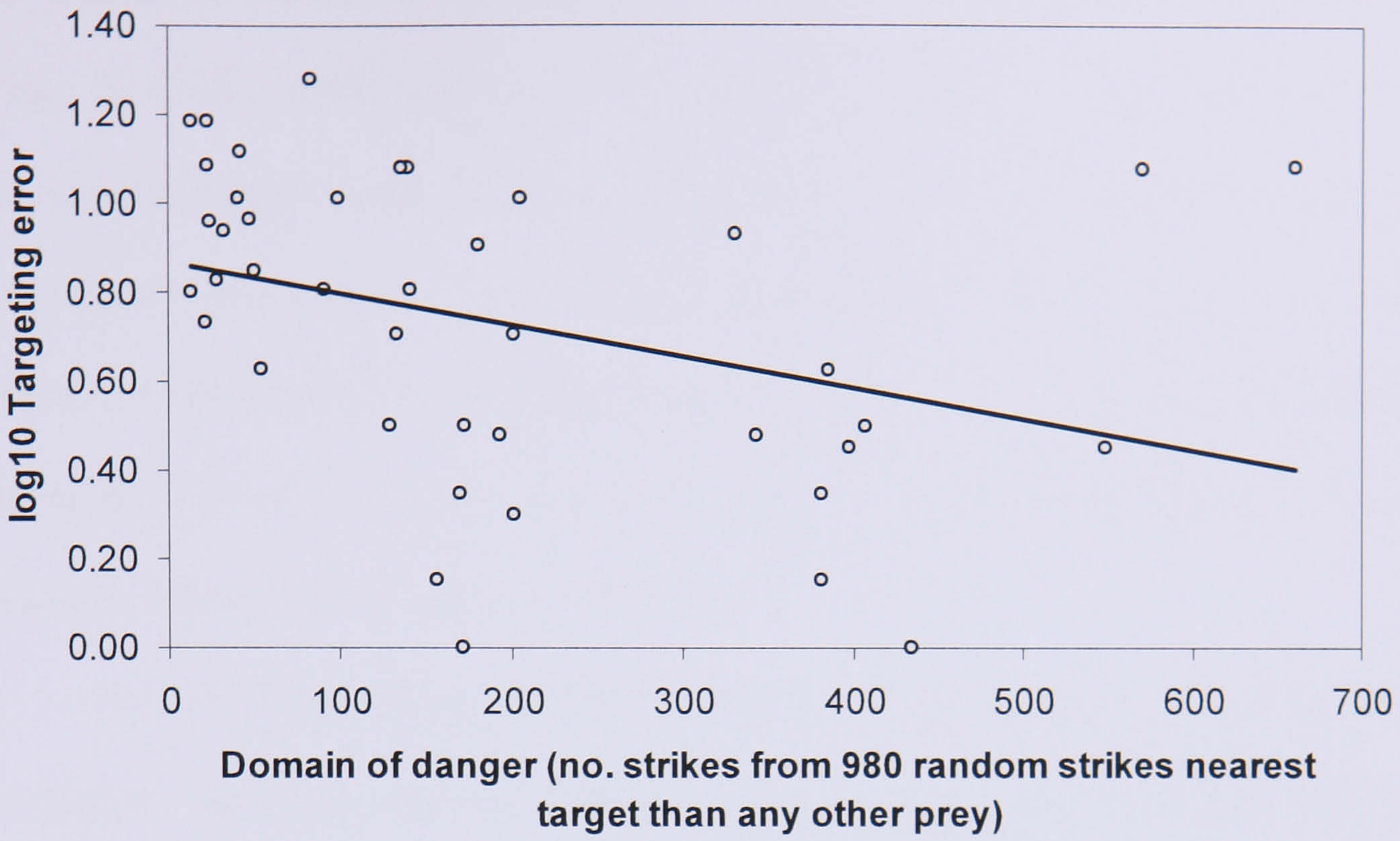


Figure 5.4. The effect of prey density on the targeting error of attacks, measured as the distance from the strike to the nearest prey. As DOD (a local scale of density) became larger, i.e. the target was in a less dense part of the group, there was a significant reduction in targeting error (solid line is from a linear regression).

Discussion

The density of *Daphnia* had a significant effect on multiple stages of predation by sticklebacks. Denser groups appeared to be more conspicuous, as were denser areas within groups, leading to targeting individuals in denser parts of the group than would be expected if the predator targeted the nearest prey. However, targeting error increased as the targeted prey distribution became denser. It appears, therefore, that prey density selects for different responses from the predator at different stages of an attack, as denser prey are more easily detected but are attacked with less accuracy. This resonates with the effect of redshank flock size on sparrowhawk and peregrine predation, where larger groups were preferentially attacked but the success of attacks was greater on smaller flocks (Cresswell 1994). The same trend was found by Krause & Godin (1995) with acara cichlids predating guppy shoals. Although this may seem maladaptive, the net intake of a predator may be maximised by frequent, but relatively unsuccessful, attacks on easily found prey. Optimal responses to prey distribution will depend on the difficulty in finding prey versus the cost of launching unsuccessful attacks, and may explain the large variation between predators in the frequency and success of attacks (Curio 1976).

In this study, however, the apparent conflict between detecting and successfully attacking prey was further complicated by spatial scale: the effect of density on conspicuousness occurred only at a global measure of density (average inter-individual distance), while the effect on targeting error was only evident at a local measure of density (domain of danger). Prey density, therefore, has effects on at least two stages of a predation event, but these effects operate at different spatial scales. A possible explanation lies in the number of prey present in the visual field of the predator at different stages of predating a group. Generally a predator will detect a group at a distance, where a relatively large number of prey individuals will be in the visual field. At this stage, the detection of a particular prey individual will be influenced by more than just its nearest neighbours, and hence a large spatial scale of density will be appropriate. However, after approaching the group to launch an attack, the number of prey in the visual field will be greatly reduced, and only the local neighbours of the target prey will be visible to influence attack success via the confusion effect. The importance of spatial scale has been ignored by models of detection rate, target selection and attack success although it makes intuitive sense that the view a predator perceives changes as an attack unfolds.

There are few studies on predator-prey behaviour which have examined the effect of spatial scale, even though, for example, it may explain why some studies have found an effect of prey density on the confusion effect (Milinski 1977b) whereas others have not (Ruxton et al. 2007; chaper IV). A reason for this may be that the importance of spatial distribution in behavioural predator-prey studies has focused on prey group size (Krause and Ruxton 2002), where the group is easily defined by sharp boundaries and does not vary with spatial scale. In contrast, ecologists dealing with population density do not often have this luxury, and have recognised the importance of spatial scale in plant and animal ecology for some time (Heads and Lawton 1983; Ives et al. 1993), including its ability to explain disagreement in the literature on subjects as important as density dependence (Ray and Hastings 1996). For example, spatial scale has effects on the relationship between host density and parasitoid aggregation (Heads and Lawton 1983) and also plant density and slug herbivory (Gunton and Kunin 2007). My results provide a perceptual mechanism for a spatial scale effect on density-dependent predator-induced mortality, and hence a behavioural basis for the importance of spatial scale in population and community dynamics.

Existing theory on the effect of spatial distribution on detection rate, i.e. the greater visual angle produced by larger groups (Vine 1973; Treisman 1978), cannot explain why denser prey were more conspicuous. As the density within a group increases, either the visual angle declines (if the visual angle is produced by the boundaries of the group) or remains constant (if it is the sum of the angles produced by all group members). This assumes that the whole prey group is within the visual field of the predator, and my experiments were designed to present all the prey to the predator simultaneously. However, it has been shown that the focus of attention can be a small subset of the total visual field when searching for cryptic prey (Dukas 2002 and references therein), which has been used to explain why the effect of group number on detection rate is greater than the effect of group size (Ioannou and Krause 2008; chapter III). A small focus of attention could explain the observed effect of prey density on conspicuousness within a visual angle framework. When prey are at a low density, few will be within the limited focus of attention at any one time, producing a small visual angle, and hence low detection rates. In contrast, a large visual angle will be produced when prey are dense, as numerous prey will fall within the focus of attention. Alternatively, the neural network approach could be applied to the detection of groups (as well as the selection of prey; Tosh et al. 2006) to give a neurobiologically more realistic model of prey detection.

The effect of density on conspicuousness remained even when prey individuals were isolated from one another; thus, increased per individual activity (Grand and Dill 1999) could not explain this density effect alone. An alternative explanation to conspicuousness, that predators prefer to attack dense prey to maximise feeding rates (Holling 1959), can be ruled out by the results of Milinski (1977b). Although primarily concerned with the confusion effect, Milinski found sticklebacks initially attacked the dense part of a *Daphnia* group when the prey were cryptic, but initially attacked strays when the prey were conspicuous. This demonstrates density has a positive effect on attracting initial attacks but only when prey were harder to detect, which would not be expected from an optimal foraging mechanism.

How does the influence of prey density on predators affect the optimal spacing strategy for prey? To minimise the chance of being the victim of a successful attack, prey should avoid globally dense areas (i.e. maximise their inter-individual distances), but at the same time minimise their domain of danger. Prey may face a trade-off in the optimal spatial position as domain of danger and inter-interindividual distances will often be positively correlated. However, despite denser parts of the group being attacked more than would be expected if predators targeted the nearest individual, the net effect was that prey at median densities were actually targeted. Thus, although denser parts of groups are more conspicuous, violating Hamilton's (1971) assumption of always attacking the nearest prey, the selfish herd effect was not completely negated: prey will still benefit from being in the densest parts of groups. In addition, knowledge of the spatial positions of other prey within groups will often be limited to nearest neighbours (Ballerini et al. 2008), and furthermore, the encounter-dilution effect (Turner and Pitcher 1986) should still presumably apply to prey in dense patches as well as large groups. Minimising the domain of danger may still be the most effective strategy to reduce overall risk, and these factors can explain the ubiquity of group compaction as a response to predation (*Daphnia*: Pijanowska 1994; minnows: Magurran and Pitcher 1987; tadpoles: Spieler and Linsenmair 1999; fiddler crabs: Viscido and Wethey 2002) as individuals attempt to reduce their domains of danger by moving closer to other individuals (Hamilton 1971; Krause 1993; Krause and Tegeder 1994). The movement rules prey use to achieve this reduction in domain of danger are still debated, and have recently been shown to be sensitive to population size, density, the attack distance of the predator and the time taken to make the attack (Morrell and James 2008).

The influence of prey density on predation is sensitive to the spatial scale density is measured at, and even methods for determining density based on detailed spatial data may not be suitable to reveal effects on both prey detection and the success of attacks simultaneously. Examining effects at multiple spatial scales is clearly required, and where this is not possible, the scale most appropriate to the hypothesis being tested should be used. This study demonstrates that an important behavioural and ecological effect, the influence of prey density on predation, can be overlooked by using a single, inappropriate spatial scale.

Chapter VI - Ecological consequences of the bold-shy continuum: the effect of predator boldness on prey risk

Abstract

Although the existence of different personality traits within and between animal populations has been relatively well established, the ecological implications of this variation remain neglected. In this study I tested whether differences in the boldness of pairs of three-spined sticklebacks led to differential predation risk in their prey, Chironomidae larvae. Bolder pairs, those that left a refuge and crossed the tank mid-line sooner, ate a greater proportion of prey in ten minutes than less bold fish (therefore prey were at a greater *per capita* risk). Fish crossed the mid-line more rapidly when a larger number of prey were presented, suggesting they accepted greater risk in return for a larger foraging reward. Perception of predation risk also affected the differences between fish in boldness, as larger fish crossed the mid-line sooner after leaving the refuge (larger fish are less at risk from predation). Hence, an interesting trophic interaction occurs, where the risk experienced by the chironomid larvae is determined by the risk perceived by their predators. Through the variation generated by boldness, a form of behaviourally mediated trophic cascade can occur within (as well as between) communities.

Introduction

Variation between individuals is a key condition for evolution through natural selection and has recently been shown to include inter-individual differences in temperament. A major axis in the study of temperament is the bold-shy continuum (Réale et al. 2007), where individuals within and between populations show consistent differences in their degree of risk prone behaviour. The degree of boldness is determined by a trade-off between foraging gains (and/or mating opportunities) and the associated risk (Wilson et al. 1994). Hence, variation in boldness is driven by the balance of costs and benefits, and is affected as such by metabolic rate (Krause et al. 1998), food deprivation (Godin and Smith 1988) and the perception of predation risk (Coleman and Wilson 1998). The importance of boldness as a behavioural strategy is also highlighted by boldness having a heritable component (Wilson et al. 1994).

Although numerous studies have demonstrated bold-shy continua across a range of taxa (Wilson et al. 1994), few studies have examined the ecological consequences of the effect (Réale et al. 2007; see Sih et al. 2004 for the ecological consequences of behavioural syndromes, a closely related subject). Generally, boldness and how it responds to ecological factors has been explored on a species level (for example, Dingemanse et al. 2004). A growing number of studies have identified patterns with likely ecological consequences, such as inter-individual differences in boldness being linked to dispersal (killifish: Fraser et al. 2001; great tits: Dingemanse et al. 2003), growth rate (Ward et al. 2004), survival (Réale and Festa-Bianchet 2003) and reproductive success (Réale et al. 2000; Both et al. 2005). However, none have yet shown direct effects on competing species or species at other trophic levels. If predators show variation in boldness, the risk experienced by prey may be determined by the boldness of sympatric predators (and thus indirectly by the factors determining this boldness). Bolder predators are likely to spend more time searching for prey (increasing encounter rate), approach prey sooner after detection (giving them less time to escape), and eat a greater proportion of encountered prey groups.

As individuals balance the degree of perceived risk with the perceived reward (reviewed by Lima 1998), predators may demonstrate a greater degree of boldness when they detect a larger number of prey. For example, Godin and Smith (1988) demonstrated food deprivation and higher food concentrations increased feeding rate by guppies, although feeding at a higher rate increased the chance of being captured by their predator, the jewel cichlid. In contrast, the value of large prey numbers may be significantly reduced by a reduction in attack success through the vigilance, group defence and confusion effects associated with large groups of prey (reviewed in Krause and Ruxton 2002). These effects can depend on coordination between prey individuals, with more social, highly coordinated groups generally enjoying a greater protection from predation. Anti-predatory grouping mechanisms make predicting predator responses to prey group size difficult, and may explain the variation observed in the relationship between group size and attack rate. Whereas larger bird flocks tend to be attacked more often by raptors (e.g. Cresswell 1994), FitzGibbon (1990) observed cheetahs preferentially attacking smaller groups, probably reflecting their greater attack success with smaller group sizes.

In this study, I examined whether the risk experienced by prey (Chironomidae larvae) is influenced by the boldness of their predators, the three-spined stickleback. Boldness was quantified as the time taken to first leave a refuge (Krause et al. 1998) and the time taken between leaving the refuge and crossing the mid-line of the tank. The number of prey presented was varied to examine its direct effect on prey risk, and whether it affected the level of boldness demonstrated by the predators. By using a prey species that does not demonstrate coordinated behaviour between individuals to avoid predation, the effect of prey number on group vigilance, defence and predatory confusion was minimised.

Method

Although assessing boldness in individuals is necessary for determining its social importance (i.e. interactions between conspecifics, e.g. Ward et al. 2004), ecological context was of primary concern in this study. Thus, as this species is naturally gregarious, pairs were used during the experiment to improve ecological realism. Forty sticklebacks (standard body length mean±s.d. = 48±3.5mm) were paired approximately according to standard body length. As in the previous chapters, sticklebacks were not sexed as they do not show sexual differences in behaviour until the breeding season. The pairs were habituated in tanks (39 x 24 cm, water depth 16 cm) for three days before trials began. White paper around and below the tanks concealed the pairs from disturbance, and simulated conditions experienced during the experiment. The pairs were fed 4-5 defrosted bloodworm per fish at 16:00 daily.

The experimental tank (91 x 64 cm, depth 61cm) was white in order to facilitate recording of fish foraging activity. A refuge (green rubber mesh attached to a semicircular piece of clear Perspex 9.5cm in radius) was mounted at one end of the tank against the wall, 12cm above the bottom of the tank. There was an unobstructed line of sight from the fish under the refuge to the prey. Two cameras mounted 170cm above the tank with overlapping fields of view allowed monitoring of the whole tank. A white sheet was draped over the top of the tank to minimise disturbance and reduce light reflections at the water surface.

The tank was filled to a depth of 12cm with fresh, aged tap water. Live bloodworms (Chironomidae larvae, 13±0.7mm) were used as prey. Prey were placed at the opposite end of the tank from the refuge, and allowed to habituate for 2 minutes before exposure to the fish. The larvae were free to move, although they mostly remained near the back wall of the tank, and never crossed the mid-line of the tank. A pair of fish was transferred to the tank, and invariably swam under the refuge whereupon timing began. The activity of the pair was monitored remotely, recording the time taken for a fish to leave the refuge for the first time (time to leave refuge) and the time taken from this point to crossing the mid-line of the tank for the first time (hesitation time). Only when the whole body of the fish emerged from the refuge or crossed the mid-line was the time recorded. The mid-line was 36cm from the refuge and 45.5cm from the back wall of the tank. As the fish often returned to the refuge after first leaving it, the fish to first cross the mid-line was not necessarily the same fish to first leave the refuge. Both of the response variables can be considered negatively correlated to boldness, i.e. it is expected bolder fish explore the whole tank sooner than shyer individuals (similar variables were used by Brown et al. 2005). Crossing of the mid-line for the first time was followed immediately by consumption of prey in 78% of trials with prey present.

Typically, the fish made multiple visits from the refuge to the other end of the tank to consume prey. The number of prey consumed within ten minutes from first crossing the mid-line was recorded. Trials were ended at this point or if neither fish crossed the mid-line in 20 minutes. For a control treatment of 0 prey, trials were ended when a fish crossed the mid-line. At the end of a trial, the fish were returned to their habituation tank. A complete water change was carried out between trials to remove fish and prey odours from the previous trial.

Each pair was tested at four numbers of prey, 0, 2, 10 and 50 chironomids, in a randomly assigned order. The trials were conducted one trial per pair each day, over four consecutive days. After completing all trials, pairs were measured and returned to the source site. 20 pairs of fish were tested at all four treatments, a total of 80 trials.

Data analysis

Initially I examined whether the two measures of boldness were correlated. To do this, the time taken to leave the refuge was averaged for the four trials of each pair, as was the hesitation time, then correlated using Pearson's correlation coefficient (both averaged variables were normally distributed after log10 transformation). I then determined whether the time to leave the refuge and the hesitation time were affected by average pair body length and the number of prey. Mixed models were used with prey number as a fixed factor, average body length as a covariate, and pair identity as a random variable.

To determine consistent differences in boldness between pairs across trials, mixed models with pair identity as a random factor were compared to the equivalent linear models without this random factor (Réale et al. 2007). This avoided the statistical problems generated by carrying out multiple correlations between trials for both measures of boldness (a total of 12 tests). When there was no effect of removing the random factor, a null mixed model (i.e. without body length and prey number, but with the random factor of pair identity) was compared to a null linear model (without factors or the random factor). Any change in the significance of removing the random factor could then be attributed to the exclusion of body length and the number of prey.

The proportion of the prey eaten within 10 minutes (i.e. per capita risk) was arcsine square-root transformed. A main-effects mixed model was used with prey number as a fixed factor and average pair body length, the time to leave the refuge and hesitation time as covariates. Pair identity was used as a random variable. Control trials with no prey were excluded from this analysis. Statistical analyses were run in R, version 2.4.1, or SPSS, version 13.

Results

The fish demonstrated a large amount of variation between pairs in the time taken to leave the refuge for the first time (figure 6.1a) and also in the time taken to first cross the mid-line of the tank after leaving the refuge, i.e. hesitation time (figure 6.1b). There was a significant positive correlation between the (log10) mean time taken to leave the refuge and the (log10) mean hesitation time (mean of the four trials of each pair; Pearson's correlation coefficient: $r=0.50$, $n=20$, $P<0.05$). The time to leave the refuge was not significantly affected by the number of prey (Mixed model, $F_{3,57}=0.86$, $P=0.47$) nor by average fish body length ($F_{1,18}=1.40$, $P=0.25$). When pair identity was removed from the model, however, there was a significant decrease in the goodness-of-fit of the model, suggesting there were consistent differences between pairs in the time taken to leave the refuge (figure 6.1a: Likelihood ratio=11.65, $P<0.001$). In contrast, hesitation time significantly decreased with increasing prey number (figure 6.2a; $F_{3,57}=3.33$, $P<0.05$) and average fish body length (figure 6.2b; $F_{1,18}=6.79$, $P<0.05$). There was no effect, however, of removing pair identity as a random variable from the model (Likelihood ratio=2.08, $P=0.15$). Pair identity did have an effect, however, when removed from a null mixed model compared to a null linear model (Likelihood ratio=3.90, $P<0.05$), suggesting the variation caused by pair identity was included with the average body length in the full model. No interactions were significant ($P>0.05$ in both cases).

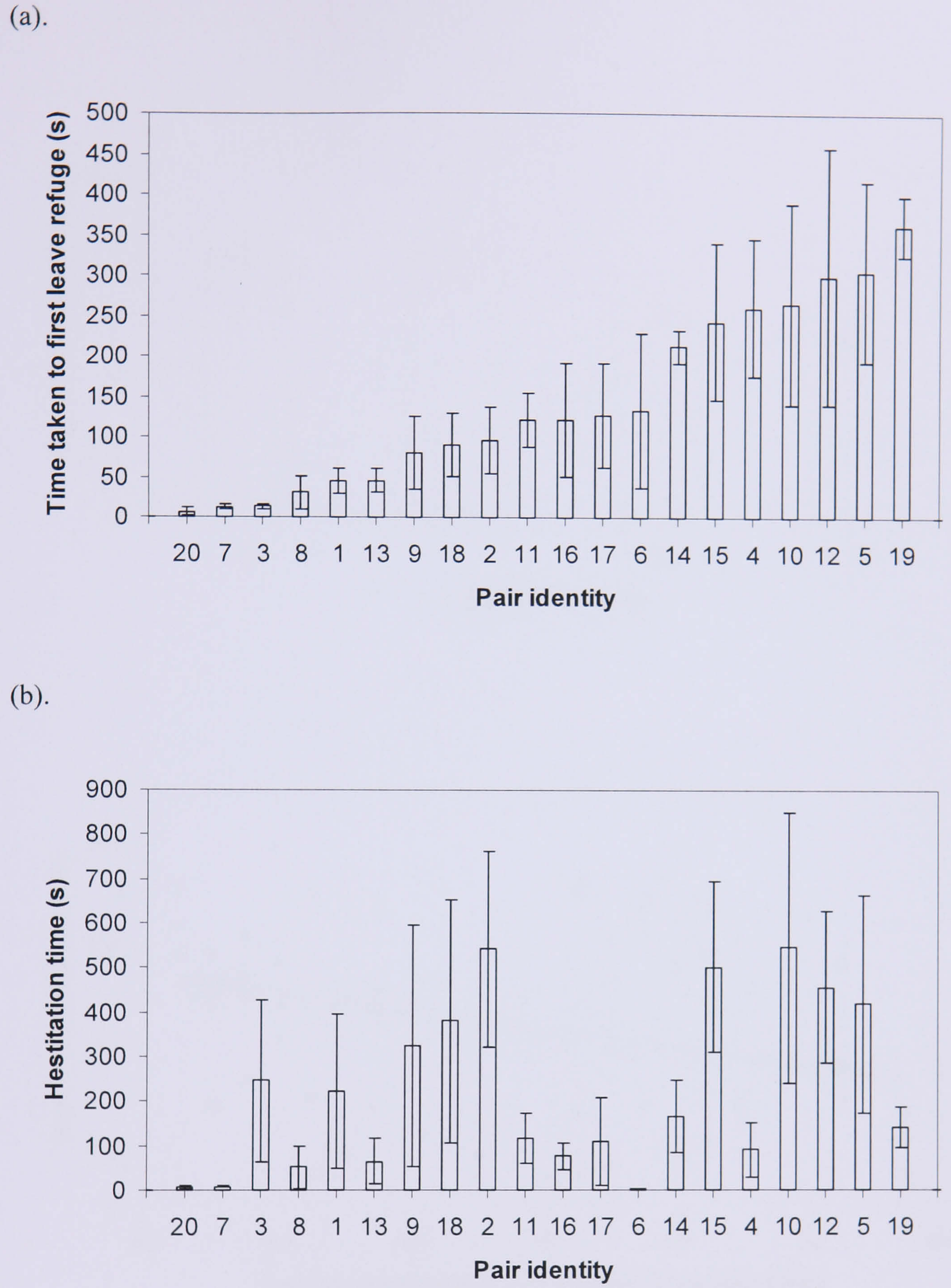
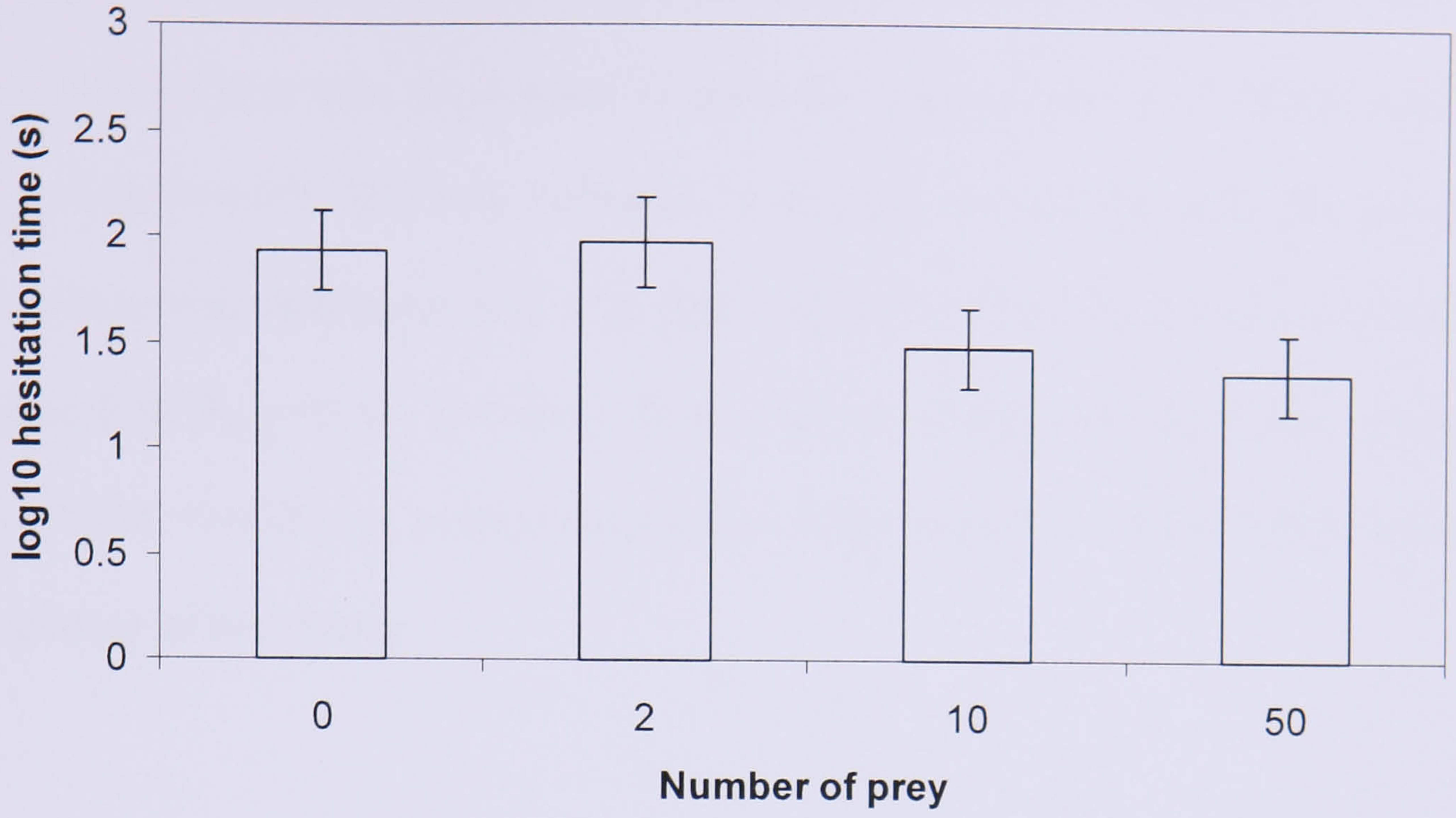


Figure 6.1. (a) The mean time taken to first leave the refuge and (b) the mean time taken to first cross the mid-line of the tank (hesitation time) for twenty pairs of sticklebacks (± SE, n=4), for each pair. For clarity, the pairs are arranged into increasing time taken to leave the refuge.

(a).



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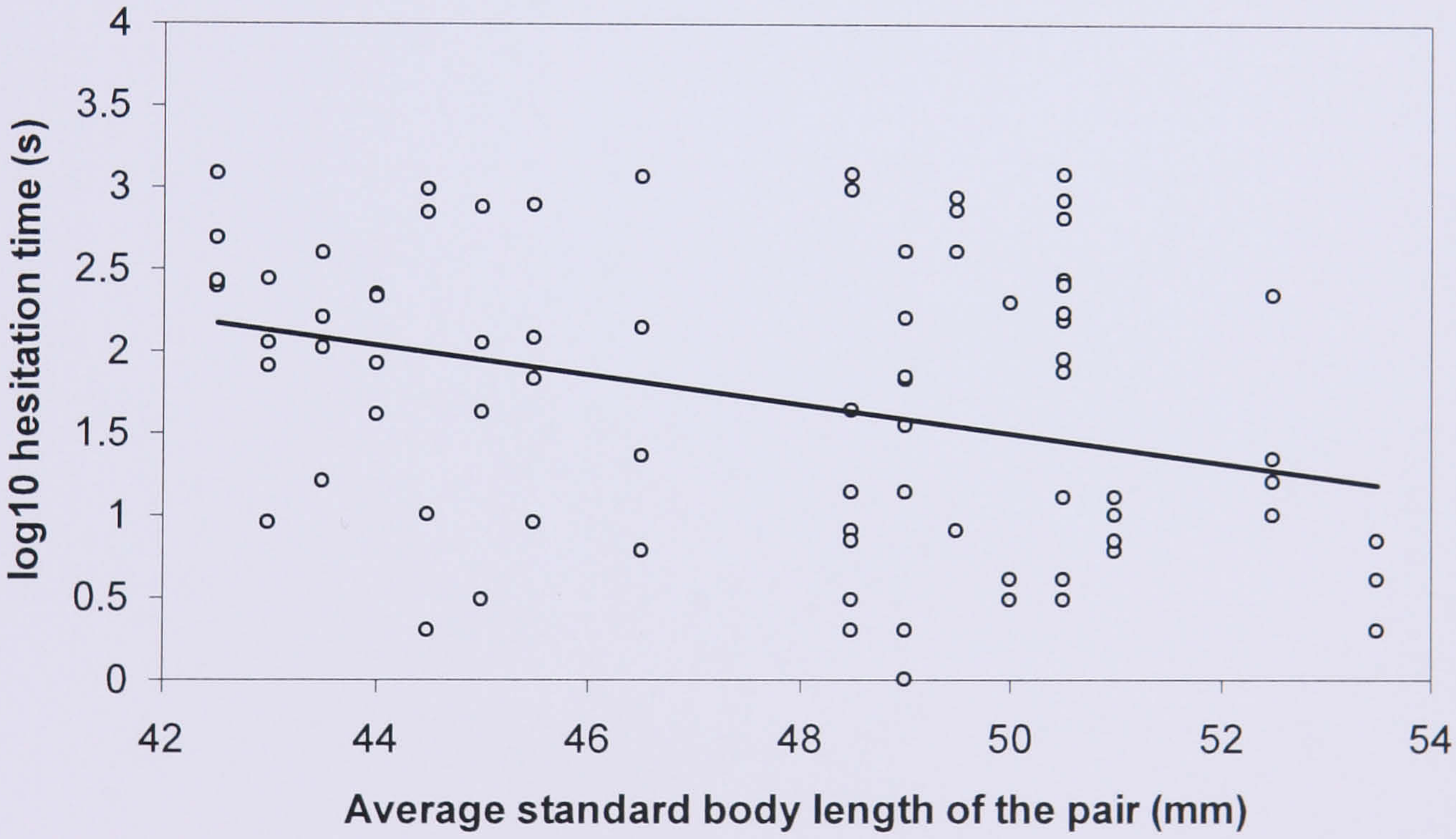
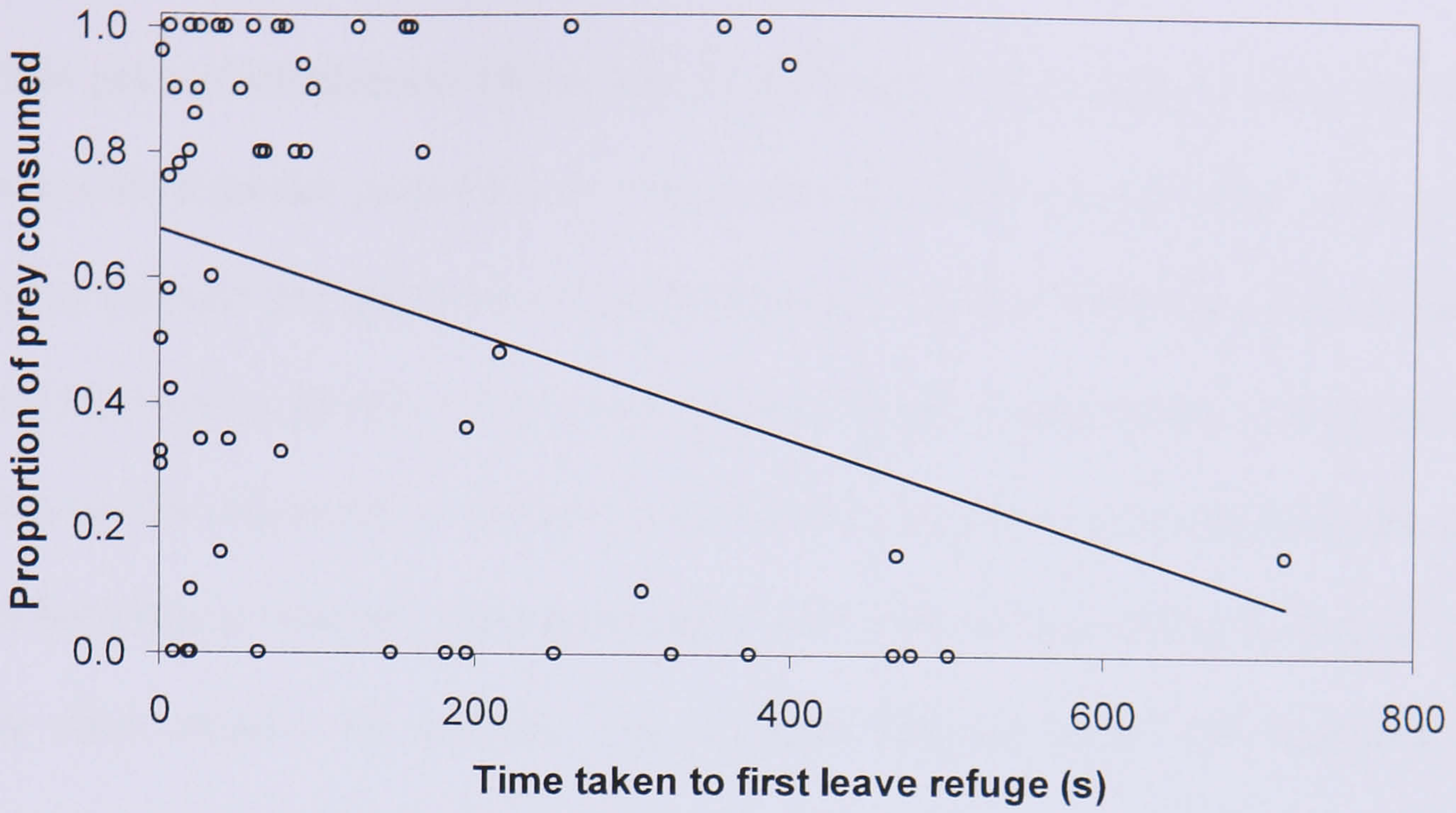


Figure 6.2. The time taken from leaving the refuge to crossing the mid-line of the tank (hesitation time) is significantly affected by (a) number of prey (mean ± SE) and (b) average body length of the pairs (with best-fit line from linear regression, r^2=0.11).

In the second half of the analysis, I examined which factors affected the risk to prey. Pairs taking more time to leave the refuge consumed a significantly smaller proportion of prey than those quick to leave the refuge (figure 6.3a; Mixed model: $F_{1,38}$=4.48, $P<0.05$). Similarly, hesitation in crossing the mid-line after first leaving the refuge was associated with a smaller proportion of the prey being consumed (figure 6.3b; $F_{1,38}$=10.57, $P<0.005$). There was no significant effect of prey number ($F_{2,36}$=2.56, P=0.09) nor average body length of the pairs ($F_{1,18}$=0.69, P=0.42) on the proportion of prey eaten.

(a).



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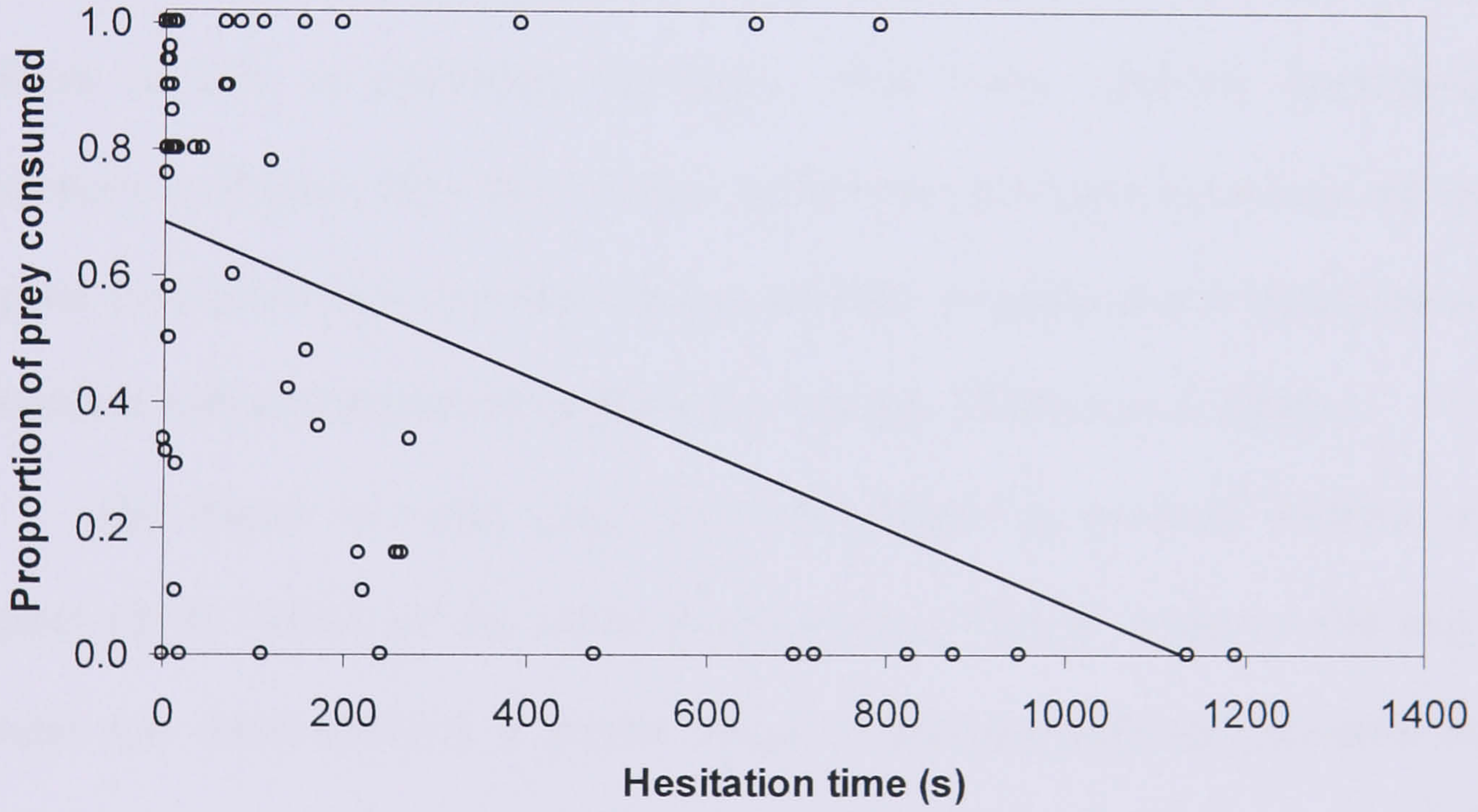

Figure 6.3. The effect of boldness on the proportion of prey consumed (i.e. per capita predation risk). Boldness is measured as (a) the time taken to first leave the refuge and (b) the time taken to first cross the mid-line of the tank (hesitation time). Note that for clarity the proportion of prey consumed are untransformed on the y axis but arcsine square-root transformed in the statistical analysis.

Discussion

Bolder pairs of sticklebacks (those leaving the refuge and crossing the tank mid-line sooner) ate a greater proportion of chironomid prey than less bold fish. Thus, prey were at a greater per capita risk when being preyed upon by bolder predators. In fact, other factors such as prey number and the body length of the predator had no direct effect on risk. The boldness effect was not due to a decrease in the time available for less bold fish to consume prey, as all pairs were given an equal time to feed on the prey after crossing the mid-line (10 minutes). Instead, bolder fish appeared to perceive a greater reward relative to the associated risk, taking less time to explore the tank, and then consumed a greater proportion of prey once feeding began. This demonstrates there can be significant variation within a single predator species in the amount of risk an individual represents, even under relatively homogenous laboratory conditions. How this variation affects prey strategies is unclear, and may explain such behaviours as predator inspection that are carried out to assess the risk associated with an encountered predator (for example, Botham et al. 2006).

The degree to which prey risk is determined by predator boldness will depend on the details of the predator-prey system. Clearly, boldness will play a greater role when there is a greater range of boldness between predators; this variation is expected to be much greater under natural conditions than the standardised conditions of my experiment. Increased risk from predators, for example, can magnify the variation in boldness between individuals within a population. Coleman and Wilson (1998) found the relative differences in the time spent in open water between three classes of pumpkinseed sunfish (bold, intermediate and shy) increased dramatically in the presence of largemouth bass.

The time to leave the refuge and the hesitation time (time taken between leaving the refuge and cross the midline) were influenced by different factors, even though they were correlated with one another and had similar relationships with the proportion of prey eaten. Whereas prey number and average body length of the pairs affected hesitation time, neither of these variables influenced the time spent under refuge. A greater number of prey items led to bolder behaviour by the sticklebacks, in agreement with studies demonstrating increased risk-prone behaviour in exchange for a greater foraging reward (reviewed by Lima 1998). Larger fish spent less time exploring the tank before crossing the mid-line and initiating feeding, suggesting boldness was influenced by predation risk (as smaller fish are more prone to predation; Sogard 1997). Alternatively, bolder fish may consume more prey and hence reach a larger size (if they manage to avoid predation up to this point). It is also possible that larger fish, with greater visual acuity, detected the prey after leaving the refuge sooner than smaller fish (McGill and Mittelbach 2006). However, if this were the case, I would expect this to be influenced by the conspicuousness of the prey (i.e. prey number; Ioannou and Krause 2008), and thus to find a significant interaction between the number of prey and body length. There was no refuge near to the prey, the white background gave a high contrast to the fish, and the water level was shallow relative to the holding tank and the conditions at the source site. It would not be surprising, therefore, that the fish perceived the trial arena as a high risk environment.

Although average pair body length did have a significant effect on hesitation time, and hesitation time was associated with prey risk, there was no direct effect of body length on risk. As the relationship between body length and hesitation time suggests variation in boldness is driven by perception of predation risk, an interesting trophic interaction occurs. Fewer prey are consumed when the risk perceived is high, i.e. when the fish are less bold. Thus, in environments with a high predation risk for the sticklebacks (for example due to frequent encounters with predators or a lack of refuges), their prey may be safer than a similar environment without this high predation risk. This is effectively a trophic cascade, as there is a positive, indirect effect of predators on the prey species two trophic levels below them.

The importance of behaviourally mediated trophic cascades has been relatively well established (e.g., Beckerman et al. 1997), with the effect often demonstrated by varying the presence of top predators in isolated communities. For example, Huang and Sih (1991) demonstrated increased refuge use by salamanders in the presence of predatory fish significantly reduced predation of isopods, the prey of the salamander. My results demonstrate that variation in boldness between predators can lead to a similar effect but within a single community. The next step is to integrate both approaches, and explore the importance of differences between individuals in the presence and absence of top predators.

Contrary to expectations, the number of prey (2 to 50 individuals) had no effect on per capita risk. Chironomid larvae rely on withdrawing into the substrate when threatened by predators (Hölker and Steif 2005) rather than anti-predatory grouping mechanisms such as vigilance, group defence and the confusion effect. Hence, the number of prey had little effect on the safety of individuals, and being unable to burrow limited the natural response to predation. It was still expected, however, that individuals would be safer at greater numbers, as the number of prey the fish could consume in 10 minutes is finite (the dilution effect; Turner and Pitcher 1986). It seems, therefore, that chironomids benefit from aggregation only when they are able to respond to predation by burrowing, which limits the number of prey that can be eaten, or are found in numbers large enough to satiate their predators.

Instead of predators being unresponsive sources of risk they are often modelled as (Lima 2002), I have demonstrated that boldness differences within a predator population can have a strong effect on prey risk. Variation in boldness arises because predators themselves are subject to the selective forces balancing risk and foraging. The optimal behavioural strategy for prey, including boldness, will then partially depend on their predator's behaviour. Thus, a game theoretical approach (Sih 1998) could be applied to explore optimal levels of boldness in multiple trophic levels. Moreover, existing models of habitat selection by predators and prey are likely to be affected by inter-individual variation in boldness as these models rely on individuals balancing predation risk and foraging benefits (for example, Hugie and Dill 1994; Genkai-Kato 2007). Especially illuminating would be considering these interactions over ecological time, where population densities respond to and affect these interactions.

Chapter VII: General Discussion

The aim of this thesis was to explore how predator behaviour affects prey risk. This is a rather broad and ambitious task which would easily fill a book, let alone a thesis, especially if one includes predator behaviours which have indirect effects on prey risk. Instead, I have focused on a handful of contemporary issues in behavioural ecology which may be illuminated by considering the behaviour of predators as well as that of their prey. Chapters II to V examine how the spatial distribution of prey interacts with predator behaviour at progressive stages of a predation event, from encountering and detecting prey to making a successful attack. I have considered only the confusion effect as a mechanism for grouped prey to avoid successful attacks, as, to my knowledge, group vigilance and defence are not used by swarms of *Daphnia* as a defence against sticklebacks. Finally, in chapter VI, I explore how the boldness of predators affects the risk to prey, and whether this too is sensitive to prey group size.

A possible way to measure the success of the current undertaking is to consider the limitations of the work and what implications the findings have for prey risk. Chapter II demonstrates that increases in prey density will not be accompanied by a proportional increase in encounter rate with predators, so per capita prey risk should decline. Moreover, prey were rarely attacked at the beginning of a search when search rate was low (in contrast to Gendron and Staddon 1984). This suggests that when predators are searching a novel habitat, attention given to the detection of predators can lead to little attention being given to the detection of prey. Prey at low density, in contrast, were encountered faster than expected from their density, and were then likely to be attacked when encountered. However, the experiment was not designed to assess subsequent encounters after the first, as behaviour is known to change after encounter (Thomas 1974) and the models of Ruxton (2005) and Travis and Palmer (2005) only dealt with the first encounter. It remains unclear whether the predator's search is 'reset' after the first encounter, or as is more likely, the search continues accelerating. Alternatively, area restricted search may occur where the search slows and becomes more spatially autocorrelated, improving the probability of discovering aggregated cryptic prey. This then raises the question of when a search really begins and ends in terms of acceleration, and how sensitive this is to the novelty of the habitat searched (the novelty of a habitat should be determined by both the time spent there previously, and also the time delay since it was last explored). Data on large-scale movements of predators and the densities of their prey, both of which should be fairly easily obtained, should provide a substantial test of my findings and those of the other study using captive blue tits (Mols et al. 2004). Another yet unexplored factor is the movement of prey, which would also be expected to accelerate over time, but should decrease rapidly after escaping an encounter with a predator.

If the spatial distribution of prey is changed via aggregation, rather than total density, the increased group size (or local density) of prey may make the prey easier to find once encountered. I found mixed results for this in my experiments. In chapter II, there was no significant effect of group size on the probability of detecting prey, although chapters III and V showed increased detection with increased group size and density, respectively. A lack of test power in chapter II could account for this difference, where the change in attention caused by the search of the fish dominated any group size effect. Interestingly, chapter III shows that although larger groups are detected more quickly, this effects saturates at small group sizes, and the number of groups had a greater effect on the time to detection than group size (as prey was detected from two groups of two as rapidly as a single group of thirty). At least in this experiment, to maximise the time to detect prey, prey should form a single, large group. This indicates that the effect of encounter is greater than that of detection, and I am currently working to formalise and test this idea using the effect of aggregation on visual angle, where aggregation increases the distance to the prey (by decreasing the number of groups) while increasing the width of the group (by increasing group size).

After encountering and detecting prey, the probability an attack is launched and is successful can also be affected by prey distribution. My results confirm the confusion effect as a benefit to living in groups, but in chapter V it is shown that this effect can be sensitive to spatial scale. Although the agreement between the results from chapter IV and predictions of neural network models is good evidence for a perceptual basis for the confusion effect, specific neurological studies have yet to be carried out. The methods necessary for measuring neural activity in optic nerves and other parts of the nervous system are available (e.g. O'Carroll et al. 1996). Ideally, an experiment would measure the response of neural activity to increasing prey group size, determining where along the pathway from perception to motor response is overloaded first, and link this to the success of attacks. Further progress in understanding the confusion effect relies on such studies, since both the theoretical and behavioural studies are well developed.

The multiple stages involved in a predation event (Lima and Dill 1990) can select for different prey strategies (some of which may be mutually exclusive) at different stages. Prey should avoid being detected, for example by keeping still and matching the background, but once detected, should conduct avoidance behaviour which often involves intimidation behaviour and bright colouration (Vallin et al. 2006). My experiments show this is very much the case with group size and density: larger groups and denser prey are more easily detected, although once an attack is launched, risk is reduced (for instance, due to the confusion effect). The net anti-predation benefit of any spacing strategy will depend on relative costs and benefits. If, for example, prey pay a high cost when disturbed by predator attacks from lost foraging or breeding opportunities, they should avoid being detected even if they are at a greater per capita risk when attacked. Prey should also favour avoiding detection where predators are able to consume large numbers of prey per encounter, as any dilution effect will be lost (Turner and Pitcher 1986).

In chapter V, this issue of balancing the different stages of predation was addressed in terms of prey density. Denser prey were attacked more quickly, although with less accuracy. However, this effect was sensitive to spatial scale, with a large scale measure of density having a significant effect on detection, and a small scale measure having an effect on attack accuracy. This illustrates a major problem in the study of group living and the adaptive spacing of animals: when is a group a group or the periphery distinct from the centre (Stankowich 2003; Christman and Lewis 2005)? Spatially distinct, highly coordinated animals can be easily classified as a group, but even in this case, if a nearby 'group' reacts to a fright response by the first, just as individuals respond to within-group fright responses, can the groups really be considered separate? This may explain why some studies have failed to demonstrate group size effects compared to other published work (e.g. confusion effect: Milinski 1977b; Jeschke and Tollrian 2007; effect on detection rate: Treherne and Foster 1982), including work presented here (e.g. chapter II). The more continuous distributions of plants and most animals have been dealt with by ecologists by examining effects at multiple spatial scales (e.g. Ray and Hastings 1996). Given that I present evidence showing the importance of spatial scale to predation, I recommend a rejection of group size as a fixed number. Instead, it may be more constructive to consider the spatial distribution most relevant to the mechanism or function under question. For example, active information transfer between individuals can take place over large spatial scales (Danchin et al. 2004), whereas inadvertent information transfer through subtle changes in speed and direction will only be detected by nearest neighbours (Couzin et al. 2005). In neither case is there a need for a definition of absolute group size.

The results of chapter V suggest these spatial scale effects can be driven by the properties of the visual system of predators, where large scales of density are appropriate when the predator is far from the prey, and small scales appropriate when the prey are close. This is because prey within the visual field when the predator is distant are excluded as the prey are approached. Additionally, a limited attentional visual field of predators (Dukas 2002) also provides the most parsimonious explanation for the greater effect of group number on detection rate compared to group size, and the effect of prey density on detection rate. These conclusions demonstrate that the perceptual constraints of predators can have an important effect on the advantages of prey spacing in terms of risk. There is some evidence that the size of the attentional field is flexible and can be adjusted to the frequency of detecting prey (O'Brian and Showalter 1993; Dukas 2002). Large fields allow a high rate of search (area searched per unit time), but at a low resolution. Conversely, small fields are more finely resolved, but the rate of search is much reduced; hence, the field of attention is a result of a trade-off between search rate and resolution (Gendron and Staddon 1984). Quantifying the field of attention, and its flexibility, in sticklebacks should be straightforward by measuring reaction times to prey at different angles around the axis of the fish. Furthermore, repeating experiments in chapters III to V with the crypticity of prey as an additional variable should reveal how altering the size of the visual field affects the importance of spatial scale, the effect of prey density on detection rate, and the balance between group size and number. Such experiments would be necessary to test the proposed perceptual mechanisms underlying the conclusions drawn from these experiments.

The visual angle produced by prey has dominated research into the mechanistic basis of prey detection (Vine 1971; Triesman 1978), with behavioural (Confer and Blades 1975) and anatomical (Hairston and Li 1982) support. Although also well established in the psychological literature (Murray et al. 2006), a larger visual angle is only one characteristic that makes an animal more conspicuous, and these other characters can interact with visual angle. For example, a group size effect on detection can be reduced if prey are conspicuously coloured (Riipi et al. 2001) or can be reversed if smaller groups are more active (Krause and Godin 1995).

Throughout these experiments I have concentrated on the importance of constraints to a predator's visual system. However, other sensory modalities are also used to locate and attack prey. Crypsis, for example, can involve olfactory (Akino et al. 2004) or acoustic (Belwood & Morris 1987) background matching, which is supported by evidence of olfactory search images developing to improve detection rate (Nams 1997). Animals may even attempt to match multiple characters of their habitat (visual, olfactory, acoustic), which should give the best overall protection from detection. How the probability of detection is affected by group size when predators search using non-visual modalities has been considered theoretically by Triesman (1978) and Kunin (1999), although there is no experimental test to date. Similarly, few studies have demonstrated the confusion effect in any modality other than vision, and of these, only tactile predators have been investigated (all of which demonstrated a confusion effect; Jeschke and Tollrian 2007). Generally, studies in predator-prey behaviour have focused primarily on vision, and other sensory modalities have been neglected. The ecological and behavioural implications of interactions between sensory modalities should also be a fertile area of future research (Duncan et al. 1997; Frye et al. 2003), such as how animals adjust use of different modalities with varying environmental conditions.

Distinguishing between patterns driven by constraints in predator perception versus optimal foraging decisions has been an issue throughout my experiments. Chapters II to V attempted to isolate the effect of perceptual constraints by presenting prey to naïve predators. In contrast, the fish in the experiment of chapter VI were likely to be aware of the prey before leaving the refuge, which allowed me to test the importance of risk-sensitive behaviour, i.e. boldness, on prey risk. As optimal foraging involves the balance of rewards (food intake) versus costs (travel time, risk from predation; Pitcher et al. 1988), this last experiment demonstrates the importance of individual differences in this calculation, and its consequences for prey risk.

Due to the fundamental difference between the underlying mechanisms, researchers tend to focus on either optimal foraging or perceptual constraints, but rarely integrate the two (although see Abrahams 1986). Optimal foraging has its basis in economics (Stephens and Krebs 1986), i.e. the optimal solution based on the available information, while perceptual constraints depend on the biological restrictions in gaining and processing this information (Dukas 2002). Animals, however, have to deal with both the acquiring of information and how it should be used to find the optimal solution (Dall et al. 2005). Sometimes the ideal response of predators will be similar whether based on optimal foraging or perceptual constraints: for example, larger prey groups are more conspicuous, and also will often allow a greater rate of predator feeding when prey are unable to escape the encounter, as in chapter VI. Conversely, when prey are highly coordinated, success rate may be lower in large groups, but the increased difficulty in detecting small groups may lead to preferential attacking of large groups (Cresswell 1994). Ideally, future experiments would vary the degree of information available and hence explore the transition from decisions based on restricted information to optimal decisions based on a greater volume of information. In addition, the uncertainty in acquired information, i.e. its reliability, is also another important consideration (Dall et al. 2005).

A final consideration can be given to how environmental variables effect the relationship between predator behaviour and prey risk. Encounter rates between predator and prey can decrease due to water turbidity, and as prey density and productivity (leading to turbidity) are linked, encounter rates can decrease even as prey density increases (Turesson and Brönmark 2007). The effect of group size on detection rate is also predicted to be altered by turbidity (Triesman 1978), and I would expect the greater effect of group number on detection time compared to group size (chapter III) would be magnified in turbid conditions. This is because turbidity decreases the contrast between an object and the background, so although groups may be larger, they may also be effectively cryptic under turbid conditions. Presumably other obstructions to vision, such as macrophytes, will have similar effects. Beyond encountering and detecting prey, the success of attacks under differing environmental conditions has not been investigated. I would predict the confusion effect to be reduced with turbidity, as turbidity will reduce the number of prey in the visual field (an equivalent effect to approaching the prey in chapter V). The benefits of group vigilance and defence should also be affected, as they involve the detection of a predator at a distance, and then a coordinated response between individuals which could be hampered by turbidity.

In summary, I have shown a range of ways in which the visual constraints of predators can effect prey risk and hence prey behaviour (especially spatial distribution) and evolution. Additionally, the degree of boldness predators show can have a direct effect on the risk to prey. Ignoring either predator or prey behaviour can lead to incorrect conclusions being drawn about predator-prey relationships, and hence incorrect predictions in ecological and evolutionary models.

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