Effects of helminth infections on the shoaling behaviour of small freshwater fishes

by

Iain Barber

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Institute of Biomedical and Life Sciences

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DEDICATION

This work is dedicated to my parents, Maurice and Pauline Barber, and to the memory of David William Searle: a fine scientist and a good friend, who enriched the lives all who knew him for too short a period of time. I will treasure my memories of him, and his wonderful ideas, forever.

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SUMMARY

The findings of laboratory and field investigations into various aspects of the effects of helminth parasites on the ecology and behaviour of minnows *Phoxinus phoxinus* and three spined sticklebacks *Gasterosteus aculeatus* are reported.

A sampling programme was implemented at Loch Maragan over a 16 month period between October 1992 and January 1994 (Chapter 2). The overall prevalence of *L. intestinalis* in minnows from the loch was 17.8%; plerocercoids were found to be weakly overdispersed within the minnow population, although one plerocercoid generally dominated multiple infections whenever they occurred. Both total parasite weight, and the weight of the largest plerocercoid present in an infection were found to be positively and highly significantly correlated with host length, with older, larger fish exhibiting higher parasite burdens. This suggests that fish become infected with *L. intestinalis* at Loch Maragan during a temporally-limited period during early life. No significant effect of the parasite on host body condition was detected. A qualitative model for transmission of *L. intestinalis* at the site, based on the available epidemiological data and the prevailing ecological conditions, is proposed.

The shoaling and investigative behaviours of minnows from two sites in central Scotland were found to differ, and possible reasons for this variation, based on the ecological disparity between the two sites, are suggested (Chapter 3). Both small and medium-sized fish formed more cohesive shoals and schooled more frequently when water depth was reduced, but small fish polarised more frequently than medium-sized fish for any given water depth.

The effects of visual oddity on individual shoaling behaviour were investigated (Chapter 3). Size-oddity had little influence on shoaling behaviour in the experimental trials, since the non-uniformity of individual small minnows, when placed in a tank with a group of medium-sized minnows, did not affect their shoaling tendency. The effects of species-oddity were more marked, since medium-sized three spined sticklebacks, when placed in a tank with medium-sized minnows, showed a consistent reduced tendency to join the shoal.

The effects of hunger and helminth parasitism on the shoaling decisions and foraging behaviour of minnows and sticklebacks were studied (Chapter 4). Hunger level was found to have a significant effect on shoaling behaviour, with satiated fish of both species spending more time with the shoal and less time out of visual contact with the shoal. The effects of *S. solidus* on the shoaling

decisions of stickleback hosts was complex. When satiated, infected fish spent less time than uninfected fish within one body length of the shoal, preferring to remain outside of the shoal, yet within visual contact; however, when deprived of food there was no difference in the proportion of time spent by infected and uninfected fish close to the shoal. The possible ecological significance of this change in behaviour is discussed with reference to the manipulation hypothesis of host-parasite interactions.

The effects of *L. intestinalis* infection on the schooling behaviour of minnows in an experimental flow pool was investigated (Chapter 5). *L. intestinalis*-infected minnows were able to 'keep up' with schooling uninfected conspecifics, but the *L. intestinalis*-infected minnow in each group consistently exhibited a larger NND than its uninfected schoolmates. *L. intestinalis*-infected minnows were more likely to be found on the periphery of a school than would be expected by chance if all school members assorted randomly. Whether infected fish are forced to occupy such positions because of their low competitive ability, or whether they actively chose them to maximise food intake is unclear. Minnow schools responded to a simulated avian attack by performing an instant 'flash expansion', which was generally followed by a dramatic reduction in school area.

Under direct competition for individual food items, *S. solidus*-infected sticklebacks were just as successful at capturing and ingesting consecutively-introduced prey as uninfected sticklebacks. However, *S. solidus*-infected fish were only successful at capturing those items to which they were the first, the closest or the only responders. Where food was limited, and items presented simultaneously in a patch, *S. solidus*-infected fish exhibited a lower patch depletion rate than uninfected fish, ingesting fewer prey over the course of the experimental trials. Uninfected fish took the first few prey items in rapid succession, with prey intake rate eventually slowing down exponentially, whereas infected sticklebacks fed at a slow, but constant, rate. The former strategy was the most successful under the experimental conditions.

Infection with the plerocercoid larvae of *L. intestinalis* and *S. solidus* has gross effects on host morphology, with perhaps the most obvious feature of infected fish being their massively-swollen abdomen, caused by the growing worm inside the body cavity. A simple photographic technique is described that allows the measurement of the dorsal profile area (DPA) of small freshwater fish. Infection with *S. solidus* and *L. intestinalis* plerocercoids was found to have a significant effect on the relationships between DPA and fork length of sticklebacks and minnows, with parasitised fish exhibiting significantly larger DPAs than length-matched uninfected conspecifics. A logistic regression

technique that allows accurate discrimination between uninfected and infected individuals and a predictive model, for accurate determination of the parasite load of infected fish (both based on DPA) are described.

The epidemiology of *Diplostomum phoxini* infection in minnows from two ecologically-dissimilar sites in central Scotland (a lowland river, the River Endrick, and a highland loch, Loch Maragan) is described (Chapter 8). *D. phoxini* prevalence approached 100% in both minnow populations, with an overdispersed distribution at both sites. Highly significant relationships between fork length ($\propto$ age) and *D. phoxini* intensity were found in both populations, but fish from the River Endrick site acquired flukes at a much quicker rate than those from Loch Maragan. The most common sites of accumulation of *D. phoxini* metacercariae were the cerebellar cavity, the optic lobes and the medulla oblongata, with sites of secondary importance being the superior lobe of the cerebellum and the anterior part of the spinal cord. A number of metacercariae were found either free in the cerebrospinal fluid, or loosely attached to the brain or the inside of the cranium.

The findings of the complete study are discussed in Chapter 9, with reference to host-parasite coevolution, the 'manipulation hypothesis', and the potential ecological consequences of parasite infection-associated behavioural change.

Chapter 1. General introduction.

1.1 FISH SHOALS AND PARASITE INFECTION

The formation of natural aggregations, or shoals, is the primary defence mechanism of many small prey fish that inhabit open waters. However, shoaling behaviour may have costs, as well as benefits, for individual shoal members. For instance, although being a member of a shoal provides protection against certain types of predators, such dense aggregations of potential prey are more visible to others, and therefore more likely to attract their attention. Although shoal members are known to be more efficient at locating patchily-distributed prey than lone foragers, once food has been located there is likely to be intense competition for it. Clearly, whether a fish forms part of a shoal or not should depend on the net result of these costs and benefits, which in turn will be determined by many internal (physiological, morphological, developmental) and external (ecological, environmental) factors.

Many fish species harbour the larvae of parasitic organisms that can only reach maturity once the fish host has been ingested by a predator. Since parasites whose intermediate hosts die without being consumed by a definitive host do not pass on their genes to the next generation, natural selection should select for parasite phenotypes that influence intermediate hosts in ways such that parasite stages are transmitted more efficiently to the next host in the life cycle. Such parasites have the potential to alter the costs and benefits involved with shoaling, since they frequently impair the nutritional status of infected fish and are associated with morphological and behavioural changes. Since shoaling is of great importance to the avoidance of predation in such fish, it is possible that by altering the value of shoal membership for infected individuals, the parasite may expose hosts to increased predation pressure, and this may in turn result in increased transmission efficiency.

1.2 THE CONSEQUENCES OF GROUP MEMBERSHIP - A SHOAL-BASED REVIEW

(Although this review concentrates specifically on fish shoals, research on non-fish groups has been mentioned where either similar work on fish shoals is unavailable, or where these examples are thought to be useful in developing the discussion).

1.2.1 Terminology

The study of fish aggregation has benefited greatly from the precise definitions provided by Pitcher (1983) for the terms 'shoal' and 'school'. Previously the literature had suffered from ambiguities in terminology, with Keenleyside (1955), Breder (1967) and Radakov (1973) all adopting

different organisational levels at which to describe a group of fish as a school. Pitcher's (1983) definition describing the word 'shoal' as a general term meaning any social group of fish, with 'schooling' meaning simply a more organised and polarised type of shoaling behaviour has clarified the situation, and these are the terms and definitions which will be used throughout this thesis.

1.2.2 Introduction: why study shoaling behaviour ?

That certain fishes form shoals and polarised schools is the principle around which the vast majority of commercial fisheries are based (Pitcher & Hart, 1982; Wardle, 1993). If fish were randomly distributed and equally spaced throughout the oceans of the world, then many modern fishing techniques would not be financially viable, due to the increased fishing effort that would be required to land even a modest catch. Yet the factors determining which species shoal, and how these factors are influenced by various ecological and physiological conditions have only recently been investigated, and their relative importance and interactions are still poorly understood. To examine the mechanisms that govern shoaling behaviour, the majority of workers have found it convenient to study less commercially-important freshwater fish species. Such species are more easily maintained in the laboratory, where conditions can be manipulated in a scientific and controlled manner (Smith, 1991).

1.2.3 Potential advantages of shoaling

Three non-exclusive hypotheses have been proposed for the adaptive function of shoaling behaviour in teleosts, suggesting that it may confer a foraging advantage (e.g. Brawn, 1969; Magurran & Pitcher, 1983), an anti-predator advantage (e.g. Magurran, 1990; Parrish, 1992) and / or hydrodynamic advantage (e.g. Weihs, 1973, 1975). Although no proper phylogenetic study of shoaling has been carried out, it seems likely that the evolution of such behaviour in 5000 teleost species (Shaw, 1978) will have been influenced by various combinations of these factors. The three hypotheses have been tested experimentally to various degrees, but results have not always been unambiguous.

1.2.3.1 Foraging advantages

Possible mechanisms

Organisms may benefit from group foraging in many ways. Co-operation between social carnivores enables large prey to be brought down and killed (Fanshawe & Fitzgibbon, 1993), and

groups of killer whales *Orcinus orca* have been shown to benefit through enhanced prey detection (Hoelzel, 1993). Individuals in both goldfish *Carassius auratus* and minnow *Phoxinus phoxinus* shoals spend a greater proportion of time foraging when in larger groups (Magurran & Pitcher, 1983), presumably because animals in small groups tend to be more timid and spend more time being vigilant (e.g. Seghers, 1981). Individual fish also locate hidden food quicker when in larger groups (Pitcher *et al*, 1982; Street & Hart, 1985). When food is distributed patchily in an open habitat, shoaling fish may also benefit by increasing the area of search per unit time, as proposed for bird flocks (Cody, 1974) and through the transfer of information regarding the foraging environment. Because of morphological constraints on feeding behaviour, which mean that foraging fish inevitably betray the fact that they have found food (Magurran, 1984), and because fish are known to prefer to feed in the company of other actively foraging conspecifics (Pitcher & House, 1987), any fish locating and exploiting a food patch is quickly joined by the rest of the shoal. This effect was demonstrated by Pitcher & Magurran (1983), who were able to show that when goldfish with prior knowledge of the changed location of a good quality food patch were made to forage with naïve conspecifics, passive transfer of this information enabled the shoal to locate the new patch more quickly. Such inadvertent transfer of information regarding the foraging environment allows fish that have been unsuccessful in searching to exploit the food finding abilities of other shoal members. However, as yet there is no evidence that some individuals are constantly better at locating food than others, and it is unclear whether the 'producer-scrounger' relationships that occur in bird flocks (Barnard, 1984) might also be present in fish shoals.

Although socially-foraging fish may gain through enhanced food location, this benefit of shoal membership is probably not shared equally. When foraging in shoals and polarised schools, the associated benefits accrued by individuals appear to be determined, at least in part, by their position in the group, and experimental work has shown that fish at the front of a shoal tend to gain a higher food intake rate than those further back (O'Connell, 1972; Krause *et al*, 1992).

Direct evidence for increased foraging efficiency

Although the various studies outlined above suggest that foraging in groups might be beneficial for fish, experimental work demonstrating a resultant effect on growth is scarce, and contradictory. Although studies on minnows *Phoxinus phoxinus* have suggested that growth rate is independent of shoal size (Cui & Wootton, 1989), research on growth in Japanese medaka *Oryzias latipes* found that

individuals in groups grew faster than solitary individuals (Kanda & Itezawa, 1978). More recent experiments have demonstrated that, when food is unlimited, individual three-spined stickleback fry that live in shoals have a higher specific growth rate than those that are raised in isolation (Peuhkuri *et al*, 1995). Such results may be a consequence of increased foraging efficiency in shoals, but it is also possible that normally-social fish become stressed when maintained in isolation.

1.2.3.2 Antipredator advantages

Pitcher & Parrish (1993) describe the many proposed mechanisms by which shoaling in fish might confer an antipredator advantage. It has been suggested that the combined effect of some or all of these advantages has been the major force driving the evolution of shoaling and schooling behaviour in fishes, and perhaps the strongest evidence for this are the population comparison studies of Seghers (1974) who worked on guppies *Poecilia reticulata*, and Magurran (1986), Magurran & Pitcher (1987) and Leversley & Magurran (1988), who worked on minnows. These studies present convincing data suggesting that individuals selected from areas suffering high predation show a much higher schooling tendency when compared with conspecifics from populations experiencing lower predation levels.

Detection avoidance

Aggregation of prey causes 'patchiness' in the foraging environment of a predator. A randomly-moving predator is therefore less likely to encounter prey if they are aggregated than if they are dispersed evenly throughout the habitat. This simple logic has led many authors to suggest that shoaling may have arisen to counter detection by predators (see references in Pitcher & Parrish, 1993). However, for any *individual*, the chances of detection by an in-water predator are equal whether they shoal or not (Pitcher, 1986); the protective benefits of the group only occur once a predator is in visual contact. In addition, shoals are more visible and attractive to many types of predator, so it seems highly unlikely that detection avoidance has played a major role in the evolution of shoaling behaviour.

Increased vigilance

When prey form aggregations (for example flocks of bird or shoals of fish) the vigilance rate of individuals drops significantly, allowing group members to maximise foraging time (Powell, 1974; Siegfreid & Underhill, 1975); moreover, the total vigilance of the group is heightened since 'many

eyes' are available at any one time to scan for predators (Bertram, 1980; see Lima, 1995, for a recent discussion of the 'group size effect'). It is predicted that as groups of animals increase in size, up to a point they should detect predators more effectively (Bertram, 1978), and experimental evidence from bird flocks (Powell, 1974; Siegfried & Underhill, 1975; Kenward, 1978) and fish shoals suggests this to be the case. Larger shoals of minnows *Phoxinus phoxinus* detected an approaching model of a pike *Esox lucius* more quickly than shoals composed of fewer fish (Magurran *et al*, 1985) but the reaction distance (at which the prey fish begin evasive behaviour) was not reduced, allowing the members of the larger group to optimise their foraging during the early stages of an attack.

Once the predator is detected, the subsequent benefit of attack dilution is likely to be important, since predators tend to prey only on one or a few individuals in a shoal. If the shoal is very large then the chance of individual predation, even after detection, is slight. Foster & Treherne (1981) tested the dilution effect using fish preying on flotillas of surface insects. Individuals in larger groups did indeed suffer a lower predation rate even in this situation where it is proposed that there no possibility for alarm calls or increased vigilance, since fish are apparently not detected until they have attacked.

Deterring attack

Both Kruuk (1964) and Hoogland & Sherman (1976) have shown that by mobbing potential predators, black-headed gulls *Larus ridibundus* could effectively deter attacks on their eggs and young, and anecdotal records suggest that some fishes may attempt to mob a detected predator by physically attacking it (Dominey, 1983; Motta, 1983; Pitcher & Parrish, 1993, p.396; F. A. Huntingford, University of Glasgow, U.K., personal communication). Such attacks are assumed to be rare (Pitcher & Parrish, 1993), and probably only occur in instances where the sizes of predator and prey are similar; however, fish may inhibit potential attackers in other ways.

'Flash displays', observed when silvery-sided fish synchronously turn in sunlight, have been observed to startle predators and scuba divers alike (Pitcher, 1979a, cited in Pitcher & Parrish, 1993), though this has not been experimentally tested. However, a more widely-spread mechanism of attack inhibition, predator inspection behaviour, has been extensively investigated (Pitcher, 1992). In the presence of a predatory fish, individuals shoal members are often seen to 'break ranks' in a seemingly suicidal bid to approach the predator (Pitcher *et al*, 1986). This may inhibit potential predators because

inspecting fish actively display their knowledge of its existence, and the element of surprise is then taken out of the attack (Magurran, 1990). Fish are able to distinguish satiated from hungry predators (Licht, 1989), and inspecting individuals may gain valuable information regarding the satiation status of a potential predator (Pitcher, 1979b). However, since all shoal members benefit from the knowledge gained by the inspecting fish, via passive or active information transfer (Magurran & Higham, 1988), why do some fish put themselves at apparently greater risk by performing inspection behaviour ? It may be that they are able to gain more accurate information regarding the state of the potential predator, and so are able to alter their subsequent actions more effectively to maximise their survival. Inspecting fish appear to avoid entering the areas of highest risk, such as the space around the head of the predator (Magurran & Seghers, 1990a); consequently the risks they take appear to be minimised, and this may be a key to understanding the net benefit to inspecting fish.

Attack mitigation

Experimental data show that the success of lone predators attacking aggregated prey decreases with increasing prey group size (e.g. goshawks: Kenward, 1978; cephalopods and fish: Neill & Cullen, 1974; sticklebacks: Milinski, 1979). On detecting a predator, fish shoals tend to become more cohesive (Andorfer, 1980) and elective group size (the number of fish in a naturally-formed shoal [Pitcher *et al*, 1983]) increases dramatically (Pitcher *et al*, 1986b) as a result of many small, loose shoals merging to form one compact group. Predator evasion normally requires that the fish maintain a 'minimum approach distance' of about 15 body lengths between themselves and the predator (Magurran & Pitcher, 1987). This leads to many of the observed group behaviours, including the formation of 'vacuoles' around potential predators that infiltrate large shoals, and the 'hourglass' formation, where a shoal streams past a stationary predator via a thin neck of fish only a few individuals wide (Pitcher & Wyche, 1983). The 'fountain effect' (Potts, 1970; Magurran & Pitcher, 1987) is frequently observed around divers and fishing gear as well as fish predators (Pitcher & Parrish, 1993) and is achieved by fleeing fish turning through 180 degrees to pass by either side of the predator and reassemble behind it. However, when fish from two or more groups join together, a 'zone of confusion' is created which momentarily shows tail-beat asynchrony, leaving some fish at risk from predators. Shoals should therefore split only as a last resort, and this has been shown to be true for sandeels, *Ammodytes* spp. (Pitcher & Wyche, 1983) and minnows (Magurran & Pitcher, 1987). 'Flash expansion', during which a

shoal suddenly explodes in all directions with all fish separating, is an extreme evasion behaviour reserved for the final strike of a predator (Potts, 1970; Nursall, 1973; Pitcher, 1979b; Pitcher & Wyche, 1983).

Shoals of pelagic fish, for instance sandeels *Ammodytes* spp. and herring *Clupaea* spp., have been observed to form extremely compact writhing masses under predation threat in the absence of cover (a behaviour known as 'ball formation'). This is an illustration of the 'selfish herd effect' (Hamilton, 1971) whereby fish on the periphery of a shoal perceive themselves as being most at risk from predators and attempt to take up a more central position.

These group responses may be rendered more effective by the confusion effect, whereby the predator's visual analysis channels are temporarily overloaded (Broadbent, 1965). Since shoaling fish tend to be of the same species and often the same size (Pitcher *et al*, 1985), confusion is maximised during synchronous behaviour, as all individuals look the same, making it difficult for a predator to track the movements of any particular individual. Milinski (1977a) and Ohguchi (1981) have both shown how sticklebacks *Gasterosteus aculeatus* prey less effectively on swarming daphniid prey than on lone individuals, whilst Landeau & Terborgh (1986) demonstrated that largemouth bass *Micropterus salmoides* quickly caught individual silvery minnows *Hybognathus nuchalis*, but that as shoal density increased so did the proportion of failed attacks. The inclusion of blue-dyed individuals to the prey shoal, which reduced phenotypic homogeneity and thus confusion, greatly increased the ability of the predator to capture both 'odd' and uniform prey, suggesting that the presence of phenotypically-odd individuals may have a cost for all shoal members.

1.2.3.3 Hydrodynamic advantage

One of the most striking features of schooling fish is the apparent order, polarisation and synchrony that is maintained within groups, and for species that spend a large proportion of their time schooling in open water, the hypothesis that this regular arrangement might confer some hydrodynamic function has been frequently postulated (Breder, 1965; Belyayev & Zuyez, 1969; Weihs, 1973, 1975). Passive avoidance of the swirling vortices that are created by the beating tails of fish immediately in front (Pitcher *et al*, 1985), or even active positioning of fish to exploit these wakes, have, as a result, been proposed as causal mechanisms in the evolution of schooling behaviour.

Weihs (1973) postulated the first testable hypothesis of the wake exploitation theory after experimental evidence had suggested that the increased endurance of fish in schools was a result of decreased oxygen consumption (reviewed by Parker, 1973). A theoretical 65% reduction in energy expenditure could be made if fish schooled in the correct formation. However, Partridge & Pitcher (1979) found that in three schooling marine species, individuals in cruising schools failed to take up the positions predicted to allow them to gain the maximum predicted benefit. A more serious criticism of Weihs' model is its intrinsic reliance on altruism, since the leading row of a school cannot benefit; indeed, only every second row would be able to benefit from the thrust vortices which are created (Partridge & Pitcher, 1979). Regular shuffling of fish positions within the school could spread the benefits and costs (Blake, 1983), but organisms are interested only in their own fitness, and this situation would be open to the evolution of a 'cheating' phenotype, and thus cannot be evolutionarily stable (Maynard-Smith & Price, 1973; Dawkins, 1976). Observations of fish in stable schools also find no evidence for regular rearrangement, nor for the expected scramble away from the worst hydrodynamic positions (Pitcher & Parrish, 1993). In addition, the lack of any detectable hydrodynamic effect on fish involved in looser shoaling behaviour strongly suggests that hydrodynamic benefits are unlikely to have been a primary reason for the evolution of fish shoals (Pitcher & Parrish, 1993), from which polarised schools were presumably initially derived.

Moreover, although the oxygen consumption rate of school members has been shown to be reduced when compared with loners of the same species (Parker, 1973; Abrahams & Colgan, 1985), adequate controls have never been used in these experiments, and since it is known that fish in groups tend to be more relaxed and less timid, the assumption that this provides incontrovertible evidence for a hydrodynamic function of schools must be viewed with a degree of scepticism.

1.2.4 Potential costs of shoaling

When compared with the substantial amount of experimental and field data that has been collected regarding the beneficial effects of shoaling and schooling in fishes, little research has been done to investigate the associated costs. In general, the disadvantages of grouping are understood to include the attraction of predators, the increased spread of disease and competition for resources (Bertram, 1978). Of these, competition for resources, and in particular food, is likely to be the most important cost of group membership for shoaling fish in the majority of habitats.

1.2.4.1 Costs associated with increased predation

Attraction of predators

Large shoals of fish are located easily by piscivorous birds and by man *via* modern echolocation equipment (Pitcher & Hart, 1982; Pitcher & Parrish, 1993; Wardle, 1993), making them a readily-exploitable food source. However, because avian predators generally only take a very small proportion of any shoal, predator attraction is likely to have been of less evolutionary significance than the benefits that are accrued by shoaling. The modern fishing techniques of *Homo sapiens*, on the other hand, are devastatingly efficient.

Successful predators on fish shoals

The ways in which such predators circumvent the defences of fish shoals are diverse. Marlin *Makaira nigricans*, tuna *Thunnus* spp., sawfish *Pristis* spp., sailfish *Istiophorous albicans*, swordfish *Xiphias* spp. and some sharks (including the thresher shark *Alopius vulpinus*) attack whole shoals of fish, thrashing their rostra or tails to injure or stun individual members that are then swiftly devoured (Breder, 1967; Pitcher & Parrish, 1993). Because they do not focus an attack on an individual fish, such predators are not affected by the confusion effect. Other predators may increase success when preying on shoaling prey by forming groups themselves, by using sound to stun individual members, by adopting a conspicuous coloration or pattern, by attacking from below, or by 'herding' the fish into a conveniently-dense 'ball' that can be efficiently attacked (see Pitcher & Parrish, 1993, for a detailed review). Clearly, in the evolutionary 'arms race' between predators and shoaling prey, certain types of predators are ahead, and in habitats where they are provide a significant threat, shoaling may not always offer the best protection for potential prey.

1.2.4.2 Foraging costs

Increased competition

As the number of individuals in a foraging group increases, the competition within that group for any located food goes up (Bertram, 1978). Indirect evidence for the existence of high levels of competition has been provided by experiments with Japanese medaka *O. latipes* (Uematsu & Takamori,

1978) and goldfish *C. auratus* (Street *et al.* 1984), which demonstrated that fish fed quicker in larger groups, presumably in an attempt to avoid interference from other shoal members. Similar evidence has been provided by experiments with birds (Barnard, 1984). It has been suggested that this 'bolting' of food, which allows individuals to maximise individual intake when competition levels are high, may have digestive costs for group members and hence potentially provide a further cost associated with group membership (Pitcher & Parrish, 1993). For a full consideration of the consequences of competition for shoaling fish, see Chapter 5.

1.3. PARASITES AND HOST BEHAVIOUR

1.3.1. Introduction : "The Manipulation Hypothesis"

The majority of macroparasite species do not complete their life cycle in or on one single host, and most require at least two host species for the adult stage to be realised. Modern biological theory acknowledges maximisation of individual fitness as the driving force of evolution, and correspondingly it is assumed that parasites should be selected to maximise their efficiency of transmission from host to host. One way in which this requirement may be met is through parasite-mediated modification of host behaviour that increases the frequency or efficiency of transmission of the parasite to the next host. This idea is usually referred to as the Manipulation Hypothesis, although there has been controversy over the validity of this term.

Diseased and sick animals are more easily captured by predators, and if the effects of parasite infection manifest themselves in this way then in situations where definitive (or next intermediate) hosts are the only, or most abundant, predators this may be sufficient to ensure effective transmission. Generally, however, a variety of predators exist in natural environments and more subtle behavioural changes are required to ensure that the 'correct' predator consumes the parasite by eating its host. Much experimental work has concentrated on the behaviour of invertebrate hosts of various parasites, and this has been extensively reviewed by Holmes & Bethel (1972), Moore (1984, 1987, 1995), Dobson (1988), Moore & Gotelli, (1990) and Hurd (1990). In this review, I concentrate on the effects of parasites of teleost fish on host behaviour.

1.3.2 Effects of fish parasites on host behaviour

Infection with parasites has been shown to affect the behaviour of fish hosts in a variety of ways, many of which have been plausibly suggested to result in selective predation of infected hosts by increasing their frequency of detection and /or capture by the parasite's definitive (or next intermediate) host. Such behavioural changes fall into six broad categories: increased conspicuousness, altered habitat selection, disorientation, altered social behaviour, the exhibition of risk-averse behaviours and reduced stamina.

1.3.2.1 Behaviour changes mediated by increased metabolic demand

The metabolic demands placed on fish by certain parasitic infections may be enormous. Mature plerocercoids of *Ligula intestinalis* and *Schistocephalus solidus* (Cestoda) regularly attain the weight of their host, and grow at rates of up to 300% dry weight per week (Meakins & Walkey, 1973). Lester (1971) demonstrated that *S. solidus*-infected sticklebacks had a higher basal metabolic rate than uninfected conspecifics, and during swimming their extra oxygen consumption increased more quickly. These increased oxygen requirements are sufficient to cause noticeable behavioural changes in the habitat use and time budgets of parasitised fish; under experimentally-controlled hypoxic conditions, Giles (1987a) showed that three spined sticklebacks infected with *S. solidus* spent more time in the upper surface layers of water performing aquatic surface respiration than did uninfected fish (see also Smith & Kramer (1987) who measured the respiration rate of nine-spined sticklebacks *Pungitius pungitius* infected with *S. solidus*). These findings provide a potential explanation for field observations that suggest infected fish spend a greater proportion of time in the surface waters or shallow margins of lakes (Lester, 1971; Jakobsen *et al.* 1988). By occupying such habitats, parasitised fish may expose themselves to an elevated risk of avian predation.

Infection with parasites that have a significant effect on the energy budget of infected fish is often associated with changes to host foraging behaviour. Giles (1983, 1987b) showed that after a frightening stimulus, *S. solidus*-infected sticklebacks were quicker to recover and resume foraging than uninfected conspecifics. 'Risky' foraging behaviour has also been demonstrated in *S. solidus* parasitised sticklebacks by Godin & Sproul (1988). It has been suggested that by taking risks, *S. solidus*-infected sticklebacks may be able to mitigate the effects of their proposed reduced competitive ability (Milinski, 1984, 1985; see Milinski, 1990, for a discussion).

Parasites may have more direct effects on host behaviour by reducing the stamina of infected fish. *L. intestinalis*-infected shiners, *Notropis* sp., and perch, *Perca* sp., have been noted swimming behind shoals of uninfected conspecifics, apparently unable to 'keep up' with the shoal during bursts of speed (Holmes & Bethel, 1972), and many authors have commented on the sluggish movement of helminth-infected fish (Dence, 1958; Arme & Owen, 1967; Meakins & Walkey, 1975). Steelhead trout *Oncorynchus mykiss* and coho salmon *Oncorhynchus kisutch* show both decreased swimming speed and stamina when cercariae of the 'salmon poison' fluke *Nanophyetus salminicola* are actively migrating through host tissues, but once encysted, these parasites have little effect on host stamina (Butler & Millemann, 1971). In contrast, parasite infection caused no change in the swimming behaviour of bluegill sunfish *Lepomis macrochirus* (Lemly & Esch, 1984), or swimming speed or stamina of rainbow trout (Russell, 1980).

1.3.2.2 Increased conspicuousness

Certain parasites induce alterations in host colour, shape or size, making them more visible to potential predators (Holmes & Bethel, 1972). Brassard *et al* (1982a) noted a darkening of body coloration in guppies infected with metacercaria of the eyefluke *Diplostomum spathaceum*, and such darkening is also reported in dace *Leuciscus leuciscus* when infected with the same parasite (J. M. Behnke, personal communication cited in Milinski, 1990). If the coloration of prey fishes is assumed to have evolved, at least in part, to make them less visible to potential predators, then any parasite-induced alteration to their hue will tend to make infected individuals more conspicuous.

Both *L. intestinalis* and *S. solidus* cause distension of the body cavity of their hosts (Arme & Owen, 1967; Sweeting, 1977; McPhail & Peacock, 1983; Milinski, 1985), and when seen from above the plerocercoid-induced swelling is visible as two parallel pale stripes flanking either side of the fish, which may make it more visible to avian predators foraging against a dark background (Holmes & Bethel, 1972). Rothschild (1962) suggested that the accumulation of pigment around the metacercariae of *Neascus cuticola*, which encysts in the skin and fins of certain freshwater fish, increases the conspicuousness of hosts and hence could create a basis for their selective predation.

Behavioural oddity may also expose individuals in groups to selective predation. Pejerrey *Basilichthys* sp., when infected with metacercariae of the trematode *Australodiplostomum mordax*, cease normal swimming and tumble about on the water surface (Szidat, 1969) as do bleak *Alburnus*

alburnus when heavily infected with *L. intestinalis* (Harris & Wheeler, 1974) and spottail shiners *Notropis hudsonius* when infected with *Tylodelphys podicipina* (Holmes & Bethel, 1972).

1.3.2.3 Habitat selection and use

Habitat use may be altered by parasites directly, by changes in the physiological requirements of infected individuals (see above), or more indirectly, by altering some other aspect of host biology. Dace *Leuciscus leuciscus* are shoaling fish that spend a proportion of time feeding at the water surface (Maitland & Campbell, 1992). However, when infected with lens-dwelling metacercariae of the digenean trematode *Diplostomum spathaceum*, individuals suffer from reduced feeding efficiency, and this requires them to spend more time in the foraging zone where they are particularly susceptible to avian predation (Crowden, 1976; Crowden & Broom, 1980)

When salmon were introduced to a Norwegian lake, *S. solidus*-parasitised sticklebacks distributed themselves in an area with an increased risk of predation, and as a result of selective predation, the salmon (a non-host predator) caused an almost total disappearance of the parasite from the lake Jakobsen *et al* (1988). *L. intestinalis*-infected gudgeon *Gobio gobio* and roach *Rutilus rutilus* were found slightly higher in the water column of Lough Neagh than uninfected conspecifics during summer months and infected fish also delayed their early winter migration to deeper waters, remaining in shallow areas as overwintering water birds, including potential definitive hosts, arrived (Bean & Winfield, 1989, but see Bean & Winfield, 1992). *L. intestinalis*-infected common shiners *Notropis cornutus fontinalis* migrated shorewards during periods of calm weather in summer (Dence, 1958) and infected rudd have been shown to fail to join spawning groups, instead preferring shallower water where they swim close to the surface (Orr, 1967).

Parasitised and injured adult menhaden *Brevoortia* sp. have been observed to form separate schools and leave their undamaged conspecifics in the open ocean, returning instead to estuarine 'nursery areas' where it is proposed that their recovery is facilitated by the slower swimming speeds achieved by schooling with the juveniles already present (Guthrie & Kroger, 1974).

Not all parasites change the behaviour of their hosts in ways that may be postulated to increase predation pressure. Sticklebacks infected by the microsporidean *Glugea anomala*, which become highly conspicuous due to the large white cysts formed by the parasite, were found to be more 'fearful' of a predator (*Tilapia* sp.), foraging preferentially at a greater distance from the predator than non-

infected or *S. solidus*-infected conspecifics (Milinski, 1985). This may be attributed to the direct nature of the parasite's life cycle, whereby any behavioural change making the host more susceptible to predation would be maladaptive for the parasite, and in this instance, the observed behavioural change probably serves to reduce, rather than increase, predation pressure on infected fish.

1.3.2.4 Altered social behaviour

Both laboratory experiments and field observations have demonstrated effects of parasites on the social behaviour of their hosts. The shoaling behaviour of fathead minnows *Pimephales promelas* is known to change when infected with the brain-encysted larvae of the fluke *Ornithodiplostomum ptychocheilus*. Parasitised fish assumed positions closer to the water surface and formed looser shoals that divided more frequently (Radabaugh, 1980) - modifications that would be likely to reduce the effectiveness of the antipredator function of shoaling behaviour. Infected common shiners "generally adhere to the gregarious mode of existence until they become cumbersome from the development of larval *L. intestinalis*", suggesting that individuals and small groups of parasitised fish leave healthy shoals, taking up a more solitary life (Dence, 1958). This would be advantageous for the parasite, since it has been demonstrated that solitary fish are a much simpler target for predators (see section 1.2.3.2, above).

1.3.3 The manipulation hypothesis - a final note

Although many workers have shown detrimental effects of parasites on host behaviour, many of which are likely to bring about an increased risk of predation, very few studies have successfully demonstrated selective predation by definitive hosts on parasitised prey, a prerequisite for the manipulation hypothesis. A notable exception to this is the work of van Dobben (1952) who demonstrated that cormorants *Phalacrocorax carbo* in the Dutch polders preyed selectively on *L. intestinalis*-infected roach, but conclusive work since then has been scarce. Selective predation by non-host predators has been demonstrated by Coble (1970) (largemouth bass *Micropterus salmoides* preying on fathead minnows *Pimephales promelas* infected with *Clinostomum* spp.), Brassard *et al* (1982b) (brook trout *Salvelinus fontinalis* preying on guppies infected with *Diplostomum spathaceum*) and by Sweeting (1976) (pike preying on *Ligula*-infected roach), who suggests that losses of parasites to non-

target predators is an unavoidable consequence when a parasite-induced behaviour change renders hosts less able to escape potential predators.

The lack of evidence for selective predation by definitive hosts is the major problem facing the validation of the manipulation hypothesis. When parasite-associated changes in the behaviour of hosts are observed, it is frequently assumed that the modification enhances parasite transmission without any experimental evidence that it actually does so. Clearly, more data on selective predation, or at least on the attack strategies of definitive host predators, is required before the manipulation hypothesis can be advocated in the majority of cases.

1.4 AIMS OF THE PROJECT

This project examines a number of features of shoaling behaviour in relation to parasite infection in small freshwater fish. The specific aims of this study are:

Experimental work

- To investigate the shoaling and schooling behaviour of minnows and sticklebacks under controlled experimental conditions (Chapter 3).
- To investigate the effects of infection with the helminth parasite *Schistocephalus solidus* on the shoaling decisions taken by host sticklebacks (Chapter 4).
- To investigate the schooling behaviour of individual minnows infected with the helminth parasite *Ligula intestinalis* in schools of otherwise uninfected conspecifics (Chapter 5).
- To determine whether food competition is a more significant cost of shoal membership for *S. solidus*-infected sticklebacks than non-parasitised fish (Chapter 6).
- To examine the effects of *S. solidus* and *L. intestinalis* infection on the morphology of infected stickleback and minnow hosts, and to develop predictive models based on simple morphometric measurements that allow parasite load to be accurately, and non-invasively, estimated (Chapter 7).
- To investigate the distribution of a brain-dwelling helminth parasite of minnows, *Diplostomum phoxini*, in order to determine whether site-selection by individual parasites may be responsible for reported changes in host behaviour (Chapter 8).

Field work

- To examine the epidemiology of *L. intestinalis* infection in the minnow population of a small, Scottish highland loch and to develop a qualitative model describing the probable transmission ecology of the parasite in this atypical habitat (Chapter 2).
- To study the epidemiology of *D. phoxini* in two ecologically-distinct habitats (Chapter 8).

Chapter 2. The ecology of the minnow-*Ligula intestinalis* host-parasite system in a Scottish highland loch

2.1 INTRODUCTION

2.1.1 Background to the present study

Plerocercoids of the pseudophyllidean cestode *Ligula intestinalis* were found in the body cavities of minnows *Phoxinus phoxinus* from Loch Maragan, in the central highlands of Scotland, by Lassière (1989). In the present study, a regular sampling programme was undertaken at the site to provide uninfected and *L. intestinalis*-infected minnows for laboratory-based behavioural experiments. Taking advantage of this regular programme, the monthly minnow samples were analysed to provide information on the biology of *L. intestinalis* infection at the site. Although the biology and ecology of *L. intestinalis* infection have been examined in previous studies, such investigations have usually concentrated on different host species (frequently roach *Rutilus rutilus*) occupying eutrophic lowland freshwater habitats. The ecology of the parasite has rarely been studied in upland regions, where the density of potential hosts, and therefore transmission rates, are likely to be lower than in these more typical cyprinid habitats.

2.1.2 Biology of the host-parasite system

2.1.2.1 Taxonomy and distribution of *L. intestinalis*

Ligula intestinalis is a widely-distributed pseudophyllidean cestode parasite of piscivorous birds in the Northern hemisphere (Dubinina, 1966, cited in Rausch, 1983), and is classified, according to the scheme of Williams & Jones (1994), as detailed below

Phylum	:	**Platyhelminthes**
Class	:	**Cestoidea**
Order	:	**Pseudophyllidea**
Family	:	**Diphyllobothriidae** Luhe, 1910
Genus	:	***Ligula*** Bloch, 1782
Species	:	***intestinalis*** (Linnaeus, 1758)

Pseudophyllidean cestodes exhibit life cycles based on transmission between three different hosts. The first intermediate host is a freshwater crustacean (usually a copepod), the second intermediate host is normally a fish, and the third, definitive, host may be either a fish, bird or mammal, depending on the particular parasite species. *L. intestinalis* utilises a piscivorous bird as its definitive

host, and the second intermediate host is generally a cyprinid or catastomid fish, although infrequent reports of *L. intestinalis* in salmonids and other groups of fish do exist (see below).

2.1.2.2 Life cycle and ecology

Plerocercoids of *L. intestinalis* have been recorded from the body cavities of 8 species of cyprinid fish in Britain, namely minnows *Phoxinus phoxinus* roach *Rutilus rutilus*, bream *Abramis brama*, chub *Leuciscus cephalus*, dace *Leuciscus leuciscus*, bleak *Alburnus alburnus*, gudgeon *Gobio gobio* and rudd *Scardinus erythrophthalmus* (see Kennedy, 1974). Although cyprinid fish are the common hosts of *L. intestinalis* in the U.K. and mainland Europe, in New Zealand, Weekes & Penlington (1986) recorded the parasite from rainbow trout *Oncorhynchus mykiss* and eleotrid fish such as the common bully *Gobiomorphus cotidianus*. In Australia, Pollard (1974) recorded *L. intestinalis* plerocercoids from the galaxid fish *Galaxias maculatus*, and in North America, catostomids are regularly used as second intermediate hosts (Hoffman, 1967). The only British record of a non-cyprinid host of *L. intestinalis* is the brown trout *Salmo trutta*, although this inclusion may have been the result of a plerocercoid escaping from a recently-ingested cyprinid (Baylis, 1939, cited in Arme & Owen, 1968).

The life cycle of *L. intestinalis* requires a bird, a fish and a copepod as hosts (Figure 2.1). Operculate eggs pass out with the faeces of piscivorous birds into the water and, following a temperature-dependent period of development (5-6 days at 24-28 C; 8-9 days at 15-18 C, Dubinina, 1953, cited in Bauer, 1959), the terminal opercular seal fractures on exposure to light, releasing a ciliated, free-swimming coracidium larva. These larvae are phototactic and propel themselves to the water surface, in doing so increasing their chances of active or passive ingestion by planktonic copepods. A certain degree of host specificity is exhibited at this stage, and whilst common experimental hosts for *L. intestinalis* have included *Eucyclops serrulatus*, the closely related *E. leuckarti* has proved an unsuitable host in laboratory experiments (Dubinina, 1953, cited in Bauer, 1959). In addition, even though Tierney (1991) used *Acanthocyclops viridis* successfully as an intermediate host of the closely related pseudophyllidean cestode *Schistocephalus solidus*, attempts to infect this species artificially with coracidia of *L. intestinalis* have proved unsuccessful; however, when the copepod species is substituted by *Cyclops strenuus abyssorum*, infection is readily achieved (I. Barber, unpublished data). On ingestion by a susceptible copepod, the coracidium sheds its ciliated

Figure 2.1 A diagrammatic representation of the life cycle of *Ligula intestinalis*. Operculate eggs pass out with the faeces of infected piscivorous birds (a) into the aquatic environment, and after a period of development, the first stage coracidium larva hatches (b). This is ingested by a planktonic copepod (c) and undergoes further development to form a procercoid. When an infected copepod is ingested by a suitable predatory fish (d), the parasite burrows through the gut and into the body cavity of the fish where it undergoes extensive growth and development to form a large, infective plerocercoid larva, that may cause severe abdominal swelling (e). If an infected fish is consumed by a piscivorous bird (f), then the plerocercoid rapidly becomes sexually mature, and eggs are produced.

a
b
c
d
e
f

membrane to reveal a six-hooked hexacanth, or oncosphere, that penetrates the gut and enters the body cavity (Bauer, 1959 and personal observations). After 9-10 days the oncosphere develops into an infective procercoid, complete with hooked cercomer. If the infected copepod is ingested by a susceptible host fish; the procercoid is released from the copepod haemocoel and bores through the gut wall of the fish into its body cavity, where it undergoes rapid growth and development to form a plerocercoid. *Ligula intestinalis* plerocercoids become infective to definitive hosts at a weight of approximately 0.5g (Wyatt and Kennedy, 1988); however, the time taken to reach this weight depends on the growth rate of the fish host, which is in turn known to be affected by many environmental factors (Jobling, 1994). Generally, the plerocercoid larvae require a period of not less than six months in the fish host before they become infective. If, after that time, a living or recently deceased infected fish (Wyatt and Kennedy, 1988) is consumed by a piscivorous bird, the final stage of development - segmentation and sexual maturation - is rapidly achieved. The adult worm attaches to the inside of the small intestine of the avian host (normally a gull, merganser or heron) and maturation takes place, with egg production starting after 48-60h (Bauer, 1959).

Ligula intestinalis has been described as an 'opportunistic' parasite species (Kennedy & Burrough, 1981), since it appears to have the ability to colonise new sites rapidly, using its avian host as a vector. The majority of definitive hosts of *L. intestinalis* are migratory, and because of this the parasite has a wide distribution. The rapid growth rate of the cestode allows high intensities and prevalences to be established very quickly (Wilson, 1971; Kennedy & Burrough, 1981). However, various abiotic features such as mean water temperature and basin topography (Black & Fraser, 1984) appear to be of great importance to the success of *L. intestinalis* in freshwater systems, as large areas of warm shallow water are required for efficient transmission of stages between hosts, providing a basis for habitat-dependent variation in epidemiological aspects of infection. Conflicting evidence exists as to whether *L. intestinalis* can achieve an equilibrium state in small lakes (FOR: Black & Fraser, 1984; AGAINST: Kennedy & Burrough, 1981), though it is likely that in larger lakes equilibrium is achieved, and that such systems act as 'reservoirs' for new colonists (Kennedy, 1985)

2.1.2.3 Pathology

Various effects of *L. intestinalis* on the health of the fish host have been extensively reviewed (Arme & Owen, 1968; Sweeting, 1977; Hoole & Arme, 1983). Heavy infections are fatal for the host, resulting in mass mortality in some situations (e.g. Burrough & Kennedy, 1979).

Host body distension is the most obvious external symptom of ligulosis. As the parasite grows, the body wall of infected fish becomes thin due to muscular atrophy (Sweeting, 1977). The resultant stretching of the skin causes the scales to become separated in extreme cases (Arme & Owen, 1968), and this may predispose ligulosed fish to secondary bacterial and fungal infection (Sweeting, 1977).

Parasitic castration has been noted by many workers (Dence, 1958; Arme & Owen, 1968; Wilson, 1971; Harris & Wheeler, 1974; Sweeting, 1976, 1977; Bean & Winfield, 1989, 1992) and it is likely that host gonad development is reduced in parasitised fish as the parasite becomes the major receptacle for the energy that would normally further sexual maturation. Tierney (1991) also found that the overall effect of a closely-related ligulid (*Schistocepalus solidus*) on gonad development in the three-spined stickleback was to prevent the host becoming sexually mature. However, this contrasts with the findings of McPhail & Peacock (1983) who found no significant changes in the gonads of infected sticklebacks, suggesting that by delaying the most severe effects of infection until post-spawning, any selection pressure for the fish to acquire an immune response against the cestode is reduced. It is argued that this may be a result of the complex co-evolution of host and parasite.

The immunological tissue reaction of cyprinids to *L. intestinalis* plerocercoids is almost ubiquitous, with a proliferation of macrophages, fibroblasts, polymorphonuclear leucocytes and connective tissue fibres being observed in all hosts except the gudgeon (Arme & Owen, 1968). Experimental implantation and cellular response analysis suggest that in the gudgeon, *L. intestinalis* evades detection by becoming coated in host proteins, thereby rendering itself 'invisible' to the host's immune system (Hoole & Arme, 1983).

Both blood packed cell volume and haemoglobin content are reduced in parasitised fish, and these two factors are negatively correlated with the parasitisation index (P.I. = [Weight of parasites per host / Weight of host plus parasites] x 100) (Arme & Owen, 1968). Mean liver weight has also been shown to be significantly reduced in infected fish and the structure of the liver has been noted as becoming more diffuse (Arme & Owen, 1968; Sweeting, 1977). Infection has been shown to be associated with a reduction in the fat reserves of bleak *Alburnus alburnus* (Harris & Wheeler, 1974), yet

although several workers (e.g. Wyatt & Kennedy, 1988; Szalai *et al.*, 1989) have noted a reduction in body condition of infected fish, still others (e.g. Weekes & Penlington, 1986; Bean & Winfield, 1989) have recorded no effect of the parasite on body condition.

2.1.2.4 European minnows *Phoxinus phoxinus* as intermediate hosts of *L. intestinalis*

The minnow *P. phoxinus* is the smallest species of cyprinid found in Britain, attaining an average adult fork length of 60-90mm, and reaching a maximum of just 120mm in favourable habitats. It is distributed almost ubiquitously from western Europe to Asia, but in Great Britain it is absent from the northern highlands of Scotland. It is probably only native to Southeast England, but its distribution has greatly expanded, perhaps because of its use by anglers as a livebait (Maitland & Campbell, 1992).

It has been said of the minnow '...he is never to be found, save in company with his kind...' (Maxwell, 1904) and the shoaling capabilities of this small fish are great. Minnows are found in almost every freshwater habitat, but have a high minimum oxygen requirement (7mg/l) and a need for clean gravel to allow spawning. Because of these constraints, they are often found with salmonids in faster flowing streams and rivers, and in this situation they form a major component of the diet of both brown trout *Salmo trutta* and salmon *Salmo salar*. Other predators include pike and perch, as well as piscivorous birds such as herons, black-headed gulls, kingfishers and sawbill ducks. The minnow itself is omnivorous, taking small crustaceans, insect adults and larvae, molluscs, worms and both algae and higher plants.

Because of the small size of minnows, and the comparatively large size of *individual L. intestinalis* plerocercoids, the phenotypic effects of the parasite on the host are greatly enhanced, and infected fish are easily distinguished from uninfected conspecifics by their swollen abdomens (Arme & Owen, 1968). In heavy infections the skin of the abdomen becomes thin and translucent as it stretches to accommodate the growing plerocercoids.

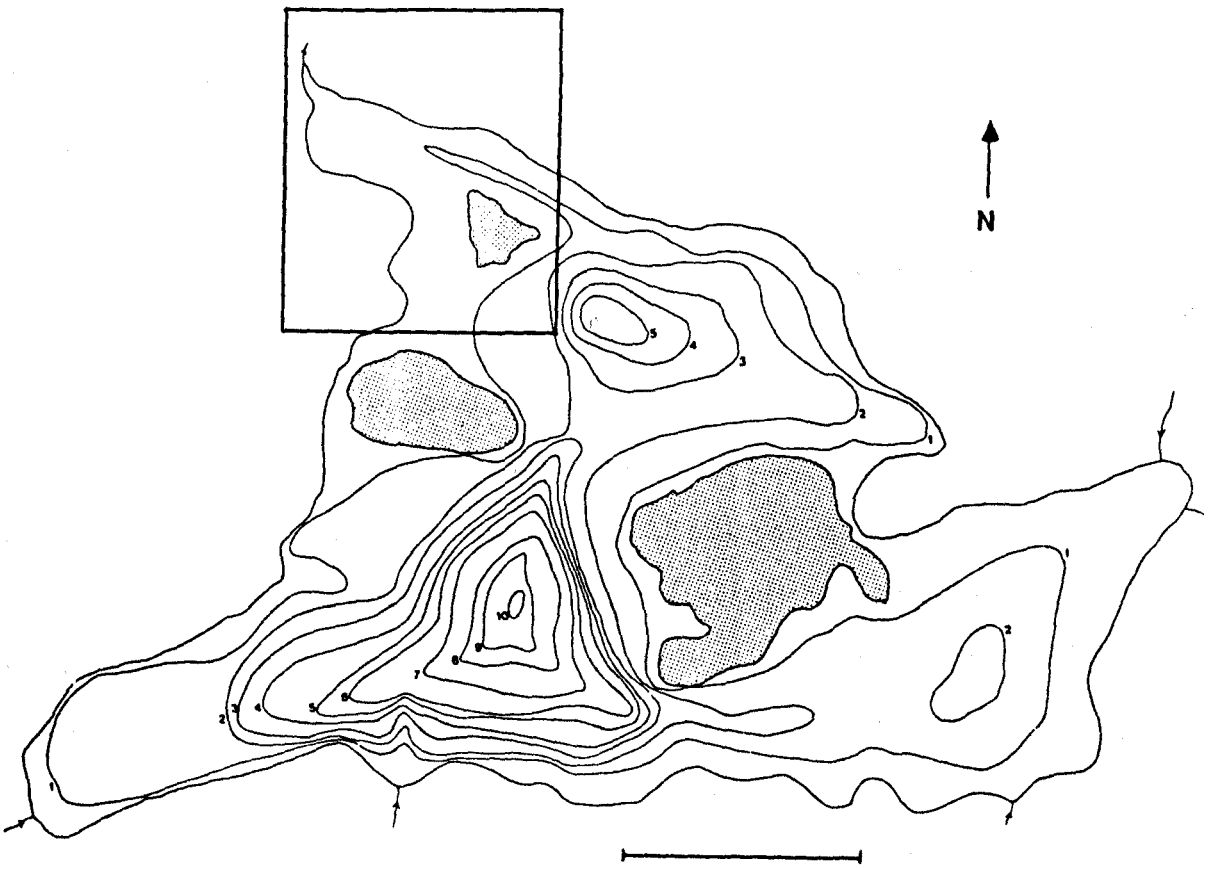
2.1.3 The ecology of Loch Maragan

2.1.3.1 Location and physical characteristics

Loch Maragan is situated in the western central highlands of Scotland, 3.2km NE of Crianlarich (Grid reference NN 402 278, Ordnance Survey 2nd Series, Sheet 51). The loch lies at an altitude of 472m, covers an area of 7.3ha and is shallow, having a maximum depth of 10.2m with a

Figure 2.2a Map of Loch Maragan, showing 1m depth contours, islands (shaded) and burns that flow into and out of the loch (After Lassière, 1989). Scale bar represents 100m.

Figure 2.2b Detail of NW corner of Loch Maragan, showing the area that was sampled for minnows on a monthly basis between October 1992 and January 1994. The island on which the common gulls nested each year is shown (**cg**) as is the outlet burn that flows into the River Fillan. Regularly-used seining transects are shown by the dotted lines (- - - - - - -).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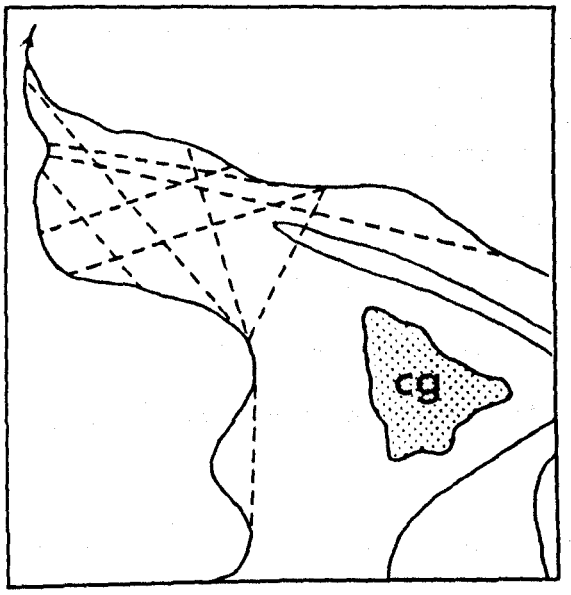

cg

large area in the NW corner of the loch being less than one metre deep (Lassière, 1989; Figure 2.2a). Because of its altitude and location, the loch freezes over during winter months.

2.1.3.2 Piscifauna

Loch Maragan supports populations of brown trout and minnows as well as an unknown number of eels *Anguilla anguilla*. The trout are wild, following an initial stocking ca. 1900, and it is likely that eels enter the loch from the small outlet burn (Inverhaggernie burn) that flows from the NW corner of the loch southwards towards the River Fillan, a tributary of the Tay. Minnows are not endemic to the region, and their presence at Loch Maragan can be best accounted for by their introduction by anglers using them as live bait whilst fishing for the trout; this has generally been the most important factor in determining the spread of these small fish throughout the U.K. (Maitland & Campbell, 1992). Individuals from both the trout and minnow populations of Loch Maragan are known to be the second intermediate hosts of pseudophyllidean cestode parasites that have avian definitive hosts; the trout are regularly infected with larval stages of *Diphyllobothrium* spp. whilst a proportion of the minnows harbour the plerocercoid larvae of *L. intestinalis* (Lassière, 1989).

2.1.3.3 Avifauna

Because of its location and altitude, when compared with more regular cyprinid habitats (e.g. lowland lakes and ponds, slow moving rivers), piscivorous birds are comparatively uncommon at Loch Maragan. Bird species known to use the loch for feeding are mainly summer visitors; red-throated diver *Gavia stellata*, heron *Ardea cinerea*, teal *Anas crecca*, mallard *Anas platyrhynchos*, tufted duck *Aythya fuligula*, dunlin *Calidris alpina*, snipe *Gallinago gallinago*, and common and black-headed gulls *Larus canus* and *L. ridibundus*, have all been recorded during sampling visits. Of these, herons, tufted duck, divers and gulls are known to include fish in their diet, and all could theoretically serve as definitive hosts of *Diphyllobothrium* and *L. intestinalis* at the loch. However, most of these species are assumed to be irregular visitors to the loch (each having been seen only once during all sampling visits), and the only piscivorous birds present in any number are a breeding colony of common gulls (a nest count carried out in May 1994 revealed 16 nesting pairs) that nest annually on one of the small islands in the loch (see Figure 2.2b). The gulls are in residence for a period of approximately two and a half months, between mid-April and the end of June each year (Lassière, 1989; personal observations).

Analysis of faecal samples taken from around the gull colony in late April 1993 revealed the presence of pseudophyllidean cestode eggs resembling those of both *L. intestinalis* and *Diphyllobothrium* sp. Since common gulls are known to include small fish in their diet, it seems probable that they feed on both minnows and small trout in the loch, and because of their numbers in comparison to other piscivorous birds at the site, and the fact that gulls are common definitive hosts of pseudophyllidean cestodes in Northern Europe (e.g. Pemberton, 1963; Bakke, 1985), it seems likely that they are the most important definitive hosts of both parasites at Loch Maragan. Further circumstantial evidence that the gulls feed on the small fish in the loch was provided by dissection of oral pellets taken from around the gull colony, which revealed the presence of otoliths and scales matching in appearance those of minnows (I. Barber, unpublished observations).

2.1.4 Objectives

The work described in this chapter is concerned with assessing the prevalence, intensity and distribution of *L. intestinalis* plerocercoids in the minnow population of Loch Maragan, with a view to gaining insight into the transmission ecology of the parasite at the site. The specific aims of this chapter are:

- To study the ecology of minnows at Loch Maragan, and to determine their year class structure.
- To investigate the epidemiology of *L. intestinalis* infection in the minnow population at Loch Maragan.
- To study the effects of *L. intestinalis* infection on the body condition of minnows from Loch Maragan.
- To examine the effects of single and multiple *L. intestinalis* infections on the growth of plerocercoids in the body cavities of infected minnows.
- To develop a qualitative model, based on available data, regarding patterns of infection and transmission of *L. intestinalis* in the minnow population at Loch Maragan.

2.2 MATERIALS AND METHODS

2.2.1 Population sampling

The minnow population was sampled at the end of each month between October 1992 and January 1994. Fish were caught using a portable trawl net (mouth dimensions: 90cm wide x 65cm

deep) that was dragged across the loch at a fast walking pace (approximately 2m/s). In order to maximise sampling efficiency, trawl-netting took place in the shallow NW region of the loch (see Figure 2.2b for regularly-used seining transects). Mean water depth in this region is less than 1m, and for much of each transect the lead line of the net was touching the substrate. It is therefore unlikely that any differences in the preferred local water column position of parasitised and unparasitised fish, such as those found by Crowden & Broom (1980) and Bean & Winfield (1989), would have had a significant effect on their 'catchability' using this method, and infected and uninfected fish of catchable size present in the line of the transect should be represented proportionally in the sample. At the end of each trawl, any fish caught were removed and placed in an insulated container with water from the loch. These fish were maintained in laboratory aquaria for a period of two months, during which they were fed *ad libitum* with live and frozen bloodworms and commercial flake food; this quarantine period enabled any recently-acquired *L. intestinalis* plerocercoids to develop so that parasite status could be assessed accurately. Without this, it is possible that some fish would have been diagnosed as uninfected when they actually harboured very small plerocercoids.

2.2.2 Laboratory dissection and examination of minnows

Following the two-month quarantine period, fish were exposed to terminal anaesthesia followed by severing the spinal cord behind the opercula. The fish were then surface dried, weighed (in g) and measured (fork length (in mm): length from the tip of the snout to the fork of the tail) using callipers before the body cavity was opened by making a ventral incision through the body wall from the vent (posterior) to the heart (anterior). The body wall was then removed from one side of the fish and, with the aid of a dissecting microscope, the body cavity was examined for the presence of *L. intestinalis* plerocercoids. Any worms recovered were surface dried and weighed (g). The head of each fish was then removed for a separate study of the intensity of diplostomatid metacercariae in the brain (see Chapter 8).

2.2.3 Calculation of condition factor, carcass weight and parasite index

Condition factor

The weight and length of fishes is generally related by a power relationship, of the form

$$w = a \,.\, l^b.$$

where w is weight, l is length, $b \cong 3$, and a is a species-specific constant (Pitcher & Hart, 1982). If length and weight are transformed by taking logarithms, the above equation becomes

$$\log_{10}(w) = \log_{10}(a) + b \,.\, \log_{10}(l),$$

which gives a linear relationship, with slope b and intercept $\log_{10}(a)$.

The length-weight regression can be used as a simple index of fish body condition (Pitcher & Hart, 1982), and in this chapter it is used to investigate the effect of *L. intestinalis* on body condition of minnows from Loch Maragan. Although an annual cyclic change in fish body condition is known to occur as gonads develop and are exhausted (Le Cren, 1951), the period of *ad libitum* feeding under constant conditions in the laboratory following capture served to minimise seasonal variation in body condition, allowing monthly samples to be combined for analysis.

Carcass weight

In order to study the effects of *L. intestinalis* on the growth and body condition of infected fish, it is necessary to calculate the weight of host tissue remaining once the parasite weight has been determined. This measurement is referred to as carcass weight (CW), and is calculated as follows:

CW (g) = Total weight of undissected infected fish (g) - Total *L. intestinalis* weight (g)

Parasite Index

A major feature of *L. intestinalis* infection in fishes is the relatively massive size individual plerocercoids may attain in relation to that of their hosts. Because many of the effects such parasites have are more likely to be dependent on their relative rather than their absolute size, parasitisation indices indicating what proportion of an infected fish's weight is contributed by the parasite have heuristic value. Two such indices are available, devised by Arme & Owen (1968) and by Kennedy & Burrough (1981).

Parasitisation index, PI (Arme & Owen, 1968)

PI = Total weight of parasites per host x 100 / weight of host + parasites

Index of parasitisation, IP (Kennedy & Burrough, 1981)

IP = Total weight of parasites per host x 100 / weight of host - weight of parasites

In the present study, Arme & Owen's Parasitisation Index is used to express the relative weight of *L. intestinalis* infections.

Table 2.1 The prevalence of *Ligula intestinalis* in samples of minnows collected from Loch Maragan, October 1992 - January 1994 (†, loch frozen; Samples of 20 fish or more shown in **bold**).

Year	Month	No. of minnows in sample	No. infected with *L. intestinalis*	Prevalence of *L. ntestinalis* infection
1992	October	14	2	14.3
	November	0†	-	-
	December	0†	-	-
1993	January	0†	-	-
	February	0†	-	-
	March	16	7	43.8
	April	**29**	**7**	**24.1**
	May	8	1	12.5
	June	**85**	**10**	**11.8**
	July	0	0	-
	August	**26**	**4**	**15.4**
	September	7	1	14.2
	October	6	2	33.3
	November	0	0	-
	December	0†	-	-
1994	January	0†	-	-

2.3 RESULTS

2.3.1 Length-frequency analysis of monthly minnow samples

The length-frequency distribution of minnows taken from the loch over the sixteen month period is shown in Figure 2.3. In the sample from October 1992, three frequency peaks can be identified at fork length 26-30mm, 46-50mm and 61-65mm, and from published age-length data for minnows (Frost, 1943; Mann, 1971; Crisp *et al.*, 1975) these peaks appear to relate to 0+, 1+ and 2+ year classes. It is possible to follow the 0+ peak though the following monthly samples, but larger fish appear to be absent. Only one large fish was caught in the shallow region of the loch, in April 1993; this fish measured 94mm and weighed 12.540g and analysis of its stomach contents revealed that it had been feeding exclusively on 0+ minnows. This fish was also infected with *L. intestinalis*.

2.3.2 The prevalence of *L. intestinalis* infection

The overall prevalence of *L. intestinalis* infection in the minnows sampled from Loch Maragan between October 1992 and January 1994 was 17.8% (34 infected fish / 191 total). Although the small samples caught during many months reduce the value of a month-by-month analysis, in months when larger samples were taken (April, June and August, 1993), the prevalence of the parasite was observed to range between 11.8 and 24.1% (see Table 2.1).

2.3.3 The distribution and intensity of *L. intestinalis* plerocercoids

Although *L. intestinalis* was found to be overdispersed in the minnow population at Loch Maragan (see Figure 2.4), the degree of overdispersion was not pronounced (variance : mean ratio = 1.78), and infected fish harbouring more than two plerocercoids were rare, comprising only 1.6% of all fish examined. No minnows from Loch Maragan were found to harbour more than five plerocercoids. Strong correlations existed between the fork length of the host fish and both total parasite weight ($F_{1,32}$ = 165.86, r^2 = 0.83, $p < 0.0005$) and, in multiple infections, the weight of the largest plerocercoid present ($F_{1,}$ = 63.17, r^2 = 0.76, $p < 0.0005$) (Figures 2.5a and 2.5b). Small plerocercoids were never found alone in large fish, only being found in multiple infections. Analysis of covariance revealed that, once host size had been taken into account, there was no significant difference between the weights of the largest plerocercoid in multiple infections, and that of plerocercoids in single infections ($F_{1,31}$ (slope)=0.12, p=0.730, N.S.; $F_{1,31}$ (elevation)=0.93, p=0.342, N.S. Figure 2.5c).

Figure 2.3 The length-frequency distribution of minnows caught in monthly samples from Loch Maragan between October 1992 and January 1994. Months in which no fish were caught have been included.

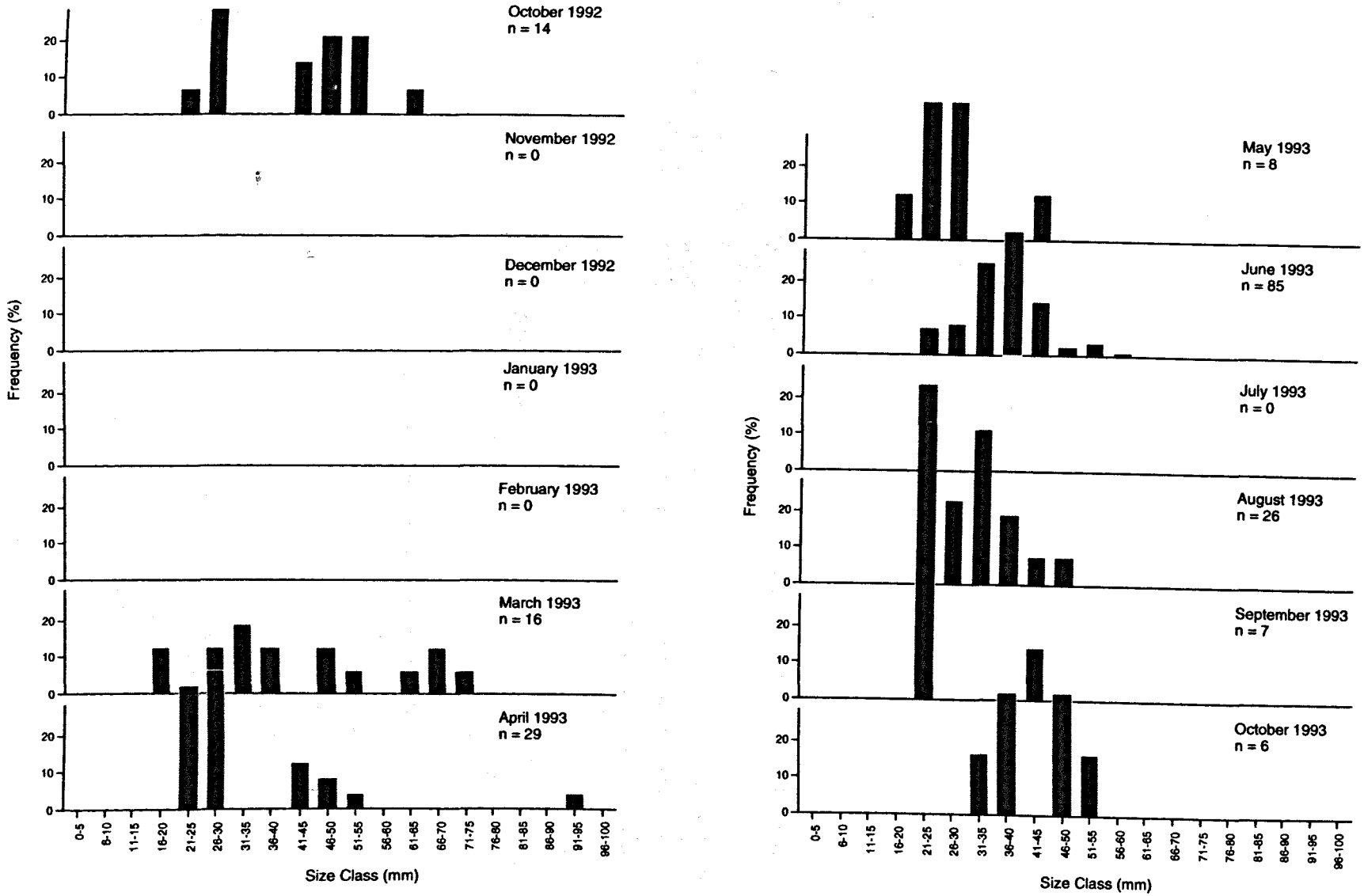
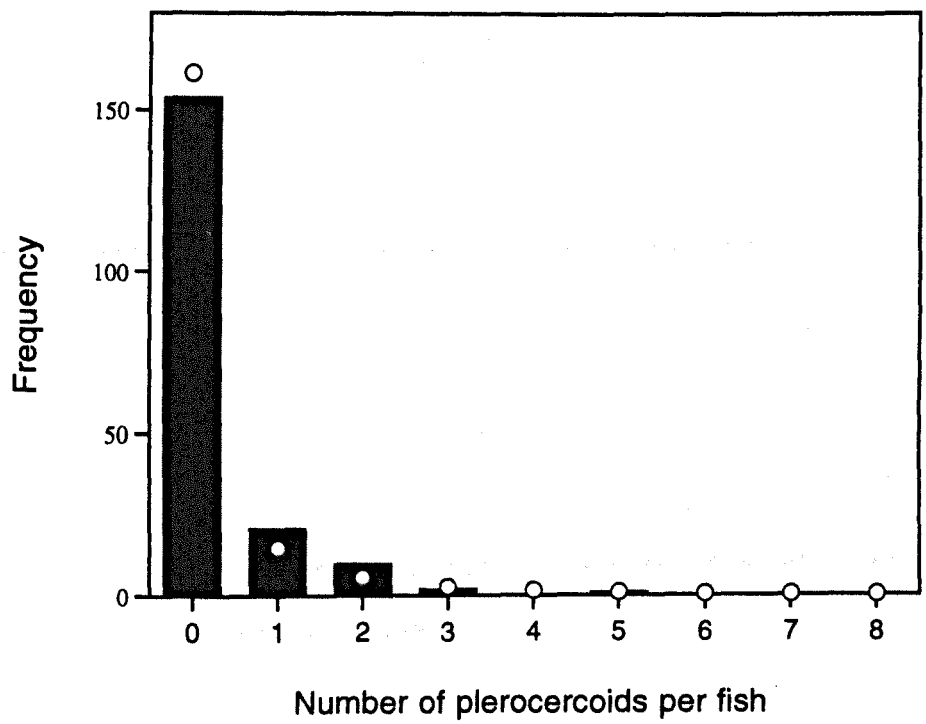

October 1992
n = 14
November 1992
n = 0
December 1992
n = 0
January 1993
n = 0
February 1993
n = 0
March 1993
n = 16
April 1993
n = 29
May 1993
n = 8
June 1993
n = 85
July 1993
n = 0
August 1993
n = 26
September 1993
n = 7
October 1993
n = 6
Frequency (%)
0
10
20
0-5
6-10
11-15
16-20
21-25
26-30
31-35
36-40
41-45
46-50
51-55
56-60
61-65
66-70
71-75
76-80
81-85
86-90
91-95
96-100
Size Class (mm)

Figure 2.4 The frequency distribution of *Ligula intestinalis* plerocercoids in the body cavities of minnows sampled from Loch Maragan between October 1992 and January 1994. The distribution of plerocercoids is overdispersed, and conforms closely to the expectations of a negative binomial distribution. Observed (■) and expected frequencies calculated from the negative binomial function (O) are shown.


Frequency
150
100
50
0
0
1
2
3
4
5
6
7
8
Number of plerocercoids per fish

In fish harbouring two *L. intestinalis* plerocercoids, a marked asymmetry in size was generally observed, with one of the plerocercoids present contributing more to the total parasite load than the other, suggesting that one plerocercoid generally dominated the infection (Figure 2.6a). Multiple infections occurred infrequently, but similar trends towards dominance by one individual were observed in infections with 3 and 5 worms (Figures 2.6b and 2.6c).

2.3.4 Minnow length / weight profiles and the effect of *L. intestinalis* infection

No differences existed in the length / weight profiles of minnows captured in different months (Figure 2.7), and so all fish were lumped together for analysis. The overall regression equation describing the relationship between the length and the weight of uninfected minnows from Loch Maragan was :

$$\log_{10}(\text{weight, } w \text{ (in g)}) = -4.865 + 2.884 \,.\, \log_{10}(\text{fork length, } l \text{ (in mm)}),$$

rearranged gives

$$w = (1.36 * 10^{-5}) \,.\, l^{2.884}.$$

The regression equation describing the relationship between the length and weight of *L. intestinalis*-infected minnows from Loch Maragan was :

$$\log_{10}(\text{weight, } w \text{ (in g)}) = -5.778 + 3.507 \,.\, \log_{10}(\text{fork length, } l \text{ (in mm)}),$$

rearranged gives

$$w = (1.67 * 10^{-6}) \,.\, l^{3.507}.$$

These relationships intercept each other at a fork length of 30.3mm, and above this length, *L. intestinalis* infected minnows were found to become increasingly heavier than uninfected conspecifics (Figure 2.8).

Figure 2.5 The correlations between fork length and a) total *Ligula intestinalis* plerocercoid weight and b) largest *L. intestinalis* plerocercoid weight. c) shows the relationship between fork length and largest *L. intestinalis* plerocercoid weight in single (□) and multiple infections (■) separately.

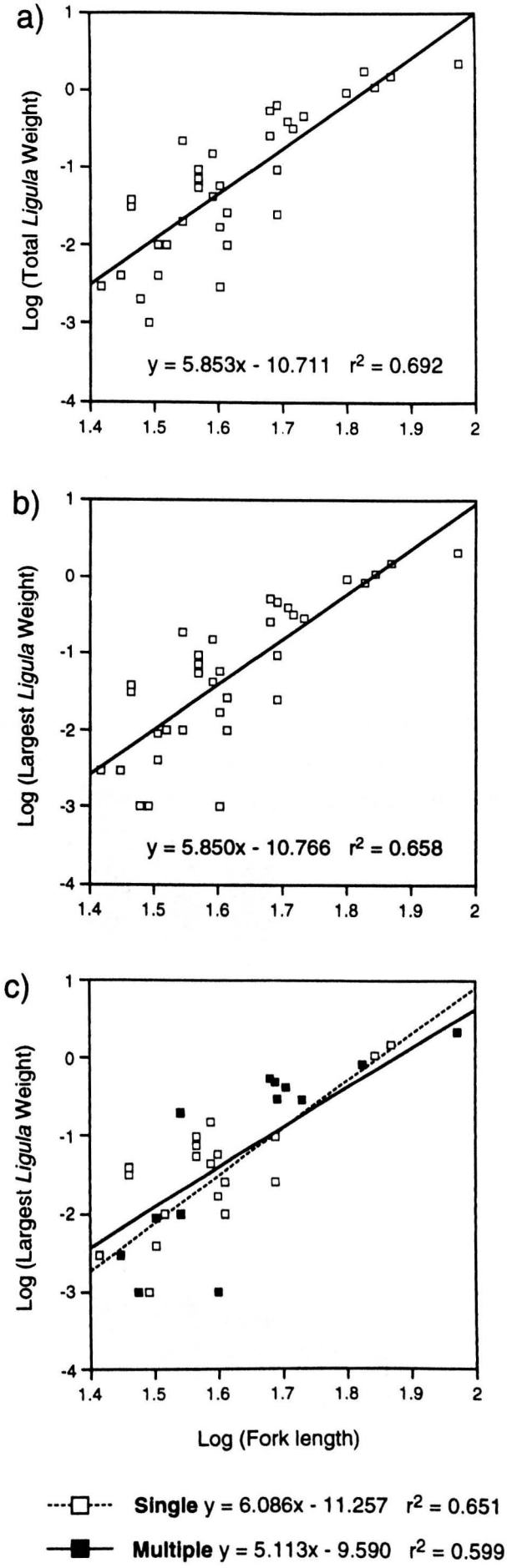

a)
Log (Total *Ligula* Weight)
y = 5.853x - 10.711 r² = 0.692
b)
Log (Largest *Ligula* Weight)
y = 5.850x - 10.766 r² = 0.658
c)
Log (Largest *Ligula* Weight)
Log (Fork length)
Single y = 6.086x - 11.257 r² = 0.651
Multiple y = 5.113x - 9.590 r² = 0.599

Figure 2.6 The proportion of total *Ligula intestinalis* weight contributed by each plerocercoid present in multiple infections of minnows sampled from Loch Maragan between October 1992 and January 1994. a) double infections (2 worms), N=10, b) infections with 3 worms, N=2, c) infections with 5 worms. N=1. In the graphs, plerocercoid 1 is the heaviest, 2 the next heaviest and so on.

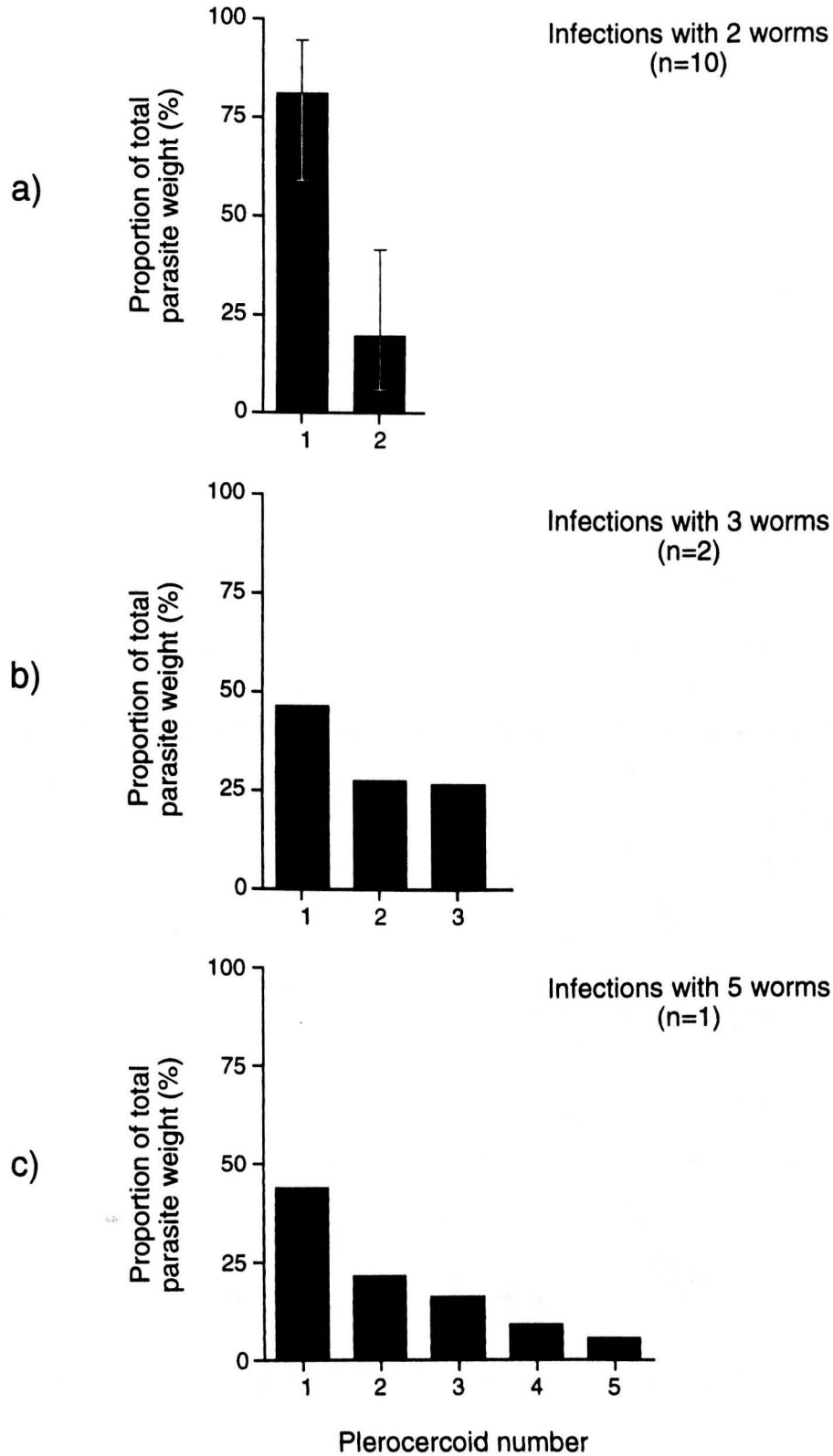

a)
Infections with 2 worms
(n=10)
Proportion of total parasite weight (%)
100
75
50
25
0
1
2
b)
Infections with 3 worms
(n=2)
Proportion of total parasite weight (%)
100
75
50
25
0
1
2
3
c)
Infections with 5 worms
(n=1)
Proportion of total parasite weight (%)
100
75
50
25
0
1
2
3
4
5
Plerocercoid number

Figure 2.7 Length - weight profiles for minnows caught in monthly samples from Loch Maragan between October 1992 and January 1994. Data points for uninfected (□) and *Ligula intestinalis*-infected minnows (■) are shown separately. The regression lines, equations and regression coefficients relate to uninfected minnows only. All fish were kept in the laboratory for two months, during which they were fed *ad libitum* (see text) prior to being weighed and measured.

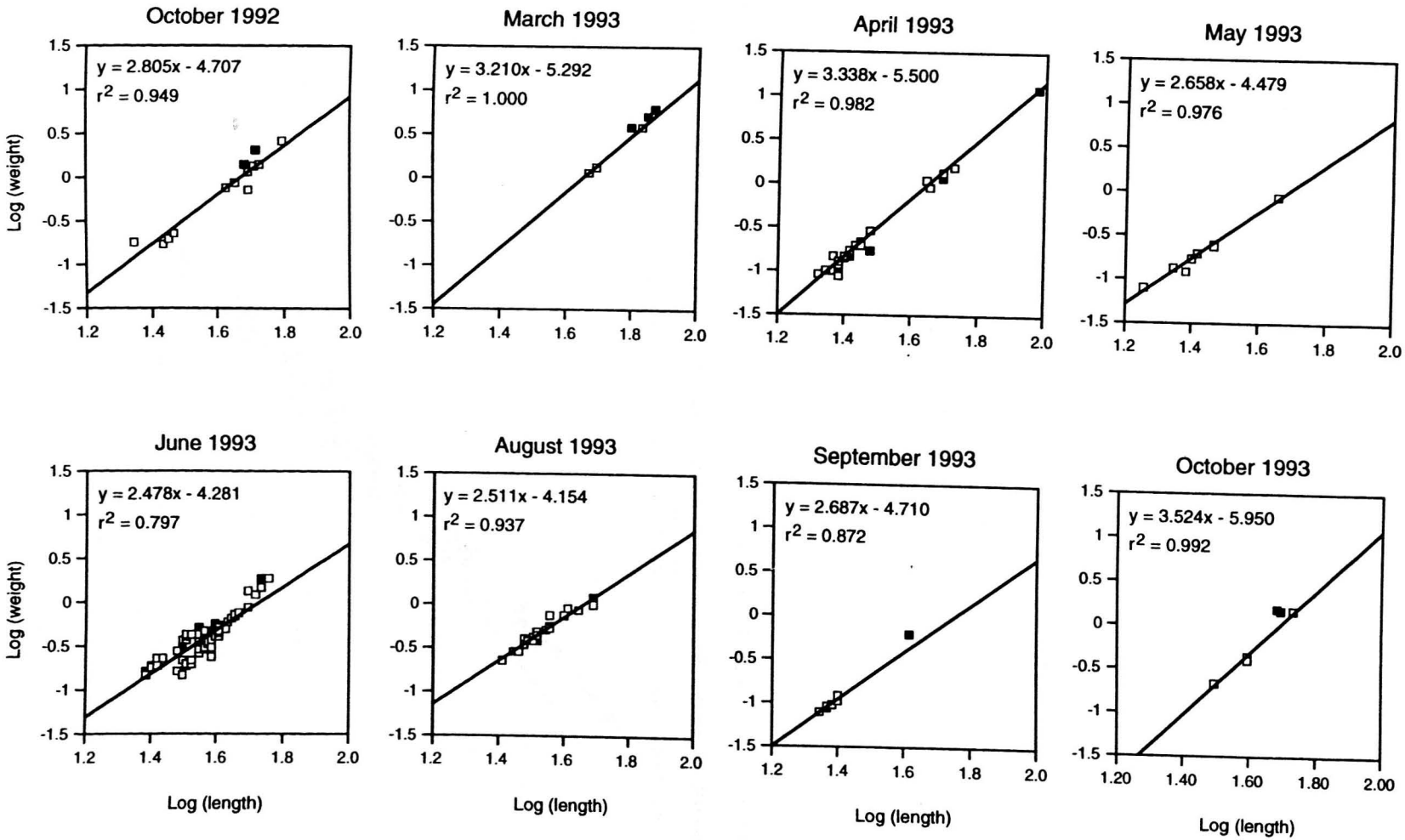
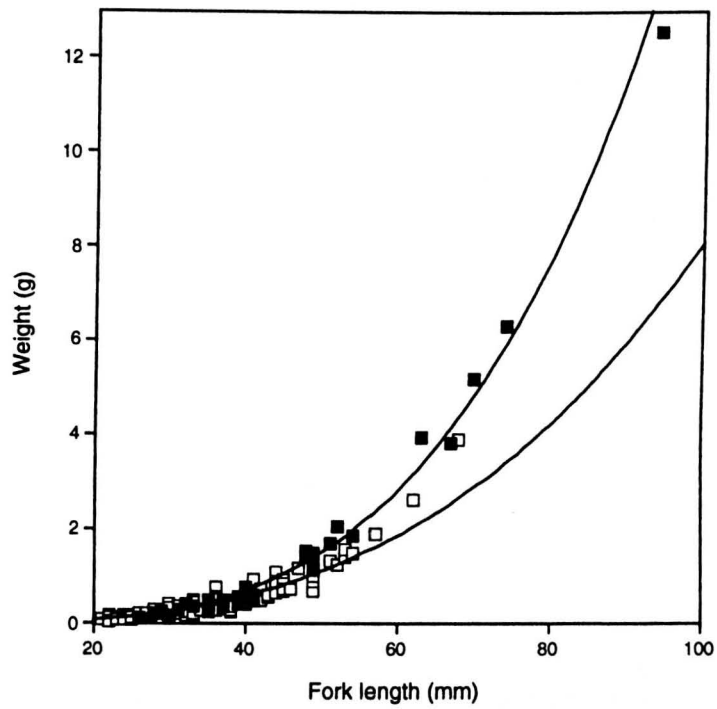

October 1992
y = 2.805x - 4.707
r2 = 0.949
March 1993
y = 3.210x - 5.292
r2 = 1.000
April 1993
y = 3.338x - 5.500
r2 = 0.982
May 1993
y = 2.658x - 4.479
r2 = 0.976
June 1993
y = 2.478x - 4.281
r2 = 0.797
August 1993
y = 2.511x - 4.154
r2 = 0.937
September 1993
y = 2.687x - 4.710
r2 = 0.872
October 1993
y = 3.524x - 5.950
r2 = 0.992
Log (weight)
Log (length)

Figure 2.8 Combined length - weight profiles for all uninfected (□) and *Ligula intestinalis*-infected minnows (■) sampled from Loch Maragan between October 1992 and January 1994. a) plot of untransformed data, and b) plot of log-transformed data.

a)


Weight (g)
0
2
4
6
8
10
12
20
40
60
80
100
Fork length (mm)

b)

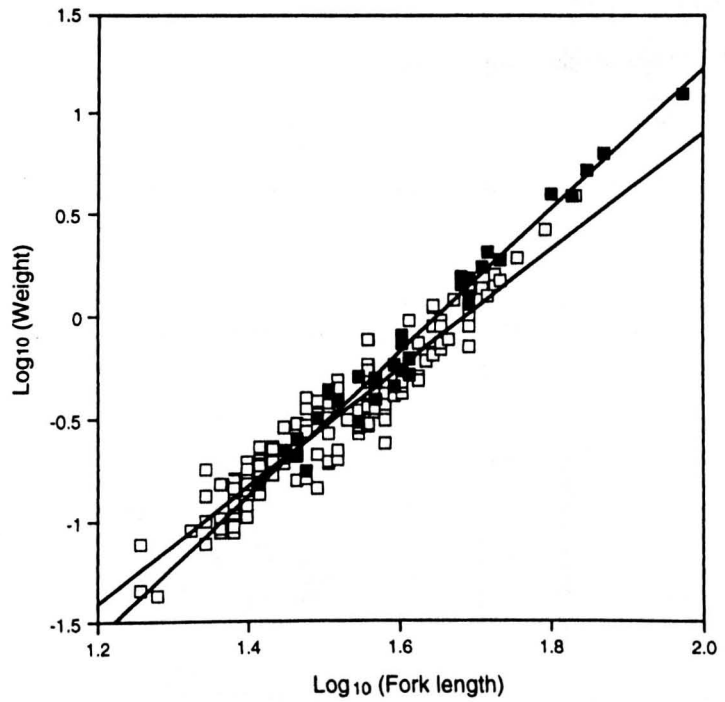
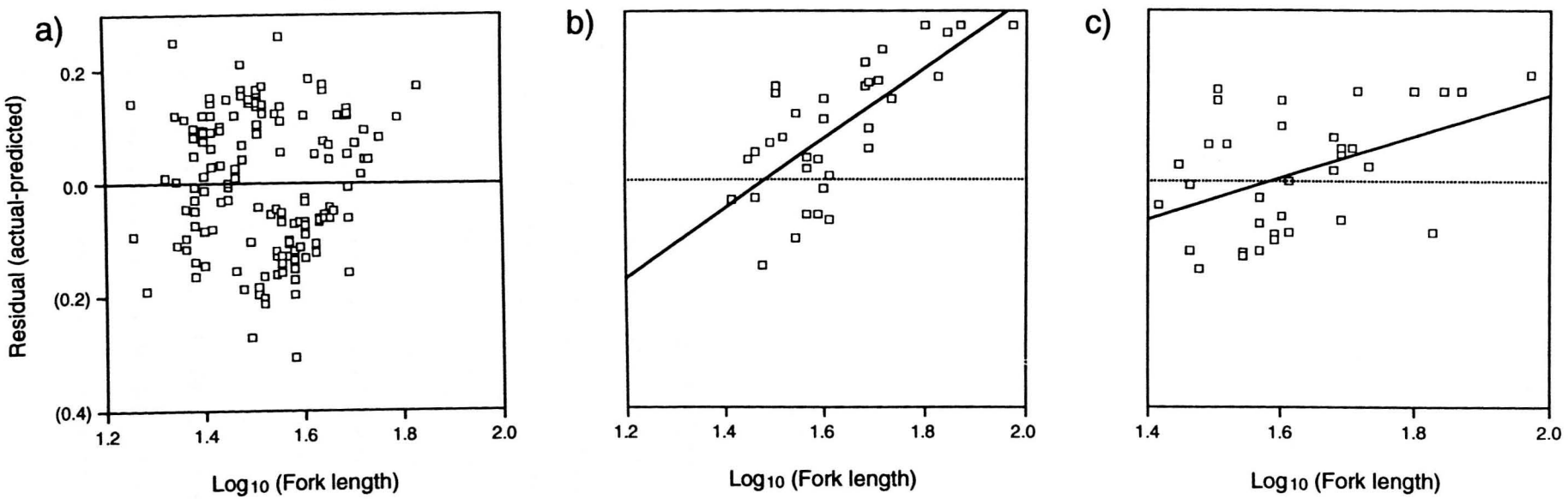

Log10 (Weight)
1.5
1
0.5
0
-0.5
-1
-1.5
1.2
1.4
1.6
1.8
2.0
Log 10 (Fork length)

Figure 2.9 Plots of the residual weight values of a) uninfected and b) *Ligula intestinalis*-infected minnows from the length - weight regression of uninfected minnows with fork length, plotted against fork length. c) Shows the residual plot for infected minnow carcass weight (infected fish weight - plerocercoid weight) against fork length.


a)
Residual (actual-predicted)
0.2
0.0
(0.2)
(0.4)
1.2
1.4
1.6
1.8
2.0
Log10 (Fork length)
b)
1.2
1.4
1.6
1.8
2.0
Log10 (Fork length)
y = 0.623x - 0.923 r2 = 0.512
c)
1.4
1.6
1.8
2.0
Log10 (Fork length)
y = 0.367x - 0.583 r2 = 0.209

Plotting the residual values for both infected and uninfected minnows from the regression line describing the length / weight relationship of uninfected fish shows this effect more clearly. When plotted against fork length, the residual values of uninfected fish form random scatter around the regression line (Figure 2.9a), whereas a positive relationship exists between the residual and fork length for infected minnows (Figure 2.9b). This suggests that, as *L. intestinalis*-infected minnows grow, they deviate further from the expected weight of uninfected fish. When the carcass weight of infected fish is calculated by subtracting the weight of *L. intestinalis* plerocercoids from that of the infected host, and the residual of infected fish carcass weight is plotted against fork length, a less clear, though still significant, positive relationship exists (Figure 2.9c).

2.4 DISCUSSION

2.4.1 The minnow population and *L. intestinalis* infection at Loch Maragan

Length-frequency analysis of minnows taken from Loch Maragan suggests that three year classes of minnows (0+, 1+ and 2+) inhabit the shallow NW corner of the loch. The absence of larger minnows in samples taken from this region of the loch may be accounted for by one of two reasons; either post-2+ fish were absent from the area or scarce enough not to feature in the samples taken, or they were able to avoid being caught in the trawl net, perhaps by being able to swim quickly enough to escape the mouth. The latter explanation is unlikely, since escape would require even a large minnow to swim at a speed of greater than 20 body lengths per second for a sustained period (twice the generally-accepted maximum speed for small fishes, Bone & Marshall, 1982). Since large minnows have been caught at Loch Maragan in gill nets set in deeper water for trout in other investigations (D.W.T. Crompton, personal communication: personal observations) the absence of post 2+ fish from the shallow sampling site appears to suggest that after their second year of growth, minnows move out of the shallow region of the loch and into deeper water. Because of its reduced depth, the temperature of the water in the shallow, sampled region of the loch will rise more quickly than in deeper parts during the spring and will also reach higher temperatures during the summer, allowing fish to grow faster and for longer. As well as enhancing growth rates, shallow, well-vegetated areas also offer a degree of protection from piscivorous fish. For these reasons such freshwater habitats frequently serve as nursery areas for fry and juvenile fish (Wootton, 1990). It appears that the shallow region of Loch

Maragan serves as a nursery area for small minnows, which then recruit into the older population in the main loch.

The overall prevalence of *L. intestinalis* infection in minnows sampled from Loch Maragan was similar to that found by Lassière (1989), who sampled the loch at a variety of locations and depths, in a survey carried out between 1986 and 1989, yet both of these prevalence values are low when compared with previous studies of the parasite in more natural cyprinid habitats, where often over 90% of available hosts are infected (Dubinina, 1953, cited in Bauer, 1959; Arme & Owen, 1968; Wilson, 1971; Harris & Wheeler, 1974; Sweeting, 1976, 1977). This suggests that the prevalence of *L. intestinalis* in the minnow population at Loch Maragan has remained relatively low and constant for the past decade, a pattern of infection that contrasts markedly with that of *L. intestinalis* in other cyprinid populations. It also suggests that there may be one or more factors limiting the transmission of *L. intestinalis* at Loch Maragan.

Plerocercoids were recovered from individuals of all age classes of minnows at Loch Maragan, demonstrating that they may be infected with the parasite from an early age; the smallest infected fish had a fork length of just 26mm and harboured a plerocercoid weighing 3mg, suggesting that it had acquired the infection very early in its life. This is perhaps unsurprising, since minnows and many other fish (sticklebacks: Ukegbu & Huntingford, 1988; trout: Rundle & Hildrew, 1992) feed solely on plankton for the first few months of life, switching to more energetically-profitable benthic and pelagic macroinvertebrates after this period (Maitland & Campbell, 1992). For such fish, there would appear to be a short period during the first year of life when they are most susceptible to infection by pseudophyllidean cestodes, and after which time infection is unlikely to occur. The strong correlation between host fork length and *L. intestinalis* burden at Loch Maragan, and the absence of small plerocercoids in singly infected large fish, support this hypothesis.

In multiple infections, one plerocercoid was generally found to weigh more than any of the rest of the infrapopulation. This may arise following simultaneous acquisition of parasites, if one outcompetes the others for resources and grows quicker and attains a larger size, or it may be a result of plerocercoids being acquired sequentially throughout the life of an individual fish, with the difference in size simply reflecting plerocercoid age. If the latter were true then recently-acquired small plerocercoids should be found in singly-infected fish of all sizes; however, this is not the case with minnows from Loch Maragan since they appear to become infected at a specific time in their life

history (see above). It appears that competition between individual plerocercoids for nutrients within the host, and competitive asymmetry between plerocercoids for those nutrients, may be more likely to cause the observed variation in parasite size. This contrasts with the situation in roach, where asymmetry in plerocercoid size has been attributed to a sequential acquisition of parasites (Tobin, 1990). However, roach are larger fish, and competition for available nutrients is unlikely to be as severe, since there is generally enough space for more than one parasite to reach an infective size. In minnows, this would be possible in only the largest fish, and any asymmetry in the ability of plerocercoids to compete for nutrients or space would be more likely to result in growth differences.

Since all fish were held in the laboratory under an *ad libitum* feeding schedule for two months following capture, any seasonal effects on body condition (including food availability) were effectively controlled. After this time, body condition reflected only the recent food intake of individual fish and their ability to assimilate that food, and would not have been a reflection on individual foraging success in the wild. However, an analysis of body condition, and the impact of parasitism on it, is still of value, since it allows us to examine the effect of *L. intestinalis* on the host in the absence of confounding ecological factors. Under these conditions, ligulosed minnows from Loch Maragan did not suffer a reduction in body condition (measured as weight / length) as may have been expected, and as has been demonstrated for *S. solidus*-infected sticklebacks in the wild (Pennycuick, 1971). In larger ligulosed fish, carcass weight actually exceeded that of uninfected fish of the same size. This may be because the parasite depletes the energy reserves of infected fish, and when food is readily and cheaply-available, infected minnows overcompensate in an attempt to become satiated. Under these conditions, sufficient nutrients may be available to maximise parasite growth and also allow somatic growth of the fish. Under constant laboratory conditions, the observed reduced swimming activity of ligulosed minnows may also maximise host weight gain, since little energy appears to be expended.

2.4.2 The epidemiology of *L. intestinalis* infection at Loch Maragan

In the UK and throughout Europe, cyprinids are the most commonly ligulosed group of freshwater fishes, and of these, roach have been described as the most important host. Roach are medium-sized cyprinids, attaining an average adult size of 20-25cm, that inhabit lowland lakes and rivers, and they are of great importance in coarse fisheries throughout Europe (Maitland & Campbell, 1992). For these reasons, the majority of studies of *L. intestinalis* transmission ecology have focused on

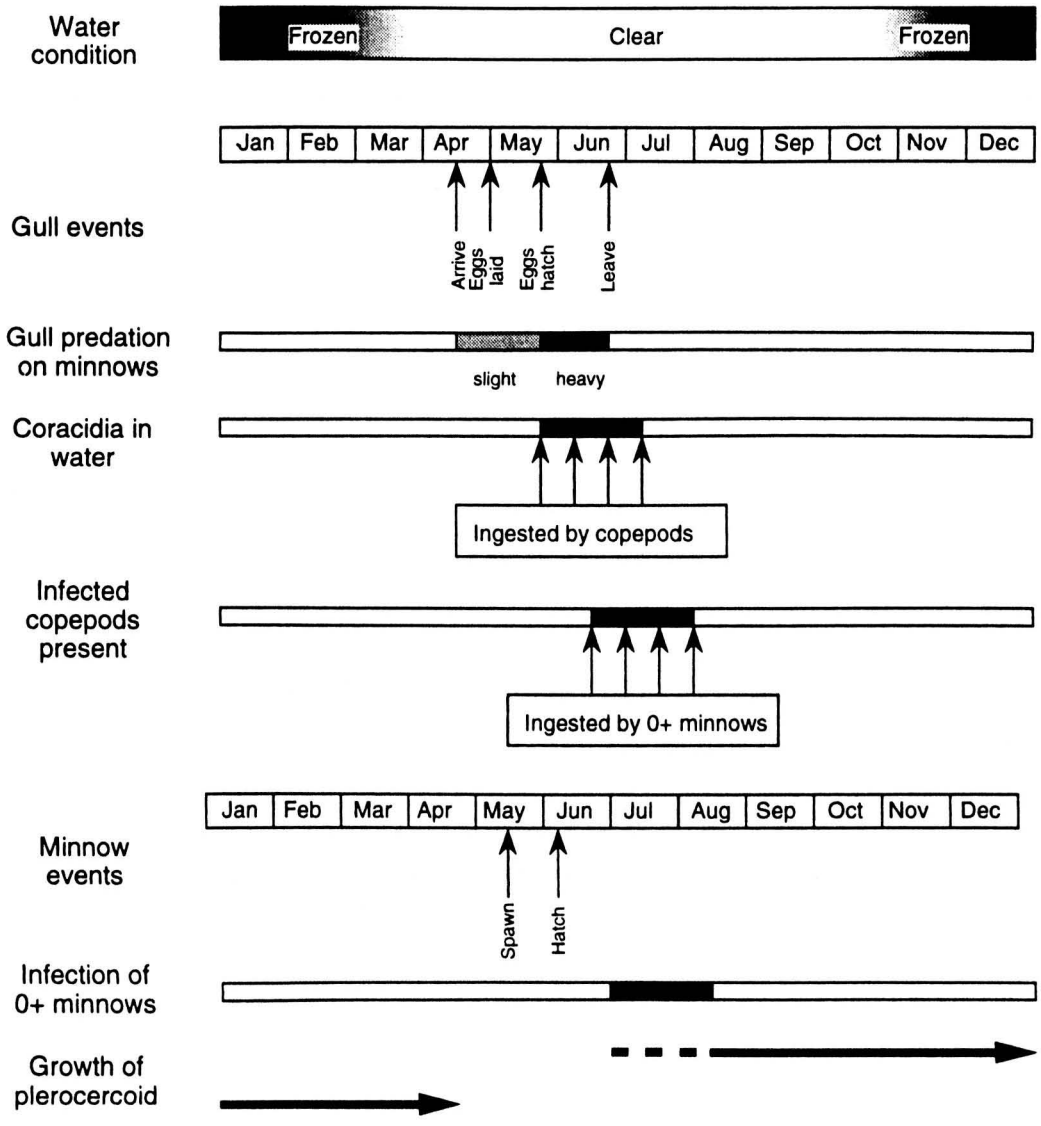
roach inhabiting these typical cyprinid habitats. Perhaps the most extensive, long-running study of *L. intestinalis* in a roach population has been that of Kennedy and co-workers at Slapton Ley, Devon, UK (summarised in Kennedy *et al*, 1994). Slapton Ley is a eutrophic lowland lake that has a wide range of resident avian predators (Burton & Mercer, 1978) and hence a year-round high level of avian predation. *Ligula intestinalis* was recorded for the first time in roach from the ley in 1973, its introduction being attributed to the arrival and subsequent successful breeding of great-crested grebes *Podiceps cristatus*, a major definitive host of the parasite, for the first time since 1945. Once introduced, the parasite was observed to spread rapidly throughout the roach population, reaching a prevalence of 28.4% two years later and causing mass mortality. The resulting crash in the roach population was followed closely by that of *L. intestinalis*, which became gradually less prevalent and eventually locally extinct by 1985 (Kennedy *et al*, 1994).

Despite intensive sampling of the roach population, *L. intestinalis* was not detected again at Slapton Ley until 1990, but just 12 months later the prevalence of the parasite had risen to 71.4% (Kennedy *et al*, 1994). This second outbreak typified the destructive and opportunistic nature of the parasite in such lowland systems, where intense, year-round avian predation on the host population ensures rapid transmission of *L. intestinalis* following its chance introduction to the system. The epidemiology of the parasite at Loch Maragan appears to differ greatly from this and other studies of the parasite, having apparently been present at low prevalence for almost a decade in the minnow population. Kennedy (1985) suggests that in small lakes the parasite may only survive in a short-lived non-equilibrium state before becoming locally extinct, whereas in larger bodies of water, *L. intestinalis* may persist for longer periods and even reach a state of equilibrium. He concludes that differences in the persistence and epidemiology of parasite infections between lakes should largely reflect differences in the parasite transmission rate. The state of *L. intestinalis* at Loch Maragan would appear to support this hypothesis: however, the parasite appears to exhibit stability at the site. This goes against the predictions of Kennedy & Burrough (1981), and illustrates the importance of studying parasites in marginal habitats in order to understand further their ecology.

2.4.3 The proposed transmission ecology of *L. intestinalis* at Loch Maragan

Major events concerning the transmission of *L. intestinalis* at Loch Maragan over a twelve month period are shown in Figure 2.10 (see legend for a detailed description of the proposed

Figure 2.10 A diagrammatic representation of the proposed route of transmission of *Ligula intestinalis* at Loch Maragan. Gulls arrive at the loch in mid-April, soon after the ice melts, and eggs are laid in early May. These eggs will have hatched by late May, and by late June the colony have left the loch. The gulls are therefore in residence for only two and a half months. Gull predation on small fish is known to increase during chick provisioning (Cramp & Simmons, 1983), and so the gulls are most likely to harbour adult *L. intestinalis* tapeworms during this period. Eggs will be passed into the water, and free-swimming coracidia will emerge during the late phase of gull residence and for some time after the gulls have left the loch. During this time they will be ingested by cyclopoid copepods (including *Cyclops abyssorum*, whose numbers are known to increase at this time in Scottish lochs (M. Dorucu, University of Glasgow, U.K., unpublished manuscript) and so following a temperature-dependent period of development, *L. intestinalis*-infected copepods will be present in the population. 0+ minnows, that will have hatched in early June, are solely planktivorous at this time, and feed extensively on copepods. Any 0+ minnows consuming infected copepods will become infected during this period, and the parasite will grow inside their body cavities before they are consumed by the gulls returning to breed at the loch in subsequent years.


Water condition
Frozen
Clear
Frozen
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Gull events
Arrive
Eggs laid
Eggs hatch
Leave
Gull predation on minnows
slight
heavy
Coracidia in water
Ingested by copepods
Infected copepods present
Ingested by 0+ minnows
Minnow events
Spawn
Hatch
Infection of 0+ minnows
Growth of plerocercoid

transmission model). The correlation between host length and parasite weight suggests that there is a brief period early in the life of 0+ minnows when they become infected at Loch Maragan, and it is proposed that this is associated with the short, highly seasonal presence of a significant number of piscivorous birds, the colony of common gulls that are resident for only about two and a half months in the spring and early summer of each year. During this time, it is likely that infected fish suffer a high mortality through avian predation, and this would result in a pulse of infective parasite stages entering the aquatic environment during the gulls' residency period. It seems tenable that transmission, and therefore prevalence, of the parasite at Loch Maragan is limited by the highly seasonal predation pressure of the gulls on infected minnows. The fact that *L. intestinalis* infection has remained at a relatively stable, low level over the past decade, rather than spreading rapidly amongst the host population as is a more common pattern for the parasite, is likely to be a consequence of the hypothesised low rates of parasite transmission at the site.

2.5 SUMMARY

- The epidemiology of the plerocercoid stage of the pseudophyllidean cestode *L. intestinalis* in its second intermediate host, the European minnow, *Phoxinus phoxinus*, in a Scottish highland loch is described.
- Length-frequency analysis was utilised to identify three year classes of minnows in a shallow region of the loch, sampled over a 16-month period. The absence of large fish from the sampled region suggests it may be a nursery area.
- The overall prevalence of the parasite at the loch was 17.8%; plerocercoids were found to be weakly overdispersed within the minnow population, with one plerocercoid dominating multiple infections when they occurred.
- Highly significant relationships were found between the weight of the total and the largest plerocercoid present and host length, suggesting that the period during which fish became infected with *L. intestinalis* was temporally limited, with fish becoming infected at a uniform early age.
- Pre-dissection *L. intestinalis*-infected fish were significantly heavier than size-matched conspecifics, and their increased weight was significantly related to their length, suggesting that older (longer) fish had higher parasite burdens. However, probably due to the period of *ad libitum* feeding captured fish

were exposed to in the laboratory, no significant effect of the parasite on host body condition was detected.

- The epidemiology of *L. intestinalis* infection at Loch Maragan appears to differ from that exhibited at other sites. This is attributed to the fact that Loch Maragan is an atypical habitat for cyprinid fish, and for *L. intestinalis* in the U.K.

Chapter 3. Preliminary laboratory studies on the shoaling behaviour of minnows from two Scottish populations

3.1 INTRODUCTION

3.1.1 Background

This chapter describes pilot studies designed to develop experimental techniques for use in examining the effects of parasites on the shoaling behaviour of fish. Methods for the quantification of shoaling behaviour have been proposed by many workers (Breder, 1954; Cullen *et al.*, 1965; Pitcher, 1973; Partridge, 1980), but many of the techniques that have been developed require the analysis of three-dimensional co-ordinates, and generate a prohibitive amount of data. In order to carry out meaningful research on fish shoaling in the present study, it was necessary to limit the shoaling of fish to two dimensions, thereby allowing data to be more readily collected (T. J. Pitcher, University of British Columbia, Canada, personal communication).

The shoals formed by fish in the laboratory can theoretically be limited to two-dimensional structures by the reduction of water depth, but although small fish such as minnows and sticklebacks frequently aggregate in water only a few centimetres deep in natural environments (Maitland & Campbell, 1992), the potential effects of artificially reduced depth on shoaling tendency and shoal structure under laboratory conditions remained unclear. In order to investigate whether reduction of water depth might be a viable method of constraining shoals to two dimensions in the laboratory, experiments were designed to identify its effects on shoaling behaviour.

3.1.2 Objectives

Experimental data from experiments examining the effects of depth, interpopulation variation, size class and visual 'oddity' on shoaling behaviour of minnows are presented. The specific aims of this chapter are:

- To investigate the effects of water depth on the shoaling behaviour of minnows in order to ascertain whether shallow water could be used to limit the formation of shoals to two dimensions.
- To identify any population differences in the shoaling behaviour exhibited by minnows from two ecologically-distinct sites in central Scotland, and to study the shoaling behaviour of two different size classes of fish.
- To investigate the effects of size- and species-oddity on the shoaling behaviour of minnows.

3.2 EXPERIMENT 3.1 : TANK USE BY SHOALING MINNOWS

3.2.1 Introduction

3.2.1.1 Inter-population behavioural variation

The comparative approach to the study of behaviour, which involves examining groups of related animal species in an attempt to find out exactly how differences in their behaviour reflect differences in their ecology (Krebs & Davies, 1987) has provided valuable insights into the adaptive nature of animal behaviour. However, when comparisons are made at the species level, it is important to ensure that all of the species within a particular study are phylogenetically isolated (Pagel & Harvey, 1988). Comparisons between the behaviour of individuals from different populations of the same species circumvent this problem, and are an ideal tool for examining the effects of natural selection on behaviour, since no phylogenetic inferences need to be made.

3.2.1.2 Inter-population differences in the behaviour of fishes

Population-level differences in fishes have been the subject of considerable investigation, and variation in reproductive strategy and life history traits (Reznik & Endler, 1982; Reznik *et al.*, 1990; Baker, 1994), shoaling behaviour (Seghers, 1974; Magurran & Pitcher, 1987), aggression (Bakker *et al.*, 1988; Huntingford *et al.*, 1990) and antipredator response (Liley & Seghers, 1975; Huntingford *et al.*, 1994) have been observed in a wide variety of species.

Fish species have traditionally been classified as 'shoaling' or 'non-shoaling', depending on the frequency with which they are observed to form social groups in their natural habitat. However, such a dichotomous classification is over-simplistic, since it is now clear that even within a single species, the extent to which individuals aggregate is dependent on a wide range of environmental variables. Such factors are known to include the local abundance of predators (Seghers, 1974; Liley & Seghers, 1975; Magurran & Pitcher, 1987), the particular types of predators normally encountered (Magurran & Seghers, 1991), the time of day (McFarland *et al.*, 1979; Helfman, 1981; Helfman, 1993), prey type and availability (Smith & Warburton, 1992) and, for those fish that spawn in shoals, the time of year. Variation in shoaling tendency is known to be heritable (Seghers, 1974; Breden *et al.*, 1987), and so this intraspecific variation in shoaling tendency is probably a result of the evolutionary history that has shaped the adaptive behaviour of geographically-isolated populations of a single species.

3.2.1.4 Location and characteristics of two study population sites

River Endrick

The River Endrick drains agricultural land around the Campsie Fells in western central Scotland and flows into the south-east corner of Loch Lomond (Maitland, 1966). At the site where the study population was sampled, Drumtian Ford near Killearn (Grid Ref. 519 877 O. S. Second Series, Sheet 57), the Endrick is a shallow lowland river. As well as minnows, other common fish species in this stretch of the river include brown trout *Salmo trutta*, Atlantic salmon *Salmo salar*, pike *Esox lucius*, stone loach *Noemacheilus barbatula* and three-spined sticklebacks *Gasterosteus aculeatus* (Maitland, 1966; Huntingford *et al.*, 1994b). Piscivorous birds commonly observed at the site include herons *Ardea cinerea*, red-breasted mergansers *Mergus serrator*, kingfishers *Alcedo ispida* and black-headed gulls *Larus ridibundus*. Small prey fish species, such as minnows and sticklebacks, at this site are important components of the diet of these birds (Giles, 1981), and also of the trout, salmon and pike that inhabit the river (Maitland, 1965; Maitland, 1966; Giles, 1981), and there is experimental evidence that small prey fish from this site display well-developed anti-predator behaviour (Giles & Huntingford, 1984; Tulley & Huntingford, 1988; Huntingford *et al.*, 1994b).

Loch Maragan

The ecological situation at Loch Maragan differs enormously from that at the River Endrick. Loch Maragan is a small highland loch with comparatively few predatory birds, and the only predatory fish present are brown trout, which appear to be restricted to deeper water and are rarely encountered in the shallow regions of the loch where the minnows spend the initial phase of their life (see Chapter 2 for site description and a detailed description of the ecology of minnows at Loch Maragan). Because of the relative scarcity of predators at this site, it is likely that the predation pressure experienced by minnows at Loch Maragan is significantly lower than that in the River Endrick population.

3.2.2 Materials and methods

3.2.2.1 Collection and husbandry of fish

Approximately 30 minnows were hand- and trawl-netted from each of the two freshwater sites described above. Following capture, fish were transferred to the laboratory where they were maintained in holding tanks for approximately one month. During this period, the fish were fed on a mixture of

flake food and frozen and live bloodworm, at a water temperature of 12°C and exposed to a 12h:12h photoperiod.

3.2.2.2 Experimental design

The experimental tank (Figure 3.1a) comprised a 54-litre glass aquarium, filled with dechlorinated tap water to a depth of 15cm. White coral sand formed the substrate, and small (c. 5mm dia.) black-painted pebbles were used to delineate a 5cm grid on the bottom. 5cm grids were also marked onto the front and back panels of the tank, and a plane glass mirror was suspended over the tank at an angle of 45°. An S-VHS video camera, positioned in front of the experimental tank, was used to film the shoaling behaviour of the minnows.

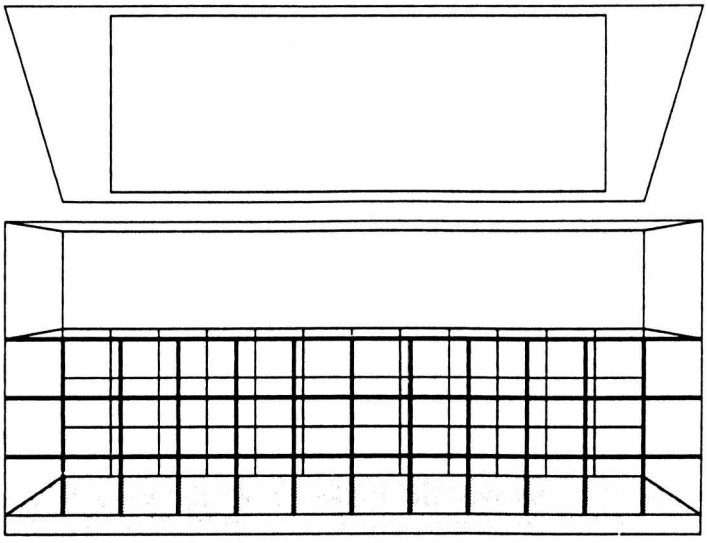
Six size-matched fish from one of the populations were selected at random from one of the stock tanks and transferred to the experimental tank, where they were left to settle for 20 minutes prior to experimentation. Following the settling period, the behaviour of the fish was recorded for 15 minutes using a S-VHS video recorder, positioned so that both frontal and plan views of the tank were clearly visible. Sixteen experimental trials were carried out each with fish from the River Endrick and from Loch Maragan. Because the groups of six fish from the two stock populations used in each experimental trial were selected at random from the stock tanks, the likelihood of pseudoreplication was reduced, as it was unlikely that any of the schools used in separate trials comprised exactly the same individuals.

3.2.2.3 Video analysis

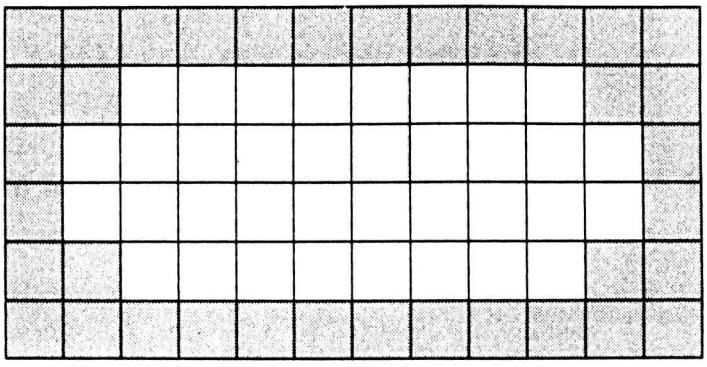
The three-dimensional cube of water that enclosed the head of each minnow in the experimental tank was recorded at sixty second intervals over the 15 minute experimental period, on specially-prepared data sheets. Elective group size (EGS; the number of fish found in a naturally-formed shoal [Pitcher *et al.* 1983]) was recorded in each analysed frame of film; a fish was classed as a shoal member if it was separated from other fish in the aquarium by less than one complete 5 x 5 x 5cm cube of water. For each trial, composed of 16 analysed frames of film, the mean EGS was calculated. In addition, the frequency with which fish occupied 'central' or 'edge' 5 x 5cm basal squares (see Figure 3.1b) was recorded, and any preference for one over the other was assessed by calculating the ratio of edge : central square occupancy.

Figure 3.1 The design of the experimental tanks used in Experiments 3.1 - 3.3. a) The 54-litre aquarium used in Experiment 3.1, showing the 5 x 5cm grids on the front and back panels, and the 5 x 5cm grid delineated by small, black painted pebbles on the base of the tank. The mirror, angled at 45° above the tank, provided a plan view of the fish. b) Diagrammatic view of the base of the tank, showing squares that were classed as 'edge/corner' (shaded) and those classed as 'central' (unshaded). c) The 54-litre aquarium used in Experiments 2 and 3, showing the 3 water depth regimes that shoaling behaviour was measured under.

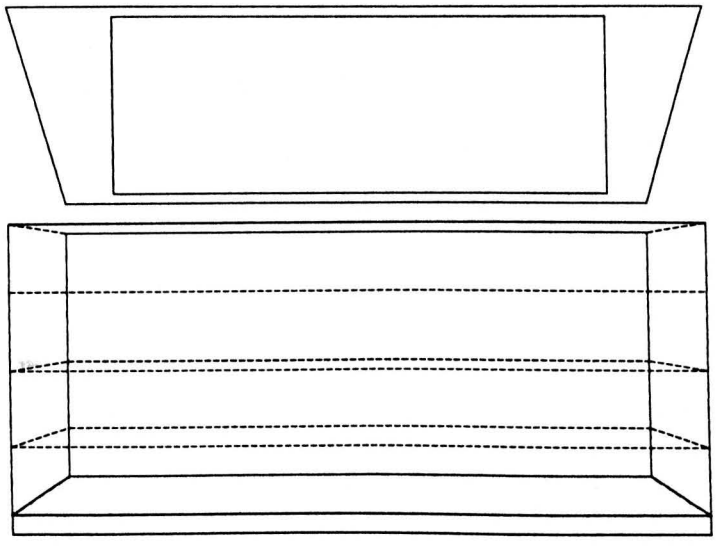
a)



b)



c)



3.2.3 Results

The study showed that the mean EGSs of minnows from the River Endrick were larger than those exhibited by Loch Maragan fish (Wilcoxon-Mann-Whitney test, W=198.5, m=16, n=16, P=0.0143; Figures 3.2a and 3.2b). Minnows from both populations showed a significant preference for certain positions in the experimental tank, occupying the edge and corner basal squares more often than would be expected if they used all of the tank space equally (Wilcoxon signed ranks test, Endrick: W=91.0, n=13, P=0.002; Maragan: W=114.0, n=16, P=0.019; Figure 3.3). However, this preference for the margins of the tank was much stronger in fish from the River Endrick (Wilcoxon-Mann-Whitney test, W=144.0, m=13 [3 values = ∞], n=16, P<0.0001). Endrick fish were also found to move around the experimental tank less than those from Loch Maragan, visiting on average fewer basal squares (Wilcoxon-Mann-Whitney test, W=363.5, m=16, n=16, P=0.0002; Figure 3.4).

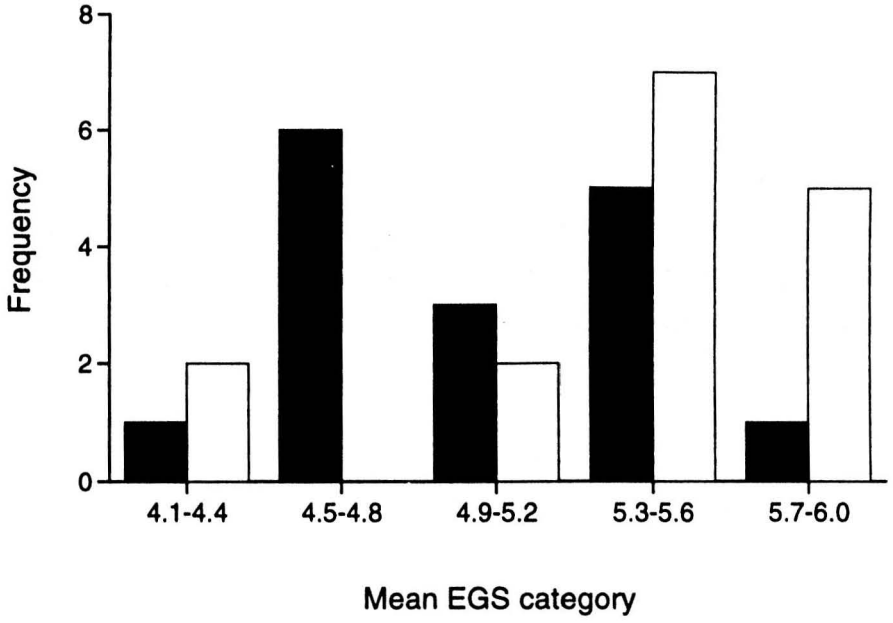
3.3 EXPERIMENT 3.2 : THE EFFECT OF DEPTH AND INDIVIDUAL SIZE ON SHOALING BEHAVIOUR

3.3.1 Introduction

For small prey fish, such as minnows and sticklebacks, use of shallow water habitats, in natural environments, is likely to increase the risk of predation by both avian and terrestrial predators, since the fish may be highly visible, because they are limited to two dimensions in which to escape, and because such conditions are generally found close to the margins of water, where they may be accessible to a range of non-aquatic predators. The advantages gained by occupying such habitats in the wild include the fact that in very shallow water, fish are relatively safe from piscivorous fish. In addition, such areas are maximally-lit, so foraging on algae or associated invertebrates (the primary foods of such fish) may be improved and, because shallow waters warm up most quickly, they may provide optimal conditions for efficient growth. Although these benefits are likely be relatively constant over an, albeit narrow, range of water depths, predation risk may be more strongly dependent on the 'shallowness' of a particular habitat.

This experiment was designed to examine the shoaling behaviour of minnows in a range of shallow water depths (5cm, 10cm and 15cm) where the perceived benefits are proposed to be relatively constant, in order to determine the response of fish to such conditions.

Figure 3.2 The mean elective group sizes (EGSs) formed by minnows from Loch Maragan (■) and the River Endrick (□). a) Frequency distribution of categories of mean EGSs formed by the fish during the experimental trials (n=16 for both Maragan and Endrick fish). b) Median values of the mean EGSs formed by the fish from either site during the experimental trials (error bars represent interquartile ranges).


8
6
4
2
0
Frequency
4.1-4.4
4.5-4.8
4.9-5.2
5.3-5.6
5.7-6.0
Mean EGS category

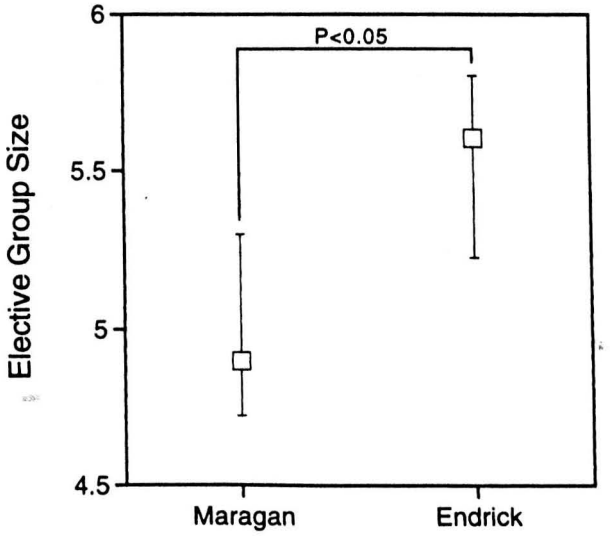
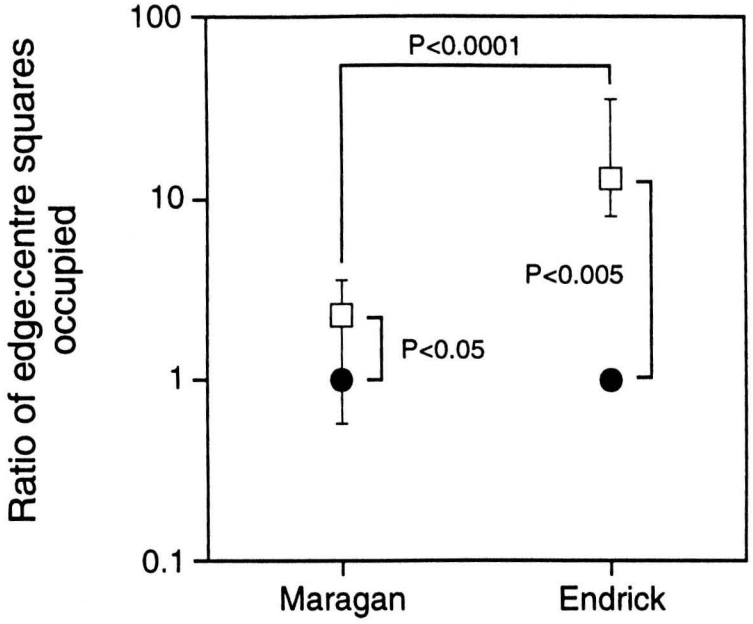
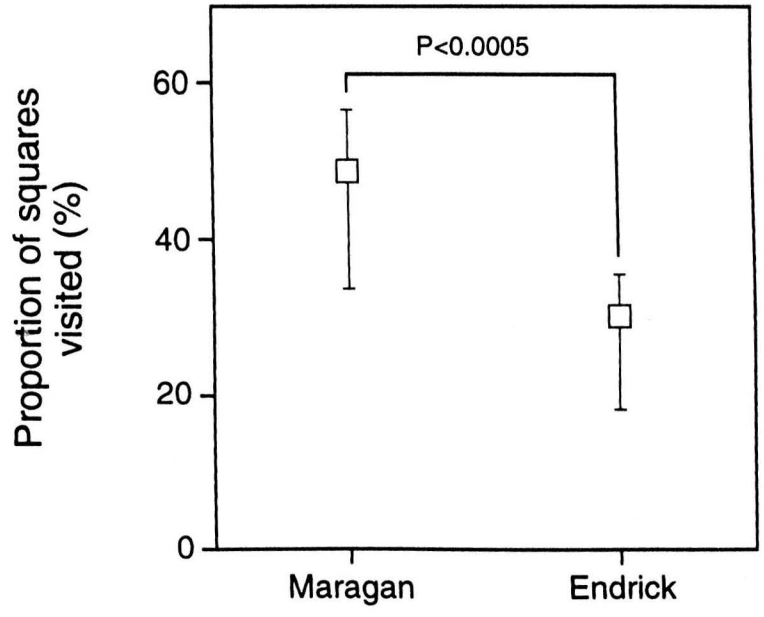

6
5.5
5
4.5
P<0.05
Elective Group Size
Maragan
Endrick

Figure 3.3 The expected (●) and observed (□) ratios of edge : centre squares occupied by minnows from Loch Maragan and the River Endrick. Observed values are medians, with error bars representing interquartile ranges (n=16 for Maragan, 13 for Endrick).

Figure 3.4 The proportion of basal 5cm x 5cm squares visited by minnows from Loch Maragan and the River Endrick. Values are medians, with error bars representing interquartile ranges (n= 16 for both Maragan and Endrick).


Ratio of edge:centre squares occupied
100
10
1
0.1
P<0.0001
P<0.005
P<0.05
Maragan
Endrick


Proportion of squares visited (%)
60
40
20
0
P<0.0005
Maragan
Endrick

3.3.2 Materials and methods

3.3.2.1 Collection and husbandry of fish

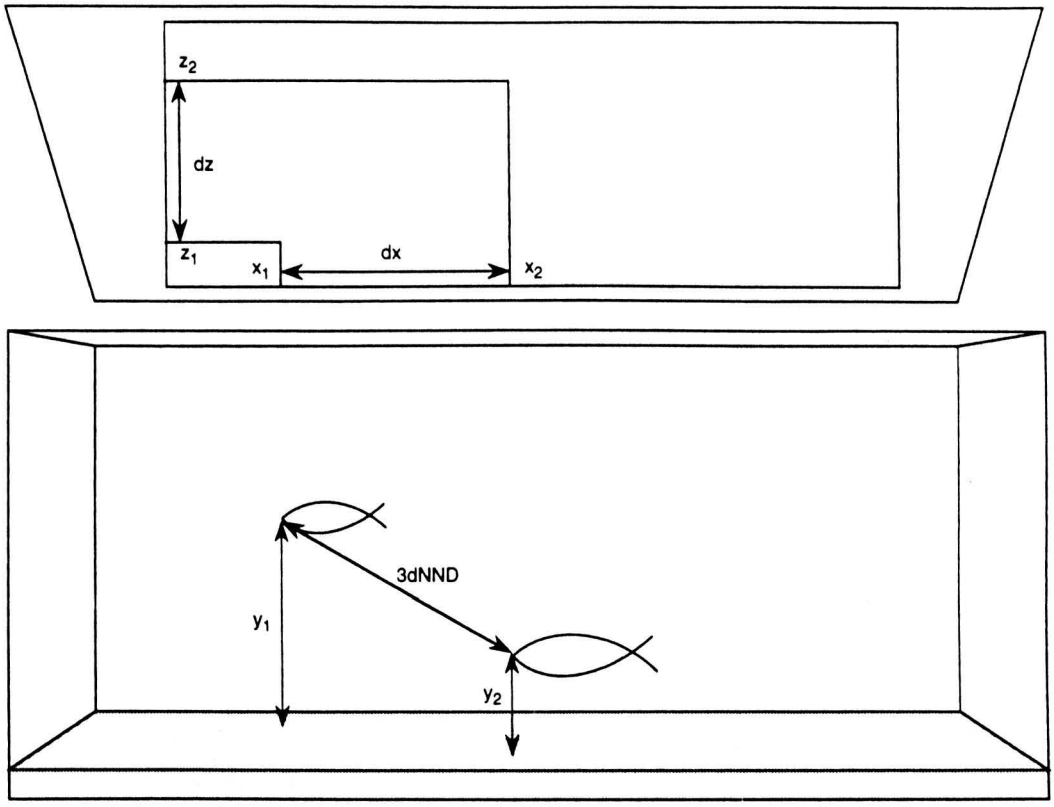
Minnows were hand netted from the River Endrick during late January 1993. They were immediately transferred to holding tanks in the laboratory and sorted into size classes ('small' [20-35mm], 'medium' [36-50mm]); a few fish belonging to a third size class ('large' [>50mm]) were also caught, but in numbers too small to form an experimental group, and so were returned. The small and medium-sized fish were maintained in laboratory aquaria for one month, as described in Experiment 3.1. After this time, three groups, each comprising six fish, were selected from each of the holding tanks containing the small and medium-sized fish and the six groups of fish were held separately in aerated aquaria for 24 hours before experimentation began. Feeding was continued as previously described in the holding tanks, and they were held under the same light regime. The individuals selected from the two size classes of fish used in this experiment differed significantly in length (mean fork $length_{small}$ ± SD = 23.5 ± 2.8mm (n=18); mean fork $length_{medium}$ ± SD = 44.1 ± 3.3mm (n=18), ANOVA, $F_{1, 35} = 415.14$, $P < 0.001$).

3.3.2.2 Experimental design

A 54-litre glass aquarium was again used as the experimental arena (Figure 3.1c), and as in Experiment 3.1 white coral sand formed the substrate. The tank was filled with dechlorinated, aerated tap water to a depth of 5, 10 or 15cm depending on the subsequent experimental trial, and was illuminated by a fluorescent tube, fitted with a diffuser, positioned above the tank. A cardboard screen surrounded the experimental tank, ensuring that no external movement could distract the fish, and a plane glass mirror (60 x 20cm), suspended above the tank at an angle of 45°, facilitated accurate recording of co-ordinates. A video camera, mounted on a tripod, was positioned so that the front elevation of the tank, as well as the image reflected by the angled mirror, was clearly visible

The six fish from one of the groups selected from the holding tanks were netted and transferred together to the experimental tank. Following a 20-minute settling period, filming began after the on-screen stopwatch had been started. The operator left the room and filming continued for 15 minutes, after which time the experiment was stopped and the fish were returned to their 'home' tanks. The experimental procedure was carried out with each group of six fish at 5, 10 and 15cm water depths, to allow for individual variation in shoaling tendency. Because each group of fish was subjected to all

Figure 3.5 A diagrammatic summary of the method used to calculate 3-dimensional nearest neighbour distances (3dNND) of fish in the experimental tank. The two sets of 3-dimensional co-ordinates of a fish and its nearest neighbour (x1, y1, z1 and x2, y2, z2) were recorded, and used to calculate their spatial differences in each plane (dx, dy, dz). The horizontal (or 'flat') x-z distance between the fish was calculated using trigonometry ($\mathbf{h_1}$), and to this was then added the vertical component (again using trigonometry) to determine the actual 3d NND ($\mathbf{h_2}$).

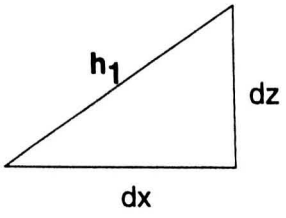


Calculation of 'flat' x-z distance

dx = x1-x2 dz = z1-z2

$$\tan = \frac{dz}{dx}$$

$$h_1 = \frac{dz}{\sin}$$

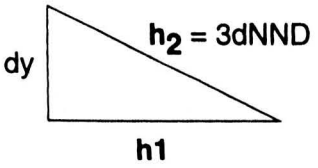


Calculation of 3dNND

dy = y1-y2

$$\tan = \frac{dy}{h_1}$$

$$h_2 \text{ (= 3dNND)} = \frac{dy}{\sin}$$



three water depth regimes, the order of treatments was randomised to prevent any learning effects affecting the results. With this in mind, no single group of six fish was subjected to the experimental condition more than once in 24h.

3.3.2.3 Video analysis

Measurement of three-dimensional nearest neighbour distance

The video film was analysed using the freeze frame facility on the VCR. Acetate screen overlays, onto which was drawn a grid corresponding to a 'real size' 1cm matrix, allowed the accurate recording of the x, y and z co-ordinates of individual fish. A 10 minute section of each 15 minute film was chosen for analysis, and the precise position of each fish in the tank was recorded at 30s intervals. The snouts of the fish were used as reference points, since they were easily distinguished from above and from the side. Recursive trigonometric methods (see Figure 3.5 and legend) were used to calculate the three-dimensional distance from each fish in the group to its to the nearest neighbour ('nearest neighbour distance', NND). Of the fish in the tank, the NNDs of those that were classified as 'shoaling' (see below for definition) were recorded, and the mean NND of each shoal analysed was calculated using the following equation:

$$\text{Mean NND} = \frac{\sum \text{NND}i}{n}.$$

(where NNDi = nearest neighbour distance of ith member classified as a shoal member and n = number of individuals in the shoal).

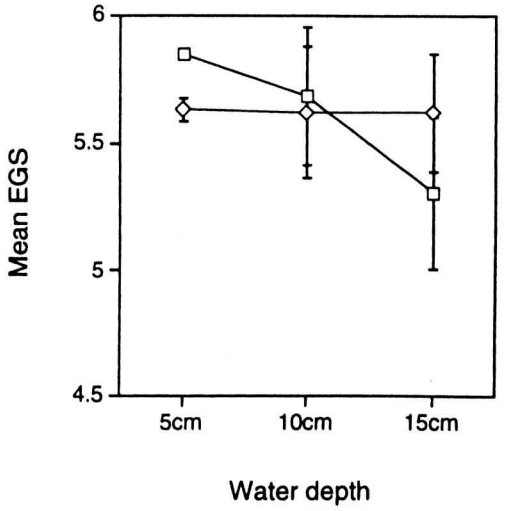
Shoal size

A fish was considered to be a member of a shoal if it occupied a position within four body lengths of its nearest neighbour (Magurran & Pitcher, 1987). Conversely non-shoaling fish were those that were separated from shoal members by a distance of greater than four body lengths. Shoal size in the experimental tank could therefore vary between two and six fish, and the number of members in the largest shoal observed in each analysed frame of film was recorded. Although the six fish generally formed a single shoal within the experimental tank, occasionally two separate shoals were formed, and in these cases the shoal with the greatest number of members was analysed.

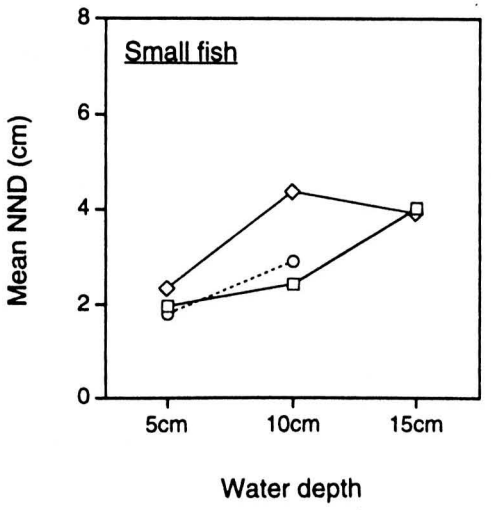
Figure 3.6 The mean elective group sizes (EGSs) of small (□) and medium-sized (◈) minnows under 5cm, 10cm and 15cm water depth regimes. Values shown are means of the 3 groups, with error bars representing standard deviations.

Figure 3.7 The mean nearest neighbour distances (NNDs) of minnow shoals under 5cm, 10cm and 15cm water depth regimes. a) The mean NNDs exhibited by three shoals of small minnows (mean length = 23.5mm) under each water depth regime. b) The mean NNDs exhibited by three shoals of medium-sized minnows (mean length = 44.1mm) under each water depth regime. Values shown are means of 20 observations. Standard errors, removed for clarity, are given in Table 3.1.

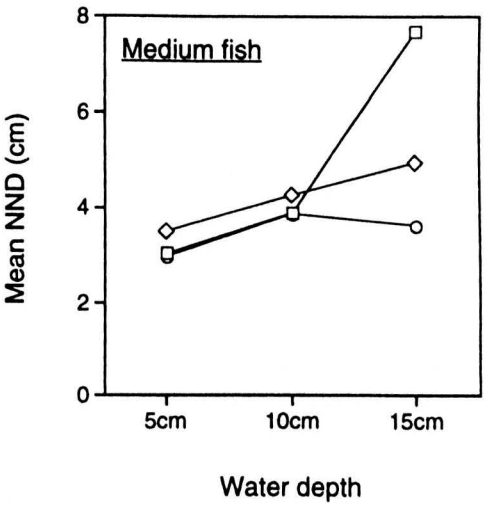
Figure 3.8 The proportion of time spent performing polarised schooling behaviour by small (□) and medium-sized (◇) minnows under 5cm, 10cm and 15cm water depth regimes. Values shown are means of the 3 groups, with error bars representing standard deviations.

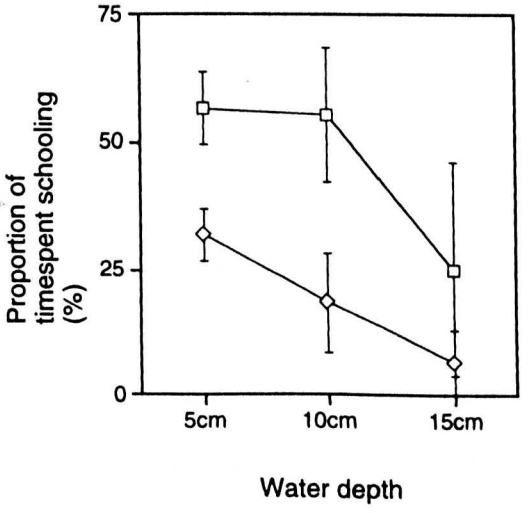

6
5.5
5
4.5
Mean EGS
5cm
10cm
15cm
Water depth

a)


Small fish
8
6
4
2
0
Mean NND (cm)
5cm
10cm
15cm
Water depth

b)


Medium fish
8
6
4
2
0
Mean NND (cm)
5cm
10cm
15cm
Water depth


75
50
25
0
Proportion of timespent schooling (%)
5cm
10cm
15cm
Water depth

Proportion of time spent shoaling / schooling

Once information regarding NND and shoal size had been extracted, the same 10 minute section of video tape was re-analysed to determine the proportion of time that the fish in the experimental tank spent performing each of three exclusive group behaviours: 'schooling', (defined as >3 fish performing synchronised, polarised shoaling), 'non-polarised shoaling' (where >3 fish were observed to associate closely, moving around the tank, though not schooling) and 'still' (where the fish remained motionless resting on the substrate). If the proportion of time spent performing 'still' behaviour (typically shown by distressed minnows) exceeded 10% of the filming period then the results of that trial were discarded.

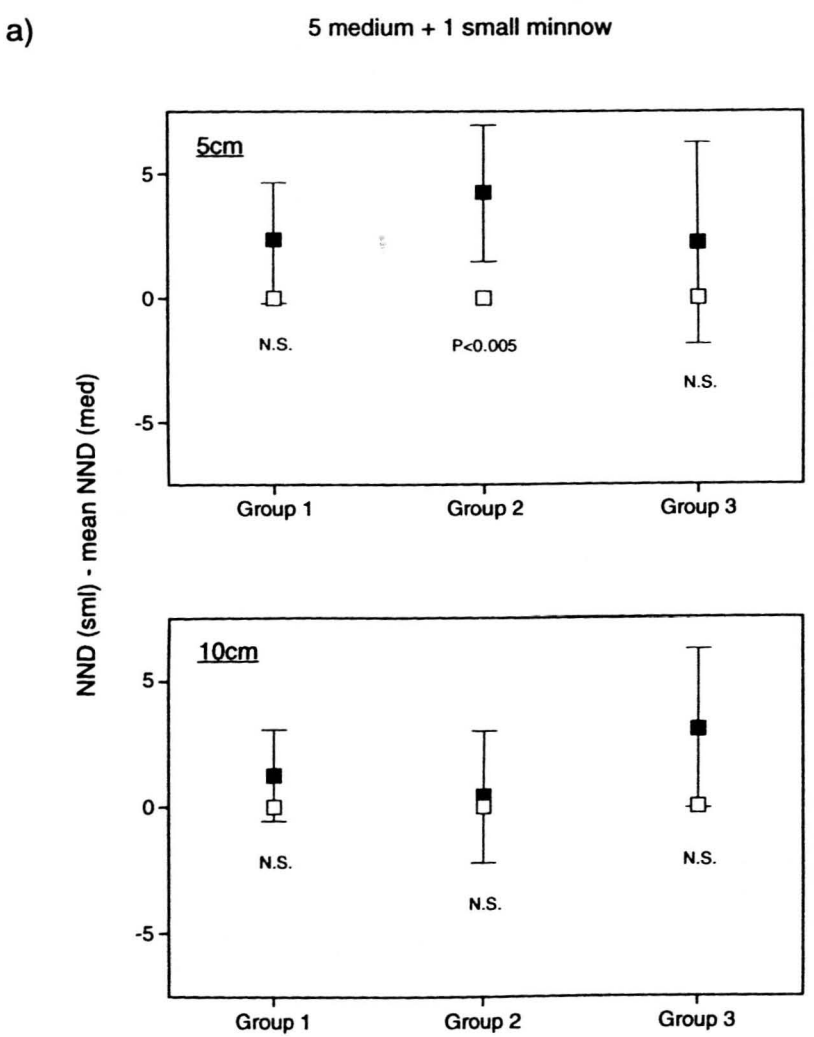
3.3.3 Results

Elective group size was independent of individual fish length and water depth, remaining high with a mean value of >5 in all runs of the experiment (see Figure 3.6 and Table 3.2 for results of Two-Way ANOVA). The majority of shoals observed in all experimental trials comprised all six fish in the tank.

Variation in the cohesiveness, measured as mean NND, of shoals formed by the separate six-fish groups within any particular [size class * water depth] trial meant that mean NND values from each group within a particular treatment could not be combined. Because of this, and also to avoid problems associated with pseudoreplication, combined means were taken from each experimental run (comprising approximately 20 shoal mean NNDs) and analysed by Two-Way ANOVA, using the General Linear Model (GLM) to allow for the unbalanced experimental design, with depth of water and size class as variables. Nearest neighbour distance increased with both water depth and the mean fork length of individual shoal members in the group (see Figure 3.7a and 3.7b and Table 3.1), demonstrating that fish tended to form more cohesive shoals in shallower water and that, on average, small fish had closer nearest neighbours than did medium-sized fish. The level of interaction between the two factors was found to be non-significant (see Table 3.2 for results of Two-Way ANOVA).

The proportion of time the fish in each group spent performing polarised schooling behaviour was also dependent on both individual fish length and water depth, with shoals of small fish polarising more frequently than shoals of medium-sized individuals, and both sizes of fish schooling more often in shallower water (see Figure 3.8 and Table 3.2 for results of Two-Way ANOVA).

Figure 3.9 The nearest neighbour distances (NNDs) exhibited by phenotypically 'odd' individuals in otherwise homogenous shoals. a) The effect of size oddity. b) The effect of species oddity. In both cases, the observed (■) and expected (□) values for the difference between the NND of the small minnow in the shoal and the mean NNDs of the rest of the shoal are shown. 'Observed' values are means of 3 trials, with error bars representing standard deviations, and P values refer to significance levels associated with paired t-tests (see text).


a)
5 medium + 1 small minnow
NND (sml) - mean NND (med)
5cm
5
0
-5
N.S.
P<0.005
N.S.
Group 1
Group 2
Group 3
10cm
5
0
-5
N.S.
N.S.
N.S.
Group 1
Group 2
Group 3

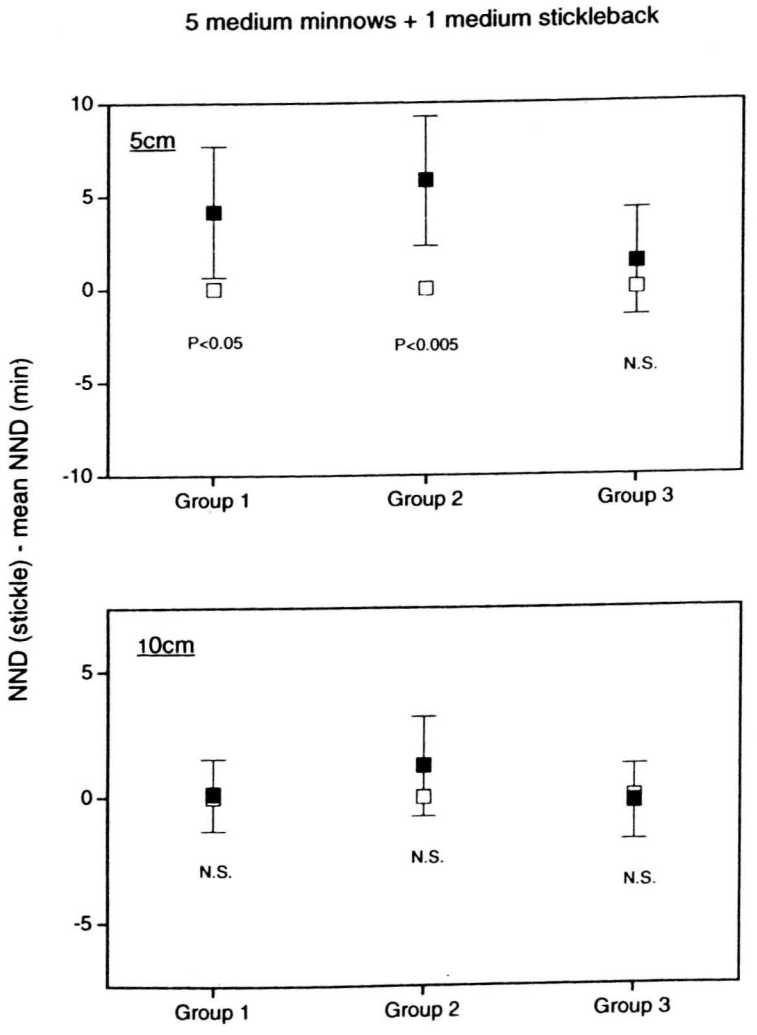

b)
5 medium minnows + 1 medium stickleback
NND (stickle) - mean NND (min)
5cm
10
5
0
-5
-10
P<0.05
P<0.005
N.S.
Group 1
Group 2
Group 3
10cm
5
0
-5
N.S.
N.S.
N.S.
Group 1
Group 2
Group 3

Table 3.1 The mean nearest neighbour distances (NNDs) exhibited by three groups of small and three groups of medium-sized minnows, shoaling under the three experimental water depth regimes (* = data unavailable).

Size class	Water depth	Group	*n*	Mean NND (cm)	SEM
Small	5cm	1	20	1.95	0.18
		2	20	2.33	0.21
		3	20	1.81	0.12
	10cm	1	20	2.43	0.16
		2	26	4.36	0.33
		3	21	2.91	0.27
	15cm	1	21	4.05	0.34
		2	26	3.92	0.29
		3	*	*	*
Medium	5cm	1	20	3.03	0.27
		2	21	3.49	0.26
		3	20	2.97	0.34
	10cm	1	22	3.90	0.38
		2	20	4.26	0.32
		3	20	3.83	0.32
	15cm	1	22	7.70	0.81
		2	20	4.92	0.39
		3	20	3.59	0.26

Table 3.2 Results of Two-Way ANOVAs on various aspects of the shoaling behaviour of small and medium-sized minnows under three water depth regimes. Significant P-values are shown in **bold type**. Percentage data were arcsin-transformed prior to statistical analysis.

Factor		mean NND		shoal EGS		% time spent schooling	
	df	F	P	F	P	F	P
Water depth	2,16	4.41	**0.039**	0.89	0.438	9.69	**0.004**
Size class	1,16	5.58	**0.038**	0.00	0.950	20.89	**<0.001**
*Water depth * size class*	2,16	0.35	0.710	0.80	0.473	0.47	0.637

3.4 EXPERIMENT 3.3 : THE EFFECT OF VISUAL 'ODDITY' ON SHOALING BEHAVIOUR

3.4.1 Introduction

Both laboratory-based experiments (Pitcher *et al.* 1986b; Ranta & Lindström, 1990; Krause & Godin, 1994) and field studies (Springer, 1957; Ehrlich & Ehrlich, 1973; Alevizon, 1976; Krause *et al.*, in press) have demonstrated that shoals of fish tend to be composed of phenotypically uniform individuals. The majority of naturally-forming shoals comprise individuals of a single, conspecific year class, and so are uniform with respect to both size and appearance. However, whether the sorting process, a prerequisite to the existence of such uniform groups, is active or passive in fish shoals is unclear.

Active sorting may be based on either the reluctance of non-uniform individuals to join a shoal, or the exclusion of the same by phenotypically-matched shoal members. An experiment was designed to investigate the effects of two types of visual non-uniformity ('oddity') on the shoaling behaviour of fish. By examining the shoaling behaviour of single small minnows and medium-sized sticklebacks with shoals of medium-sized minnows, the experiment aimed to examine the effects of both size-oddity and species-oddity, and to provide an indication of their relative importance in shaping shoal structure.

3.4.2 Materials and methods

3.4.2.1 Collection and husbandry of fish

Minnows and three-spined sticklebacks were hand-netted from the River Endrick and maintained in laboratory aquaria as described above. Six groups of six fish were selected from the holding tanks: three comprising five size-matched medium-sized minnows (mean lengths 43.2mm, 43.4mm, 43.2mm) and a single small minnow (lengths, 26mm, 27mm, 29mm) and three comprising five size-matched medium-sized minnows (mean lengths 42.3mm, 42.9mm 44.0mm) and a single medium-sized stickleback (42mm, 39mm, 42mm). The six groups were held separately in aerated aquaria for 24 hours before being netted and transferred to the experimental tank, where their behaviour was video-taped, as described in Experiment 3.2.

3.4.2.2 Experimental design

As described for Experiment 3.2

3.4.2.3 Video analysis

The video tape was analysed in the same way as described for Experiment 3.2. Shoal size data were recorded at sixty second intervals, and when the fish adopted a 5+1 configuration, with 5 individuals taking up a position within 5 group mean body lengths of one another and one fish remaining outside of the shoal, the frequency with which either the small minnow, or the medium-sized stickleback (i.e. the odd fish in each group) was the lone fish was calculated. When the odd fish was a shoal member, its NND was measured and compared with the mean NND calculated for the non-odd fish within the shoal.

3.4.3 Results

3.4.3.1 The effects of size oddity on shoaling

Water depth had no effect on the mean shoal size formed by the three groups of six fish in the experimental tank (Mean shoal $size_{5cm}$ ± SEM = 5.18 ± 0.31; Mean shoal $size_{10cm}$ = 5.15 ± 10, t-test t=0.10, df=2, P=0.93, N.S.). When the group adopted a 5+1 configuration there was no consistent effect of size oddity on shoal membership / non-membership. The small minnow was a loner more often than expected by chance in two of the six trials carried out at 5cm and 10cm water depth, but in one of the trials (Group 1, 10cm) odd fish were loners significantly less often than expected by chance (see Table 3.3 for results of χ^2 tests).

When the odd small minnow was a shoal member, its NND was significantly greater than the mean NND of the medium-sized minnows within the shoal in only one of the six experimental trials (Paired t-tests, Group 1_{5cm} t=1.96, n=18, p=0.067, N.S.; Group 1_{10cm} t=1.48, n=20, p=0.16, N.S.; Group 2_{5cm} t=3.27, n=18, p=0.0045; Gp2_{10cm} t=0.32, n=19, p=0.75, N.S.; Group 3_{5cm} t=1.20, n=12, p=0.25, N.S.; Group 3_{10cm} t=2.15, n=13, p=0.053, N.S.; Figure 3.9a).

3.4.3.2 The effect of species oddity on shoaling

Water depth had no significant effect on the mean shoal size formed by the three groups of 5 medium-sized minnows and single medium-sized stickleback in the experimental tank (Mean shoal $size_{5cm}$ ± SEM = 5.33 ± 0.07; Mean shoal $size_{10cm}$ = 5.80 ± 0.06, t-test t= –3.02, df=2, P=0.057, N.S.). When the group adopted a 5+1 configuration, with 5 individuals taking up a position within 5 group mean body lengths of one another and one fish remaining outside of the shoal, the stickleback was the

'loner' more often than expected by chance in each of the trials carried out at 5cm and 10cm depth respectively (see Table 3.4 for results of χ^2 tests).

When the 'odd' medium-sized stickleback was a member of a shoal, no consistent effect of oddity on NND was observed; the NND of the stickleback was significantly greater than the mean NND of the medium-sized minnows individuals within the shoal in just two of the six experimental trials (Paired t-tests. Group 1_{5cm} t=2.50, n=18, p=0.023; Group 1_{10cm} t=0.14, n=16, p=0.89, N.S.; Group 2_{5cm} t=3.55, n=16, p=0.0029; Group 2_{10cm} t=1.28, n=19, p=0.22, N.S.; Group 3_{5cm} t=1.03, n=17, p=0.32, N.S.; Group 3_{10cm} t= -0.34, n=20, p=0.74, N.S.; Figure 3.9b).

Table 3.3 Results of Chi-square tests to determine whether lone fish observed during experimental trials were the 'odd' small minnows more often than expected by chance. Significant P-values are shown in **bold type**.

Depth	Group	n	Expected	Observed	χ^2	P
5cm	1	2	0.33	1	1.63	N.S.
	2	5	0.83	1	0.04	N.S.
	3	10	1.70	7	19.91	**<0.001**
10cm	1	20	3.33	0	4.00	**<0.05**
	2	5	0.83	0	1.00	N.S.
	3	5	0.83	4	14.52	**<0.001**

Table 3.4 Results of Chi-square tests to determine whether lone fish observed during experimental trials were the 'odd' sticklebacks more often than expected by chance. Significant P-values are shown in **bold type**.

Depth	Group	n	Expected	Observed	χ^2	P
5cm	1	3	0.50	2	5.40	**P<0.05**
	2	4	0.67	4	19.89	**P<0.001**
	3	6	1.00	3	4.80	**P<0.05**
10cm	1	1	0.17	1	4.88	**P<0.05**
	2	3	0.50	2	5.40	**P<0.05**
	3	0	-	-	-	-

3.5 DISCUSSION

3.5.1 Experiment 3.1: Population differences in shoaling behaviour

In Experiment 3.1, under identical experimental conditions, minnows from the River Endrick exhibited larger EGSs, occupied edge positions more frequently and moved around the experimental tank to a lesser extent than similarly-sized minnows from Loch Maragan. This behaviour in controlled laboratory experiments suggests that minnows from the River Endrick have a stronger tendency to form shoals and a reduced propensity to explore the novel environment than their counterparts from Loch Maragan. Since fish from each population were exposed to identical conditions following capture and in the laboratory prior to experimentation, the differences demonstrated in these laboratory experiments most likely reflect variation in the environmental variables that shaped the development of schooling behaviour in the respective fish populations prior to capture.

The extent to which individuals in fish populations shoal is known to be dependent on a wide range of ontogenetic, evolutionary and temporally-fluctuating variables (e.g. hunger state). Extensive research carried out on various wild populations of the guppy, *Poecilia reticulata*, has demonstrated that local predation regimes are important determinants of the social strategy adopted by a particular population (Magurran, 1993). Geographically-isolated guppy populations suffer diverse predation regimes; the major predation threat in many habitats is the pike cichlid *Crenicichla alta*, an ambush predator, whereas in other populations, the main predation threat is from a palaemonid prawn that uses chemical and tactile cues in addition to vision when hunting (Magurran & Seghers, 1990b). Guppies from habitats where pike cichlids are sympatric form cohesive schools (Seghers, 1974), and stream observations have shown that in these populations, the majority (>90%) of guppies are members of shoals (Magurran & Seghers, 1991). This contrasts with populations sympatric with the prawn, where 70% of the individuals observed were solitary (Magurran & Seghers, 1991). The success of ambush predators is known to be reduced against shoaling prey (Neill & Cullen, 1974), whereas grouping would appear to be a less useful strategy against predators that rely on chemical cues such as the prawn (Magurran, 1993), so the observed patterns of shoaling in guppy populations appears to be adaptive.

The increased frequency with which minnows from Loch Maragan 'broke ranks', either singly or in small groups, compared with minnows from the River Endrick may be attributable to differences in the local predation regimes experienced in their natural habitats. Small prey fish in the River Endrick, such as minnows and sticklebacks, are subject to a wide variety of predators, including

piscivorous fish and birds, and it is likely that shoaling has a more important antipredator function in the ecology of these fish than in that of minnows from Loch Maragan, which appear to be subjected to only a limited risk of predation (see Chapter 2). This observation supports the results of previous studies carried out by Magurran and co-workers, who found stronger schooling tendency in minnow populations sympatric with pike when compared with predator-naïve populations (Magurran, 1986a; Magurran, 1989; Magurran & Pitcher, 1987).

Variation in local predation levels in their natural habitats may also be responsible for the observed differences in tank use by individuals from the two populations. The more cautious, timid behaviour of minnows from the River Endrick population, which moved around the experimental tank to a lesser extent, and remained close to the edges of the tank more often than conspecfics from Loch Maragan, may reflect their higher natural perceived risk of predation. Movement around a novel habitat has potential costs and benefits for exploring fish, since valuable information can be gained regarding the local distribution of food, shelter and predators. Conversely, the probability that that they might encounter a foraging predator is also increased when individuals explore a new habitat. In the experiments described above, group size, a factor known to govern the timidity of fish in shoals, was controlled and so the observed variation in movement around the experimental tank is probably reflective of the degree of predation threat that they perceive themselves to be under. Where predators are abundant, neophobia (avoidance of new objects) and agrophobia (avoidance of open areas) would be expected to be maximised in prey organisms. The greater propensity of minnows from Loch Maragan to explore their new environment following transfer from holding tanks to the experimental aquarium, and their increased occupancy of central, more open positions within the tank strongly suggest that these fish perceive the predation threat differently from River Endrick minnows.

However, the extent to which shoaling behaviour is exhibited by individuals of any species is not only controlled by the type and abundance of predators normally encountered. Whether fish form shoals is dependent on a trade-off between the diverse benefits and costs involved with being a shoal member (Pitcher & Parrish, 1993; Barber *et al.*, 1995; Chapter 4) and clearly there are problems when attempting to account for variation in the behaviour of individuals from two very different habitats. For instance, variation in the abundance, distribution or type of prey at either site may favour different levels of social behaviour (e.g. Smith & Warburton, 1992). Alternatively, if there is a hydrodynamic advantage associated with the formation of polarised schools, there may also have been a greater

selection for the development or maintenance of cohesive schooling ability and stronger shoaling tendency in the River Endrick population, where they constantly experience moving water conditions, compared with the population at Loch Maragan which inhabit a still water habitat. However, until a large cross-population study is undertaken, examining the shoaling behaviour of individuals from a wide variety of well-documented populations, any of these proposed hypotheses must be regarded as purely speculative.

Although it is evident that various components of the shoaling and exploratory behaviour differ between individuals from the two populations, clearly, difficulties arise when attempting to fully understand this variation without a complete knowledge of the evolutionary forces which have shaped their respective behavioural adaptations. Nevertheless, because of population-level variation in the shoaling behaviour of minnows, it is at least clear that future work on the shoaling and schooling behaviour of parasitised and non-parasitised individuals will need to be carried out with fish from within the same population in order to make a meaningful assessment of any observed differences in behaviour.

3.5.2 Experiment 3.2 : The effect of depth and individual size on shoaling behaviour

In natural habitats, minnows frequently inhabit shallow water, particularly during summer months when large size-matched shoals congregate in the shallow reaches of streams and rivers, which warm up quickly and may provide the best conditions for feeding and growth, but where water depth may only be a few centimetres (Maitland and Campbell, 1992). Occupancy of such shallow habitats may also take minnows out of the reach of predatory fish, which are generally restricted to deeper water because of their larger size, yet it is likely that they become increasingly more visible, and therefore susceptible, to avian, mammal and possibly invertebrate predators. Results obtained from this experiment suggest that the use of shallow water in experiments is valid, since it does not detrimentally affect the behaviour of the fish. Shoaling tendency is maintained, even enhanced, in depths of only 5cm, and shoals formed by both small and medium-sized minnows become more cohesive in shallow water. The strong shoaling behaviour observed in these shallow depths perhaps demonstrates that the fish perceive themselves to be at a greater risk of predation in such conditions. This hypothesis is strengthened by the observation that shoals spent more time polarising in shallower depths of water, a behaviour known to maximise levels of confusion in potential predators (Pitcher & Parrish, 1993).

Importantly, however, mean shoal size was not affected by the reduced water depths, and even when the water depth in the experimental tank was reduced to just 5cm, individuals only rarely broke ranks.

3.5.3 Experiment 3.3 : The effect of individual 'oddity' on shoaling behaviour

In Experiment 3.3, the effects of visual oddity on shoaling behaviour were investigated experimentally by studying shoals composed of medium-sized minnows and either a single small minnow (size-oddity) or a single medium-sized stickleback (species oddity). The effect of size oddity on shoal membership and NND within a shoal were inconsistent, and in most trials not significant; however, the effects of species oddity were more substantial, and more uniform. When lone fish were observed in the experimental tank, they were significantly more likely to be the 'odd' sticklebacks than minnows in all of the trials undertaken. Although these preliminary results require further substantiation, they initially suggest that being a similarly-sized member of a different species is more prohibitive to shoal membership than being a differently-sized member of the same species.

If the value of shoal membership is determined, at least in part, by an individual's phenotypic similarity to other shoal members (see Chapter 4, section 4.1.2) then fish that are in some way visually-odd might be expected to show a reduced tendency to join shoals comprised of phenotypically-uniform fish. Odd fish may be reluctant to join uniform shoals for several reasons. Firstly, because their nutritional requirements may differ (Lindström & Ranta, 1993; Krause 1994), odd individuals might not gain the foraging benefits generally associated with shoal membership (see Chapter 1, section 1.2.3.1). Secondly, phenotypically-odd individuals in otherwise homogenous groups are known to be more susceptible to certain types of predators (Pielowski, 1959; Hobson, 1963; Mueller, 1972, 1975; Ohguchi, 1978, 1981; Visser, 1982; Wolf, 1985; Landeau & Terborgh, 1986; Theodrakis, 1989) since they do not benefit from the confusion and dilution effects as phenotypically-similar fish do. Shoaling for odd fish is therefore less likely to be a viable antipredator strategy. In addition, it is thought that by maximising uniformity, individuals become more effective at evading predators, since escape responses become more efficient when all shoal members are phenotypically-matched (Pitcher & Parrish, 1993).

An exception to the 'phenotype-matching' that normally occurs in social groups is shown by the often large foraging shoals of cyprinids in freshwater habitats, where several species may join up to maximise foraging efficiency (see Pitcher & Parrish, 1993). However, experimental studies have shown that when such mixed species shoals are placed under predation threat they either split up into

single-species shoals, or odd individuals abandon the shoal altogether (Wolf, 1985; Allan & Pitcher, 1986; Theodrakis, 1989), further strengthening the hypothesis that uniformity maximises the antipredator function of shoals.

3.6 SUMMARY

- Methods for the quantification of shoaling behaviour were developed and tested, generating experimental data on the effects of water depth, individual size, population differences and oddity on various aspects of shoal structure.
- Population-level variability in the shoaling behaviour exhibited by minnows from two sites in central Scotland was discovered; possible reasons for these differences, based on the ecological disparity between the two sites, are suggested.
- The shoaling tendency of minnows was shown to be dependent on water depth, with both small and medium-sized fish forming more cohesive shoals, and schooling more frequently, as water depth was reduced. Individual size was also found to be important in determining how often fish formed schools, with small fish polarising more frequently than medium-sized fish for any particular water depth.
- The use of shallow water depths to reduce shoaling to two dimensions in experimental systems was vindicated, since such conditions were found not to impair the formation of shoals, or their structure.
- The effect of size- and species-oddity on the shoaling behaviour of individual fish was investigated. Size-oddity appeared to have little influence on shoaling behaviour in the experimental trials, but the effects of species-oddity were more marked. Medium-sized three-spined sticklebacks, when placed in a tank with uniform medium-sized minnows, showed a consistent, reduced tendency to join the shoal.

Chapter 4. The effects of hunger and helminth parasitism on the shoaling decisions of small freshwater fish

4.1 INTRODUCTION

4.1.1 Animals as decision-makers

One approach to the study of animal behaviour involves viewing the study organism as an economic decision-maker whose behaviour is adapted to maximising its own inclusive fitness (i.e. the total number of genes passed on to future generations, primarily by the production of offspring). This approach has provided insight into the adaptive nature of animal behaviour, and is currently a commonly-used tool of behavioural ecologists, allowing the development of our understanding of why animals do what they do, and the potential ecological and evolutionary significance of their actions. Although the term 'decision-making', when applied to non-human animals, may seem apparently anthropomorphic, it is generally used in this context as an abbreviation for the existence of an evolutionarily-successful preference for one particular behaviour over possible alternatives under any particular set of internal and external conditions. As such, the concept of decision-making in animals does not require that organisms apply conscious thought before making a behavioural decision, and no level of cognition is assumed. If animals do behave in order to maximise their lifetime inclusive fitness, then we should expect that the outcome of any behavioural decisions made during the course of that life to be dependent on the ratio of the potential 'costs' (negative implications) and 'benefits' (positive implications) of performing any of the alternatives.

Although there are some instances when only one behavioural option could realistically be seen to serve to increase lifetime reproductive success (e.g. avoiding a striking predator), animals are more commonly placed in situations where each available option may have both benefits and costs for their fitness. In order to ensure fitness maximisation, individuals should choose the option that maximises the ratio of benefits gained to costs endured, and trade-offs frequently need to be made between the costs and benefits associated with making a particular behavioural decision.

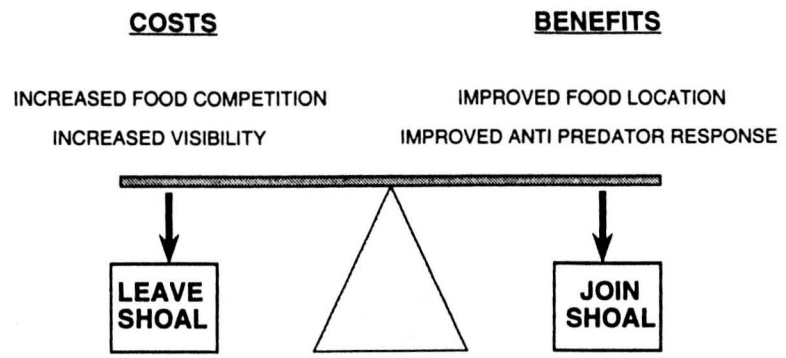
4.1.2 The shoaling behaviour of prey fish species : a cost-benefit analysis

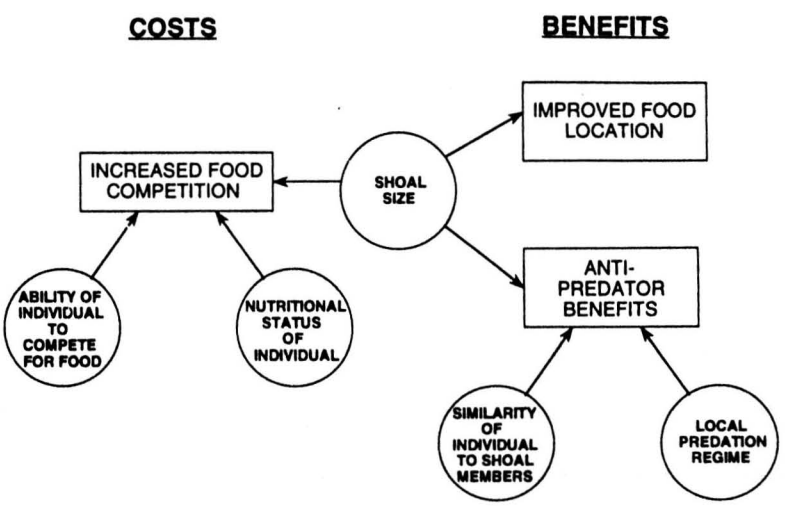
The formation of natural aggregations, or shoals (*sensu* Pitcher, 1983) is an effective antipredator adaptation of many prey fish species (Magurran, 1990), but a great deal of inter-population variation in shoaling tendency has been documented in a variety of fish species (see Magurran, 1993, for a review). However, even within a single population shoaling is unlikely to be equally attractive for all individuals. This is because the costs and benefits of shoal membership for individual fish depend

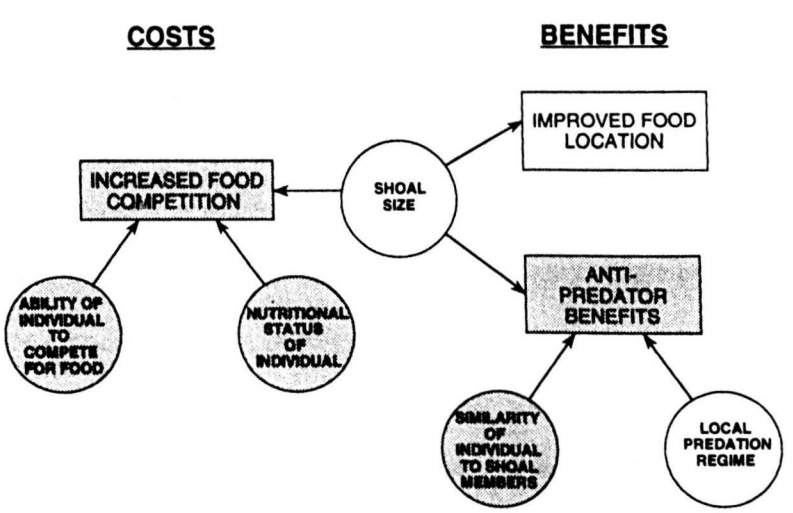
Figure 4.1a A diagrammatic summary of the main costs and benefits associated with shoal membership for small prey fish species. If shoaling is adaptive, then when the benefits outweigh the costs, an individual fish should remain with, or join a shoal of conspecifics, and when the costs outweigh the benefits, an individual fish should leave, or not join, the shoal.

Figure 4.1b A diagrammatic summary of the various ecological and physiological factors (circles) that may indirectly affect the outcome of shoaling decisions taken by small prey fish by altering the costs and benefits (rectangles) involved with joining or remaining with a shoal.

Figure 4.1c A diagrammatic summary of the various ecological and physiological factors that may indirectly affect the outcome of shoaling decisions taken by small prey fish, indicating those factors that may be affected by *Schistocephalus solidus* infection (shaded).


COSTS
BENEFITS
INCREASED FOOD COMPETITION
INCREASED VISIBILITY
IMPROVED FOOD LOCATION
IMPROVED ANTI PREDATOR RESPONSE
LEAVE SHOAL
JOIN SHOAL


COSTS
BENEFITS
INCREASED FOOD COMPETITION
SHOAL SIZE
IMPROVED FOOD LOCATION
ANTI-PREDATOR BENEFITS
ABILITY OF INDIVIDUAL TO COMPETE FOR FOOD
NUTRITIONAL STATUS OF INDIVIDUAL
SIMILARITY OF INDIVIDUAL TO SHOAL MEMBERS
LOCAL PREDATION REGIME


COSTS
BENEFITS
INCREASED FOOD COMPETITION
SHOAL SIZE
IMPROVED FOOD LOCATION
ANTI-PREDATOR BENEFITS
ABILITY OF INDIVIDUAL TO COMPETE FOR FOOD
NUTRITIONAL STATUS OF INDIVIDUAL
SIMILARITY OF INDIVIDUAL TO SHOAL MEMBERS
LOCAL PREDATION REGIME

on various environmental, physiological and morphological factors that fluctuate over time, and as they do so we may expect the subsequent shoaling behaviour of individuals to change in accordance.

Although shoal membership confers antipredator and foraging advantages, these benefits are countered by the costs associated with group living, including competition for mates, increased risk of disease transmission and increased visibility to certain predators (see Chapter 1 for a review of the costs and benefits of shoal membership). However, for most shoaling fishes, the most important cost of group living is probably the increased competition for limited food resources (Pitcher & Parrish, 1993).

If organisms have evolved to maximise their own fitness (Hamilton, 1964), then the decision of individual fish to join, remain with or leave a shoal should be expected to reflect the relative values of these costs and benefits (Figure 4.1a), and the resultant behaviour will therefore be the product of various trade-offs between these conflicting demands. One such trade-off that can be envisaged involves the increased competition costs of joining a large conspecific shoal versus the antipredator benefits of doing so. Evidence for the existence of this particular trade-off has been provided by van Havre & FitzGerald (1988), who experimentally manipulated the hunger levels of three-spined sticklebacks (*Gasterosteus aculeatus* L.) and found that hungry fish preferred to join shoals of 15 rather than 45 fish, whilst satiated fish spent more time with the larger shoals. In a natural habitat, however, it is unlikely that a fish will be within visual range of two differently-sized shoals between which it has a choice of joining, as naturally-occurring shoals have been shown to be optimally sized for any particular habitat (FitzGerald & van Havre, 1985).

4.1.3 Potential causes of variation in individual shoaling behaviour

The perceived ratio of costs endured : benefits gained, and therefore the value of shoal membership, is clearly subject to considerable variation, both temporally, for any particular individual, and also between individuals at any given time. Figure 4.1b shows, diagrammatically, some of the factors that may affect the decision of an individual fish to join a shoal of conspecifics.

In natural populations, individual fish show considerable variation in dominance and competitive ability (see review by Magurran, 1993). Since competitively-successful individuals are known to be less affected by higher competition levels (Rubenstein, 1981; Milinski, 1982), the higher food competition costs encountered when joining a shoal are likely to be more important to nutritionally-stressed, poor competitors than to well-fed dominant fish. In order to receive an adequate

food supply, fish of low competitive ability may need to leave the shoal, temporarily or permanently, to exploit individual foraging opportunities.

Many of the antipredator benefits of shoaling depend on all shoal members having identical behaviour and appearance, since individuals that are distinguishable from the rest of a group are known to suffer a higher risk of predation (Ohguchi, 1981; Landeau & Terborgh, 1986), and so phenotypic similarity to other shoal members is likely to be an important consideration when shoaling decisions are made. Although no two individuals of any species are ever completely identical in appearance, certain types of phenotypic 'oddity' - such as deviant size, colour, shape, behaviour or movement - make individuals exhibiting these characters highly visible when observed against a background of homogeneity. Such oddity is frequently associated with illness, disease, physical injury or parasite infection.

4.1.3 The importance of shoaling in the ecology of minnows and sticklebacks

European minnows and three-spined sticklebacks are the most abundant freshwater prey fish species in the UK, and feature significantly in the diets of piscivorous fishes, birds, reptiles, mammals and invertebrates (Giles, 1981; Maitland & Campbell, 1993; Reimchen, 1994). They occupy a wide variety of freshwater habitats and are frequently found sympatrically. Both species have well-developed antipredator responses. Minnows frequently inhabit shallow, open water with little cover, and the protection offered by the formation of cohesive shoals is their primary antipredator defence mechanism. In the UK, freshwater sticklebacks typically inhabit less open habitats, and the shoals they form tend to be less cohesive than those formed by minnows. It is possible that, because of their protective spines and body armour, which are an effective deterrent against predators (Hoogland *et al*, 1957; Reimchen, 1994), sticklebacks rely less on the group protection offered by the shoal, and more on individual protection.

4.1.4 Parasite-associated phenotypic variation

In habitats that facilitate their transmission, a proportion of individuals in three-spined stickleback and minnow populations are infected with the parasitic plerocercoid larvae of the pseudophyllidean cestodes *Schistocephalus solidus* and *Ligula intestinalis* respectively (Kennedy, 1974; see Chapter 2 for details of *L. intestinalis* infection). Both parasites grow rapidly in the body cavity of

infected fish, and cause marked swelling of the abdomen (see Chapter 6). The metabolic demands of growing *S. solidus* plerocercoids (Walkey & Meakins, 1970) reduce their hosts' energy reserves (Pascoe & Mattey, 1977) and have been demonstrated to cause an increased requirement for oxygen (Lester, 1971). In addition, fish that harbour heavy infections exhibit impaired swimming movements, altered foraging and reduced antipredator behaviours (see Milinski, 1990 and Barber & Huntingford, in press, for reviews of *S. solidus*-associated changes in stickleback behaviour) and, in some populations, infection is associated with a deterioration of skin pigment, causing localised albinism (LoBue & Bell, 1993). Infection with such parasites is clearly associated with phenotypic changes that make infected individuals appear 'odd' when compared with uninfected conspecifics. For infected fish, such changes have the potential to alter the value of shoal membership. Many predators are known to prey selectively on odd individuals in otherwise homogeneous groups (see references in Chapter 3, section 3.5.3), and so increased predator protection through the dilution and confusion effects may not be available to infected fish. Thus, parasite-associated morphological modification, allied with the apparent reduced competitive ability (see Chapter 6) and the higher nutritional requirement of infected hosts may potentially have a significant effect on their shoaling behaviour by changing the costs and benefits associated with group membership (see Figure 4.1c).

4.1.5 Objectives

The specific aims of this chapter are:

- To investigate natural differences in the shoaling behaviour of two freshwater fish species; namely minnows and three spined sticklebacks.
- To investigate the hypothesised trade-off between individual foraging and shoaling by examining the shoaling decisions of minnows and sticklebacks under different feeding regimes.
- To investigate the effects of the parasitic cestode *S. solidus* on the shoaling decisions of their stickleback hosts under different feeding regimes.

4.2 MATERIALS AND METHODS

4.2.1 Supply and husbandry of animals

Minnows from the River Endrick at Killearn, Stirling District (see Chapter 3 for site description) and three-spined sticklebacks from Inverleith pond, Edinburgh (Grid ref. 74N 27E O.S.

Second Series Sheet 66) were hand netted and transferred to the laboratory. Inverleith pond is situated in an urban park on the outskirts of Edinburgh, and is known to hold a dense population of sticklebacks (Tulley & Huntingford, 1987; Tierney, 1991). The fish were kept in separate aerated aquaria at 15°C under a 12:12 light:dark photoperiod for two months prior to experimentation, and fed daily on a mixture of live and frozen bloodworm (*Chironomus* sp.). A week prior to experimentation, size-matched minnows and both uninfected and *S. solidus*-infected sticklebacks were selected from their holding tanks, individually marked (minnows: alcian blue dye marks (Kelly, 1967); sticklebacks: coloured dorsal spine rings) under Benzocaine anaesthesia, and housed in separate aquaria under the same conditions as above. A previous study of *S. solidus*-infected sticklebacks, carried out by Tierney and co-workers (Tierney *et al*, 1993), demonstrated that altered host behaviour was only evident when plerocercoids reached a threshold weight of 50mg (the weight at which they become infective to the definitive host [Tierney & Crompton, 1992]). In order to ensure that only *S. solidus*-infected fish harbouring plerocercoids of a size infective to the definitive host were tested in the present study, only moderately heavily infected individuals were selected from the stock tanks.

The marked fish were exposed to one of three feeding regimes; satiation, 24h food deprived and 48h food deprived. Under the satiation regime, fish were fed excess food until immediately before the trial; 24h food-deprived fish were not fed on the day of the trial and 48h food-deprived fish were not fed on the day of the trial, nor on the day preceding that. The fish in the captive shoal (see below) were selected from the same stock tanks, introduced to the experimental environment a week before the trials began, and fed daily on the same diet as previously. The fork lengths of the fish forming the shoals did not differ significantly from that of test fish (mean fork lengths ± S.D; minnows: shoal = 42.3 ± 2.2mm, test fish = 42.4 ± 2.4mm; sticklebacks: shoal = 52.3 ± 2.3mm, uninfected test fish = 51.9 ± 2.2mm, infected test fish, 49.0 ± 4.3mm). After the trials had been completed, all fish were subjected to a lethal dose of Benzocaine anaesthetic before being weighed (to 0.001g) and measured (total length, to 1mm) prior to dissection to determine infection status. Any *S. solidus* plerocercoids recovered were weighed (to 0.001g) and the total plerocercoid weight and the Parasite Index (see Chapter 2, section 2.2.3) was calculated for each individual fish.

4.2.2 The experimental tank

The experimental tank (Figures 4.2a and 4.2b) was a 60cm x 30cm x 30cm aquarium filled with water to a depth of 10cm and divided into five sections (A-E) by opaque plastic partitions. In the centre of section A, which occupied half of the tank area, was located the captive shoal, which comprised six size-matched conspecific fish housed within a transparent, perforated Perspex cylinder (internal diameter 12.5cm; perforations (3mm diameter) at a density of one per cm^2). The other half of the tank comprised a maze, created by small offset openings (40mm x 40mm) in the opaque partitions, through which the test fish was free to pass. At the base of each opening was a food patch, comprising four live bloodworm tethered with fine nylon thread to a plastic mounting (Figure 4.3). This ensured that the prey items provided a stimulating moving food source without straying from position. The bottom of the tank was covered with a layer of washed white coral sand, to form a substrate against the fish would be readily visible. An opaque screen surrounded the experimental tank to prevent any external movement disturbing the experimental fish, which were observed via a mirror suspended above the tank, angled at 45°. Light level, photoperiod and water temperature in the experimental tank were as described for the stock tanks.

4.2.3 Experimental protocol

Individual fish were introduced to the transparent settling chamber in section A and released after five minutes. The subsequent movements and feeding behaviour of the test fish were recorded on a multi-event recorder (Epson HX-20) for a species-dependent period of time. Trials lasted 30 minutes for sticklebacks, whereas minnows were tested for only 15 minutes in an attempt to prevent their inability to associate closely with conspecifics causing distress.

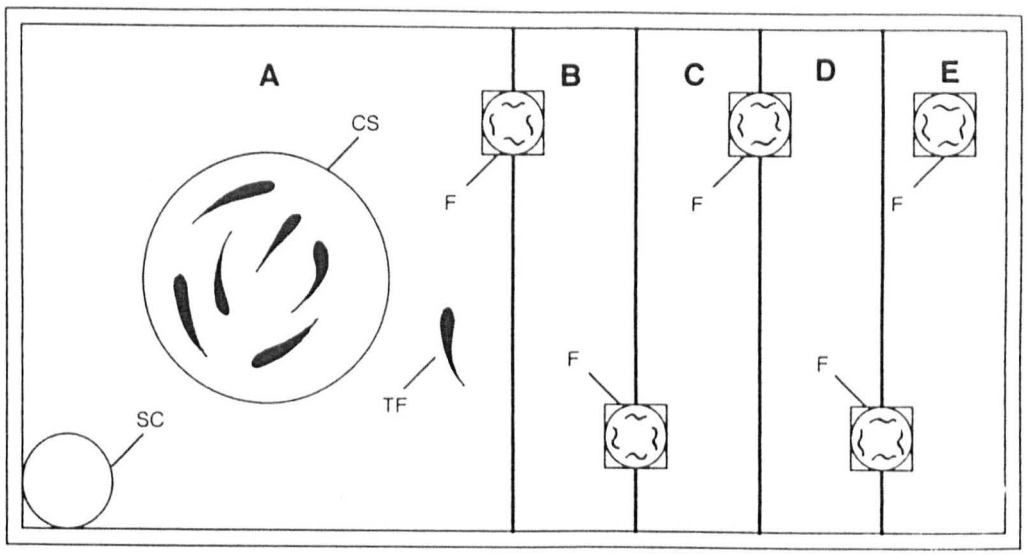
4.2.4 Data analysis

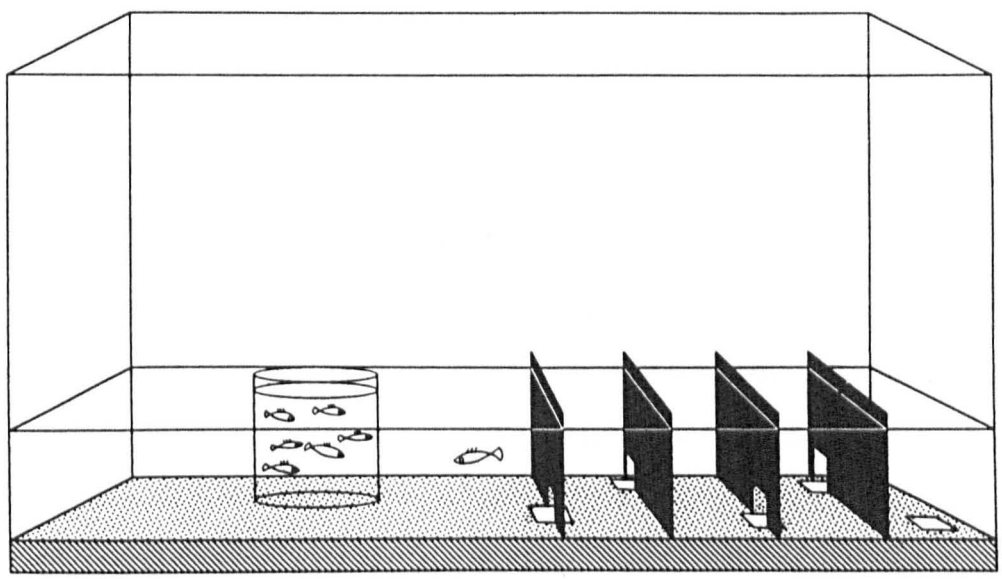
To allow for intraspecific variation in metabolic activity when examining the effect of food deprivation on shoaling behaviour, individual fish were tested under all three satiation states. Because fish were subjected to three separate experimental trials following different feeding regimes, the order of treatments was randomised to prevent any possible learning effects biasing the results. The time individual fish spent performing 'close shoaling behaviour' - defined as taking up a position within one body length of the captive shoal - and the proportion of time spent out of visual contact with the shoal

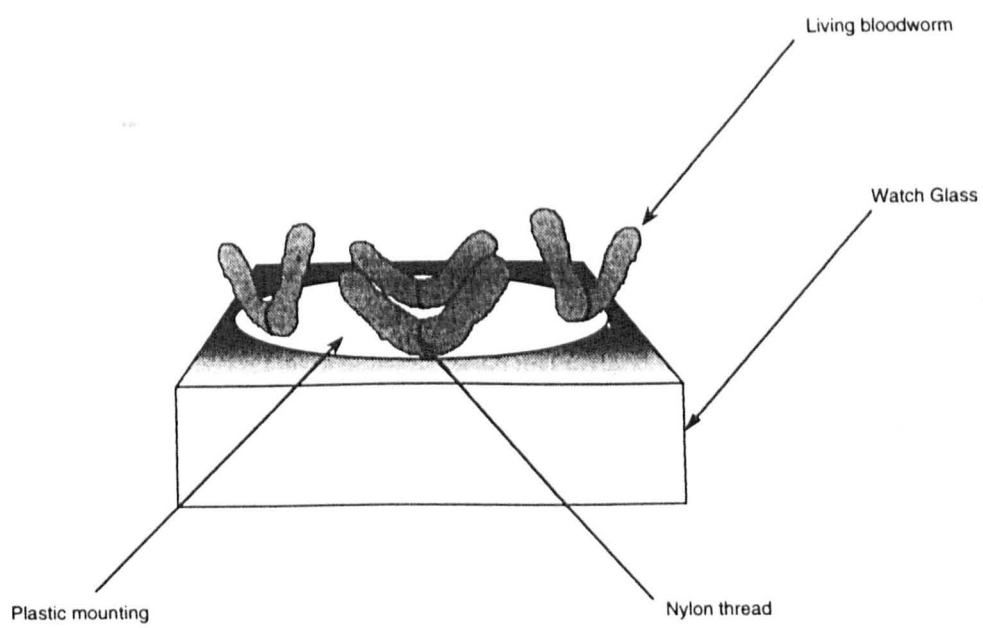
Figure 4.2a Plan view of the experimental tank designed to examine the individual shoaling decisions of minnows and sticklebacks, showing the captive shoal (CS) housed within the transparent Perspex cylinder. On release from the settling chamber (SC), test fish (TF) are free to move around the sections of the tank (A-E) via small openings in the opaque partitions above the bloodworm feeders (F). Water depth in the experimental tank was 10cm, and white coral sand formed the substrate.

Figure 4.2b Elevated view of the experimental tank designed to examine the individual shoaling decisions of minnows and sticklebacks.

Figure 4.3 Detail of the bloodworm feeder used in the shoaling decisions experiment. Four live bloodworms were tethered to a plastic disc which was mounted on a 40mm x 40mm watch glass, providing a moving stimulus for the test fish, whilst ensuring that they did not stray from position.


A
B
C
D
E
CS
F
F
F
F
F
TF
SC




Living bloodworm
Watch Glass
Plastic mounting
Nylon thread

(i.e. outside of section A, in the maze system) were calculated. A simple index to indicate the degree of risk at which a fish was placing itself during each experimental trial was also calculated. This 'Risk Index' is based on the assumptions that the perceived risk of predation are positively related to a) the total amount of time an individual spends away from the shoal, and b) the average distance of the individual from the captive shoal. Section A, being closest to the shoal, is assumed to be the 'safest' section in the experimental tank, with sections B, C, D and E being progressively more distant from the shoal and, therefore, more 'risky'. To account for these inherent differences in risk, the time spent by an individual test fish in each section needs to be multiplied by a 'risk factor' to calculate the total risk it is exposed to during a trial. The Risk Index was therefore calculated by the following equation:

$$\text{Risk Index, RI} = \frac{\sum_{E}^{A}(\text{Time spent in section} * \text{Section risk factor})}{\text{Duration of Trial}},$$

where section risk factors for sections A-E were as follows: A=1, B=2, C=3, D=4 and E=5. By choosing these risk factor values, the 'Risk Index' calculated from this equation is directly related to the area underneath the graphs depicting the movements of individual fish around the experimental tank (see Figures 4.6a, 4.6b and 4.6c). The minimum possible value of RI is therefore unity, and this score would mean that the test fish spent all of its time within section A, in visual contact with the shoal. Conversely, the maximum possible value of RI would be 5, since this would involve the test fish spending all of its time in section E, as far away from the shoal as possible.

The total number of foraging trips into the maze was recorded and used to calculate the mean length of individual foraging trips.

4.3 RESULTS

4.3.1 Parasite status of experimental fish

All 14 infected sticklebacks used in the trials contained at least one infective plerocercoid, the mean weight (± S.D.) of the largest plerocercoid present in each fish being 0.165 ± 0.069g. The mean *S. solidus* load (total weight of all plerocercoids present in the body cavity) of infected sticklebacks was 0.299 ± 0.148g, which translates into a mean Parasite Index of 25.2 ± 7.0%.

None of the sticklebacks used in the captive shoal, or as uninfected test fish, contained *S. solidus* plerocercoids. None of the minnows used in the trials harboured plerocercoids of *S. solidus* or the closely related cestode *Ligula intestinalis*. Apart from large numbers of metacercariae of the trematode *Diplostomum phoxini*, which were found in the brain and cranial cavity of all minnows examined during the present study (see Chapter 8), no other internal or external macroparasites were recorded from the experimental fish.

4.3.2 The effect of food deprivation on foraging behaviour

The number of bloodworms consumed by both uninfected and *S. solidus*-infected sticklebacks was dependent on the satiation status of the fish (Friedman ANOVA: uninfected, S=23.80, d.f.=2, $P<0.0005$; infected, S=21.42, d.f.=2, P<0.0005; Figures 4.4a and 4.4b). When deprived of food for 24h and 48h periods, test fish ate more worms than when satiated (both treatments, P<0.05). The numbers of bloodworms consumed during the minnow trials were not recorded, and so no data are available on the effects of food deprivation on the food intake of minnows in this study. However, since the test fish were held under identical conditions and were of a comparable size to the sticklebacks tested, the effects of food deprivation on appetite are likely to be similar.

The proportion of food items encountered and subsequently eaten by both uninfected and *S. solidus*-infected sticklebacks was higher in food-deprived fish, which appeared to become less selective in their foraging behaviour when food was withheld for longer periods (Figures 4.5a and 4.5b). This finding agrees with the observations of Beukema (1968), and along with the previous observation, confirms that the periods of food deprivation implemented were sufficient to elicit changes in foraging behaviour, and suggests that they caused some nutritional deficit within the fish.

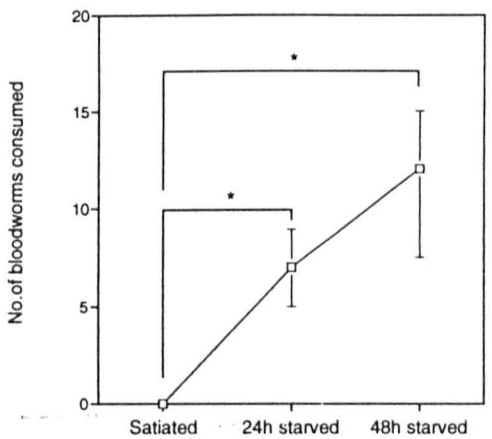
4.3.3 The effect of food deprivation on shoaling behaviour

[The spatio-temporal movements of all individual minnows, uninfected sticklebacks and *S. solidus*-infected sticklebacks around the five sections of the experimental tank, under each of the three hunger states, are shown diagrammatically in Figures 4.6a, 4.6b and 4.6c, respectively]

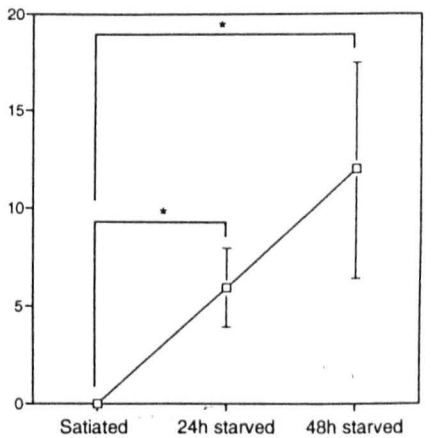
Figure 4.4 The number of bloodworms consumed during the experimental trials by a) uninfected and b) *Schistocephalus solidus*-infected sticklebacks after being fed to satiation and after periods of 24h and 48h of food deprivation. Median values are shown with error bars representing interquartile ranges ($N_{uninfected}$ = 13, $N_{infected}$ = 14; * = $P<0.05$, pairwise comparisons following Friedman ANOVA).

Figure 4.5 The proportion of bloodworms consumed once located by a) uninfected and b) *Schistocephalus solidus*-infected sticklebacks during the experimental trials after being fed to satiation and after periods of 24h and 48h of food deprivation. Median values are shown with error bars representing interquartile ranges ($N_{uninfected}$ = 13, $N_{infected}$ = 14; * = $P<0.05$, pairwise comparisons following Friedman ANOVA).

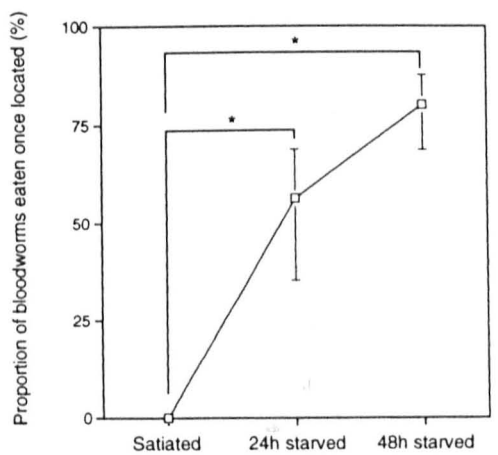
a)


No.of bloodworms consumed
20
15
10
5
0
*
*
Satiated
24h starved
48h starved

b)


20
15
10
5
0
*
*
Satiated
24h starved
48h starved

a)


Proportion of bloodworms eaten once located (%)
100
75
50
25
0
*
*
Satiated
24h starved
48h starved

b)

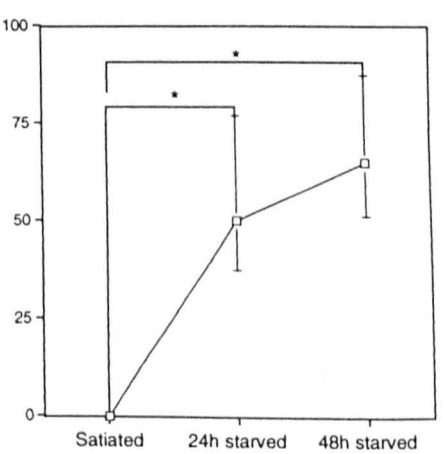

100
75
50
25
0
*
*
Satiated
24h starved
48h starved

Figure 4.6a The spatio-temporal movements of minnows around the five sections (A-E) of the experimental tank during experimental trials when satiated, and after 24h and 48h periods of food deprivation.

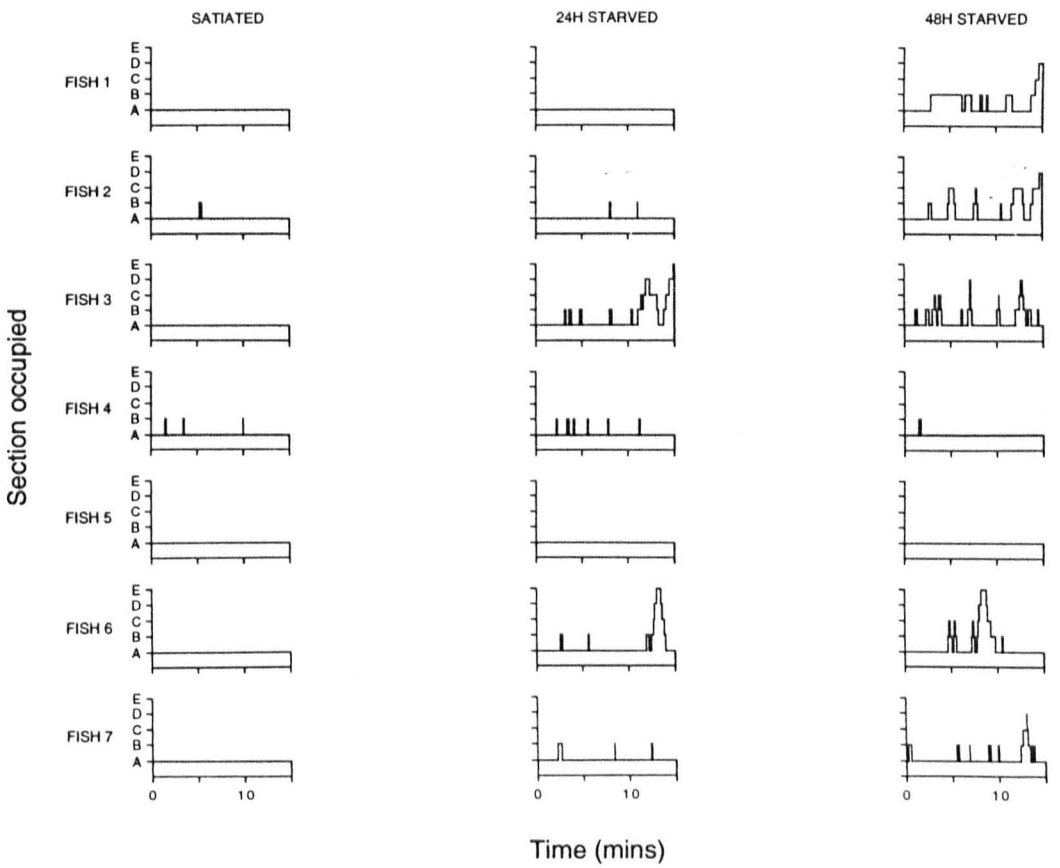

SATIATED
24H STARVED
48H STARVED
FISH 1
FISH 2
FISH 3
FISH 4
FISH 5
FISH 6
FISH 7
E
D
C
B
A
0
10
Section occupied
Time (mins)

Figure 4.6b The spatio-temporal movements of uninfected sticklebacks around the five sections (A-E) of the experimental tank during experimental trials when satiated, and after 24h and 48h periods of food deprivation.

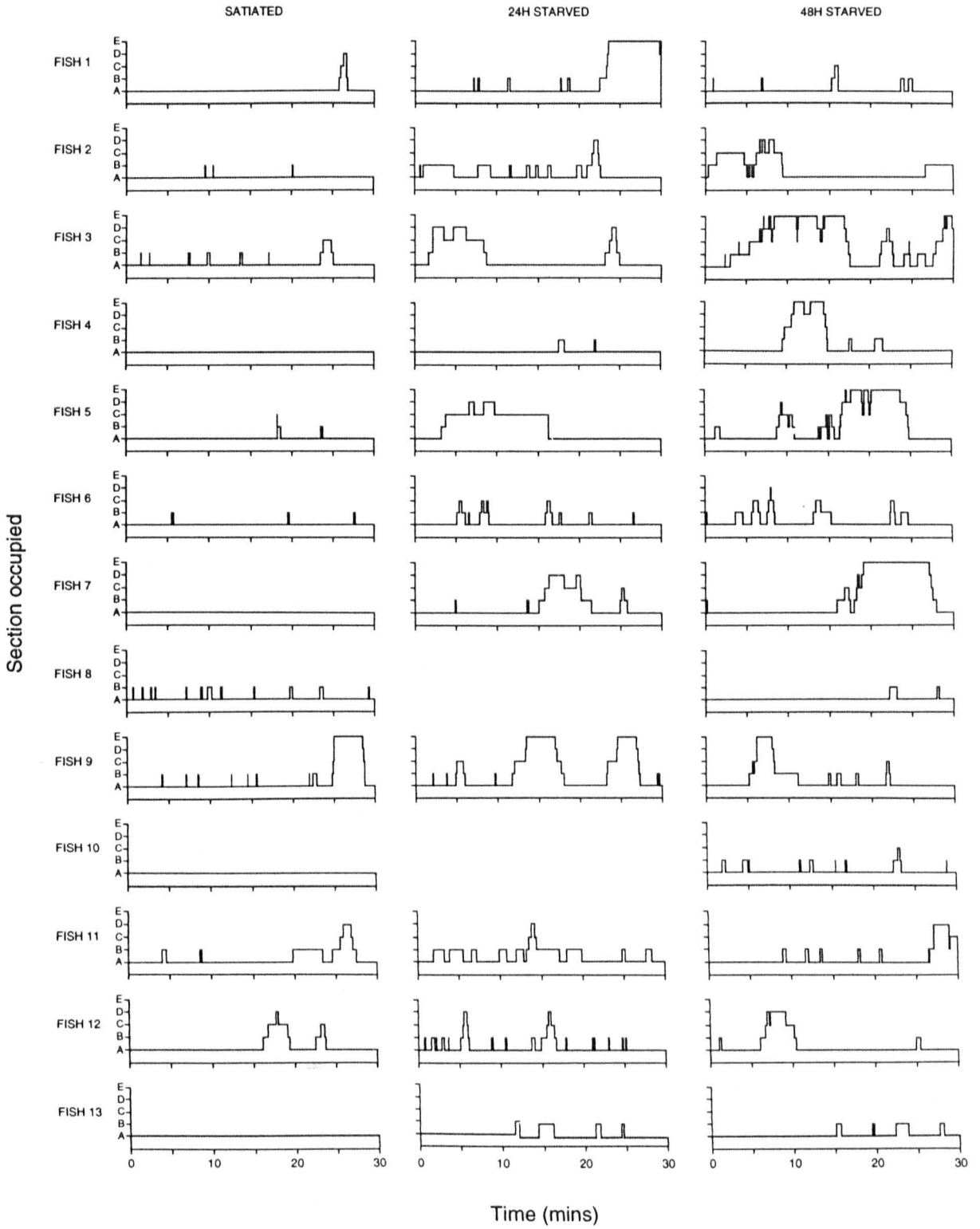
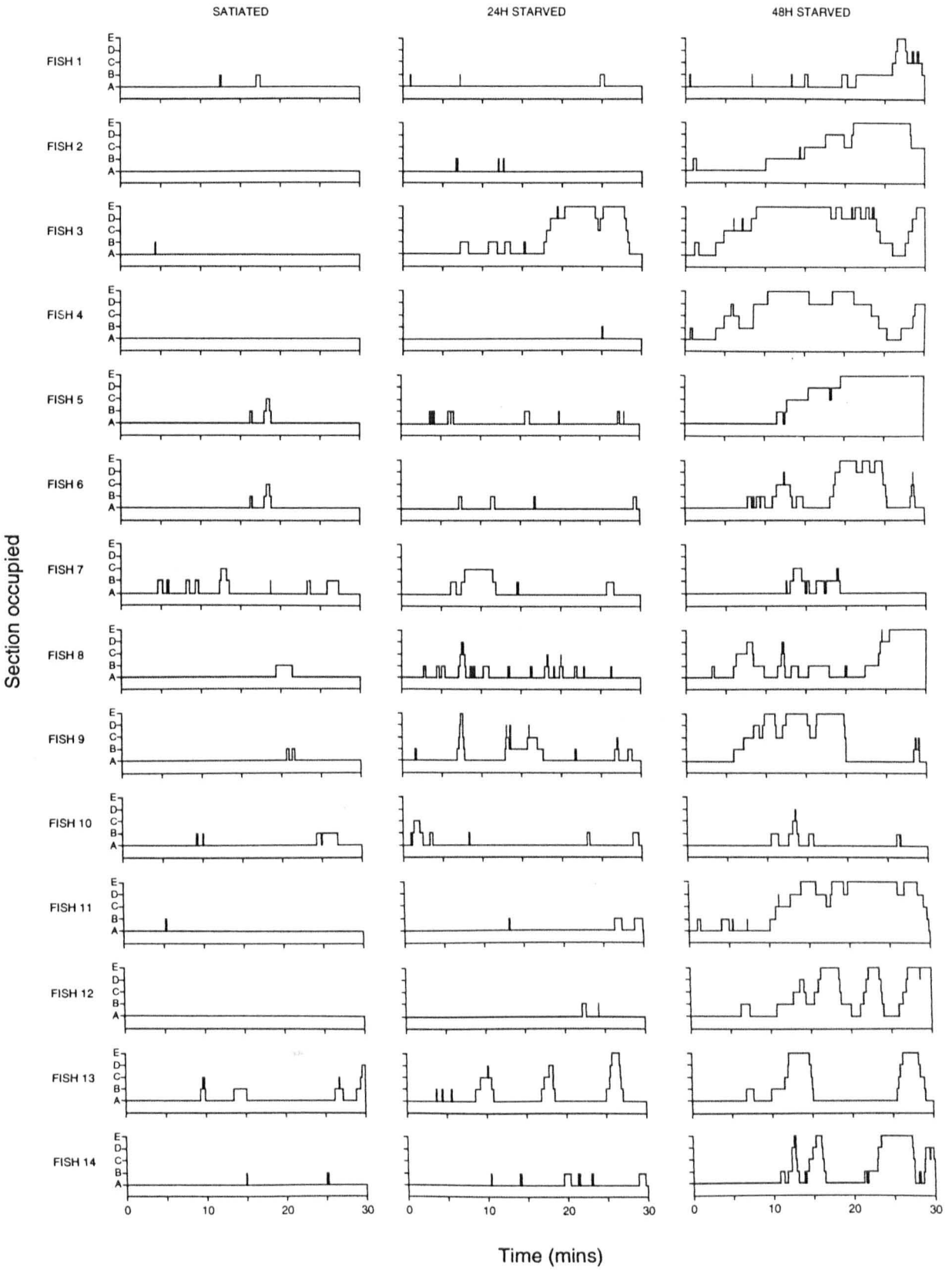

SATIATED
24H STARVED
48H STARVED
FISH 1
FISH 2
FISH 3
FISH 4
FISH 5
FISH 6
FISH 7
FISH 8
FISH 9
FISH 10
FISH 11
FISH 12
FISH 13
E
D
C
B
A
0
10
20
30
Section occupied
Time (mins)

Figure 4.6c The spatio-temporal movements of *Schistocephalus solidus*-infected sticklebacks around the five sections (A-E) of the experimental tank during experimental trials when satiated, and after 24h and 48h periods of food deprivation.


SATIATED
24H STARVED
48H STARVED
FISH 1
FISH 2
FISH 3
FISH 4
FISH 5
FISH 6
FISH 7
FISH 8
FISH 9
FISH 10
FISH 11
FISH 12
FISH 13
FISH 14
E
D
C
B
A
0
10
20
30
Section occupied
Time (mins)

The proportion of time spent by individuals of both species performing close shoaling behaviour during the experimental trials was dependent on satiation status (Friedman ANOVA, minnows, $S=14.00$, d.f.=2, $P=0.001$; sticklebacks, $S=20.46$, d.f.=2, $P<0.0005$; Figures 4.7a and 4.7b). *A posteriori* testing showed that both minnows and sticklebacks spent a greater proportion of time close shoaling when satiated than following periods of food deprivation (both species, $P<0.05$). Conversely, when deprived of food for 24h and 48h periods, uninfected sticklebacks spent more time out of visual contact with the shoal, inside the maze system, than when they were satiated ($P<0.05$), as did 48h food deprived minnows ($P<0.05$) (Figures 4.8a and 4.8b).

Although the overall amount of risk taken by minnows, as calculated by the 'risk index', was found to be slightly affected by the period without food (Friedman ANOVA, $S=6.38$, df=2, $P=0.042$; Figure 4.9a), multiple comparisons between treatments revealed no significant differences between any pairs of results ($P>0.5$ in all cases). However, individual sticklebacks significantly increased the amount of 'risk' at which they placed themselves when deprived of food for 24h or 48h periods (Friedman ANOVA, $S=14.2$, df=2, $P=0.001$, pairwise comparisons $P<0.05$; Figure 4.9b).

When deprived of food for 48h, fish of both species visited more distant sections in the experimental tank than when satiated (Friedman ANOVA, minnows, $S=6.71$, d.f.=2, $P=0.035$, sticklebacks $S=11.53$, d.f.=2, $P=0.003$; pairwise comparisons $P<0.05$) (Figure 4.10).

4.3.4 Interspecific differences in shoaling behaviour

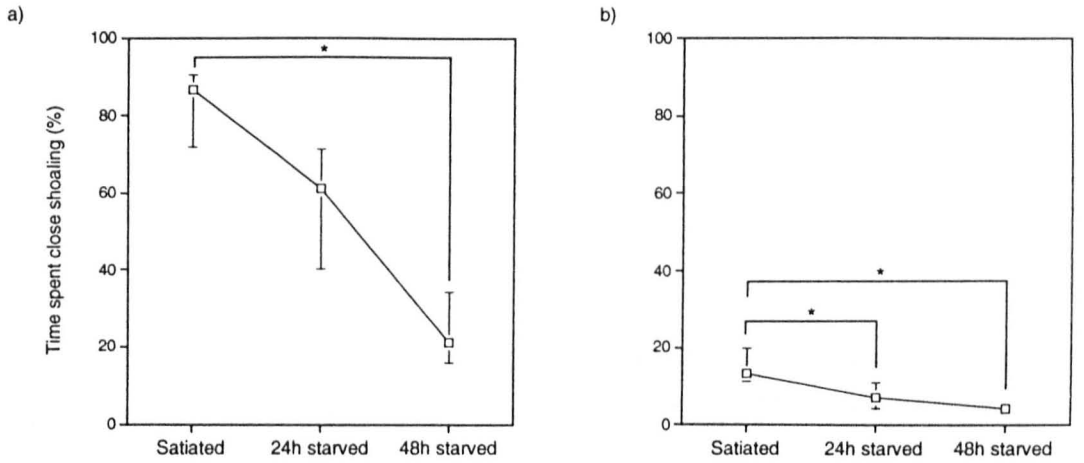
Natural interspecific differences in shoaling tendency were demonstrated in the experimental trials. At all levels of satiation, individual minnows spent more time performing close shoaling behaviour (Wilcoxon-Mann-Whitney test, $W_{satiated}=119.0$, $P_{satiated}=0.0004$; $W_{24h}=119.0$, $P_{24h}=0.0004$; $W_{48h}=119.0$, $P_{48h}=0.0004$; Figure 4.7) and less time out of visual contact with the shoal (Wilcoxon-Mann-Whitney test, $W_{satiated}=44.0$, $P_{satiated}=0.0141$; $W_{24h}=32.0$, $P_{24h}=0.0012$, $W_{48h}=48.0$, $P_{48h}=0.0476$; Figure 4.8) than did sticklebacks.

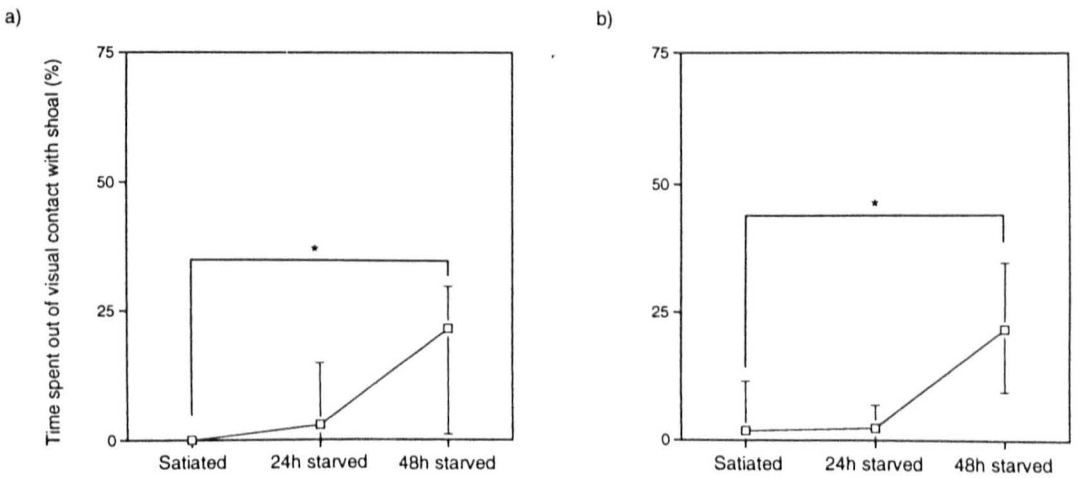
Although both minnows and sticklebacks adjusted their foraging behaviour following periods of food deprivation, the ways in which they did so differed. Minnows achieved longer total foraging time when deprived of food for longer periods by making more individual foraging trips (i.e. leaving section A more frequently), whereas sticklebacks increased the average duration of each individual foraging trip (defined as the time elapsed between leaving and returning to section A), but showed little

Figure 4.7 The proportion of time spent by a) minnows and b) uninfected sticklebacks performing close shoaling behaviour during the experimental trials after being fed to satiation and after periods of 24 and 48h of food deprivation. Median values are shown with error bars representing interquartile ranges ($N_{uninfected}$ = 13, $N_{infected}$ = 14; * = $P<0.05$, pairwise comparisons following Friedman ANOVA).

Figure 4.8 The proportion of time spent by a) minnows and b) uninfected sticklebacks out of visual contact with the shoal during the experimental trials after being fed to satiation and after periods of 24 and 48h of food deprivation. Median values are shown with error bars representing interquartile ranges ($N_{uninfected}$ = 13, $N_{infected}$ = 14; * = $P<0.05$, pairwise comparisons following Friedman ANOVA).

Figure 4.9 The degree of risk, measured using a simple index (see text for details), exhibited by a) minnows and b) uninfected sticklebacks during the experimental trials after being fed to satiation and after periods of 24 and 48h of food deprivation. Median values are shown with error bars representing interquartile ranges ($N_{uninfected}$ = 13, $N_{infected}$ = 14; * = $P<0.05$, pairwise comparisons following Friedman ANOVA).


a)
b)
Time spent close shoaling (%)
100
80
60
40
20
0
*
*
*
Satiated
24h starved
48h starved


a)
b)
Time spent out of visual contact with shoal (%)
75
50
25
0
*
*
Satiated
24h starved
48h starved

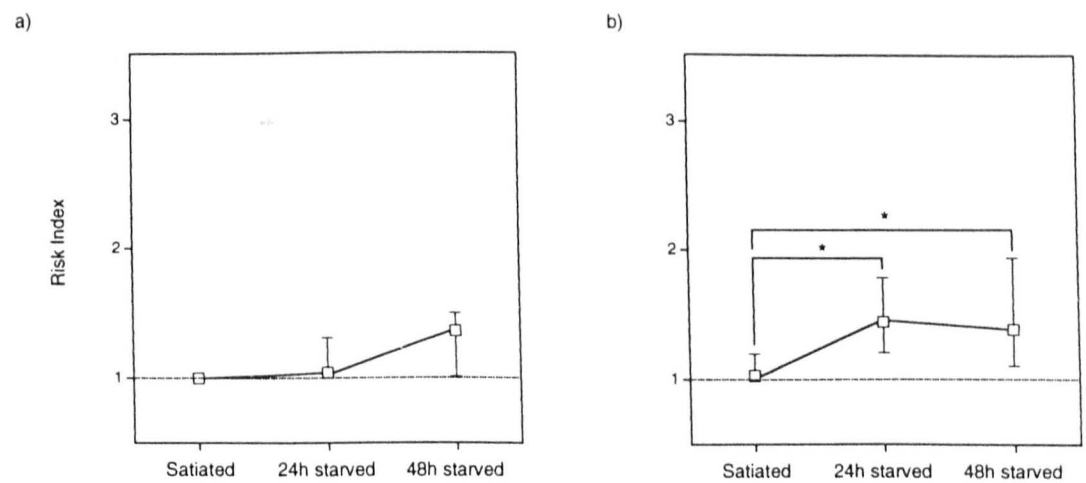
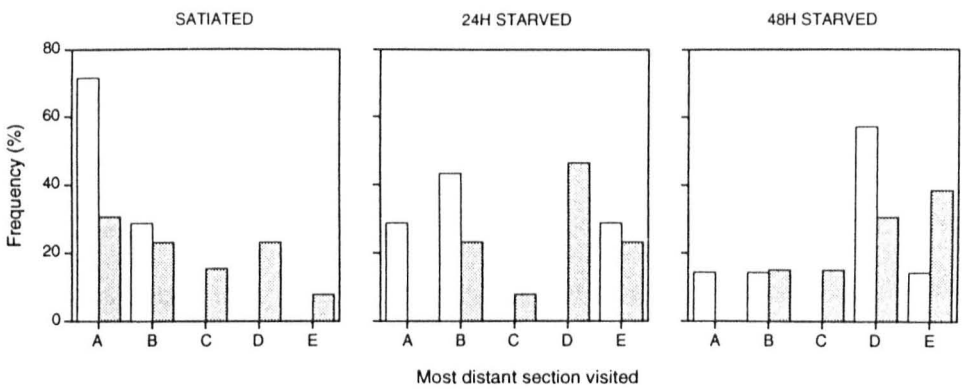
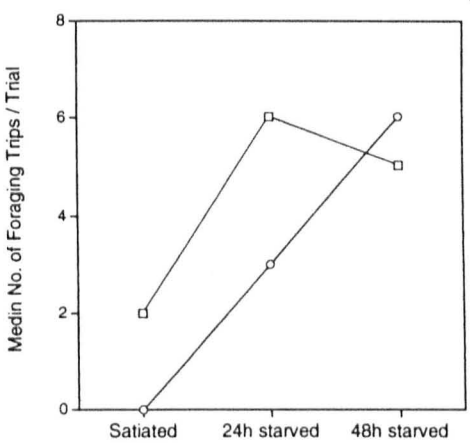

a)
b)
Risk Index
3
2
1
*
*
Satiated
24h starved
48h starved

Figure 4.10 The most distant sections visited during the experimental trials by minnows (open bars) and uninfected sticklebacks (shaded bars) when satiated and after 24h and 48h periods of food deprivation.

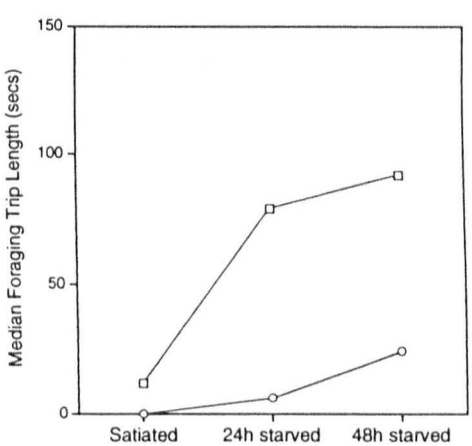
Figure 4.11 Aspects of the foraging behaviour of minnows and sticklebacks; a) the number of foraging trips and b) the mean length of foraging trips undertaken during the experimental trials when satiated, and after 24h and 48h of food deprivation. Median values are shown with error bars representing interquartile ranges ($N_{uninfected} = 13$, $N_{infected} = 14$).


SATIATED
24H STARVED
48H STARVED
Frequency (%)
80
60
40
20
0
A
B
C
D
E
Most distant section visited

a)


Medin No. of Foraging Trips / Trial
8
6
4
2
0
Satiated
24h starved
48h starved

b)


Median Foraging Trip Length (secs)
150
100
50
0
Satiated
24h starved
48h starved

change in the number of foraging trips undertaken during a trial (Figures 4.11a and b). In addition to increasing the duration of foraging trips when satiated, sticklebacks also ventured further into the maze system than did minnows, reaching more distant sections (Wilcoxon-Mann-Whitney test, W=49.0, P=0.0439) (Fig. 4.10).

4.3.5 The effect of *Schistocephalus solidus* infection on the shoaling behaviour of sticklebacks

Periods of food deprivation also altered the behaviour of *S. solidus*-infected sticklebacks; however, differences in the way in which they behaved under food deprivation were observed when compared with uninfected conspecifics. Although the proportion of time that infected fish spent close shoaling was reduced after 48h without food, there was no significant difference in the close shoaling behaviour between the 'satiated' and '24h food-deprived' treatments, even though this was the time when the biggest changes in behaviour had been observed both in minnows and uninfected sticklebacks (pairwise comparisons $P<0.05$). When satiated, *S. solidus*-infected sticklebacks spent significantly less time performing close shoaling behaviour than uninfected fish (Wilcoxon-Mann-Whitney test, W=117.0, P=0.0001), but the proportion of time allocated to close shoaling when deprived of food for 24h or 48h periods did not differ significantly from that of uninfected conspecifics (Wilcoxon-Mann-Whitney test, W_{24h}=211.0, P>0.05, N.S.; W_{48h}=176.5, P>0.05, N.S.; Figure 4.12).

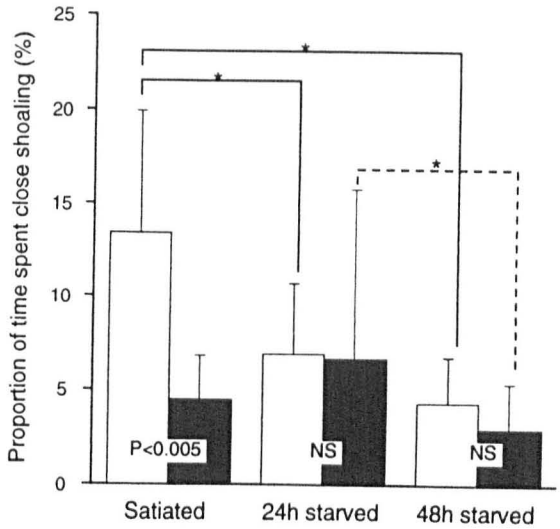
Despite spending less time 'close shoaling', satiated infected sticklebacks did not spend more time out of visual contact with the shoal than similarly-treated uninfected fish, as may have been expected (Wilcoxon-Mann-Whitney test, W=189.0, P>0.05, N.S.; Figure 4.13). Both 24h and 48h food deprivation treatments were associated with a greater tendency for infected fish to occupy positions out of visual contact with the shoal (pairwise comparisons, $P<0.05$); when deprived of food for 24h, infected fish spent significantly less time than uninfected fish out of visual contact with the shoal (Wilcoxon-Mann-Whitney test, W=142.0, P=0.0094), but this situation was reversed after 48h of food deprivation (Wilcoxon-Mann-Whitney test, W=253.0, P=0.0061)

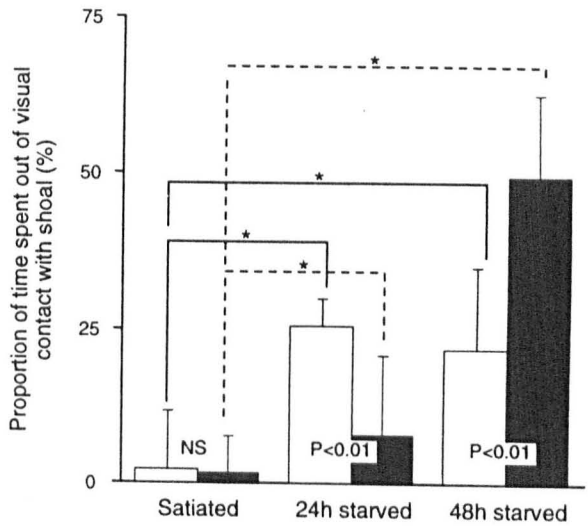
The degree of risk, as defined above, that infected and uninfected sticklebacks placed themselves at during the experimental trials was also affected by the food deprivation period, with both classes of fish becoming more risk-prone as they became more hungry (Friedman ANOVA, infected sticklebacks, S=19.00, d.f.=2, $P<0.001$; pairwise comparisons $P<0.05$). No significant difference in the risk taken during the trial by uninfected and infected sticklebacks was observed when satiated

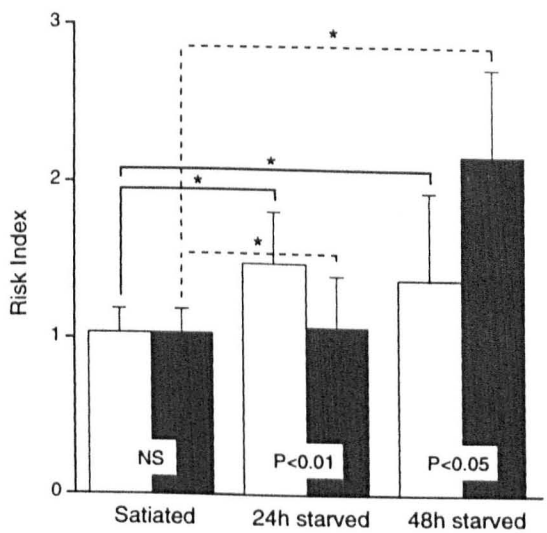
Figure 4.12 The proportion of time spent by uninfected (□) and *Schistocephalus solidus*-infected (■) sticklebacks performing close shoaling behaviour (i.e. within one body length of the shoal) when satiated, and after 24h and 48h periods of starvation. Median values with upper interquartile ranges are shown. Friedman ANOVA with *a posteriori* testing was used to calculate statistical differences between satiation treatments of uninfected and infected fish ($N_{uninfected}$ = 13, $N_{infected}$ = 14; * = $P<0.05$) and Wilcoxon-Mann-Whitney tests were used to show any statistical differences between uninfected and infected fish within any particular satiation treatment.

Figure 4.13 The proportion of time spent by uninfected (□) and *Schistocephalus solidus*-infected (■) sticklebacks out of visual contact with the shoal (i.e. inside the maze) when satiated, and after 24h and 48h periods of starvation. Median values with upper interquartile ranges are shown. Friedman ANOVA with *a posteriori* testing was used to calculate statistical differences between satiation treatments of uninfected and infected fish ($N_{uninfected}$ = 13, $N_{infected}$ = 14; * = $P<0.05$) and Wilcoxon-Mann-Whitney tests were used to show any statistical differences between uninfected and infected fish within any particular satiation treatment.

Figure 4.14 The degree of risk (measured in terms of the 'risk index' [see text]) taken by uninfected (□) and *Schistocephalus solidus*-infected (■) sticklebacks whilst foraging in the experimental tank when satiated, and after 24h and 48h periods of starvation. Median values with upper interquartile ranges are shown. Friedman ANOVA with *a posteriori* testing was used to calculate statistical differences between satiation treatments of uninfected and infected fish ($N_{uninfected}$ = 13, $N_{infected}$ = 14; * = $P<0.05$) and Wilcoxon-Mann-Whitney tests were used to show any statistical differences between uninfected and infected fish within any particular satiation treatment.


Proportion of time spent close shoaling (%)
25
20
15
10
5
0
P<0.005
NS
NS
Satiated
24h starved
48h starved


Proportion of time spent out of visual contact with shoal (%)
75
50
25
0
NS
P<0.01
P<0.01
Satiated
24h starved
48h starved


Risk Index
3
2
1
0
NS
P<0.01
P<0.05
Satiated
24h starved
48h starved

(Wilcoxon-Mann-Whitney test, W=188.0, P=0.7865). After a 24h period of food deprivation, uninfected fish appeared to be slightly more 'risky' (Wilcoxon-Mann-Whitney test, W=235.0, P=0.01), but again this situation reversed after 48h of food deprivation (Wilcoxon-Mann-Whitney test, W=139.5, P=0.0415; Figure 4.14)

4.4 DISCUSSION

4.4.1 Interspecific differences in shoaling behaviour

The differences in behaviour shown by minnows and sticklebacks in the experimental trials suggest that the costs and benefits associated with joining a shoal of conspecifics differ between species. European minnows are a strongly shoaling species which naturally inhabit fast-flowing rivers and streams (Maitland & Campbell, 1992). The shoal is the minnows' primary defence against predators, whereas three-spined sticklebacks, which form less cohesive groups appear to rely more on the individual protection offered by their dorsal and lateral spines and bony plates (Hoogland *et al.*, 1957). This is reflected in the apparently more 'bold' behaviour of the sticklebacks in the experimental trials, and suggests that for sticklebacks, the antipredator advantages of shoaling are not always sufficient to overcome the possible fitness gains of leaving the group. It seems likely that without complex shoaling mechanisms that enhance the antipredator benefits of shoaling, such as those possessed by minnows, the increased food competition in shoals causes individual sticklebacks to leave the shoal more readily.

4.4.2 The trade off between individual foraging opportunity and shoaling

Food deprivation was found to have a significant effect on the shoaling behaviour of both minnows and sticklebacks, with individuals shoaling more strongly when satiated than when food deprived. As the period without food was increased, individual fish reduced the amount of time spent performing antipredatory shoaling behaviour and increased the time spent foraging. This suggests that when making shoaling decisions, fish trade the need to acquire food against that of avoiding predation, and will leave the shoal in order to forage alone if feeding motivation is strong enough. Van Havre and FitzGerald (1988) showed that satiated sticklebacks chose to join larger shoals than those chosen by hungry fish; presumably satiated individuals have a reduced feeding motivation and are able to take advantage of the increased protection offered by the shoal without suffering from the elevated level of

competition for food. Another example of the effects of hunger on shoaling behaviour is provided by Krause (1993a), who examined the positional preferences of experimentally food-deprived juvenile cyprinids in a natural mixed species shoal. Hungry fish were observed to take up positions at the front of the shoal, where feeding rates are known to be highest (O'Connell, 1972; Krause *et al.*, 1992) in return for possible increased predation risk or hydrodynamic costs. Both sticklebacks (Keenleyside, 1957) and bluntnose minnows *Pimephales notatus* (Morgan, 1988) have been reported to form less cohesive shoals when hungry than when well fed.

4.4.3 The effects of parasites on shoaling behaviour

The behaviour of *S. solidus*-infected sticklebacks in these trials suggests that, even when satiated, and therefore apparently unaffected by the increased levels of food competition, parasitised fish do not attempt to join the shoal. They do, however, prefer to remain in visual contact with shoal members. This behaviour can be explained by three non-exclusive hypotheses. The first two hypotheses (indirect and direct parasite-mediated behavioural modification) infer the manipulation hypothesis, and assume the behaviour change to be of survival value to the parasite (see Moore & Gotelli, 1990, for a critical review). The third relies on the resulting behaviour being of survival value to the infected host, which would imply that it may be a successful counter adaptation of the host to infection.

4.4.3.1 The indirect, parasite-mediated behavioural modification hypothesis

The growth of *S. solidus* plerocercoids in the body cavity of a fish causes a significant demand on host energy reserves, which increases both the metabolic rate and feeding motivation of infected fish (Giles, 1983, 1987b). *S. solidus*-infected fish appear to be poor competitors for food (Milinski, 1984; see Chapter 6), and so may be forced outside of the shoal to avoid the higher level of food competition associated with shoal membership. In such a case, the effect of the parasite would be indirect as it changes the host's behaviour by modifying the costs and benefits of being in a shoal. However, this hypothesis does not explain why infected fish should spend less time close shoaling when satiated, as presumably this would be a situation in which a poor competitor could accrue the benefits of shoaling without suffering through increased food competition levels.

4.4.3.2 The direct, parasite-mediated behavioural modification hypothesis

Helluy & Holmes (1990) demonstrated experimentally that the acanthocephalan parasite *Polymorphus paradoxus* affects the antipredator behaviour of its host, the crustacean *Gammarus lacustris*, and that an identical behaviour change could be induced by the injection of serotonin, a neurotransmitter chemical. This suggests a possible direct parasite-mediated change in host behaviour, whereby the parasite modifies the nervous control of movement by direct interference. If *S. solidus* has evolved a similar mechanism, then direct manipulation could be responsible for altered shoaling behaviour, although it seems unlikely that neural pathways controlling a complex behaviour such as shoaling would be effectively changed by as simple a mechanism as that proposed for the *Polymorphus-Gammarus* system.

4.4.3.3 The host-mediated response to infection hypothesis

Although predation by a piscivorous bird is a necessity for the parasite, it is fatal for the host fish, and, so long as infected fish were able to successfully reproduce, there should be an equally high selection pressure on the host population to evolve a strategy to counter any such parasite-induced self-destructive behaviour. Many predators select prey through oddity-based mechanisms that are a successful counter-adaptation to the 'confusion effect' experienced by predators feeding on swarming, shoaling or flocking prey (Ohguchi, 1981; Landeau & Terborgh, 1986). When viewed from above *S. solidus*-infected sticklebacks are strikingly different in outline from similarly-sized uninfected fish (see Chapter 6), and this offers a potential basis by which they could be selected by a predator against a homogeneous background of uninfected fish. Their slower swimming speed and impaired escape response may also result in infected fish taking a disproportionate amount of risk once a shoal is attacked. Many avian predators are attracted to shoaling fish (Pitcher & Parrish, 1993) and if this is true of sticklebacks then the strategy employed by infected fish, namely remaining outside the shoal yet within visual range of shoal members, may help reduce the elevated risk of predation yet still allow the fish to take advantage of increased vigilance and other benefits associated with shoaling.

However, it is unclear whether *S. solidus*-infected sticklebacks reproduce successfully in the wild, a prerequisite for the evolution of conditional behavioural strategy such as that outlined above. McPhail & Peacock (1983) suggest that *S. solidus* infection has little effect on gonad development, and that this is a result of host-parasite co-evolution. However, recent studies suggest that in sticklebacks

from Inverleith pond, gonad development is affected by *S. solidus* infection (Tierney *et al.*,in press). There have also been anecdotal accounts of hugely-swollen, *S. solidus*-infected females destroying males' nests as they attempt to deposit their eggs. In conclusion, at Inverleith pond at least, it seems unlikely that *S. solidus*-infected sticklebacks often produce offspring.

A different approach may be needed to determine whether the observed behavioural differences shown by infected fish are due to parasite manipulation or host adaptation. Determining whether the altered behaviour of infected sticklebacks results in increased or reduced avian predation on infected fish in the field would give a strong indication of whether the behaviour change was more likely to favour host or parasite survival, and hence whether any changes are more likely to be host- or parasite-mediated. Such direct experimental tests of the ecological significance of behavioural changes observed in the laboratory are urgently required in this, and other proposed examples of the manipulation hypothesis.

4.5 SUMMARY

- An experiment was designed to provide individual minnows and three spined sticklebacks with a mutually exclusive choice between joining a shoal of conspecifics and foraging alone in a maze.
- The shoaling decisions and foraging behaviour of individual fish were studied when the fish were satiated and after 24h and 48h periods of food deprivation.
- Hunger level was found to have a significant effect on shoaling behaviour. When satiated, fish of both species spent a greater proportion of time within one body length of the shoal and spent less time out of visual contact with the shoal than after periods of food deprivation.
- The effect of the cestode parasite *S. solidus* on the shoaling behaviour of stickleback hosts was complex. When satiated, infected fish spent less time than uninfected fish within one body length of the shoal, preferring to remain outside of the shoal, yet within visual contact, although when food deprived there was no difference in the proportion of time spent by infected and uninfected fish close to the shoal.
- The possible ecological significance of this change in behaviour is discussed with reference to the manipulation hypothesis of host-parasite interactions.

Chapter 5. The effect of *Ligula intestinalis* infection on the schooling behaviour of minnows

5.1 INTRODUCTION

5.1.1 The importance of schooling in the ecology of small freshwater fish

Many species of fish that occupy open water habitats form polarised schools. The ability to polarise, and to maintain the strong synchrony and speed that typify the behaviour of such schools is acquired quickly during the early development of individuals of schooling fish species (Magurran, 1986b; Magurran & Seghers, 1990b), and evidently the formation of schools has an important role in their ecology. However, the selection pressures that have led to the evolution of schooling behaviour and its complex sensory and mechanistic basis in such fish have been the subject of much debate. Schooling behaviour is currently viewed as a specialised form of more general shoaling behaviour (defined simply as 'temporal and spatial aggregation' [Pitcher, 1983]), from which it is assumed to have evolved (Pitcher & Parrish, 1993). The two hypothesised functional benefits of schooling in the ecology of fishes, over and above those of non-specialised shoaling (discussed in Chapter 4), have been its possible hydrodynamic advantage (see Chapter 1, section 1.2.3.3), and its increased antipredator value.

The polarised and synchronised swimming actions of individual members, which both characterise and define fish schools, may maximise the 'confusion effect' experienced by many predators when attacking aggregated prey (Broadbent, 1965; Ohguchi, 1981; Milinski, 1990), thereby enhancing any advantages gained through simple aggregation (Pitcher & Parrish, 1993). Schools tend to be compact aggregations and it is thought that the close packing of individuals in schools facilitates the performance of synchronised manoeuvres, since it potentially allows very rapid communication between individual school members. This is possible since local pressure changes caused by movements of adjacent school members can be detected by the lateral line and otolith systems of schooling fish, and instantaneous responses to these signals can be made (Gray & Denton, 1991). In addition, many schooling fishes are either silvery in colour or longitudinally-striped (Pitcher & Parrish, 1993), both of which may serve to further increase the confusion factor by 'dazzling' predators as they perform synchronous turns (Springer, 1957; Hobson, 1968).

5.1.2 Measurements of school compactness

The study of spatial relationships within animal aggregations is made simpler by the use of indices that are able to accurately describe the 'compactness' of any social group. Possibly the most

familiar of these measurements is the nearest neighbour distance (NND). This is calculated for each member of a population or social group by measuring the distance from each individual to its closest neighbour within that group. By calculating the mean nearest neighbour distance ($\bar{x}$ NND; see equation below) and the standard deviation from this mean for a group of organisms, and assuming the distribution of NNDs within the group to be normal, a reasonable estimate of the compactness of any social group can be obtained.

$$\text{Mean nearest neighbour distance} = \bar{x}\text{NND} = \frac{\sum \text{NND}i}{n},$$

where NNDi = NND for the ith individual in the group and n = the number of individuals in the group.

Although two-dimensional measurements of NND are sufficient to give an accurate picture of the true spatial relationships between individuals of terrestrial, ground dwelling species, for the analysis of free swimming fish schools it is also necessary to take into account a third dimension, depth, when calculating the NNDs of individual group members. In order to measure the three-dimensional NNDs of individuals within groups, experimental techniques incorporating geometrical theory have been developed (e.g. Cullen *et al.*, 1965; Pitcher, 1973; Partridge, 1980). However, because many small freshwater fish that form schools spend a large proportion of time in very shallow water, the three-dimensional structure of these groups is often constrained to one plane, with the NNDs of individual group members having a negligible depth component. The analysis of these 2-dimensional schools is more conducive to the manual collection of the large amount of NND data that is required in experimental studies, and the use of a minimal water depth under experimental conditions has been validated in this project (see Chapter 3). For these reasons, and on the advice of Dr. Tony J. Pitcher (University of British Columbia, Canada, personal communication), it was decided that the analysis of schooling behaviour in the present study be carried out in 2-dimensions only.

5.1.3 Fitness consequences of school position

The notion that fish schools provide a perfect example of leaderless, egalitarian society (Breder, 1954; Shaw, 1962; Radakov, 1973) has been replaced with the modern paradigm that

individuals only remain with such groups for as long as it is beneficial for them to do so (Pitcher, 1986; Pitcher & Parrish, 1993). However, despite the revelation that animals are social only through individual selfishness, many authors have failed to recognise the fact that the costs and benefits of group living are, as a consequence, unlikely to be shared equally between all members of a group, since each animal will be competing for the largest share of the available benefits (Krause, 1994). There is now growing evidence that the particular spatial positions occupied by individuals within groups may have important consequences for various components of fitness, such as food intake rate (Petit & Bildstein, 1987; Black *et al.*, 1992; O'Connell, 1972; Krause *et al.*, 1992; Krause, 1993a), predation risk (Hamilton, 1971; Parrish 1989) and the cost of locomotion (Weihs, 1973, 1975). Differential fitness returns in relation to spatial positioning in animal groups has recently been the subject of a major review (Krause, 1994).

5.1.3.1 Peripheral versus central positions in fish schools

By definition, any aggregation of animals comprises some individuals that are on the periphery and others that are in the centre. Peripheral individuals in groups are presumed to suffer the highest risk of predation, since they are closest to potential predators. This concept of marginal predation, formalised by the 'selfish herd' theory of Hamilton (1971), is supported by a great deal of indirect evidence from studies of fish schools; *direct* evidence for the increased predation risk of occupying peripheral school positions has proved somewhat elusive.

Few studies have examined predation on naturally-formed fish schools with a view to determining any preference of predators for individuals occupying edge or central positions. Such studies are difficult to undertake, and any data on capture success perhaps even more difficult to analyse. A general problem in studies examining possible differential fitness returns to individual group members is defining shoal positions, and subjectivity in position allocation may account for some of the observed ambiguity in results (see Krause, 1994). [The problem of accurately and unambiguously allocating individual group members to either category can be largely overcome by the use of a geometrical algorithm; see 5.2.5.3].

The few studies of predation on peripheral and central fish in schools have actually suggested that central fish may be more at risk than those on the periphery (gaftopsail pompano *Trachinotus rhodopus* feeding on flat-iron herring *Harengula thrissina*, Hobson, 1963; various piscivorous fish

feeding on *H. thrissina*, Parrish *et al.*, 1989; black seabass *Centropristis striata* feeding on Atlantic silversides *Menidia menidia*, Parrish, 1989). However, each of these studies are differently faulted. As pointed out by Krause (1994), the findings of Hobson (1963) and Parrish *et al.* (1989) are difficult to put into context, since in the former, 'edge' and 'centre' positions are not clearly defined, and in the latter although *more* prey occupying central positions were shown to be captured, the *per capita* risk (the chance that any one individual in either position will be captured) of peripheral and central fish is not given. In addition, although Parrish (1989) recorded over 1000 seabass attacks on schooling silversides, only 5 fish (3 central / 2 peripheral) were successfully captured, so the validity of the claim that this demonstrates selective predation is questionable.

Indirect studies of the 'selfish herd' theory, in the absence of real data on marginal predation in fish schools, has provided circumstantial evidence that fish on the periphery may perceive themselves as being at a greater risk than centrally-positioned school members. By using alarm substance ('Schreckstoff'), Krause (1993b) was able to elicit a fright response in a previously unexposed minnow *Phoxinus phoxinus*, swimming with a school of dace *Leuciscus leuciscus* that had been previously habituated to the chemical signal, and did not respond to it. On addition of the alarm substance, the Schreckstoff-naïve minnow showed a significant preference for positions in the centre of the school, whereas no such preference was observed prior to the trial. Such a result demonstrates that central positions may indeed be perceived as more secure by schooling fishes. Further evidence from non-fish aggregations also provides a convincing argument for the existence of marginal predation in certain natural systems (see reviews in Hamilton, 1971 and Krause, 1994).

In addition to the proposed differential predation risks associated with the occupancy of peripheral and central positions in schooling fish, there could also be an effect on the levels of competition for food. Fish at the front (and therefore at the periphery) show a higher feeding rate than those further back (see 5.1.3.2), but whether all peripheral fish make such foraging gains is unclear (although this has been shown to be the case in several studies of invertebrates and non-fish vertebrates [see Krause, 1994]). Peripheral fish have fewer near neighbours than central fish, and so under certain foraging conditions this may lead to them experiencing reduced levels of competition for food (see Chapter 6, section 6.4.1). They are also presumably better positioned to make short trips away from the school to exploit nearby foraging opportunities (e.g. Major, 1978), although such behaviour is almost certainly associated with an increased predation pressure (Pitcher & Parrish, 1993). The

potential benefits (reduced competition, increased prey availability) of occupying the periphery of schools may, in some circumstances, mediate the hypothesised associated increased predation risk. Assuming there is a discrepancy in the fitness payoffs associated with occupying peripheral and central positions in social groups, then there ought to be strong competition for occupancy of the most profitable sites. Because it is physically impossible for all individuals to be in the centre of a school, then evidently a proportion of the individuals present will be forced to occupy peripheral positions, and if variation in the ability of individuals to compete successfully for preferred positions exists, we should expect less competitive fish to be found in the least favoured positions, i.e. on the periphery. Such variation in competitive ability may be a result of differences in size, manoeuvrability, sensory ability, or general health status of group members. In the absence of such competitive variation then it would appear possible that regular shuffling of peripheral and central fish would occur as all members attempted to maximise the proportion of time spent in the centre. When the benefits of central positions are highest, e.g. in the presence of a predator known to attack peripheral individuals, it would seem that this turnover rate should increase, with a subsequent reduction in school volume. Conversely, in situations where the costs of peripheral occupancy are lowest, we may expect to observe less frequent shuffling of positions.

The process of changing position must incur some cost, since fish moving from the edge to the centre of a school perform complex manoeuvres, probably requiring some reduction in vigilance. In addition, changing position also requires fish to swim at a different angle to the rest of the fish present, which would have a hydrodynamic cost as well as increasing the visual oddity of the fish. This hypothesised cost of changing position may limit the extent to which fish move from the outside to the inside of a school under perceived safe conditions.

5.1.3.2 Front versus back positions in fish schools

As well as being classified as peripheral or central, members of a polarised group of animals can also be described as occupying front or back positions. There is evidence that in polarised schools fish occupying front positions receive additional foraging benefits. In species that feed on items at the surface and in the water column, front fish have been shown to exhibit higher food intake rates, and to get better quality food than fish elsewhere in the shoal (O'Connell, 1972; Krause *et al.*, 1992; Krause, 1993a). This is because individuals situated at the front of a unidirectional polarised group come into

contact with water-born food items first, removing a large proportion of them and depleting the supply for fish in rear positions. Just as fish schools have to be comprised of central and peripheral fish, so they also need to have front and back members, since it is impossible, for example, for all fish to be in the front.

Few experimental tests of school position preference have been carried out, with the notable exception of Krause (1993a) who found that, after being removed and deprived of food, a proportion of a wild school of roach showed an immediate preferences for front positions when they were returned to the school from which they had been originally been removed. This strong preference shown by the food-deprived fish was observed to disappear after 24h, presumably once any nutritional deficit had been repaid. This strongly suggests that nutritional status has an important effect on positional preferences within a group.

Evidently, individual foraging success can be increased by the occupancy of frontal positions. In a group of fish that differ in individual competitive ability, it would seem that dominant fish should exclusively occupy frontal positions, yet the previous example has illustrated that front fish are more likely to be those that are hungry, rather than those that are dominant. In other words, fish only appear to occupy frontal positions when they have to. This could be explained if occupying front positions incurred some significant cost, as well as conferring an advantage; however, the potential costs of occupying frontal, rather than simply peripheral, positions have not yet been quantified, and their identity remains unclear.

5.1.3 Potential effects of *Ligula intestinalis* on the schooling behaviour of minnows *Phoxinus phoxinus*

A striking characteristic of *L. intestinalis* infection in cyprinids, and of the closely related pseudophyllidean cestode *Schistocephalus solidus* in the three-spined stickleback *Gasterosteus aculeatus*, is the enormous relative size to which individual worms grow compared to the host fish that they parasitise. This often results in the parasite contributing a very high proportion of the total weight of infected fish, and in heavily-infected individuals, the total weight of parasites harboured frequently approaches that of the remaining host. Associated with these large parasite loads, are significant effects on host morphology, physiology, and behaviour (see Chapter 7, and reviews by Milinski, 1990, and Barber & Huntingford, in press).

Changes in the appearance and behaviour of parasitised individuals are likely to be associated with increased predation risk, since infection with a debilitating parasite is often allied to a reduction in morphology or crypsis, sensory capability, escape response or stamina (see Chapter 1). Transmission of *L. intestinalis* plerocercoids in natural ecosystems relies on host fish being consumed by piscivorous birds, the definitive hosts of the parasite, so parasites may be expected to have evolved mechanisms to increase the susceptibility of their fish hosts to predation by such predators.

The behaviour of parasitised fish in polarised schools, the primary anti-predator defence of small prey fishes that occupy open water habitats, could potentially provide a mechanistic basis for selective predation on infected fish in schools composed of otherwise healthy individuals, as described by van Dobben (1952) for roach infected with *L. intestinalis*.

5.1.4 Objectives

In the present study, an experiment was devised to test whether the schooling behaviour exhibited by individual minnows *P. phoxinus* infected with *L. intestinalis* differed from that shown by the other, uninfected, individuals in the school. The specific objectives of this chapter are:

- To design an experimental technique for the accurate measurement of various aspects of school structure, including nearest neighbour distance, school area, and elective group size.
- To investigate any temporal changes in the structure of minnow schools formed over a 6h period of residency in the previously-novel experimental arena.
- To determine the effect of a simulated avian attack on school structure.
- To investigate the behaviour of *L. intestinalis*-infected minnows in schools of otherwise uninfected individuals, and to compare it with that of the uninfected fish.
- To determine the potential for infection-associated traits in the schooling behaviour of minnows harbouring *L. intestinalis* plerocercoids to cause increased predation pressure on parasitised fish.

5.2 MATERIALS AND METHODS

5.2.1 Supply and husbandry of fish

Experimental fish were collected from Loch Tarsan (Grid reference NS 078 840, Ordnance Survey 2nd Series, Sheet 56), an artificial reservoir situated between Dunoon and Colintraive, Argyll, Scotland, owned by Hydro-Electric plc. The loch is known to hold large populations of *L. intestinalis*-

infected minnows and *S. solidus* -infected sticklebacks, as well as both wild and stocked brown trout, *Salmo trutta* (J. R. McInnes, Dunoon and District Angling Club, personal communication), and is home to a large resident population of common and black-headed gulls (*Larus canus* and *L. ridibundus* respectively), which are the most numerous potential definitive hosts of both parasites at the site. Minnows were caught by trapping or by hand-netting from a dam at the western end of the loch and transferred to holding tanks in the laboratory, where they were maintained for two months prior to experimentation. During this period, fish were fed *ad libitum* with both live and frozen bloodworm and flake food, in order to standardise nutritional status as far as possible. Any recently-acquired *L. intestinalis* infections became visible during this time, so preventing fish being incorrectly classified as uninfected when they may actually have harboured small plerocercoids (see Chapter 2). After this quarantine period, fish were sorted with respect to infection status, and six size-matched groups, each composed of nine uninfected and one *L. intestinalis*-infected minnow, were selected and maintained in separate 54-litre aquaria.

5.2.2 The experimental flow pool

Although minnows school almost constantly in their natural environment, they were rarely observed to form polarised schools in holding tanks in the laboratory. In order to study the schooling behaviour of minnows, it was necessary to provide them with an environment in which they would form schools. A 160cm diameter, rigid-walled wading pool with a water depth of 7cm provided the large area (20 000 cm^2) required by the fish for the formation of schools. The shallow water depth regime was implemented so as to limit the formation of schools to a single plane, thereby allowing two-dimensional analysis to be carried out (see Chapter 2). In order to maintain uniformity of the direction of polarisation within schools, the pool was adapted to provide a constant, unidirectional water current against which the fish would swim. A plastic 'island' (diameter 30cm) was created in the centre of the pool to house two connected water pumps which drew water in through a mesh-covered inlet, and forced it out through a horizontal perforated spray bar which lay just under the water surface of the main pool, generating a uniform, unidirectional water current in the pool of 0.12ms^{-1}, a current speed that is known to be conducive to the formation and maintenance of cohesive schools of minnows (Pitcher, 1973). The pool was illuminated by ten diffused fluorescent tubes, fixed 1.5m above the water surface, and was surrounded by a black plastic screen to minimise disturbance to the fish caused by

Figure 5.1a The experimental flow pool designed to examine the schooling behaviour of minnows. A 180cm diameter wading pool was filled to a depth of 7cm and fitted with pumps and a spray bar to provide a constant, unidirectional current of approximately 12cm.s-1. A 10cm x 10cm grid was marked onto the base of the pool to facilitate the accurate recording of the co-ordinates of the minnows in the pool. The movements of fish were filmed simultaneously from two positions, by the 'Plan' and the 'I.D' cameras.

Figure 5.1b Diagrammatic representation of the simulated avian attack. A cardboard silhouette of a kingfisher was released from a position 1.5m above the pool ('A') and allowed to travel down a guide wire, at an angle of 45°, to a position just above the water surface at the opposite side of the arena ('B'). The time taken for the model to pass from 'A' to 'B' was approximately 1s.

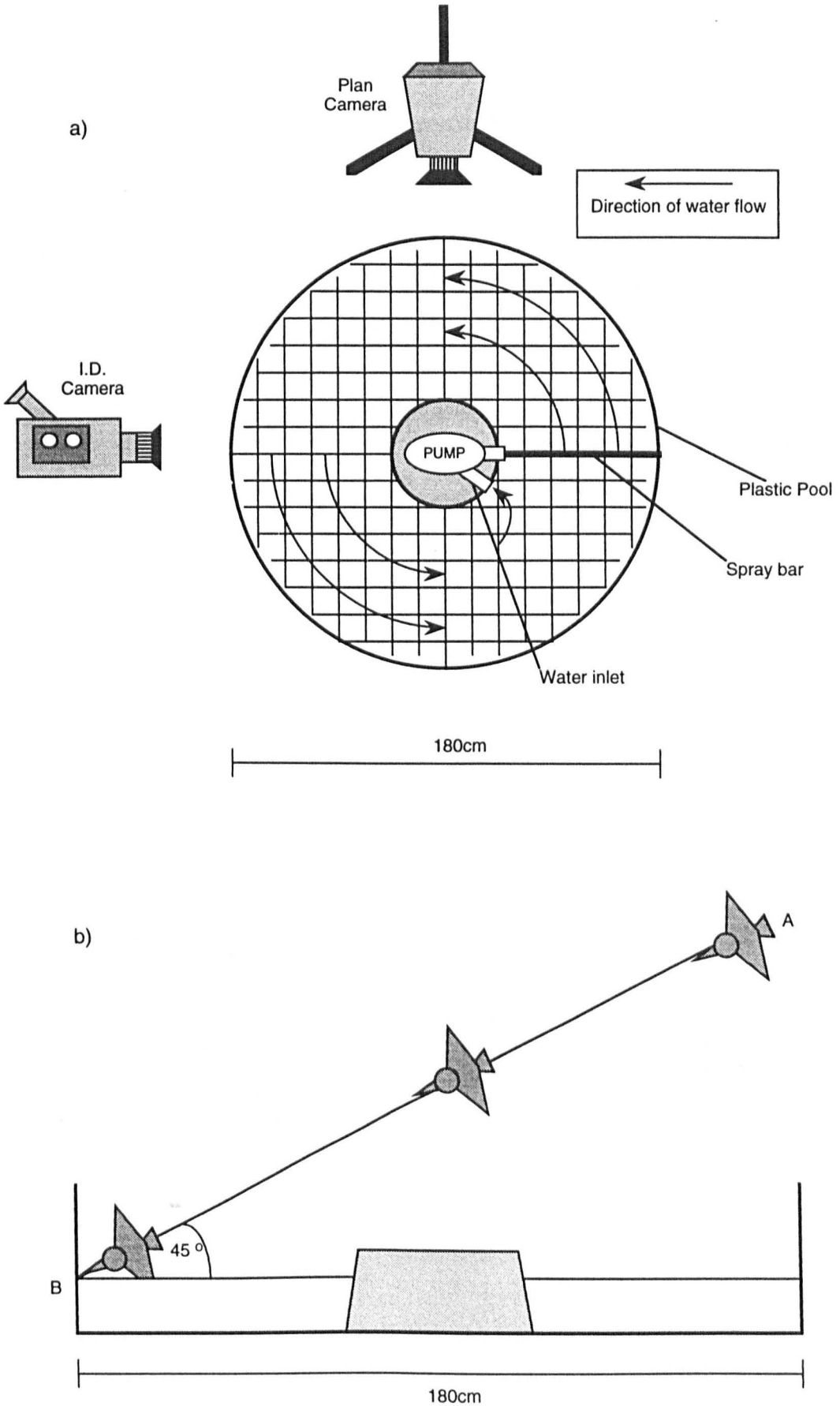
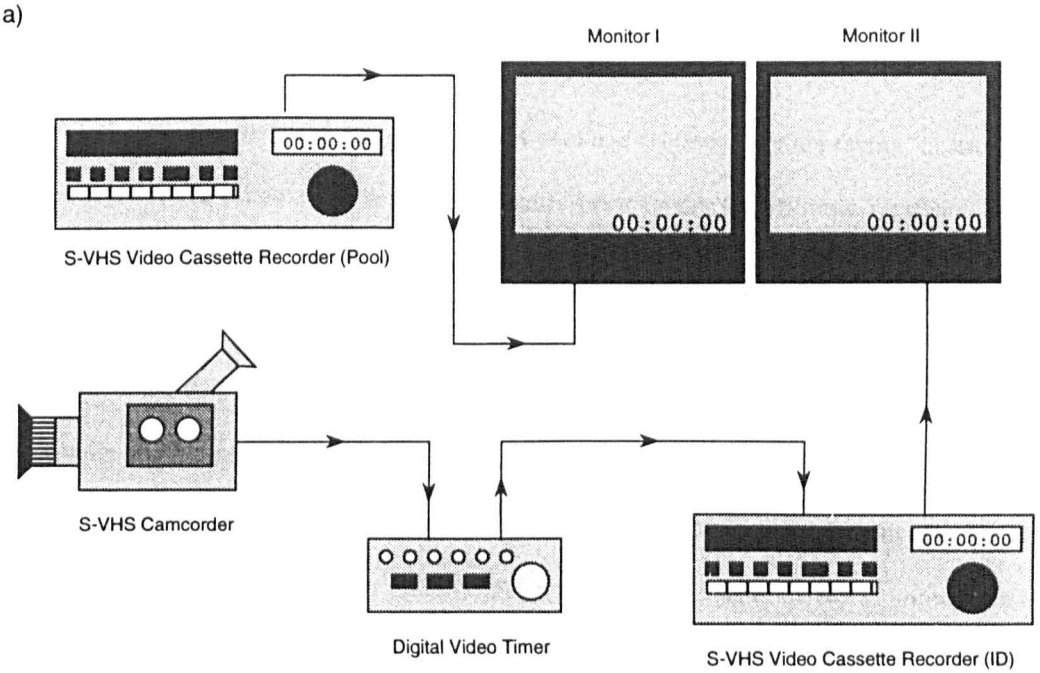

a)
Plan Camera
Direction of water flow
I.D. Camera
PUMP
Plastic Pool
Spray bar
Water inlet
180cm
b)
A
45 °
B
180cm

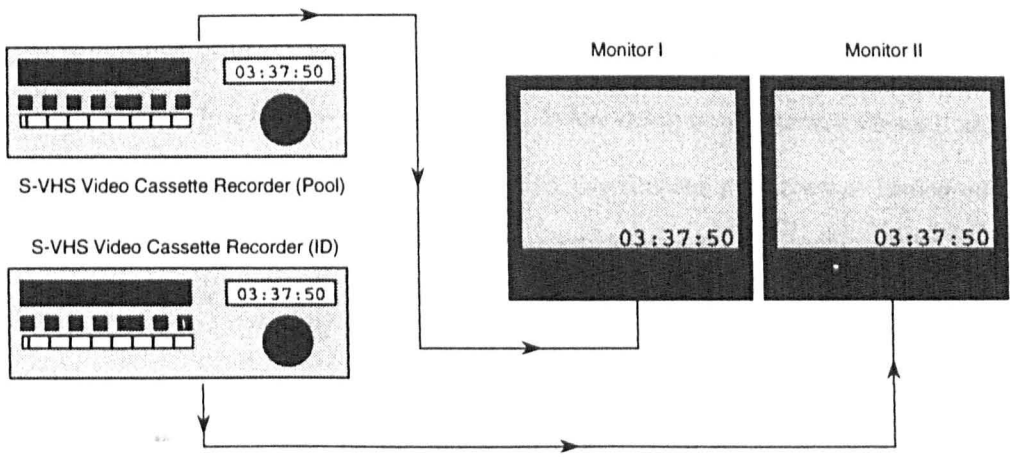
Figure 5.2a Schematic diagram illustrating the method employed to add synchronous timing signals to the videotape. The VHS-C tape from the plan camera, complete with on-screen stopwatch display, was recorded onto a standard VHS format videotape, and replayed on a monitor (Monitor I). The VHS-C tape from the ID camera, which had no on-screen display, was replayed on the second monitor (Monitor II) via a digital video timer and synchronised with the image from the plan camera. When the two tapes were synchronised, the digital video timer was used to add a corresponding on-screen timing signal to the standard VHS format videotape onto which ID image was being recorded.

Figure 5.2b Schematic diagram illustrating the equipment used to analyse the plan and ID videotapes synchronously.

a)


Monitor I
Monitor II
00:00:00
00:00:00
00:00:00
S-VHS Video Cassette Recorder (Pool)
S-VHS Camcorder
Digital Video Timer
00:00:00
S-VHS Video Cassette Recorder (ID)

b)


03:37:50
Monitor I
Monitor II
S-VHS Video Cassette Recorder (Pool)
03:37:50
03:37:50
S-VHS Video Cassette Recorder (ID)
03:37:50

external movements. A 10cm x 10cm grid was marked onto the base of the pool to facilitate the recording of the precise positions of individual fish.

5.2.3 Experimental protocol

The ten fish were transferred to the experimental pool and released together facing 'upstream'. The fish formed a polarised school, and were left to settle prior to the first filming session. Each filming session lasted five minutes, and the fish were filmed after 1h, 2h, 3h, 4h and 5h of residence in the experimental pool (Fig. 5.1a). During each filming session the fish were filmed simultaneously by two cameras; one camera, the 'plan camera', (a Panasonic NV-MS 95 S-VHS camcorder, fitted with a Hama HR 0.5x wide-angle lens) was mounted on a tripod 1.3m above the water surface, providing a complete view of the whole pool, and the other, the 'I.D. camera', (a JVC GRM-7 camcorder) was hand-held by the operator, and was used to identify and track the movements of the *L. intestinalis*-infected individual. Immediately prior to the fifth filming session (after 5h of residence) an avian attack was simulated by releasing a cardboard silhouette of a kingfisher from a position 1.5m above the tank along a guide-wire to a position just above the surface of the water at the opposite side of the pool, at an angle of 45° (Figure 5.1b) (Licenced procedue, see page iii). Because of methodological constraints, the behaviour of the fish in the pool could only be recorded by the plan camera during the simulated attack, preventing the identification of the position of the infected fish during the attack.

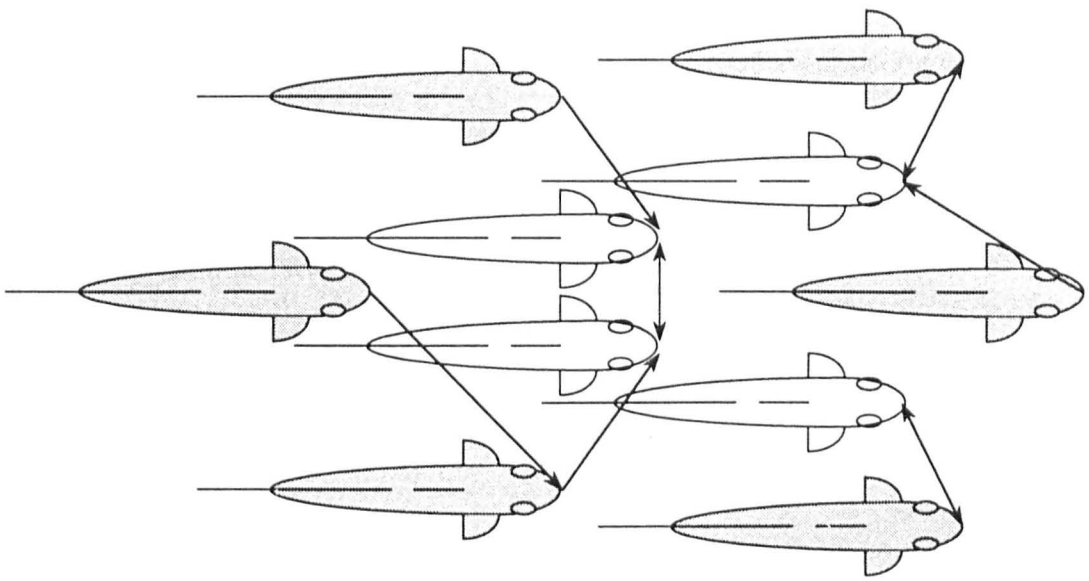
After the termination of the fifth and final filming session, the fish were removed from the experimental pool and exposed to a lethal dose of anaesthetic, before being weighed and measured, and finally dissected to ascertain *L. intestinalis* parasite status. The experimental protocol was carried out with each of the six groups of fish.

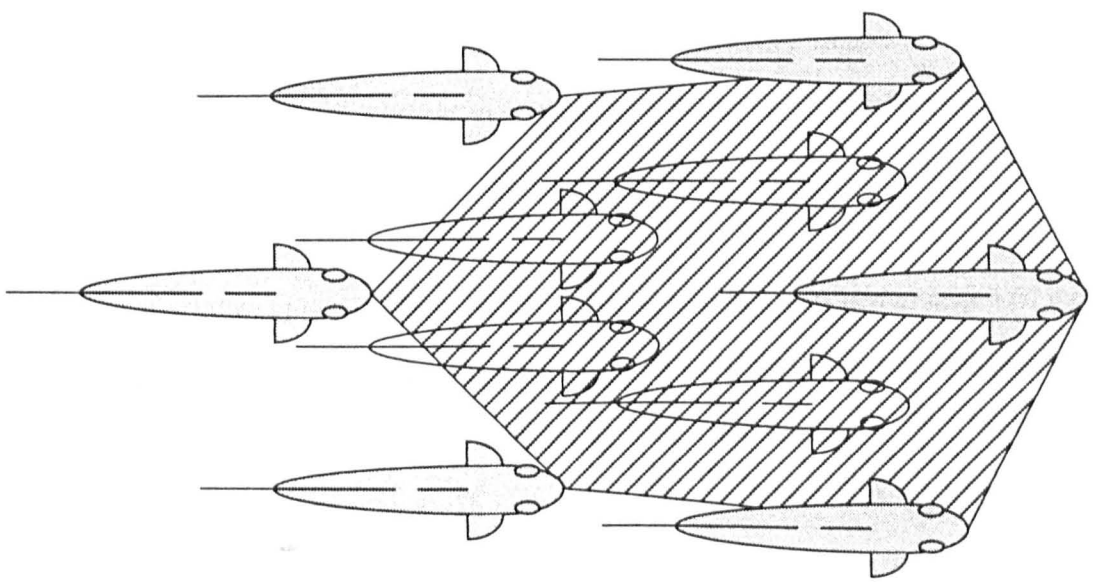
5.2.4 Video analysis

In order to collect accurate data on the individual school positions of uninfected and *L. intestinalis*-infected fish, it was necessary to analyse the videotape from the plan and I.D. cameras simultaneously. To ensure that the two films ran synchronously, the on-screen timing signal from the plan camera was matched and copied onto the film from the I.D. camera (which previously had no on-screen signal) using a video-timing unit (see Figure 5.2a and legend for a detailed description of the technique employed).

Figure 5.3a A diagrammatic summary of the method used to calculate nearest neighbour distance (NND). The position of the snout of each fish in the school was represented by a point, and the distance from each point to its closest neighbour (illustrated by an arrow in the diagram) was measured using a digitising tablet. The mean NND was calculated for the uninfected fish in the school, and compared with the NND for the *Ligula intestinalis*-infected fish.

Figure 5.3b A diagrammatic summary of the method used to calculate school area. Individuals on the periphery of the school were identified (shaded in the diagram) and the points denoting the positions of their snouts joined to form a convex polygon, illustrated in the diagram by the hatched area. This area was then measured using a digitising tablet.





Once the synchronous timing signals had been added, the videotapes were then analysed simultaneously using the freeze-frame and frame advance facilities of the two video cassette recorders (Figure 5.2b), with the positions of each fish in the pool being taken from the plan film, and the identification of the *L. intestinalis*-infected fish being made from the I.D. film. Frames were analysed at 15s intervals throughout each five minute filming period. The precise co-ordinates of each fish in the school were represented on a pool map, with the snout of each fish being represented by a point, and the location of the infected fish in the school clearly identified.

During the avian attack, the positions of each fish in the pool were recorded every 0.1s, beginning 2s before the model was released, to allow the rapid movements of the startled fish to be recorded accurately. Analysis of the fifth filming period was carried out as previously, with frames being analysed at 15s intervals.

5.2.5 Spatial analysis of fish school data

Fish were classified as school members if they were within five body lengths of another individual. From qualitative analysis of the schooling behaviour of fish in the arena, individuals within five body lengths of their nearest neighbour appeared to maintain speed and synchrony with the rest of the school, whereas those outwith this distance appeared to be behaving independently of the group. The chosen criterion is close to that of four body lengths used by Magurran & Pitcher (1987).

5.2.5.1 Nearest neighbour distance

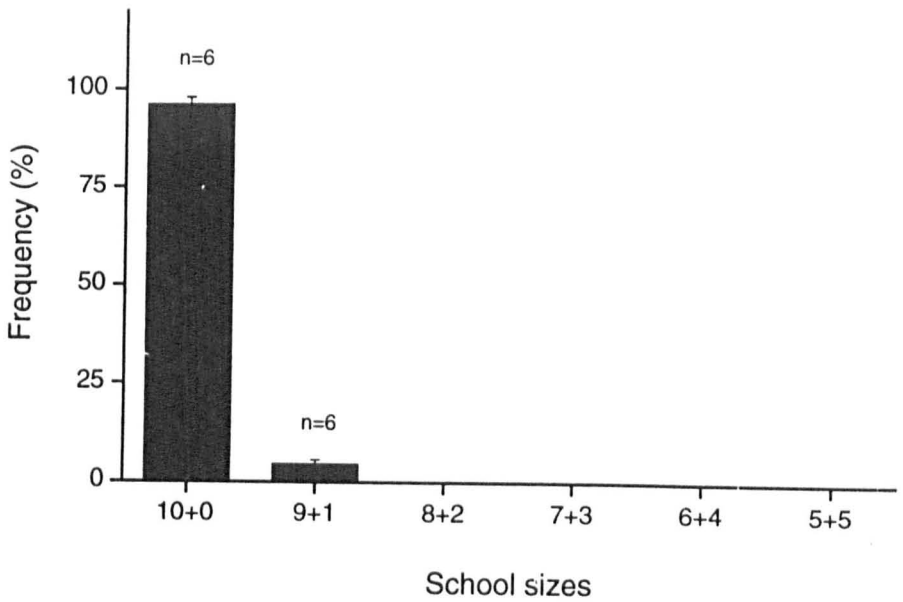
For each analysed frame of film, the distance from each fish to its nearest neighbour (nearest neighbour distance, NND; Figure 5.3a) in the school was measured using a calibrated digitising tablet (Cherry Electrical Products Ltd., Harpendon, Herts, U.K.) connected to a BBC Master series microcomputer (software written by M. D. Burns, University of Glasgow, U.K.). The mean nearest neighbour distance of uninfected fish in the school, and the standard deviation from this mean, was calculated and compared with the NND of the *L. intestinalis*-infected fish.

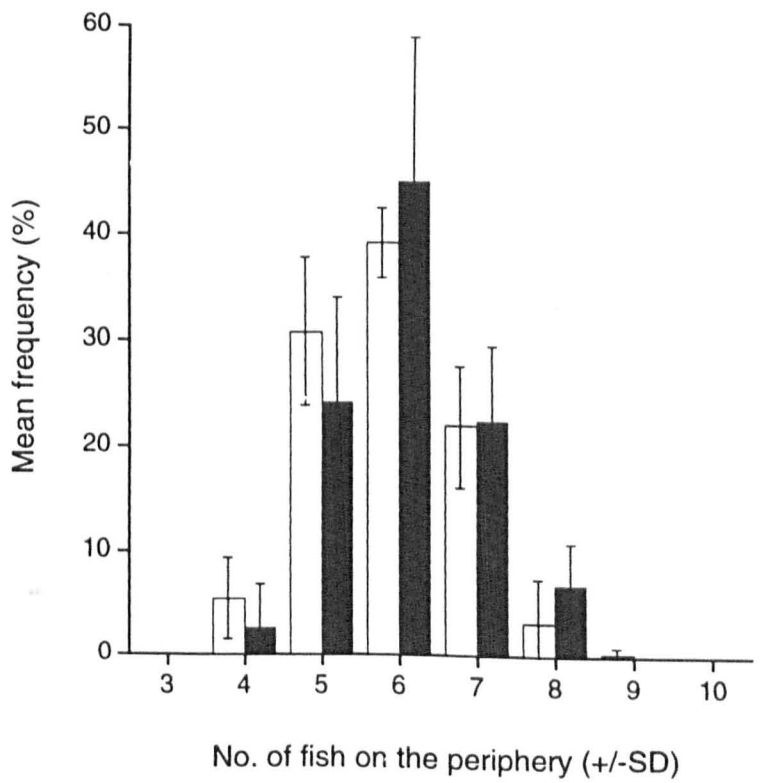
5.2.5.2 School area

Using the minimum-area convex polygon technique described by Krause & Tegeder (1994), the fish on the periphery of the school in each frame for which points had been plotted were identified

Figure 5.4 The frequency with which the ten fish in the experimental flow pool formed schools of various sizes during the six experimental trials. Mean values, with standard deviations, are shown.

Figure 5.5 The frequency with which each school configuration, based on the number of peripheral fish, occurred in schools comprising ten individuals from the six trials. The distributions of pre-strike (□) and post-strike (■) configurations are shown. Bar heights are medians, with error bars representing interquartile ranges.


n=6
n=6
100
75
50
25
0
Frequency (%)
10+0
9+1
8+2
7+3
6+4
5+5
School sizes


60
50
40
30
20
10
0
Mean frequency (%)
3
4
5
6
7
8
9
10
No. of fish on the periphery (+/-SD)

and joined together to form a polygon (Figure 5.3b). The area enclosed by this polygon, which represented the two-dimensional area covered by the school, was measured using the digitising tablet and microcomputer (see above).

5.2.5.3 Peripheral vs central position occupancy

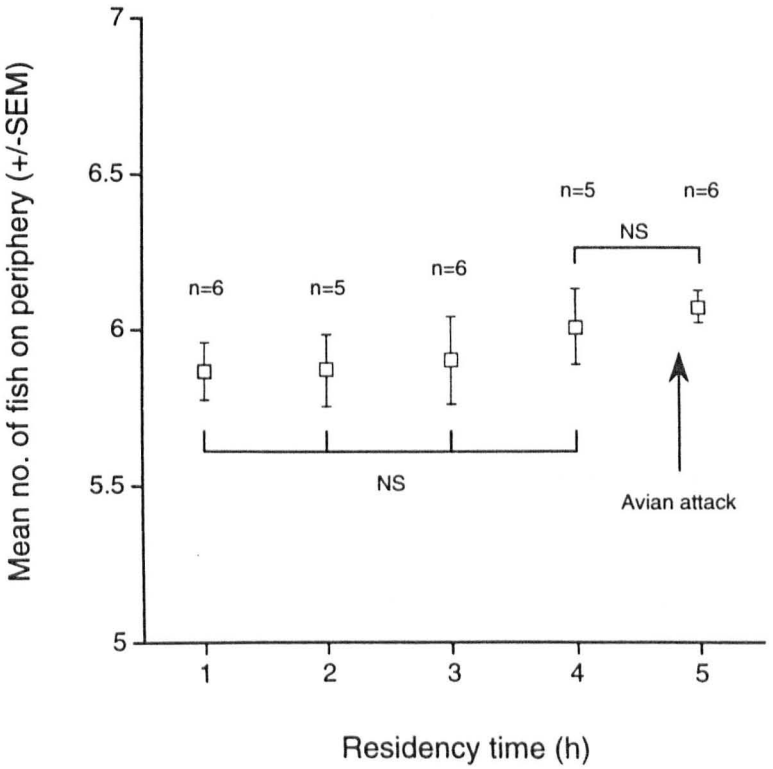
For each analysed frame of film, the number of peripheral fish in the school (i.e. those at the vertices of the polygon described above) was determined. For schools comprising ten fish, the minimum number of fish that could be present on the periphery as described above is three, and the maximum, ten. The frequency with which schools had each possible number of peripheral members was calculated for each five minute filming period. The frequency with which infected fish occupied the school periphery under each school configuration (i.e. number of peripheral fish) was also calculated. For a school composed of any number of individual members under any configuration, it is possible to calculate the expected frequency (F_{exp}) with which any one individual should be found on the periphery, assuming random assortment of school members within the school, using the following equation:

$$F_{exp} = \frac{\text{Number of peripheral fish}}{\text{Total number of fish in school}} * 100 .$$

5.2.6 Statistical Analysis

Statistical analysis of the experimental data needs to take into account the fact that certain features of schools may possibly not alter between successive individual frames within a trial, and therefore individual frames are unlikely to be independent observations. For example, a fish that is on the periphery of a school in one frame is likely to be on the periphery of the school in the next frame, and the second observation is probably at least partly determined by the first. For this reason, the experiment was designed so that frames to be analysed were taken from film taken in discrete temporal blocks - for five minutes after 1h, 2h, 3h and 4h of pool residency - and individual data for schools within each five minute filming period (a 'run') were combined and averaged for certain analyses. It was proposed that data analysis would take place at this level, following statistical testing to prove independence between runs.

Figure 5.6 The effect of residency time and the simulated avian attack on school configuration based on the number of fish occupying peripheral positions. Combined mean values of the six experimental trials are shown, with error bars representing SEMs.


Mean no. of fish on periphery (+/-SEM)
7
6.5
6
5.5
5
n=6
n=5
n=6
n=5
n=6
NS
NS
Avian attack
1
2
3
4
5
Residency time (h)

5.3 RESULTS

5.3.1 Number of fish in the school

Of the frames of film analysed from each trial, 96.1 ± 1.4% (mean ± SEM; n=6) showed schools comprised of all ten individuals present in the pool, with the remainder of frames showing schools comprising nine fish and one loner. None of the analysed frames showed schools comprising fewer than nine members (Figure 5.4). When the school was composed of nine fish, the lone fish was the *L. intestinalis*-infected minnow 25.0 ± 17.1% (mean ± SEM; n=6) of the time, which is not statistically more often than would be expected by chance, i.e. 10% (t-test t=0.88, n=6, P=0.42, N.S.). Because of the small frequency of schools comprising fewer than ten individuals, they were excluded from the rest of the analysis.

5.3.2 School configuration

Based on the number of fish classified as *peripheral* or *central*, a school comprising ten individuals may exhibit one of eight possible configurations (see Table 5.1). The observed number of peripheral fish in the school in the experimental trials ranged from four to nine with a normal distribution between these extremes and a modal configuration of six peripheral and four central members both before and after the simulated avian attack (Figure 5.5). The simulated attack had no significant effect on the distribution of peripheral occupancy frequencies (Kolmogorov-Smirnov 2-sample test, $D_{max}=0.096$, $\chi^2=1.8432$, $P>0.1$, N.S.). 91.5 ± 1.7% (combined mean ± SEM; n=6) of frames analysed from all pre-attack filming sessions in each trial and 90.7 ± 2.0 (combined mean ± SEM; n=6) analysed from all post-attack filming sessions in each trial showed schools with five, six or seven peripheral members (Figure 5.5). The number of peripheral fish observed in each school during the pre-attack period was unaffected by residency time (ANOVA, $F_{3,23}=0.16$, P=0.920, N.S.) and although the number of peripheral members appeared to increase slightly following the simulated avian attack, there was no significant difference in the number of peripheral members in the school before and immediately after the attack (paired t-test, t=1.31, n=6, P=0.25, N.S.) (Figure 5.6).

5.3.3 School area

A significant positive correlation existed between residency time and the area covered by the school in the pool for five out of the six experimental trials, demonstrating that as the fish adapted to

their new surroundings they gradually formed less compact schools (Figure 5.7). A significant negative relationship existed between residency time and school area in one trial, trial 2, and no reason for this apparent anomaly can be given, other than that the smaller, more uniform school areas after 3 and 4 hs of residence in this trial were similar to those of the other fish schools following the simulated avian attack (see later), suggesting that the fish may have been startled unintentionally between the second and third filming sessions.

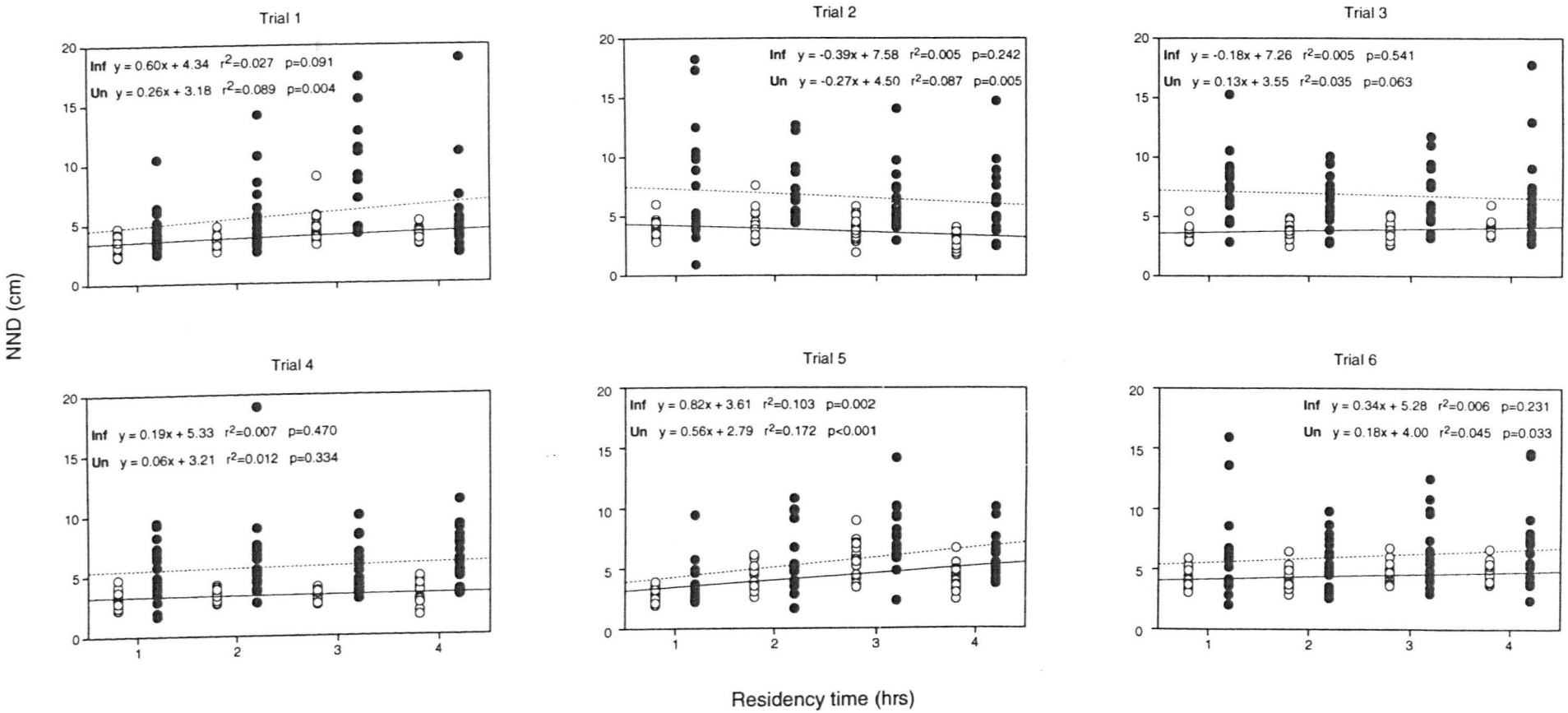
Table 5.1 The eight possible peripheral / central configurations that can be formed by a school comprising ten individuals

No. of peripheral fish	No. of central fish	Diagram	Frequency (%) (pre attack / post attack)
3	7		0% / 0%
4	6		5.3% / 2.5%
5	5		30.5% / 23.7%
6	4		39.0% / 44.9%
7	3		21.9% / 22.0%
8	2		3.1% / 6.8%
9	1		0.2% / 0%
10	0		0% / 0%

Figure 5.7 The effect of residency time and the simulated avian attack on the area covered by a school of minnows. The areas covered by the school in the 20 frames analysed from each 15-minute filming after 1h, 2h, 3h, and 4h of residency are shown. Significant positive relationships exist between residency time and school area in all but one of the trials. Regression equations are given in the individual figures.

y=22.0x + 109 r²=0.091 p=0.006
Trial 1
y= -31.6x + 263 r²=0.143 p<0.001
Trial 2
y=20.2x + 143 r²=0.071 p=0.013
Trial 3
y=12.3x + 103 r²=0.084 p=0.006
Trial 4
y = 51.0x + 84 r²=0.125 p=0.001
Trial 5
y = 21.9x + 183 r²=0.076 p=0.008
Trial 6
School area (cm²)
Residency time (hrs)

Figure 5.8 The effect of residency time on the nearest neighbour distances (NNDs) of individual *Ligula intestinalis*-infected minnows (●), and the mean NNDs of uninfected minnows (O) in schools observed during the six experimental trials. Regression equations are given in the individual figures.


Trial 1
Inf y = 0.60x + 4.34 r²=0.027 p=0.091
Un y = 0.26x + 3.18 r²=0.089 p=0.004
Trial 2
Inf y = -0.39x + 7.58 r²=0.005 p=0.242
Un y = -0.27x + 4.50 r²=0.087 p=0.005
Trial 3
Inf y = -0.18x + 7.26 r²=0.005 p=0.541
Un y = 0.13x + 3.55 r²=0.035 p=0.063
Trial 4
Inf y = 0.19x + 5.33 r²=0.007 p=0.470
Un y = 0.06x + 3.21 r²=0.012 p=0.334
Trial 5
Inf y = 0.82x + 3.61 r²=0.103 p=0.002
Un y = 0.56x + 2.79 r²=0.172 p<0.001
Trial 6
Inf y = 0.34x + 5.28 r²=0.006 p=0.231
Un y = 0.18x + 4.00 r²=0.045 p=0.033
NND (cm)
Residency time (hrs)

5.3.4 Nearest neighbour distance, NND

5.3.4.1 The effect of residency time on NND

Significant positive relationships were found between residency time and the mean NND of uninfected fish in the school in the pool in four out of the six experimental trials (Figure 5.8). Uninfected fish in trial 2, previously described as being apparently anomalous, exhibited a significant negative relationship, whereas uninfected fish in trial 4 exhibited a non significant positive relationship. Infected fish appeared to adjust their NND to a lesser extent as residency time increased. Only in one of the experimental trials (trial 5) was there a significant positive relationship between residency time and the NND of the infected fish in the school (figure 5.8).

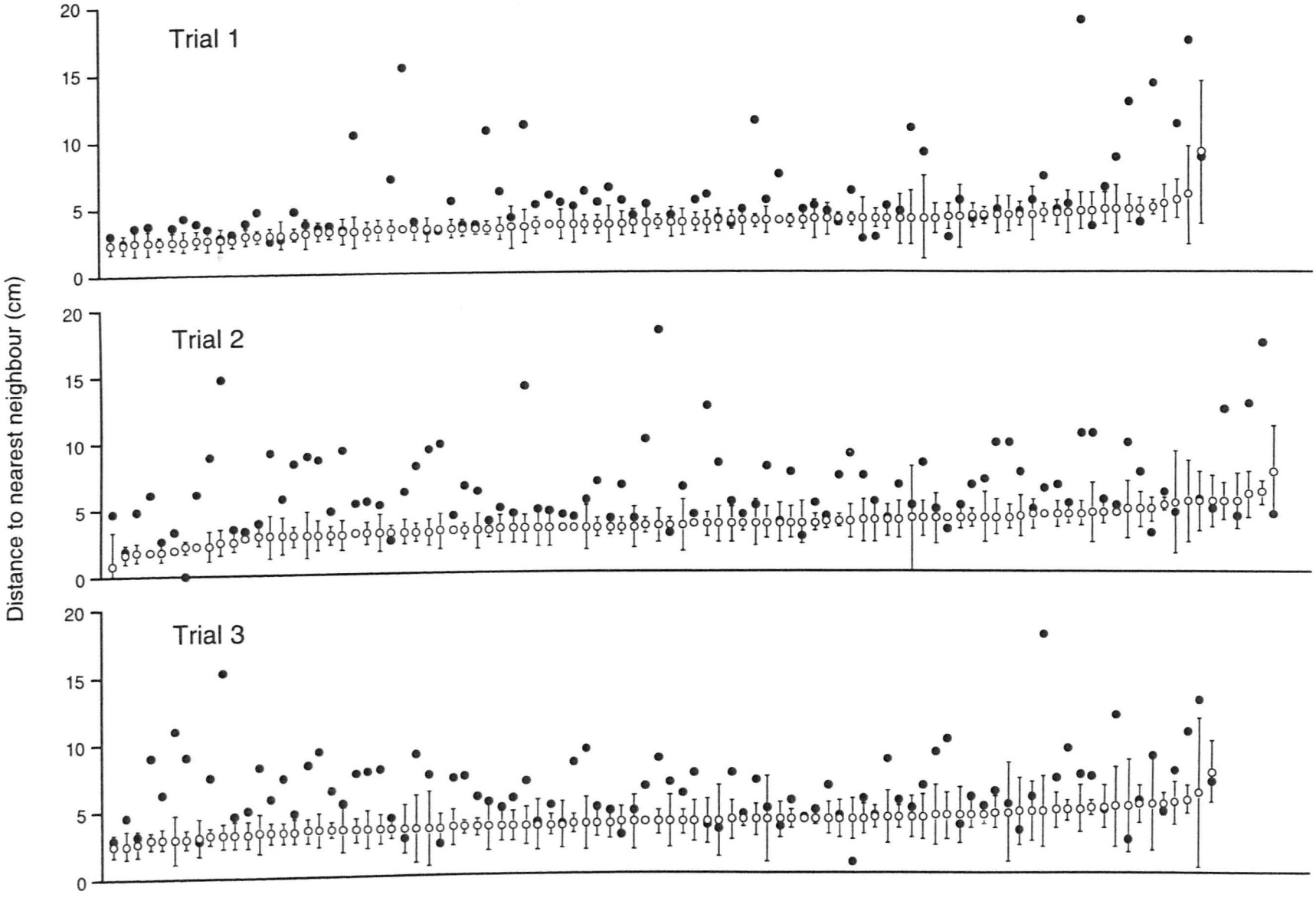
5.3.4.2 The NND of *L. intestinalis*-infected and uninfected school members

The NND of the *L. intestinalis*-infected school member was greater than the mean NND of the nine uninfected fish in the school more often than would be expected by chance in all six runs of the experiment (Figure 5.9; for results of χ^2 tests, see Table 5.2). The magnitude of the difference between the mean NND of uninfected school members and that of infected fish was expressed by calculating the ratio of infected NND : mean uninfected NND for each analysed frame of film. The cumulative frequency of this ratio plotted for each experimental trial was plotted (Figure 5.10), and from this plot, the minimum value, maximum value and median value of the ratio ($Ratio_{min}$, $Ratio_{max}$ and $Ratio_{50}$, respectively) were calculated. Mean and individual trial values of these ratios are given in Table 5.3.

5.3.4.2 The relationship between NND and school area for uninfected and infected minnows

Predictably, a strong positive relationship was found to exist between the mean NND of uninfected school members and school area in each of the six trials. There was also a significant positive relationship between the NND of the *L. intestinalis*-infected minnow in the school and school area in each trial (see Figure 5.11 and Table 5.3 for regression analyses). However, in each trial the regression lines defining the relationships between NND and school area for uninfected and *L. intestinalis*-infected minnows differed significantly, with infected fish showing, on average, a significantly higher NND than uninfected fish for any given school area (see Table 5.3 for results of analysis of covariance).

Figure 5.9 The nearest neighbour distance (NND) of *Ligula intestinalis*-infected minnows (●) compared with the mean NND of uninfected school members (O ; error bars represent ± 1 SD from the mean NND of the uninfected fish) for schools observed in each frame analysed from the six experimental trials. For heuristic reasons, schools have been ranked in ascending order along the x-axis with respect to mean NND for the uninfected school members.


Trial 1
Trial 2
Trial 3
Distance to nearest neighbour (cm)
0
5
10
15
20

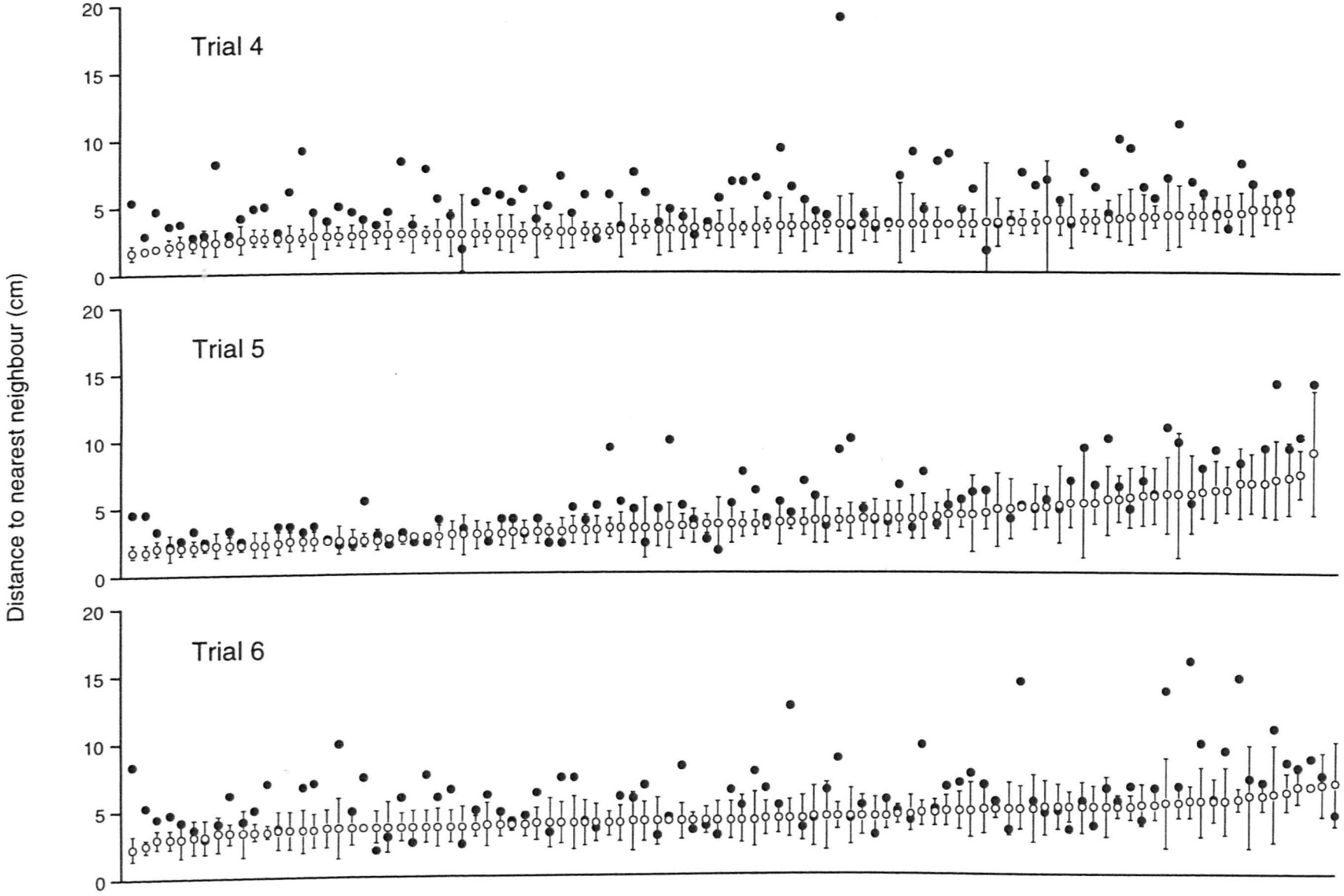

Trial 4
Trial 5
Trial 6
Distance to nearest neighbour (cm)
0
5
10
15
20

Figure 5.10 The ratio of the NND of parasitised minnows to the mean NND of unparasitised minnows in schools analysed in the six trials, plotted as cumulative frequencies.

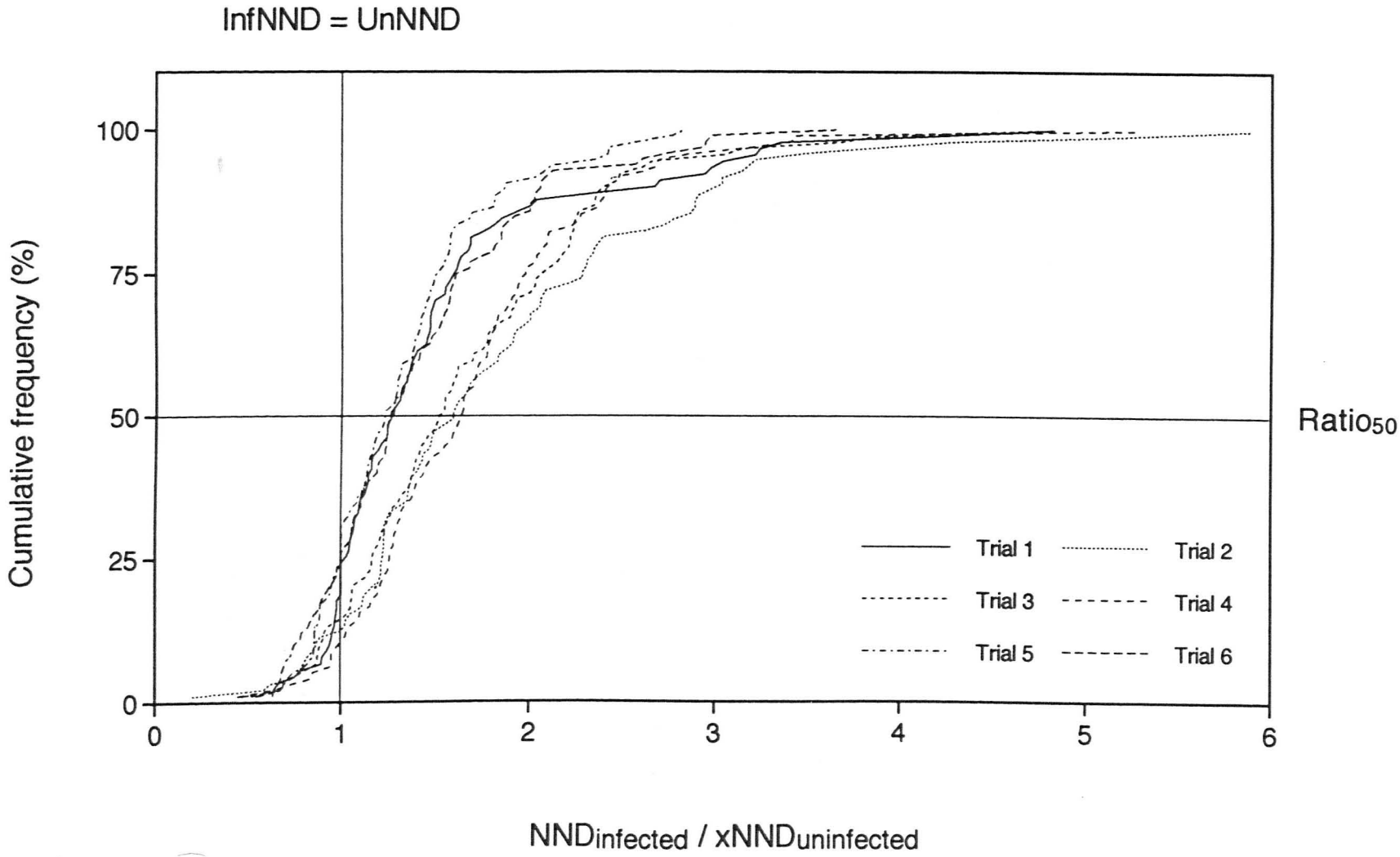

InfNND = UnNND
Cumulative frequency (%)
100
75
50
25
0
0
1
2
3
4
5
6
Ratio50
Trial 1
Trial 2
Trial 3
Trial 4
Trial 5
Trial 6
NNDinfected / xNNDuninfected

Figure 5.11 The relationship between the area covered by the school and the nearest neighbour distance (NND) of *Ligula intestinalis*-infected minnows (●) and uninfected minnows (O ; mean values for all uninfected fish in each analysed school) in each of the six trials. Regression equations and results of covariance analysis are given in Table 5.5.

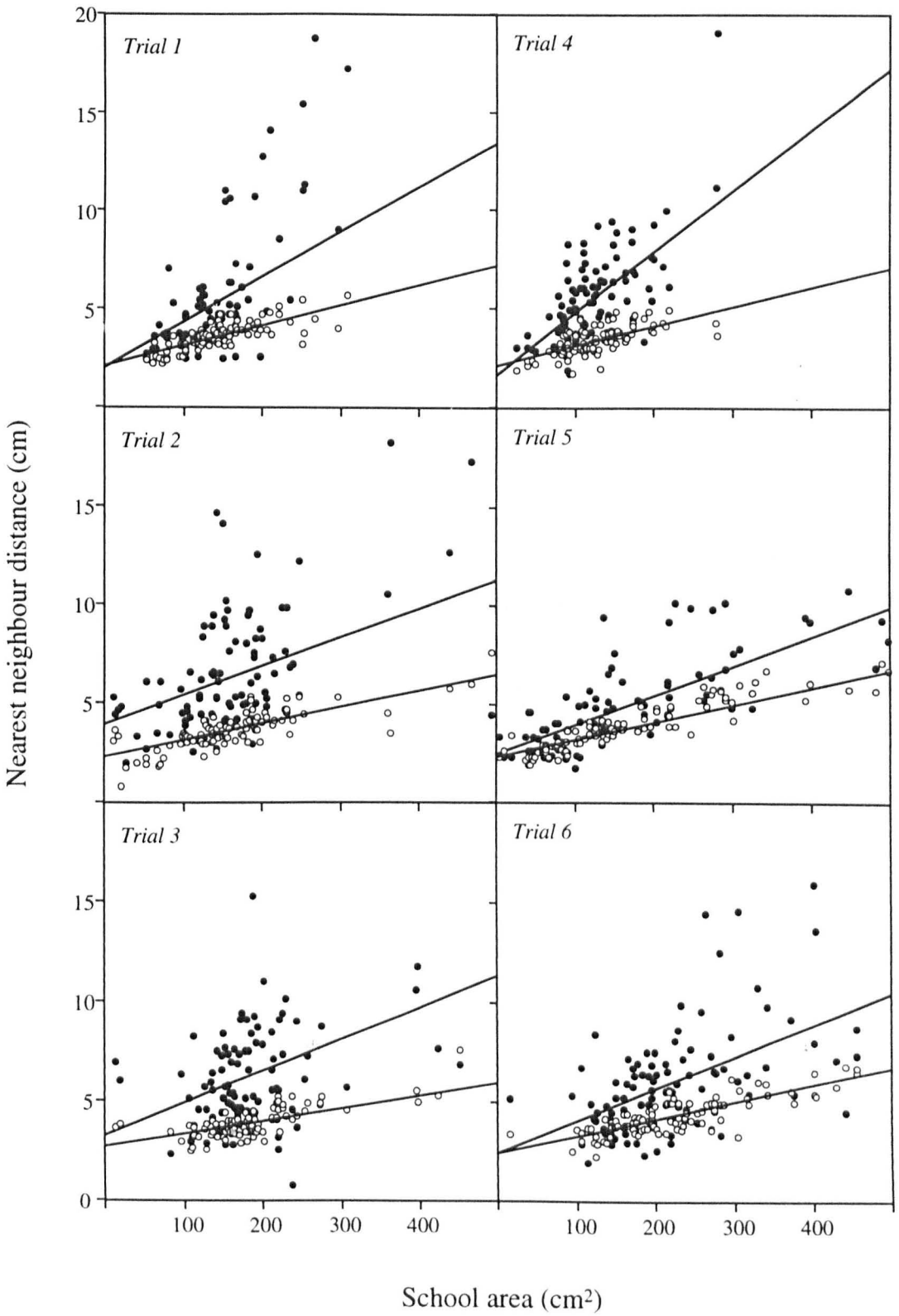

Trial 1
Trial 4
Trial 2
Trial 5
Trial 3
Trial 6
Nearest neighbour distance (cm)
School area (cm²)
0
5
10
15
20
100
200
300
400

Figure 5.12 The expected (——) and observed (O) frequencies of *L. intestinalis*-infected individuals occupying peripheral positions in schools with varying numbers of peripheral fish (see text for calculation of expected values). Observed values given are means for the six experimental trials. with error bars representing ± 1 SD from the mean.

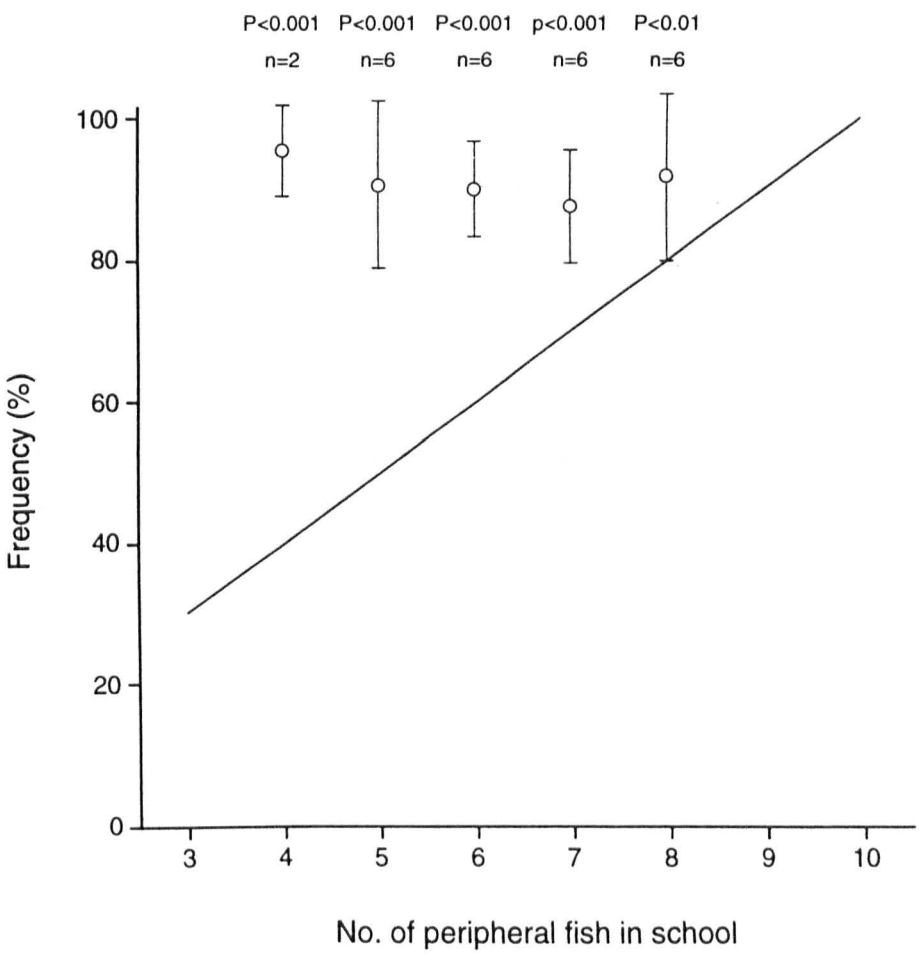

P<0.001
n=2
P<0.001
n=6
P<0.001
n=6
p<0.001
n=6
P<0.01
n=6
100
80
60
40
20
0
Frequency (%)
3
4
5
6
7
8
9
10
No. of peripheral fish in school

Figure 5.13 Changes in the area covered by the school during the simulated avian attack. The kingfisher model was released from its resting position at time = 0, marked by arrows in the figure.

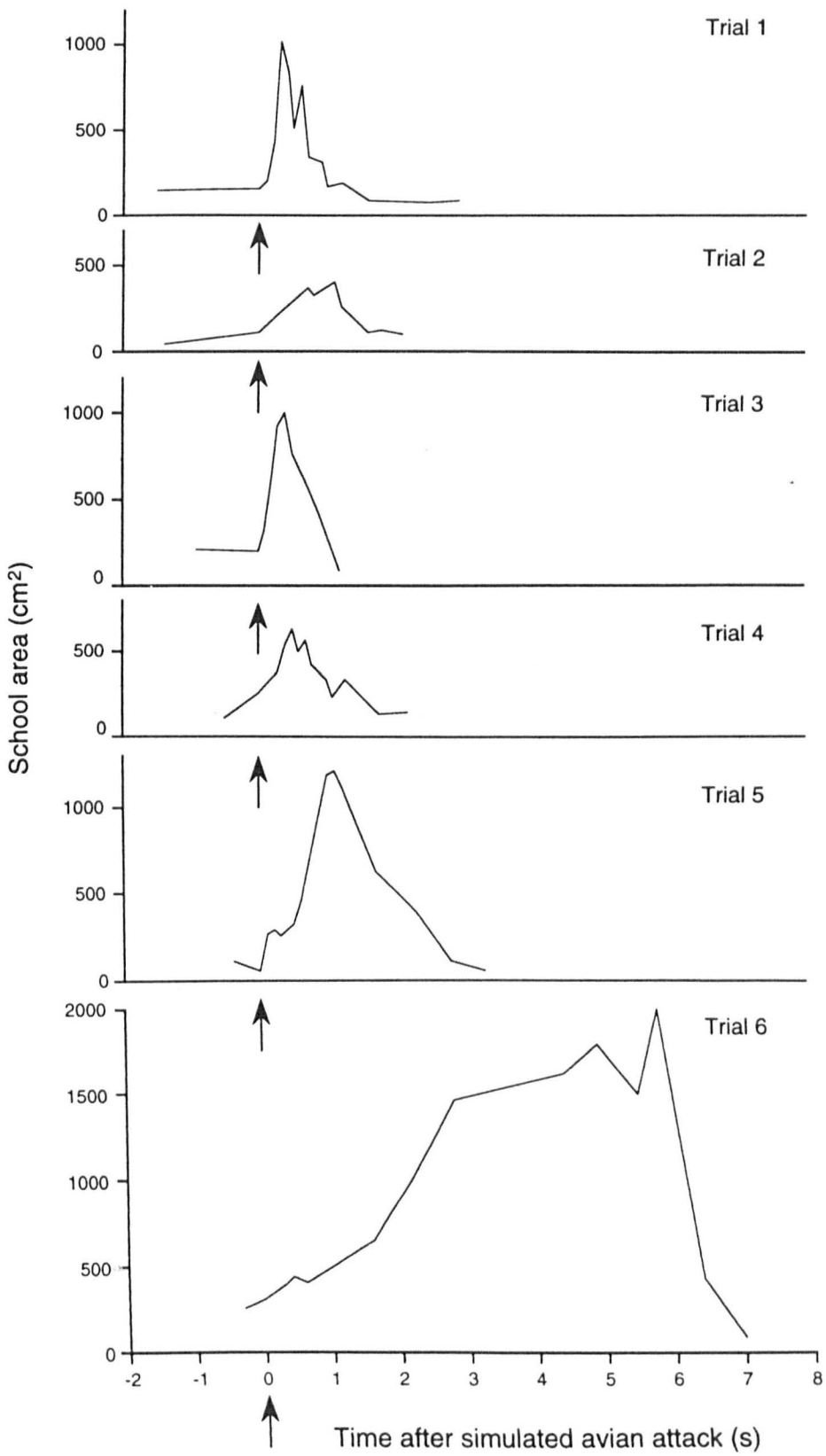
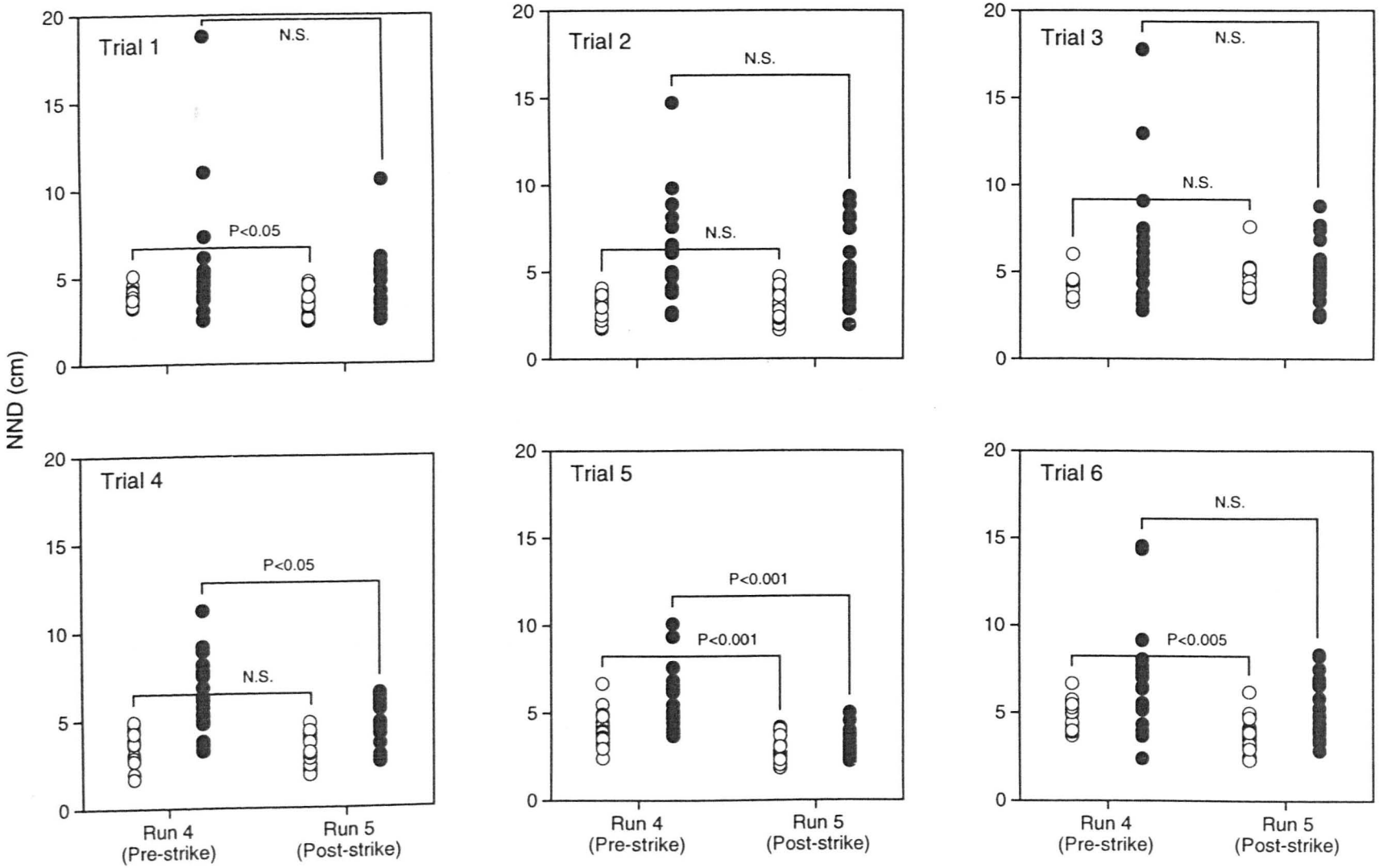

Trial 1
Trial 2
Trial 3
Trial 4
Trial 5
Trial 6
School area (cm²)
Time after simulated avian attack (s)
1000
500
0
2000
1500
-2
-1
1
2
3
4
5
6
7
8

Figure 5.14 The effect of the simulated avian attack on the area covered by minnow schools. The school areas measured before (Run 4) and immediately after the simulated attack (Run 5) are shown for each of the six experimental trials. Significance values refer to analysis of variance tests, details of which are provided in the text (section 5.3.6).

School area (cm2)
600
400
200
0
Trial 1
P<0.001
Trial 2
N.S.
Trial 3
N.S.
Trial 4
P<0.001
Trial 5
P<0.001
Trial 6
P<0.001
Run 4
(pre-strike)
Run 5
(post-strike)

Figure 5.15 The effect of the simulated avian attack on the nearest neighbour distances (NNDs) of *Ligula intestinalis*-infected minnows (●) and by uninfected minnows (O ; mean values of all uninfected fish in each school). NNDs were measured before (Run 4) and immediately after the simulated attack (Run 5) and are shown for each of the six experimental trials. Significance values refer to analysis of variance tests, details of which are provided in the text (section 5.3.6).


NND (cm)
Trial 1
N.S.
P<0.05
Trial 2
N.S.
N.S.
Trial 3
N.S.
N.S.
Trial 4
P<0.05
N.S.
Trial 5
P<0.001
P<0.001
Trial 6
N.S.
P<0.005
0
5
10
15
20
Run 4
(Pre-strike)
Run 5
(Post-strike)

5.3.5 Occupancy of peripheral positions

Analysis of school positions during the pre-attack period was made at the level of hourly samples which appear to be independent observations, since there was no significant difference in the variance within samples and the total variance (mean variance within samples (n=24) = 0.8331, total variance (n=456) = 0.8640, t = 0.55, P=0.59, N.S.).

In a school comprising ten individuals, assuming random assortment of school positions, any particular individual school member should be expected to be found on the periphery 30% of the time when there were three peripheral members, 40% when there were four, 50% when there were five and so on (see 5.2.5.3). In the experimental trials, *L. intestinalis*-infected minnows were found on the periphery of the school more often than would have been expected by chance, had random assortment been taking place, for each school configuration (4 on periphery: χ^2=128.34, d.f.=1, P<0.001; 5 on periphery: χ^2=66.26, d.f.=1, P<0.001; 6 on periphery: χ^2=37.25, d.f.=1, P<0.001; 7 on periphery: χ^2=14.75, d.f.=1, P<0.001; 8 on periphery: χ^2=8.56, d.f.=1, P<0.01. Figure 5.12).

5.3.6 Effect of the simulated avian attack

5.3.6.1 During the attack

Immediately following the release of the kingfisher silhouette from its pre-attack position, school area was observed to increase rapidly to a size several times the pre-attack area as the schooling fish scattered (median maximum school area during attack = 1004.0cm^2; median school area increase factor[1] = 5.6; see Table 5.4). This marked expansion was followed in each trial by an equally rapid contraction, returning to the pre-attack school area quickly (median time to return to pre-attack school area from commencement of attack = 1.45s; see Table 5.4) (Figure 5.13).

5.3.6.2 After the attack

During the five minute filming session immediately following the simulated avian attack, school area was significantly reduced from pre-attack levels in four out of the six experimental trials

[1] = Maximum post-strike school area / Pre-strike school area

Table 5.2 Results of Chi-square goodness-of-fit tests to investigate the NNDs of infected and uninfected school members in frames analysed from each experimental trial

Trial no.	n	Infected NND > mean				
		Expected	Observed	χ^2	df	P
1	91	45.5	75	38.25	1	P<0.001
2	97	48.5	88	64.34	1	P<0.001
3	92	46.0	79	47.34	1	P<0.001
4	96	48.0	87	63.38	1	P<0.001
5	98	49.0	75	27.59	1	P<0.001
6	100	50.0	77	29.16	1	P<0.001

Table 5.3 The values of the minimum ($Ratio_{min}$), maximum ($Ratio_{max}$) and median values ($Ratio_{50}$) of the ratio of infected NND : mean uninfected NND for each experimental trial.

Trial no.	$Ratio_{min}$	$Ratio_{max}$	$Ratio_{50}$
1	0.63	4.81	1.26
2	0.20	5.88	1.59
3	0.52	4.78	1.51
4	0.45	5.28	1.62
5	0.47	2.83	1.22
6	0.54	3.65	1.26
Mean ± SD	0.47 ± 0.15	4.54 ± 1.11	1.41 ± 0.18

Table 5.4 Characteristics of the escape response of the school during the simulated avian attack in each of the six experimental trials

Trial No.	Latency (s)	$Area_{max}$	$\frac{Area_{max}}{Area_{orig}}$
1	1.30	1005.0	6.9
2	1.59	395.0	3.4
3	1.09	1003.2	4.9
4	1.04	630.5	2.5
5	3.24	1211.8	20.4
6	6.63	2003.9	6.3
Median (95% CI)	1.45 (1.08-4.09)	1004.0 (572-1410)	5.6 (3.2-10.3)

Table 5.5 Results of analysis of covariance tests describing the differences in the relationships between the school area and nearest neighbour distance (NND) for uninfected and the *L. intestinalis*-infected school members

Trial No.	Uninfected minnows			*L. intestinalis*-infected minnow			ANCOVA		Ratio of slopes (m_{inf}/m_{un})
	Regression equation	r^2	P	Regression equation	r^2	P	$F_{(df1,df2)}$	P	
1	$y = 0.0102x + 2.16$	0.744	P<0.001	$y = 0.0227x + 2.10$	0.274	P<0.001	$10.34_{(1,178)}$	P=0.002	2.225
2	$y = 0.0088x + 2.18$	0.623	P<0.001	$y = 0.0157x + 3.83$	0.193	P<0.001	$4.41_{(1,190)}$	P=0.037	1.784
3	$y = 0.0069 + 2.62$	0.562	P<0.001	$y = 0.0181x + 2.94$	0.259	P<0.001	$14.68_{(1,180)}$	P<0.001	2.623
4	$y = 0.0098x + 2.12$	0.448	P<0.001	$y = 0.0310x + 1.69$	0.369	P<0.001	$24.73_{(1,188)}$	P<0.001	3.163
5	$y = 0.0090x + 2.26$	0.865	P<0.001	$y = 0.0149x + 2.42$	0.696	P<0.001	$30.55_{(1,192)}$	P<0.001	1.656
6	$y = 0.0084x + 2.36$	0.674	P<0.001	$y = 0.0168x + 2.24$	0.278	P<0.001	$8.38_{(1,196)}$	P=0.004	2.000

(one-way ANOVA: Trial 1 $F_{1,39}$=25.84, p<0.001; Trial 2 $F_{1,39}$=0.05, p=0.825, N.S.; Trial 3 $F_{1,36}$=0.47, p=0.499, N.S.; Trial 4 $F_{1,39}$=18.58, p<0.001; Trial 5 $F_{1,39}$=23.00, p<0.001; Trial 6 $F_{1,39}$=29.84, p<0.001; Figure 5.14).

In trials 2 and 3, no significant reduction in school area was observed, although in each case the mean area covered by the fish in the school in the filming session before the simulated attack exceeded that exhibited in the post attack filming session. Following the simulated avian attack, the mean NND of uninfected fish in the school was significantly reduced in 3 of the 6 trials (one-way ANOVA: Trial 1 $F_{1,39}$=6.06, p=0.019; Trial 2 $F_{1,39}$=0.00, p=1.00, N.S.; Trial 3 $F_{1,36}$=0.94, p=0.338, N.S.; Trial 4 $F_{1,3}$=0.39, p=0.536, N.S.; Trial 5 $F_{1,39}$=20.61, p<0.001; Trial 6 $F_{1,39}$=13.10, p=0.001; Figure 5.15). The NND of infected fish in the school was significantly reduced in 2 of the 6 trials (one-way ANOVA: Trial 1 $F_{1,39}$=1.29, p=0.263, N.S.; Trial 2 $F_{1,39}$=0.25, p=0.618, N.S.; Trial 3 $F_{1,36}$=2.53, p=0.121, N.S.; Trial 4 $F_{1,37}$=6.17, p=0.018; Trial 5 $F_{1,39}$=26.68, p<0.001; Trial 6 $F_{1,39}$=4.11, p=0.05, N.S.; Figure 5.15).

5.4 DISCUSSION

5.4.1 The general behaviour of minnows during the experimental trials

During each 5h experimental trial, the minnows in the pool were observed to school continuously against the 0.12ms^{-1} current, completing one circuit of the pool, a linear distance of about 3m, approximately each minute. This translates to a free swimming speed of roughly 0.17ms^{-1}, which is equivalent to approximately 3 body lengths s^{-1}. This is within the range of normal swimming speeds for a small fish such as the minnow in its natural habitat (Bone & Marshall, 1982). The vast majority of schools formed in the experimental trials were composed of all ten fish present in the pool, and when a lone fish was observed it was not the *L. intestinalis*-infected school member any more frequently than would have expected by chance. These results strongly suggest that ligulosed minnows are able to keep up with the rest of the school over normal swimming speeds in the experimental pool, at least over the 5h duration of each experimental trial.

During the experimental trials, schools were most frequently composed of approximately equal numbers of peripheral and central members, with extreme school configurations occurring with very low frequency. Since peripheral positions have traditionally been thought of as more risky, yet more profitable, than central positions, it was originally thought that as the school became more relaxed, after

undisturbed residency in the pool, the number of individuals occupying peripheral positions might increase. However, although there seemed to be a slight trend in this direction, school configuration was found not to change significantly over the pre-attack phase of the experiment.

Evidence for the school settling down over the pre-attack residency period was found, however, since significant increases in both the area covered by the school and the mean NND of school members were observed as more undisturbed time was spent in the experimental arena. After the simulated avian attack, following the initial 'flash expansion' - an extreme predator avoidance behaviour reserved for the final strike of a predator (Pitcher & Parrish, 1993) - school area was observed to be significantly reduced below that of pre-strike levels as school members closed ranks by reducing their individual NNDs. This increase in cohesiveness of schools under apparent predation threat has been recorded before (Major, 1978; Krause & Tegeder, 1994), and it is thought that by reducing the distance between itself and its closest neighbour, an individual reduces its 'domain of danger'; in other words, the closer a fish is to others, the less likely it is to be the target of an attacking predator (Hamilton, 1971). When all members of a school carry out this type of behaviour, the result is a reduction in mean NND, and consequently in the area covered by the group.

5.4.2 Possible mechanisms for the differences in schooling behaviour shown by parasitised minnows

The fact that *L. intestinalis*-infected minnows were found to occupy peripheral positions more often than would be expected, suggests that they are either forced out to the edges of the school by more dominant, uninfected fish (a *penalty* associated with school membership), or that they choose to occupy such positions (a *preference*). If infected fish choose to occupy peripheral positions, then it may be that they prefer these positions because they allow them to maximise some aspect of their immediate fitness, such as increased foraging rate. *L. intestinalis* plerocercoids cause a reduction in the body condition of infected fish in natural habitats (Harris & Wheeler, 1974; Sweeting, 1977), and they are likely to cause nutritional demands as has been described for plerocercoids of *S. solidus* infecting three spined sticklebacks *Gasterosteus aculeatus* (see Chapter 4, section 4.1.4). In addition, individuals infected with plerocercoids of pseudophyllidean cestodes may be poor competitors for food (see Chapter 7), and so it may be that infected fish are only able to achieve sufficient energy intake by occupying peripheral positions, which offer better foraging opportunities and a reduced level of competition (see 5.1.3) in return for an increased predation risk. However, it is possible that by

remaining as members of the school infected minnows may reduce the predation pressure which they would otherwise suffer if they leave the group completely.

In each experimental trial, the *L. intestinalis*-infected minnow was consistently further away from a nearest neighbour than uninfected fish in the school. This was not a generally a result of infected fish being unable to 'keep up' with the school, since they have been shown to be within five body lengths of the other fish in the vast majority of analysed frames. Rather, it suggests that the parasitised fish are less able to demonstrate the synchrony that is required (as is shown by the uninfected fish) to maintain the ordered lattice-type structure that typifies minnow schools (Pitcher, 1973; Partridge, 1980). The NNDs of uninfected fish in schools tended to be highly uniform (note the small error bars in Figure 5.9), whereas the NNDs of infected fish generally deviated greatly from these, suggesting a sensory or motor-driven inability to conform to the structure of the school.

5.4.3 Potential ecological consequences of infection-associated changes in schooling behaviour

The apparent inability of infected fish to school with the same synchrony as uninfected schoolmates, and their propensity for occupying peripheral positions in schools potentially has profound consequences for their susceptibility to predators, and thereby also for the transmission of the parasite. Schooling freshwater fish, such as minnows, feature significantly in the diet of a wide variety of predators, including piscivorous fish, birds, reptiles, mammals and larger invertebrates, any combination of which may prey on such fish in a particular habitat. Of these, only birds frequently serve as definitive hosts for *L. intestinalis* ('target' predators, for the parasite); ingestion by the others ('non-target' predators) is generally fatal for the parasite.

The strategies of piscivorous fish and birds when feeding on schooling prey are poorly understood, but it is clear that there exists great variety in the way that schools are attacked. Although there are reports of certain piscivorous fish showing position-based selection, such studies do not provide conclusive evidence for or against the phenomenon of marginal predation (see 5.1.3.1). In addition, a number of fish predators are known to attack whole schools, either as individuals, or in groups, forcing school members to split up and thereby become more susceptible to predation. Occupation of specific school positions is unlikely to have a significant effect on the ability of individuals to avoid being caught by such predators, since all positions appear intuitively to equally susceptible to attack. On the other hand, against stalking predators that select prey and lunge at them

from close distance, such as the pike *Esox lucius*, occupation of peripheral positions is likely to be risky.

Birds, such as kingfishers (*Alcedo* spp.), terns (*Sterna* spp.) and gulls (*Larus* spp.), often hunt in open habitats, diving from above the water to catch prey; others, such as herons (*Ardea* spp.), generally ambush fish in shallow margins, whilst still others, such as mergansers (*Mergus* spp.) and grebes (*Podiceps* spp.) dive and swim underwater to catch their prey. Against the latter group, which tend to break up schooling fish, and to outswim individuals that split off, any preferences shown by potential prey for particular school positions are unlikely to be of value in reducing predation risk.

The inability to conform to a typical lattice structure is less likely to cause infected fish to appear 'spatially-odd' to in-water predators, especially where schools approximate to two-dimensional structures. The lattice structure of schooling minnows is more obvious when observed from above, and deviations from this lattice, such as those exhibited by infected fish, constitute a type of 'spatial oddity', which is known to increase the success of certain predators by reducing the confusion effect generated by massed, regularly-spaced prey (Milinski, 1977a). Although the confusion effect has only been studied experimentally in relation to piscivorous fish (see Chapter 1, section 1.2.3.2), it is likely that certain types of avian predators also suffer from the information overload that it causes (Broadbent, 1965), and if this is the case, then the spatial oddity of *L. intestinalis*-infected minnows may increase their susceptibility to predation by piscivorous birds that are normally affected by 'confusion'.

Whether spatial positioning of fish in schools has any bearing on predation risk, and if so, whether peripheral or central positions are likely to be most risky, will probably depend on the abundance and range of predatory species present at any site, and more importantly, their criteria for prey selection and their mode of prey capture. Further studies on the predatory behaviour of piscivorous fish and birds attacking schooling fish prey are required to identify and quantify the behavioural costs and benefits of positioning behaviour in these aggregations. Until these are understood, the consequences of the observed behaviour for the transmission of *L. intestinalis* in natural environments will remain unclear. Evidently, before the manipulation hypothesis can be advocated in this or any other instance, it will be essential to gain a more complete understanding of the prey selection mechanisms of both target and non-target predators.

5.5 SUMMARY

- The schooling behaviour of 6 separate groups of 10 minnows *Phoxinus phoxinus* in an experimental flow pool was investigated over 5h periods. Each group was composed of 1 *Ligula intestinalis*-infected and 9 uninfected fish.
- A stereo video system was used to to record the number of fish in schools formed, their configuration and the area they covered and nearest neighbour distances (NNDs). These indices of schooling behaviour were used to investigate the effect of residency time, parasite status and a simulated avian attack on the schooling behaviour of individual fish in the pool.
- More than 95% of all frames analysed (N=583) showed schools composed of all 10 fish in the pool. Infected fish were found to be members of these schools more than 95% of the time.
- Increased residency time in the pool was generally associated with an increase in the mean NND for uninfected school members, and consequently an increase in school area. The relaxation of school cohesiveness over time suggests that the minnows may have been responding to the perceived 'safe' conditions in the flow pool. Infected fish, however, did not generally show this change, and their NNDs remained steady, and large, throughout the period.
- *L. intestinalis*-infected individuals consistently exhibited larger NNDs than uninfected schoolmates (on average, infected fish had a NND of 1.4x that of the mean of uninfected fish).
- A strong positive relationship was found between school area and the mean NND of uninfected fish in all 6 trials. Significant, though less strong, positive correlations were also found between school area and the NND of *L. intestinalis*-infected fish. However, within each trial uninfected and *L. intestinalis*-infected fish differed significantly, with parasitised fish exhibiting increasingly larger NNDs than uninfected fish as school area increases.
- Infected fish were also more likely to be found on the periphery of a school of any particular configuration than would be expected by chance if all school members assorted randomly.
- Schools responded to the simulated avian attack by performing an instant 'flash expansion', generally followed by a reduction in school area below that of pre-attack levels. A significant reduction in the NNDs of uninfected fish was observed in 3, and of infected fish in 2, of the 6 trials following the simulated avian attack.
- The observed behavioural effects of *L. intestinalis* infection are discussed with reference to possible mechanisms, and potential ecological, and especially predator-prey, interactions.

Chapter 6. The effects of *Schistocephalus* infection on the competitive ability of three-spined sticklebacks

*"**Competition** occurs when two or more organisms, or other organismic units such as populations, interfere with or inhibit one another. The organisms concerned typically use some common resource which is in short supply. Moreover, the presence of each organismic unit reduces the fitness ... of the other."*

(Pianka, 1981).

6.1 INTRODUCTION

6.1.1 The importance of food competition for social animals

Heightened intraspecific competition for resources is frequently cited by behavioural ecologists as the major cost of group living (Bertram, 1978; Pulliam & Caraco, 1984; Trivers, 1985; Krebs & Davies, 1987; Milinski & Parker, 1991; Pitcher & Parrish, 1993). Social groups may form for a variety of different reasons including antipredator defence or improved foraging efficiency (see Chapter 4). Members of such groups pay a cost because the locally high density of individuals within the group is seldom matched by similarly constant high densities of prey. As a result the *per capita* food intake of group members is often less than that potentially achievable by solitary foragers; however, social groups frequently persist under such conditions because they offer other benefits. Even so, as group size increases there must be a point at which competition becomes prohibitive to membership, and individuals may be predicted to leave, or fail to join, such groups under such conditions.

6.1.2 The influence of prey distribution and abundance on competition within social groups

Ecologists have defined a range of competition types, based on the extent to which competitors come into contact with each other (Pianka, 1981; Krebs, 1985; Begon *et al*, 1990). At one end of the scale lies *exploitation* (or *resource*) competition, where individuals rarely encounter one another, yet suffer through the reduction of available resources by unseen competitors, and at the other end lies *total interference* (or *contest*) competition, where direct physical contact (i.e. fighting) is involved in securing resources. Animals living in groups may experience a variety of competition types, from exploitation through to interference (Rubenstein, 1978), but the particular type of competition experienced at any given time is likely to depend substantially on the spatial distribution of available prey. In habitats where locally abundant, randomly dispersed prey items are fed on by aggregated

predators (e.g. shoaling fish feeding on drifting planktonic prey), individual group members suffer minimally from interference competition ; conversely, when prey is distributed patchily in natural environments, either with respect to space or time (or both), interference competition is likely to become more severe since a roaming group is likely to come upon food patches that are locally dense, yet of insufficient size to satiate all group members. In the latter case, a further form of competition, *scramble* competition, is likely to prevail. In scramble competition, individual competitors do not interfere with each other directly, and each may attempt to elevate its rate of feeding in order to maximise food intake over a short period of time (Uematsu & Takamori, 1976; Barnard, 1984; Street *et al*, 1984), possibly to the detriment of some other aspect of fitness such as antipredator behaviour or digestive efficiency. As a food patch becomes severely depleted following scramble competition, contest competition may prevail over the last remaining items.

6.1.3 Intraspecific variation in competitive ability

Intraspecific competition is likely to be the most severe type of food competition, since individuals of the same species tend to have the same specific nutritional requirements and prey preference (Begon *et al*, 1990). However, even though food requirements are likely to be the same for individuals within monospecific groups, individuals are unlikely to be equally successful in food acquisition. That individuals of the same species frequently differ in their ability to compete with one another for food is undoubted, and an abundance of field and laboratory studies have provided data showing the effects of competition on individual growth and survival to be differential in their severity. Rubenstein (1981) showed that the individual variation in growth rate of Everglades pygmy sunfish *Elassoma evergladei* exhibited even in the absence of competition was intensified as competitor density increased. At the highest competitor densities, some fish were found to show negative growth rates, eventually starving to death, whereas others grew well, apparently unaffected by the elevated competition levels. In a study of oystercatchers *Haematopus ostralegus* foraging on mussel *Mytilus edulis* beds in the Exe estuary, Ens & Goss-Custard (1984) demonstrated that individuals higher up in the dominance hierarchy were relatively unaffected by increased levels of competition and continued to feed with a high rate, whereas the foraging rate of subordinates dropped significantly in the face of more severe competition. Competition may also have more subtle effects on the feeding behaviour of poor competitors, and changes in foraging strategy have been postulated as a result. By showing a

preference for less profitable prey, poor competitors can avoid energetically-expensive contests for larger prey that they would lose anyway (Milinski, 1982). Poor competitors will often switch to feeding on more profitable prey items when competition for them is reduced. Following the experimental removal of larger individuals from a shoal of humbug damselfish *Dascyllus aruanas* at a coral head feeding station, the remaining smaller fish consumed larger and more profitable individual prey items than they had prior to the removal (Coates, 1980).

Membership of groups of conspecifics may be differentially profitable for good and poor competitors. Poor competitors may leave the shoal, temporarily or permanently, to exploit risky individual foraging opportunities, or they may form separate shoals, matched with respect to competitive ability. Although phenotype-matching (the tendency of individuals with similar behavioural or morphological traits to aggregate) with respect to other characters is known to be a common factor governing the membership of social groups in many species (see Krause *et al.*, in press, for a review), shoals composed of fish of uniform competitive ability are unlikely to occur in nature, since shoals of poor competitors would be open to colonisation by stronger competitors that may be able to exploit their subordinance. Indeed, recent experimental evidence has demonstrated that European minnows *Phoxinus phoxinus* are able to assess the competitive ability of other fish in separate shoals and that they show a marked preference for joining shoals comprising individuals of poor competitive ability (Metcalfe & Thomson, 1995). Because of this phenomenon, natural shoals are more likely to be stratified with respect to competitive ability (see Ranta *et al*, 1993), with groups comprising individuals with a wide variety of competitive ability.

The factors responsible for the observed variation in individual competitive ability are likely to be highly diverse, and have been shown to include age (Persson, 1983), size (e.g. Wilson, 1975; Ranta & Lindström, 1990), feeding motivation (Krause *et al*, 1992), innate 'fierceness' (Huntingford *et al*, 1990), social dominance (Metcalfe, 1986), experience (Ware, 1971) and the adoption of alternative strategies (Milinski & Parker, 1991). However, in natural populations, individuals often vary significantly with respect to their general health, and in particular parasite load. Infection with parasites is potentially one of the most important causes of heterogeneity in competitive ability within size- and age-matched monospecific groups in natural environments. Additionally, because parasites are frequently overdispersed within a host population, i.e. the majority of parasites are harboured by a

disproportionately small number of hosts, then they are potentially important causal agents of competitive heterogeneity within social groups.

6.1.4 The effects of parasites on the ability of their hosts to compete for food

In Park's pioneering experiments on competition in cultures of *Tribolium confusum* (Park, 1948), the debilitating effect of a protozoan parasite on host performance and subsequent survival were clearly shown, with infected cultures being outcompeted by uninfected animals under conditions of limiting resources. Parasite infection may alter an individual's competitive ability indirectly, for instance by changing its feeding motivation, or more directly by affecting co-ordination, manipulative skill, manoeuvrability, speed of movement or stamina. Metacercariae of the digenean trematode *Diplostomum spathaceum* cause eyefluke disease in a number of freshwater fish species as they aggregate in the lenses of infected individuals (Chappell *et al*, 1994). The efficiency with which dace *Leuciscus leuciscus* prey on gammarids (Crowden, 1976; Crowden & Broom, 1980) and the reactive distance of sticklebacks *Gasterosteus aculeatus* to live cladocerans are both reduced by eyefluke infection (Barber, 1992; Owen *et al*, 1993), almost certainly reducing the competitive ability of infected hosts. Other fish parasites that affect host mobility (see Chapter 1) almost certainly have similar effects on host competitive ability.

In an experiment designed to test the effect of *S. solidus* on the foraging efficiency of sticklebacks, Cunningham *et al* (1994) found that infected fish spent more time than uninfected individuals handling prey items prior to consumption. When combined with the detrimental effect of *S. solidus* on energy reserves (Pascoe & Mattey, 1977) and manoeuvrability (Arme & Owen, 1967), this strongly suggests that infected fish should be poor competitors for food. In this chapter, the effects of *S. solidus* on the ability of stickleback hosts to compete for food with an uninfected conspecific are examined.

6.1.5 Objectives

In Chapter 4, *S. solidus* infection was found to be associated with an increased tendency to leave a shoal to exploit individual, risky, foraging opportunities. It was suggested that the elevated competition levels within shoals may be responsible for the adoption of this strategy by parasitised fish, since they may be poor competitors for food under such circumstances. The present chapter describes

experiments designed to investigate how *S. solidus* infection affects the food intake of stickleback hosts under different types of competition and whether, by affecting the competitive ability of its host, the parasite may change the costs and benefits associated with shoal membership. Specifically, the aims of this chapter are:

- To investigate experimentally the effects of *S. solidus*-infection on the competitive ability of stickleback hosts under direct competition with uninfected fish for single, consecutively-presented prey items (i.e. under *interference* competition conditions).
- To investigate experimentally the effects of *S. solidus*-infection on the total prey intake of sticklebacks when feeding on a food patch of finite size in direct competition with uninfected fish (i.e. under *scramble* competition)

6.2 EXPERIMENT 6.1: EFFECT OF *S. SOLIDUS* ON INTERFERENCE COMPETITION IN STICKLEBACKS

6.2.1 Introduction

Where single, indivisible food items are encountered randomly in the environment by aggregated predators, there is likely to be direct competition between two or more individuals as they pursue or contest the prey item. Since successful foraging in this situation relies on recognising the prey item as such, on moving quickly towards the prey item and on being able to compete successfully with similarly-motivated conspecifics, this may be a situation in which parasitised fish may lose out. An experiment was designed to investigate the foraging success of *S. solidus* infected sticklebacks under this type of competition with a single uninfected conspecfic. The aims of Experiment 6.1 were:

- To investigate the dynamics of pairwise competition between uninfected and *S. solidus* infected sticklebacks feeding on sequentially-introduced prey..
- To determine the total foraging success of both classes of competitor, measured as total food intake during the experiment.
- To examine the events leading to prey capture by either competitor in order to determine whether subtle differences in the way in which prey are caught might influence the foraging success of infected fish in larger groups.

6.2.2 Materials and methods

6.2.2.1 Fish collection and husbandry

Approximately 200 three-spined sticklebacks were hand-netted from Inverleith pond, Edinburgh during August 1994. The population has been extensively studied, and a large proportion of individuals are known to be infected with the plerocercoid stage of *S. solidus* (Tierney, 1991; Tierney *et al*, in press). The fish were maintained in stock tanks for a quarantine period of two months before any trials were run, to allow any newly-acquired infections to reach a size at which they would be infective to the definitive host (piscivorous birds). After this two month period it was possible to classify fish as infected or uninfected with a high degree of accuracy on the basis of appearance alone.

6.2.2.2 Protocol

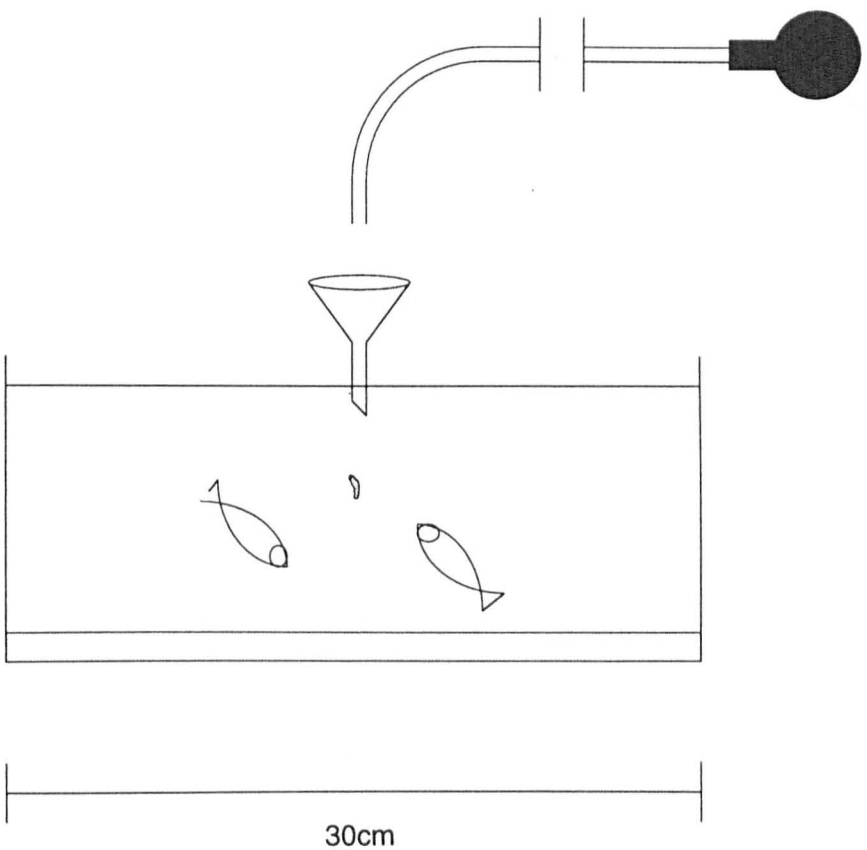
Twenty pairs of sticklebacks, each comprising one infected and one uninfected fish, were selected from the stock tanks and housed in separate 1800cm^3 cells in a shallow fibreglass tank. As relative size is known to be an important factor in determining competitive ability, each pair was matched with respect to fork length. The paired fish were fed on live bloodworms *ad libitum* for two days before feeding was stopped 24h prior to the start of the trial, in order to ensure a degree of feeding motivation. Each fish pair was transferred in turn to a 12-litre glass aquarium (30 x 20 x 20cm) and left to settle until both fish began to display typical foraging activity, e.g. probing the coral sand substrate. Individual prey items (halved bloodworms) were then introduced to the experimental tank via an extended pipette from behind a screen (Figure 6.1) at 90s intervals. The responses and feeding behaviour of both fish were recorded on videotape for subsequent analysis.

6.2.2.3 Video analysis

For each prey item introduced to the tank, the following variables were recorded:

- Which of the fish reacted first to the prey item (infected / uninfected / simultaneous response).
- In the case of a simultaneous response, which fish was closest to the prey item (infected / uninfected / equidistant).
- Which of the fish consumed the prey item (infected / uninfected / neither).
- What was the total number of prey items consumed by either fish in the pair by the end of the trial.

Figure 6.1 The design of the experimental tank used to examine pairwise food competition between *Schistocephalus solidus*-infected and uninfected sticklebacks in Experiment 6.1. Individual prey items (halved bloodworms) were introduced from behind a screen via a pipette, and directed by a funnel to standardise presentation.


30cm

Once all 10 prey items had been introduuced sequentially, the experiment was terminated and fish were exposed to a lethal dose of Benzocaine anaesthetic and dissected immediately to confirm their parasite status.

6.2.3 Results

6.2.3.1 General behaviour

As each prey item was introduced, either one or both of the sticklebacks immediately oriented and swam quickly towards the food. Because both fish were moving freely around the tank, differences in either the distance of the fish from the introduced food and in the direction in which each was facing were common, and this frequently resulted in one fish responding to the prey before its competitor. Following these asymmetric responses, either the primary responder quickly ingested the prey item before its competitor showed any sign of being aware of the presence of the food, or the other fish was alerted to the presence of the food either by the prey item itself or by the movements of the primary responder, and also oriented and swam quickly towards the food. Following either a simultaneous response of both fish to the food (i.e. within the same frame of film, approximately 0.05s) or an asynchronous response, the prey item was subsequently ingested by one of the two fish. If one fish arrived at the prey item before the other, ingestion was generally swift and uncontested; however, if the two fish arrived simultaneously, ingestion was often only achieved following a contest over the prey item; with each fish either biting repeatedly at the prey item before it was ingested by the successful fish, or seizing one end of the prey item in its mouth and attempt to pull it away from the other. These two types of contest will be referred to as 'Battle' and 'Tug-of-War' respectively.

Based on the manner in which individual prey were captured, each item was classified as 'easy', 'difficult' or 'contested', as defined by the criteria in Table 6.1.

6.2.3.2 The effects of *S. solidus* on host competitive ability

Over the twenty trials, there was no consistent effect of *S. solidus* infection status on the frequency with which fish responded first to the introduced prey (Wilcoxon signed rank test, $T^{+} = 79.5$, n=19, P=0.546, N.S.; Figure 6.2). Following non-simultaneous responses to introduced prey items, the fish that responded first generally ingested the prey item, irrespective of infection status (Wilcoxon-

Mann-Whitney test, carried out on the proportions of prey ingested by infected and uninfected primary responders in the 20 trials, W=421.5, n=19, P=0.1404, N.S.; Figure 6.3).

Table 6.1 Definitions of the difficulty categories of prey taken by sticklebacks in Experiment 6.1.

Prey category	Definition
'Easy'	Only successful fish reacts to prey item, *or* Successful fish reacts first, *or* Simultaneous reaction of both fish; successful fish closest to prey item.
'Difficult'	Successful fish reacts second, *or* Simultaneous reaction of both fish; fish equidistant, *or* Simultaneous reaction of both fish; successful fish furthest from prey item.
'Contested'	'Battle': both fish bite rapidly at the prey item before it is consumed by the successful fish, *or* 'Tug-of-War': each fish has one end of the prey item in its mouth before it is consumed by the successful fish.

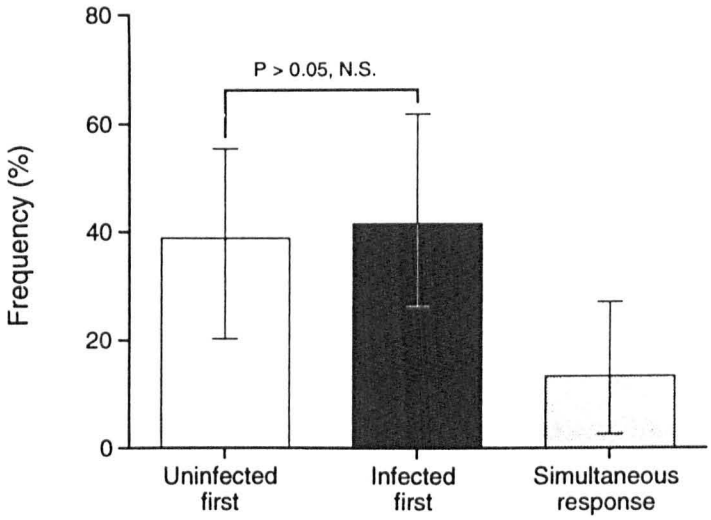
When the response to an introduced prey item was simultaneous, and the fish differed in their distance from the food, the closest fish (infected or uninfected) was not consistently successful in ingesting the food (Paired t-tests, carried out on [Frequency of being closest and capturing - Frequency of being closest and not capturing]: Uninfected fish closer, t=0.00, n=4, P=1.00, N.S.; Infected fish closer, t= −0.26, n=7, P=0.80, N.S.). However, when both fish responded simultaneously and were also equidistant from the introduced prey item, the uninfected individual was successful in capturing and ingesting the food significantly more often than the infected fish (Paired t-test, t=5.58, n=10, P=0.0003; Figure 6.4).

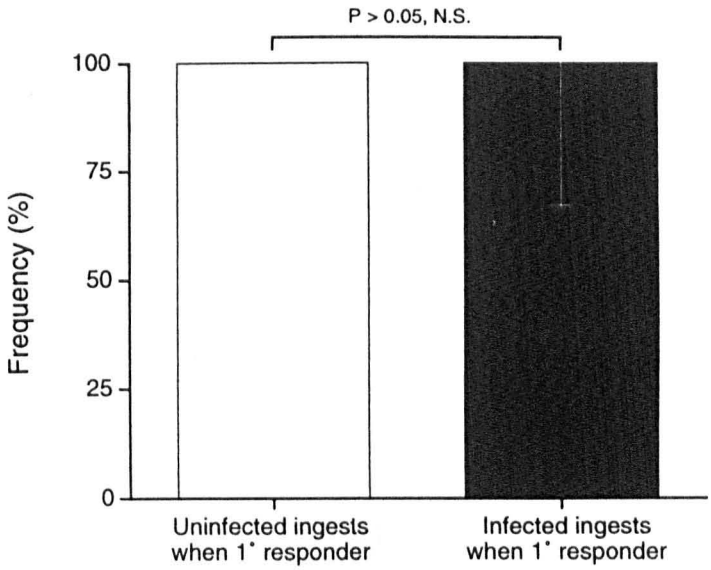
Schistocephalus solidus infection status did not significantly affect the total foraging success of the competing fish, measured as the total number of prey items ingested during the trial (Wilcoxon signed ranks test, T⁻=111.5, n=17, P=0.102, N.S.; Figure 6.5). However, a more detailed analysis of the manner in which the prey were captured revealed significant differences. On average, a higher proportion of the food items ingested by infected fish were classified as 'easy' items, i.e. those that the fish would have been expected to capture because they either responded quicker or were closer to the food (Wilcoxon-Mann-Whitney test, W=319.0, n=20, P=0.0121). Conversely, a higher proportion of the prey items captured by uninfected fish were 'difficult' items (Wilcoxon-Mann-Whitney test,

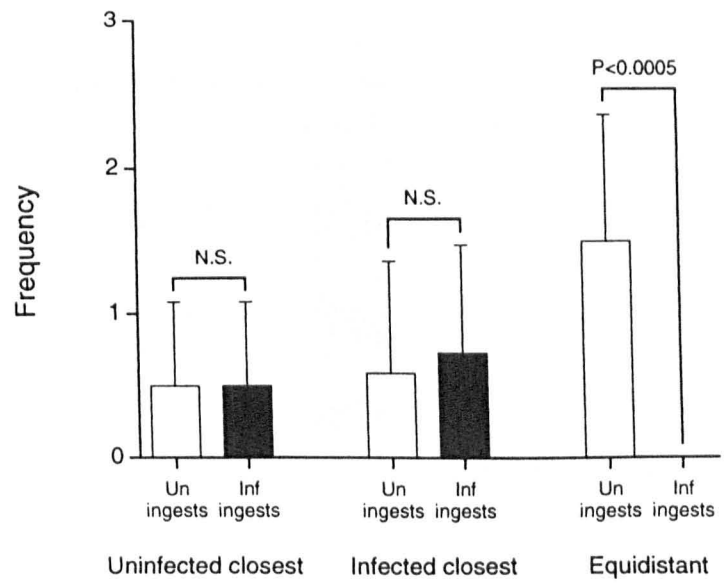
Figure 6.2 The proportional frequencies with which the different responses to introduced prey were observed. Medians of 20 trials are shown, with error bars representing interquartile ranges. The probability value refers to the results of a Wilcoxon Signed Ranks test (see text).

Figure 6.3 The proportional frequencies with which uninfected and infected fish successfully ingested prey items after making the primary response. Medians of 20 trials are shown, with error bars representing interquartile ranges. The probability value refers to the results of a Wilcoxon-Mann-Whitney test (see text).

Figure 6.4 The absolute frequencies with which uninfected and infected fish successfully ingested prey items following each type of response. Median values are shown, with error bars representing interquartile ranges. Probability values refer to the results of paired t-tests (see text).


80
60
40
20
0
Frequency (%)
P > 0.05, N.S.
Uninfected first
Infected first
Simultaneous response


100
75
50
25
0
Frequency (%)
P > 0.05, N.S.
Uninfected ingests when 1° responder
Infected ingests when 1° responder


3
2
1
0
Frequency
N.S.
N.S.
P<0.0005
Un ingests
Inf ingests
Un ingests
Inf ingests
Un ingests
Inf ingests
Uninfected closest
Infected closest
Equidistant

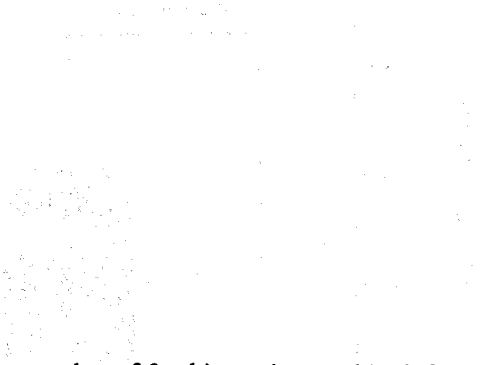


Figure 6.5 The differences in the number of food items ingested by infected and uninfected fish in each of the 20 trials. The final point shows the median of these differences, with error bars representing interquartile ranges. The probability values refer to the results of a paired t-test.

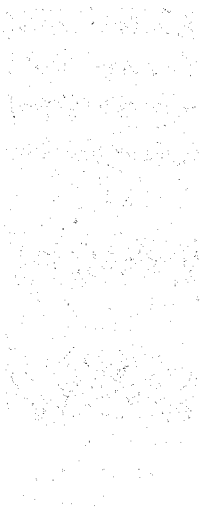
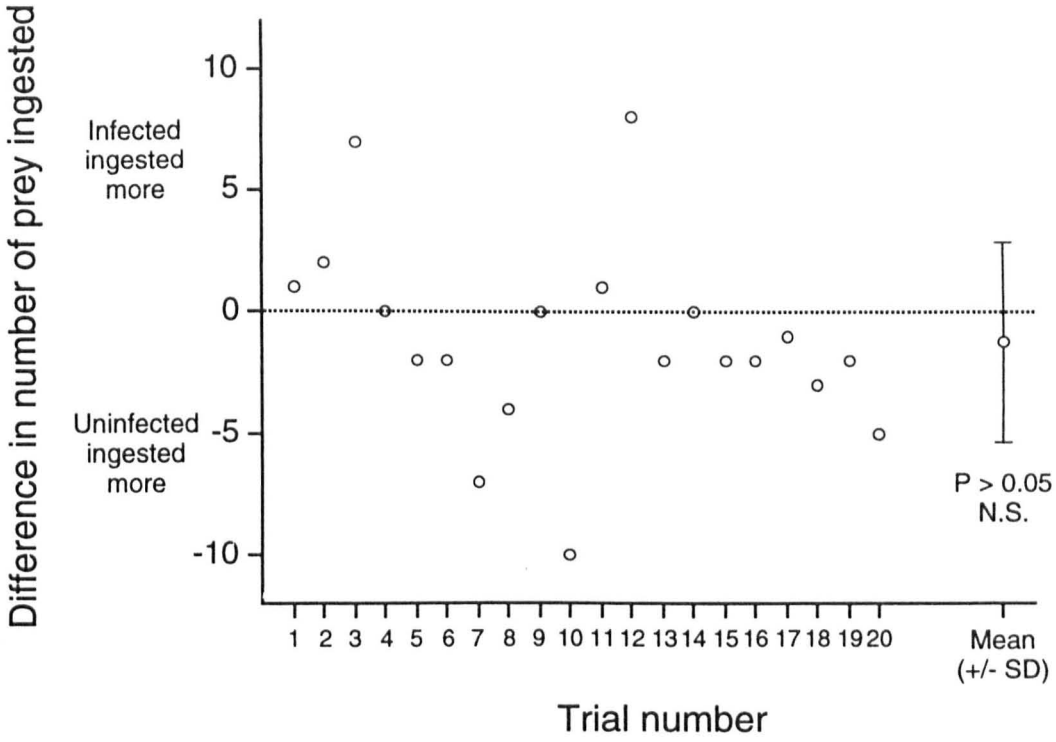
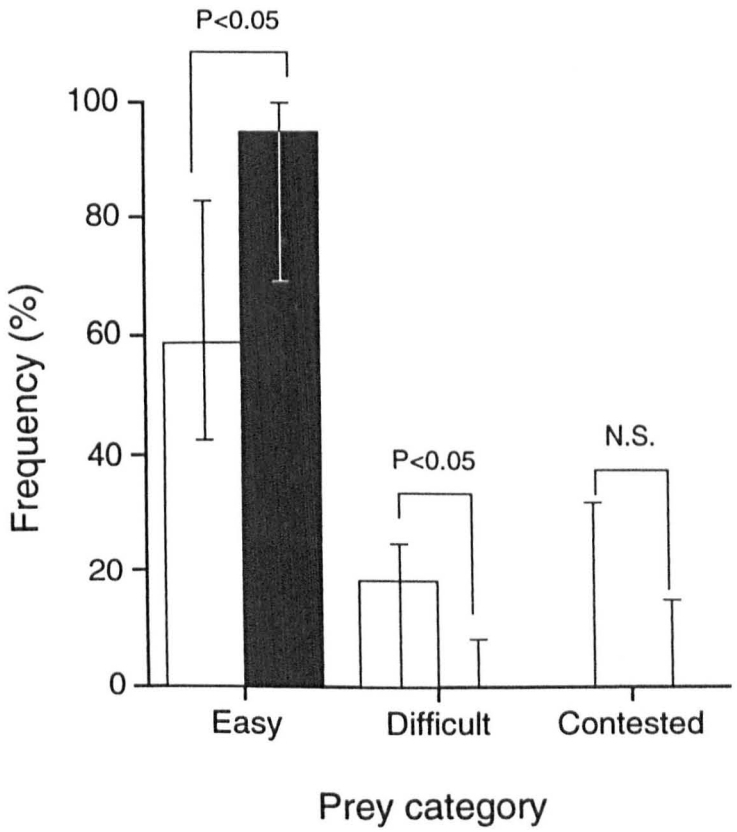


Figure 6.6 The proportional frequencies with which infected (■) and uninfected (□) sticklebacks ingested differently-contested prey items (see text for category definitions). Medians of 20 trials are shown, with error bars representing interquartile ranges. Probability values refer to the results of Wilcoxon Signed Ranks tests.


Difference in number of prey ingested
10
5
0
-5
-10
Infected ingested more
Uninfected ingested more
P > 0.05
N.S.
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Mean (+/- SD)
Trial number


P<0.05
100
80
60
40
20
0
Frequency (%)
P<0.05
N.S.
Easy
Difficult
Contested
Prey category

W=486.5, n=20, P=0.0242). The ability of competitors to secure contested prey items was not dependent on *S. solidus* infection status in this experiment (Wilcoxon-Mann-Whitney test, W=464.0, n=20, P=0.0889, N.S. Figure 6.6).

6.2.4 Discussion

In this experiment, where individual prey items were encountered sequentially, infected fish in pairs do not appear to suffer with respect to their total food intake. The way in which infected fish acquired their food items, however, was different to that of uninfected fish, with their diet comprising almost totally of 'easy' items, since they did not compete well when prey was contested. This suggests that in natural habitats, where there is likely to be stronger competition for prey items, shoal membership for infected fish may be more costly than for uninfected conspecifics.

6.3 EXPERIMENT 6.2: EFFECT OF *S. SOLIDUS* INFECTION ON SCRAMBLE COMPETITION IN STICKLEBACKS

6.3.1 Introduction

The results of Experiment 6.1, above, suggest that infected fish may be poor competitors for food under certain types of foraging conditions, i.e. when food items are encountered singly and randomly in the environment. However, shoaling fishes are known to often forage on *patches* of food, rather than individual items, and successful foraging under these circumstances probably requires different skills. Patch foraging involves scramble competition, where there is little direct interference or contest for individual prey items, and it was hypothesised that *S. solidus* infected fish may benefit from the reduced levels of interference over individual food items. An experiment designed to test this hypothesis is described below. The aims of Experiment 6.2 were:

- To investigate the rate of patch depletion by both uninfected and *S. solidus* infected sticklebacks.
- To determine the total foraging success of both classes of competitor
- To determine whether joining shoals foraging on patches of food may be less costly to *S. solidus*-infected sticklebacks than joining a group where interference competition is higher (e.g. as in Experiment 6.1).

6.3.2 Materials and methods

6.3.2.1 Fish collection and husbandry

Fish were collected from the same site and using the same techniques as described in Experiment 6.1, above.

6.3.2.2 Experimental set-up

The experimental tank was a 12-litre glass aquarium with a white coral sand substrate, fitted with a feeder that held 8 live bloodworms (Figure 6.7a). The design of the feeder was an important part of the experimental set-up, since it was intended that the food patch should mimic the natural habitat of such worms, with individuals being presented in a semi-natural manner. In natural habitats, chironomid larvae spend a large part of their lives burrowing, or half buried, in mud or silt (Fitter & Manuel, 1986), and this is certainly the case at Inverleith pond, where they are found in abundance, half-embedded in the muddy substrate (personal observations). In an attempt to imitate this natural habit in the experiment, 8 live bloodworms were anchored vertically in individual petroleum jelly-filled cells of a 4 x 4-cell section (measuring 4cm x 4cm) cut from a 96-well microtitre plate (Figure 6.7b). The feeder design ensured that individual bloodworms were presented to the fish, and were removed with similar ease by them, as they would be in the natural environment. Prior to the beginning of each experimental trial, the feeder was hidden by a remotely-operated opaque cover. The experimental tank was covered on all sides by an opaque screen to prevent any external movements causing disturbance to the fish.

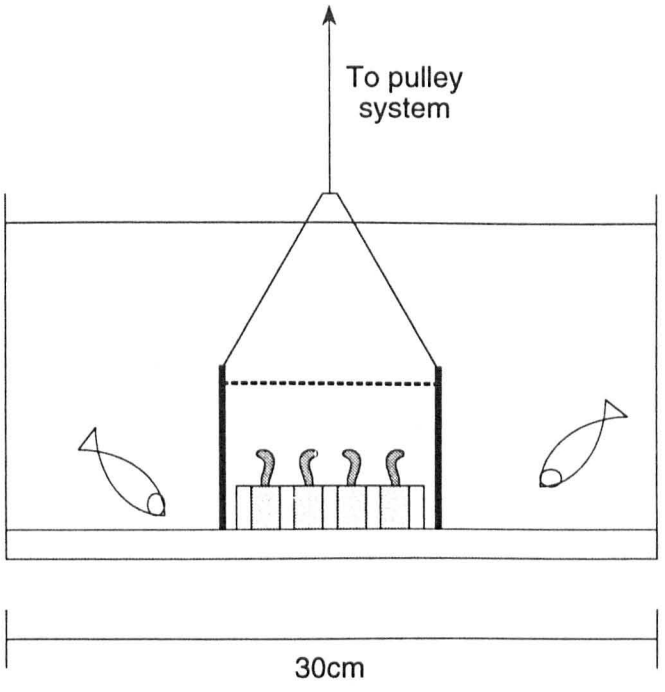
6.3.2.3 Protocol

Eleven pairs of infected and uninfected sticklebacks, matched with respect to fork length, were selected from the stock tanks and housed as described above (section 6.2.2.2). Following *ad libitum* feeding with live bloodworms for 2 days, food was withheld from the fish for 24h prior to experimentation. In order to get fish used to the experimental set-up, on the second day of feeding, a number of bloodworms were presented in the feeder to be used in the trial.

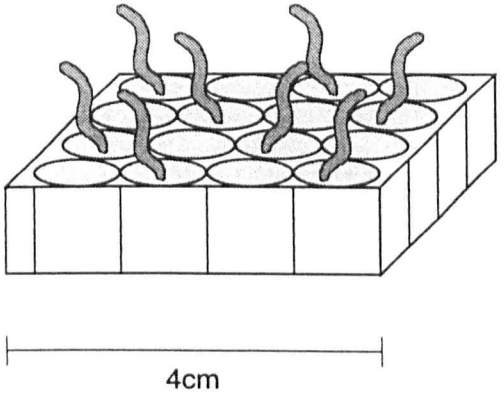
At the beginning of the trial, a pair of sticklebacks (one uninfected and one infected) were introduced to the tank and allowed to settle for five minutes, after which the cover was slowly removed to reveal the food patch. The subsequent feeding behaviour of both the uninfected and infected fish on the patch was recorded on videotape until all of the bloodworms had been consumed or until 5 minutes

Figure 6.7 The experimental tank used to examine the effects of *Schistocephalus solidus* on host prey intake rate in Experiment 6.2. a) Diagram of the experimental tank prior to the removal of the feeder cover. b) Detail of the bloodworm feeder (see text for explanation).

a)



b)



had elapsed with no further signs of foraging. In no trial were fish observed to leave bloodworms in the feeder. In 5 of the 11 trials, only 7 bloodworms were ingested in total, although eight were available at the start of each trial, because one escaped from the feeder and buried into the coral sand substrate where it lay undetected.

6.3.2.4 Video analysis

On analysis of the videotape, the time following the beginning of the trial at which each bloodworm was consumed by either fish was recorded. By using the freeze frame facility on the VCR, elapsed time could be measured accurately with the on-screen timing signal to 0.1s.

6.3.3 Results

6.3.3.1 Time taken to begin feeding

The time taken for individual fish to commence feeding on the bloodworms presented in the food patch following the remote removal of the opaque cover was not dependent on *S. solidus* infection status (Wilcoxon-Mann-Whitney test, W=144.0, n=11, m=11, P>0.05, N.S.; Figure 6.8), both fish commencing feeding within a few seconds of the start of the trial. Infection status did not predict which of the pair first ingested a prey item (5/11 uninfected fed first; 4/11 infected fed first; 2/11 simultaneous first feeding; Chi-square goodness-of-fit test, χ^2=0.111, d.f.=1, P=0.739, N.S.).

6.3.2.2 Foraging success of competitors

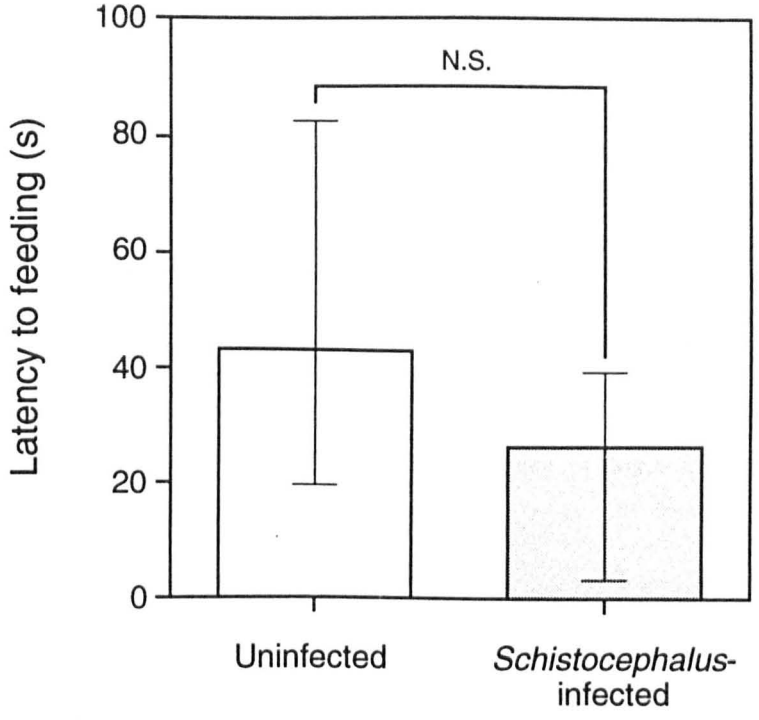
Although the latency period prior to feeding did not differ, uninfected fish consumed more bloodworms than their infected counterparts during the trials (Wilcoxon signed ranks test, T^+=2.5, n=8, P=0.036; Figure 6.9).

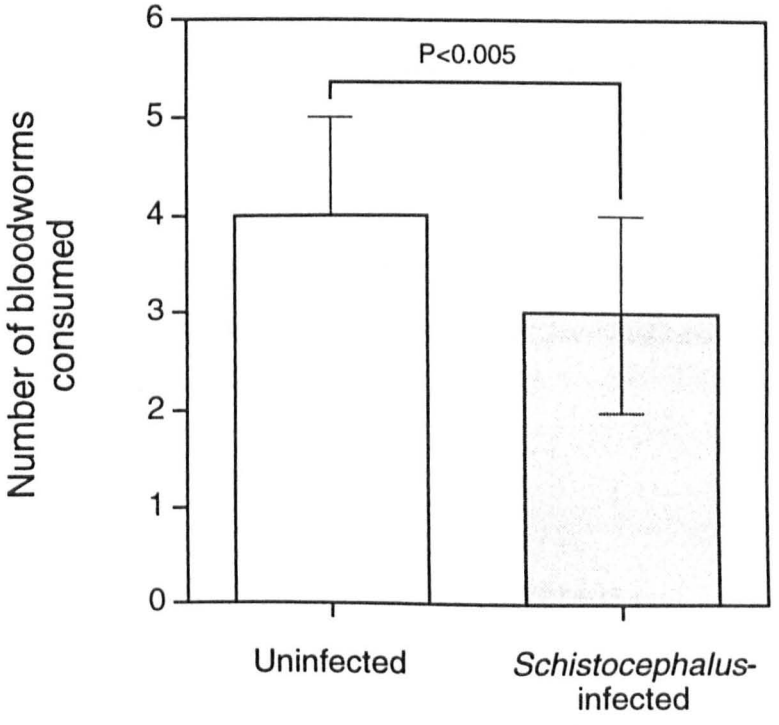
6.3.2.3 Temporal aspects of prey ingestion

Plots of the number of prey ingested over time for both the uninfected and *S. solidus*-infected member of each pair are shown in Figure 6.10. In general, it appears that uninfected fish are able to achieve a higher total food intake in these experiments by ingesting consecutive prey items more effectively than their infected competitors. However, it is only when the data are reanalysed, and the probability that uninfected and infected fish will have ingested their first, second, third etc. prey item in

Figure 6.8 The latency periods of uninfected and *Schistocephalus solidus*-infected sticklebacks to begin feeding on bloodworms in the feeder. Median values of the 20 trials are shown, with error bars representing interquartile ranges.

Figure 6.9 The total number of bloodworms consumed by uninfected and *Schistocephalus solidus*-infected sticklebacks competitng with one another during Experiment 6.2. Median values of the 20 trials are shown, with error bars representing interquartile ranges.


Latency to feeding (s)
100
80
60
40
20
0
N.S.
Uninfected
Schistocephalus-infected


Number of bloodworms consumed
6
5
4
3
2
1
0
P<0.005
Uninfected
Schistocephalus-infected

x amount of time, that the effect of *S. solidus* on the prey ingestion of hosts can be fully understood (Figure 6.11a and 6.11b). From these probability plots, it is possible to calculate the time at which an nth prey item has been ingested by infected and uninfected fish in 50% of the trials (the T_{50}). The T_{50}'s of uninfected fish increase logarithmically with time, with the first item being taken quickly and the second, third etc. with ever increasing intervals (Figure 6.12). However, the T_{50}'s of infected fish differ; they show more evenly-spaced ingestion of the first 4 successive food items, which leads to a more linear relationship between elapsed time and T_{50}. Whereas uninfected fish appear to bolt food initially, slowing after the first couple of items, infected fish seem to take their time and achieve a more steady rate of food intake. However, infected fish were never observed to ingest more than four bloodworms.

6.3.4 Discussion

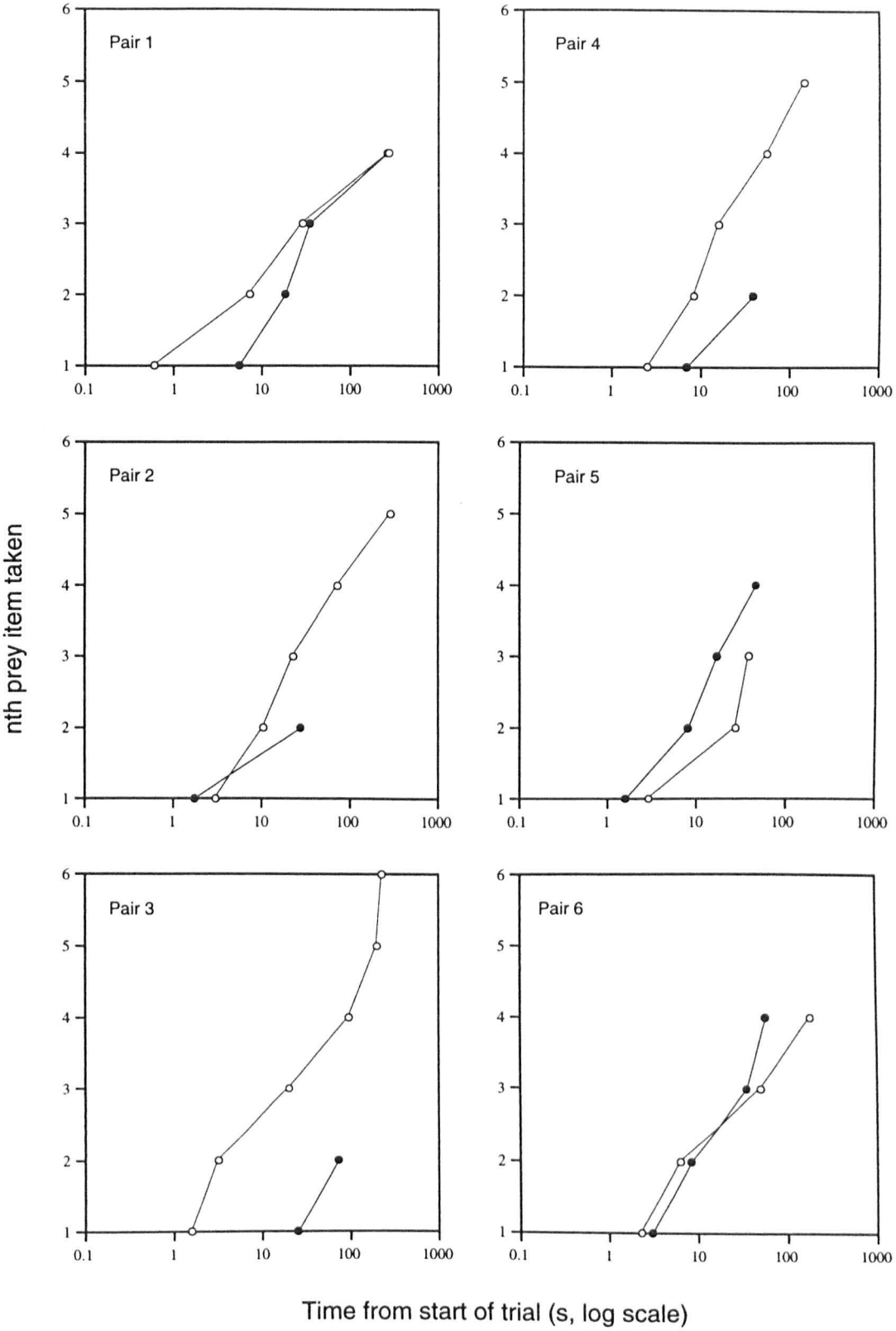
Foraging on patchily-distributed food is a common feature of certain grouped predators, including stickleback shoals (Manzer, 1976; Hart & Gill, 1994). Clearly, when food patches contain numbers of prey items that exceed the number of individual predators, the most successful foragers will be those that able to catch, handle and ingest prey quickest, enabling them to take items in quick succession. Foraging *rate* is therefore important. In Experiment 6.2, uninfected sticklebacks clearly outcompeted *S. solidus*-infected fish early in the trials, being able to take the first few items in quick succession. Although the slow, steady rate of foraging exhibited by the infected fish appeared to 'pay off' later in the trial, in larger shoals it is unlikely that any prey would remain after this time. It seems, therefore, that joining a shoal of fish that specialise on patchily-distributed prey would also be costly for a *S. solidus*-infected individual.

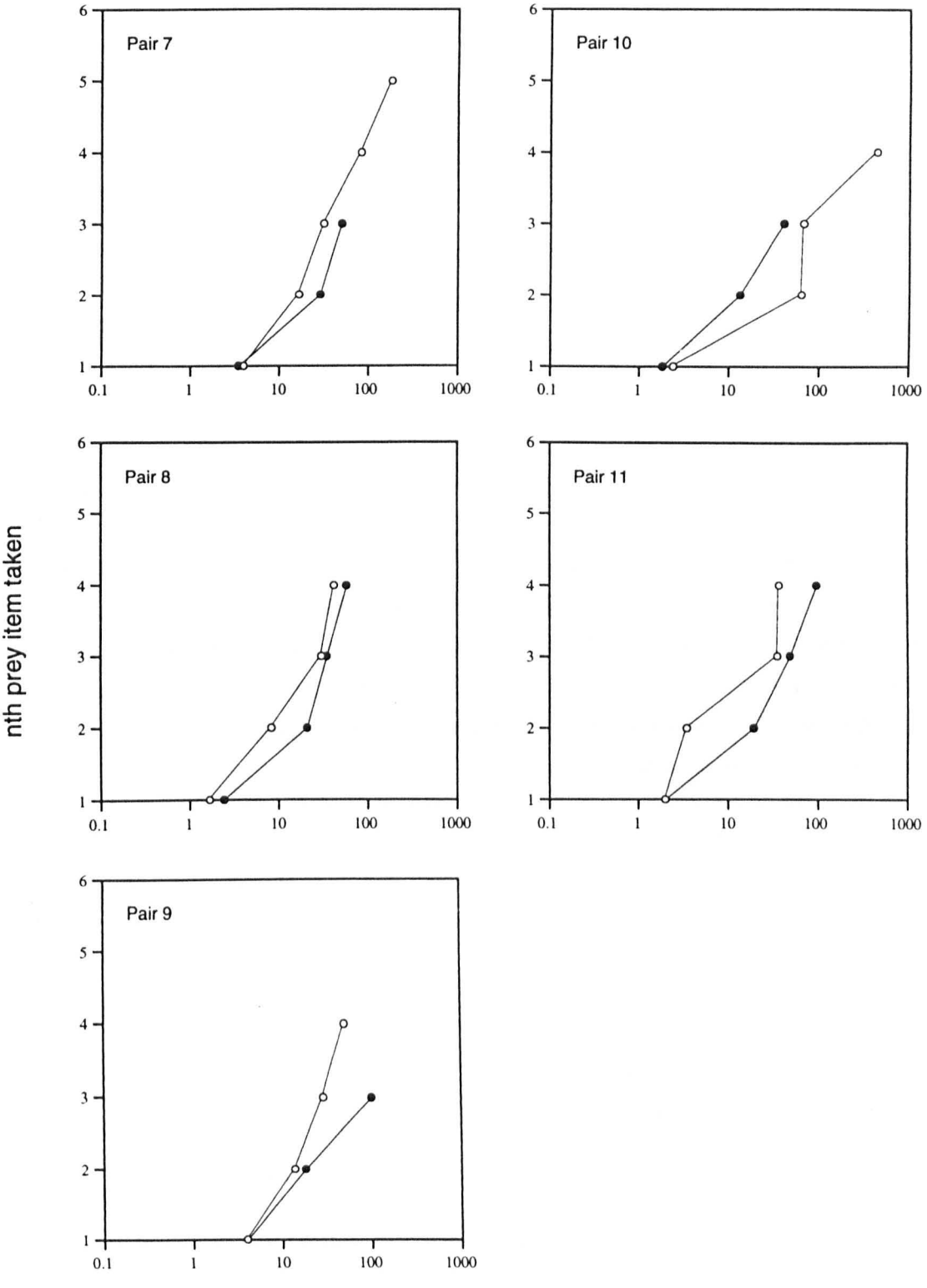
6.4 DISCUSSION

6.4.1 *Schistocephalus solidus* infection and the competitive ability of sticklebacks

In natural habitats, shoaling fish may encounter food in one of two different ways. If prey are randomly distributed and encountered with a high frequency, as with surface drift items, individual shoal members may frequently suffer through interference or contest competition, since more than one individual will often respond to a single indivisible prey item (Figure 6.13a), and under such conditions, it may be that peripheral fish suffer less from food competition than those in the centre. On

Figure 6.10 The times taken by uninfected (O) and *Schistocephalus solidus*-infected (●) competing sticklebacks to ingest successive prey items in each of the 11 trials.


Pair 1
Pair 4
Pair 2
Pair 5
Pair 3
Pair 6
nth prey item taken
Time from start of trial (s, log scale)
0.1
1
10
100
1000
1
2
3
4
5
6


Pair 7
Pair 10
Pair 8
Pair 11
Pair 9
nth prey item taken
Time from start of trial (s, log scale)
0.1
1
10
100
1000
1
2
3
4
5
6

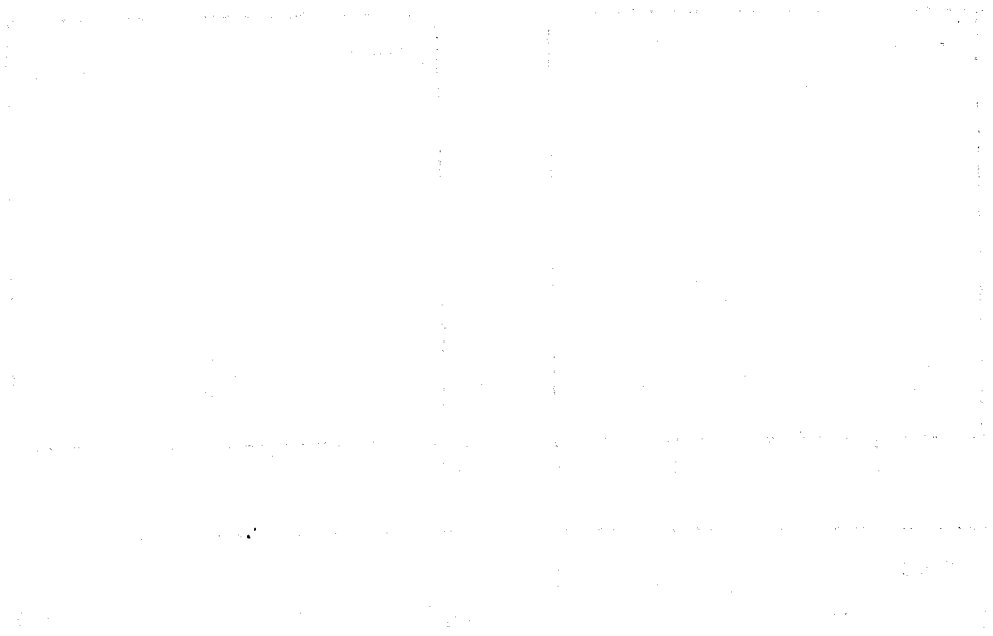
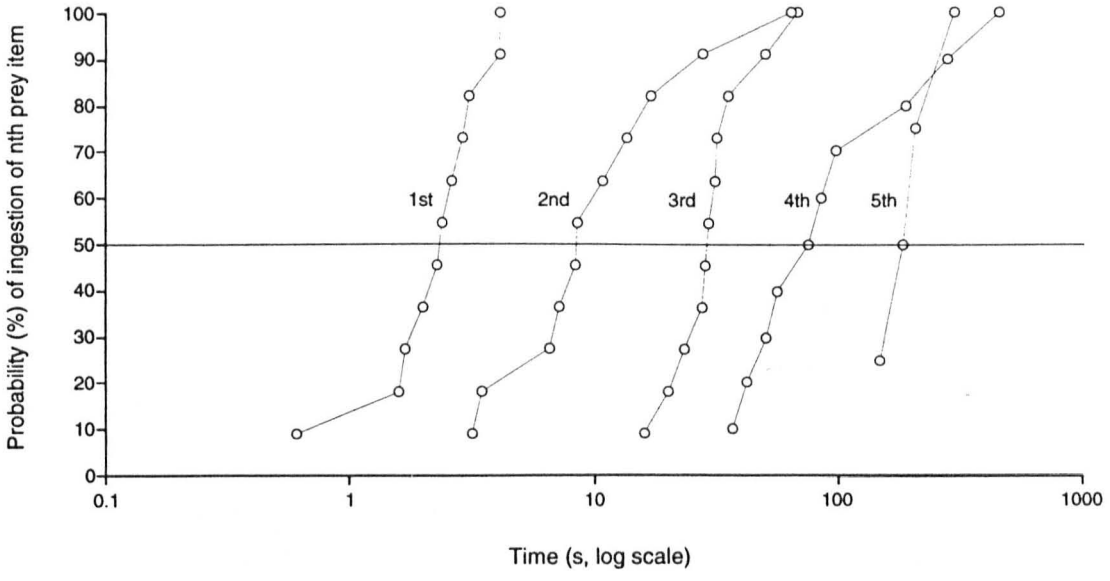


Figure 6.11 Graphical explanation of the technique employed to calculate the time taken for a) uninfected (O), and b) *Schistocephalus solidus*-infected (●) sticklebacks in 50% of the trials to consume their nth prey item (the T_{50}). The probabilities (y-axis) that n prey items had been ingested after a period of elapsed time (x-axis) were plotted using data from the 11 experimental trials, and the T_{50}'s associated with consecutive ingestion of prey items calculated by interpolation or direct observation.


Probability (%) of ingestion of nth prey item
100
90
80
70
60
50
40
30
20
10
0
1st
2nd
3rd
4th
5th
0.1
1
10
100
1000
Time (s, log scale)

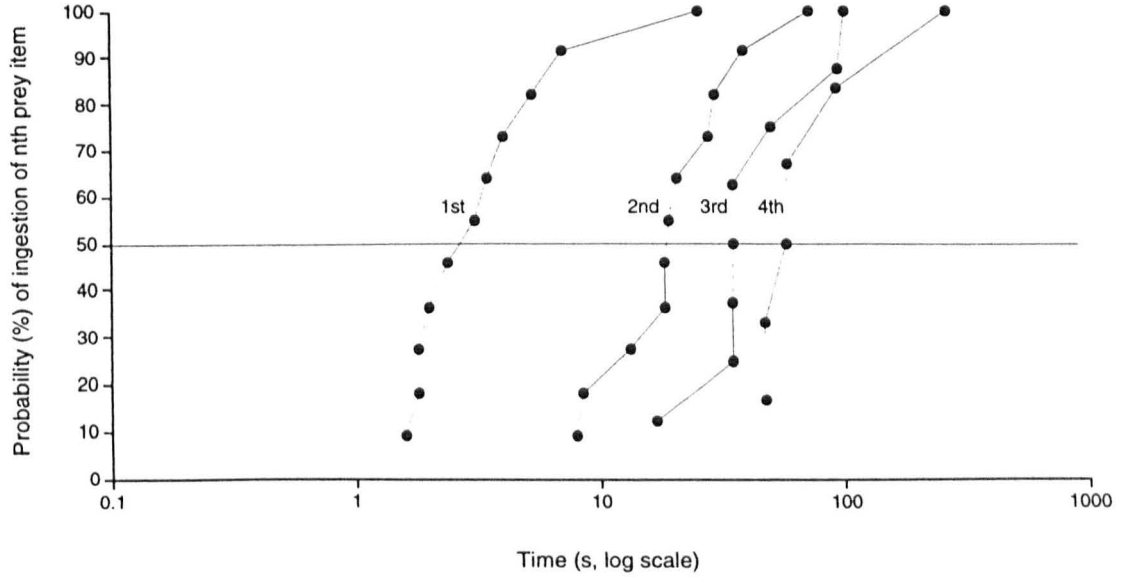

Probability (%) of ingestion of nth prey item
100
90
80
70
60
50
40
30
20
10
0
1st
2nd
3rd
4th
0.1
1
10
100
1000
Time (s, log scale)

Figure 6.12 The time taken for uninfected (O) and *Schistocephalus solidus*-infected (●) sticklebacks to consume their nth consecutive prey item in 50% of the trials (T_{50}). The relationship is best described by an exponential function for uninfected sticklebacks ($y=0.848 * 10^{0.481x}$, $r^2=0.991$) and by a linear function infected sticklebacks ($y = 18.425x - 17.050$, $r^2=0.992$).

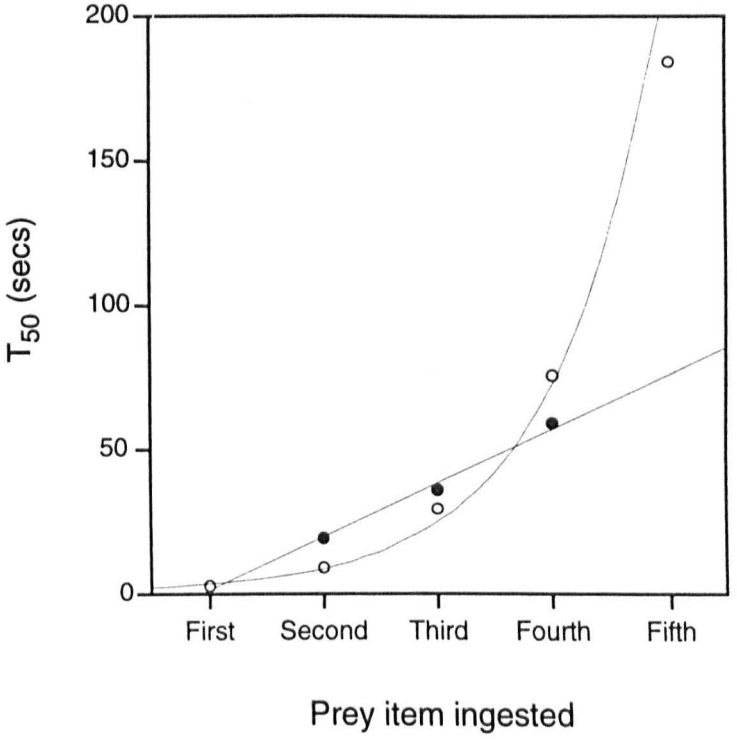
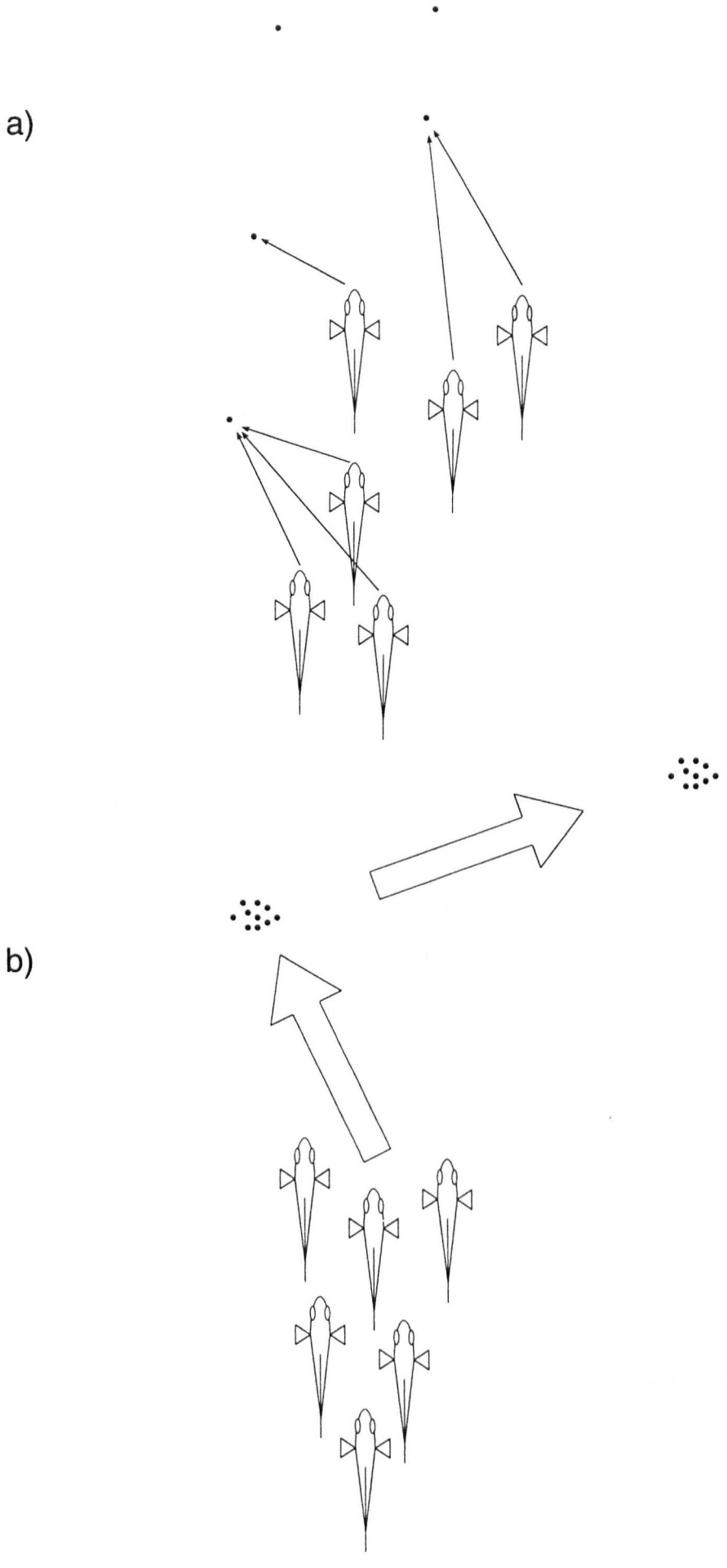

200
150
100
50
0
T_{50} (secs)
First
Second
Third
Fourth
Fifth
Prey item ingested

Figure 6.13 A diagrammatic summary of two ways in which distribution of food might affect competition in shoaling fish . a) When food items are randomly distributed in the habitat, and b) when food items are patchily-distributed, or clumped. See text for further explanation.


a)
b)

the other hand, where prey items show a patchy, or clumped, distribution (e.g. Manzer, 1976) shoals of fish tend to move slowly around their habitat seeking out such patches of food, depleting them and moving on to the next (Figure 6.13b). Following the discovery of such a patch, scramble competition is likely to occur, with each shoal member attempting to maximise its short-term foraging rate to enable it to consume as large a proportion of the available food as possible. Experiments 1 and 2 were designed to mimic these two types of competition, using pairs of sticklebacks only, in order to assess the effect of *S. solidus* infection on host foraging success.

The results of Experiment 6.1 show that when prey items were presented sequentially to pairs of fish, the total number of prey items ingested by infected sticklebacks was equivalent to that ingested by their uninfected conspecifics. However, although infected fish appear to do rather well in Experiment 6.1, it may be that the total number of prey ingested in this experiment is not be the best indicator of competitive ability. Because both infected and uninfected fish were equally likely to respond first to the introduced prey (suggesting that *S. solidus* had no effect on host vigilance), and because the first fish to respond to the prey item was generally successful in ingesting the food irrespective of infection status (largely because of the small size of the tank), measurement of the *total* prey intake tells us little about the actual competitive ability of either class of fish. The most meaningful assessment of competitive ability in the experiment was gained by examining the outcome of competition following a simultaneous response of both competitors to introduced prey, especially those cases when the fish were equidistant from the prey item when they responded. Following equidistant, simultaneous responses (which occurred a total of 26 times), *S. solidus*-infected competitors *never* successfully ingested the prey item, *always* losing out to uninfected fish. This is presumably because of either their slower swimming speed or their reduced manoeuvrability and co-ordination whilst approaching the prey. As a consequence, the diet of uninfected fish comprised mainly 'easy' prey, whereas the diet of uninfected fish included a greater proportion of 'difficult' prey items. It is likely that as the number of competitors increases, the number of undetected food items (on which infected fish appear to rely) will decrease, so although infected fish appear initially to fare rather well in this type of experiment with only one competitor, the situation in the field may be more severe. As a result, in shoals comprising both classes of fish, uninfected fish may be outcompeted because of their inability to secure 'difficult' prey items.

In Experiment 6.2, the ability of infected and uninfected sticklebacks to deplete patches of food was investigated. The experimental design ensured that both fish discovered the food patch at exactly the same time, and that the effort required to remove individual prey items was similar to that in the natural environment. The results show that, when foraging under scramble competition conditions, uninfected sticklebacks ingest more of the available prey items than infected counterparts, apparently because they are able to 'get through' the first few food items quicker. At the onset of foraging, uninfected fish outcompete parasitised fish, but later on infected fish 'catch up' and even 'overtake' uninfected competitors, with those that eventually ingest their fourth item doing so, on average, before uninfected fish. However, the usefulness of being able to eat a fourth item depends wholly on whether there is a fourth item to eat. If the infected fish were competing with several uninfected fish all feeding at a high rate, it is likely that the food patch would be bare by the time they were ready to 'catch up'. Rapid bolting of food by uninfected fish appears to be a more effective strategy in these trials, since it resulted in uninfected fish acquiring more food. So why did infected fish not 'bolt' prey items in the same way ?

Arme & Owen (1967) describe the gross pathology of *S. solidus* infection in the stickleback to include displacement of the host viscera as the plerocercoid grows in the body cavity of infected fish. Since, in heavy infections, the parasite can weigh at least as much as the host (Arme & Owen, 1967; personal observations), this is likely to place a severe limitation on space availability within the body cavity. After periods of intensive feeding, the greatly distended stomach of uninfected sticklebacks normally expands to fill most of the available space in the body cavity (Hart & Gill, 1992). It is likely that the stomachs of infected fish have much less room available for distension, reducing the volume of prey which could be consumed during one feeding bout. Experimental evidence supports this hypothesis, and *S. solidus*-infected sticklebacks have been shown to consume fewer prey items than size-matched uninfected conspecifics by various workers (Milinski, 1985; Cunningham *et al*, 1994), strongly suggesting that the "appetite" - the number of prey items taken before voluntary cessation of feeding (Wootton, 1990) - of infected fish is greatly reduced by the physical presence of plerocercoids in the body cavity. It may be that infected fish are unable to bolt food in the same way as uninfected fish, because of the physical restrictions of the parasite on gut expansion.

6.4.2 The effect of *S. solidus* on host foraging strategy

As an alternative to the suggestion that the physical presence of *S. solidus* plerocercoids reduce the ability of stickleback hosts to 'bolt' food, it may be that the reduced success of infected fish in scramble competition with uninfected conspecifics is a result of the adoption of a different feeding strategy. *Schistocephalus solidus*-infected fish are known to have higher nutritional demands than uninfected size-matched conspecifics (e.g. Pascoe & Mattey, 1977), yet experimental evidence suggests that they are poor physical competitors for food. One solution would be for the infected fish to maximise the efficiency with which they digested any prey items consumed, and it has been suggested that 'bolting' of food may have digestive costs. By 'pacing' their food intake, although infected fish inevitably lose out in direct competition with uninfected fish, it may allow them to maximise energetic gain from foraging.

As Milinski (1990) points out, poor physical competitors need to be "clever" to obtain sufficient food for vital functions, and parasitised sticklebacks appear to mediate their survival through alterations to their foraging strategy. These might involve foraging in habitats that offer low levels of competition in return for a higher risk of predation, or changing prey selection criteria to concentrate on less contested prey. Experimental evidence for the first possibility is provided by Giles (1983), who demonstrated that sticklebacks infected with *S. solidus* would return to food patches more quickly than uninfected conspecifics after a simulated avian attack. Presumably the infected fish were prepared to accept a perceived elevated risk of predation in return for the possibility of increasing their food intake rate by foraging in a competitor-free environment. Later experiments by Milinski (1985) showed that infected sticklebacks would feed in close proximity to a live predatory cichlid in order to maintain a constant rate of food intake, whereas uninfected fish fed only on the prey items most distant from the predator, and even then with a reduced intake rate. Evidently, under risk of predation the apparent 'boldness' of infected fish may be a mechanism enabling them to compensate for any competitive disadvantage the parasite may convey.

Where some heterogeneity exists in prey phenotype, infected fish may maximise their food intake rate by selecting less preferred prey types. When feeding on two size classes of *Daphnia*, in direct competition with unparasitised fish in the absence of any predatory threat, *S. solidus* infected sticklebacks were found to select the smaller prey items preferentially (Milinski, 1984), in much the same way as uninfected poor competitors had in previous experiments (Milinski, 1982). This strategy

allowed infected fish to avoid energetically-expensive competitions for the larger prey, which were preferred by the unparasitised fish. Ranta (1995) found that although infected fish had a lower food intake rate than uninfected fish, infected fish consume a relatively greater proportion of the larger prey. By selecting the larger prey, infected fish were able to achieve equivalent short-term energy intake rates to uninfected fish, which fed at a higher rate and preferred the smaller individuals. Ranta (1995) accounts for the discordance between his and Milinski's (1984) results by pointing out that the sticklebacks used by Milinski had been starved prior to experimentation, whereas his own study fish had been allowed to feed *ad libitum* for 4 hours prior to the trials.

It would appear in both of the above studies that, when competition is high, infected fish 'make the best of a bad situation' and select the smaller, apparently less profitable food items. However, foraging on these small food items may not always be sub-optimal for infected sticklebacks. The increased prey handling time of *S. solidus* infected sticklebacks when partially-full results in the same sized prey being differentially profitable to fish of different infection status, and in an experimental test of prey choice, fish infected with *S. solidus* exhibited a preference for smaller prey items that were more profitable on a Joules per second basis (Cunningham *et al.*, 1994). These results demonstrate that prey choice reflects prey profitability, which is in turn dependent on both infection status and the level of stomach fullness of the individual concerned.

6.4.3 Effects of *S. solidus* on the feeding ecology of sticklebacks in natural environments

Do the differences in competitive ability and prey preferences demonstrated in the laboratory relate to the field situation, where the variety of prey encountered is inevitably more varied ? Data on the effects of *S. solidus* on the diet composition and foraging success of sticklebacks in the wild is scarce, although research by Tierney (1994) and Jakobsen *et al* (1988) has detected differences in both the stomach fullness and prey selection of *S. solidus*-infected and uninfected fish at particular times of the year when food competition was likely to be at its highest. However, it is not clear whether these differences in apparent foraging success were a direct result of increased feeding competition, or whether some other factor, such as low oxygen tension, restricted infected fish to less productive parts of the habitat, and the effects of parasites on host foraging in the wild provides a potentially fruitful area for further research.

It appears that the physical and energetic restrictions imposed by *S. solidus* change the foraging decisions taken by stickleback hosts by increasing their nutritional requirements whilst a) reducing their ability to compete successfully with others for food, b) decreasing the amount of food which can be consumed in one feeding bout, and c) having a detrimental effect on their foraging efficiency. It is likely that in the field these changes in foraging behaviour may have broader implications for the ecology of parasitised hosts than simply altering diet selection. For instance, by altering the foraging efficiency of infected sticklebacks, *S. solidus* may have an effect other facets of its host's behaviour. Social foraging in many species of fish, including the three-spine stickleback (Keenleyside, 1957; Wootton, 1984) is known to be common, and the formation of shoals is an evolutionary successful adaptation to the combined pressures of predator evasion and the location of patchily-distributed food resources. By altering the foraging behaviour of infected fish, *S. solidus* could theoretically change the value of being a shoal member.

6.5 SUMMARY

- Experiments were designed to examine the ability of *S. solidus*-infected sticklebacks to compete with uninfected conspecifics for food under direct competition for individual items (*interference* competition) or indirect competition via patch depletion (*scramble* competition).
- Under direct competition for individual food items, *S. solidus*-infected sticklebacks were just as successful at ingesting capturing single introduced prey as uninfected sticklebacks. However, *S. solidus*-infected fish were only successful at capturing those items to which they were the first, the closest or the only responders.
- *S. solidus*-infected sticklebacks were shown to exhibit a lower patch depletion rate than uninfected fish when feeding in direct competition with them on an artificial food patch, ingesting fewer prey over the course of the experimental trials. Uninfected fish take the first few prey items in rapid succession, eventually slowing down prey intake exponentially, whereas infected sticklebacks feed at a slow, but constant rate.
- The possible consequences of the observed behaviour on the ecology of uninfected and infected fish, and in particular the potential of *S. solidus*-mediated competitive disability to affect the social behaviour of infected fish, are discussed.

Chapter 7. A non-invasive technique for measuring the cestode plerocercoid load of small freshwater fish

7.1 INTRODUCTION

7.1.1 Background

The investigation of the effects of macroparasites on the behaviour of their hosts has been, in recent years, a productive area in the study of behaviour, yielding large amounts of data on parasite-associated behavioural modification, and demonstrating that such changes have the potential to alter the ecology of infected individuals (Holmes & Bethel, 1972; Moore & Gotelli, 1990; Moore, 1995). Frequently, such observed behavioural modification is associated in some way with the parasite load, with more heavily-infected individuals showing more severe behavioural changes. Although some of the fish parasites that have been studied in such investigations are ectoparasitic, or at least externally-visible (e.g. the fish louse, *Argulus* spp., or the subcutaneous encysted metacercariae of the trematode *Crassiphiala bulboglossa*), other parasites, such as the plerocercoid larvae of pseudophyllidean tapeworms, occupy body compartments that cannot be accessed without the need for destructive sampling. Accurate evaluation of the parasite loads of fish infected with such species is therefore necessarily terminal for the host.

Although terminal diagnosis of parasite load is currently the only method presently available for fish biologists, there are some objections, both scientific and ethical, to this procedure. As well as having potentially adverse effects on natural habitats (since large numbers of individuals often need to be sacrificed to gain adequate data on parasite intensity and prevalence), the reliance on terminal dissection for assessment of parasite load is prohibitive to many potentially interesting experiments regarding *in vivo* parasite growth and nutrient partitioning during parasite infection. In addition, biologists are, quite rightly, under constant pressure to devise techniques to minimise the suffering caused to animals during experimental procedures, and to reduce the numbers of terminal procedures undertaken (Stamp Dawkins & Gosling, 1992). Accurate, non-destructive, non-invasive approaches to parasite load determination therefore have both scientific and ethical value, and are increasing of use in the fields of aquaculture and fish biology, where morphometric indices have recently been developed to determine lipid content (Simpson *et al*, 1992) and maturation status (Kadri, in press).

7.1.2 Effects of *S. solidus* and *L. intestinalis* on the general appearance of their fish hosts

Schistocephalus solidus and *L. intestinalis* are two parasites that inhabit the body cavities of three-spined sticklebacks *Gasterosteus aculeatus* and certain cyprinid fish species respectively

(Williams & Jones. 1994). Both are common parasites of three-spined sticklebacks and cyprinid fish respectively in the U.K. (Kennedy, 1974). As *S. solidus* and *L. intestinalis* plerocercoids grow the skin of the abdomen grows to accommodate them and the abdomens of infected fish become characteristically distended (Arme & Owen, 1967; Arme & Owen, 1968; Sweeting, 1977). The swelling caused by the parasite is especially noticeable when the host is observed from above, since it causes disruption to the generally streamlined shape of the fish, and it has been suggested that this increase in the dorsal profile makes infected fish swimming at the same depth as uninfected fish appear higher in the water column, and therefore potentially more vulnerable to avian predators (McPhail & Peacock, 1983) although, according to LoBue & Bell (1993), infected fish may *actually* swim higher in the water column, rather than this being a simple 'optical illusion'). Although many workers have noted the abdominal distension of *L. intestinalis*- and *S. solidus*-infected fish, no attempts have been made to quantify the swelling.

7.1.3 The value of studying the behaviour of *S. solidus*- and *L. intestinalis*-infected fish

The stickleback-*S. solidus* host-parasite system has been a widely used tool in the experimental investigation of the effects of parasites on host decision-making (Milinski, 1985, Cunningham *et al*, 1994; Barber *et al*, 1995), host antipredator behaviour (Giles, 1983; Tierney *et al*, 1993), host respiration (Walkey & Meakins, 1970; Lester, 1971; Meakins & Walkey, 1975), host reproduction (Meakins, 1974; Pennycuick, 1971; McPhail & Peacock, 1983; Tierney *et al*, in press), diet (Tierney, 1994), the energetic costs of parasite infection (Walkey & Meakins, 1970; Orr & Hopkins, 1969; Pennycuick, 1971; Körting & Barrett, 1977) and the effects of parasites on the ecological habitat selection of hosts (Giles 1987a & b, Smith & Kramer, 1987; Jakobsen *et al*, 1988).

Investigations into the effects of *L. intestinalis* on the behaviour of cyprinid hosts have been largely restricted to field studies, with few controlled, experimental laboratory-based studies having been undertaken. However, investigations of the epidemiology of *L. intestinalis* infection have increased understanding of how such parasites may control, and may be controlled by, host population dynamics (see Chapter 2 and references therein). In addition, detailed studies have led to a full understanding of *L. intestinalis* infection on the reproductive physiology of cyprinid hosts, and the *L. intestinalis*-cyprinid system has also been used extensively in the investigation of the cellular and humoral immunological responses mounted by fish against such parasites. In summary, studies of the

L. intestinalis-cyprinid system have contributed a great deal to the understanding of host-parasite relationships, and the system continues to be an extremely useful experimental tool for fish biologists, parasitologists and behavioural ecologists alike.

Both sticklebacks and minnows are intermediate hosts in the life cycles of the parasites described above, and the behavioural changes observed in infected fish are often proposed to facilitate transmission of the parasite to the definitive host which, in the case of *S. solidus* and *L. intestinalis*, is usually a piscivorous bird (Hopkins & Smyth, 1951; Yamaguti, 1958). Recent research suggests that behavioural modification is often not apparent until the parasite reaches a weight where it is infective to the definitive host (Tierney *et al*, 1993).

Clearly, for fish infected with such parasites, the *magnitude* of the parasite load harboured is more likely to be important in determining whether there are any associated effects on host behaviour than infection status alone. Parasite load in such infections is normally expressed as either the total weight of parasite tissue, or as the 'Parasite Index', which expresses what proportion of an infected fish's weight is contributed by parasite tissue (see Chapter 2, section 2.2.3, for further explanation). Determination of both of these measurements of parasite load is currently only possible following terminal dissection, which makes certain types of potentially interesting and valuable studies impossible to carry out.

For instance, in order to gain a clearer picture of how parasites alter behaviour over the course of an infection, experiments need to be designed to monitor the behaviour of individual infected fish throughout the growth phase of the parasite, from artificial infection to the point where the parasite reaches its maximum size. This type of experiment requires the availability of a non-destructive, non-invasive and stress-free method that will allow the accurate, regular determination of parasite load.

In addition, the availability of such a method would allow investigations of relative growth rates of parasites and hosts, and thereby further the understanding of how energy resources of the host are partitioned during parasitic infection. A non-destructive method for parasite load assessment would also allow behavioural ecologists to design experiments using fish of known parasite loads, for instance to examine the behaviour of fish with infective and non-infective worms, and to ensure that sample sizes of each group were approximately equal.

7.1.4 Objectives

A sampling and morphometric analysis programme was undertaken with the aim of developing predictive models that would allow the non-destructive determination of cestode plerocercoid loads in small freshwater fishes. The specific aims of this chapter are:

- To develop a photo-morphometric system for the accurate measurement of the dorsal profile area (DPA) of small freshwater fish.
- To quantify the relationship between fish length and DPA for minnows and three-spined sticklebacks.
- To quantify the relationships between parasite index and the DPA for *S. solidus*-infected sticklebacks and *L. intestinalis*-infected minnows.
- To develop predictive models to discriminate accurately between uninfected and infected fish, and to assess the weight of parasite tissue in infected fish, without the need for destructive sampling.

7.2 MATERIALS AND METHODS

7.2.1 Supply and husbandry of fish

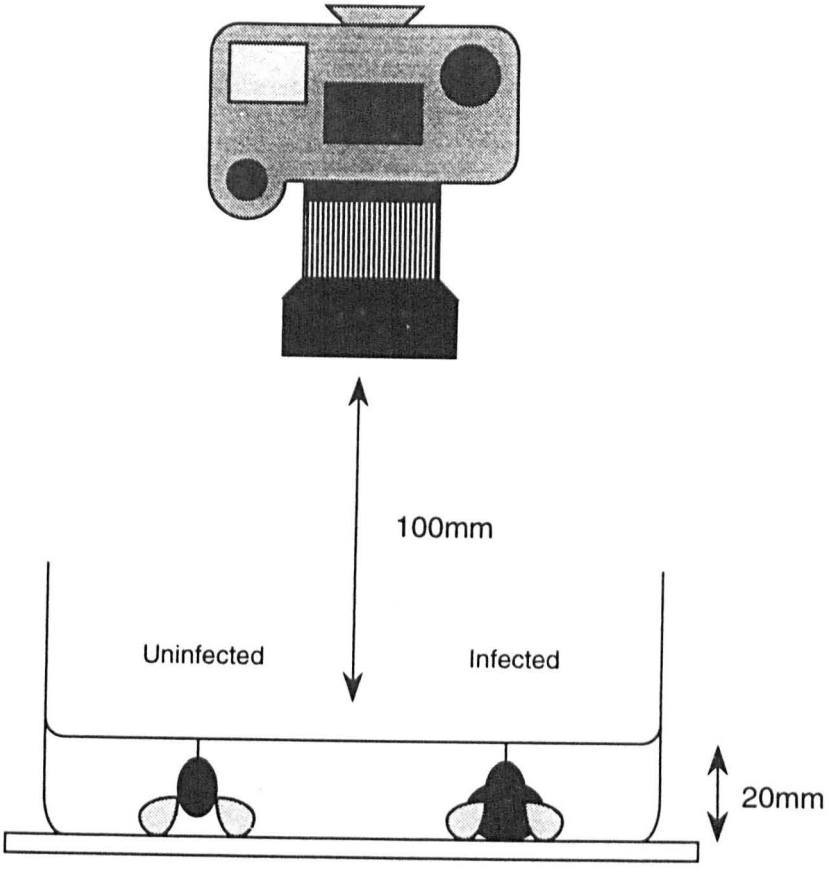
Uninfected and *S. solidus*-infected sticklebacks were collected by hand netting or by trapping from Inverleith pond, Edinburgh. Uninfected and *L. intestinalis*-infected minnows were collected by trawl-netting from Loch Maragan and by trapping from Loch Tarsan (see Chapters 2, 3 and 5 for site descriptions). All fish were caught during the autumn and winter of 1994/95, and immediately transferred to laboratory holding tanks where they were maintained at 10°C, under a 12h:12h photoperiod in order to prevent females becoming gravid. Fish were maintained in the stock tanks for a period of several weeks, during which they were fed *ad libitum* to excess with both live and frozen bloodworms and artificial flake food in order to standardise, as far as possible, body condition.

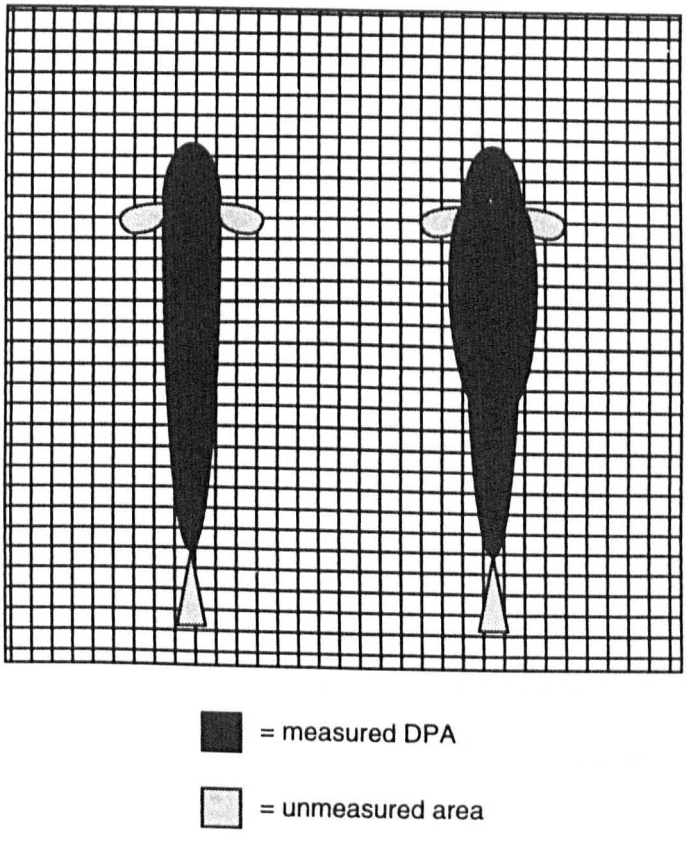
7.2.2 Measurement of the Dorsal Profile Area (DPA)

Individual sticklebacks and minnows were placed in a shallow glass crystallising dish (diameter: 200mm) which was filled to a depth of 20mm with clean, aerated tap water. Beneath the glass dish was a 5mm reference grid. Individual fish were photographed from directly above, using a Canon T90 SLR camera fitted with a macro lens, mounted on a tripod (see Figure 7.1). To achieve maximum definition, black and white film was used (Ilford Pan-F) and, once printed, the photographs were enlarged by 100% on a standard photocopier. A digitising tablet (Cherry Products, Harpendon,

Figure 7.1 The apparatus used to photograph fish for the subsequent determination of Dorsal Profile Area (see text).

Figure 7.2 Diagrammatic representation of the area measured to provide the values of the Dorsal Profile Area for infected and uninfected fish, showing the calibration grid below the fish.


100mm
Uninfected
Infected
20mm


= measured DPA
= unmeasured area

Essex, U.K.) connected to a BBC microcomputer was used to measure the DPA of each individual fish in the enlarged photographs (Figure 7.2), using the reference grid to calibrate the equipment (digitising software written by M. D. Burns, University of Glasgow, U.K.).

7.2.3 Determination of infection status

After being photographed, individual fish were immediately exposed to a lethal dose of Benzocaine anaesthetic and weighed (to 0.001g) and measured (fork length: to 1mm) prior to dissection to determine parasite status. Any plerocercoids recovered were surface dried and weighed (to 0.001g) and the parasite intensity, total parasite weight and Parasite Index were calculated for each fish.

7.2.4 Rationale for the development of the predictive model

Theoretically, the DPA of *L. intestinalis* and *S. solidus*-infected fishes should serve as a reliable predictor of the parasite load, for the following reasons:

a) The DPA of both uninfected and infected fish should increases as a species- and infection status-dependent factor of fork length.

b) Fish can be photographed from above and the DPA measured accurately using a digitising tablet

c) The parasite is more efficient than its host in assimilating available nutrients, and it grows disproportionately quickly, with a resultant effect on the swelling of the abdomen. Therefore a fish with a larger parasite index should have a DPA that deviates further from that of size-matched uninfected fish than an infected fish with a smaller parasite index (Figure 7.3).

d) This deviation can be calculated by subtracting the expected DPA for an uninfected fish of given fork length from the observed DPA of the infected fish, and expressed as a residual ('R') of DPA (Figure 7.4).

e) The residual value should be proportional to the Parasite Index of the infected fish.

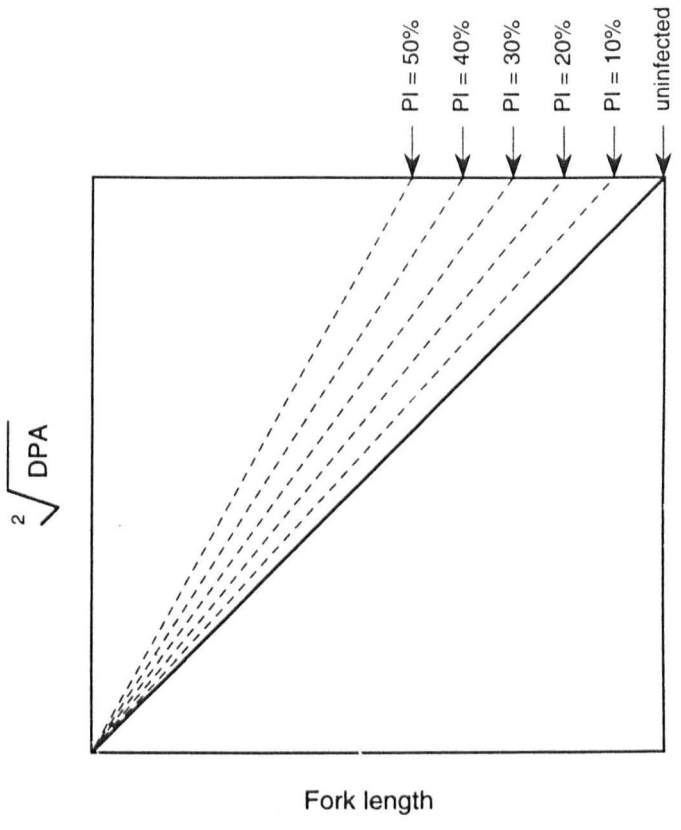
4.3 RESULTS

4.3.1 The relationship between fork length and DPA for uninfected and infected fish

Highly significant linear relationships were found to exist between fork length and the square root of the measured DPA for both uninfected sticklebacks and uninfected minnows (Figures 7.5 & 7.6; see Table 7.1 for details of regression equations).

Figure 7.3 Schematic representation of the hypothesised combined effects of increasing fork length and parasite load, measured as Parasite Index (PI), on the Dorsal Profile Area (DPA) of minnows and sticklebacks infected with the plerocercoid larvae of pseudophyllidean cestodes.

Figure 7.4 Diagrammatic explanation of the method used to calculate the residual Dorsal Profile Area (DPA) values exhibited by infected fish. The hypothetical DPAs of three infected fish are shown, creating residual values of R_1, R_2 and R_3.


PI = 50%
PI = 40%
PI = 30%
PI = 20%
PI = 10%
uninfected
$\sqrt[2]{\text{DPA}}$
Fork length

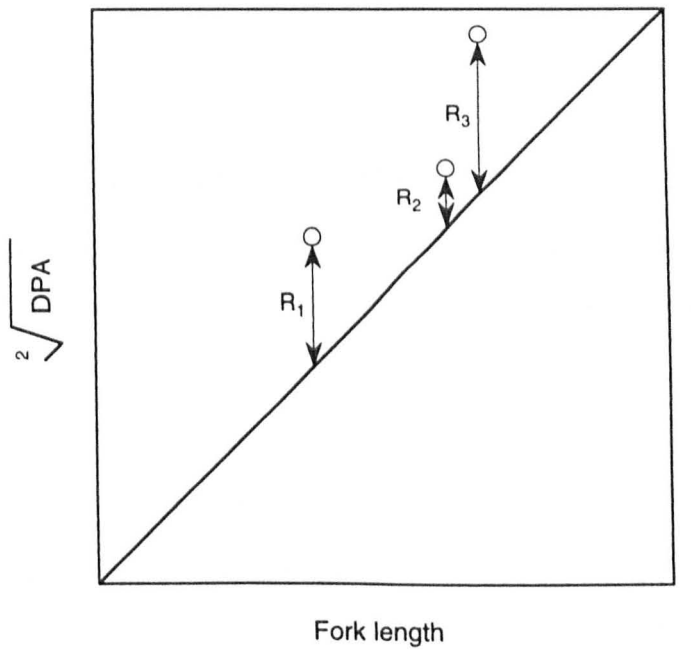

R_1
R_2
R_3
$\sqrt[2]{\text{DPA}}$
Fork length

Table 7.1 The relationships between DPA and fork length for uninfected and infected sticklebacks and minnows

Group	Regression equation	r^2	P
Uninfected sticklebacks	$\sqrt[2]{DPA} = 0.290(\text{fork length}) - 1.95$	0.934	**<0.001**
S. solidus-infected sticklebacks	$\sqrt[2]{DPA} = 0.277(\text{fork length}) - 0.257$	0.911	**<0.001**
Uninfected minnows	$\sqrt[2]{DPA} = 0.273(\text{fork length}) + 0.538$	0.979	**<0.001**
L. intestinalis-infected minnows	$\sqrt[2]{DPA} = 0.209(\text{fork length}) + 5.39$	0.723	**0.043**

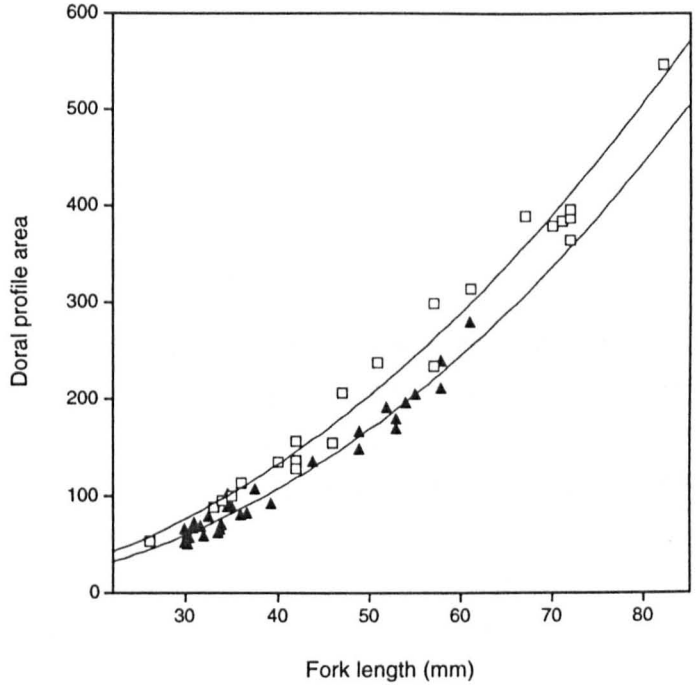
Analysis of covariance, using the General Linear Model (GLM), showed that the regression lines describing these two relationships differed significantly from one another in elevation (ANCOVA, $F_{1,50} = 57.38$, $p<0.001$), meaning that for any particular fork length, minnows presented a larger DPA than sticklebacks. DPA was positively and highly significantly related to fork length in both *S. solidus*-infected sticklebacks and *L. intestinalis*-infected minnows (Figures 7.7 and 7.8, Table 7.1); however for both minnows and sticklebacks the relationships between DPA and fork length for infected and uninfected fish differed significantly, with infected fish having larger DPAs than uninfected conspecifics for any given fork length (ANCOVA, sticklebacks, $F_{1,60} = 68.14$, $P<0.001$; minnows, $F_{1,24} = 8.37$, $P = 0.008$).

7.3.2 The relationship between parasite index and residual DPA

By using the linear equations describing the relationship between fork length and DPA for uninfected individuals detailed above, a set of predicted DPA values for uninfected fish of precisely the same fork length as the infected fish photographed were generated. Residual values were calculated by subtracting the observed DPA measurement for infected fish from the predicted DPA measurement expected for an uninfected fish of the same size (see Figure 7.4). This residual value, which indicated to what extent the observed DPA of an infected fish deviated from the expected DPA of a hypothetical uninfected, size-matched conspecific, was found to be related to the (arcsine transformed) Parasite Index of both *S. solidus*-infected sticklebacks and *L. intestinalis*-infected minnows (Figures 7.9 and 7.10) by the equations below;

Figure 7.5 The relationships between fork length and Dorsal Profile Area for uninfected three spined sticklebacks (▲) and uninfected minnows (□). Both relationships are described by a square function (see text for equations).

Figure 7.6 The relationships between fork length and the square root of Dorsal Profile Area for uninfected three spined sticklebacks (▲) and uninfected minnows (□). The relationships conform to linear functions, and differ from one another significantly (see text for equations and statistical analysis).


600
500
400
300
200
100
0
Doral profile area
30
40
50
60
70
80
Fork length (mm)

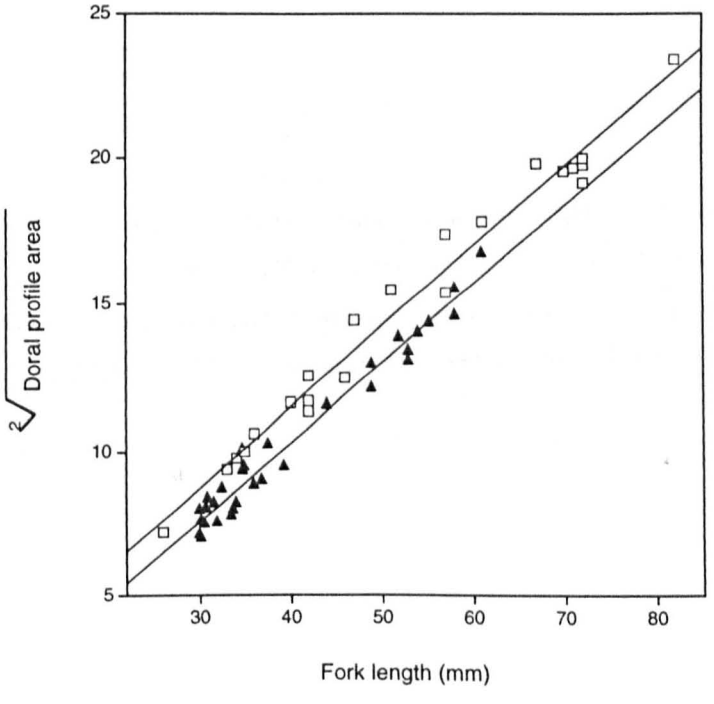
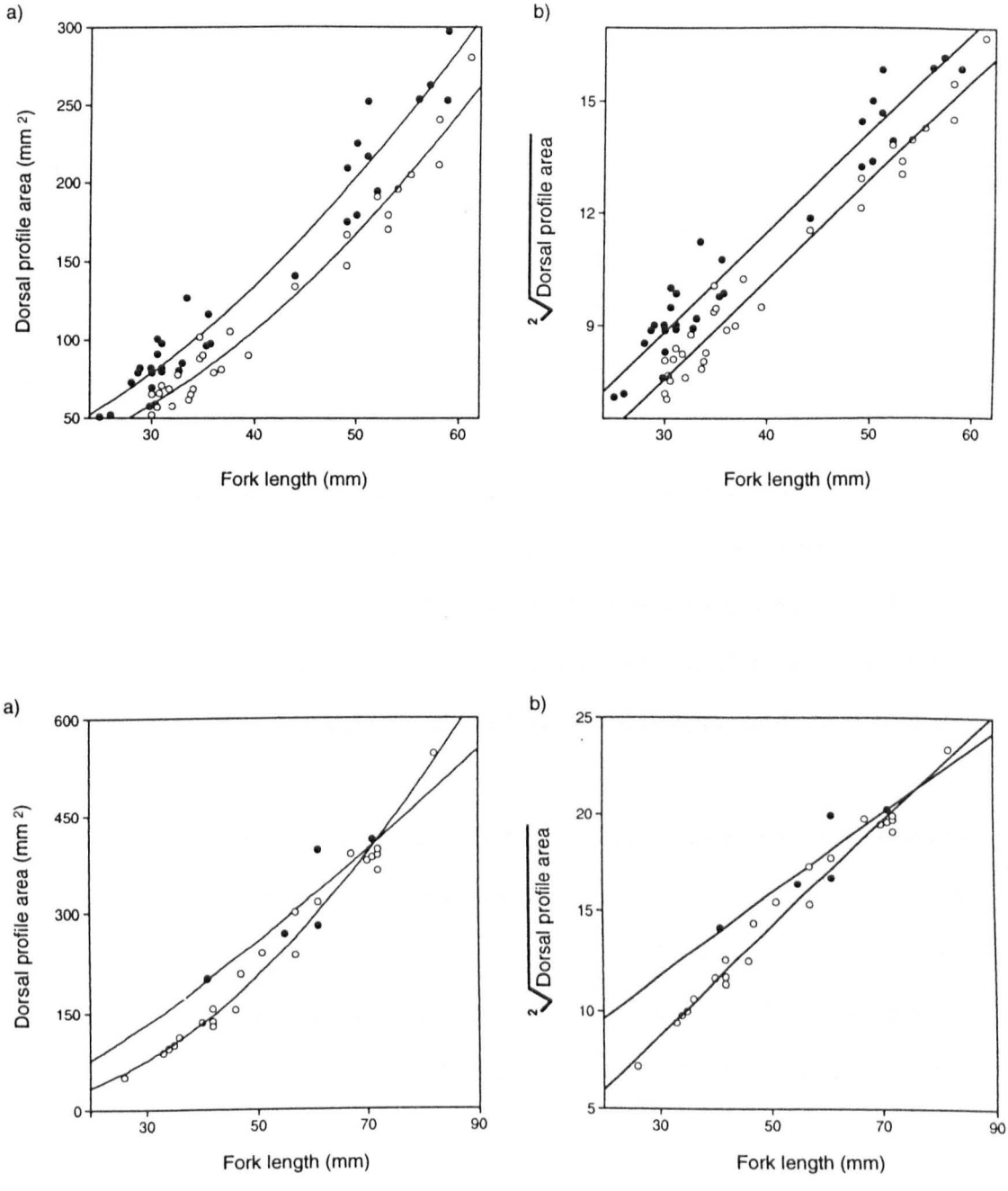

25
20
15
10
5
Doral profile area
30
40
50
60
70
80
Fork length (mm)

Figure 7.7 The relationships between fork length and Dorsal Profile Area (DPA) for uninfected (O) and *Schistocephalus solidus*-infected (●) three spined sticklebacks. a) The relationship between fork length and DPA. b) The relationships between fork length and the square root of DPA. The regression equations describing the relationships of infected and uninfected fish differ significantly (see text).

Figure 7.8 The relationships between fork length and Dorsal Profile Area (DPA) for uninfected (O) and *Ligula intestinalis*-infected (●) minnows. a) The relationship between fork length and DPA. b) The relationships between fork length and the square root of DPA. The regression equations describing the relationships of infected and uninfected fish differ significantly (see text).


a)
Dorsal profile area (mm 2)
300
250
200
150
100
50
30
40
50
60
Fork length (mm)
b)
2√Dorsal profile area
15
12
9
30
40
50
60
Fork length (mm)
a)
Dorsal profile area (mm 2)
600
450
300
150
0
30
50
70
90
Fork length (mm)
b)
2√Dorsal profile area
25
20
15
10
5
30
50
70
90
Fork length (mm)

Infected sticklebacks

$$\sin^{-1}\sqrt[2]{\text{Parasite Index}} = 29.908(\text{Residual}^{0.159}),$$

rearranged, gives:

$$\text{Parasite Index (\%)} = \sin[29.908*(\text{Residual}^{0.159})]^2 * 100.$$

Infected minnows

$$\sin^{-1}\sqrt[2]{\text{Parasite Index}} = 32.649(\text{Residual}^{0.115}),$$

rearranged, gives:

$$\text{Parasite Index (\%)} = \sin[32.649*(\text{Residual}^{0.115})]^2 * 100.$$

7.3.3 Testing the models

7.3.3.1 The efficacy of DPA measurement as a predictor of infection status

Logistic regression analysis was employed to ascertain the efficacy of DPA measurement as a predictor of *S. solidus* and *L. intestinalis* infection status of sticklebacks and minnows respectively. Overall, using logistic regression on residual DPA values, 87.5% of sticklebacks were assigned to the correct infection status group overall (84.4% accuracy in assigning to infected group [n=32]; 90.6% accuracy in assigning to uninfected group [n=32]; Figure 7.11), which is highly significant (Chi-square test, $\chi^2 = 49.89$, df = 1, $P<0.0001$).

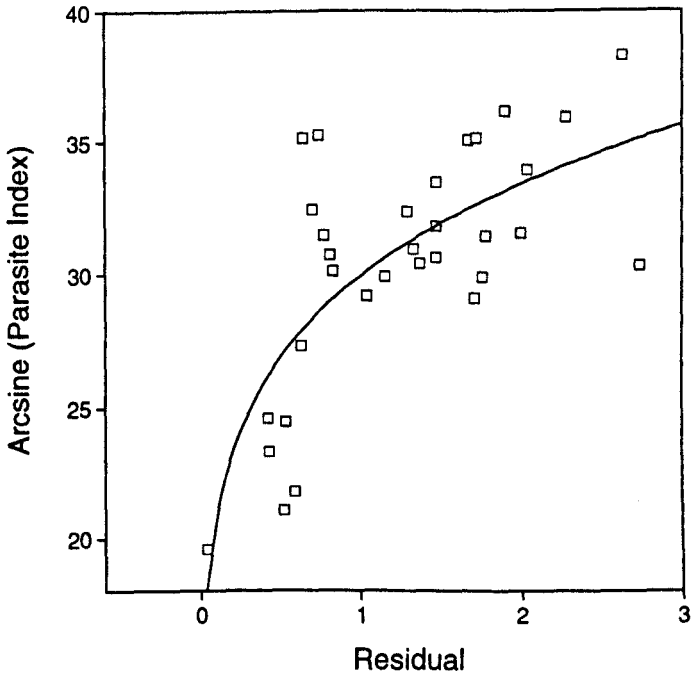
Similar logistic regression techniques on residual DPA values assigned 88.9% of minnows to the correct infection status group overall; however, most of this success was due to the accurate assignment of the more numerous uninfected fish, and 3 out of the 5 infected fish were assigned to the wrong group (40.0% accuracy in assigning to infected group [n=5]; 100.0% accuracy in assigning to uninfected group [n=22]; Figure 7.12). The overall result is highly significant (Chi-square test, $\chi^2 = 6.20$, df = 1, P=0.0128).

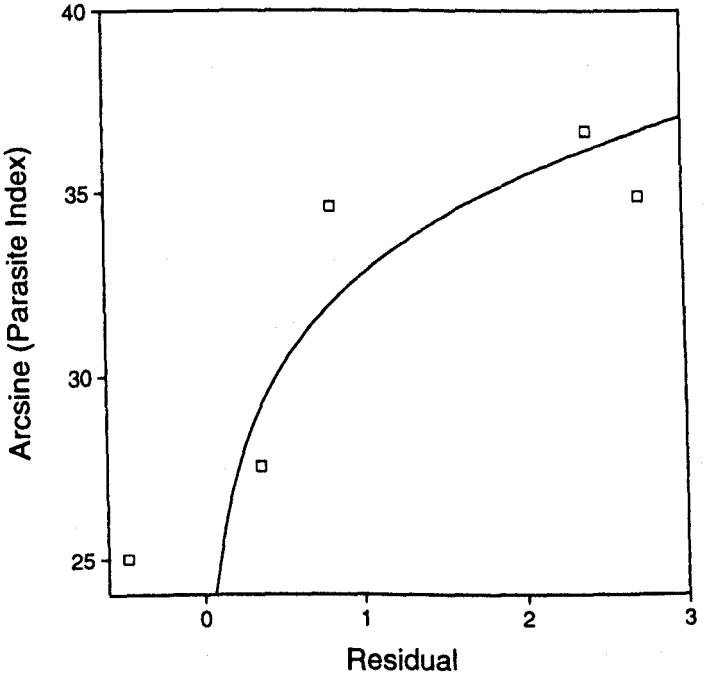
7.3.3.2 The efficacy of DPA measurement as a predictor of parasite load

Using the model described above, residual DPA was found to be an accurate predictor of the Parasite Index of *S. solidus*-infected sticklebacks. The difference between the measured PI and the predicted PI for each parasitised fish was calculated, and the cumulative frequency of these differences

Figure 7.9 The relationship between residual Dorsal Profile Area and arcsine-transformed Parasite Index for *Schistocephalus solidus*-infected three spined sticklebacks. See text for details of the equation describing the relationship.

Figure 7.10 The relationship between residual Dorsal Profile Area and arcsine-transformed Parasite Index for *Ligula intestinalis*-infected minnows. See text for details of the equation describing the relationship.


Arcsine (Parasite Index)
40
35
30
25
20
0
1
2
3
Residual


Arcsine (Parasite Index)
40
35
30
25
0
1
2
3
Residual

is shown in Figure 7.13. This analysis demonstrates that the predicted PI of 80% of infected sticklebacks were within 5% of the actual PI. The same analysis for *L. intestinalis*-infected minnows demonstrated that that the predictive ability of the model was reduced, probably because of the small sample size (see Discussion). For infected minnows, 80% (4/5) of predicted PIs were within 15% of the actual value, (see Figure 7.14).

However, in order to ascertain how predictive such models are, it is necessary to express the difference between the observed and predicted PIs as a percentage of the observed PI value, rather than as an absolute value. This is because an absolute difference between predicted and observed PI of 5% would be likely to cause more concern when the observed PI was small (for instance, 10%) than if it were large (say, 60%). When the cumulative frequency of the differences, expressed as a proportion of observed PI, is plotted for infected sticklebacks (Figure 7.15), it can be seen that the predicted PIs of approximately 75% of fish fell within 20% of the observed value of PI. The same analysis for *L. intestinalis*-infected minnows again shows the reduced predictive value of the model, with 80% of predicted PIs falling within 40% of the observed values (Figure 7.16).

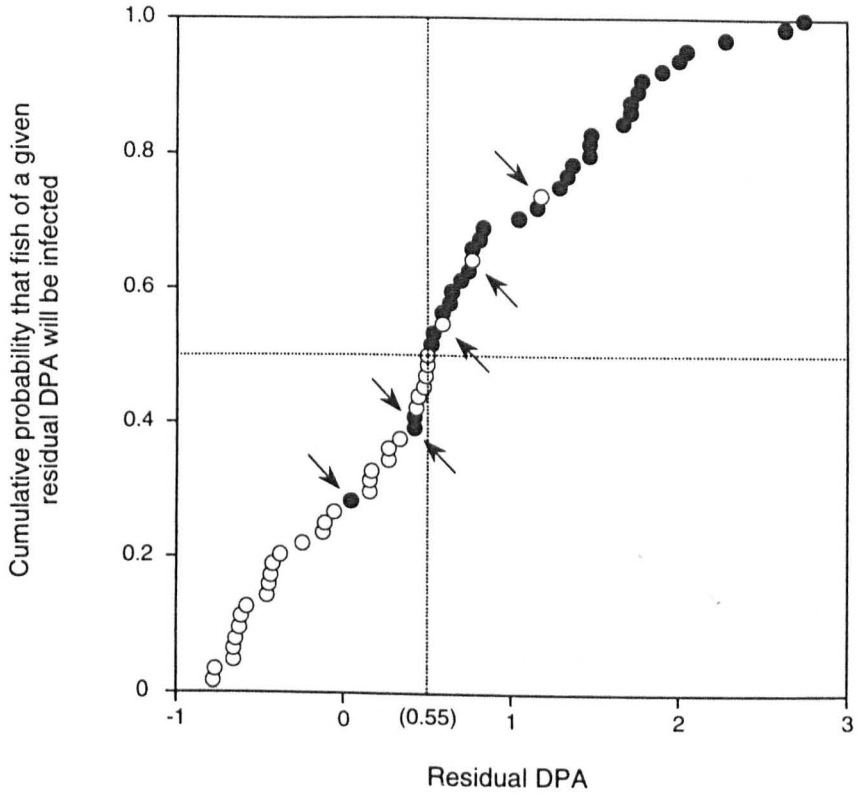
7.4 DISCUSSION

7.4.1 Interspecific and infection-associated differences in the DPA of minnows and sticklebacks

Although the majority of teleost species are generally streamlined in shape, based on the typical 'torpedo' dorsal profile that is known to offer minimal drag whilst moving through water, various modifications to allow for specialised modes of feeding and locomotion are exhibited by many species, resulting in the variety in shape observed between species (Bone & Marshall, 1982). Minnows are adapted for prolonged schooling in open water, and have a correspondingly highly streamlined body shape, whereas sticklebacks tend to spend less time in active 'tail' swimming, instead moving slowly about their environment and primarily using their enlarged pectoral fins for propulsion (Wootton, 1976; Wootton, 1984). The body form of sticklebacks is more laterally-compressed (Wootton, 1976) and less 'torpedo-shaped' than that of minnows, possibly reflecting these differences in the general mode of locomotion. The interspecific differences in body form are clearly revealed by the measurement of DPA, since uninfected minnows presented a larger area in dorsal profile than length-matched uninfected sticklebacks.

Figure 7.11 The results of logistic regression analysis devised to determine the predictive power of the stickleback model. The cumulative probability that individual fish will exhibit a particular residual Dorsal Profile Area (DPA) is plotted, with the predicted cut-off point shown by the dotted line. The residual DPA values of *Schistocephalus solidus*-infected (●) and uninfected (O) three spined sticklebacks are shown. Individuals incorrectly assigned to wrong infection status groups (uninfected classed as infected, or infected classed as uninfected) are arrowed.

Figure 7.12 The results of logistic regression analysis devised to determine the predictive power of the minnow model. The cumulative probability that individual fish will exhibit a particular residual Dorsal Profile Area (DPA) is plotted, with the predicted cut-off point shown by the dotted line. The residual DPA values of *Ligula intestinalis*-infected (●) and uninfected (O) minnows are shown. Individuals incorrectly assigned to wrong infection status groups (uninfected classed as infected, or infected classed as uninfected) are arrowed.


Cumulative probability that fish of a given residual DPA will be infected
1.0
0.8
0.6
0.4
0.2
0
-1
0
(0.55)
1
2
3
Residual DPA

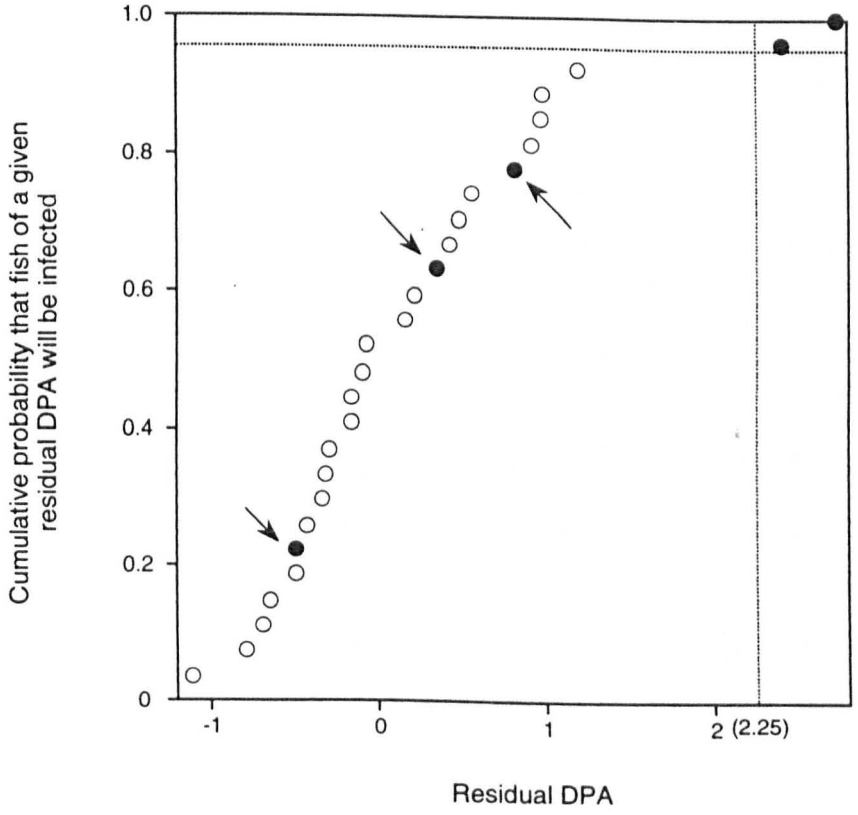
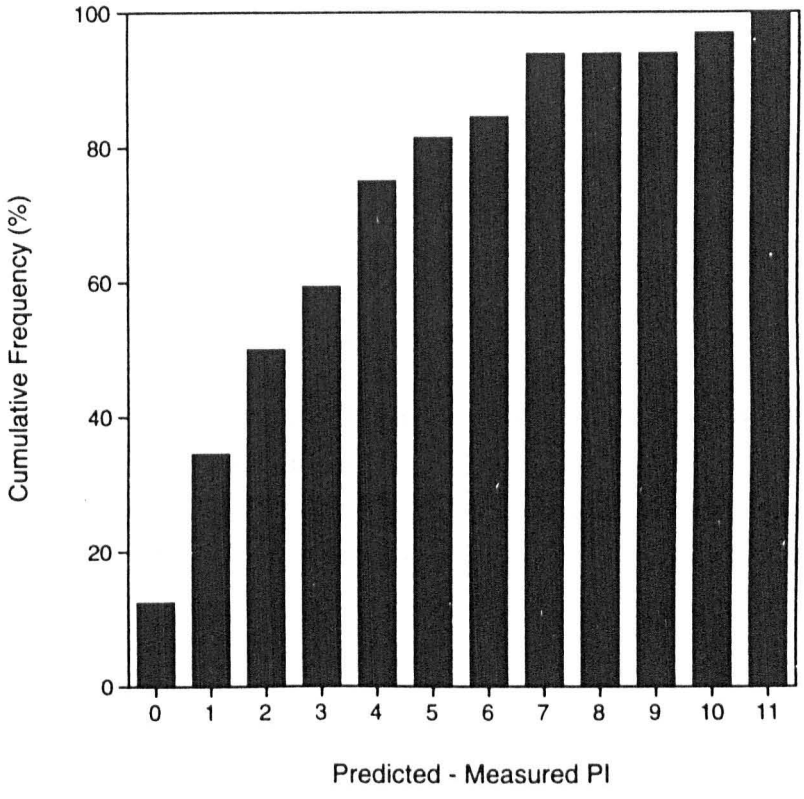

Cumulative probability that fish of a given residual DPA will be infected
1.0
0.8
0.6
0.4
0.2
0
-1
0
1
2 (2.25)
Residual DPA

Figure 7.13 Testing the accuracy of the stickleback model, I. The cumulative frequency of the differences between the Parasite Index (PI) predicted by the residual Dorsal Profile Area and that measured following dissection of each *Schistocephalus solidus*-infected stickleback, is shown (N=32).

Figure 7.14 Testing the accuracy of the minnow model, I. The cumulative frequency of the differences between the Parasite Index (PI) predicted by the residual Dorsal Profile Area and that measured following dissection of each *Ligula intestinalis*-infected minnow, is shown (N=5).


100
80
60
40
20
0
Cumulative Frequency (%)
0 1 2 3 4 5 6 7 8 9 10 11
Predicted - Measured PI

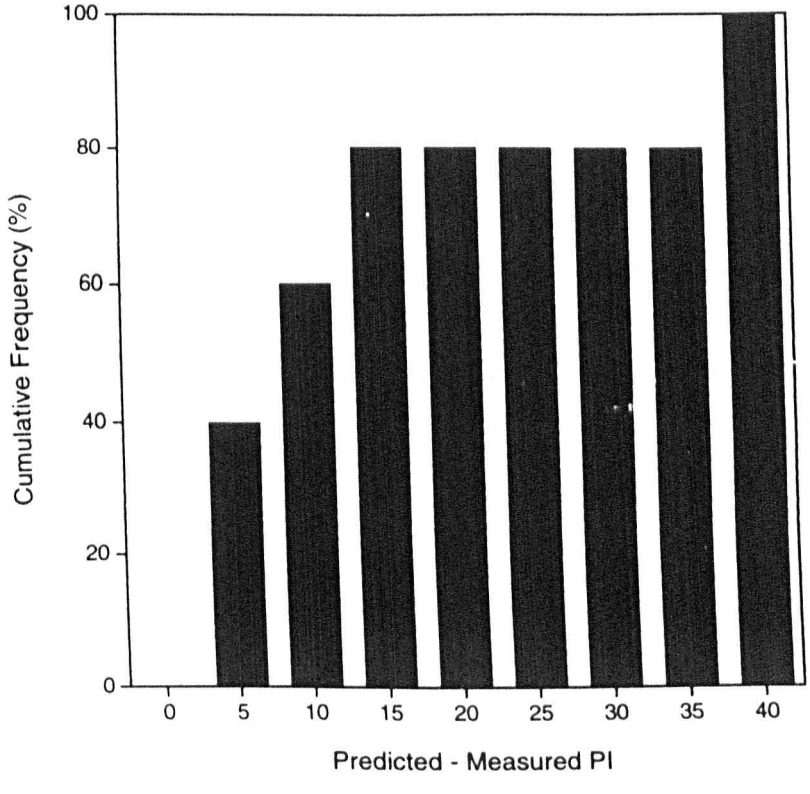
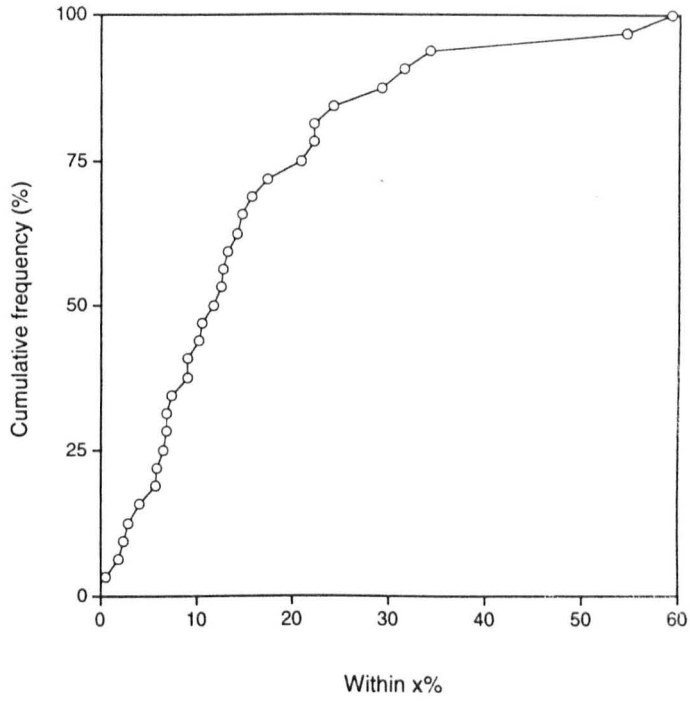
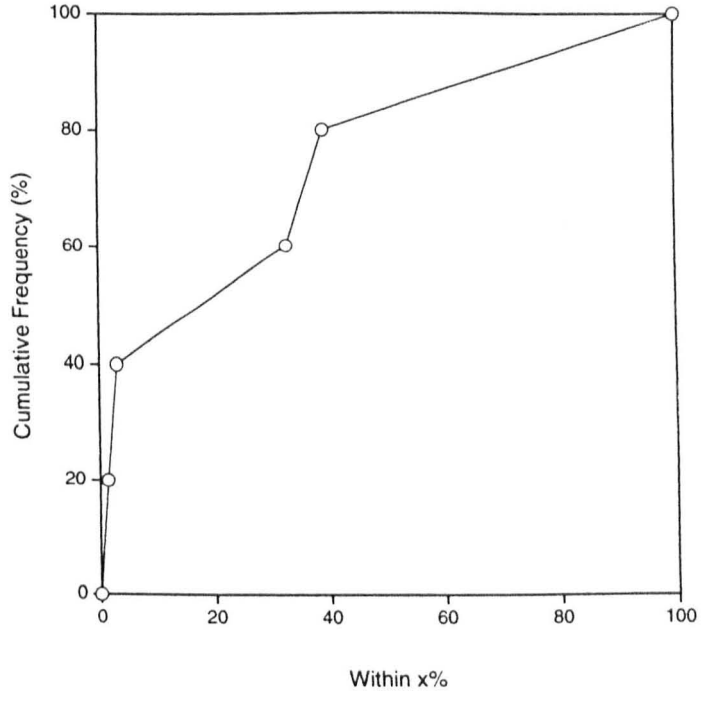

100
80
60
40
20
0
Cumulative Frequency (%)
0 5 10 15 20 25 30 35 40
Predicted - Measured PI

Figure 7.15 Testing the accuracy of the stickleback model, II. The cumulative frequency of the differences between the Parasite Index (PI) predicted by the residual Dorsal Profile Area and that measured following dissection of each *Schistocephalus solidus*-infected stickleback, expressed as a percentage of the measured value, is shown (N=32).

Figure 7.16 Testing the accuracy of the minnow model, II. The cumulative frequency of the differences between the Parasite Index (PI) predicted by the residual Dorsal Profile Area and that measured following dissection of each *Ligula intestinalis*-infected minnow, expressed as a percentage of the measured value, is shown (N=5).


100
75
50
25
0
Cumulative frequency (%)
0
10
20
30
40
50
60
Within x%


100
80
60
40
20
0
Cumulative Frequency (%)
0
20
40
60
80
100
Within x%

The results described above suggest that DPA is a viable way of determining whether a fish is infected or not, and for infected fish, to give a reasonably accurate estimate of the parasite load. Because the model developed for sticklebacks was based on morphometric measurements obtained from a larger number of uninfected and, in particular, infected fish, than the minnow model, it has more predictive value. Unfortunately, the minnow model was, of necessity, developed with data from only a very small number of infected fish (a consequence of an inability to locate a reliable supply of *L. intestinalis*-infected minnows), and so has little predictive use as it stands. However, it is interesting to note that even though it was developed with such a small sample size, the minnow model appears to be very similar in form to the stickleback model. It seems likely that with more data on infected minnows exhibiting a range of PIs, similar predictive accuracy to that offered by the stickleback model should be obtained.

There are several possible problems associated with the use of this model, including the fact that the body condition (i.e. 'fatness') of fish is known to vary seasonally on a cyclical basis, as gonads develop and are exhausted. Other temporal changes in the fat reserves of fish may be expected in those populations that live in habitats where food availability is seasonal (e.g. Tierney, 1991, 1994). Population differences may also pose a problem for successful transference of the model, since variation in body form (and hence, potentially the factor linking fork length and DPA) in freshwater stickleback populations is widespread, and frequently marked (Bell, 1976; Wootton, 1976; Bell & Foster, 1994). In developing the stickleback model, all fish were taken from the same population, on the same date, and exposed to *ad libitum* feeding in the laboratory for several weeks following capture before being photographed to minimise any natural variation in body condition, which may have been apparent when the fish were caught due to individual heterogeneity in foraging success. Because of these precautions, the effects of temporal variation, population differences and individual variation were effectively controlled. However, in order to make the model more transferable, both over time and between populations, certain modifications would need to be made.

7.4.3 Extracting further information regarding parasite infection

Once the PI of infected fish has been estimated using the model, the calculation of total parasite weight can be made. In many stickleback populations, parasitised fish are known to generally harbour a single plerocercoid (e.g. Tierney, 1991; LoBue & Bell, 1993). In such populations, or in fish

artificially infected with a single *S. solidus* procercoid under controlled conditions, knowledge of the total parasite weight will be sufficient to determine whether an infected fish is carrying an infective worm. For fish harbouring single, experimentally-induced infections, because the weight of both plerocercoid and host can be readily ascertained, the model also makes it theoretically possible to study the growth rates of infected fish and their parasites separately, potentially allowing dynamic studies into resource partitioning between parasite and host.

The models described above rely on the morphological changes that these particular parasites cause to their fish hosts by growing to such a massive size in the body cavity, and it seems that the usefulness of this approach is probably limited to hosts of *S. solidus* and *L. intestinalis* (or other proposed members of the *Ligula* genus) (see Kennedy, 1974, for a list of host species). Also, the technique may be limited to smaller species, or at least to smaller individuals of all species, since these are more likely to harbour infections with sufficiently high PIs to affect the DPA. Although the abdomens of small roach *Rutilus rutilus* infected with *L. intestinalis* are known to become noticeably distended, the same is not true of larger infected fish (D. Hoole, Keele University, U.K., personal communication).

7.4.5 Potential ecological effects of parasite-associated changes in DPA

Infection with the plerocercoid larvae of pseudophyllidean cestodes have been demonstrated in this study to increase the area presented by infected sticklebacks in dorsal profile by up to 40%. In addition, the normally-streamlined profile of the fish is transformed into a rather more 'bulky' shape. Any infected fish shoaling with a group of uniform, uninfected conspecifics would therefore be phenotypically-distinct when viewed from above. For many predators that aim to maximise foraging efficiency in terms of energy gained per unit time, or per unity energy expended, feeding on larger prey may be beneficial. This, linked to the fact that many predators are known to be more successful when attacking phenotypically-odd individuals in otherwise homogeneous groups, may lead to the infection-associated increase in DPA serving to make infected fish more susceptible to predation, and may be a reason why infected fish are more reluctant to join uninfected shoals than uninfected conspecifics (Chapter 4).

The increase in dorsal profile will be especially noticeable to those predators that attack from above, such as piscivorous birds. Since the parasites that have been shown to cause such morphological

modification are those that require transmission to avian definitive hosts in order to reach sexual maturity and reproduce, it may be hypothesised that the increased DPA exhibited by *L. intestinalis*- and *S. solidus*-infected fish might serve facilitate transmission of the parasite.

7.5 SUMMARY

- A simple photographic technique is described that allows the measurement of the dorsal profile area (DPA) of small freshwater fish.
- The measured DPAs of uninfected minnows and three spined sticklebacks were strongly correlated with individual fork length, but the regression lines describing the relationships between DPA and fork length for the two species differed significantly.
- Infection with the plerocercoids of *S. solidus* and *L. intestinalis* had significant effects on the relationship between DPA and fork length of sticklebacks and minnows respectively, with parasitised fish exhibiting significantly larger DPAs than length-matched uninfected conspecifics.
- A logistic regression technique is described that exploits the increased DPA of infected fish to allow accurate discrimination between uninfected and infected individuals, without the need for destructive sampling of fish.
- A predictive model that allows accurate determination the parasite index of infected fish, based on DPA measurement, is also described.
- The generality and transferability of the model, with particular reference to time of year, population and different host species-parasite systems, and possible changes to the model to account for such variation, are discussed.
- The potential ecological significance of the increased DPA of infected fish are discussed with reference to the predator-prey relationship of hosts, and the possible effects on parasite transmission.

Chapter 8. Ecological and histological studies of *Diplostomum phoxini* (Diplostomatidae: Trematoda) infection in minnows *Phoxinus phoxinus* from two Scottish populations.

8.1 INTRODUCTION

8.1.1 Factors governing the distribution and abundance of parasites in natural habitats

The distribution of parasites in natural environments is constrained primarily by the availability of susceptible hosts, the distributions of which are determined by many environmental and ecological factors (Krebs, 1985; Begon *et al*, 1990). For parasites with life cycles requiring transmission between several host species before sexual maturity can be reached, the availability of hosts restricts the types of habitats in which the life cycle can be maintained. In addition, the distribution of those parasites with life cycles involving free-living developmental or transmission stages may also be directly affected by the same local factors that govern the distribution of free-living organisms. Parasites therefore exhibit both spatial and temporal heterogeneity in abundance in the same way as do free-living organisms. Even when all of the necessary hosts are present, transmission between them is likely to be less effective if any of the host populations are in some way resistant to infection, or do not come into contact with each other.

8.1.2 Ecological terms descriptive of parasite infection characteristics

The ecological terms used by parasitologists to describe the extent of parasite infection within a particular population of a host species have been standardised and strictly defined by the American Society of Parasitologists (Margolis *et al*, 1982). The proportion of individuals within a population of a host species that are infected with a particular parasite species at any given time is described as the **prevalence** of the parasite. This parameter is calculated using the equation below, and is usually expressed as a percentage;

$$\text{Prevalence} = \frac{\text{No. of individuals infected}}{\text{No. of hosts examined}} * 100.$$

The number of individual parasites of a particular species harboured by any individual host within a population is defined by the term **intensity**, which may be determined directly (by counting individual parasites) or indirectly (e.g. by counting eggs passed with host faeces). The **mean intensity** of a particular population, which is equivalent to the mean number of individuals of a particular parasite species per infected host, is a term used commonly to indicate the 'typical' parasite load of individuals within a particular host population. However, since the distribution of parasites in host populations is

frequently overdispersed, with the majority of individual parasites being harboured by a disproportionately small number of hosts, this index is of limited heuristic value. A more useful concept is that of **median intensity**, which is a more robust statistic since it is less affected by the small number of heavily -infected individuals commonly found in overdispersed parasitic infections.

Both prevalence and mean (or median) intensity may be calculated for a population as a whole, or alternatively may be calculated individually for different sub-populations with respect to a variety of characteristics including sex, age class, size etc. By analysing the parasite status of sub-populations, important information may be gathered regarding the local epidemiology of the particular parasite infection (Anderson, 1993).

8.1.3 Inter- and intra-population variation in parasite infection

Variation in parasite intensity may occur at individual, sub-population and population levels, whereas variation in parasite prevalence is frequently evident between sub-populations or populations.

8.1.3.1 Intrapopulation variation

Differences in the parasite intensities exhibited by individuals within a parasitised population, or sub-population, are commonly observed in epidemiological studies and may arise from variation in exposure to infective parasite stages or from variation in the immune response shown towards infective parasites.

Frequently, the distribution of parasites within a host population is overdispersed, with the majority of individuals being concentrated in relatively few hosts (Schmidt & Roberts, 1989). Although overdispersion of microparasites is frequently due to localised reproduction of parasite stages within a host, such heterogeneity in the distribution of macroparasites is more likely to be caused by a variety of factors that include variability in host behaviour, host- or parasite-mediated spatial accumulation of infective stages or differences in the ability of individual hosts to mount effective immunological responses to parasite invasion (Anderson, 1993). An estimate of the degree of overdispersion is obtained by calculating the variance-to-mean ratio for the observed parasite intensities exhibited by all members of the population under study, or a sample thereof. Values exceeding unity are suggestive of the infection being overdispersed within the population. Typically, when the frequencies of observed

parasite intensities in an overdispersed infection are plotted as a histogram, the resultant distribution is best described by a negative binomial function (see Chapter 2, Figure 2.4).

8.1.3.2 Inter-population variation

Certain populations of a potential host species may not act as hosts for a particular parasite in certain environments, because they are either totally resistant to infection, or environmental conditions do not allow the other hosts of the parasite to exist. Within habitats where conditions allow a parasite life cycle to persist, both the prevalence and intensity exhibited by a particular parasite species within any of its host populations is governed, at least in part, by the rate of transmission between successive hosts (Anderson, 1993). The transmission rate is determined, in turn, by other ecological factors including the density of host populations, the behaviour of both the host and the parasite species involved, their spatio-temporal relationships and, in certain instances, climatic conditions.

For parasites that rely on predator-prey interactions for successful transmission, the frequency with which the life cycle is completed in any particular habitat (and hence the local abundance of the parasite in any of the host populations) is determined by the relative densities of definitive host and non definitive host predators, the prey selection behaviour of definitive host predators and the antipredator behaviour of infected and uninfected intermediate hosts. Many parasites transmitted in this way are thought to alter the behaviour of their intermediate hosts in ways that make them more susceptible to definitive host predators; it has been hypothesised that such deviant behaviour is maladaptive and may serve to enhance the chances of successful transmission and probably result in the parasite being more prevalent in the population than would normally be expected if transmission was purely random (see Moore & Gotelli (1990) for a critical review of the manipulation hypothesis). Evidently, the ability for parasites to influence host behaviour in either of these ways is dependent largely on the selection of particular sites within the host.

8.1.4 Site selection in parasites

The particular sites occupied by macroparasites for growth and development may have important consequences for the biology of their hosts (Crompton, 1973). Many parasites of fish and other vertebrates are localised in particular organs, tissues or body fluids (see Crompton, 1976, for a tabulated review), and frequently this affinity for a particular site is so strong that it can be used in the

identification of morphologically-similar parasite species of the same genus (Sukhdeho & Sukhdeho, 1994). Affinity for particular sites may be the result of specific environmental or nutritional requirements which can only be met in certain locations within the host, the result of interspecific competition and subsequent niche separation (Holmes, 1973; Holmes & Price, 1985), or an attempt by the parasite to evade the host's chemical and cellular immune defences (Crompton, 1973; Holmes & Price, 1985; Kennedy, 1985; Price, 1987; Sukhdeho & Mettrick, 1987). Some sites are understood to convey a reduced immune response to parasites: these sites are generally accepted to be the eye and the central nervous system (e.g. Cox, 1994), where the blood-tissue barrier is seldom breached. These immunologically-privileged sites should be attractive to helminth parasite stages that do not need a direct connection with the external environment for release of eggs, but the extent to which they are used varies across host phyla. Although helminths of non-fish vertebrates show no real predilection for these sites (their occasional presence being described as "fortuitous wandering" by O'Connor, 1976), in fishes such sites are frequently used, and Williams & Jones (1994) tabulate data for 22 helminth species commonly found within the brain and sense organs of fishes. Strigeid metacercariae are the most common metazoan parasites of the brain and nervous system of fishes (Williams & Jones, 1994). Such helminths do not attain sexual maturity until their host is consumed by a suitable definitive host, usually a piscivorous bird (Schmidt & Roberts, 1989).

The accumulation of parasites in the central or peripheral nervous system, or in sensory organs, may cause localised tissue physical damage which may, in turn, affect the sensory physiology or behaviour of their host. Direct parasite-mediated changes in host behaviour have been demonstrated experimentally for *Diplostomum spathaceum* metacercariae, which are found inside the lenses of the eyes of various species of freshwater fish (Kennedy, 1974; Chappell *et al*, 1994). The presence of the parasite causes damage to the lens, altering its optical properties and changing the foraging behaviour of infected individuals (Dace *Leuciscus leuciscus*: Crowden & Broom, 1962; three spined stickleback *Gasterosteus aculeatus*: Owen *et al*, 1993).

8.1.5 Biology of *Diplostomum phoxini*

Metacercariae of *Diplostomum phoxini* (Faust 1918) are common and widespread parasites of European minnows *Phoxinus phoxinus* in the U.K. (Kennedy, 1974) and are found in the brain and cranial cavity of infected fish (Ashworth & Bannerman, 1927; Rees, 1955; Bibby, 1972). Minnows are

the second intermediate host in the life cycle of *D. phoxini*, and become infected when cercariae released from parasitised lymneid snails penetrate the fishes' skin. The parasites are thought to migrate to the brain in the bloodstream, passing into the cerebral cavity via the choroid plexus (Erasmus, 1959) where they develop into infective metacercariae. These metacercariae may survive for five years or more (Dönges, 1969, cited in Ballabeni & Ward, 1993), and appear to accumulate throughout the fish's life. Within individual minnow populations, *D. phoxini* load is strongly and positively correlated with fish length, and larger individuals in certain populations frequently harbour heavy infections (Ashworth & Bannerman, 1927; Rees, 1955). Although an assortment of vertebrate species have been used in laboratory studies as definitive hosts, including young chickens *Gallus gallus* (Berrie, 1960), domestic ducks *Anas boschas domestica* (Rees, 1955; Bell & Hopkins, 1956; Berrie, 1960), herring gulls *Larus argentatus* (Berrie, 1960) and laboratory mice (Berrie, 1960), it is likely that the most important definitive hosts in natural habitats are piscivorous birds (Yamaguti, 1958). Following ingestion by a suitable definitive host, metacercariae undergo development and sexual maturation in the gut, producing eggs after 3-4 days (Bell & Hopkins, 1956).

8.1.6 Objectives

This chapter describes an investigation of the epidemiology of *D. phoxini* infection in minnows from two ecologically-distinct sites in central Scotland. In addition, data regarding the specific sites occupied by metacercariae in the brains of minnows from each location are described. Specifically, the objectives of this chapter are:

- To investigate the prevalence and intensity of *D. phoxini* metacercariae in minnows from populations at two ecologically-distinct sites.
- To examine the variation in the environmental conditions at the two habitats, and to ascertain whether this variation may account for any observed differences in the characteristics of infection at either site.
- To investigate, using histological and three-dimensional mapping techniques, the positions of individual metacercariae in the brains of minnows from both sites, in order to determine whether metacercariae aggregate in particular regions of the brain.

8.2 MATERIALS AND METHODS

8.2.1 Fish collection and husbandry

Minnows were hand- and trawl-netted from two sites in central Scotland: the River Endrick at Killearn, Stirling District and Loch Maragan in the central highlands (see Chapters 2 & 3 for site descriptions) and were immediately transferred to the laboratory, where they were maintained in aerated aquaria for several weeks to allow any newly-acquired *D. phoxini* to migrate to the brain and develop into infective metacercariae (Arvy & Buttner, 1954, 1955, cited in Berrie, 1960).

8.2.2 Determination of parasite intensity

Groups of fish were sacrificed by exposure to terminal dose of Benzocaine, weighed (wet weight: to 0.001g) and measured (fork length: to nearest 1mm). Following cranial dissection, the brain of each fish was removed and washed with distilled water to remove any metacercariae loosely attached to its surface. The cranial cavity was then flushed thoroughly with distilled water to displace any remaining flukes, which were counted together with those washed from the brain surface. The brain was then placed between two glass microscope slides and squashed gently, to reveal flukes in the brain by means of examination under 100x magnification. Each brain preparation was then examined at this magnification using a scanning technique, moving the microscope stage across the top edge of the preparation, then moving the stage down by one field of view and repeating the procedure until all of the brain tissue had been examined. Any *D. phoxini* metacercariae encountered were counted using a manual tally counter, and the total parasite intensity of each individual host was calculated by summing the numbers recovered from inside the cranium, from the outside of the brain and from inside the brain.

8.2.3 Histological techniques

8.2.3.1 Tissue collection and preparation

Four size-matched fish from each population were sacrificed by exposure to an overdose of Benzocaine anaesthetic, weighed and measured (mean fork length $_{Endrick}$ = 48.3 ± 1.5mm SD; mean fork length $_{Maragan}$ = 48.0 ± 1.5mm SD; T = 0.25, df = 3, P = 0.81). The brain of each fish was removed, as described above, and washed with distilled water to remove any metacercariae loosely attached to its surface. The cranial cavity was then flushed thoroughly with distilled water to displace any remaining flukes, which were counted together with those washed from the brain surface. Each brain was then

fixed in 5% buffered formalin solution and processed for light microscopy, using an automatic tissue processor (2L Processor Mk. II, Shandon Southern Products Ltd., Astmoor, Cheshire).

8.2.3.2 Sectioning and analysis

Following processing, brains were embedded in paraffin wax and oriented so that transverse (sagittal) sections could be cut. Serial sections of each brain (thickness : 7μm) were taken from the olfactory bulb (anterior) to the spinal cord (posterior) and stained using a standard haematoxylin and eosin technique (Bancroft & Stevens, 1990). The serial sections were then examined sequentially under the compound microscope for the presence of metacercariae, and the co-ordinates of each fluke were plotted on dorsal- and lateral-view maps of the minnow brain, to represent their three-dimensional positions diagrammatically.

8.3 RESULTS

8.3.1 The epidemiology of *D. phoxini* infection in the two populations

The overall prevalence of *D. phoxini* infection in minnows sampled from the two populations over the total sampling period (November 1992-October 1993) were 100% for fish from the River Endrick (n=78) and 97.8% for fish from Loch Maragan (n=139). All size classes in each sample exhibited 100% prevalence, except the smallest size class (18-29mm) of fish from Loch Maragan, which included a small number of uninfected individuals in two of the samples (see Table 8.1).

Significant, positive relationships were found to exist between individual fork length and the intensity of infection with *D. phoxini* metacercariae for minnows from both of the populations sampled (see Figure 8.1). Regression equations describing the relationships between fork length and *D. phoxini* intensity for minnows from either site were as follows;

River Endrick

$$\text{Intensity} = 6.92 * [\text{Fork length (in mm)}] - 97.8 \qquad (r^2 = 0.552, p < 0.001)$$

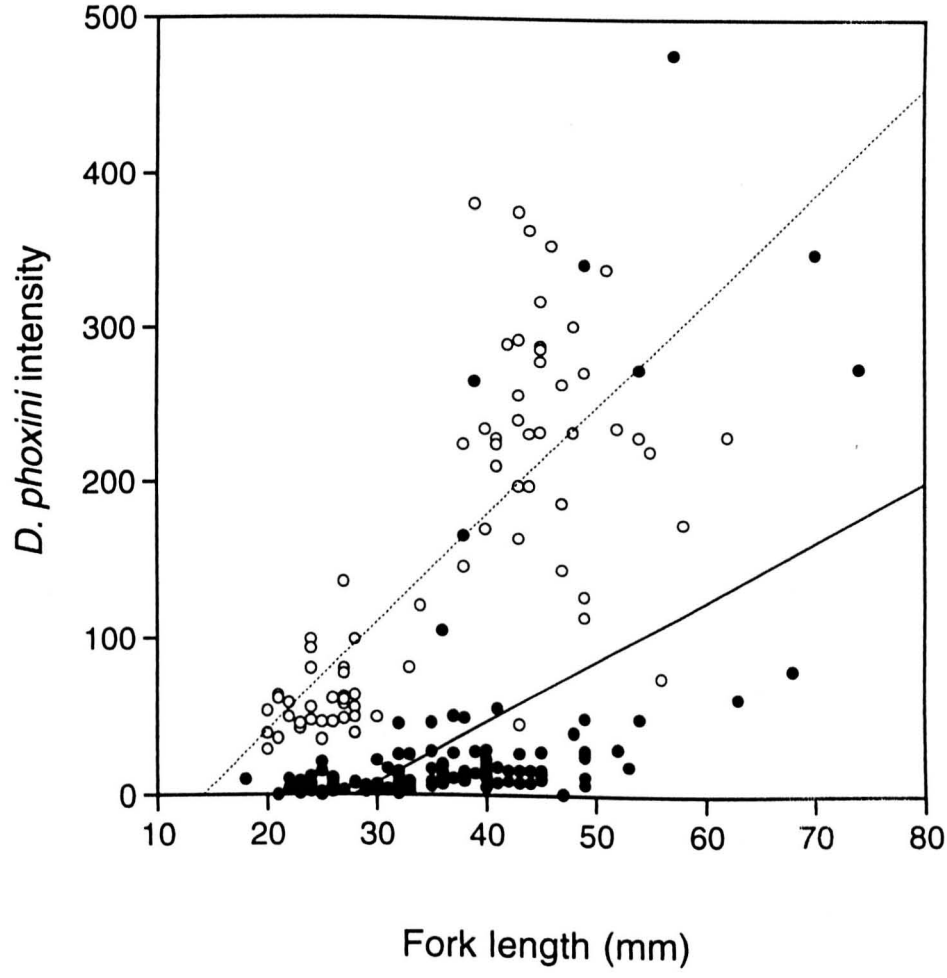
Loch Maragan

$$\text{Intensity} = 3.85 * [\text{Fork length (in mm)}] - 107 \qquad (r^2 = 0.283, p < 0.001)$$

Table 8.1 The monthly prevalence of *Diplostomum phoxini* infection in samples of different size classes of minnows taken from the River Endrick and from Loch Maragan.

Location	Size class	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Total
River Endrick	18-29mm	4/4 100%	-	-	-	-	-	18/18 100%	-	-	13/13 100%	-	-	35/35 100%
	30-41mm	5/5 100%	-	-	-	-	-	4/4 100%	-	-	2/2 100%	-	-	11/11 100%
	42-53mm	9/9 100%	-	-	-	-	-	18/18 100%	-	-	-	-	-	27/27 100%
	54-65mm	4/4 100%	-	-	-		-	1/1 100%	-	-	-	-	-	5/5 100%
	Total	**22/22** **100%**	-	-	-	-	-	**41/41** **100%**	-	-	**15/15** **100%**	-	-	**78/78** **100%**
Loch Maragan	18-29mm	-	-	-	-	-	11/13 84.6%	7/7 100%	-	-	3/3 100%	5/6 83.3%	-	26/29 89.7%
	30-41mm	-	-	-	-	-	-	-	38/38 100%	-	20/20 100%	1/1 100%	-	59/59 100%
	42-53mm	-	-	-	-	2/2 100%	3/3 100%	1/1 100%	11/11 100%		3/3 100%	-	2/2 100%	22/22 100%
	54-65mm	-	-	-	-	1/1 100%	-	-	3/3 100%	-	-	-	-	4/4 100%
	66-77mm	-	-	-	-	3/3 100%	-	-	-	-	-	-	-	3/3 100%
	Total	**22/22** **100%**	-	-	-	**6/6** **100%**	**14/16** **87.5%**	**8/8** **100%**	**52/52** **100%**	-	**26/26** **100%**	**6/7** **85.7%**	**2/2** **100%**	**136/139** **97.8%**

Figure 8.1 The relationships between individual fork length and the intensity of *Diplostomum phoxini* infection (measured as the number of metacercariae per host) in minnows sampled from populations in the River Endrick at Drumtian Ford (O) and at Loch Maragan (●) (see text for regression equations).


500
400
300
200
100
0
10
20
30
40
50
60
70
80
D. phoxini intensity
Fork length (mm)

Analysis of covariance showed that the slopes of the two regression lines differed significantly (ANCOVA $F_{1,191} = 11.89$, $P = 0.001$). Assuming that the growth rate of minnows in the River Endrick is at least equal to that of conspecifics from Loch Maragan (see Discussion), this strongly suggests that Endrick fish acquire *D. phoxini* metacercariae at a faster rate than Maragan fish.

When intensity of *D. phoxini* infection was analysed by size class, minnows from the River Endrick were found to harbour more metacercariae than conspecifics from Loch Maragan for the majority of size classes for which sufficient data were available for statistical analysis (see Table 8.2; Figure 8.2).

The distribution of *D. phoxini* metacercariae was found to be overdispersed in both of the minnow populations studied in this investigation, with the majority of metacercariae being harboured by relatively few hosts (Figure 8.3). However, population differences in the variance-to-mean ratios were observed, suggesting that differences existed in the degree of overdispersion exhibited by minnows at either site (River Endrick variance : mean = 71.49, Loch Maragan variance : mean = 163.42).

Table 8.2 Summary table of parasite intensities by size class, and results of Wilcoxon-Mann-Whitney Tests to examine differences between size-matched minnows from the two study populations.

Size Class	River Endrick		Loch Maragan		Wilcoxon-Mann-Whitney Test	
	n	median	n	median	W	P
18-23mm	10	47	9	4	145.0	0.0003
24-29mm	25	61	20	6.5	825.0	<0.0001
30-35mm	3	81	27	8	87.0	0.0057
36-41mm	8	225	32	16	284.0	0.0001
42-47mm	20	260.5	12	15	450.0	<0.0001
48-53mm	7	236	10	28	91.0	0.0073
54-59mm	4	197.5	3	274	14.0	0.5959
60-65mm	1	231	1	62	†	†
66-71mm	0	-	2	214	†	†
72-77mm	0	-	1	275	†	†

† denotes categories where insufficient data are available for statistical analysis

Figure 8.2 The intensity of *Diplostomum phoxini* infection (measured as number of metacercariae per host) in 10 size classes of minnows sampled from populations in the River Endrick at Drumtian Ford (□) and from Loch Maragan (■). Bar heights represent median values, with error bars showing interquartile ranges. Figures above each bar are sample sizes, and significance values refer to the results of Wilcoxon-Mann-Whitney tests (see Table 8.2 for details). † denotes categories where insufficient data are available for statistical analysis.

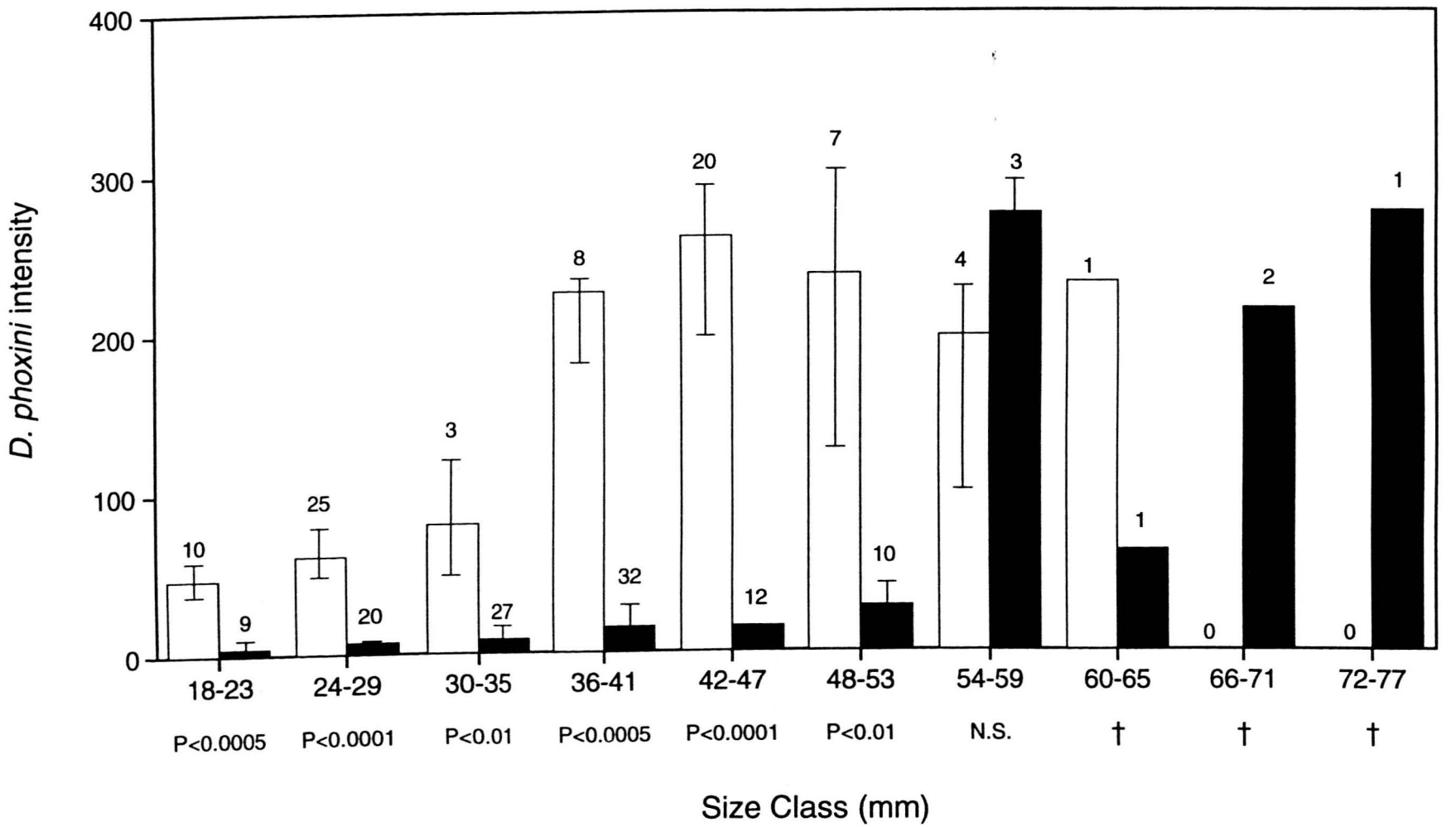
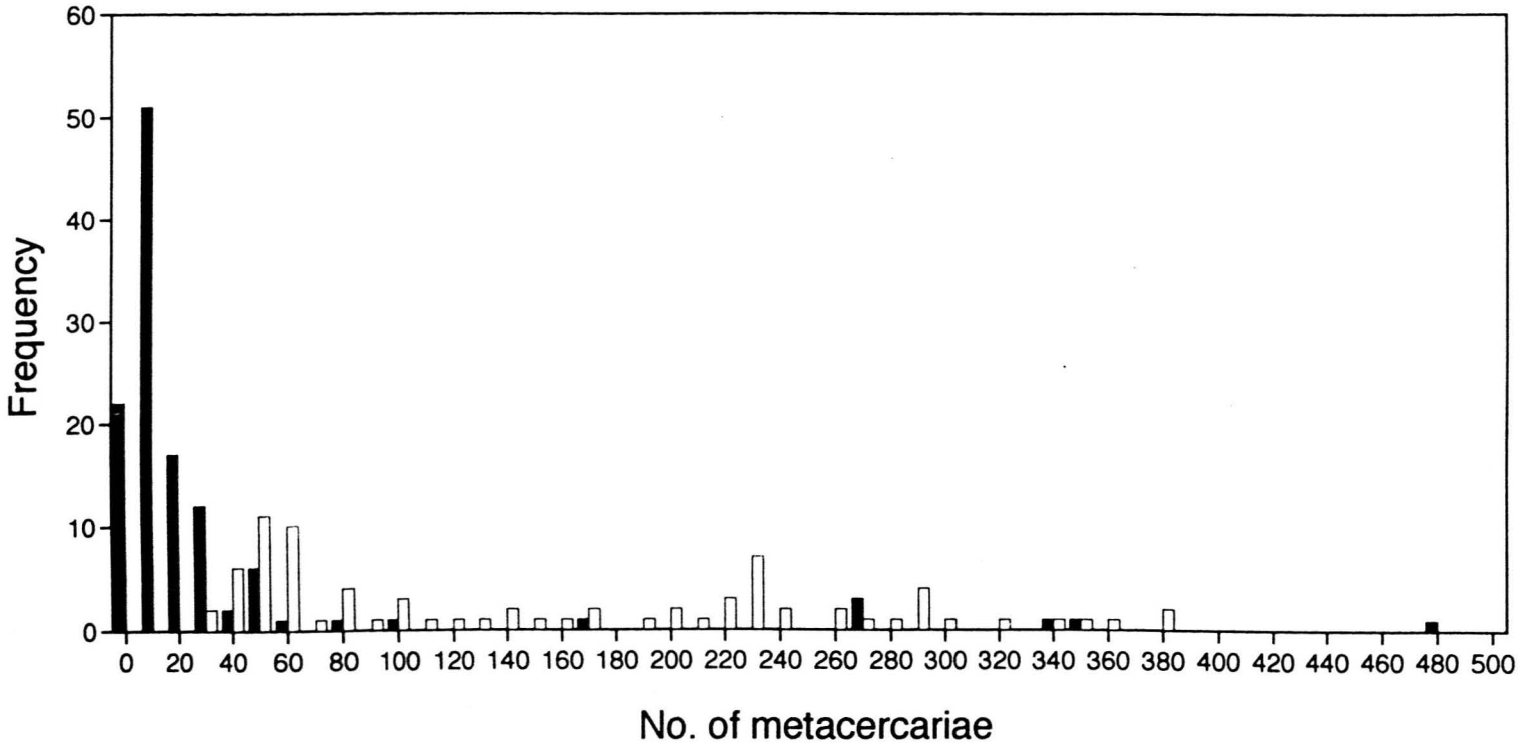

D. phoxini intensity
400
300
200
100
0
10
9
25
20
3
27
8
32
20
12
7
10
4
3
1
1
0
2
0
1
18-23
24-29
30-35
36-41
42-47
48-53
54-59
60-65
66-71
72-77
P<0.0005
P<0.0001
P<0.01
P<0.0005
P<0.0001
P<0.01
N.S.
†
†
†
Size Class (mm)

Figure 8.3 The frequency distribution of numbers of metacercariae of *Diplostomum phoxini* in minnows from Loch Maragan (■) and the River Endrick (□). The parasite is overdispersed at each site (see text for details).


Frequency
60
50
40
30
20
10
0
0 20 40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400 420 440 460 480 500
No. of metacercariae

8.3.2 The sites occupied by *D. phoxini* metacercariae

The number of metacercariae counted in the brain and neurocranium of the size-matched minnows selected for the histological studies from the two sites also differed, with fish from the River Endrick harbouring more metacercariae than those from Loch Maragan (Wilcoxon-Mann-Whitney test, Endrick median intensity = 220, Maragan median intensity = 21, $W = 18.0$, $P<0.05$).

Figure 8.4 shows the various compartments of the brain of the minnow. Graphical representations of the three-dimensional distribution of *D. phoxini* metacercariae within the brains of minnows sampled from the River Endrick and Loch Maragan populations are shown in Figures 8.6 and 8.7 respectively. Despite the differences in the metacercarial intensity of fish from either population, no significant differences were found in the distribution of metacercariae between the different regions of the brain (see Figure 8.7 and Table 8.3; results of Wilcoxon-Mann-Whitney tests, carried out on percentage data are given in Table 8.4). The most frequently-occupied sites included the medulla oblongata, the cavity between the superior lobe of the cerebellum and the anterior part of the medulla oblongata (the cerebellar cavity), the cavity between the optic tecta and the cerebellum (the optic vesicles) and the superior lobe of the cerebellum itself; these sites harboured 98% of all metacercariae counted within the brains of infected minnows.

Only in the heavily-infected fish from the River Endrick were a small number of metacercariae found in the anterior part of the spinal cord and the olfactory lobes, suggesting that these regions might only be used when the more frequently-utilised parts of the brain are crowded. The inferior lobe of the cerebellum, pituitary, optic lobes and olfactory bulbs were largely free of parasites. On average, 25.9 % of the metacercariae recorded from River Endrick minnows, and 26.5 % of those from Loch Maragan fish were dislodged by washing when the brain was removed from the neurocranium, though it is not clear what proportion of these were originally loosely attached to the outside of the brain, and what proportion were free in the cerebrospinal fluid or attached to the inside of the cranium.

8.4 DISCUSSION

8.4.1 Variation in the infection dynamics of *D. phoxini* in the two minnow populations : possible causes In the present study, as well as in those of previous workers (Ashworth & Bannerman, 1927, Rees, 1955; Berrie, 1960; Bibby, 1972), strong correlations have been found between fish size and the intensity of *D. phoxini* infection within each specific population. This in itself in not surprising, since

Figure 8.7 The sites occupied by *Diplostomum phoxini* in the brains of minnows *Phoxinus phoxinus* from populations at Loch Maragan (■) and the River Endrick (□).

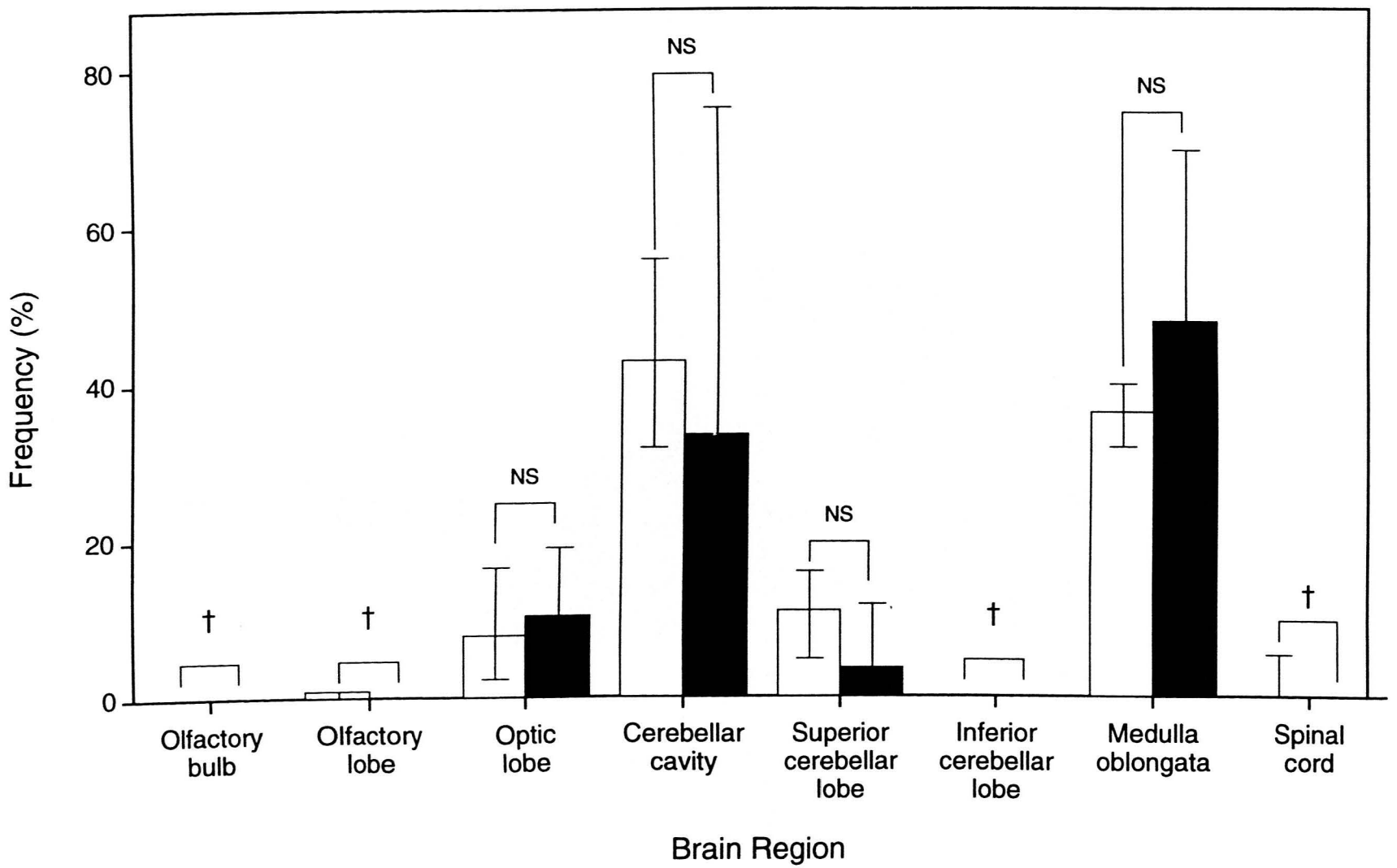

Frequency (%)
80
60
40
20
0
NS
NS
NS
NS
†
†
†
†
Olfactory bulb
Olfactory lobe
Optic lobe
Cerebellar cavity
Superior cerebellar lobe
Inferior cerebellar lobe
Medulla oblongata
Spinal cord
Brain Region

Figure 8.4 A representation of the brain of the minnow, showing the various compartments referred to in the text. ob. olfactory bulb; ol. olfactory lobe; opl, optic lobe; cc, cerebellar cavity; scl, superior cerebral lobe; icl, inferior cerebral lobe; m, medulla; sc, spinal cord. Scale bar represents 1mm.

sc
m
scl
c
orl
ol
ob

Figure 8.5 The positions of *Diplostomum phoxini* metacercariae in the brains of three minnows from the River Endrick at Drumtian Ford, analysed by serial histological sectioning techniques. The location of each metacercaria is shown in two planes (lateral elevation and dorsal elevation) to give an impression of the three-dimensional distribution of the parasites within the brain.

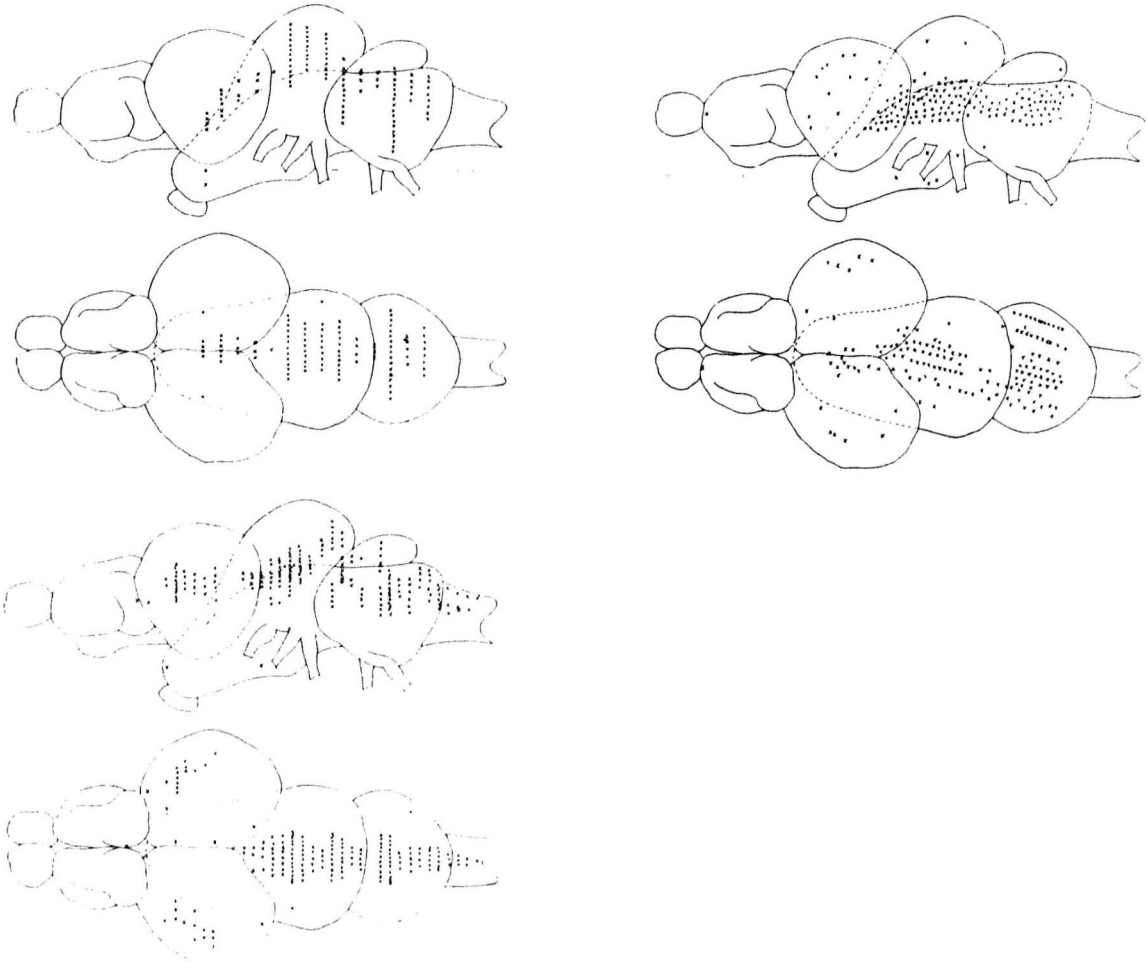


Figure 8.6 The positions of *Diplostomum phoxini* metacercariae in the brains of four minnows from Loch Maragan, analysed by serial histological sectioning techniques. The location of each metacercaria is shown in two planes (lateral elevation and dorsal elevation) to give an impression of the three-dimensional distribution of the parasites within the brain.

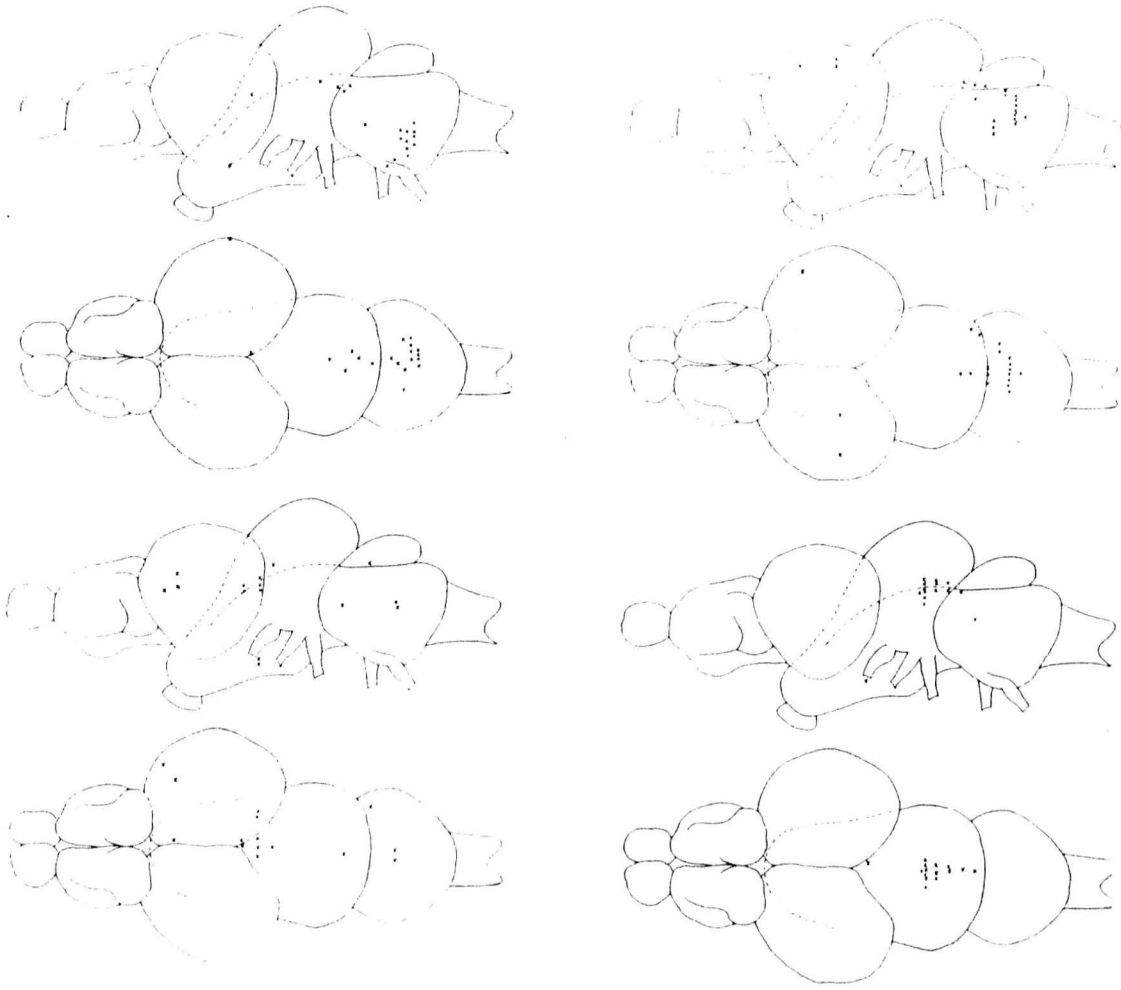


Table 8.4 Results of Wilcoxon-Mann-Whitney tests calculated to test between the preferred accumulation sites of *Diplostomum phoxini* in the brains of minnows *Phoxinus phoxinus* from two sites in Scotland.

Brain Region	Endrick median (n=3)	Maragan median (n=4)	W	P
Olfactory bulb	0	0	†	†
Olfactory lobe	0	0	†	†
Optic lobes	7.7	10.1	12.0	1.00, N.S.
Cerebellar cavity	42.6	33.2	14.0	0.60, N.S.
Superior cerebral lobe	10.7	3.4	15.0	0.37, N.S.
Inferior cerebral lobe	0	0	†	†
Medulla oblongata	36.0	47.6	12.0	1.00, N.S.
Spinal cord	0	0	†	†

† unable to compute Mann-Whitney tests due to all values being equal

Table 8.5 A summary of the functions of the major compartments of the teleost brain, indicating those that were observed to be regularly invaded by *Diplostomum phoxini* metacercariae in the present study.

Brain region	Function	*D. phoxini* invasion
Prosencephalon		
Olfactory bulb	Primary olfactory centres	no
Olfactory lobe	Secondary olfactory centre + non-olfactory functions including courtship behaviour, learning and appetitive behaviour	no
Mesencephalon		
Optic lobes	Integration of vision	no
Rhombencephalon		
Superior lobe of cerebellum	Postural control and autonomic regulation. Alerting mechanisms	yes
Inferior lobe of cerebellum	Homeostatic and appetitive behaviour	no
Medulla oblongata	Somatic & visceral sensory and motor areas	yes
Facial lobe	External taste and touch	no
Vagal lobes	Oropharangeal taste and touch sensitivity	no

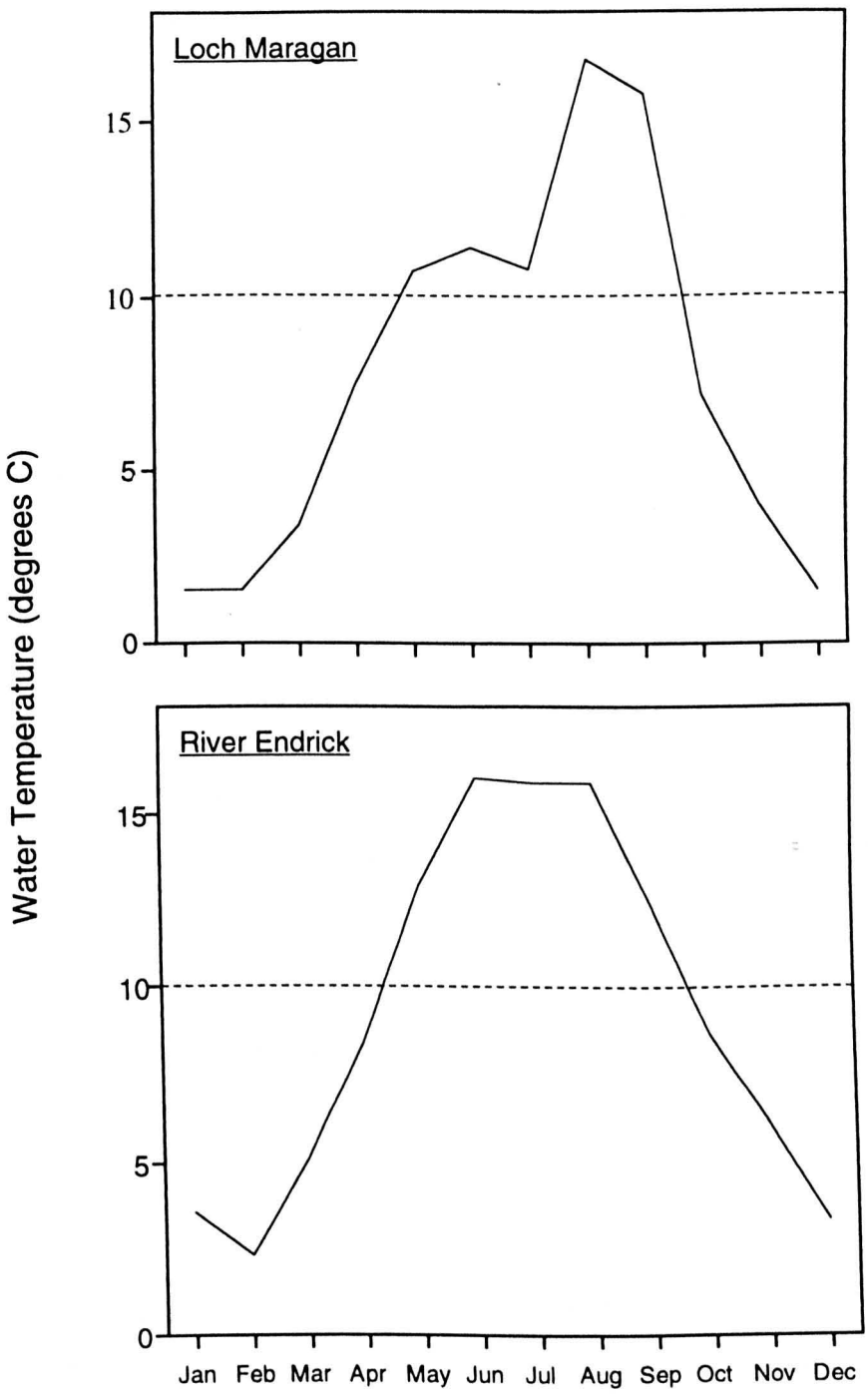
the parasites are known to be acquired continuously throughout the life of the minnow, and because metacercariae are known to survive for at least five years within the brains of infected fish and would therefore effectively never be lost during their lifetime. The rates of metacercarial acquisition, the median intensity and the distribution of *D. phoxini* in the two minnow populations under study, however, were found to differ greatly, and possible causes of this variation are suggested below.

8.4.1.1 Climatic factors

The rate at which diplostomatid metacercariae accumulate in the brains of minnows is likely to be affected by a multitude of ecological factors. Active transmission of *Diplostomum* spp. parasites to both snails and fish is temperature dependent, with a lower limit of around 10°C (Wootten, 1974; Stables & Chappell, 1986). The amount of time water temperature is above this threshold level for infection will determine the length of the period during which fish are be exposed to infection. In addition, there may also be a correlation between actual water temperature and the rate of diplostomatid acquisition, as Höglund & Thulin (1990) have demonstrated that the rate of accumulation of *Diplostomum* spp. metacercariae in perch *Perca fluviatilis* in an artificially-heated lake far exceeded that in an unheated control lake.

The monthly changes in the water temperature of Loch Maragan are compared with those of the River Endrick at Drumtian Ford (Figure 8.8). During the spring, water temperatures rise more quickly at the River Endrick site, which is 40m above sea level, than at Loch Maragan, which is at an altitude of 472m, and the 10°C threshold level for *Diplostomum* spp. infection is reached correspondingly earlier. Water temperature appears to decline at similar rates at the two sites after summer, and the 10°C threshold is crossed at approximately the same time. The >10°C infection period appears to last slightly longer in the River Endrick. In addition, the post-winter water temperature increase at Loch Maragan appears to stall during early summer, and did not peak until August, whereas temperatures in the Endrick peaked in June, and remained high in July and August. The number of 'degree-days' in the Endrick therefore far exceeds that at Loch Maragan, and if infection rate is closely linked to water temperature, as has been suggested, then this may provide an explanation for some of the variance in prevalence and intensity levels at the two sites

Figure 8.8 The monthly changes in water temperature at the two sites where minnow populations were sampled. a) The water temperatures at Loch Maragan, measured 10cm below the water surface in the shallow part of the loch were minnow population was sampled. b) The water temperatures in the River Endrick at Drumtian Ford (from data in Maitland, 1966).


Loch Maragan
15
10
5
0
River Endrick
15
10
5
0
Water Temperature (degrees C)
Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec

8.4.1.2 Physico-chemical factors

The distribution of freshwater molluscs, including lymneid snails (the first intermediate host of *D. phoxini*), is largely governed by water chemistry, and the most important predictor of species diversity and abundance is the availability of calcium ions (Russell-Hunter, 1964). The River Endrick at Drumtian Ford is very base-rich, with a pH of 7.6 and an associated alkalinity of 41mg $CaCO_3.l^{-1}$ (Maitland, 1966; Doughty & Maitland, 1994), whereas Loch Maragan is more acidic, with a pH of 6.4 and an alkalinity of just 0.12meq.l^{-1} (Lassière, 1989), equivalent to 6mg $CaCO_3.l^{-1}$ (C. Adams, University of Glasgow, U.K., personal communication), and it seems that differences in water chemistry may be responsible for the differential abundances of snails at either site (personal observations), since the relatively soft water found at Loch Maragan is likely to be less conducive to successful growth and reproduction of the snail when compared to the situation in the River Endrick, and the reduced availability of snail hosts may be a major factor limiting the success of the parasites life cycle at Loch Maragan.

8.4.1.3 Ecological factors

Diplostomum phoxini only achieves maturity naturally in the gut of a piscivorous bird. The frequency with which avian predators ingest infected minnows therefore limits the frequency with which the parasite life cycle is completed, and this will determine the temporal and spatial density of eggs and infective miracidia present in the water and, to a large extent, control the prevalence and intensity of the parasite within the local snail population.

Avian predation on the minnow population at Loch Maragan is thought to be limited, due to its geographical position and to its altitude (see Chapter 2), whereas piscivorous birds are known to be abundant at the River Endrick (Maitland, 1966; Huntingford *et al*, 1994b and personal observations), and are significant predators of small fish at this site (Giles, 1981). It would appear that the opportunity for completion of the parasite life cycle would be higher at the River Endrick than at Loch Maragan, and these differences in the predator regime at either site may be responsible for some of the observed variation in the infection dynamics at the two sites.

Although only two sites are being compared in this study, and these proposed hypotheses require verification (probably by the use of a large study of infection in a wide range of sites) it seems

clear that environmental differences have the potential to contribute significantly to the observed variation in the dynamics and the epidemiology of diplostomatid infection.

8.4.2 Site selection in diplostomatid metacercariae: an adaptation for transmission ?

Parasites may have either direct or indirect effects on host behaviour; direct effects include the changes in appearance caused by certain cestodes on the morphology of fish (see Chapter 7), and the manipulation of neurotransmitter levels, or damage to neural tissue (reviewed by Holmes & Zohar, 1990), whereas indirect effects include the manipulation of host behaviour by altering its internal state, such as raising hunger levels (see Chapter 4, and review by Milinski, 1990). Clearly, an important determinant of how a parasite might alter host behaviour is the particular site that it occupies within a host.

The occupancy of sites within the brain potentially allows parasites to have direct effects on the behaviour of their host. In experimental trials, North American fathead minnows *Pimephales promelas* infected with the brain-encysted metacercariae of *Ornithodiplostomum ptychocheilus* were shown to form less compact shoals than uninfected conspecifics (Radabaugh, 1980); presumably the parasites affect either the sensory systems or motor control of infected fish. Fish infected with *Myxobolus cerebralis*, a protozoan parasite found in the cranium of certain salmonids, and the causative agent of 'whirling disease', display erratic circular swimming behaviour at the water surface as the growing cysts expand and put physical pressure on the nervous system. Rees (1955) observed similar disorientation and aberrant swimming behaviour in a small number of minnows infected with *D. phoxini* in her laboratory stock tanks. On dissection these fish were found to contain up to 1300 metacercariae in the brain and neurocranium, whereas a sample of the normally-behaving fish were all found to contain fewer than 600 metacercariae. This is suggestive of some intensity-dependent effect of the parasites on host behaviour.

If *D. phoxini* does have such an intensity-dependent effect on host behaviour, then the highly divergent *D. phoxini* intensities exhibited by the two minnow populations examined in the present study might have been expected to show associated population-level differences in behaviour. However, even though differences in the shoaling and exploratory behaviour of fish from the two populations concerned have been discovered (Chapter 3), it is impossible to say whether metacercarial load had an effect, or whether the observed behavioural variability was a result of differences in the evolutionary

and development of behaviour in minnows from the two populations. None of the fish examined from either population have been found to harbour so many metacercariae as those exhibiting the aberrant behaviour described by Rees (1955), and it seems plausible that the parasites have no significant effect on host behaviour until they reach a critical intensity.

Although specific behavioural tests have rarely been carried out, studies of aspects of infection with brain-inhabiting parasites have often not recorded any aberrant behaviour of parasitised fish. Hoffman & Hoyme (1957, 1958), examining the histopathology of *Diplostomum baeri eucaliae* in the brains of brook sticklebacks *Eucalia inconstans* noted no impairment in the reflexes of fish exposed to sub-lethal doses of the parasite. Mitchell *et al* (1985) reported no obvious detrimental effects of the protozoan parasite *Myxobolus hendricksoni* on the behaviour of infected *Pimephales promelas*, even in heavy infections where large parts of the brain had been replaced by parasite tissue.

Individual metacercariae would appear to be ineffective at bringing about a change in the nervous control of behaviour, since they are small and readily encapsulated by host tissue. However, in heavy infections strigeid metacercariae cause proliferation of host tissue in the regions in which they aggregate, and have a significant pathological effect on the nervous system (Heckman, 1992). In the present study, and in other investigations (Ashworth & Bannerman, 1927; Rees, 1955; Heckman, 1992), diplostomatid metacercariae have been found to accumulate in regions of the brain associated with sensory function and motor control (see Table 8.5). Accumulation in these specific regions of the brain may be a means by which, once enough individuals have assembled, the behaviour of infected fish might be altered. It may therefore benefit parasites to aggregate in those regions of the brain that, once damaged, would be most likely to have a detrimental effect on the anti-predator behaviour of their host. The fact that *D. phoxini* metacercariae have been shown to aggregate in specific regions of the brain concerned with sensory systems and motor control would appear to support this hypothesis.

8.5 SUMMARY

- The epidemiology of *D. phoxini* infection in minnows from two ecologically-dissimilar sites in central Scotland (a lowland river, the River Endrick, and a highland loch, Loch Maragan) was investigated. In addition, histological techniques were used to study the distribution of *D. phoxini* metacercariae within the brains and neurocraniums of individual minnows from the two populations.

- *D. phoxini* prevalence approached 100% in minnow populations from both the River Endrick and Loch Maragan. Distribution of the parasite was overdispersed in both fish populations.
- Although highly significant relationships between fork length ($\propto$ age) and *D. phoxini* intensity were exhibited by minnows sampled from both sites, fish from the River Endrick site acquired flukes at a much quicker rate than those from Loch Maragan.
- The most common sites of accumulation to be the cerebellar cavity, the optic lobes and the medulla oblongata, with sites of secondary importance being the superior lobe of the cerebellum and the anterior part of the spinal cord. The inferior lobe of the cerebellum, the pituitary, the optical and olfactory lobes and the olfactory bulbs were largely free of parasites. A proportion of metacercariae were found either free in the cerebrospinal fluid, or loosely attached to the brain or the inside of the cranium.
- Possible explanations for the observed variation in epidemiology of the parasite in the two minnow populations are discussed with reference to the environmental and ecological conditions at the two sites.
- A hypothesis that could potentially explain the apparent accumulation of *D. phoxini* metacercariae in specific regions of the brain is presented.

Chapter 9. General discussion

Parasite infection : cause or consequence of deviant host behaviour ?

Care has been taken throughout this account to avoid the temptation of assuming the existence of causal relationships between parasite infection and the observed behavioural changes of the hosts. Correlational or assocational evidence such as that presented here is generally insufficient to prove that the one is dependent on the other. There always remains, for instance, the possibility that fish exhibiting deviant behaviour may be predisposed to parasite infection as a result of correlations between the particular behaviour and factors that determine the probability of becoming infected (e.g. either exposure to infective parasite stages, or effectiveness of the immune response to them). Should this be the case, then deviant behaviour would be a determinant, rather than a consequence, of parasite infection. For example, variability in behavioural patterns is known to be an important predictor of differential exposure to infection, and consequently differential parasite loads, in humans (see review by Bundy & Blumenthal, 1990).

However, circumstantial evidence suggests that infection is more likely to play a causal role in altered host behaviour than *vice versa*. Tierney *et al.* (1993) demonstrated that altered (apparently risk-prone) behaviour in *Schistocephalus solidus*-infected sticklebacks *Gasterosteus aculeatus* was only observed in those fish that harboured infective plerocercoids; those fish harbouring non-infective worms behaved as uninfected fish in the tests. Similar findings are presented by Bethel & Holmes (1990) for the altered behaviour of *Polymorphus paradoxus*-infected *Gammarus lacustris* (Crustacea). Although both investigations examined only aspects of antipredator behaviour, such studies show that the behaviour of parasitised hosts is correlated with the ontogeny of the parasites they harbour, and so they demonstrate that parasites have the ability to modify host behaviour.

In the light of the undoubted effects of the parasites under investigation on the morphology and physiology of infected hosts, it seems intuitive, and it is the author's opinion, that cestode infection in this case is more likely to be a cause of the observed altered behaviour than a result of it, or merely the result of a fortuitous correlation. Such possibilities, however, are difficult to rule out using an experimental approach.

Ecological consequences of the behaviour of parasitised fish

In experiments described in this study, *S. solidus*-infected sticklebacks have been shown to exhibit a reduced shoaling tendency and a reduced ability to compete for food when compared with

uninfected conspecifics, whereas *Ligula intestinalis*-infected minnows *Phoxinus phoxinus* exhibited a reduced ability to occupy close positions to neighbours in polarised schools, and were found on the periphery of these schools more often than expected by chance. In addition, both parasites have significant effects on host morphology, quantified for the first time in this study. All of these aspects of helminth infection potentially have important consequences for the ecology of parasitised hosts.

The formation of shoals and schools is known to be an adaptive response of individuals of small prey fish species in freshwater habitats to the combined pressures of predation avoidance and efficient foraging, and group members benefit through enhanced food-finding and improved antipredator responses. They also suffer associated costs, the most important of which are increased visibility to certain predators, and increased competition for food items. Clearly, the food availability and predation regimes in any particular habitat will determine the relative importance of these costs and benefits, and thereby probably determine the extent to which fish form shoals and schools. For species such as sticklebacks that have other forms of predator defence, shoaling tendency is less strong, and individuals appear to leave the shoal more readily to forage alone, than in strongly shoaling and schooling species. In natural environments, the effect of *S. solidus* of further reducing the shoaling tendency of their hosts would theoretically lead to infected fish being less often members of shoals or schools, and more often existing as 'loners' than uninfected conspecifics. This may allow them to increase their food intake, since they would not suffer through their reduced ability to compete for food, but probably also has consequences for predator-prey relationships. Lone individuals are known to be captured by certain predators with more success than shoaling fish, and *S. solidus* sticklebacks that do not join conspecific groups may be more readily-available to these predators in natural habitats. By avoiding the shoal, however, infected fish may escape detection by other types of predators that may be more likely to spot aggregated prey than single individuals. In addition, the demonstrated effects on the morphology of infected fish probably make them more visible to some predators than to others.

Minnows exhibit a stronger tendency than sticklebacks to join conspecific groups, and are probably more reliant on shoal and school formation for antipredator defence. Although *L. intestinalis*-infected minnows were rarely observed as non-schooling loners in experimental trials, the inability of parasitised minnows to maintain spatially-correct positions in schools and their propensity for peripheral positions suggest that helminth infection is, for them too, likely to influence predator-prey interactions. Studies have shown that predators preying on grouped prey are known to select visually-

odd group members, since it appears to reduce the confusion effect from which they normally suffer when attacking such aggregations. Since oddity in fish schools may be associated with deviant size, shape, spatial position or behaviour, the fact that *L. intestinalis*-infected minnows deviate from uninfected fish in all of these factors leads one to expect that parasitised shoal members may suffer from selective predation. However, since predation regimes in natural environments are complex, the ecological consequences of shoal membership, and the shoaling changes associated with *S. solidus* infection, are likely to be strongly dependent on the particular types of predators present in any particular habitat.

The consequences of host behaviour for host, parasite and uninfected conspecifics: who wins ?

In his book, 'The Extended Phenotype', Richard Dawkins describes parasitised hosts as exhibiting a 'shared' phenotype, reflecting the effects of both host and parasite genes (Dawkins, 1982). This view is particularly well-illustrated by the parasitised fish considered in this study, where parasite infection is associated with significant and measurable differences in host morphology, behaviour and physiology. Although the effects of both host and parasite genes are reflected in the observed phenotype, these genes have conflicting ultimate interests. For instance, the genes of *S. solidus* parasites will only be replicated if infected sticklebacks are ingested by a piscivorous bird, whereas avoidance of predation is probably one of the primary aims of the stickleback genome. This genetic conflict of interests is likely to lead to an evolutionary 'arms race' for the control of the behaviour of infected hosts, with parasites being selected to maximise host susceptibility to predation by definitive hosts, and hosts co-evolving to minimise the effects of the parasite, or at least delaying their effects until the host has reproduced successfully.

Since the ability to form cohesive groups is an effective antipredator response to many types of predators, any changes in the shoaling behaviour of individual fish are likely to be allied to a change in their predation pressure. Litvak (1993) demonstrated experimentally that the responses of shoaling prey to a simulated avian attack probably serve to protect group members from predation by piscivorous birds, and this suggests that the deviant shoaling and schooling behaviour exhibited by *L. intestinalis*- and *S. solidus*-infected fish may result in their selective predation by avian predators. If altered shoaling behaviour does result in selective predation of parasitised fish by piscivorous birds, transmission of the parasite would be enhanced, and the ability to induce such behavioural change

would be selected for during parasite evolution. On the other hand, if the infection-associated behaviour change in some way increased the survivial of infected hosts, by reducing the predation pressure they would face by shoaling 'normally', then the behavioural phenotype would be selected for during evolution of the host.

The phenotypic consequences of parasite infection may have further implications for group structure in natural habitats. The fact that many predators known to select prey that are visually-distinguishable from the rest of a group suggests that shoals composed of otherwise uninfected, near-identical individuals could, theoretically, benefit by including parasitised members amongst their ranks. If, for instance, a predator was known to be 5 times more likely to attack a shoaling *L. intestinalis*-infected minnow than an uninfected shoal member, the inclusion of an infected fish in a shoal of, say, 10 fish would reduce the odds of any uninfected individual being attacked from 1 in 10 to 1 in 14. Therefore, by using the infected fish as a 'fall guy', uninfected minnows would be able to effectively reduce their *per capita* risk of attack, yet avoid the heightened competition for resources that would normally be associated with an increase in shoal size. In fact, food competition for uninfected members may even be decreased by the inclusion of infected fish, since they have been shown to exhibit a reduced competitive ability. Since fish infected with pseudophylliean cestodes cannot pass the parasite to uninfected conspecifics, there would appear to be little cost to shoaling with them (which appears not to be the case with fish harbouring contagious ectoparasites, since they are actively avoided by uninfected conspecifics [Dugatkin *et al*, 1994]), and in situations where belonging to a shoal or school is a more attractive option for an infected fish than being a loner, uninfected shoal members might benefit from both of these mechanisms. However, there is evidence that such inclusion a small number of visually-distinctive fish may have less beneficial effects for other shoal members. Landeau & Terborgh (1986) demonstrated that the inclusion of blue-dyed (and therefore visually-distinct) silvery minnows *Hybognathus nuchalis* into a school of non-dyed conspecifics significantly increased the rate of successful predation by largemouth bass *Micropterus salmoides* on *all* fish, not just the odd individuals. It may be that the presence of visually-distinct individuals reduces the total confusion normally generated by the school, and allows visually-hunting predators to focus more clearly on specific fish, irrespective of whether they are actually odd or not.

Whose genes ultimately benefit from the differences in shoaling and schooling behaviour exhibited by *S. solidus*- and *L. intestinalis*-infected fish will clearly depend on whether such differences result in increased predation of hosts by definitive host predators (parasite genes win), or decreased predation by all predators on hosts (host genes win). If the behavioural phenotype of infected fish resulted in infected fish being consumed more often by non-definitive hosts, then both genotypes would lose out, and this phenotype would be strongly selected against by both host and parasite evolution. Studies are required that will determine the consequences of the behaviour exhibited by parasitised fish, whether the observed behaviour is more likely to have been the result of parasite 'manipulation' or of host adaptation, and therefore which partner is currently 'ahead' in this evolutionary arms race.

Infection-associated host behaviour change: possible consequences for habitat suitability

Because the ingestion of infected fish by piscivorous birds is a prerequisite for completion of the *L. intestinalis* life cycle, the frequency with which this final transmission stage occurs is likely to play an important role in determining the epidemiological characteristics of infection in any particular habitat. The rate at which this final transmission stage occurs at any particular site is likely to be related to both the density of suitable avian predators, and the density of infected fish. In the types of habitats generally occupied by cyprinid fish in the U.K., piscivorous birds are abundant, and predation on the local fish populations frequently intense providing ample opportunity for the completion of pseudophyllidean life cycles (e.g. Winfield, 1990). Once introduced to such a system, *L. intestinalis* infection typically spreads rapidly through the local cyprinid population, with the increased parasite prevalence showing positive feedback until almost all of the fish are infected.

Because of its altitude and geographic location, Loch Maragan is not a 'typical' habitat for cyprinid fish, and therefore for *L. intestinalis* infection, in the U.K., and avian predators are scarce at the site. Epidemiological studies of *L. intestinalis* infection in the minnow population at Loch Maragan described in this study have revealed that the parasite exhibits a low, apparently constant level of infection at the site when compared with other studies of its epidemiology in more typical sites. Because the parasite is more frequently associated with a highly destructive pattern of infection, it is perhaps surprising that the parasite can persist at Loch Maragan with such apparent stability and equilibrium, whilst exhibiting a relatively low level of infection. Because of the losses inevitably suffered at each transmission stage of such a parasite, the life cycle must need to be completed with a

certain frequency for the parasite to persist. Because of the spatial and temporal scarcity of piscivorous birds at Loch Maragan, and the low frequency of *L. intestinalis*-infected minnows at the site (a number of which will harbour worms too small to infect definitive hosts), then if birds preyed on the minnow population randomly, with uninfected and infected fish being taken in numbers proportional to their occurrence in the population, it may be that successful completion of the parasite life cycle would take place too infrequently for the parasite to survive at the site. Under such severe conditions, it may be that selective predation on ligulosed fish (such as that shown by van Dobben, 1952) is required for sufficient transmission of the parasite.

Behavioural or morphological changes associated with parasite infection, such as those described in this project, may form the basis for the selective predation of parasitised intermediate hosts by definitive hosts and consequently allow parasites to invade habitats where transmission rates would generally be expected to be too infrequent, due to the spatial or temporal scarcity of these predators, to allow the parasite to survive. If *S. solidus- or L. intestinalis*-infected fish are ingested more often by definitive hosts, and less often by non-definitive hosts than would be expected if prey were taken in direct proportion to their frequency in the population, then such parasites, once introduced by a chance event, may become established in such habitats. *L. intestinalis* was probably introduced to Loch Maragan by either the introduction of parasitised fish by anglers, or by the introduction of viable parasite eggs passed into the loch with faeces of definitive hosts that had become infected elsewhere. Once introduced, the scarcity of definitive hosts and initial low prevalence of the parasite would probably have predicted that the parasite would have been unlikely to become established at the site. Instead, the parasite has been present in the minnow population for at least the past decade, exhibiting an apparently stable, low prevalence. It may be that the ability of *L. intestinalis* to survive at this site and exhibit a stable prevalence in the minnow population, rather than rapidly becoming locally-extinct, as may have been predicted, has been due to selective predation by definitive hosts, possibly as a result of the altered behaviour associated with infection.

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APPENDIX 1. PUBLISHED PAPERS

Published articles removed due to copyright issues.

Behaviour (ISSN 0005-7959) 132 (15-16): 1223-1240

The effect of Schistocephalus solidus (Sestoda: Pseudophyllidea) on the foraging and shoaling behaviour of three-spined sticklebacks, Gasterosteusaculeatus. Iaian Barber, Felecity A. Huntingford

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The effect of hunger and cestode parasitism on the shoaling decisions of small freshwater fish. Barber, F. A. Huntingford, D. W. T. Crompton.

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