POPULATION DIFFERENCES IN THE ORIENTATION BEHAVIOUR OF THREE-SPINED STICKLEBACKS

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Preface

This thesis has been composed by me, is the result of my own research and contains no work done in collaboration except where stated otherwise. The text does not exceed 70,000 words. No part of this thesis has been submitted to any other university in application for a higher degree.

Abstract

An animal that remembers the position of shelter or the location of a potential mate is likely to have higher fitness compared with a more forgetful conspecific. Previous research has shown that an animal's behaviour can be influenced by its ecology. By studying the interaction between ecology and learning and memory we gain a better understanding of the functional significance of animal cognition. The aim of this thesis was to determine how ecology shapes spatial learning and behaviour in different populations of three-spined sticklebacks (*Gasterosteus aculeatus*). These fish occupy a wide range of marine and freshwater habitats and their behaviour is, therefore, subject to a range of different selective pressures.

Under experimental conditions, fish sampled from three river habitats exhibited preferences for developing algorithmic behaviour (i.e. learning a series of turns or movements), while fish from two pond habitats showed a preference for visual landmark information. Furthermore, two river populations were found to be more adept at using direction of flow to orientate than two pond populations. Habitat surveys for each of the populations tested were carried out. These surveys were used to assess candidate ecological correlates that may be responsible for the observed population learning and memory differences. In addition, morphometric assessment of the different populations revealed a degree of morphological variation. Inferences about feeding ecology and predation pressures in the various habitats were drawn from these results.

To determine whether the observed variable spatial ability arose through genetic differences between the populations or was learned anew by each generation, artificial breeding and rearing experiments were carried out. These revealed that the spatial cue preferences and abilities of the three-spined sticklebacks were influenced by an interaction between experience during development and inherited factors.

The results of this work have revealed intra-specific variation in both spatial cognition and morphology among natural populations of three-spined stickleback. The variation in spatial cognition was related to both inherited factors and ontogeny within a particular type of habitat.

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1.1. Introduction

1.1.1. The benefits of learning

In a changing environment, learning and memory are essential for an animal's survival and reproduction. Animals which learn to avoid poisonous prey, to avoid potential predators or to behave appropriately with the opposite sex will prolong their life and have increased chances of reproductive success. Experimental work has revealed the importance of learning in some of the basic processes of life. For example, orange chromids, *Etrophus maculatus*, reared in isolation from other fish, failed to learn correct courtship displays. They developed some of the correct movements, but could not perform them appropriately, and instead performed openly aggressive acts to potential mates rather than the usual non-contact displays (Wyman & Ward, 1973). The absence of social learning, therefore, reduced their chance of successful mating. Croy & Hughes (1991) showed that in fifteen-spined sticklebacks, *Spinachia spinachia*, handling skill of a particular prey species improved with practise. In this case, learning enabled the fish to exploit as efficiently as possible the particular type of prey they came across most often in their environment.

Orientation learning has been shown to increase survival in white-footed mice, *Peromyscus leucopus*. Mice which had learned about, and become familiar with their surroundings were less prone to predation from owls than those which were in unfamiliar surroundings (Metzgar, 1967). Presumably, experienced mice could locate shelter much more quickly than inexperienced ones. Prey handling skills, courtship displays and predator avoidance can all therefore be influenced by learning.

1.1.2. Learning and the environment

The influence of ecology on learning and memory has recently received considerable attention, and evidence that the environment an animal inhabits influences what it learns and remembers is mounting (for recent review see Dukas, 1998). Several studies have examined the impact of different environments on learning by comparing closely related species occupying different types of habitat. For example, foraging efficiency and memory retention have been shown to vary between closely related species of stickleback (*Gasterosteidae*) from marine, estuarine and freshwater habitats (Mackney & Hughes, 1995). Those forms inhabiting more variable environments, and therefore experiencing greater prey diversity (i.e. marine and estuarine forms), exhibited a shorter memory for foraging skills associated with particular prey types than the freshwater population. The freshwater population was sampled from a landlocked pond which was presumed to contain a smaller diversity of prey species, a circumstance under which the ability to remember particular prey handling skills for longer would be adaptive.

In another study, Micheli (1997) showed that blue crabs, *Callinectes sapidus*, which range widely over a variety of habitats were able to adapt their feeding behaviour in accordance with previous experience during experimental trials. Atlantic mud crabs, *Panopeus herbistii*, a much more sedentary species which does not range over many different habitat types did not show any modification to their feeding behaviour despite having the same previous experience. It seems that the species which experienced a diversity of habitats was able to modify its foraging behaviour to suit the current environmental conditions, whereas the species which experienced less environmental heterogeneity was less able to, and indeed had less need to, be able to adapt its behaviour.

1.2. Three-spined sticklebacks - a model system

Although many studies suggest that the environment has an effect on the information which is learned and remembered, much of the evidence that ecology may influence learning and memory typically comes from interspecific comparisons employing two, or at most a few species (Mackney & Hughes, 1995, Micheli, 1997, Potting *et al.*, 1997). Many characteristics other than learning and memory, however, will differ between species, for example genetic compliment or life history, and so it may be difficult to attribute behavioural variation to any one particular cause. To overcome this problem, either comparisons between large numbers of species should be made, or alternatively, comparisons should be made between populations of one species. This would perhaps give greater insight into the influence of habitat on behaviour. An ideal species for an intraspecific comparison investigating the effects of ecology on learning and memory is the three-spined stickleback, *Gasterosteus aculeatus*. Since the retreat of the last ice age, 9000-13000 years BP (before present), this species has colonised a wide range of post glacial freshwaters, and now occupies many different aquatic habitats such as small ponds, larger lakes and rivers (Bell & Foster, 1994). These habitats differ greatly in nature, and may provide the potential for a range of behavioural adaptations to develop within the species. The variability in habitat along with the huge wealth of literature from studies on sticklebacks

(Wootton, 1976, Bell & Foster, 1994) makes this species an ideal choice for a behavioural comparison. Indeed, some behavioural and morphological differences between Scottish populations of freshwater three-spined stickleback have already been documented (Campbell, 1985, Ibrahim PhD thesis 1988).

1.2.1. Overview of stickleback biology

The three-spined stickleback has been studied in depth for over fifty years. Behavioural and phylogenetic studies have shown that this species is extremely variable not only in behaviour, but also in morphology (Bell & Foster, 1994, Foster *et al.*, 1994, Foster & Baker, 1994). Such variation confers a high degree of flexibility and enables this species to survive in a huge range of environments, from open oceans to estuaries, rivers, lakes and ditches. This variation is perhaps the feature that has attracted so many researchers to use this species as a model in evolutionary, ecological and behavioural studies. Studies include work on reproductive and courtship behaviour and display (McLennan, 1996), parental behaviour (Black, 1971), predator avoidance (Giles & Huntingford, 1984, Tulley, 1985, Huntingford & Wright, 1989), foraging (Tugendhat, 1960, Croy & Hughes, 1991), social behaviour (Larson, 1976), competitive ability (Gill & Hart, 1996), learning (Huntingford & Wright, 1989), memory (Milinski, 1994, Mackney & Hughes, 1995), seasonal and spatial distribution (Bentzen *et al.*, 1984), the effect of parasites on behaviour (Milinski, 1984, Barber *et al.*, 1998) behavioural and trophic specialisation to different niches (Bentzen & McPhail, 1984, Lavin & McPhail, 1985), morphological differentiation (Reimchen *et al.*, 1985, Campbell, 1984), divergence (Lavin &

McPhail, 1992, Thompson *et al.*, 1997), and phylognetics (McLennan, 1993, Bell & Foster, 1994). So widespread is the research interest of the stickleback that the *third* international conference centred on this species in fifteen years is being planned for June 1999.

These fish have not only been studied in depth, but they also adapt well to laboratory conditions, owing to their small size and basic feeding and husbandry requirements. They have a reasonably short generation time (<1 year), and are conducive to being bred and reared under artificial conditions. They are therefore an ideal choice of species for laboratory based comparative studies.

1.3. Spatial learning and memory

When choosing the kind of behaviour around which to base a comparative study of the effect of the environment on behaviour, several factors should be considered. The behaviour should be ecologically important, and likely to be affected by environmental pressures. Spatial behaviour fulfils these criteria. The ability to orientate in the environment is vital in allowing animals to perform many important functions. Finding the way back to certain locations, such as a safe place to hide, a good food source or the location of potential mates confers obvious benefits. Clarke *et al.* (1993) showed that when threatened, the eastern chipmunk, *Tamius striatus*, was much better at locating shelter in terms of speed and efficiency when in a familiar home range than when in an unfamiliar area. The chipmunk, therefore, appeared to have built up a knowledge of its spatial surroundings which improved its survival chances. A study by Smith & Dawkins (1971) revealed that spatial learning was used by great tits, *Parus major*, to allow them to return to the most profitable foraging patches. When foraging patterns were analysed, the birds were found to have spent most time foraging at the locations in which they had previously experienced most success. Similarly, the foraging efficiency of rufous hummingbirds, *Selasphorous rufus*, was shown to be improved by the use of spatial learning (Hurly & Healy, 1996). Hummingbirds remembered the exact location of flowers they had recently depleted of nectar. Thus spatial learning helped the birds to avoid wasting time and energy by revisiting recently depleted flowers before they had replenished their supply of nectar.

Orientation behaviour has also been shown to be subject to selection pressures exerted by the environment in several studies involving closely related species of birds and mammals. For example, the spatial ability of two closely related species of voles seemed to be influenced by ecology and life history (Gaulin & Fitzgerald, 1989). One species of vole, *Microtus pennsylvanicus,* was polygynous, with the males ranging widely during the breeding season to locate many mates. During this time, males of this species exhibited better spatial ability than females which did not range around looking for mates. The other species, *Microtus ochrogaster,* was monogamous. Consequently, the males did not range more widely than the females, and no difference in spatial ability between the sexes was recorded.

Several species of bird store food in the late summer and autumn to help them survive the winter. A good spatial memory is required to enable birds to relocate previously hidden items or caches. Indeed, it has now been shown that birds not only remember specific locations, but also whether the cache has been removed, the type of food which was hidden there (Shettleworth, 1995), and when it was stored (Clayton & Dickinson, 1998). Sherry *et al.* (1992) reviewed studies which revealed the effect of ecology on spatial ability and hippocampal size (the hippocampus is the part of the brain which is involved in spatial memory - see Sherry & Healy, 1998 for recent review). For example studies of food-storing and non-food-storing families of related birds revealed that food-storers usually possess a hippocampus over twice the size of non-storers (Krebs *et al.*, 1989). In some species of food-storing bird, food-storing behaviour peaks just before the winter, when it is most needed. Smulders *et al.* (1995) showed that the hippocampus of the black-capped chickadee, *Parus atricapillus*, is at its greatest volume at that time of year. Since hippocampal size is related to spatial performance (Sherry *et al.*, 1992), ecological pressures exerted by the seasons appear to have resulted in appropriate seasonal changes in spatial ability. Thus the ecological pressures which produced food-storing have also enhanced spatial memory.

Spatial learning and memory, therefore, provide great adaptive benefits to many species of animal. Researchers have been attracted to the varied and complex nature of spatial behaviour as a useful tool with which to investigate not only the cognitive ability such learning and memory processes demand, but also the mechanisms underlying spatial behaviour. There are many mechanisms which animals use to orient themselves. A short overview of these is given below.

1.3.1. Spatial behaviour

Some simple orientation behaviours such as kinesis and taxis are brought about purely by reflex, with no learning or cognitive effort involved. Kinesis is a very simple kind of orientation behaviour in which organisms such as flat worms or wood lice alter their rate of turning in response to the detection of adverse or desirable stimuli (Roberts, 1986). If unpleasant conditions are being experienced, movement and turning increase until, by random chance, the organism comes to a more favourable environment. In order to remain in the favourable area, movement and turning rate decrease until the organism remains still. Taxis involves an organism detecting a stimulus from a particular direction, for example, light, and moving towards it. Species of *Daphnia* and *Euglena* exhibit this phototactic response, *Euglena* in order to photosynthesise, and *Daphnia* to locate photosynthetic algal prey. The result is that the optimum habitat is likely to be occupied.

Higher organisms have the opportunity to use their more complex receptors and nervous systems to guide their movements. Orientation in higher organisms is often closely linked to learning, as animals must learn to associate particular movements with particular outcomes. A number of orientation strategies have now been observed in a wide range of animals as they orientate within their environment.

1.3.1.1. Use of olfactory cues

Odour following is a basic mechanism for place finding, and is exhibited by many taxonomic groups from molluscs to mammals. Many animals release pheromones from scent glands, which can be used in communication, mating and orientation, for example, insects such as male parasitoid wasps, *Aphelinus asychis*, follow trails of pheromones through host vegetation to locate sexually receptive females (Fauvergue *et al.*, 1998). Some species of ant (Formicidae) lay down a scent trail as they move out from their nesting area to locate a food source (Wehner, 1992). They can then use the trail to find their way back to the nest and to direct other con-specifics to the food source. It has been shown that some mammals also orientate using scent, for example, Peters & Mech (1975) found that wolves, *Canis lupus*, orient in their home range by remembering the spatial relationships of different scent markings.

Perhaps one of the more famous examples of scent following comes from evidence that salmon (Salmonidae) returning to their natal streams use olfaction to locate the correct route up to five years after migrating to the sea (for review see Dittman & Quinn, 1996). Different streams are believed to possess unique odours from the soil and vegetative properties of the drainage basin which the fish imprint on just before they leave their natal waters. Early investigations of this process used hatchery reared Coho salmon, *Onchyhnchus kisutch*, that were conditioned to the organic chemical, morpholine before being released into a lake (Hasler & Wisby, 1951). Just prior to the time when the fish were due to begin migrating up river to mate, one of the inlet streams flowing into the lake was treated with morpholine. The vast majority of fish subsequently observed in the morpholine treated inlet stream were the same tagged individuals that had been reared with morpholine.

With olfaction being used by a wide range of animals, experiments testing the use of other types of orientation strategy should always control for the possibility that olfaction could be involved.

1.3.1.2. Visual landmarks

Setting a course using the known relationship between visual landmarks and a goal is known as piloting (Pearce, 1997). Many types of animals have been shown to pilot, amongst them the hoverfly (Collett & Land, 1975), the Cataglyphis ant (Collett *et al.*, 1992), the honey-bee (Cartwright & Collett, 1983), the eastern chipmunk (Clarke *et al.*, 1993), the gerbil (Collett *et al.*, 1986) the rat (Beigler & Morris, 1996) and the pigeon (Cheng, 1988, 1995). Perhaps the most widely known example of piloting is that provided by Tinbergen (1951). Digger wasps, *Philanthus trangulum*, dig a hole in the ground for a nest. Emerging females fly around the entrance for a few seconds, presumably to learn the configuration of visual landmarks, and their relation to the hole. Tinbergen placed a ring of pine cones around a nest, and found that if the ring was displaced by 12 inches after the female emerged, returning wasps searched for the nest inside the ring of pine cones, instead of in the correct location. This showed that the wasps used the cones as visual guides to the entrance of their nest.

The simplest use of visual information is to head towards a landmark which has become associated with a goal close to it. In such a case, the landmark acts as a beacon, guiding the animal to the appropriate location. If the goal is not located right next to the landmark, but some distance away from it, the animal must learn the geometric relationship between the landmark and the goal i.e. they must learn to go to a point a certain distance to one side of the landmark. Different groups of animals achieve this is different ways, for example, bees, seem to match their current view of the landmark to a retinal snapshot, or memory, of what the landmark should look like from the goal. Cartwright & Collett (1983) found that if the size of the landmark was manipulated, bees, *Apis mellifera*, altered their search position accordingly. If the landmark was made larger, they searched further away from it, and *vice versa*. Gerbils, *Meriones unguiculatus*, however, did not behave in this way (Collett *et al.*, 1986). Manipulations of the landmark size did not cause them to alter the distance of search. The gerbils appeared to be using an unidentified method of judging the distance they had to travel to find food on the basis of the course dictated by the landmark.

When more than one landmark was available, i.e. when a geometric arrangement of landmarks surrounded the goal, bees and gerbils differed again in the way they located the goal. Gerbils located a hidden reward by learning the geometric relationship between the landmarks and the goal (Collett *et al.*, 1986), while bees appeared to take compass bearings from the goal to each landmark, and move around until the compass bearings of each landmark matched their previous record (Cartwright & Collett, 1983).

1.3.1.3. Stereotypic movements

There are some examples of stereotypic movements, or algorithmic behaviour patterns being exhibited by animals performing spatial tasks. Algorithmic behaviour involves animals learning a series of movements (often repeated) which enables them to reach a goal. For example, Roitblat *et al.* (1982) found that in an aquatic radial arena Siamese fighting fish, *Betta splendens*, searched each arm sequentially i.e. upon exiting one arm, they always turned in the same direction, and entered the next arm in the series. This means that instead of having to remember the relative positions of each arm that had already been depleted, the fish only had to remember one rule - always turn in the same direction and enter the next arm. Since only one small piece of information need be remembered, this might therefore prove to be a less costly spatial process in terms of neural capacity than, for example, forming a map of the environment (see below). While the use of visual landmarks is regarded as an allocentric orientation strategy since it requires the use of information not connected to the animal, the use of a behavioural algorithm is an egocentric strategy, because it depends on the movement of the subject itself rather than memory of the arrangement of the surroundings. Rodriguez *et al.* (1994) carried out a spatial experiment using goldfish, *Carassius auratus*, which revealed that fish learned to use an allocentric strategy more quickly than an egocentric one. Visual landmarks might therefore be more important to goldfish as a spatial cue than developing algorithmic behaviour.

1.3.1.4. Path integration

Path integration, or dead reckoning, allows animals (or early sailors) to navigate without using any visual information. Velocity and time are integrated to give an idea of the current location in relation to the starting point. For example, if a blindfolded person walked some distance into an arena, they would probably have some idea of their current position, and be able to walk back to the vicinity of the starting point without the aid of visual information. Dead reckoning has been demonstrated experimentally in ants (Wehner & Srinivasan, 1981), bees (Von Frisch, 1950), hamsters (Georgakopoulos & Etienne, 1994), and indeed, provided early sailors with a method by which to keep track of position. Wehner & Srinivasan (1981) trained desert ants (genus *Catagliphis*) to use a food source 20 m from the nest. Subsequently, ants were transplanted by 600 m as they left the food source. They continued to home as if they had not been displaced, and travelled to an area that would have been within 50 cms of the nest had they not been displaced. This showed that the ants were not following a chemical trail, or using familiar visual landmarks, but instead, some internal measure of direction and distance i.e. path integration.

1.3.1.5. Flow

Animals living in flowing water may be able to use the direction of flow to orient themselves. Although the use of ocean currents, upwellings and tides to orientate has been demonstrated in plaice (Metcalfe & Buckley, 1997), lobsters (Phillips, 1981) and eels (Power & McLeave, 1983), the use of continuous uni-directional flow in a river habitat has been studied much less. Fish might be able to use flow in a manner not unlike path integration, but instead of integrating direction with time to keep track of the current location, fish might be able to integrate direction of water flow in relation to the body with time to achieve the same result. For example, if a fish travels forwards with the water flowing directly towards it, it might be aware that it is now upstream of the starting point. Similarly, if a fish travels a certain distance out from the river bank with the flow coming from the right, then in order to return to the bank, the fish should turn until the flow is coming from the left, and travel the same distance. This type of orientation mechanism might be useful when seeking shelter from predators under the riverbanks. To determine whether three-spined sticklebacks can rely on this cue to orientate, the ability to distinguish direction of flow, and to use it to orientate is investigated in chapter 4,.

1.3.1.6. Maps

Maps are internal representations of an area which bear no relation to the current position or movements of the animal. Less evidence has been found for the existence of maps than for other orientation methods, and considerable controversy surrounds their study (Bennett, 1996). This is in part owing to the difficulty associated with experimentally demonstrating the use of a map without some simpler explanation fitting the observations. In general, it is believed that if animals have a map they should be able to find novel routes to a goal and plan detours around introduced objects. Some evidence for maps has been found in rats (Morris, 1981), but a more convincing study was carried out using the blind cave fish, *Anopthychthis jordani*, as a subject (Teyke, 1989). These fish exhibit exploratory swimming behaviour when placed in an unfamiliar environment. As the fish are blind, they detect their environment through the lateral line organ (a line of sensory receptors on each flank). The exploratory swimming is of a higher than normal velocity to optimally stimulate the lateral line organ. Water movements between the lateral line organ and objects in the surroundings allow the fish to learn about the position of objects in their surroundings. In addition to increasing swimming velocity, the fish also glide more frequently alongside objects in a novel environment compared with a familiar one. Having explored the surroundings, fish revert back to normal swimming velocity and do not investigate objects as often. Since the cave fish has no vision, this behaviour was taken as evidence that the exploratory behaviour allowed them to form a map of their surroundings.

1.3.1.7. Summary

Orientation strategy	Description
Kinesis and taxis	Instinctive movements e.g. towards light or favourable conditions
Olfaction	Orientating towards stronger olfactory stimuli, following scent trails, or forming an 'olfactory map'
Visual landmarks	Visual cues used as beacons or landmarks - some animals learn geometric relation between visual cues and goals - an allocentric spatial strategy
Stereotypic movements	Patterns of movements (or algorithm) sometimes repeated - an egocentric spatial strategy
Path integration	Integrate direction with time and speed to calculate current position in relation to starting position
Flow	Orientating using continuous water flow (e.g. a rivers current) as a directional cue
Maps	Internal representations of the spatial arrangement of an area

1.3.2. Multiple behavioural responses

Animals have a range of behavioural responses available to cope with variation they may experience in their environment. Werner & Hall (1974) and Kislalioglu & Gibson (1976) conducted experiments which show that as preferred prey decline in number and consequently hunger increases, the foraging behaviour of bluegill sunfish, *Lepomis macrochirus*, and fifteen-spined stickleback, *Spinachia*

spinachia, respectively, changes to accept a wider range of prey types. Foraging behavioural responses therefore can depend on prey availability. Changes in predation risk can also give rise to an alteration in foraging behaviour. Mittelbach (1981) found that when predators are absent, bluegill sunfish of all size classes move out at the appropriate time to open waters where optimal foraging can take place. However, when predatory largemouth bass, *Micropterus salmoides*, were present, only those fish large enough to avoid being eaten moved out. The smaller fish remain in the vegetated littoral zone where foraging is suboptimal, but the risk of predation is reduced. Two responses were available to these smaller fish. Without the alternative response of remaining in the littoral zone, survival would be greatly decreased.

Multiple behavioural strategies have also been observed in spatial behaviour. For example, the type of spatial cues used depends on current environmental conditions. Migrating birds might use the star compass at night and the sun compass during the day. On overcast days when the sun is obscured, birds can orientate using patterns of polarised light or a magnetic compass. Indeed, many studies have shown that a range of orientation cues can be used by animals (see Able, 1993 for review). The orientation strategy preferred by an animal must result from the cognitive capability of the species itself and on the type of information available in the environment. For example, visual information is unlikely to be important to fish living at depths where no light can penetrate, or those inhabiting only the pelagic zone of a deep water body. Similarly, the benefit of forming a visual spatial map of the environment will be short-lived for fish living in very changeable environments e.g. fast flowing rivers or tidal estuaries.

Experimental evidence has revealed the simultaneous acquisition of several types of spatial information. Vallortigara *et al.* (1990) trained chicks, *Gallus gallus domesticus*, to locate a food reward in a rectangular arena, near a landmark. When the landmark was moved around the arena, the chicks followed it, and continued to search near it regardless of were it was placed. When the landmark was made unavailable, by removing it from the arena altogether, the chicks relied on the shape of the arena to continue searching in the correct place. The chicks had therefore simultaneously learned to use both the visual landmarks and the shape of the arena to locate the goal. They relied primarily on the visual landmark, following it as it was moved around and ignoring the shape of the arena. When the landmark was removed, the chicks could then rely on the shape of the arena as a secondary spatial strategy.

Braithwaite *et al.* (1996) found that juvenile Atlantic salmon, *Salmo salar*, were able to use visual landmarks to track a moving food source when they were available, but when the landmarks became unavailable, the fish appeared to revert to an alternative strategy, perhaps using chemosensory cues to maximise foraging success.

Three-spined sticklebacks used in the current thesis may also be able to use more than one type of orientation cue simultaneously. Like the chicks and the salmon above, they might have a preference for a particular type of cue, but if necessary, be able to switch to whatever type of cue is available. The use of more than one strategy is investigated in experiments 2.a. and 3.

1.3.3. Orientation behaviour in fish

Many species of fish exhibit well developed spatial ability. From salmon and eels migrating thousands of miles to locate their ancestral breeding sites (McDowall, 1988), to the twilight movements of grunts into their traditional feeding areas (Helfman & Schultz, 1984), the ability to return to a certain area is vital for maximising fitness. We now know that fish can employ a range of different strategies and cues to orientate in their environment (see Braithwaite, 1998 for review). For example, Warburton (1990) demonstrated that goldfish are able to locate a food patch by forming an association between the patch and visual landmarks. In addition, Braithwaite *et al.* (1996) showed that juvenile Atlantic salmon can use visual landmark to track a moving food source. Roitblat (1982) found that Siamese fighting fish use stereotypical algorithmic behaviour when foraging in an eight-arm radial maze, i.e. upon leaving one arm, the fish always turned left or right and entered the next arm in the sequence. Metcalfe & Buckley (1997) found that plaice, *Pleuronectes platessa*, can use tidal gradients to migrate to spawning areas. Sun compasses have been demonstrated in bluegill sunfish, *Lepomis macrochirus*, by Goodyear & Bennet (1979), and electrolocation was shown to be used by elephantnose fish, *Gnathonemus petersii*, (Cain, 1995).

Some researchers have suggested that fish can form a map of their surroundings (Rodriguez *et al.*, 1994, Markel, 1994). For example, Noda *et al.* (1994) found that stout body chromis, *Chromis chrysurus*, exhibited intensive searching at set locations in their feeding territory, and travelled directly between them. The set locations yielded a high prey capture rate in general, but intensive searching was observed even when the patches did not contain food, and no currents were present which could indicate their position, suggesting that the fish located their feeding patches by memorising a map of the area. Perhaps the most famous, and convincing evidence for a map in fish comes from the research of Aronson (1951, 1971). Rock gobies, *Bathygobius soporator*, are sometimes trapped in tide pools when the tide goes out. In order to reach the sea, these fish can jump accurately from one pool to the next, even when they cannot see into the next pool. In an artificially constructed series of pools, gobies that were unfamiliar with the area jumped inaccurately, and were stranded on dry land. Gobies that had explored the area at 'high tide' jumped accurately between pools when the 'tide' was later out. This suggests that the fish knew the geometric relationships of the pools.

1.4. Aims of the thesis

It is interesting that so many types of spatial cue can be used by many different kinds of animal, but what factors decide which strategy is preferred, how many strategies can be used, and what influence does the environment have on orientation? The aim of this thesis was to investigate spatial strategies used by three-spined stickleback sampled from different populations. The use of visual landmarks, stereotypic movements and direction of flow were investigated, as was the effect of the environment on the extent to which these cues are used. In addition, artificial breeding experiments were carried out to determine if orientation cue preferences are inherited or are learned by each new generation. Behavioural variation is discussed with reference to environmental and morphological variation to gain an insight into how the populations of sticklebacks have diverged.

1.5. Structure of the thesis

In chapter 2, experiment 2.a. investigates the orientation strategies four populations of three-spined stickleback use to navigate a simple line maze. Two pond populations and two river populations were chosen for the experiment to test fish from a range of different habitat types. Two types of spatial information were made available. Visual landmarks could be used like beacons to guide fish through the maze, or a particular algorithmic response could be developed which would enable fish to learn the correct sequence of left and right turns to solve the maze without visual information. The experiment was designed to investigate how fish from the different habitats used these different types of spatial information.

While an inter-population comparison provides a strong base for a study of the effect of environment on behaviour, there still remain a few factors which should be considered. Any alternative explanations for observed behavioural differences should be ruled out. Experiments 2.b. and 2.c. were designed to this end. Experiment 2.b. was a different, simpler type of learning task which was designed to determine if the gross cognitive ability, adaptation to laboratory conditions and general boldness during the experiment could explain any observed behavioural variation between the populations. Experiment 2.c. investigates the use of olfaction in solving the tasks. This was necessary because the motivation to learn all the tasks reported in this thesis was maintained by the presence of a food reward. It was important to rule out whether the fish were able to locate such a reward using olfaction.

The orientation strategies introduced in experiment 2.a, and some of the reinforcing factors responsible for performance, are further investigated with a different type of discrimination / spatial task in experiment 3. This task resembles the typical eight-arm maze apparatus commonly used in spatial experiments involving rats.

Chapter 4 reports on one particular type of orientation cue which has rarely been studied directly. Directional information is provided by a strong polarising cue available only to fish inhabiting moving water - the direction of water flow. Experiment 4.a. studies the use of this particular cue by the same four populations as experiment 2.a. In addition, experiment 4.b. tests the performance of fish when given visual and flow information which was subsequently manipulated to provide conflicting spatial information.

Having revealed behavioural variation between populations of stickleback from different habitats, I wanted to determine which of two possible factors controlled this variation. Was spatial behaviour genetically controlled, or was it learned as each population developed in different habitats? Some kinds of orientation behaviour are influenced by learning during development, while others appear to be under genetic control. The orientation behaviour of pigeons has been shown to be related to the experience of the birds during their early life after fledging (Wiltschko & Wiltschko, 1989, Braithwaite & Guilford, 1995). In contrast, long-distance migration in birds has been shown to be genetically controlled. Schuz (1971) showed that European storks (Ciconiidae) inherit the information as to the direction they should fly around the Mediterranean. Those in western Europe fly via Gibraltar, while in the east, they fly round to the east of the Mediterranean. Eggs displaced from the east to west showed the tendency to fly east instead of flying *via* Gibraltar.

To determine whether an observed behaviour is attributable to learning or genetic compliment, artificial breeding and rearing under controlled conditions is required. The subsequent behaviour of such artificially reared animals can then be compared with that of wild individuals. Chapter 5 describes artificial breeding and rearing of several populations of stickleback whose behaviour was studied in chapters 2, 3 and 4. Artificially reared fish were made to perform some of the same spatial tasks as wild caught fish in earlier chapters. This enabled me to investigate the extent to which orientation behaviour in these populations was controlled by inheritance or environmental factors.

The main subject of the thesis has been to determine the effect of different environments on behaviour. There is little point, however, in studying the effect of different environments on behaviour if there is no difference between the environments sampled. To investigate the ways in which the selected habitats differ, habitat surveys were carried out, involving different aspects of the environment. The findings are presented in chapter 6 which gives detailed comparisons between the habitats occupied by stickleback populations used in this thesis.

Using populations of the same species for a behavioural comparison reduces the extent to which genetic rather than environmental effects could possibly explain behavioural variation, however, within-species genetic variation does occur. One way to gain an insight into the genetic variation between populations is to determine the extent to which morphology has diverged. Chapter 7 is a morphological analysis and comparison of populations used in previous chapters.

The results from chapters 2 to 7 are drawn together in the final chapter (8). In this chapter, the findings of the thesis are summarised and ideas for future research are suggested.

Chapter 2

Population differences in spatial learning, but not discrimination learning

Experiments 2.a, b and c are reported in Girvan, J. R. & Braithwaite, V. A. 1998. Population differences in spatial learning in three-spined sticklebacks. Proc. R. Soc. Lond. Series B, vol. 265, 913-918.

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2.1. Introduction

Many animals inhabit changeable, unpredictable environments. The ability to modify behaviour to suit conditions through learning is an adaptive benefit to animals living in changing conditions that has been illustrated in several recent studies. For example, the reproductive success of parasitic wasps (Braconidae) is related to the quality and number of hosts parasitised. Evidence has been found that many species of these wasps which occupy variable environments learn the olfactory and visual cues associated with finding suitable hosts (Turlings *et al.*, 1990). Potting *et al.* (1997), however, reported on a particular species of wasp, *Cotesia flavipes*, which occupies predictable, homogeneous environments e.g. fields of maize or grasses. In such a predictable environment, learning might be considered to have a lower adaptive value than in heterogeneous environments. Indeed, Potting *et al.* found that this particular species did not exhibit learning when locating host plants, but instead relied on innate responses to specific cues.

In addition to learning behaviour being influenced by the changeability of the environment occupied, memory has also been shown to be affected by heterogeneity. It might be expected that having a long memory for a particular behaviour would not be adaptive if the environment is constantly changing. Instead, obsolete memories should be discarded and new ones learned. An example of memory retention being influenced by environmental heterogeneity is provided by Mackney and Hughes (1995). Closely related forms of stickleback (Gasterosteidae) from marine, estuarine and freshwater habitats exhibited differences in foraging efficiency and the duration of memories for different foraging tasks. Those forms inhabiting more variable environments, and therefore experiencing greater prey diversity (i.e. marine and estuarine forms) had a shorter memory for foraging skills associated with particular prey types compared to the freshwater population. The freshwater population was sampled from a landlocked lake which was presumed to contain a smaller diversity of prey species. These results reflect that with less changeability in the available prey species, the ability to remember particular prey handling skills for longer is desirable.

More recent studies have taken the approach of using inter-population comparisons to investigate the effect of the environment on learning. One such example is the work of Carlier & Lefebvre on Zenaida doves, *Zenaida aurita*, living in Barbados. These can either forage as a group, or defend a feeding territory depending on the habitat occupied. Group foragers associate most often with conspecifics, while territorial birds must defend against both con- and heterospecifics. As a result of these different experiences, differences have arisen between the learning behaviours of the two populations. The group feeders learn foraging techniques more readily from the conspecifics they are familiar with, while in contrast, the territorial birds are slower to learn, but they learn more readily than group foragers from a pre-trained heterospecific tutor (Carlier & Lefebvre, 1997). These behavioural adaptations are doubtless adaptive to maximise foraging success of the doves from each of the two foraging types.

Habitat selection and foraging efficiency are therefore influenced by ecology. Orientation behaviour is also influenced in this way. Scapini & Borgioli (1997) compared the orientation behaviour of different populations of sandhoppers, *Talitrus saltator*. In this case, orientation strategy seemed to relate to the changeability of the environment, with more than one strategy being required to cope with changeable conditions. Sandhoppers from a narrow, clear beach primarily used the sun compass to orient seaward, while those from a wide beach covered in detritus, mainly used visual cues to orient towards sheltering microhabitats provided by the detritus.

The hypothesis I wanted to address in the first experiment was whether different habitats similarly influence the spatial strategies preferred by three-spined sticklebacks to solve two types of maze task. One type of maze was designed to determine whether sticklebacks used a learned sequence of turns, or algorithm to negotiate their way through the maze, and the second type, to see if they could rely on using visual cues acting as beacons marking the desired route.

The aim of experiment 2.b. was to test whether there were any differences between the populations in their general cognitive ability, boldness, adaptation to laboratory conditions or gross visual perception which could account for any behavioural variation discovered in experiment 2.a, or in the remainder of the project. This experiment investigated a type of non-spatial learning, discrimination learning. Discrimination learning is the ability to learn to distinguish between two different stimuli e.g. two different colours, patterns, flavours or sounds. Experiment 2.b. was a simple colour discrimination task, in which the same four populations as experiment 2.a. were required to learn to discriminate between two colours and associate the correct colour with a food reward. There is evidence to suggest that discrimination learning in fish and mammals is controlled by a different neural network than spatial learning (Packard *et al.*, 1994, Beigler & Morris, 1996, Salas *et al* ., 1996). The neural basis of one of these types of learning could therefore become modified by environmental pressures without the other type necessarily being similarly affected. Both types of behaviour were compared across the four populations in these first two experiments.

Experiment 2.c. was included as a control test to examine whether sticklebacks use olfaction to locate a food reward. Sticklebacks are known to have good vision, and a poor sense of smell (Wootton, 1976), however, it was necessary to ensure that the orientation tasks were being solved using spatial strategies rather than the fish simply following the odour of a food reward.

EXPERIMENT 2.a.

2.2. Aim

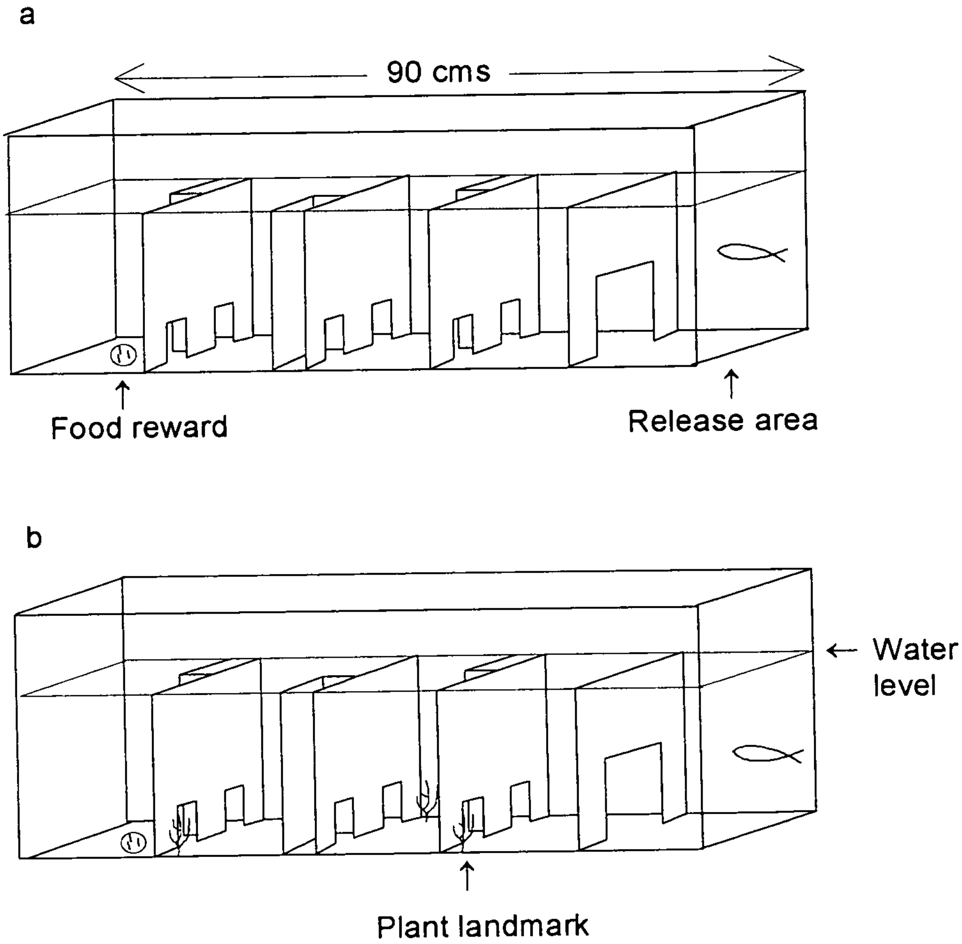
Experiment 2.a. was designed to test the ability of different populations of sticklebacks to negotiate a simple form of maze. This particular design of maze will be referred to as a 'line maze' (see figures 2.1a & b) to distinguish it from other forms of maze. Different types of orientation cue were available to the fish. Plant landmarks were available to half the fish from each population. These could be used as beacons to guide fish through the maze. Without the landmarks, the simple nature of the maze allowed the use of a learned sequence of turns, or behavioural algorithm, to find the correct route. Two pond and two river populations were tested to determine if habitat differences had any effect on preferences for the visual or non-visual orientation strategies.

2.3. Methods

Twelve three-spined sticklebacks were collected from four geographically distinct sites. Two pond and two river habitats were selected; Inverleith pond in the centre of Edinburgh next to the botanical gardens, Balmaha pond on the banks of Loch Lomond, the river Kelvin flowing through the centre of Glasgow to the Clyde, and the river Endrick which flows into the southern end of Loch Lomond (full habitat descriptions and precise locations provided in chapter 6). Fish were sampled outside the breeding season, which peaks between June and August, to prevent reproductive behaviour affecting the results. Each population was held in a different 30x40x30 cm holding tank, on a diet of chironomid larvae. All fish were given a four week settling period in the laboratory. The temperature was constant at 11°c, with a constant light:dark cycle of 10:14. All four samples were of similar mean body length (Inverleith: 4.40cm; Balmaha: 5.03cm; Endrick: 4.59cm; Kelvin: 4.77cm). The fish were individually tagged with coloured plastic rings attached to their spines during the fourth week of the settling period.

A line maze was set up in a 90x30 cm tank in 20 cm depth of water. To reduce the use of extra-maze cues, the tank was surrounded by black card. On one side, the card was raised 10 cm from the tank thus enabling the observer to watch the fish through a small slit in the card without disturbing the fish. Lighting was provided by two fluorescent lights which ran across the plane of the experimental tank but could not be seen from it. The tank was divided across its width by three plain walls made from plastic coated white card, 15 cm apart (see figure 2.1a). Each wall had two evenly spaced doors (6x3 cm). At one end of the tank there was a 15x30 cm release site with a trap-door which could be raised using a pulley system with minimum disturbance to the fish. To begin a trial, a single fish was carefully dip-netted from its holding tank and allowed to settle in the release area for five minutes. The trap door was then raised, but timing did not begin until the fish chose to swim out through the trap-door. At the other end of the tank a food reward of three bloodworms, secured in a Vaseline filled petri-dish of 3 cms diameter, was used to encourage the fish to swim through the maze. Each population was divided into two groups of six fish. Group 1 was tested in a plain maze with no landmarks present (NoLM) (figure 2.1a), while group 2 was tested in a maze containing visual landmarks placed next to open doors (LM) (figure 2.1b).

Figures 2.1a & b. Experimental set-up of line maze, (a) without plant landmarks and (b) with plant landmarks placed next to open doors



2.3.1. Pre-training

During a pre-training period, fish were familiarised with the mazes. Single fish were transferred daily from the holding tank to the release area, and after a 5 minute settling period, allowed to swim the length of the tank to obtain a food reward. At this stage, all the doors were open. For group 1, the maze had no landmarks present (NoLM). For group 2, landmarks (small plastic plants) were placed next to all the doors (LM). Five minutes after their first contact with the food reward, fish were returned to their holding tanks. The only opportunity to feed during the entire course of the experiment was while the fish were in the experimental apparatus, as fish were never fed in their holding tanks. After seven pre-training runs the fish had learned to swim through the doors and find the food. The maze was then modified for the training phase.

2.3.2. Training phase

During training, one door on each wall was modified to lead into a dead-end so that the fish now had to swim along a particular route in order to pass through the maze without getting caught in a dead end. Group 1 (NoLM) remained without landmarks (figure 2.1a), while for group 2 (LM) landmarks were only placed next to the open doors (figure 2.1b). Fish were given trials to negotiate the series of open and closed doors until they reached a criterion performance level of three consecutive trials where food was located in less than 150s, without any mistakes being made i.e. without any of the dead ends being entered. At the beginning of the training phase, some fish took up to 1200s to complete the task, while at the end of the training phase all fish took less than 150s to do so. Performance, therefore, improved greatly during training. Trials were between 36 and 48 hours apart to maintain a reasonable level of hunger in all fish, to sustain their motivation to complete the mazes. The number of trials taken to reach criterion performance level, the time from leaving the release area until first contacting the food and the number of times an incorrect choice was made upon first approaching a wall was recorded. When criterion was reached, the maze was subjected to one final modification for a single test trial.

2.3.3. Test trial

The open and closed doors were reversed, so that previously open doors now led to dead-ends, and vice versa. For group 2 (LM), the landmarks were moved to the new open doors thereby remaining reliable indicators of the route through the maze. Fish were given only one test trial in the modified maze. Again, the time from leaving the release area until first contact with the food reward, and the number of times an incorrect choice was made on the first approach to a wall were recorded for comparison with performance in the last trial of the training phase.

2.3.4. Data analysis

Data were tested for normality using a Kolmogorov-Smirnov test. Analysis of variance (ANOVA) and Fishers protected least significant difference (PLSD) *post hoc* tests were used to compare the four populations. These comparisons were run using Statview^R software.

2.4. Results

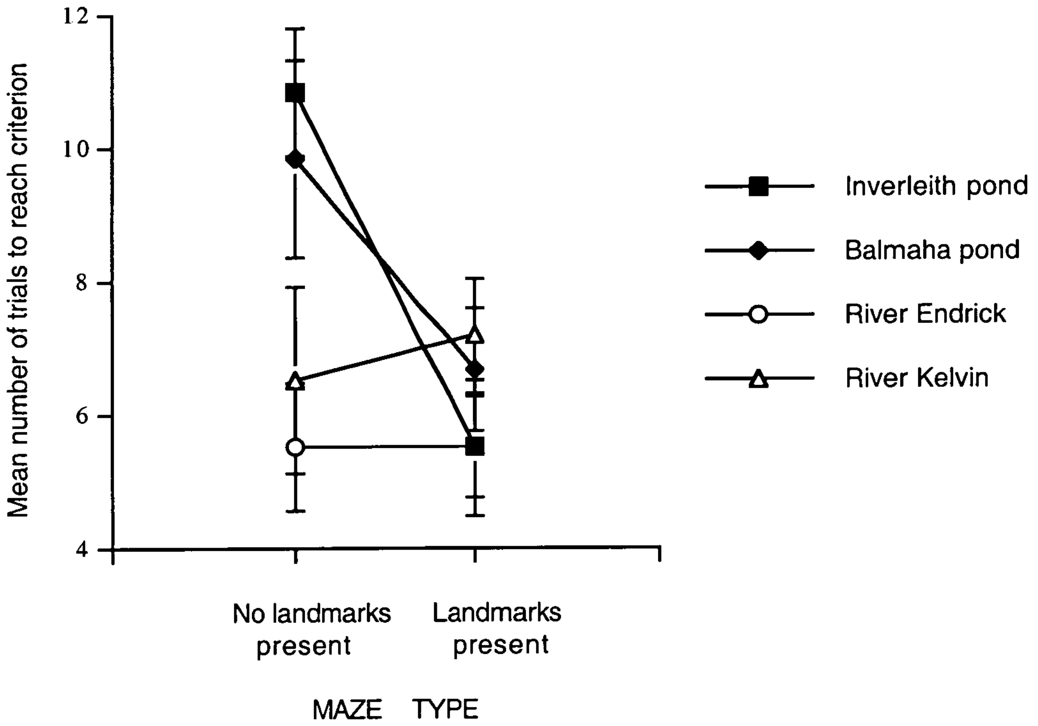
2.4.1. Training

The number of trials required to reach criterion was found to be normally distributed using a Kolmogorov-Smirnov test for normality (χ^2=2.042, p=0.721) i.e. there was no significant difference between the data and an ideal normal distribution. Parametric statistical tests could therefore be used. A two-way analysis of variance (ANOVA) with the number of trials to reach criterion as the dependent variable revealed a significant main effect of population ($F_{3,40}$=2.96, p=0.044). The type of maze (i.e. LM or NoLM) also affected performance ($F_{1,40}$=6.93, p=0.012) and there was an interaction between population and maze type ($F_{3,40}$=3.28, p=0.031).

To determine which populations differed in the number of trials to learn the task, a *post hoc* Fisher's (PLSD) test was carried out. This revealed that Inverleith and Balmaha pond populations took longer to learn the task without plant landmarks (noLM) than they did when they were present (LM). Both the river Endrick and Kelvin populations, however, were equally efficient at learning the task with and without plant landmarks in the maze.

The Endrick fish learned both mazes in significantly fewer trials than Inverleith and Balmaha pond populations ($p<0.02$ in both cases) (see figure 2.2).

Figure 2.2. Mean number of trials required by each group to learn the task ± standard error (the lines joining the points are not meant to suggest any relationship between them, but are simply intended to help comparisons be drawn between the different populations)



2.4.2. Test phase (reversal)

The time to complete the maze before and after the reversal was found to be normally distributed (Kolmogorov-Smirnov test: before; $\chi^2=2.667$, $p=0.527$: after; $\chi^2=2.042$, $p=0.721$). A t-test for paired data was used to compare performance before and after the reversal. Although all groups showed some increase in time to complete the reversed maze, this increase was only significant for the two river populations, regardless of landmarks (see table 2.1).

Table 2.1. t-test comparing mean time for each group to complete task before and after reversal (asterisks denote level of significance: * $p < 0.05$, ** $p < 0.02$).

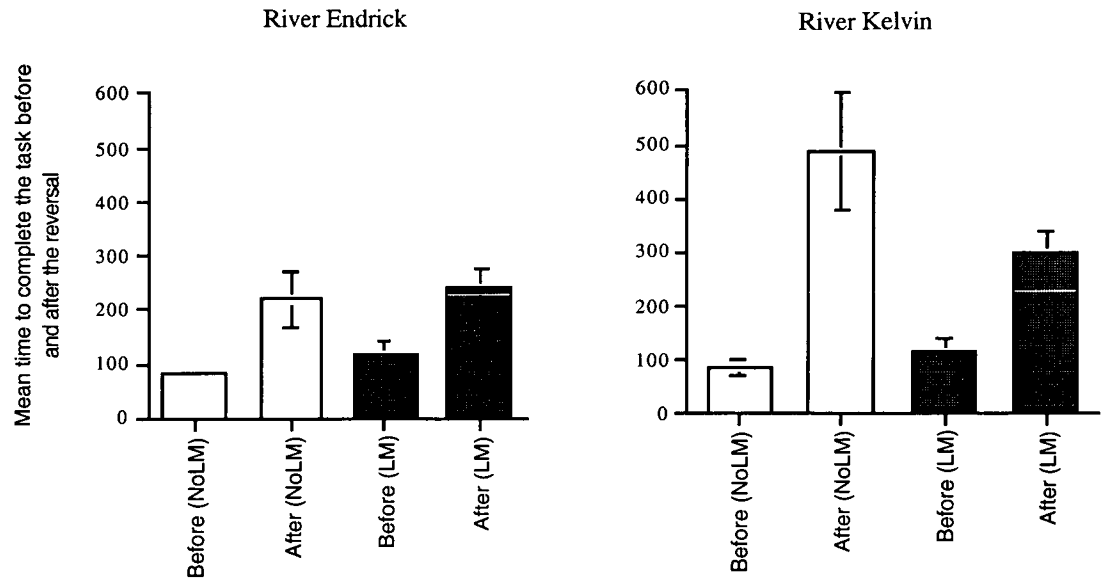
	No landmarks present		Landmarks present	
	t value	p value	t value	p value
Inverleith pond	-1.62	0.17	-1.57	0.18
Balmaha pond	-1.67	0.16	-1.49	0.20
River Endrick	-2.59	0.048 *	-2.61	0.047 *
River Kelvin	-3.71	0.014 **	-5.58	0.003 **

The two river populations exhibited significant increases in the time to complete both mazes after the reversal. To determine whether there were any differences in the extent of these time increases, an ANOVA was carried out comparing the two river populations. There was a significant main effect of population ($F_{1,20}=5.775$, $p=0.026$), but no effect of maze type on the change in performance, and no interaction between population and maze type ($F_{1,20}=2.988$, $p=0.099$; $F_{1,20}=2.274$, $p=0.147$; respectively).

To determine which groups differed, a Fisher's PLSD *post hoc* test was carried out. The Kelvin population without the landmarks present (NoLM), exhibited a significantly larger increase than all of the other groups ($p<0.035$ in each case) (see figure 2.3. overleaf).

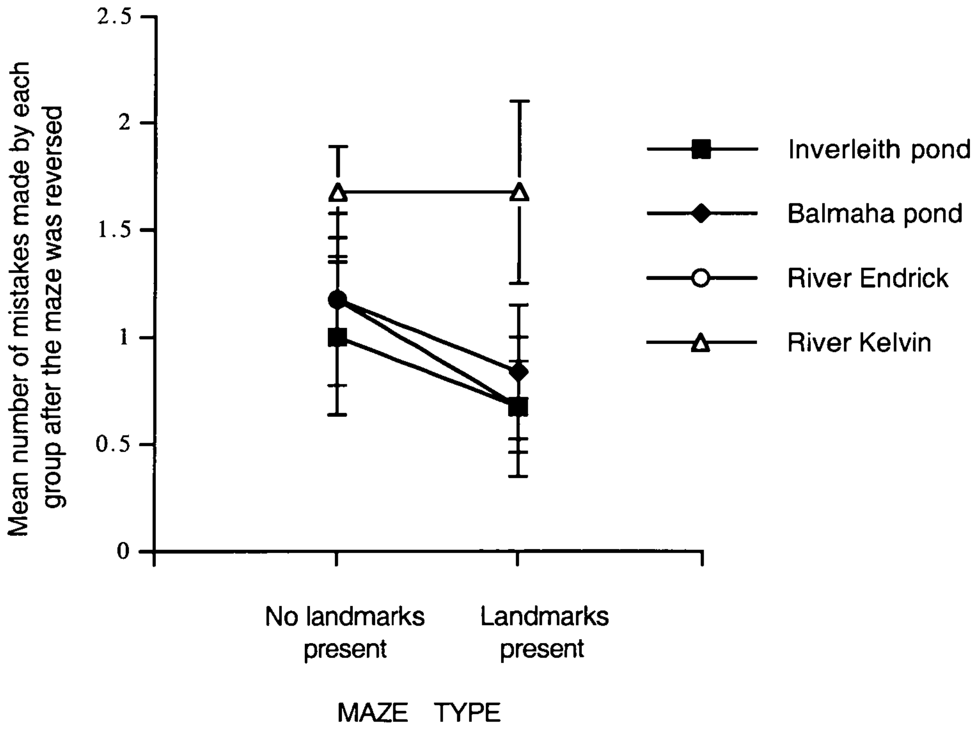
When the number of mistakes made by each of the four populations was compared between groups using an ANOVA, it was found that there was no difference between any of the groups before the maze was reversed ($F_{3,40}=0.29$, $p=0.83$). The same is not true after the reversal. There was a significant effect of population ($F_{3,40}=2.93$, $p=0.045$) (see figure 2.4. overleaf). A Fishers (PLSD) post hoc test revealed that Kelvin fish in both mazes (NoLM and LM) made significantly more mistakes than the other three populations ($p<0.05$ in each case).

Figure 2.3. Mean time for the two river populations to perform the task before and after the reversal ± standard error.



N.B. The standard error of the NoLM river Endrick group performing the maze before the reversal was 7, an error too small to be seen on this graph.

Figure 2.4. Mean number of mistakes made by each group after the maze was reversed ± standard error (the lines joining the points are not meant to suggest any relationship between them, but are simply intended to help comparisons be drawn between the different populations)



2.5. Discussion

When the number of trials to learn the task were compared, it was found that both pond populations were significantly slower at learning the route through the maze when they did not have the plant landmarks available (NoLM). Both river populations, however, performed equally well regardless of the landmarks. This suggests that the pond fish were paying attention to the landmarks, and used them to improve their orientation ability, while the river fish apparently ignored them. The river fish were presumably relying on some alternative strategy to negotiate the maze, such as learning the sequence of turns required i.e. turn left, turn right, turn left.

That the river fish were learning the sequence of turns is supported by their behaviour when the mazes were reversed. Fish that were learning a sequence of turns should be confused when the sequence is reversed, while fish following landmarks should not be confused by the reversal as the landmarks remain reliable predictors of the open doors. Considering the two river populations first, they both took significantly longer to complete the reversed mazes, even when landmarks were available. This suggests that they were not paying much attention to the landmarks, and were instead learning the sequence of turns. The two pond populations on the other hand, did not take significantly longer to complete the either mazes after the reversal (for both maze types). When plant landmarks were present, they could continue to follow these beacons to guide their path through the maze. Interestingly, without landmarks (NoLM), the two pond populations continued to perform the reversed maze task quickly. How can fish from Inverleith and Balmaha ponds still be able to complete the reversed plain maze quickly without the aid of landmarks? One possible explanation could be that the fish were able to detect and use a more subtle visual cue. Looking at the way the maze was constructed revealed that the amount of light passing through the open and closed doors differed. The closed doors were in shadow from the dead ends, while open doors were relatively lighter. Examination of the maze after the experimental work confirmed that doors leading to dead ends were on average 40% darker than open doors (measured by a Weston Master V light meter). It is possible that the Inverleith and Balmaha fish may have used these differences in door light levels as a form of landmark. This explanation implies that these populations preferred to rely on landmark information, either as conspicuous plastic plants or more subtle differences in light levels, rather than learning a sequence of turns. The difference in light levels could conceivably be more difficult to distinguish as a reliable landmark than the plants, and this may have increased the difficulty of the plain maze. This would translate into the increased number of trials that the Inverleith and Balmaha pond fish spent learning the correct route through the maze without plant landmarks.

When the mean increase in time for each group to complete the reversed maze was examined, one group showed a significantly greater increase than any other group. The Kelvin fish, without landmarks available (NoLM), took significantly longer than the other groups after the reversal. This result might be expected if the Kelvin fish were developing a behavioural algorithm to negotiate the maze, as suggested above. The large time increase suggests a high level of confusion after the reversal which would result if fish had been relying heavily on performing the sequence of turns. That the river Kelvin fish should develop the strongest algorithmic behaviour might be explained by the nature of the Kelvin habitat. This subject is developed further in the discussion section (2.13).

The Kelvin fish did not only show the greatest increase in time after the reversal, but they also made more mistakes than the other populations regardless of the presence of landmarks. This lends weight to the suggestion that they were relying on algorithmic behaviour to solve both mazes. When the mazes were reversed, they became very confused, making several mistakes and taking longer to solve the task when the mazes were reversed.

Differences in the spatial strategies used by the different populations seem to be explained by differences in the nature of the habitat occupied. It is important to ensure, however, that other unforeseen factors do not account for the behavioural variation.

For example, if there should be any differences in boldness or ability to adapt to and perform under laboratory conditions, then the strength of the above observations will be reduced. Indeed, differences in gross cognitive ability might also be able to account for the behavioural variation. These alternative explanations seem unlikely, as each of the four populations required a similar number of trials to learn the (NoLM) maze. However, it was thought appropriate that a simpler, different kind of task should be carried out to ensure that no differences in boldness or gross cognitive ability exist. To investigate this, a discrimination task was carried out in experiment 2.b.

EXPERIMENT 2.b.

2.6. Introduction

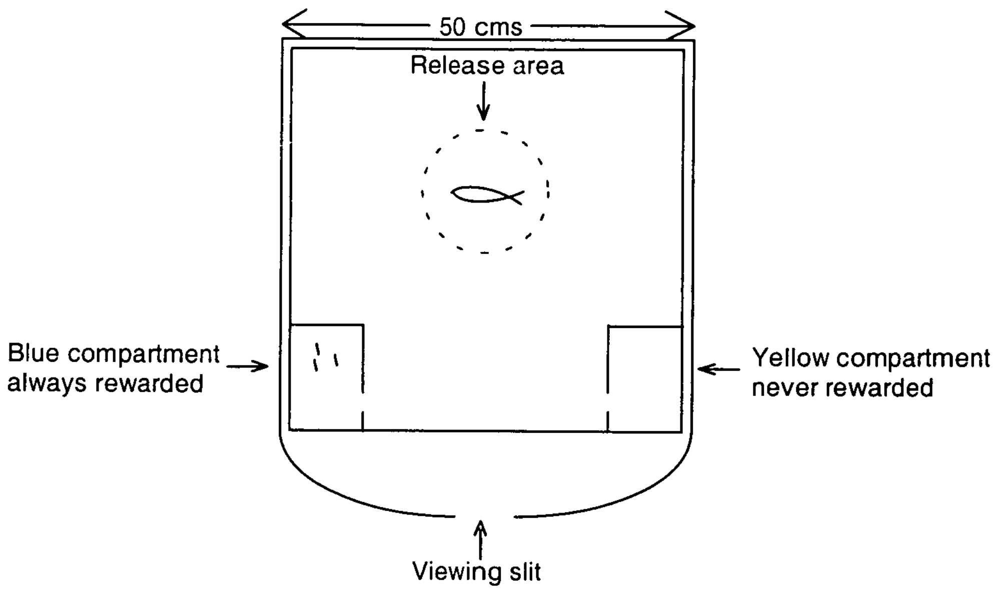
In order to investigate if any factors other than spatial ability could account for the population differences revealed in experiment 2.a, a simple discrimination experiment was designed. In experiment 2.b, fish had to discriminate between two different colours, and associate one with a food reward. There are examples from the literature citing evidence that fish can discriminate between visual stimuli. For example, Warburton & Lees (1996) found that guppies, *Poecilia reticulata*, used mainly visual information to discriminate between familiar and unfamiliar conspecifics when choosing shoal-mates, and Braithwaite *et al.* (1996) showed that juvenile Atlantic salmon, *Salmo salar*, could discriminate between visual patterns; crosses and straight lines of different colours. The discrimination test described here was designed to provide less of a challenge to the fish than the line maze of experiment 2.a, with only one foraging decision being made before the acquisition of the food reward. This contrasts with the line maze, where fish were required to make three consecutive correct choices of door before they could obtain the reward. I designed a simple discrimination task where fish needed to discriminate two colours of compartment, swimming into a blue compartment to collect the food reward, while ignoring a similar but unrewarded yellow compartment.

2.7. Methods

Ten fish were sampled from each of the same four populations in used in experiment 2.a. (the rivers Kelvin and Endrick, and Inverleith and Balmaha ponds). They were allowed to settle for four weeks in the laboratory under the same conditions (11°c, 10:14 L:D). Each sample was of similar mean body length (Inverleith: 4.48cm; Balmaha: 5.31cm; Endrick: 4.47cm; Kelvin: 4.80cm). During the settling period, fish were maintained on a diet of bloodworm, and were individually tagged as before.

The test tank was cubic (50x50x30 cm) and contained two compartments (3x4x15 cm) built from plastic building bricks (Lego). These were positioned in the two front corners of the tank (see figure 2.5) in a depth of 20 cms of water.

Figure 2.5. Plan view of experimental apparatus



The blue compartment always contained a food reward consisting of three bloodworm secured in a petri-dish of vaseline (3cm diameter). This petri-dish was attached to the wall above the level of the door, so that it was not visible from outside the compartment. The yellow compartment contained a similarly suspended unrewarded petri-dish. Pilot work revealed an observed preference for the fish to enter the yellow compartment. So that this apparent preference would not bias the results, the blue compartment was chosen as the one in which the food reward was always found. The two colours were randomly switched from side to side of the tank between trials to prevent the fish associating a position rather than a colour with the food reward. The test tank was screened using black card as described for experiment 2.a, with a narrow viewing slit through which the fish could be observed.

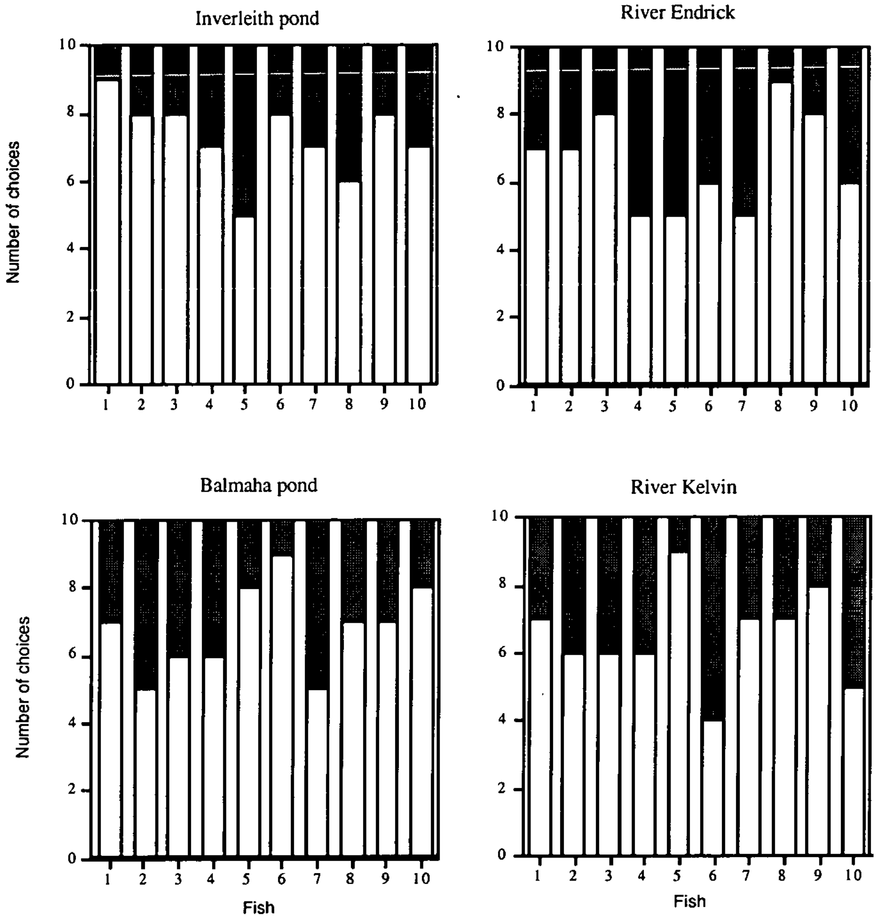
Separately, each population spent five 24-hour settling periods in the experimental tank, with the blue compartment baited copiously. During this time, the fish learned to enter the blue compartment, and became used to performing the task.

The test phase consisted of ten trials, one trial per fish per day (fish rested at least 36 hours between trials to maintain a high level of motivation to find the food reward). Fish were netted individually from the holding tank and placed in a cylindrical release chamber (see figure 2.5) A five minute settling period was allowed before fish were released into the experimental tank. The order in which the compartments were visited was recorded. After their first contact with the food reward, fish were left in the test tank for five minutes to reduce the chance of an association arising between completion of the task and stress associated with being netted from the experimental set-up. The number of correct choices of compartment was recorded for each fish.

2.8. Results

A Cochran test (Armitage & Berry, 1987) was used to test if the populations entered the correct compartment first in significantly more test trials than would be expected if the fish were selecting the compartments at random. In all four populations, sticklebacks entered the rewarded blue hut first on a significant proportion of the trials. Inverleith χ^2=11.3, $p<0.01$; Balmaha χ^2=4.34, $p<0.05$; Kelvin χ^2=5.21, $p<0.05$; Endrick χ^2=14.22, $p<0.01$ (see figure 2.6). When the four populations were compared to one another, no significant difference in the mean number of correct choices made by each population was found (Kruskal-Wallace test; K=0.25; p=0.969).

Figure 2.6. Number of correct and incorrect choices of colour compartments made by fish from each population. White bars represent the number of correct choices: grey bars represent the number of incorrect choices.



2.9. Discussion

No population differences were observed in the performance of the visual discrimination task. Each population was equally able to discriminate between the two coloured compartments, and to associate the correct colour with a food reward. That all four populations could perform this task to the same standard shows that no differences in boldness, adaptation to laboratory conditions or in gross cognitive ability exist between the populations which could explain the behavioural variation documented in experiment 2.a.

It is interesting to note that spatial ability differs between the four populations inhabiting different environments while discrimination ability does not. The environmental effects which apparently influenced orientation learning did not similarly lead to differences in discrimination learning. The apparent dissociation between spatial and discrimination learning and memory has been documented by previous researchers (Packard *et al.*, 1994, Beigler & Morris, 1996, Salas *et al* ., 1996). There is evidence to suggest that discrimination learning is controlled by a different neural network than spatial learning, and so it is quite possible that one of these learning systems should differ between populations of animals without the other necessarily doing so. For example, by administering *d*-amphetamine (a memory retention enhancing chemical) to different structures of the brain, Packard *et al.* (1994) discovered that rats, *Rattus norvegicus*, have two separate areas of the brain which deal independently with the retention of the two types of memory. Memory for a spatial task, where rats had to remember the spatial location of a submerged escape platform in a water maze, was enhanced by post-training injections into the hippocampus but not the caudate nucleus. Memory for a recognition task, however, where rats had to recognise a striped escape platform, was enhanced by injections to the caudate nucleus, but not the hippocampus. This showed that the hippocampus of the rat is involved in performing spatial tasks, but that the caudate nucleus is involved in controlling recognition ability.

Evidence in unmanipulated animals that dissociation between spatial and discrimination learning and memory exists has been provided by Beigler & Morris (1996). In an arena where only one visual landmark reliably predicted the location of a hidden food reward (i.e. the food reward was always in the same position in relation to the one correct landmark), rats were able to discriminate the reliable landmark from the unreliable one when both were moved around randomly between 49 possible positions. However, although the rats searched in the vicinity of the reliable landmark, they were unable to locate the exact position of the reward in relation to it. They remembered which landmark to search near, but they could not remember the correct distance from it, or even the correct side of the landmark on which to search despite its being asymmetrical. The same experiment was carried out with both landmarks always in the same position in relation to each other and the arena surroundings. In the stable set-up, the rats could not only locate the correct landmark, but also the correct relative position at which to search. This experiment suggested a dissociation between discrimination and spatial learning with respect to geometric stability because, while geometric instability did not affect the ability of the rats to discriminate the correct landmark from the others, it did affect their ability to find the correct position of the hidden reward in relation to the correct landmark. If the same learning system was being used for both types of learning and memory, then either both or neither of the two types should have been affected by stability.

The same trend for dissociation between spatial and discrimination learning and memory has been observed in fish. The fish brain differs greatly in structure from that of the mammal, and until recently, less was known about the function of the different structures. However, research has now shown that the fish telencephalon is responsible for spatial awareness, in the same way as the hippocampus in mammals. For example, Salas *et al.* (1996) showed that telencephalic ablation reduced the accuracy of performance of a complex spatial task in goldfish. In addition, although ablation interfered with performance of a spatial task, it had no effect on visual discrimination ability. Experiments 2.a & b. have provided results to support the conclusions of Salas *et al.*, that the same kind of dissociation found between discrimination and spatial learning in rats also occurs at some level in fish.

One important alternative explanation for the performance of the fish in experiments 2.a. and 2.b. has not yet been investigated in full. In experiment 2.a, spatial learning was suggested to be the skill controlling the performance of the fish, and in experiment 2.b, discrimination ability was implied. If the fish were able to locate the food reward using olfaction, then the strength of the observations on spatial and discrimination ability would be weakened. This was investigated in experiment 2.c.

EXPERIMENT 2.c.

2.10. Introduction

Although sticklebacks are known to have good vision, and to rely primarily on that sense during hunting (Wootton, 1976), it was necessary to ensure that the fish were not using olfaction to locate the food reward, rather than using their orientation skills to do so. If olfaction was involved, then less could be concluded about orientation or discrimination ability. Experiment 2.c. was designed to investigate this. Nine naive fish from Inverleith pond were given two compartments, one of which was baited with a food reward (chosen at random), in the same way as experiment 2.b. In this case, instead of having to discriminate between two colours, both compartments were white. If fish were still able to consistently locate the rewarded compartment, then olfaction would clearly be influencing performance.

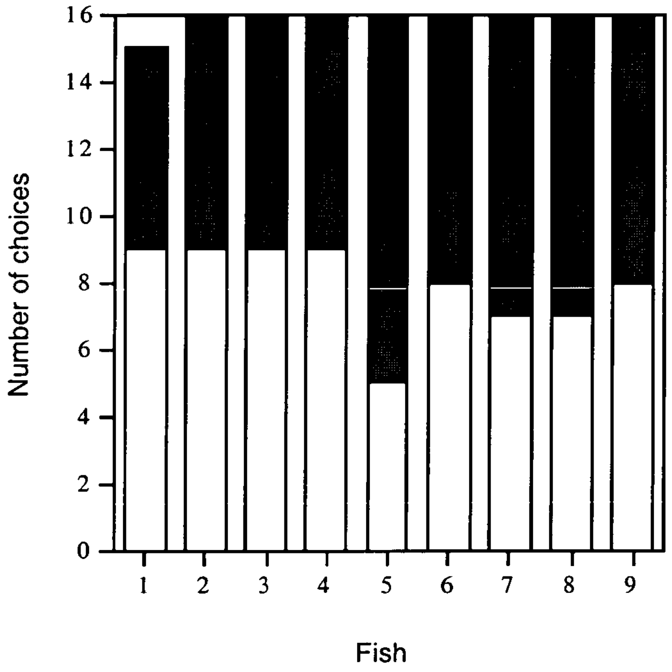
2.11. Methods

Aside from the colour of the compartments, experiment 2.c. was carried out in the same way as experiment 2.b. (see section 2.6), and under the same laboratory conditions.

2.12. Results

A Cochran test was carried out to see if the number of correct choices was different to that expected with random performance. Fish did not choose the rewarded compartment any more often than expected by random choice (Cochran test: $\chi^2=0.083$; $p>0.05$) (see figure 2.7).

Figure 2.7. Number of correct and incorrect choices of compartment. White bars represent correct choices: grey bars represent incorrect choices.



NB. Fish number 1 died after the fifteenth choice.

2.13. Discussion

Fish were not able to locate the food reward reliably in experiment 2.c. Rewarded and unrewarded compartments were entered a similar number of times, and so there cannot not have been any cues, olfactory or visual, available to the fish to indicate which compartment to enter.

No evidence was found that fish from the population tested (Inverleith pond) were using olfaction. The same findings were reported for both Inverleith pond fish and river Endrick fish in a similar experiment (Freer, unpublished data). This, in conjunction with the knowledge that this species has a poorly developed sense of smell, and the absence of olfaction being used in experiment 4.a. (see chapter 4) allow the conclusion that it is unlikely that the sticklebacks were using olfactory information to solve any of the tasks.

2.14. General discussion

Clear differences in spatial ability were found between the four populations tested in experiment 2.a. Fish from Inverleith and Balmaha pond populations took significantly fewer trials to reach criterion when the landmarks were present in the line maze than when they were absent. This suggests that the two pond populations were using the conspicuous plant landmarks to guide them through the (LM) maze. Without the visual landmarks (NoLM), they were still able to learn the correct route to the food reward, but it took them longer to do so. After the reversal, the pond fish continued to perform the mazes quickly, even without plant landmarks present. It was suggested that these fish could be following a more subtle visual cue to solve the NoLM maze, that of the different light levels between open doors and dead ends. This difference in light level may have been more difficult to learn than the conspicuous plant landmarks, and this might be the reason that the NoLM pond fish took longer to learn the task. Future experiments should remove the incidental visual cue of the differences in light levels of open and closed doors by shading the open doors with a similar construction as the dead ends which did not completely block off the door, or by constructing the dead ends from clear plastic, or plexiglass, which would not change the light level.

The river Kelvin and Endrick populations took the same number of trials to learn the mazes regardless of the presence or absence of the landmarks. This suggests that they did not rely on the plant landmarks to the same extent as the two pond populations, but may have used an alternative strategy. As the sides of the tank containing the maze were covered by black card and the tanks were under a uniformly white ceiling, the fish were unlikely to have been using global, extra-maze cues to learn the correct route. I suggested that the fish were likely to be using a behavioural algorithm i.e. a learned series of turns (turn left at first wall, right at the second wall etc.) to find the correct route through the mazes.

To investigate whether fish were using this algorithmic behaviour, all groups were given one test trial where the sequence of open and closed doors was reversed.

Only fish relying on the behavioural algorithm should be affected by this reversal. The two pond populations were not significantly affected by the reversal of either type of maze (NoLM and LM), but both of the river populations took significantly longer to perform the reversed task in both mazes, even when the landmarks were present to indicate the correct route. This would appear to be further evidence that the river fish did not learn to navigate the LM maze by following the plant landmarks, but instead learned the sequence of turns.

That the pond fish should rely on visual landmarks while the river fish prefer a different, non-visual cue might be explained by differences in their respective habitats. A river, with continuous current, would be likely to have less stable visual surroundings than a pond habitat without flow in it (see chapter 6 for full details). Fish inhabiting a changeable environment like a river might therefore learn not to rely on visual information when orientating, preferring instead a non-visual cue like the algorithm, while those in a more relatively stable pond habitat might learn to rely heavily on the arrangement of their surroundings to find their way around.

Differences became evident between the two river populations when the increase in time to perform the reversed task was compared between them. River Kelvin fish tested in the reversed plain maze (NoLM) showed a significantly greater time increase than any other river group (figure 2.3). This suggests that these fish developed the strongest algorithmic behaviour, as they were most confused by the reversal. After the reversal, both Kelvin groups (NoLM & LM) also made more mistakes than any of the other populations. Why should the Kelvin fish have developed stronger algorithmic behaviour than the river Endrick fish? The answer could lie in the nature of the two river habitats. The Kelvin fish were sampled from a eutrophic, river with a silty (i.e. unstable) substrate, poor visibility, much aquatic vegetation and a noticeable level of pollution. Such conditions may not promote the use of visual information for orientation since the visual surroundings are unlikely to be stable or indeed visible (see chapter 6 for details). The Endrick river, however, is clear and oligotrophic with little submerged vegetation and a largely mineral (i.e. stable) substratum. These conditions might not totally preclude something being learned about visual information since there is likely to be less variation in the surroundings, and more visual stability than the Kelvin. Fish from the Endrick might not therefore rely to such a high degree on non-visual orientation strategies as Kelvin fish.

Despite the strong algorithmic behaviour developed by the river Kelvin fish, some effect of plant landmarks was evident. Figure 2.3. compared the time the river populations took to perform both mazes before and after the reversal. The river Kelvin fish exhibited a significantly larger increase in time without landmarks than they did with landmarks present. This shows that these fish may have been paying some attention to plant landmarks after all. The effect of landmarks was, however, masked during acquisition of the task by a strong reliance on learning the sequence of turns.

Differences between pond and river fish in the degree to which they use visual cues might also be due to perceptual differences in the vision of the fish rather than differences in spatial strategy preferences. For example, some of the populations might have more acute vision, or better colour vision than others which would make the green plant landmarks or light level differences more obvious. The results from experiment 2.b, however, suggest that this is not the case. The four populations, in contrast to their spatial ability, exhibited no differences in a colour discrimination task. All four populations were able to discriminate between two different colours, and associate the correct colour with a food reward i.e. they could all recognise the two different colours to the extent that they could consistently discriminate between them. Even though in experiment 2.a, the two river populations did not rely primarily on visual information to negotiate the line mazes, the results from experiment 2.b. suggest that this was not the result of gross perceptual differences in the vision of the fish, but rather of preferences for different spatial strategies.

The environmental differences which appear to have led to variation in spatial behaviour did not have an equivalent effect on discrimination behaviour. Perhaps fish from all four habitats retained the same need to discriminate. For example, regardless of the differences between the pond and river habitats, fish from all the populations still need to recognise prey, and that is achieved by discriminating the colour and shape of potential prey items. Indeed, that spatial behaviour can evolve and vary between populations while discrimination ability does not, tends to support previous evidence that the two types of learning and memory are separate systems, controlled by separate parts of the brain.

Experiment 2.c, along with several other sources of evidence mentioned in section 2.12 suggests that olfaction is not being used by the sticklebacks to solve the learning tasks. This allows me to conclude that the tasks which fish were trained to perform in this thesis were likely to have been solved using purely spatial and discrimination ability.

2.15. Summary

In conclusion, it has been shown that differences in preferred orientation strategy exist between populations of three-spined stickleback which may relate to differences in the respective habitats occupied. Two pond populations preferred to rely on visual cues to negotiate a line maze, while two river populations preferred a non-visual strategy. There was evidence that one of the populations (the Kelvin) was noticing more than one type of orientation cue, with one type (the algorithm) being strongly favoured over the other (visual landmarks). In contrast to orientation ability, no evidence was found that there were any differences in discrimination ability. That one type of learning should differ, possibly owing to environmental influences, while the other type of learning did not differ, provides some support to the body of evidence suggesting that spatial and discrimination learning are controlled by separate systems. The results reported here also provide evidence that at least one of the populations was definitely not performing the tasks using olfaction to locate the food reward, and any behavioural variation between all four populations is unlikely to be explained by basic differences in boldness, adaptation to laboratory conditions or visual perception.

Chapter 3

Population differences in spatial strategy in a radial arena

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EXPERIMENT 3

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3.1. Introduction

In experiment 2.a, clear differences were found in the spatial strategies employed by different populations of three-spined sticklebacks negotiating a line maze. The fish were required to make a series of three sequential orientation choices which, when performed correctly, resulted in the acquisition of a food reward. Two pond populations relied primarily on visual landmarks to perform the task, while two river populations preferred to develop the necessary behavioural algorithm. In order to investigate the upper limits of the spatial ability of the sticklebacks, I designed the more complex spatial task, reported in this chapter. In this new task, the fish were trained to locate four potential foraging patches in an octagonal arena that presented a total of eight patches. Spatial *and* discrimination learning could be used by fish to locate the rewarded patches. To forage optimally in the arena, fish should develop an orientation strategy that allows them to visit the four rewarded patches while avoiding the unrewarded ones. Unlike experiment 2.a, in which fish progressed to a new part of a maze at each foraging decision, in the eight-sided arena, fish returned to the same central area after each decision. This could prove to be more confusing for the fish as it might be more difficult to remember which patches have already been visited. To investigate how two populations achieved this, each population was split into two groups. One group was given distinctive landmarks that could be associated with the rewarded patches. The second group did not have any distinctive landmarks, and had to rely on learning an algorithm to visit the correct patches. The pattern they needed to learn was to visit alternate patches as rewarded and unrewarded patches were arranged in an alternating pattern.

The eight-sided arena designed for experiment 3 had many features in common with the often-used eight-arm radial maze designs like those frequently used to test laboratory rats (see figure 3.1). There were eight possible choices of door, arranged around a common central platform like the spokes of a wheel, and like many of the original eight-arm maze experiments an item of food was located at certain arms

(Pearce, 1997). Various manipulations of the original eight-arm maze such as switching depleted arms with undepleted ones (Olton & Samuelson, 1976), confining rats to the central platform for a period between choices (Olton, Collison & Werz, 1977), forcing the choice of some of the arms (Roberts & Dale, 1981) and interrupting the choices with forced delays (Brown *et al.,* 1993) have suggested that the rats performance is based mainly on internal representations of spatial information. Interestingly, the rats did not select arms sequentially, and frequently had very irregular patterns in their sequence of arms visited (Olton, 1978). This irregularity has been taken to imply that rats solve the radial maze by forming an internal representation, or a map of the maze.

A contrasting result, however, was drawn from work with fish. Roitblat *et al.* (1982) found that in an aquatic version of the radial arm maze, Siamese fighting fish, *Betta splendens*, visited the eight arms with a high level of accuracy (i.e. visiting the different arms without repetition), but unlike rats, the fish usually visited the arms sequentially. This strong stereotypic response pattern suggested that the fish were relying mainly on the learned algorithm of always turning the same way upon leaving an arm, and entering the next one in the series. So it seems that Siamese fighting fish behaved differently to the rats in that they did not appear to form an internal representation or map of the maze.

I designed experiment 3 to determine how different populations of three-spined sticklebacks compared in their use of algorithm or visual cues to learn a task similar to an eight-arm radial maze. An octagonal arena was made, with each side of the octagon leading to a small compartment (food patch) through a door. One group (Group 1), consisting of half of the fish from two different populations, were given green plant landmarks placed above all eight doors. For a second group (Group 2), brown plant landmarks were placed above doors to rewarded compartments to indicate their position, with green plants above unrewarded doors.

To visit only rewarded, undepleted compartments, fish could develop algorithmic behaviour. The behavioural algorithm of always turning in the same direction upon leaving a rewarded compartment, and entering the next door-but-one could be used. Any fish using the algorithm will be revealed by their visiting the rewarded compartments sequentially. Developing a behavioural algorithm is regarded as being an egocentric strategy since it involves learning an internal response to the stimulus of the surroundings i.e. always turn left (Rodriguez *et al.,* 1994). Alternatively, for Group 2 (disLM) an association between visual landmarks and profitable patches could be formed. The use of visual landmarks is an external or allocentric strategy because the fish must attach importance to landmarks separate from themselves. There is also a chance that the fish could develop an internal map of the arena, memorising the relative positions of rewarded and unrewarded compartments. The formation of such a map would be implied by irregular (non-sequential), yet accurate visiting of the profitable patches in the absence of visual beacons.

EXPERIMENT 3

3.2. Methods

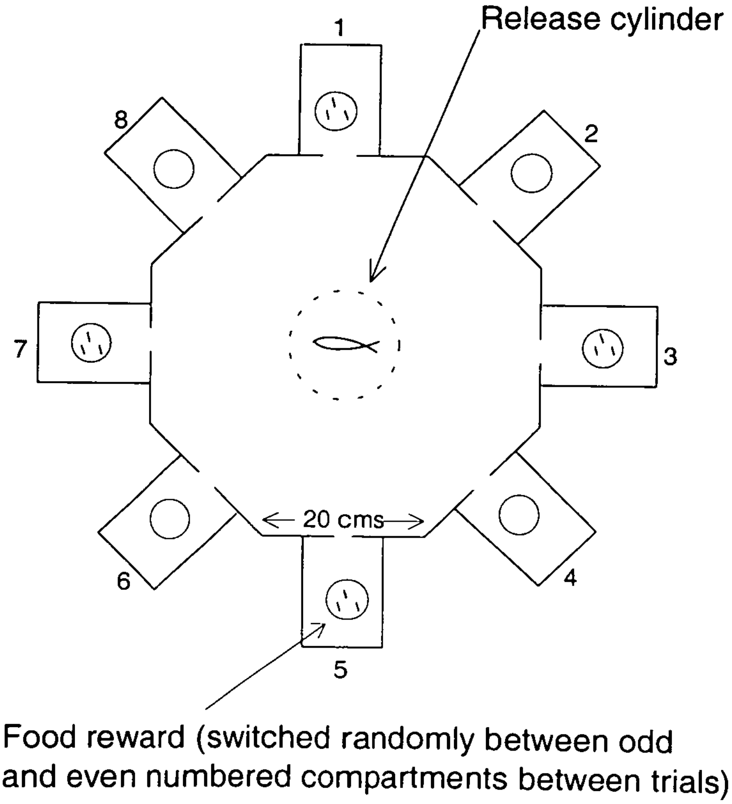
3.2.1. Subjects

Sticklebacks were collected from each of two geographically distinct populations. One sample was taken from Inverleith pond, Edinburgh (n=16), and one from the river Esk at Roslin Glen 6 miles south of Edinburgh (OS 626 267) (n=16). The habitats and their locations are described in full in chapter 6. The river Esk population was sampled instead of one of the two original river populations used in experiment 2.a, because at the time of collection, insufficient numbers of fish were found in the sampling areas of the rivers Kelvin and Endrick. In the laboratory, each population was housed in a 30x40x30 cm holding tank at 12°c, with a 12:12 light: dark cycle, and maintained on a diet of chironomid larvae (bloodworm). Fish were settled in the laboratory for four weeks before the experiment began and tagged in the same way as previous experiments (see appendix 1).

3.2.2. Apparatus

The octagonal arena was constructed from 3 mm dark green matt plastic board. It was placed in a 1.5 m diameter pool and filled with water to a depth of 18 cm. Each side of the arena measured 20 cm in length and height, and had a central 5 x 3 cm door (see figure 3.1). The doors led into 15 x 20 cm compartments or 'foraging patches' in the centre of which were opaque petri-dishes (3 cms diameter) filled with Vaseline. The dishes had high sides to prevent any food reward they contained being seen from outside the doors. In the centre of the arena was a circular, transparent release cylinder that was raised using a pulley system. Situated 1m above the centre of the arena was a Vantage CCD camera with a Computar 2.6 mm wide angle lens allowing the movements of the subjects to be viewed on a black and white video monitor set up next to the pool.

Figure 3.1. Plan view of octagonal arena.



3.2.3. Task

Fish were required to locate bloodworm secured in the Vaseline filled petri-dishes in alternate compartments of the arena. Each population was divided into two groups of eight. For Group 1 (no distinguishing landmarks; NodisLM), green plant landmarks were secured above all eight doors. There were therefore no visual landmarks indicating the rewarded compartments. For Group 2 (distinguishing landmarks; disLM), brown plant landmarks were secured above the doors of rewarded compartments, while green landmarks were secured above the doors to unrewarded compartments. The distinguishing brown plants indicated the rewarded compartments, hence (disLM). Group 1 (NodisLM), therefore, had no visual guidance to the correct compartments, while Group 2 (disLM) could potentially learn to use the colour difference in the plant landmarks to guide them to the food.

All groups from each population separately spent a 24 hour period in the arena. This pre-training procedure helped to reduce freezing or hiding behaviour when the fish were placed individually in the arena during training.

3.2.4. Training

Fish were trained individually until they reached a criterion performance level (see below). Each time a fish was given a trial in the arena, the position of the four rewarded compartments was assigned randomly between odd and even compartment numbers (see figure 3.1). This meant that global landmarks (visual cues outside the arena) did not reliably indicate the position of the rewarded compartments.

At the beginning of a training trial, fish were dip-netted individually from their holding tank to the release chamber and allowed to settle for 5 minutes. The release chamber was then raised, and the fish released into the arena. The time to enter or leave a compartment and the time at which a bloodworm was eaten were recorded. In addition, the order in which the compartments were entered was recorded to determine if fish visited the patches sequentially. When all four bloodworm had been eaten, a period of five minutes elapsed before fish were netted back to their holding tanks. Fish were given trials in the arena at an average of 64 hour intervals until they had reached a criterion performance level on three consecutive trials. The number of trials required by each group i.e. the speed with which the task was learned, could then be compared between groups. The criterion performance level differed slightly for Groups 1 and 2. In order to perform a trial correctly, Group 1 (NodisLM) had to enter all four rewarded compartments, and eat all four rewards with no more than one unrewarded compartment entered. In addition, if the first compartment entered was unbaited, then this choice was disregarded since Group 1 (NodisLM) had no way of knowing which compartments were baited until the first compartment had been entered. Group 2 (disLM) had to enter all four rewarded compartments and take the rewards with no more than one unrewarded compartment entered.

During pilot work carried out prior to experiment 3, it became clear that the fish seemed to reach a plateau in the level of accuracy with which they performed the task. As a result, the asymptotic level of at least three correct choices of the first four was chosen. One mistake was permitted for each group since the task appeared to test the fish to the limits of their ability.

3.2.5. Probe trial

Once fish reached criterion performance level, probe trials were carried out. These were different for the two groups. For Group 1 (NodisLM), the probe trial involved the food reward being absent from all compartments. For Group 2 (disLM) all eight of the plant landmarks were green, with alternate compartments baited as before. The probe trials were used to investigate which cues the fish were using to complete the task correctly. If the performance of Group 1 (NodisLM) decreased when the food rewards were absent, then the fish were presumably using the reinforcement of finding a food item in order to continue working through the algorithm. If the performance of Group 2 (disLM) decreased when all the plant landmarks were made the same colour, then these fish were presumably relying on the distinguishing plant landmarks to locate the correct compartments.

3.3. Results

To compare the performance of each population under the different conditions, the mean number of trials required to reach criterion was compared between groups using a two-way analysis of variance (ANOVA) with the number of trials to reach criterion as the dependant variable (parametric tests could be used because the data was normally distributed - Kolmogorov-smirnov test; c2=3.879, p=0.288). A significant main effect of population ($F_{1,29}$=22.02, p=<0.001) and group ($F_{1,29}$=10.81, p=0.003) was found. There was also an interaction between population and group ($F_{1,29}$=5.55, p=0.026). A Fishers *post hoc* PLSD test revealed that the Inverleith (NodisLM) group took significantly longer to learn the task than the other three groups, with a p value of <0.0005 in each case (see figure 3.2. overleaf).

Accuracy of search for a group was defined as the mean number of correct choices (to previously unvisited rewarded compartments) in the first four choices made. Mean choice accuracy increased over the first, third, fifth and the final trial of training for each group (see figure 3.3. overleaf) indicating that learning was indeed taking place.

By the end of training, all groups searched accurately, with a mean accuracy greater than 3. This means that all fish entered the four rewarded compartments without making more than 1 mistake. A number of fish from each group not only searched accurately, but also entered the rewarded compartments in sequential order e.g. visited compartments 2, 4, 6 then 8. In both populations, more fish searched sequentially when there were no distinguishing landmarks present (see table 3.1. on page 61). A Fishers exact test, however, revealed that this difference between Groups 1 and 2 was not significant for either population (Esk, p=1.37; Inverleith, p=0.18).

Figure 3.2. Mean number of trials to reach criterion performance for each group ± standard error.

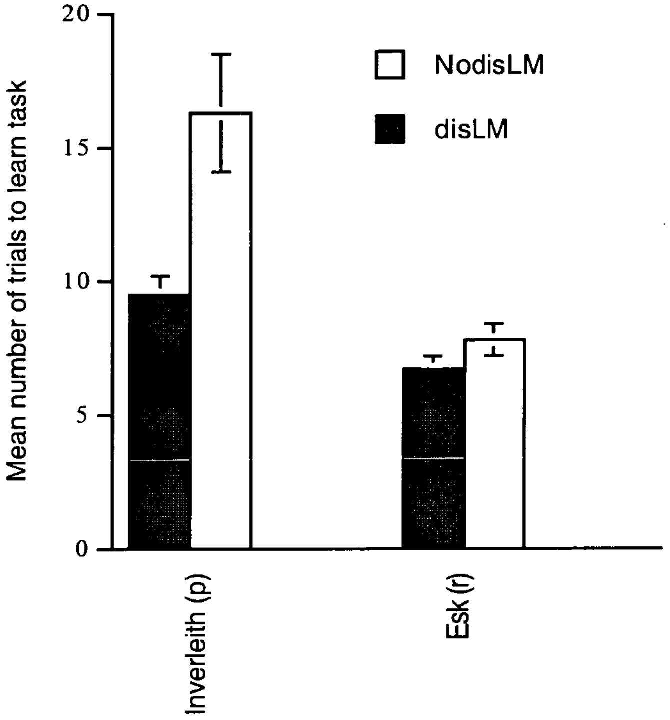
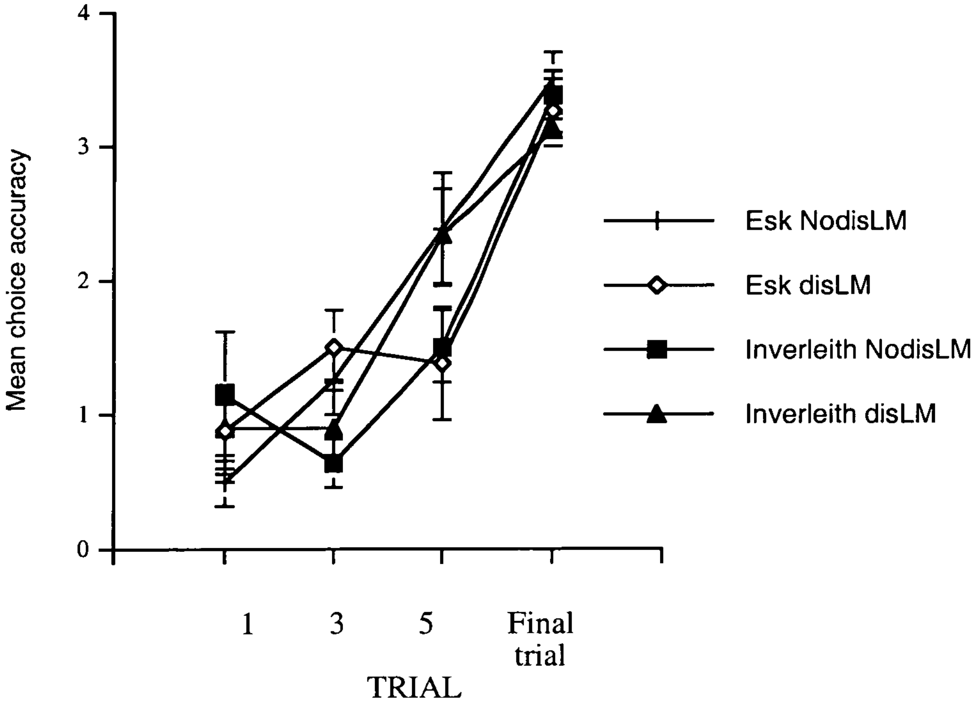


Figure 3.3. Choice accuracy on the first, third, fifth and final learning trial.



3.3.1. Probe trial: effects on searching behaviour

With the arena modified, very few fish from either Group 1 or 2 continued to search in the methodical, sequential pattern (see table 3.1).

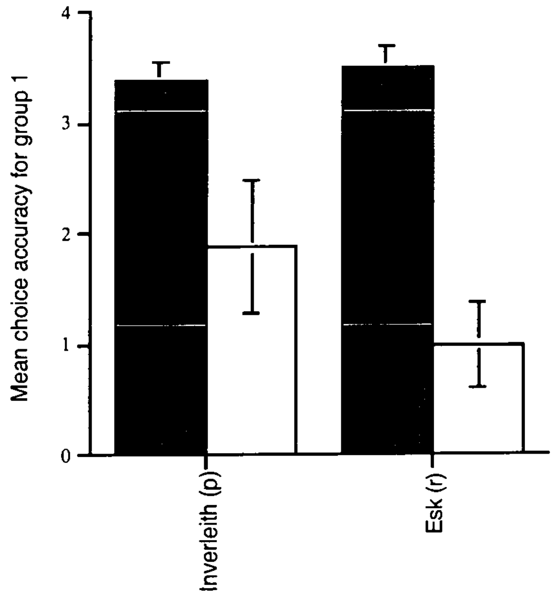
Table 3.1. Number of fish in each group entering the rewarded patches in sequential order in the last training trial, and the probe trial.

	End of training	Probe trial	Probe trial modification
Inverleith pond Group 1 (NodisLM)	6/8	3/8	Food reward removed
Inverleith pond Group 2 (disLM)	4/9	1/9	All plant LMs green
Esk river Group 1 (NodisLM)	6/8	2/8	Food reward removed
Esk river Group 2 (disLM)	3/8	1/8	All plant LMs green

The number of fish doing this was reduced in every group. A McNemar change test was carried out to determine if the decreases in the number of fish searching sequentially were significant. No significant effect of the probe trial was found (d.f.=1; $P>0.05$ in each case).

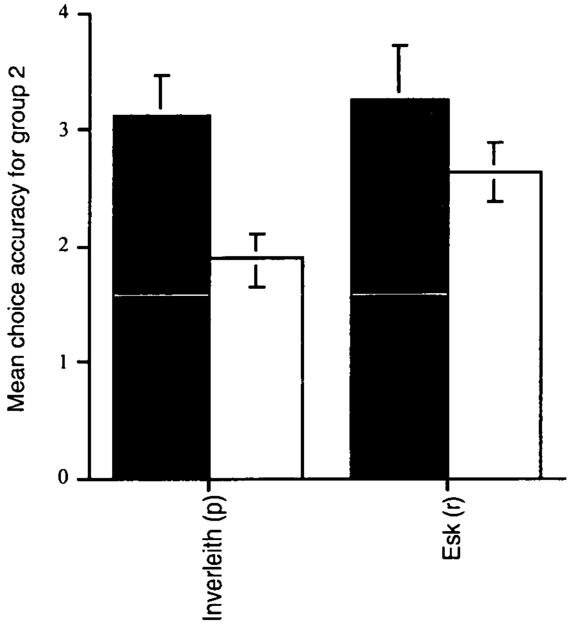
The modifications introduced in the probe trial did not only affect whether the fish searched sequentially, but also the accuracy with which they searched. The choice accuracy of Group 1 (NodisLM) for each population was compared in the last training trial and the probe trial (when the food rewards were absent from the arena). With the food present, all the fish were able to enter all of the rewarded chambers making no more than one mistake. Mean choice accuracy for the two populations was 3.38 (Inverleith) and 3.5 (Esk) correct choices out of a possible 4. However, when the food reinforcement was removed during the probe trial, accuracy dropped to 1 and 1.88 respectively (see figure 3.4. overleaf). To determine if accuracy during the probe trial was significantly lower than that of the final training trial, t-tests for matched pairs were carried out on each group. For Group 1 (NodisLM), the decrease in accuracy was significant for both populations (Inverleith; t=2.393, p=0.048: Esk; t=5.916, p=0.001).

Figure 3.4. Mean choice accuracy for Group 1 in the final training trial (grey bars), and in the probe trial (white bars).



For Group 2, the removal of the brown plant landmarks during the probe trial also reduced accuracy. Before the probe trial, the pond and river fish had a choice accuracy of 3.11 (Inverleith) and 3.25 (Esk), and in the probe trial, this was reduced to 1.89 and 2.63 respectively (see figure 3.5. overleaf). For Group 2 (disLM), the change in accuracy was significant for the Inverleith, but not the Esk population (t-test for matched pairs: Inverleith; t=-4.40, p=0.002: Esk; t=1.67, p=0.14). The removal of the distinguishing visual landmarks did not have as much effect on the accuracy of the river fish as it did on the pond fish.

Figure 3.5. Mean choice accuracy for Group 2 in the final training trial (grey bars) and the probe trial (white bars).



3.4. Discussion

When the number of trials required to learn the task was compared for the Inverleith population, it was found that Group 2 Inverleith fish (disLM) learned the task significantly more quickly than Group 1 Inverleith fish (NodisLM). Since the only difference between the groups was the presence or absence of distinguishing brown plant landmarks, Inverleith fish (disLM) are likely to have been using this visual cue to improve their performance and increase the speed with which they learned the task. A similar conclusion was drawn from the performance of Inverleith fish in experiment 2.a. The same is not true for the Esk fish which learned the task just as quickly regardless of the presence or absence of distinguishing landmarks. This suggests that, like the two river populations in experiment 2.a, they did not pay much attention to the landmarks while they searched for the food rewards. It seems unlikely that the Group 2 Esk fish did not pay attention to the brown landmarks because they could not distinguish them from the green ones. It is known that sticklebacks have good colour vision (Wootton, 1976), and other Scottish populations had no trouble discriminating blue and yellow in experiment 2.c.

No contrasting plant landmarks were present for Group 1 fish (NodisLM), yet both Esk and Inverleith populations were still able to learn the task. This could have been achieved by fish adopting a behavioural algorithm which resulted in only rewarded patches being visited i.e. upon finding a rewarded patch, fish left the compartment, always turned in the same direction and entered the next door-but-one. Any fish adopting this algorithm would enter the rewarded patches sequentially i.e. patches 3, 5, 7, 1, or patches 8, 6, 4, 2. Indeed, it can be seen from table 3.1 that in Group 1 (NodisLM) of both populations, 75% of trained fish searched the arena in this sequential pattern. The majority of Group 1 fish from both populations, therefore, appeared to adopt the behavioural algorithm to forage in the (NodisLM) arena.

Group 1 Inverleith pond fish took significantly longer than Group 1 Esk fish to learn the behavioural algorithm. This suggests that their preferred orientation cues are visual landmarks. When these are not available, a less preferred strategy has to be adopted. It is perhaps not surprising that Inverleith fish were slower at learning an arena with no distinguishing visual landmarks.

In general, the Inverleith pond fish appeared to be able to rely on two strategies to forage in the arena. They relied primarily on the contrasting plant landmarks to guide them, but when these were unavailable (i.e. for Group 1), they were also eventually able to learn the behavioural algorithm which would take them to the rewarded patches. The river Esk population did not seem to be able to use two different strategies. Esk fish appeared to rely only the behavioural algorithm, even with distinguishing landmarks present (disLM).

These results support the hypothesis put forward in experiment 2.a, that the use of visual information by fish during orientation is encouraged by the relatively stable visual surroundings found in a pond. The less stable surroundings found in a river might reduce the advantage of relying on a visual orientation strategy, and encourage river fish to rely on the non-visual strategy of the behavioural algorithm. A related experiment with interesting results has been carried out by Rodriguez *et al.*

(1994). Goldfish, *Carassius auratus*, were trained to locate a rewarded arm in a three armed maze by using either the allocentric (external) cue of global landmarks outside the apparatus, or the egocentric (internally linked) cue of always turning in the same direction out of the release arm (algorithmic behaviour). They found that goldfish were quicker at learning to rely on the visual landmarks to orientate than the algorithm of always turning in the same direction. This resembles the behaviour of the pond fish reported in experiments 2.a. and 3. In both experiments they, too, were quicker at learning a spatial task using visual cues than by learning the algorithm. Since the river fish did not exhibit this same performance, it is possible that this preference for using visual cues is a feature of fish inhabiting still water such as a pond.

During the probe trial, the distinguishing plant landmarks were removed from the disLM arena. Choice accuracy of these Group 2 (disLM) fish was affected by this change (figure 3.5). Accuracy decreased significantly for Group 2 Inverleith fish, but not for Group 2 Esk fish. This, again, supports the observation that Inverleith fish relied more upon the contrasting plant landmarks than the Esk fish since they were more affected by the removal of the distinguishing plant landmarks.

The choice accuracy of Group 1 (NodisLM) fish decreased significantly in both populations during the probe trial. The probe trial involved removing the food rewards from the arena. Without this reinforcement, fish from both populations could not continue to search the arena accurately.

There was a trend for some fish from each of the four groups to enter the rewarded patches in sequential order e.g. 2, 4, 6 then 8 (with approximately half of them moving clockwise around the arena, and half anti-clockwise). From table 3.1, it can be seen that although some fish in all the groups searched the arena in a sequential pattern, there was a trend for more fish in Group 1 (NodisLM) to follow this sequence, than those in Group 2 (disLM) which had the landmarks to guide them (true for both populations). It might be expected that Group 1 from each population, with no distinguishing landmarks, would be more likely to enter the patches sequentially, as they had to rely on memory for where they had just been, in order to decide where to go next. This memory task would be made easier if patches were searched in a methodical or sequential manner. Group 2 fish might have less need to enter the chambers sequentially since they always had the brown plant landmarks to guide them and therefore had to rely less on remembering where they had just been.

By the end of training, some fish were searching sequentially, while others were not. The sequential pattern may help the fish to remember which compartments have already been depleted, but for those fish which were performing the task accurately without the sequential pattern, and without the benefit of the plant beacons, some other spatial mechanism could have been influencing their performance. It has already been suggested that searching in a non-sequential pattern implies the use of an internal map, allowing animals to locate undepleted patches without having to search completely methodically. It is possible that fish that did not search sequentially, and yet still performed the task accurately without the distinguishing plant landmarks, were using an internal map of the arena to ensure that they did not revisit depleted patches.

That fish learned and remembered information about the task during training can be seen if choice accuracy is compared across a selection of trials during the training period (figure 3.3). Choice accuracy begins at a much lower level in trial 1 and increases gradually until the criterion level of accuracy is exhibited in the final trial of training. This suggests that fish were not using some other non-spatial method to find the food reward such as olfaction or by seeing the food items from outside the compartments. If fish had been doing so, accuracy would have been high even near the beginning of training.

Fish from the Inverleith population were involved in both experiments 2.a. and 3. Both Inverleith groups, with or without distinguishing landmarks, required more trials to learn experiment 3 than the corresponding groups in the line maze task of experiment 2.a (see table 3.3. overleaf).

Table 3.3. Mean number of trials for both groups of Inverleith pond fish (with and without distinguishing landmarks) to learn to perform experiments 2.a. and 3.

Exp 2.a. Inverleith (NoLM)	10.83 ± 0.95
Exp 2.a. Inverleith (LM)	5.5 ± 1.02
Exp 3. Inverleith (NodisLM)	16.25 ± 2.23
Exp 3. Inverleith (disLM)	9.4 ± 0.71

In addition, fish could be expected to perform the line maze (experiment 2.a) without making any mistakes, while for the eight-sided arena (experiment 3), an asymptotic level allowing one mistake was accepted. This suggests that fish found experiment 3 more difficult than experiment 2.a. In the arena, fish returned to the central area after every foraging decision. This may have created a certain amount of confusion as to which patches had already been visited. In the line maze, however, fish progressed on to another section of the maze after each decision, and so may have become less confused about where they had already been, and where they had to go next.

The results reported here show a similar pattern as experiment 2.a. Different populations of stickleback, again, preferred to rely primarily on different orientation strategies to perform a spatial task. Without visual cues available, fish relied on learning a behavioural algorithm, with a river population developing this behaviour much more quickly than a pond population. The pond fish performed much better when visual cues where available than when they were not, while the river fish did not appear to pay very much attention to them. It seems likely that the characteristics of the environments occupied by the different populations play a role in determining which strategy is preferred. It was also found that more than one orientation strategy can be used by Inverleith fish, depending on the types of information available.

River fish did not rely heavily on visual information, preferring instead the non-visual strategy of the behavioural algorithm. Another type of non-visual directional cue may also be used more effectively by river fish. The next chapter evaluates the importance of direction of water flow as a cue for orientation for two river and two pond populations.

Chapter 4

Water flow as a cue for orientation, and cue preferences in a conflict situation

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4.1. Introduction

4.1.1. Flow as a cue for orientation

Experiments 2.a and 3 have revealed population differences in the orientation ability of three-spined sticklebacks. In both experiments there was evidence that fish from two pond populations preferred to rely on visual landmarks to solve different types of maze task, while three river populations appeared to rely on alternative, non-visual methods, for example, learning the sequence of turns required to negotiate the line maze, or a more complex algorithm in the radial arena. One of the problems associated with testing fish in laboratory aquaria, however, is that any population sampled from a river will lose a very strong polarising cue, the direction of water flow.

Although few studies have expressly investigated whether fish can use direction of flow for orientation, some previous work does suggest that certain kinds of water movements can be used for orientation. For example, patterns of water movements between a fish and the surroundings can be picked up by the lateral line organ (lines of mechanoreceptors running along each side of the fish body) from which the direction and distance of stationary objects can be ascertained (Jobling, 1995). It has been shown that blind cave fish, *Anoptichthys jordani*, using the lateral line, can sense water movement between themselves and surrounding objects, and can use that information to learn about the layout of their environment (C. von Campenhausen *et al.*, 1981). In the example of the blind cave fish, water movements are generated by the fish itself but other examples show that naturally moving water can be used as an orientation cue. In plaice, *Pleuronectes platessa*, Metcalfe & Buckley (1997) found that the tidal stream (powerful horizontal currents created by tidal pressure gradients) is used to orientate during pre- and post-spawning migrations. Atlantic salmon smolts, *Salmo salar*, make downstream migrations, and some authors maintain that these are not entirely passive (Solomon 1978 & Kennedy *et al.* 1984). There is evidence that smolts actively seek out stronger currents to avoid being caught in sloughs and backwaters (Hansen & Jonsson 1985). The suggestion is therefore that these fish can distinguish direction and speed of water flow. Indeed, we assume that fish which migrate between fresh and marine waters must recognise the direction of water flow in relation to their own movements.

It is not yet known, however, whether non-migrating fish such as resident freshwater three-spined sticklebacks use direction of flow for local orientation movements. When attempting to catch sticklebacks in river habitats by sweep-netting near the banks, I observed that as the fish were disturbed, they quickly disappeared under the banks for shelter. In order to locate the banks quickly, fish could possibly be using flow as a directional cue. The ability to discriminate between upstream and downstream movement may, in the past, have been very important to the ancestors of freshwater sticklebacks as they migrated between marine and freshwater to colonise new habitats.

To determine whether sticklebacks from the populations tested in chapter 2 are able to orientate using direction of flow, I designed experiment 4.a. Here, the same four populations used in chapter 2 were required to locate a food reward when flow was the only reliable orientation cue. All other visual cues were randomised which made them unreliable and redundant.

4.1.2. Effect of habitat on behaviour

Two of the populations used (Kelvin and Endrick) were sampled from rivers, and therefore had extensive experience of flow, and two (Inverleith and Balmaha) were sampled from ponds and therefore had little or no previous experience of continuous flow. As a result, it was possible to compare the effects of inhabiting flowing and standing water on the ability of fish to use flow as a cue for orientation. Giles & Huntingford (1984) illustrated that the behaviour of three-spined stickleback can vary between populations occupying different habitats. Fish sampled from a habitat with many stickleback predators exhibited more effective fright responses than those sampled from a 'low-risk' site with few predators. A similar pattern might be observed in the orientation behaviour of sticklebacks. Those which occupy a habitat without flow (i.e. the two pond populations) may be less effective at using flow to orientate than those occupying a habitat where they are constantly exposed to flow (i.e. the two river populations).

In the experiment of Giles and Huntingford, with continual exposure to predator attacks, the fish from the 'high-risk' site may have *learned* better escape responses. Alternatively, fish with poor predator responses may have been *selected out* of the population over the generations. Similarly, in the current thesis, any observed differences in the use of flow to orientate may be learned during development in a pond or river habitat, or they could have become genetically encoded. This aspect of orientation behaviour is studied in Chapter 5 which investigates if any observed behavioural variation between the populations of stickleback used in this thesis is due to experience, or is a result of genetic differences.

4.1.3. Cue preferences

Having a degree of behavioural flexibility allows animals to adapt their behaviour to suit the demands of the particular environment they occupy. Animals will therefore be selected to develop behaviour which is useful and relevant in their surroundings. Such flexibility will result in different populations preferring to rely on different types of orientation cue. An example of this is provided by Wiltschko & Wiltschko (1989). They found that the environment under which pigeons, *Columba livia*, were raised influenced the type of orientation cue they subsequently used to help them orientate home. The cue that they experienced most in the environment in which they were raised was the type preferred in later homing experiments. Pigeons raised in roof lofts with access to the wind and olfactory cues from all directions relied more on olfactory cues than siblings reared in sheltered ground level lofts. The ground loft birds while sheltered from the wind, were forced to fly around the area regularly, becoming familiar with their surroundings. It was suggested that they were able to home successfully using visual cues.

Experiment 4.b was designed to determine whether sticklebacks from different habitats relied, like the pigeons of Wiltschko & Wiltschko's experiments, upon the particular cues which they experienced most regularly and reliably during their development. In this experiment, both flow and visual information predicted the location of a food reward for fish from a river and a pond population. Subsequently, a conflict situation was designed, to allow the extent of any cue preferences of the populations to be established.

4.1.4. Summary

Experiment 4.a investigates the use of flow as an orientation cue with the same four populations used in experiment 2.a. Experiment 4.b takes a subset of these populations (one river and one pond) to investigate whether any preferences exist for flow or visual information. It was intended that experiment 4.b should involve all four populations used in experiment 2.a, but sampling took place in January, a time of year when it is difficult to catch large numbers of fish. I was unable to obtain enough fish from all four habitats, and so had to confine experiment 4.b. to two populations.

EXPERIMENT 4.a

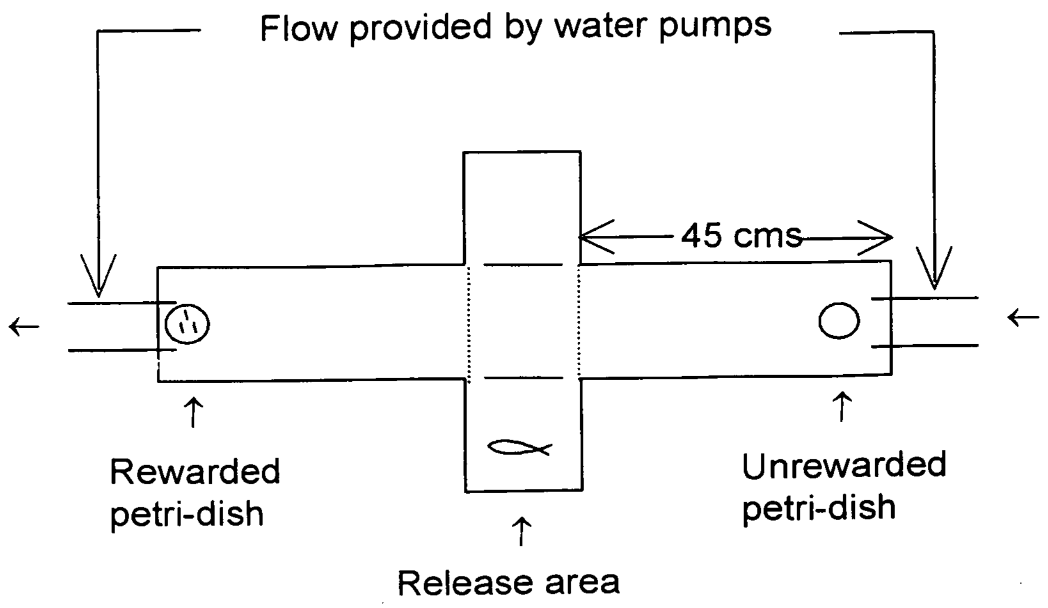
4.2. Methods

Ten three-spined sticklebacks were sampled from each of the same four sites as experiment 2.1 :- Inverleith and Balmaha ponds, and the rivers Kelvin and Endrick (see chapter 6 for full descriptions of sites). In the laboratory, each population was housed in a 30x40x30 cm tank and allowed to settle for 4 weeks with a 12:12 L:D cycle at 10°c. Fish were individually marked as before by the attachment of coloured plastic rings to the dorsal or pelvic spines (see appendix 1). Each group had a similar mean length (Inverleith; 4.5cms: Balmaha; 5.0cms: Endrick; 4.3cms: Kelvin; 4.7cms), and each population was divided into two groups of five fish. One group was required to swim upstream to find a food reward, and the remaining group was required to swim downstream.

4.2.1. Apparatus

The experimental apparatus consisted of a flume placed in a 1.5m diameter circular pool. The flume was 1m long and 15 cm wide constructed from 3 mm green matt plastic board. Two 15x15 cm release areas were located on either side of the mid-section of the flume (see figure 4.1). These were screened from the flume by trap doors which could be raised remotely by the experimenter. Water flow was provided by two water pumps (Hagan Aquaclear powerhead 402), one at each end. These could be set to pump or suck water, and so the flow through the flume could be set in either direction. The maximum flow in the flume was 0.7 m/s which was measured using a mini bucket wheel flow meter. The end walls of the flume were perforated so that the water level did not rise within the flume. Situated 1m above the centre of the flume was a Vantage CCD camera with a Computar 2.6 mm wide angle lens allowing the movements of the subjects to be viewed on a black and white video monitor next to the pool. Each population spent five 24 hour settling periods in the flume, with each population having one period, and then three days off while the other three populations took their turns. During these periods, a copious amount of bloodworm was placed at one randomly chosen end. Flow was absent on these occasions, and subsequently, fish were always tested individually.

Figure 4.1. Plan view of experimental flume.



4.2.2. Phase 1

Individual fish were given trials in the flume until they learned the task. At the beginning of a trial, a fish was introduced to one of the two release areas (varied randomly between trials) and after five minutes, the trap door was raised and the fish allowed to enter the flume at will. It is possible that some fish might have a preference for swimming up or downstream. This could bias the results, so each population was divided into groups that had to find food either upstream or downstream. Up and downstream groups will be compared to see if there are any differences in their performance.

The food reward was placed at the end of one of the flume arms secured in a 3 cm diameter petri-dish with a layer of Vaseline. To avoid the reward being seen from a distance, the dish was opaque, and 1.5 cms deep. An identical but empty petri- dish was placed at the unrewarded end of the flume. Thus fish were prevented from choosing the correct end simply by observing the presence of a dish at one end. Direction of flow through the flume was assigned randomly for each trial, so that visual cues either within the apparatus, or the experimental room, did not reliably predict the location of the reward. The only reliable cue was the direction of flow.

To complete a trial correctly, fish had to enter the rewarded arm and take the food before entering the unrewarded arm. The 'entrance' to each arm is indicated by a dotted line (see figure 4.1). Having taken the reward, fish were allowed two minutes before being netted and placed back in the holding tank. The delay was designed to avoid an association arising between eating the reward and any stress that may be induced by being netted. Trials continued at an average of 42 hour intervals until a criterion performance level was reached. This consisted of three consecutive trials performed correctly. The time to leave the release area, reach either end of the flume and take the food reward were recorded.

4.2.3. Phase 2

Once the criterion performance level for phase 1 was reached, the task was reversed for phase 2. Fish which previously had learned to swim upstream to obtain the reward now had to swim downstream and *vice versa.* Again trials continued until the same criterion performance level was reached.

4.2.4. Control trial

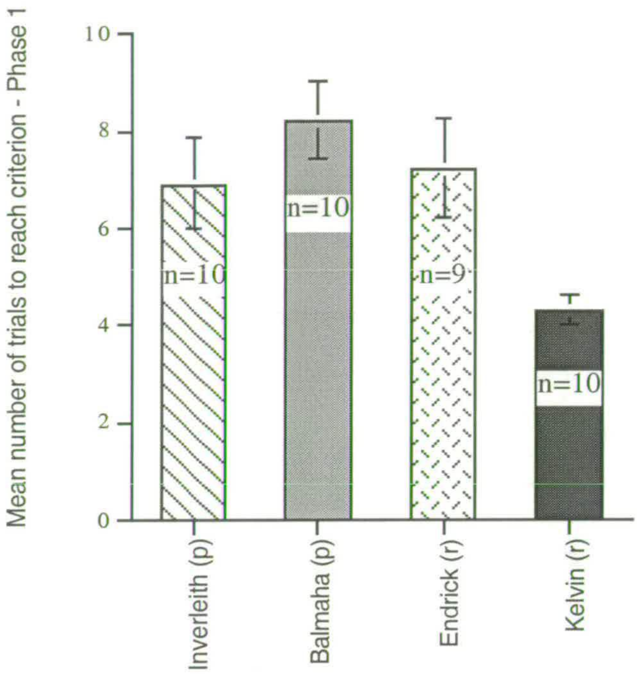
A single control trial was carried out at the end of phase 2. The water pumps were switched off for this trial, so there was no flow through the flume, and the reward was placed randomly at one end. The aim of this trial was to confirm that no factors other than the direction of water flow were being used by the fish to solve the task. The choices of arm made by the fish were recorded.

4.3. Results

PHASE 1: Using a Kolmogorov-Smirnov test, I found that the number of trials to learn phase 1 was normally distributed (χ^2=2.513, p=0.569). A parametric test was therefore suitable to determine whether the populations differed in the time they took to learn the task. A one way analysis of variance (ANOVA) was carried out with the number of trials to reach criterion as the dependant variable. This revealed a significant main effect of population ($F_{3,35}$=4.55, p=0.0085). A Fisher protected least significant difference (plsd) *post hoc* test showed that the population effect was due to the river Kelvin population learning phase 1 in significantly fewer trials than the other three populations (see figure 4.2. overleaf).

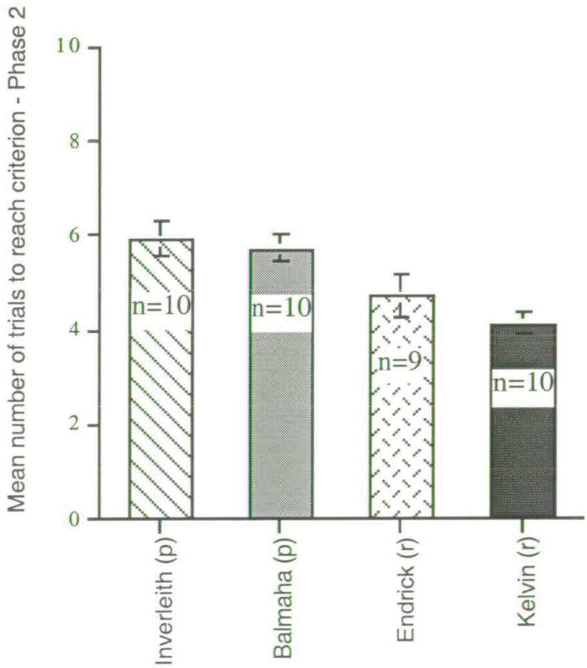
PHASE 2: The number of trials to learn phase 2 was also normally distributed (χ^2=3.282, π=0.388), and a second ANOVA carried out on phase 2 data. Again, there was a significant main effect of population ($F_{3,35}$=6.68, p=0.001). A Fisher plsd *post hoc* test revealed that both rivers Endrick and Kelvin learned phase 2 in significantly fewer trials than both pond populations (see figure 4.3. overleaf)

Figure 4.2. Phase 1: Mean number of trials the four populations required to learn to use flow to locate a food reward ± standard error bars (p = pond; r = river).



N.B. One of the Endrick fish died before completing phase 1

Figure 4.3. Phase 2: Mean number of trials all four populations required to learn to use flow in the opposite direction as phase 1, to locate a food reward ± standard error bars (p = pond; r = river).



COMPARING PHASES 1 & 2: To determine the effect of the reversal of the task, the mean number of trials required by each population to reach criterion in phase 1 was compared to those required in phase 2. Two of the populations learned phase 2 significantly more quickly than phase 1. These were Balmaha pond and the river Endrick (t-test for matched pairs: see table 4.1).

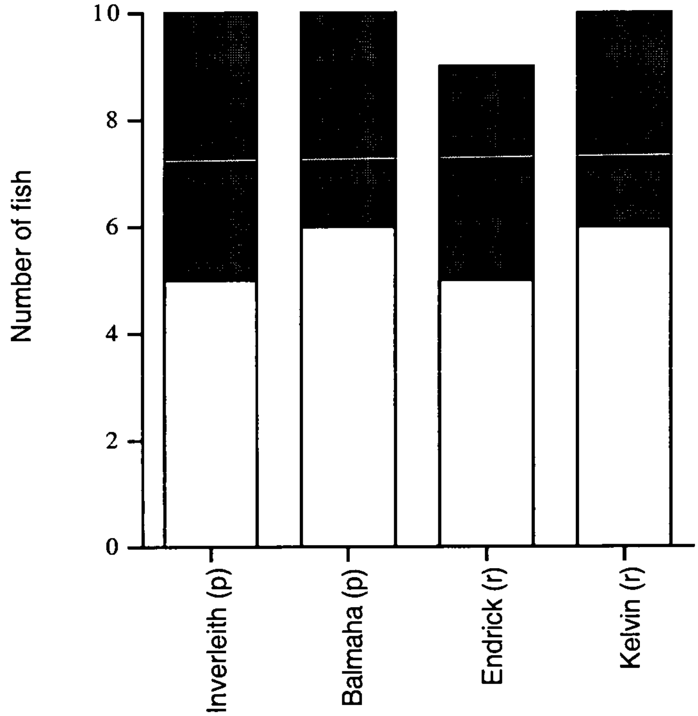
Table 4.1. Table showing t-test comparing the number of trials to learn phase 1 and phase 2, ** = $p<0.05$.

	Mean number of trials to reach criterion		
	Phase 1	Phase 2	t and p values
Inverleith pond	6.9	5.9	t=0.913, p=0.385
Balmaha pond	8.2	5.7	t=3.049, p=0.0138 **
River Endrick	7.2	4.7	t=2.234 p=0.050 **
River Kelvin	4.2	4.1	t=0.514, p=0.619

The river Kelvin population learned both phases in around the minimum number of trials possible (i.e. 3 - 4 trials). Fish from Inverleith pond learned phase 2 slightly, but not significantly faster than phase 1 (table 4.1).

CONTROL TRIAL: To compare the performance (the number of correct and incorrect choices) of the four populations in the control trial, a χ^2 contingency test was used. No association between population and performance was found ($\chi^2 = 0.61$, d.f. = 3, $p>0.05$). With no heterogeneity between populations, the data for the four populations were pooled, and a Cochran test carried out to determine whether fish were choosing the rewarded or unrewarded ends randomly (Armitage & Berry, 1987). Performance did not differ from that expected by chance alone ($\chi^2 = 0.82$, d.f. = 1, p>0.05) (see figure 4.4).

Figure 4.4. Number of fish choosing the rewarded and unrewarded ends of the flume when there was no flow. White bars represent the number of correct choices: grey bars represent the number of incorrect choices.



To determine if there was any preference for swimming upstream or downstream, a one-way ANOVA was performed on the number of trials to reach criterion in each phase with the direction of travel (upstream/downstream) as the dependant variable. No effect of direction on the number of trials to reach criterion in either phase was found (Phase 1; $F_{1,37}$=2.63, p=0.11: Phase 2; $F_{1,37}$=1.08, p=0.31).

4.4. Discussion

The river Kelvin fish took significantly fewer trials to reach the criterion performance level for phase 1 than the other three populations (see figure 4.2). In phase 2, both river populations, the Endrick and the Kelvin required significantly fewer trials to reach criterion compared to the two pond populations (see figure 4.3). The two river populations were therefore more adept at using direction of flow to orientate than the two pond populations. It seems likely that this is because the river fish were sampled from an environment where flow is a regular feature.

The Endrick fish were not as quick as the Kelvin fish to learn phase 1, although there was no difference in their performance of phase 2. This small difference in the ability of the two river populations to learn the task might relate to habitat differences between the two rivers. Section 4.9. discusses how the higher levels of pollution and vegetation in the Kelvin might encourage reliance on non-visual orientation cues such as flow. The Endrick, however, is clean and clear of vegetation and so may allow a certain degree of reliance on visual cues which could detract from the exclusive use of flow.

When the performance of each population was compared between the two phases, it was found that of the two pond populations, one improved performance significantly in the second phase (Balmaha), while the other one did not (Inverleith). No difference was expected between the ability of the two pond populations to use flow since neither was likely to have had experience of flow in their natural surroundings. Performance of the second phase, however, depended not only on the ability to orientate using flow, but also on the speed with which the first phase was forgotten, or discarded. The difference between them in their speed to learn phase 2 might be explained by a difference in the duration of memory due to a slight difference in the relative stability of the two pond habitats. This point is developed further in the general discussion (see section 4.9).

During the control trial, with no flow through the flume, performance in all populations was reduced to chance levels. This confirms that fish were using only flow to locate the rewarded end of the flume.

No preference for swimming up or downstream was discovered. Fish were just as quick to learn the task regardless of the direction in which they were required to swim. This result adds weight to the conclusion of experiment 2.c, where Inverleith pond fish did not use olfaction to locate a rewarded chamber (see section 2.9.). In the current experiment, fish which learned to swim upstream to reach the food reward, began each trial downstream of the reward, and yet these fish were no faster at learning to locate this reward than fish which began each trial upstream of the reward. Olfaction was therefore unlikely to have been used by the fish to perform this task.

EXPERIMENT 4.b

4.5. Introduction

The behavioural flexibility which benefits animals living in unpredictable environments can result in differences in the type of orientation cue being preferred by the adult animal. Able (1993) suggests that 'differences in cue weighting could reflect the results of developmental flexibility in response to varying availability or reliability of orientation information'. This is well illustrated by Wiltschko & Wiltschko (1989) who found pigeons preferred to use olfactory cues to home when they had been reared in roof lofts with access to the wind from all sides. Siblings reared in a sheltered location did not rely so heavily on olfaction (see section 4.1.3).

Experiments 2.a, 3 and 4.a. have revealed a trend for sticklebacks to prefer to use the type of orientation cues which are present in their natural habitat. For example, experiment 4.a showed that river fish were more adept at using direction of flow, a regular feature of their environment, to orientate compared with the pond fish. The same two river populations were, however, less adept at using visual information in experiment 2.a. This may be because visual information is less reliable in a habitat possessing a current. Furthermore, pond populations in experiments 2.a. and 3 showed preferences for using visual cues to solve spatial tasks.

To investigate these preferences for different types of cue further, experiment 4.b. was designed. The fish were trained to find a food reward with both flow and visual information predicting the location. Once the fish had learned the food location and were consistently visiting this site, the two types of cue were placed in conflict with each other. The flow and visual landmarks now directed the fish to different locations. The choices made by fish in this conflict situation could therefore reveal the extent of any orientation cue preferences.

4.6. Methods

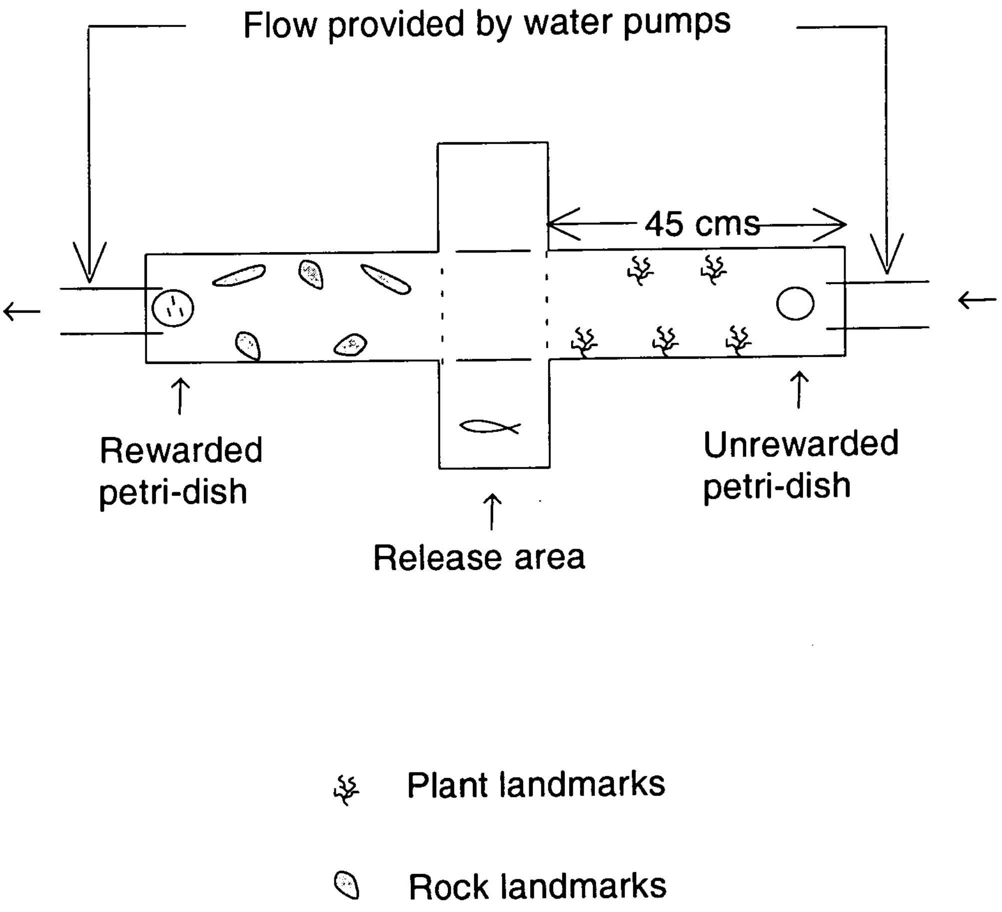
4.6.1. Subjects

Ten three-spined sticklebacks were sampled from two populations, the river Kelvin and Balmaha pond. These were the only two sites from which enough fish could be sampled. The fish were accustomed to the usual laboratory conditions and individually marked as usual. Each group had a similar mean length (Balmaha; 4.6cms: Kelvin; 4.4cms).

4.6.2. Apparatus

The experimental apparatus was the same as that used in experiment 4.a, with the addition of small visual cues (rocks and plants) placed inside the flume (see figure 4.5).

Figure 4.5. Plan view of experimental apparatus



Flow was provided in the same way as experiment 4.a (described in section 4.2.1). One arm of the flume was lined with five rock landmarks, while the other was lined with five plant landmarks. As in experiment 4.a. the apparatus was viewed using a camera situated 1 meter above the centre of the flume, which allowed the movements of the fish to be remotely viewed on a video monitor. During five 24 hour settling periods in the flume each group was fed copiously with bloodworm placed at one end of the flume which altered randomly between settling periods. Flow and landmarks were absent on these occasions.

4.6.3. Task

Fish were introduced singly to one of the two release sites (chosen randomly) and after five minutes, the trap door was raised and the fish was allowed to enter the flume at will. All fish were required to swim downstream to the rock end of the flume to locate the food reward (three bloodworm). The populations were not divided into up and downstream swimming groups as they were in experiment 4.a. because it has already been shown that there is no preference for swimming up or downstream. The food reward was secured in an opaque 1.5 cm deep petri- dish, and so could not be viewed from a distance. An empty petri- dish was placed at the unrewarded end of the flume so fish could not choose the correct end of the flume simply by the presence of a dish. The direction of flow through the flume was assigned randomly for each trial. This precluded the use of any visual information in the experimental room for orientation. Only direction of flow and the internal visual landmarks reliably indicated the location of the reward. To complete a trial correctly, fish had to reach the correct end and take the food reward before entering the unrewarded end. The dotted lines in figure 4.5. indicate the 'entrance' to each arm of the flume. Having taken the reward, two minutes passed before fish were netted and placed back in their holding tank. Trials continued at 48 hour intervals until a criterion performance level had been reached. This consisted of three correct consecutive trials. The time to begin the task, reach either end of the flume and complete the task was recorded.

4.6.4. Probe trial

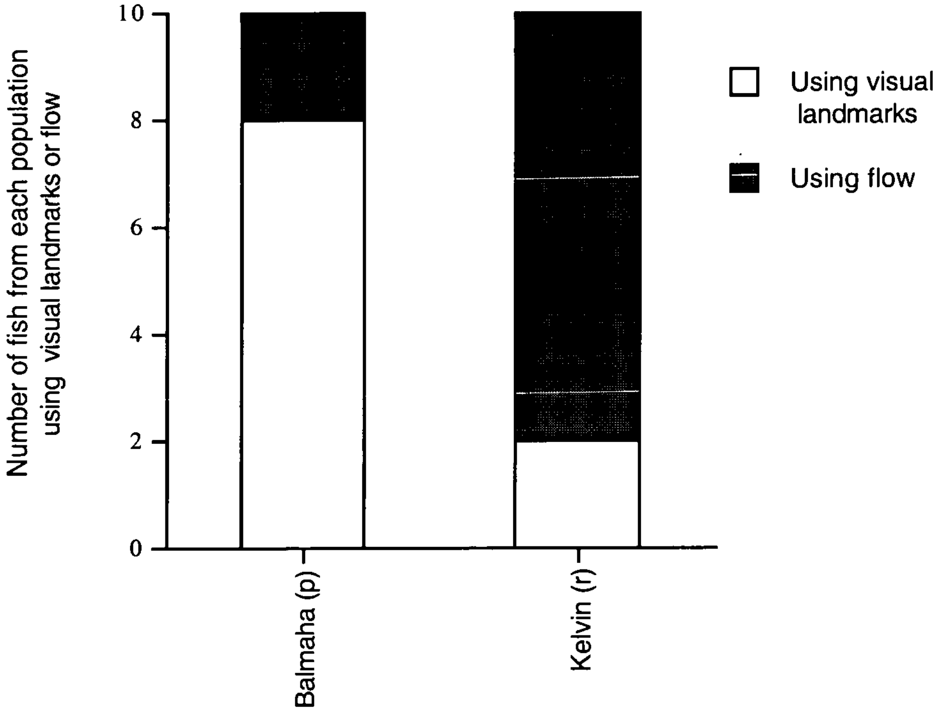
Having learned the task, fish were subjected to one conflict test trial. Here, the two possible orientation cues (flow and landmarks) conflicted with each other. The rock landmarks were switched with the plant landmarks so that the rocks were now upstream instead of downstream of the release areas. No food was present, and the first flume arm which each fish chose to swim down was recorded. Fish could either choose an arm in relation to the correct landmarks, or the correct direction of flow. The number of fish in each population making each choice was recorded.

4.7. Results

The number of trials required by fish to learn the task was normally distributed (Kolmogorov-Smirnov test; $\chi^2=2.5$, $p=0.57$). A parametric t-test was therefore used to determine whether the two populations differed in the time they took to learn the task. There was no significant difference between the two populations ($t=-021$, $p=0.84$).

There was, however, a significant difference between the number of fish in each population choosing to follow a particular type of cue (Fisher's exact test for 2 x 2 tables; $p=0.01$). The majority of Balmaha pond fish (8/10) followed the visual landmarks, and the majority of river Kelvin fish (8/10) followed the direction of flow (see figure 4.6).

Figure 4.6. Number of fish from each population preferring to follow visual landmark or flow information in the probe trial. White bars represent number of fish using visual landmarks: grey bars represent number of fish using flow.



4.8. Discussion

There was a significant difference between the number of river and pond fish choosing to follow the directional information indicated by flow. Significantly more of the pond fish preferred to rely on visual information, while significantly more of the river fish relied on direction of water flow. Both populations therefore preferred the particular cue which is likely to be most reliable in their natural habitat.

In addition, while in experiment 4.a. river Kelvin fish were quicker than Balmaha pond fish at learning to locate a food reward using flow, a contrasting result was obtained in experiment 4.b. In 4.b, visual information was available as well as flow, and as a result there was no difference in the number of trials each population required to learn the task.

4.9. General Discussion

The results from experiments 4.a. and 4.b. revealed that direction of flow can be used as an orientation cue by sticklebacks, the extent to which flow is relied upon varies between populations, and given the choice, the river Kelvin fish showed a clear preference for flow over visual cues while Balmaha pond fish preferred visual cues over flow.

In experiment 4.a, the river Kelvin fish took significantly fewer trials to reach the criterion performance level for phase 1 than the other three populations (figure 4.2). In phase 2, both river populations, the Endrick and the Kelvin required significantly fewer trials to reach criterion than the two pond populations (figure 4.3). Taken together, these results show that the river fish were more adept at using direction of flow to locate the reward than pond fish.

When the two river populations are compared with each other, it can be seen that the Kelvin fish were slightly better than the Endrick fish at learning the task since they learned phase 1 in significantly fewer trials. This difference in performance between the two river populations could be explained by differences between the two river habitats. The Kelvin fish were sampled from a polluted urban river (Clyde River Purification Board, 1994) which implies poor visibility (see chapter 6 for full details). Visual landmarks such as rocks and debris would frequently be obscured by suspended solids, and the vegetation and silty substrate would potentially make unreliable visual cues since they would be shifted over time by the current. This being the case, the direction of water flow may be the only consistently reliable orientation cue available to Kelvin fish. The Endrick, however, is an oligotrophic water body (low nutrient content, low primary productivity) with good quality clear water (Best & Traill, 1994). There is therefore good visibility, and the substrate is made up of stable bedrock and large rocks and virtually no aquatic vegetation. Although both the Endrick and Kelvin fish inhabit flowing water, the Endrick fish may in addition, have been able to learn something about the use of visual information. With some access to visual cues which are not available to the Kelvin fish, the Endrick fish might have a slightly lower preference for using direction of water flow as a spatial cue and may pay at least some attention to visual cues, and this might explain the slight difference in their ability to learn to use flow for orientation.

Both river populations learned phase 2 of experiment 4.a. significantly more quickly than both pond populations, and this could again be explained by their having more experience of flow. In order to learn phase 2, however, the information learned during the first phase must be discarded. The ease with which phase 2 is learned could be affected not only by previous experience of flow, but also by the duration of a fish's memory. Fish with shorter memories will be able to discard previously learned information more quickly, enabling them to learn new information. Mackney & Hughes (1995) provide evidence that sticklebacks inhabiting niches made relatively unstable by tidal and wave action (an estuary and the sea) exhibit shorter memory for learned tasks than sticklebacks taken from a more stable habitat (a land-locked lake).

A long-term memory for an event or a location may not be adaptive if the surroundings are constantly changing. The riverine sticklebacks used in the current study inhabit an environment which is relatively more changeable than that occupied by the pond populations owing to the river's current disturbing the physical arrangement of the surroundings. Riverine fish might therefore have shorter memory for a learned task. In other words, they may forget previous learned information and acquire new memories more quickly than pond fish under changing circumstances.

Balmaha pond fish learned phase 2 of experiment 4.a. in significantly fewer trials than phase 1, while Inverleith pond did not learn phase 2 any more quickly than phase 1. It is interesting that the two pond populations did not show the same improvement in phase 2. As both pond habitats have the same lack of flow, no differences in previous experience of flow could explain why one of the pond populations should learn phase 2 significantly more quickly than it learned phase 1 while the other pond population did not. This could instead reflect a slight difference in the two pond habitats. A comparison of the different habitats in chapter 6 reveals that Balmaha pond might be slightly less visually stable than Inverleith due to the growth and dying back of the large amount of vegetation which Balmaha pond supports. This difference in visual stability between the two pond habitats could translate into a slight difference in memory duration. If Balmaha pond is a little less stable than Inverleith pond, Balmaha fish might discard obsolete memories slightly more quickly, and this might provide a possible explanation for their better performance in phase 2.

The control trial at the end of experiment 4.a. was included to determine whether any factors other than flow were being used. Here fish were required to locate the food reward without the aid of flow. The results show that fish from all populations chose the rewarded end no more often than expected by chance alone. This indicates that flow was indeed the primary orientation cue being used to solve this task.

The ease with which fish learned to swim up- and downstream to locate the food reward was examined. In each phase of experiment 4.a, half the fish from each population were required to swim upstream, and half downstream to locate the food reward. The number of trials to reach criterion for fish swimming in each direction were compared, and no effect of direction on performance was found. This shows that fish did not exhibit any preference in this experiment for swimming up- or downstream. This result also emphasises that olfactory cues did not appear to aid the performance of fish which started each trial downstream of the food reward.

In experiment 4.b, with both visual and flow information available, river Kelvin and Balmaha pond fish learned the task in a similar number of trials. This contrasts to the results of experiment 4.a. With only flow available, the river Kelvin fish learned the task in significantly fewer trials than Balmaha pond fish. It seems that in experiment 4.b, each population was able to learn the task, presumably relying mainly on the orientation cue which they had a preference for. The probe trial revealed that this was indeed the case, with a significant number of the river fish relying more heavily on flow, and the pond fish relying on visual landmarks.

These results lend weight to previous suggestions that the environment may have a part to play in determining orientation behaviour. Given the choice, fish from the river environment, with reliable unidirectional flow and unstable visual surroundings preferred to rely on flow rather than visual landmarks to locate the food source. Fish from the pond habitat, with relatively more stable visual surroundings, and no unidirectional flow, showed a preference for visual information to indicate the position of the food source.

In conclusion, experiments 4.a and 4.b have again demonstrated that behavioural variation exists between populations of three-spined stickleback. These differences seem to adapt the different populations to the particular type of habitat they occupy. Experiment 2.a revealed differences in the ability of the four populations of sticklebacks to use visual information when solving a spatial task, with two pond populations relying to a greater extent on visual landmarks than two river populations. It has now been shown that direction of water flow can also be used for orientation and that the two river populations, with their extensive experience of water flow, appear to be better at using this cue compared with the two pond populations. Differences in the abilities of the two river populations to learn the task were found which could relate to habitat differences. Furthermore, it is possible that the duration of memory might be affected by the relative stability of the environment that the sticklebacks inhabit.

Chapter 5

Behavioural variation: a result of differential experience or inherited traits?

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5.1. Introduction

The experiments reported in chapters 2, 3 and 4 have revealed differences in the orientation behaviour of five distinct populations of three-spined stickleback. After comparing behaviour in these experiments, and studying habitat differences between the populations (reported in chapter 6), it would appear that this behavioural variation has arisen through different selection pressures exerted by the respective habitats. The variation could be genetically encoded and inherited by each generation, or it could be a result of learning during development in different habitats. There is evidence to suggest that the circumstances under which an animal develops affects many aspects of its subsequent behaviour, for example, deprivation experiments, where an animal is isolated from others during development, have revealed subsequent adverse effects on adult behaviour in cats and rhesus monkeys (Wiesel & Hubel, 1965, Chamove *et al.*, 1973). In addition, there is evidence to suggest that early experience of the environment allows animals to adapt their behaviour to the particular habitat occupied. For example, early experience affected the subsequent use of orientation mechanisms in pigeons. Wiltschko & Wiltschko (1989) performed experiments which revealed that a pigeons use of orientation cues depended on its early experience. Pigeons raised in a roof loft had access to the winds from all directions, while those raised at ground level were sheltered from the wind. The roof pigeons were not forced to fly around, while those on the ground were forced to fly every day, so they could become familiar with the spatial configuration of their surroundings. When the birds were eventually taken 10 km from the lofts and released while anosmic (unable to smell due to anaesthetised nasal membranes), the birds from the roof loft were disorientated while the ground birds homed more successfully. The roof loft birds which were raised where they could smell the wind from all sides appeared to rely on olfaction for homing. When they were deprived of their sense of smell, they homed less successfully. Those which were raised on the ground, in shelter from the wind, however, continued to home successfully even when deprived of their sense of smell. They must have relied on some other strategy, possibly their knowledge of the arrangement of their surroundings. Many of the birds used in this experiment were siblings, making the genetic compliment of the two experimental groups similar. This provides compelling evidence that it was experience during development which determined the adult behaviour of these pigeons.

An elegant illustration of the interaction between learned and inherited traits in determining behaviour is provided by studies on song learning in birds. The song of the American white-crowned sparrow, *Zonotrichia leucophrys*, for example, varies geographically along the Pacific coast, with birds from different areas varying systematically in the songs they produced. To study the genetic component of this variation, Marler & Tamura (1964) obtained young males as soon as they hatched, and reared them in isolation. These birds subsequently sang very similar, simple songs instead of their local 'dialect'. The basic, simple song must therefore be genetically encoded, with regional variation being acquired through the experience of listening to adult birds from the appropriate area during development.

There is evidence that the behaviour of fish can also be shaped by early experience. Warburton & Lees (1996) showed that juvenile guppies, *Poecilia* *reticulata*, prefer to associate with conspecifics that they recognise from their early development, than with unfamiliar fish. Furthermore, the guppies were visually attracted to and shoaled near swordtails, *Xiphothurus helleri*, only if they had been reared with swordtails. These results were interpreted as evidence that the recognition template formed in early life is flexible, rather than controlled by a closed genetic program i.e. the type of shoal mates preferred was not genetically encoded, but depended on early experience.

Another example of where both experience of the environment and genetic compliment seem to influence behaviour is provided by studies of Scottish three-spined sticklebacks. Giles & Huntingford (1984) found that sticklebacks sampled from a habitat where the risk of predation was high (the river Endrick) showed more effective fright responses than fish from a 'low risk' site (Inverleith pond). Responses to an overhead threat ranged from an immediate jump away from the simulated predator with a longer recovery time, exhibited by the 'high risk' populations, to complete disregard of a simulated predator by fish from 'low risk' sites. The behaviour of these fish therefore appeared to be adapted to suit the different levels of predation risk experienced in the two habitats. It was not, however, evident whether the behavioural traits were learned by each generation, or passed between generations through the genes. To study this, a further experiment was carried out using laboratory reared individuals from each population. Offspring of fish sampled from the 'high risk' site still exhibited more effective predator avoidance than offspring of fish from the 'low risk' site even though neither laboratory-reared population had ever experienced a predator attack (Tulley &

Huntingford, 1987a). The suggestion was, therefore, that the predator avoidance traits were inherited, and developed without experience of predators. It was then found, however, that the development of good predator responses in offspring from the 'high risk' population depended not only on inherited traits, but also upon a certain feature of the environment - that of the presence of the father at the nest during the first eight weeks of life (Tulley & Huntingford, 1987b). In the three-spined stickleback, interactions between father and offspring appear to provide the offspring with experiences similar to a predator attack. When a fry attempts to leave the nest, the father chases it, captures it in his mouth and returns it to the nest. This process may resemble a predator attack closely enough to enable offspring to develop appropriate predator avoidance behaviour. When the populations were reared in the laboratory with the father present, the 'high risk' fish developed better responses than the 'low risk' fish. Predator response behaviour in this case is controlled by an interaction between inherited characteristics and environmental experience.

5.1.1. Controlling development

Like the populations of sticklebacks and sparrows mentioned above, the populations tested in the current thesis each exhibited behaviour particular to their surroundings e.g. fish sampled from flowing water were better at using direction of flow to orientate than those from sampled from standing water. To tease apart the influence of the two possible controlling factors on the behaviour of the sticklebacks (the environment or inherited behavioural traits), artificial breeding and rearing was carried out. This allowed the conditions experienced during development to be controlled. Subsequent adult behaviour was then compared between artificially reared populations, and reflected back to the behaviour of the wild caught fish.

Fish from some of the populations used in earlier chapters were bred and reared under controlled conditions, and tested in experiments identical to 2.a. and 4.a. Stability has been suggested in previous chapters to have some influence on the type of orientation strategy adopted. To determine how visual stability affected orientation behaviour, groups of fish were raised in aquaria with unstable geometric arrangements of objects, while others were reared in aquaria in which the arrangement of objects remained stationary for the entire period of development. In addition, a single group of fish from the river Kelvin were allowed to breed in a small outdoor pond. Their offspring therefore developed in non-flowing water. If orientation behaviour is controlled by experience of the environment, then the behaviour of the pond-reared offspring should differ from wild fish caught in the Kelvin. If, however, the orientation behaviour is genetically controlled, then the pond-reared offspring should behave in the same way as the wild caught fish regardless of having developed in such different surroundings.

EXPERIMENT 5.a.

5.2. Subjects

Three artificial populations were used in experiment 5.a. When fish were large enough, they were tested in the line mazes described in experiment 2.a.

POPULATION 1 - Kelvin pond (Kel p): consisted of Kelvin river fish (six for each type of maze - landmarks and no landmarks) bred and raised in the Gordon MacKenzie Memorial pond at ICAPB, University of Edinburgh. The pond was installed in early 1997, approximately 2.5 m^2 and 0.5 m deeps. Five adult three-spined stickleback from the river Kelvin were placed in the pond in late spring 1997. In March 1998 (ten months later), 25 offspring were removed from the pond and placed in the laboratory for 4 weeks and kept under the same conditions as fish in experiment 2.a. (12^0c and L:D 12:12). This group therefore had the Kelvin population genetic compliment, but the fish had never experienced a riverine habitat, i.e. had no experience of flow or visual instability of landmarks.

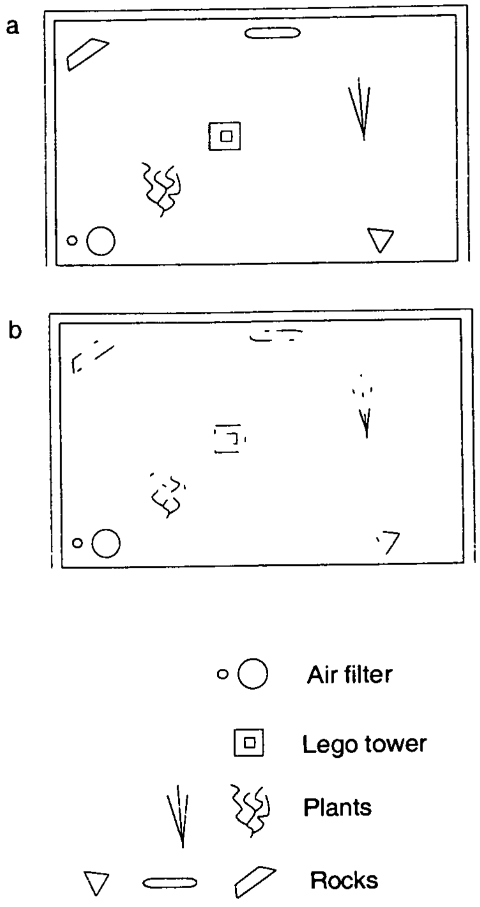
POPULATION 2 - Inverleith stable (Inv st+): 12 fish were bred artificially in the laboratory from Inverleith pond stock (6 fish for each maze type), using the artificial breeding and incubation protocol described in Appendix 1. This group of fish were reared in a holding tank (30 x 40 x 30 cms) with six visual landmarks which were always in the same stable geometric arrangement (see figure 5.1a. on page 103). The fry were therefore raised in a visually stable environment. The bloodworm diet was always provided in the same location in the tank (the front right-hand corner). It was hoped that fish would learn to associate this particular location, identifiable by its relative position amongst a stable landmark arrangement, with food. Fish were reared for ten months before being used in the experiment. Each weekday, I disturbed population 2 by placing my hand in the aquarium and moving it around. This was to ensure that these fish experienced the same level of disturbance as population 3 which had the landmark arrangement changed around each weekday (see below).

POPULATION 3 - Inverleith unstable (Inv st-): 12 Inverleith fry were bred in the laboratory (6 for each maze type) and reared in a similar holding tank as population 2, with six identical visual landmarks, but without any stable geometric arrangement. The landmarks were moved around randomly every weekday (see figure 5.1b. overleaf). The fry were therefore raised in a visually unstable environment. Food was scattered across the tank randomly to prevent the fish from learning to relate a regular location with a food reward. Although there was no flow present in the aquaria, the arrangement of the physical surroundings was constantly changing. Fish in this population were expected to learn to rely less on visual landmarks because their experience would imply visual landmarks are not particularly reliable as spatial predictors. Again, fish were reared for ten months prior to the experiment.

5.3. Methods

Artificially bred and reared sticklebacks from the three populations described above were tested using the same procedures as experiment 2.a (see figures 5.2 a&b. on page 104). Fish had to learn to find the correct route through the maze with plant landmarks or without plant landmarks present in the maze. Having learned the task, fish were given one trial with the maze reversed to investigate which orientation cues they were relying on to find the route through the maze. Each population had a similar mean length (Kel p: 3.87cms; Inv st+: 4.12cms; Inv st-: 3.76cms). The number of trials to learn the task, time to complete the task before and after the maze was reversed, and number of mistakes made before and after the reversal were recorded.

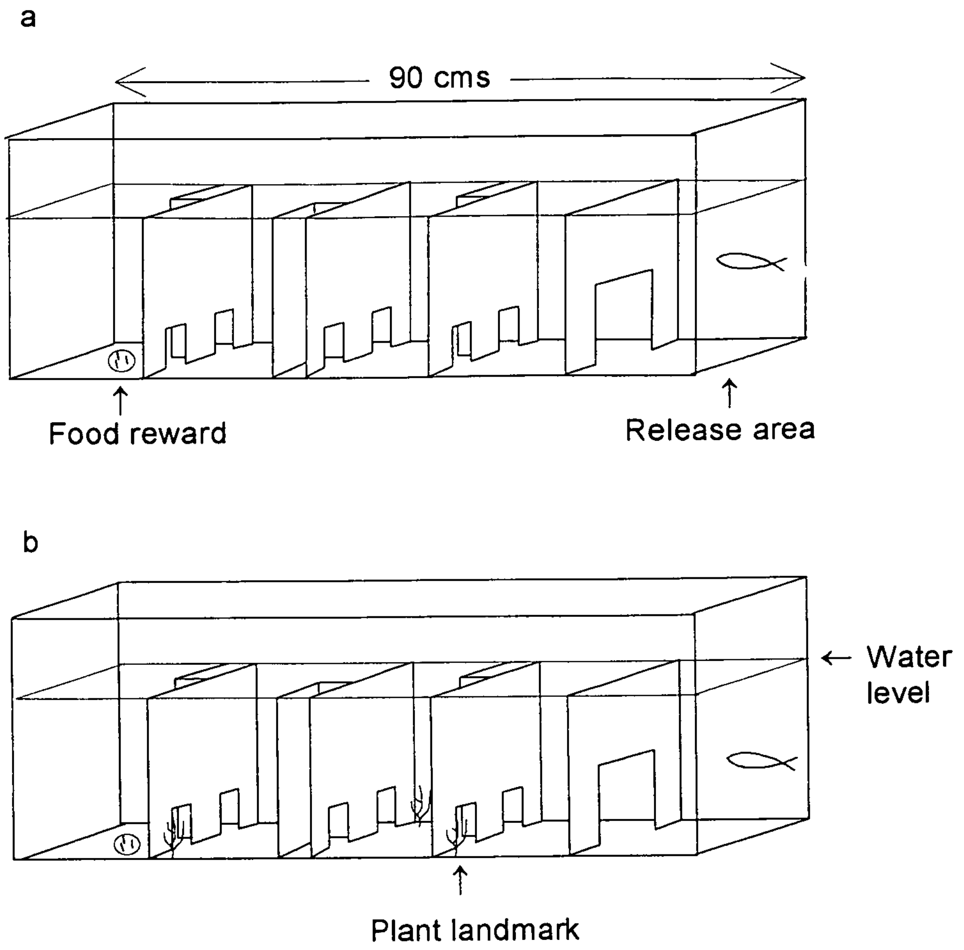
Figures 5.1.a & b. Plan view of (a) the stable landmark arrangement experienced by population 2 (Inv st+) and (b) dotted lines represent moving landmarks experienced by population 3 (Inv st-).



5.4. Results

The normality of the data was tested using a Kolmogorov-Smirnov test for normality. Because the number of trials required by each population to reach criterion was not normally distributed (p=0.047), and were unsuitable for transformation due to the irregular distribution, non-parametric statistical tests were used. A Kruskal-Wallis test revealed no effect of population on number of trials to

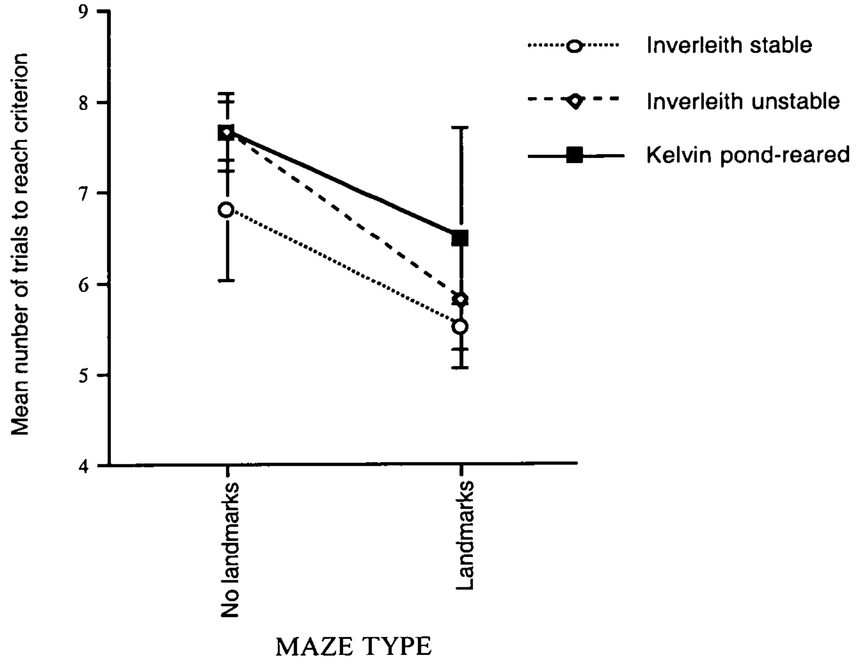
Figures 5.2.a & b. Experimental set-up of line maze (a) without plant landmarks, and (b) with plant landmarks.



learn the task (H=3.914, p=0.012). There was, however, a significant effect of maze type (NoLM and LM) in two of the populations, revealed by Mann-Whitney tests for un-matched samples. The Inverleith (unstable) population, and the Kelvin fish reared in the pond learned the task significantly more quickly when the maze contained landmarks (Inv st-: z=-2.472, p=0.013; Kel p: z=-1.986, p=0.047). There was no significant effect of maze type on the Inverleith (stable) population (z=-

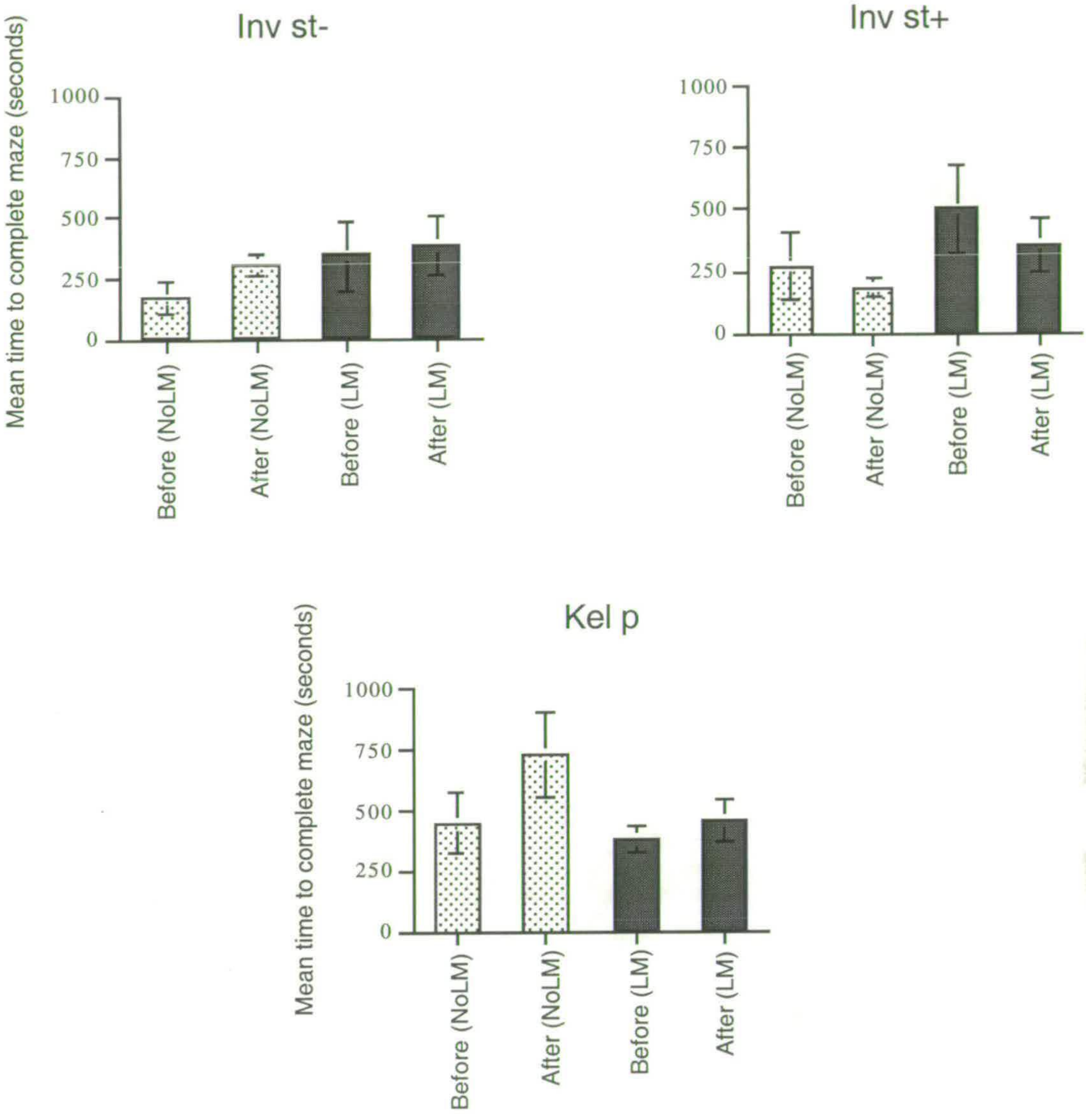
1.269, p=0.204), however, note the large standard error for the Inverleith stable population when there were no landmarks in the maze (see figure 5.3).

Figure 5.3. Mean number of trials for each population to reach criterion performance level ± standard error.



To examine the effect of reversing the maze, the mean time to complete the maze for each group before the reversal was compared with that after the reversal using a Wilcoxon test for matched pairs. One group took significantly longer to perform the task after the reversal, the Inverleith unstable group tested in the plain maze (NoLM) (z=-1.997, p=0.046). The other five groups did not (Inv st+; NoLM, z=-0.105,p=0.916; LM, z=-1.572, p=0.116: Inv st-; LM, z=-0.105, p=0.917: Kel p; NoLM, z=-1.153, p=0.249; LM, z=-0.524, p=0.600) (see figure 5.4).

Figure 5.4. Mean time for each population to complete both types of maze before and after the reversal ± standard error.



To compare the number of mistakes made by each of the six groups (3 populations, 2 treatments) before the reversal, a Kruskal-Wallis test was carried out. No difference was found between the groups (H=6.25, p=0.28). After the maze was reversed, however, there was a significant effect of group on the number of mistakes made (H=19.34, p=0.002). To investigate the effect of landmarks on the number of mistakes, each population was examined individually using a Mann-Whitney test for un-matched pairs. This revealed that only in the Kel p population, tested in a plain maze (NoLM) did the presence or absence of landmarks have an effect on the number of mistakes made (z=-2.70, p=0.007). In the other two populations, maze type (NoLM and LM) had no effect on the number of mistakes made (Inv st+; z=-1.92, p=0.056: Inv st -; z=-1.90, p=0.058).

5.5. Discussion

There were no significant differences in the number of trials any of the three populations required to reach criterion in either maze (NoLM and LM). This contrasts with the original experiment 2.a. in which wild caught Kelvin fish required significantly fewer trials than Inverleith pond fish to learn the NoLM maze, yet in experiment 5.a, the Kelvin and the two Inverleith populations required similar numbers of trials to learn the NoLM maze. It was suggested in chapter 2 that differences between the Kelvin and Inverleith habitats might explain why the river fish learned the NoLM maze more quickly than the pond fish. For example, the greater changeability of a river environment compared to a pond might explain why the river fish were faster at learning. If the environment is changeable, it would be beneficial to learn about features of the environment quickly (and indeed discard old memories quickly) before things change. The view that the environment can affect the speed of learning is supported by the results from experiment 5.a. When the Kelvin and the Inverleith fish were both reared in stable, current-free habitats, there was no difference in the number of trials required to learn the plain maze.

The performance of two of the artificial populations in experiment 5.a. was affected by the presence or absence of plant landmarks. The Kel p and Inv st-populations both learned the maze significantly faster when landmarks were present. This suggests that both of these populations were relying on the landmarks to guide their path through the LM maze. In the original experiment 2.a, the wild caught Kelvin fish did not pay any attention to landmarks in the LM maze while the Inverleith pond fish did appear to rely on them. Here, the pond reared Kel p fish behaved more like the wild caught Inverleith pond fish of experiment 2.a. This suggests that the environment experienced by developing sticklebacks does influence the subsequent use of visual landmarks for orientation.

Comparing the two Inverleith populations tested in experiment 5.a, the unstable (Inv st-) population was quicker at learning the maze when landmarks were present, while the stable (Inv st+) population learned each maze equally quickly. Perhaps, then, the Inv st+ fish learned the sequence of turns to negotiate the maze since they did not appear to pay any attention to the landmarks. A possible alternative explanation for this could be that a feature of the maze (that of the open doors being slightly lighter than the dead ends) was being noticed by the fish from the Inv st+ populations. It was suggested in chapter 2 that some fish could rely on this subtle visual cue to find the correct route through the mazes. Perhaps the Inv st+ population became so used to relying on their stable visual surroundings for orientation that they quickly learned to rely on a very subtle cue like light level differences. The latter explanation is supported by the performance of the Inv st+ fish when the maze was reversed. They did not take longer to swim through the maze, or make more mistakes, in either type of maze (NoLM and LM). This supports the hypothesis that they were using the differences in light levels to solve the NoLM maze.

The Inv st- population performed better with the plant landmarks present, and when the maze was reversed, took longer to perform the NoLM maze. They did not take longer to complete the LM maze after the reversal, and so were likely to be following the landmarks. These results suggest that the Inv st- fish were not relying on subtle visual information like the differences in light level as the Inv st+ did, but they were able to learn to rely on the plant landmarks when these were available.

The Kel p fish did not take longer after the reversal even in the NoLM maze. At first sight, this seems to suggest that they too could follow the differences in light levels. When the number of mistakes made is examined, however, it is clear that without landmarks (NoLM), the Kel p population made significantly more mistakes than any other group. When a mistake was made, fish quickly chose the alternative door (pers. obs.). In this way, the time to complete the task did not increase. When available, the Kel p population used the landmarks to guide them through the maze, making few mistakes and taking no more time. This contrasts with the wild caught Kelvin fish of experiment 2.a. which took longer to complete the task after the reversal regardless of the presence or absence of landmarks.

The Kel p and Inv st- populations respectively made more mistakes and took longer to complete the NoLM maze after the reversal. They were confused when the NoLM maze was reversed, suggesting that they had learned the sequence of turns required, in the absence of plant landmarks to guide them.

In figures 5.3. and 5.4, the standard errors are quite large, revealing that there is quite a lot of individual variation within each group. Larger sample sizes would have decreased this variation. The experimental procedure was very intensive and time-consuming and so it was difficult to run large numbers of fish. Even with small sample sizes, however, significant differences between the populations were found.

The behaviour of the Kel p fish differs with that of wild caught Kelvin fish in experiment 2.a. in that visual landmarks seem to be much more important to the fish that were reared in the pond. Inverleith fish reared in stable and unstable habitats exhibited differences to each other in the extent to which subtle visual cues were used, with the fish reared in the stable environment being even more adept at using visual landmark than those reared in the unstable environment. It would therefore appear that the environment experienced during development does play a large role in influencing the subsequent orientation behaviour of adult three-spined sticklebacks.

A summary of the different cues believed to be used by each population in the two types of maze can be found in table 5.1.

Table 5.1. Summary of orientation cues believed to be used by the three populations

	MAZE TYPE	
	NoLM	LM
POPULATION 1 Kel p	Sequence of turns	Plant landmarks
POPULATION 2 Inv st+	Differences in light levels	Differences in light levels
POPULATION 3 Inv st-	Sequence of turns	Plant landmarks

EXPERIMENT 5.b.

5.6. Introduction

Experiment 4.a. revealed that wild caught fish from the river Kelvin were consistently quicker at learning to locate a food reward by using water flow as a directional cue compared to fish from Inverleith pond. The suggestion was that fish sampled from flowing water (the Kelvin) benefited from having had extensive experience flow during their development. To determine if it was purely the experience of flow which resulted in this greater aptitude for using flow to orientate, river Kelvin fish that had been bred and reared in the pond (in the absence of flow) were tested in the same flume in experiment 4.a. If experience shaped this orientation behaviour, then the pond reared Kelvin fish would not be expected to perform better than Inverleith pond fish. If, however, this particular behaviour is genetically controlled, then the pond reared Kelvin fish should continue to learn to locate the food reward using flow to orientate more quickly than the pond fish.

In addition, a group of Kelvin fry sampled from the wild when only a few weeks old and raised in the laboratory, and a similar group of Inverleith fry were included for comparison.

5.7. Subjects

The following three populations of artificially reared fish were bred for use in experiment 5.b:

POPULATION 1(Kel p): 11 Kelvin river fish bred and reared in the Gordon MacKenzie pond. These fish were sampled for use in experiment 5.b. at the same time as population 1 of experiment 5.a.

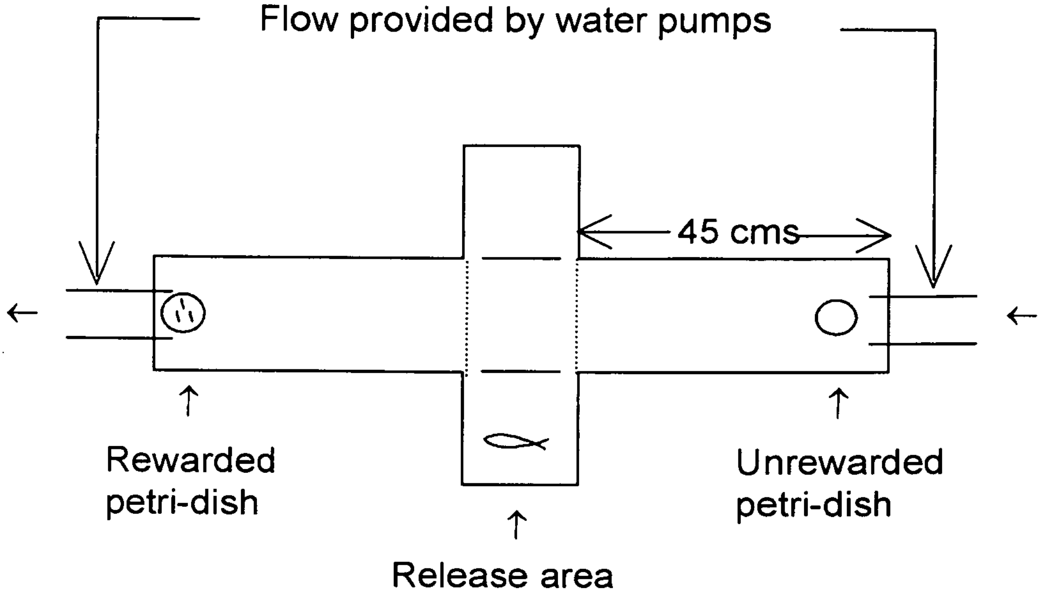
POPULATION 2 (Kel fry): In June 1997, 12 fry were sampled from the river Kelvin at the vet school location by sweeping dip nets along and under the banks. The smallest fry were judged by their length (< 1 cm) to be less than a few weeks old. These were collected and brought back to the laboratory where they were reared for ten months. Fish were housed in an aquarium measuring 30x40x30 cms, which contained only a thin layer of gravel and a small Algarde air biofilter, and no landmarks.

POPULATION 3 (Inv fry): 10 Fry were also sampled, in the same week that fry were collected from the Kelvin, from Inverleith pond. As above, the smallest fry were chosen and were estimated by their size to be less than a few weeks old. The fry were raised in an aquarium in the laboratory, under identical conditions to population 2.

5.8. Methods

The experiment was carried out in the same way as the original experiment 4.a. described in chapter 4 (section 4.3). Fish from each of the three populations were around ten months old when they were used in experiment 5.b. The mean lengths for each were as follows (Kelvin pond: 4.41cms; Kelvin fry: 5.46cms; Inverleith fry: 5.03cms). The experiment was carried out at a temperature of 12°c and 12:12 L:D using the same procedure as described in experiment 4.a. (see figure 5.5).

Figure 5.5. Plan view of flume apparatus

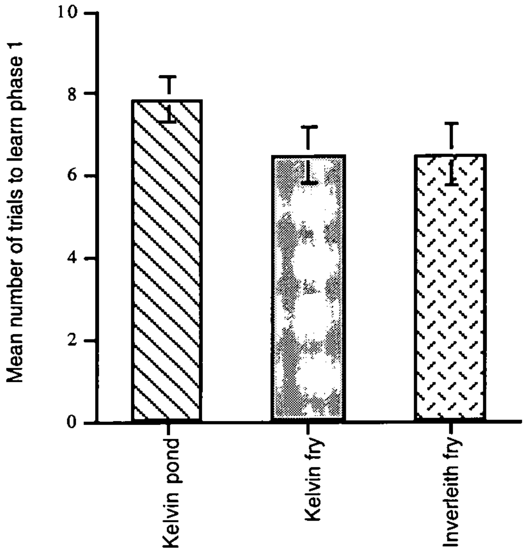


In phase 1, fish had to learn to always swim up or downstream to obtain a food reward. These directions were reversed in phase 2. A final probe trial was carried out, with no water flow in the flume to determine whether the fish could still locate the reward without the cue of direction of water flow. The number of trials to acquire the task in phase 1 and 2, the difference in the number of trials between the two phases for each group, and the choices of fish in the probe trial were recorded.

5.9. Results

The time to learn phase 1 was compared between the three populations using a Kruskal-Wallis test for multiple samples (non-parametric tests were used as the data was not normally distributed, and was unsuitable for transformation). This revealed that there was no difference in the performance of the three populations (H=5.539, p=0.063) (see figure 5.6).

Figure 5.6. Mean number of trials for each population to learn phase 1 ± standard error.



A second Kruskal-Wallis test was carried out on the number of trials for each population to learn phase 2. Again, no significant effect of population was found (H=0.040, p=0.980) (see figure 5.7).

When phase 1 was compared with phase 2, it was found that all populations learned the second phase significantly faster than the first (Wilcoxon test for matched pairs: see table 5.2).

Figure 5.7. Mean number of trials for each population to learn phase 2 ± standard error.

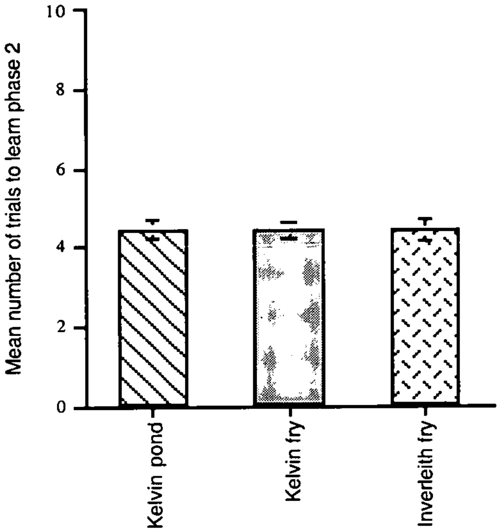
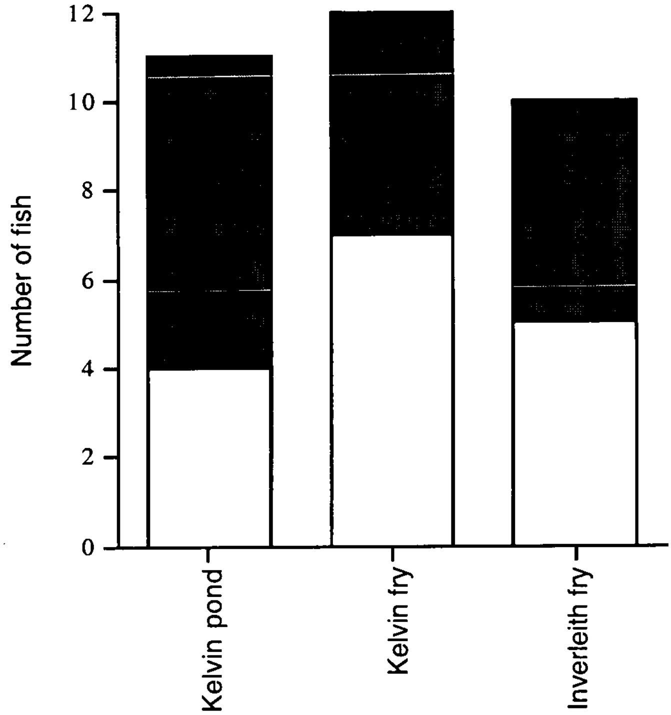


Table 5.2. Mean number of trials to learn phase 1 and phase 2, compared using a Wilcoxon test for matched pairs. ** = $p< 0.02$, *** = $p < 0.01$.

	Phase 1	Phase 2	p-value
Kelvin pond	7.83	4.45	0.001 ***
Kelvin fry	6.50	4.42	0.015 **
Inverleith fry	6.50	4.40	0.007 ***

To compare the performance of the three populations in the control trial, a c^2 contingency test was used. Figure 5.8. shows the number of fish from each population visiting the rewarded and unrewarded ends of the flume first. No association between population and performance was found (c^2=1.11, d.f.=2, $p>0.05$). With no heterogeneity between populations, the data for the three populations were pooled, and a Cochran test carried out to determine whether fish were choosing the rewarded or unrewarded ends randomly (Armitage & Berry, 1987). Performance did not differ from that expected by chance alone (c^2=0.38, d.f.=1, p>0.05) (see figure 5.8).

Figure 5.8. Number of fish from each population visiting the rewarded (correct) and unrewarded (incorrect) arms of the flume first. White bars represent correct choices; grey bars represent incorrect choices.



The number of trials required by fish learning to swim upstream was compared in each phase to those swimming downstream using Mann-Whitney tests. This revealed that there was no effect of direction on number of trials to learn either phase (Phase 1; z=-0.402, p=0.688: Phase 2; z=-0.903, p=0.367).

5.10. Discussion

Experiment 5.b. shows that none of the three populations exhibited any difference in their ability to locate a food reward by learning its location in relation to the direction of water flow. This contrasts with the results reported in the original experiment 4.a. In that case, the wild caught fish from the river Kelvin learned both phases of the flume task in fewer trials than wild caught fish from Inverleith pond. This was thought to be due to their greater experience of flow in their natural habitats. The results from the current experiment support this suggestion. Fish from Inverleith pond and the river Kelvin, both reared in environments without unidirectional flow, subsequently exhibited no difference in their ability to navigate using flow as a directional cue.

All three populations learned phase 2 in significantly fewer trials than phase 1. Having learned to pay attention to the direction of flow during phase 1, they then simply had to reverse the direction they swam in phase 2, so this result it is perhaps not surprising. It should be noted, however, that in the original experiment (experiment 4.a) one of the wild caught populations (Inverleith pond) did not learn phase 2 any faster than phase 1. It was hypothesised that this was a result of the Inverleith fish inhabiting a very stable environment which may not encourage the fish to discard obsolete memories and acquire new ones quickly i.e. discard phase 1 and learn phase 2 quickly. That hypothesis is not supported by the results of experiment 5.b. All three populations of the current experiment were reared in very stable surroundings, and yet were able to discard phase 1 and learn phase 2 very quickly. Some other, unknown factor, must account for the poor performance of the Inverleith fish in phase 2 of experiment 4.a.

In the control trial, flow was absent from the flume. If fish were still able to locate the food reward in their first choice of arm, then orientation cues other than the direction of flow must be available to the fish. The results showed that performance was random during the control trial. Without flow, fish were no longer able to reliably locate the food reward.

Half of the fish from each population were required to swim upstream to locate the reward, and the other half downstream in each phase. No evidence was found for any preferences for swimming up or downstream. Fish swimming in either direction were equally quick to learn the task.

5.11. General discussion

River Kelvin fish were bred and reared in the Gordon MacKenzie pond. They shared the same genetic compliment as wild caught Kelvin fish, but experienced a different environment during their development. In experiment 5.a. (the line mazes) these pond reared river fish behaved differently to the wild caught Kelvin fish of experiment 2.a. The wild fish in experiment 2.a. did not pay any attention to plant landmarks while learning to negotiate the line mazes. The artificially reared fish, however, learned the task significantly more quickly when the plant landmarks were present. Developing in the pond environment increased the reliance of these fish on visual landmarks. In support of this observation, pond reared Kelvin fish did not take longer to complete the reversed maze when landmarks where present, and did not make more mistakes while doing so. Wild caught Kelvin fish in experiment 2.a, in contrast, did take longer to complete the maze after the reversal even when landmarks were present. In chapter 2, it was suggested the wild caught Kelvin fish were learning the sequence of turns instead.

Without landmarks (NoLM), the pond reared Kelvin fish were confused and made more mistakes in the reversed maze trial, suggesting that without landmarks to follow, the fish had learned the sequence of turns instead. The pond reared Kelvin fish, therefore, relied on the landmarks when they were available, in the manner of pond fish. Without landmarks available, they learned the sequence of turns, in the manner of the original wild caught Kelvin fish. Having been reared in a pond, they acquired some of the characteristic behaviour of the original pond fish, but also retained some of the features of the original Kelvin fish.

It was suggested in chapters 2, 3 and 4 that environmental stability might have an effect on orientation behaviour, particularly on the degree to which visual information is relied upon. To investigate this, two Inverleith pond groups (Inv st+ and Inv st-) were bred and reared in the laboratory under different conditions of stability. One group experienced stable surroundings (Inv st+); six visual landmarks were present in the aquarium, and were kept in the same place. Food was always found in the same position in relation to the stable geometric arrangement of landmarks. The other group experienced unstable surroundings (Inv st-); a duplicate set of six visual landmarks was moved around each weekday, and food was located in different, random, positions. Despite the unstable surroundings of their development, the Inv st- population were able to rely on the plant landmarks to negotiate the landmark maze (they were quicker to learn the (LM) maze than the (NoLM) maze). In addition, after the reversal, the Inv st- (NoLM) group were confused and took longer to complete the task while the Inv st- (LM) group continued to navigate the maze quickly. They were presumably following the landmarks through the maze to achieve this.

The Inv st+ group may have been even better at using visual cues to negotiate the LM maze. These fish were just as quick to learn both types of maze (LM and NoLM). The (LM) group may have been using the plant landmarks to guide their path through the maze, but the (NoLM) group were just as quick to learn without the benefit of the plant landmarks. The two groups (LM and NoLM) taking the same time to learn could have come about in two ways. Both groups could have developed the behavioural algorithm to navigate the maze, or they could have developed such a strong reliance on visual information that they could rely on more subtle visual cues i.e. the open doors being lighter than the dead ends. The second explanation seems more likely considering the stability of these fish's environment during development. That the second explanation is correct is supported by the performance of these Inv st+ fish when the maze was reversed. With or without landmarks, these fish did not take longer to navigate the mazes, or make more mistakes in doing so than they did before the reversal. The fish did not appear to be learning the sequence of turns, as the reversal trial did not cause confusion. Visual cues outside the tank were screened off from view, and the ceiling above the experimental tank was uniformly white. It seems that the light levels were the only possible cue the fish could have been using. If this is the case, then the effect of a stable environment on the Inverleith fish has been to increase their reliance on any possible reliable visual information to orientate.

Experiment 5.b. also revealed that the behaviour of artificially reared fry differed from that of wild caught fish. The three artificial populations consisting of

Kelvin and Inverleith fish all required the same number of trials to learn to use direction of flow to locate a food reward. The pond reared Kelvin fish were no quicker than Inverleith fish at learning the task. In the original experiment 4.a, however, Kelvin fish were consistently quicker than Inverleith fish at learning the task. A notable environmental difference between the wild caught and laboratory reared Kelvin fish is that the laboratory reared fish did not have any experience of flow during their development. It seems, therefore, that developing in an environment of flowing water increases subsequent ability to orientate according to direction of flow.

5.12. Summary

The experiments reported in this chapter have shown that the environment experienced during development affects subsequent orientation behaviour of sticklebacks. Behaviour is therefore controlled to a large extent by the particular environment occupied. Some of the results, however, indicate that there are also others factors, presumably genetic, in shaping orientation behaviour. This was illustrated by some of the fish developing in an artificial laboratory environment, and yet clearly exhibiting behaviour typical of fish from their population's natural environments, for example, Kelvin fish reared in a pond used landmarks to guide them through the landmark maze. This behaviour is typical of the wild caught pond fish used in experiment 2.a, yet in the (NoLM) maze, these pond reared river fish used the behavioural algorithm to find the correct route, a behaviour displayed by the wild caught river fish in experiment 2.a. The results show that, in this case at least, there appears to be a combination of factors controlling the fish's behaviour.

Chapter 6

Comparing the habitats of sticklebacks used in this thesis

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6.1. Introduction

Species which occupy a range of different habitats can diverge in both morphology and behaviour as different populations become better adapted to their particular niches. For example, the body size of the North American white-tailed deer, *Odocoileus virginianus*, increases with latitude across most of its range (Futuyma, 1986). This pattern which has been found in many different species of bird and mammal, and has been dubbed 'Bergman's rule', arose as a result of endotherms adapting to colder climates. The smaller surface area to volume ratio of a larger body allows less heat to be lost (Begon *et al.*, 1990).

An example of behavioural variation between populations of the same species has been documented in Zenaida doves, *Zenaida aurita*, in Barbados (Carlier & Lefebvre, 1997). These birds usually feed on seeds and berries which are usually relatively predictably and evenly dispersed. One of two populations studied occupied an area of coastal woodland and recreational land, and exhibited the typical behaviour of the species, with individuals defending feeding territories all year round. Another population, however, inhabited a harbour area where the primary food source, grain and legume spillage, was unpredictable and concentrated in one place. Instead of trying to defend territories, a group feeding strategy was adopted with all the birds scrambling to get as large a share of food as possible (scramble competition). These birds had therefore adapted their foraging behaviour to this unusual type of niche.

Morphological and behavioural divergence has also been documented in three-spined sticklebacks found in North America and Canada occupying different habitats.

Bentzen & McPhail (1984) discovered two distinct types of three-spined stickleback in a Vancouver lake. One type inhabited the water column, and was morphologically adapted for planktivory (limnetic type), while the other associated closely with the substrate, and was better adapted for feeding on benthic invertebrates (benthic type). The two types were not only morphologically distinct, but also exhibited behavioural differences in foraging ability which increased their success when foraging in the appropriate niche. Under experimental conditions, the benthic type made more successful attempts at capturing prey from the substrate than the limnetics. Indeed, the female limnetics did not even attempt to feed on the substrate at all (Bentzen & McPhail, 1984). Such differentiation reduces competition for food and living space, and may be the basis for speciation events.

The current thesis has documented differences in the spatial behaviour of three-spined sticklebacks sampled from five different Scottish populations. It is possible that in the same way as the cases cited above, the differences in spatial behaviour have arisen because of the varying nature and characteristics of the different habitats occupied. To investigate this further, it was necessary to document the extent to which the habitats differed, concentrating on features which could conceivably have an effect on orientation behaviour. I therefore carried out habitat surveys. Four of the sites were surveyed in summer 1997 and early spring 1998. The fifth site was included after these surveys were established, and so was surveyed separately in summer 1998. The surveys focused on the nature of the substrate, visibility, fauna, vegetation and the presence or absence of flow. Owing to the descriptive nature of these surveys, I combine the results with discussion in section 6.3., and draw conclusions in section 6.4. (summary).

6.1.1. Fish sampling sites

Fish were sampled from the river Kelvin, a tributary of the Clyde, the river Endrick, which flows into the southern end of Loch Lomond, and the river Esk which flows past Edinburgh to the Forth estuary. The Kelvin was sampled at Glasgow vet school campus in north west Glasgow (OS 702 556), the Endrick at the ford of Killearn (OS 879 517), 20 miles west of Stirling and the Esk at Roslin Glen road bridge, 4 miles south of Edinburgh (OS 626 267) . All sampling took place within a 100 meter stretch of river, and each stretch was approximately 5 meters wide. (See figures 6.1a-1c).

Figure 6.1.

a. The river Kelvin at the Glasgow vet school, winter.

b. The river Endrick at the ford of Killearn, summer,

c. The river Esk upstream of the bridge at Roslin Glen car park, winter.

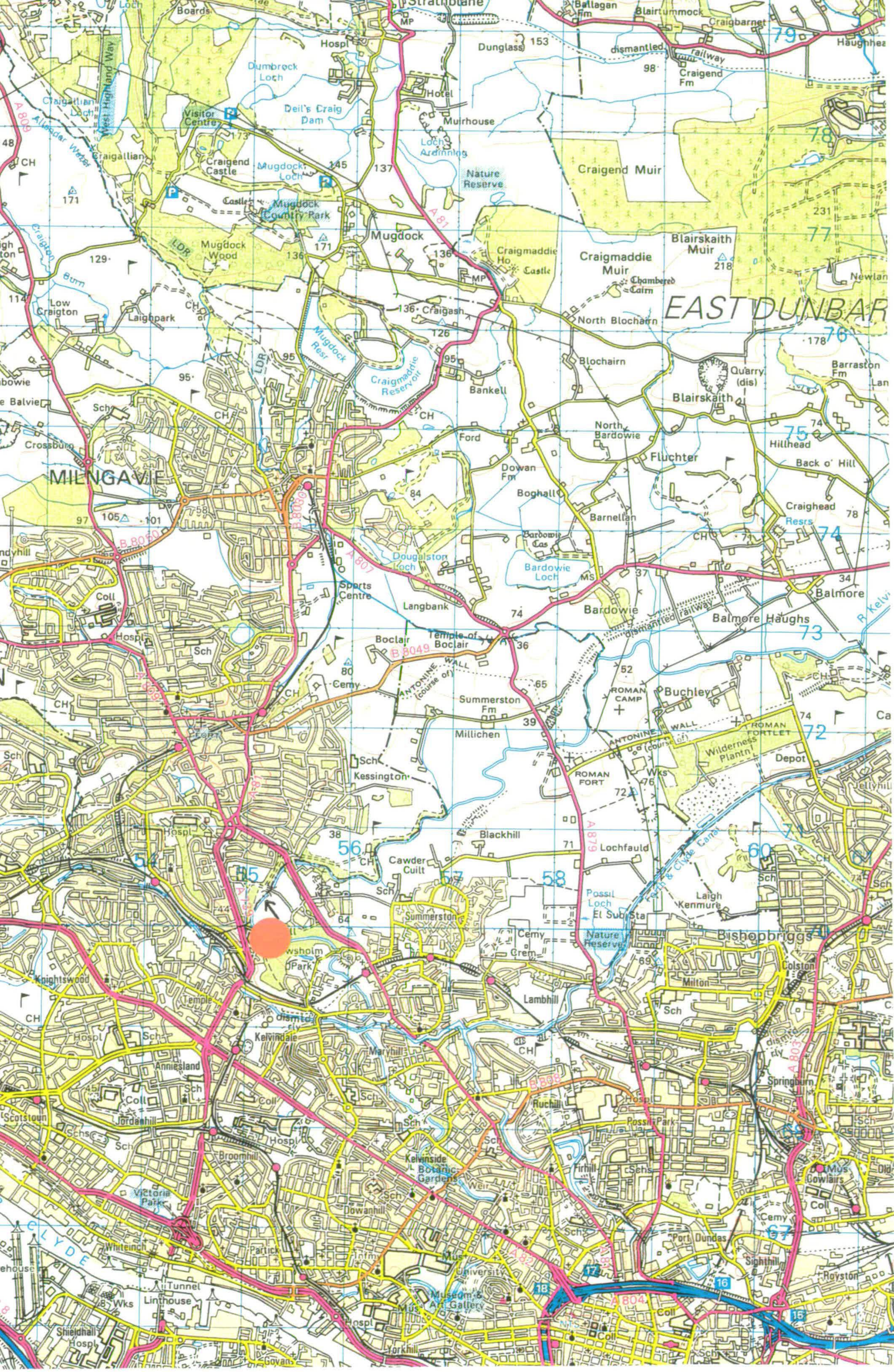
Fish were also sampled from two ponds, Inverleith pond near the botanical gardens in central Edinburgh (OS 742 241), and Balmaha pond on the south eastern shores of Loch Lomond (OS 911 421). The two ponds differ in their nature. Inverleith is a large, artificial pond, around 100 m long, 50 m wide, and 1 m deep across its width (pers. com. Edinburgh district council park patrol). Balmaha pond, however, is a naturally occurring pond of around 10 x 20 m^2. It supports emergent vegetation across its width and is therefore shallow enough to prevent light levels becoming attenuated by suspended solids which might prevent rooted vegetative growth. (See figures 6.1d & 1e).

Figure 6.1.

d. Inverleith pond near Edinburgh's Botanical Gardens, winter.

e. Balmaha pond near Balmaha car park, summer.

OS maps of each of the five sites are provided overleaf. The field sites are indicated by black arrows next to prominent orange circles.

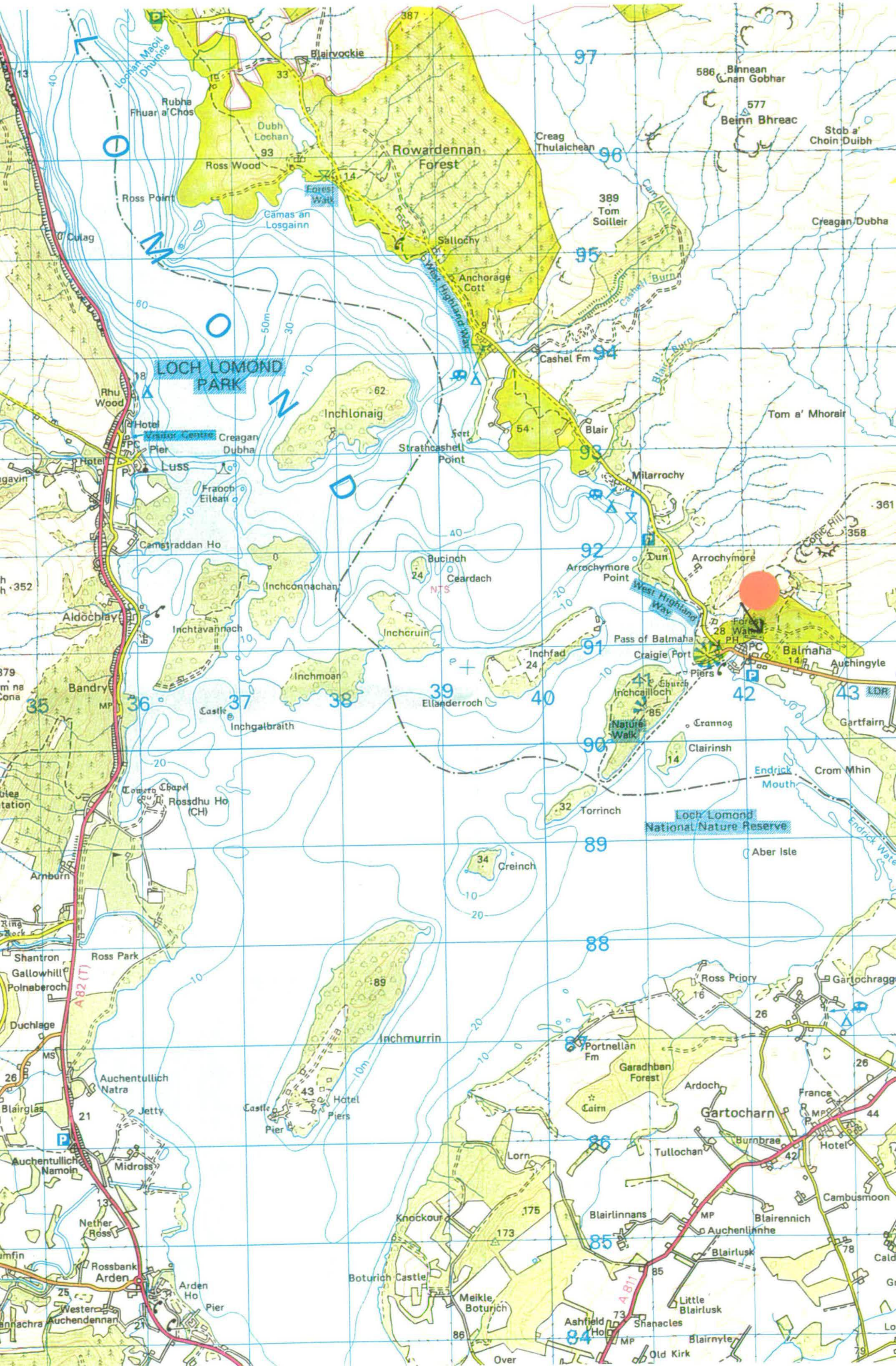


Strathblane
Boards
Ballagan Fm
Blairtummock
Craigbarnet
79
Haughhead
Hospl
Dunglass
153
dismantled railway
98
Craigend Fm
West Highland Way
Dumbrock Loch
Hotel
Craigallian Loch
Visitor Centre
Deil's Craig Dam
A 809
Allander Water
Muirhouse
78
173
Loch Ardinning
48
Craigallian
Craigend Castle
Mugdock Loch
145
137
Nature Reserve
Craigend Muir
CH
171
Castle
Mugdock Country Park
A 81
231
77
LDR
Mugdock Wood
171
Mugdock
Blairskaith Muir
Craigton
129
136
136
Craigmaddie Ho
Castle
Craigmaddie Muir
218
Chambered Cairn
Newlan
Burn
MP
114
Low Craigton
Laighpark
CH
136
Craigash
EAST DUNBAR
126
North Blochairn
76
Mugdock Resr
178
LDR
95
95
Craigmaddie Reservoir
Blochairn
Quarry (dis)
Barraston Fm
95
Bankell
Blairskaith
Balvie
Sch
CH
CH
North Bardowie
75
74
Crossburn
Ford
Hillhead
Fluchter
Dowan Fm
Back o' Hill
MILNGAVIE
84
Boghall
B 8030
Barnellan
Craighead
78
97
105
101
58
Resrs
B 8050
Bardowie Cas
CH
74
Dougalston Loch
A 807
Bardowie Loch
MS
37
34
Balmore
Coll
Sports Centre
Langbank
74
Bardowie
dismantled railway
R Kelvin
Balmore Haughs
Hospl
Boclair
Temple of Boclair
36
73
Sch
B 8049
ANTONINE WALL (course of)
80
Cemy
52
CH
65
ROMAN CAMP
Buchley
A 809
CH
Summerston Fm
74
CH
Millichen
39
ANTONINE WALL (course of)
ROMAN FORTLET
72
Fort
Sch
Wilderness Plantn
Depot
Sch
Kessington
A 81
ROMAN FORT
Wks
76
72
Jellyhill
A 879
Hospl
38
Blackhill
71
71
56
Lochfauld
60
54
CH
Cawder Cuilt
55
57
58
A 739
Forth & Clyde Canal
CH
Possil Loch
Sch
Laigh Kenmure
44
Sch
64
Summerston
El Sub Sta
Bishopbriggs
Dawsholm Park
Cemy
Crem
Nature Reserve
69
Colston
Knightswood
Temple
dismtd rly
Lambhill
Milton
CH
Sch
Hospl
Sch
Kelvindale
dismtd rly
Maryhill
CH
Annieslnd
A 803
Sch
Sch
B 808
Ruchill
Springburn
Scotstoun
Coll
Hospl
Jordanhill
Schs
Sch
Possil Park
Hospl
Sch
Broomhill
Kelvinside Botanic Gardens
Firhill
Schs
Mus
Cowlairs
Victoria Park
Weir
Sch
Dowanhill
Cemy
Coll
CLYDE
A 82
Port Dundas
67
Whiteinch
Partick
Sch
A 81
Sighthill
Mus
17
Royston
University
16
18
Tunnel
Linthouse
Wks
Museum & Art Gallery
A 804
Mus
Hospl
Coll
15
Shieldhall
Hospl
Govan
Yorkhill
Sch


Back Borland
Borland Fm
Borland Ho
Crooks
Offerance
Flanders Moss
Nether Easter Offerance
Chapelarroch
26
Dalmary
East Gartfarran
59
19
Moat
MP
Wester Offerance
Gartentruach
dismantled
Green Burn
Ward
Turner
44
Garchell
Kepdowrie
Woodend
Wester Mye
Easter Mye
28
Broch (remains of)
Gartclach
B835
Cretlevane
34
Gartinstarry
B835
Buchlyvie
PO
Oxhill
Ballamenoch
Forest Walks
72
Drymen Road Cottage
Gartcurrachan
Gartachoil
St Patrick's College
Cashley
PH
Thirds Heights
Park of Auchentroig
MP
Wester Auchentroig
Ballochneck
P
Badivow
Upper Balwill
Gowston
Easter Culbowie
Hoish
Douchlage
Lower Ballaird
Whiteleys
Wester Culbowie
82
89
MP
Garrauld
MP
A875
Kepculloch
Upper Ballaird
175
Buchlyvie Muir
116
Ballat
82
Gaisland
Knockingalle
Little Drumtie
Glenfoot
A81
51
52
53
54
55
56
57
58
192
lairfad
Spittal Ballat
MP
191
Templetea
Tombrake
Cairnhall
dismtd rly
Loaninghead
Camoquhill
125
Shian
Rinnans
Ballindalloch Muir
Upper Blairmore
A81
98
Balfunning Ho
Balfron Station
Camoquhill Douglas
Cemy
98
Sch
Balfron
Westec
Lednabra
Edinbellie
Motte
Mountain Rescue Post
Ballochearn
Cairnlea
Clachanry
Kilfasset
Dalfoil
68
Ballindalloch
Endrick Water
41
Drumtian
Old Ballikinrain
Ballochruin
Endrick Br
86
41
MP
Little Drumquharn
Boquhan
Parkhall
Clayland
Ballikinrain
Killearn Br
Carbeth Ho
Home Fm
Boquhan Old Ho
67
Ballikinrain Castle
A875
B818
84
Weir
Redyett
Mount
Drumore
Gartness
Waterfall
Heron's Court
Millstone Wood
Cemy The Firs
MP
Boquhan Glen
Sir Archibald's Plantation
Balglass Burn
87
Killearn
Spittal
Mon
105
Ibert
B834
Inn
Auchenibert
Kilmorie
Croy Cunningham
MP
Ballikinrain Muir
Machar Burn
Ballikinrain Burn
West Highland Way
23
MS
Killearn Ho
478
Cou Ba
Fm
A81
Laighparks
High Lettre
Allanrowie
Blane Water
Westerton
Lettre Cottage
67
Carston
Cairn
Earl's Seat
578
A875
Waterfall
MS
Moss
MP
P
Baptiston
543
Blaneswell
PH
Garloch Hill
C
ualt
Dumgoyne
Clachertyfarlie Knowes
A
M
P
S
dismtd rly
Stockiemuir
Ledlewan
427
Little Earl
Blairgar
Dumgoyne
Graham's Cairn
Craigbarnet Muir
96
95
94
93
92
91
90
89
88
87
86
84
83

Swanston
Lothianburn
Morton Ho
Broomhills
Burdiehouse
Straiton
West Edge Fm
Melville Grange
Melville Mains
Wester Melville
Sand Pit
Edgefield
Hospl
LOANHEAD
Ski Centre
Country Park
Caerketton Hill
Damhead Holdings
Old Pentland
Tigh-na-geat
Pentland Mains
Niven's Knowe
Woodfield
Mavisbank Ho
Polton
Burghlee
Boghall
Woodhouselee Hill
Woodhouselee
Pentland Grove
Seafield
Bilston
Dryden Tower
Langhill Fm
Fulford
Danger Area
Ranges
Easter Howgate
Fort & Souterrain
Centre of Rural Economy
Research Centre
Springfield Ho
Weir
Midfield Ho
Mountmarle
Hawthornden Castle
Hawthornden
Dalhousie Chesters
Crosshouse
Castlelaw
Bush Ho
Gowkley Moss
Roslin
Gortonlee
Wallace's Cave
Gorton Ho
White Bog
Upper Dalhousie
Visitor Centre
Glencorse Ho
Glencorse Burn
New Milton
Cemy
B 7003
Roslin Glen
Castle
House O' Muir Fm
Loganbank
Glencorse Mains
Milton Bank
Milton Bridge
Sand Pit
Eskhill
Oatslie
Auchendinny
Weir
Cycle Route
dismantled railway
Rosewell
St Joseph's Hospl
Capielaw
Bellwood
Greenlaw Mains
Mauricewood
Firth Ho
Old Woodhouselee Castle
Auchendinny Ho
Kirkettle
Gourlaw
Thornton
Broachrigg Fm
Parduvine
Grange Dell
Beeslack
Penicuik
Cuiken
Cornbank
Crystal Wks
Kirkhill
Maybank
Auchendinny Mains
Rosslynlee Hospl
Firth Mains
Newbigging
Shewington
Stonefieldhill Fm
Loan Burn
Loanstone
Edgelaw Moor
Uttershill Castle
Pomathorn
B 7026
B 6372
A 6094
A 701
A 703
A 702
A 720
B 7006
B 701
Upper Firth
Cauldhall Moor
Lilyburn
Edgelaw
Edgelaw Resr
Ravensneuk Fm
Fallhills
Ankrielaw
Cauldhall
Halls Fm
Howgate
Dykeneuk
Black Burn
Milkhall Pond
Herbertshaw Fm
Walltower
Chapel (rems of)
Fullarton
Fullarton Water
Saw Mill
Mount Lothian
Mosshouses
Wellington Sch
Rose View
Newstead
Fountainside
Upper Side
Kingside
Leadburn
Gladhouse Mains
Cockmuir
Spurlens Rig
Toxsidehill
Quarry (dis)


Granton Harbour
Granton Point
Caroline Park
Gas Wks
Granton
Pilton
Drylaw
Martello Tower
Black Rocks
Middle Craigs
Eastern Craigs
Port of Leith
Newhaven
Leith
Trinity
Sewage Works
Inverleith
Royal Botanic Garden
Warriston
Pilrig
Leith Links
Craigentinny
Stockbridge
Broughton
Craigleith
Lochend
Restalrig
Ravelston
Dean
New Town
Calton Hill
Abbeyhill
Meadowbank
Abbey & Palace of Holyroodhouse
Art Gallery
Art Galleries
Castle
Waverley Sta
Old Town
Haymarket Sta
Murrayfield
Northfield
EDINBURGH
Holyrood Park
Salisbury Crags
Arthur's Seat
Dunsapie Loch
Duddingston
Bingham
Roseburn
Rugby Gd
Dalry
Meadow Park
Bruntsfield Links
Commonwealth Pool
Duddingston Loch
Prestonfield
Hotel
Gorgie
Weir
Merchiston
Peffermill House
Newington
Craigmillar
Slateford
Cameron Toll
Inch House
The Inch
Craigmillar Castle
Craig Ho
Morningside
Blackford Hill
Royal Observatory
Univ
Craiglockhart
Hermitage of Braid
Little France
Edmonstone
Pentecox
Moredun
Liberton Tower
Braid Hills
Liberton House
Liberton
Barracks
Colinton
Oxgangs
Comiston
Meadowhead Fm
Gracemount
Gilmerton
Dreghorn
Swanston
Fairmilehead
Mortonhall
Kaimes
Morton Ho
Broomhills
West Edge Fm
Melville Grange
Melville Mains
Burdiehouse
Lothianburn
Straiton
Wester Melville
Sand Pit
Ski Centre
Country Park
Hillend
Damhead Holdings
Old Pentland
Edgefield
Allermuir Hill
Caerketton Hill
Tigh-na-geat
Pentland Mains
LOANHEAD
A 901
A 902
A 199
A 7
A 701
A 702
A 703
A 720
B 701
Braid Burn
Burdiehouse Burn
Swanston Burn
Howden Burn



LOCH LOMOND
LOCH LOMOND PARK
Lochan Maoil Dhuinne
Blairvockie
387
Rubha Fhuar a' Chos
Dubh Lochan
Ross Wood
93
33
14
Rowardennan Forest
Forest Walk
Ross Point
Camas an Losgainn
Culag
Sallochy
West Highland Way
Anchorage Cott
Creag Thulaichean
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96
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89
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87
86
85
84
586 Binnean nan Gobhar
577
Beinn Bhreac
Stob a' Choin Duibh
389 Tom Soilleir
Creagan Dubha
Cashell Burn
Cashel Fm
Blair Burn
Tom a' Mhorair
Blair
54
Milarrochy
361
Conic Hill
358
60
50m
30
10
40
20
18
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Rhu Wood
Hotel
Visitor Centre
Creagan Dubha
PC
Pier
Luss
Inchlonaig
62
Fort
Strathcashell Point
Fraoch Eilean
Camstraddan Ho
Bucinch
24
Ceardach
NTS
Inchconnachan
Arrochymore Point
Arrochymore
Dun
West Highland Way
Forest Walk
28
PH
PC
Balmaha
Pass of Balmaha
Craigie Fort
Piers
Church
Inchcailloch
85
Nature Walk
Crannog
Clairinsh
14
Auchingyle
LDR
Gartfairn
Crom Mhin
Endrick Mouth
Endrick Water
352
Aldochlay
Inchtavannach
Inchcruin
Inchmoan
Inchfad
Ellanderroch
Castle
Inchgalbraith
379
Bandry
MP
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36
37
38
39
40
41
42
43
Torrinch
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Loch Lomond National Nature Reserve
Aber Isle
Chapel
Rossdhu Ho (CH)
Amburn
Creinch
34
Ring Rock
Shantron
Ross Park
Gallowhill
Polnaberoch
A 82 (T)
Duchlage
MS
Inchmurrin
89
43
Castle
Pier
Hotel
Piers
10m
Ross Priory
16
26
Gartochraggen
Portnellan Fm
Garadhban Forest
Cairn
Ardoch
Gartocharn
France
MP
P
44
Hotel
Burnbrae
42
Tullochan
Auchentullich Natra
Jetty
Blairglas
21
Auchentullich Namoin
Midross
Lorn
Knockour
175
173
Blairlinnans
Auchenlinnhe
Blairenrich
Cambusmoon
78
Blairlusk
A 811
Little Blairlusk
Nether Ross
Rossbank
Arden
Arden Ho
Pier
25
Wester Auchendennan
Boturich Castle
Meikle Boturich
86
Ashfield Ho
73
Shanacles
MP
Blairnyle
Old Kirk
Over
79

6.1.2. Possible environmental factors that could affect orientation behaviour

Orientation using visual landmarks, algorithm formation and direction of water flow has been demonstrated in chapters 2, 3, 4 and 5. The environmental factors which could conceivably influence such orientation behaviour in fish relate to the stability of the substrate, the level of aquatic vegetation and visibility. Visual stability in a habitat might promote the reliance on visual landmarks for orientation, however, in a visually unstable habitat i.e. one with a current, visual landmarks may be less reliable. There is evidence to suggest that the reliability of the geometric arrangement of the surroundings affects the use of visual information during orientation (Biegler & Morris, 1996). Rats were unable to learn the relationship between a visual landmark and a food reward if the landmark did not remain stable with respect to the rest of the surroundings. If the visual surroundings are unstable, which is likely in a river habitat, then some other kind of non-visual cue, such as the directional information provided by the current, might be preferred.

The nature of the substrate, vegetative growth and turbidity of the water could all affect visual stability. If large boulders form the substrate of a river, it would be less likely to be altered by currents or turbulence than if it was composed of fine silts and sediments. Season and spate alter the distribution and occurrence of vegetation to a great extent. The presence of vegetation in a habitat could therefore decrease visual stability. In the shorter term, weeds and grasses would also decrease visual stability by continually being moved around if a current is present. In polluted, peat stained or deep water, visibility would decrease, and the visual surroundings would become less accessible to fish. When visual information is unavailable or unreliable, alternative non-visual strategies might be favoured. These main factors are considered below for each habitat.

6.2. Methods

Each site was sampled in mid-summer 1997 and early spring 1998 except the river Esk which was sampled in summer 1998.

6.2.1. Substrate

The substrate type of each pond and section of river was determined by observations from the banks and from the water. The composition of each substrate was partly determined visually, but also during wading. Feeling the substrate with the feet gave an idea of the size of the particles when visibility was poor.

6.2.2. Aquatic plants and bank vegetation

To identify the compliment of aquatic plants and bank vegetation at each river habitat, a 50m stretch was slowly waded along, while every new species of plant was noted and identified *in situ* using several field guides for identification. These were :- the Collins Photo Guide to Lakes, Rivers, Streams and Ponds of Britain and North-West Europe (Fitter & Manual, 1995), the Collins Gem guide to ponds and streams (Manuel & Shields, 1991) and the Collins Pocket guide to Wild flowers of Britain & Northern Europe (Fitter, Fitter & Blamey, 1996). The vegetation at Balmaha pond was identified from the west bank which was clear of trees and vegetation, and allowed a clear view of the pond.

6.2.3. Water quality and chemistry

The turbidity or clarity of water is affected by its load, i.e. suspended algae and debris held in the water (Jeffries & Mills, 1995). Effluent suspended in water increases turbidity and thereby decreases visibility. Staining of the water by peat also reduces visibility. One method of measuring turbidity is known as the Secchi disk method. The Secchi depth is that depth at which a white disc ceases to be visible to an observer at the surface (Moss, 1997). Due to the shallow water of the pond habitats, and the flowing water of the river habitats, this method was unsuitable for the current study. Instead, the relative turbidity of the water of the five field sites was estimated through many direct observations at different times. In addition, information was gathered from various other sources outlined in the results and discussion section (6.3.4.).

Water from each site was chemically tested for nitrogen and phosphorous compounds. High levels of these compounds suggest a large amount of organic debris suspended in the water, and therefore decreased visibility. Samples were collected from the four main sites in summer 1997, early spring 1998, and from the river Esk in summer 1998. The samples were analysed for the following chemical measures in the laboratory of Glasgow University field station at Rowardennan, Loch Lomond:- nitrogen contained in ammonia (NH_3-N), nitrite (NO_2-N), and nitrate (NO_3-N), and phosphorous contained in phosphate (PO_4-P). A Palin test kit was used to analyse the samples. The kit provides the appropriate chemicals which need to be dissolved in the water sample. The sample is then placed in a self calibrating colorimeter. The intensity of colour of the sample accurately relates to the quantity of the particular component being tested for. High nitrogen and phosphorous levels indicate organic pollution. The source of this type of pollution is often agricultural run off or deliberate input of sewage effluent. The bacteria that colonise and multiply on the organic matter require much oxygen to decompose it, and so organically polluted water becomes deoxygenated. This results in the loss of many species of invertebrates and fish. Eventually, the community may be dominated by filamentous algae, and pollution tolerant oligochaete worms and chironomid larvae.

In addition, total dissolved oxygen was measured on site using an HI 9143 Microprocessor Auto Cal Dissolved Oxygen Meter (Hanna instruments) and pH was measured on-site using a New Tech Aqua test kit for pH (6.0-7.6). Low oxygen levels can be detrimental to invertebrates, and fish can suffocate. Low oxygen levels also induce changes in the substrate e.g. the release of inorganic ions such as iron, manganese and phosphate which are usually locked into insoluble oxidised complexes in the sediment (Moss, 1980). Daily fluctuations occur in oxygen level, with lower levels after a night of plant respiration, and higher levels after a day of photosynthesis. All the measures above were taken at the same time of day (noon). However, if a more accurate profile of a rivers status is required, more frequent (daily) measurements of dissolved oxygen levels should be obtained.

Changes in pH can have diverse effects on freshwater life. The lowering of the pH due to the input of hydrogen ions (H^+), from acid rain for example, disrupts ionic regulation which is of huge importance in freshwaters owing to the osmotic difference between organisms and the surrounding medium. In addition, as the proportion of H^+

ions increase, toxic metals such as mercury and aluminium are more readily dissolved into the acidic water. Many species of invertebrates and plankton cannot survive at low pH, and the eggs of fish and amphibians are vulnerable at pH levels less than 5.

6.2.4. Benthic macro-invertebrates

The array of benthic invertebrates present on a stream or pond bed provides a good indication of the long term water quality. Chemical measures reflect only the instantaneous water quality, and might overlook any damage caused by a chemical spill or pollution event unless carried out at the exact moment of the event. However, the survival of benthic invertebrates depends on the continuing quality of the water, and so benthic invertebrate samples better reflect water quality in the long term. Benthic kick samples were collected from the four main sites between 12th June and 3rd July 1997, and from the fifth site (the river Esk) on 24th June 1998. Each kick sample involved wading slowly upstream for 30 seconds while kicking the substrate to disturb the fauna. A dip net was positioned just behind the disturbed area, barely touching the substrate, to collect the dislodged invertebrates. The samples were preserved immediately in 90% alcohol. The diversity and abundance of invertebrate families present was later assessed. Freshwater Biology Association guides and the Collins Photo Guide to Lakes, Rivers, Streams and Ponds of Britain and North-West Europe (Fitter & Manual, 1995) were used to identify the invertebrates to family level. The National Water Council provides a biological scores system which can be used to work out a score to indicate the quality of a habitat (Moss, 1997). Each group of invertebrate families is appointed a score depending on how pollution tolerant they are. For example caddis flies (Order Trichoptera) and mayflies (Order Ephemeroptera) are awarded the highest points as they are very intolerant of pollution and can only exist in higher quality water. Oligochaete worms, however, are extremely tolerant of pollution , and so are awarded the lowest score (see section 8.3.6.).

6.3. Results and Discussion

6.3.1. Substrate

RIVERS - The substrate of the river Kelvin is composed of a variety of particle sizes. Silt and mud are the main constituent with a few large boulders (>40 cms diameter) and small rocks (2-40 cms). The river Kelvin might therefore be prone to substrate instability. The small size of the particles comprising the substrate renders them easily moved by the current and continually washed downstream. In contrast, the substrate of the river Endrick is composed mainly of bedrock, large (>40 cms) and intermediate sized (10-40 cms) boulders, with a very small amount of silty sediment and gravels. The large size of the rocks making up the substrate, and the lack of fine sediments suggests that the substrate of the Endrick is relatively stable despite the current. The Esk had a rocky substrate, with mainly small size (< 10 cms) and some larger (10 - 50 cms) boulders embedded in much less silt and gravel than that observed at the river Kelvin.

PONDS - The substrate of Inverleith pond consists to a large extent of fine silt, mud, leaf litter and gravel. The occasional large rock and item of rubbish, for example a shopping trolley, is also present! Balmaha pond has a very thick, spongy layer of leaf litter from the many overhanging trees and high level of aquatic vegetation. However, there are no currents present in either pond to move the substrate around, and so unlike the rivers, the silty substrates do not indicate instability.

6.3.2. Aquatic plants and bank vegetation

RIVER KELVIN - During the course of the project, many aquatic plants were observed in the Kelvin. Pond weeds (Potamogetonaceae) tape grass, *Vallisneria spiralis*, emergent rushes, and large amounts of filamentous algae were widespread especially in the summertime. After heavy rainfall, when the banks were scoured, much less evidence of aquatic vegetation was observed. A few trees were present on the banks, and many overhanging ferns and flowering plants such as marsh stitchwort, *Stellaria palustris*, common field speedwell, *Veronica persica,* and spearworts (Ranunculaceae) dipped into the water providing plenty of shelter for small fish and fry.

RIVER ENDRICK - The Endrick river did not support much submerged vegetation even in summer. Only small amounts of yellow flag, *Iris pseudacorus*, and emergent grasses were seen occasionally at the margins. A silty bed is required to support aquatic vegetation, since most of a plants nutrients are gained from sediments rather than the surrounding water. The rocky, silt free substrate of the Endrick is therefore not conducive to the occurrence of aquatic macrophytes. The banks supported many wild flowers including lesser water parsnip, *Berula erecta*, meadow buttercup, *Ranunculus acris*, red campion, *Silene dioica*, monkey flowers, *Mimulus guttatus*, and green alkanet, *Pentaglottis sempervirens*. Wild rhubarb, *Rheum hybridum*, wild garlic, *Allium ursinum*, and common nettles, *Urtica dioica*, covered the banks extensively. Many large trees were situated on or near the banks, and overhanging the water.

RIVER ESK - The Esk contained a few common or Norfolk reeds, *Phragmites australis*, in one small discrete area, and on some stretches of the bank a few emergent grasses were present. The rocky substrate appeared to limit the occurrence of aquatic macrophytes. The banks, however, were covered in a rich compliment of flowers, shrubs and trees. A similar compliment of wild flowers and shrubs as the Endrick was present, with the addition of water forget-me-not, *Myosotis scorpioides*, pink purslane, *Claytonia sibirica* and herb robert, *Geranium robertianum*,

INVERLEITH POND - Inverleith pond contained no macrophytes, with plant life being restricted to algae. The pond was surrounded by a continuous 1m wide concrete and tarmac path, and so no bank vegetation was present next to the water. However, many trees were present in the park, at more than 1m from the waters' edge.

BALMAHA POND - Balmaha contrasted strongly with this. Emergent plants occurred across the entire width of the pond. Water horsetail, *Equisetum fluviatile*, was widespread, along with broad leafed pond weed, *Potamogeton natans*, Canadian pond weed, *Elodea canadensis*, and yellow flag. The banks were filled with trees overhanging the water. Seasonal growth and dying back of vegetation would change the visual surroundings of Balmaha pond to some extent, whereas a pond such as Inverleith with no vegetation might be relatively more visually stable.

6.3.3. Water quality

The Kelvin was observed to be very turbid, especially when compared to the Endrick. In 1994, the Clyde river purification board, responsible in the past for protecting and improving the water quality of the Kelvin reported that "the major pollution problem in the wider Kelvin catchment is sewage effluent from the sewage treatment works serving towns and villages in the area". Although some areas of the Kelvin improved recently, deteriorations in quality were recorded at Kelvingrove and the botanical gardens which are not far downstream from the vet school area (Gardiner & Armstrong, 1996). According to the classification scheme used for describing water quality published by the Scottish Office Environment Department (SOED), 1992, the Kelvin is described as class C or poor condition (Clyde River Purification Board, 1994).

The Endrick was found in recent years to be of very high water quality, and oligotrophic (with low primary productivity) along its length (Best and Traill, 1994), with satisfactorily treated sewage released into the river from several small settlements. The SOED classified the Endrick and its tributaries as class A1 (Rivers unpolluted and recovered from pollution, pers. com., SOED).

The waters of the Esk appeared to be slightly stained with peat. The area sampled was approximately 50 meters upstream from a sewage works, and was of good quality (SOED classification B, pers. com. SOED).

Inverleith pond is located in the middle of a built up urban area, and has very turbid, poor quality water. Large amounts of rubbish float on the surface and sit on the bottom. Algal blooms occur from time to time in the summer, and no plant life survives. In contrast, Balmaha pond is extremely clean and clear, supporting plenty of aquatic vegetation.

6.3.4. Water chemistry

Table 6.1 shows the results of the water analyses carried out at each habitat.

Table 6.1. Chemical and physical measures made at each field site.

	River Endrick		River Kelvin		River Esk		Inverleith pond		Balmaha pond	
S=summer 1997 Sp=spring 1998	S	Sp	S	Sp	S	-	S	Sp	S	Sp
Temperature at time of sample (°c)	14.8	9.2	16.3	6.7	15.8	-	18.6	10.1	15.6	8.9
Dissolved oxygen (%)	105.5	117.6	38.7	90.7	98.0	-	73.8	84.1	46.5	77.5
Total ammonia (N) mg/l	0.04	0.03	0.00	0.29	0.08	-	0.06	0.43	0.05	0.06
Nitrite (N) mg/l	0.00	0.021	0.245	0.00	0.04	-	0.00	0.04	0.00	0.00
Nitrate (N) mg/l	0.13	0.48	0.85	0.80	0.31	-	0.05	0.45	0.08	0.11
Phosphate(P)mg/l	0.23	0.236	0.78	0.07	0.58	-	0.08	0.08	0.08	0.05
pH	7.6	7.6	7.6	7.2	7.8	-	7.6	7.4	6.6	6.6

At higher temperatures, water can hold less oxygen. Accordingly, table 1 shows that for each of the habitats, the summer measures of oxygen are lower than the spring measures. In general, river habitats have a higher level of dissolved oxygen than ponds due to turbulence and mixing. This trend can be seen in the above table except for the summer Kelvin sample, where the extremely low level of dissolved oxygen might be explained by the high temperature coupled with the high organic content of the river due to sewage releases.

Most of the habitats had a neutral pH. Balmaha pond had a slightly depressed pH (6.6) This could have arisen due to anthropogenic acidification from industrial emissions from nearby Glasgow (acid rain). However, some aquatic habitats have a naturally low pH (5-6.9) caused by an acidic catchment surrounded by forest or bog.

Table 6.1 shows that for most habitats, nitrogen and phosphorous levels, were relatively low. However, the levels of nitrogen and phosphorous were high in the summer Kelvin sample, presumably from sewage inputs. If nitrogen and phosphorous levels are high, and the stream bottom is muddy, as is the case in the Kelvin, an abundance of oligochaete worms and chironomid larvae predominate. This was indeed the case revealed by the Kelvin benthic samples (see below). The poorer quality and observed turbidity of the Kelvin suggests that this river experiences the lowest visibility of the five habitats.

6.3.5. Benthic macro-invertebrates

The families of benthic invertebrates found at each site were identified. Table 6.2 overleaf lists the number of individuals of each family found at each site, the benthic invertebrate group to which each family belongs, and the number of points awarded to each represented group (in brackets).

In order to calculate the National Water Council score for each of the five sampling sites, points are awarded to each site for each group of invertebrates represented. For example, if the family Hydrobiidae (snails and bivalves (3)) was present at a site, three points would be awarded. If both Hydrobiidae and Sphaeridae were found, three points would still be awarded. Points are allocated for each invertebrate group represented rather than for each family. The scores calculated for each of the five sites sampled are shown in table 6.3, in descending order of quality:

Table 6.3. National Water Council biological scores system: scores for each of the five sampling sites.

Sampling site	Score
River Endrick	73
River Esk	65
Balmaha pond	42
River Kelvin	30
Inverleith pond	26

Table 6.2. Number of individuals of each family of benthic invertebrates present at each sampling site with the number of points awarded to each represented group in brackets.

Invertebrate group	Family	River Endrick	River Kelvin	River Esk	Inverleith pond	Balmaha pond
Mayflies (10)	Siphlonuridae	40	-	33	-	4
	Ephemerellidae	37	-	79	-	1
	Leptophlebidae	-	12	1	-	-
	Ephemeridae	1	-	-	-	-
Stoneflies (10)	Taeniopterygidae	1	-	-	-	-
	Capniidae	1	-	-	-	-
	Leuctridae	-	-	2	-	-
	Perlolidae	9	-	-	-	-
Caddis-flies (10)	Phryganeidae	1	-	1	-	-
	Letpoceridae	1	-	-	5	-
	Sericostomatidae	9	-	-	-	-
Dragonflies (8)	Lestidae	-	-	-	-	1
Net-spinning caddis-flies (8)	Psychomyiidae	3	-	-	-	-
Mayflies (7)	Caenidae	-	-	-	11	-
Snails (6)	Ancylidae	1	3	1	-	-
	Neritidae	-	249	-	-	-

cont......

Table 6.2. continued

Invertebrate group	Family	River Endrick	River Kelvin	River Esk	Inverleith pond	Balmaha pond
Crustacea (6)	Gammeridae	20	-	28	-	-
Dragonflies (6)	Platycnemidae	-	-	-	-	2
Beetles (5)	Dytiscidae	46	-	11	-	2
	Elminthidae	3	-	-	-	-
Caddis-flies (5)	Hydropsychidae	1	-	1	-	-
Dipteran flies (5)	Simulidae	-	6	-	-	-
Mayflies (4)	Baetidae	20	-	88	-	-
Alderflies (4)	Sialidae	-	-	-	-	5
Snails & bivalves (3)	Hydrobiidae	396	29	3	13	-
	Sphaeriidae	1	22	-	382	-
	Lymnaeidae	-	-	-	-	2
	Physidae	-	2	-	-	-
Leeches (3)	Glossiphonidae	-	-	-	31	2
Crustacea (3)	Asellidae	2	136	4	1	-
Diptera (2)	Chironomidae	5	93	22	85	247
Whole class (1)	Oligochaeta	44	67	156	165	3

The NWC scores in table 6.3. verify that the river Kelvin and Inverleith pond are the most polluted habitats, with the lowest scores. The benthic fauna of these two habitats consisted mainly of a great abundance of tolerant invertebrates (at the lower end of table 6.2) such as chironomids and oligochaete worms. The Endrick, Esk and Balmaha pond, however, are higher quality habitats, with higher scores. More families of less tolerant families of invertebrate (at the upper end of table 6.2) were present in less abundance, in addition to the less tolerant families. A greater diversity of benthic invertebrates with less abundance of each family signifies higher quality water.

6.4. Summary

Table 6.4 summarises the features of each habitat.

Table 6.4. Summary of the nature and character of the five sampling sites.

Location	Substrate	Flow	Aquatic Vegetation	Clarity	Proposed visual stability
Endrick river	Mainly rocky	Yes	Very little	Very good	Quite unstable
Kelvin river	Large amounts of silt	Yes	Large amounts	Poor	Very Unstable
Esk river	Rocky, some silt	Yes	Very little	Stained	Quite unstable
Inverleith pond	Silt and gravel	No	Very little	Quite poor	Stable
Balmaha pond	Thick leaf litter	No	Large amounts	Very good	Stable

The river Endrick could be regarded as being quite visually unstable owing to the presence of flow, however, the good visibility, lack of vegetation and solid substrate prevents this habitat from being extremely unstable, and so the possibility for the use of visual information might not be completely precluded. The river Kelvin could be regarded as being visually and physically unstable owing to the combination of flow, silty substrate, poor visibility and vegetation. Under such conditions, visual information is unlikely to be of much use for orientation. The river Esk falls in between these two with regard to visual stability. Although there is little aquatic vegetation and the substrate is quite solid, visibility is reduced by staining. Both ponds are considered visually stable due to the lack of flow. In Balmaha pond, only the growth and dying back of vegetation causes some variation in the surroundings. Inverleith pond has no aquatic vegetation, and so remains relatively unchanging.

Chapter 7

Morphological variation in the three-spined stickleback: adaptations to a range of environments

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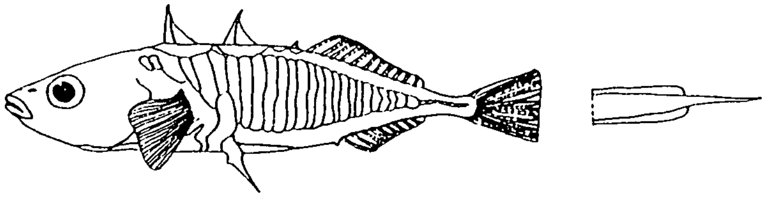
7.1. Introduction

The previous chapters have detailed behavioural variation between five populations of Scottish sticklebacks. A study of the habitats occupied by these populations (chapter 6) revealed that they are likely to exert different selection pressures on the resident sticklebacks. Such pressures appear to have influenced behaviour, causing it to become adapted to the particular niche occupied. Selection pressures might not have only affected behaviour, but might also have caused genetic differentiation as well. One way to gain an insight into the genetic variation between populations is to determine the extent to which morphology has diverged. There are many cases of stickleback morphology diversifying as a result of different selection regimes (see below), and so I decided to carry out a morphological analysis of the populations used in this thesis. The previous chapter revealed substantial differences between the habitats the populations occupy, but if the populations should exhibit morphological variation as well, then this could be taken as further evidence that they are subject to diversifying effects in their natural environments.

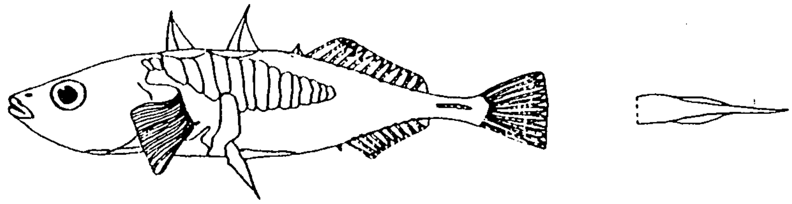
7.1.1. Morphological variation

The three-spined stickleback exhibits a high level of morphological diversity. Indeed, it is often regarded not as a species, but as a species complex, ranging throughout marine, coastal and fresh waters of the northern hemisphere. Three main morphs exist. The marine or *trachurus* form is widely regarded as the ancestral state of the three-spined stickleback, with the mainly estuarine (*semi-armatus*) and freshwater (*leiurus*) forms having diverged from this basic template as they colonised post glacial brackish and freshwater habitats. The marine form possesses a full compliment of dorsal spines, lateral armour plates along the entire length of the flanks, and a broad caudal keel (see figure 7.1). The fossil record indicates that this form has changed little in the last 10 million years (Bell, 1994).

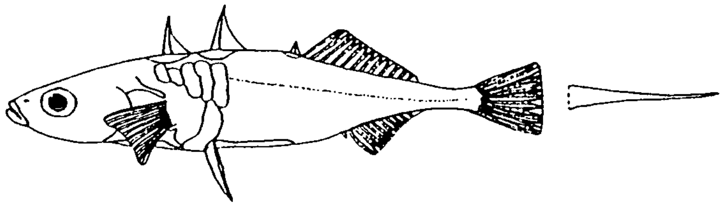
Figure 7.1. Trachurus (a), semi-armatus (b) and leiurus forms (c) (taken from Wootton, 1976).



(a) Trachurus



(b) Semiarmatus

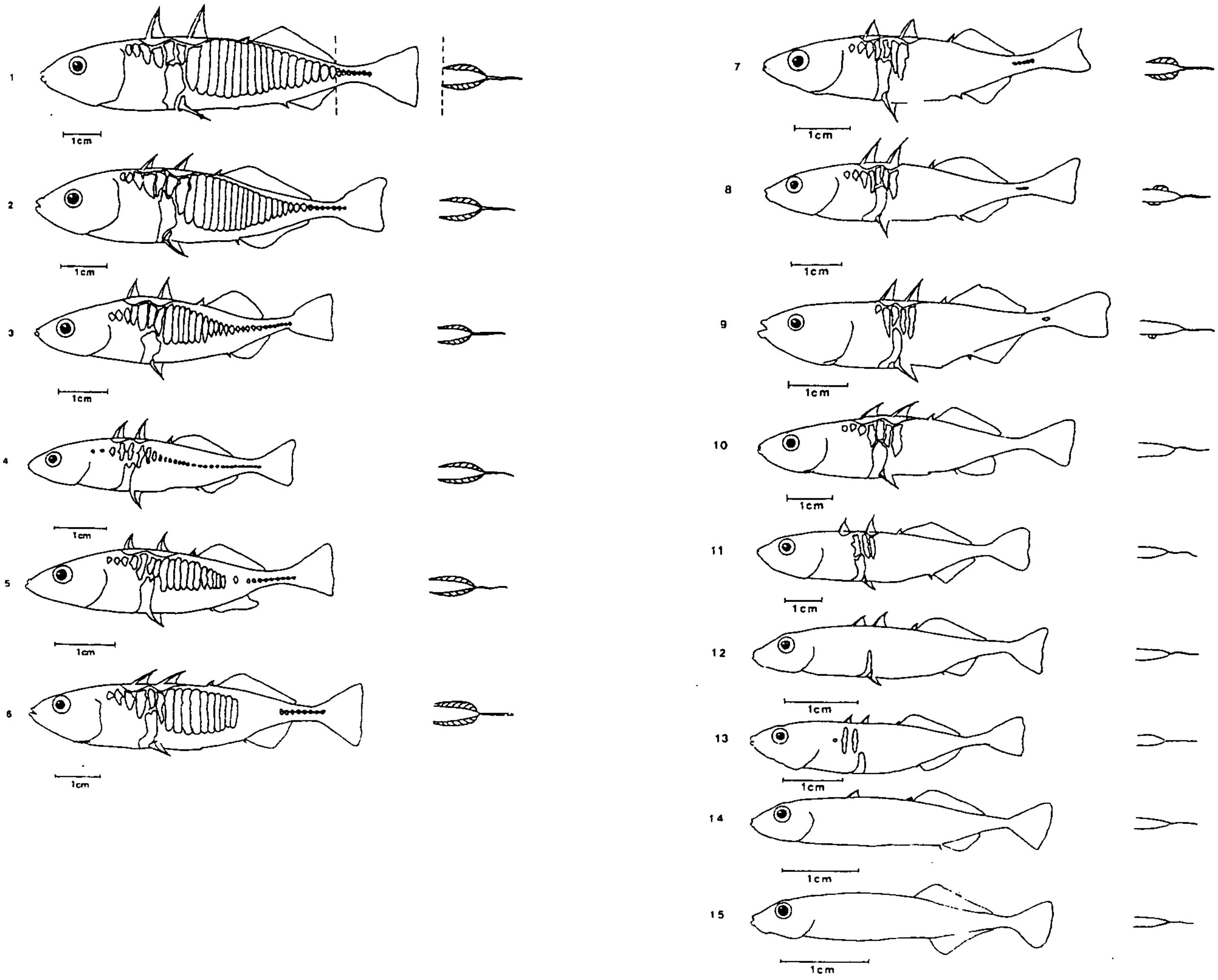


(c) Leiurus

The freshwater form has lost all but a few anterior lateral plates, and the broad caudal keel. An intermediate form occurs mainly in estuaries, retaining the keel but with an intermediate number of plates. Even within these three main forms, a continuous range of morphological diversity can occur. Figure 7.2 illustrates the wide range of diversity found in Scottish sticklebacks, as described by Campbell (1985).

Indeed, the three-spined stickleback has been described as being 'among the most variable species ever studied' (Foster, 1995).

Figure 7.2. Wide range of lateral plate variation in Scottish sticklebacks (taken from Campbell & Maitland, 1992).



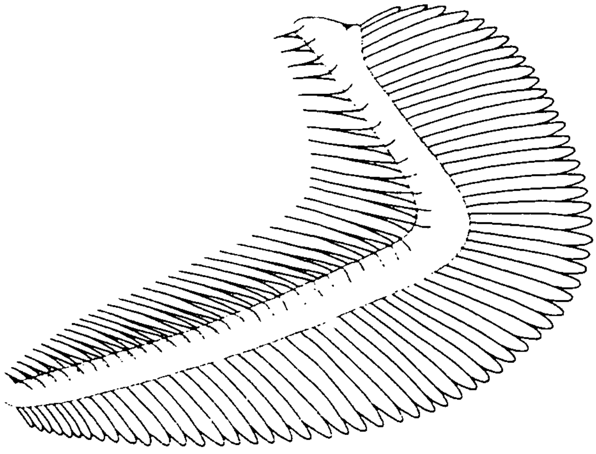
Fish used in the experiments of this thesis are taken from freshwater habitats, and are therefore the freshwater *leiurus*, or low plated forms. The functional advantage of lateral plate variation is not yet certain, for example, it is difficult to suggest the advantage of reducing the number of lateral armour plates. Attempts to relate plate number with environmental calcium, temperature or predation pressure have remained inconclusive (Gross, 1977, Giles, 1983, Klepaker, 1995). However, it has been observed that in populations of Norwegian freshwater sticklebacks, that the longer the particular habitat has been isolated from the sea, the lower the number of plates of the resident stickleback (Klepaker, 1995). There may therefore be some selective advantage for the low plate morph in freshwater.

Evidence supports the theory that the freshwater form has evolved locally innumerable times from multiple invasions by marine fish, rather than having evolved once and spread by dispersal through post glacial freshwaters (for review see chapter 1, Bell & Foster, 1994). Many freshwater populations occur on isolated islands and peninsulas, and these are unlikely to have arisen through freshwater migration routes in the short time since the last ice age. In addition, the marine form enters coastal marine or freshwater habitats to breed, so the capacity for frequent colonisation of freshwater exists. Thus, the world geographical distribution of freshwater populations is unlikely to have arisen from dispersal only through freshwater.

Evidence that certain freshwater populations were derived from nearby marine populations is provided by Reimchen *et al.* (1985). They carried out intensive morphological analysis on lake, pond, river and marine sticklebacks sampled from the river Sangan catchment area on the Queen Charlotte islands in Canada. Under the assumption that phenotypic similarity provides insight into descendant relationships, the marine (*trachurus*) sample taken from the coast near the mouth of the river was shown to be more closely related to the river fish than the lake fish. This suggests that a stream form arose from a population of marine fish that moved up into the river mouth to breed, and then did not all return to the sea. The stream form could then have colonised the lakes and ponds further upstream, resulting in further divergence. Geological evidence (Sutherland-Brown, 1968) suggests that these two evolution events took place in the last 3000 - 5000 generations. This is a rapid rate of speciation comparable to that of the African cichlids (Greenwood, 1965).

Aside from the level of plating, other types of morphological variation have also been documented. McPhail (1984) described two different forms of three-spined stickleback living sympatrically in Enos lake, Vancouver island, one of which inhabited the body of the water (limnetic form), and the other which associated with the substrate in the shallows (benthic form). The mouth parts, body shape and eyes varied between the two forms. The limnetic type that occupied the water column and fed on plankton had a more slender, streamlined body, a narrower snout for feeding on small plankton prey and protrusive eyes which give better distance vision for fixing prey. The benthic type living near the substrate or benthos fed mainly on benthic invertebrates. It had a deeper body allowing greater manoeuvrability, a broader snout for the larger benthic prey, and less protrusive eyes. The two forms, or 'ecotypes' (after Foster, 1995), also exhibited different gill raker designs which are associated with benthic and planktivorous feeding. Gill rakers are a series of protrusions which extend from the first gill arch across the opercular gap (for example, see figure 7.3).

Figure 7.3. Gill rakers of a herring (from Maitland & Campbell, 1992).



The function of the gill rakers is to prevent prey being washed out with expelled water while at the same time not impeding the flow of water through the gap. Fish feeding on large prey such as benthic invertebrates need not have so many or so closely spaced gill rakers. The gill rakers of the planktivores, however, were found to be longer, more closely spaced and more numerous, to prevent the smaller prey items escaping. By diversifying in such a way, populations can become trophically segregated, thereby reducing competition for food and space. The same pattern of differentiation between benthic and limnetic sticklebacks has been documented in many lakes of British Columbia (Bentzen & McPhail, 1984, Lavin & McPhail, 1985), Alaska (Foster, 1995), and Scotland (Ibrahim & Huntingford, 1988).

Fish inhabiting lakes, ponds and streams within the same watershed can also show this pattern of variation. Reimchen *et al.* (1985) compared the morphology of 16 populations of river, pond and lake stickleback from the Sangan watershed, Queen

Charlotte Islands, Canada. A high degree of morphological variation, including differences associated with benthic and limnetic types, was observed between sticklebacks living in lakes, ponds and streams (all *leiurus* forms). For example, body depth was greatest in fish living in streams, lower in ponds, and lowest in lakes, while for gill raker number, a reciprocal relationship was found. Stream fish therefore showed adaptations common to the benthic morph, whereas the lake fish were similar to the limnetic type, with the shallower pond fish intermediate. The degree of variation did not depend on the geographical distance between populations, but rather on the type of habitat occupied, with no consistent relationship to the degree of sympatry or allopatry.

7.1.2. Behavioural variation

Behavioural variation is also widespread between populations. Many instances of behavioural differences relating to ecological and trophic segregation have been documented. For example, feeding behaviour was found to differ between the limnetic and benthic forms in Enos lake, Vancouver Island (Bentzen & McPhail, 1984). Under experimental conditions, benthic fish foraged more successfully on the substrate, consuming more, larger prey than the limnetic fish. Indeed, female limnetic fish could not be induced to forage on the substrate. Limnetic fish were much better than benthic fish at feeding on small zooplankton. These behavioural differences are inextricably linked to the morphological differences described above (7.1.1).

In Scotland, a similar study using sticklebacks from Balmaha pond and nearby Loch Lomond showed differences in the foraging choices of the two populations

(Ibrahim & Huntingford, 1988). Fish from the Loch achieved higher profitabilities when feeding on plankton, whereas those from the small, shallow pond, which did not contain as much plankton, did better with benthic prey. In terms of feeding behaviour, the Loch fish more closely resembled the limnetic morph, while the pond fish resembled the benthic morph.

A different study at Paxton Lake, British Columbia (Larson, 1976) showed that the benthic morph was more aggressive than the limnetic type. It was proposed that the spatial distribution of different prey types may explain this. Limnetic forms forage on fairly uniformly and continuously distributed planktonic prey which are hard to defend, whereas benthic forms forage on a more limited and discontinuous type of prey on the substrate. The benthic form can therefore defend food patches from con-specific competitors. In addition, the benthic type typically occupy the same habitat where nests are built, and young are reared (in the shallows). Higher aggression may therefore help the defence of the nest, and reduce the danger of cannibalism which is widespread among the benthic type during the breeding season (Foster, 1995). The limnetic fish, however, are non-cannibalistic and do not usually inhabit the area suitable for nesting unless in the act of breeding. The chances of a limnetic coming across and cannibalising a conspecific nest may be much less likely. Consequently, lower levels of aggression are required.

Foraging and cannibalism tendencies also relate to differences across populations in courtship behaviour. For example, in North American lakes, cannibalistic populations have been seen to exhibit a less conspicuous zig-zag display when courting females, paying more attention to dorsal pricking instead (Foster, 1995). It is thought that this altered display draws less attention to the location of the nest. Ward & Fitzgerald (1987) found that sticklebacks do not recognise nests visually from their structural characteristics. Instead, males rely upon remembering its location relative to the surroundings, and females rely on the male revealing the location. Nesting males from cannibalistic populations have also been observed to exhibit diversionary displays to lead other fish away from the nest (Foster, 1988). This is done by making rooting movements in the substrate away from the vicinity of the nest. These mimic feeding and attract any onlooking fish towards that area away from the nest. The non-cannibalistic limnetic fish have a more prominent, prolonged display, beginning to zig-zag while the potential mate is further away, and they do not exhibit the diversionary behaviour. These differences in courtship behaviour suggest that a certain degree of reproductive isolation might exist between the forms. At Drizzle Lake, Queen Charlotte, Canada, stream and lake forms overlap in their distribution where the inlet stream connects to the lake. These forms were found to be in behavioural reproductive isolation because of different habitat and mate preferences (Stinson, 1983).

It can therefore be seen that behavioural and morphological variation result from ecological differences, and the need to adapt to the particular environment occupied. The work reported in this thesis has examined behavioural differences between three river and two pond populations. It is possible these populations might show similar morphological divergence to that described in the Sangan river study (Reimchen *et al.*, 1985) since they have been sampled from a similar range of habitats. Morphological analyses were therefore carried out to determine if the populations resembled mainly benthic or limnetic morphs. This morphological comparison should reveal whether the populations have diverged morphologically, and will also provide information on the lifestyle (benthic or limnetic living) and feeding preferences of each population.

7.1.3. Morphological analysis of populations used in chapters 2, 3, 4 & 5

The populations used in this thesis have had since the end of the last ice age to adapt to their different habitats. This is not considered to be a long time in evolutionary terms, but there is a chance that there may be considerable genetic differences between the different populations. Quite recently, the work of Thompson *et al.* (1997) revealed that two populations of three-spined sticklebacks genetically diverged substantially within that time frame. Their study compared the mitochondrial DNA (mtDNA) of a lake-stream pair of three-spined stickleback which evolved post glacially in Misty Lake, Canada. The lake sticklebacks occupied the body of the lake, while the stream fish occupied the inlet stream to the lake, with some degree of overlapping. The lake and stream forms exhibited differences in trophic morphology (body parts involved in feeding), body shape and colour. It was found using mtDNA restriction site assays that the two lineages (lake and stream) were 'highly divergent', differing by 2.7%.

Morphological differences between the two pond and three river populations used in experiments 2, 3, 4, and 5 (the rivers Kelvin, Esk and Endrick, and Balmaha and Inverleith ponds) were assessed. The data that was collected included measurements of trophic characters such as gape width, gill raker number, length and separation and features of body shape to indicate degree of streamlining and manoeuvrability (important for feeding on benthic prey). Using these analyses it was possible to gain some insight into the extent to which the populations have diverged.

7.2. Methods

Ten individuals were collected from the rivers Kelvin and Endrick, and Balmaha and Inverleith ponds (for full details of these habitats, see chapter 6). Only five specimens could be sampled from the river Esk. These will be compared to the other populations in the results and discussion sections, but will not be included in the overall statistical analysis as the sample size of n=5 was too small. The pond fish were sampled using minnow traps constructed from 1 litre plastic bottles, while the river fish were captured by dip netting under the banks. The fish were euthanased using MS222, and preserved in 90% alcohol. The following external measures were obtained using Vernier callipers (0.05mm): Standard length (not including tail fin), body depth and body width (both measured at base of pectoral fin), length 1st and 2nd dorsal spine, gape width, gape height, and left and right eye diameters (including iris). In addition, left and right lateral plate number, and the number of fin rays in the fork (tail fin) were counted. The left hand gills were dissected using bow-handled scissors so that gill raker number, length (of the 5th gill raker), and separation (of the 5th and 6th raker) could be measured using a binocular microscope and a PZO microscope with a micrometer eye piece at x10 and x40 magnification. A comparative estimate of the degree of crenation of the dorsal and pelvic spines was also made. Crenation ranged from zero (on very smooth spines) to 3 (on highly crenated spines).

All lengths were expressed as a proportion of standard length, to control for differences in age and size.

7.2.1. Statistical analysis

Multiple analysis of variance (MANOVA) were carried out on the morphological data from the rivers Kelvin and Endrick populations, and Balmaha and Inverleith pond populations using Statview^R software. Fisher's PLSD *post hoc* tests were carried out to determine which morphological characters differed between the different populations. Lateral plate number and degree of crenation were not included in the MANOVA. Lateral plate number was unsuitable since it was not continuously distributed, and the degree of crenation was a subjective comparative estimate.

7.3. Results

Morphological measurements expressed as a proportion of standard length (except fork length) for all characters are recorded in appendix 3. The MANOVA revealed that there was a significant effect of population on morphology (Wilk's Lambda: $F_{51,37}=4.92$, $p<0.001$). The Fisher's PLSD *post hoc* test revealed that fish from the Endrick had significantly broader and deeper bodies than the other three populations (table 7.1).

Table 7.1. Significant p-values show that Endrick fish had significantly broader and deeper bodies than the other three populations. *p<0.05, **p<0.01. Mean widths and depths are expressed as a proportion of standard body length.

Comparison	Respective mean widths	p-value for width	Respective mean depths	p-value for depth
Endrick *vs* Inverleith	0.147 vs 0.126	0.0105 *	0.256 vs 0.236	0.0107 *
Endrick *vs* Balmaha	0.147 vs 0.117	0.0002 **	0.256 vs 0.239	0.0148 *
Endrick *vs* Kelvin	0.147 vs 0.119	0.0016 **	0.256 vs 0.237	0.0385 *

Balmaha pond fish had shorter spines than Inverleith pond and river Kelvin fish (1st dorsal, p=0.0297 & 0.0034 respectively; 2nd dorsal, p=0.0237 & 0.0082 respectively). Both Endrick and Kelvin had thicker 1st dorsal spines than both Inverleith and Balmaha (see table 7.2. overleaf).

The Kelvin fish had the largest gape height, followed by Inverleith pond. Endrick and Balmaha had the smallest gape heights (table 7.3. overleaf).

Inverleith had more gill rakers than the Kelvin, Balmaha and Endrick populations (p=0.0001, 0.0003 & <0.0001 respectively), while Endrick fish had wider gill raker spaces than Inverleith and Balmaha fish (p=0.0247 & 0.0134 respectively).

Table 7.2. Significant p-values show that both river populations had thicker 1st dorsal spines than both pond populations. Mean thickness is expressed as a proportion of standard body length.

Comparison	Respective mean thickness'	p-value
Endrick *vs* Inverleith	0.0807 vs 0.0817	0.0295 *
Endrick *vs* Balmaha	0.0807 vs 0.0734	0.0049 **
Kelvin *vs* Inverleith	0.0894 vs 0.0817	0.0261 *
Kelvin *vs* Balmaha	0.0894 vs 0.0734	0.0057 **

Table 7.3. Significant differences in gape height. Mean gape height expressed as a proportion of standard body length.

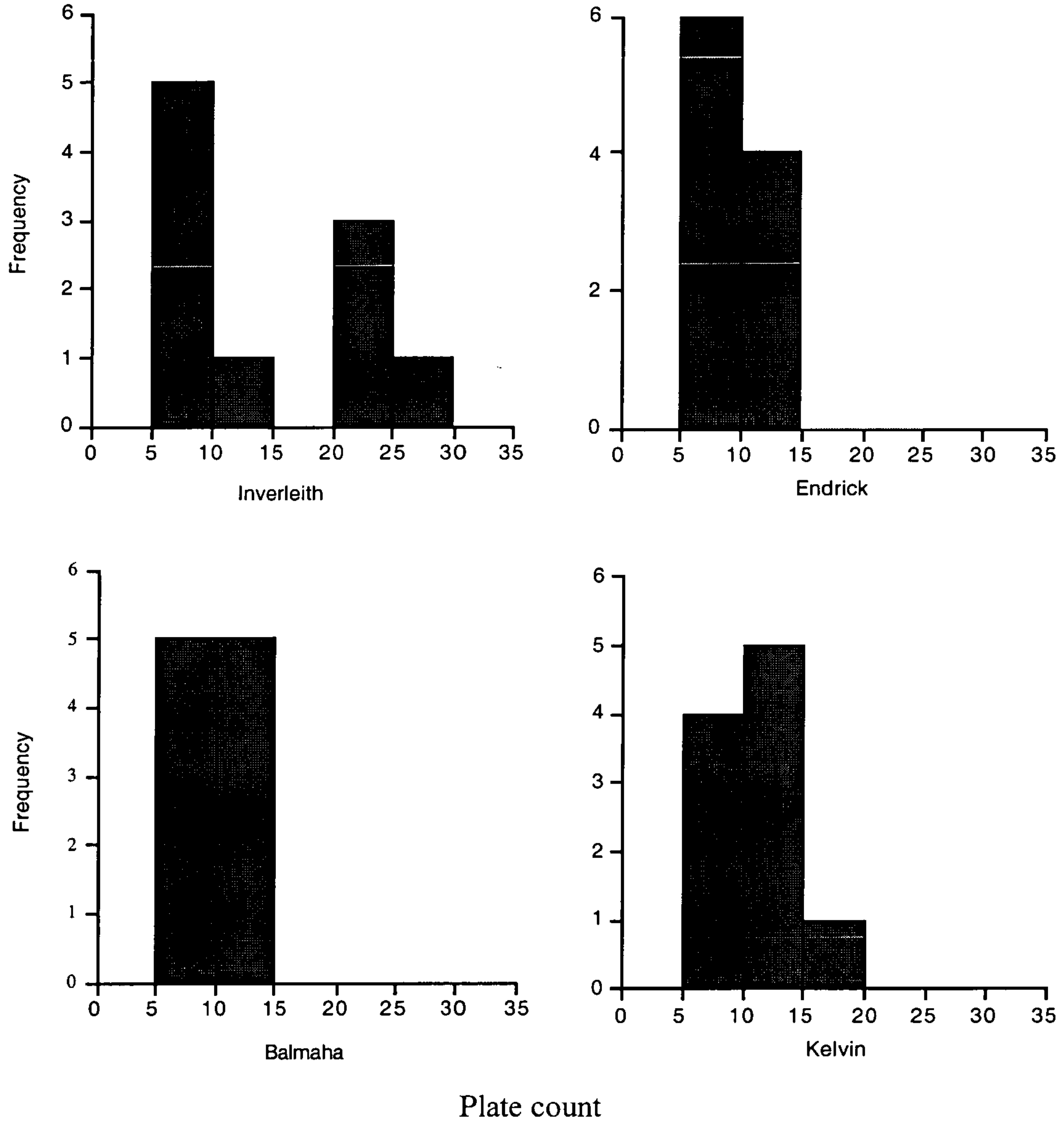
Comparison	Respective mean heights	p-value
Endrick *vs* Inverleith	0.0677 vs 0.0784	0.0002 **
Endrick *vs* Kelvin	0.0677 vs 0.0891	<0.0001 **
Inverleith *vs* Balmaha	0.0784 vs 0.0682	<0.0001 **
Inverleith *vs* Kelvin	0.0784 vs 0.0891	0.0037 **
Balmaha *vs* Kelvin	0.0682 vs 0.0891	<0.0001 **

No differences were revealed between the standard length of the samples, the size of the eyes, gape width, number of fork rays, or gill raker length.

Lateral plate number was not included in the MANOVA, but was graphed instead for a comparison between populations. One population (Inverleith pond) showed a bi-modal distribution of plate counts (figure 7.4. overleaf).

The degree of crenation was also not included in the MANOVA because the measure was a subjective estimate. Degree of crenation appeared to be similar between populations, although Balmaha pond had a few more fish with zero crenation than the other populations (see appendix 3). All five Esk fish had smooth spines with no crenation.

Figure 7.4. Frequency of total lateral plate counts for each populations.



7.4. Discussion

Significant morphological variation between the four populations was revealed. Some of the morphological variation included trophic features, for example, the Endrick population had broader and deeper bodies than the other three populations. Greater body depth was cited in the introduction to this chapter as a feature of benthic foraging sticklebacks which allowed greater manoeuvrability when foraging on the benthos. The suggestion is therefore, that Endrick fish forage to a greater extent on the benthos than the other populations. It is possible that the river Endrick contains less plankton than the standing water bodies (ponds) and so the Endrick fish must rely on the benthos for all their feeding activity. Indeed, the work of Reimchen *et al.* (1985) which was discussed in the introduction, revealed that river fish of the Sangan river system in Canada were most like benthic forms in that they had the deepest bodies, while shallow lake or pond fish had less deep, more streamlined bodies. It is more difficult, however, to explain why the Endrick fish should have deeper bodies than the Kelvin fish since both habitats are rivers. It would be useful to have a more thorough analysis of the prey types available in the two river habitats, and the foraging preferences of the fish to try and explain this difference.

Endrick fish not only had the deepest bodies, but also more widely spaced gill rakers than Inverleith and Balmaha fish. This is another feature of sticklebacks that feed on larger benthic prey rather than planktonic prey suspended in the body of the water. Both of the pond populations may have the opportunity to feed on small planktivorous prey, and this would select for more closely spaced gill rakers.

Inverleith pond, but not Balmaha pond fish, had more gill rakers than the river populations. Having more numerous gill rakers is an adaptation to enable some degree of planktivorous feeding (see introduction). Why Inverleith fish should have more than Balmaha pond fish, however, is not clear. Perhaps there is a greater supply of plankton in Inverleith pond compared to Balmaha pond. Kelvin and Inverleith fish had the highest gapes. This suggests that these two populations are capable of taking larger prey than the other two populations. To explain why Inverleith pond fish should have more gill rakers for planktivorous feeding, and yet have a high gape for consuming large benthic prey suggests that these fish exhibit both feeding strategies to some extent. Indeed, I have observed both benthic and planktonic prey species in Inverleith pond. however, further investigation would be beneficial before further conclusions can be drawn.

Balmaha fish had shorter spines than Inverleith and Kelvin fish, and the two pond populations had thinner 1st dorsal spines than the two river populations. Predation pressures, especially exerted by piscivorous fish (Wootton, 1976), seem to influence spine length and thickness, with thicker, longer spines being more effective at avoiding predation. Although not an absolute defence against predators, spines greatly increase the difficulty predators have in handling them. When attacked, the spines of the stickleback lock in the upright position, making the stickleback very difficult to swallow. Pike, *Esox lucius*, and perch, *Perca fluviatilis*, given the choice in the laboratory, preferred to eat minnows, *Phoxinus phoxinus*, first, followed by nine-spined sticklebacks, *Pungitius pungitius*, (which have shorter spines) and finally three-spined sticklebacks (Hoogland *et al.*, 1957). With the spines cut off, there was no longer any preference for a particular species. In addition, Hagen & Gilbertson (1972) found a positive correlation between stickleback spine length and numbers of predatory fish species present in the same habitat. In the current thesis, Balmaha fish had shorter, thinner spines than the other populations. Balmaha pond is a small, isolated, relatively shallow pond, and these circumstances may not be conducive to the presence of large piscivorous fish. There was also a slight trend for the Balmaha fish to have less crenation on their spines than the other populations, and this could also relate to the lower predation pressure which has been suggested for this population. Inverleith pond is visited by many seagulls which seem to prey quite heavily on stickleback (pers. obs.), and this could explain why longer spines have been selected for in this population. Kelvin fish, however, had the longest, thickest spines, suggesting that they are subject to greater predation pressure than the other populations. To support this hypothesis, Gardiner & Armstrong (1996) found trout, *Salmo trutta*, a predator of sticklebacks (Wootton, 1976), to be widely distributed throughout the Kelvin. Endrick fish had intermediate spine length, but their 1st dorsal spine was thicker than the two pond populations. These morphological results suggest that this population is subject to some predation pressure. Indeed, sea trout are known to come up into the Endrick from Loch Lomond (Mills & Graesser, 1992), and the rural surroundings are likely to be conducive to the presence of piscivorous birds..

Total lateral plate number was strongly bi-modal in the Inverleith pond population (Figure 7.4), with peaks in frequency at 5-15, and 20-30 lateral plates. The suggestion here, is that the Inverleith population is a mixture of *leiurus* (freshwater) and *semi-armatus* forms. The *semi-armatus* form is associated mainly with estuarine habitats, although it is not unknown for *semi-armatus* or even *trachurus* (marine) forms to exist in freshwater (Maitland & Campbell, 1992). To investigate how this form could have arrived in Inverleith pond, I rang the Park Patrol of Edinburgh District Council, who revealed that Inverleith pond drains into a nearby river (the Water of Leith) which runs on only for a further 3 kms before reaching the Port of Leith and the Firth of Forth. The *semi-armatus* fish could have come upstream from the sea, and entered the pond through the drainage system. Unfortunately, it is not known if any of the Inverleith fish used in the experiments were of the *semi-armatus* form. Since the Inverleith pond fish generally exhibited behaviour typical of the pond fish of this project, either there were no *semi-armatus* forms in the experimental samples, or they had developed the same orientation behaviours as the *leiurus* forms in response to occupying a pond habitat. This could be considered to be much the same process as seen in the river Kelvin fish reared in a pond habitat, discussed in chapter 5.

Two of the other populations occupied habitats which do not connect with the sea at all (The Endrick water rises in the Gargunnock hills west of Stirling, and drains into the south-eastern end of Loch Lomond and Balmaha pond is isolated on the banks of Loch Lomond), while the other habitat (Kelvin) was sampled a great distance from the sea (at least 20 kms). This might explain why it was just Inverleith pond, at only 3 kms from the sea, which had the semi-armatus form present.

The same morphological measures as the four main populations were carried out on a small sample of Esk fish (n=5). From this small sample, it appeared that many of the measurements did not differ greatly from the four main populations (see table A1 in appendix 3). The dorsal spines, however, although not crenated, were long like those of the Kelvin sample, and were also the widest of any of the populations. This could reflect a high predation pressure on the Esk fish.

7.5. Summary

A degree of morphological divergence has been revealed. Of the four populations, the river Endrick fish had the most pronounced adaptations for benthic foraging, a deeper body and more widely spaced gill rakers. This fits to previous work with sticklebacks in Canada (Reimchen *et al.*, 1985) which revealed that stream fish were more like the benthic form than pond or lake fish. Balmaha fish had the shortest, dorsal spines. They were least adapted to avoid predation, suggesting that few piscivorous fish are present in Balmaha pond. This is perhaps not surprising since Balmaha pond is small and isolated. The river populations, including the Esk, seemed the best adapted to protect against predation, judging by spine length and thickness. Certainly, they are likely to have more piscivorous fish predators than the two ponds. The Inverleith population was revealed to be a mixture of two of the main forms of stickleback, *leiurus* and *semi-armatus*.

These populations have diverged, therefore, not only in their orientation behaviour, but also in their morphological adaptations to the different habitats they occupy.

Chapter 8

General conclusions

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8.1. Conclusions

Spatial learning experiments reported in this thesis show that three-spined sticklebacks use more than one orientation strategy to solve spatial tasks, and different populations use these different strategies to varying extents. For example, two pond populations relied more heavily than three river populations on visual landmarks to orientate (experiments 2.a. and 3). In addition, two river populations were more adept than two pond populations at relying on direction of water flow to orientate (experiment 4.a). Differences between the various habitats appeared to account for this behavioural variation. Work with artificially reared sticklebacks (experiments 5a and 5b) suggested that these behavioural differences are, to a considerable extent, learned by each new generation during development in a particular habitat type. A small effect of inheritance was also suggested.

8.1.1. Orientation using visual landmarks and stereotypic movements

The results from experiments 2.a. and 3 revealed that two pond populations (Inverleith and Balmaha ponds) relied on visual landmarks to a greater extent than three river populations (Kelvin, Endrick and Esk rivers) to solve laboratory maze tasks. The performance of the two pond populations in these spatial tasks improved when visual landmarks were present. It was suggested that the relatively visually stable surroundings of a pond habitat might promote reliance on visual landmarks. Such landmarks would remain reliable indicators of location owing to the lack of any strong currents which might move objects and the substrate around. The river fish did not show the same trend when landmarks were present. Their performance was not affected by visual landmarks, suggesting that they relied primarily on an alternative method to learn the task. The alternative method appeared to be the adoption of stereotypical behaviour i.e. learning the pattern of turns, usually a repeated sequence, required to successfully complete an orientation task. That this method was being used by river populations was supported by their performance in experiment 2.a, when the line maze was reversed. The strong currents characteristic of river habitats would prevent visual landmarks being reliable indicators of location as they would be continually moved around. The opportunity to rely upon visual landmarks to orientate may therefore not arise in a river habitat. A non-visual method of orientation such as stereotypic movements might therefore be more adaptive.

8.1.2. Orientation using direction of water flow

Two river populations were better at using direction of flow to locate a food reward than two pond populations (experiment 4.a). One of the river populations was particularly adept at using this particular type of cue (the river Kelvin). It was suggested that due to the poor visibility and high level of visual instability present in the Kelvin (full details of habitat in chapter 6), direction of flow was perhaps the only possible cue fish from this habitat could use to orientate. The river Endrick population was slightly less adept at using direction of flow to orientate than the river Kelvin population. The Endrick contrasts with the Kelvin in that it has extremely good visibility (see chapter 6), and is also more visually stable than the Kelvin due to its rocky substrate. In this case, the use of visual information as a secondary tool for orientation may not be completely precluded, and so less emphasis need be placed on being able to navigate entirely by direction of flow.

The two pond populations were consistently slower to learn this task. Their poorer performance was suggested be due to their lack of experience of flow, since they were sampled from standing waters.

8.1.3. Conflict between visual landmarks and direction of water flow

When two types of orientation cue were provided for one river and one pond population (experiment 4.b), it was revealed that a significant proportion of the pond fish preferred to orientate according to visual landmarks, while a similar proportion of the river fish preferred direction of water flow. The majority of fish from each population had a preference for the type of orientation cue which is likely to be more reliable and accessible in their natural habitat.

8.1.4. Experiments with artificially reared fish

It was revealed that the behavioural differences were influenced to a considerable extent by experience of the environment during development. Artificial rearing of the fish altered behaviour in predictable ways. In experiment 5a, river fish bred and reared in a pond environment exhibited similar behaviour previously found in pond fish in experiment 2.a. i.e. they relied on visual landmarks to solve a spatial task.

Experiment 5b revealed that a river and a pond population reared in aquaria in the absence of flow both learned to associate direction of flow with a food reward in a similar number of trials. This contrasts with the wild caught fish from the same river and pond populations which exhibited differences in the speed with which they learned experiment 4a.

8.1.5. General cognitive and olfactory abilities of the fish

Experiments 2b and c showed that olfaction was not used by the fish to help them locate a food reward. This was further backed up by experiment 4.a (flume experiment). I therefore concluded that the performance of the fish depended on the orientation strategies described for each experiment, rather than the food reward simply being located by its odour. Indeed, the literature reports that olfaction is not a well developed sense in these fish whereas vision is the primary sense (Wootton, 1976).

In addition, experiment 2.b, a simple discrimination task, revealed that there were no differences in the ability of the four main populations (excluding the Esk) to adapt to laboratory conditions and to perform a learning task. Other experiments with fish from the river Esk suggest that these fish are similar to the other two river populations in terms of boldness and adaptation to laboratory conditions (Wiener, pers. comm.). Thus, it is unlikely that differences in boldness, adaptation to laboratory conditions or gross cognitive ability could account for the behavioural variation reported in the spatial learning experiments (2.a, 3, 4.a & 4.b).

8.1.6. Ecological and morphological differences between the populations

Chapter 6 compared the habitats occupied by the five study populations. Differences were found between the various habitats that could affect spatial cue preferences, for example, some habitats were more changeable than others owing to such factors as the presence of flow, vegetation or an unstable substrate. In addition, visibility was likely to vary due to the different levels of pollution in each habitat. Both factors could affect the degree to which visual and non-visual spatial cues are used by sticklebacks.

Morphological variation between the populations was revealed in chapter 7. Morphological features relating to predator avoidance (spine length and thickness), prey availability (planktonic *versus* benthic prey) and plate number varied between the populations. This implied that a degree of genetic divergence had occurred between the study populations, perhaps as a result of the different selection regimes imposed by the different habitats.

8.2. Future research

This thesis has demonstrated the use of several different orientation strategies by the same species. The particular type of habitat occupied seems to influence which type of strategy is of primary importance. Several related lines of further study readily suggest themselves.

1. Behavioural differences exist between different populations of stickleback. These differences appear to be related to several environmental factors, not least whether a population occupies standing or flowing water. It would be interesting to

see if the behavioural differences are typical for pond and river fish in general (not just the five populations tested here), by testing a larger number of pond and river populations in their ability to use the orientation strategies described here.

2. It has now been shown that rearing river fish in the absence of flow substantially alters their adult orientation behaviour. Other factors of the environment such as habitat complexity may similarly be involved in fine tuning different aspects of behaviour. How factors affect different behaviours could be studied using habitat manipulation experiments involving the rearing of fish in artificial habitats which could be made to differ in various ways.

3. Although it has been demonstrated here that some populations of stickleback use landmarks to orientate, it has not been determined exactly how the landmarks were used. For example, the fish could have been swimming towards them, following them like beacons. This would presumably involve fish forming some kind of association between moving towards the plant landmarks and finding a food reward. Alternatively, they could have been forming a cognitive map, encoding the geometric relationships between the landmarks and the reward which enabled them to approach the correct location. Landmark manipulation experiments involving detour or novel route finding could be carried out in order to study this further.

4. Orientation ability might be affected by seasonal changes. The experiments reported here were conducted using non-breeding condition individuals at a constant low temperature of 10-12^{O}c and short day length (12:12), to avoid the possibility that seasonal shifts could affect behaviour. During the breeding season the behaviour of sticklebacks changes in accordance with the demands of courtship and breeding

success. Female sticklebacks may sample many nests and then return to the one of the highest quality, and males must defend a nest, constantly chase intruders and subsequently return to the nest. These activities could require superior spatial ability since nests are not easily recognisable from their physical structure, but rather from their location in relation to the surroundings (Ward & Fitzgerald, 1987). It has been shown that season can affect spatial ability. The males of a polygynous species of vole, *Microtus pennsylvanicus*, must range around, locating potential mates during the breeding season. This requires good spatial awareness. The higher spatial demands on the males during the breeding season resulted in their having a better spatial ability than females of the same species at that time of year (Gaulin & Fitzgerald, 1989). Similarly, spatial ability of sticklebacks could change during the breeding season. To investigate this, the same populations could be tested simultaneously under breeding and non-breeding environmental conditions thereby revealing any seasonally induced improvements in orientation ability.

The work of this thesis has shown that Scottish freshwater three-spined stickleback exhibit a degree of behavioural flexibility which allows them to adapt to the wide range of different habitats they occupy. Many aspects of the interaction between animal behaviour and the environment remain to be studied, and will provide future workers with challenging lines of research.

Appendices

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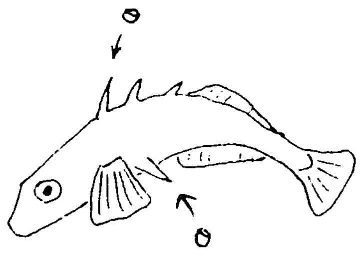
A.1. Tagging fish

Fish were individually tagged using short lengths of electrical wire. A length of electric cable was stripped of the outer plastic coating, and the different coloured wires inside revealed. These were cut into short lengths (4 cms). A small piece of coloured plastic coating was stripped off one end of the length using a razor blade, revealing the copper wires inside. These could be gripped with forceps and pulled out leaving a hollow plastic tube. Each coloured tube could be made into a 2 mm long tag. The tubing was just the right diameter to fit tightly onto a dorsal or pelvic spine.

During marking, the fish were held gently in a net. A mounted needle held between the teeth was used to hold the selected spine upright while the coloured tube was slipped on with just a little pressure to avoid breaking the spine or damaging the membrane running between the spine and the body (see figure A.1). Fish could be identified by specifying the colour of the tubing, and the particular spine which was marked, for example, right pelvic yellow or left pelvic yellow. For experiments requiring large numbers of individuals, more than one spine was tagged.

Fig. A.1. The coloured cylinder is pressed gently onto a dorsal or pelvic spine.



A.2. Artificial breeding and rearing

Adult sticklebacks were collected from the main four populations used in the project (Inverleith pond, Balmaha pond, river Endrick and river Kelvin) at various times during the academic year 96/97 and held in a temperature and light controlled environment (12°c, L:D 12:12) to resemble winter-like conditions and prevent breeding changes taking place. Up to 16 fish were contained in 30x40x30 cm holding tanks. In May 1997, the temperature of the laboratory was raised over a period of four weeks to 20^0c, the day length was increased from 12 to 18 hours, and the quantity of food provided was increased. These environmental changes led to the onset of sexual coloration in males, and egg production in females. When males began to show sexual coloration, they were placed individually in 20x12x12 cm tanks containing fine sand, courser gravel and lengths of grey and green cotton thread (3-10 cms). The males usually began nest building using these items within a few days of isolation.

All remaining fish (females and juvenile or non coloured males) continued to be fed copiously in their original holding tank. It was observed that when coloured males were removed from the holding tank, previously uncoloured males in the holding tank developed breeding coloration. The holding tanks were also checked daily for the appearance of gravid (sexually receptive) females. Gravid females exhibited lateral swelling of the abdominal area caused by egg production. With experience these fish could be easily distinguished from those which were simply gorged on food or which were infected with *Shistocephalus solidus*, a parasitic worm inhabiting the body cavity which can swell the abdomen of a fish to huge proportions.

Twice a day, gravid looking fish were taken from the holding tanks and placed individually in glass jars 2/3 filled with water. These could be floated in the tanks of isolated males. The apparent intrusion of another fish into the males territory elicited the typical aggressive response of the breeding male, and stimulated very intense nest building behaviour, intensification of the breeding colours and frantic mating displays.

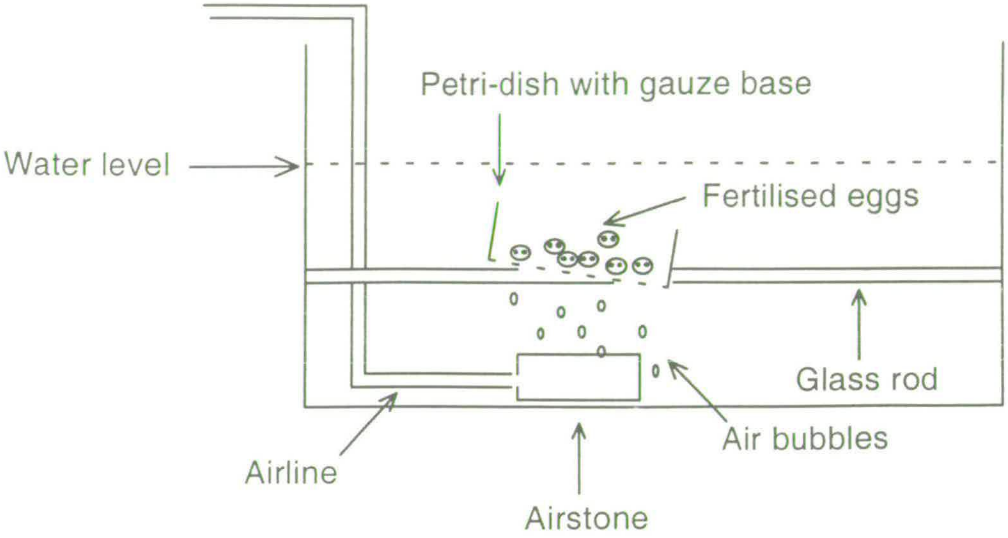
These displays involve vigorous zig-zagging and mouth gaping towards the intruder in the jar (see plate A.1).

Plate A.1. A highly coloured male showing aggression towards a gravid looking female (in the jar). Notice the males' gaping mouth.

If the fish in the jar reacted with fright to this display, they were returned to the holding tank, but if they assumed the typical 'head up' posture of the sexually receptive female, then they were used in an artificial fertilisation procedure. An artificial procedure had to be used because for some reason, males with fry in their nests repeatedly cannibalised their young.

Instead of releasing the female into the males tank to allow them to mate, the male was euthanased, and his gonads dissected. These were crushed in a watch glass with a little distilled water. The female was carefully stripped of eggs into the watch glass. To ensure the female was ready to lay her eggs, a gentle pressure was applied to the abdomen and if eggs began to appear from the ano-genital opening, then the female was selected for use in the artificial fertilisation process. The egg/sperm mixture was agitated by gently shaking the watch glass and then allowed to sit for 15 mins while fertilisation occurred. The fertilised eggs were transferred into special incubators where they could be gently aerated under controlled conditions (see figure A.2).

Figure A.2. Artificial incubator. The dish is placed at an angle to the surface of the water so that the air bubbles pass gently along the base instead of forcing their way up through the eggs.



Each day, the eggs were examined for any dead or infected eggs which were removed (these appeared opaque, white, mouldy or hard instead of translucent and soft). Eyes developed in the eggs and soon became visible within a week. Shortly afterwards, the fry hatched. Great care was taken to transfer the fry to small gently aerated plastic containers where they were fed copiously on newly hatched brine shrimp. The procedures and apparatus involved in breeding and incubating fry using the artificial fertilisation method were originally designed by Iain Barber. When around 1.5 cms, the fry were then moved to small glass tanks and fed bloodworm (coarsely chopped and whole) until they were large enough to take part in the learning experiments described in chapter 5.

A.3. Morphological measurements

In chapter 7, morphological measurements of five populations of stickleback were taken. These are recorded fully in table A1. below, including means, standard deviations and standard errors. All measurements were expressed as proportions of standard length except fork length, number of fins rays in pectoral and tail (fork) fins, number of lateral plates, degree of crenation and number of gill rakers on the left gill.

Table A1. Morphological measurements for the five populations.

Inverleith pond

	Standard length (mm)	Fork length (mm)	Depth	Width	Length 1st dorsal
	44.70	52.90	0.237	0.123	0.0828
	51.60	60.80	0.225	0.114	0.0717
	38.40	46.00	0.229	0.115	0.0964
	38.30	46.20	0.245	0.120	0.0914
	43.00	41.00	0.250	0.112	0.0853
	31.80	38.10	0.236	0.110	0.1006
	47.20	47.20	0.263	0.169	0.0742
	40.00	40.00	0.189	0.117	0.0775
	52.00	52.00	0.254	0.163	0.0673
	35.60	35.60	0.230	0.121	0.0702
Mean	**42.26**	**45.98**	**0.236**	**0.126**	**0.0817**
SD	**6.6872**	**7.7150**	**0.0204**	**0.0213**	**0.0115**
SE	**2.1162**	**2.4415**	**0.0065**	**0.0067**	**0.0036**

	Length 2nd dorsal	Length left pelvic	Length right pelvic	Gape width	Gape height
	0.0962	0.1432	0.1476	0.0738	0.0738
	0.0911	0.1221	*	0.0620	0.0698
	0.1146	0.1536	0.1562	0.0651	0.0703
	0.1018	0.1645	0.1593	0.0757	0.0835
	0.1059	0.1735	0.1618	0.0676	0.0765
	0.1101	0.1667	0.1698	0.0692	0.0786
	0.0932	0.1377	0.1356	0.0741	0.0869
	0.0850	0.1375	0.1400	0.0775	0.0875
	0.0865	0.1115	0.1308	0.0731	0.0731
	0.0758	0.1292	0.1376	0.0815	0.0843
Mean	**0.0960**	**0.1440**	**0.1487**	**0.0713**	**0.0784**
SD	**0.0121**	**0.0204**	**0.0136**	**0.2119**	**0.0067**
SE	**0.0038**	**0.0064**	**0.0043**	**0.0671**	**0.0021**

Inverleith pond

	Left eye diameter	Right eye diameter	No. fin rays left pectoral	No. fin rays right pectoral	No. fin rays fork
	0.0805	0.0783	10	10	12
	0.0736	0.0775	9	10	12
	0.0911	0.0859	10	10	12
	0.0888	0.0940	10	10	12
	0.0853	0.0971	10	10	12
	0.0975	0.0912	10	10	12
	0.0826	0.0932	10	10	12
	0.0925	0.0925	10	10	12
	0.0942	0.0904	6	10	12
	0.0983	0.0955	10	10	12
Mean	**0.0884**	**0.0896**	**9.5**	**10**	**12**
SD	**0.0079**	**0.0069**	**1.3**	**0.0**	**0.0**
SE	**0.0025**	**0.0022**	**0.4**	**0.0**	**0.0**

	No. lateral plates, left	No. lateral plates, right	Degree of crenation	Width 1st dorsal spine	No. gill rakers, left
	4	5	2	0.0380	11
	13	11	1	0.0291	10
	11	11	1	0.0286	13
	13	14	1	0.0365	11
	4	5	1	0.0265	12
	4	4	1	0.0220	14
	4	6	1	0.0339	11
	4	4	1	0.0325	11
	10	11	1	0.0327	10
	4	4	0	0.0225	12
Mean	**7.1**	**7.5**	**1**	**0.0302**	**11.5**
SD	**4.09**	**3.81**	**0.47**	**0.0055**	**1.27**
SE	**1.30**	**1.21**	**0.15**	**0.0017**	**0.40**

	Gill raker length	Gill raker separation
	0.01897	0.00268
	0.01072	0.00507
	0.01247	0.01247
	0.01686	0.00567
	0.01904	0.00798
	0.01663	0.00510
	0.00923	0.00593
	0.01467	0.00489
	0.01625	0.00030
	0.01226	0.00545
Mean	**0.01471**	**0.00555**
SD	**0.00341**	**0.00317**
SE	**0.00108**	**0.00100**

Balmaha pond

	Standard length (mm)	Fork length (mm)	Depth	Width	Length 1st dorsal
	46.1	55.3	0.252	0.126	0.0586
	45.1	54.3	0.259	0.126	0.0599
	48.4	57.9	0.240	0.124	0.0764
	42.2	50.8	0.223	0.104	0.0616
	39.8	47.8	0.231	0.113	0.0879
	40.1	47.8	0.222	0.110	0.0723
	37.2	43.9	0.231	0.113	0.0887
	38.8	46.3	0.242	0.113	0.0696
	42.5	51.6	0.242	0.118	0.0706
	38.3	45.5	0.245	0.123	0.0888
Mean	**41.85**	**50.12**	**0.239**	**0.117**	**0.0734**
SD	**3.6948**	**4.6293**	**0.0120**	**0.0076**	**0.0118**
SE	**1.1692**	**1.4650**	**0.0038**	**0.0024**	**0.0037**

	Length 2nd dorsal	Length left pelvic	Length right pelvic	Gape width	Gape height
	0.0737	0.1388	0.1410	0.0824	0.0694
	0.0687	0.1197	0.1153	0.0776	0.0687
	0.0826	0.1364	0.1322	0.0826	0.0702
	0.0829	0.1185	0.1185	0.0711	0.0664
	0.1080	0.1382	0.1432	0.0678	0.0603
	0.0873	0.1446	0.1372	0.0698	0.0673
	0.0941	0.1478	0.1478	0.0753	0.0780
	0.0799	0.1237	0.1237	0.0747	0.0670
	0.0800	0.1318	0.1341	0.0776	0.0612
	0.0992	0.1540	0.1488	0.0705	0.0731
Mean	**0.0856**	**0.1354**	**0.1342**	**0.0749**	**0.0682**
SD	**0.0119**	**0.0120**	**0.0118**	**0.0052**	**0.0052**
SE	**0.0038**	**0.0038**	**0.0037**	**0.0016**	**0.0016**

	Left eye diameter	Right eye diameter	No. fin rays left pectoral	No. fin rays right pectoral	No. fin rays fork
	0.0976	0.0911	10	10	12
	0.0842	0.0931	10	10	12
	0.0888	0.0826	10	10	12
	0.0995	0.0948	10	10	12
	0.1080	0.1055	11	10	12
	0.0923	0.0873	10	10	12
	0.0914	0.1022	10	10	12
	0.0954	0.0902	10	10	12
	0.0894	0.0988	10	10	12
	0.0914	0.0966	10	10	10
Mean	**0.0938**	**0.0942**	**10.1**	**10**	**11.8**
SD	**0.0067**	**0.0069**	**0.3**	**0.0**	**0.6**
SE	**0.0021**	**0.0022**	**0.1**	**0.0**	**0.2**

Balmaha pond

	No. lateral plates, left	No. lateral plates, right	Degree of crenation	Width 1st dorsal spine	No. gill rakers, left
	5	5	1	0.0390	11
	4	4	0	0.0200	9
	5	5	1	0.0310	12
	5	5	0	0.0213	9
	4	4	1	0.0226	8
	4	4	1	0.0299	10
	4	4	1	0.0269	8
	4	5	1	0.0361	9
	5	5	0	0.0212	9
	5	5	1	0.0209	10
Mean	**4.5**	**4.6**	**0.7**	**0.0269**	**9.5**
SD	**0.53**	**0.52**	**0.48**	**0.0069**	**1.27**
SE	**0.17**	**0.16**	**0.15**	**0.0022**	**0.40**

	Gill raker length	Gill raker separation
	0.01562	0.00586
	0.01042	0.00576
	0.01033	0.00413
	0.01421	0.00592
	0.01507	0.00678
	0.01496	0.00549
	0.01881	0.00538
	0.01417	0.00644
	0.01412	0.00706
	0.01775	0.00653
Mean	**0.01455**	**0.00594**
SD	**0.00269**	**0.00084**
SE	**0.00085**	**0.00027**

River Endrick

	Standard length (mm)	Fork length (mm)	Depth	Width	Length 1st dorsal
	38.00	45.50	0.271	0.179	0.0684
	40.50	47.60	0.264	0.141	0.0815
	37.90	45.10	0.245	0.140	0.0844
	39.20	46.50	0.265	0.140	0.0842
	40.00	47.40	0.248	0.143	0.0900
	37.00	43.80	0.227	0.135	0.0730
	37.70	45.60	0.276	0.167	0.0928
	40.40	48.80	0.282	0.158	0.0668
	39.80	47.90	0.234	0.133	0.0729
	34.60	41.60	0.249	0.130	0.0925
Mean	**38.51**	**45.98**	**0.256**	**0.147**	**0.0807**
SD	**1.8484**	**2.1509**	**0.0183**	**0.0161**	**0.0098**
SE	**0.5849**	**0.6807**	**0.0058**	**0.0051**	**0.0031**

	Length 2nd dorsal	Length left pelvic	Length right pelvic	Gape width	Gape height
	0.0842	0.1211	0.1290	0.0763	0.0658
	0.0864	0.1333	0.1407	0.0543	0.0617
	0.0923	0.1398	0.1451	0.0528	0.0712
	0.0918	0.1480	0.1451	0.0587	0.0714
	0.0925	0.1600	0.1600	0.0625	0.0725
	0.0946	0.1324	0.1270	0.0649	0.0730
	0.1061	0.1592	0.1512	0.0649	0.0690
	0.0743	0.1114	0.1114	0.0644	0.0619
	0.0829	0.1357	0.1231	0.0503	0.0578
	0.0954	0.1561	0.1532	0.0694	0.0723
Mean	**0.0901**	**0.1397**	**0.1386**	**0.0619**	**0.0677**
SD	**0.0086**	**0.0163**	**0.0154**	**0.0080**	**0.0055**
SE	**0.0027**	**0.0052**	**0.0049**	**0.0025**	**0.0017**

	Left eye diameter	Right eye diameter	No. fin rays left pectoral	No. fin rays right pectoral	No. fin rays fork
	0.1000	0.1000	10	10	12
	0.0889	0.0864	10	10	12
	0.0871	0.0818	10	10	12
	0.0867	0.0918	10	10	12
	0.0925	0.0875	10	10	12
	0.0838	0.0865	10	10	12
	0.0875	0.0875	10	10	11
	0.0941	0.0965	10	10	12
	0.0829	0.0854	10	10	12
	0.0896	0.0896	10	10	12
Mean	**0.0893**	**0.0893**	**10**	**10**	**11.9**
SD	**0.0051**	**0.0054**	**0.0**	**0.0**	**0.32**
SE	**0.0016**	**0.0017**	**0.0**	**0.0**	**0.10**

River Endrick

	No. lateral plates, left	No. lateral plates, right	Degree of crenation	Width 1st dorsal spine	No. gill rakers, left
	7	6	1	0.0395	8
	4	4	2	0.0272	9
	5	5	1	0.0607	8
	4	4	1	0.0357	9
	5	4	1	0.0400	9
	4	4	1	0.0405	7
	4	4	1	0.0371	8
	5	5	1	0.0371	10
	4	5	0	0.0402	6
	5	5	1	0.0318	7
Mean	**4.7**	**4.6**	**1**	**0.0390**	**8.1**
SD	**0.95**	**0.70**	**0.47**	**0.0087**	**1.197219**
SE	**0.30**	**0.22**	**0.15**	**0.0028**	**0.3789**

	Gill raker length	Gill raker separation
	0.01315	0.00395
	0.01438	0.00111
	0.01715	0.00396
	0.01403	0.00380
	0.01375	0.00375
	0.01270	0.00351
	0.01326	0.00398
	0.01404	0.00108
	•	•
	0.02023	0.00434
Mean	**0.01474**	**0.00328**
SD	**0.00242**	**0.00126**
SE	**0.00077**	**0.00040**

River Kelvin

	Standard length (mm)	Fork length (mm)	Depth	Width	Length 1st dorsal
	41.4	50.2	0.251	0.126	0.0966
	39.6	48.8	0.240	0.124	0.0859
	37.3	44.8	0.247	0.121	0.0858
	35.3	42.4	0.224	0.105	0.0935
	37.4	46.4	0.243	0.107	0.0856
	39.1	46.3	0.240	0.138	•
	40.5	47.9	0.230	0.114	0.0889
	37.5	44.5	0.237	0.104	0.0880
	39.8	48.4	0.239	0.111	•
	38.4	46.8	0.221	0.138	0.0911
Mean	**38.63**	**46.65**	**0.237**	**0.119**	**0.0894**
SD	**1.8074**	**2.3134**	**0.0096**	**0.0127**	**0.0040**
SE	**0.5720**	**0.7321**	**0.0030**	**0.0040**	**0.0013**

	Length 2nd dorsal	Length left pelvic	Length right pelvic	Gape width	Gape height
	0.1159	0.1643	0.1787	0.0580	0.0894
	0.0934	0.1616	0.1667	0.0657	0.0909
	0.0911	0.1314	0.1367	0.0590	0.0885
	0.0992	0.1331	0.1360	0.0680	0.0935
	0.0962	0.1578	0.1711	0.0668	0.0882
	0.1100	0.1662	0.1637	0.0614	0.0869
	0.1086	0.1555	0.1457	0.0494	0.0815
	•	0.1413	0.1440	0.0640	0.0933
	0.0955	0.1507	0.1507	0.0653	0.0879
	•	0.1693	0.1615	0.0677	0.0911
Mean	**0.1012**	**0.1531**	**0.1555**	**0.0625**	**0.0891**
SD	**0.0090**	**0.0136**	**0.0149**	**0.0058**	**0.0035**
SE	**0.0029**	**0.0043**	**0.0047**	**0.0018**	**0.0011**

	Left eye diameter	Right eye diameter	No. fin rays left pectoral	No. fin rays right pectoral	No. fin rays fork
	0.0870	0.0918	10	10	12
	0.0934	0.0960	10	10	12
	0.0911	0.0938	10	10	12
	0.0850	0.0822	10	10	12
	0.0882	0.0909	10	10	12
	0.0895	0.0870	10	10	12
	0.0839	0.0839	10	10	12
	0.0933	0.0960	10	10	12
	0.0905	0.0955	10	10	12
	0.0833	0.0859	9	9	12
Mean	**0.0885**	**0.0903**	**9.9**	**9.9**	**12**
SD	**0.0037**	**0.0052**	**0.3**	**0.3**	**0.0**
SE	**0.0012**	**0.0016**	**0.1**	**0.1**	**0.0**

River Kelvin

	No. lateral plates, left	No. lateral plates, right	Degree of crenation	Width 1st dorsal spine	No. gill rakers, left
	5	5	2	0.0362	10
	4	5	1	0.0581	8
	5	5	1	0.0375	8
	4	5	1	0.0368	9
	7	8	1	•	7
	5	5	0	•	12
	4	4	1	0.0346	9
	3	3	1	0.0453	9
	5	5	1	•	10
	5	5	1	0.0286	11
Mean	**4.7**	**5**	**1**	**0.0396**	**9.3**
SD	**1.06**	**1.25**	**0.47**	**0.0095**	**1.49**
SE	**0.34**	**0.39**	**0.15**	**0.0030**	**0.47**

	Gill raker length	Gill raker separation
	0.02174	0.00604
	0.01386	0.00405
	0.01743	0.00536
	0.01983	0.00708
	0.02139	0.00668
	0.01963	0.00462
	0.01489	0.00249
	0.02133	0.00533
	0.01743	0.00218
	0.01042	0.00521
Mean	**0.01780**	**0.00490**
SD	**0.00376**	**0.00162**
SE	**0.00119**	**0.00051**

River Esk

	Standard length (mm)	Fork length (mm)	Depth	Width	Length 1st dorsal
	43.4	37.7	0.190	0.101	*
	37.2	41.5	0.214	0.103	0.0944
	37.8	41.4	0.209	0.097	0.0953
	32.9	37.3	0.213	0.105	0.0827
	33.8	37.3	0.204	0.094	0.0744
Mean	**37.0**	**39.0**	**0.201**	**0.096**	**0.0863**
SD	**4.14**	**2.21**	**0.008**	**0.005**	**0.0100**
SE	**1.85**	**0.99**	**0.004**	**0.002**	**0.0045**

	Length 2nd dorsal	Length left pelvic	Length right pelvic	Gape width	Gape height
	0.0834	0.1342	0.1376	0.069	0.067
	*	0.1635	0.1685	0.061	0.091
	*	0.1857	0.1900	0.066	0.114
	0.1003	0.1586	0.1601	0.052	0.116
	0.0839	0.1143	0.1144	0.062	0.012
Mean	**0.0870**	**0.1513**	**0.1541**	**0.0627**	**0.084**
SD	**0.0115**	**0.0276**	**0.0291**	**0.0064**	**0.0429**
SE	**0.0053**	**0.0123**	**0.0130**	**0.0028**	**0.0191**

	Left eye diameter	Right eye diameter	No. fin rays left pectoral	No. fin rays right pectoral	No. fin rays fork
	0.0736	0.0668	10	10	12
	0.0918	0.0974	10	10	12
	0.0825	0.0742	10	10	12
	0.0858	0.0973	10	10	12
	0.0833	0.0922	10	10	12
Mean	**0.0825**	**0.0842**	**10**	**10**	**12**
SD	**0.0076**	**0.0164**	**0**	**0**	**0**
SE	**0.0034**	**0.0073**	**0**	**0**	**0**

	No. lateral plates, left	No. lateral plates, right	Degree of crenation	Width 1st dorsal spine	No. gill rakers, left
	4	4	0	0.0412	10
	9	9	0	0.0576	9
	5	5	0	0.0133	9
	6	6	0	0.0627	9
	5	5	0	0.0811	8
Mean	**5.8**	**5.2**	**0**	**0.0512**	**9**
SD	**1.924**	**0.837**	**0**	**0.0255**	**0.7071**
SE	**0.860**	**0.374**	**0**	**0.0114**	**0.3162**

River Esk

	Gill raker length	Gill raker separation
	0.01165	0.00213
	0.01276	0.00265
	0.01147	0.00354
	0.01125	0.00387
	0.01089	0.00628
Mean	**0.0116**	**0.0037**
SD	**0.0007**	**0.0016**
SE	**0.0003**	**0.0007**

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