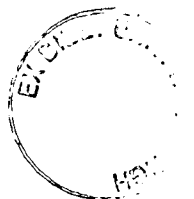


The influence of habitat ecology on spatial learning by the threespine stickleback

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degree of Doctor of Philosophy

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Declaration

This thesis is the result of my own research and contains no work done in collaboration except where otherwise stated. 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

Despite its potentially crucial role in improving the fit between an organism's phenotype and the environment, relatively little is known about exactly when and how animals should use learning within their natural habitats. This thesis integrates the comparative approach with techniques traditionally developed in experimental psychology to assess how divergent habitat conditions shape learned responses in the threespine stickleback, *Gasterosteus aculeatus*. Fish collected from different habitats (ponds and rivers) were trained to learn a simple spatial task in which both landmarks and turn direction (turn left or right) reliably indicated reward location. Pond fish used both landmarks and turn direction while river fish showed a preference for using turn. In rivers, flow and turbulence may make local visual features unstable and therefore unreliable as positional cues. However, both pond and river fish fail to learn to use landmarks as goal directing cues when they are the only predictor of reward location and unstable with respect to all other sources of spatial information. A controlled rearing experiment was carried out to investigate the causal basis of cue preferences in pond and river fish. The results suggest both genetic and environmental factors may influence cue preference in the threespine stickleback. A comparison of spatial learning by sympatric species of threespine stickleback that occupy different microhabitats (pelagic and littoral zones) within the same lakes, revealed a species difference in the rate at which they learnt the task. The two species may therefore be equipped with learning abilities that best suit them to either a littoral or pelagic lifestyle, even within the same macrohabitat. Together, these results suggest that learned behaviour is fine-tuned or adapted in response to local habitat conditions on a fine scale. Learning appears to operate in close conjunction with genetic and or developmental processes that enable and direct it in response to particular ecological problems.

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Chapter 1

Introduction

A problem that faces almost all animals is that of environmental uncertainty. A capacity to learn enables animals to modify their response to the environment on the basis of experience. Such behavioural plasticity allows animals to adapt to ecological factors that vary too rapidly to effect adaptive changes in the gene pool. Despite its potentially crucial role in improving the fit between the phenotype of organisms and their environment, surprisingly little is known about exactly when and how animals should use learning within their natural environments. To understand more about the adaptive function and evolution of learning, we need to investigate the relationship between learning and the selective environment in which it has evolved, an approach that has been rarely taken in the past. Population and species variation in learning provides a powerful tool for probing this relationship. This thesis integrates ethological comparative approaches with laboratory techniques traditionally developed in experimental psychology to assess how divergent habitat conditions shape learned behaviour in the threespine stickleback, *Gasterosteus aculeatus*.

The introduction presents an overview of past and contemporary approaches to the study of learning. It is argued that much present-day disagreement and confusion may be avoided by precisely clarifying the issues that are being addressed. This thesis concentrates on spatial learning; therefore an introduction to the topic is followed by a brief review of the literature on spatial learning in fish. Finally, I

discuss why the threespine stickleback is a particularly suitable subject for comparative studies of learning. The chapter concludes with an outline of the thesis.

1.1 WHAT IS LEARNING?

As yet no rigorous and all-encompassing definition of learning exists (Mackintosh, 1983; Papaj & Prokopy, 1989). Shettleworth (1998b) opted for a very general definition of learning as “ a change in state resulting from experience” (p100).

Obviously this will include phenomena other than learning e.g. growth or maturation. However, more restrictive definitions exclude phenomena intuitively considered to be learning. For example, “ a reversible change in behaviour with experience” excludes phenomena such as imprinting, in which the modification caused by some experience is fixed and resistant to further change (Papaj & Prokopy, 1989). Until more is known about the neurological bases of learning, it is difficult to make a precise and all-embracing definition. This thesis is principally concerned with questions regarding the function and evolution of learning. As such a looser definition of learning that makes few assumptions about the processes involved is appropriate. For the purposes of this thesis, I specify learning as a form of phenotypic plasticity demonstrated when individual behaviour changes in a repeatable way as a consequence of experience.

1.2 TWO APPROACHES TO THE STUDY OF LEARNING

1.2.1 Traditional approach: General learning theory

“General learning theory” encompasses the theoretical basis behind learning research traditionally undertaken by experimental psychologists. The goal of this tradition was

to identify and describe general principles of learning that transcended both species and learning tasks (Davey, 1989). Although Darwin addressed learning and other cognitive issues to some extent in *The Origin of Species* (Darwin, 1859), for much of the nineteenth and twentieth century learning failed to be taken up by evolutionary biologists and instead remained the province of experimental psychologists. Public sensitivity concerning the origin of human intelligence is likely to have been a major factor that steered evolutionary biologists away from cognitive issues (Dukas, 1998a). Although experimental psychology was itself strongly influenced by Darwin's ideas on evolution, the greatest influence came from Darwin's insight into the historical continuity of animals and man expressed most clearly in *The Descent of Man and Selection in Relation to Sex* (Darwin, 1871). The search for general principles of learning was undoubtedly influenced by Darwin's claims that other animals are likely to share with humans, cognitive abilities such as reasoning and memory. Ironically, Darwin's other fundamental insight that dealt with the diversity of species and their remarkably adaptive fit with their environment was virtually ignored during the next hundred years of research on animal learning. Two consequences of the "general learning theory" approach to learning are discussed below.

i) The search for general-process mechanisms

In response to the principle of continuity, experimental psychologists tended to search for a few general-process mechanisms of learning that were believed to apply across the board regardless of the species being studied or the nature of the task the animal was being trained to perform (see box 1.1). This approach contributed

significantly to our understanding of learning mechanisms, and revealed a great many general phenomena of learning that transcend particular sensory, motor and motivational contexts (Bitterman, 2000). However, the general-process approach limited our understanding of learning as a biological adaptation.

1) Nonassociative learning

Habituation: An animal stops reacting to a novel event if it occurs repeatedly without any important consequences.

Sensitization: The response to a stimulus is enhanced for some time after initial exposure, if the stimulus is a salient one such as food.

2) Associative learning

Pavlovian or classical conditioning: An animal is exposed to a consistent relationship between two events and its behaviour changes because of the properties of that relationship.

Operant or instrumental conditioning: The predictive event is some behaviour that the animal performs; that is the animal learns about the relationship between its own response and the consequences of that response.

Box 1.1. General learning mechanisms believed to transcend species and task (Mackintosh, 1983).

The assumption that general principles of learning found in one species could be extrapolated to all others led to the intensive study of learning in two or three species, for example the laboratory rat and pigeon. Focusing on so few species in an artificial laboratory environment diverted attention away from species-specific differences. In addition, laboratory procedures were designed largely to eliminate the species-specific traits of subjects that might interfere with the extraction of general

principles (Davey, 1989). However, it is these differences combined with an understanding of ecological relevance that are likely to reveal the relationship between learning abilities and biological fitness (Kamil & Yoerg, 1982).

ii) The search for a “*scala naturae*” of intelligence

A second consequence of interest in the continuity between the animal and human mind was to encourage psychologists working with animals to approach learning from an anthropocentric point of view. Much of experimental psychology still focuses on whether animals can do what people do and if so how (Shettleworth, 1998a). There is clearly practical value to this approach. For example, animals can be used as model systems for studying general processes of learning or the neural bases of learning and memory, assuming basic behavioural and brain processes are the same across the species being compared (Miller, 1985). However, assigning animals to human-based tasks detracts attention away from investigating those learning abilities likely to be relevant in the context of the animal’s natural ecology.

Furthermore, the choice of species used for such comparisons is often based more on convenience than on sound evolutionary considerations (Shettleworth, 1998a). More alarming has been the tendency to embrace the concept of a “*scala naturae*”; that is that some sort of linear scale of intelligence exists from lower to higher animals, culminating in ourselves (Campbell & Hodos, 1991).

There are several reasons why a search for some arbitrary unit of intelligence that increases on moving up the evolutionary tree has contributed little to our understanding of the adaptive function of learning:

- 1) Rather than occupying positions on a ladder of progressive complexity, organisms are adapted to their own particular ecological circumstances.
- 2) Investigating the adaptive function of learning has tended to be overshadowed by controversy over the matching of labels to phenomena in an attempt to classify learning into “simple” and “more complex” forms. An example of this is social learning, which has been sub-named as true imitation, local enhancement, social facilitation etc. (Galef, 1988; Heyes, 1993).
- 3) Animal intelligence has been treated as some sort of abstract ability in isolation from a consideration of its biological function. For example, Bitterman (1965, 1975) used the performance of species in a variety of learning paradigms to classify each species as either rat-like or fish-like in its learning abilities. Programs of study such as these have tended to adopt a human-oriented criterion of intelligence that is unrelated to the demands imposed on a particular animal within its specific niche (Davey, 1989).
- 4) The concept of a “*scala naturae*” of intelligence encourages the view that learning is only of interest in a few “complex species” (those closely related to ourselves). This has further distracted attention from the widespread role learning is likely to play across the animals kingdom, as an adaptive response to environmental variability.

1.2.2 Contemporary approach: Cognitive ecology

Over the past few decades, attempts have increasingly been made to consider the other side of the evolutionary coin in the study of learning, diversity and adaptability. This began in the late 1960's and early 1970's when animal psychologists began to find exceptions to the rules specified by general learning theory. For example, work by Garcia and colleagues showed that in the case of illness-based aversions in rats, associations between a flavour and a toxin could be acquired when illness occurred hours after the flavour was sampled, and flavours were readily associated with illness while auditory events or visual properties of food were not (Garcia & Koelling, 1966; Garcia *et al.*, 1966). Their findings appeared to make adaptive sense given that many foods can be identified by their flavors, and toxins may act slowly. However, these traits appeared to be “special properties” not predicted by general learning theory. The first theoretical attempt to incorporate learning “anomalies” into a formal research program was the loosely labeled “biological constraints” approach (reviewed in Davey, 1989). This promoted awareness of the involvement of evolutionary and ecological factors in learning but failed to revolutionize the traditional methods of learning research (Domjan & Galef, 1983). The biological constraints approach clung to the general theory approach by simply cataloguing special – case scenarios or “exceptions to the rules” specified by general learning theory (Domjan & Galef, 1983; Davey, 1989). As with general learning theory research, studies searching for “biological constraint phenomena” were carried out in the absence of relevant ethological information on the behaviour and lifestyle of the species concerned and focused on equally arbitrary laboratory tests. More recently, calls have been made for collaboration between experimental psychology and

behavioural ecology (Domjan & Galef, 1983; Shettleworth, 1984; Davey, 1989; Yoerg, 1991; Real, 1993; Francis, 1995; Dukas, 1998c). For example, Domjan & Galef (1983) were amongst the first to propose introducing modern comparative methods into the study of animal learning. They argued that comparisons of taxonomically similar but ecologically disparate species (or vice versa in the case of convergent phenomena) used to demonstrate the adaptive specialisation of morphological features or fixed behaviour could equally well be applied to the study of learning.

Recent years have witnessed increasing application of the techniques developed in behavioural ecology to the study of learning. This “ecological approach” asks how learning is fine-tuned to the details of the individual’s environment and how this fine-tuning influences fitness. Interest is primarily in understanding when and how animals ought to learn based on an examination of their life history strategies (Shettleworth, 1984). Some argue that “cognitive ecology” has emerged as a new field of research (Dukas, 1998a; Chittka, 1999; Healy & Braithwaite, 2000), although it is principally an approach based on an amalgamation of previously established and successful techniques (Shettleworth, 2000).

1.3 A QUESTION OF INTEREST?

The “ecological approach” to the study of learning has recently received criticism perhaps predominantly because of confusion over the questions it claims to be able to answer. The modern ethological approach originally put forward by Tinbergen (1963) emphasizes the necessity of understanding behaviour at different levels, in

terms of its proximate mechanism, development, phylogeny and adaptive function. It is generally agreed that the study of animal behaviour should involve all four levels of enquiry. However, as Bolhuis and Macphail (2001) clearly state “a functional interpretation of why an animal performs a specific behaviour does not explain the cognitive and neural mechanisms governing that behaviour” (p 426). Conclusions drawn from the wealth of comparative data on food-storing birds in particular has been criticized for making assumptions about superior performance reflecting specialised learning and memory abilities. The message of Bolhuis and Macphail’s (2001) recent critique of the “neuroecology of learning and memory” is clear; “questions of mechanism cannot be solved by functional considerations” (p428). On the basis of this, Macphail & Bolhuis (2001) argue that the general process view of learning and the techniques developed within this tradition should be pursued in favour of the ecological approach in the study of learning and memory.

Debates over the relative value of the general process approach versus the ecological approach to learning and memory critically overlook the fact that the two approaches aim at different types of explanation. A century of research has revealed a great many phenomena of learning that transcend particular sensory, motor, and motivational contexts, which are found in many different species and which point to the operation of common processes (Bitterman, 2000). Psychologists working in the general learning theory tradition and neurologists are likely to be best placed to investigate these processes and provide mechanistic accounts of learned behaviour (Bitterman, 2000). The ecological approach to learning seeks explanation in terms of adaptive function. As Macphail and Bolhuis (2001) argue, observations that species

or populations differ in their performance in a laboratory-based task does not allow conclusions to be drawn about the mechanisms and processes responsible. Species may differ in their motivation, or in their ability to learn, remember or perceive information. However, such species or population differences in performance in learning tasks warrant functional explanation. An evolutionary approach to the study of learning is likely to be crucial in understanding how organisms appropriately match their phenotypic response to a variable environment.

1.4 HOW TO STUDY LEARNING FROM AN ECOLOGICAL APPROACH

While presenting a considerable advance in terms of treating learning as a biological adaptation, there are pitfalls associated with applying traditional ethological methods to the study of learning. The study of flexible learned behaviour presents several unique difficulties that do not necessarily apply to the study of fixed traits. This thesis employs both experimental and comparative approaches to the study of learning. In the next section, some of the findings generated from the application of these two ethological approaches to learning are discussed along with the potential pitfalls associated with each method of study.

1.4.1 Experimental approach

The experimental approach is based on manipulating the variable of interest and comparing animals randomly assigned to different treatments. This approach is limited in its application to learning mainly because of the difficulty of manipulating cognitive ability. For example, brain lesions (e.g. Krushinskaya, 1966) raise serious ethical problems and represent fairly crude manipulations, the effects of which are

notoriously difficult to interpret. A number of studies have attempted to quantify the fitness benefits of learning by using a restriction of the opportunity to learn to simulate an inability to learn (Hollis, 1984, 1999; Hollis *et al.*, 1997; Dukas & Duan, 2000; Dukas & Bernays, 2000). For example, Dukas & Duan (2000) showed that parasitoid wasps allowed to select host substrate based on experience laid more eggs and produced more adult offspring than wasps forced to lay on host substrates at random. Similarly, male gouramis, *Trichogaster trichopterus*, defend their territories more efficiently when the appearance of a rival is signaled by a coloured light than when it is unpredictable. Moreover when the arrival of a potential mate is signaled, conditioned males are able to spawn with females sooner, clasp females more often and produce more young than males that do not have the benefit of a signal (reviewed in Hollis, 1999). One drawback of this approach is that it does not allow an assessment of the costs likely to be associated with possessing the capacity to learn. Those individuals deprived of the opportunity to learn presumably still pay energetic costs associated with maintaining the neurological machinery required for learning and memory.

Aside from manipulating cognitive ability, another experimental technique that has been applied to learning involves manipulating the developmental environment of the animal. This approach has been used extensively to investigate the role of environmental and genetic influences on the properties of a learned response. For example, substantial evidence indicates that rats reared in an enriched environment, with toys and other rats, show superior spatial learning ability in laboratory tests compared to rats reared in isolation (Seymour *et al.*, 1996 and refs

therein). A full discussion of this controlled rearing approach to the study of learning is given in chapter 6.

1.4.2 Comparative approach

Questions concerning the adaptive function of learning have been addressed most extensively using the comparative method. In this approach, species or populations are chosen for study based on their natural history and phylogeny, taking advantage of the processes of divergence and convergence (Harvey & Pagel, 1991; Harvey & Purvis, 1991). Much of our current understanding about when and how animals should be expected to learn, based on an analysis of their life histories and habitat ecologies comes from the results of comparative studies.

i) When to learn?

Several comparative studies have addressed the question of when an organism should be expected to learn (e.g. Papaj, 1986; Micheli, 1997). Attention to this problem is likely to have been motivated in part by theoretical models that investigate the kinds of environmental unpredictability that should select for learning (McNamara & Houston, 1987; Stephens, 1987, 1991, 1993; Mangel, 1990; Bergman & Feldman, 1995; Luttberg & Warner, 1999). In general, theoretical models predict that the evolutionary value of learning will be determined by the pattern of unpredictability in relation to the animal's life history (Stephens, 1991). Empirical tests of this "environmental unpredictability hypothesis" have tended to compare learning in two species occupying environments differing in predictability (Papaj, 1986; Micheli, 1997). For example, Micheli (1997) reported a capacity to learn in the highly mobile

blue crab, *Callinectes sapidus*, which experiences a wide variety of prey types. In contrast, experience had no effect on the foraging behaviour of a more specialist and less mobile forager, the Atlantic mud crab, *Panopeus herbstii*.

However, when only two species are compared, it is difficult to assign any differences found between them to a particular cause since many factors are likely to vary between species (Papaj & Prokopy, 1989). A major constraint on the use of the comparative method is therefore the availability of data from a sufficiently large number of species. Similarly, the comparative method must be applied together with information about phylogeny so that similarity owing to common adaptation can be distinguished from similarity owing to common descent (Harvey & Pagel, 1991). In general, conclusions about adaptation based on the comparative method should be made with caution. Differences in learning between species could be caused by factors other than natural selection (e.g. differences in gene flow between or genetic drift within populations). Moreover, unexpected outcomes may result if selective factors operating in the past were different from those currently observed (Papaj & Prokopy, 1989).

ii) Is learning adaptively specialised?

Aside from testing predictions about the occurrence of learning, the comparative approach has been used extensively in assessing whether learning is adaptively specialised for specific life histories and habitat ecologies. Several studies have compared learning abilities and rates of memory loss in food handling tasks in species naturally exposed to different levels of prey variability (Johnson *et al.*, 1994;

Mackney & Hughes, 1995). Species experiencing more variable food types either learn to forage on a novel food type faster and, or forget prey-specific handling techniques more rapidly. Sociality has similarly been advocated as a potential selection pressure for increased learning and memory capabilities in bees (Dukas & Real, 1991). In some cases, species and population differences in the types of information used during learning appears to correlate with cue availability and, or reliability within natural habitats (Huntingford & Wright, 1989; Brodbeck, 1994; Carlier & Levebvre, 1997; Girvan & Braithwaite, 1998; Odling-Smee & Braithwaite, in press). Many of the comparative studies investigating whether learning and memory abilities are adaptively specialised focus on food-storing parids and corvids, predominantly because of the impressive spatial learning abilities and memory these birds are expected to have in order to re-locate, in some cases, thousands of cache sites within a single season (Balda *et al.*, 1998). With some notable exceptions (Macphail & Bolhuis, 2001), food-storing species frequently outperform non-storers or less specialist storers in a range of laboratory based spatial learning and memory tasks (Balda & Kamil, 1989; Krebs *et al.*, 1990; reviewed in Krebs *et al.*, 1996).

Many of these studies suffer from the problems outlined above. It is all too easy to come up with a plausible adaptive explanation for a difference in behaviour but where only two or a few species or populations are compared, it is difficult to rule out with any certainty, the role of confounding factors. However, these problems apply in general to the comparative method. A fundamental problem that confronts the study of cognitive processes is the “learning-performance” problem (Macphail, 1987). Assessment of learning abilities can only ever be made by measuring

behaviour but performance is likely to be affected by many factors other than learning ability such as motivation, susceptibility to stress etc. Several solutions have been proposed. Kamil (1988) advocated testing species with a battery of tests assessing the same cognitive ability, arguing that a species difference that holds across a wide variety of tasks is more convincing evidence of a real difference in cognitive ability. However, this is unlikely to eliminate all contextual variables. For example, some species may be more adaptable than others to the laboratory environment. An additional step proposed to control for this possibility is to test the species in a task as similar as possible to the one demonstrating species differences but that tests a different cognitive ability (Kamil, 1998). Lefebvre (1996) argues for a yet more rigorous approach by which interspecific variation in general aspects of memory, learning, neophobia and motivation are quantitatively removed from the adaptive specialisation test. Clearly, the possibility of contextual variables presents a serious problem in the application of the comparative approach to learning (Macphail, 1987).

An alternative to estimating cognitive abilities from performance in laboratory-based tasks is to measure qualitative and quantitative features of the neural substrates thought to underpin learning and memory. For example, variation in relative brain size appears to correspond at least in part with the need to store, process and retrieve spatial information. In mammals ranging from bats to primates, differences in relative brain size have been associated with diet, or more precisely with foraging strategy (Eisenberg & Wilson, 1978; Clutton-Brock & Harvey, 1980; Mace *et al.*, 1981; Sawaguchi, 1992). Larger brains may reflect a greater need for spatial learning

resulting from the large range sizes demanded by certain diets coupled to the spatial and temporal unpredictability of certain food types. However, overall brain size is a very global and unsatisfactory measure and likely to be associated with many factors other than learning ability (Aboitiz, 1996; Barton, 1996; Keverne *et al.*, 1996). In fact, there is surprisingly little evidence for the widely held assumption that brain size and cognitive abilities are linked (Macphail, 1982). Potentially more informative are studies that focus on particular brain regions such as those required for spatial learning and memory.

There is evidence to suggest that the hippocampus is the part of the brain required for spatial learning and memory in birds and mammals (Olton & Papas, 1979; Morris *et al.*, 1982; Sherry *et al.*, 1989; Bingman, 1992; Biegler *et al.*, 2001; but see Bolhuis & Macphail, 2001). For example, hippocampal damage disrupts memory-based retrieval of stored food in food-storing passerines (Krushinskaya, 1966; Sherry *et al.*, 1989). Evidence for a relationship between spatial learning and the hippocampus has spurred numerous comparative studies. These investigate whether quantitative and qualitative differences in the hippocampus correlate with variation in dependence on spatial learning and memory predicted from an analysis of the animals' lifestyle and ecology. With some exceptions (Bolhuis & Macphail, 2001), species differences in hippocampal volume, adjusted for body or total brain size correlate with dependence on cached food in parids and corvids (Krebs *et al.*, 1989; Healy & Krebs, 1993; Healy *et al.*, 1994). In fact, both birds and mammals that cache food for future consumption, negotiate a complex nesting environment, or have experienced artificial selection for spatial memory, have larger hippocampi

and /or more hippocampal neurons and qualitatively different neurons than closely related species that do not exhibit such spatially demanding behaviour (Rehkämper *et al.*, 1988; Krebs *et al.*, 1989; Sherry & Vaccarino, 1989; Healy & Krebs, 1992; Jacobs, 1992; Basil *et al.*, 1996; Abbott *et al.*, 1999). Sexual dimorphisms in hippocampus size similarly appear to correspond to sexual dimorphisms in the need to process spatial information (Jacobs *et al.*, 1990; Sherry *et al.*, 1993; Reboreda *et al.*, 1996).

Given the problems associated with measuring and interpreting performance in learning tasks, the analysis of a morphological structure is clearly appealing. However, there are concerns over the validity of this approach. The most notable objection is that measuring hippocampal volume may not present a considerable advance on measuring total brain size. Many other factors could be affected by hippocampal size aside from spatial learning and memory abilities. Until the relationship between spatial learning ability and specific features of the hippocampus has been more clearly elucidated, the results of these studies present suggestive but as yet inconclusive evidence for a relationship between learning ability and ecological demand (Macphail & Bolhuis, 2001).

1.5 SPATIAL LEARNING – DO FISH SPATIALLY LEARN?

Most ecologically important behaviour; foraging, locating receptive mates, predator avoidance, nest guarding and parenting etc. will require animals to move through space and keep track of their location with respect to an external point of reference. Unsurprisingly, questions about how animals find their way around their

environments have attracted considerable research interest (Healy, 1998; Capaldi *et al.*, 1999). Much of this research involves assessing how animal navigation and orientation is dependent upon the formation, storage and retrieval of spatial memories, which permit repeated visits to fixed points in the environment. Perhaps more than any other field of behavioural research, studies of navigation and spatial orientation have acknowledged and addressed the involvement of learning and memory. As discussed above, numerous comparative studies suggest information use and spatial learning abilities may differ in animals exposed to divergent habitat conditions (Krebs *et al.*, 1990; Brodbeck, 1994; Clayton & Krebs, 1994b). Therefore, spatial learning presents an ideal model system on which to base a study that investigates how ecology shapes learned behaviour.

In the next section I briefly review what is currently known about spatial learning in fish, paying particular attention to issues raised in subsequent chapters of the thesis. Specifically, attention is focused on when, what and how fish should be expected to learn based on the navigational problems and environmental conditions they are likely to encounter within their natural environments. Past research on fish orientation and migration has tended to focus on genetically hard-wired patterns of behaviour, and overlook the possibility that orientation is a flexible process relying on experience and the learning of environmental relationships (Dodson, 1988). However, for most species of fish, biologically important locations as well as the physical environment used for obtaining fixed reference points will be subject to varying degrees of change favoring a capacity to learn (Braithwaite, 1998).

1.5.1 Why should fish keep track of location?

Most ecologically important behaviour, including tracking resources, predator avoidance, and re-locating natal home areas for reproduction, will require fish to move through space and keep track of their location with respect to an external point of reference. Food is often distributed among sites that vary spatially and temporally in profitability and food patches may differ in the likelihood of renewal after depletion. As long as there is some degree of predictability, foraging efficiency will increase if fish can map the status and renewal rates of individual food patches onto their location (Noda *et al.*, 1994; Hughes & Blight, 1999). Spatial information may similarly be used to predict the location of receptive mates. For example, in certain reef fish, spawning aggregations draw fish to specific locations from disparate areas of the reef (Mazeroll & Montgomery, 1998).

Many species display territoriality and a tendency to remain in a restricted area or “home range” (Ogden & Buckman, 1973; Green & Fisher, 1977; Ogden & Ehrlich, 1977; Matthews, 1990a; Mazeroll & Montgomery, 1995). This may reduce intra-species competition and aggression (Gerking, 1959) and allow fish to keep track of the location of shelters or refuges for rapid escape in the event of a predatory attack (Aronson, 1951, 1971). Site fidelity may also be important for the defence of nests and territory boundaries associated with reproduction (Clarke, 1970).

In addition to keeping track of site-specific resources, fish may need to monitor locations associated with risk. Some predators are associated with particular microhabitats or locations that are best avoided by potential prey (Goodyear, 1973).

Others have predictable movements for example many reef-based piscivores concentrate on coral reefs at night and move away from the reefs during the day (Ogden & Quinn, 1984; Mazeroll & Montgomery, 1995). Predator avoidance strategies may therefore involve daily migrations requiring fish to keep track of their location with respect to both feeding areas and protective refuges (Ogden & Buckman, 1973; Ogden & Quinn, 1984; Mazeroll & Montgomery, 1995). Predators themselves may need to be equipped with information about the spatial structure of their home range in order to avoid being recognized by potential prey (Brown *et al.*, 1995). Some species of fish return to their natal area for reproduction, otherwise known as “homing”. In order to achieve accurate return to their natal sites, fish must relocate their natal area from impressive distances, in some cases thousands of kilometers (Quinn & Dittman, 1990).

1.5.2 Do fish use learning and memory in orientation?

Observations of fish movements within their natural habitats imply a capacity for learning and memory without providing hard evidence or revealing what exactly might be learnt. For example, Noda *et al.* (1994) tracked the search behaviour of a planktivorous reef fish, *Chromis chrysurus*, by marking individual fish with acrylic paint and following their movements after release. The fish showed local search behaviour at three distinct foraging areas, swimming slowly and in a stereotypic pattern within each foraging site before swiftly moving off to the next patch. In this case, spatial memory may allow the fish to concentrate foraging at relatively high densities of zooplankton and avoid revisiting depleted areas.

Many mark-and-observation programs involve the displacement of marked fish at various distances from their home range in order to investigate their ability to return home (Hasler & Wisby, 1958; Green, 1971; Carlson & Haight, 1972; Hallacher, 1984; Quinn, 1984). Some species can return home from considerable distances and after several months in captivity, implying a long-term memory for orientation routes (Green, 1971; Carlson & Haight, 1972). Tracking methods such as ultrasonic telemetry, in which signals are detected from transmitters carried by individual fish (e.g. Matthews, 1990b), or the attachment of buoys or floats to the body of the fish (Hasler *et al.*, 1958; Hasler & Wisby, 1958; Winn *et al.*, 1964) provide continuous data on fish movements. These have shown that displaced fish often make direct return paths to their home range.

Overall, observations of fishes' movements in their natural environment indicate remarkable spatial abilities. However, they reveal little about the precise spatial strategies used and in particular, the extent to which learning and memory is involved. Most of our knowledge about the role of learning and memory in fish orientation and the types of spatial information used, comes from controlled experiments. These involve manipulations of fish sensory systems or spatial cues, or laboratory based spatial tasks whereby fish are trained to learn particular associations.

1.5.3 Learning about landmarks and maps

In many aquatic landscapes, local visual or olfactory features are likely to be highly changeable components of the environment requiring that they be stored in memory

and updated on the basis of experience. Limited evidence that some species may memorize visual landmarks comes from field studies in which fish blinded by eyecaps or retinal injections fail to return to home ranges or to their natal sites for reproduction (LaBar, 1971; Goff & Green, 1978; Ueda *et al.*, 1995). However, sensory manipulation may affect other aspects of behaviour that reduce the ability to return home, for example blind fish may be more susceptible to predation. A less intrusive approach has been to manipulate the position of landmarks in the natural migrating paths of fish (Reese 1989; Mazeroll & Montgomery, 1998). For example, Reese observed that butterfly fish, *Chaetodon trifasciatus*, spent some time searching in an area from which coral heads had been removed, before continuing along their original foraging path. However, the disruption of migratory routes by shifted landmarks does not prove that landmark relationships were originally learnt to guide orientation. An alternative explanation is that fish simply react to environmental novelty. Moreover, in field studies, it is difficult if not impossible to rule out the potential role of additional navigational cues such as compass cues. In controlled laboratory experiments fish can be trained to use specific landmarks and additional cues, such as compass or global place cues, can be more easily removed by screening or rotating the test tank (e.g. Warburton 1990). Many species of fish have been trained to use landmarks as goal-directing cues in a range of laboratory based spatial tasks (Huntingford & Wright, 1989; Braithwaite *et al.*, 1996; Salas *et al.*, 1996a; Girvan & Braithwaite, 1998; López *et al.*, 1999; López *et al.*, 2000b; Hughes & Blight, 2000).

In addition to using landmarks as direct cues or beacons, fish appear to be capable of generating an internal map-like representation, which is independent of any particular view of the surroundings. This enables fish to take short cuts or choose between alternative routes to a goal without having to rely on a particular sequence of locations. For example, goldfish can remember the spatial position of food patches in a tank (Pitcher & Magurran, 1983) and are capable of discriminating spatial relationships in the environment (Ingle & Sahagian, 1973; Warburton, 1990; Rodríguez *et al.*, 1994). The performance of Siamese fighting fish, *Betta splendens*, in an eight-arm radial maze similarly suggests some amount of spatial memory is involved in recognizing which of the eight arms have already been depleted of the food reward (Roitblat *et al.*, 1982). The fact that fish can detect environmental modifications (Welker & Welker, 1958) and show an organized pattern of exploration when they are introduced into a novel environment (Kleerokoper *et al.*, 1974) also suggests some degree of spatial memory.

A classic demonstration of the ability of fish to use spatial maps is provided by Aronson's (1951, 1971) experiments on the gobiid fish, *Bathygobius sutorator*. When threatened, gobiids jump from their home tide-pool to an adjacent pool with impressive accuracy. In order to investigate whether gobiids acquire memories of the local topography around their home pools, Aronson constructed three artificial pools and manipulated the water level to simulate low and high tides. Only fish that were given experience of the spatial distribution of the pools at "high tide" successfully escaped a simulated attack at "low tide" by jumping into the appropriate pool.

Where vision is precluded by lifestyle or habitat conditions, information about landmarks and spatial boundaries may be acquired through alternative sensory channels. Some fish have been shown to learn landmark locations using electrolocation (Cain *et al.*, 1994; Cain, 1995). Others rely on the detection of water movements by the lateral line organ (Campenhausen *et al.*, 1981; Teyke, 1985, 1989). Olfactory cues present an additional type of landmark information, that can be learned to relocate home areas or natal streams. Depriving fish of the ability to smell has suggested a role for olfactory learning in orientation and site recognition in a number of species (LaBar, 1971; Goff & Green, 1978; Halvorsen & Stabell, 1990). Perhaps most acclaimed is the ability of mature salmon to relocate their natal streams based on its unique olfactory composition (Quinn & Dittman, 1990).

1.5.4 Other sources of spatial information

Fish are likely to rely on more than one source of spatial information when learning about locations or routes, although cue hierarchies may be established with fish weighting more attention or importance to the most reliable or abundant sources of information. The use of multiple cues will provide back up points of reference if changes in the environment make one cue unavailable or unreliable. Compasses provide another source of spatial information, which may often be used in combination with landmarks (Goodyear, 1973; Goodyear & Bennet, 1979; Hawryshyn *et al.*, 1990). In habitats where visual and compass cues are unavailable or unreliable, fish may resort to acquiring spatial information from the body of water that surrounds them. Some species can learn to locate landmarks based on information from water movements occurring between stationary objects in the

environment and their own bodies (Teyke, 1985, 1989). There is also some evidence that threespine sticklebacks can learn to use flow direction as an orientation cue (Girvan & Braithwaite, 1999). A “back-up” strategy that may be more resistant to environmental fluctuations is the use of inertial or body-centered information (Etienne *et al.*, 1998). Although it is not clear to what extent fish use this strategy when orienting in the wild, a diverse array of species solve maze tasks by learning a body-centered pattern of movement. For example, in the absence of spatial cues, fifteen-spined sticklebacks, *Spinachia spinachia*, and corkwing wrasse, *Crenilabrus melops*, improve their foraging efficiency in an 8-arm radial maze, by developing the algorithm of visiting every third arm (Hughes & Blight, 1999; Roitblat *et al.*, 1982). The use of a body centered turn response or a sequence of turns has similarly been observed in threespine sticklebacks and goldfish (Rodríguez *et al.*, 1994; Salas *et al.*, 1996a; Girvan & Braithwaite, 1998). Finally, by observing and following the behaviour of “informed” conspecifics, individuals may acquire spatial knowledge while avoiding many of the costs thought to be associated with individual learning, such as making mistakes or wasting time (Laland *et al.*, 1996). There is evidence from both field and laboratory studies that fish can learn new foraging or migratory routes by following conspecifics (Helfman & Schultz, 1984; Laland & Williams, 1997).

1.5.5 Is learning “adaptively specialised”?

Fish are clearly exposed to an enormous diversity of potential cues from which they can extract information about their spatial location within their natural environments. Across the diverse array of aquatic habitats occupied by fish, different sources of

spatial information will differ in availability and, or reliability. Equally diverse will be the range of spatial problems encountered by different species, populations, individuals and even different life stages. Rather than learning all possible environmental relationships throughout life, individuals might be expected to pay preferential attention to those cues that are most reliable within their particular habitats and invest time and energy in learning during those developmental stages that require maximum flexibility. In the next section, I review the limited evidence that suggests learned orientation responses in fish differ between species, populations and individuals exposed to different ecological conditions.

i) When to learn?

Fish that remain in a restricted area or home range may need to continuously update stored representations of local topography throughout life. However, in species that return to their natal site to breed, particularly those with an anadromous lifestyle (fish that migrate from salt to fresh water to spawn), sensory contact with the home site may be lost for prolonged periods of life. In these fish, imprinting may be the mechanism by which young learn characteristics of the home site allowing recognition later in life (Morin *et al.*, 1989a, b). Although the concept of imprinting and the validity of some of the criteria are controversial issues, imprinting is thought to be a specialised type of learning which takes place during a restricted period known as a sensitive period, and results in relatively long-lasting memory (Immelmann & Suomi, 1981). In migratory fish, long-term memory that is resistant to change is likely to be essential if the natal site is to be successfully recognized at the end of the return migration (Dodson, 1988).

In some cases, physiological changes in perceptual systems may restrict learning to specific stages in development. For example, Hawryshyn *et al.* (1990) noted that rainbow trout lose the ability to learn to orient using polarized light at a developmental stage characterized by the disappearance of UV-sensitive cones. A possible explanation is that the availability of celestial cues in the natural habitat of trout is restricted to an early developmental stage, for example when the young occupy shallow streams and rivers prior to migrating into deeper waters or into the sea. Where plasticity is not restricted to early developmental stages, it may still be that associations learned early in life have a greater impact on subsequent behaviour than those learned later. In Goodyear's (1973) study of sun-compass orientation in mosquitofish, offspring and adults displayed different retention times of laboratory-learned shoreward directions. Young mosquitofish appear to imprint on the direction of shore, forming a long-term memory, while adults that have to lose their original orientation and learn a new shoreward direction appear to do so on a short-term basis only. Aside from salmon, the importance of sensitive periods and the effect of early experience on learned orientation responses in fish have been virtually unexplored.

ii) What to learn?

Control over when to learn may be accompanied by mechanisms that predispose fish to use specific types of information or to learn certain associations in preference to others. For example, Huntingford & Wright (1989) observed population differences in the use of local visual cues by threespine sticklebacks collected from two sites of high and low predation risk. Fish from the high risk site used local landmark cues to learn an avoidance task more often than fish from the low risk site. However, other

factors are likely to vary between two sites in addition to predation pressure, which could affect the use of visual cues (see chapter 3).

iii) How much to learn?

Evidence from lesion studies suggests that the fish telencephalon is the part of the brain specifically required for spatial learning and memory, in a similar manner to the hippocampus of terrestrial vertebrates (Salas *et al.*, 1996a, b; López *et al.*, 2000a, b; Rodríguez *et al.*, 2002). Although relationships between learning ability and brain morphology are difficult to assess, preliminary evidence suggests that differences in telencephalon morphology may correlate with differences in the ecological demand for spatial learning (Van Staaden *et al.*, 1994; Huber *et al.*, 1997; Kotrschal *et al.*, 1998; Carneiro *et al.*, 2001). For example, Carneiro *et al.* (2001) recently discovered what may be an ecologically driven sex difference in the telencephalon of Azorean rock-pool blennies. In this species, males establish nests in crevices and almost never leave their nest area during the entire breeding season, while females must travel relatively long distances in order to visit different nests and spawn with males. Females may need to retain a spatial map of the area and remember the location of previously visited nest sites, a requirement that may explain why the dorso-lateral region of the telencephalon is larger in females. In cichlids, variation in telencephalon size appears to relate closely to the challenges of spatial, environmental complexity (Kotrschal *et al.*, 1998).

So far, preliminary evidence suggests that in fish, as appears in terrestrial vertebrates (Sherry, 1998), spatial learning may be modified or fine-tuned in

response to particular ecological conditions. Some species appear predisposed to “know” when to learn or what stimuli to attend to. Furthermore, suggestive but as yet inconclusive evidence suggests that fish may invest only as much into spatial learning capacity as their ecologies and lifestyles demand.

1.6 THE STUDY SPECIES

The threespine stickleback has been extensively studied by behaviourists and evolutionary biologists and detailed information on its ecology, behaviour and evolution provides an exceptional background for comparative studies (Wootton, 1984; Bell & Foster, 1994; McKinnon & Rundle, 2002). It is a species complex that comprises thousands of phenotypically diverse allopatric populations and biological species that are distributed widely in coastal marine and freshwaters of the northern hemisphere (Bell & Foster, 1994; McPhail, 1994). Its broad geographical and ecological distribution and the fragmentation of its gene pool into many thousands of isolated or semi-isolated demes in freshwater habitats have generated an extraordinary range of phenotypic diversity (Bell & Foster, 1994). This diversity offers an exceptional opportunity for investigating how divergent ecologies may shape learned responses.

The threespine stickleback presents an ideal model system for a comparative approach. First, much of the phenotypic diversity within the *Gasterosteus aculeatus* complex has been placed in a phylogenetic context. This is crucial for accurate interpretation of results, since both common ancestry and adaptation may contribute to phenotypic similarity among related groups (Harvey & Pagel, 1991; Bell, 1995).

Freshwater populations have been derived locally from marine and anadromous ancestral populations innumerable times (Bell & Foster, 1994; Bell, 1995). Colonization has been followed by divergence in numerous traits, including morphology, behaviour, physiology and life history. Second, threespine sticklebacks are ubiquitous in lowland habitats of the boreal Holarctic, providing ample opportunity for selection of appropriate samples for comparative studies. Third, many freshwater populations occur in recently (i.e. $\leq 25,000$ years ago) deglaciated regions, limiting the time for dispersal through freshwater (Bell & Foster, 1994; McPhail, 1994).

Preliminary evidence suggests learned responses may differ between populations and species of threespine stickleback in response to variation in habitat conditions. Species and population-level differences in diet variability correlate with differences in learning rates and rates of memory loss of prey-specific handling skills (Mackney & Hughs, 1995). Moreover, higher predation risk and greater habitat stability have been associated with a greater use of local landmark information during spatial learning (Huntingford & Wright, 1989; Girvan & Braithwaite, 1998).

Finally, sticklebacks have a number of practical advantages. They are easy and inexpensive to keep in small populations, as a consequence of their small size and simple feeding requirements, allowing increased sample sizes and replicate samples at the population level.

1.7 OUTLINE OF THE THESIS

In this chapter, it has been argued that our understanding of the evolution and adaptive function of learning lags behind that of fixed behavioural traits, such that relatively little is known about exactly when and how animals should use learning within their natural habitats. The following chapters describe comparative studies of spatial learning in the threespine stickleback, which investigate how or indeed whether learned responses are fine-tuned or adapted to particular habitat conditions. Many of the problems associated with the comparative approach discussed in section 1.4.2 are avoided or at least reduced by testing multiple populations within a species and by comparing species whose phylogeny is well established.

The thesis is comprised of seven chapters. General experimental and statistical methods are described in chapter 2. Chapter 3 details a comparative study that compares the use of local landmarks relative to an alternative cue (a body-centered movement) during spatial learning by populations of threespine sticklebacks originating from habitats differing in stability (ponds and rivers). In chapter 4, the use of landmarks by pond and river fish is compared in a different experimental context. In this case landmarks are the only reliable indicator of reward location and unstable with respect to all other sources of spatial information. An additional experiment investigates the role of landmark stability in affecting the use of landmarks during spatial learning by pond fish. Chapter 5 details a comparative study that compares cue use and spatial learning in two sympatric species of threespine stickleback that inhabit divergent microhabitats within the same lakes. Chapter 6 examines the possible causal basis for population differences in the use of spatial

cues by pond and river fish using a controlled rearing approach. The final chapter summarizes the principal findings, discusses the main implications and comments on future directions for the study of learning.

Chapter 2

General methods

2.1 EXPERIMENTAL DESIGN

2.1.1 Introduction

In chapters 3-6, threespine sticklebacks are trained to locate a hidden reward in one arm of a cross-maze by using one or more types of spatial information. The cross-maze paradigm was originally introduced by Tolman and colleagues (Tolman *et al.*, 1946, 1947) and has since been established as an effective tool for analysing learning and memory (Rodríguez *et al.*, 1994; Salas *et al.*, 1996b; López *et al.*, 2000a, b, c). In all the experiments described in this thesis, only 3 out of the 4 arms of the maze are ever used during any one trial. Therefore, throughout the thesis the maze will more usefully be referred to as a T-maze.

In several experiments described in chapters 3 to 6, after a period of training with two or more cues consistently indicating the reward location in one arm of the T-maze, fish are exposed to a series of “probe trials”. In a probe trial, one of the spatial cues is presented in the opposite arm from where it had been during training such that the spatial cues now conflict. The arm of the T-maze selected by fish during a probe trial is used to establish whether any preferences exist for the different types of spatial information. One difficulty with this approach is in deciding exactly when fish can be said to have learnt the task. In some studies, all animals are given a fixed number of trials or training sessions before being exposed to a spatially disruptive probe trial (e.g. Packard & McGaugh, 1996; Salas *et al.*, 1996b). This

ensures that all individuals have equal experience of the apparatus and procedure prior to being tested, but risks slow-learning individuals being tested for cue preference before they have acquired the spatial task with a sufficiently high level of accuracy. Randomly selected arm choices by animals that have not yet learnt may be wrongly interpreted as reflecting a lack of preference for any particular spatial cue. An alternative approach that takes individual variation into account is to expose subjects to probe trials only after they have reached a pre-determined criterion performance (e.g. López *et al.*, 2000a, b, c). This approach is taken in the experiments described in this thesis since individual variation in performance by the threespine stickleback when trained to learn a spatial task was found to be considerable. The results of the experiment described in this chapter were used to establish an appropriate criterion performance evident of learning for use in later experiments.

Assessing the types of spatial information that determine the direction taken by fish depends critically on being able to control and manipulate all the available cues that could potentially be used by fish to locate the reward. In experiments where fish are trained to use local visual cues or body-centred movements to locate a goal, attempts are often made to eliminate extra-maze cues (visual cues outside the maze such as shadows, differences in light levels etc.), for example by using curtains or screens (Warburton, 1990; Rodríguez *et al.*, 1994). In some cases, the position of the goal in the maze or the maze itself is switched or rotated between trials to disrupt any possible association between global extra-maze cues and reward location (e.g. López *et al.*, 2000a, b). Both these measures were applied to the experimental design used

here. A further possibility is that fish can locate a reward in the maze by using olfactory or uncontrolled visual cues within the maze. The experiment described in this chapter was designed to investigate this possibility. Fish were trained to locate a goal in one arm of a T-maze by learning a turn direction out of the start box. After a period of training in the maze, fish were exposed to a single probe trial in which the reward was re-positioned to the opposite arm from where it had been during training. If fish were attending to odour traces or other uncontrolled visual cues, I predicted that they should be able to track the new position of the reward. Threespine sticklebacks collected from a pond and a river were tested in this experiment to ensure that there were no differences between fish collected from different habitats in their ability to use olfactory cues etc.

2.1.2 Methods

i) Subjects

Threespine sticklebacks were collected from the River Kelvin (NS 54 70) and from Balmaha pond (NS 42 91) in southern Scotland, in September 1999 outside their reproductive season, using 3 mm-mesh dip nets and standard minnow traps. All fish were given a settling period in the laboratory of 4-8 weeks, maintained on a diet of defrosted frozen bloodworm (*Chironomid* spp.). Pairs of fish that could be distinguished from size differences and individual markings were housed in holding aquaria of size 34 x 20 cm and 20 cm high. Holding tanks were lined with gravel and furnished with a plant and terracotta refuge. The temperature was maintained at $12 \pm 1^\circ\text{C}$ and overhead lighting was provided by 40-W fluorescent tubes, operating on a 12:12 hour light:dark cycle.

ii) Apparatus

Two four-arm mazes were constructed from 3-mm green plastic, each arm being 30 cm long, 12 cm wide and 20 cm high (Fig. 2.1). The mazes were lined with white coral gravel and submerged into aerated and filtered water to a depth of 15 cm within a rectangular pool (1.7 x 1.2 m and 0.3 m high). Grooves into which a 15 x 20 cm high removable screen could be slid, enabled any one of the arms to be shut off, producing a T-maze. A trap door (12 x 20 cm) placed 15 cm away from the central stem of the T-maze was used to close the start box for each trial. This was attached to nylon filament and controlled remotely by a hand-operated pulley system. Food rewards were provided by securing bloodworms into Vaseline filled petri-dishes (3 cm in diameter and 1.5 cm in depth), which were placed 5 cm away from the ends of each arm. The worms were sunk into a cavity in the Vaseline such that the fish were not able to see the food until they were a few centimetres away. Two opaque PVC partitions (12 x 20 cm) were positioned in the maze 15 cm from the ends of each arm. Mid-way and at the bottom of each partition was a small hole 4 cm high and 2 cm wide through which the fish could swim to reach the end of the arm. Trap doors (12 x 20 cm) held in grooves just behind the PVC partitions could be raised and lowered remotely. If a fish swam into the wrong arm, the trap door in the opposite arm could be lowered, preventing the fish from swimming back and accessing the food. Rewards could therefore be restricted to fish whose first choice was correct. The end walls of each arm were made of transparent Perspex. A transparent bottle housing 2 randomly selected, non-experimental sticklebacks could be placed alongside the Perspex to simulate the presence of a shoal. The shoal was only visible to the fish once it had passed through the hole in the PVC partition, and was designed

to reduce stress caused by training sticklebacks in isolation. Fish therefore received a double reward on accessing the goal arm of food and shoal mates. Ambiguity as to whether fish respond to the food reward or to the shoal mates is unimportant since the experiment is investigating how fish learn the route to the rewarded end, regardless of what motivates them (Fig. 2.1). Situated 1 m above the centre of the pool was a Vantage CCD camera with a Computer 2.6 mm wide angle lens allowing the movements of the fish to be viewed on a black and white monitor next to the pool. The entire apparatus was surrounded by white curtains in order to avoid disturbance from the observer and to minimise the availability of global extra-maze cues.

The T-maze

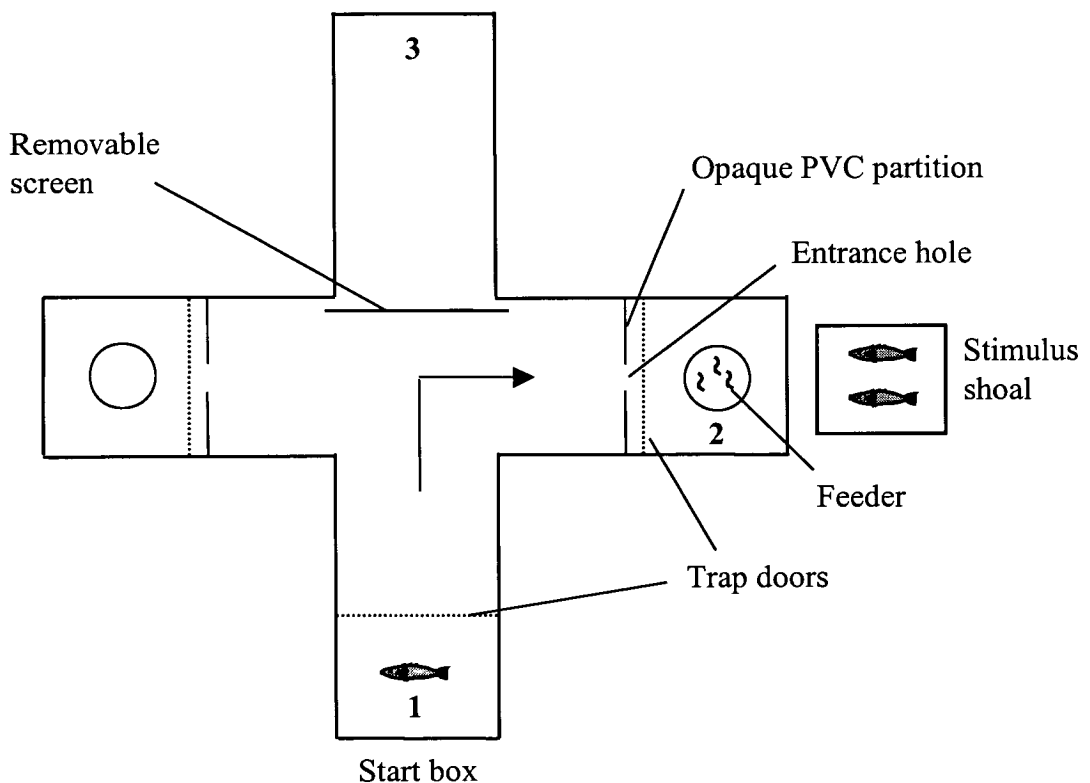


Figure 2.1. Diagrammatic representation of the spatial task. The arrow indicates the correct route a right-trained fish had to take to obtain food and shoal mates. The numbers indicate the sequence of start box positions for a run of three consecutive trials starting at position 1.

iii) Procedure

Thirty-two fish (16 Kelvin and 16 Balmaha fish) were trained to locate the reward in the T-maze by learning a turn direction out of the start box. Kelvin and Balmaha fish were divided into two groups (8 fish in each), which were trained sequentially.

Pre-training

The purpose of the pre-training was to familiarise fish with the apparatus and procedure. During pre-training the trap doors were raised, allowing fish free access through both holes in the PVC partitions and to both ends of the T-maze. Petri-dishes containing copious amounts of bloodworm were placed in both arms of the maze but the simulated shoal was not used as fish were not yet in isolation. Both mazes were used simultaneously and fish were pre-trained in groups of four, each group having four 24 hour periods in a maze alternated with 24 hours in their home tanks. In any one session, groups of Kelvin and Balmaha fish were pre-trained simultaneously. Four fish were individually transferred in a clear plastic cup from their holding tanks to a start box. For each session, the maze and start box were randomly selected with the constraint that no same maze or start box was used more than twice in a row. An opaque cover was placed over the start box to minimise disturbance. After 5 minutes, the door was raised remotely and the fish were given free access to both arms of the T-maze. Feeding only took place in the maze. After 4 pre-training sessions, the fish swam out of the start box, and found food in the ends of the T-maze in under a minute. Fish were food-deprived for two days between pre-training and training, a procedure that has no ill-effects on the fish which can survive at this temperature without food for several days (Bell & Foster, 1994).

Training

Fish were given 3 trials a day, once every two days, and the order in which the fish were trained was randomised for each day. In each trial two petri-dishes were placed in the maze, only one of which contained a food reward (three bloodworms). The simulated shoal in the bottle was placed at the end of the rewarded arm. Half the fish from each site were trained to turn right and half were trained to turn left in order to control for any directional bias the fish might have. Each fish was introduced into a start box and left for 5 minutes with the opaque cover in position. For each 3-trial session, the maze and start box were randomly selected with the constraint that no same maze or start box was used more than twice in a row. After 5 minutes, the door was raised and the time taken for the fish to leave the start box, to enter an arm, and the first arm choice were recorded. Arm entry was judged to have occurred when the base of the caudal fin had passed through the hole. Each trial was terminated after the fish had fed, or after 10 minutes had elapsed. Fish were left for 3 minutes after feeding before being gently encouraged to swim into the rewarded end if not already there. The trap door was then lowered, and the apparatus manipulated such that the previously rewarded end became the start box for the next trial. In this way, fish rotated around the arms of the maze; anti-clockwise for right-turners and clockwise for left-turners. This allowed individuals to be trained in runs of 3 trials, with minimum handling between trials.

In the first 12 trials, the trap doors in the arms of the T-maze were raised allowing fish access to both ends. For the remainder of the experiment, after the fish entered a hole in the partition at one end, the trap door raised above the entrance to

the opposite end was lowered. Fish were then only rewarded on making a correct first choice. Throughout the period of training, feeding only took place in the maze.

iv) Probe trial

After 24 trials, fish were exposed to a single probe trial in which the food and simulated shoal were re-positioned to the opposite side from where they had been during training.

v) Statistical methods

In order to assess the factors affecting performance, I calculated the proportion of correct trials across runs of 6 consecutive trials, from henceforth referred to as blocks (see section 2.2.4). To meet the assumptions of parametric procedures, the data were arcsine transformed. I then carried out a four-factor (population, order of training, turn direction, block) analysis of variance (ANOVA) with block as a repeated measure. In each model, fish was nested within population, turn direction and order of training. The results of the probe trial were analysed using the Fisher's exact test.

2.1.3 Results

There were no significant differences in performance between the counterbalanced right-trained and left-trained conditions within groups ($p > 0.1$) therefore these data were collapsed when calculating the group averages.

i) Acquisition

One Balmaha fish failed to leave the start box in the first 6 trials and was dropped from the experiment, leaving a sample size of 31 fish (15 Balmaha and 16 Kelvin fish). Fish from both sites learnt to find the rewarded arm with performance significantly improving over the 4 blocks (24 trials) of training (ANOVA, block: $F_{3,81} = 24.4$, $p < 0.001$, Fig. 2.2). However, there was a significant interaction between the order of training, population and block (ANOVA, pop*order*block: $F_{3,81} = 3.3$, $p = 0.02$). The performance of Balmaha fish trained in the first phase of the experiment was worse than any other group (Fig 2.2). It is not clear why Balmaha fish in the first group were poorer learners than any other group. Slight differences in handling at the time of collection and transferral of fish to the laboratory may have resulted in this group being more stressed than any other group.

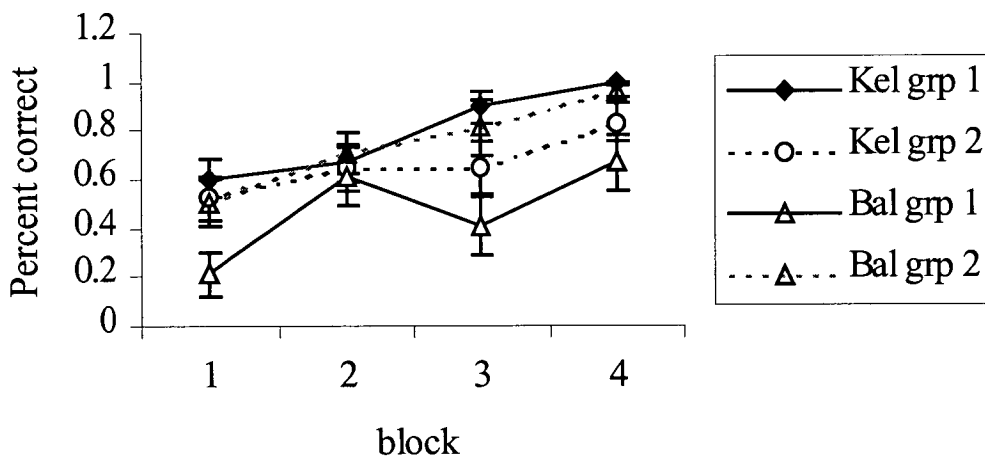


Figure 2.2. Mean \pm S.E. percentage of correct choices across the first 4 blocks (24 trials) of training (1 block = 6 trials).

ii) Probe trial

During the probe trial, fish showed a significant preference for turning in the direction to which they had been trained even though the food reward and the simulated shoal had been re-positioned to the opposite arm (Fisher's exact: $p < 0.01$, Fig. 2.3). Eighty-seven percent of the fish trained to turn right turned right in the probe trial and 75% of the fish trained to turn left turned left in the probe trial. There were no significant differences between Balmaha and Kelvin fish in the arm choices they made in the probe trial (Fisher's exact: $p = 0.99$).

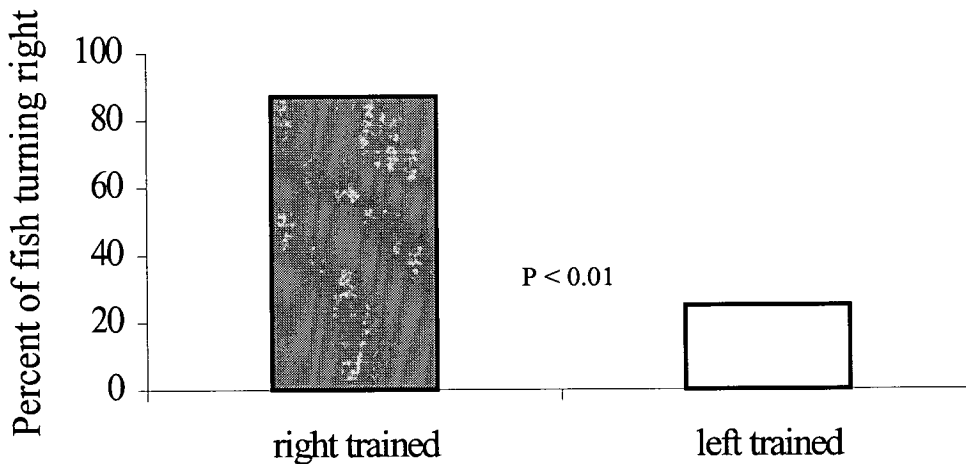


Figure 2.3. Percentage of right trained and left trained fish selecting the right-hand arm of the T-maze in the probe trial.

2.1.4 Discussion

i) Establishing a criterion for learning

The repeated measures analysis revealed that overall, fish improved their performance over the 4 blocks of training providing evidence of learning. However,

there was considerable individual variation in performance (mean percentage of correct trials: 67%; range: 67; see Appendix I). To ensure that in subsequent experiments fish would be tested for cue preference only once they had learnt the task, the choice scores from this experiment were used to establish a criterion performance evident of learning. Previous experiments training fish to learn a two-choice task have used varying criteria. Girvan (1999) tested sticklebacks for cue preference in a T-maze similar in design to this one after they had performed 3 correct trials in a row, while in studies by López and colleagues the acquisition criterion for a two choice spatial task was set at 21 correct trials out of 25 (López *et al.*, 2000a) or 13 correct trials out of 15 (López *et al.*, 2000c).

Since accurate interpretations of choice responses made during probe trials depends on subjects having learnt the task, it is crucial to select an appropriate criterion performance. A performance of 3 correct trials in a row was found not to provide sufficiently stringent evidence for learning in this experimental set-up. Twenty-eight fish out of 31 chose the correct arm of the T-maze in three consecutive trials in a mean of 12 trials. Simulations found that if arm choice is random, a mean of 14 trials are required before a run of three consecutive correct trials will be observed by chance (T. Vines, pers. com.). Therefore, a performance of 3 correct trials in a row by fish in the 24 trials of this experiment could have arisen without learning (Fig. 2.4). In order to ensure that fish have learnt the task before they are subjected to probe trials, a conservative criterion was selected of at least 9 correct trials in a run of 10. Simulations show that if there is no learning (probability of being correct = 0.5), the proportion of fish from any sample that would be expected

to perform 9 correct trials out of 10 in 24 trials is 0.066 (T. Vines, pers. com., Fig 2.5).

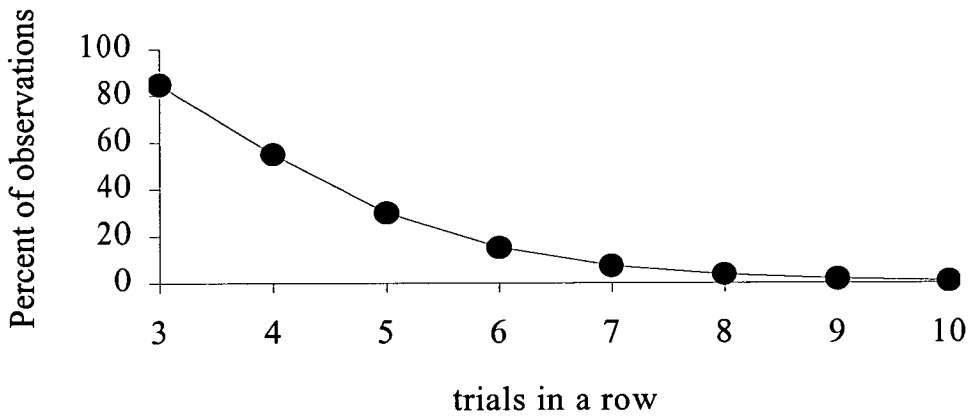


Figure 2.4. Percentage of times a run of 3 to 10 consecutive correct trials will be observed within 24 trials without learning (i.e. where the probability of selecting the correct arm in the T-maze is 0.5).

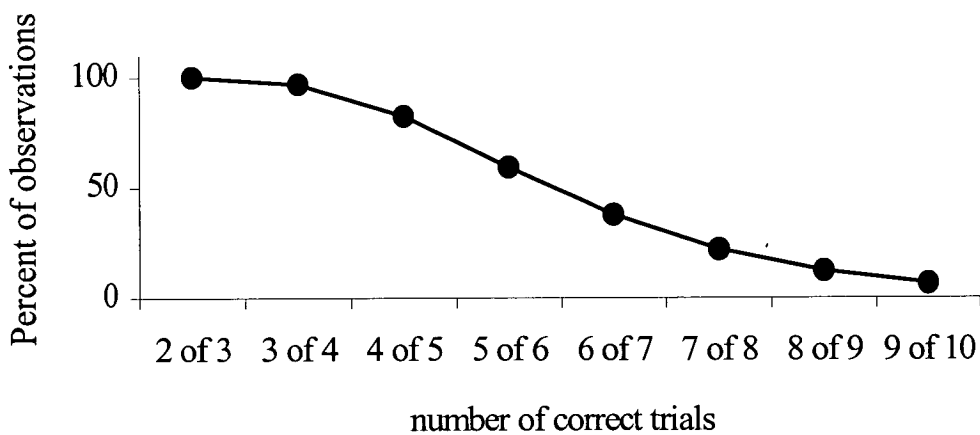


Figure 2.5. Percentage of times a number of correct trials (n) will be observed out of $n + 1$ trials within 24 trials without learning (i.e. where the probability of selecting the correct arm in the T-maze is 0.5).

ii) Control over spatial cues

The results of the probe trials indicate that sticklebacks collected from two different habitats, a pond and a river, cannot use olfactory cues or uncontrolled visual cues within the maze to locate the goal arm in the T-maze. Fish that had been trained to turn either left or right into an arm tended to turn in the same direction even when the reward had been repositioned to the opposite arm from where it had been during training. All the fish that achieved the criterion performance of at least 9 correct trials out of 10 during training, turned in the direction to which they had been trained in the probe trial. This further supports the claim that a performance of 9 correct trials out of 10 demonstrates learning.

2.2 GENERAL METHODS

In chapters 3 to 6, the T-maze is used to compare performance and cue use during spatial learning in different populations of threespine sticklebacks and under different experimental conditions. To avoid repetition, general methodological procedures common to these studies are given here.

2.2.1 Subjects

With the exception of the Canadian species of threespine stickleback studied in chapter 5, all threespine sticklebacks used in this thesis were collected from sites in southern and central Scotland. Figure 2.6 shows the location of the sites used in this thesis. Grid references taken from the Landranger Series of Ordnance Survey maps are listed in Table 2.1. All fish collections were made using 3 mm-mesh dip nets and standard minnow traps.

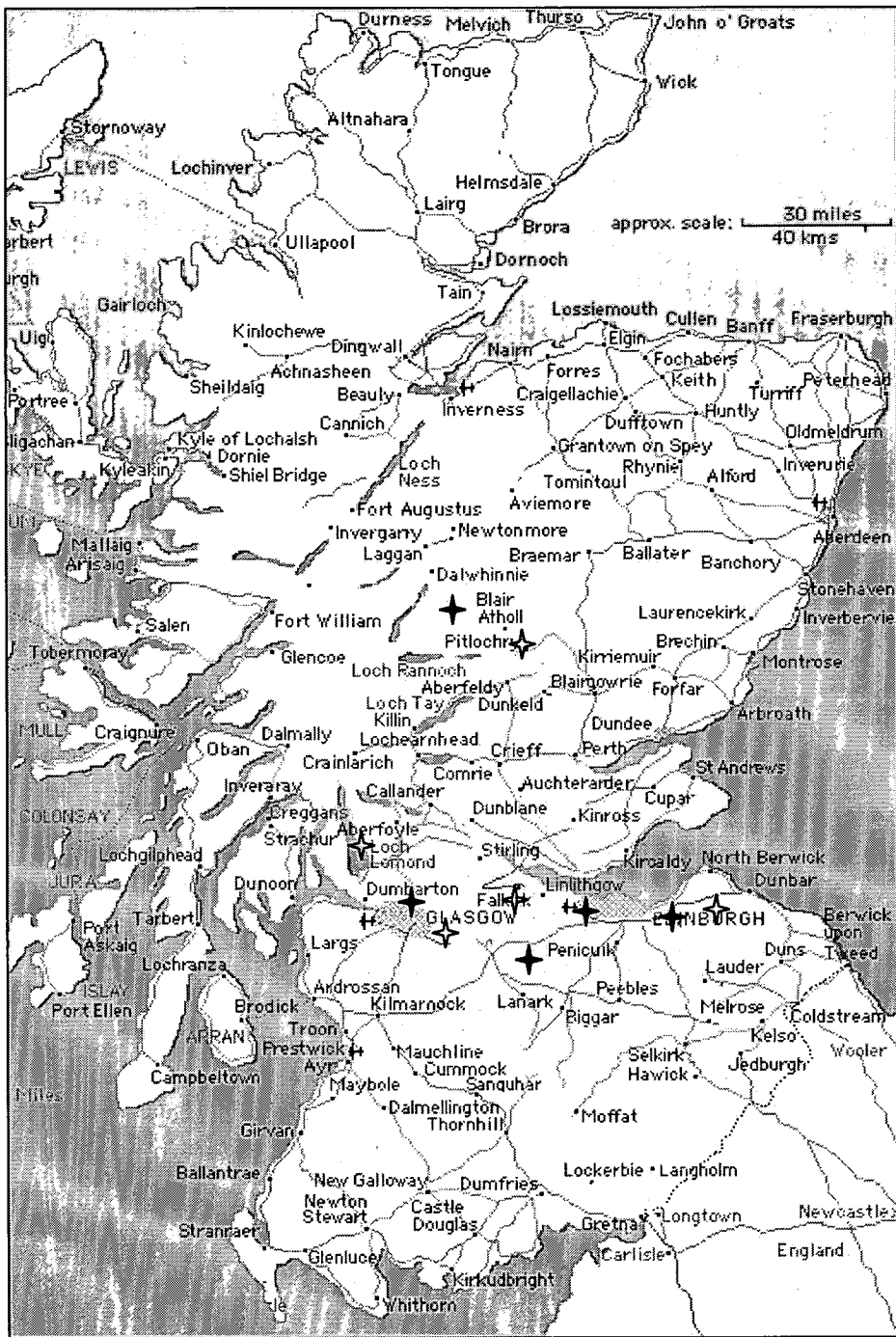


Figure 2.6. Map of Scotland showing the location of the 10 sites where threespine sticklebacks were collected. Ponds are indicated by the white symbols and rivers by the black symbols.

Site name	Habitat type	O.S. reference
Kelvin	River	NS 54 70
Garry	River	NN 90 63
Avon	River	NS 97 78
Almond	River	NS 96 76
Water of Leith	River	NT 23 73
Balmaha	Pond	NS 42 91
Inverleith	Pond	NT 24 75
Cuilc	Pond	NN 93 58
Queenspark	Pond	NS 57 62
Becraig	Pond	NS 99 74

Table 2.1. Grid references of the collection sites used in this thesis taken from the Landranger Series of Ordnance Survey maps.

2.2.2 Housing conditions

In all experiments, fish were given a settling period in the laboratory of at least 3 weeks prior to being tested. Sticklebacks were housed in holding tanks ranging in size from 30 x 19 x 20 cm to 75 x 45 x 30 cm. Holding tanks were generally fitted with a Fluval foam filter and airstone (Animal House Ltd., UK) and were cleaned before each experiment began to ensure that subjects only fed on the food provided and did not feed on algae etc. All tank water was aerated and treated with aquarium salt before any fish were transferred. Holding tanks were lined with gravel and typically contained a plant and terracotta refuge. Fish were generally maintained on a

diet of defrosted frozen bloodworm (*Chironomid* spp.), occasionally supplemented with newly hatched brine shrimp (*Artemia* spp.) and live *Daphnia*.

2.2.3 Welfare

In several experiments, fish were food-deprived for a maximum of two days. Threespine sticklebacks can survive without food for several days (Bell & Foster, 1994) and no ill-effects or increased mortality were observed during or after food deprivation. Fish were transferred between tanks and mazes in a clear plastic cup, which was found to cause less stress than catching fish with hand-nets. Threespine sticklebacks are a social species and frequently show characteristic stress reactions such as freezing or hiding if placed alone in a tank. To reduce stress, fish were never housed in isolation in their home tanks. The general health of the fish was checked daily and any illnesses were treated with Protozin and Myxazin water treatments (Animal House Ltd., UK). At the end of each experiment, fish were sacrificed with the anaesthetic MS222.

2.2.4 Statistical methods

Statistical procedures were in general taken from Sokal and Rohlf (1995) and Fowler *et al.* (1998). The significance level (α) was set at 0.05 and tests were two-tailed unless otherwise stated. Parametric tests were conducted unless the data did not meet the assumptions of parametric procedures, in which case appropriate transformations were applied to the data, or non-parametric statistics employed. Success of the transformation was confirmed with the Kolmogorov-Smirnov test. Unless otherwise stated, in order to affect the factors affecting performance in the spatial task, I

calculated the proportion of correct trials across runs of 6 consecutive trials, referred to as blocks. This converted the binary choice data (correct *versus* incorrect) into scores that were suitable for repeated measures analyses of variance (ANOVA).

Probe trials and trials in which fish failed to enter either end of the maze within the ten minutes of testing were excluded. When conducting ANOVAs, full models were fitted to the data initially, including all explanatory variables and their interactions.

Terms were then removed from the model by stepwise deletion. Non-significant effects of interactions and main factors are reported at the point of their removal from the model.

Chapter 3

Population differences in landmark use during spatial learning in the threespine stickleback

3.1 INTRODUCTION

Theoretical models that investigate the kinds of environmental unpredictability that should select for learning assume that there must be costs and limits to being plastic that constrain its evolution (Mangel, 1990; Stephens, 1991, 1993; Bergman & Feldman, 1995). Although few attempts have been made to critically examine the costs of learning, proposed costs include the cost of making mistakes, the energetic cost of processing information, delayed reproductive success, increased juvenile vulnerability, and increased parental investment (Johnston, 1982). However, little is known about how, or indeed whether animals optimise the use of learned behaviour within their natural environments such that potential costs are minimised.

In some cases, plasticity of brain structures and neurological machinery may enable animals to reduce energetic expenditure when there is a reduced demand for information processing. For example, seasonal fluctuations in the rate of neurogenesis and hippocampal volume in black-capped chickadees follow seasonal changes in foraging behaviour and space use (Barnea & Nottebohn, 1994; Smulders, 1995). Similarly, enhancement and regression of spatial learning ability in polygynous rodents appears to track seasonal shifts in the demand for this behaviour (Galea *et al.*, 1994; Gaulin, 1995). Interpretations of the adaptive function of

seasonal changes in brain structures have been criticised for lacking experimental evidence (Bolhuis & Macphail, 2001). However, it is likely that at least some animals are highly economical with their investment in neural tissue, building and maintaining neurological machinery only when the metabolic costs can be offset by the benefits of increased efficiency in acquiring food or mates.

More wide-ranging are mechanisms that guide and direct learning and associated perceptual processes in response to specific ecological problems. Animals are often pre-programmed to pay attention to certain cues in preference to others. For example, when exposed to songs of multiple species, juveniles of a number of bird species preferentially learn conspecific song (Thorpe, 1961; Marler & Tamura, 1964; Immelmann, 1969; Marler & Peters, 1977, 1988). Moreover, certain associations may be learnt more readily than others (Garcia & Koelling, 1966). Hummingbirds can easily learn to discriminate colours but fail to learn to distinguish between objects of differing brightness (Goldsmith *et al.*, 1981). Similarly, hummingbirds reinforced for visiting a flower location can more easily learn to choose a different location during a subsequent foraging effort than learn to return to the same location (Cole *et al.*, 1982). Differences in performance may be caused by differences in the ability of animals to learn and remember, or result from differences in associated processes such as perception or motivation. Either way, such predispositions may filter out uninformative or unreliable sources of information such that the risk of making mistakes, wasting time, and processing redundant information is minimised.

Developing a deeper understanding of the nature and degree to which perceptual processes or learning and memory biases guide and enable learned behaviour, is likely to be central to understanding how learning is used in its natural context in a cost-effective way. In this chapter, I investigate whether different habitat conditions shape learned behaviour in populations within a species, asking specifically whether habitat stability influences the use of visual cues in orientation in the threespine stickleback. Several studies imply that habitat stability can influence the use of visual cues in spatial learning (Taube & Burton, 1995; Biegler & Morris, 1996a, b). For example, there is evidence that food storing-bird species differ from non-storing species in responding preferentially to spatial position over local visual cues (Brodbeck, 1994; Clayton & Krebs, 1994b). One explanation is that local visual cues are more prone to change between storage and retrieval of food than is spatial location. However, many factors differ between species that could potentially explain differences in their use of local visual cues. These confounding factors are typically reduced in intraspecific comparisons (Papaj & Prokopy, 1989; Carlier & Lefebvre, 1996).

In habitats such as rivers and streams, disturbance of the visual landscape by flow and currents is likely to render local visual landmarks unreliable indicators of location for use in orientating to feeding patches or nests. I predicted that fish from unstable river habitats should show reduced reliance on visual cues compared to fish inhabiting visually stable habitats such as ponds. In support of this, Girvan and Braithwaite (1998) showed that sticklebacks collected from two ponds learned an orientation task faster if landmarks were present than when they were absent, but this

trend was not observed in fish collected from two rivers. Although intuitively compelling, it is difficult to rule out the role of alternative variables when only two or a few populations are compared. In Girvan and Braithwaite's study, adaptation to differences in water clarity was also suggested as a factor that might account for variation in the use of visual cues observed between the two river populations. My aim here was to clarify this ambiguity and investigate whether the relationship between habitat stability and the use of visual cues persists in a comparison of ten populations. In this study, I tested fish collected from five fast-flowing rivers and five ponds in order to reduce the possibility of variables other than stability, such as predation level or water clarity, co-varying in a non-random manner with pond and river habitats.

Pond and river fish were trained to learn a simple orientation task whereby they had to locate a goal arm in a T-maze. Fish could locate the goal arm either by using an algorithmic behaviour (turn left or right) or by tracking the position of plant landmarks. Probe trials, in which the cues conflicted, investigated the types of spatial information used and assessed whether differences existed between pond and river fish in their propensity to use landmarks.

3.2 METHODS

i) Subjects

Threespine sticklebacks were collected from ten sites in southern and central Scotland. The rivers were River Kelvin, River Garry, River Avon, River Almond, and Water of Leith. These ranged from 4–10 m in width and 0.5–2.5 m in depth. All were in spate at the time of sampling. The ponds were Balmaha pond, Inverleith pond, Cuilc pond, Queenspark pond, and Beecraig pond. The ponds ranged from 10-100 m in diameter and 0.5-2 m in depth. All fish were collected in September 2000 outside their reproductive season, using 3 mm-mesh dip nets and standard minnow traps.

Forty fish were used for the experiment (4 fish from each site). All fish were given a settling period in the laboratory of 4 weeks maintained on a diet of defrosted frozen bloodworm. Fish were maintained in groups of 4 in holding aquaria of size 46 x 30 cm and 30 cm high. The temperature was maintained at $12 \pm 1^\circ\text{C}$ and overhead lighting was provided by 40-W fluorescent tubes, operating on a 12:12 hour light:dark cycle. In the fourth week of the settling period, fish were individually tagged with coloured plastic rings (see Appendix II). Tagging did not affect the health of the fish and did not appear to have any effect on their behaviour.

ii) Apparatus

The apparatus used for this experiment was the same as that described in chapter 2 (p 36). An important difference was that in this experiment, two plastic plant landmarks were additionally placed in each maze. One was situated directly next to

the hole in the PVC partition leading to the food reward. The second was situated in the correct arm just in front of the removable barrier and was visible from the exit of the start box (Fig. 3.1). In addition, a VCR was connected to the monitor so that the movements of the fish could be recorded on video.

The T-maze

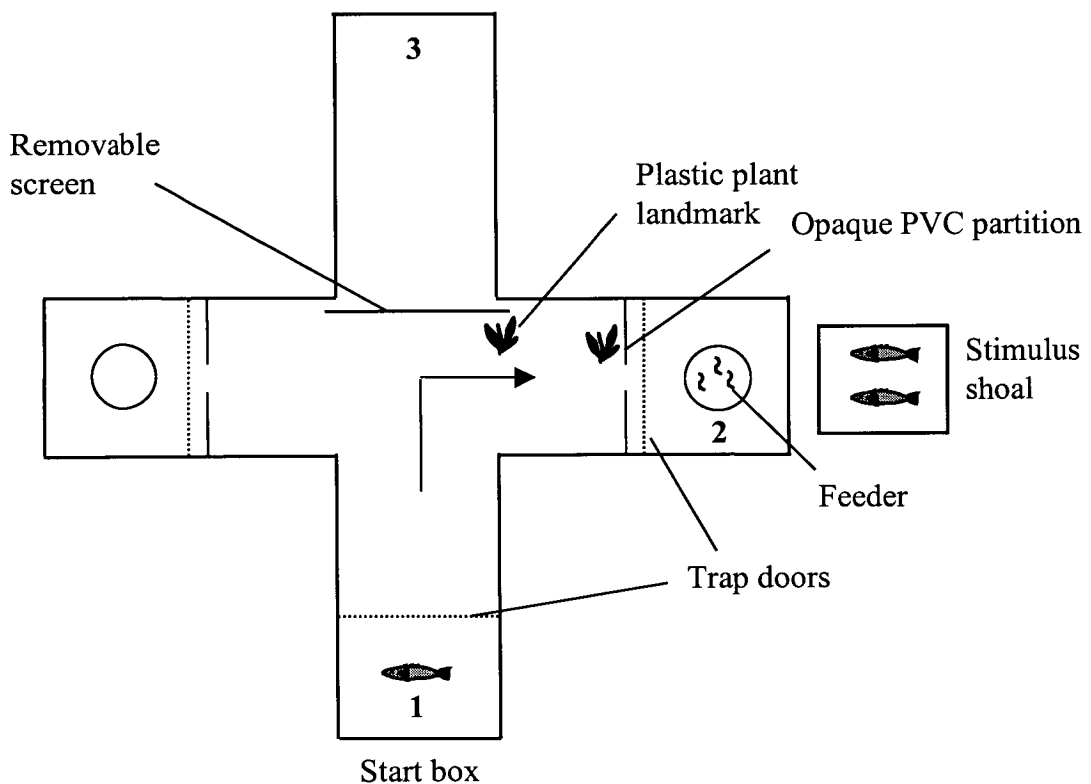


Figure 3.1. Diagrammatic representation of the spatial task showing the position of plant landmarks, which were always presented in the rewarded arm of the maze. The arrow indicates the correct route a right-trained fish had to take to obtain food and shoal mates. The numbers indicate the sequence of start box positions for a run of three consecutive trials starting at position 1.

iii) Procedure

Pre-training

As described in chapter 2 (p 38), the purpose of pre-training is to familiarise fish with the apparatus and experimental procedure. Pre-training was carried out using the same method as that described in chapter 2 except that in this experiment, fish were pre-trained in groups of ten (1 fish from each site). In addition, each group was given five instead of four 24 hour periods in a maze alternated with 24 hours in their home tanks. The number of pre-training sessions was increased to ensure all fish were sufficiently familiar with the procedure before being trained in isolation. No landmarks were present during pre-training and fish were food deprived for two days between pre-training and training as described in chapter 2 (p 38).

Training

Training followed the same procedure as that described in chapter 2 (p 39) with several important differences. As before, half the fish from each site were trained to turn right and half were trained to turn left in order to control for any directional bias the fish might have. However, in addition to learning a turn direction out of the start box, the fish in this experiment could use landmarks to locate the reward since plant landmarks were always presented in the goal arm of the T-maze. For each trial, the two plastic plant landmarks were positioned in the right arm or left arm accordingly. In addition, in this experiment the learning criterion was established as 9 correct trials out of 10. Fish were trained until they had reached criterion or for a maximum of 45 trials. When the fish reached criterion, additional post-criterion sessions were conducted interspersed by probe trials. A minimum of 4 correct trials out of a run of

5 had to be achieved between any two probe trials. The final training trial before the first probe trial was recorded on VCR.

iv) Probe trials

Probe trials were designed to determine the spatial strategy employed by the fish.

During probe trials, food and shoals were placed at both arms of the maze to avoid punishing the fish for selecting one or other of the available cues. In each probe trial, the plant landmarks were repositioned to the opposite side from where they had been during training. For fish trained to turn right, the landmarks were now positioned in the left arm of the maze and vice versa for fish trained to turn left. Fish were allowed to enter an arm and feed before being removed from the maze. A turn response was recorded if, regardless of the position of the landmarks, the fish turned in the same direction as it had done during training. A landmark response was recorded when the fish selected the arm presenting the plastic plant landmarks. All fish were subjected to 3 probe trials interspersed between at least 5 training trials. All probe trials were recorded on VCR.

v) VCR recordings

Two behaviours indicating “hesitancy” or “confusion” were scored from video recordings of the three probe trials and of the last training trial prior to the first probe test. A “pause” was scored if a fish remained completely stationary for a minimum of 3 seconds. A “reversal” was scored if a fish, having swum in one direction, made a 180° turn and swam in the opposite direction. Behaviours were scored immediately after the fish left the start box and before it entered the end of an arm. During video

analysis, fish were identified on the monitor from codes, which gave no information as to whether they were pond or river fish.

vi) Statistical analysis

Where appropriate, data were log, square root or arcsine transformed to meet required assumptions of homogeneity of variance and normality of residuals.

Parametric statistical tests were used throughout except for the results of the probe trials, which were analyzed using the G-test. In order to assess the factors affecting performance, I carried out a four-factor (habitat type, population, turn direction, block) ANOVA with block as a repeated measure (1 block = 6 trials). In each model, population was nested within habitat type and fish was nested within turn direction, habitat type, and population.

3.3 RESULTS

There were no significant differences between the counterbalanced right-trained and left-trained conditions within groups in any phase of the experiment (all p 's > 0.1) therefore these data were collapsed when calculating the group averages.

i) Acquisition

Figure 3.2 shows the percentage of pond and river fish performing the task correctly during the first 24 trials (4 blocks) of training. Fish from all ten sites learnt to find the rewarded arm with performance significantly improving over the first 24 trials of training (block: $F_{3,114} = 6.8$, $p < 0.001$). Pond and river fish showed no significant differences in performance (habitat type: $F_{1,8} = 0.62$, $p = 0.55$; habitat type x block:

3. Population differences in landmark use during spatial learning

$F_{3,114} = 2.36, p = 0.08$). There was no significant effect of population on performance (pop: $F_{8,30} = 0.44, p = 0.64$; block x pop: $F_{24,87} = 1.37, p = 0.15$).

Seven individuals did not reach criterion in 45 trials leaving a sample size of 15 pond fish and 18 river fish ($n = 33$) that reached criterion. A one way ANOVA with the number of trials taken to reach criterion as the dependent variable revealed no significant effect of habitat type on performance (ANOVA, habitat type: $F_{1,31} = 0.42, p = 0.52$; Pond: $\bar{X} \pm S.E = 22.5 \pm 2.94$ trials; River: $\bar{X} \pm S.E = 25.1 \pm 2.59$ trials). There were too few fish from each site to look at the effect of population within a full model including habitat type and population as factors. However, a one-way ANOVA on the collapsed data revealed no significant effect of population on the number of trials taken to reach criterion (ANOVA: $F_{9,23} = 0.98, p = 0.5$).

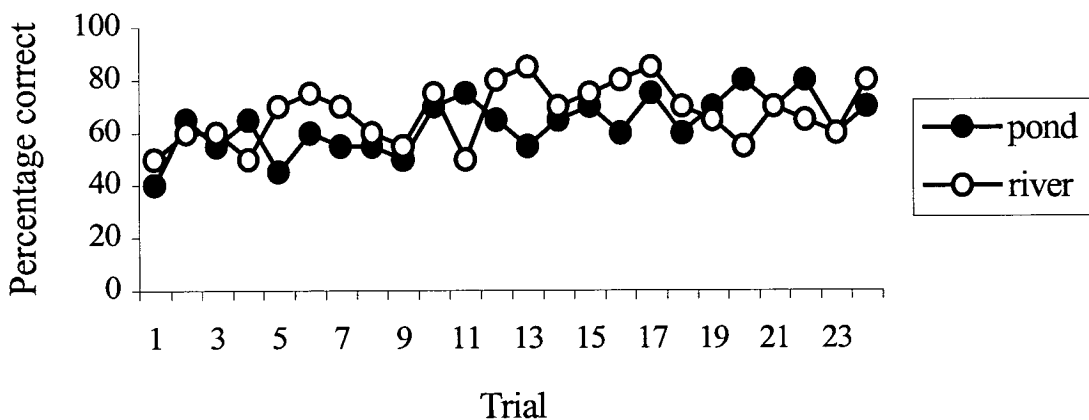


Figure 3.2. Percentage of correct choices by pond and river fish during the first 24 trials of training.

ii) Post-criterion performance

Throughout the post-criterion trials during which probe trials were interspersed, pond and river fish maintained a high and steady level of accuracy (pond: $\bar{X} \pm \text{S.E} = 85.8 \pm 0.03\%$ correct; river: $\bar{X} \pm \text{S.E} = 94.9 \pm 0.02\%$ correct). However, performance of the pond fish appeared to be significantly more disrupted by the probe trials than that of river fish. A one-way ANOVA carried out with the number of post-criterion errors as the dependent variable revealed a significant difference between pond and river fish in the number of errors made during post-criterion trials (ANOVA, habitat type: $F_{1,31} = 6.35, p = 0.02$). Pond fish made significantly more errors than river fish during post-criterion training (pond: $\bar{X} \pm \text{S.E.} = 1.73 \pm 0.35$ errors; river: $\bar{X} \pm \text{S.E} = 0.57 \pm 0.19$ errors, Fig. 3.3).

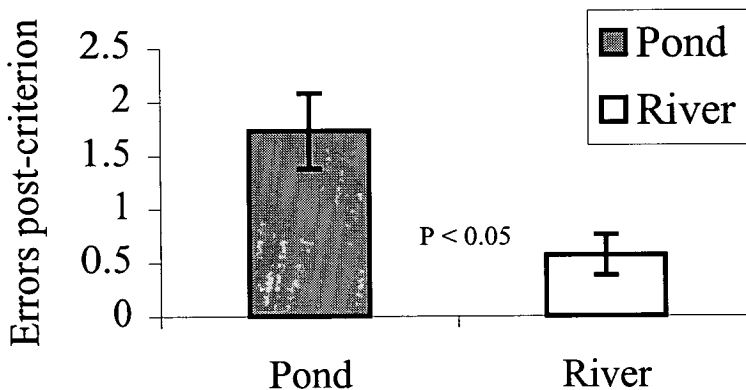


Figure 3.3. Mean \pm S.E. number of post-criterion errors made by pond and river fish.

iii) Probe trials

Figure 3.4 shows the results of the probe trials. A G-test compared the distribution of fish using one of three possible strategies: i) landmarks only across all three probe trials; ii) landmarks and turn iii) turn only, to a random expectation assuming no preference for either cue. Pond and river fish appeared to differ in the strategies they used to solve the spatial task. Although pond fish used turn across all three probe trials (33 %) more often than they used landmarks across all three probes (7 %), overall, pond fish showed no significant preference for using turn direction over landmarks (G-test: $G_2 = 2.18$, $p > 0.2$). Sixty percent of pond fish used a combination of turn and landmarks across the three probe trials. In contrast, river fish showed a significant preference for using turn direction over landmarks (G-test: $G_2 = 16.46$, $p < 0.001$). Seventy-two percent of river fish consistently used turn across all three probe trials, 22 % used turn and landmarks and 6 % consistently used landmarks.

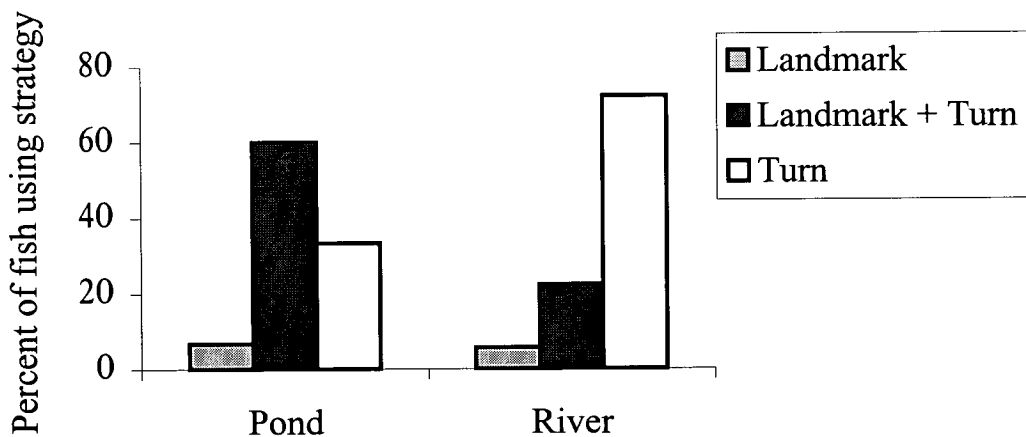


Figure 3.4. Percentage of pond and river fish using three strategies: landmarks across all three probe trials (Landmark), both landmarks and turn (Landmark + Turn) or turn across all three probe trials (Turn).

iv) Pauses and reversals

For each fish, the mean number of pauses or reversals made across the three probe trials (probe score) was compared with the number of pauses or reversals made in the final training trial before the probe tests began (criterion score). Figures 3.5 a and b show the probe and criterion scores for pond and river fish. Pond fish paused significantly more often during the probe trials than at criterion (paired t-test: $t_{14} = -3.75$, $p = 0.002$). This difference was not observed in river fish (paired t-test: $t_{17} = -0.44$, $p = 0.66$). A two-way ANOVA carried out with the probe score as the dependent variable revealed that pond fish paused significantly more often than river fish (ANOVA, habitat type: $F_{1,30} = 12.47$, $p < 0.001$). Fish using a mixed strategy to solve the task (i.e. landmarks and turn), did not pause significantly more often than fish using a consistent strategy of turn only or landmarks only (ANOVA, strategy: $F_{1,30} = 2.49$, $p = 0.13$). This suggests that pond fish paused significantly more often than river fish, regardless of which spatial strategy they used.

Neither pond fish nor river fish showed more reversals across probe trials than at criterion (paired t-test, pond: $t_{14} = -0.99$, $p = 0.34$; river: $t_{17} = -1.54$, $p = 0.14$). Although pond fish showed more reversals than river fish during probe trials, this was not statistically significant (ANOVA, habitat type: $F_{1,30} = 0.03$, $p = 0.87$). There was no effect of spatial strategy on the number of reversals made during probe trials (ANOVA, strategy: $F_{1,30} = 0.82$, $p = 0.37$).

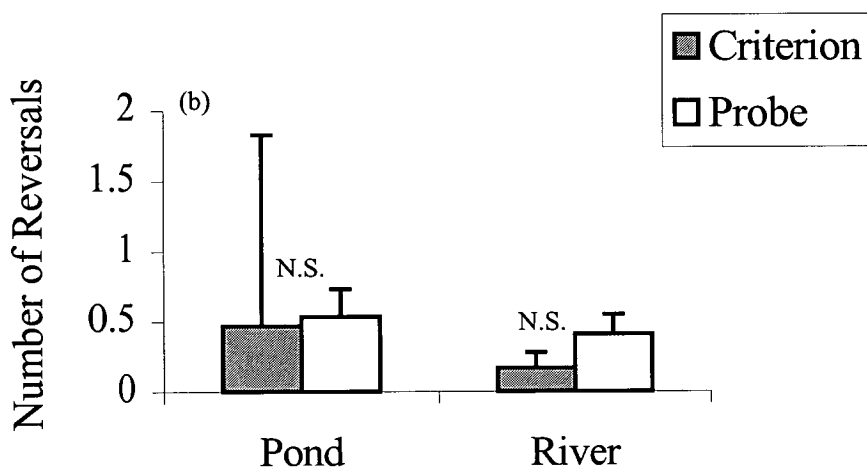
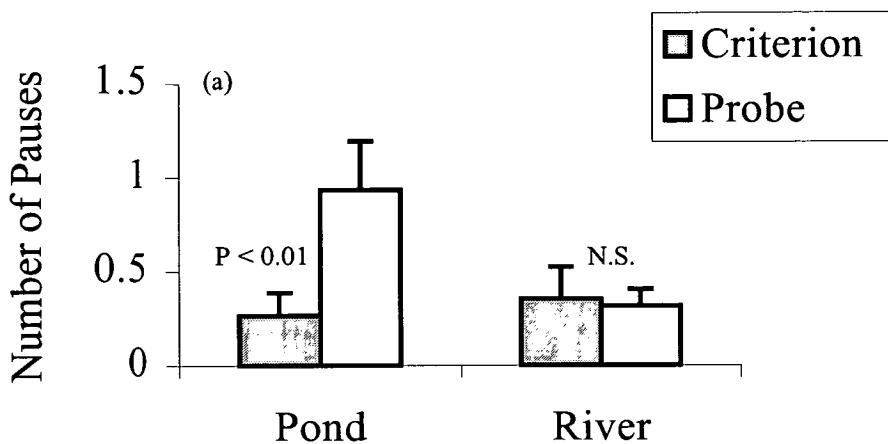


Figure 3.5. Mean \pm S. E. number of pauses (a) and reversals (b) made at criterion and during probe trials.

3.4 DISCUSSION

The results of this experiment suggest that populations exposed to different environmental conditions differ in the types of information they use to solve a spatial task. Although pond and river fish learn a spatial task at similar rates, several lines of evidence suggest that they employ different strategies. First, during probe trials, pond fish show no significant preference for either cue, while river fish preferentially rely on turn direction. Second, in the training trials following the first probe trial, pond fish make more mistakes than river fish. Disrupting the relationship between the cues in the probe trials might be expected to cause confusion if fish are paying attention to both types of cue and to the relationship between the cues. Differences in swimming behaviour also suggest that pond fish are more disorientated by the re-positioning of plant landmarks in the probe trials. Pond fish pause more often during the probe trials than river fish and unlike river fish, more often during the probe trials than at criterion. These results are consistent with the hypothesis that populations experiencing stable habitats show greater reliance on local landmarks as positional cues compared to fish living in unstable habitats, where local landmarks are likely to be unreliable.

The differences observed between pond and river fish may be specific to the use of local visual cues and not extend to the use of all available visual information. River fish may be unable to learn to use local landmarks as goal directing beacons but they may be capable of locating a goal by referring to a spatial array of visual cues (Hughes & Blight, 1999) or by using global cues external to the maze. The experiment described in chapter 2 showed that threespine sticklebacks collected from

a pond and river can not track the rewarded end by responding to olfactory or uncontrolled visual cues within the maze. However, I cannot eliminate the possibility that fish may have been using global features outside the maze. Learning the movements around the arms of the maze may have been aided by tracking global features such as differences in light level, the camera lens etc. This is unlikely for two reasons. First, enclosing the entire apparatus within a uniform white tent-like structure meant that very few global cues were available. Previous experiments testing spatial learning in fish have effectively eliminated the use of extra-maze cues by using similar curtains or screens (Warburton, 1990; Rodríguez *et al.*, 1994). In addition, both the maze and the start box for each 3-trial session was randomized such that the fish were presented with a different array of global cues each day. However, it may be that large scale features of rivers such as overhanging trees or the spatial arrangement of a river bend, present a stable and reliable source of visual information which can be effectively used in orientation. Further experiments are needed to elucidate whether the differences observed between pond and river fish in their use of local visual cues extends to their use of spatial arrays of landmarks and extra-maze global cues.

By including ten populations in this analysis, I aimed to reduce the possibility that an additional ecological factor could co-vary in a consistent way with the pond-river dichotomy other than habitat stability. A river and pond site was selected from each geographical area within a ten mile radius, to remove geographic location as a confounding variable. Three of the five ponds were highly eutrophic or sediment-rich, with visibility being considerably less in these ponds than in any of the five

rivers. It is therefore unlikely that differences in water clarity could explain why pond fish use local visual cues to a greater extent than river fish. There is limited evidence suggesting that exposure to different levels of predation can influence the use of visual cues in the threespine stickleback (Huntingford & Wright, 1989). I cannot speculate on this since the predation risk associated with each of the ten sites is not known.

So far, habitat stability remains the most compelling ecological factor that can account for differences in the use of local visual cues by pond and river fish. In turbulent and fast-flowing rivers, local features within microhabitats are likely to be subject to continual change. Equally likely are displacements of the fish themselves to new locations within the river system and to unfamiliar arrays of local landmarks. Learning to orientate back to feeding or nest sites could prove extremely costly if fish extract information from unreliable sources such as moving or unfamiliar landmarks. In addition to making more mistakes, fish using landmarks in unstable habitats may waste exploration time if there is a delay between a change in the environment and a shift in behavioural response. For example, in goldfish, previously relevant landmarks have been shown to inhibit attentional shifts to new patch-related stimuli following a change in the spatial relationship between food and landmarks (Warburton, 1990).

Differences in the use of landmarks by pond and river fish may be genetic and, or the result of differential experience. Human-mediated movements of stickleback populations combined with the close proximity of sites (< 40 miles apart)

make genetic isolation unlikely. However, stickleback populations have recently been found to be genetically divergent among lakes and rivers separated by similar distances (Reusch *et al.*, 2001). Therefore, it is possible that river fish have been selected to use alternative mechanisms other than visual cues for orientating. Alternatively, cue preference may be flexible with fish learning to respond differently to landmarks in different habitat conditions. Within rivers, habitat stability may vary between microhabitats with certain regions cut off from the main channel remaining relatively stable. Equally likely is seasonal variation in stability, with turbulence and flow rate being considerably reduced in dry summer months. It may therefore pay fish to continually update their assessment of cue reliability. If cue preference is itself flexible and learned, population differences might be expected to disappear soon after introducing the fish to a stable laboratory environment. However, the fish used in this experiment had been housed in tanks containing rocks and plants in fixed positions for 2-3 months by the time they were tested in the probe trials. Despite this, the possibility remains that the differences observed between pond and river fish were reduced compared to what would have been observed had they been tested immediately on bringing them into the laboratory. A further possibility is that cue preference is fixed during a restricted plastic period in development. Early experience of a more stable habitat may result in greater reliance on visual landmarks later in life.

Equally open to speculation is whether genetic and, or environmental influences act directly on the ability to learn and remember or on associated sensory input systems. River fish may differ from pond fish in their ability to perceive visual

3. Population differences in landmark use during spatial learning

landmarks or in their ability to learn associations based on this type of information. If learning and memory processes are involved, populations may differ in the storage and, or the retrieval of memory or by a difference in the way they respond to their memories. Based on the current data, I cannot differentiate between these possibilities.

In conclusion, local visual landmarks may provide reliable indicators of location for threespine sticklebacks inhabiting ponds but not rivers. In river fish, landmark information appears to be largely ignored when learning an orientation task. Potential costs of learning such as the cost of making mistakes, may be significantly reduced by genetic or developmental programs that guide perceptual and learning processes even at the population level. Understanding how animals maximise the benefits of being behaviourally plastic while minimising the potential costs, will demand a closer look at the interplay between learning, genetics and development.

Chapter 4

Is landmark stability a prerequisite for landmark use in spatial learning by the threespine stickleback?

4.1 INTRODUCTION

Animals can simultaneously use a variety of navigational strategies to locate a goal in their environment. Experiments in which one set of cues is played against another have revealed that birds, fish, mammals and insects use information from multiple sources. For example, several species of migratory birds use multiple orientation cues including the earth's magnetic field, stars, patterns of polarised skylight and the sun (Able, 1993). Food storing and non-storing bird species use multiple cues to locate caches, including the spatial position and features of local landmarks (Vander Wall, 1982; Brodbeck, 1994; Clayton & Krebs, 1994b). Goldfish can learn a turn direction, or use local landmarks or global "place" cues to solve a spatial task (Rodríguez *et al.*, 1994; López *et al.*, 1999). In mammals, external visual cues are often used in concurrence with internally based sensory information (Teroni *et al.*, 1987; Etienne *et al.*, 1990). In most insects, landmark guidance similarly supplements computations of current position and direction based on self generated sensory inputs (Collet, 1996; Collett & Zeil, 1998). Across a diverse range of species, where more than one solution to a given navigational problem exists, multiple strategies appear to be pursued in parallel.

4.1.1 Why use multiple cues?

An obvious advantage to using multiple cues is that if one cue becomes unavailable, an alternative source of information can be used. For example, in a series of experiments (Teroni *et al.*, 1987; Etienne *et al.*, 1990), golden hamsters were trained to leave a nest box and retrieve food from the middle of a circular arena. In minor cue conflict situations, when dead reckoning and distal landmarks diverged up to 90°, visual landmarks were preferentially used to locate the food. Deprived of visual cues however, hamsters used route-based information. Similarly, juvenile Atlantic salmon use visual landmarks to track a moving food source, but revert to using an alternative strategy, possibly chemosensory cues, in the absence of visually distinct landmarks (Braithwaite *et al.*, 1996).

Using combinations of cues may also reduce the error that accumulates from using any one cue in isolation. For example, many animals have been shown to use path-integration or dead reckoning. Animals keep track of their directional heading by reference to an “internal navigation system” i.e. inertial forces and other sensory signals generated by the animal’s own movements (Etienne *et al.*, 1998). However, no purely inertial directional system is free from error and such errors are cumulative (Etienne *et al.*, 1990; McNaughton *et al.*, 1991; Dyer, 1998). Visual “fixes” on landmarks may be necessary to correct for cumulative error (Etienne *et al.*, 1990; Collet, 1996). In some cases multiple cues appear to be used additively (see Sutherland & Mackintosh, 1971 for a review of additivity). Salas *et al.* (1996b) observed that goldfish trained to use both turn and place strategies simultaneously to solve a spatial task performed more accurately and steadily than fish trained to use one cue in isolation. Similarly, Diez-Chamizo *et al.* (1985) found that rats trained to

use both extra- and intra-maze cues, learned a discrimination task faster than rats trained with each component cue on its own.

In addition, different strategies may have complementary strengths and weaknesses that make them considerably more effective when they work in tandem. The synergistic use of landmarks with path-integration may increase the flexibility of path finding by allowing animals to interpolate from known scenes and vectors to slightly different ones, thus helping them to home correctly from new locations (Collet, 1996).

Although there are clear advantages to using multiple cues, there are likely to be constraints on the amount of spatial information that can be stored and remembered by an animal (Dukas, 1998b). Little is known about how animals react to multiple sources of information in the most economical and adaptive manner. Are animals physiologically constrained to pay attention to certain cues at the expense of others? Is the choice of cues used weighted according to their reliability and accuracy as goal-directing cues? In this chapter, I attempt to address some of these issues, focusing on the factors affecting the weight given to local landmarks.

4.1.2 Factors affecting the use of landmarks

Animals use local landmarks in orientation in a variety of different ways and to different extents. The degree of dependence on local landmarks in preference to other cues may in part be determined by genetically or developmentally acquired biases related to the animal's ecology. For example, as discussed in chapter 3, species

experiencing visually stable habitats may show greater dependence on local landmarks compared to those experiencing unstable habitats (Brodbeck, 1994; Clayton & Krebs, 1994b). Relatively fixed preferences for environmental cues such as landmarks will suffice if environments change slowly (and are the same for each generation) but animals may need to have flexible responses to cues that vary in reliability and quality over a shorter time scale (Stephens, 1993). There is substantial evidence from laboratory studies on rats and to a lesser extent from studies on birds and insects, that the weighting given to any particular landmark is flexible and affected by a) constraints on information processing (e.g. Diez-Chamizo *et al.*, 1985; Biegler & Morris, 1999) and b) how accurately and reliably they are perceived to direct the animal towards the goal (Biegler & Morris, 1996b).

Constraints on the amount of visual information that can be stored could be responsible for “overshadowing” effects between different types of visual cues. Animals trained with both extra-maze and intra-maze visual cues relevant, learn less about each type of cue than those trained with one cue in isolation (Wagner *et al.*, 1968; Diez-Chamizo *et al.*, 1985; March *et al.*, 1992; Redhead *et al.*, 1997). Furthermore, learning about a novel spatial cue can be “blocked” by prior learning about pre-existing cues, suggesting that animals do not waste resources processing redundant information but instead, remember the best indicator of a goal at the expense of other stimuli. For example, Biegler and Morris (1999) trained rats to search for food hidden near landmarks in an open field arena. After the rats had learned to use the first array, an additional landmark was noticed and explored but failed to gain control over the rats’ search performance (see also Rodrigo *et al.*,

1997; Roberts & Pearce, 1999). Learning about intra-maze cues can similarly be blocked by prior learning about extra-maze cues (Diez-Chamizo *et al.*, 1985), again implying that rats learn only as much as they need to.

Animals also appear to weight attention to landmarks based on their perceived accuracy as goal-directing cues. Landmarks near the goal supply the most precise information in specifying goal location (Cheng, 1990). A variety of species ranging from invertebrates to humans rely preferentially on nearer landmarks (Vander Wall, 1982; Cheng *et al.*, 1987; Cheng, 1989; Bennett, 1993; Spetch & Wilkie, 1994; Spetch, 1995), although this is not always the case (Margules & Gallistel, 1988).

In addition, several lines of evidence suggest that for many species the weighting given to a particular landmark depends on whether it is perceived as being geometrically stable with respect to at least one other source of spatial information, and therefore reliable. Experiments with rats have found spatial learning to be impaired under conditions in which landmarks are not geometrically stable (Biegler & Morris, 1993, 1996b; but see Roberts & Pearce, 1998). Varying the position of a landmark also decreases the control over search location exerted by that landmark (Biegler & Morris, 1996a). Other studies have investigated the effect of landmark stability on the firing of place cells and thalamic head direction cells in rats. These are cells in the hippocampus that discharge in relation to the animal's location (O'Keefe & Dostrovsky, 1971; Muller *et al.*, 1987; Jung & McNaughton, 1993). The strength of landmark control over place and head direction cells depends on the rats

learned perception of the stability of the landmarks (Knierim *et al.*, 1995). Jeffrey (1998) showed that if a cue card was moved in full view of the rat and therefore seen to be mobile, place cells ceased to be controlled by it and became orientated instead by self-generated idiothetic cues.

Landmarks may not have to be absolutely stable in order for them to be used in spatial learning. Often the amount of discrepancy between visual landmark information and a second source of spatial information influences the weight given to landmarks (reviewed in Biegler & Morris, 1996b). Cheng (1988) trained pigeons to find food hidden in an enclosure. When the landmark was moved, the search distribution shifted proportionally to landmark displacement, but only up to an asymptote, after which point the weight given to the landmark decreased. Chittka and Gieger (1995) found that the influence of landmarks on the flight direction of honeybees decreased as the discrepancy between the orientation of the landmark array and the bees' sun compass increased. Golden hamsters trained to leave a nest box and retrieve food from the middle of a circular arena preferentially use visual landmarks to locate the food when dead reckoning and distal landmarks diverge up to 90°. When the discrepancy is increased to 180°, the influence of the landmark decreases (Etienne *et al.*, 1990). In addition, landmark stability may only need to be local rather than global in order for animals to weight attention to landmark cues. For example, in training rats to use a moving landmark as a predictor of reward location, Biegler and Morris (1996a) found that adding a second landmark at a distance from the first improved performance, by adding a stable spatial relationship within the landmark array.

4.1.3 Aims of study

Taken together, these results imply that for a range of species, attention is weighted to landmarks depending on their perceived stability with respect to at least one other source of spatial information, although this conclusion is by no means consistently supported by all the available data (Roberts & Pearce, 1998). As yet we have virtually no understanding about whether these findings extend to the use of local landmarks in fish. Several studies have shown that fish can use landmarks in locating a goal (Reese, 1989; Warburton, 1990; Salas *et al.*, 1996a, b; Girvan & Braithwaite, 1998; Hughes & Blight, 2000; López *et al.*, 1999, 2000a; Odling-Smee & Braithwaite, in press.). However, the role of landmark stability in spatial learning has not yet been formerly investigated in controlled laboratory experiments. In the experiment described in chapter 3, pond fish appeared to show a greater reliance on local landmarks compared to river fish. The aim of the experiments reported here was to further investigate the use of local landmarks by pond and river threespine sticklebacks in different experimental contexts.

In experiment 4a, I investigated whether there are differences between pond and river fish, collected from multiple sites, in their ability to learn a spatial task when landmarks are the only predictor of reward location, and unstable with respect to all other sources of spatial information. In experiment 4b, a single population of pond fish was trained to locate a goal in the T-maze using three possible cues; turn direction, local landmarks and global place cues. Probe trials in which one cue was placed in conflict with the others, tested how fish weight attention to local landmarks

relative to the other cues, having been trained with landmarks remaining in fixed, stable positions.

4.2 EXPERIMENT 4A

The aim of experiment 4a was to investigate whether pond and river fish differ in their ability to locate a reward, by tracking landmarks that are in a shifted position with respect to other spatial cues during each trial. Pond and river fish collected from ten different sites in southern and central Scotland, were trained to locate a goal arm in a T-maze by tracking the position of plant landmarks (task 1). The position of the goal was randomized with respect to all other sources of spatial information (turn direction and global place cues). On reaching a criterion performance (9 correct trials out of 10), the same fish were then trained to learn a second task (task 2), in which the turn direction out of the start box was the only predictor of goal location. During this task, the position of the plant landmarks was randomized with respect to the goal.

4.2.1 Methods

i) Subjects

The threespine sticklebacks used in this experiment were collected on the same dates and from the same ten sites as those used in chapter 3. Forty fish (4 from each site) were given a settling period in the laboratory of 4 months in the same conditions as those described in chapter 3 (p 54). Two weeks before the start of the experiment, fish were individually tagged with coloured plastic rings (Appendix II).

ii) Apparatus and procedure

The experimental set up used in this experiment and the procedure taken for pre-training the fish and familiarizing them with the apparatus was exactly the same as that used in chapter 3 (p 54-56).

Task 1: local landmarks predict reward location

Fish were trained in the same way as described in chapter 3 (p 56), except that for each trial, the maze and the start box and the position of the goal (i.e. in the left or in the right-hand arm), were randomly selected with the constraint that no same selection could be made more than twice in a row. The two plastic plant landmarks were always placed in the rewarded arm, one directly next to the hole leading to the food reward, and the second in the correct arm just in front of the removable barrier. The landmarks provided the only reliable cues as to the location of the reward (food and shoal). On reaching a criterion performance (at least 9 correct trials out of 10), the same fish were trained to learn a second task.

Task 2: turn direction predicts reward location

The training procedure was the same as that described above except that now turn direction was the only reliable cue predicting the position of the reward. Landmarks were still presented in one arm of the T-maze but their position was now randomised with respect to the goal. Landmark position was randomised with the constraint that landmarks did not agree with turn direction for more than two trials in a row. For each population of pond and river fish, half the fish were trained to turn right and half the fish were trained to turn left. This was in order to control for any directional

bias the fish might have. Fish were trained to a criterion performance of at least 9 correct trials out of 10 or for a maximum of 35 trials.

iii) Statistical analysis

Where necessary, to ensure normality and homogeneity of variance the data were log or arcsine transformed. In order to assess the factors affecting performance in task 1, I carried out a three-factor (habitat type, population, block) ANOVA, with block as a repeated measure (1 block = 6 trials). In each model, population was nested within habitat type and fish was nested within habitat type and population.

4.2.2 Results

In all measures of performance, there were no significant differences between the counter-balanced right-trained and left-trained conditions (all p 's > 0.2). Therefore, these data were collapsed when calculating the group averages.

i) Task 1: local landmarks predict reward location

Figure 4.1 shows the performance of pond and river fish during the first 5 blocks (30 trials) of training. Three pond fish and one river fish failed to leave the start box in the first 6 trials and were dropped from the experiment, leaving a sample size of 36 fish (17 pond fish and 19 river fish). Both pond and river fish showed a poor ability to track the reward using landmarks, with only 36% (7 pond fish and 6 river fish) reaching criterion. Overall, fish did not significantly improve their performance across the first 5 blocks (30 trials) of training (ANOVA, block: $F_{4,129} = 1.48$, $p = 0.21$). Performance did not significantly differ between pond and river fish

4. Landmark stability: a prerequisite for landmark use?

(ANOVA, habitat type: $F_{1,8} = 0.15$, $p = 0.92$; habitat type x block: $F_{4,125} = 1.11$, $p = 0.36$). Nor was there an effect of population on performance (ANOVA, pop: $F_{8,26} = 1.61$, $p = 0.29$; pop x block: $F_{32,93} = 1.43$, $p = 0.09$). Restricting the analysis to fish that reached criterion, a one-way ANOVA with the number of trials taken to reach criterion as the dependent variable revealed no significant effect of habitat type on performance (habitat type: $F_{1,11} = 0.01$, $p = 0.91$).

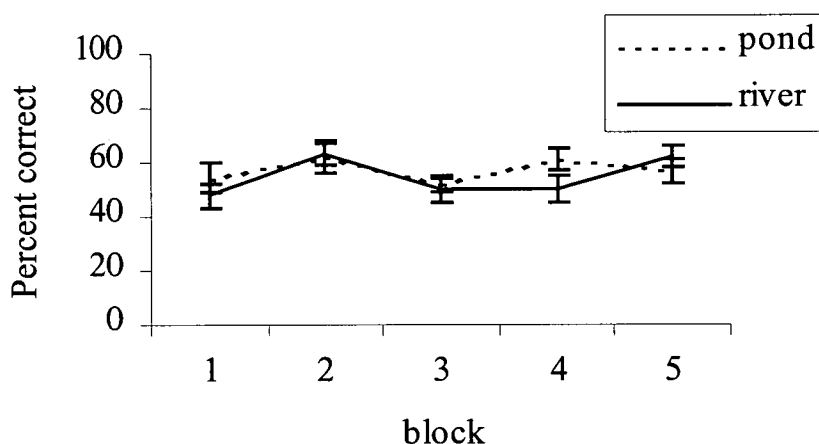


Figure 4.1. Mean \pm S.E. percentage of correct choices by pond and river fish during the first 5 blocks (30 trials) of training in task 1 (1 block = 6 trials).

ii) Task 2: turn direction predicts reward location

Eight of the thirteen fish (62%) trained to learn a turn direction in task 2, reached criterion within the maximum training period of 35 trials. A one-way ANOVA with the proportion of correct trials as the dependent variable showed there were no significant differences between pond and river fish in their performance ($F_{1,11} = 0.02$, $p = 0.9$). Restricting the analysis to fish that reached criterion only, with the number of trials taken to reach criterion as the dependent variable, did not change this result

(habitat type: $F_{1,6} = 0.18$, $p = 0.69$). With a sample of 8 fish, it was not possible to assess whether performance differed between different populations.

iii) Comparing task 1 with task 2

A comparison including only those fish that reached criterion both in task 1 and in task 2, revealed that fish took significantly longer to reach criterion in task 1 when trained to use landmarks, than in task 2 when trained to use turn (paired t-test: $t_7 = 3.79$, $p = 0.007$). The same pattern was observed when all 13 fish were included in the analysis with scores in task 2 being capped at 35 trials i.e. fish that failed to reach criterion in task 2 were given a maximum score of 35 trials (paired t-test: $t_{12} = 2.36$, $p = 0.04$; Pond: $\bar{X} \pm S.E = 32.86 \pm 1.74$ trials in task 1 and 13.25 ± 0.9 trials in task 2; River: $\bar{X} \pm S.E = 33.67 \pm 2.31$ trials in task 1 and 14.5 ± 1.18 trials in task 2, Fig. 4.2).

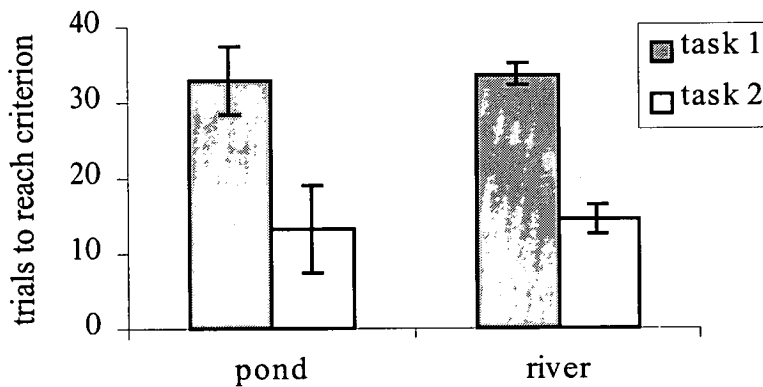


Figure 4.2. Mean \pm S.E. number of trials taken to reach criterion by pond and river fish in task 1 (landmarks only reliable cue) and in task 2 (turn only reliable cue) with scores in task 2 capped at 35 trials.

4.2.3 Discussion

The results of the experiment described in chapter 3 suggest that pond fish rely on local landmarks in locating a goal in a T-maze to a greater extent than river fish. It was argued that in pond habitats, local visual cues are more likely to provide reliable indicators of location than in unstable, fast-flowing rivers. However, in experiment 4a, when local landmarks are the only cue predicting a goal location and are unstable with respect to all other sources of spatial information, there appear to be no differences in the ability of pond and river fish to use the landmarks as beacons to track the rewarded end. Both pond and river fish show limited ability to learn this task, with fewer fish reaching criterion than was reported in chapter 3 and with those that reach criterion taking longer to do so.

There are at least three possible explanations for these findings: 1) The laboratory environment has a detrimental effect on learning ability; 2) the presence of two mutually reinforcing cues facilitates learning compared to when only one cue is available; 3) pond and river fish weight attention or importance to landmarks according to their perceived stability and so reliability. I will consider each of these possibilities in turn.

The fish used in this experiment had been held in the laboratory before testing for 3 months longer than those tested in chapter 3. Housing fish in a constant laboratory environment may have a detrimental effect on their ability to learn (Seymour *et al.*, 1996). However, when the fish that reached criterion in task 1, were trained to use turn direction in task 2, the same fish showed much faster rates of

learning, indicating that these fish are indeed capable of faster learning (but see below). In addition, rates of learning comparable to those reported in chapter 3 were observed in experiments in which sticklebacks have been housed in the laboratory for up to 6 months prior to testing (Odling-Smee, unpublished data).

Several lines of evidence suggest that animals may use cues additively. The presence of two cues often improves performance compared to when the animal is trained with either cue in isolation (see introduction). The poor learning ability displayed in task 1 compared to that observed in chapter 3 may be explained by the fact that only one spatial cue was available in task 1 (local landmarks). In the experiment described in chapter 3, two spatial cues (local landmarks and turn direction) were simultaneously available. However, fish trained to use turn direction as the only available cue in task 2 of this experiment showed rates of learning comparable to those displayed when two cues are available. Caution should be taken when comparing performance in task 1 with that in task 2, as these are not independent measures. Prior training in task 1 may facilitate learning in the second task, by providing additional experience of the maze etc. It is worth noting however, that fish trained from the outset to use turn direction as the only predictor of goal location in chapter 2 were able to successfully learn the task at a comparable rate to that shown here in task 2.

Taken together, the results suggest that pond and river threespine sticklebacks can be successfully trained to orientate to a goal using turn direction. Training sticklebacks to track landmarks that are unstable with respect to all other cues

appears to be considerably more difficult. This is consistent with the suggestion that some property specific to local landmarks reduces the likelihood of them being used as spatial cues when they are unstable with respect to all other sources of information.

4.3 EXPERIMENT 4B

Taking the results reported in chapter 3 together with the findings of experiment 4a, it appears that pond sticklebacks may pay attention to local landmarks as positional cues, but only if they agree with at least one other source of spatial information. In experiment 4b, a single population of pond fish was trained to locate a goal in a T-maze using three possible spatial cues. The aim here was to ask how fish that appear to use turn and landmark information (chapter 3) weight attention to local landmarks when trained with three types of spatial information reliably indicating the goal location. Only one population of pond fish was used in order to simplify the experimental design and allow the performance of fish exposed to different types of probe trial in a different sequence to be compared. Fish could locate the goal by learning a turn direction out of the start box, by tracking plant landmarks or by paying attention to global place cues external to the maze. All three types of spatial information reliably indicated the position of the goal. Therefore in this case, local landmarks are in a fixed, stable position throughout training. On reaching a criterion performance, fish were exposed to different types of probe trials in which one spatial cue was placed in conflict with the other two. I predicted that fish should weight as much attention to landmarks as to the other sources of spatial information, since landmarks have remained geometrically stable throughout training. I also predicted

that reliance on landmarks would vary between the different types of probe trial, with less weighting given to landmarks that are placed in conflict with all other spatial cues compared to when they are reinforced by a second source of spatial information.

4.3.1 Methods

i) Subjects

Threespine sticklebacks were collected from a pond in Beecraig National Park in June 2001, using standard minnow traps. Fish were given a settling period in the laboratory of 2 months in conditions designed to accelerate their transition out of breeding condition. This was to ensure that changes in behaviour associated with reproduction would not interfere with performance in the T-maze. The temperature was maintained at $10 \pm 1^\circ\text{C}$ and overhead lighting was provided by 40-W fluorescent tubes, operating on a 10:14 hour light:dark cycle. By the end of the settling period males had lost their breeding colouration and were not displaying behaviours associated with reproduction (territory defense, chasing or nest building). Similarly, gravid females had reabsorbed their eggs and were not displaying interest in male conspecifics through head up postures or following (Wootton, 1984). Fish were maintained in groups of 4, in holding aquaria of size 46 x 30 cm and 30 cm high. Each aquarium was divided into two equal sections by a transparent Perspex partition measuring 30 cm x 26 cm. Two fish that could be distinguished from size differences or body markings were housed in each section. Separating fish into pairs was found to be a preferable method for identification to tagging, since tags tended to drop off after about 4 weeks of training in the T-maze. Both sides of each tank were furnished

with a plastic plant and terracotta refuge. All fish were maintained on a diet of defrosted frozen bloodworm.

ii) Apparatus

The apparatus used in this experiment was the same as that described in chapter 3 (p 54).

iii) Procedure

Pre-training

Thirty-two fish were subjected to pre-training, 30 of which were selected for further training. Two “spare” fish were included to keep the numbers of fish in the maze the same for each pre-training session. The procedure used for pre-training was the same as that described in chapter 3 (p 56) except that 1) fish were pre-trained in groups of eight, 2) each fish was randomly assigned to a maze which remained fixed throughout the experiment, and 3) only the south start box was used (the start box nearest the accessible side of the pool). Fish were familiarised with the start box and the maze to which they would be assigned for the duration of training, but there was no opportunity to associate spatial information with the position of the goal, since food rewards were placed in both arms of the maze.

Training

Thirty fish were selected for training. The procedure for training was the same as that used in chapter 3 (p 56) except that each fish was trained in the maze to which it had been assigned for pre-training and was always released from the south start-box.

Plastic plant landmarks were positioned in the goal arm. Fish pre-trained in maze 1 were trained to turn right, and fish pre-trained in maze 2 were trained to turn left. This was to control for any directional bias the fish might have or for a possible preference for one side of the pool over the other. Throughout training, the position of the goal arm in the T-maze was reliably indicated by the turn direction out of the start box, the position of plant landmarks and the position of extra-maze global cues (differences in light levels, camera lens etc.). Between each trial, the gravel was disturbed and the PVC partitions and removable screens were swapped between the two mazes to minimise the possibility that fish would pay attention to intra-maze visual cues other than the plant landmarks. When the fish reached a criterion performance of at least 9 correct trials out of 10 they were subjected to a probe trial, after which additional post-criterion sessions were conducted during which further probe trials were interspersed. A minimum of 4 correct trials out of a run of 5 had to be achieved between any two probe trials. The final training trial before the first probe trial was recorded on VCR.

iv) Probe trials

Fish were given three types of probe trials (Fig 4.3). In type 1 probes, the plant landmarks were repositioned to the opposite side from where they had been during training. In these probes, the correct turn direction agreed with place information but local landmark information conflicted with both. In type 2 probes, fish were placed in the north start box (opposite the start box used in training) and the apparatus was manipulated such that local landmarks agreed with the correct turn direction but place information conflicted with both. In type 3 probes, fish were placed in the

4. Landmark stability: a prerequisite for landmark use?

north start box and the apparatus was manipulated such that place cues agreed with local landmarks but the correct turn direction conflicted with both. The 30 fish were randomly divided into three groups of 10 fish. In each group, five fish had been trained to turn right and five trained to turn left. Each group received the probe trials in a different order to control for the possibility that the order in which probe trials are received might affect the use of spatial cues. During probe trials, food and shoals were placed at both arms of the maze to avoid punishing the fish for selecting one or other of the available cues. All probe trials were recorded on VCR.

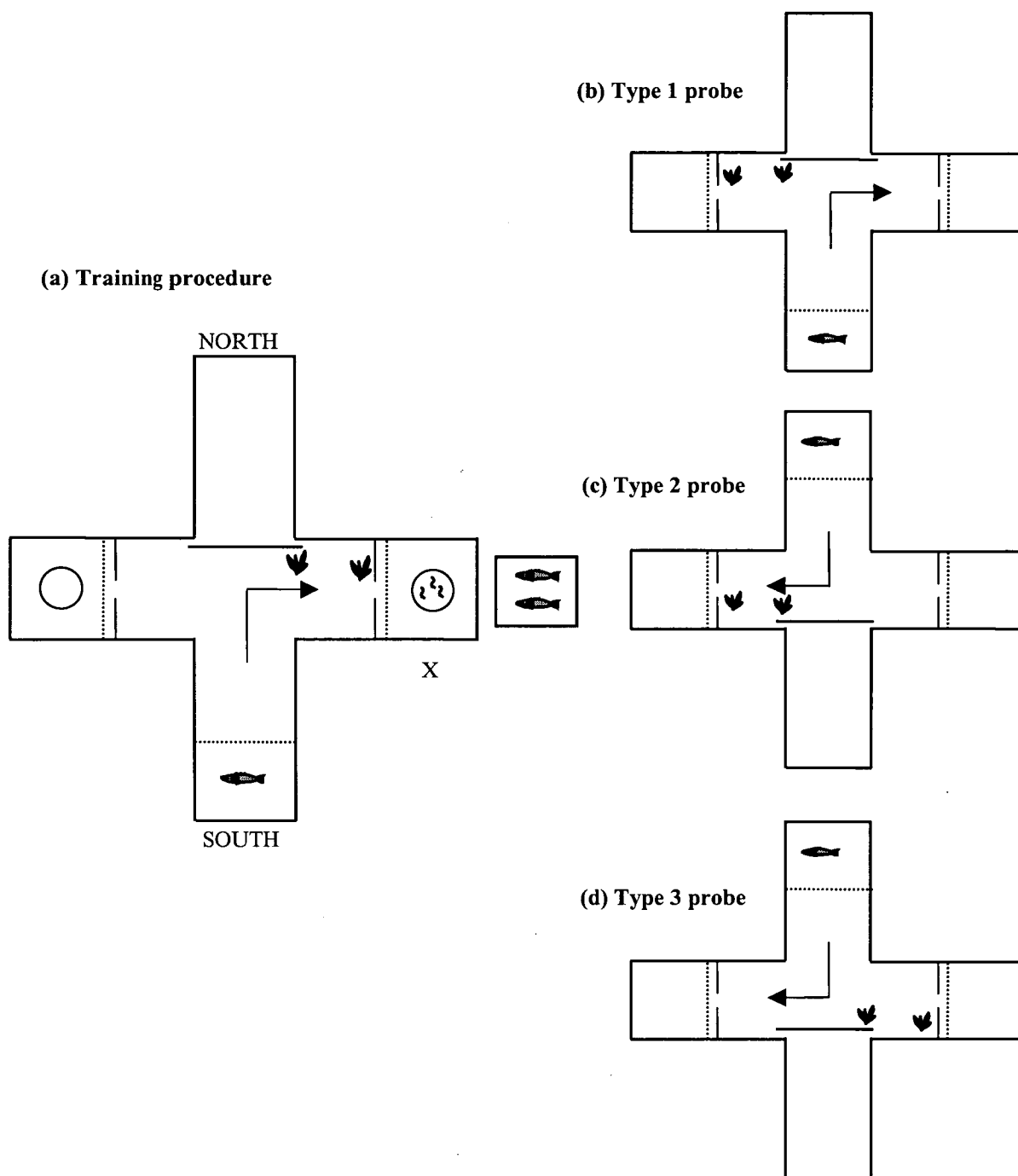


Figure 4.3. Diagrammatic representation of the training procedure and the three types of probe trial for right-trained fish. During training the goal is always in the same position (X) and fish are always released from the south start box (a). In type 1 probes (b), turn agrees with place information but landmarks conflict with both. In type 2 probes (c), landmarks agree with turn direction but place information conflicts with both. In type 3 probes (d), landmarks agree with place information turn direction conflicts with both.

v) VCR recordings

Pauses and reversals were scored from video recordings of the three probe trials and of the last training trial prior to the first probe test.

vi) Statistical analysis

Where necessary, to ensure normality and homogeneity of variance the data were log, arcsine or square root transformed. Parametric statistical tests were used throughout, except for the results of the probe trials which were analysed using the G-test. In order to assess the factors affecting performance in the T-maze, I calculated the proportion of correct trials across blocks of 5 trials. I then carried out a two factor (turn, block) ANOVA with block as a repeated measure. Fish was nested within turn direction.

There were no significant differences between the counter-balanced right-trained and left-trained conditions within groups in any phase of the experiment (all p 's > 0.2). Nor were there any significant differences in performance or in cue preference between groups of fish that received probe trials in a different order (all p 's > 0.2). Therefore, these data were collapsed when calculating the group averages.

4.3.2 Results

i) Acquisition and post-criterion performance

Figure 4.4 shows the performance of fish during the first 15 trials of training. All fish learnt to find the rewarded arm with performance significantly improving over the first 3 blocks (15 trials) of training (ANOVA, block: $F_{2,58} = 8.79$, $p < 0.001$). All 30 fish reached the criterion performance in 16 ± 5.9 trials, and maintained a high and

steady level of accuracy ($\bar{X} \pm \text{S.E} = 92.8 \pm 1.73$ % correct trials) throughout the post-criterion training during which probe trials were interspersed.

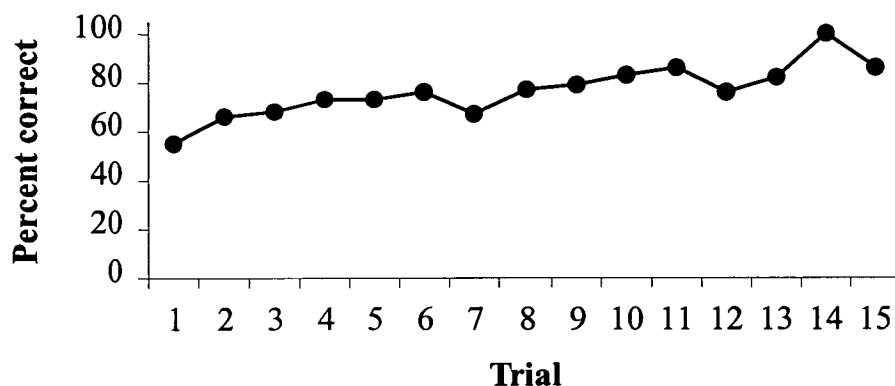


Figure 4.4. Percentage of fish performing the task correctly during the first 15 trials of training.

ii) Probe trials

Figure 4.5 shows the results of the probe trials. In all three types of probe trial, fish showed a significant preference for using turn direction, both in combination with a second cue and when it was in conflict with all other spatial cues. G-tests compared the distribution of fish selecting one or other of the arms in each type of probe to an expected distribution based on random choice. In all three types of probe trial, the distribution of choices differed significantly from expected (all p 's < 0.05, see table 4.1). This rules out the possibility that fish are simply confused by the probe trials and select one arm of the maze at random.

A more specific null hypothesis is that fish use all three types of spatial information, but have no preference for any particular cue. If fish use all three types

4. Landmark stability: a prerequisite for landmark use?

of spatial information independently, with no preference for any particular cue or combination of cues, on any one probe trial each fish would be expected to use one of the spatial cues with a probability of 33%. The probability that any fish will follow a route marked by two cues is 66%. The expected distribution of fish using 2 cues versus 1, is therefore in a 2:1 ratio. In all three types of probe trial, the distribution of choices differed significantly from expected (all p 's < 0.05, see table 4.2). In type 1 probes where landmark cues conflicted with place and turn, 83% of fish ignored the new position of the landmarks and selected the arm corresponding to place and turn information. In type 2 probes where place conflicted with landmark and turn direction, 90% of fish ignored the shifted place cues and selected the arm corresponding to landmark and turn direction. In type 3 probes where turn direction conflicted with place and landmark cues, 70% of fish ignored the shifted landmark and place cues and made the correct turn direction.

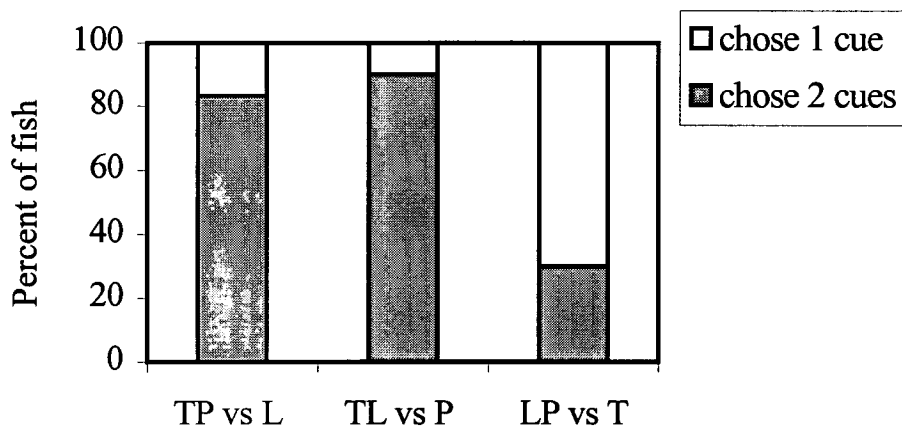


Figure 4.5. Percentage of fish selecting arm presenting two cues versus one for type 1 probe (landmark (L) conflicts with turn (T) and place (P) information), type 2 probe (place conflicts with turn and landmark information), and type 3 probe (turn conflicts with landmark and place information).

	PROBE - TYPE 1		PROBE - TYPE 2		PROBE - TYPE 3	
	Landmark	Turn + Place	Place	Turn + Landmark	Turn	Landmark + Place
O	5	25	3	27	21	9
E	15	15	15	15	15	15
G_{adj}	14.27		21.65		4.84	
Probability (d.f. = 1)	P < 0.001		P < 0.001		P < 0.05	

Table 4.1. Results of G-tests comparing the observed distributions of fish selecting each arm of the T-maze (O) with the expected distributions (E) based on random choice.

	PROBE - TYPE 1		PROBE - TYPE 2		PROBE - TYPE 3	
	Landmark	Turn + Place	Place	Turn + Landmark	Turn	Landmark + Place
O	5	25	3	27	21	9
E	10	20	10	20	10	20
G_{adj}	4.15		8.98		4.15	
Probability (d.f. = 1)	P < 0.05		P < 0.01		P < 0.05	

Table 4.2. Results of G-tests comparing the observed distributions of fish selecting each arm of the T-maze (O) with the expected distributions (E) based on no cue preference.

Because of the strong preference for using turn over the other types of spatial cue, it was not possible to assess whether landmarks presented in agreement with place or turn cues, present a more salient cue than landmarks placed in conflict with all other spatial information.

iii) Pauses and reversals

Figures 4.6a and b show the mean scores for “pause” and “reversal” behaviour at criterion (in the final training trial before probe tests began) and across the probe trials for the three groups of fish receiving probe trials in a different order. There was no significant effect of group or of probe type on any of the reversal scores (all p 's > 0.2). No group showed more reversal behaviour during the probe trials than they had done at criterion (all p 's > 0.5).

Fish from the three groups paused more often during the probe trials than they did at criterion, although this was only significant for group 3 (paired t-tests, group 1: $t_9 = -1.45$, $p = 0.18$; group 2: $t_9 = -1.49$, $p = 0.17$; group 3: $t_9 = -3.25$, $p = 0.01$). A one-way ANOVA revealed a significant effect of probe type on the number of pauses made in the first probe trial ($F_{2,27} = 5.14$, $p = 0.01$). Fish paused more often, when both landmark and turn cues were in conflict with place information (type 2 probe) than they did in any other type of probe trial. There was a significant effect of group on the mean number of pauses made across all three probes (ANOVA: $F_{2,27} = 3.4$, $p = 0.05$). Group 3, which received probe type 2 as the first probe trial, paused more often than any other group.

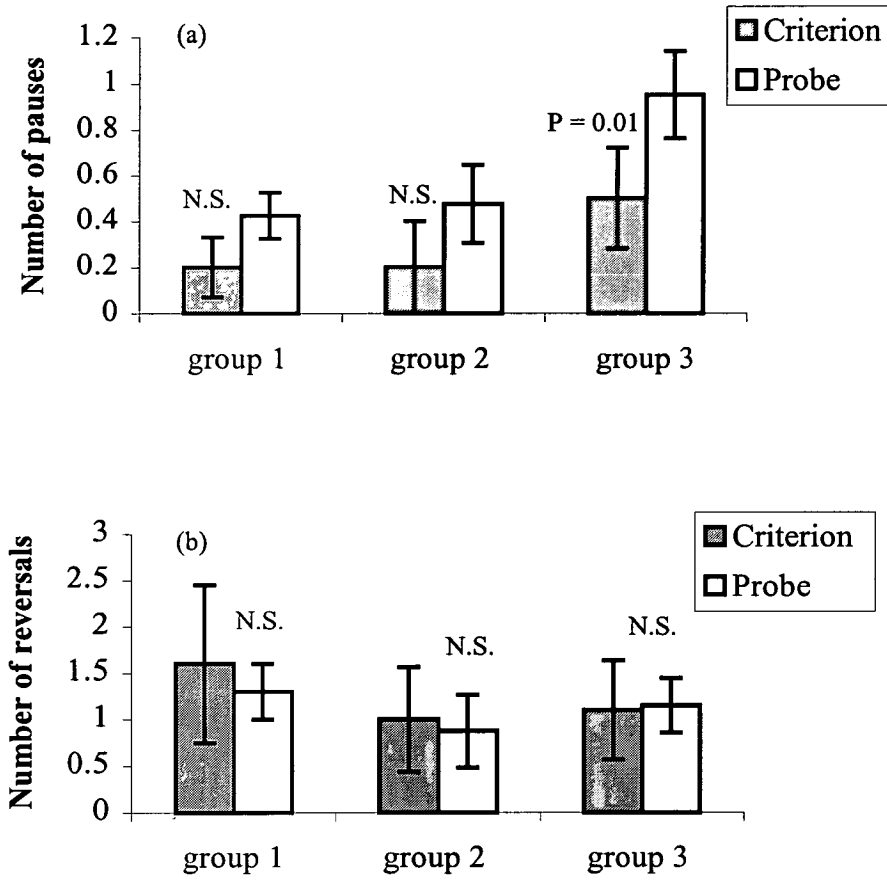


Figure 4.6. Mean S.E. number of pauses (a) and reversals (b) made at criterion and across probe trials for the three groups of fish receiving probe trials in a different order.

4.3.3 Discussion

The results of experiment 4b fail to provide support for the prediction that fish should weight the same attention to local landmarks as to the other types of spatial information, landmarks having been in a fixed, stable position throughout training. During the different types of probe trials, fish showed a preference for using turn direction over local landmarks and over global place cues. However, several

observations tentatively suggest that the presence of all three cues during training facilitated learning and improved performance compared to what would have been expected if turn direction was available alone. Although between experiment comparisons should be treated with caution, since different populations have been tested at different times of the year, the rate of learning displayed in this experiment was faster than that observed when fish are trained to use turn direction alone (see chapter 2), or when fish are trained to use turn direction simultaneously with local landmarks (see chapter 3). However, it remains a possibility that fish collected from Beecraig pond are simply faster learners than populations previously tested.

More compelling evidence for the use of cues in addition to turn direction, is provided by the fact that the fish paused more often during the first probe trial than they did at criterion, and significantly so for group 3. Although this could simply be a dishabituation effect (a reaction to novelty), if fish are paying attention to more than just turn direction they might be expected to be confused by the disruption of spatial relationships during the probe trials. Presenting landmark and turn cues in conflict with place information (probe type 2) appeared to cause more confusion than any other cue conflict situation. It is not clear why this type of probe trial should generate more confusion than any other. If fish have a preference for turn but are also paying attention to all three types of spatial cue, probe type 3 might be expected to generate most confusion, when turn is in conflict with all other cues. It is possible that group 3 simply consisted of fish that were more sensitive to a change in their spatial environment than the other two groups.

An explanation as to why fish weight less importance to place cues compared to turn direction might lie in the fact that the array of available visual cues external to the maze was limited, since the entire apparatus was surrounded by white sheets. As far as possible continuity was maintained between the experiment described in chapter 3 and the set-up used here, as a highly salient extra-maze visual cue might have blocked the use of local landmarks entirely (Diez-Chamizo *et al.*, 1985). Extra maze visual cues such as differences in light levels, the position of the camera lens etc. were available in the current set-up, but the presence of a more salient cue such as a coloured cue card, may have increased the weighting given to place cues. Evidence from studies on rats suggests the nature of the testing environment can affect the relative expression of place and response learning (reviewed in Restle, 1957).

What is less clear is why the fish in this experiment used turn direction in the probe trials significantly more often than they used the plant landmarks, even in probe trials in which plant landmark cues were placed in agreement with global place cues. A possible explanation is that making a body centered turn became an automated response with little attention being paid in later trials to the additional sources of spatial information that were available. Although mammals are likely to behave quite differently from fish, several studies have shown that rats switch from using local landmarks during early learning to making an automated turn response in later trials (Hicks, 1964; Packard & McGaugh, 1996). This could be tested here by exposing the fish to probe trials at an earlier stage in training. However, the fact that fish paused more often during the first probe trial than they did at criterion implies

that they were paying attention to the array of spatial cues, and were confused by the disruption of spatial relationships in the probe trials.

Because the fish learned to locate a goal within an environment that remained stable throughout the period of training, it is possible that re-locating the fish to the opposite side of the maze during the probe trials type 2 and 3, disorientated the fish to such an extent that visual local and global cues were no longer trusted as being reliable positional cues. As discussed in the introduction, the amount of discrepancy perceived between local landmarks and a second source of spatial information can affect the weighting given to landmarks (reviewed in Biegler & Morris, 1996b). For fish that have been trained in an environment that is stable throughout training, a 180° discrepancy between local landmarks and other cues during probe trials may be particularly striking. The fish in this experiment may have weighted equal importance to local landmark cues as to the other sources of spatial information throughout training, but confusion caused by re-positioning the landmarks in the probe trials may have caused a shift in cue use. On perceiving landmarks as unstable, fish may have switched to using reliable body-centered turn information. This raises the problem that probe trials may not accurately reveal which types of cue the fish are using during task acquisition.

A further possible explanation for the preference for turn direction shown by Beecraig fish is that the pond fish from this particular site show reduced reliance on local landmarks compared to other populations of pond fish because of differences in local habitat conditions. There is no reason to expect this since Beecraig pond is a

stable, clear-water habitat in which landmarks should be both clearly visible and stable.

The bias in cue preference towards turn direction meant that it was not possible to assess whether fish preferentially use landmark cues when they are in combination with additional spatial information, over landmark cues that are in conflict with all other spatial cues. This would have been supported if the number of fish using place and landmark cues in combination, or turn and landmark cues in combination, exceeded the sum total of fish using each component cue. Additional experiments are needed to identify whether two cues in agreement represent a more salient directional cue to sticklebacks compared to one cue when it is in conflict with all other sources of spatial information.

4.4 GENERAL DISCUSSION

As has been shown for other species, the weight given to local landmarks by threespine sticklebacks may be dependent on landmark stability. A common feature that applies to habitats occupied by groups as diverse as mammals, birds, insects and fish is that landmarks that move are unlikely to be reliable indicators of location and should be ignored. Although the experiment described in chapter 3 suggested that pond sticklebacks pay more attention to landmark information than river fish, this may be conditional on landmarks being perceived as geometrically stable with respect to at least one other source of spatial information. River fish inhabiting unstable habitats may pay a heavy cost in learning orientation routes if they use unreliable moving landmarks as positional cues. Equally for pond fish, there will be

features of the local environment that are less stable than others. Pond fish may benefit from using landmarks in orientation only if they are equipped with an additional level of plasticity allowing them to distinguish and respond appropriately to stable and therefore reliable landmarks and unstable, unreliable ones.

Although no studies as yet have directly addressed the role of landmark stability in spatial learning in fish, it is worth considering the results of these experiments in the context of others that have investigated the use of landmarks by fish. A number of laboratory tests suggest that fish can use local landmarks as orientation cues. However, in many of these experiments, landmarks are geometrically stable with respect to extra-maze spatial cues or body centered turn information during task acquisition (Huntingford & Wright, 1989; Warburton, 1990; Salas *et al.*, 1996b; Girvan & Braithwaite, 1998; López *et al.*, 1999, 2000a). Interestingly, in contrast to Warburton's (1990) study in which goldfish were trained to use landmarks as spatially fixed beacons, Douglas (1996) found that goldfish trained to track moving landmarks randomly re-positioned between each trial, found the task "extremely difficult" with the 3 out of 9 fish that learnt the task taking 175 trials to do so. In addition Mazeroll and Montgomery (1998) found the reliance on particular landmarks during migrations by brown surgeonfish was reduced when landmarks were moved more than 6 m from their original location.

In other studies, fish have been shown to successfully track landmarks that are unstable with respect to all other sources of spatial information. In Braithwaite *et al.*'s (1996) study on juvenile Atlantic salmon, landmarks and associated food

rewards were randomly positioned on different sides of the test tank, and the salmon were able to track the moving reward. López *et al.* (2000b) successfully trained goldfish to use coloured panels to locate an exit door from a diamond-shaped enclosure. Fish were released from different start boxes, and the enclosure and the cues were rotated such that the panels were the only relevant cues available. Similarly Hughes and Blight (2000) trained fifteen-spined sticklebacks and corkwring wrasse, to track a food reward that was randomly positioned in one arm of a starburst maze. The only cue indicating the correct arm was a red tile. Therefore the conclusion that landmarks should not move if they are to be used as an effective point of reference for navigating to a goal is by no means supported by all the data on landmark learning in fish. However, in none of these studies has a direct comparison been made that investigates the relative importance that fish attach to stable as opposed to unstable landmarks.

The second experiment in this chapter (4b) highlights a serious problem in identifying the extent to which fish rely on landmarks by inserting spatially disruptive probe trials in between training trials. If landmarks remain stable with respect to other sources of spatial information throughout training, they may well be perceived as reliable and therefore used in task acquisition. However probe trials designed to reveal cue preferences, in which the position of landmarks is often switched (Huntingford & Wright, 1989; Girvan & Braithwaite, 1998; López *et al.*, 2000a), may cause a corresponding shift in cue use. If landmarks are now perceived as being unstable because of the disruption of spatial relationships, fish may resort to using an alternative source of spatial information such as place cues or a body

centered turn. Furthermore, landmarks perceived as unreliable during spatially disruptive probe trials, may be ignored throughout subsequent training. The results of probe trials may therefore not always accurately reveal the types of cues used during task acquisition.

An alternative approach is to compare the rates of learning when animals are trained to use stable landmarks versus unstable landmarks. However, this is confounded by the fact that the addition of a reference spatial cue e.g. global place cues by which landmark stability is measured, may itself facilitate learning over and above the stability of the local landmarks per se. Several studies have attempted to counter this problem by carrying out extinction trials, in which the second source of spatial information is made unreliable in the hope that it will lose importance as a predictor of spatial location (Biegler & Morris, 1996b). However it is difficult to rule out the possibility that learning is facilitated by the presence of additional spatial information as opposed to increased landmark stability (Roberts & Pearce, 1998). A more effective way of investigating the role of landmark stability in spatial learning may be to carry out a titration procedure whereby the discrepancy between landmarks and other sources of spatial information is steadily increased. The extent of discrepancy needed before landmarks are ignored may provide a measure of the relative importance animals attach to landmark stability (e.g. Cheng, 1989; Etienne *et al.*, 1990).

In conclusion, the extent to which landmark stability is a prerequisite for their use in spatial learning in fish is unclear and more studies need to be undertaken in order to address to what extent this might be true. It may be that species and

populations vary quite considerably in the degree of importance they place on landmark stability, depending on the nature of their habitats. However, across a range of diverse habitats, being able to respond flexibly to cues that vary in reliability is likely to play an important role in reducing the probability and so the costs associated with learning the wrong thing.

Chapter 5

Do local habitat conditions influence spatial learning by sympatric species of threespine stickleback?

5.1 INTRODUCTION

5.1.1 Habitat ecology, information use, and learning ability

As discussed in chapter 3, animals often appear equipped with mechanisms that guide and direct learning and associated processes in response to specific ecological problems (Garcia & Koelling, 1966; Goldsmith *et al.*, 1981; Cole *et al.*, 1982).

Predispositions that direct attention to certain cues or associations in preference to others may filter out uninformative or unreliable sources of information such that the risk of making mistakes, wasting time, and processing redundant information is minimised (Brodbeck, 1994; Clayton & Krebs, 1994b; Odling-Smee & Braithwaite, in press).

Since a capacity to learn is likely to incur costs, such as the energetic costs of producing, maintaining and operating neural machinery, investment in the capacity to learn might similarly be predicted to match the ecological demand for this ability. Some support for this prediction comes from studies comparing animals' investment in the neural substrate thought to be required for spatial learning and, or their performance in laboratory based spatial tasks. As discussed in chapter 1, several studies suggest the hippocampus is the part of the brain required for spatial learning and memory in birds and mammals (Olton & Papas, 1979; Morris *et al.*, 1982;

Sherry *et al.*, 1989; Bingman, 1992; Biegler *et al.*, 2001). Spurred by these findings, numerous studies have investigated the relationship between hippocampal volume and the expected demand for spatial learning based on an examination of life histories (e.g. Krebs *et al.*, 1989; Healy & Krebs, 1993; Healy *et al.*, 1994). Birds and mammals that cache food for future consumption, negotiate a complex nesting environment, or have experienced artificial selection for spatial memory, have larger hippocampi and, or more hippocampal neurons and qualitatively different neurons than closely related species that do not exhibit such spatially demanding behaviour (Rehkämper *et al.*, 1988; Krebs *et al.*, 1989; Sherry & Vaccarino, 1989; Healy & Krebs, 1992; Jacobs, 1992; Basil *et al.*, 1996; Reboreda *et al.*, 1996; Abbott *et al.*, 1999). Sexual dimorphisms in hippocampus size have similarly been proposed to correlate with sexual dimorphisms in the need to process spatial information (Jacobs *et al.*, 1990; Sherry *et al.*, 1993; Reboreda *et al.*, 1996). A serious problem with these claims however, is the possibility that neuroanatomical differences are not directly linked to differences in spatial learning ability (Bolhuis & Macphail, 2001; Macphail & Bolhuis, 2001). For example, hippocampal volume is a very global measure and is likely to affect a great many factors aside from spatial learning ability (Kamil, 1998).

A different approach has been to compare the performance of animals in a range of laboratory based spatial tasks. For example, food-storing birds frequently outperform non-storers or less specialist storers at memory and spatial tasks (Balda & Kamil, 1989; Krebs *et al.*, 1990; reviewed in Krebs *et al.*, 1996). Sex differences in spatial ability in mazes favouring males have also been well documented across species (Gaulin & FitzGerald, 1989; Jacobs *et al.*, 1990; Gaulin, 1992; Bucci *et al.*,

1995; Gaulin, 1995). These sex differences have been proposed to reflect the different spatial demands presented by sexually dimorphic ranging patterns (Gaulin & FitzGerald, 1989).

As discussed in Chapter 1 (section 1.4.2), where only two or a few species are compared (e.g. Balda & Kamil, 1989; Gaulin *et al.*, 1990; Krebs *et al.*, 1990), it is difficult to rule out with any certainty, confounding factors that may affect performance in a spatial task. Moreover, comparative studies of spatial learning abilities often generate plausible hypotheses that warrant further testing. For example, Gaulin and FitzGerald's (1989) study is one of the few studies of mammalian sex differences that attempts to quantify the different spatial demands experienced by male and female voles by using radiotelemetry tracking methods to assess sexually dimorphic ranging patterns. However, a wealth of studies have revealed a positive relationship between performance in spatial tasks or neuroanatomical measures, and the demand for spatial learning predicted from an analysis of life histories. These provide preliminary support for the argument that investment in the capacity for spatial learning should mirror ecological demand.

5.1.2 A comparison of benthic and limnetic species

Several lakes in the Strait of Georgia region of south-western British Columbia, Canada, are inhabited by a young species pair of threespine sticklebacks (Schluter & McPhail, 1992; McPhail, 1993, 1994). The two species have morphological differences associated with distinct habitat preferences and diets. One species, referred to as “benthic” is deep bodied, with a few short gill rakers, wide mouth and

short broad snout. Benthics live predominantly in the spatially structured, vegetated littoral zone of the lakes, where they feed mainly on littoral invertebrates in sediments or attached to vegetation. The other species known as “limnetic” is morphologically and behaviourally better adapted for pelagic prey with a slender body, a narrower mouth, and longer, more numerous gill rakers (Schluter, 1996, Fig 5.1). Limnetics live in a comparatively homogenous environment in terms of spatial complexity, in the open water column where they feed mainly on plankton. As well as being divergent in feeding morphology, benthics and limnetics have diverged in armour characteristics, which is likely to reflect their divergent predation pressures (Hatfield, 1997; Vamosi & Schluter, 2002). Benthics display reduced pelvic and dorsal spines and fewer lateral plates compared to limnetics (McPhail, 1994). It has been proposed that limnetics have higher encounter rates with predators such as piscivorous birds or the cutthroat trout, while benthics may be more susceptible to insect predators (Vamosi & Schluter, 2002).

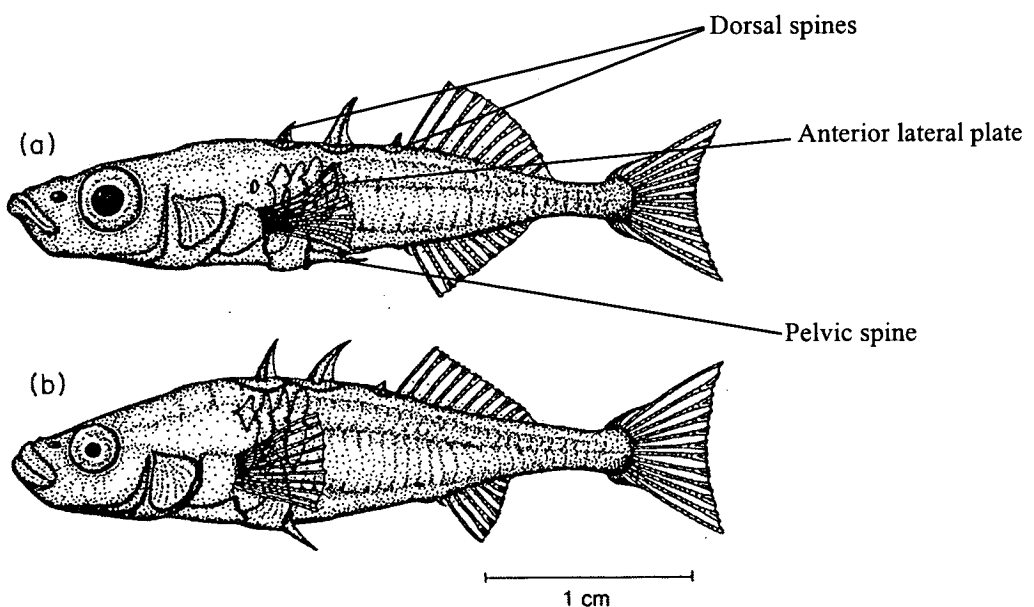


Figure 5.1. The Enos Lake species pair: (a) limnetic and (b) benthic species, showing some of the morphological features characteristic of the two species (taken from Hart & Gill, 1994).

The postglacial history of this region of British Columbia indicates that these species have coexisted for no more than 12,000 years (McPhail, 1994). In fact, divergence of benthics and limnetics is so recent that little genetic incompatibility has built up and post-mating isolation is primarily ecological. Assortative mating and divergent nesting sites of parental males appear to contribute to the low incidence of hybrids in the wild (McPhail, 1994; Hatfield & Schluter, 1996; Vamosi & Schluter, 1999). Two geographical models of species origins are consistent with the present distribution of species pairs; double invasion, whereby lakes were colonised by a marine ancestor at two distinct times or from two different sources, and sympatric speciation within lakes (Schluter & McPhail, 1992; McPhail, 1993). The present morphological and habitat differences appear to result from competition-induced character displacement (Schluter & McPhail, 1992; McPhail, 1994; Schluter, 1996) with the two habitats presenting distinct selective environments. Transplant experiments have shown benthics and limnetics to grow at about twice the rate of the other in their preferred habitat (Schluter, 1993, 1995) and hybrids do significantly worse in either of the two main habitats compared to the parental species (Schluter, 1995; Hatfield & Schluter, 1999).

Although much is known about the divergent foraging and mate choice behaviour shown by these species pairs (Schluter & McPhail, 1992; Schluter, 1993, 1995, 1996; Day *et al.*, 1994; Day & McPhail, 1996; Vamosi & Schluter, 1999; Rundle *et al.*, 2000), until the present study nothing has been known about their spatial learning capacities. The aim of the experiments described in this chapter was to investigate whether different local habitat conditions, even within the same lakes,

generate differences in spatial learning by sympatric species of threespine stickleback. The benthic-limnetic species pairs present an ideal system on which to base a comparative study of learning. First, the evolutionary history of the species pairs is relatively well known. Second, multiple speciation events allow replicate species pairs to be compared, thereby facilitating the elimination of confounding factors that could affect performance in a learning task.

5.1.3 Predictions

There are two ways in which spatial learning might be expected to differ between benthics and limnetics based on an examination of their divergent microhabitats. First, benthics occupying the vegetated littoral zone of lakes are likely to encounter a greater abundance of visual features and potential landmarks compared to limnetics living in the open water column. Although data on fish is lacking, several studies suggest that for a number of bird species, the availability of orientation information during development affects the weighting given to cues later in life. For example, Wiltschko and Wiltschko (1989) reported that homing pigeons raised on a rooftop where wind flow was unobstructed relied on odors to determine the homeward direction from unfamiliar release sites. Pigeons housed in a sheltered garden relied on alternative cues. Similarly, pigeons reared with a full view of their surroundings are more dependent on visual cues compared to siblings reared in a loft with opaque windows (Braithwaite & Guilford, 1995). If benthics experience a greater availability of visual landmarks within the vegetated littoral zone, they may be expected to show a greater propensity to use local landmarks when learning a spatial task compared to limnetics.

Second, the fact that benthics occupy a more complex, spatially structured environment, may result in them being better able to learn a spatial task compared to limnetics. There is some evidence suggesting that the level of “environmental complexity” experienced during development can affect subsequent spatial learning abilities in mammals. For example, rats reared in a complex environment (with toys and conspecifics) display superior performance in learning a variety of mazes compared to rats raised alone in standard laboratory cages (Juraska et al, 1984; Seymoure *et al.*, 1996 and refs therein). Relatively little is known about how an ecological need for spatial learning may affect non-mammalian, non-avian species (Day *et al.*, 1999). However, brain measurements tentatively suggest a relationship may exist between spatial learning capacity and environmental spatial complexity in fish. Van Staaden *et al.* (1994) and Huber *et al.* (1997) examined the brains of 189 species of cichlids from the East African Lakes and Madagascar. Species living in complex habitats created by shallow rock and vegetation, as opposed to the open waters of the pelagic, feature comparatively large telencephalons, the part of the brain thought to be required for learning spatial relationships (Salas *et al.*, 1996a, b; López *et al.*, 2000a, b; Vargas *et al.*, 2000; Rodríguez *et al.*, 2002). These findings should be interpreted with caution, since the telencephalon is likely to govern many other cognitive abilities aside from spatial learning. So far these observations lack support from behavioural studies directly comparing spatial learning ability.

In order to test these predictions, benthics and limnetic species pairs were collected from two lakes in British Columbia where they are known to have derived independently of one another; Priest and Paxton Lakes (Taylor & McPhail, 2000). In

the same way as described in chapter 3, fish were trained to locate a goal arm in the T-maze either by using an algorithmic behaviour (turn left or right) or by tracking the position of plant landmarks. Probe trials in which the turn and landmark cues conflicted, investigated the types of spatial information used and assessed whether differences exist between benthics and limnetics in their propensity to use the two types of spatial information. In addition, a second experiment was designed to compare the motivation of benthics and limnetics to access the rewards used to train the fish in the T-maze. Both these experiments were conducted in Dolph Schluter's laboratories at the University of British Columbia, Vancouver.

5.2 EXPERIMENT 5A – SPATIAL TASK

5.2.1 Methods

i) Subjects

Benthic and limnetic threespine sticklebacks were collected from two lakes in separate drainages on Texada Island, British Columbia, Canada: Paxton Lake ($49^{\circ}43'N$, $124^{\circ}30'W$), and Priest Lake ($49^{\circ}45'N$, $124^{\circ}34'W$, Fig 5.2). Both species were collected from each lake. All fish were collected in October 2001 outside their reproductive season, using dip nets and standard minnow traps.

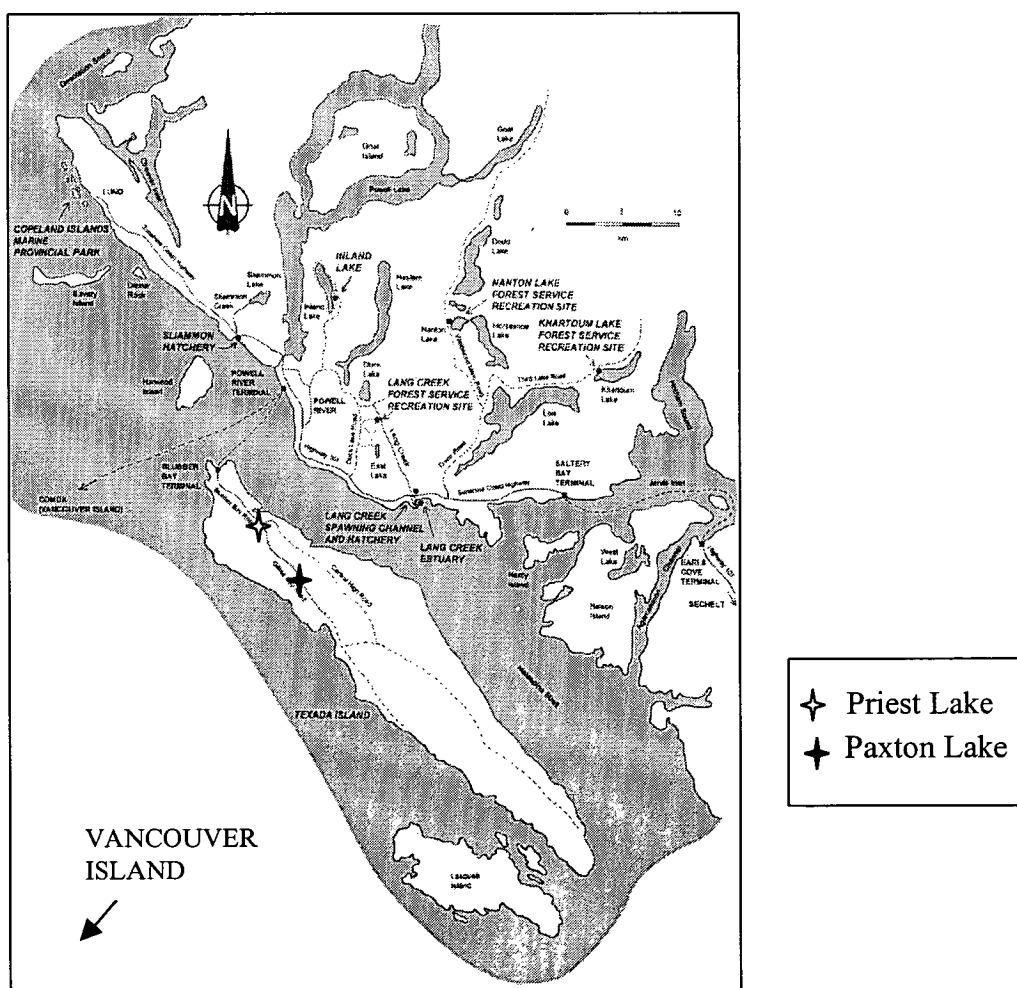


Figure 5.2. The central Strait of Georgia region showing the location of Priest Lake and Paxton Lake on Texada Island.

Forty fish were used for the experiment, 10 benthics and 10 limnetics from each lake. All fish were given a settling period in the laboratory of 3 weeks maintained on a diet of defrosted frozen bloodworm and newly hatched brine shrimp. Fish were maintained in groups of 20-30 at $16^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and on a 12:12 hour light:dark schedule in holding aquaria of size 75 x 30 cm and 45 cm high. A temperature of 12°C matching the conditions of experiments described in chapters 2-4 could not be provided since cooling fans were not installed in the laboratory. In the third week of the settling period, fish were transferred in groups of 10 to four holding aquaria. Each aquarium was divided into four equal sections by three transparent Perspex partitions measuring 29.5 x 40 cm. Two or three fish that could be distinguished from size differences or body markings were housed in each section.

ii) Apparatus

The apparatus used in this experiment was the same as that described in chapter 3 (p 54) except that for each fish tested, the shoal fish consisted of con-specifics selected from the same lake of origin. In addition, in this experiment a RCA PRO843 8 mm Camcorder was used to monitor the movements of the fish.

iii) Procedure

Pre-training

Pre-training followed the same procedure as that described in chapter 3 (p 56) with several modifications. Fish were pre-trained in groups of ten but because of time constraints, each group was given five 4 hour periods in the maze over a period of 5 days. During pre-training, fish showed exploration behaviour in the maze

predominantly during the first two hours after release from the start box. Therefore, reducing the time spent in the maze in each session from 24 hours to 4 hours was unlikely to have a significant impact on the overall experience acquired during pre-training. Ten fish (10 benthics or 10 limnetics from each lake) were transferred in a clear plastic cup from their holding tanks to a randomly selected start box. Paxton or Priest fish were pre-trained in the morning or in the afternoon at random. Since sticklebacks are more active at warmer temperatures and require a greater intake of food, all fish were fed a mixture of chopped bloodworm and newly hatched brine shrimp in their home tanks in the two days between pre-training and training.

Training

Fish were trained using the same procedure as that described in chapter 3 (p 56) except that all fish were fed a mixture of chopped bloodworms and newly hatched brine shrimp in their home tanks on the days that they did not receive training. This was partly because of the warmer laboratory temperature but also because the limnetics from both lakes were found to take the bloodworm food reward in only 50% of the trials in which the correct arm of the T-maze was entered (see discussion, section 5.2.3).

iv) Probe trials and VCR recordings

After acquiring the criterion performance of at least 9 correct trials out of 10, benthics and limnetics were exposed to three probe trials during which the plant landmarks were repositioned to the opposite side from where they had been during training. The procedure for conducting probe trials is described in chapter 3 (p 57).

Pauses and reversals were scored from video recordings of the three probe trials and of the last training trial prior to the first probe test, as described in chapter 3 (p 57). During video analysis, fish were identified on the monitor from codes, which gave no information as to their species or lake of origin.

At the end of the experiment, all fish were sacrificed with the anaesthetic MS222 and weight and length measurements were taken.

v) Statistical analysis

Where necessary, to ensure normality and homogeneity of variance the data were log, arcsine or square root transformed, or non-parametric statistics were employed.

In order to assess the factors affecting performance, I carried out a four-factor (site, species, turn direction, block) ANOVA with block as a repeated measure (1 block = 6 trials). In each model, fish was nested within turn direction, site, and species. To assess whether benthics and limnetics differ in the types of spatial information they use during the probe trials, I use the “likelihood approach” (Edwards, 1972).

Applying standard frequency tests such as the G-test or the chi-square test is precluded by the low expected values generated from the small sample sizes. I use the binomial distribution to quantify the use of the two cues (landmarks or turn) in the probe trials. Specifically, this describes the cumulative probability that a fish will use turn x times in n probe trials, given its probability t of using turn in a single probe trial. Normally, one would be interested in the probability of observing x turns given t (denoted $\Pr[x | t]$). Here I infer t from observed values of x . The probability of t

given x is known as the likelihood (denoted $L[t | x]$). The value of t that gives the data with the highest likelihood (L) is the best fitting value of t and is known as the maximum likelihood estimate (MLE). The relative plausibility of two alternative hypotheses (two values of t) is given by the ratio between their likelihoods, or more conveniently, by the difference between the natural logarithms of their likelihoods (denoted here by $\Delta\text{Log}L$). For large samples, the difference in log likelihood between two hypotheses that differ by ν degrees of freedom ($\Delta\text{Log}L_\nu$) is approximately distributed as $(1/2)\chi^2_\nu$ (Edward 1972). I use this test here but acknowledge that it is at best a rough approximation for the small sample sizes.

5.2.2 Results

There were no significant differences between the counterbalanced right-trained and left-trained conditions within groups in any phase of the experiment (all p 's > 0.2), therefore these data were collapsed when calculating the group averages.

i) Length and weight measurements

Table 5.1 shows the lengths and weights of benthic and limnetic fish collected from the two lakes.

Population	Length: $\bar{X} \pm \text{S.E. (cm)}$	Weight: $\bar{X} \pm \text{S.E. (g)}$
Paxton Benthic	42.7 ± 0.5	1.04 ± 0.03
Priest Benthic	46.6 ± 0.5	1.36 ± 0.04
Paxton Limnetic	36.2 ± 0.4	0.52 ± 0.02
Priest Limnetic	37.1 ± 0.5	0.48 ± 0.01

Table 5.1. Mean \pm S.E. lengths and weights of benthic and limnetic fish from Paxton and Priest lakes.

ii) Acquisition

Four limnetics died (2 from each lake) leaving a sample size of 36 fish (20 benthics and 16 limnetics). Figure 5.3 shows the percentage of benthics and limnetics from the two lakes performing the task correctly across the first 4 blocks (24 trials of training). Benthics and limnetics from both lakes learnt to find the rewarded arm with performance significantly improving over the first 4 blocks of training (ANOVA, block: $F_{3,105} = 25.34$, $p < 0.001$). Overall, the performance of benthics was significantly better than that of limnetics (ANOVA, species: $F_{1,32} = 14.97$, $p = 0.001$). There was no significant effect of species on the change in performance over the first four blocks of training (species x block: $F_{3,99} = 1.62$, $p = 0.19$). Nor was there a significant effect of lake on performance (ANOVA, lake: $F_{1,32} = 0.24$, $p = 0.62$; lake x block: $F_{3,102} = 1.82$, $p = 0.15$).

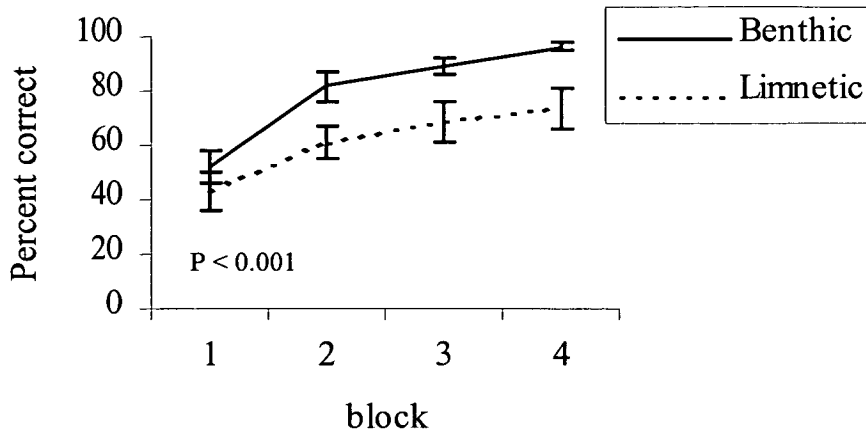


Figure 5.3. Mean \pm S.E. percentage of correct choices during the first 4 blocks (24 trials) of training (1 block = 6 trials).

All benthics and limnetics ($n = 36$) achieved the criterion performance of at least 9 correct trials out of 10. A two-way ANOVA with the number of trials taken to reach criterion as the dependent variable revealed the same pattern as described above.

Again, there was no significant effect of lake on performance (ANOVA, lake: $F_{1,33} = 0.41$, $p = 0.53$) but benthics took significantly fewer trials to reach criterion than limnetics (ANOVA, species: $F_{1,34} = 14.84$, $p < 0.001$; benthics: $\bar{X} \pm \text{S.E.} = 16.2 \pm 1.1$ trials; limnetics: $\bar{X} \pm \text{S.E.} = 26.5 \pm 2.8$ trials, Fig. 5.4).

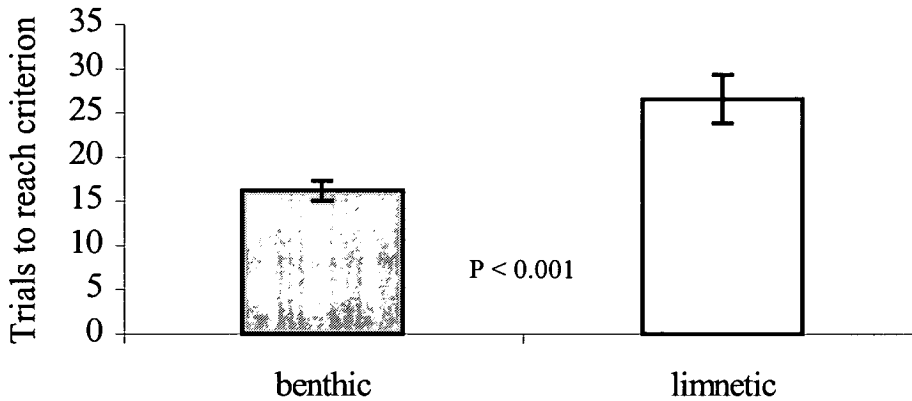


Figure 5.4. Mean \pm S.E. number of trials taken to reach criterion by benthics and limnetics.

iii) Propensity to feed on entering the correct arm

There were no significant differences between the two benthic populations or between the two limnetic populations in the number of times they fed, after entering the rewarded end of the T-maze (all p 's > 0.6). Therefore, these data were collapsed to compare feeding behaviour by the two species. Benthics and limnetics significantly differed in the number of times they took the bloodworm food reward after they had accessed the rewarded end of the maze (Mann-Whitney U-test: $U = -74$, $p < 0.01$, Table 5.2).

Population	Mean \pm S.E. percentage of trials fish fed after entering the correct arm of the T-maze
Paxton benthic	$\bar{X} \pm \text{S.E.} = 96.7 \pm 1.05 \%$
Priest benthic	$\bar{X} \pm \text{S.E.} = 96.1 \pm 3.02 \%$
Paxton limnetic	$\bar{X} \pm \text{S.E.} = 53.9 \pm 1.15 \%$
Priest limnetic	$\bar{X} \pm \text{S.E.} = 54.9 \pm 1.78 \%$

Table 5.2 Mean \pm S.E. percentage of trials in which benthics and limnetics fed, on entering the correct arm of the T-maze.

iv) Assessing “boldness”

Differences in how benthic and limnetic fish coped with the stress of the experimental procedure could have affected their ability to learn the spatial task. The time taken for fish to enter the arms of the maze in the first trial is likely to reflect boldness and propensity to explore a novel environment. This measure did not significantly differ between fish from the different lakes or between benthics and limnetics (ANOVA, lake: $F_{1,33} = 0.14$, $p = 0.71$; species: $F_{1,34} = 0.35$, $p = 0.56$; benthics: $\bar{X} \pm \text{S.E.} = 64 \pm 22.1$ seconds; limnetics: $\bar{X} \pm \text{S.E.} = 36 \pm 6.17$ seconds). Furthermore, stressed fish tend to freeze or hide in the start box or in the plant landmarks with the result that they fail to pass through the hole in the PVC partition and enter either end of the T-maze during a 10-minute trial (pers. obs.). The occurrence of this stress-related behaviour differed between the four populations but was not consistently associated with species (ANOVA, species x lake: $F_{1,32} = 8.92$,

$p < 0.01$, Fig. 5.5). Paxton limnetics failed to enter an arm more often than Paxton benthics but the opposite trend was observed in fish from Priest Lake.

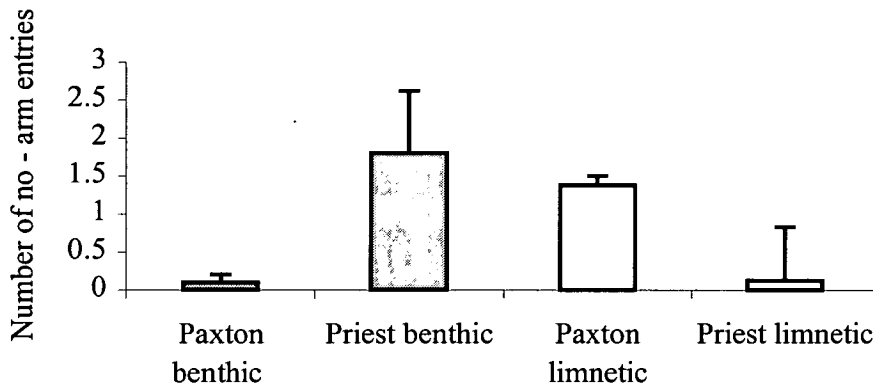


Figure 5.5. Mean \pm S.E. number of times benthics and limnetics from the two lakes failed to enter an arm of the T-maze during a ten-minute trial.

v) Post-criterion performance

Throughout the post-criterion trials, during which probe trials were interspersed, benthics and limnetics maintained a high and steady level of accuracy (benthics: $\bar{X} \pm \text{S.E.} = 96.53 \pm 1.27$ % correct; limnetics: $\bar{X} \pm \text{S.E.} = 86.9 \pm 2.05$ % correct). A one-way ANOVA with the number of post-criterion errors as the dependent variable revealed that limnetics made significantly more mistakes than benthics (ANOVA, species: $F_{1,34} = 15.64$, $p < 0.001$; Limnetics: $\bar{X} \pm \text{S.E.} = 1.4 \pm 0.24$ errors; Benthics: $\bar{X} \pm \text{S.E.} = 0.35 \pm 0.13$ errors, Fig. 5.6). There was no significant effect of lake on the number of errors made during post-criterion trials (ANOVA, lake: $F_{1,33} = 2.37$, $p = 0.13$).

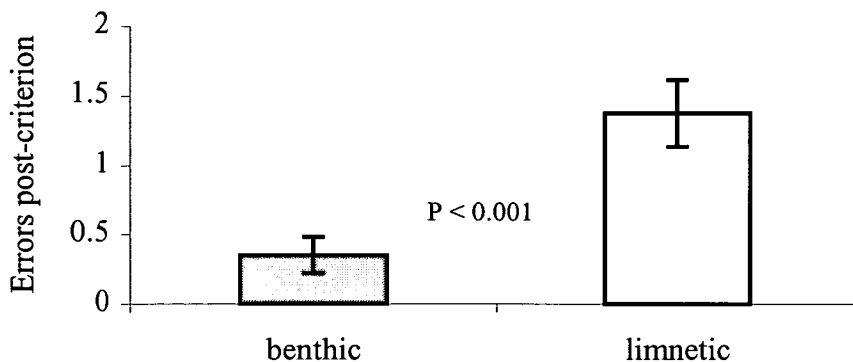


Figure 5.6. Mean \pm S.E. number of post-criterion errors made by benthics and limnetics.

vi) Probe trials

The results of the probe trials did not significantly differ between lakes (all p 's > 0.2), therefore the data were collapsed to investigate species differences. Benthics and limnetics did not differ in their use of the two cues during the three probe trials; that is, there were no significant differences in the MLE of t for the different species ($\Delta\text{Log}L_1 = 0.23$, $p = 0.63$). The proportion of benthic and limnetic fish from the two sites using one of three possible strategies: i) landmarks only across all three probe trials; ii) turn and landmarks; iii) turn only is shown in figure 5.7. These proportions and the MLE of t for each population is shown in table 5.3. If fish have no preference for using turn or landmarks, t is expected to be 0.5. The overall MLE of t (0.54) is not significantly different from 0.5 ($\Delta\text{Log}L_1 = 0.296$, $p = 0.44$). Thus, all fish regardless of site of origin or species appear to use both landmark and turn information with no significant preference for either cue.

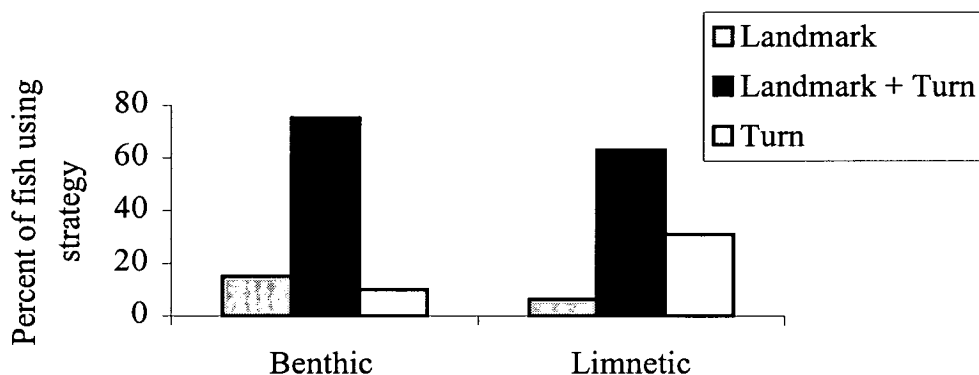


Figure 5.7. Percentage of benthic and limnetic fish using three strategies: landmarks across all three probe trials (Landmark), both landmarks and turn (Landmark + Turn) or turn across all three probe trials (Turn).

Population	Landmark only	Landmark and Turn	Turn only	MLE of t
Paxton Benthic	10%	90%	0%	0.5 ($p = 0.99$)
Priest Benthic	20%	60%	20%	0.53 ($p = 0.71$)
Paxton Limnetic	0%	88%	13%	0.5 ($p = 0.99$)
Priest Limnetic	13%	38%	50%	0.62 ($p = 0.22$)

Table 5.3. Percentage of benthic and limnetic fish from the two lakes using each of the three possible strategies and the maximum likelihood estimate of t for each population. The probability that the observed data were generated by $t = 0.5$ (predicted if there is no preference for either cue) is shown in brackets.

vi) Pauses and reversals

For each fish, the mean number of pauses and reversals made across all three probe trials (probe score) was compared with the number of pauses and reversals made in the final training trial before the probe tests began (criterion score). Figures 5.8a and b show the probe and criterion scores for benthics and limnetics from the two lakes. There was no effect of lake or of species on any of the reversal scores (all p 's > 0.2). Neither benthics nor limnetics from either lake showed more reversal behaviour during the probe trials than they had done at criterion (all p 's > 0.1). Benthics from both lakes paused significantly more often during the probe trials than they had done at criterion (paired t-tests, Paxton: $t_9 = -4.26$, $p = 0.002$; Priest: $t_9 = -3.87$, $p = 0.004$). Paxton limnetics paused more often during the probe trials than they had done at criterion, but this was not true for Priest limnetics (paired t-tests, Paxton: $t_7 = -2.83$, $p = 0.03$; Priest: $t_7 = -1.03$, $p = 0.34$). There was no effect of lake or species on the mean no of pauses fish made across the three probe trials (ANOVA, lake: $F_{1,33} = 0.01$, $p = 0.9$; species: $F_{1,34} = 0.25$, $p = 0.62$).

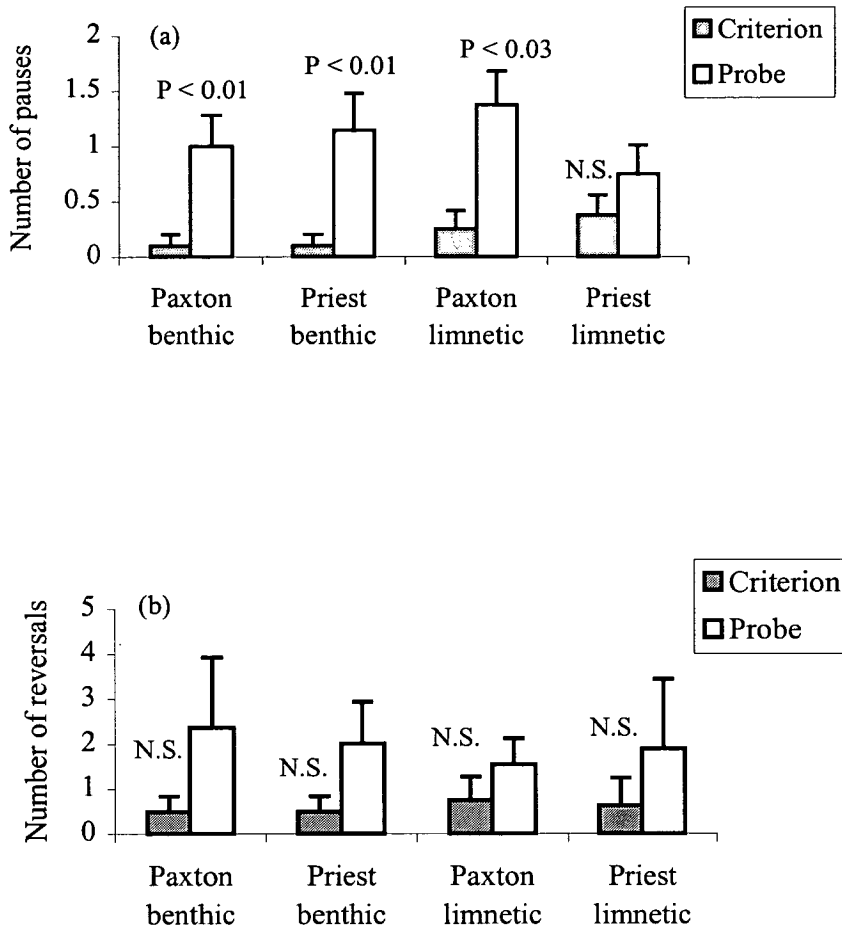


Figure 5.8. Mean \pm S.E. number of pauses (a) and reversals (b) made at criterion and during probe trials.

5.2.3 Discussion

Regardless of their lake of origin, performance by limnetics in the T-maze was considerably worse than that of benthics. Limnetic sticklebacks took longer than benthics to learn the spatial task and consistently made more mistakes than benthics throughout the entire period of training.

Significantly more benthics than limnetics reached criterion within the first 4 blocks (24 trials) of training, which means that for benthics but not for limnetics, the last two blocks in the repeated measures analysis include post-criterion trials. However, this is unlikely to be important in terms of affecting the results. Any confusion caused by spatially disruptive probe trials would be predicted to disrupt learning. Therefore, the inclusion of post-criterion trials for benthics but not for limnetics in the repeated measures analysis should reduce the species difference in performance rather than enhance it.

Many factors in addition to learning ability could affect performance in this spatial task. The lack of species differences in the time taken to enter an arm of the T-maze in the first trial or in the number of times fish showed stress-related behaviour (by failing to enter an arm of the maze), suggests differences in activity or boldness are unlikely to explain such dramatic differences in their rate of learning (see general discussion, section 5.4). However, there was a striking difference in the feeding behaviour of benthics and limnetics throughout the experiment. During training, benthics and limnetics differed in the number of times they took the bloodworm food reward on accessing the rewarded arm in the T-maze. Benthics fed in approximately 96% of trials, while limnetics only fed in approximately 54% of trials. This may have been because benthics are naturally specialised for feeding on littoral invertebrates such as the bloodworms used in this experiment while limnetics are behaviourally and morphologically adapted to feed on plankton (McPhail, 1994). Differences in motivation to access the reward might therefore account for the differences in performance by benthics and limnetics in the T-maze. However, an

additional reward was present in the goal arm; the simulated shoal. Motivation to locate shoal mates by limnetics may have compensated for their relative lack of motivation to access the food reward. In order to investigate this, an additional experiment was carried out (experiment 5b), which was designed to assess the motivation of benthics and limnetics to access the two types of reward, food and shoal mates.

5.3 EXPERIMENT 5B – CHOICE TEST

5.3.1 Methods

i) Subjects

Benthics and limnetics collected from the same two lakes and on the same dates as the ones used in experiment 5a were housed for 2 months in groups of 20 in the same conditions as those described in section 5.2.1. (p 111-112). A week before being tested, groups of 10 fish (10 benthics or 10 limnetics from each lake) were transferred to separate holding tanks of size 75 x 30 cm and 45 cm high.

ii) Apparatus

The experimental tank of size 75 x 30 cm and 45 cm high was lined with white coral gravel and filled with aerated and filtered water to a depth of 30 cm. Three sides of the tank were covered with black plastic to prevent experimental subjects from seeing fish outside the test tank. The front of the tank was left uncovered to allow the behaviour of test fish to be observed. The tank was divided into two sections by a transparent Perspex partition placed about 19 cm away from one end of the tank

(Fig. 5.9). Five non-experimental sticklebacks (conspecifics from the same lake of origin as the test fish) could be transferred to the smaller section of the tank. Lines were marked on the front of the tank dividing the remainder of the tank into three “zones”; two smaller zones measuring about 19 cm in width and a central larger zone measuring about 37 cm in width. The zone adjacent to the Perspex partition enclosing the five conspecifics was designated the “shoal zone”. The zone at the opposite end of the tank was designated the “food zone”. Food rewards were provided in this zone by securing copious amounts of bloodworm into a Vaseline filled Petri-dish which was propped up against the side of tank. Test fish could access, but not deplete this food reward during the five minute period of testing (see below). A transparent cylindrical chamber, weighted at the bottom, could be placed on the gravel in the centre of the central zone designated the “start zone”. This was attached to nylon filament and controlled remotely by a hand-operated pulley system (Fig. 5.9).

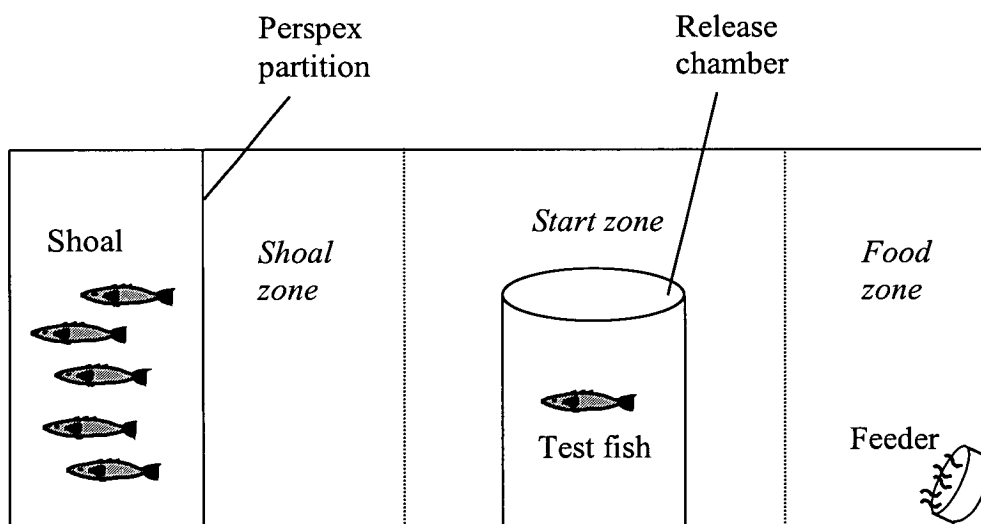


Figure 5.9. Diagrammatic representation of the choice test. The test fish is released from the central chamber and the proportion of time the fish spend in each of the three zones within a five minute trial period is recorded.

iii) Procedure

Once a day for three days, a Vaseline filled petri-dish in which copious amounts of bloodworm had been fastened, was placed in the home tanks of the experimental fish. Fish were not otherwise fed during this period. This was to encourage the fish to recognise the petri-dish as a food resource when exposed to it in the test tank. After 24 hours of exposure, the petri-dishes in all the home tanks had been depleted of bloodworms. After three days of being conditioned to feed from the petri-dishes, fish were food deprived for 24 hours.

Five non-experimental shoal fish were transferred to the smaller section of the tank and left to settle for 2 hours. Individual test fish were then transferred to the cylindrical chamber in the central start zone. After five minutes, the chamber was slowly raised, allowing the test fish access to the tank. Each fish was given 5 minutes to explore the three zones of the tank; the start zone, food zone and shoal zone. For each fish, the proportion of time spent in the three zones was measured by recording which zone was entered and the time of entry. Zone entry was judged to have occurred when the base of the caudal fin had passed across the line drawn on the front of the tank. Forty fish were tested over a period of 2 days and the order of testing was randomised.

5.3.2 Results

Figure 5.10 shows the amount of time benthics and limnetics from the two lakes spent in the shoal zone as a percentage of the total time spent in both the shoal and

food zones. Limnetics showed a significant preference for staying near shoal mates compared to feeding ($\chi^2 = 67.1$ and 43.8 for Paxton and Priest limnetics respectively, $p < 0.001$ for both). The two benthic populations differed in their behaviour. Priest benthics showed a significant preference for staying near shoal mates compared to feeding ($\chi^2 = 20.1$, $p < 0.001$) while Paxton benthics spent similar amounts of time in the shoal and food zones ($\chi^2 = 0.06$, $p > 0.2$). Paxton limnetics spent a significantly greater proportion of time in the shoal zone compared to Paxton benthics (Mann-Whitney U-test: $U = -4$, $p < 0.01$). There were no significant differences between Priest benthics and limnetics in the proportion of time they spent in the shoal zone (Mann-Whitney U test: $U = 46$, $p > 0.2$).

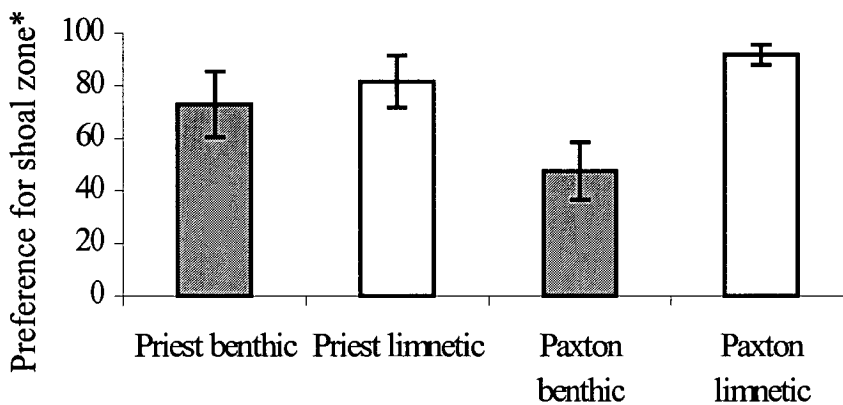


Figure 5.10. Mean \pm S.E. percentage of time fish spent in the shoal zone out of the total time spent in both the food and shoal zones for Priest and Paxton species pairs.

$$\text{* preference for shoal zone} = \frac{\text{time in shoal zone}}{\text{time in shoal zone} + \text{time in food zone}} \times 100$$

5.3.3 Discussion

In experiment 5b, limnetics from both lakes showed a strong preference for staying in the shoal zone near to conspecifics and spent little time exploring the rest of the tank or feeding. Although the apparatus differs between the spatial task (experiment 5a) and the choice test (experiment 5b), the procedures likely to cause stress to the fish are similar. In both cases fish are individually trapped in a plastic cup and given five minutes to settle before being released into a novel environment. The motivational drives indicated in the choice test are therefore likely to reflect the motivational drives present when fish are tested in the T-maze. For the limnetics, strong motivation to locate shoal mates indicated in the choice test may compensate for a relative lack of motivation to access the bloodworm food reward during training in the T-maze (see general discussion, section 5.4).

The two benthic populations displayed interesting differences in their behaviour in the choice test. Priest benthics showed a significant preference for staying in the shoal zone while Paxton benthics did not spend more time in the shoal zone than in the food zone. This may reflect the different predation levels present in the two lakes. Priest benthics are naturally exposed to higher levels of predation than Paxton benthics (D. Schluter, pers. com.), which may have selected for a stronger tendency to shoal (Seghers, 1973). Differences in predation pressure may also explain the variation in the occurrence of stress-related behaviour observed in experiment 5a (i.e. a failure to enter either arm of the maze). Priest benthics may show more stress-related behaviour than Paxton benthics as a result of being naturally more prone to predatory attacks. However, it is unclear why Paxton

limnetics should show more stress-related behaviour than Priest limnetics, given that both populations are exposed to similar levels of predation.

5.4 GENERAL DISCUSSION

Benthics and limnetics do not show differences in their propensity to use local landmarks when learning a spatial task, contrary to the first prediction made in section 5.1.3. However, they show striking differences in the rate at which they learn the task, providing support for the second prediction that benthics should be better spatial learners than limnetics. Each of these results will be discussed in turn.

The arm choices made during the probe trials suggest that both benthics and limnetics recognise and use local landmarks in combination with a body-centred pattern of movement. Three out of the four populations paused significantly more often during the probe trials than they had done at criterion implying that they were disorientated by the re-positioning of plant landmarks in the probe trials. This might be expected if during training, fish pay attention to both types of cue and to the relationship between the cues. Both benthics and limnetics are littoral breeders with males building and defending nests and territories on the bottom sediment during the summer months (McPhail, 1994). Therefore, the ability to use structural features as goal-directing cues may play an important role for both species in facilitating the location of nests and potential mates. Limnetics may similarly use visual features within the water column such as floating logs and surface vegetation, as positional cues indicating profitable feeding grounds, for example concentrated patches of

zooplankton (Noda *et al.*, 1994). Therefore, it is possible that limnetics benefit from using landmarks as positional cues as and when they are available, even if they encounter landmarks less frequently than benthics.

The second prediction that fish experiencing a complex, spatially structured littoral environment (benthics) will be better spatial learners than fish experiencing a more homogenous environment (limnetics), is supported by the results of experiment 5a. Two independently derived populations of limnetic threespine sticklebacks take considerably longer than benthics from the same lakes to learn a simple spatial task and consistently make more mistakes than benthics throughout the entire period of training.

As discussed in chapter 1 (section 1.4.2), a classic problem in comparative studies of learning is the possibility that contextual variables rather than differences in ability are responsible for species differences in performance (Shettleworth, 1993). By using relatively young species pairs that inhabit the same lakes and for whom hybridisation is still possible (Hatfield & Schluter, 1999; Vamosi & Schluter, 1999), many of the confounding factors normally associated with between species comparisons are removed. However, it is possible that the divergent conditions of the pelagic and littoral zones of the lakes generate behavioural differences aside from the ability to learn that could affect performance in a laboratory based spatial task.

First, benthics and limnetics are likely to be exposed to different types of predator and may be differentially vulnerable to predation. This could affect their susceptibility to stress when being tested in the laboratory environment. Insect predators tend to hunt in the covered littoral habitat exploited by benthics, which may explain why defensive armour has been reduced in benthics. Individuals with little or no armour offer few sites for these predators to grab hold (Reimchen, 1994). Limnetics are excluded from the protective littoral zone and have more extensive body armour than benthics (McPhail, 1994). This suggests that they have higher encounter rates with predators such as piscivorous birds or the cutthroat trout, a predator present in both Paxton and Priest Lakes (J. Boughman, pers. com.). A recent study by Vamosi and Schluter (2002) showed that benthics survive better than limnetics when trout are added to ponds containing the species pairs. Greater vulnerability to piscivorous fish and bird predators may translate into limnetics being more prone to stress on being brought into the laboratory environment, which could affect their performance in the T-maze. Against this, the occurrence of stress-related behaviour (a failure to enter either arm of the maze) was not consistently associated with species; Paxton limnetics showed this behaviour more often than Paxton benthics but the opposite trend was observed in fish from Priest Lake. Similarly, there were no differences between species, in the time they took to enter an end of the maze during the first trial. This suggests that benthics and limnetics do not differ in “boldness” indicated by their propensity to explore a novel environment when placed in isolation.

A second possibility is that benthics and limnetics differ in their motivational drive to reach the reward. Although not completely specialised (Schluter, 1993), limnetics are behaviourally and morphologically adapted to feed on plankton, while benthics, with their wide gape and fewer, shorter gill rakers, are specialised for exploiting littoral invertebrates such as the bloodworms used in this experiment (McPhail, 1994; Day & McPhail, 1996; Schluter, 1996). Limnetics were able to recognise the bloodworms in the petri-dish as food since food rewards were taken during group pre-training and in about 50 % of the trials in which the correct arm was entered. However, the tendency to ignore food rewards suggests bloodworms are a less salient reward for limnetics than for benthics. Although the fish were of the same age class (D. Schluter, pers. com.), the limnetics used in this experiment were considerably smaller than the benthics, which may have further contributed to their poor motivation to forage. However, an additional reward was present at the goal end, the simulated shoal. In the choice test (experiment 5b), limnetics from both lakes displayed a strong preference for staying near shoal mates. This suggests that poor motivation to forage on bloodworms in the T-maze may have been compensated by a strong drive to locate and stay with shoal mates. Shoaling is likely to be a particularly important defence strategy for this species that forages in the open and is excluded from the protective littoral zone. In addition, all of the limnetics eventually displayed the stringent criterion performance of at least 9 correct trials out of 10, which is difficult to explain if limnetics are consistently poorly motivated to access the correct arm.

Together these results support the argument that differences in activity, boldness or motivation are unlikely to account for such dramatic differences in the rate of learning by benthics and limnetics. However, eliminating the possibility that motivation differences could account for the difference in performance by benthics and limnetics in this spatial task, would require repeating experiment 5a using a stimulus which is known to have equal salience to both species. For example, benthics and limnetics could be trained to learn an avoidance task using a simulated predatory attack to signal an incorrect choice in the T-maze (e.g. Huntingford & Wright, 1989).

A further possibility is that the two species differ in their performance in the T-maze because of perceptual biases. For example, being able to recognise and use plant landmarks more easily may enhance performance in the spatial task. Against this, as already discussed, both benthics and limnetics appear to use local landmark information in combination with turn direction. Thus, it seems unlikely that benthics are better equipped than limnetics to perceive landmark information or monitor a body-centred pattern of movement. However, it is possible that limnetics are better adapted to learn a spatial task that relies on the use of alternative types of spatial information such as polarized light or sun compass information (Goodyear, 1973; Hawryshyn *et al.*, 1990).

A plausible explanation based on the current data is that the difference between benthics and limnetics in their ability to learn a spatial task directly results

from the different demands for spatial learning made by benthic and pelagic lifestyles. In addition to foraging and anti-predator specialisations specific to each habitat, benthics and limnetics may be equipped with learning abilities that best suit them to either a littoral or pelagic lifestyle. The distribution of benthic invertebrates at the littoral edges of the lake is likely to be more clumped and patchy than the distribution of plankton throughout the water column. Benthics may therefore need to process greater amounts of spatial information than limnetics in order to learn and remember the location of food patches (Hughes & Blight, 1999, 2000; but see Noda *et al.*, 1994). While shoaling appears to be an important predator defence strategy for limnetics, benthics may rely to a greater extent on rapid escape responses and hiding. Benthics may therefore need to learn and remember the location of protective refuges within the littoral habitat (Markel, 1994; Brown & Warburton, 1997). Similarly, although males of both species build and defend nests in the littoral zone during the breeding season, benthic males nest in dense cover, while limnetic males tend to nest on open sediments (McPhail, 1994). As a result, benthics may rely to a greater extent on learning spatial relationships and complex environmental features in order to relocate their nests within dense vegetation. As yet, it remains open to speculation which particular features of a littoral lifestyle might be expected to require an enhanced capacity for spatial learning compared to a pelagic one.

The energetic costs likely to be associated with a capacity to learn may mean fish only invest as much into spatial learning capacity as they need to. Habitats divergent in spatial complexity, even on a local scale may therefore result in differential investment in the capacity for spatial learning. Genetically based

differences in learning ability may have been selected in these species pairs, assuming they are exposed to relatively constant conditions across generations. Learning adaptations to local habitat conditions may therefore present a further important source of ecological adaptation in these species pairs, that maximise the fitness of parent species in their respective habitats and contribute to hybrid disadvantage (Hatfield & Schluter, 1999). Alternatively, experience may be responsible for generating this divergence in learning ability (Juraska *et al.*, 1984; Seymoure *et al.*, 1996). If this is the case, individual fish may be selected to specialise during their lifetime on habitats that make consistent demands on their spatial learning and memory abilities (Dukas, 1999).

In chapter 3, the use of particular cues by the threespine stickleback during spatial learning appeared to be adapted in response to specific habitat conditions. Here, flexible learned behaviour again appears to be adapted, in this case in response to different local habitat conditions even within the same lakes. Genetic or developmental constraints may result in plastic learned responses being appropriately tailored to deal with specific ecological problems in the most cost-effective way.

Chapter 6

Causal basis of population differences in landmark use during spatial learning by the threespine stickleback

6.1 INTRODUCTION

In chapters 3 and 5, it was shown that populations and species of threespine stickleback experiencing different habitat conditions, appear to differ either in the cues that they use to learn a spatial task (chapter 3) or in their ability to learn the task (chapter 5). These results support the claim that learned behaviour is adapted for the tasks and environmental conditions that an animal is likely to encounter (Healy & Braithwaite, 2000). However, it is rarely known whether individual, population, or species differences in learned behaviour are themselves learned and reversible, genetically fixed, or the result of interacting genetic and environmental influences. In sufficiently predictable environments, genetic predispositions may be selected to shape learned behaviour in response to specific ecological conditions. In more changeable environments, the properties of learned behaviour may be more plastic and directed or fine-tuned by experience.

In practice, it is notoriously difficult to partition the roles of genes and experience in the development of behaviour patterns (Bateson, 1983). Invariably, their effects overlap and interact with no clear boundary between their relative contributions. What can be investigated, however, is whether genes or environmental influences play any role at all in the development of individual, population and species differences in behaviour, and the nature of their interactions.

6.1.1 Genetic influences on learned behaviour

Some support for a genetic component to individual and population differences in learning ability has come from artificial selection experiments. In strains of laboratory rats and mice, selected differences in hippocampal morphology correlate with differences in performance in laboratory based spatial tasks (Lipp *et al.*, 1989; Schwegler & Crusio, 1995). Artificial selection for homing ability in pigeons similarly correlates with a heritable enlargement of the hippocampal complex (Rehkämper *et al.*, 1988).

A different approach is to effectively remove all environmental influences and observe whether individual or population differences in learning persist. In practice, a complete removal of environmental influences is impossible. However, the persistence of differences in learned behaviour following extreme deprivation of environmental stimuli strongly implies that ontogenetic effects play a limited role compared to that of fixed genetic instruction (Gaulin & Wartell, 1990). A controlled rearing approach has been used to tease apart the effects of genes and experience on learned anti-predator responses that differ in magnitude between local populations in a number of fish species (Tulley & Huntingford, 1987; Magurran, 1990). For example, Magurran, (1990) found genetically inherited anti-predator behaviour shown by two laboratory-reared populations of minnows could be modified by early experience. However, the greatest adjustment in anti-predator behaviour occurred in the minnows naturally sympatric with pike predators. Fish from this population appear to be genetically predisposed to respond more strongly to early experience of a predatory attack.

6.1.2 Manipulating experience

Experiments in which animals are exposed to specific types of experience further reveal the complexity of gene-environment interactions in the development of learning “modifications”. Kinsley and colleagues (1999) have recently suggested that pregnancy and exposure to a litter of pups may combine to enhance spatial learning ability and memory in female rats. Preparatory to mating, steroid hormones initiate morphological changes in the hippocampus. In addition, the sensory stimulation provided by a litter of pups appears to reorganise hypothalamic connections. These changes apparently enhance subsequent learning and memory since the performance of pregnant and suckling female rats in spatial tasks is significantly improved compared to controls. Thus genetically instructed and developmentally regulated hormone activity combined with the external stimulation provided by pups, may literally reshape the brain and enhance learning and memory abilities. General environmental enrichment and training in laboratory-based tasks similarly leads to measurable changes in rat brains, which are likely to affect learning and memory (Bennett *et al.*, 1964; Rosenzweig, 1984). Rats reared in an enriched physical environment exhibit superior performance in learning a variety of mazes compared with rats raised alone in standard laboratory cages (Seymoure *et al.*, 1996).

The potential for experience to produce changes in the functional architecture of the brain, which affects subsequent learning and memory, may be under fixed genetic control. In rats, the brains of some genetic strains are more modifiable than others (Bennett *et al.*, 1964). Furthermore, Clayton and Krebs (1994a) showed that experience of storing and retrieving food caused an increase in hippocampal volume

in hand-raised marsh tits but had no effect on the hippocampus of blue tits (a non food-storing species).

Seasonal shifts in learning may provide a further example of where animals are genetically predisposed to respond to an external signal (a change in day length) that modifies learning and memory. Male polygynous rodents raised under long (breeding) photoperiods show superior maze performance compared to females raised under the same conditions, while males caught in the winter, or reared under a winter photoperiod show no enhanced ability relative to females (Galea *et al.*, 1994; Gaulin, 1995). Seasonal changes in the neural substrates required for spatial learning have also been reported (Barnea & Nottebohn, 1994; Smulders, 1995; Clayton *et al.*, 1997), although it is not clear whether these seasonal neuroanatomical changes are directly linked to shifts in the demand for spatial learning.

6.1.3 Genetic and environmental influences on information use

In addition to influencing learning ability, genetic and environmental influences may affect the types of information that are used in learning. For example, as already discussed in chapter 5 (section 5.1.3), the rearing conditions experienced by homing pigeons, appears to affect their use of cues when learning homing routes as adults (Wiltschko & Wiltschko, 1989; Braithwaite & Guilford, 1995). In addition, Clayton (1995) has reported that after being given food-storing experience, hand-raised marsh tits respond preferentially to the spatial relationship between cues as opposed to their individual features, when solving a spatial task. Blue tits (a non food-storing species)

given the same experience respond equally to both spatial position and object specific cues.

Whether a predisposition to use certain types of information in preference to others is under genetic or environmental control, may itself depend on environmental variability. If cues are sufficiently reliable within and across generations, cue preferences may be fixed and under genetic control. Alternatively, individuals may need to continually update their use of particular cues, if cue availability and reliability is variable (see chapter 4, section 4.4). Investigating how flexible learning interacts with genetic and developmental instruction is therefore likely to be central to understanding how animals optimise their use of learned behaviour in the face of varying levels of environmental change.

6.1.4 Aims of study

The experiments described in this chapter were designed to investigate whether genetic, and or environmental influences play a role in the development of cue preferences by pond and river threespine sticklebacks. In chapter 3, pond and river threespine sticklebacks were found to differ in the types of information that they appeared to use to learn a spatial task. Pond fish appeared to use both turn direction and landmarks while river fish showed a significant preference for using turn direction. In an earlier study, Girvan and Braithwaite (2000) raised pond and river threespine sticklebacks in an array of different conditions to investigate the mechanisms underlying cue preferences in wild pond and river sticklebacks. In Girvan & Braithwaite's (2000) study, fish were exposed to five different rearing

treatments, but these were not replicated making it difficult to conclude whether differences in the behaviour of fish subjected to the different treatments was the result of treatment or due to an arbitrary factor such as the position of the tank in the room etc. In addition, the behaviour of the reared fish was not compared to that of the wild parent populations tested at the same time. Given the potential for seasonal and other environmental influences on learned behaviour, the absence of the wild comparison in the same experiment, risks misinterpretation of the results.

The experiments described here use a similar “controlled rearing approach” to that taken by Girvan & Braithwaite (2000). In these experiments, purpose-built outdoor ponds provided rearing environments, since a stimulus deficient laboratory environment has been reported to depress learning ability in several species (Rosenzweig, 1984). The aim of these experiments was to investigate the effects of genetic and environmental influences on cue preference, not on the ability to learn *per se*. Here the cue preferences of wild fish collected from a pond and a river were compared with the offspring of pond and river fish originally bred in the laboratory and reared in replicate pond habitats. If pond and river fish raised under identical conditions exhibit the behavioural differences displayed by their wild parent stock then there is good evidence that cue preference is an inherited trait. Alternatively, if the rearing environment dictates which cues are used to learn a spatial task, both pond and river fish reared in the replicate pond habitats should show an equal use of turn direction and landmarks, matching the behaviour of wild pond fish.

6.2 EXPERIMENT 6A

6.2.1 Methods

A summary of the procedure is provided in figure 6.1. A more detailed description of the methods used for rearing and training the fish is provided below.

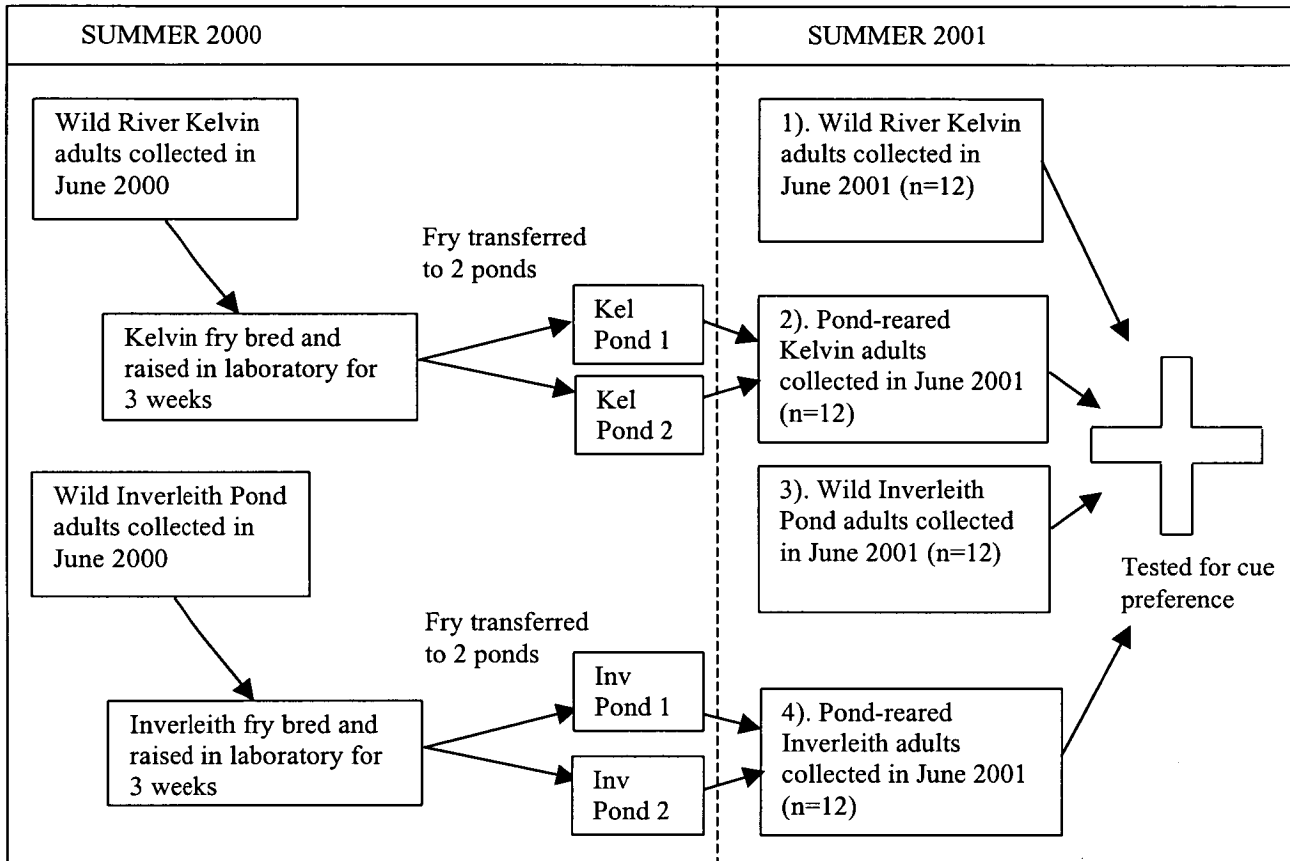


Figure 6.1. Flow chart summarising the experimental procedure.

i) Procedure for rearing fry

Threespine sticklebacks were collected from the River Kelvin (NS 54 70) and from Inverleith pond (NT 24 75) during their reproductive season in June 2000, using 3 mm-mesh dip nets and standard minnow traps. Egg clutches were obtained from spawnings between at least 8 male and female pairs from each site (see Appendix

III). Fry were fed for several days on *Paramecium* and were then switched to a diet of newly hatched brine shrimp and later, finely chopped de-frosted frozen bloodworm. After 3 weeks of growth, the fry (measuring about 1.5 cm in length) were transferred to four replicate ponds, 20-30 individuals being transferred to each one. Inverleith fry were transferred to two replicate ponds and Kelvin fry were transferred to a further two ponds. In each case, the pond was selected at random. The ponds were constructed from Lotus pond liner stapled to wooden frames (see Appendix IV) and were 115 x 180 cm and 55 cm high. Each pond was sunk into a previously existing concrete walled rectangular pond of size 7.5 x 7.5 m and 0.5 m high, and supported by a scaffolding framework (Figure 6.2; Appendix IV). The ponds were lined with gravel and furnished with rocks and weed. All ponds were given 6 weeks to mature before any fry were transferred. After transferral of the fry, the ponds were covered in wire mesh to protect the fish from aerial predation. Fry continued to be fed in the ponds once every 2-3 days, initially on chopped defrosted frozen bloodworm, and after 3 months on whole bloodworm.



Figure 6.2. Photograph of replicate ponds. Strips of pond liner were stapled to an upper and lower wooden frame to form identical rectangular ponds. These were then sunk into a pre-existing concrete walled pond and the upper wooden frames were supported by a scaffolding framework (see Appendix IV).

ii) Experimental subjects

The following summer (May 2001), threespine sticklebacks were collected from the River Kelvin and from Inverleith Pond. Fish that had been bred in the laboratory and allowed to develop in the replicate ponds (from henceforth referred to as pond-reared Inverleith and pond-reared Kelvin fish) were collected from the four ponds using standard minnow traps, also in May 2001.

Forty-eight fish were used for the experiment; 12 wild Kelvin fish, 12 wild Inverleith fish, 12 pond-reared Kelvin fish (6 fish from each pond) and 12 pond-reared Inverleith fish (6 fish from each pond). All fish were given a settling period in the laboratory of 4 weeks, maintained on a diet of defrosted frozen bloodworm. Fish were maintained in groups of 6, in holding aquaria of size 46 x 30 cm and 30 cm high. Each aquarium was divided into two sections by a clear perspex partition measuring 30 x 26 cm. Three fish that could be distinguished from size differences or body markings were housed in each section. Both sides of each tank were furnished with a plastic plant and terracotta refuge. The temperature was maintained at $12 \pm 1^\circ\text{C}$ and overhead lighting was provided by 40-W fluorescent tubes, operating on a 12:12 hour light:dark cycle, matching the experimental conditions described in chapter 3 (p 54). The fish used in this experiment were in breeding condition at the time of collection and after the 4-week settling period in the laboratory, some males had retained their breeding colouration and several of the females were slightly gravid. However, the low temperature and reduced daylength provided in the laboratory appeared to inhibit behaviour associated with reproduction since the fish

did not display “head-up postures”, territory defence, chasing or nest building (Wooton 1984).

iii) Apparatus

The apparatus used for this experiment was the same as that described in chapter 3 (p 54).

iv) Procedure

The procedure for pre-training was the same as that described in chapter 3 (p 56) except that fish were pre-trained in groups of six over a period of ten days. This was to allow fish from the same rearing condition to be pre-trained together, removing the need to tag individuals. Each group was given five 4 hour periods in a randomly selected maze every other day. On any one day, groups of Kelvin and Inverleith pond-reared fish or Kelvin and Inverleith wild fish were pre-trained simultaneously in the morning or afternoon at random. Two days after the last pre-training session, fish were individually trained to locate the goal in the T-maze by using turn direction and plant landmarks in the same way as that described in chapter 3 (p 56).

v) Probe trials and VCR recordings

In order to assess which types of information the fish used to solve the spatial task, fish were exposed to three probe trials where the position of the plant landmarks was switched to the opposite side from where it had been during training. The procedure for conducting probe trials is described in chapter 3 (p 57). Pauses and reversals were

scored from video recording of the three probe trials and of the last training trial prior to the first probe test as described in chapter 3 (p 57).

vi) Statistical analysis

Where necessary, to ensure normality and homogeneity of variance the data were log, arcsine or square-root transformed. In order to assess the factors affecting performance in the T-maze, I carried out a four-factor (habitat type, rearing treatment, turn direction, block) ANOVA with block as a repeated measure (1 block = 6 trials). In each model, fish was nested within turn direction, habitat type (pond or river), and rearing treatment (wild or pond-reared). To assess whether pond and river fish exposed to different rearing treatments differ in the types of spatial information that they use during the probe trials, I use the likelihood approach (Edwards, 1972). As discussed in chapter 5 (section 5.2.1), applying standard frequency tests such as the G-test or the chi-square test is precluded by the low expected values generated from the small sample sizes. A more detailed statistical discussion is presented in chapter 5 (p 114).

6.2.2 Results

There were no significant differences between the counterbalanced right-trained and left-trained conditions within groups in any phase of the experiment (all p 's > 0.2). Within the two pond-reared treatments (Kelvin pond-reared and Inverleith pond-reared), fish from the two replicate ponds did not significantly differ in their

performance (all p 's > 0.1). Therefore, these data were collapsed when calculating the group averages.

i) Acquisition

One wild Kelvin fish died leaving a sample size of 47 fish. Figure 6.3 shows the percentage of fish performing the task correctly during the first 4 blocks (24 trials) of training. All fish learnt to find the rewarded arm with performance significantly improving over the first 4 blocks (24 trials) of training (ANOVA, block: $F_{3,138} = 14.32$, $p < 0.001$). There was no significant effect of habitat type (pond or river) or of rearing treatment (wild or pond-reared) on performance (ANOVA, habitat type: $F_{1,43} = 0.48$, $p = 0.62$; rearing treatment: $F_{1,43} = 0.09$, $p = 0.82$; habitat type x rearing treatment: $F_{1,43} = 0.80$, $p = 0.38$).

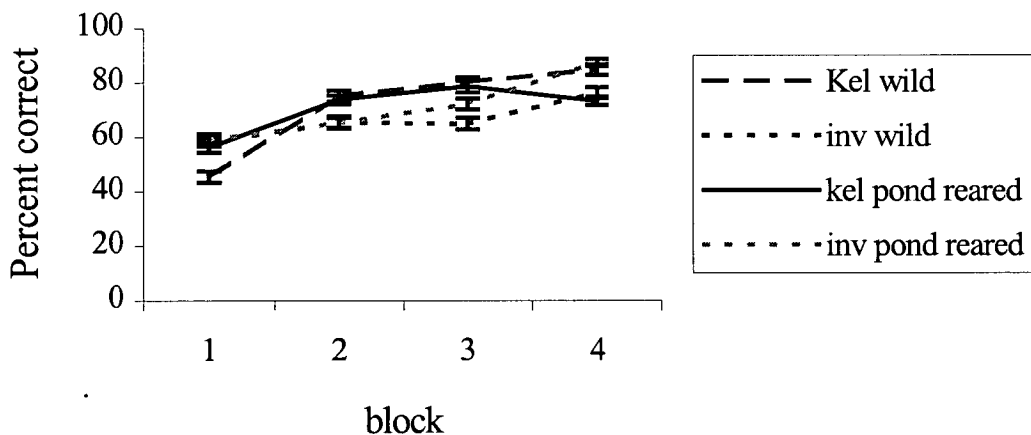


Figure 6.3. Mean \pm S.E. percentage of correct choices during the first 4 blocks (24 trials) of training (1 block = 6 trials).

Forty-two fish (11 wild Kelvin, 10 wild Inverleith, 10 Kelvin pond-reared and 11 Inverleith pond-reared) achieved the criterion performance of at least 9 correct trials out of 10. A two way ANOVA revealed no significant effect of habitat type or of rearing treatment on the number of trials fish took to reach criterion (ANOVA, habitat type: $F_{1,39} = 1.11$, $p = 0.30$; rearing treatment: $F_{1,39} = 0.31$, $p = 0.58$; habitat type x rearing treatment: $F_{1,38} = 3.09$, $p = 0.09$, Table 6.1).

Treatment	Mean \pm S.E. trials to reach criterion
Kelvin wild	17.6 \pm 1.68
Inverleith wild	29.6 \pm 5.42
Kelvin pond-reared	20.7 \pm 2.16
Inverleith pond-reared	19.5 \pm 2.19

Table 6.1. Mean \pm S.E. number of trials taken to reach criterion by wild and pond-reared Kelvin and Inverleith fish.

ii) Post-criterion performance

Throughout the post-criterion trials during which probe trials were interspersed, fish maintained a high and steady level of accuracy (Table 6.2). A two-way ANOVA with the number of post-criterion errors as the dependent variable revealed no significant effect of habitat type or of rearing treatment on the number of mistakes fish made during post-criterion trials (ANOVA, habitat type: $F_{1,39} = 0.31$, $p = 0.58$; rearing treatment: $F_{1,39} = 0.77$, $p = 0.39$; habitat type x rearing treatment: $F_{1,38} = 2.71$, $p = 0.11$ Table 6.2).

Treatment	Mean \pm S.E. % of correct trials post-criterion	Mean \pm S.E. errors post-criterion
Kelvin wild	89.3 \pm 4.81	1.6 \pm 0.24
Inverleith wild	83.52 \pm 4.96	2.6 \pm 0.41
Kelvin pond-reared	77.6 \pm 4.87	3.7 \pm 0.34
Inverleith pond-reared	87.2 \pm 3.99	1.55 \pm 0.16

Table 6.2. Mean \pm S.E. percentage of correct trials and number of mistakes made after reaching criterion and until the end of training by wild and pond-reared Kelvin and Inverleith fish.

iii) Probe trials

Figure 6.4 shows the results of the probe trials. There were no significant differences between fish from the 4 populations (wild Kelvin, pond-reared Kelvin; wild Inverleith and pond-reared Inverleith) in their use of the two cues during the three probe trials; that is, there were no significant differences in the maximum likelihood estimate (MLE) of t between populations ($\Delta\text{Log}L_3 = 1.34$, $p = 0.44$). The proportion of fish using one of 3 possible strategies: i) landmarks only across all three probe trials; ii) turn and landmarks; iii) turn only, and the MLE of t for each population is shown in table 6.3. If fish have no preference for using turn or landmarks, t is expected to be 0.5. The overall MLE of t (0.58) was not significantly different from 0.5 ($\Delta\text{Log}L_1 = 1.59$, $p = 0.07$). Therefore, all fish regardless of habitat type and rearing treatment appear to use both landmark and turn information with no significant preference for either cue.

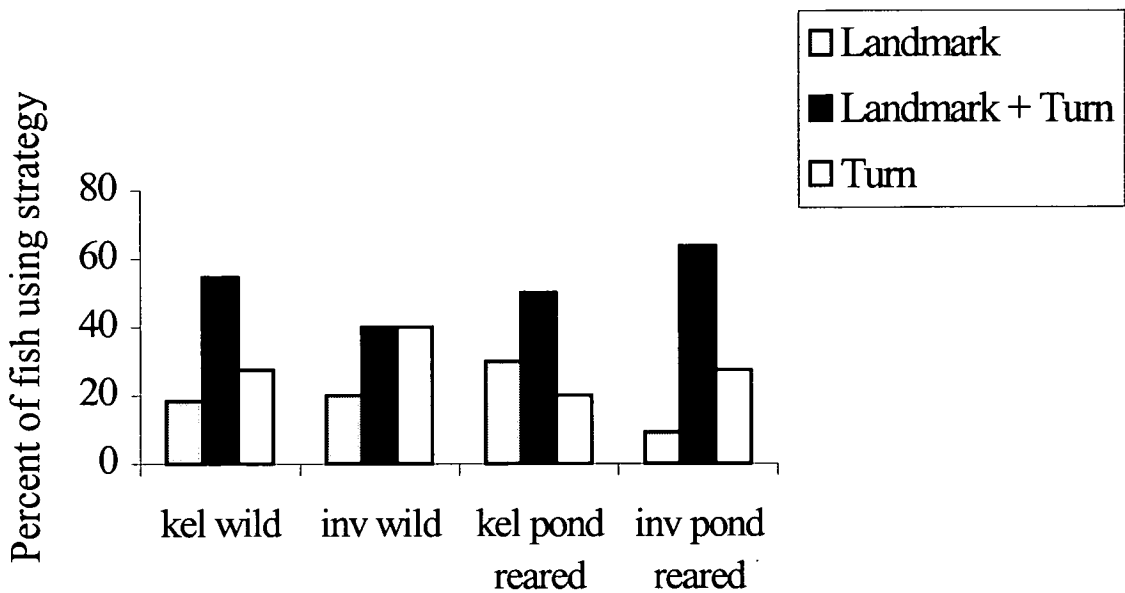


Figure 6.4. Percentage of wild and pond-reared Kelvin and Inverleith fish using three strategies: landmarks across all three probe trials (Landmark), both landmarks and turn (Landmark + Turn) or turn across all three probe trials (Turn).

Treatment	Landmark only	Landmark and Turn	Turn only	MLE of t
Wild Kelvin	18%	55%	27%	0.6 (p = 0.22)
Wild Inverleith	20%	40%	40%	0.56 (p = 0.46)
Pond reared Kelvin	30%	50%	20%	0.46 (p = 0.13)
Pond reared Inverleith	9%	64%	27%	0.66 (p = 0.053)

Table 6.3. Percentage of wild and pond-reared Kelvin and Inverleith fish using each of the three possible strategies and the maximum likelihood estimate of t for each treatment. The probability that the observed data were generated by $t = 0.5$ (predicted if there is no preference for either cue) is shown in brackets.

iv) Pauses and reversals

For each fish, the mean number of pauses and reversals made across all three probe trials (probe score) was compared with the number of pauses and reversals made in the final training trial before the probe tests began (criterion score). Figures 6.5a and b show the probe and criterion scores for wild and pond-reared Kelvin and Inverleith fish. Kelvin wild fish did not show significantly more pause or reversal behaviour across probe trials than they had done at criterion (paired t-test; pauses: $t_{10} = -0.65$, $p = 0.53$; reversals: $t_{10} = -1.20$, $p = 0.26$). Inverleith wild fish did not pause significantly more often across probe trials than they has done at criterion (paired t-test: $t_9 = 0.11$, $p = 0.92$) but they did show significantly more reversal behaviour across probe trials than at criterion (paired t-test: $t_9 = -4.45$, $p = 0.002$). Kelvin pond-reared fish paused more often across probe trials than at criterion (paired t-test: $t_9 = -4.37$, $p = 0.002$). This was not true for reversal behaviour (paired t-tests: $t_9 = -1.26$, $p = 0.24$). Inverleith pond-reared fish showed significantly more pause and reversal behaviour across probe trials than at criterion (paired t-test; pauses: $t_{10} = -2.60$, $p = 0.03$; reversals: $t_{10} = -3.32$, $p = 0.008$).

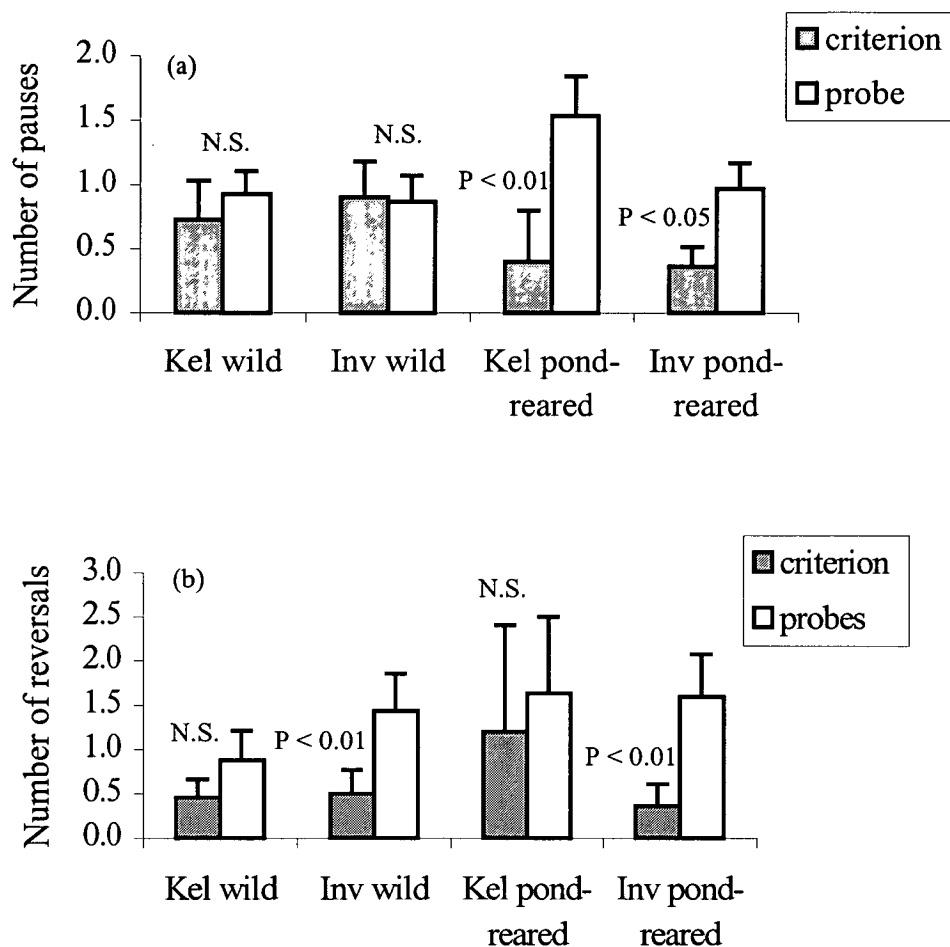


Figure 6.5. Mean \pm S. E. number of pauses (a) and reversals (b) made at criterion and during probe trials.

There was no significant effect of habitat type (pond or river) or of rearing treatment (wild or pond-reared) on the mean number of pauses or reversals made during the probe trials (for pauses: ANOVA, habitat type: $F_{1,39} = 1.90$, $p = 0.18$; rearing treatment: $F_{1,39} = 2.49$, $p = 0.12$; habitat type \times rearing treatment: $F_{1,38} = 1.27$,

$p = 0.27$; for reversals: ANOVA, habitat type: $F_{1,39} = 1.63$, $p = 0.21$; rearing treatment: $F_{1,39} = 1.42$, $p = 0.24$; habitat type x rearing treatment: $F_{1,38} = 0.93$, $p = 0.34$).

6.2.3 Discussion

The results of experiment 6a do not allow an assessment of genetic and environmental influences on the cue preferences shown by pond and river sticklebacks in chapter 3, since all fish, regardless of treatment, showed the same use of cues. In contrast to the findings presented in chapter 3, here there were no differences between wild river and wild pond fish in their propensity to use landmarks. In this experiment, both Inverleith Pond and River Kelvin fish, whether wild or pond-reared, appear to use turn direction and landmarks during the probe trials, with no significant preference for either cue.

The suggestion that all fish use both types of cue is supported by the fact that there were no significant differences between the four populations in the number of mistakes fish made after they had reached criterion, or in the number of pauses or reversals they made during the three probe trials. If all fish are paying attention to both turn direction and landmarks, there should be no between population differences in the extent to which their performance is disrupted by spatially disruptive probe trials. Only wild River Kelvin fish did not show significantly more pause or reversal behaviour during the probe trials than they had done at criterion. Fish from all other sites showed more pause and, or reversal behaviour during the probe trials than they

had done at criterion. “Confusion” caused by the disruption of spatial relationships in the probe trials might be expected if, during training, fish pay attention to both turn direction and landmarks and to the relationship between the cues.

Why do wild River Kelvin fish use landmarks and turn direction in this experiment when in the experiment described in chapter 3, river fish from five different sites appeared to ignore landmarks and show a significant preference for using turn direction? One possibility is that ecological conditions specific to the River Kelvin favour the use of landmarks. For example, the River Kelvin may be a relatively stable habitat compared to other rivers if flow rate and turbulence is comparatively low. In the experiment described in chapter 3, only 4 fish from each of the five rivers sampled were tested for cue preference, therefore between site differences may not have shown up. This possibility seems unlikely given the results of Girvan & Braithwaite’s (1998) study. They reported a preferential use of turn direction over landmarks by River Kelvin fish in a comparison that tested 12 fish from each population.

A second possibility is that there is seasonal variation in the use of landmarks by River Kelvin fish. As far as possible, conditions were kept constant between the experiment described in chapter 3 and experiment 6a. However, a clear difference between these experiments was in the time of year that fish were collected and tested. In the experiment described in chapter 3, fish were tested in November, whereas in experiment 6a, fish were tested in June. In summer, a more structured use of space

associated with reproduction may favour the use of landmarks. Moreover, habitat stability may increase in summer, making landmarks more reliable indicators of location (see general discussion, section 6.4).

To investigate these possibilities, a comparison of wild River Kelvin fish and pond-reared Kelvin fish was carried out in winter in experiment 6b. If conditions specific to the River Kelvin favour the use of landmarks regardless of season, the use of landmarks by wild Kelvin fish should persist during the winter. Alternatively, a preference for turn direction in wild Kelvin fish tested in winter would support the possibility of seasonal variation in landmark use.

6.3 EXPERIMENT 6B

6.3.1 Methods

i) Subjects

Threespine sticklebacks were collected from the River Kelvin and from the two replicate ponds containing fish bred from Kelvin adults, in January 2002 using 3 mm-mesh dip nets and standard minnow traps. Twenty-four fish were used for the experiment; 12 wild Kelvin fish, and 12 pond reared-Kelvin fish (6 fish from each pond). All fish were given a settling period in the laboratory of 4 weeks under the same conditions as those described in section 6.2.1.

ii) Procedure

The fish in this experiment were trained to locate a goal in the T-maze using exactly the same procedure as that described for experiment 6a, section 6.2.1.

6.3.2 Results

There were no significant differences between the counterbalanced right-trained and left-trained conditions within groups in any phase of the experiment (all p 's > 0.2). Where the behaviour of fish from the two ponds differs (in task acquisition), they are treated as separate populations. Post-criterion performance, cue use and pause and reversal scores did not significantly differ between fish from the replicate ponds (all p 's > 0.1). Therefore, these data were collapsed to look at the effect of rearing treatment on performance. "Treatment" refers to the two rearing conditions: pond-reared or wild.

i) Acquisition

One wild Kelvin and one pond-reared Kelvin fish died leaving a sample size of 22 fish. Figure 6.6 shows the percentage of fish performing the task correctly during the first 4 blocks (24 trials) of training. All fish learnt to find the rewarded arm with performance significantly improving over the first 4 blocks (24 trials) of training (ANOVA, block: $F_{3,63} = 5.55$, $p = 0.002$). The two pond-reared populations significantly differed in their performance (ANOVA, pop: $F_{1,9} = 5.88$, $p = 0.04$) with fish from pond 1 making fewer correct choices across trials than fish from pond 2. However this effect was small since it disappeared when all three populations were compared (ANOVA, pop: $F_{2,19} = 1.75$, $p = 0.2$).

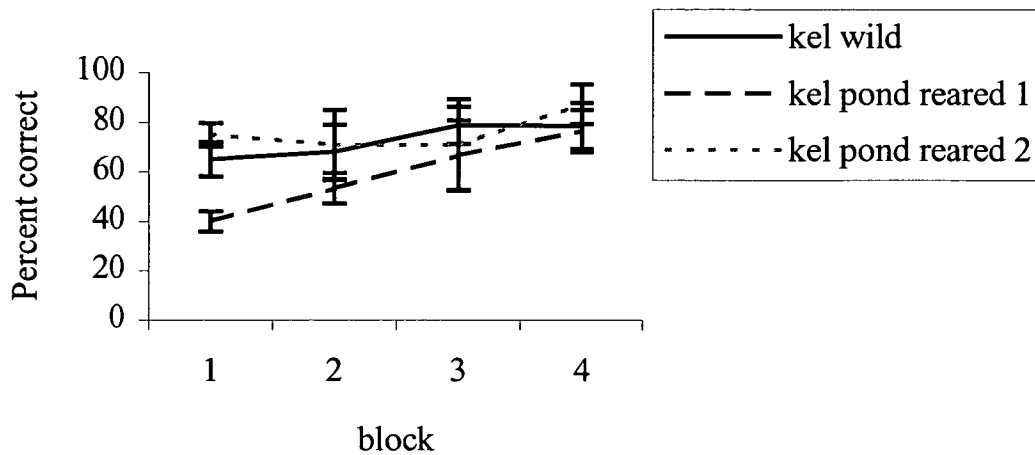


Figure 6.6. Mean \pm S.E. percentage of correct choices during the first 4 blocks (24 trials) of training (1 block = 6 trials).

One fish from each site failed to reach criterion, leaving a sample size of 19 fish. A one way ANOVA with the number of trials taken to reach criterion as the dependent variable revealed no significant effect of treatment (wild or pond-reared) on the number of trials fish took to reach criterion (ANOVA, treatment: $F_{1,17} = 0.87$, $p = 0.36$; Kelvin wild: $\bar{X} \pm \text{S.E.} = 18.2 \pm 3.23$ trials; Kelvin pond-reared: $\bar{X} \pm \text{S.E.} = 22.4 \pm 3.18$ trials).

ii) Post-criterion performance

Throughout the post-criterion trials during which probe trials were interspersed, fish maintained a high and steady level of accuracy (Kelvin wild: $\bar{X} \pm \text{S.E.} = 95.6 \pm 2.27$ % correct; Kelvin pond-reared: $\bar{X} \pm \text{S.E.} = 90.7 \pm 4.67$ % correct). A one-way ANOVA with the number of post-criterion errors as the dependent variable revealed no significant effect of treatment on the number of mistakes made during post-

criterion trials (ANOVA, treatment: $F_{1,17} = 0.54$, $p = 0.5$; Kelvin wild: $\bar{X} \pm S.E = 0.6 \pm 0.31$ errors; Kelvin pond-reared: $\bar{X} \pm S.E = 1.33 \pm 0.73$ errors).

iii) Probe trials

Figure 6.7 shows the results of the probe trials. There were no significant differences between wild River Kelvin fish and pond-reared Kelvin fish in their use of the two cues during the three probe trials; that is there were no significant differences in the maximum likelihood estimate (MLE) of t between the two treatments ($\Delta\text{Log}L_1 = 0.5$, $p = 0.32$). The overall MLE of t (0.81) was significantly different from 0.5 ($\Delta\text{Log}L_1 = 11.55$, $p < 0.001$). Thus both wild caught River Kelvin fish and pond-reared River Kelvin fish showed a significant preference for using turn across the three probe trials (Table 6.4).

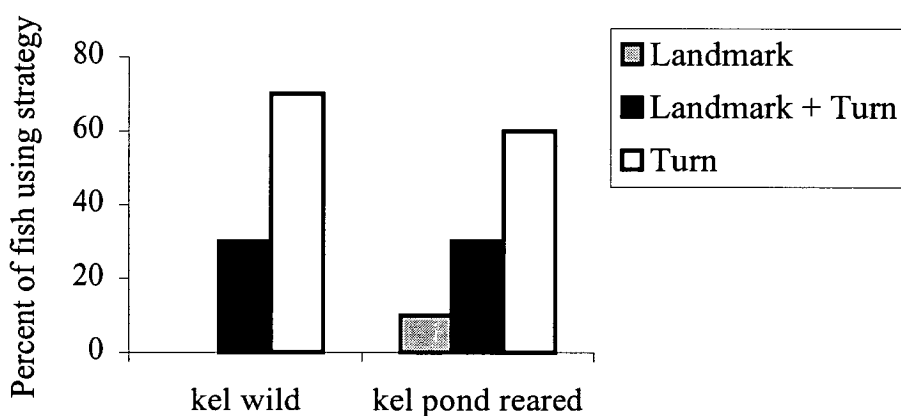


Figure 6.7. Percentage of fish from the two treatments using three strategies: landmarks across all three probe trials (Landmark), both landmarks and turn (Landmark + Turn) or turn across all three probe trials (Turn).

Treatment	Landmarks only	Turn and Landmarks	Turn only	MLE of t
Wild Kelvin	0%	30%	70%	0.86 ($p < 0.001$)
Pond reared Kelvin	11%	33%	57%	0.74 ($p = 0.01$)

Table 6.4. Proportion of fish from the two treatments using each of the three possible strategies and the maximum likelihood estimate of t for each population. The probability that the observed data were generated by $t = 0.5$ (predicted if there is no preference for either cue) is shown in brackets.

iv) Pauses and reversals

Figures 6.8a and b show the mean number of times wild Kelvin and pond-reared Kelvin fish showed pause and reversal behaviour at criterion and across the three probe trials. Wild River Kelvin fish did not pause significantly more often across the probe trials than they had done at criterion (paired t-test; $t_9 = -1.22$, $p = 0.25$).

However, pond-reared Kelvin fish paused significantly more often during the probe trials than at criterion (paired t-test: $t_8 = -2.79$, $p = 0.02$). Neither wild nor pond-reared fish showed significantly more reversal behaviour across the probe trials than they had done at criterion (all p 's > 0.1).

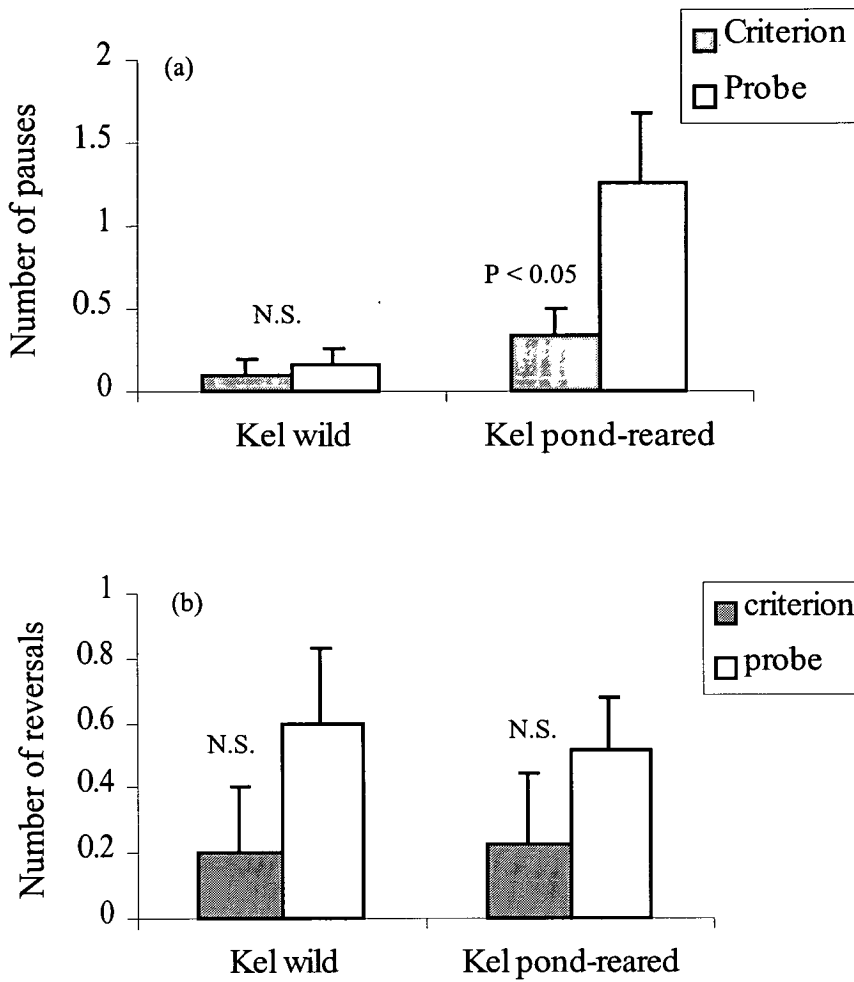


Figure 6.8. Mean \pm S.E. number of pauses (a) and reversals (b) made at criterion and during probe trials.

There were no significant differences between wild and pond-reared River Kelvin fish in the number of reversals they made across the probe trials (ANOVA; pop: $F_{1,17} = 0.08$, $p = 0.78$). However, pond-reared River Kelvin fish paused significantly more often during the probe trials than wild River Kelvin fish (ANOVA; pop: $F_{1,17} = 6.27$, $p = 0.02$).

6.3.3 Discussion

The results of experiment 6b support the suggestion that the use of landmarks by wild River Kelvin fish varies at different times of the year. Wild River Kelvin fish collected and tested in the T-maze in winter show a significant preference for using turn direction. These fish perform with a high level of accuracy after reaching criterion and do not show more pause or reversal behaviour during the probe trials than they did at criterion. This suggests that their performance is not significantly disrupted by the re-positioning of landmarks during the probe trials. This would be expected if, in winter, wild River Kelvin fish pay little attention to landmarks during training. However, there was no significant effect of probe trials on pause or reversal behaviour in the wild River Kelvin fish tested in summer (experiment 6a, p154), even though these fish appear to use both landmarks and turn direction in the probe trials. Therefore, the extent of pause or reversal behaviour during the probe trials may not consistently reflect the types of information being used to solve the task.

The River Kelvin fish that had been bred in the laboratory and reared in ponds also show a significant preference for using turn direction when tested in winter. This raises the possibility that Kelvin fish are genetically predisposed to alter their use of landmarks from summer to winter, although the current data do not allow alternative explanations to be ruled out (see general discussion, section 6.4). Pond-reared Kelvin fish pause more often during the probe trials than wild River Kelvin fish and, unlike wild fish, more often during the probe trials than at criterion. This may be because they are more “confused” than wild fish, by the re-positioning of landmarks in the probe trials. Being reared in a stable pond environment may result

in pond-reared Kelvin fish paying slightly more attention to landmark information than wild Kelvin fish when tested in winter.

6.4 GENERAL DISCUSSION

The results of experiment 6a and 6b provide suggestive but inconclusive evidence for both environmental and genetic influences playing a role in the development of cue preferences by pond and river threespine sticklebacks. Two possibilities are raised: 1) River Kelvin fish may vary their use of landmarks on a seasonal basis and 2) River Kelvin fish may be genetically predisposed to alter their use of landmarks at different times of the year. The following discussion will consider each of these possibilities in turn.

First, the difference in cue use displayed by wild River Kelvin fish tested in winter from that of wild Kelvin fish tested in summer, may reflect a seasonal change in the use of landmarks. A possible confounding factor is an age difference in fish collected at different times of the year. In the wild, sticklebacks generally survive for 1-4 years and catches made in the summer may include adults spanning this age range, although most are likely to be 1-2 years (Bell & Foster, 1994). The winter catches made from the River Kelvin and from the artificial ponds may also include sub-adults that had hatched in the previous spring (aged 6-10 months). It seems unlikely that different age classes within this range should differ in their use of landmarks. However, confirmation of a seasonal change in the use of landmarks by River Kelvin fish would require this experiment to be repeated over multiple seasons, ideally with known age classes. One way of achieving this might be to sacrifice fish

after testing them and age individuals by counting the annual rings on their otoliths (Wooton, 1984).

Seasonal changes in the properties of learned behaviour have been reported in a number of species (e.g. Davachi *et al.*, 1992). However, these studies tend to report a change in learning ability potentially associated with seasonal shifts in the demand for learning and memory as opposed to a change in the types of information used in learning. Why should River Kelvin fish be expected to modify their use of landmarks on a seasonal basis? It is likely that within rivers, habitat conditions differ at different times of the year. An indication of this is shown by the seasonal variation in mean flow rates measured in the Luggie Water, a tributary of the River Kelvin (Figure 6.9).

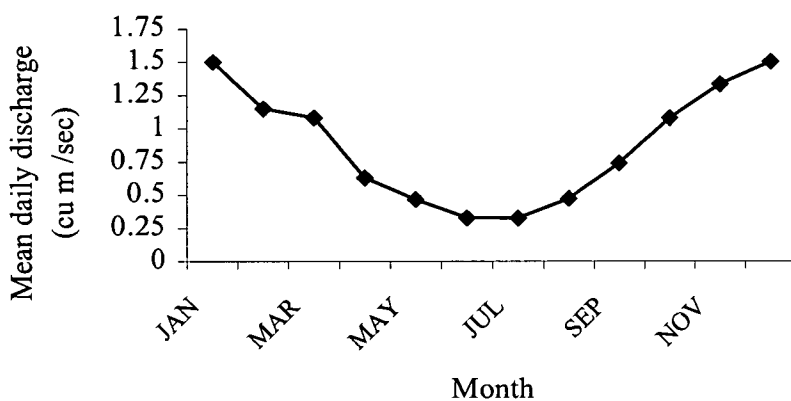


Figure 6.9. Flow data collected from October 1966 to December 1999 for the Luggie water (tributary of the River Kelvin) taken from the National River Flow Archive.

Increased flow rate and turbulence associated with flooding and high water levels common to autumn and winter, may continually disrupt the visual landscape making local landmarks unreliable indicators of location. In summer, decreased water levels and reduced flow and turbulence may mean some areas remain

relatively stable. Within stable microhabitats, local landmarks may present a reliable source of information for locating resources such as refuges and food patches. Moreover, during the summer, male sticklebacks build nests and defend territories while females may need to learn and remember the location of multiple nests in order to spawn with preferred males (Wooton, 1984). Therefore, a more structured use of space associated with reproductive activity may favour the use of local landmarks in learning orientation routes. If landmark use is directly associated with reproduction, it is unclear why Inverleith pond fish appear to use landmarks both during and outside the reproductive season (chapter 3; experiment 6a). However, it is possible that several season-related changes including greater habitat stability and a more structured use of space interact to favour the use of landmarks by River Kelvin fish during the summer.

A possible mechanism by which River Kelvin fish may modify their use of local landmarks is to respond to changes in day length. Artificially manipulated photoperiods affect performance in a number of rodent species trained to learn laboratory based spatial tasks (Davachi *et al.*, 1992). Although in both experiments 6a and 6b the day length was held constant at 12:12 light:dark hours, it may be that the longer-term exposure to natural day lengths prior to being housed and tested in the laboratory, generated the observed differences in the use of landmarks. It is also possible that the difference in cue use displayed by River Kelvin fish tested at different times of the year, would have been even greater had the daylength in the laboratory been matched to that of natural conditions.

The second principle finding from these experiments was that the use of cues by pond-reared Kelvin fish did not significantly differ from that of wild River Kelvin fish tested at the same time of year. Like the wild fish, pond-reared Kelvin fish tested in the summer appear to use both landmarks and turn direction, while pond-reared Kelvin fish tested in the winter show a preference for using turn. This raises the possibility that River Kelvin fish are genetically predisposed to modify their use of landmarks as orientation cues at different times of the year. However, alternative explanations could account for this change in behaviour. One possibility is that conditions in the artificial ponds change from summer to winter such that the availability or reliability of local landmarks varies between seasons. For example, the visual landscape may be substantially altered by season-related changes in leaf litter deposits, plant growth etc. Therefore, pond-reared Kelvin fish may modify their use of visual cues purely on the basis of experience. However, given the results reported in chapter 3, this seems unlikely. Sticklebacks collected from an array of 5 ponds divergent in size and water clarity and exposed to the same external environmental influences as the replicate ponds used in this experiment, appear to use landmark information when tested during the winter (see chapter 3, section 3.3).

It is worth noting that in winter the pond-reared Kelvin fish use landmarks slightly more often than wild River Kelvin fish, although this is not significant. There is also some evidence that the performance of pond-reared Kelvin fish is more disrupted by the insertion of spatially disruptive probe trials than that of wild Kelvin fish. Pond-reared fish paused significantly more often during the probe trials than did wild fish and more often during the probe trials than they had at criterion. Therefore

it may be that experience of a stable pond environment results in pond-reared Kelvin fish paying slightly more attention to landmark information than wild Kelvin fish when tested in winter.

In conclusion, the results of this study tentatively suggest a complex role for both genetic and environmental influences in affecting the propensity of sticklebacks to use local landmarks during spatial learning. First, River Kelvin fish may modify their use of landmarks on a seasonal basis, in response to a change in environmental conditions. Second, the similarity between the behaviour of pond-reared Kelvin fish and that of wild Kelvin fish raises the possibility that Kelvin fish are genetically predisposed to alter their behaviour in response to a seasonal change in conditions. Although more experiments are need to confirm these findings, the suggestions raised from these experiments add support to the claim that learned behaviour is likely to be tightly regulated throughout life, by subtle and complex interactions with genetic and developmental processes.

Chapter 7

General discussion

7.1 INTRODUCTION

In this chapter, a summary of principle findings is followed by a brief discussion of implications, unresolved issues and suggestions for future work. I then consider this work in the context of a wider theme that, for just over a century, has been much discussed but rarely tested; that is the role of learning as a driving force in generating evolutionary change. Two related issues are considered: 1) the evolution of ecological specialisation and 2) the role of learning in influencing rates of genetic change. It is argued that focus on the genotype as the key player in evolutionary processes has overshadowed attention to the phenotype. Consensus is beginning to be established that phenotypes shaped by both genetic and environmentally induced effects are likely to play a considerably more important role in evolutionary processes than has previously been appreciated. Investigations of how learned responses, and genetic and developmental processes interact to shape the phenotype in response to the local environment are likely to shed light on these central, unresolved issues. The chapter concludes with proposals for future directions in the study of learning, highlighting the value of pursuing an integrated approach.

7.2 SUMMARY OF PRINCIPLE FINDINGS

i) Population differences in landmark use

- Threespine sticklebacks originating from ponds use local landmarks in combination with a body-centered turn direction, while river fish show a preference for using turn (chapter 3).
- River Kelvin fish show variation in their propensity to use landmarks when tested at different times of the year. The cue preferences of pond-reared River Kelvin fish match those of the wild parent population tested in winter and in summer (chapter 6).

ii) Landmark stability: A prerequisite for landmark use?

- When trained to use landmarks as goal-directing beacons that are unstable with respect to all other sources of spatial information, most fish regardless of whether they originate from ponds or rivers fail to learn the task (chapter 4; experiment 4a).
- Fish collected from a pond show a preference for using turn direction over local landmark cues or global place cues in cue-conflict probe trials after being trained with all three cues reliably indicating reward location (chapter 4; experiment 4b).

iii) Learning differences in sympatric species pairs

- Benthic and limnetic threespine sticklebacks from two lakes show no differences in the types of information they use to learn a spatial task;

both use local landmark and turn information, with no preference for either cue (chapter 5; experiment 5a).

- Benthics from both lakes learn the spatial task at a faster rate than limnetics collected from the same lakes (chapter 5; experiment 5a).

7.3 IMPLICATIONS, UNRESOLVED ISSUES AND FUTURE DIRECTIONS

i) Population differences in landmark use

It was proposed that differences between pond and river fish in their propensity to use local landmarks when learning a spatial task result from differences in the stability and so reliability of landmarks encountered in their natural habitats. In rivers, fish may periodically be displaced to new and unfamiliar locations and flow and turbulence may continually disrupt the visual landscape making local landmarks unreliable indicators of location.

The pond-river differences in landmark use described in chapter 3 corroborate the findings of Girvan & Braithwaite (1998) and Girvan (1999) who compared the use of landmarks by sticklebacks from two ponds and three rivers in a range of different spatial tasks. In these studies, pond fish appeared to use local landmarks in preference to alternative sources of information (body-centered turns or the direction of water flow) and, or performed better when local landmarks were present than when they were absent. These trends were not observed in river fish.

The possibility that river fish modify their use of landmarks on a seasonal basis warrants further investigation. Comparisons carried out across multiple seasons

testing fish collected from multiple rivers are needed to confirm the predicted correlation between season and propensity to use local landmarks and to reveal the generality of this pattern. Many species alter their use of space on a seasonal basis and, or experience seasonal changes in the requirement for spatially demanding behaviour (Barnea & Nottebohn, 1994; Galea *et al.*, 1994; Smulders, 1995). Seasonal modifications of spatial learning may therefore be a more widespread phenomenon than is currently appreciated. This possibility should be considered when interpreting species or population differences in learning from comparisons made at restricted times of the year.

Although further experimental work is required to confirm a genetic influence on the propensity to use landmarks, the possibility that cue preference in sticklebacks is partly under genetic control raises some interesting issues that are worth considering here. As discussed in the introduction, the threespine stickleback presents a particularly appropriate model organism on which to base a comparative study. Multiple freshwater populations have evolved repeatedly from marine and anadromous ancestral populations and their colonization of recently deglaciated regions limits the time they have had to disperse through freshwater (Bell & Foster, 1994; Bell, 1995). Bell (1995) argues that where there is sufficient geographical isolation between stickleback populations, and where the phenotypic trait being compared is absent in the ancestral marine or anadromous forms, it is reasonable to assume that the trait evolved independently in each population. Therefore, phylogeny can be factored out and similarity between populations used to infer adaptive causes for variation among populations (Harvey & Pagel, 1991). However, the phylogenetic

relationships between the populations compared in this thesis have not been established. As such it remains a possibility that any genetically based phenotypic-environment interactions that are present, evolved only once and spread by dispersal from the point of origin to many similar habitat patches (Bell, 1995). An interesting avenue for future research would be to establish the phylogenetic relationships between the populations compared in this study or to compare additional pond and river populations whose phylogenetic relationships are known. Moreover, an inclusion of marine or anadromous ancestral populations would indicate the evolutionary direction of any genetically based changes in cue preference that do exist. Evidence is growing that natural selection can favour subtle phenotypic differences at the intraspecific level in vertebrates over very small scales and that populations can evolve extremely rapidly (Robinson & Wilson, 1996; Orr & Smith, 1998; Reusch *et al.*, 2001). Therefore, genetic divergence between the populations studied in this thesis is not implausible.

ii) Landmark stability: A prerequisite for landmark use?

The differences observed between pond and river fish in their use of landmarks during spatial learning appears to be conditional not only on the time of year when fish are tested but also on the details of the experimental procedure used to train the fish. When fish are trained to locate a goal by tracking landmarks that are unstable with respect to all other sources of spatial information, most fish regardless of whether they originate from ponds or rivers, fail to learn the task.

It was suggested that pond fish are predisposed to ignore landmarks when they are perceived as being unstable with respect to all other sources of spatial information. A variety of similar biases have been shown to influence the use of landmarks in spatial learning by a range of vertebrates including a preference for using near versus far landmarks (Cheng, 1990; Bennett, 1993; Cheng & Spetch, 1998) or for preferentially referring to the spatial array of landmarks as opposed to their individual features (Cheng, 1986; Brodbeck, 1994; see chapter 4, section 4.2.2). However, it is rarely established whether these predispositions are genetically fixed or whether they are themselves acquired by learning which aspects of a landmark array are most reliable within a natural habitat. A genetically fixed predisposition to ignore landmarks that move might be predicted since, under most circumstances, unstable landmarks are likely to be unreliable indicators of location. One way of testing this would be to investigate whether pond fish, artificially reared in stable laboratory conditions, show the same predisposition to ignore unstable landmarks as their wild parent stock, even without prior experience of unstable visual features in their environment.

The experiments described in chapter 4 raise the possibility that in winter, pond and river fish differ in the degree of landmark stability that must be perceived for landmarks to be used during spatial learning. River fish tested in winter appear to ignore landmarks, even when they are reinforced by a second source of spatial information (turn direction) but this is not true for pond fish. Had pond and river fish been trained to use landmarks that remained stable throughout training with respect to all other sources of spatial information (turn direction and global place cues

outside the maze), fish from both types of habitat may have weighted equal importance to the local landmark cues. Evidence against this possibility is provided by Girvan & Braithwaite's (1998) study. In their experiments, sticklebacks collected from two ponds learned an orientation task faster if landmarks were present than when they were absent, but this trend was not observed in fish collected from two rivers. For both pond and river fish, the landmarks remained in fixed stable positions throughout training. Conversely, the differences reported in chapter 3 between pond and river fish in their use of landmarks, may have been enhanced had the fish been trained with landmarks that remained in a stable position with respect to turn direction and global place cues. Many questions concerning the role of landmark stability in affecting landmark use by both pond and river threespine sticklebacks remain open to investigation.

The results of the experiments presented in chapter 4, investigating landmark stability, reveal the extent to which cue preferences in laboratory based learning tasks can be dramatically altered by what appear to be small differences in experimental procedure. Studies investigating ecologically driven differences in information use during spatial learning should therefore involve a battery of experiments that test cue preferences under a range of experimental contexts and whose design is based on a detailed understanding of the habitat ecology and spatial problems likely to be encountered by the animal in its natural habitat.

iii) Learning differences in sympatric species pairs

Benthic and limnetic species show equal use of both landmark cues and a body centered turn direction. If the encounter rate with potential landmarks does differ between the species, this does not appear to affect their propensity to use landmarks during spatial learning as and when they are available. This would make sense given frequent observations that animals tend to use all the available information to orientate, selectively ignoring only those cues that are unreliable (Able, 1993; Collet, 1996; Von der Emde & Bleckmann, 1998).

It was suggested that the considerable difference in performance by benthics and limnetics in the rate at which they learn a spatial task may reflect the different demands for spatial learning ability made by pelagic and littoral lifestyles. The main difficulty with this study is the problem of removing contextual variables that could also affect performance in a laboratory based spatial task (Macphail, 1982; Kamil, 1998). How can a species difference in performance caused by a difference in learning ability be distinguished from that resulting from differences in motivation or stress? The possibility of contextual variables affecting performance can never be ruled out, but attempts can be made to minimize the problem. So far, suggested methods involve extensive research programs that compare species in a battery of different tests (Kamil, 1988, 1998). A less demanding approach, in terms of the number of experiments needed, is to measure numerous aspects of performance that are likely to reveal species differences in variables other than in learning ability. For example, in chapter 5 it was argued that the lack of differences found between benthics and limnetics, in measures of performance likely to be affected by stress or

boldness, support the suggestion that benthics and limnetics differ in their ability to learn. However, the findings from this study would be strengthened by comparing the performance of benthics and limnetics in a learning task which uses a reward or aversive stimulus known to have equal salience to both species (see chapter 5, section 5.5). In addition, the two species should be tested in a learning task where no species difference is predicted, for example in a simple avoidance task (Brown & Warburton, 1999). This would establish whether the two species differ in their adaptability to laboratory conditions and, or in their general learning abilities.

As discussed in chapter 5, the benthic-limnetic species pairs have been used extensively as a model system for research in studies of speciation and ecological specialisation. Phylogenetic relationships are generally well established. Moreover, experimental techniques have been developed for assessing to what extent the two species are adapted to their respective habitats. For example in “cage experiments”, benthics and limnetics can be reared in enclosures and confined to the two main habitats: the open water and shallow littoral habitat (Schluter, 1995, 1996). Reciprocal transplant techniques can be used to assess the relative influence of genetic and environmental effects on the development of morphological traits and the fitness benefits associated with these traits (e.g. Schluter, 1995; Robinson & Wilson, 1996). In principle, these experimental techniques could be applied to the study of “learning specialisations” in order to investigate the functional significance of species differences in learning abilities. Moreover, where phylogenetic relationships have been established (Taylor & McPhail, 1999, 2000), the evolutionary direction of any genetically based differences in learning abilities that exist between benthics and

limnetics could be traced. In general, the role of phenotypic plasticity including learning has tended to be neglected in studies of adaptive specialisation (see below). Therefore, the incorporation of learning into study systems such as these could prove a useful starting point for understanding the role of behavioural plasticity in evolution.

7.4 OVERVIEW

The overriding message that comes from the main findings of this thesis is that flexible learned responses appear to be fine-tuned or adapted in response to local habitat conditions on a very fine-scale. Populations occupying different habitats show differences in the types of information that they appear to use during spatial learning and species exposed to different microhabitats, even within the same lakes, appear to differ in their ability to learn a spatial task.

In recent years, it has become increasingly apparent that attempts to categorise behaviour as learned or “innate” are likely to prove fruitless (Bateson, 1983; Huntingford, 1993; Shettleworth, 1998a). The results presented in this thesis support this view and suggest that where an association in the environment is sufficiently predictable, flexible learning is guided and fine-tuned by less flexible developmental and, or genetic processes. As such plastic learned responses can not easily be separated from the developmental and genetic influences that are likely to enable, guide and instruct them. In the next section, I will briefly consider how an understanding of the interactions between learned responses, genetic instruction and

developmental processes may help to inform studies addressing wider evolutionary issues, in particular the potential role of learning in generating evolutionary change.

7.5 THE ROLE OF LEARNING IN EVOLUTION

i) Evolution of ecological specialisation

A fundamental endeavor by ecologists is to understand how and why organisms evolve to become specialised to particular niches (Futuyma & Moreno, 1988). Phenotypic plasticity including learning is generally assumed to facilitate a generalist lifestyle and enable expansion of niche breadth (West-Eberhard, 1989; Kelley & Farrell, 1998). For example, insects that learn to recognize suitable host plant species on which to lay eggs are more likely to incorporate a novel potential host species into their search program than those expressing genetically fixed preferences (Parmesan *et al.*, 1995). However, constraints on learning and memory may result in individual learners specialising on resources even when equally valuable alternatives are available in the environment (Heinrich *et al.*, 1977; Lewis, 1986). For example, the cabbage butterfly, *Pieris rapae*, shows flower constancy by continuing to visit the flower species with which it has had experience even when other equally rewarding flower types are available (Lewis, 1986). In this case, learning to extract nectar from a second species interferes with the ability to extract nectar from the first.

Predispositions and perceptual biases that enable and direct learning may encourage specialisation. Indeed such predispositions are likely to have been selected because of the advantages associated with concentrating efforts on the most rewarding resources. In Heinrich *et al.*'s (1977) study, bumblebees showed a

preference for blue over white flowers. Blue, in contrast to white, is learned rapidly and the bees trained to blue do not switch to white flowers even when the white is rewarded with more food than the blue (see also Weiss, 1997). Within environments that consist of distinct habitat patches, the costs of interference to learning incurred by habitat switching may result in individuals specialising on particular patches. For example bluegill sunfish, *Lepomis macrochirus*, are generalist predators, capable of foraging on a wide array of prey types in the littoral vegetation and open water limnetic habitats of North American freshwater lakes (Ehlinger, 1990). However, each habitat requires different searching techniques for successful foraging and appropriate adjustment of these techniques through learning can take several days. Experiments presenting fish with artificial littoral and open water habitats have shown individuals to differ in their habitat selection. Habitat choice correlates with differences in morphology associated with habitat-specific foraging efficiency (Ehlinger, 1990). Therefore, individual bluegills appear to select the habitat for which they are morphologically and behaviourally best adapted. Moreover, individuals are not equally flexible in making learned adjustments to specific habitats, implying some degree of specialisation of learning for the different habitat types (Ehlinger, 1989).

Overall, the way in which learning may affect resource use and habitat selection is likely to be complex but for the most part remains a neglected aspect in studies of the evolution of ecological specialisation (Futuyma & Moreno, 1988). It has become increasingly apparent that the effects of learning on foraging efficiency can cause foragers to choose diets and patches that are not predicted by conventional

foraging theory (Hughes, 1979; Johnson, 1991). This will almost certainly have implications for the evolution of ecological specialisation. The extent to which an ability to learn can facilitate niche shifts and enable generalized lifestyles is likely to depend on the extent to which costs of interference to learning, exceed the costs associated with specialisation such as increased travel time between resources, reduced resource availability etc (Papaj, 1990). Studies addressing issues concerning resource and habitat specialisation are likely to benefit from a more thorough understanding of the limits to learning and memory in addition to the extent to which learned behaviour is genetically constrained and fine-tuned in response to particular ecological problems.

ii) The effect of learning on rates of genetic change

Learning has been proposed to both slow down and speed up rates of genetic change. Phenotypic plasticity including learning may act as a “buffer” which shields genetic variants from selective pressure. The uncoupling of the genotype from the phenotype may therefore limit the impact of natural selection on the genetic structure of populations by reducing the selective differential between genotypes (Anderson, 1995). However, in changing environments, some of these protected variants may turn out to be better adapted to the new environmental conditions. Hence learning could also facilitate the process of adaptive genetic change (Anderson, 1995). A related idea is that new selection pressures induced by changes in behaviour which may or may not be learned, will often drive subsequent morphological changes facilitating occupation of the new ecological niche or adaptive zone (West-Eberhard, 1989; Wcislo, 1989).

Theoretical models have also investigated whether an originally learned response can evolve into a genetically fixed trait provided the environmental conditions favouring that trait remain constant (Papaj, 1993). Moreover, learning has been proposed as a potential agent in facilitating speciation either through sexual imprinting (Irwin & Price, 1999; Owens *et al.*, 1999) or through increasing fidelity to sites where both mate selection and egg laying occur (Papaj & Prokopy, 1989).

The idea that learning may act as a driving force in evolution is not new and was originally proposed over a century ago by Baldwin (1896) and independently by Morgan (1896) and Osborn (1896). Suspicions of it being Lamarckist meant this line of theory faded with the rediscovery of Mendel and the subsequent development of the modern evolutionary synthesis. Recently, renewed interest has led to theoretical models that provide some support for a role of learning in directing and generating evolutionary change (Hinton & Nowlan, 1987; Anderson, 1995; Ancel, 1999). However, given the magnitude of these claims, the potential role of learning as a driving force in evolution has received surprisingly little empirical investigation (Wyles *et al.*, 1983; Lefebvre *et al.*, 1997). A major difficulty is the scarcity of biological systems likely to provide the requirements that a test of this hypothesis would demand. However, as discussed in section 7.3, learning could in principle be incorporated into evolutionary studies of adaptive specialisation and speciation. Inclusion of learning into studies such as these, combined with empirical investigations of how animals use learned behaviour within their natural environments and how flexible learning interacts with genetic instruction are likely

to provide crucial starting points in establishing the plausibility of what so far have remained theoretical propositions.

7.6 A MANIFESTO FOR THE STUDY OF LEARNING

As implied in the previous section, phenotypic plasticity including learning has tended to remain a neglected area in studies of adaptive systems and evolutionary processes. Several authors have repeatedly argued that focus on genetically determined traits as the key players in evolutionary processes has occurred at the expense of recognizing the potentially important role played by phenotypic plasticity (Stearns, 1989; Wcislo, 1989; Schlichting & Pigliucci, 1998). Developing a better understanding of the adaptive function and evolution of learning is likely to be an important starting point for understanding its potential role in driving genetic change. In the final section of this discussion, future directions in the study of learning from an evolutionary and ecological perspective are briefly proposed, addressing each of Tinbergen's (1963) four questions of ethology in turn.

i) Mechanism

Current understanding of the neural mechanisms and substrates that underlie learning generally lags behind our understanding of learning at the behavioural level.

Considerable effort has been directed towards identifying specialist neural systems and brain areas necessary for spatial learning, birdsong learning and imprinting (Sherry *et al.*, 1989; Bolhuis, 1991; Bingman, 1992; Brenowitz & Kroodsma, 1996).

However, many of these studies have raised as many questions as they have answered (Bolhuis & Macphail, 2001). For example, sex differences and seasonal

changes in the size of a number of “song control nuclei” (brain regions traditionally thought to underpin song learning) were originally interpreted to reflect variation in the demand for learning song repertoires (Nottebohm, 1981, 1989). However, the relationship between the size of these neural substrates and song learning remains ambiguous. For example, seasonal changes in the song control nuclei have been found to occur in species that sing all year round and in adult birds that learn songs only during a restricted period in their development (Brenowitz *et al.*, 1998). Likewise, the relationship between qualitative and quantitative features of the hippocampus and spatial memory remains contentious. Bolhuis & Macphail (2001) reviewed recent evidence that suggests the hippocampus is not crucial for memory storage, but that it might be involved in processing contextual or spatial input. Indeed, the extent to which adaptively specialised brain regions designated for particular learning tasks are likely to exist is itself some matter of debate (Bolhuis & Macphail, 2001). However, given the difficulty of interpreting learning abilities based on performance in cognitive tasks, being able to make qualitative and quantitative measurements of specific neural substrates presents an appealing alternative. Therefore, evolutionary studies of learning are likely to benefit considerably from a better understanding of the neural structures that underpin specific learning abilities.

ii) Development

The influence of genetic, developmental and environmental influences on learning is generally poorly understood. There is considerably more scope for experiments that employ artificial selection techniques or the “controlled rearing approach” described

in chapter 6 to identify the causal basis of differences in a learned response. Studies addressing the ontogeny of a learned behaviour may do well to concentrate on a relatively simple behavioural response in an organism with a short lifespan, conducive to artificial selection and controlled rearing procedures. Insect taxa that learn host plant cues for ovipositing may prove ideal for such research (Papaj & Lewis, 1993).

iii) Function

The adaptive value of learning has received relatively little empirical attention. The technique of manipulating the trait of interest and assessing the fitness advantages associated with the presence or absence of the trait has been well established in behavioural ecology but rarely applied to learning. The few studies that have been carried out suggest applying this approach may prove informative. For example, growth or reproductive success have been reported to be higher in experimental environments where important resources like mates or oviposition sites can be associated with simple cues compared to when the same resources are unpredictable (Hollis, 1984, 1999; Hollis *et al.*, 1997; Dukas & Bernays, 2000; Dukas & Duan, 2000). A similar approach would be to compare fitness measures in closely related species that differ in their ability to learn, for example, generalist and specialist bumblebees (Lavery & Plowright, 1988). Manipulating environmental variability, such as the number of nectar-rewarded flower species (in the case of bees), might enable an assessment of both the adaptive function and potential costs of learning under different environmental conditions.

iv) Evolution

Numerous theoretical models have investigated the kinds of environmental variability under which learners will be at a selective advantage compared to non-learners (McNamara & Houston, 1987; Stephens, 1987, 1991, 1993; Mangel, 1990; Bergman & Feldman, 1995; Luttberg & Warner, 1999). However, these models have received little empirical support (e.g. Papaj, 1986). At least in some cases, it may be possible to quantify environmental variability for example by using measures such as diet breadth or foraging patch duration (Laland *et al.*, 1996). Using an appropriate measure of learning ability, the relationship between environmental variability and learning across species or between populations could in principle be analysed using the comparative method. Alternatively, it may be possible to carry out selection experiments. In species with a relatively short life-time such as *Drosophila* it may be possible to manipulate environmental variability in the laboratory and assess what effect this has on the inherited propensity to learn (e.g. Mery & Kawecki, 2002).

Very few attempts have been made to critically examine the costs of learning despite the fact that relationships drawn between ecological demand and learning and memory abilities imply that a species' learning and memory capacity represents some trade-off between benefits and costs. Johnston (1982) conducted a thorough review of the possible costs of learning but emphasized the scarcity of direct empirical evidence. At least two of the costs of learning proposed by Johnston (greater complexity of the genome and developmental fallibility) seem highly speculative given how little is still known about the physiological and neural bases of learning and memory in most animals. More recently, Dukas (1999) discussed the

probable costs of memory drawing parallels with molecular and physiological information systems such as cellular DNA whose integrity is known to involve an active and costly process of maintenance and repair. Again, this is a rather speculative account unsupported by empirical observations.

The lack of direct evidence for the costs of learning is likely to be due in part to the difficulty of manipulating learning ability. Laverty & Plowright (1988) evade this problem by comparing the foraging performance of a learning and non-learning species of bumblebee on a particular species of flower. Initially specialist foragers were able to locate the nectar reward at a dramatically faster rate than generalist bees that relied on learning, even though none of the bees had had previous foraging experience. Although learners eventually reached the same level of performance as the non-learning specialist bees, they took considerably longer to do so.

The fact that animal learning and memory abilities are limited and that animals show differences in learning ability and biases in what they learn provides perhaps the most compelling indication yet that a capacity to learn entails costs (Kamil, 1998). However, studies that directly address the costs of learning are notably lacking and so far this remains a relatively untested assumption of comparative studies that draw relationships between learning and memory abilities and ecological demand.

There is clearly considerable scope for future research on learning from an ecological and evolutionary perspective. An integrated approach, considering learning at all four levels of enquiry, is likely to provide the most instructive route for establishing exactly how and when animals should use learning in order to adapt to environments that are subject to varying degrees of change. Moreover, a greater effort to incorporate learning into evolutionary studies is likely to reveal new insights on a wide range of topics ranging from optimal foraging in behavioural ecology to the adaptive function and evolutionary consequences of phenotypic plasticity in a variable environment.

7.7 SYNOPSIS

This thesis describes a series of experimental studies that investigate learning from an ecological and evolutionary perspective. Studies based on this approach provide evidence that learned responses are fine-tuned or adapted in response to local habitat conditions on a fine-scale. The precise nature of this “fine-tuning” differs between species and populations exposed to different environmental conditions. Overall, the results of these experiments support the growing consensus that behavioural plasticity and genetically fixed instruction lie at two ends of a continuum. Learning appears to operate in close conjunction with genetic and, or developmental processes that enable, and direct it in response to particular ecological problems. Learning and phenotypic plasticity in general has been a neglected aspect in studies of adaptive systems and evolutionary processes. Approaching learning from an ecological and evolutionary perspective is likely to prove valuable not only in understanding how organisms use learning to improve the match between their phenotype and the environment but also in shedding light on the evolutionary consequences of phenotypic plasticity in a variable environment.

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Appendix I

Runs of correct scores by fish in 24 trials

Fish	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	% correct
1	1	1	0	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	92
2	1	0	0	1	1	0	0	1	0	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	71
3	1	0	1	1	0	1	1	1	1	0	1	1	0	1	0	1	1	1	1	1	1	1	1	1	79
4	0	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	88
1*	1	1	1	1	1	1	1	1	1	1	0	1	0	1	0	1	1	0	1	1	1	1	1	1	83
2	0	0	1	0	0	1	0	1	0	1	0	1	0	0	0	0	1	0	1	1	1	1	1	0	46
3	0	0	1	0	0	1	1	0	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0	1	71
4	0	1	0	1	1	1	0	1	1	1	1	0	0	1	1	1	1	1	0	1	1	0	0	1	67
1	0	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	88
2	1	1	0	1	1	1	1	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	88
3	0	1	0	0	0	1	0	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	71
4	0	1	0	1	0	0	0	1	0	1	1	0	1	1	0	1	0	0	1	1	1	1	1	1	58
1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	92
2	1	1	1	1	0	0	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	88
3	0*	0	1	0	0	1	0	1	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	52
4	0	0	0	1	1	0	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0	1	1	1	33
1	0	0*	0	0*	1*	*	1*	*	1	1*	0	0	0	1	1	0	1	1	1	1	1	1	1	1	58
2	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	1	0	1	1	25
3	1	0*	0	1	0	0	1	1	0	0	1	0	1	0	0	0	0	0	0	1	0	0	0	0	30
4	1	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	79
1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	92
2	0	1	1	1	1	0	0	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	83
3	0	1	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	1	0	1	1	1	1	1	42
4	1*	1	0*	0	1	1	0	0	0	0	0	0	1	0	1	0	1	0	1	0	1	1	1	1	55
1	0	0*	0	0	0	0	1*	1	1	1	0	1	0	1	0	1	0	1	0	1	0	0	1	1	45
2	1	1*	0	0	1	0	0	1	0	0	0	0	0	0	0	1	0	1	0	1	1	1	0	0	39
3	1	0	0	0	0	1	0	1	0	0	1	1	0	0	1	0	1	1	0	1	1	1	1	1	54
1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	92
2	1	0	1	0	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	83
3	1	0	1	1	1	0	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	88
4	0	0	0	0*	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	65

Table A1. The arm choices made by fish tested in the preliminary experiment described in chapter 2 (1 = correct choice; 0 = incorrect choice; * = no arm entered in 10 minutes of testing). The last column shows the percentage of trials performed correctly across the 24 trials.

Appendix II

Tagging fish

In the experiments described in chapters 3 and 4, fish were individually tagged with coloured plastic rings. These rings were obtained from the coloured plastic that coats the wire inside electric cables. First, the outer sheath was stripped from the cable to reveal the inner plastic-coated wires. Short lengths of these smaller wires were cut using a razor, and forceps were used to remove the copper wires from inside, leaving a hollow plastic sheath. Each coloured sheath was cut into sections of 2 mm in length to form rings. The rings were dropped in 70 % ethanol before being used to tag fish, to minimise the risk of causing infection.

For marking, fish were gently held in a hand-net and a mounted needle was used to hold the selected spine upright, while the coloured plastic ring was slipped on using forceps. A variety of colours were available and rings could be applied to different spines; the 1st or 2nd dorsal or the left or right pelvic spine, thereby allowing the identification of large numbers of individuals. Each ring was dabbed in superglue before being transferred to the spine since applying too much pressure to secure the tags risks damaging the delicate membrane that runs between the spines. To further minimise the risk of infection, tagged fish were transferred to a water bath containing a high concentration of Protozin anti-fungal water treatment (Animal House Ltd., UK) for several minutes being returned to their home tanks.

Appendix III

Breeding protocol

The procedures and apparatus involved in breeding fry were originally developed by Iain Barber. Adult male and female sticklebacks were collected from the River Kelvin (NS 54 70) and from Inverleith Pond (NT 24 75) during their reproductive season in June 2000, using 3 mm-mesh dip nets and standard minnow traps. Males were housed singly in holding aquaria of size 46 x 30 cm and 30 cm high, and maintained on a diet of defrosted frozen bloodworm. Each aquarium was provided with nest-building materials; three to four sand-filled petri-dishes, cotton thread and filamentous pond weed (*Spirogyra* spp.). Females were housed in groups of 5 in aquaria of size 30 x 92 cm and 39 cm high and fed copious amounts of bloodworm and live daphnia 3 times a day. The temperature was maintained at 17-20°C and overhead lighting was provided by 40-W fluorescent tubes, operating on a 16:8 hour light:dark cycle. After a few days of exposure to these conditions, males showed intense breeding colouration; a greenish-blue back, bright blue irises, and a red anterior ventral surface (Wooton, 1984). Females simultaneously became gravid, which was indicated by a lateral swelling of the abdominal area caused by egg production.

In order to stimulate the males to build nests, gravid females were placed in transparent glass jars and presented to males by floating the jar in the males' aquaria for 5 minutes, three times a day. Within two weeks, 12 males had established nests in their home tanks. If females displayed a receptive "head-up" posture on being presented to the males (Wooton 1984), they were gently released from the glass jar.

Females were removed immediately after spawning or after 20 minutes had elapsed, and returned to their home tanks. Males were left to care for the eggs for the first 24 hours after spawning, unless they were observed to cannibalise eggs in which case the clutch was immediately removed from the nest. Males ventilate clutches by fanning and remove eggs that have died and become mouldy (Wooton, 1984). Since males tended to cannibalise eggs after this period, egg clutches were removed from the nests 24 hours after spawning and transferred to 1-mm mesh-lined petri-dishes (3 cm in diameter and 1.5 cm in depth). These were suspended in plastic trays of size 22 x 18 cm and 8 cm high and gently aerated from below (Fig. A3.1). The airstone was placed slightly to one side of the clutch as a direct air stream can result in air bubbles being trapped in the egg clutch which can cause the eggs to float to the surface and dry out. Eggs that exhibited arrested or abnormal development were removed daily.

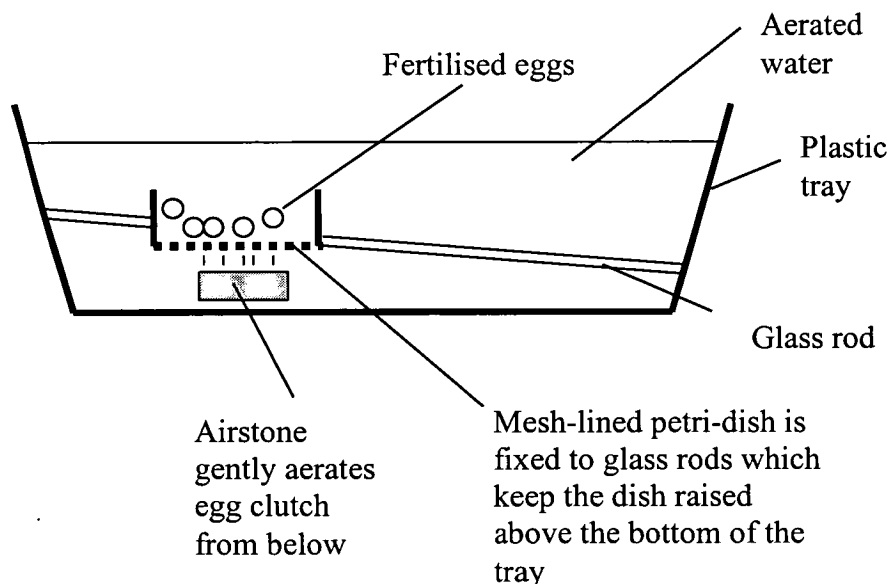


Figure A3.1. Diagrammatic representation of egg incubator

Eyespots developed within 2 days and the fry hatched in about a week. Fry were transferred to small tanks of size 30 x 19 cm and 20 cm high and fed for several days on *Paramecium* before being switched to a diet of newly hatched brine shrimp (*Artemia* spp., box A3). After 2 weeks of growth, hatchlings were fed a combination of brine shrimp and finely chopped defrosted frozen bloodworm. Any excess food, dead fry or debris was removed daily by suction. At least one third of the water in each tank was replaced daily with water that had been aerated for at least 4 days.

Brine shrimp cultures were obtained by adding small capfuls (2 cm in diameter x 1.5 cm in height) of brine shrimp eggs (INVE Aquaculture, inc., USA) to jars containing freshly collected sea-water. These jars were maintained at a temperature of 28°C in a water bath heated with an electric aquarium heater (Tetrathec Heater Thermostat, Animal House Ltd., UK). The water in each jar was strongly aerated such that the eggs were continually suspended and prevented from sinking to the bottom of the jar (Fig A3.2). The jars were situated directly under 60-W fluorescent tubes, operating on a 16:8 hour light:dark cycle. The combination of warmth and light, generally induced hatching after 24 to 48 hours. In order to rinse the shrimp in freshwater and remove excessive traces of seawater, which would damage the fry, the entire contents of each jar was poured through a series of filters. Shrimp and egg cases were rinsed and transferred to a new jar of freshwater. After a few minutes the shrimp sank to the bottom of the jar and the egg cases floated to the top. Samples of shrimp relatively uncontaminated by noxious egg cases could be obtained by pipetting the bottom sediment.

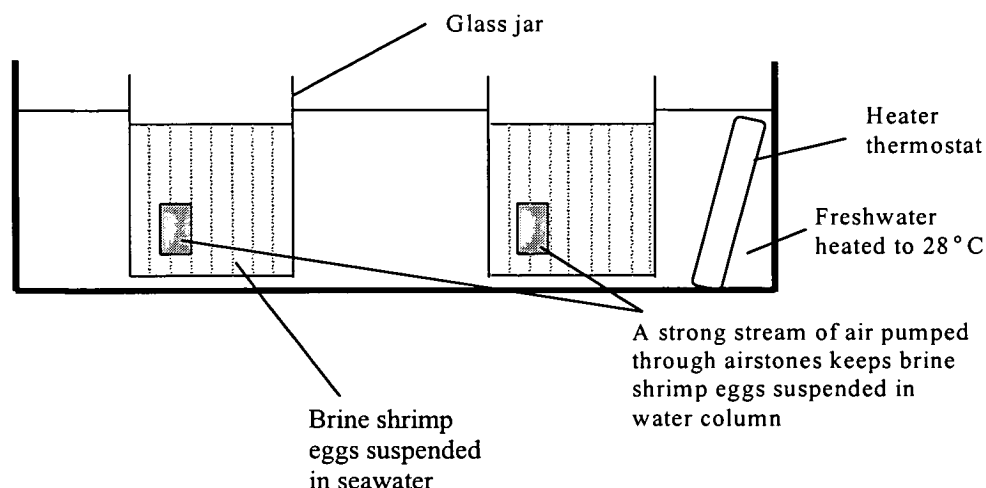


Figure A3.2. Diagrammatic representation of apparatus used to cultivate brine shrimp.

Box A3.1. Procedure used to cultivate brine shrimp.

Appendix IV

Artificial ponds

Eight replicate ponds were constructed, four of which were used for the experiment described in chapter 6. These ponds were sunk into a previously existing concrete walled rectangular pond of size 7.5 x 7.5 m and 0.5 m high, and supported by a scaffolding framework (Mitie suppliers, UK).

Scaffolding framework

Figure A4.1 shows a diagrammatic representation of the scaffolding framework that was constructed over the pre-existing pond. Five short lengths of scaffolding (3.6 m long) were placed across the pond. Two of these lengths were placed alongside the edges of the pond (lengths A). The other 3 lengths (lengths B) were placed across the pond, supported at one end by the walled surround and at the other end by scaffolding “feet” (Box A4.1). Three longer lengths of scaffolding (lengths C), measuring 7.75 m were placed across the pond perpendicular to the short lengths. One long length (C) was fastened to the three feet supporting the short lengths (B). The other two long lengths were positioned midway along the short lengths. A further three feet supported these long lengths of scaffolding and attached them to the shorter lengths. Scaffolding lengths were fastened to each other and to the feet using universal clamps (Mitie suppliers, UK). Six planks of wood (0.13 x 0.05 m and 3.87 m long) were placed over the scaffolding framework between the two central long lengths of scaffolding to make a central walkway (Fig 6.1, chapter 6). Pairs of planks were fastened together at their mid-point using brackets.

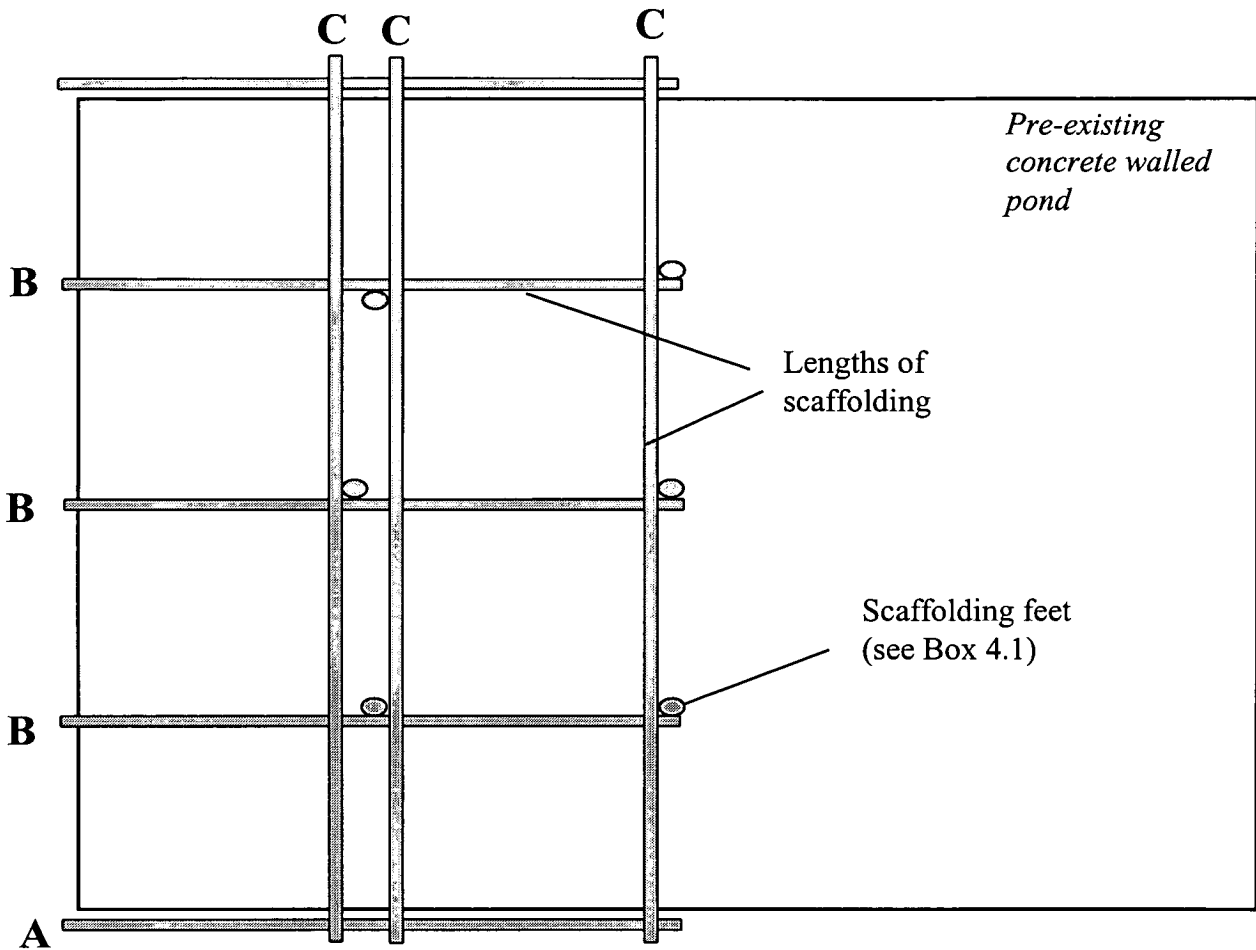
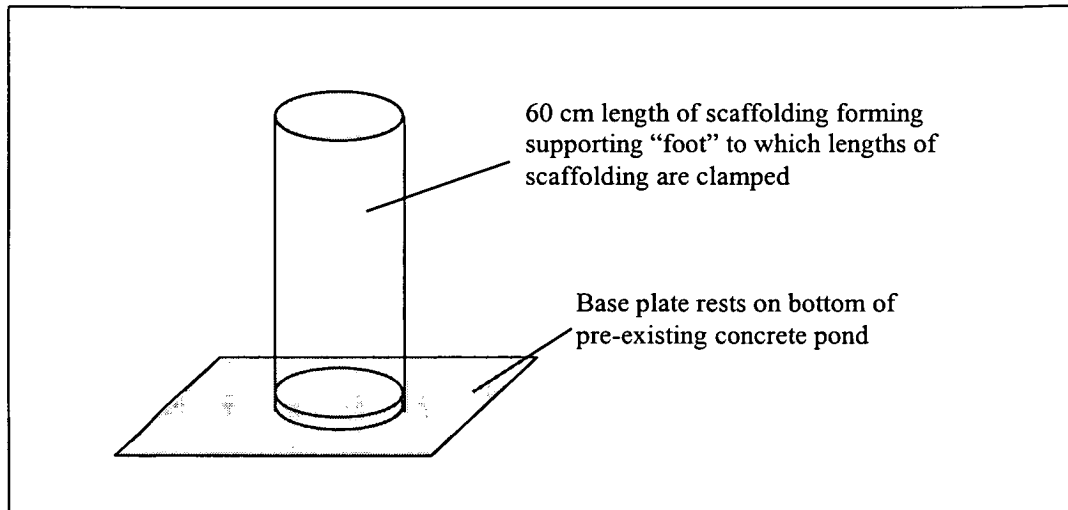


Figure A4.1. Diagrammatic representation of scaffolding framework built over pre-existing concrete walled pond.



Box A4.1. Diagrammatic representation of scaffolding feet

Artificial ponds

Replicate ponds were constructed by stapling Lotus pond liner to wooden frames. For each pond, two wooden frames were made from lengths of (2 x 5 cm) garden timber (B&Q suppliers, UK). For the base, two lengths of wood measuring 1.75 m were nailed to two shorter lengths measuring 1.12 m to form a rectangle. A sheet of pond liner (1.8 x 1.20 m) was stapled to the frame and pulled taut. For the upper frame, two lengths of wood measuring 1.84 m in length were nailed to two smaller lengths measuring 1.12 m. Overhanging edges of 4 cm in length were created so that the frames could be supported on the scaffolding framework (Fig. 4A.2). Rectangular strips of pond liner measuring 0.6 x 5.76 m were stapled to the inside perimeter of the upper frame to form the four walls of the pond. The meeting edges of the pond liner were stapled to a wooden strut (55 cm in length). The bottom edge of the pond liner strip was then stapled to the pond base (Fig 4A.2). Silicone Aquaseal (Animal House Ltd., UK) was applied to the stapled surfaces of the pond liner to ensure there were no holes present through which the fry could escape. The ponds were filled

with tap water and submerged into the pre-existing concrete walled pond, with the overhanging edges of the wooden frames resting on the scaffolding framework. Each pond was lined with gravel and furnished with rocks and weed. The water in each pond was left to mature and de-chlorinate for at least 6 weeks before any fry were transferred.

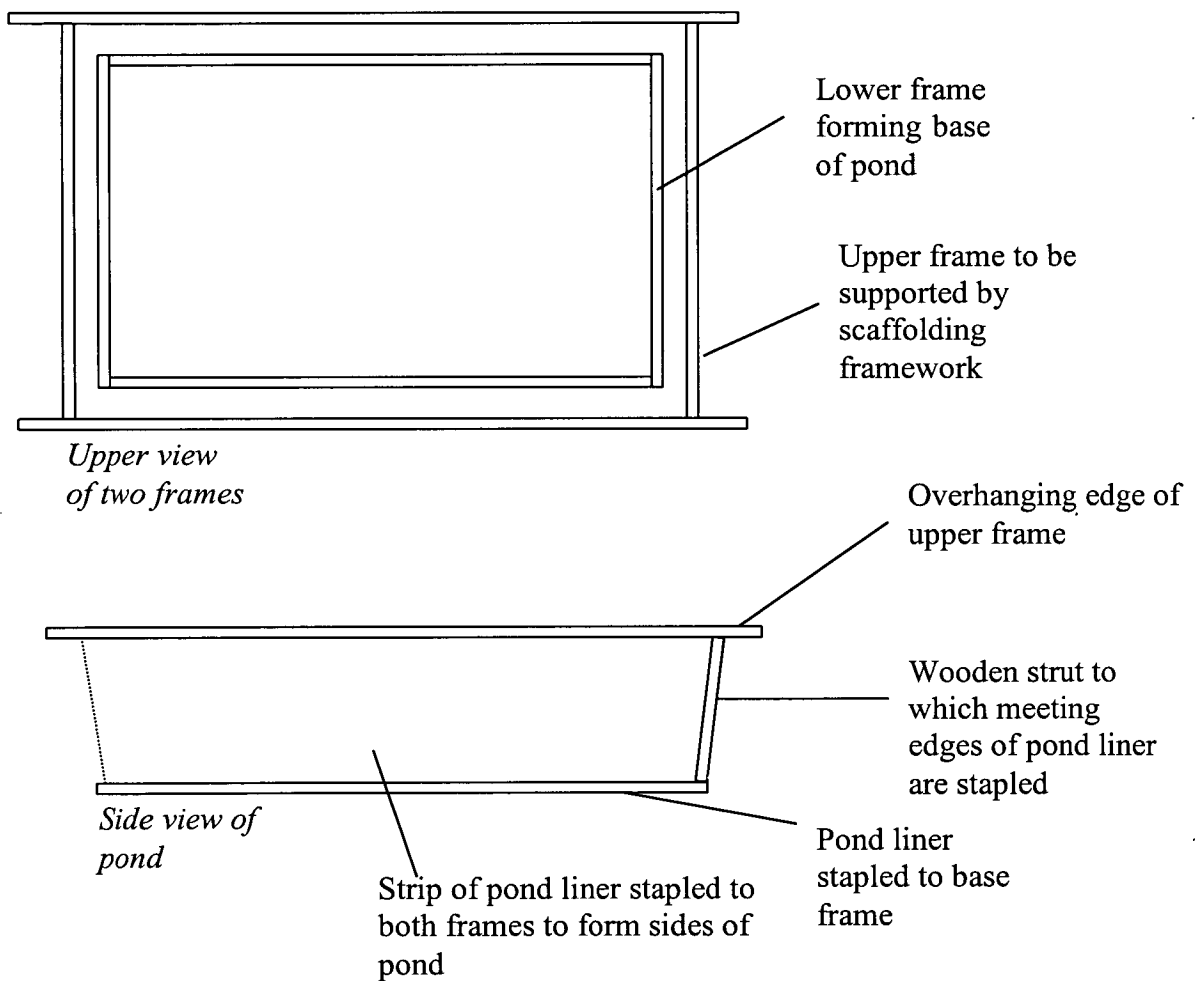


Figure 4A.2. Diagrammatic representation of the ponds, constructed by stapling strips of pond liner to two wooden frames.