

# Sympatric species of threespine stickleback differ in their performance in a spatial learning task

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Received: 10 March 2008 / Revised: 10 June 2008 / Accepted: 3 July 2008 / Published online: 1 August 2008  
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**Abstract** Increasing evidence suggests that cognitive function is under selection in diverse taxa and that this results in different cognitive abilities in taxa experiencing different selective environments. For example, species inhabiting spatially complex environments might be expected to have good spatial learning ability. We investigated whether local habitat conditions influence learning by comparing the performance of two recently diverged species of threespine stickleback (*Gasterosteus aculeatus* complex) in a spatial learning task. The two species reside sympatrically in several lakes. Benthics occupy the spatially structured vegetated littoral zone, whereas limnetics occupy the spatially homogenous open-water pelagic zone. We trained fish to locate a hidden reward in a T-maze and asked whether they did so by learning a body-centred pattern of movement (turn left or right) or by using plant landmarks. Both benthics and limnetics used turn and landmark cues, but benthics learnt the task almost twice as quickly as limnetics. This difference is consistent with the hypothesis that benthic and limnetic sticklebacks are equipped with

spatial learning abilities well suited to the spatial complexity of their littoral and pelagic habitats. Our findings add to the understanding of the evolution of learning.

**Keywords** Spatial learning · Stickleback · Cognitive ecology

## Introduction

Behavioural ecology has been dominated by studies that investigate the adaptive value of genetically determined traits. Less attention has been given to acquired or learned behaviours and to the dynamic interactions that may occur between an organism and its environment throughout its life. However, this has been changing in recent years. The evolutionary significance of phenotypically plastic traits, including behavioural plasticity, has been a focus of recent inquiry (Schlichting and Pigliucci 1998; Stamps 2003; Jablonka and Lamb 2005). Not surprisingly, the expression of behaviours has been shown to depend on ecological conditions in diverse taxa (Dukas 1998). For example, several studies show that females modify their choosiness in response to costs of choice (Milinski and Bakker 1992), and males modify courtship behaviour in response to threats of predation (Godin 1995). However, the role of environment in shaping learning ability has received less attention.

The interaction between environment and learning is beginning to be addressed in studies that take an ecological approach to learning (Dukas 1998; Shettleworth 1998; Healy and Braithwaite 2000) and ask how an animal should learn based on an analysis of its ecology and life history. For example, several studies have revealed a positive relationship between the size of the hippocampus, the part of the brain required for processing spatial information in

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Communicated by T. Bakker

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birds and mammals, and the expected demand for spatial learning based on an analysis of life-history strategies (Jacobs et al. 1990; Healy and Krebs 1993; Rebores et al. 1996; but see Bolhuis and Macphail 2001). Comparing performance in laboratory-based learning tasks is a more direct approach and reveals similar correlations. In particular, food-storing bird species often outperform non-storers or less specialist storers in a range of laboratory-based spatial learning and memory tasks (reviewed in Krebs et al. 1996; but see Bolhuis and Macphail 2001). In addition to differences in learning and memory abilities, species differences in the types of information used when learning have been correlated to the availability or reliability of cues within the species' natural habitats (Cole et al. 1982; Brodbeck 1994; Clayton and Krebs 1994; Shettleworth and Westwood 2002).

Central to these comparative studies is the assumption that a species' learning and memory capacity represents a trade-off. Producing, maintaining and operating the neural machinery required for cognitive abilities is expected to be energetically costly such that animals should invest in these abilities only as much as their ecologies and lifestyles demand (Robinson and Dukas 1999; Mery and Kawecki 2003). Moreover, species' predispositions to pay attention to certain cues or to learn certain associations in preference to others are likely to filter out uninformative or unreliable sources of information, reducing the risk of making mistakes, wasting time and processing redundant information.

A better understanding of the developmental and selective forces that shape learning is likely to inform our general understanding of phenotypic evolution (Schlichting and Pigliucci 1998). However, despite claims that a growing area of research concerned with cognitive evolution warrants the naming of a new field (Healy and Braithwaite 2000), our understanding of the evolutionary and developmental processes that shape learning is surprisingly limited. Comparative studies of natural populations are key to improving this understanding (e.g. Krebs et al. 1990; Healy et al. 1994; Micheli 1997). We can strengthen our inferences of the selective causes of learning by replication and appropriate controls and by considering the phylogenetic relationships among taxa studied. Furthermore, comparative studies have tended to concentrate on a few 'special-case' taxonomic groups, in particular food-storing parids and corvids, mainly because of the impressive 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). Our current understanding leaves open the possibility that learning is modified in response to ecological conditions only in such specialists and is not a general phenomenon. Broadening our scope to include other taxa will allow us to test the generality of findings.

Fish are an ideal taxonomic group in which to address such questions. They are the largest group of vertebrates and live in diverse habitats, exposing them to varying intensities of selection for learning. Furthermore, they show learning in various contexts, including learning foraging skills (Mackney and Hughes 1995; Warburton 2003) and learning to recognize predators (Chivers et al. 2002), kin (Frommen et al. 2007), rivals (Jenkins and Rowland 1996), and potential mates (Jenkins 1997). Threespine sticklebacks have been a particular focus for such studies. For example, threespine sticklebacks may learn aspects of display behaviour (Losey and Sevenster 1995) and modify mate choice based on short-term memory of previously encountered potential mates (Milinski and Bakker 1992). Spatial learning has also been demonstrated for threespine sticklebacks (Braithwaite and Girvan 2003).

Here, we use a microevolutionary approach to investigate local adaptation for spatial learning in threespine stickleback species pairs (*Gasterosteus aculeatus* species complex). This approach avoids many of the problems associated with comparative studies of learning (Kamil 1998; Lefebvre 1996; Bolhuis and Macphail 2001). These species pairs reside sympatrically in several lakes in the Strait of Georgia region of south-western British Columbia (Schluter and McPhail 1992; McPhail 1993). Genetic, behavioural and ecological data indicate that the limnetics and benthics within a lake are good biological species (McPhail 1984; Hatfield and Schluter 1999; Taylor and McPhail 2000; Rundle et al. 2000; Gow et al. 2007). A key advantage of this system is that multiple speciation events provide evolutionary replication. Each limnetic population has evolved from the marine ancestors independently yet arrives at a similar endpoint, suggesting that all limnetic populations have experienced similar selection pressures. This is also true for benthics. Thus, we can compare multiple, independently derived limnetic populations and multiple benthic populations. This evolutionary replication lends power to testing evolutionary hypotheses.

The two species show extensive morphological, behavioural and habitat differences (McPhail 1984, 1992, 1994; Schluter and McPhail 1992; Schluter 1996). Benthics are large and deep bodied, with a few short gill rakers, wide mouth and short broad snout. They 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. Limnetics are morphologically and behaviourally better adapted for pelagic prey with a small and slender body, a narrower mouth and longer, more numerous gill rakers (Schluter 1996). They tend to be more social than benthics (Vamosi 2002). Limnetics live in a relatively homogenous environment in terms of spatial complexity, predominantly in the water column where they feed mainly on plankton.

We tested two predictions about how the two different habitats may shape spatial learning, based on the fact that benthics encounter a greater abundance of visual features and a more spatially structured habitat in the vegetated littoral zone compared to limnetics: first, that benthics should use landmarks to a greater extent than limnetics, and second, that benthics would be better at spatial learning than limnetics. To test these predictions, we trained fish from two benthic and two limnetic populations to locate a goal arm in a T-maze, a procedure that has often been used to analyse learning and memory (Olton 1979; Rodríguez et al. 1994). The four populations were collected from two lakes on Texada Island where each species pair is known to have descended from the marine ancestor independently of one another (Taylor and McPhail 2000). 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 turn and landmark cues conflicted assessed whether there were differences between benthics and limnetics in their propensity to use the two types of spatial information. Differences between benthics and limnetics in their motivation to access the goal arm of the T-maze (defined as propensity to reach the reward) could affect their rate of learning. We therefore designed a second experiment to compare the motivation of benthics and limnetics to access the rewards used to train the fish in the T-maze.

## Materials and methods

### Subjects

Benthic and limnetic threespine sticklebacks were collected from two lakes in separate drainages on Texada Island,

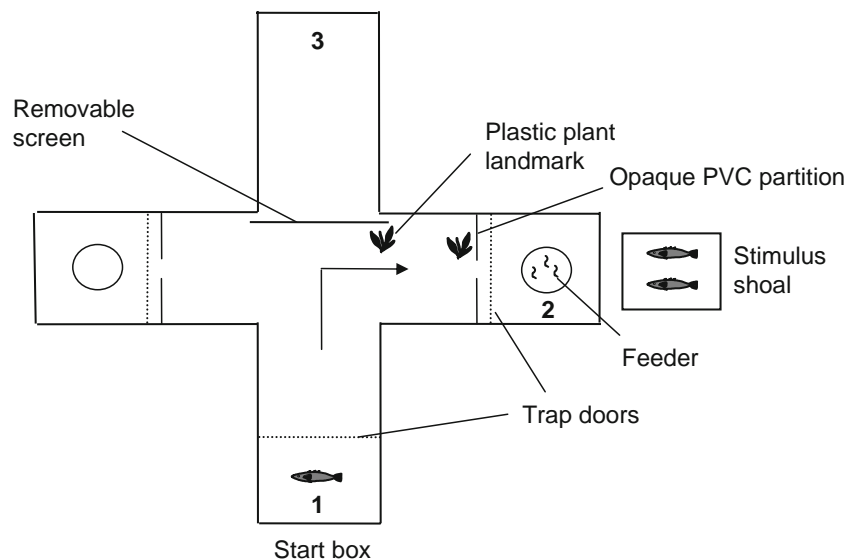
British Columbia: Paxton Lake (49°43' N, 124°30' W) and Priest Lake (49°45' N, 124°34' W). Both species were collected from each lake using dip nets and standard minnow traps. All fish were juveniles approximately 4 months of age and caught in October.

### Experiment 1: spatial learning task

#### Apparatus

Details of the apparatus used in this experiment can be found in Odling-Smee and Braithwaite (2003). In brief, two four-arm mazes were submerged into aerated and filtered water within a rectangular pool. Any one of the arms could be shut off by lowering a screen to produce a T-maze. A remotely controlled trap door placed in the central stem of the T-maze was used to close the start box for each trial. Two opaque polyvinyl chloride (PVC) partitions cut off the ends of each arm into which we could place bloodworm food rewards secured into petri dishes. Both species readily feed on bloodworms in captivity. The end walls of each arm were made of transparent Perspex behind which we placed two randomly selected, conspecific sticklebacks in a transparent bottle to simulate the presence of a shoal. The fish therefore received a double reward (food and shoalmates) when it reached the goal. Other studies show that juvenile sticklebacks of both species readily associate with a shoal and do not differ in their propensity to do so (Kozak and Boughman 2008). Two plastic plant landmarks were also placed in the maze, one directly next to the hole leading to the food reward and the other in the correct arm just in front of the removable barrier, visible from the exit of the start box (Fig. 1). An RCA PRO843 8 mm Camcorder 1 m above the centre of the pool allowed us

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



to view the movements of the fish on a black-and-white monitor next to the pool without being seen by the fish. The entire apparatus was surrounded by white curtains to avoid disturbance from the observer and to minimize the availability of global extramaze cues.

### Procedure

Fish were given a settling period in the laboratory of 3 weeks in holding aquaria, maintained on a diet of defrosted frozen bloodworm (*Chironomus* spp.) and newly hatched brine shrimp (*Artemia salina*). The photoperiod was held at a constant 12 h light:12 h dark cycle, and the temperature was maintained between 16°C and 20°C. We have successfully housed thousands of limnetic and benthic sticklebacks in these conditions in the past. After 3 weeks, we transferred groups of ten benthics or ten limnetics from each lake to one of four 100 l holding aquaria ( $n=40$ ). Each aquarium was divided into four equal sections by three transparent Perspex partitions. Two or three fish that could be distinguished from size differences or body markings were housed in each section. Four limnetics died during the experiment (two from each lake), leaving a sample size of 36 fish (20 benthics and 16 limnetics).

### Pretraining

The pretraining, training and probe trials were conducted in much the same way as that described in Odling-Smee and Braithwaite (2003). The purpose of pretraining was to familiarize fish with the apparatus and procedure. During pretraining, no landmarks were present, and petri dishes containing large amounts of bloodworm were placed in both arms of the maze, but we did not use the simulated shoal. During pretraining, groups of ten fish had free access to the whole T-maze for 4 h every day for five consecutive days. Paxton or Priest fish were pretrained in the morning or in the afternoon at random. After five pretraining sessions, the fish swam into the ends of the T-maze in less than 1 min. All fish were fed a mixture of chopped bloodworm and newly hatched brine shrimp in their home tanks in the 2 days between pretraining and training.

### Training

Fish were given three trials a day, once every 2 days, and we randomized the order in which the fish were trained each day. They received all their food during trials on trial days. 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. We trained half the fish from each population to turn right and half to turn left in

order to control for any directional bias. The plastic plant landmarks were positioned in the right arm or left arm accordingly. Each fish was introduced into a start box and left for 5 min with the opaque cover in position. For each three-trial session, the maze and start box were randomly selected with the constraint that no maze or start box was used more than twice in a row. After 5 min, the door was raised, and we recorded the time taken by the fish to leave the start box and to enter an arm and which arm the fish chose first. Each trial was terminated after the fish had fed or after 10 min. Fish were left for 3 min after feeding, then the trap door was lowered and the apparatus manipulated such that the previously rewarded end became the start box for the next trial. This allowed us to train individuals in blocks of three trials, with minimum handling between trials.

In the first 12 trials from this part of the experiment, we trained the fish to associate the landmark and one direction (right or left) with the reward. During these early training trials, we raised the trap doors in the arms of the T-maze allowing fish access to both the correct (rewarded and marked) and incorrect (unrewarded and unmarked) ends. We do not use these trials in our data analysis. For the training trials, throughout the remainder of the experiment, we rewarded fish only for making a correct first choice by lowering the trap door to the unchosen end after the fish entered a hole in the partition at one end. The learning criterion was established as nine correct trials out of ten. Fish were trained until they had reached criterion or for a maximum of 45 trials. When the fish reached criterion, we conducted additional post-criterion trials interspersed by probe trials. A minimum of four correct trials out of a run of five had to be achieved between any two probe trials for the fish to be considered at criterion. All fish achieved this performance level.

### Probe trials

We used probe trials to determine the fish's spatial strategy. In these trials, the direction and landmarks conflicted. By noting which direction the fish turned, we could assess whether they were relying on landmarks or were using an algorithmic strategy (i.e. turn right). 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. We allowed fish to enter an arm and feed before we removed them from the maze. We recorded a turn response if 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 received three probe trials interspersed between at least five training trials.

## Experiment 2: motivation test

Potential differences in motivation to access the reward could cause differences in performance by benthics and limnetics in the T-maze. We tested this possibility in two ways. First, we compared the time required to complete the task during several points in training using *t* tests. Second, we designed an additional experiment to assess the motivation of the two species to access the two types of reward (shoalmates and bloodworm). Although the apparatus differs between the spatial task and the motivation test, we handled fish in the same way. In both cases, fish are individually trapped and given 5 min to settle before being released into a novel environment. The motivational drives indicated in this second experiment, or choice test, are therefore likely to reflect the motivational drives present when fish are tested in the T-maze.

### Apparatus

A 100-l tank was lined with white coral gravel. 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, and we worked behind a blind. The tank was divided into two sections by a transparent Perspex partition placed 19 cm from one end of the tank (Fig. 2). Five conspecifics from the same lake of origin as the test fish were 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’. The 19-cm zone adjacent to the five conspecifics was designated the ‘shoal zone’. The 19-cm zone at the opposite end of the tank was designated the ‘food zone’. Food rewards were provided in this zone by securing large amounts of bloodworm into a petri dish. A transparent cylindrical release chamber was placed in the centre of the ‘start zone’.

This was controlled remotely by a hand-operated pulley system (Fig. 2).

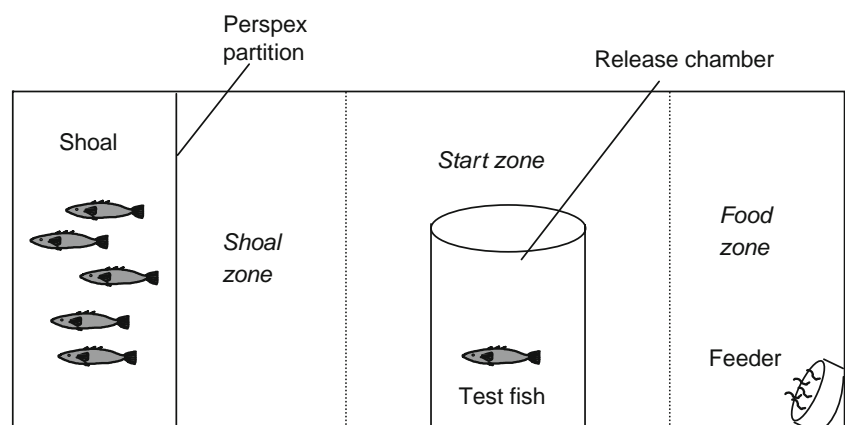
### Procedure

This experiment was conducted after the spatial learning experiment. We transferred groups of ten benthics or ten limnetics from each lake to one of four holding tanks. Once a day for 3 days, a petri dish with large amounts of bloodworm was placed in the holding tanks and left for 24 h to encourage the fish to recognize the petri dish as a food resource. After 24 h, the petri dishes in all the holding tanks had been depleted of bloodworms. On the fourth day, we transferred five nonexperimental shoal fish to the smaller section of the experimental tank and left them to settle for 2 h. We then transferred individual test fish to the cylindrical chamber in the central start zone. After 5 min, we slowly raised the chamber and allowed the test fish to swim into the tank. Each fish was given 5 min to explore the three zones of the tank (start zone, food zone and shoal zone). During this period, we measured the amount of time each fish spent in the three zones. We judged zone entry to have occurred when the base of the caudal fin had passed across the line drawn on the front of the tank. We tested 40 fish over a period of 2 days and randomized the order of testing. Observations were done behind a blind, and fish were not fed before their trial.

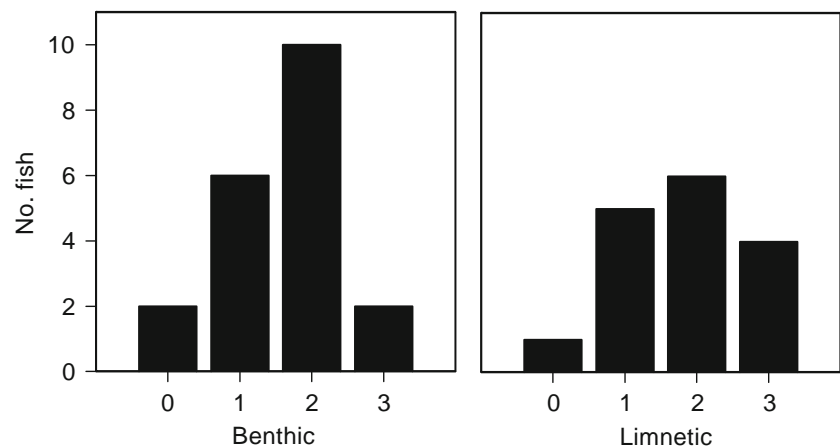
### Statistical analyses

Where necessary, to ensure normality and homogeneity of variance, the data were arcsine or log transformed, or non-parametric statistics were employed. We used a chi-square test of independence to assess whether benthics and limnetics differed in the types of spatial information used (landmarks or turn) during the probe trials. This test asked whether the proportion of benthics using landmarks (zero,

**Fig. 2** Diagrammatic representation of the choice test. The test fish was released from the central chamber, and the proportion of time the fish spent in each of the three zones within a 5-min trial period was recorded



**Fig. 3** Cue use by benthic and limnetic sticklebacks. Number of probe trials in which each species used turn cues from 0 (all landmarks) to 3 (all turns). Each species used both cues more than expected by chance ( $p < 0.0001$  for both), and the species did not differ in cue use ( $p = 0.54$ ). See text for details of statistical tests



one, two or three times) was different than limnetics and was based on a  $2 \times 4$  contingency table. We found no significant differences between the counterbalanced right-trained and left-trained fish ( $F_{1,34} = 0.16$ ;  $p = 0.69$ ); we therefore collapsed these data for analysis. To assess the factors affecting performance, we calculated the proportion of correct trials across runs of six consecutive trials, referred to as sets, and conducted a three-factor (species, lake, set) analysis of variance (ANOVA) with set as a repeated measure. We did not find significant differences in performance between fish from the two lakes ( $F_{1,32} = 1.44$ ;  $p = 0.24$ ), and therefore, we collapsed these data to investigate species differences. This gave a two-way repeated-measures ANOVA. We compared benthic and limnetic performance for each set with post hoc Tukey tests. We report means  $\pm$  SE in text. Bonferroni corrections were applied to chi-square tests of proportions and Mann–Whitney  $U$  tests for the motivation data but did not change any conclusions.

## Results

### Experiment 1: spatial learning task

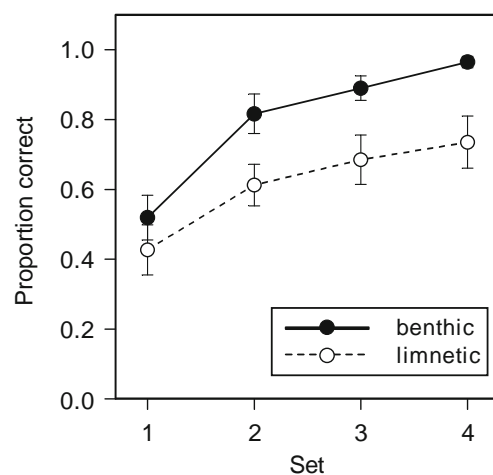
#### Use of spatial cues

The number of benthic and limnetic fish using zero to three turns is shown in Fig. 3. Fish who use zero turns always use landmarks, whereas fish using three turns always use the turn cue. We found that 80% of benthics and 68% of limnetics used both cues, which is more than expected by chance (binomial test: benthic  $p < 0.0001$ ; limnetic  $p < 0.0001$ ). Benthics and limnetics did not differ in their use of the two cues during the three probe trials ( $\chi^2_3 = 2.115$ ;  $p = 0.54$ ). With our sample sizes, our power to detect the predicted difference between species in cue use is 0.78. Thus, regardless of their lake of origin, both benthics and

limnetics appear to use both landmark and turn information with no significant preference for either cue.

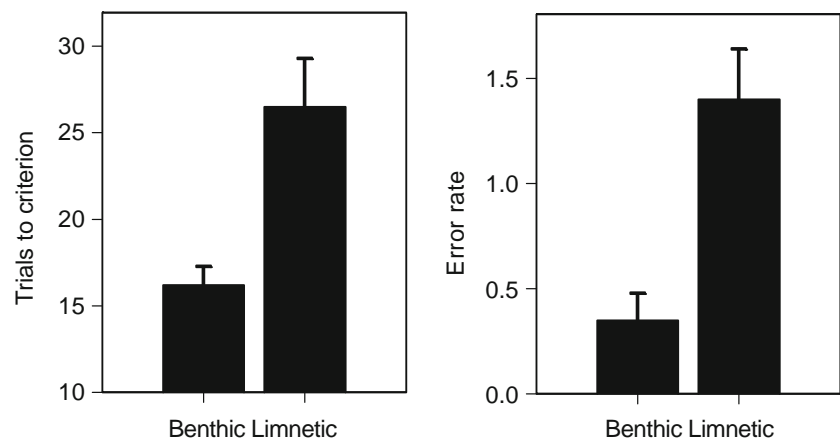
#### Task acquisition and post-criterion performance

Figure 4 shows the proportion of benthic and limnetic individuals performing the task correctly across the first four sets (24 trials of training). Benthics and limnetics from both lakes learned to find the rewarded arm with performance significantly improving over the first four sets of training (ANOVA, set:  $F_{3,105} = 25.34$ ,  $p < 0.001$ ). Overall, the performance of benthics was significantly better than that of limnetics (ANOVA, species:  $F_{1,32} = 14.97$ ,  $p = 0.001$ ). Benthics and limnetics did not differ in performance at the start of the experiment (set 1:  $F_{1,34} = 0.91$ ,  $p = 0.3$ ). However, benthics performed better than limnetics at all other time points (set 2:  $F_{1,34} = 6.04$ ,  $p = 0.02$ ; set 3:  $F_{1,34} = 7.66$ ,  $p = 0.009$ ; set 4:  $F_{1,34} = 11.1$ ,  $p = 0.002$ ).



**Fig. 4** Mean  $\pm$  SE proportion of correct choices by benthic and limnetic individuals during the first four sets of training trials (one set = six trials). The species do not differ in the first set, but ANOVA shows that benthics have a significantly higher proportion correct in sets 2, 3, and 4

**Fig. 5** Performance of benthics and limnetics in the spatial task. **a** Mean±SE number of trials to criterion and **b** mean±SE error rate for each species. All individuals of both species achieved criterion and maintained good performance. However, benthics required fewer trials to reach criterion and made fewer errors than limnetics



All benthics and limnetics achieved the criterion performance of at least nine correct trials out of ten. A one-way ANOVA with the number of trials taken to reach criterion as the dependent variable revealed that benthics took significantly fewer trials to reach criterion than limnetics (ANOVA, species:  $F_{1,34}=14.84$ ,  $p<0.001$ ; Fig. 5a). Throughout the post-criterion trials, during which probe trials were interspersed, benthics and limnetics maintained a high and steady level of accuracy (benthics:  $96.53\pm 1.27\%$  correct; limnetics:  $86.9\pm 2.05\%$  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:  $F_{1,34}=15.64$ ,  $p<0.001$ ; Fig 5b).

#### Contextual variables—motivation, boldness and susceptibility to stress

We found no significant differences between species in the time required to start or complete the spatial task throughout training (Table 1). The time taken for fish to enter an arm of the maze in the first trial is likely to reflect boldness and propensity to explore a novel environment (Fraser et al. 2001; Brick and Jakobsson 2002). This measure did not significantly differ between benthics and limnetics (ANOVA, species:  $F_{1,34}=0.35$ ,  $p=0.56$ ; benthics:

$64\pm 22.1$  s; limnetics:  $36\pm 6.17$  s). Early in training, limnetics completed the task slightly faster than benthics but not significantly so (Table 1). Later in training, benthics were non-significantly faster. We have a power of over 0.80 to detect a difference of 14 s, which is the average difference we found throughout the experiment.

Fish rarely showed signs of stress during the experiment. Stressed fish tended to freeze or hide in the start box or in the plant landmarks with the result that they failed to pass through the hole in the PVC partition and enter either end of the T-maze during a 10-min trial. This was not consistently associated with species (three benthics and three limnetics). Paxton limnetics failed to enter an arm more often than Paxton benthics, but the opposite trend was observed in fish from Priest Lake (ANOVA, species  $\times$  lake:  $F_{1,32}=8.92$ ,  $p<0.01$ ).

In the spatial task, benthics and limnetics differed in the number of times they took the bloodworm food reward after they had accessed the rewarded end of the T-maze (Mann–Whitney  $U$  test:  $N_{\text{benthic}}=20$ ,  $N_{\text{limnetic}}=16$ ,  $U=-74$ ,  $p<0.01$ ; benthics:  $96.4\pm 0.76\%$  of trials; limnetics:  $54.4\pm 1.7\%$  of trials), suggesting that the two species may differ in their motivation to feed on the bloodworm food reward.

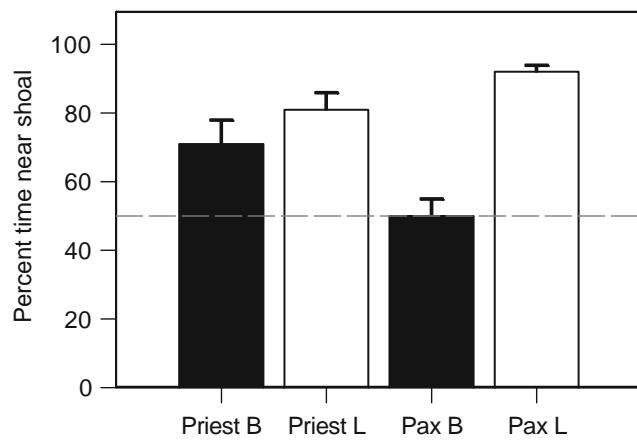
#### Experiment 2: motivation test

Figure 6 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 spent a greater proportion of time in the shoal zone than in the food zone ( $\chi^2$  test of proportions= $67.1$  and  $43.8$  for Paxton and Priest limnetics, respectively,  $p<0.001$  for both). The two benthic populations differed in their behaviour. Priest benthics spent longer in the shoal zone than in the food zone ( $\chi^2$  test of proportions= $20.1$ ,  $p<0.001$ ), while Paxton benthics spent similar amounts of time in the shoal and food zones ( $\chi^2$  test of proportions= $0.06$ ,  $p=0.25$ ). Paxton limnetics spent a significantly greater

**Table 1** Tests of differences in the time required to complete the task for limnetics and benthics

Trial number	$t_{34}$	$P$
1	1.11	0.3
2	1.16	0.3
6	-0.22	0.8
12	-0.87	0.4
24	-1.35	0.2

Negative  $t$  values indicate that benthics were faster than limnetics.



**Fig. 6** Motivation for food and social reward in benthics and limnetics. Mean $\pm$ SE proportion of time benthics (B) and limnetics (L) from Priest and Paxton lakes (Pax) spent in the shoal zone out of the total time spent in both the food and shoal zones. Paxton benthics spent an equal amount of time with the shoal and food; whereas the other groups spent more time with the shoal

proportion of time in the shoal zone compared to Paxton benthics (Mann–Whitney  $U$  test:  $N_{\text{benthic}}=10$ ,  $N_{\text{limnetic}}=8$ ,  $U=-4$ ,  $p<0.01$ ). 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:  $N_{\text{benthic}}=10$ ,  $N_{\text{limnetic}}=8$ ,  $U=46$ ,  $p=0.63$ ). This suggests that shoalmates were an effective reward for both species.

## Discussion

### Use of spatial cues

The arm choices made during the probe trials suggest that both benthics and limnetics can recognise and use landmarks in combination with a body-centred pattern of movement to locate a goal. This is contrary to our first prediction—that limnetics would show a preference for turn and benthics for landmarks on the basis that limnetics probably encounter landmarks less frequently than benthics. Previous studies have revealed differences between stickleback populations in their use of landmark cues in a spatial learning task (Girvan and Braithwaite 1998; Odling-Smee and Braithwaite 2003); in both these studies, fish collected from pond habitats used landmarks to a greater extent than fish collected from rivers. It was argued that in unstable fast-flowing rivers, landmark cues are likely to be unstable and therefore unreliable as directional cues. It may be that cue reliability as opposed to cue availability is a more important factor in determining the preferential use of cues in spatial learning. When visual features are available in the water column, limnetics may benefit from using these as additional reference points, for example to

locate concentrated patches of zooplankton (Noda et al. 1994). Certainly, a common observation in studies of animal spatial learning is that where more than one solution to a given navigational problem exists, multiple strategies are pursued in parallel (Etienne et al. 1990; Able 1993; Collett and Zeil 1998).

### Spatial learning ability

Despite using similar cues to navigate, limnetics from both lakes learned the spatial task more slowly than benthics. They also made more mistakes than benthics throughout the entire period of training. A combination of evolved differences and experience may be responsible for differences in learning ability.

Experience could be important in generating differences in spatial learning (Juraska et al. 1984; Seymoure et al. 1996) because the species may have had different spatial experience before we collected them from the wild. If juvenile benthics have more experience with spatially complex habitats, they may learn subsequent spatial tasks more readily. We cannot rule out this possibility with our data; however, this seems unlikely to fully explain the difference for two reasons. First, the fish collected in October were approximately 4 months old. Very young juveniles of both species are found in the littoral zone, and subsequently both move into the open water to forage on plankton in late summer (Bentzen et al. 1984). Therefore, their life histories have been relatively similar up to the age at which we collected them, and both species have experienced both habitats. The species become more specialized in habitat and resource use as individuals mature (Bentzen et al. 1984; Bentzen and McPhail 1984; McPhail 1994), and thus, spatial experience is likely to diverge as fish age. Second, all the fish tested had shared the same laboratory conditions for a month before being trained in the T-maze and for 3 months by the end of the experiment. Therefore recent spatial experiences were similar.

The species difference in performance may also reflect an evolved difference in central cognitive processes, resulting from benthic and pelagic habitats selecting for differential investment in spatial-learning ability. Within the spatially complex littoral habitat, adult benthics may well rely on learning and remembering spatial information in order to locate clumped and patchy distributions of invertebrates (Hughes and Blight 1999, 2000). The different anti-predator tactics shown by benthics and limnetics (Vamosi 2002) are also likely to place different demands on spatial learning; benthics may need to learn and remember the spatial position of protective refuges (Markel 1994; Brown and Warburton 1999), but this will be less important for limnetics that rely on rapid escape or schooling.

Similarly, males of both species build and defend nests in the littoral zone during the breeding season; but benthic males nest in dense cover, while limnetic males nest on open sediments (McPhail 1994). As a result, benthics may rely to a greater extent on learning spatial relationships and complex environmental features to relocate their nests and defend territorial boundaries within dense vegetation.

This finding is consistent with the suggestion that a species' learning and memory capacity represents a trade-off (Dukas 1998, 1999). The energetic and fitness costs likely to be associated with producing, maintaining and operating the neural machinery required to learn (Mery and Kawecki 2003, 2004, 2005) mean that investment in a particular learning ability should be closely tailored to ecological demand. Studies testing for differences in learning between species that experience different selective environments are consistent with this trade-off (reviewed in Dukas 1999). Our study is in keeping with these. We did not test costs of learning here; however, we suggest that habitats differing in spatial complexity may select for differential investment in the capacity for spatial learning.

Such local adaptation for learning could have important implications for habitat choice and subsequent ecological specialisation. In the benthic–limnetic species pairs, the difficulty of navigating through dense cover may impair male limnetics' ability to defend territories against competing males and to lead receptive females to the nest. Likewise, female limnetics may be less proficient than benthic females at finding males and returning to previously sampled males for mating, reducing the value of vegetated mating territories for limnetics. Different spatial learning abilities might, therefore, be one factor influencing choice of breeding habitat, and this may also contribute to the observed pattern of limnetics nesting in open and benthics in vegetated areas (McPhail 1994). Divergent nesting sites will strengthen reproductive isolation and drive further ecological specialisation.

#### Possible confounding factors

A classic problem in comparative studies of learning is the possibility that contextual variables (such as stress or activity level), rather than differences in learning ability per se, are responsible for species differences in performance (Shettleworth 1993; Lefebvre 1996; Kamil 1998). Differences between benthics and limnetics, aside from their ability to learn, could affect their performance in a spatial task.

This appears unlikely here because 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', as indicated by their propensity to explore a novel environment when placed in isolation (Fraser et al. 2001; Brick and Jakobsson 2002). Overall, the behavioural measures taken during training in the T-maze and in the motivation test suggest that contextual variables such as differences in activity or boldness are unlikely to account for the striking differences in the rate of learning by benthics and limnetics.

Although differences in motivation are more difficult to rule out, our evidence suggests that they do not fully explain species differences in performance. Limnetics responded to the bloodworms in the petri dish as food rewards, as they fed in over 50% of the trials in which the correct arm was entered. Also, an additional reward was present: the simulated shoal. In the second experiment designed to measure the relative motivation of the two species to access the two types of reward, limnetics from both lakes displayed a very strong preference for staying near shoalmates. Shoals appear to be a highly salient reward to limnetics. Indeed shoaling appears to be a particularly important defence strategy for open-water foragers like limnetics that are excluded from the protective littoral zone (Vamosi 2002). Moreover, there was no significant difference in the time limnetics and benthics took to complete the task; limnetics were even slightly faster in early trials. This is contrary to expectations if limnetics were poorly motivated. A final possibility is that the fish were using other cues to navigate. Future studies could investigate the differential use of other cues, such as odor, in spatial tasks.

In conclusion, emphasis on learning as mediating phenotypic flexibility has tended to detract attention away from considering how learning operates at a species-specific level and, as such, how it may be adapted for particular habitat conditions (Shettleworth 1998). However, the results presented here suggest that habitat-specific adaptations in the expression of learning traits may be more widespread and exist on a finer scale than is currently appreciated. We suggest that a better understanding of the extent to which learning traits are adaptively specialised in response to local habitat conditions is likely to open up further questions about the potentially widespread role learning could play in evolutionary processes (Irwin and Price 1999; Owens et al 1999; Anderson 1995; Ancel 1999).

**Acknowledgments** We thank D. Schluter for his assistance and generous provision of facilities. We thank S. Vamosi, D. Taylor and H. Rundle for technical assistance; T. Vines for statistical advice; and C. Brown and S. West for valuable comments on earlier drafts of the manuscript. This research was supported by the Biotechnology and

Biological Sciences Research Council, the Fisheries Society of the British Isles and The University of Edinburgh Student Travel Fund and Development and Alumni Services. This research was conducted in accordance with animal care guidelines of the University of British Columbia and University of Edinburgh.

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