

# Spatial learning ability of the threespine stickleback (*Gasterosteus aculeatus*) in relation to inferred ecology and ancestry

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## ABSTRACT

**Background:** Spatial learning is the ability to learn and use features in space to navigate within an environment. In fishes, it is associated with residence in structurally complex habitat, but very little is known about its ancestral condition in adaptive radiations.

**Goal:** To investigate the relationship between foraging mode and spatial learning in derived populations of a well-studied evolutionary model fish species, and to test the importance of experience on spatial learning in an ancestral analogue of this species.

**Organism:** Threespine stickleback (*Gasterosteus aculeatus*) occur as sea-run (ancestral) and divergent freshwater (derived) forms. Freshwater populations occupy an ecological continuum, with benthic stickleback at one extreme, residing and foraging in shallow, structurally complex lakes, and limnetics at the other, living and feeding in the open water of deep lakes that lack structure.

**Methods:** I used a T-maze to measure spatial learning. In Experiment 1, I compared five benthic and five limnetic field-caught stickleback populations to explore ecological divergence of spatial learning. In Experiment 2, I used a sea-run population to infer the ancestral condition of spatial learning; I studied laboratory-reared sea-run fish raised in spatially complex or simple aquaria because adequate samples of field-caught sea-run adults proved difficult to attain.

**Results:** In Experiment 1, benthics exhibited better spatial learning than limnetics. These differences were independent of differences in boldness, exploratory behaviour, activity level, or other performance variables that are independent of spatial learning. In Experiment 2, no differences were detected between rearing treatments, but a number of fish from either group still solved the maze, indicating that even fish reared in spatially simple conditions were capable of spatial learning. However, the relative contributions of inheritance and experience remain unclear.

**Keywords:** cognitive map use, ecotypic variation, forebrain, genetics, hippocampus, phenotypic plasticity, telencephalon.

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## INTRODUCTION

Spatial learning can be defined broadly as the ability to learn and use information concerning features in space to navigate within an environment (Gallistel, 1989; Hughes and Blight, 1999; Shettleworth, 2010). Most tests of spatial learning are conducted on spatio-visual information. In fishes, spatial learning based on visual information has been demonstrated for foraging (Hughes and Blight, 1999), predator evasion (Aronson, 1951, 1971; Markel, 1994; Burns and Rodd, 2008), territoriality (Lamanna and Eason, 2003), and migration (Fukumori *et al.*, 2010). In birds and mammals, it is associated with food acquisition (Gaulin and FitzGerald, 1986, 1989; Sherry and Duff, 1996), maintaining a home range (see Sherry, 1998; Spritzer *et al.*, 2005), and homing and migration (Healy *et al.*, 2005, Bingman *et al.*, 2006).

As in birds and mammals (for reviews, see Sherry, 1998; Shettleworth, 2010), teleost fishes can find a goal using at least three different vision-based spatial learning strategies (Hughes and Blight, 1999; López *et al.*, 1999; Braithwaite and de Perera, 2006). Fish can reach a goal by learning that it is in the proximity of visual landmarks – ‘cue learning’, *sensu* Salas *et al.* (1996a; see also Durán *et al.*, 2008) – or by learning an algorithm such as a sequence of turns – ‘turn discrimination’, *sensu* López *et al.* (2000; see also Salas *et al.*, 1996b). In contrast, cognitive map use employs a map-like representation of multiple features (e.g. visual cues, environmental geometry) that may be learned in isolation or simultaneously, and it can incorporate reciprocal relationships among several nearby (local) and distant (global) visual landmarks to guide navigation (see Gallistel, 1989; López *et al.*, 1999; Jacobs, 2003; Shettleworth, 2010). Cognitive map use is particularly interesting because, unlike the other strategies, it is associated with the size of the hippocampus of birds and mammals (Healy *et al.*, 2005) and is processed within the dorsolateral region of the telencephalon in fishes (Salas *et al.*, 1996a, 1996b; Vargas *et al.*, 2000, 2009; Rodríguez *et al.*, 2002).

Although proximate mechanisms underlying spatial learning have been intensely investigated in fishes (Salas *et al.*, 1996a, 1996b; Vargas *et al.*, 2000, 2009; Rodríguez *et al.*, 2002; Odling-Smee and Braithwaite, 2003a), their evolution in fishes remains poorly understood. The threespine stickleback (*Gasterosteus aculeatus*) is an exceptionally useful model to explore the evolution of spatial learning strategies. Stickleback biology and evolution have been studied extensively (Wootton, 1976, 1984; Bell and Foster, 1994; Paepke, 1996; Östlund-Nilsson *et al.*, 2007; Hendry *et al.*, 2013). The threespine stickleback is primitively marine or anadromous but has repeatedly colonized and adapted to diverse freshwater habitats (Bell, 1995). Because resident freshwater populations evolved from anadromous stickleback, which appear to be phenotypically conservative (Bell *et al.*, 2009) and geographically homogeneous (Walker and Bell, 2000), anadromous populations can be used to infer ancestral spatial learning abilities. In derived lake populations, adaptation to different foraging demands has resulted in predictable ecological and phenotypic divergence among stickleback populations. Benthic (bottom-feeding) and limnetic (open-water planktivore) stickleback populations represent extremes along a dietary continuum (Schluter and McPhail, 1992). Benthic stickleback prey on invertebrates on highly structured, shallow lake bottoms, whereas limnetic populations feed above deep, open waters on plankton. Benthics and limnetics are strongly divergent for ecologically important morphological (McPhail, 1984, 1992, 1994; Lavin and McPhail, 1985, 1986; Schluter and McPhail, 1992; Walker, 1997; Caldecutt *et al.*, 2001; McKinnon and Rundle, 2002; Vamosi, 2002; Aguirre, 2009; Park and Bell, 2010; Willacker *et al.*, 2010) and behavioural traits (Bentzen and McPhail, 1984; Foster, 1994; Mackney and Hughes, 1995; Scotti and Foster, 2007).

In Experiment 1, allopatric freshwater benthic and limnetic populations from Cook Inlet, Alaska were used to investigate differences in spatial learning ability in relation to

divergence of foraging mode. Compared with limnetics, benthics may use spatial information to a greater extent because they forage in shallow, structurally complex habitats with numerous landmarks and stable food locations (Odling-Smee *et al.*, 2008). A previous study using benthic–limnetic sympatric species pairs from lakes in British Columbia revealed ecotypic differences in the ability to use nearby landmarks to solve a spatial task (Odling-Smee and Braithwaite, 2003a; Odling-Smee *et al.*, 2008). Both benthics and limnetics learned and used local visual landmarks, and both preferred local cue learning to turn discrimination (Odling-Smee *et al.*, 2008). However, consistent with the above prediction, benthics made fewer errors during training trials in the spatial task and learned it sooner than limnetics. These findings strongly suggest that benthics are more efficient spatial learners, but the extent to which they use distant landmarks or a cognitive map is uncertain. The current work extends the previous study in two ways. First, it explores whether benthics are better than limnetics at using distant landmarks when both local landmark use and turn discrimination are made unreliable. Second, independently derived allopatric freshwater populations from a geographically distant region (Cook Inlet, Alaska) are used instead of sympatric species pairs. Although ecological and morphological differences between allopatric benthic and limnetic ecotypes are not as extreme as sympatric benthic–limnetic species pairs (Schluter and McPhail, 1992), sympatric benthic–limnetic species pairs occur in only a handful of lakes (McPhail, 1994; Gow *et al.*, 2008) and have only been observed in one lake in Alaska (Willacker *et al.*, 2012). Therefore, ecological differences among derived, allopatric lake populations are a very good representation of the threespine stickleback radiation as a whole.

Experiment 2 was a separate study using laboratory-reared fish from one sea-run (anadromous) population to determine if spatial learning was likely to have been present in the common ancestor of freshwater populations. Fish from an anadromous population that breed in Rabbit Slough, Cook Inlet, Alaska were selected to represent the putative ancestral state of spatial learning. If it was an ancient trait, it should be present in contemporary sea-run populations. Although the Rabbit Slough population may not represent all sea-run populations, there is little morphological variation among sea-run threespine stickleback populations worldwide (Bell and Foster, 1994; Walker and Bell, 2000; Colosimo *et al.*, 2005) and limited variation in genomic architecture (Hohenlohe *et al.*, 2012), suggesting limited ecological heterogeneity. The fossil record also indicates that morphological traits of marine threespine stickleback have not changed considerably in the last 13 million years (Bell, 1994; Bell *et al.*, 2009).

Recent investigations into the spatial learning behaviour of fishes have primarily focused on the importance of local visual landmarks (see Warburton, 1990; Girvan and Braithwaite, 1998), but the use of distant landmarks may be just as important for solving spatial tasks in nature (see Rodriguez *et al.*, 1994; Salas *et al.*, 1996a, 1996b). Because it can prove difficult to distinguish between the use of local and distant cues in experimental individuals if they are not spatially decoupled, I employed a ‘spatial constancy’ (see Salas *et al.*, 1996a) approach that randomized local landmarks but kept a constant spatial relationship between distant ones and the food reward. Although spatial learning using local landmarks has been demonstrated in numerous distantly related fish species (Aronson, 1951, 1971; Markel, 1994; Salas *et al.*, 1996a, 1996b; Hughes and Blight, 1999; de Perera, 2004), this is the first intra-specific study to explore ecotypic variation in spatial learning behaviour in the absence of local cue learning and turn discrimination.

## MATERIALS AND METHODS

### Populations and sampling

Stickleback were collected using 3.18 mm or 6.36 mm mesh, unbaited Gee minnow traps set overnight in heterogeneous microhabitats submerged at less than 1 m depth within 3 m of shore; ambient water temperature was between 15° and 20°C. For Experiment 1, live field-caught fish from five benthic and five limnetic stickleback populations were collected to test for ecotypic differences in spatial learning. To increase the chance of detecting a difference, populations with extreme benthic–limnetic differences were chosen. In June 2006, threespine stickleback were sampled from Corcoran, Walby, and Willow lakes, which contain benthic stickleback, and from Long, Lynne, and Matanuska lakes, which contain limnetic stickleback. In June 2008, Mud and Tern lakes, which contain benthic populations, and South Rolly and Stormy lakes, which are inhabited by limnetics, were sampled. Table 1 and Park and Bell (2010) present dietary, phenotypic, geographic, and ecological details for these populations and lakes. The characteristics of sampled benthic and limnetic populations were consistent with what is typically true of each ecotype. Compared with the benthics, the limnetic populations sampled generally occur in deeper lakes and have shallower body (Aguirre, 2009) and skull (Willacker *et al.*, 2010) shapes, higher gill raker numbers (Park and Bell, 2010), and more planktivorous dental microwear (Purnell *et al.*, 2006) and foraging behaviour (S.A. Foster and J. Baker, personal communication) (Table 1). Populations with the same putative ecotype came from different drainages because similar populations from different drainages are unlikely to share derived characteristics due to common ancestry. For Experiment 2, anadromous threespine stickleback from Rabbit Slough (for geographic and ecological details of this site, see Aguirre *et al.*, 2008; Park and Bell, 2010) were collected in June 2006 and used to generate laboratory-reared fish to study the proposed ancestral state of spatial learning. Sampling, husbandry, and treatment of all experimental fishes were approved by the Alaska Department of Fish and Game and by the Institutional Animal Care and Use Committees (IACUC) at the University of Alaska Anchorage and Stony Brook University.

### Transport, housing, and rearing

#### *Experiment 1: Ecotypic comparisons of field-caught samples*

Due to high mortality of senescing field-caught adults in captivity, pre-reproductive one-year-old threespine stickleback were collected. Live pre-reproductive field-caught fish were transported to the University of Alaska Anchorage and kept in aged tap water in outdoor pools for 24–48 h and prepared for shipping. They were placed into shipping bottles that were aerated with an air stone and cooled down to 5–8°C. The bottles were sealed and placed into an ice chest with freezer packs and shipped overnight to Stony Brook University. Upon arrival, the fish were acclimated for water temperature over several hours and then transferred to 60-litre aquaria. Each aquarium contained only pre-reproductive fish ( $n \leq 30$ ) from a single population. The fish were fed once per day with thawed frozen adult brine shrimp. As a standard procedure, after 14 days of captivity in Stony Brook, all fish were treated for potential pathogens that typically compromise the welfare of field-caught stickleback held in captive conditions for several months. No mortality was observed during medical treatment. Experiments with these fish commenced 3 weeks after their arrival to Stony Brook University.

**Table 1.** Criteria for ecotype (B = benthic, L = limnetic) classification of study populations

Population	Region	RLA <sup>1</sup>	Gill rakers <sup>1</sup>	Stomach contents <sup>5</sup>	Microwear <sup>5</sup>	Body <sup>6</sup>	Skull <sup>7</sup>	Foraging <sup>8,9</sup>
Long	Mat-Su	Limnetic (30%)	Limnetic (21.9)	(32.6% B, 67.4% L)	Limnetic	Limnetic	Limnetic	Limnetic
Lynne	Mat-Su	Limnetic (51%) <sup>2</sup>	Limnetic (21.1) <sup>3</sup>	N.A.	N.A.	N.A.	Limnetic	Limnetic
Matanuska	Mat-Su	Limnetic (10%) <sup>2</sup>	N.A.	N.A.	N.A.	Limnetic	Intermediate	Limnetic
South Rolly	Mat-Su	N.A.	Limnetic (21.4) <sup>3</sup>	N.A.	N.A.	Limnetic	Limnetic	Limnetic
Corcoran	Mat-Su	Benthic (100%)	Uncertain (20.5)	(79.2% B, 20.8% L)	Benthic	Benthic	Benthic	N.A.
Mud	Mat-Su	Benthic (100%)	Benthic (18.0)	(93.4% B, 6.6% L)	Benthic	Benthic	Benthic	N.A.
Willow	Mat-Su	Benthic (100%) <sup>2</sup>	Benthic (19.9) <sup>4</sup>	(57.2% B, 42.8% L) <sup>4</sup>	Benthic	Benthic	Intermediate	N.A.
Walby	Mat-Su	Benthic (78%) <sup>2</sup>	Benthic (19.8) <sup>3</sup>	N.A.	N.A.	Benthic	Benthic	N.A.
Stormy	Kenai	Limnetic (33%)	Uncertain (20.4)	N.A.	N.A.	Limnetic	Intermediate	Limnetic
Tern	Kenai	Benthic (100%) <sup>2</sup>	Benthic (17.7) <sup>3</sup>	N.A.	N.A.	Benthic	Benthic	N.A.
Rabbit Slough	Mat-Su	N.A.	Limnetic (22.4) <sup>4</sup>	(72.8% B, 27.2% L) <sup>4</sup>	N.A.	N.A.	N.A.	N.A.

*Note:* Lake names with map coordinates were provided by Bell and Orti (1994), and the regions are the Matanuska-Susitna Borough (Mat-Su) and Kenai Peninsula (Kenai). Criteria include relative littoral area of lakes (RLA), gill raker number, stomach contents (% B, % L by item count), dental microwear, lateral body and skull shape, and foraging behaviour. The relative littoral area of lakes (RLA) is the fraction of lake bottom area deeper than the isobath for euphotic zone depth [higher numbers indicate more benthic habitat (see Walker, 1997; Park and Bell, 2010)]. Except for gill raker number, inferences of ecotype are entered. Sources of information on lake or stickleback properties are described in the Materials and Methods. N.A. = data not available. Table modified from Park and Bell (2010). R.L.A.: <sup>1</sup>Walker (1997) or <sup>2</sup>M.A. Bell (unpublished data). Gill rakers: <sup>3</sup>Walker (1997), <sup>4</sup>P.J. Park (unpublished data), or <sup>5</sup>M.P. Travis (unpublished data). Stomach contents: <sup>6</sup>M.P. Travis (unpublished data) or <sup>7</sup>Purnell *et al.* (2006). Microwear: <sup>8</sup>Purnell *et al.* (2006). Body: <sup>9</sup>W.E. Aguirre (unpublished data). Skull: <sup>10</sup>Willacker *et al.* (2010). Foraging: <sup>11</sup>S.A. Foster (personal communication, 2006) and <sup>12</sup>J. Baker (personal communication, 2008).

*Experiment 2: Plasticity in a sea-run sample*

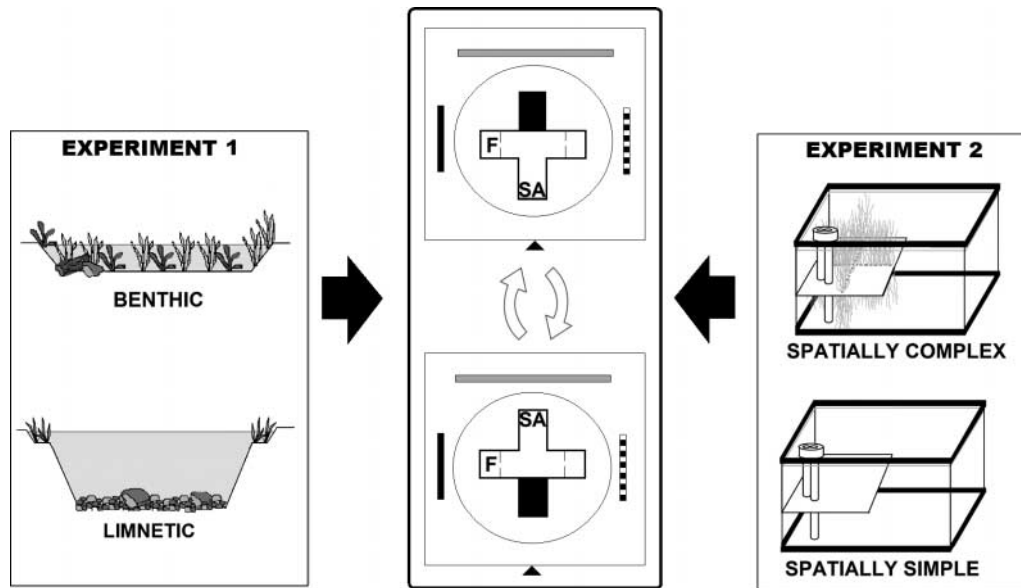
Only laboratory-reared fish from this population could be used because of high mortality of senescing field-caught adults in captivity and the lack of known sites from which to collect a large sample of wild sea-run pre-reproductive individuals. Ten sexually mature sea-run threespine stickleback were collected from Rabbit Slough and used in a mass cross to generate laboratory-reared fish (for detailed methods, see Park *et al.*, 2012). Fourteen-day-old fry were transferred from culture dishes to either spatially complex (SC) or spatially simple (SS) aquaria (Table 2; Fig. 1). Both types of aquaria were constructed by fastening a 29 × 29 cm black plexiglass platform to the aquarium walls, dividing it into upper and lower halves along one half of its length; the opposite half of the aquarium remained open. In the SC aquarium, the plexiglass platform had 15 cm long frayed white polypropylene rope protruding above and below the platform, creating four equal-size quadrants. In the SS aquarium, no rope was used. A bare platform was placed in the SS aquarium in case there were any unanticipated effects of having the platform during rearing. Each aquarium was filtered using a sponge filter (Hydro-Sponge II, Aquarium Technology, Inc., Decatur, GA) and maintained on a 12 hour light/12 hour dark photoperiod at 18°C with 3 ppt saltwater.

Both types of aquaria were isolated by a white shower curtain to eliminate external visual cues during rearing. For both rearing treatments, a feeding apparatus was constructed using 12 cm and 28 cm long white polyvinyl chloride pipes (2.2 cm diameter) fastened together with silicone glue (Fig. 1). The apparatus allowed food to be inserted into either of the pipes to deposit the food onto the platform or aquarium bottom without the fish knowing in advance where it would be delivered. All fish were forced to forage off the bottom to

**Table 2.** Results for spatial learning experiments by lake name and ecotype

Experiment/sample	Ecotype	S	NS	E	A	Year
<b>Experiment 1</b>						
Long	Limnetic	10	3	3	0	2006
Lynne	Limnetic	7	3	6	0	2006
Matanuska	Limnetic	8	7	1	0	2006
South Rolly	Limnetic	2	0	9	0	2008
Stormy	Limnetic	3	0	6	2	2008
Corcoran	Benthic	10	5	1	0	2006
Mud	Benthic	5	1	5	0	2008
Walby	Benthic	11	4	1	0	2006
Willow	Benthic	10	6	0	0	2006
Tern	Benthic	2	1	5	3	2008
<b>Experiment 2</b>						
Rabbit Slough (Complex)	Sea-Run	8	1	4	0	2007
Rabbit Slough (Simple)	Sea-Run	5	1	7	0	2007

*Note:* The experiment number is shown on the left above corresponding samples. Four categories of results are shown: the number of fish that reached the 'six-out-of-seven' criterion (S), fish that could not solve the maze in 50 trials (NS), fish that were terminated early because they were 'encouraged' ten times (E), and fish lost due to attrition (A). Year of experiment (Year) is listed on the right. 'Complex' and 'Simple' refer to the spatial conditions under which Rabbit Slough stickleback (Experiment 2) were reared.

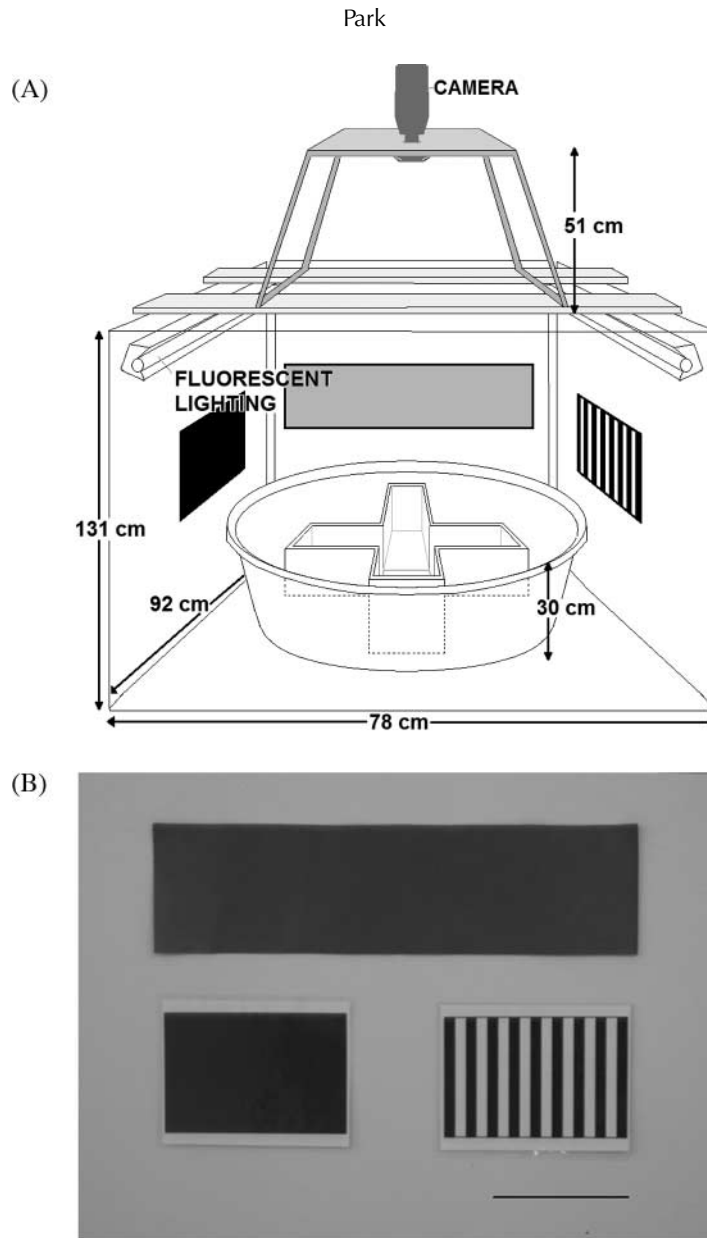


**Fig. 1.** Schematic outline of experiments and T-maze protocol for testing spatial learning. Experiment 1 explored ecotypic differences in spatial learning in field-caught samples. Experiment 2 explored the influence of experience in laboratory-reared fish from an anadromous population reared in spatially contrasting aquaria. The T-maze protocol (centre) required the randomization of the starting area (SA) between positions 180° apart; the set-up for a fish assigned food to the left side of the enclosure entrance (▲) is shown (i.e. ‘left-assigned fish’). To facilitate spatial learning, three extra-maze cues (solid black short, banded black-and-white short, and solid grey elongate boxes) were placed on the walls within the experimental enclosure. The spatial relationship of the food reward (F) to the extra-maze cues remained unchanged for each fish through trials. Not drawn to scale.

eliminate any confounding effects of variation in food location in the water column. In the SS aquarium, food was deposited simultaneously on both the platform and aquarium bottom. In contrast, in the SC aquarium, all food was deposited at only one randomly pre-determined level, and brine shrimp liquid with no food was deposited in the other tube. The SC feeding regimen ensured that some fish did not remain in the feeding quadrant during non-feeding periods; all SC fish had to negotiate the frayed rope between levels to find their food on a daily basis. Experimentation with these fish commenced at approximately one year of age.

#### Experimental enclosure and maze apparatus

A high steel framed experimental enclosure (92 cm long, 78 cm wide, and 131 cm high) lined with opaque white shower curtains was constructed to minimize disturbances occurring outside the maze (Fig. 2). A fluorescent lighting fixture (Lights of America, Model No. 8045: 118 V, 60 Hz, 70 W, 8 amps, Walnut, CA) with one bulb (Sylvania Gro-Lux, 40 W, Danvers, MA) was attached along the upper lengths of the top frames. A 51 cm tall video camera stand that rested atop the enclosure was constructed from PVC, plastic, and wood. The distance from the camera lens to the top of the maze was 119 cm. Entry into the



**Fig. 2.** Experimental enclosure and extra-maze cues. (A) Experimental enclosure, camera stand, extra-maze cues, and pool with four-arm maze (not to scale). See text for dimensions of four-arm maze. (B) The three extra-maze visual cues used for all experiments. Scale bar is 20 cm.

enclosure was through a slit between overlapping shower curtains along one width of the enclosure.

A four-arm maze similar to that used by Odling-Smee and Braithwaite (2003b) was constructed from 1 cm thick black plexiglass (Fig. 2). Each arm was 30 cm long, 10 cm wide, and 20 cm high. Vertical grooves cut along the height of each arm near their intersection



made it possible to close any arm with a 3 mm thick, black plexiglass sliding door to produce the T-maze. The ends of two of the three remaining arms located 180° apart in the T-maze were formed by black plexiglass walls (3 mm thick) with 51 × 25 mm cut-out doors in the centre. Each of these two walls with a door was located 15 cm away from the end of the arm, creating a room in which food could be placed (see Fig. 1). A sliding trap wall located 15 cm away from the end of the third arm in the 'T' created the starting area. This trap wall could be lifted from a distance by the observer by pulling on a clear, monofilament thread. The maze was submerged in a circular pool (100 cm diameter, 30 cm high) filled with 3 ppt artificial seawater; the water level was kept at 1 cm below the top of the walls of the maze.

### **Pre-training**

During pre-training trials, fish were housed in 60-litre aquaria, one population per aquarium (Experiment 1) or one rearing treatment per aquarium (Experiment 2). For each 2006 Experiment 1 sample and each 2007 Experiment 2 rearing treatment group, 20 fish were randomly selected from the stock population in their general housing aquarium (see 'Transport, rearing, and housing') and isolated for pre-training; 14 fish were used from 2008 Experiment 1 samples because they had smaller sample sizes in general housing aquaria. Pre-training trials were administered to familiarize all fish with the experimental apparatus. All four arms of the maze were accessible and no walls with doors were used (see Fig. 2A). Thawed frozen adult brine shrimp were placed in a plastic culture dish at the end of all four arms. Fish were motivated by withholding food for 24–36 h prior to pre-training; fish fed only in the maze. Each population was divided into two sets of 10 fish, except for the 2008 samples, which were limited to seven per set. Fish from a set were allowed to swim together freely for one hour during a pre-training trial. One pre-training trial was administered every other day; each set received a total of three pre-training trials.

### **Experimental trials**

Experimental trials commenced after the third pre-training trial. Each fish was placed in its own 0.5 cm thick plexiglass holding compartment (13 × 8 × 26 cm) to keep track of it during the experiment. Each compartment had a circular hole (radius = 37 cm) covered with 1 mm mesh on each side of the compartment to allow filtered water to pass through all the compartments constantly. Twelve of these compartments were suspended side-by-side in each of up to eight 60-litre aquaria with 3 ppt artificial saltwater. Each fish was housed in the same compartment throughout the experiment. The compartments were randomly rearranged among the aquaria on a daily basis such that each aquarium had fish from different samples and any potential confounding effects of water quality differences across aquaria were eliminated. Each aquarium was filtered continuously with a sponge filter (Hydro-Sponge II, Aquarium Technology, Inc., Decatur, GA) and hanging power filter (Aquaclear 30 power filter, Rolf C Hagen Corp., Mansfield, MA) and maintained on a 12 hour light/12 hour dark photoperiod at 18°C.

The T-maze was constructed by permanently closing off one arm, creating two rooms that were 180° apart, and creating the starting area in the perpendicular arm. During all experimental trials, three conspicuous distant (global) visual, extra-maze cues were located outside of the maze but within the experimental enclosure to facilitate spatial learning

(Fig. 2). A  $71.5 \times 19.5$  cm piece of blue poster board was located on the shower curtain opposite the enclosure entrance, a  $27 \times 18.5$  cm black and white-banded board was located to the right of the entrance, and a  $27 \times 18.5$  cm solid black board was to the left of the entrance (Figs. 1, 2). Blue and black colours were used because threespine stickleback can distinguish between them (Rowe *et al.*, 2004). The video camera was connected to a TV monitor and VCR with which all trials were recorded.

Before each trial, four thawed, frozen adult brine shrimp were placed into the food reward room. Every sample in an experimental series was divided into two sets of fish to test for side bias in the maze. The fish in one set were given their food reward in the room to the left of the enclosure entrance ('▲' in Fig. 1) during the experiment (i.e. left-assigned fish, see Fig. 1), while fish from the other set were fed to the right (i.e. right-assigned fish). Subjects were given two trials per day, and this constituted a 'trial-day'; each trial-day was administered every 2 days. Food was available only in the maze during trials, thus fish were motivated by denial of food for 24–36 h between trial-days. Upon completion of the first of two trials on a trial-day, the subject was placed back in its holding compartment; its second trial was not administered until after all other subjects completed that same trial. The order of subjects run in the maze was randomized among trials using a random number generator.

Individual fish were expected to adopt spatial learning to the exclusion of local cue learning or turn discrimination by randomizing the location of the starting area by  $180^\circ$  between trials (Fig. 1). A random number generator was used to select the order in which each of the two orientations was used, except that no more than three consecutive trials with the same orientation were allowed. When the starting area was rotated  $180^\circ$ , the pool and maze within it were rotated together to ensure that no local visual intra-maze cues (e.g. scratches on the maze, conspicuous gravel) contributed to learning the food reward location using local cue learning. However, because the spatial relationship of the distant (global) visual extra-maze cues and the food reward never changed during the entire duration of an experiment, subjects had to use global visual cues to solve the maze.

To eliminate the possibility that fish followed an odour plume, half of the water in the maze was replaced with water from the pool and then mixed after every individual run – this was done for every single fish. In addition, a complete water change for the pool and maze was done after a trial – this was done after all fish of an experiment were run for that trial. Although non-visual information could still have been used, validation experiments indicated that odour (Girvan and Braithwaite, 1998; also see Odling-Smee and Braithwaite, 2003b; Park, 2011) and sound (Park, 2011) appear to be unimportant to stickleback to navigate in the T-maze.

At the start of every experimental trial, a single fish was placed in the starting area for 60 s, after which the trap wall was raised. The time that each fish first left the starting area was recorded (hereafter 'starting time'; except for Trial 1, see below). The fish was allowed to swim freely through the maze. As an indicator of 'activity level' (except for Trial 1, see below), total movement (also called ambulation) in the maze was recorded as the sum of entries a fish made into either arm or back into the starting area. A trial was completed when the fish entered the food reward room and fed, after which the fish was left for an additional 5 min before being gently netted and returned to its individual holding compartment. All fish had to feed before being removed from the maze. The time interval between first leaving the starting area and entering the food reward room (hereafter 'food time') was recorded. All performance measures were based on the passage of the

caudal peduncle [i.e. base of the tail fin (see Hughes and Blight, 1999)] through the relevant plane for the starting area, base of the T-maze arm, door, or food reward room.

The occurrence of six out of seven trials in which the subject found the food reward room without entering the non-reward room was the criterion for achievement of the spatial learning criterion. This criterion occurs within a range of successful criteria used by Odling-Smee and colleagues (Odling-Smee and Braithwaite, 2003b; Odling-Smee *et al.*, 2008) to investigate vision-based learning differences in threespine stickleback. Any correct choices made during the first two trials were excluded from the criterion because these could have occurred by chance; in other words, the six out of seven could not include the first two trials. A maximum of 50 trials per fish was used. Comparable studies with stickleback have used 45 trials as a maximum limit (see Odling-Smee and Braithwaite, 2003b; Brydges *et al.*, 2008). A trial ended if a fish did not leave the starting area within 10 min or did not find the food reward within 10 min after initially leaving the starting area. In either case, the fish was 'encouraged' by gently prodding it with a net to the food reward. Encouragement using this approach is a standard procedure employed in behavioural studies with fishes (see Salas *et al.*, 1996a), and all fish that had been encouraged were observed feeding in the food reward room before being transferred to their holding compartment. Whether they were encouraged or found the food reward room on their own, all fish fed only in the maze to maintain their motivation to reach the criterion. A limit of 10 min per trial to solve the maze was used in a similar study using fishes (Brown and Braithwaite, 2005), and fish that were encouraged were still able to complete subsequent trials within 10 min without encouragement. All subjects solved the first two trials without necessitating encouragement, thus it is unlikely that encouragement biased some fish not to solve the maze at the start of an experiment. Preliminary studies (cf. Park, 2011) indicated that reaching ten encouraged trials was associated with the inability to solve the maze before 50 trials, and for logistical purposes experimentation was terminated for fish that were encouraged at least ten times. Observers were not given any information about the ecotype or source population while behaviours were recorded. Immediately after completion of the experiment, each fish was sacrificed by overdose with MS-222. The sex of subjects, which is usually identified based on incision of the ventral body wall and inspection under a dissecting scope, could not be determined because gonads were immature and difficult to characterize reliably.

In Experiment 1, two fish from Stormy Lake (limnetic) and three fish from Tern Lake (benthic) did not survive before completing experimental trials (Table 2). These data were not omitted because statistical methods that can account for potentially informative data lost due to mortality before conclusion of an experiment (i.e. progressively censored data) were used to compare spatial learning differences (see 'Statistical analyses'). Final sample sizes used in experimental trials are listed in Table 2. It is very unlikely that welfare differences among samples were present during trials because all subjects were housed under identical conditions and shared the same 3 ppt artificial saltwater. Therefore, the loss of five fish in Experiment 1 was probably due to factors that were random with respect to treatment group. There was no attrition in Experiment 2.

### **Trial 1: Boldness and exploratory behaviour**

The performance of each fish during its first trial in the T-maze was used to estimate differences in boldness and exploratory behaviour (Table 3). During this trial, an individual fish was exposed for the first time to a starting area, moving trap wall, and a room with

**Table 3.** Behavioural variables for spatial learning experiments and their descriptions

Variable name (Y)	Measure	Transform.	Subjects	Trial(s)	Test for:
Trials to reach criterion	Trials to reach criterion	N.A.	S + NS + E	N.A.	Spatial learning
No. of fish (not) reaching criterion	No. of fish (not) reaching criterion	N.A.	S + NS + E	N.A.	Spatial learning
Boldness	No. of freezes	$\sqrt{(Y)}$	S + NS + E	1	Bold or shy behaviour
Exploratory behaviour	Novel ambulation	$\sqrt{(Y)}$	S + NS + E	1	Tendency to explore
Latency to emerge	Novel latency to emerge	Log(Y)	S + NS + E	1	Boldness–exploratory behaviour conflict
Activity level	Experienced ambulation	$\sqrt{(Y)}$	S	Criterion	Active or passive behaviour
Starting time	Experienced latency to emerge	Log(Y)	S	Criterion	Time leaving starting area
Food time	Time to complete trial	Log(Y)	S	Criterion	Time to reach food reward

*Note:* Boldness, exploratory behaviour, and latency to emerge were measured only during the first trial of experiments. ‘Criterion’ values refer to the results from the six successful criterion trials. Transformations (Transform.) used to normalize residuals of data used in ANOVAs are given (N.A., not applicable). The categories of subjects used in statistical analyses are as follows: subjects that solved the maze, S; that did not solve the maze, NS; that were encouraged ten times before reaching the maximal 50 trials, E.

a door at either end of the arms that were 180° apart. Subjects were acclimated for 60 s before the trap wall was opened.

Boldness is ‘the willingness to accept risk in return for potentially higher foraging or reproductive gain’ (Ward *et al.*, 2004), and is typically not measured in novel situations (Réale *et al.*, 2007). However, it is possible that bolder individuals will likely put themselves in new surroundings (see Budaev, 1997), potentially biasing them to learn visual aspects of their surroundings sooner than shy individuals (see Burns and Rodd, 2008). Freezing is a standard behavioural measure used to determine tendency for boldness in fishes (Walsh and Cummins, 1976; Budaev, 1997; Templeton and Shriner, 2004; Burns, 2008; Burns and Rodd, 2008). Freezing is a reliable indicator of boldness in stickleback because it is a strategy used to evade detection by predators (Wootton, 1984; Huntingford and Coyle, 2007). Boldness was measured as the residual of the number of total freezes regressed on the log of the time interval between when a fish left the starting area and first entered a door into a food reward room (see Burns and Rodd, 2008).

An animal’s reaction to a novel situation occurs along an exploration–avoidance continuum (see Réale *et al.*, 2007). As ambulation is a quantitative measure of total movement, it indicates activity level. In a novel environment, however, it may also indicate general exploratory behaviour (Walsh and Cummins, 1976; Gervai and Csányi, 1985; Budaev, 1997; Burns, 2008), which may favour the learning of spatial cues. Subjects that explore by re-visiting areas may

generate a better internal representation of their new environment than those that do not (see Capaldi *et al.*, 2000; Eilam *et al.*, 2003). Ambulation is typically measured as the time spent in motion (Budeav, 1997; Burns, 2008) or the number of times a subject crosses regular spatial intervals (Gervai and Csányi, 1985). In the current study, ambulation was recorded as the number of times the subject entered either arm or re-entered the starting area from their intersection during Trial 1. Because many aspects of the maze (e.g. trap wall, rooms with doors) were novel to subjects during this trial, ambulation was a better proxy for exploratory behaviour than activity level. Activity level (ambulation in non-novel situations) is typically measured under conditions in which subjects have had considerable experience or are under low stress (Burns and Rodd, 2008; also see below).

Emergence tests measure how willing the subject is to leave a starting area (Gervai and Csányi, 1985; Burns, 2008). While latency to emerge has been considered a measure of boldness (Brown and Braithwaite, 2004; Brown *et al.*, 2005), it may more accurately reflect a conflict between shyness and willingness to explore (see Réale *et al.*, 2007; Burns, 2008). Therefore, in the current study, 'latency to emerge' was used as a composite measure of shyness and exploratory behaviour. Latency to emerge was recorded as the time from lifting the trap wall of the starting area until a fish left it and entered the maze for the first time (see Odling-Smee *et al.*, 2008). During Trial 1, the time that a fish left the starting area after the trap wall was raised could be treated as an emergence test because all fish emerged slowly from the starting area, as if they were inspecting their surroundings, and most of them returned to the starting area before entering a room (Experiment 1, 84%,  $n = 135$ ; Experiment 2, 78%,  $n = 18$ ). Therefore, it is reasonable to assume that subjects treated the starting area as an artificial refuge and the newly exposed area of the maze as a novel environment.

### **Criterion trials: starting time, food time, and activity level**

Performance differences among or within samples of successful learners (i.e. excluding fish that could not achieve criterion) may provide insight into behaviours that contribute to spatial learning differences. Fish that achieved criterion were analysed further to determine whether performance differences were present between ecotypes (Experiment 1) or rearing treatment (Experiment 2). The six successful (out of seven) criterion trials were analysed for starting time, activity level, and food time (Table 3). This dataset included more-or-less similarly experienced individuals that did not make wrong door errors. The starting time was the time that each fish initially left the starting area. Just as in Trial 1, activity level was recorded as the sum of entries a fish made into either arm or back into the starting area. Fish that reached the criterion are experienced and presumably under less stress in the T-maze than during Trial 1. Therefore, total movement during criterion trials should be more indicative of activity level than exploratory behaviour. Finally, food time was recorded as the time interval between first leaving the starting area and entering the food reward room.

### **Statistical analyses**

Each experiment was analysed separately. Statistica version 9.1 and Biomstat version 3.30q were used to test for differences in spatial learning and several other measures of performance between ecotypes (Experiment 1) or rearing treatments (Experiment 2). Analyses of variance (ANOVA) were used if data could be transformed such that sample residuals

conformed to a normal distribution (see Sokal and Rohlf, 1995) (Table 3). Fish assigned food in the room to the left or to the right of the enclosure entrance were tested for side bias whenever it was possible.

For each experiment, subjects were placed into one of three categories (Table 2): fish that solved (S) the maze, did not solve (NS) the maze within 50 trials, or were encouraged ten times (E) before reaching 50 trials. Spatial learning was inferred using two analyses. The number of trials to reach the criterion is time-to-event (or waiting time) data, which are not expected to be normally distributed, thus tests that compare waiting time data were used to analyse samples for this variable (see Lee and Wang, 2003). These tests do not make any assumptions about the waiting time distribution for a sample. In the second analysis, a *G*-test of independence was used to infer whether the proportion of fish that solved the maze differed statistically from the number of those that did not (i.e. NS and E fish).

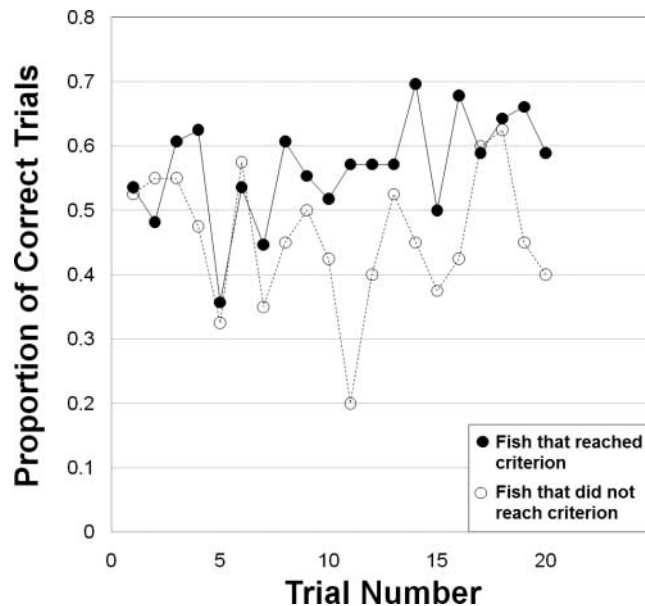
All subjects were used for tests of boldness, exploratory behaviour, or latency to emerge (i.e. boldness–exploratory behaviour conflict) (Table 3). To measure boldness, analysis of covariance (ANCOVA) was used to compare relative number of freezes with log of the time until first entry into a door as the covariate. Nested ANOVA was used to test exploratory behaviour and emergence times. Fish that achieved criterion were investigated further for ecotypic differences in other maze performance measures (Table 3). Results for their six successful (out of seven) criterion trials for starting time, food time, and activity level were analysed using repeated-measures ANOVA to compare ecotypes (Experiment 1) or rearing treatments (Experiment 2).

## RESULTS

### Validation of spatial learning criterion

The 2006 dataset of Experiment 1 provided the largest and most complete dataset to validate use of the criterion (i.e. correctly choosing the food room in six of seven trials) for both experiments. To determine whether fish that achieved the criterion (i.e. S fish) made fewer mistakes than those that did not reach the criterion (i.e. E and NS fish), wrong-door error (i.e. choice of non-reward room) data for the first 20 trials were analysed for all subjects. Wrong-door error data were not available for all 50 trials for every fish because trials were terminated once a fish reached criterion or were encouraged at least ten times. However, 20 trials were sufficient in detecting learning improvement in some spatial task studies (see Salas *et al.*, 1996b). The relative proportion of correct choices made by S fish that achieved this criterion was plotted against trial number for the first 20 trials (Fig. 3). If subjects were learning to avoid the unrewarded room in later trials, the proportion of correct choices made by subjects was expected to increase with trial number. Angular transformation (i.e. the inverse sine of the square-root of the proportion) was used for proportional data (Sokal and Rohlf, 1995). Regression of transformed proportions on trial number was statistically significant for S fish ( $N = 56$ ,  $F_{1,18} = 5.88$ ,  $P < 0.05$ ) but not for E + NS fish ( $N = 40$ ,  $F_{1,18} = 0.066$ ,  $P = 0.800$ ). A greater proportion of fish that achieved criterion avoided the non-reward room during the first 20 trials.

The behaviour of S fish and E + NS fish did not differ during their first exposure to the T-maze. A two-level nested ANOVA with side as the subgroup did not detect a difference between S fish and E + NS fish for exploratory behaviour (group:  $F_{1,22} = 0.99$ ,  $P = 0.332$ ; side:  $F_{22,72} = 0.39$ ,  $P = 0.992$ ) or latency to emerge (group:  $F_{1,22} = 1.02$ ,  $P = 0.323$ ; side:

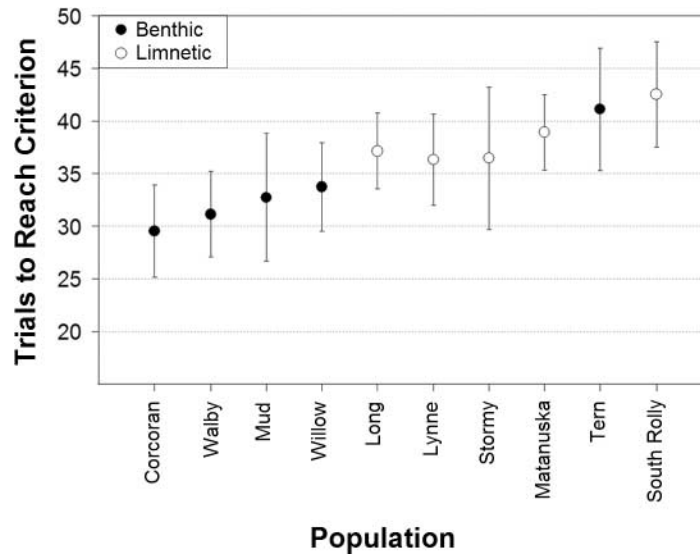


**Fig. 3.** Evaluation of the ‘six-out-of-seven’ criterion. The relative proportion of correct choices made by fish that achieved the criterion of correctly choosing the food room in six out of seven trials was plotted for the first 20 trials. The proportion of correct choices made by S fish increased with trial number, indicating that S fish were learning to avoid the non-reward room as trials progressed. No trend was detected for E + NS fish.

$F_{22,72} = 1.66$ ,  $P = 0.06$ ), and an ANCOVA failed to detect differences in boldness ( $F_{1,93} = 0.16$ ,  $P = 0.688$ ). To determine if E + NS fish still learned to negotiate the maze (even though they could not learn to avoid the non-reward room), performance during criterion trials of S fish and comparable trials of E + NS fish were tested against their own performance during Trial 1. For this analysis, the dataset of all ( $\leq 50$ ) trials completed by subjects was used. Food times for Trial 1 of subjects were compared with the mean food times of their last six trials without a wrong-door error. Compared with their performance on Trial 1, both S fish (Mann-Whitney  $U$ -test:  $U = 2526$ ,  $P < 0.05$ ) and E + NS fish (Mann-Whitney  $U$ -test:  $U = 971$ ,  $P < 0.05$ ) completed the maze in less time during the later trials. Moreover, the mean food times of these later trials did not differ between S fish and E + NS fish (Mann-Whitney  $U$ -test:  $U = 115$ ,  $P = 0.41$ ). In summary, both S fish and E + NS fish responded to novel surroundings in similar ways and learned that food was provided in the maze, but compared with E + NS fish, S fish made fewer mistakes. Therefore, using six of seven trials without a wrong-door error is an effective criterion for learning to find food efficiently in the maze.

### Experiment 1

This experiment was used to test for an association between spatial learning and ecotype. Tests that compare waiting time distributions were used to analyse the number of trials to reach criterion. Fish that did not reach the criterion (NS and E fish) were designated the maximal 50 trials but treated as singly censored data in the analysis. For individuals that did



**Fig. 4.** Spatial learning in field-caught samples (Experiment 1). Mean values and standard errors of cumulative trials to reach the criterion of correctly choosing the food room in six out of seven trials are shown for field-caught samples of benthic and limnetic populations. Individuals lost due to attrition (see Table 2) are not included.

not survive before reaching criterion (A fish), which only occurred in Experiment 1 (see Table 2), the trial number reached just prior to attrition was incorporated into the analysis as progressively censored data (see Table 2 for sample sizes). A Gehan's generalized multi-sample Wilcoxon test failed to detect differences for number of trials to reach criterion among the ten lake samples ( $\chi^2 = 8.77$ , d.f. = 9,  $P = 0.458$ ; Fig. 4), across benthic samples ( $\chi^2 = 2.60$ , d.f. = 4,  $P = 0.628$ ; Fig. 4), or across limnetic samples ( $\chi^2 = 2.33$ , d.f. = 4,  $P = 0.676$ ; Fig. 4). Therefore, results from separate populations were pooled within ecotype. Benthics ( $N = 70$ ) reached the criterion in fewer trials than limnetics ( $N = 70$ ) (Cox's  $F$ -test:  $F_{60,82} = 1.57$ ,  $P < 0.05$ ; Log-Rank test:  $L = -2.07$ ,  $P < 0.05$ ; Peto and Peto Wilcoxon test:  $W = -1.98$ ,  $P < 0.05$ ). While this analysis accounted for attrition, it does not rule out the possibility that non-surviving fish ( $N = 5$ ) were ill and thus had impaired performance. Therefore, it was re-run without non-survivors. Benthics ( $N = 67$ ) still took fewer trials than limnetics ( $N = 68$ ) based on the Log-Rank test ( $L = -1.99$ ,  $P < 0.05$ ). However, the Peto and Peto Wilcoxon test ( $W = -1.91$ ,  $P = 0.0556$ ) and Cox's  $F$ -test ( $F_{60,76} = 1.479$ ,  $P = 0.053$ ) were marginally not statistically significant. Rationale for choosing among these tests is provided in the Discussion. Finally, although slightly more benthic fish ( $N = 38$  of 67) reached criterion than limnetic fish ( $N = 30$  of 68), a  $G$ -test of independence did not detect a statistical difference (benthics,  $N = 67$ ; limnetics,  $N = 68$ ;  $G_{adj} = 2.13$ ,  $P > 0.05$ ).

Boldness, exploratory behaviour, and latency to emerge could have influenced these results. Unlike trials to reach criterion, these variables did not include any censored data because all fish participated in the first trial. An ANCOVA did not detect boldness differences among benthic ( $F_{4,64} = 1.80$ ,  $P = 0.141$ ) or limnetic ( $F_{4,64} = 0.53$ ,  $P = 0.715$ ) samples, thus populations within ecotype were pooled. ANCOVA failed to detect differences between ecotypes ( $F_{1,137} = 0.14$ ,  $P = 0.705$ ). A three-level nested ANOVA with



side $\subset$ population $\subset$ ecotype (i.e. 'within' noted hereafter as  $\subset$  for nested ANOVA) as groups was used to compare exploratory behaviour. Nested ANOVA failed to detect an effect of ecotype ( $F_{1,8} = 0.0014$ ,  $P = 0.971$ ), population ( $F_{8,10} = 1.44$ ,  $P = 0.288$ ), or side ( $F_{10,120} = 0.39$ ,  $P = 0.951$ ). A three-level nested ANOVA with side $\subset$ population $\subset$ ecotype as groups and latency to emerge as the dependent variable also failed to detect an effect of ecotype ( $F_{1,8} = 1.84$ ,  $P = 0.213$ ), population ( $F_{8,10} = 2.70$ ,  $P = 0.072$ ), or side ( $F_{10,120} = 0.99$ ,  $P = 0.460$ ). Thus, generally, boldness, exploratory behaviour, and latency to emerge did not differ between populations, the side on which the food was placed, or ecotypes.

To compare performance during criterion trials among successful learners, ecotypic differences for other behavioural measures were tested in fish that achieved the criterion (S fish). Lake populations were pooled within each ecotype because limited sample sizes did not allow an analysis among populations. A repeated-measures ANOVA with ecotype as the factor and activity level during the six successful criterion trials as the dependent variable was not statistically significant ( $F_{1,66} = 1.12$ ,  $P = 0.295$ ). Similarly, a repeated-measures ANOVA did not detect an effect of ecotype for starting time ( $F_{1,66} = 1.11$ ,  $P = 0.296$ ) or food time ( $F_{1,66} = 0.006$ ,  $P = 0.939$ ). Thus, starting time, food time, and activity level did not differ between ecotypes.

## Experiment 2

This experiment was designed to infer the role of experience on spatial learning in a putatively ancestral, anadromous population and tested laboratory-reared fish from Rabbit Slough. Tests to compare waiting time distributions did not detect a difference between the spatially complex (SC) and spatially simple (SS) rearing treatments (Log-Rank test:  $L = 1.09$ ,  $P = 0.27$ ; Cox's  $F$ -test:  $F_{10,16} = 1.89$ ,  $P = 0.125$ ; Peto and Peto Wilcoxon test:  $W = 1.01$ ,  $P = 0.311$ ). Rearing treatments were also compared with field-caught benthics and limnetics from Experiment 1 (lake populations were pooled within ecotype). A Gehan's generalized multi-sample Wilcoxon test failed to detect differences for waiting time distributions among SC anadromous fish, SS anadromous fish, and benthics ( $\chi^2 = 2.45$ , d.f. = 2,  $P = 0.294$ ), or among SC anadromous fish, SS anadromous fish, and limnetics ( $\chi^2 = 1.03$ , d.f. = 2,  $P = 0.597$ ).

A  $G$ -test of independence ( $G_{\text{adj}} = 1.32$ ,  $P > 0.05$ ) and multinomial exact test (SC:  $N_{\text{Solved}} = 8$ ,  $N_{\text{Not Solved}} = 5$ ,  $P = 0.157$ ; SS:  $N_{\text{Solved}} = 5$ ,  $N_{\text{Not Solved}} = 8$ ,  $P = 0.157$ ) failed to detect a difference between rearing treatments in the number of fish that solved the maze. A multinomial exact test was used here because it is statistically more powerful than the  $G$ -test when sample sizes are small (Conahan, 1970).

ANCOVA failed to detect boldness differences between rearing treatments ( $F_{1,24} = 0.15$ ,  $P = 0.705$ ). A two-level nested ANOVA with side $\subset$ rearing as groups was used to compare exploratory behaviour and latency to emerge. Using exploratory behaviour as the dependent variable, nested ANOVA failed to detect an effect of rearing ( $F_{1,2} = 2.36$ ,  $P = 0.264$ ) or side ( $F_{2,23} = 0.59$ ,  $P = 0.562$ ). Similarly, using latency to emerge as the dependent variable did not detect an effect of rearing ( $F_{1,2} = 4.21$ ,  $P = 0.177$ ) or side ( $F_{2,23} = 0.55$ ,  $P = 0.584$ ). Thus, boldness, exploratory behaviour, and latency to emerge did not differ between rearing treatments.

Differences between rearing treatments for other maze performance measures were tested in fish that achieved the criterion of six correct out of seven consecutive trials to compare performance among successful learners. Repeated-measures ANOVA with activity level

as the dependent variable did not detect an effect of rearing ( $F_{1,11} = 0.40$ ,  $P = 0.543$ ). Repeated-measures ANOVA also failed to detect an effect of rearing for starting time ( $F_{1,11} = 0.44$ ,  $P = 0.519$ ) or food time ( $F_{1,11} = 0.42$ ,  $P = 0.533$ ). Thus, starting time, food time, and activity level also did not differ between rearing treatments.

## DISCUSSION

Spatial cognition creates the ability to recognize, learn, remember, and use spatial cues in the environment, which can allow a navigator to re-use efficient and safe routes or to create novel routes to a goal (Tolman, 1948; Jacobs, 2003; Braithwaite and de Perera, 2006; Shettleworth, 2010). O'Keefe and Nadel (1978) identified several operational criteria for spatial strategies that López *et al.* (1999) confirmed in fishes. The evolution of spatial learning was explored in the current study using threespine stickleback (*Gasterosteus aculeatus*) from populations of an endemic Alaskan adaptive radiation (Bell *et al.*, 1993; Bell and Orti, 1994; Walker, 1997; Aguirre, 2009; Arif *et al.*, 2009; Willacker *et al.*, 2010). While others have explored ecological variation of vision-based spatial learning strategies in threespine stickleback (see Girvan and Braithwaite, 1998, 2000; Odling-Smee and Braithwaite, 2003b; Brydges *et al.*, 2008; Odling-Smee *et al.*, 2008), this study is the first to do so while controlling for local cue learning and turn discrimination. The present research shows that foraging ecotypes from allopatric freshwater stickleback populations differ in at least spatial learning of distant cues and that this trait was probably present in the anadromous ancestor.

The analysis of benthics versus limnetics for number of trials to reach criterion was administered with and without non-surviving fish. The analysis including non-surviving fish took attrition into account and found that benthics took fewer trials than limnetics to reach the criterion. However, these results could have been biased if non-surviving fish were ill, impairing their ability to solve the T-maze. Therefore, these data were re-analysed with non-survivors excluded. The overall results did not change based on the Log-Rank test but the Cox's  $F$ -test and the Peto and Peto Wilcoxon test gave marginal results. Although justification for choosing among these tests is unsettled, the Peto and Peto test is probably least accurate here for two reasons that are relevant to the current dataset. The Log-Rank test is more sensitive at detecting differences that occur at later, rather than earlier, time points (Lee and Wang, 2003), and Cox's  $F$ -test tends to be more statistically powerful when using small sample sizes (Gehan and Thomas, 1969). However, these results do suggest that the decision to include non-surviving fish in an analysis is not trivial. The cause of death of non-surviving fish remains uncertain, but the possibility that they were ill cannot be ruled out, even though very careful measures were taken both before and throughout the experiments to optimize the welfare of subjects (see 'Transport, housing, and rearing'). Overall, although similar proportions of benthics and limnetics learned the task, benthics reached the learning criterion faster, as predicted, and this result was generally supported regardless of attrition. Tests of waiting time distributions provide a very useful way to analyse behaviour because they can account for missing data that are not under the control of the investigator (e.g. attrition, unsuccessful recapture in field studies).

For fish to successfully reach the criterion in the current study, they had to use distant visual cues to the exclusion of local cue learning or turn discrimination strategies. Benthics required fewer trials than limnetics to reach the criterion, and the difference does not appear to be due to boldness or exploratory behaviour. A detailed analysis among lake samples indicated that the fewer trials of benthics were due to contributions by all benthic samples.

However, among all ten populations, Tern Lake (benthic) took the second highest number of trials, indicating that spatial learning ability in this population is strikingly poorer than those of other benthic populations. Tern Lake stickleback have extreme benthic qualities such as deep body shapes (McPhail, 1994; Walker, 1997; Aguirre, 2007), short snouts and wide skulls (Willacker *et al.*, 2010), and laterally round telencephalon shapes (Park and Bell, 2010). Thus, the behaviour of Tern Lake fish is paradoxical. One possible explanation is that spatial learning ability is considerably influenced by phenotypic plasticity (see below), and ecological factors (e.g. heavy predation, high parasitism) and/or the genetic architecture of this population might limit the expression of this learning trait from year to year (see references within Kaeuffer *et al.*, 2012). Tern Lake, located on the Kenai Peninsula, is the only lake with benthic stickleback that was sampled outside of the Matanuska-Susitna Valley region (Table 1). Tern Lake is as shallow as the other sampled lakes with benthic populations, but unlike them, it is far inland and far upstream at a high elevation. Compared with downstream populations, those that are higher in drainages tend to have lower genetic diversity (Crispo *et al.*, 2006; Caldera and Bolnick, 2008). Consistent with this expectation, the Tern Lake population has substantially less genetic diversity than other lake populations in the Cook Inlet region that are not as far inland (W.E. Aguirre, personal communication), suggesting that the anomalous behaviour of Tern Lake fish may be exceptional, not typical, of benthic populations overall. Further study of spatial and temporal variation of spatial learning in this and other benthic populations on the Kenai Peninsula and of laboratory-reared Tern Lake fish may provide key insights into the relative importance of environmental and heritable factors in this population.

The overall result that benthics outperform limnetics supports findings from a previous study, which showed that compared with sympatric limnetics, benthics use local landmarks to solve a spatial task more efficiently (Odling-Smee *et al.*, 2008). Similarly, the arm choices by subjects in this study indicate that both benthics and limnetics can recognize, remember, and use distant visual landmarks, just as sympatric counterparts can for local ones. Thus, in the wild, stickleback from many populations may learn and remember local and distant visual cues in similar ways. It is interesting that outcomes of the previous and current studies were similar even though the T-maze protocols used were very different. Odling-Smee *et al.* (2008) intentionally minimized extra-maze cues and randomized their relationships to the food room. In contrast, the procedure used in the current study included large, conspicuous distant landmarks and the spatial relationship among these landmarks and the location of the food room remained constant for each subject. Neither the maze protocol used by Odling-Smee *et al.* (2008) or in the present work can rigorously distinguish cognitive map use from local and/or distant cue learning, but it would be very interesting to know if the superior spatial learning ability of benthics compared with limnetics is at least partly due to better cognitive mapping ability.

Several fish in every lake sample were capable of spatial learning, suggesting that both ecotypes readily use distant landmarks in the wild. Although benthics live in shallow lakes dominated by littoral habitat, limnetics may use spatial learning to forage in the relatively limited littoral edges surrounding the deep, open areas of their lakes (see Schluter and McPhail, 1992; Odling-Smee *et al.*, 2008; Park and Bell, 2010). In addition, both male and female limnetics enter shallow water to breed, although males spend more time there (M.A. Bell, personal communication). This may explain the spatial learning of limnetics used in the present work. Although this study is the first to identify the use of distant visual cues to the exclusion of local cue learning and turn discrimination in threespine stickleback, further work is needed to understand individual variation within populations and its causes in natural populations.

For example, it is certain that at least some fish within a population have the capacity to use multiple spatial strategies (see Odling-Smee *et al.*, 2008), but the factors that drive preference of a particular strategy or the decision to switch strategies are unknown.

No behavioural differences were detected during the first experimental trial between fish that reached criterion (S fish) and those that could not (E + NS fish). Fish that reached criterion were analysed further to test the possibility that there were performance differences between just the successful learners in the benthic and limnetic populations. General activity level (i.e. the sum of entries a fish made into either arm or back into the starting area), starting time (i.e. the time before each fish initially left the starting area), and food time (i.e. the time it took for subjects to leave the starting area and complete the trial) were explored. This dataset included fish with comparable experience, and it did not take wrong-door errors into account. There were no differences for any of these performance variables between benthics and limnetics. Therefore, while benthics are superior spatial learners, benthic and limnetic spatial learners do not appear to differ in how quickly they left the starting area, found the food reward room, or moved around the maze during a comparable set of trials.

Boldness in threespine stickleback is more prevalent in lake populations with native predatory fish than populations from lakes without them (Huntingford *et al.*, 1994). Because all lake samples in the current study came from habitats with native predatory fishes, population differences for boldness were not expected. Consistent with expectations, ecotypic differences for boldness were not found in the present study. Ecotype also did not influence latency to emerge, which suggests that benthics and limnetics responded to introduction to the T-maze similarly. In birds and mammals, enhanced spatial learning ability is associated with residence in structurally complex habitats (Sherry, 1998). Thus, compared with limnetics, benthics may have been expected to exhibit more exploratory behaviour. However, unlike spatial learning, no differences in exploratory behaviour were detected between Alaskan threespine stickleback ecotypes. It is possible that this measure was not sensitive enough to detect subtle differences in exploratory behaviour. Alternatively, potential foraging in the relatively limited littoral edges around the deeper, open areas of lakes with limnetic populations could account for exploratory behaviour in limnetics.

The anadromous Rabbit Slough stickleback population in Experiment 2 was used to infer the proposed ancestral state of spatial learning ability (see Bell, 1995; Park and Bell, 2010). Because the resident lake populations sampled exhibit spatial learning, it is very likely that variation for spatial learning also existed in the anadromous ancestor. Rabbit Slough fish were reared in spatially contrasting aquaria to test for the importance of experience. Fish reared in the spatially complex aquarium were expected to exhibit superior spatial learning than those reared in the spatially simple aquarium. Contrary to this expectation, rearing treatment did not influence spatial learning ability significantly. There are at least three possible explanations for the negative finding: (i) experience may not be important for the expression of spatial learning; (ii) the influence of experience may be substantial, but the artificial spatially complex rearing treatment may not have been complex enough to elicit phenotypic plasticity for spatial learning ability; (iii) the absence of a rearing effect may be due to limited sample sizes. Environmental enrichment induces neurological changes associated with enhanced spatial learning in many species (Juraska *et al.*, 1985, 1989; Patel *et al.*, 1997; Rampon *et al.*, 2000; van Praag *et al.*, 2000; Vargas *et al.*, 2000; Olson *et al.*, 2006). In addition, Girvan and Braithwaite (1998) detected vision-based spatial learning differences using field-caught pond and river stickleback populations, but when they replicated this comparison using

laboratory-reared fish from spatially contrasting aquaria, these differences disappeared (Girvan and Braithwaite, 2000). Taken together, these studies strongly suggest that experience is very important for spatial learning in stickleback and that the artificial spatial complexity used in Experiment 2 in this work may have been insufficient to induce differences in spatial learning ability.

The relative importance of experience versus inheritance to spatial learning ability in lake and anadromous stickleback remains unclear. Nothing is known about the spatial experiences of field-caught fish in this study prior to capture, but experience is critical for spatial learning in fish from at least one Alaskan lake population. Field-caught fish from Corcoran Lake (benthic) took fewer trials to reach the criterion than their laboratory-reared counterparts did (see Park, 2011). Additional support for the importance of experience to spatial learning in lake stickleback comes from comparative neuroanatomy studies. In addition to benthics being superior spatial learners than limnetics (this study; also see Odling-Smee *et al.*, 2008), field-caught benthics also have rounder telencephalon shapes than field-caught limnetics (Park and Bell, 2010), a pattern that disappears in laboratory-reared counterparts (Park *et al.*, 2012). Greater telencephalon convexity may reflect greater volume of the dorsolateral (DI) region in the telencephalon, a fish forebrain substructure known to be involved in cognitive map use, a spatial learning strategy (Broglia *et al.*, 2003; Northcutt, 2006). The relationships among experience, DI size, and the extent of spatial learning abilities may even differ among closely related species. For example, in ninespine stickleback (*Pungitius pungitius*), field-caught marine populations have larger relative telencephala than pond populations (Gonda *et al.*, 2009, 2011), but the opposite was found in threespine stickleback (Park and Bell, 2010; Park *et al.*, 2012).

Even though experience is important for spatial learning, substantial genetic potential for spatial cognition was identified in the proposed anadromous ancestral population, Rabbit Slough. Despite the absence of an overall rearing effect in Experiment 2, a considerable number of Rabbit Slough fish from both rearing treatments solved the maze (Table 2). Furthermore, these laboratory-reared anadromous stickleback reached criterion just as efficiently as field-caught benthics and limnetics from Experiment 1, but these results must be interpreted with caution because data from field-caught fish were compared with those of laboratory-reared fish from a different population and ecotype. Generally, however, the patterns of phenotypic plasticity expressed in ancestral, marine stickleback tend to mirror the patterns present in benthic and limnetic populations for morphological (Wund *et al.*, 2008) and behavioural (Shaw *et al.*, 2007) traits. Thus, to the extent that experience is important for spatial learning in stickleback, it is reasonable to speculate that field-caught anadromous fish, equipped with their prior spatial experiences in the wild, could have outperformed their laboratory-reared counterparts from Experiment 2 and possibly the field-caught lake fish from Experiment 1. Anadromous and resident freshwater fish certainly share non-foraging behaviours that involve spatial cognition, such as predator evasion (Aronson, 1951, 1971; Markel, 1994; Burns and Rodd, 2008) or territoriality during the breeding season (Lamanna and Eason, 2003). However, other behaviours such as migration (see Fukumori *et al.*, 2010) may be more important to anadromous stickleback and could contribute to enhanced spatial learning ability.

Vertebrates can use an array of spatial learning strategies to solve spatial tasks (Nadel, 1994; Schacter and Tulving, 1994; López *et al.*, 1999; Shettleworth, 2010), but most previous findings are based on birds and mammals (see Dodson, 1988; Healy, 1998). The current study investigated the evolution of spatial learning to the exclusion of local cue learning or turn discrimination in threespine stickleback. Benthics exhibited superior use of distant landmarks than limnetics, and

laboratory-reared anadromous stickleback exhibited spatial learning abilities comparable to those of field-caught lake fish, supporting a deep homology of this learning trait. Multiple components of learning (e.g. motivation, reinforcement, discernment, memory) may be affected during the evolution of spatial learning (see West-Eberhard, 2003), and future work on fishes should aim to identify all these components, trade-offs among them, and their possible relationship to the dorsolateral region of the telencephalon (see Warburton, 2003).

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### REFERENCES

- Aguirre, W.E. 2007. *The pattern and process of evolutionary diversification: lessons from a threespine stickleback adaptive radiation*. PhD thesis, Stony Brook University, Stony Brook, NY.
- Aguirre, W.E. 2009. Microgeographic diversification of threespine stickleback: body shape–habitat correlations in a small, ecologically diverse Alaskan drainage. *Biol. J. Linn. Soc.*, **98**: 139–151.
- Aguirre, W.E., Ellis, K.E., Kusenda, M. and Bell, M.A. 2008. Phenotypic variation and sexual dimorphism in anadromous threespine stickleback: implications for postglacial adaptive radiation. *Biol. J. Linn. Soc.*, **95**: 465–478.
- Arif, S., Aguirre, W.E. and Bell, M.A. 2009. Evolutionary diversification of operculum shape in Cook Inlet threespine stickleback. *Biol. J. Linn. Soc.*, **97**: 832–844.
- Aronson, L.R. 1951. Orientation and jumping behavior in the gobiid fish, *Bathygobius soporator*. *Am. Mus. Novit.*, **1486**: 1–22.
- Aronson, L.R. 1971. Further studies on orientation and jumping behavior in the gobiid fish, *Bathygobius soporator*. *Ann. NY Acad. Sci.*, **188**: 378–392.
- Bell, M.A. 1994. Paleobiology and evolution of the threespine stickleback. In *Evolutionary Biology of the Threespine Stickleback* (M.A. Bell and S.A. Foster, eds.), pp. 438–471. Oxford: Oxford University Press.
- Bell, M.A. 1995. Intraspecific systematics of *Gasterosteus aculeatus* populations: implications for behavioral ecology. *Behaviour*, **132**: 1131–1152.
- Bell, M.A. and Foster, S.A. (eds.) (1994) *The Evolutionary Biology of the Threespine Stickleback*. Oxford: Oxford University Press.
- Bell, M.A. and Ortí, G. 1994. Pelvic reduction in threespine stickleback from Cook Inlet lakes: geographic distribution and intrapopulation variation. *Copeia*, **1994**: 314–325.
- Bell, M.A., Ortí, G., Walker, J.A. and Koenings, J.P. 1993. Evolution of pelvic reduction in threespine stickleback fish: a test of competing hypotheses. *Evolution*, **47**: 906–914.

- Bell, M.A., Stewart, J. and Park, P.J. 2009. The world's oldest fossil threespine stickleback fish. *Copeia*, **2**: 256–265.
- Bentzen, P. and McPhail, J.D. 1984. Ecology and evolution of sympatric sticklebacks (*Gasterosteus*): specialization for alternative trophic niches in the Enos Lake species pair. *Can. J. Zool.*, **62**: 2280–2286.
- Bingman, V.P., Jechura, T. and Kahn, M.C. 2006. Behavioral and neural mechanisms of homing and migration in birds. In *Animal Spatial Cognition: Comparative, Neural, and Computational Approaches* (M.F. Brown and R.G. Cook, eds.). Available at: [www.pigeon.psy.tufts.edu/ascl/bingman/](http://www.pigeon.psy.tufts.edu/ascl/bingman/).
- Braithwaite, V.A. and de Perera, T.B. 2006. Short-range orientation in fish: how fish map space. *Mar. Freshw. Behav. Physiol.*, **39**: 37–47.
- Broglio, C., Rodríguez, F. and Salas, C. 2003. Spatial cognition and its neural basis in teleost fishes. *Fish Fish.*, **4**: 247–255.
- Brown, C. and Braithwaite, V.A. 2004. Size matters: a test of boldness in eight populations of the poeciliid *Brachyrhaphis episcopi*. *Anim. Behav.*, **68**: 1325–1329.
- Brown, C. and Braithwaite, V.A. 2005. Effects of predation pressure on the cognitive ability of the poeciliid *Brachyrhaphis episcopi*. *Behav. Ecol.*, **16**: 482–487.
- Brown, C., Jones, F. and Braithwaite, V.A. 2005. *In situ* examination of boldness–shyness traits in the tropical poeciliid, *Brachyrhaphis episcopi*. *Anim. Behav.*, **70**: 1003–1009.
- Brydges, N.M., Heathcote, R.J.P. and Braithwaite, V.A. 2008. Habitat stability and predation pressure influence learning and memory in populations of three-spined sticklebacks. *Anim. Behav.*, **75**: 935–942.
- Budaev, S.V. 1997. 'Personality' in the guppy (*Poecilia reticulata*): a correlational study of exploratory behavior and social tendency. *J. Comp. Psychol.*, **111**: 399–411.
- Burns, J.G. 2008. The validity of three tests of temperament in guppies (*Poecilia reticulata*). *J. Comp. Psychol.*, **122**: 344–356.
- Burns, J.G. and Rodd, F.H. 2008. Hastiness, brain size, and predation regime affect wild guppies in a spatial memory task. *Anim. Behav.*, **76**: 911–922.
- Caldecutt, W.J., Bell, M.A. and Buckland-Nicks, J.A. 2001. Sexual dimorphism and geographic variation in dentition of threespine stickleback, *Gasterosteus aculeatus*. *Copeia*, **2001**: 936–944.
- Caldera, E.J. and Bolnick, D.I. 2008. Effects of colonization history and landscape structure on genetic variation within and among threespine stickleback (*Gasterosteus aculeatus*) populations in a single watershed. *Evol. Ecol. Res.*, **10**: 575–598.
- Capaldi, E.A., Smith, A.D., Osborne, J.L., Fahrbach, S.E., Farris, S.M., Reynolds, D.R. *et al.* 2000. Ontogeny of orientation flight in the honeybee by harmonic radar. *Nature*, **403**: 537–540.
- Colosimo, P.F., Hosemann, K.E., Balabhadra, S., Villarreal, G., Jr., Dickson, M., Grimwood, J. *et al.* 2005. Widespread parallel evolution in sticklebacks by repeated fixation of ectodysplasin alleles. *Science*, **307**: 1928–1933.
- Conahan, M.A. 1970. *The comparative accuracy of the likelihood ratio and  $\chi$ -squared as approximations to the exact multinomial test*. EdD dissertation, Lehigh University, Bethlehem, PA.
- Crispo, E., Bentzen, P., Reznick, D.N., Kinnison, M.T. and Hendry, A.P. 2006. The relative influence of natural selection and geography on gene flow in guppies. *Mol. Ecol.*, **15**: 49–62.
- de Perera, T.B. 2004. Spatial parameters encoded in the spatial map of the blind Mexican cave fish, *Astyanax fasciatus*. *Anim. Behav.*, **68**: 291–295.
- Dodson, J.J. 1988. The nature and role of learning in the orientation and migratory behavior of fishes. *Environ. Biol. Fish.*, **23**: 161–182.
- Durán, E., Ocãna, F.M., Gómez, A., Jiménez-Moya, F., Broglio, C., Rodríguez, F. *et al.* 2008. Telencephalon ablation impairs goldfish allocentric spatial learning in a 'hole-board' task. *Acta Neurobiol. Exp.*, **68**: 519–525.

- Eilam, D., Dank, M. and Maurer, R. 2003. Voles scale locomotion to the size of the open-field by adjusting the distance between stops: a possible link to path integration. *Behav. Brain Res.*, **141**: 73–81.
- Foster, S.A. 1994. Evolution of the reproductive behavior of three-spine stickleback. In *The Evolutionary Biology of the Threespine Stickleback* (M.A. Bell and S.A. Foster, eds.), pp. 381–398. Oxford: Oxford University Press.
- Fukumori, K., Okuda, N., Yamaoka, K. and Yanagisawa, Y. 2010. Remarkable spatial memory in a migratory cardinalfish. *Anim. Cogn.*, **13**: 385–389.
- Gallistel, C.R. 1989. Animal cognition: the representation of space, time, and number. *Annu. Rev. Psychol.*, **40**: 155–189.
- Gaulin, S.J.C. and FitzGerald, R.W. 1986. Sex differences in spatial ability: an evolutionary hypothesis and test. *Am. Nat.*, **127**: 74–88.
- Gaulin, S.J.C. and FitzGerald, R.W. 1989. Sexual selection for spatial learning ability. *Anim. Behav.*, **37**: 322–331.
- Gehan, E.A. and Thomas, D.G. 1969. The performance of some two-sample tests in small samples with and without censoring. *Biometrika*, **56**: 127–132.
- Gervai, J. and Csányi, V. 1985. Behavior-genetic analysis of the paradise fish, *Macropodus opercularis*. I. Characterization of the behavioral responses of inbred strains in novel environments: a factor analysis. *Behav. Genet.*, **15**: 503–519.
- Girvan, J.R. and Braithwaite, V.A. 1998. Population differences in spatial learning in three-spined sticklebacks. *Proc. R. Soc. Lond. B*, **265**: 913–918.
- Girvan, J.R. and Braithwaite, V.A. 2000. Orientation behaviour in sticklebacks: modified by experience or population specific? *Behaviour*, **137**: 833–843.
- Gonda, A., Herczeg, G. and Merilä, J. 2009. Adaptive brain size divergence in nine-spined sticklebacks (*Pungitius pungitius*)? *J. Evol. Biol.*, **22**: 1721–1726.
- Gonda, A., Herczeg, G. and Merilä, J. 2011. Population variation in brain size of nine-spined sticklebacks (*Pungitius pungitius*) – local adaptation or environmentally induced variation? *BMC Evol. Biol.*, **11**: 75.
- Gow, J.L., Rogers, S.M., Jackson, M. and Schluter, D. 2008. Ecological predictions lead to the discovery of a benthic–limnetic sympatric species pair of threespine stickleback in Little Quarry Lake, British Columbia. *Can. J. Zool.*, **86**: 564–571.
- Healy, S.D. 1998. *Spatial Representation in Animals*. Oxford: Oxford University Press.
- Healy, S.D., de Kort, S.R. and Clayton, N.S. 2005. The hippocampus, spatial memory, and food hoarding: a puzzle revisited. *Trends Ecol. Evol.*, **20**: 17–22.
- Hendry, A.P., Peichel, C.L., Matthews, B., Boughman, J.W. and Nosil, P. 2013. Stickleback research: the now and the next. *Evol. Ecol. Res.*, **15**: 111–141.
- Hohenlohe, P.A., Bassham, S., Currey, M. and Cresko, W.A. 2012. Extensive linkage disequilibrium and parallel adaptive divergence across threespine stickleback genomes. *Phil. Trans. R. Soc. Lond. B*, **367**: 395–408.
- Hughes, R.N. and Blight, C.M. 1999. Algorithmic behaviour and spatial memory are used by two intertidal fish species to solve the radial maze. *Anim. Behav.*, **58**: 601–613.
- Huntingford, F.A. and Coyle, S. 2007. Antipredator defences in sticklebacks: trade-offs, risk sensitivity, and behavioural syndromes. In *Biology of the Three-spined Stickleback* (S. Östlund-Nilsson, I. Mayer and F.A. Huntingford, eds.), pp. 127–156. Boca Raton, FL: CRC Press.
- Huntingford, F.A., Wright, P.J. and Tierney, J.F. 1994. Adaptive variation in antipredator behavior in threespine stickleback. In *Evolutionary Biology of the Threespine Stickleback* (M.A. Bell and S.A. Foster, eds.), pp. 277–296. Oxford: Oxford University Press.
- Jacobs, L.F. 2003. The evolution of the cognitive map. *Brain Behav. Evol.*, **62**: 128–139.
- Juraska, J.M., Fitch, J.M., Henderson, C. and Rivers, N. 1985. Sex differences in the dendritic branching of dentate granule cells following differential experience. *Brain Res.*, **333**: 73–80.
- Juraska, J.M., Fitch, J.M. and Washburne, D.L. 1989. The dendritic morphology of pyramidal



- neurons in the rat hippocampal CA3 area. II. Effects of gender and environment. *Brain Res.*, **479**: 115–119.
- Kaeuffer, R., Peichel, C.L., Bolnick, D.I. and Hendry, A.P. 2012. Parallel and nonparallel aspects of ecological, phenotypic, and genetic divergence across replicate population pairs of lake and stream stickleback. *Evolution*, **66**: 402–418.
- Lamanna, J.R. and Eason, P.K. 2003. Effects of landmarks on territorial establishment. *Anim. Behav.*, **65**: 471–478.
- Lavin, P.A. and McPhail, J.D. 1985. The evolution of freshwater diversity in the threespine stickleback (*Gasterosteus aculeatus*): site-specific differentiation of trophic morphology. *Can. J. Zool.*, **63**: 2632–2638.
- Lavin, P.A. and McPhail, J.D. 1986. Adaptive divergence of trophic phenotype among freshwater populations of the threespine stickleback (*Gasterosteus aculeatus*). *Can. J. Fish. Aquat. Sci.*, **43**: 2455–2463.
- Lee, E.T. and Wang, J.W. 2003. *Statistical Methods for Survival Data Analysis*. New York: Wiley.
- López, J.C., Broglio, C., Rodríguez, F., Thinus-Blanc, C. and Salas, C. 1999. Multiple spatial learning strategies in goldfish (*Carassius auratus*). *Anim. Cogn.*, **2**: 109–120.
- López, J.C., Broglio, C., Rodríguez, F., Thinus-Blanc, C. and Salas, C. 2000. Reversal learning deficit in a spatial task but not a cued one after telencephalic ablations in goldfish. *Behav. Brain Res.*, **109**: 91–98.
- Mackney, P.A. and Hughes, R.N. 1995. Foraging behaviour and memory window in sticklebacks. *Behaviour*, **132**: 1241–1253.
- Markel, R.W. 1994. An adaptive value of spatial learning and memory in the blackeye goby, *Coryphopterus nicholsi*. *Anim. Behav.*, **47**: 1462–1464.
- McKinnon, J.S. and Rundle, H.D. 2002. Speciation in nature: the threespine stickleback model systems. *Trends Ecol. Evol.*, **17**: 480–488.
- McPhail, J.D. 1984. Ecology and evolution of sympatric sticklebacks (*Gasterosteus*): morphological and genetic evidence for a species pair in Enos Lake, British Columbia. *Can. J. Zool.*, **62**: 1402–1408.
- McPhail, J.D. 1992. Ecology and evolution of sympatric sticklebacks (*Gasterosteus*): evidence for a species pair in Paxton Lake, Texada Island, British Columbia. *Can. J. Zool.*, **70**: 361–369.
- McPhail, J.D. 1994. Speciation and the evolution of reproductive isolation in the sticklebacks (*Gasterosteus*) of south-western British Columbia. In *The Evolutionary Biology of the Threespine Stickleback* (M.A. Bell and S.A. Foster, eds.), pp. 399–437. Oxford: Oxford University Press.
- Nadel, L. 1994. Multiple memory systems: what and why, an update. In *Memory Systems* (D.L. Schacter and E. Tulving, eds.), pp. 39–63. Cambridge, MA: MIT Press.
- Northcutt, R.G. 2006. Connections of the lateral and medial divisions of the goldfish telencephalic pallium. *J. Comp. Neurol.*, **494**: 903–943.
- Odling-Smee, L.C. and Braithwaite, V.A. 2003a. The role of learning in fish orientation. *Fish Fish.*, **4**: 235–246.
- Odling-Smee, L.C. and Braithwaite, V.A. 2003b. The influence of habitat stability on landmark use during spatial learning in the three-spined stickleback. *Anim. Behav.*, **65**: 701–707.
- Odling-Smee, L.C., Boughman, J.W. and Braithwaite, V.A. 2008. Sympatric species of threespine stickleback differ in their performance in a spatial learning task. *Behav. Ecol. Sociobiol.*, **62**: 1935–1945.
- O’Keefe, J. and Nadel, L. 1978. *The Hippocampus as a Cognitive Map*. Oxford: Clarendon Press.
- Olson, A.K., Eadie, B.D., Ernst, C. and Christie, B.R. 2006. Environmental enrichment and voluntary exercise massively increase neurogenesis in the adult hippocampus via dissociable pathways. *Hippocampus*, **16**: 250–260.
- Östlund-Nilsson, S., Mayer, I. and Huntingford, F.A., eds. 2007. *Biology of the Three-spined Stickleback*. Boca Raton, FL: CRC Press.
- Paepke, H.J. 1996. *Die Stichlinge*. Magdeburg: Westarp Wissenschaften.

- Park, P.J. 2011. *Variation of spatial strategies and the telencephalon of the threespine stickleback *Gasterosteus aculeatus* in relation to inferred ecology*. PhD Thesis, Stony Brook University, Stony Brook, NY.
- Park, P.J. and Bell, M.A. 2010. Variation of telencephalon morphology of the threespine stickleback (*Gasterosteus aculeatus*) in relation to inferred ecology. *J. Evol. Biol.*, **23**: 1261–1277.
- Park, P.J., Chase, I. and Bell, M.A. 2012. Phenotypic plasticity of the threespine stickleback *Gasterosteus aculeatus* telencephalon in response to experience in captivity. *Curr. Zool.*, **58**: 189–210.
- Patel, S., Clayton, N. and Krebs, J. 1997. Spatial learning induces neurogenesis in the avian brain. *Behav. Brain Res.*, **89**: 115–128.
- Purnell, M.A., Hart, P.J.B., Baines, D.C. and Bell, M.A. 2006. Quantitative analysis of dental microwear in threespine stickleback: a new approach to analysis of trophic ecology in aquatic vertebrates. *J. Anim. Ecol.*, **75**: 967–977.
- Rampon, C., Tang, Y.P., Goodhouse, J., Shimizu, E., Kyin, M. and Tsien, J.Z. 2000. Enrichment induces structural changes and recovery from nonspatial memory deficits in CA1 NMDAR1-knockout mice. *Nature Neurosci.*, **3**: 238–244.
- Réale, D., Reader, S.M., Sol, D., McDougall, P.T. and Dingemanse, N.J. 2007. Integrating animal temperament within ecology and evolution. *Biol. Rev.*, **82**: 291–318.
- Rodríguez, F., Durán, E., Vargas, J.P., Torres, B. and Salas, C. 1994. Performance of goldfish trained in allocentric and egocentric maze procedures suggests the presence of a cognitive mapping system in fishes. *Anim. Learn. Behav.*, **22**: 409–420.
- Rodríguez, F., López, J.C., Vargas, J.P., Broglio, C., Gómez, Y. and Salas, C. 2002. Spatial memory and hippocampal pallium through vertebrate evolution: insights from reptiles and teleost fish. *Brain Res. Bull.*, **57**: 409–503.
- Rowe, M., Baube, C.L., Loew, E.R. and Phillips, J.B. 2004. Optimal mechanisms for finding and selecting mates: how threespine stickleback (*Gasterosteus aculeatus*) should encode male throat colors. *J. Comp. Physiol. A*, **190**: 241–256.
- Salas, C., Broglio, C., Rodríguez, F., López, J.C., Portavella, M. and Torres, B. 1996a. Telencephalon ablation in goldfish impairs performance in a ‘spatial constancy’ problem but not in a cued one. *Behav. Brain Res.*, **17**: 193–200.
- Salas, C., Rodríguez, F., Vargas, J.P., Durán, E. and Torres, B. 1996b. Spatial learning and memory deficits after telencephalic ablation in goldfish trained in place and turn maze procedures. *Behav. Neurosci.*, **110**: 965–980.
- Schacter, D.L. and Tulving, E. 1994. *Memory Systems*. Cambridge, MA: MIT Press.
- Schluter, D. and McPhail, J.D. 1992. Ecological character displacement and speciation in sticklebacks. *Am. Nat.*, **140**: 85–108.
- Scotti, M.L. and Foster, S.A. 2007. Phenotypic plasticity and the ecotypic differentiation of aggressive behavior in threespine stickleback. *Ethology*, **113**: 190–198.
- Shaw, K.A., Scotti, M.L. and Foster, S.A. 2007. Ancestral plasticity and the evolutionary diversification of courtship behaviour in threespine sticklebacks. *Anim. Behav.*, **73**: 415–422.
- Sherry, D.F. 1998. The ecology and neurobiology of spatial memory. In *Cognitive Ecology: The Evolutionary Ecology of Information Processing and Decision Making* (R. Dukas, ed.), pp. 261–296. Chicago, IL: University of Chicago Press.
- Sherry, D.F. and Duff, S.J. 1996. Behavioural and neural bases of navigation in food-storing birds. *J. Exp. Biol.*, **199**: 165–171.
- Shettleworth, S.J. 2010. *Cognition, Evolution, and Behavior*. New York: Oxford University Press.
- Sokal, R.R. and Rohlf, F.J. 1995. *Biometry*. New York: W.H. Freeman.
- Spritzer, M.D., Meikle, D.B. and Solomon, N.G. 2005. Female choice based on male spatial ability and aggressiveness among meadow voles. *Anim. Behav.*, **69**: 1121–1130.
- Templeton, C.N. and Shriner, W.M. 2004. Multiple selection pressures influence Trinidadian guppy (*Poecilia reticulata*) antipredator behavior. *Behav. Ecol.*, **15**: 673–678.

- Tolman, E.C. 1948. Cognitive maps in rats and men. *Psychol. Rev.*, **55**: 189–208.
- Vamosi, S.M. 2002. Predation sharpens the adaptive peaks: survival trade-offs in sympatric sticklebacks. *Ann. Zool. Fenn.*, **39**: 237–248.
- van Praag, H., Kempermann, G. and Gage, F.H. 2000. Neural consequences of environmental enrichment. *Nature Rev. Neurosci.*, **1**: 191–198.
- Vargas, J.P., Rodríguez, F., López, J.C., Arias, J.L. and Salas, C. 2000. Spatial learning-induced increase in the argyrophilic nucleolar organizer region of the dorsolateral telencephalic neurons in goldfish. *Brain Res.*, **865**: 77–84.
- Vargas, J.P., López, J.C. and Portavella, M. 2009. What are the functions of fish brain pallium? *Brain Res. Bull.*, **79**: 436–440.
- Walker, J.A. 1997. Ecological morphology of lacustrine threespine stickleback *Gasterosteus aculeatus* L. (Gasterosteidae) body shape. *Biol. J. Linn. Soc.*, **61**: 3–50.
- Walker, J.A. and Bell, M.A. 2000. Net evolutionary trajectories of body shape evolution within a microgeographic radiation of threespine sticklebacks (*Gasterosteus aculeatus*). *J. Zool.*, **252**: 293–302.
- Walsh, R.N. and Cummins, R.A. 1976. The open field test: a critical review. *Psychol. Bull.*, **83**: 482–504.
- Warburton, K. 1990. The use of local landmarks by foraging goldfish. *Anim. Behav.*, **40**: 500–505.
- Warburton, K. 2003. Learning of foraging strategies by fish. *Fish Fish.*, **4**: 203–215.
- Ward, A.J.W., Thomas, P., Hart, P.J.B. and Krause, J. 2004. Correlates of boldness in three-spined sticklebacks (*Gasterosteus aculeatus*). *Behav. Ecol. Sociobiol.*, **55**: 561–568.
- West-Eberhard, M.J. 2003. *Developmental Plasticity and Evolution*. New York: Oxford University Press.
- Willacker, J.J., von Hippel, F.A., Wilton, P.R. and Walton, K.M. 2010. Classification of threespine stickleback along the benthic–limnetic axis. *Biol. J. Linn. Soc.*, **101**: 595–608.
- Willacker, J.J., von Hippel, F.A., Ackerly, K. and Welker, J. 2012. Morphological and trophic divergence in a phenotypically bimodal population of threespine stickleback. Paper presented at the *7th International Conference on Stickleback Behavior and Evolution*, Bainbridge Island, WA, July.
- Wootton, R.J. 1976. *The Biology of the Sticklebacks*. New York: Academic Press.
- Wootton, R.J. 1984. *A Functional Biology of Sticklebacks*. Los Angeles, CA: University of California Press.
- Wund, M.A., Baker, J.A., Clancy, B., Golub, J.L. and Foster, S.A. 2008. A test of the ‘flexible stem’ model of evolution: ancestral plasticity, genetic accommodation, and morphological divergence in the threespine stickleback radiation. *Am. Nat.*, **172**: 449–462.

