



## Can juvenile Atlantic salmon use multiple cue systems in spatial learning?

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**Abstract.** An ability to form a map or spatial representation of a home range should facilitate efficient foraging and promote the use of effective escape routes to shelter. Although a few experiments have shown some species of fish are capable of simple spatial behaviour, little is known about the behavioural mechanisms they use to orient as they forage. Whether juvenile Atlantic salmon, *Salmo salar*, have the capacity to use conspicuous visual landmarks to help them track a moving resource was investigated. The experiment was then modified to determine whether the salmon could continue to track the resource in the absence of conspicuous visual cues. The salmon followed a moveable food source in the presence and, after retraining, in the absence of distinct coloured landmarks. It is suggested that juvenile salmon may be capable of using multiple forms of cue to help them solve spatial tasks such as tracking a moving resource.

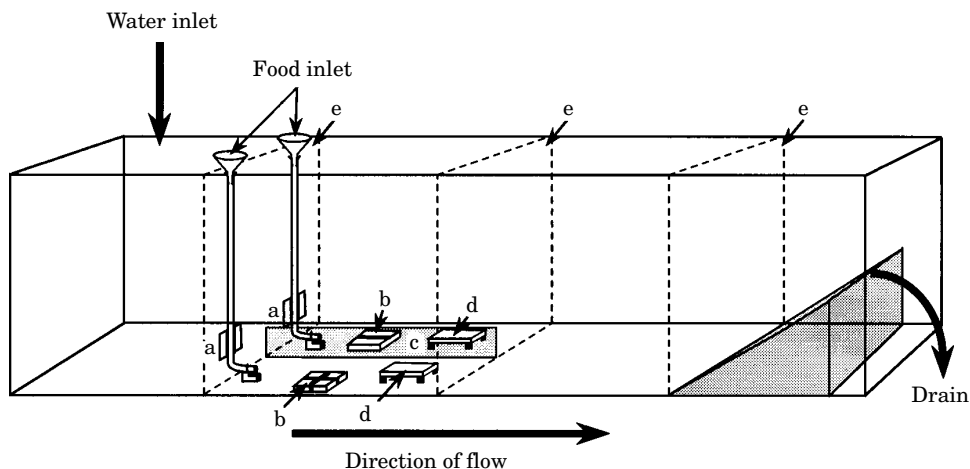
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An ability to form some sort of mental spatial representation of the distribution of patchy resources will influence the fitness of an individual and the distribution of populations. There is a wealth of literature on how animals locate objects within their environment (Gallistel 1990) and it is now clear, for many species, that there may be several techniques that can be used, depending on the past history of the animal concerned and the current nature of the spatial problem it faces (for example, locating a profitable food patch or finding shelter when threatened). Much attention has been paid to the spatial ability of birds and mammals but relatively little to fish. There is, however, some evidence that fish can perform simple spatial tasks. For example, blind cave fish, *Anoptichthy jordani*, appear to remember specific features in their environment which they detect

with their lateral line system (Teyke 1989) and inter-tidal gobies, *Bathygobius soporator*, are capable of jumping between rock pools at low tide when they are unable to see their destination pool (Aronson 1951, 1971). Furthermore, goldfish, *Carassius auratus*, can learn a simple visual discrimination (landmark versus no landmark) to find a hidden food reward efficiently (Warburton 1990) and sticklebacks, *Gasterosteus aculeatus*, will learn to avoid sites where they have experienced predatory attacks (Huntingford & Wright 1989). Although these studies suggest an ability to use and remember features in an environment, they were not designed to test whether specific landmarks are remembered or used to locate a particular goal.

Juvenile Atlantic salmon (parr), *Salmo salar*, live within home ranges during most of their period in freshwater before migrating out to sea. Within these home ranges their use of space is highly structured (Gibson 1993). They hold station, just above or resting on the substrate, in one or a few favoured sites from which they intercept drifting food items (Keenleyside 1962; Gibson 1993). Salmon move between these sites during the day (Gustafson-Greenwood & Moring 1990), presumably in response to variation in the availability of food, for example through changes

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**Figure 1.** Through-flow flume used for training and testing salmon. a: Front screen landmarks; b: base-plate landmarks; c: central baffle; d: shelters; e: screens.

in water flow (Waters 1969). Previous experiments have shown that profitable feeding sites can be followed (Gotceitas & Godin 1992), thus, it would seem likely that foraging efficiency will be increased if parr are capable of learning and using cues that signal the relative profitability of different patches.

Juvenile salmon have an excellent sense of vision (Douglas & Hawryshyn 1990) which is used to detect food, predators and competitors. It seems likely that vision might also be important in spatial orientation, but different senses may also play a role. Previous work has shown that juvenile salmon will use their sense of smell in some situations. For example, they prefer water conditioned by familiar rather than unfamiliar individuals (Stabell 1982; Brown & Brown 1992) and can discriminate between urine derived from sibling and non-sibling fish (Moore et al. 1994). Furthermore, parr appear to recognize odours extracted from gravel lining their holding tank (Stabell 1987). It is possible, therefore, that olfactory information may also be used in orientation.

Our aim in this experiment was to assess whether juvenile salmon are capable of predicting the position of a moveable food source in the presence or absence of conspicuous visual landmarks. The fish were tested over two phases. First, we assessed the ability to track the position of a moveable food source which was labelled with distinct visual landmarks. Second, we made all the landmarks visually identical to determine whether

the salmon parr could continue to track the food source despite the absence of the distinct visual cues.

## METHODS

### Tank Design

Three flow-through flume tanks supplied with an untreated freshwater supply from Loch Faskally, Pitlochry, Scotland were used to test nine hatchery reared 1+ (i.e. at least 1 year old) juvenile Atlantic salmon (between ca 50 and 80 mm fork length). Prior to the experiments the fish were housed in a grey PVC tank 1 m square with a circulating water supply. An automatic feeder dispensed commercial food into the water over the course of the day, uneaten food being quickly carried into the out-flow drain. The tests were run over two summer seasons in 1993 and 1994. Each flow-through tank (Fig. 1), received a constant flow of water delivered through a controlled valve to the first section. The water (250 mm deep) entered the main observation area through a stainless steel screen (mesh size 3 mm); from here it flowed through a second, central screen to the rear of the observation area and then passed through a third and final screen before running to a drain. Although the mesh screens filtered a lot of the background debris from the water a certain amount of silt was deposited on the floor of each tank over 24 h. This and any

excess uneaten food was removed by a siphon at least 2 h after the fish had been fed. The tanks, illuminated by natural light from a skylight, were arranged around a central hide made from black plastic. The observation area of each tank had a black plastic curtain to the front and rear preventing the fish from seeing any external cues through the sides of the tank. The front curtain contained a flap which could be raised to allow the fish to be observed. By raising only one flap at a time, the observer in the dark central hide remained invisible to the fish. A frosted Perspex lid on top of the tank observation area prevented the fish from seeing any external features above the tank but allowed natural background light to illuminate the tank.

A central baffle (100 mm in height) made from clear PVC divided the main observation area in half (i.e. two patches each of 500 × 600 mm, see Fig. 1 for detail). The fish were able to swim between the two patches over the central baffle. Each half contained a removable white PVC base plate which created a false floor to the tank. On top of each plate, we used plastic building bricks (Lego) to build three constructions. First, a shelter (65 × 125 mm) was positioned towards the centre at the back of the plate. This consisted of four legs and a flat top section. In front of the shelter there was a central Lego square landmark, or flag (80 × 80 mm). This was built from white bricks, which matched the background colour of the base plate, with bricks of a different colour inserted to produce symbols, a blue cross and a red line in two of the tanks and a yellow cross and a blue line in the third tank. The use of different coloured symbols in the experimental tanks did not affect fish performance. To the front of the flag a Lego tower (30 mm high), the same colour as the symbol on that plate, and a clip held a plastic tube (10 mm diameter) in place. The tube was connected to a funnel attached to the top of the front screen. The funnel was supplied with a constant flow of water creating a jet of water that was faster than the background flow in the tank. The water jet was positioned to run along the length of the PVC plate towards the shelter. A belt feeder was arranged to deliver a supply of fish pellets into the neck of the funnel, these were then carried down the tube and sprayed by the water jet towards the shelter. The force of the jet carried most of the uneaten pellets through the second screen into the rear half of the observation area. A further Lego

landmark, identical to the one on the floor of the plate, was positioned vertically on the front screen behind the fast water jet.

### Phase 1: Visually Distinct Landmarks

The fish were deprived of food for 3 days, put into the observation area one at a time and left to settle for 24 h. After this period, we noted their position; the base plate they were resting on for this first observation became the rewarded plate for the rest of this phase. No position bias, left or right side of the tank, or colour and shape of symbol bias were observed. The fish were given a training period during which they were taught that food pellets were delivered through the fast jet tube for 1 h a day to one side of the tank only, the one containing the rewarded plate. Food was available between 0930 and 1030 hours at the approximate rate of a pellet every 2.5 min. The central baffle prevented food drifting between the plates so the fish had to be positioned on the rewarded plate to obtain food. For phase 1, the fish could use the different shapes and colours of the landmark symbols to learn which plate was the one that received food. To prevent the fish from learning a position bias, such as the left or right side of the tank, either we moved the base plates with the Lego bricks and the front screen landmarks, as a pair, between the two sides of the tank between trials, or left them in the same position. We used a random sequence to indicate when, between trials, the plates should be moved or kept in the same place. If the base plates and front landmarks remained in the same place between a trial they were still removed to mimic a position change but were returned to their original positions. Prior to this disruptive period we raised the second, central screen and encouraged the salmon into the rear half of the observation area.

We gave the fish daily feeding trials for at least 14 days. At the end of this period all fish had been observed feeding from pellets delivered through the fast water jet and tended to forage from a position near the shelter towards the rear of the plate. Any fish that did not learn to sample on both the left and right side of the tank (i.e. were biased to one side) was not used after this training phase.

To test whether the fish could predict where food would be delivered, in the presence of a

distinct visual cue, we noted their positions prior to the delivery of food during the following 10 days. Again the positions of the plates were interchanged between trials using a random sequence.

### Phase 2: Visually Identical Landmarks

By testing whether the salmon could continue to locate the rewarded plate in the absence of distinct visual landmarks, we designed this phase to explore whether fish were capable of using information other than the coloured Lego landmarks to help them track the position of the rewarded plate. Only salmon that had successfully tracked the rewarded plate for 70% or more of the test period in phase 1 were used.

For this phase, we used the colour and symbol shape that the individual fish associated with a reward in phase 1 to create two new visually identical plates. Now the Lego shelters, landmarks (front and base plate) and tube holders were made to look identical to create two matching patches. One plate was assigned to be the rewarded plate (chosen as in phase 1). The fish were given 10 days of training and the plates and front landmarks were randomly moved between the two sides of the tank between trials as in phase 1. After this training period, we noted the fish positions prior to food delivery for a further 10 days of test observations as in phase 1.

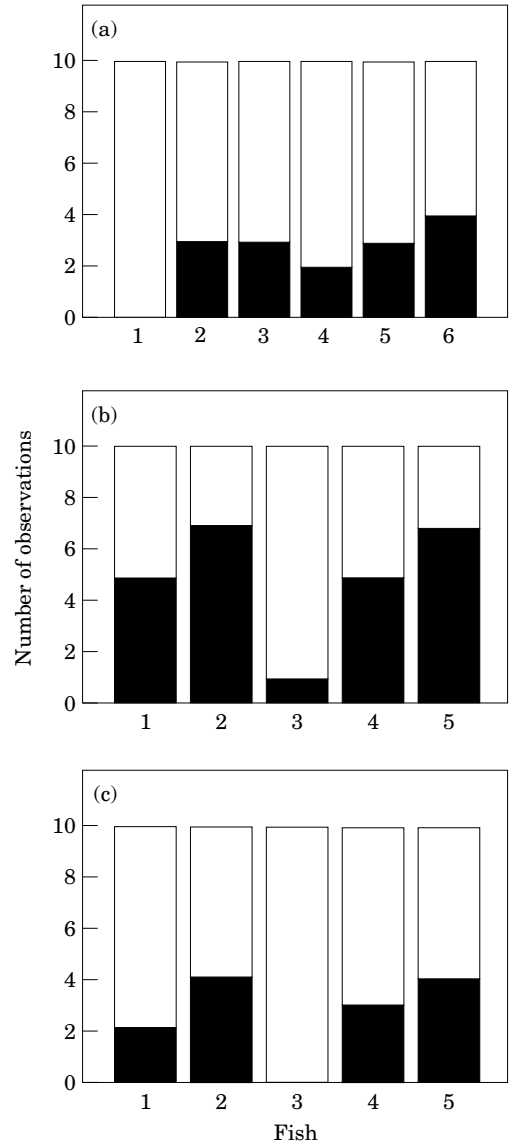
At the end of phases 1 and 2 and before the next fish was tested, all the plates and Lego bricks were soaked in a sodium hypochlorite solution and scrubbed with absolute ethanol to try to eliminate any chemical substances the salmon may have deposited onto the surface of the plates.

## RESULTS

### Phase 1: Visually Distinct Landmarks

Of the nine fish that were tested, three individuals became site-attached to either the left or the right side of the tank and were rarely, if at all, observed on the opposite side. These three fish did not progress from the training stage.

All six fish that were tested were found waiting for food on the rewarded rather than the non-rewarded plate on more than half the observations



**Figure 2.** Number of occasions fish were observed waiting on the rewarded (□) or non-rewarded (■) plate prior to food delivery. (a) Phase 1: visually distinct landmarks. (b) Training for phase 2: visually identical landmarks. (c) Phase 2: visually identical landmarks.

(a mean performance level of 75%). A Cochran test (Armitage & Berry 1987) showed that salmon were waiting for food on the rewarded plate significantly more often than on the non-rewarded plate ( $\chi^2=8.14$ ,  $df=1$ ,  $P<0.01$ ; Fig. 2a).

### Phase 2: Visually Identical Landmarks

Five fish were trained and tested in this phase. The 75% mean performance level shown during the phase 1 test period dropped to 30–50% for four of the five fish during the phase 2 training. One remaining fish continued a high performance level of 90% during this training period (Fig. 2b). During the testing period, however, all five fish were observed more than half of the time on the rewarded plate (Fig. 2c). A Cochran test showed that the salmon were again observed to be waiting for food significantly more frequently on the rewarded rather than the non-rewarded plate ( $\chi^2=4.37$ ,  $df=1$ ,  $P<0.02$ ) even though the plates were visually identical.

## DISCUSSION

These results show clearly that juvenile Atlantic salmon have the capacity to use landmarks to help them track a moveable food source. The first phase of the experiment indicated that the parr could use a coloured visual landmark as a local cue (the tank design did not permit the use of global cues) to allow them to predict the position of the rewarded plate. Although earlier work has shown some fish species can solve simple spatial problems, the experiments **we report here show that juvenile salmon are capable of more complex spatial tasks.** The fish were able to distinguish between two similar visual landmarks and could learn to track the movements of one specific landmark to predict the location of a food supply.

The second phase of the experiment showed that salmon were capable of tracking the rewarded plate despite the absence of distinct visual landmarks. During the phase 2 training period, tracking performance dropped for all but one fish when the differently shaped and coloured landmarks were substituted for identical ones (Fig. 2b). This would suggest that the majority of the fish had to learn a new method to allow them to predict the position of the rewarded plate. Fish 3, however, did not show the same drop in performance. This fish appeared to use alternative cues to the coloured landmarks during phase 1 and apparently continued using this alternative method during phase 2, thus showing no decrease in ability to find the rewarded plate. (If fish 3

is removed from the first data set [phase 1, the visually distinct landmarks] the level of significance shown by a Cochran test remains unchanged ( $\chi^2=7.40$ ,  $df=1$ ,  $P<0.01$ ) indicating that the remaining five fish still appear to be using the coloured visual landmarks to guide them to the rewarded plate). After the phase 2 training period, however, the performance of the remaining fish increased so that during the phase 2 test observations the salmon were capable of predicting which plate was rewarded.

The materials used for the plates and landmarks were intentionally chosen for their durable, hard-to-scratch surfaces, to minimize the chance that small visual differences between the patches could be used by the fish in phase 2. Although we cannot rule out the possibility that small visual inconsistencies in the plates and landmarks existed we speculate that one alternative suggestion is that the fish were using some form of chemosensory cue. There are several potential sources for such a cue. Food remnants could have been detectable either in the jets or on the base plates. However, both jets were used to deliver food at different stages during the experiment and the cleaning procedure removed all excess food from the plates. Alternatively, the fish may have been marking the substrate. Such a source of olfactory cues was originally hypothesized by Stabell (1987) after he discovered that salmon parr could recognize water containing extracts from gravel that had lined their holding tank. If the juvenile salmon are depositing some form of chemosensory cue then it would seem that such olfactory marks can function as landmarks (also see Halvorsen & Stabell 1990). It is possible that this hypothesis may explain why fish 3 appeared to be unaffected by the phase 1–phase 2 transition; this fish may have been using olfactory cues preferentially from the beginning of the experiment.

The salmon showed individual variation at every stage of this study. For example, three individuals did not progress from the training stage of phase 1 as these fish were never observed sampling between the two plates. Instead they appeared to use a simple laterality cue (stay left or stay right) to indicate where they should spend their time. Further variation in fish performance is shown by the data in Fig. 2. Some of this variation may be explained by different sampling behaviour (Shettleworth et al. 1988); however, it is

possible that differing social status as described by Gotceitas & Godin (1992) and perhaps different life-history strategies (Metcalf et al. 1990) may also contribute to the variation exhibited by the parr.

Many salmonids rely on chemosensory mechanisms during their migratory periods, and so may be expected to be better at chemosensory tracking than other fish groups. Comparative studies with other species could be used to test this idea. Work with other animal groups using cue-conflict experiments has suggested that hierarchies of cues are relatively common (see Able 1993 and references therein). Salmon parr may also use cue hierarchies. One adaptive explanation for the use of more than one form of spatial cue (i.e. visual and chemosensory) may be found in the recently discovered switch from diurnal to nocturnal behaviour (Fraser et al. 1993; Heggenes et al. 1993). It has been reported that, as day-length shortens and temperatures fall, juvenile salmon switch from diurnal feeding to predominantly nocturnal activity. Presumably as the parr move between shelter and feeding stations at night, visual cues will be largely redundant and alternative information such as olfactory cues may become more important. Further experiments are now required to tease apart the dependence these fish show for each form of spatial cue.

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