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## **DOCTOR OF PHILOSOPHY**

**Topographic learning and memory in habitat selection by *Lipophrys pholis* L.**

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**Topographic learning and memory in habitat  
selection by *Lipophrys pholis* L.**

A thesis presented to the University of Wales  
for the degree of Doctor of Philosophy

by

**Jane Dodd B.Sc., M.Sc.**

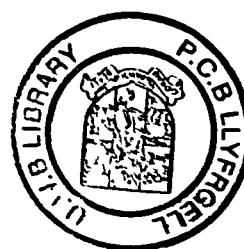
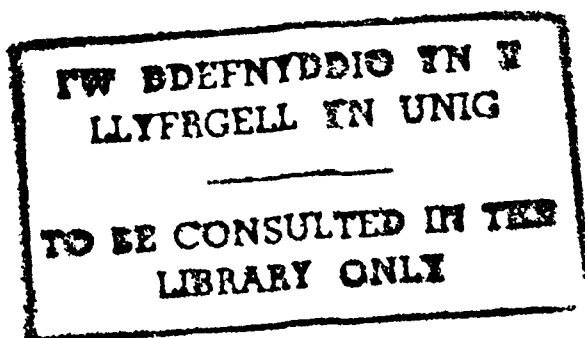
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For Mum, Dad and Sara

## SUMMARY

Experiments were carried out to test the ability of *L. pholis* to learn and remember the position of a food reward in a hierarchical maze during daily trials. This task was completed in 9 days in the presence of Lego™ landmarks. Learning did not take place in the presence of a white screen clue or in the absence of any obvious visual clues after 15 days.

An attempt was made to identify the effect of a change in conditions on *L. pholis* who had already learned to successfully navigate the hierarchical maze, and to identify the clues utilised in learning, by altering the intra and extramaze clues. Geomagnetic, olfactory / gustatory and current direction clues were eliminated as the source of spatial information, as was the use of a cognitive map (Tolman 1948, O'Keefe & Nadel 1978). Rather, evidence suggested that the fish were learning a specific route through the hierarchical maze using the Lego™ towers as beacons, and as a prompt as to which direction to turn at important stages in the journey. This memory was retained for a period of at least 30 days. A hierarchy of clue use was suggested by the fact that the subjects used the direction of entry to the experimental arena, or the direction by which the experimenter left the arena just prior to a daily test, as a directional clue to the position of the reward box in the absence of Lego™ towers. These experiments suggested that in the wild *L. pholis* uses the position of local landmarks, such as rocks and clumps of algae, to direct movements towards feeding patches.

A study of behaviour of *L. pholis* placed in a novel artificial habitat was also carried out. *L. pholis* moved along the edges of the objects placed in the arena, followed regularly used paths between refuges, and explored the arena from a series of "base" refuges. In the more active individuals, each refuge was investigated until the subject took up residence in a preferred refuge. This adopted shelter was often centrally placed and commanded a good view of a large area of the arena. Activity was concentrated in the more complex half of the arena and experienced fish directed their movement towards this area 24 hours after 6 hours exploration of the novel habitat. There was also evidence to suggest that certain individuals learned the position of a specific preferred refuge after the 6 hour exploratory period.

Finally, the ability of *L. pholis* to remember the position of a refuge was tested in an artificial habitat under the influence of different clues. *L. pholis* learned the position of the refuge in the presence of an A4 sized black screen clue only. They responded to this clue by moving towards it and pressing themselves up against it while Lego™ towers and a white screen clue did not provoke such a response. *L. pholis* continued to respond to the black screen in this way even when it was moved to another location further from the refuge. After 12 days *L. pholis* learned to use the black screen as an indirect clue and navigate to the refuge directly without first touching it. These results suggested that when placed in a novel habitat the immediate reaction of *L. pholis* is to move quickly towards the first dark area they see. Later, they systematically explore all available shelters and choose a preferred one according to complexity of the surrounding habitat. With experience, they can use the position of objects around them to navigate quickly and efficiently to their preferred refuge or the nearest suitable refuge depending on the severity of the threat.

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## **Chapter 1**

### **General introduction**

Movements in animals are generally non-random and concentrated towards areas where suitable environmental conditions of shelter, food or sexual partners are found. If such criteria are met at spatially discrete sites it is of great selective advantage to be able to orientate movements or migrations between these sites. Homing is the ability of an animal to learn and remember its surroundings and use the memory to return to a particular location or “the return to a place formerly occupied instead of going to equally probable places” (Gerking 1959).

## **1.1 Homing**

Papi (1990) classified homing phenomena based on the origin of information that is used to home. In this classification there are only three sources of information, *genetic* (or innate), sensory, and memory dependent (Jander 1975). However these can be combined in various ways and because the information from the three sources can be separated into several items the actual number of categories is much greater (Papi 1992).

### **1.1.1 Random or systematic search**

The first category of Papi’s classification of homing is random or systematic search. This strategy may consist of transecting behaviour whereby the animal undertakes radially orientated excursions from a central starting point followed by a return to this central point. Random or systematic search may also consist of convoluted search, which involves loops of increasing size with regular returns to the starting point.

### **1.1.2 Genetically-based orientation**

The basis for the second classification of homing, genetically-based orientation, is inherited information. This phenomenon was demonstrated in young birds reared in the laboratory, which were able to select the correct migratory direction and displayed restlessness for the time needed to carry out their journey, without prior experience of migration or access to older conspecifics (Berthold 1990). Genetically-based

orientation, incorporates distance and direction, therefore has been referred to as vectorial orientation or vectorial navigation.

### 1.1.3 Trail finding

The third category of homing, trail finding, in contrast to other route based mechanisms, does not include memory. Rather, it relies on the laying of a mucus or scent trail in the outward journey, for example in molluscs and ants (Papi 1992) respectively, which can be identified and followed to guide the return journey.

### 1.1.4 Route-based orientation

The fourth category, route-based orientation, can be subdivided into three further categories, route reversal, course reversal and path integration. During route reversal an animal retraces its outward path by referring to a series of reference points or landmarks memorized during the outward journey. Course reversal is slightly more complex in that it requires the animal to record the compass direction of the outward journey and orientate homeward in the opposite direction. If the distance travelled is also recorded with this method, homing is made easier (Papi 1992). The final and most complex method of route-based homing is known as path integration. This technique is characterised by a straight return to home after a winding outward journey. The straight homeward path is made possible by taking into account the direction and length of each leg of the route, and continuously updating the position with respect to the starting point in the working memory. As this method involves the calculation of distance and direction, it is sometimes known as vectorial navigation or vectorial integration. When it is based on external points of reference, path integration is referred to as allothetic. In contrast, path integration based on internal information, for example integrating the proprioceptive information generated by rotations and translations of the body on the outward journey and bringing the integrator “back to zero” on the return, is known as idiothetic path integration (Mittelstaedt & Mittelstaedt 1973). This phenomenon has been studied extensively in Mongolian gerbils *Meriones unguliculatus* (Mittelstaedt & Mittelstaedt 1982) and golden hamsters *Mesocricetus*

*auratus* (Etienne *et al.* 1988). However, without use of memory for the position of landmarks, idiothetic integration accumulates errors, and so generally is not regarded as being a likely long-distance homing mechanism (Potegal 1987, 1988, Etienne *et al.* 1988, Benhamou *et al.* 1990, Muller & Wehner 1992, Seguinot *et al.* 1993, Bennett 1996). Darwin (1873) was probably the first to hypothesise that animals could fix their position with respect to a goal or starting point by path integration. He referred to this phenomenon using the nautical term “dead reckoning” and spoke of the visual reference and “the sense of muscular movement” required. Idiothetic orientation is also referred to as kinaesthetic (Gorner 1958), endokinetic or intrinsic orientation (Jander 1975). A fifth term, inertial navigation, is a hypothetical form of idiothetic orientation which requires that an animal can record all the linear and angular accelerations during a journey and double integrate them to compute the direct route home (Barlow 1964).

### 1.1.5 Pilotage and true navigation

The fifth category of homing includes pilotage and true navigation. Pilotage is the use of a familiar array of landmarks or clues (which may be visual or olfactory), which are recognised in the appropriate sequence, and are used to guide the animal to any known site without the use of a compass. This method of navigation is employed by a great many vertebrates and presupposes the use of a topographic map (Papi 1992), a phenomenon referred to by psychologists after Tolman (1948) as cognitive mapping (Thinus-Blanc 1987). True navigation requires the use of a compass in addition to knowledge of the position of local landmarks. It allows animals to calculate the goal direction in unfamiliar areas as long as familiar landmarks are visible. True navigation can be subdivided into two categories. The first is map-based navigation whereby an animal uses experience of an array of local clues to calculate the bearing to the goal. With experience each landmark becomes associated with a bearing to a particular goal, most likely home, and direct routes to different goals can therefore be calculated by referring to these. It should be noted here that Harden Jones (1982) distinguished between the words clue and cue when discussing the landmarks used in the homing

behaviour in fishes. He suggested that “we use a *clue* in the sense of information as to where” and “*cue* be used in the sense of information as to when”, it is this usage which will be adhered to throughout the present study. The second method of true navigation is grid-based navigation, which has so far only been hypothesized and is yet to be demonstrated (Papi 1992). Grid-based navigation suggests that animals can rely on, for example, two chemical clues, the concentrations of which rise and fall steadily in given directions and cross each other to form a gradient or grid map. A comparison of the local values of these two clues with the home values, in a mechanism known as the comparative system, would allow an animal to fix its position relative to home. It is expected that the area included in a gradient map would be very large, possibly even world wide. Since each point in the grid would be determined by two coordinates, grid-based navigation has also been referred to as bicoordinate navigation.

## **1.2 Clues used in homing**

Animals can receive information concerning their environment from all of the sensory organs available to them, but the importance of the information received from each organ varies considerably depending on the environment that each animal inhabits. Olfaction is used in trail following as described earlier and, it is hypothesised, would be used in grid-based orientation, the hypothetical method of true navigation discussed above (Papi 1992).

### **1.2.1 Celestial clues and polarized light**

Vision is used in the recognition of visual landmarks for reference to the position of the sun, the moon and the stars, the celestial clues, and the pattern of polarized light in the sky. The ability to use the sun’s position for orientation was first observed by Santschi (1911) in ants, but, the ability to compensate for the sun’s daily movement across the sky (true sun compass orientation) was not reported until the 1950s in bees (von Frisch 1950), shore dwelling arthropods, (Papi 1955), birds (Kramer 1953) and spiders (Papi *et al.* 1957). In true sun compass orientation the animal takes into account the sun’s azimuth (geographical location), and compensates for the change in



azimuth of the sun during the day by varying its angle of orientation with the sun. The process is regulated by an endogenous rhythm or biological clock. Night-time navigation in various animals (for example sockeye salmon, Brannon *et al.* 1981) has been shown to improve on clear nights when the moon is visible but the mechanism by which the moon influences orientation has yet to be fully understood. In contrast to sun compass orientation, star compass orientation, first reported by Sauer (1957) and subsequently identified as the primary source of navigational information utilized exclusively by night-time migrating birds (Papi 1992), does not require compensation for the rotation of the earth (Emlen 1967). Orientation using polarized light is a separate phenomenon from sun compass orientation in which animals compare a symmetrical pattern of polarized light in the sky with an internal celestial “map”. Navigation using polarized light has the advantage of allowing orientation when only part of the sky is visible or in overcast conditions (Papi 1992).

### 1.2.2 Magnetic compass

The use of a magnetic compass has also been identified in a variety of animals belonging to all main phyla (Kirshvink *et al.* 1985). However, except in sharks and rays, which may use their electro receptors for magnetic detection (Kalmijn 1971), no evidence of the receptor or transduction mechanism of magnetic stimuli has so far been discovered in other groups.

## 1.3 The Cognitive map

As mentioned earlier the cognitive map is a term used regularly in psychology. Unfortunately there are several definitions for it and little or no unequivocal evidence for its existence (Bennett 1996). The cognitive map was first described by Tolman (1948) who defined it as “a representation of the environment which indicates the routes, paths and environmental relationships that an animal uses in making decisions about where to move”. He added that the animal must be capable of learning left from right and have the ability to make novel short cuts between any two points. Another definition which follows the original theory presented by Tolman (1948) is that of

O'Keefe & Nadel (1978) which states that "a cognitive map is a powerful memory of landmarks which allows short-cutting to occur". A third definition of the cognitive map according to Gallistel (1989) is that "a cognitive map is any representation of space used by an animal", and still a fourth definition presented by Thinus-Blanc (1988) defined a cognitive map as an "allocentrically organised representation of environmental features". Bennet (1996) argues that "no animal has been conclusively shown to have a cognitive map *sensu* Tolman and O'Keefe & Nadel because simpler explanations of the crucial short-cutting results are invariably possible". One of these simpler explanations of novel short-cutting was described by O'Keefe & Nadel (1978) themselves as a simple alternative to the cognitive map; they called it the "route". In this all-or-nothing method of navigation the goals are located at or very near to landmarks, and so animals are able to remember a long winding route and reach the goals by moving at least approximately towards subsequent landmarks in the sequence (Bennett 1996). Although the calculations involved in deciding on a "route" are simpler and travel via this method is faster, O'Keefe & Nadel suggested that the use of a "route" could be disadvantageous under certain circumstances. For example, in a dynamically changing environments the relatively inflexible "route" could be easily disrupted if just a single landmark in the sequence was removed. It was for this reason that they developed the hypothesis of the cognitive map, which would be much more resistant to the removal of several landmarks, and would be more *efficient because it* would allow different lines of movement between goals. In one short-cutting test, bees were taught to follow a "V" shaped route between goals A and B. They were eventually found to be capable of moving from site B back to site A across the "V" without following the original learned route, and were therefore presumed to possess a cognitive map (Gould 1986). However, these results were only reproduced when landmarks were clearly visible at site A (Menzel *et al.* 1990, Wehner *et al.* 1990, Wehner & Menzel 1990, Dyer 1991). Furthermore, several studies argued that there were much simpler explanations for novel short-cutting (Cartwright & Collet 1983, Collet 1987, Wehner & Menzel 1990, Dyer 1991). In order to be sure that novel short-cutting does take place one must first be certain that the animal has never crossed the

“V” before (Bennett 1996), a very difficult task when studying a wild animal. Secondly, one has to be sure that path integration is not responsible for novel short-cutting, particularly since path integration is considered to be widespread amongst animals (Potegal 1982, Wehner 1992). Finally, one has to be sure that the animal has done more than simply recognise from a new angle the landmarks at sites A and B learned in training, and move directly towards them using a “route” Bennett (1996). Bennett (1996) also contested that “even if one accepts the author’s assertions that the short-cuts were truly novel, in no cases were just the last two alternatives eliminated”, and concluded that “no animals have ever been shown to have a cognitive map *sensu* Tolman, O’Keefe & Nadel”.

#### 1.4 Short range homing in subtidal fishes

Although spectacular scale migrations between spawning grounds and feeding grounds in fishes such as salmon takes place over large distances (Quinn & Dittman 1992), many fishes confine their movements for significant periods of time to relatively small, actively defended territories or commonly-used home ranges. Experimental displacements of such fishes have revealed an ability to return to home if the displacement distance is not too great. For example Thompson (1983) described the territories of the blenny *Forsterygion varium* (a 1.5-2 m<sup>2</sup> area of subtidal rocky reef around New Zealand) and displaced 10 male blennies 700 m. Eight out of ten of these fish homed within 6 days and one individual returned a distance of 100 m in only 30 min.

Observations of the seabed surrounding the Lora I underwater habitat from windows in Lora I and using SCUBA determined that the home range of the radiated shanny *Ulvaria subbifurcata* is <3 m<sup>2</sup>, and that homing in this species was possible from distances of 270 m. Complementary experiments testing the directional preferences of *Ulvaria subbifurcata* displaced 30 m and then placed in a radially symmetrical arena, indicated that this species can orientate towards home in the absence of visual clues (Green & Fisher 1977). However, further experiments investigating homing in

control, blinded and anosmic *Ulvaria subbiturcata* showed that anosmic fish are site fixed and capable of homing, it appears therefore that a combination of vision and olfaction are used in the homing in this species (Goff & Green 1978).

Rockfishes (*Sebastes* spp.) tend to remain at the site of capture and are capable of homing to the same general habitat, if not the same crevice in a rock (Matthews 1990a), from distances of up to 22.5 km when displaced (Carlson & Haight 1972). Resightings of tagged rockfishes using SCUBA showed that size of home ranges, excursions away from the reef, and homing following experimental displacements of up to 8 km, were significantly different on high relief reefs, artificial reefs and low relief reefs (Matthews 1990a). Rockfishes on the high relief, rocky reefs generally maintained home ranges of less than 30 m<sup>2</sup>, displayed strong site fidelity that was not affected by season, did not move off the reef and returned from displacement. On artificial reefs rockfishes maintained home ranges of similar size to those on high relief rocky reefs but showed significant variation in behaviour with season. In summer the fishes made many off-reef excursions and did not return from displacements, while in autumn and winter they were more site fixed and homed following displacements. The home ranges maintained on low relief rocky reefs were generally much larger than those on high relief rocky reefs and artificial reefs; most were less than 400 m<sup>2</sup> but some were up to 1500 m<sup>2</sup>. Rockfishes only inhabited low relief rocky reefs in summer and only returned from displacements during this season (Matthews 1990a).

Further experiments using ultrasonic telemetry to investigate the home ranges and homing routes of copper (*Sebastes caurinus*) and quillback (*S. maliger*) rockfishes again showed that home ranges were generally <10 m<sup>2</sup> on high relief reefs and less than 4000 m<sup>2</sup> on low relief reefs (Matthews 1990b). No off-reef movement was detected from either reef type in the 11 copper and quillback rockfishes monitored in this experiment. Seven copper and quillback rockfishes were displaced 500 m from a high relief reef to a low relief reef. Immediately following displacement individual rockfishes were observed moving backwards and forwards over the new site for several

days before returning quite directly to the their home site. Six out of seven of the displaced fishes took between 8 and 25 days to home, and then showed no further movement suggesting home-site recognition. Analysis of the homing path indicated three phases of behaviour, i) initial movements along a bimodal northwest-southeast axis ii) movement towards the home reef in a westerly direction, iii) final location of the home site (Matthews 1990b). The initial movements of rockfishes following displacement are thought to take place as a result of habitat assessment. In the autumn kelp dies back and is damaged by storms on low relief reefs, making the habitat less attractive to the rockfishes as there is less cover available for sheltering from predators (Matthews 1990c). The change in habitat quality appears to encourage off-reef movement, probably because the fishes are searching for superior habitats. It has been suggested that rockfishes may relocate home sites by utilizing information gathered when first settling and searching for suitable habitat, but this hypothesis has yet to be tested (Hallacher 1985 in Matthews 1990b). Another explanation suggested for the homing abilities of rockfishes is piloting or true navigation. Such goal-orientated movement across unfamiliar areas would allow rockfishes to return from periodic exploratory excursions if they fail to find a habitat of higher quality than that of their home.

## 1.5 Homing in intertidal fishes

### 1.5.1 Site fidelity and the use of a home range

Experiments in which individuals from a particular pool are tagged, followed by regular resampling of that pool and its immediate neighbours have shown that several intertidal species spend most, or all, of their time in a restricted area or home range (for reviews see Gibson 1969, 1982). The most conclusive evidence of this phenomenon exists for intertidal sculpins. For example, the wooly sculpin *Clinocottus analis* in South California was found to remain in the vicinity of a particular rock pool for up to 20 weeks (Richkus 1978), but reports of homing success vary among species and studies. Green (1971a) recorded 86% recapture of *O. maculosus* in tide pools on Vancouver Island, British Columbia after 2 weeks, whereas Moring (1976) only

recaptured 7% and Yoshiyama *et al.* (1992) only 19% of the same species in rock pools in North Carolina and on the Central Oregon coast respectively.

Pool fidelity is also suggested by relatively slow recolonization of experimentally depopulated pools. For example, Bussing (1972) poisoned rock pools on Marshall Island and resampled after 3 weeks. He found that the number of individuals present had only returned to 50% of its previous level in this time, while the biomass was only at 3% because most individuals repopulating the denuded area were juveniles. The size of a home range inhabited by any species is very difficult to define. However it is thought to have a diameter much less than the distances fishes are capable of travelling at high water (Gibson 1982), to vary considerably between species and between individuals, to vary with time and to be influenced by environmental conditions (Yoshiyama *et al.* 1992). For example Green (1971b) showed that tidepool sculpins had different home range sizes which varied throughout the year and with exposure of the location.

### 1.5.2 Homing

The presence of a particular fish in the same location in subsequent samples is evidence that it is site fixed and suggests that it may home. However, the best evidence for homing is a high percentage of tagged individuals returning to a particular rock pool following experimental displacement, providing the displacement distance is greater than the diameter of the home range (Gibson 1982).

Homing has also been the subject of a number of early studies in the behaviour of intertidal fishes particularly sculpins (for reviews see R. N. Gibson 1969, 1982, 1993 Dodson 1988). The tidepool sculpin (*O. maculosus*) has been recorded as homing from distances greater than 100 m (Green 1971a, Khoo 1974). Green (1973) also recorded the mosshead sculpin *Clinocottus globiceps* as capable of homing. However, the maximum displacement distance in this experiment was only 10 m, and it has since been suggested that individuals were not transferred out of their home range, making

the experiment an invalid test of homing ability (Yoshiyama *et al.* 1992). Williams (1957) showed that the woolly sculpin *C. analis* exhibited strong fidelity to a particular pool but that they tended not to return when displaced. It was suggested, however, that this failure to home may have been due to an aversion to handling and the fish may have actively avoided the experimental area after tagging. Finally, a similar, more recent study of intertidal fishes in the Cape Arago and Bay State Parks on the Central Oregon coast has proven that site fidelity and homing are behavioural features of at least 3 species of sculpin at this locality, namely the tidepool sculpin, *O. maculosus*, the mosshead sculpin *Clinocottus globiceps* and the fluffy sculpin *O. snyderi* (Yoshiyama *et al.* 1992).

### 1.5.3 Factors affecting site fidelity and homing

Many factors are thought to affect the residence times of fishes in rock pools and their ability to return if displaced, including wave exposure and height of the particular area of the shore inhabited, seasonal conditions and body length of the individual concerned. For example, *O. maculosus* does not leave pools at high water in exposed areas, and in sheltered areas where individuals do leave their home range at high water the number of fish leaving is greatest in summer (Green 1971b). Also Sasaki & Hattori (1969, in Gibson 1982) found that 2 species of goby which inhabit different heights in the intertidal zone exhibited significantly different recapture rates 2 months after first sampling a particular pool. They found that 60 % of *Chasmichthys gulosus* inhabiting the area higher on the shore were recaptured, while only 16% of *C. dolichognathus* inhabiting the area lower on the shore were found again. The reason for the wide variation in pool fidelity is thought to be the fact that *C. dolichognathus* is immersed longer at high water and therefore has a higher probability of dispersal and a lower probability of recapture. Pool-residence time in sculpins was found to vary due to several factors, particularly fish length and pool stability. For example, in the tidepool sculpin *Oligocottus maculosus* site fidelity was greater in larger fish (possibly due to the fact that colonization is performed by juveniles and fish become more sedentary with age) and those individuals inhabiting regions of high turbulence (possibly because

fish living in conditions of high turbulence don't travel far for fear of being swept off the rocks, Craik 1981). These results lead Craik (1981) to suggest that sculpins, particularly *O. maculosus*, acquire spatial information when they are young and range over a wide area, so that later when they grow older and adopt a home pool, they are able to home if they are displaced by natural phenomena such as storms. Additional evidence for this change in homing ability with age also includes the fact that after periods in captivity young fish were unable to retain their homing ability for as long as older fish (Craik 1981). Experiments designed to test the retention of information concerning the home range, have shown that several species of intertidal fishes could still home successfully after considerable periods away from the natural home. For example *Bathygobius soporator* retained their memory after 2 weeks (Aronson 1971) and *O. maculosus* after 6 months in captivity (Craik 1981).

#### 1.5.4 Why do intertidal fishes home?

There are many suggestions for the function of homing in intertidal fishes. Homing may serve the same purpose as territoriality, to disperse individuals over a wide area (Green 1971b), or allow fishes to maintain themselves in favourable positions of food availability and shelter (Yoshiyama *et al.* 1992). Several authors have suggested that animals which inhabit home ranges and return to that area when displaced are simply attracted to areas where there are sufficient resources for survival, such as food and shelter. On the rocky shore it is inevitable that fishes (and many other organisms) collect in rock pools at low water as this is the only place they can survive (Gibson 1969). However, it has been shown that the activity of certain intertidal fishes such as *L. pholis* is altered by the rhythm of the rising and falling tide, and it has been suggested that this is the method they use to return to a rock pool in time to prevent them getting stranded in the open rather than simply relying on the receding tide to transport them to a rock pool by chance at the appropriate time (Gibson 1967a). The most likely explanation is that homing enables fishes to return to locations that provide suitable refuge from desiccation at low tide (Williams 1957, Gibson 1982, Yoshiyama *et al.* 1992).



### 1.5.5 How do intertidal fishes home?

The method by which fishes home is somewhat obscure. It has been suggested that a topographical memory is formed with the aid of visual and olfactory clues (Gibson 1982). However, some species have been shown to home from distances far greater than they would normally travel. This suggests that when displaced these fishes do not recognise the area that they are transplanted into (it is unlikely that they have been there before), and therefore that they simply wander or search until they recognise the features of familiar territory, at which point they begin to direct their movements homeward (Gibson 1982, Yoshiyama *et al.* 1992). If a fish does not reach familiar terrain within a certain time, it is possible that the “search image” for home deteriorates to a point where the fish becomes habituated to some other favourable area and settles there (Yoshiyama *et al.* 1992).

Khoo (1974) found that blinded *O. maculosus* and those with olfactory organs destroyed were less capable of homing than those with normal visual and olfactory capabilities. He concluded therefore that vision and olfaction were important in aiding homing success, olfaction being the most important of the two senses. Craik (1981) suggested that one sense was sufficient to allow homing in older individuals but both were necessary in younger ones. She also showed that neither conspicuous landmarks nor olfactory clues emanating from the home pool are recognised, suggesting the presence of a spatial map, the use of which does not require prompting by the presence of environmental clues.

## 1.6 Study species *Lipophrys pholis*, the shanny

### 1.6.1 Habitat

*Lipophrys pholis* are probably the commonest intertidal fish found on British coasts (Quasim 1957). They inhabit rocky shores from MHWN to ELWS (Milton 1983) particularly those where barnacles are present (Gibson 1969). *L. pholis* forms the highest proportion of littoral fish populations on exposed shores (Milton 1983), where

their specially adapted pectoral fin rays aid in moving along rough surfaces even in turbulent conditions (Whitear 1970).

*L. pholis* are thought to forage from a familiar area or home range at high water and return to a safe refuge such as a crevice in a rock pool at low tide to avoid desiccation (Gibson 1969, 1982). If they do not succeed in reaching shelter at low tide *L. pholis* are capable of surviving the loss of 22% of their body water and exposure to air for 4-6 hours in high humidity, shade and still air (Daniel 1971). Any available water is pumped over the gills during immersion and respiration takes place across the scaleless skin and over the surface of the highly vascularised oesophagus (Nonotte & Kirsch 1978).

### 1.6.2 Diet

Gut-contents analyses have suggested that *L. pholis* are omnivorous grazers that concentrate their diet on common sessile shore organisms such as barnacles, algae, hydrozoans and bryozoans, although evidence of amphipod, gastropod, lamellibranch, crab, isopod and polychaete remains have also been recorded (Quasim 1957). Quasim (1957) reported that barnacles were found to be the dominant food, occurring in the gut all year round along with large quantities of finely crushed barnacle shells indicating a regular habit of browsing or chewing on these animals. Diet is thought to change with increasing size, and possibly age and maturity (Milton 1983). The smallest fish appear to take the smallest prey and as gape increases in larger fish (Dunne 1977) there is a change in the diet to include limpets *Patella*, *Patina* and *Acmaea*, relatively more whole barnacles and more lower shore amphipods (*Jassa*, *Caprella*) and isopods (*Gnathia*, *Idotea*, Milton 1983). The most striking change in diet however is the increasing occurrence of algae, particularly Rhodophyceae, *Ulva* and *Enteromorpha* in the gut of larger fish.

### 1.6.3 Reproduction

Both sexes mature at approximately 7 cm when nearly 2 years old (Quasim 1957, Milton 1983). Some adults grow as large as 16 cm (Quasim 1957) however life span is relatively short, for example only a few individuals aged 10 years were found on the south west coast of England (Milton 1983) and in the Isle of Man (Bowers *et al.* 1960) while 13 year old fish were found at Carna (Dunne 1977). *L. pholis* are not generally territorial and are often found in pairs under the same boulder or in the same crevice along with other species. In the breeding season, however, each male will establish a territory containing a nest which he guards from the attention of other males (Gibson 1969, Almada *et al.* 1983). The nest is simply a hole or crevice with a suitable smooth, hard surface on to which the female lays her eggs (Quasim 1956). The breeding season is generally considered to be long (Shackley & King 1977, Milton 1983) but the exact time of spawning varies depending on location. For example Quasim (1957) reported that spawning takes place from April to August and reaches a peak in June on sheltered shores in the Menai Strait, North Wales, while Milton (1983) suggested that high recruitment in *L. pholis* in south west England in July suggests spawning in April-May. The male attracts the female into his nest where she lays her eggs and carefully sticks them to the rock surface in a single layer covering an area of several square centimetres. The male then fertilises the eggs and tends them until they hatch, often during periods of emersion (Almada *et al.* 1992). Paternal care includes attacking other animals (including the mother) that approach the nest, aerating the eggs and keeping them free of silt by creating a current through the nest by beating the tail, and protecting the eggs from fungal infection by regularly wiping them with the body (Quasim 1956).

### 1.6.4 Rhythmic behaviour

Laboratory experiments on the behaviour of *L. pholis* under controlled conditions have demonstrated the presence of persistent rhythms of approximately tidal (circatidal) frequency in their locomotory behaviour (Gibson 1967b). Also during these

experiments the behaviour of *L. pholis* was found to be suppressed during the dark period, revealing them to be diurnal.

### 1.6.5 Agonistic behaviour

*L. pholis* is known to exhibit agonistic behaviour towards conspecifics in aquaria (Gibson 1969). This behaviour seems to have an effect on the physiology of the organism as *L. pholis* increases its oxygen consumption when confronted by its reflection in a mirror (Wirtz & Davenport 1975) and a decreased growth rate when kept in visual and olfactory contact with a conspecific (Wirtz 1974, 1975). These results led Wirtz & Davenport (1975) to conclude that *L. pholis* is essentially a solitary species, that increased respiration and decreased growth rate are a consequence of a departure from normal living conditions, and aggression between individuals aids in dispersal. It is thought that aggression in *L. pholis* may also have a tidal rhythm which peaks at high water in order to disperse individuals over a wide area during feeding excursions. Later when the tide recedes aggression declines and therefore several *L. pholis* can shelter together under the same rock or in the same crevice (Gibson 1969).

### 1.6.5 Senses

Many intertidal fishes exhibit a wide range of adaptations suitable for collecting information about their habitat. For example, the eyes of many gobies and blennies are large, set high on the head and are capable of forming an extensive visual image of the fish's surroundings anteriorly, dorsally and laterally (Gibson 1969). Most of these species also have independently movable eyes (Brett 1957) that can be converged on an object in the narrow anterior field for temporary binocular vision (Roule 1926, Brett 1957). Many blennies possess a foveated retina that magnifies images. A study conducted by Bull (1935) showed that blennies can discriminate different wavelengths of light. Also, the visual pigments of 18 species of intertidal fishes show a correlation between the wavelength of maximum absorption and the spectral quality of water which they inhabit (Loew & Lithgoe 1978).

All these specialisations in vision have led some authors to conclude that intertidal species locate their prey by sight alone (Bateson 1890), although other senses appear to be highly developed. For example, the heads of many blennies often bear tentacles or other appendages, and although these are absent in *L. pholis* this species does possess fringes around the nostrils. The function of these appendages or fringes is for the most part unknown. Bayliss (1914) however, concluded they were sensitive to touch and it is possible that they are involved in species recognition. Chemosense may also be important, particularly in locating food. For example, Bull (1930) found that a conditioned response in *Blennius (Lipophrys) pholis* and *B. gattorugine* could be initiated by exposing them to seawater extracts of nereid worms or *Mytilus edulis*. Under the influence of these extracts of their food the fishes exhibited gulping and chewing movements of the mouth, while no response was evoked by an artificial olfactory stimulus, a musk. This led Bull to conclude that the gustation is important in these species whereas olfaction is poorly developed.

## 1.7 Aims

The aims of the present study were to observe the exploratory behaviour of *L. pholis* in a novel arena to ascertain how this species gains spatial information about its environment and the length of experience required to learn the position of a refuge in that environment. Also, to determine the ability of *L. pholis* to learn and remember the position of a food reward in a hierarchical maze, and to ascertain the clues the fish use to navigate by altering the intra- and extra-maze conditions once they have learned to navigate to the reward successfully. Finally, whether *L. pholis* can learn the position of a single refuge in an artificial habitat, and if so what clues they use to do so.

## **Chapter 2**

### **Spatial memory for the position of a food patch in *Lipophrys pholis***

## 2.1 INTRODUCTION

A spatial memory for the position of food patches in their habitat allows animals to move directly to the food source, therefore reducing energy expenditure during foraging and improving their overall fitness (Hughes *et al.* 1992).

There are many examples of fishes learning to complete simple spatial tasks such as relocating the position of a feeding point in an aquarium (*Gobius* and *Gasterosteus*, Goldsmith 1914; goldfish, *Carassius auratus*, Pitcher & Magurran 1983), and spatially distinguishing the more profitable of two food patches or feeding stations using visual clues. One such study showed that juvenile Atlantic salmon (parr) *Salmo salar* are capable of distinguishing two similar visual landmarks, and learning to track the movements of one specific landmark to predict the location of a food supply (Braithwaite *et al.* 1996). Juvenile Atlantic salmon live within a restricted home range during most of their time in fresh water before migrating to sea. The use of space within the home range is highly structured (R. J. Gibson 1993), each fish holding station just above or resting on the substratum in one or a few favoured sites from which they intercept food items (Keenleyside 1962, R. J. Gibson 1993). During an experiment to ascertain whether juvenile Atlantic salmon could track the position of a moveable resource labelled with a distinct visual landmark, a Lego<sup>TM</sup> plate and flag, (Phase I) the fish learned to settle on the rewarded feeding station 75% of the time, significantly more than they settled on the unrewarded feeding station. When exposed to visually indistinct landmarks (Phase II) mean performance reached 30-50% during training and > 50% during testing in all but one fish therefore Juvenile Atlantic salmon are capable of tracking the rewarded site in the absence of the visually distinct clues. Braithwaite *et al.* (1996) could not rule out the possibility that small visual inconsistencies in the plates were being used as visual landmarks. Many salmonids rely on chemosense during migration, and have been found to distinguish water conditioned by familiar rather than unfamiliar individuals (Stabell 1982, Brown & Brown 1992), to be able to discriminate urine from sibling and non sibling fish (Moore *et al.* 1992) and appear to recognize odour extracted from gravel lining their holding

tank (Stabell 1987). Therefore Juvenile Atlantic salmon may be expected to possess excellent chemosensory abilities and Braithwaite *et al.* (1996) concluded that it was most likely that the fish were using chemosensory clues emanating from the food to track the resource. Several potential sources of chemosensory clues were suggested including food remnants in jets used to deliver the food or on the base plates, or mucus secretions on the plate left by the fish themselves, a phenomenon first hypothesised by Stabell (1987). However both jets were used to introduce food at various times and extensive cleaning was carried out in an attempt to remove all excess food and secretions from the jets and the plates between experiments. One individual (Fish 3) produced a mean performance of 90% throughout the experiment. This result suggested that Fish 3 was using an alternative method of locating the rewarded feeding plate throughout Phase I and continued to use it during Phase II. It was suggested that this alternative method of locating the rewarded plate was olfaction and that while all the other individuals were relying on vision first and olfaction when the visual clues were the same, Fish 3 was relying on chemosense throughout the experiment. Hierarchies of clue use such as this (i.e. relying on vision until it is unavailable and then relying on olfaction) are relatively common (Able 1993). One adaptive explanation suggested for the presence of a hierarchy of clue use in juvenile Atlantic salmon by Braithwaite *et al.* (1996) is the recent discovery of a switch from diurnal to nocturnal activity in this species (Fraser *et al.* 1993, Heggenes *et al.* 1993). As day length shortens, and temperature falls, juvenile Atlantic salmon switch from diurnal to nocturnal feeding. Presumably as they move between shelter and feeding stations at night, visual clues will be largely redundant and alternative information such as olfactory clues may become more important.

However, the strongest experimental evidence for the importance of landmark clues to foraging fish to date comes from a laboratory study using goldfish *Carassius auratus* (Warburton 1990). In this experiment paired fish were provided with two food patches in an open arena, one containing flake food buried beneath the gravel covering the floor of the tank and the other remaining empty. Two different types of visual landmarks were provided, four columns of red Lego<sup>TM</sup> blocks or a plastic plant attached to a rock.



The position of these clues was alternated between the actual and dummy patches therefore providing direct and indirect stimuli (respectively). The ability of fish to learn and remember the position of the two food patches was measured by recording initial patch choice accuracy and latency to feeding. Four experimental treatments were tested over a total of 12 daily trials:

1. Unmarked;
2. Direct - 4 plastic Lego<sup>TM</sup> columns surrounding food patch;
3. Indirect - as for direct with Lego<sup>TM</sup> columns surrounding empty patch;
4. Natural - plastic plant attached to rock placed close to food patch.

In the case of the unmarked treatment the feeding latency improved with experience but the first choice accuracy did not. This result suggested that spatial learning is poor in the absence of clear local visual clues, and that performance is improved by faster swimming in progressively more familiar surroundings, rather than by shortening the path to food through learning. In contrast, the direct treatment group showed very high choice accuracy, less choice variability and significant improvement with experience. While the natural treatment group chose more accurately than expected by chance and their performance did not improve with experience. These results led Warburton to conclude that the clues provided increased in relevance in the order unmarked-natural-direct. He suggested that the fish responded more definitely to the Lego<sup>TM</sup> blocks because they were brightly coloured and more obvious than the natural clue (the rock and plant) and that, given more time, the fish would respond to the natural clue in the same way. The significant increase in accuracy in first finding food in the indirect treatment and following reversal indicated that the goldfish could also learn to use landmarks as indirect spatial reference points. This process appears to require more experience because the results in initial trials were close to chance. Moreover, following reversal (i.e. becoming an indirect treatment) the new food patch was recognised as such by members of the direct group only after a number of visits to, or “a period of fixation on” the old (now empty) patch over the course of seven trials. Warburton attributed this response to “overshadowing” (Mackintosh 1974) a phenomenon whereby a previously relevant strong landmark stimulus inhibits an

attention shift to a new patch related stimulus. This presents the possibility that while confusion is minimised and learning facilitated by the presence of salient visual clues in predictable environments (Olton 1978, Warburton 1990, Biegler & Morris 1993), they may inhibit adaptation to changed conditions when spatial relationships between, for example, food and landmarks are disrupted (Warbuton 1990). An increase in patch sampling following reversal was also described. However visits to empty patches were often very short therefore this phenomenon was attributed to *testing local spatial* information rather than patch quality (Warburton 1990).

Experiments using simple mazes, consisting of one or more partitions placed across the width of an aquarium and pierced by a small doorway, have shown that various species (*C. auratus*, Churchill 1916; *Gasterosteus*, V.A. Braithwaite *pers. comm.*) can learn to navigate this obstruction in order to return to a food patch or nest site relatively quickly, even when the problem is confounded by constructing the partition from glass (*C. auratus*, Goldsmith 1914).

Still more complex spatial tasks include solving a radial arm maze. These structures typically consist of a central compartment or platform from which arms radiate like spokes on a wheel, and have been used less often in the study of fish behaviour than in the study of rats in psychology. However, Siamese fighting fish *Betta splendens* remembered the position of food rewards at the end of each of eight arms in a radial maze (Roitblat *et al.* 1982). The optimum solution to the eight-arm radial maze was to visit each arm only once, and was scored by recording the number of correct choices per trial. Following a series of 55 twice-daily trials *B. splendens* achieved a performance significantly better than that of a random simulation (i.e. from that due to chance), with a maximum average of 6.63 correct choices out of 8. Despite the presence of a strongly stereoscopic pattern of movement whereby the fish were most likely to move in a clockwise direction visiting the adjacent arm to the one previously visited, Roitblat *et al.* (1992) concluded that this algorithm alone could not account for the high performance level of the fish, and therefore suggested the presence of spatial memory. Also there is evidence to suggest that other species, such as the fifteen-spined

stickleback *Spinachia spinachia* and corkscrew wrasse *Crenilabrus melops* can solve a radial maze using an algorithm in a similar way (C.M. Blight *pers. comm.*).

Goldfish trained over a period of 54 days (a total of 270 trials) in three arms of a four-arm radial maze remembered the position of a food reward (Rodriguez *et al.* 1994). The goldfish were trained under the following combinations of allocentric (a type of spatial learning whereby the subject learns their position by referring to the position of the objects around them), and egocentric (a type of spatial learning whereby the subject learns the position of the objects in the room by referring to their own position) treatments:

**Allocentric** - two opposite start positions, fish always rewarded at the extreme of the goal arm situated in the same place in the room, in order to determine whether fish could learn the position of the reward solely on the basis of extramaze clues (those present in the room outside the maze);

**Egocentric** - two opposite start arms used at random and for all trials the position of the reward arm was determined by a fixed turn response (e.g. always left) relative to the start arm, in order to ascertain whether the fish could choose the correct arm on the basis of specific turn response irrelevant to extramaze clues;

**Ego-allocentric** - start box always situated in the same place in the room, rewarded exclusively in a goal arm located in another constant position in order to allow selection of arm on the basis of turn direction and / or extramaze clues;

**Control** - start box in a fixed position but reward arm position assigned at random to one of two positions in order to account for the possibility that the fish may be responding to any odour emanating from the food.

Performance was measured both as percentage of correct choices and as days to reach criterion (which was 13 correct trials out of 15, a mean of 86.67% correct over three consecutive sessions). The Ego-allocentric group remembered the position of a food reward after 14 days (5 trials a day therefore 70 trials), while the Egocentric and Allocentric group remembered the position of the food after 16 and 20 days (80 and 100 trials) respectively and the performance of the Control group remained close to that expected by chance throughout the experiment (Rodriguez *et al.* 1994). The Egocentric group required more training sessions and made significantly more errors ( $33.57 \pm 8.8$ ) before reaching criterion than the Allocentric and Ego-allocentric groups

( $23.43 \pm 6.9$  and  $19.6 \pm 4.4$  respectively), suggesting that this may have been the more difficult task to learn. Similar results have been recorded for mammals, for example rodents trained to turn in one direction in a maze in which the environment and intramaze clues were irrelevant, were slower to learn the task than those trained in a task in which a particular position was rewarded (Hill & Thune 1952, Scharlock 1955, Tolman *et al.* 1946).

The aims of this chapter were to ascertain whether *L. pholis* can learn the position of a food reward in a hierarchical maze, and if so what clues they use to accomplish this task.

## 2.2 METHODS

### 2.2.1 Collection of *L. pholis*

Adult *L. pholis* (> 8cm, Quasim 1957) were collected during low water spring tide in the months of June, July and August in 1996 and 1997 from rock pools at Easdale on Seil Island, Argyll (Grid ref. 56°17'N5°39' W; OSM NM 884014 N169 W750). Collection was made using the anaesthetic quinaldine (2-methyl quinoline) and hand nets (Gibson 1967b). Fish were measured, sexed (according to methods described by Quasim 1956) and kept in individual holding tanks measuring 12 cm x 12 cm and filled to a depth of 25 cm with sea water in a constant flow-through system in the aquarium at Dunstaffnage Marine Laboratory. No evidence of reproductive behaviour was observed during the captive period of these fish and it was therefore assumed that behaviour during experiments was not affected by reproductive condition.

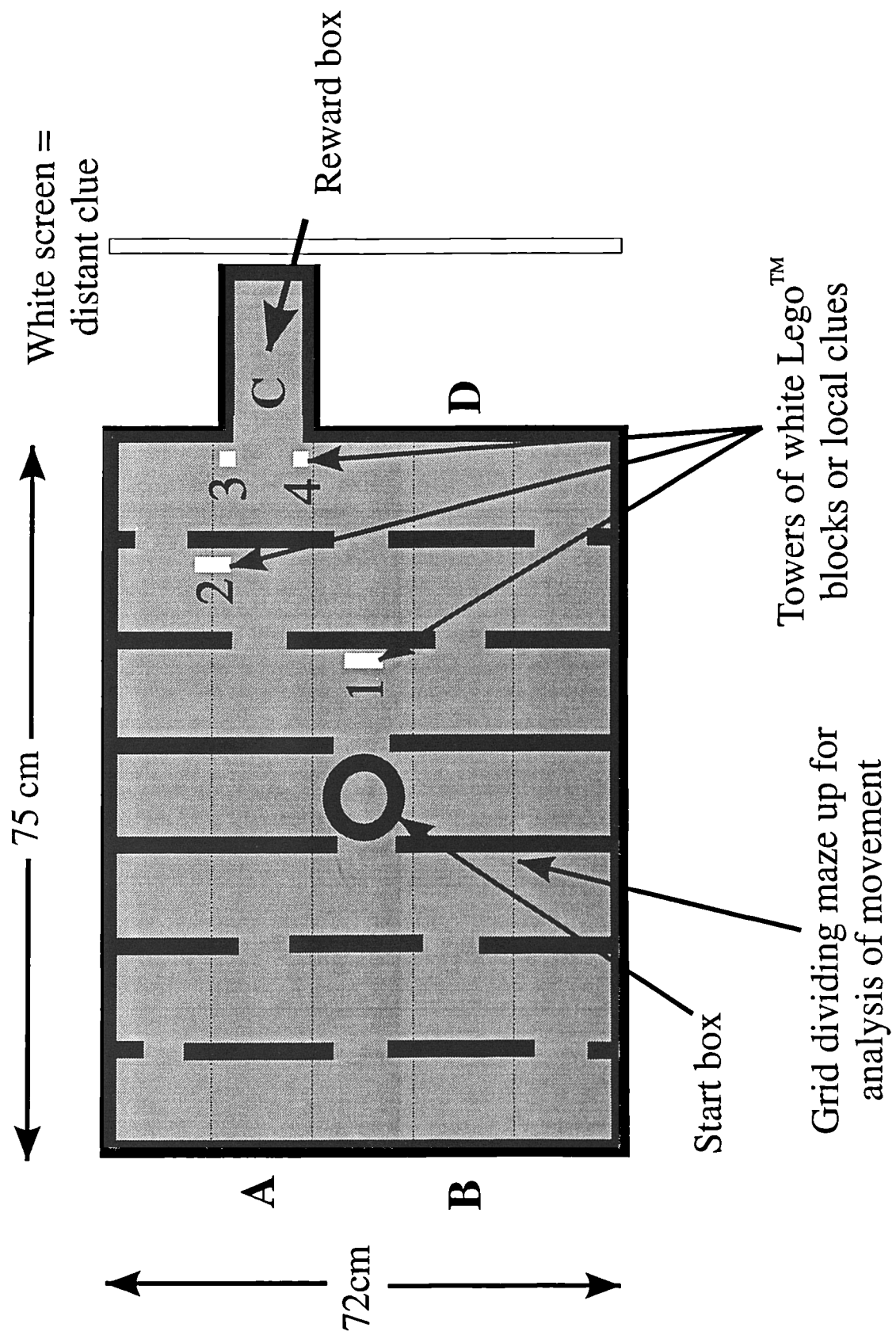
Prior to the beginning of this experiment fish were starved for one week. If, during the experiment, any individual did not complete the maze for a significant period (a few days), it was given a maintenance ration of chopped *Mytilus edulis* in the holding tank.

### 2.2.2 Equipment - The Hierarchical maze

The hierarchical maze was built from black laminated cardboard. It was designed to present the subject with a series of choices of directions to travel and doorways to pass through at different stages of a journey to a reward. It also provided the experimenter with the opportunity to manipulate the position of the reward and the visual clues present in order to observe the acquisition of spatial memory by the subject (K. Warburton *pers. comm.* Fig. 2.1).

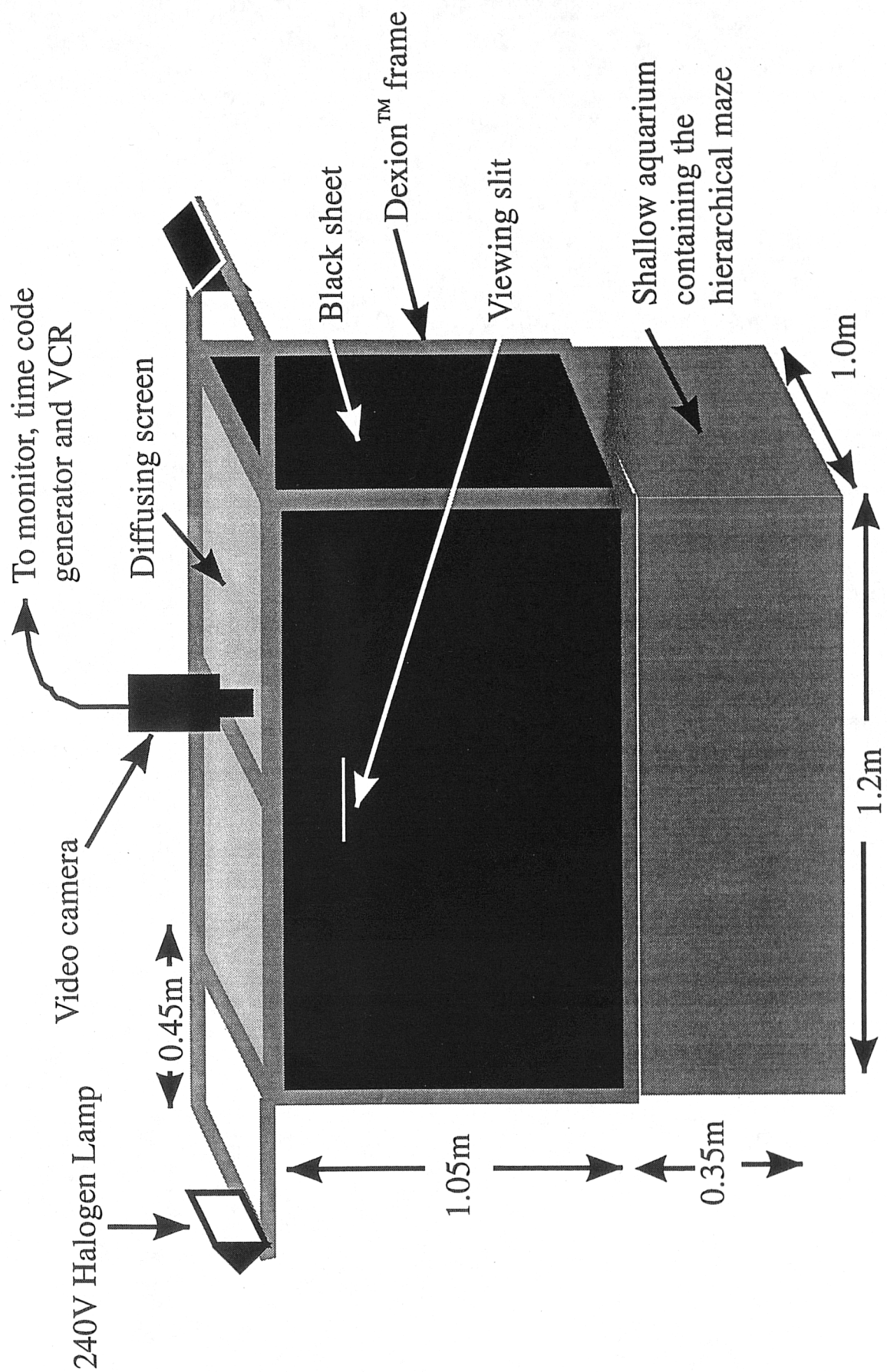
The maze was surrounded by a hide consisting of a Dexion™ frame lined with black sheets and covered from above by a diffusing screen (Fig. 2.2). Lighting was provided by 2, 240V, 500W Halogen lamps mounted on opposite corners of the frame. Throughout the experiment the maze stood on a sand bed in a shallow tank of still sea water. The movement of each individual could be observed through a viewing slit in

**Fig. 2.1** The hierarchical maze showing the positions of the start box, the reward box at Position C and other possible positions A, B and D, the white screen, the Lego™ towers marking the most efficient route to the reward and the grid which divided the maze up for analysis of movement.



**Fig. 2.2** The hide which covered the hierarchical maze showing position of the video camera, the halogen lamps and the viewing slit.





the black curtain or via a T.V. monitor connected to a time code generator and a video camera, the output from which was video recorded for subsequent analysis.

### 2.2.3 Protocol

Each fish was placed in the start box at the centre of the maze and allowed to settle for 5 min. It was then released by remotely raising the start box (by pulling on two lengths of fishing line attached to it and looped over the frame of the hide) and allowed to explore for a maximum time of 30 min. If within this time the fish reached the reward box located at one of four different sites A, B, C or D (Fig. 2.1), it was trapped there by a remotely operated door and allowed to feed on the chopped *M. edulis* for 10 min. If it did not reach the reward, it was removed from the maze using a hand net and replaced in its holding tank. Each fish was tested at the same time every day in order to eliminate the effect of any endogenous rhythm in activity, the water was replaced in the maze at the beginning of each day and the sand was raked before each fish was placed in the maze.

The experiment was carried out on consecutive days for a total of 15 days, and on four groups of eight fish, consisting of 4 males and 4 females (total 32 fish), under the influence four different treatments (Table 2.1).

### 2.2.4 Analysis

The behaviour of the fish was observed as they moved around in the maze and performance was measured as time to completion (from leaving the start box to entering the reward box), path length (number of steps taken from one grid square to another in the journey from the start box to the reward box) and number of fish that completed the maze on each day. To assess improvement in performance each treatment group was tested for a decrease in time to completion and path length and an increase in the number of fish that completed the maze over consecutive days, using Page's test, a distribution-free test for ordered alternatives (Neave & Worthington, 1988). Each of the performance criteria was tested for any significant difference between treatments using Friedman's test and subsequently Dunn's multiple

**Table 2.1** Visual clues provided and hypothesis tested for each Treatment.

Treatment	Conditions	Null hypothesis tested
1	No obvious visual clues	<i>L. pholis</i> cannot learn to navigate the hierarchical maze in the absence of any obvious visual clues
2	White screen (1 m x 0.7 m) replacing the black wall of the hide directly behind the reward box	<i>L. pholis</i> cannot learn to navigate the hierarchical maze using a distant / global clue
3	Five towers of white Lego™ blocks (base 16 mm x 16 mm, height 70 mm) marking the most efficient route to the food	<i>L. pholis</i> cannot learn to navigate the hierarchical maze using local, visual clues
4	White screen and five towers of white Lego™ blocks marking the most efficient route to the reward box	<i>L. pholis</i> cannot learn to navigate the hierarchical maze using global and local, visual clues

comparison test (Neave & Worthington 1988), to determine which visual clue aided *L. pholis* during spatial learning. Videos of days 1-15 for Treatments 3 and 4 were analysed to determine mean latency to movement for each fish on each day. Latency was subtracted from the total time to completion of each trial to ascertain the exploration time, which was then divided by the path length to give a measure of mean speed in grid squares per second traversed on each day. Both were analysed for any decreasing trend using Page's test, to ascertain any change in hesitancy of *L. pholis* with time.

As the performance and length of the subjects in Treatments 3 and 4 were not significantly different (Treatment 3 mean length = 10.29cm, Treatment 4 mean length = 11.49, Mann Whitney U test,  $P = 0.099$ ), these data were pooled and tested for any difference in performance with sex and length of subjects using the Mann Whitney U test. Correlation of mean performance for days 11-15 against sex and length was carried out in order to determine whether these variables had any effect on the spatial learning ability of *L. pholis*.

The learning ability of individuals in Treatments 3 and 4 was tested using Kendall's coefficient of concordance (Sokal & Rohlf 1969) in order to determine if there was any consistent variation in the learning ability among individuals.

All these and subsequent statistical tests were carried out using Minitab, except Dunn's test and Kendall's coefficient of concordance which were carried out using spreadsheets in Excel. Also, a computer programme was written in BASIC for Page's test (using the listing from Neave & Worthington 1988). Significance was expressed in tables as  $P < 0.05 = *$ ,  $P < 0.01 = **$ ,  $P < 0.001 = ***$ .

## 2.3 RESULTS

### 2.3.1 Fish Behaviour

Initially the fish exhibited a fright response when released from the start box, whereby they swam quickly to the end of the central corridor of the maze where they remained stationary for several minutes before beginning to move again. This behaviour decreased over several days, with many individuals beginning to move about in the maze immediately they were released from the start box after day 8.

In the absence of any clues (Treatment 1) or with only a white screen present (Treatment 2) *L. pholis* did not improve their performance in time to completion, path length and the number of fish that completed the maze after 15 days (Tables 2.2, 2.3 and 2.4, Figs 2.3, 2.4 and 2.5). Under these conditions performance remained constant and varied about a mean of time 775.60s, and standard deviation  $\pm 250.86$ , path length 26.04cm  $\pm 14.65$  and number of fish that completed the maze  $4.27 \pm 1.65$  for Treatment 1 and 617.82s  $\pm 208.52$ , 24.32cm  $\pm 9.24$  and 4.13 fish  $\pm 2.08$  respectively for Treatment 2. In contrast, the mean time to completion, mean path length and the number of fish that completed the maze when provided with Lego™ towers (Treatment 3) and Lego™ towers and a white screen (Treatment 4) showed a significant improvement (Page's test, Page's statistic = 1182, 1173, and 1084 and 1162, 1224, and 1181.5 respectively, Critical value at 5% = 1083.59, d.f. = 14, Tables 2.2, 2.3 and 2.4). In Treatment 3 asymptotes of mean time to completion 193.47s  $\pm 62.48$  mean path length 29.30cm  $\pm 9.75$  and number of fish that completed the maze  $8.00 \pm 0.00$  were reached on Day 9. While in Treatment 4 asymptotes of mean time to completion 292.48s  $\pm 167.90$ , mean path length 21.83cm  $\pm 8.44$  and number of fish that completed the maze  $7.6 \pm 0.55$ , were also reached on Day 9.

There was a significant difference between treatments in all three performance criteria (Friedman's test,  $P < 0.001$ , 0.020, and  $< 0.001$  respectively, d.f. = 3). Time to completion, path length and the number of fish that completed the maze were not significantly different in Treatments 1 and 2 (Dunn's test,  $Q = 2.174$ , 0.398, and 0.121 respectively,  $Q_{0.05} = 2.638$ ,  $k = 4$ ). Time to completion and the number of fish which

**Table 2.2** Results of analysis for a decreasing trend in mean time to completion for *L. pholis* in the hierarchical maze using Page's test, critical value 1083.59, d.f. = 14, n = 8.

Treatment	Conditions	Page's statistic	
1	No clues	981	NS
2	White screen	1078	NS
3	Lego <sup>TM</sup>	1182	*
4	White screen and Lego <sup>TM</sup>	1162	*

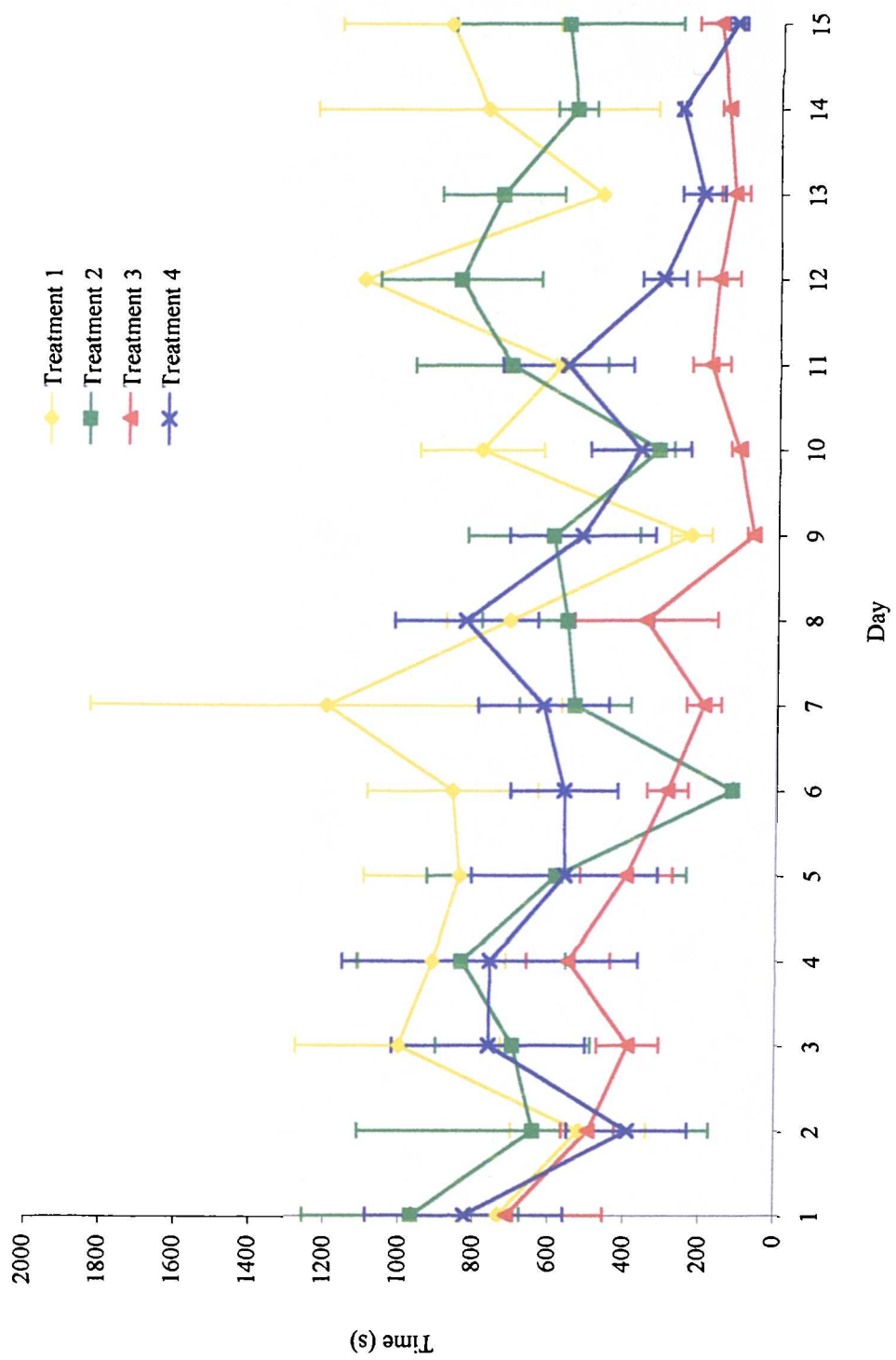
**Table 2.3** Results of analysis for a decreasing trend in mean path length to the reward for *L. pholis* in the hierarchical maze using Page's test, critical value 1083.59, d.f. = 14, n = 8.

Treatment	Conditions	Page's statistic	
1	No clues	1023	NS
2	White screen	1004.5	NS
3	Lego <sup>TM</sup>	1173	*
4	White screen and Lego <sup>TM</sup>	1224	*

**Table 2.4** Results of analysis for an increasing trend in the number of *L. pholis* which completed the hierarchical maze using Page's test, critical value 1083.59, d.f. = 14.

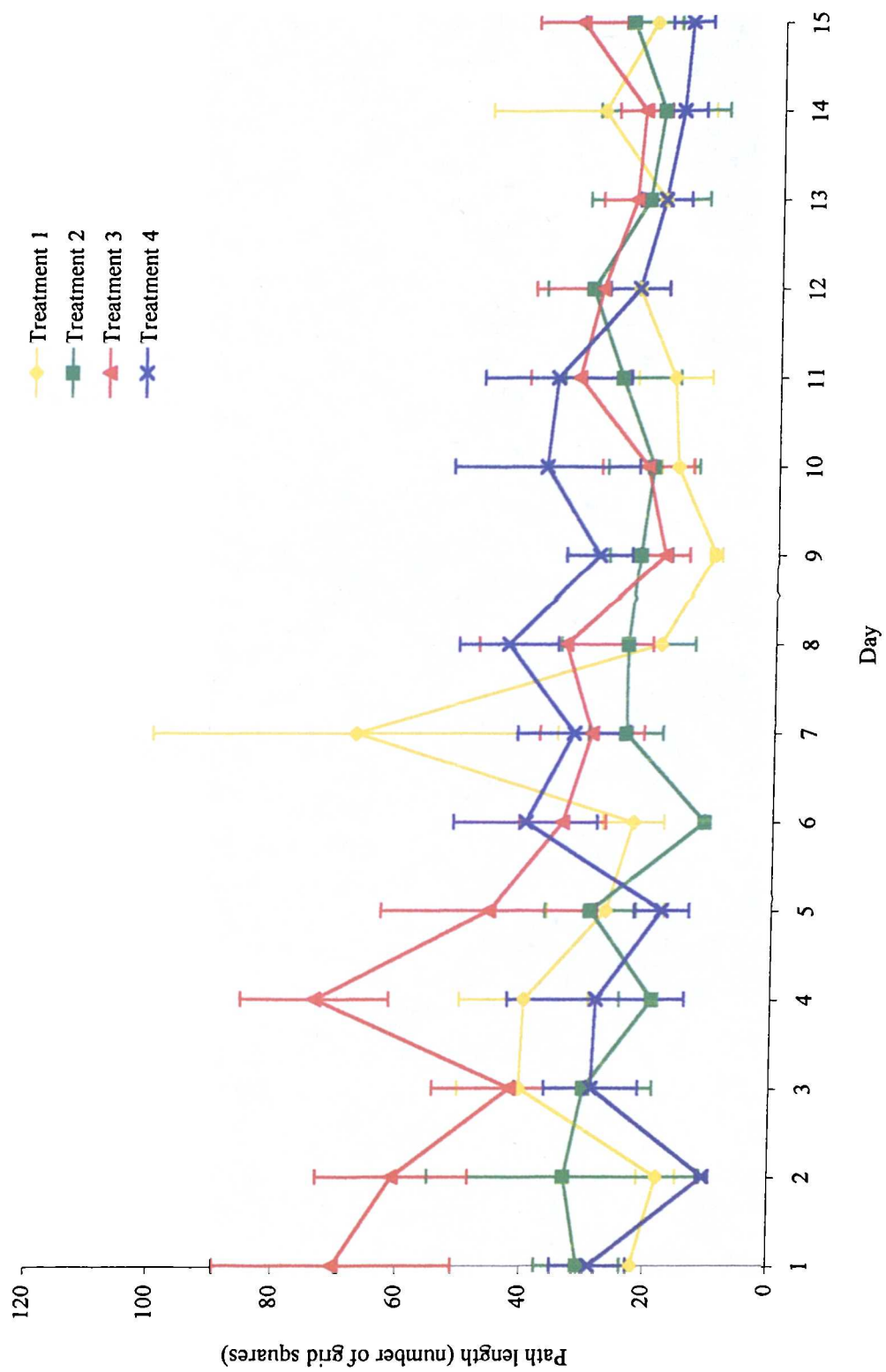
Treatment	Conditions	Page's statistic	
1	No clues	873	NS
2	White screen	1058.5	NS
3	Lego <sup>TM</sup>	1084	*
4	White screen and Lego <sup>TM</sup>	1181.5	*

**Fig. 2.3** Mean time to completion of the hierarchical maze for *L. pholis* in Treatment 1, no clues, Treatment 2, white screen marking the position of the reward, Treatment 3, 5 towers of Lego™ blocks marking the most efficient route to the reward, Treatment 4, 5 towers of Lego™ blocks and a white screen marking the position of the reward. n = 8, error bars show the standard error of the mean.

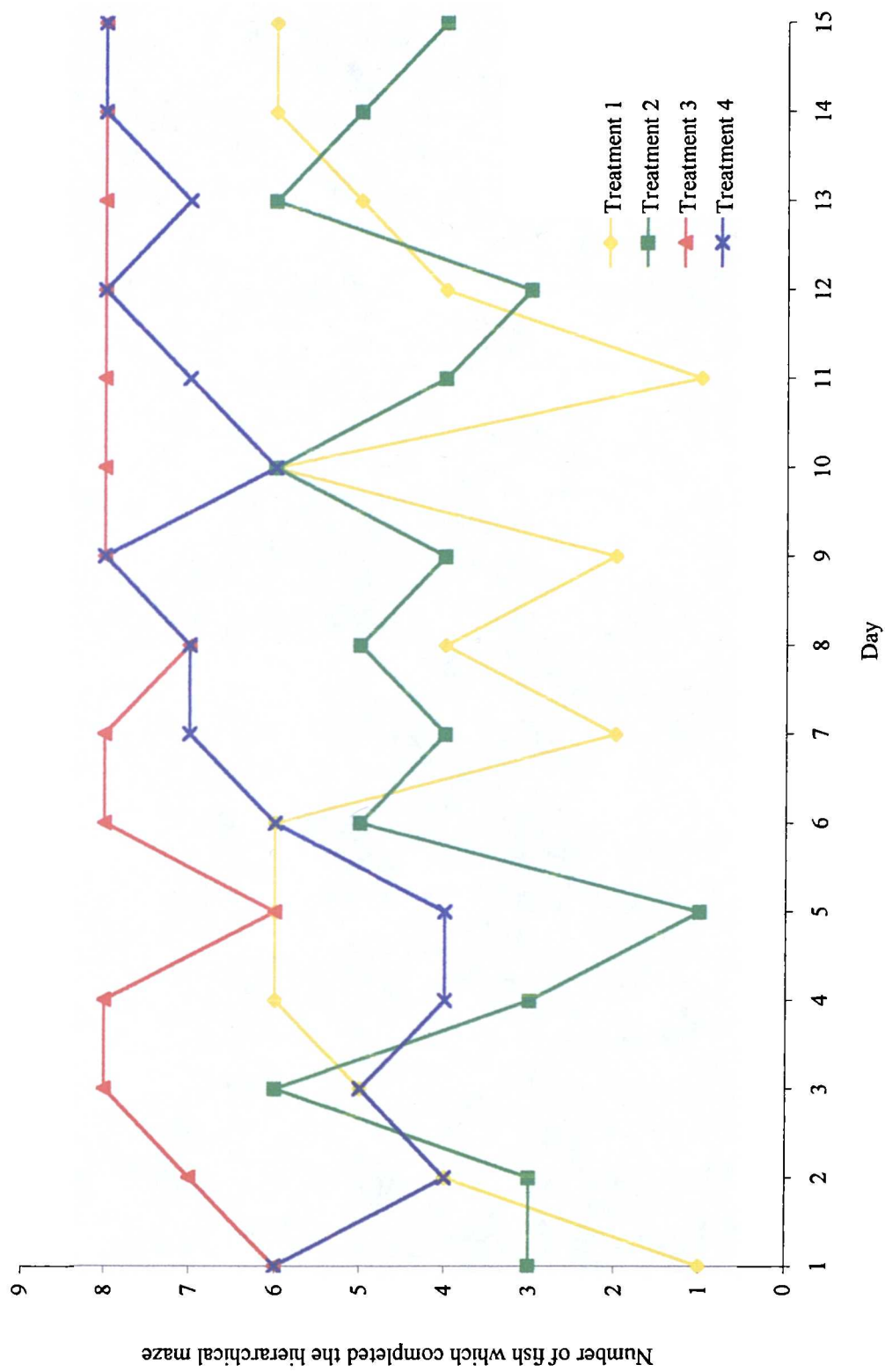




**Fig. 2.4** Mean path length to the reward in the hierarchical maze for *L. pholis* in Treatment 1, no clues, Treatment 2, white screen marking the position of the reward, Treatment 3, 5 towers of Lego<sup>TM</sup> blocks marking the most efficient route to the reward, Treatment 4, 5 towers of Lego<sup>TM</sup> blocks and a white screen marking the position of the reward, n = 8, error bars show the standard error of the mean.



**Fig. 2.5** Number of *L. pholis* which completed the hierarchical maze on each day in Treatment 1, no clues, Treatment 2, white screen marking the position of the reward, Treatment 3, 5 towers of Lego<sup>TM</sup> blocks marking the most efficient route to the reward, Treatment 4, 5 towers of Lego<sup>TM</sup> blocks and a white screen marking the position of the reward.



completed the maze was significantly lower in Treatment 1 than in Treatment 3 while the path length was not significantly different (Dunn's test,  $Q = 4.819, 3.719$  and  $2.506$  respectively). Mean time to the reward was not significantly different in Treatment 1 and Treatment 4, however mean path length to the reward and the number of fish which reached the reward were significantly different (Dunn's test,  $Q = 2.478, 2.647$  and  $3.316$  respectively,  $Q_{0.05} = 2.638, k = 4$ ). Mean time to completion in Treatment 2 was significantly longer than Treatment 3 (Dunn's test,  $Q = 2.645, Q_{0.05} = 2.638, k = 4$ ) but not significantly different from Treatment 4 (Dunn's test,  $Q = 0.303, Q_{0.05} = 2.638, k = 4$ ). Mean path length in Treatment 2 was not significantly different from Treatment 3 but was significantly shorter than Treatment 4 (Dunn's test,  $Q = 2.108$  and  $2.249$  respectively,  $Q_{0.05} = 2.638, k = 4$ ). The number of fish that completed the maze was significantly lower in Treatment 2 than in Treatments 3 and 4 (Dunn's test,  $Q = 3.598$  and  $3.196$  respectively,  $Q_{0.05} = 2.638, k = 4$ ). Mean time to completion was significantly shorter in Treatment 3 than Treatment 4 while mean path length and the number of fish that completed the maze was not (Dunn's test,  $Q = 2.341, 0.141$ , and  $0.402$  respectively,  $Q_{0.05} = 2.638, k = 4$ , Tables 2.5, 2.6, and 2.7, Figs 2.3, 2.4 and 2.5).

Performance (mean time to completion, mean path length and the number of fish that completed the maze), was not significantly different in male and female *L. pholis* (Mann Whitney U test  $P = 0.159, 1.000$ , and  $0.199$  respectively, d.f. = 14, Fig. 2.6). There was no correlation between fish length and the mean best performance on days 11-15 (Table 2.8,  $n = 8$ , Pearson product moment correlation coefficient, time to completion and fish length =  $-0.177$ , path length and fish length =  $0.053$  and number of fish that completed the maze and fish length =  $0.225$ ).

Mean latency to movement and mean velocity of the 16 fish in Treatments 3 and 4 decreased significantly over 15 days, (Page's test, Page's statistic = 1125 and 1152 respectively, Critical value at 5% = 1083.59, d.f. = 14, Figs 2.7 and 2.8) and reached an asymptote of  $66.2s \pm 1.96$  and  $6.40s \pm 1.96$  respectively on Day 9. The number of visits made to each grid square showed that certain individuals in Treatment 3, such

**Table 2.5** Results of analysis for a significant difference in mean time to completion of the hierarchical maze (n = 8) for *L. pholis* in each Treatment (Treatment 1, no clues, Treatment 2, white screen, Treatment 3, Lego™, Treatment 4, Lego™ and white screen), using Dunn's test,  $Q_{0.05} = 2.638$ , k = 4.

	Treatment 2	Treatment 3	Treatment 4
Treatment 1	2.174 NS	4.819 ***	2.478 NS
Treatment 2		2.645 *	0.303 NS
Treatment 3			2.34 NS

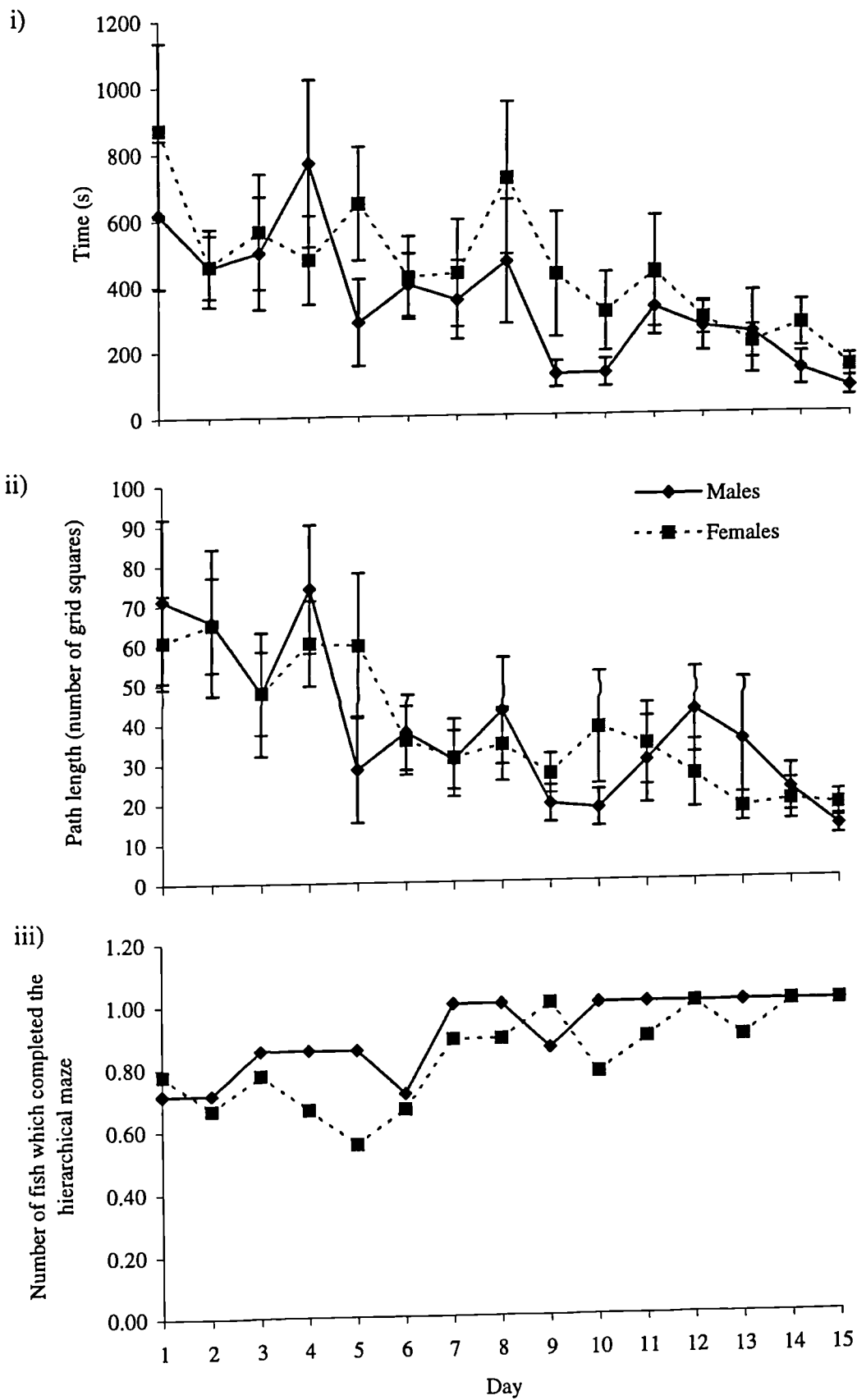
**Table 2.6** Results of analysis for a significant difference in mean path length to the reward in the hierarchical maze (n = 8) for *L. pholis* in each Treatment (Treatment 1, no clues, Treatment 2, white screen, Treatment 3, Lego™, Treatment 4, Lego™ and white screen ),using Dunn's test,  $Q_{0.05} = 2.638$ , k = 4.

	Treatment 2	Treatment 3	Treatment 4
Treatment 1	0.398 NS	2.506 NS	2.647 *
Treatment 2		2.108 NS	2.249 NS
Treatment 3			0.141 NS

**Table 2.7** Results of analysis for a significant difference in number of *L. pholis* which completed the hierarchical maze in each Treatment (Treatment 1, no clues, Treatment 2, white screen, Treatment 3, Lego™, Treatment 4, Lego™ and white screen ), using Dunn's test,  $Q_{0.05} = 2.638$ , k = 4.

	Treatment 2	Treatment 3	Treatment 4
Treatment 1	0.121 NS	3.719 **	3.316 **
Treatment 2		3.598 **	3.196 **
Treatment 3			0.402 NS

**Fig. 2.6** Comparison of learning in male and female *L. pholis* in the hierarchical maze, Treatment 3, 5 towers of Lego<sup>TM</sup> blocks marking the most efficient route to the reward and Treatment 4, 5 towers of Lego<sup>TM</sup> blocks and a white screen marking the position of the reward, pooled, **i)** mean time to completion (n = 8), **i)** mean path length (n = 8), **iii)** number of fish which completed the maze, error bars show the standard error of the mean.



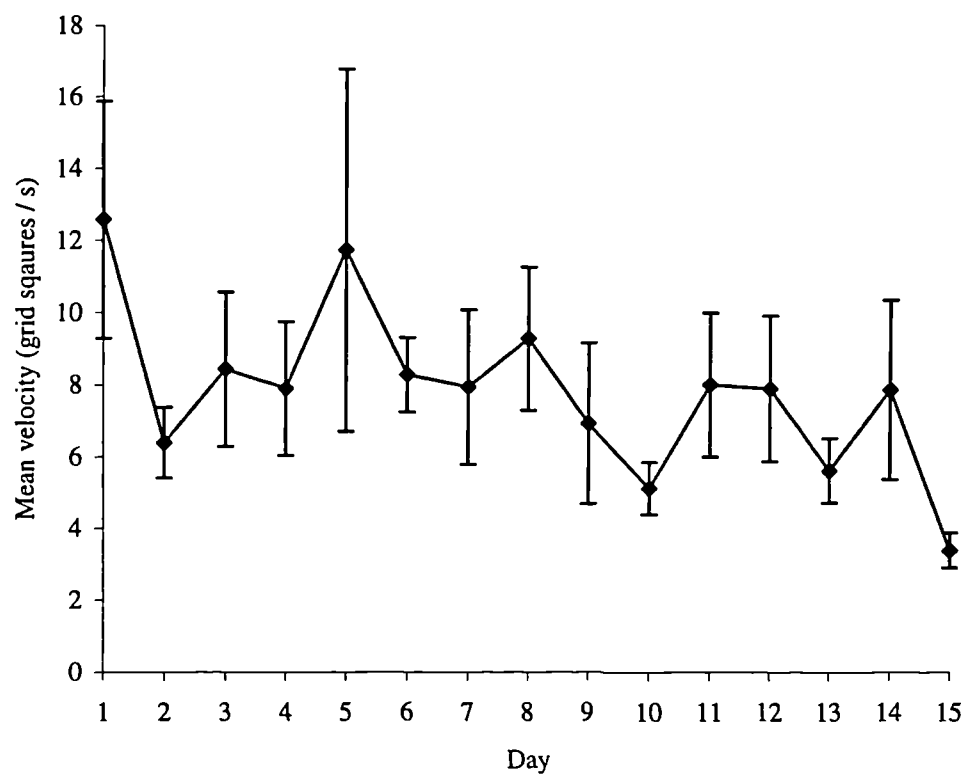
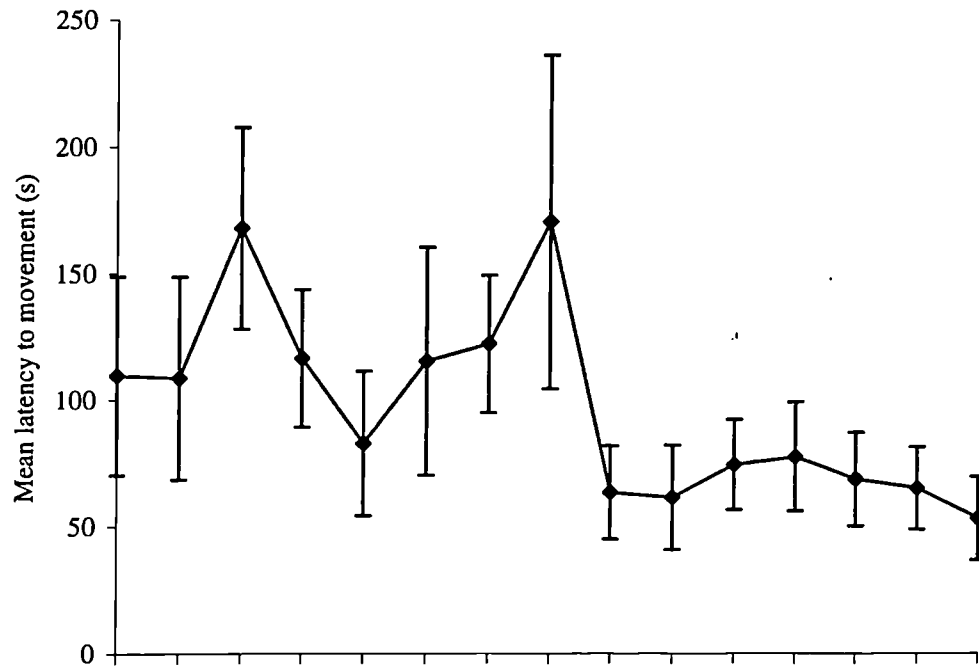


**Table 2.8** Individual fish lengths, mean time to completion and mean path length to the reward, of *L. pholis* in the hierarchical maze on Days 11-15 in Treatment 3, Lego™, and Treatment 4, Lego™ blocks and white screen, n = 8.

	Fish	Length (cm)	Mean time to completion days 11-15 (s)	Mean path length to the reward days 11-15
Treatment 3	1	13	99.77	26.27
	2	11.4	92.71	12.64
	3	12	244.47	40.64
	4	11.9	136.2	39.5
	5	11.6	290.86	37.45
	6	11.3	133.52	20.55
	7	9.1	91.07	30.09
	8	11.6	299.24	18.36
Treatment 4	1	10.6	19.26	9.8
	2	9.7	264.2	12.8
	3	10.7	156.06	16.6
	4	12	526.62	32.4
	5	12.8	275.92	19
	6	9.4	444.53	12.33
	7	8.8	179.32	21.8
	8	8.3	497.38	43.8

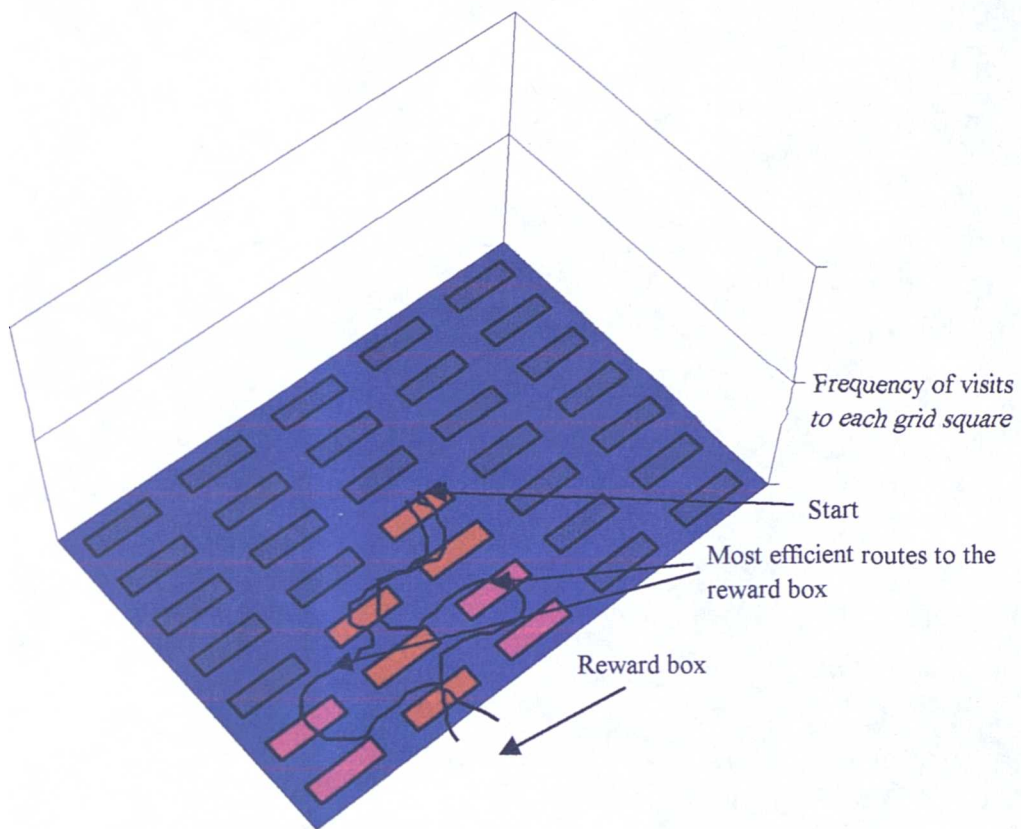
**Fig 2.7** Mean latency to movement of *L. pholis* in the hierarchical maze, Treatment 3, 5 towers of Lego™ blocks marking the most efficient route to the reward and Treatment 4, 5 towers of Lego™ blocks and a white screen marking the position of the reward, pooled between treatments, n = 16 , error bars show the standard error of the mean.

**Fig 2.8** Mean velocity of *L. pholis* in the hierarchical maze, Treatment 3, 5 towers of Lego™ blocks marking the most efficient route to the reward and Treatment 4, 5 towers of Lego™ blocks and a white screen marking the position of the reward, pooled between treatments, n = 16, error bars show the standard error of the mean.



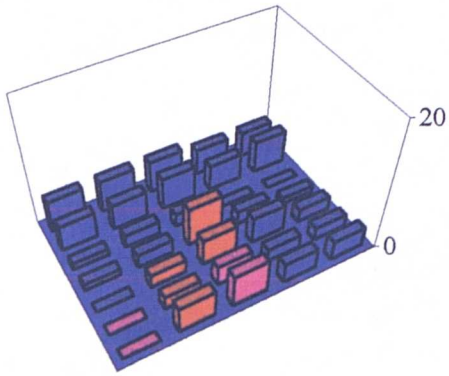
as Fish 4 and Fish 8, and certain individuals in Treatment 4, such as Fish 3 and Fish 8, favoured a particular route to the reward box (Figs 2.9 and 2.10). Mean time to completion and mean path length of individuals were ranked consistently in Treatment 4 only (Kendall's coefficient of concordance  $W = 0.514, 0.411$ ,  $X^2 = 53.978, 43.133$ ,  $\chi^2_{(0.05, 14)} = 23.68$ , Table 2.9).

**Fig. 2.9** The hierarchical maze showing the two most efficient routes from the start position to the reward box at position D. Red indicates the only grid squares that can be passed through at the beginning of the most efficient route while the pink squares illustrate the two alternative paths that can be followed later in the route.

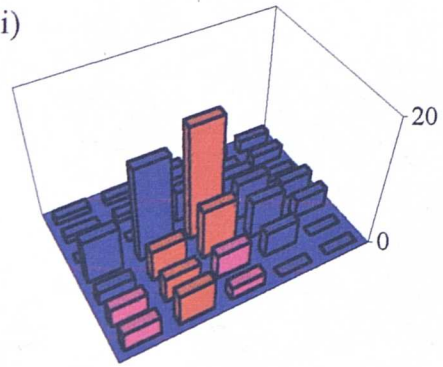


**Fig. 2.10** Frequency of visits to each grid square in the hierarchical maze showing “preferred routes” of each individual *L. pholis* in Treatment 3, Lego™ and Treatment 4, Lego™ and white screen, i - viii Treatment 3, i) Fish 1, ii) Fish 2, iii) Fish 3, iv) Fish 4, v) Fish 5, vi) Fish 6, vii) Fish 7, viii) Fish 8, ix - xvi Treatment 4, ix) Fish 1, x) Fish 2, xi) Fish 3, xii) Fish 4, xiii) Fish 5, xiv) Fish 6, xv) Fish 7, xvi) Fish 8. Red and pink bars indicate the most efficient route to the reward as described in Fig. 2.9, from the start to the reward at position D for Treatment 3 (i - viii), and from the start to the reward at position B for Treatment 4 (ix - xvi).

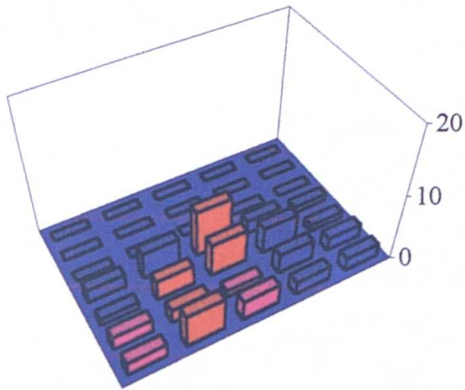
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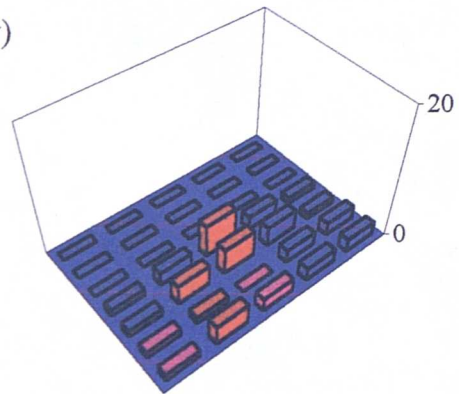
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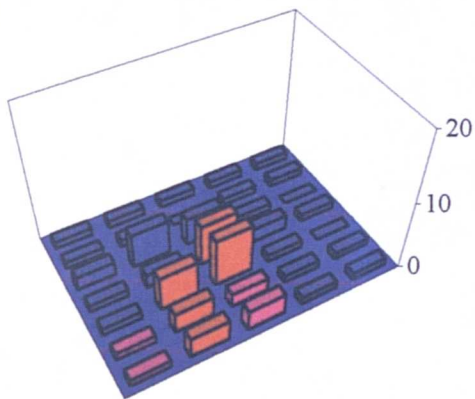
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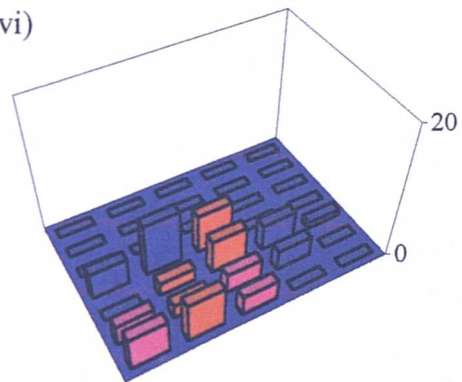
iv)



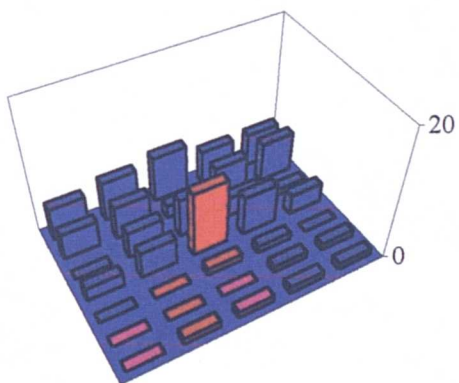
v)



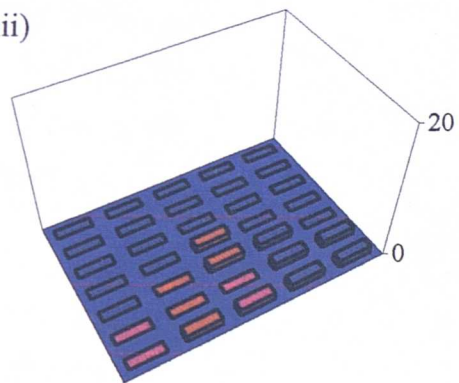
vi)



vii)

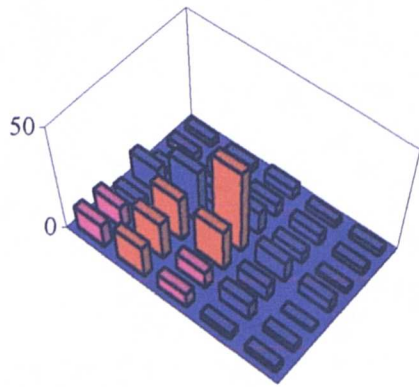


viii)

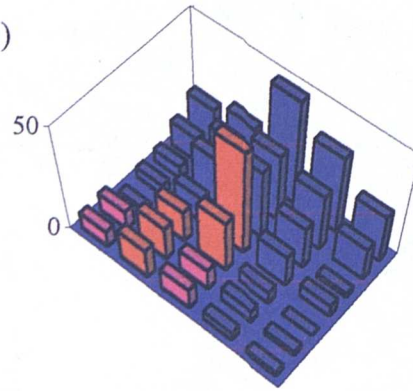




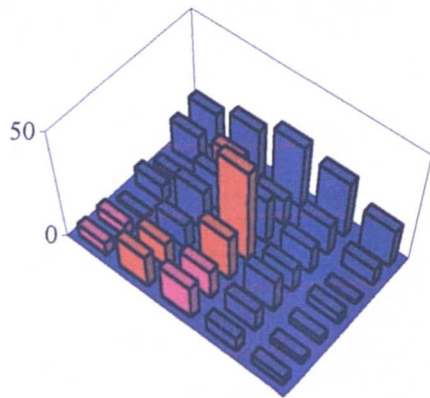
ix)



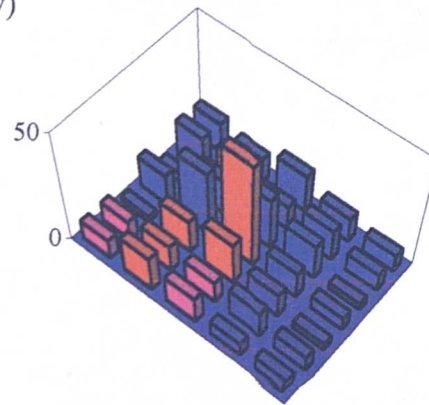
xii)



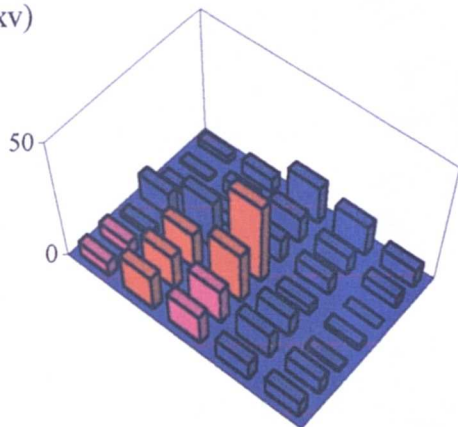
viii)



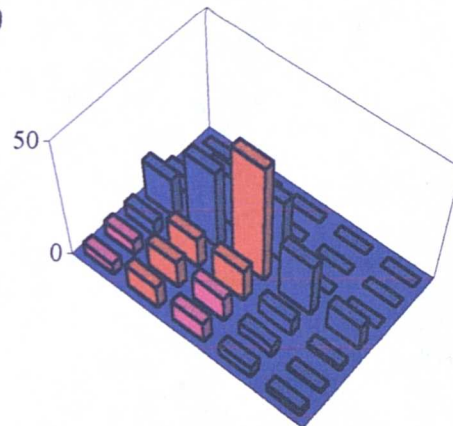
xiv)



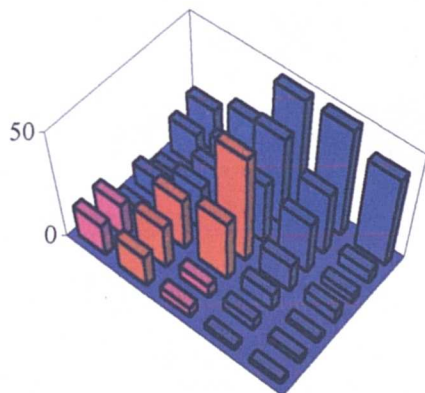
xv)



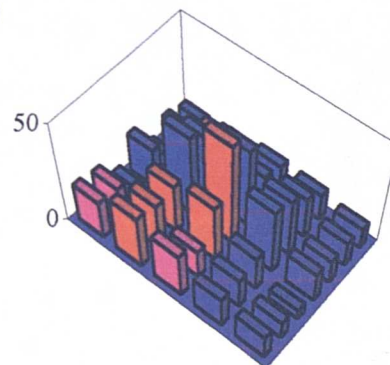
xvi)



xvii)



xviii)



**Table 2.9** Results of test for consistency in learning ability of individual *L. pholis* in Treatments 3, Lego™, and Treatment 4, Lego™ and white screen, using Kendall's coefficient of concordance,  $\chi^2_{(0.02, 14)} = 23.68$ ,  $\chi^2_{(0.05, 14)} = 29.14$ ,  $\chi^2_{(0.001, 14)} = 36.12$ , d.f. = 14.

Treatment	Performance criterion	$\chi^2$	<i>W</i>	
3	Time to completion	19.33	0.18	NS
3	Path length	-2.83	-0.03	NS
4	Time to completion	53.98	0.51	***
4	Path length	43.13	0.41	***

## 2.4 DISCUSSION

The results showed that an improvement in performance (time to completion, path length and the number of fish completing the maze) of *L. pholis* in the hierarchical maze took place only in the presence of Lego™ towers. Performance reached an asymptote on Day 9, as did the mean latency to movement and mean velocity of individuals. There was no difference in the performance of males and females, and no correlation between the length of individuals and their best performance. There was concordance in the time to completion and path length of individuals in Treatment 4.

The decrease in time to completion after just 9 days in Treatments 3 and 4 was due, in part, to a significant decrease in the latency to movement as the fish became habituated to the experimental procedure. However, velocity decreased in both Treatments over the 15 days, and the improvement in time to completion therefore was primarily due to subjects learning and remembering a more direct route to the reward box and using this consistently on later days. The fact that mean latency to movement and mean velocity of fish in Treatments 3 and 4 both reached asymptote at around Day 9, suggests either that the fish had become habituated to the experimental procedure, or that they had come to the end of an exploratory phase of their behaviour (Kleerekoper *et al.* 1970, Mikheev & Andreev 1993). Since time to completion, path length and the number of fish completing the maze also reached asymptote on Day 9, it is likely that the fish had finished exploring the maze and had learned the position of the reward at this point, and subsequently began to move more slowly and accurately towards the reward.

None of the performance criteria showed a significant decrease in Treatments 1 and 2 although path length in these Treatments was very low at the beginning of the experiment and there was therefore little scope for improvement. The short path length at the beginning of the experiment for Treatment 1 and 2 was probably due to the fact that only a small number of individuals in these treatments completed the maze throughout the experiment. Those that did, happened by chance to complete the task

via a relatively direct route, therefore reducing the average time to completion and path length throughout the experiment for these treatments.

More fish completed the maze throughout Treatments 3 and 4 than Treatments 1 and 2, possibly because the Lego™ towers within the maze created a more complex habitat and therefore encouraged *L. pholis* to explore more extensively. Whilst exploring, therefore, they were more likely to discover the food reward by chance and be motivated to search for it when they were returned to the maze the following day. Fish who did not explore and did not find the food may have been less inclined to waste energy moving about in the maze on subsequent days, if they did not realise there was a food reward available.

The absence of any difference in learning between male and female *L. pholis* and among those of different body lengths was surprising, because the male is primarily responsible for guarding the eggs in this species. It might therefore be expected that males would have a better developed spatial memory, to be able to return to the brood after foraging. Also, larger intertidal cottids have been found to be able to return to their home pool more successfully than smaller ones, with some decline in this ability into extreme old age (Craik 1981).

All the *L. pholis* seemed to favour a particular route to the reward box but this was not always the most efficient route, and the path they chose was not always clearly defined. This obscurity of the preferred route occurred because individuals often made mistakes, entered the wrong side of the maze and then swam up and down a particular corridor several times therefore increasing the number of visits to incorrect grid squares. It is possible that visits to the incorrect side of the maze were deliberate, and the fish were intentionally sampling their environment to check that no other food patches were available. This sampling of their habitat would most likely be carried out at regular intervals and would ensure that the fish would be aware if any new food supplies should appear in their environment. However, no clear evidence of sampling behaviour (for example a rhythm in the visits to either side of the maze) was found.

The fact that each fish appeared to favour a particular route to the reward box, which was not always the most efficient, suggests that *L. pholis* did not learn a map of the whole hierarchical maze but simply associated one particular route with the reward and remembered it on subsequent days.

The significant trend in learning ability of individuals in Treatment 4, showed that within the natural variation among individuals, some fish consistently learn better than others. It is possible that concordance was not found in Treatment 3 because of the low number of fish used in this experiment; larger treatment groups may have produced concordance in performance in all treatments.

## 2.5 CONCLUSION

*L. pholis* are capable of learning a specific route through a relatively complex habitat to a food reward. There was no improvement in performance in the absence of clues or in the presence of a white screen clue representing a distant landmark or light source, even after 15 days. In the presence of towers of white Lego™ blocks, however, performance improved and reached asymptote after 9 days. This suggested that salient, local landmarks such as rocks or clumps of algae are important aids to spatial memory in *L. pholis* when foraging in the wild. These clues may be used as landmarks or beacons when learning the position of a food patch, allowing individuals to employ a direct route to return to food, therefore saving energy and improving the overall fitness of the individual (Hughes *et al.* 1992). There was no difference in learning between male and female *L. pholis*, or fish of different lengths, yet there was evidence to suggest significant concordance between the learning ability of individuals.

### **Chapter 3**

**The effect on changing conditions on the  
performance of *Lipophrys pholis* in the  
hierarchical maze**

### 3.1 INTRODUCTION

The investigation of novel objects by fishes is well established (Brawn 1961, Breder 1950, Breder 1954, Hoar 1958, Russell 1967, Welker & Welker 1958). Many studies have shown that structural changes to the habitat in which a spatial task has been learned, greatly affect the subsequent ability of the subject to complete that task. For example, *Carassius auratus* learned the position of a new food patch only after “a period of fixation” on its previous location, (Warburton 1990), a phenomenon often described as “overshadowing” (Mackintosh 1974). Also, blackeye gobies *Coryphopterus nicholsi* returned to the previous site of their refuge when it was moved to a different location (Markel 1994). Often the changes in behaviour of an animal following modification of its habitat reveals the clues it was using to complete spatial tasks.

The effects of removing landmarks on the foraging behaviour of fishes was observed in the field using three reef dwelling butterfly fishes *Chaetodon trasciatus*, *C. trifasciatus* and *C. ornatissimus* (Reese 1989). These species follow a similar search path in each foraging bout and apparently navigate by recognising a sequence of coral heads along the route. Reese removed a specific coral head along the foraging path of certain easily recognisable pairs of fish. This caused them to halt mid path and search in the area from which the coral had been removed but soon resume foraging along the original path at the first familiar landmark they encountered. A similar effect was observed when fishes were frightened away from their original search path. Presumably it is advantageous to butterfly fishes to remember a successful foraging route within their own territory and use it regularly, particularly as their food source is relatively renewable and if predators are not encountered along the way (Hourigan 1987 in Reese 1989). During this study the butterfly fishes were also observed to make long excursions out of their territories to the edge of the reef some 30 m or more. The paths followed appeared to be different on the outbound and homeward legs of these journeys. Whether these were novel and there is evidence for the use of a spatial map of the area as described by Tolman (1948) and O’Keefe and Nadel (1978) is yet to be



established, although it would seem likely in such a structured environment.

Possibly the best illustration of the effects of altering the immediate surroundings of a subject on their ability to remember learned spatial information to date comes from the experiments of Rodriguez *et al.* (1994). Having trained Egocentric, Allocentric, Allo-egocentric and Control groups of *Carrasius auratus* to navigate a four-arm maze (see Chapter 2), Rodriguez *et al.* (1994) continued their experiments to determine how the goldfish were performing the task by altering the intra and extramaze clues. During these experiments all four arms of the radial maze were used and no maze arms were baited (behaviour was not reinforced). Ten trials were carried out and the following changes in conditions were made:

**Transfer tests -**

**Type 1:** the maze remained in its usual position in the room but the animals were released from a start box placed in a novel position;

**Type 2:** the maze was displaced relative to the objects in the room so that the start box remained in its previous location and the end of one arm was located in the same position in the room where the fish were rewarded during training trials;

**Probe tests -**

**Type 1:** one or more salient visual clues in the room was removed, or hidden with a brown curtain;

**Type 2:** a brown curtain was placed around the whole of the maze;

**Reversal tests -**

**Ego-allocentric group:** 180° shift in reward arm location;

**Allocentric group:** 180° shift in reward arm location;

**Egocentric group:** the turn opposite to that previously conditioned was rewarded;

**Control group:** the baited goal arm was randomly assigned.

The behaviour of each fish was described as egocentric, allocentric, or other according to the arm chosen. The results showed that the allocentric group was able to reach the

goal from novel start arms and unvisited locations in the room and to use routes without a history of previous training. Rodriguez *et al.* (1994) therefore concluded that this group have “the capacity to discriminate and represent spatial relationships in the environment independent of body centred reference position” that is, they employ a cognitive map as first described by Tolman (1948) and further discussed by O’Keefe & Nadel (1978). The Egocentric group also showed a high level of accuracy in Transfer trials, mainly by choosing the arm corresponding to 90° turn made in training, regardless of start position and maze location in the room. This led Rodriguez *et al.* (1994) to conclude that this group made arm choices without reference to environmental information available to them. The results for the Egocentric and Allocentric groups suggested that both egocentric or allocentric strategies were used independently, while the results presented for the Ego-allocentric group suggested that it is possible for both strategies to be used simultaneously. In this group 51.4%  $\pm$  22.6 of the choices made were “egocentric” while 45.7%  $\pm$  23.1% were “allocentric” in the Type 1 transfer, and 65.71%  $\pm$  19.02% of the choices made were “egocentric” and 31.95%  $\pm$  18.10% made were “allocentric” in the Type 2 transfer, therefore, the “other” arm was virtually never chosen. The use of both strategies by the Allo-egocentric group could explain the tendency of this group to perform more accurately and consistently than the Egocentric or Allocentric groups. Cooperative use of different spatial strategies have been described for other fishes (Roitblat *et al.* 1982), and mammals (Schenk & Morris 1985, Whishaw 1989, Whishaw and Mittleman 1986) in the laboratory, and animals and fishes in their natural environment (Able 1991, Reese 1989 respectively) and is in agreement with comments made by O’Keefe & Nadel (1978) who stated that “cognitive and non-cognitive systems are not mutually exclusive since they can act in concert”. Since conflicting spatial information does not occur in nature, one might expect that use of more than one behavioural system would improve navigational efficiency (Able 1991).

The results of the probe tests showed no change in performance when any single clue was removed in any of the groups but when all clues were removed, performance in the

Allocentric group decreased to levels comparable with the Control group (Rodriguez *et al.* 1994). This suggested that the Allocentric group was navigating by reference to a combination of environmental clues around the room in accordance to the cognitive map hypothesis described by O'Keefe & Nadel (1978). Similar evidence has been found in other studies involving the alteration of extramaze stimuli (Maxmanian & Roberts 1983, Morris 1981, O'Keefe and Conway 1978, Suzuki *et al.* 1980). Rodriguez *et al.* (1994) suggested that the Egocentric group are not effected by the removal of any of the distal clues because their behaviour was not controlled by external stimuli, and rather they "performed on the basis of behavioural stereotypes probably acquired with extensive training" as reported earlier for mammals (Hicks 1964, Mackintosh 1965, Restle 1957). Therefore this group were probably employing an idiothetic orientation strategy whereby they solved the task by performing a particular turn within a body-controlled reference system (O'Keefe & Nadel 1978). The Ego-allocentric group also continued to choose correctly in the absence of salient distal clues. This result suggested that goldfish can use egocentric and allocentric strategies simultaneously to solve spatial problems and, in accordance with similar studies, if the basis for one strategy is removed they can still perform successfully using the remaining one (O'Keefe & Nadel 1978, Quinn 1980, Quinn & Brannon, 1982, Schenk & Morris 1985, Sutherland and Rudy 1989, Whishaw 1989).

In the reversal trials, the Allocentric and the Ego-allocentric groups rapidly learned to navigate to the new goal position by the shortest trajectory (Rodriguez *et al.* 1994). This behaviour revealed the capacity for rapidly detecting environmental changes by animals employing these methods of navigation (Poucet *et al.* 1986, Thinus Blanc *et al.* 1987, Welker & Welker 1958), and is in concordance with results reported by Warburton (1990) for the relearning of the position of the most profitable food patch in goldfish following clue reversal. In contrast, the behaviour of the Egocentric group was much less flexible; they persistently chose the arm coinciding with same turn after which they were previously rewarded and only reached the new goal after visiting the now unrewarded, but previously rewarded, arm (Rodriguez *et al.* 1994).

Finally, Rodriguez *et al.* (1994) concluded that goldfish were able to establish novel routes to a reward from unfamiliar start points and use distal clues to navigate. They could build complex spatial representations or cognitive maps of their environment which permit flexibility in their spatial navigation. Allocentric cognitive systems work in parallel with, and can cooperate with, egocentric systems so that goldfish have the capacity to employ the most profitable strategy at any given time.

The capacity of animals to learn the position of goals under the influence of stable and unstable landmarks has been the subject of some debate (Beigler & Morris 1993, Bennett 1993, Morris 1993). In the case of the rat, varying the position of local landmarks reduces the reliance of the subject on the landmarks as a predictor of the position of the goal (Beigler & Morris 1996). However, it appears that animals are capable of learning to relate the position of a moving landmark to a reward by association (for example Atlantic salmon parr, *Salmo salar*, Braithwaite *et al.* 1996).

The aims of this chapter were a) to ascertain the effects of changing conditions within the hierarchical maze on two groups of fish who had already learned to navigate to the reward successfully, and b) to determine the clues the fish had been using to find their way.

## 3.2 METHODS

### 3.2.1 Collection of study species

*L. pholis* were collected according to the method described in Chapter 2.

### 3.2.2 Protocol

The performance of two groups of fish, both previously trained to navigate the hierarchical maze (Treatments 3 and 4 from the experiment presented in Chapter 2) was re-tested according to the protocol described in Chapter 2 under a series of changed conditions (Tables 3.1 and 3.2, Figs 3.1 and 3.2). Group 1 contained eight individuals numbered Fish 1 - 8 that were trained to navigate the hierarchical maze in the presence of Lego™ towers and white screen clue. Group 2 consisted of eight individuals numbered Fish 9 - 16 up to Day 45 but only seven thereafter as Fish 12 died. Group 2 were trained to navigate the hierarchical maze in the presence of Lego™ towers only. The performance of Fish 12, Group 2 was therefore excluded from all statistical analysis using grouped data. Treatments consisted of five daily tests or until the fish appeared to have reached asymptote in mean performance. The treatments were run consecutively in all cases except where a large decrease in performance was observed (Group 1, Treatments 3 and 4, and Group 2, Treatments 6 and 7). If performance decreased dramatically, conditions in the maze were returned to that of the treatment just prior to the change, and the fish were allowed to explore once a day for several days as before. This settlement period was included to reduce the effect of the particularly disruptive treatments on the subsequent performance of the subjects.

The behaviour of the fish was observed as they moved around in the maze and performance was measured as completion time (in seconds from leaving the start box to entering the reward box), path length (number of steps taken from one grid square to another in the journey from the start box to the reward box) and number of fish completing the maze on each day. The mean time and path length in each treatment were compared with mean time and path length in the final few days of the Pre-treatment (days -4 to 0 in Group 1 and days -9 to 0 in Group 2 because Treatment 4

**Table 3.1** Conditions within the hierarchical maze for each treatment, Group 1. The abbreviations for treatment conditions are used in all subsequent tables for Group 1.

Treatment	Days	Conditions	Abbreviation	Null hypothesis tested
Pre-treatment	-14-0	<i>L. pholis</i> trained to efficiently and consistently navigate the hierarchical maze in the presence of 4 towers of white Lego™ blocks and a white screen marking the reward box position (Fig. 3.1)	Pre <sub>1</sub>	
1	1-13	Hierarchical maze and hide rotated 180° from original position (Fig. 3.1)	M&H180°	<i>L. pholis</i> do not use geomagnetic clues to navigate the hierarchical maze
2	14-23 24-28	10 day retention period during which the fish are maintained in their holding tanks and fed every other day Five day test period for Treatment 2, conditions as for Treatment 1	R <sub>10</sub>	<i>L. pholis</i> cannot remember how to navigate the hierarchical maze after a 10 day retention period
3	29-33	Olfactory / gustatory clue removed from the food by placing it in a glass beaker half filled with sea water, the mouth of which stands clear of the aquarium water surface	- Olf	<i>L. pholis</i> do not use olfactory / gustatory clues to navigate the hierarchical maze.
4	36-41	Most efficient route to the reward box blocked using black laminated cardboard (Fig. 3.1)	Blocked	<i>L. pholis</i> do not form a spatial map of the hierarchical maze

Treatment	Days	Conditions	Abbreviation	Null hypothesis tested
5	46-50	White screen removed from the hide (Fig. 3.1)	- Screen	<i>L. pholis</i> do not use a global clue / light clue to navigate the hierarchical maze.
6	51-62	Reward box moved from position A to C, Lego™ towers moved to mark position C (Fig. 3.1)	A to C	<i>L. pholis</i> cannot relearn the position of the reward box when it is moved to the opposite side of the hierarchical maze.
7	63-82 83-87	20 day retention period which during which the fish are maintained in their holding tanks and fed every other day Five day test period for Treatment 7, conditions as for Treatment 6	R <sub>20</sub>	<i>L. pholis</i> cannot remember how to navigate the hierarchical maze after a 20 day retention period
8	88-92	Reward box moved from position C to B, Lego™ towers moved to mark position B (Fig. 3.1)	C to B	<i>L. pholis</i> cannot relearn the position of the reward box when it is moved to the opposite side of the hierarchical maze.
9	93-123 124-129	30 day retention period, during which the fish are maintained in their holding tanks and fed every other day Five day test period for Treatment 9, conditions as for Treatment 8	R <sub>30</sub>	<i>L. pholis</i> cannot remember how to navigate the hierarchical maze after a 30 day period

**Table 3.2** Conditions within the hierarchical maze for each treatment, Group 2. The abbreviations for treatment conditions are used in all subsequent tables for Group 2.

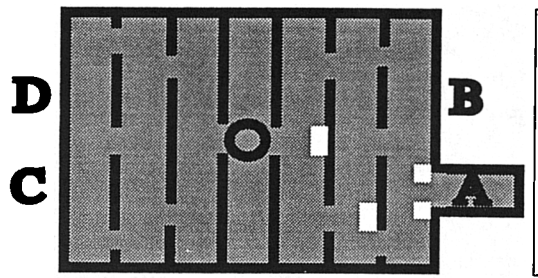
Treatment	Days	Conditions	Abbreviations	Null hypothesis tested
Pre-treatment	-19-0	<i>L. pholis</i> trained to navigate the hierarchical maze efficiently and consistently in the presence of 4 towers of white Lego™ blocks (Fig. 3.2)	Pre <sub>2</sub>	
1	1-10	Reward moved from position C to D, Lego™ towers moved to mark position D (Fig. 3.2)	C to D	<i>L. pholis</i> do not use the local clues to navigate the hierarchical maze
2	11-20	Reward moved from position D to B, Lego™ towers moved to mark position B (Fig. 3.2)	D to B	Lego™ towers do not influence the sequence of left and right turns in the journey of <i>L. pholis</i> to the reward
3	21-25	Lego™ tower 1 removed (Fig. 3.2)	- Lego™1	<i>L. pholis</i> cannot navigate the hierarchical maze in the absence of Lego™ tower 1
4	26-30	All remaining Lego™ towers removed (Fig. 3.2)	- All Lego™	<i>L. pholis</i> cannot navigate the hierarchical maze in the absence of Lego™ towers
5	31-40	Reward moved from position B to D in the absence of Lego™ clues (Fig. 3.2)	B to D	<i>L. pholis</i> cannot learn a new route to the reward in the absence of Lego™ towers
6	41-45	Hide and the position of the experimenter rotated 180° (therefore experimenter entering from Position y) in the absence of Lego™ clues	H180°Ey	<i>L. pholis</i> do not use inconspicuous visual clues from within the hide to navigate the hierarchical maze



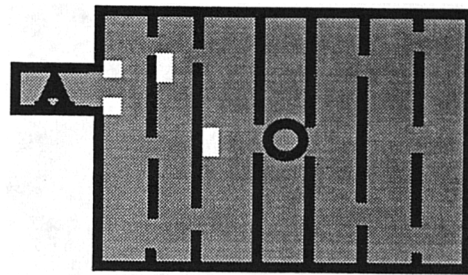
Treatment	Days	Conditions	Abbreviations	Null hypothesis tested
7	49-53	Hide rotated back to original position, experimenter entering from Position y (through a new point of entry in the side of the hide not containing the viewing slit) in the absence of Lego™ clues	E <sub>y</sub>	<i>L. pholis</i> do not use the viewing slit (in the wall of the hide originally at position x) or the position of the experimenter just prior to their release from the start box as a visual clue to navigate the hierarchical maze
8	57-61	Hide rotated 180°, experimenter entering from Position x (through original point of entry containing the viewing slit) in the absence of Lego™ clues	H180°E <sub>x</sub>	<i>L. pholis</i> do not use the position of the experimenter, just prior to their release from the start box, as a visual clue to navigate the hierarchical maze

**Fig. 3.1** Conditions within the hierarchical maze for Pre-treatment, Treatment 1, hierarchical maze and hide rotated 180°, Treatment 4, most efficient route to the food blocked, Treatment 5, white screen removed from the hide, Treatment 6, Lego™ blocks and reward box moved from position A to position C, Treatment 8, Lego™ blocks and reward box moved from position C to position B, Group 1.

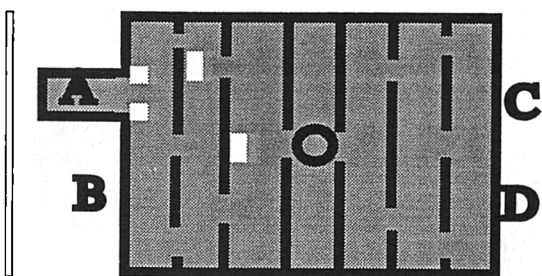
**Pre-treatment**



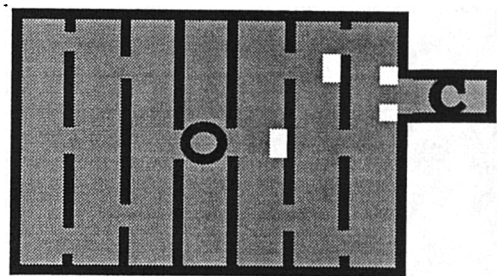
**Treatment 5**



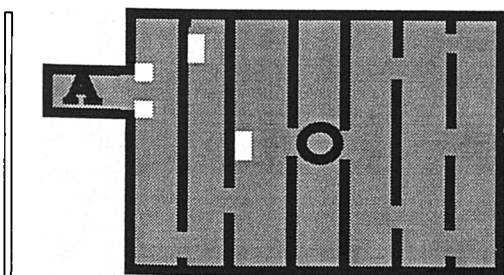
**Treatment 1**



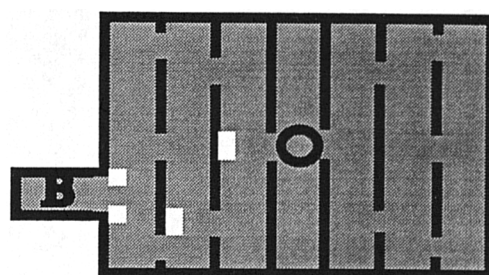
**Treatment 6**



**Treatment 4**

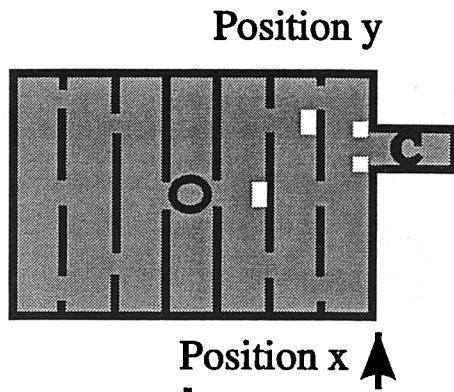


**Treatment 8**

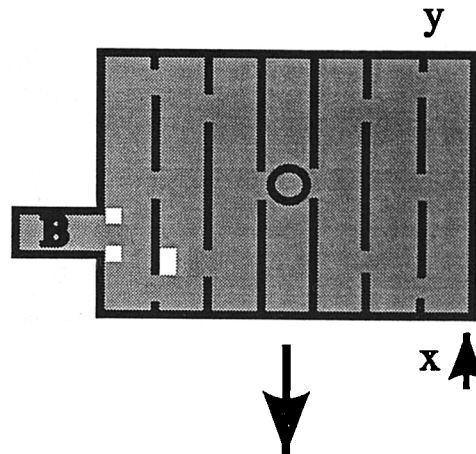


**Fig. 3.2** Conditions within the hierarchical maze for Pre-treatment, Treatment 1, reward box and Lego™ blocks moved from position C to position D, Treatment 2, reward box and Lego™ blocks moved from position D to position B, Treatment 3, Lego™ tower 1 removed, Treatment 4, all remaining Lego™ towers removed, Treatment 5, reward moved from position B to position D, Treatment 6, hide and the position of the experimenter rotated 180°, Treatment 7, hide rotated back to original position, experimenter entering from side y, Treatment 8, hide rotated 180° experimenter entering from side x, Group 2.

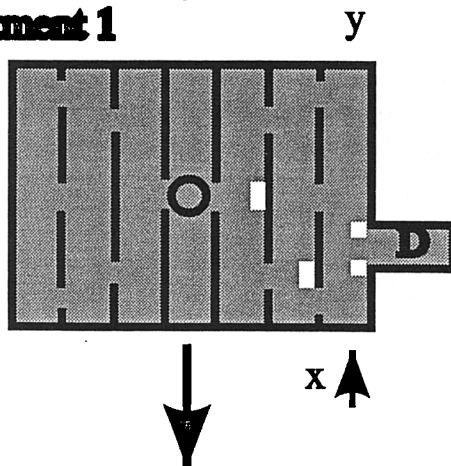
**Pre-treatment**



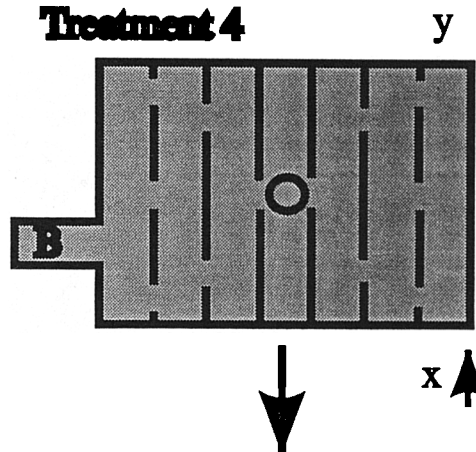
**Treatment 3**



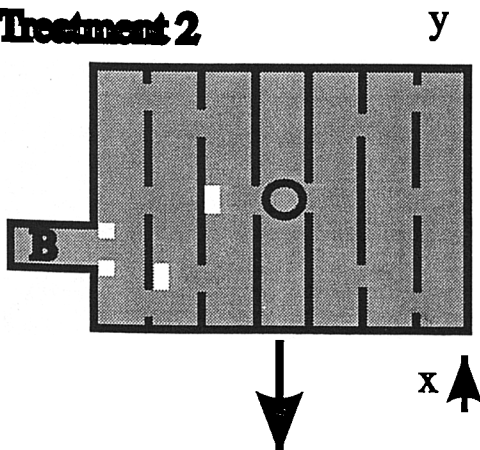
**Treatment 1**



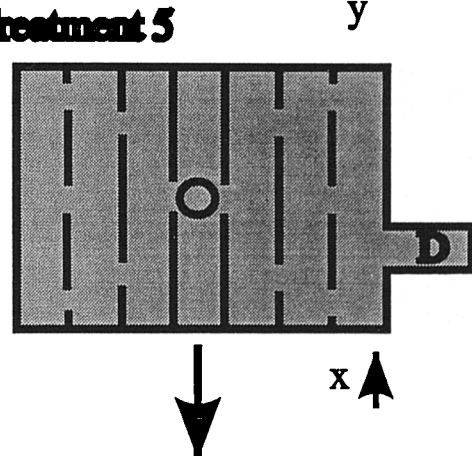
**Treatment 4**



**Treatment 2**

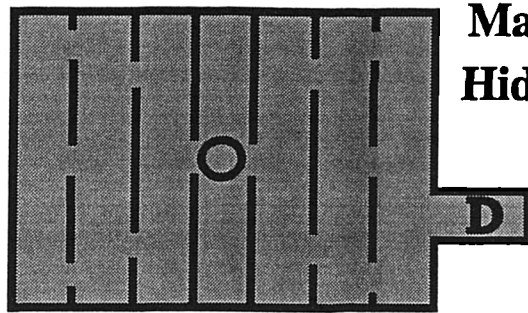


**Treatment 5**



**Treatment 6**

y ↓



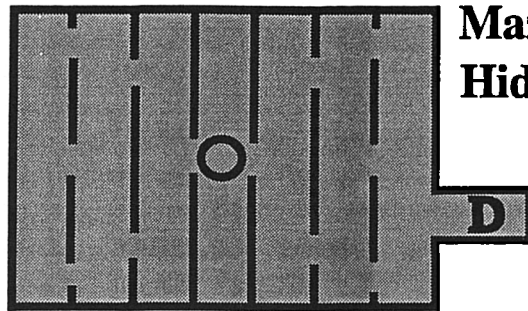
Maze in original position  
Hide rotated 180°

x

**Treatment 7**



y ↓



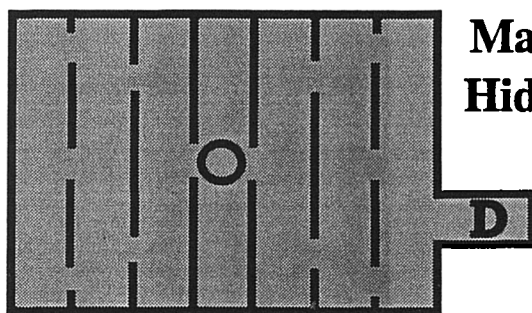
Maze in original position  
Hide in original position

x

**Treatment 8**



y



Maze in original position  
Hide rotated 180°

x ↑

x ↑ = experimenter entering from position x

y ↓ = experimenter entering from position y

(now Group 2) in the experiment presented in Chapter 2 was carried out for 20 days while Treatment 3 (now Group 1) was only carried out for 15 days and it was assumed that performance had reached asymptote on day -5 and -10 respectively) using Friedman's test. Thus it was ascertained if there was any difference between the performance reached at the end of the Pre-treatment and performance in each treatment following a change in conditions. Where a significant difference was found using Friedman's test, Dunn's test (Neave & Worthington 1988) was applied to the data, to determine where the difference between Treatments occurred. Each treatment was also tested for a decrease in time and path length to the reward over consecutive days using Page's test, in order to ascertain whether the fish had re-learned the task and therefore had improved their performance under the new conditions.

### 3.3 RESULTS

#### 3.3.1 Effect of changing conditions, Group 1

During this experiment all fish began to move around the maze almost immediately they were released from the start box and in the majority of cases all of them completed the maze within the 30 min allowed throughout all treatments (Figs 3.3 and 3.4).

There was a significant difference in time to the reward and path length between treatments in Group 1 (Table 3.3, Friedman's test,  $P = 0.001$  and  $0.001$  respectively, d.f. = 9). The mean path length to the reward was significantly higher in Treatment 4 than in the Pre-treatment (Dunn's test,  $Q = 3.67$ ,  $Q_{0.05} = 3.260$ ,  $k = 10$ , Table 3.4 and Fig. 3.6).

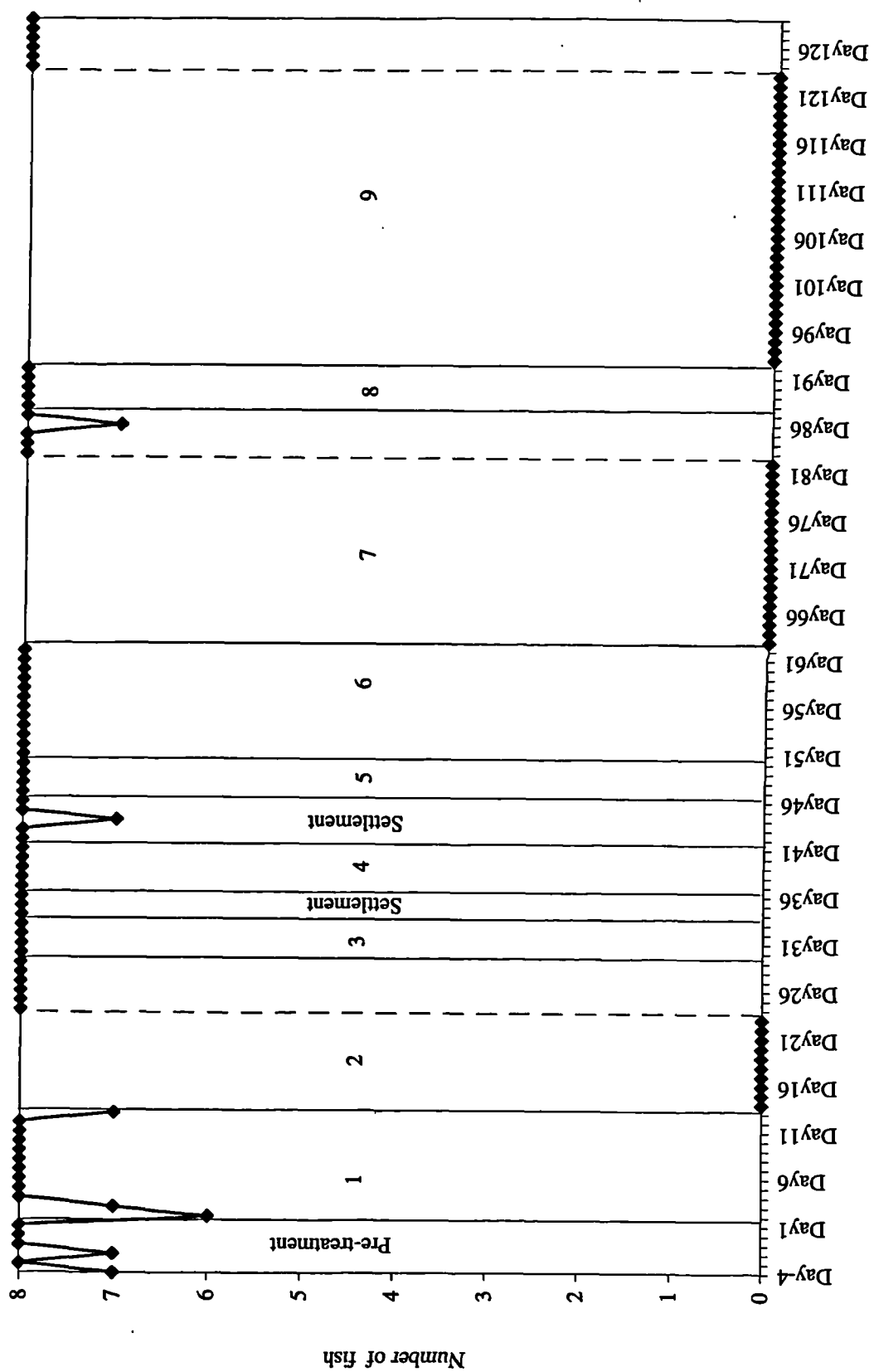
Only Treatment 5 (when the most efficient route was blocked) showed a significant decreasing trend in mean time to completion over several days (Page's test, Page's statistic = 54, critical value = 53.725 at 5%, d.f. = 4, Tables 3.5 and 3.6, Figs 3.5 and 3.6).

#### 3.3.2 Individual route variation and clue use, Group 1

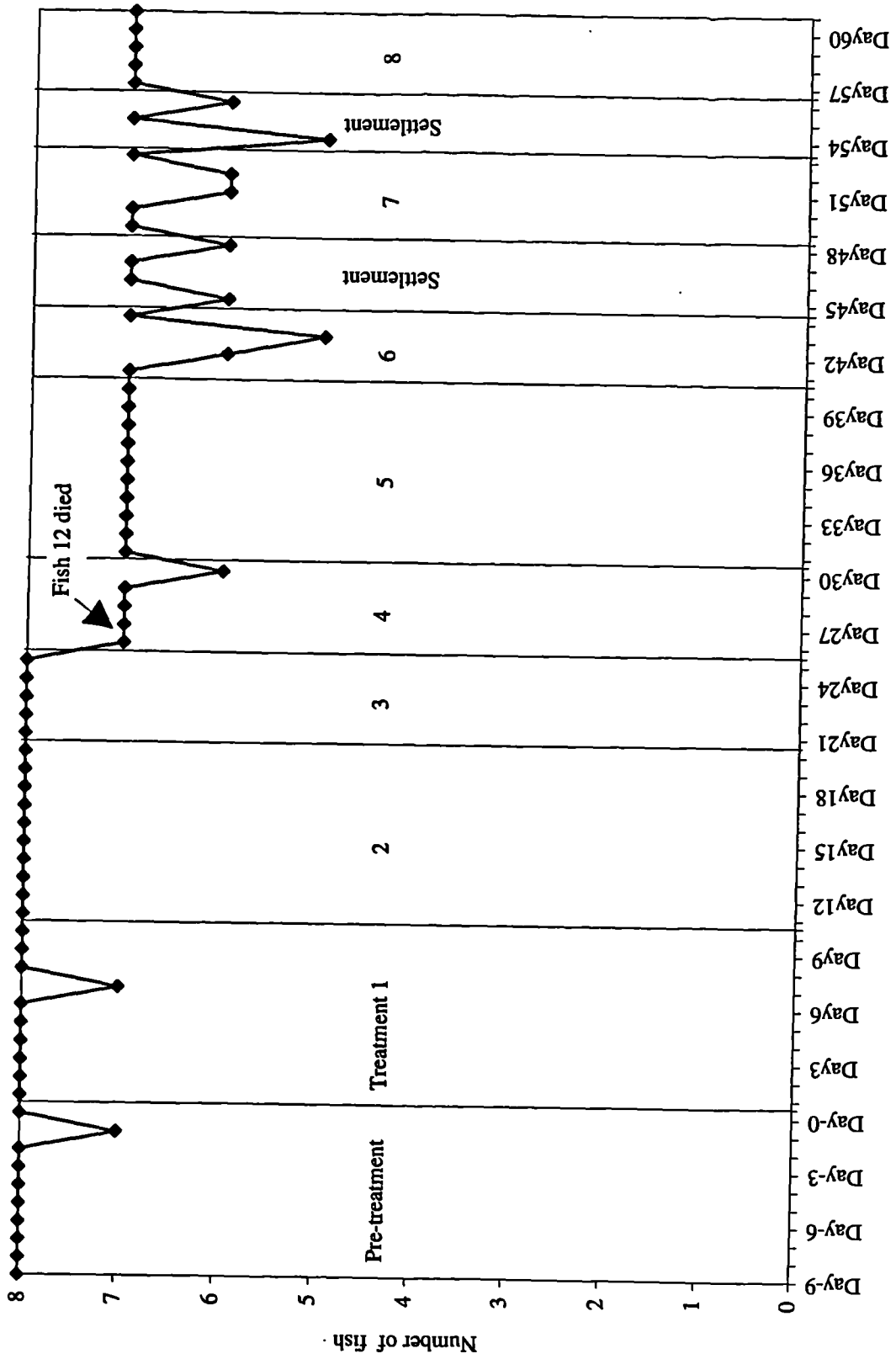
On Day 36 (immediately after the most efficient route to the reward box was blocked, Treatment 4), 2 out of 8 fish (Fish 3 and 7) chose the wrong direction out of the start box, but once they returned to the centre of the maze they followed a route avoiding the blockades and leading directly to the reward box (Fig. 3.7A). Fish 1, 4 and 6 attempted to use the most efficient route (Fig. 3.7B). When fish 4 and 6 found this blocked they became confused and visited the opposite side of the maze before correcting their path and eventually reaching the reward box. Fish 1 corrected its path and travelled directly to the reward box after this one mistake. Fish 2, 5 and 8 made several attempts to use the most efficient route, visited the wrong side of the maze and returned to the centre where they swam up and down the central corridor several times before eventually reaching the reward (Fig. 3.7C).



**Fig. 3.3** The number of *L. Pholis* completing the maze in Treatment 1, hierarchical maze and hide rotated 180°, Treatment 2, following 10 day retention period, Treatment 3, olfactory clue removed from the food, Treatment 4, most efficient route to the food blocked, Treatment 5, white screen removed from the hide, Treatment 6, Lego™ blocks and reward box moved from position A to position C, Treatment 7, following a 20 day retention period, Treatment 8, Lego™ blocks and reward box moved from position C to position B, Treatment 9, following a 30 day retention period, Group 1, Days -4 to 129. The full vertical lines indicate the beginning of a new treatment and the dashed lines indicate the end of a retention period.



**Fig. 3.4** The number of *L. pholis* completing the maze in Treatment 1, reward box and Lego™ blocks moved from position C to position D, Treatment 2, reward box and Lego™ blocks moved from position D to position B, Treatment 3, Lego™ tower 1 removed, Treatment 4, all remaining Lego™ towers removed, Treatment 5, reward moved from position B to position D, Treatment 6, hide and the position of the experimenter rotated 180°, Treatment 7, hide rotated back to original position, experimenter entering from side y, Treatment 8, hide rotated 180° experimenter entering from side x, Group 2, Days -9 to 62. The vertical lines indicate the beginning of a new treatment.



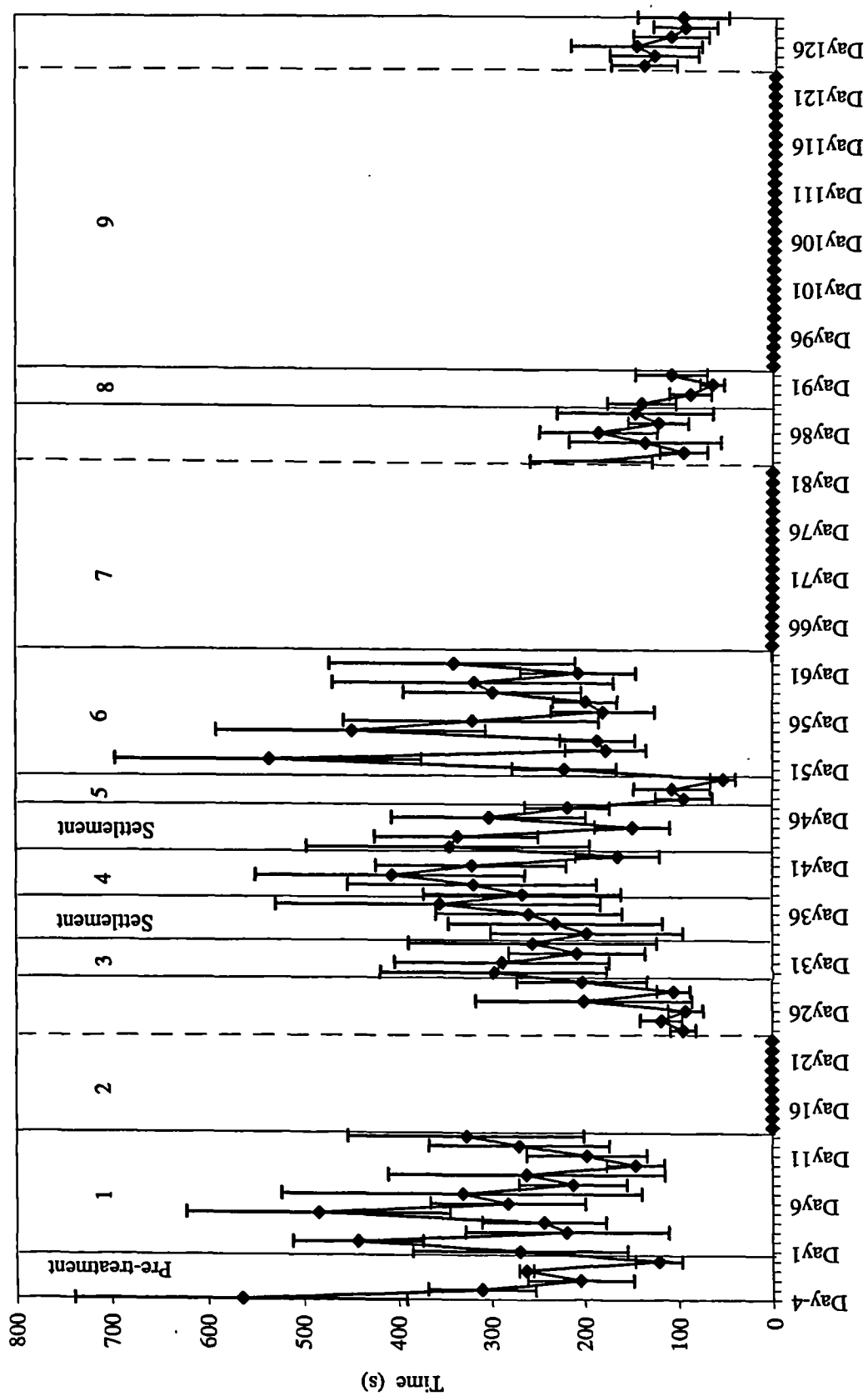
**Table 3.3** Mean time ( $\pm$  SE) to reward box ( $n = 8$ ) and mean path length ( $\pm$  SE) to reward box ( $n = 8$ ) for *L. Pholis* in each treatment, Group 1.

	Pre <sub>1</sub>	M&H180°	R <sub>10</sub>	- Olf	Blocked	- Screen	A to C	R <sub>20</sub>	C to B	R <sub>30</sub>
Time to reward box (s)	295.30 $\pm$ 180.40	284.48 $\pm$ 126.20	122.21 $\pm$ 72.25	250.73 $\pm$ 188.82	332.13 $\pm$ 260.44	154.86 $\pm$ 61.56	294.76 $\pm$ 116.66	148.94 $\pm$ 71.77	112.20 $\pm$ 58.24	139.34 $\pm$ 146.00
Path length	21.06 $\pm$ 11.60	29.80 $\pm$ 12.23	18.75 $\pm$ 5.92	34.33 $\pm$ 19.37	49.54 $\pm$ 18.26	25.63 $\pm$ 6.47	8.24 $\pm$ 10.67	33.13 $\pm$ 10.85	29.40 $\pm$ 9.80	40.91 $\pm$ 48.01

**Table 3.4** Results of Dunn's test comparing the mean time to the reward box ( $n = 8$ ) for *L. pholis* in the Pre-treatment with the mean time to the rewardbox ( $n = 8$ ) in each treatment, and mean path length to the reward box in the Pre-treatment with mean path length in each treatment Group 1,  $Q_{0.05} = 3.260$ ,  $k = 10$ .

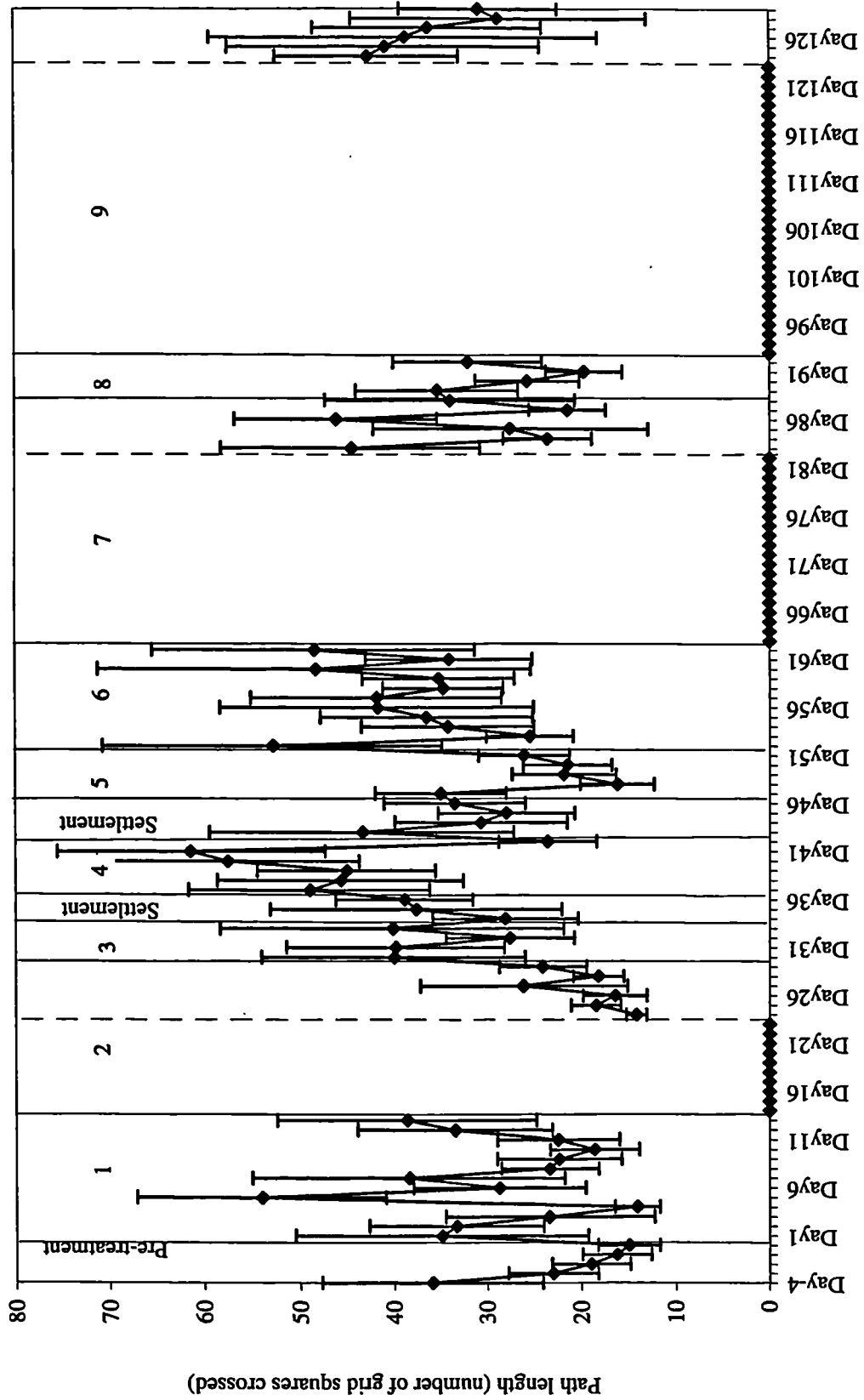
	M&H180°	R <sub>10</sub>	- Olf	Blocked	- Screen	A to C	R <sub>20</sub>	C to B	R <sub>30</sub>
T	0.14	2.52	0.72	0.14	1.64	0.29	1.85	2.66	2.42
Pre <sub>1</sub>	NS	NS	NS	NS	NS	NS	NS	NS	NS
PL	1.58	0.34	1.71	3.67	1.02	2.87	2.13	1.68	1.17
Pre <sub>1</sub>	NS	NS	NS	*	NS	NS	NS	NS	NS

**Fig. 3.5** Mean time to the reward box for *L. pholis* in Treatment 1, hierarchical maze and hide rotated 180°, Treatment 2, following 10 day retention period, Treatment 3, olfactory clue removed from the food, Treatment 4, most efficient route to the food blocked, Treatment 5, white screen removed from the hide, Treatment 6, Lego™ blocks and reward box moved from position A to position C, Treatment 7, following a 20 day retention period, Treatment 8, Lego™ blocks and reward box moved from position C to position B, Treatment 9, following a 30 day retention period, Group 1, Days -4 to 129. Error bars show the standard error of the mean, the full vertical lines indicate the beginning of a new treatment and the dashed lines indicate the end of a retention period.





**Fig. 3.6** Mean path length to the reward for *L. pholis* in Treatment 1, hierarchical maze and hide rotated 180°, Treatment 2, following 10 day retention period, Treatment 3, olfactory clue removed from the food, Treatment 4, most efficient route to the food blocked, Treatment 5, white screen removed from the hide, Treatment 6, Lego™ blocks and reward box moved from position A to position C, Treatment 7, following a 20 day retention period, Treatment 8, Lego™ blocks and reward box moved from position C to position B, Treatment 9, following a 30 day retention period, Group 1, Days -4 to 129. Error bars show the standard error of the mean, the full vertical lines indicate the beginning of a new treatment and the dashed lines indicate the end of a retention period.



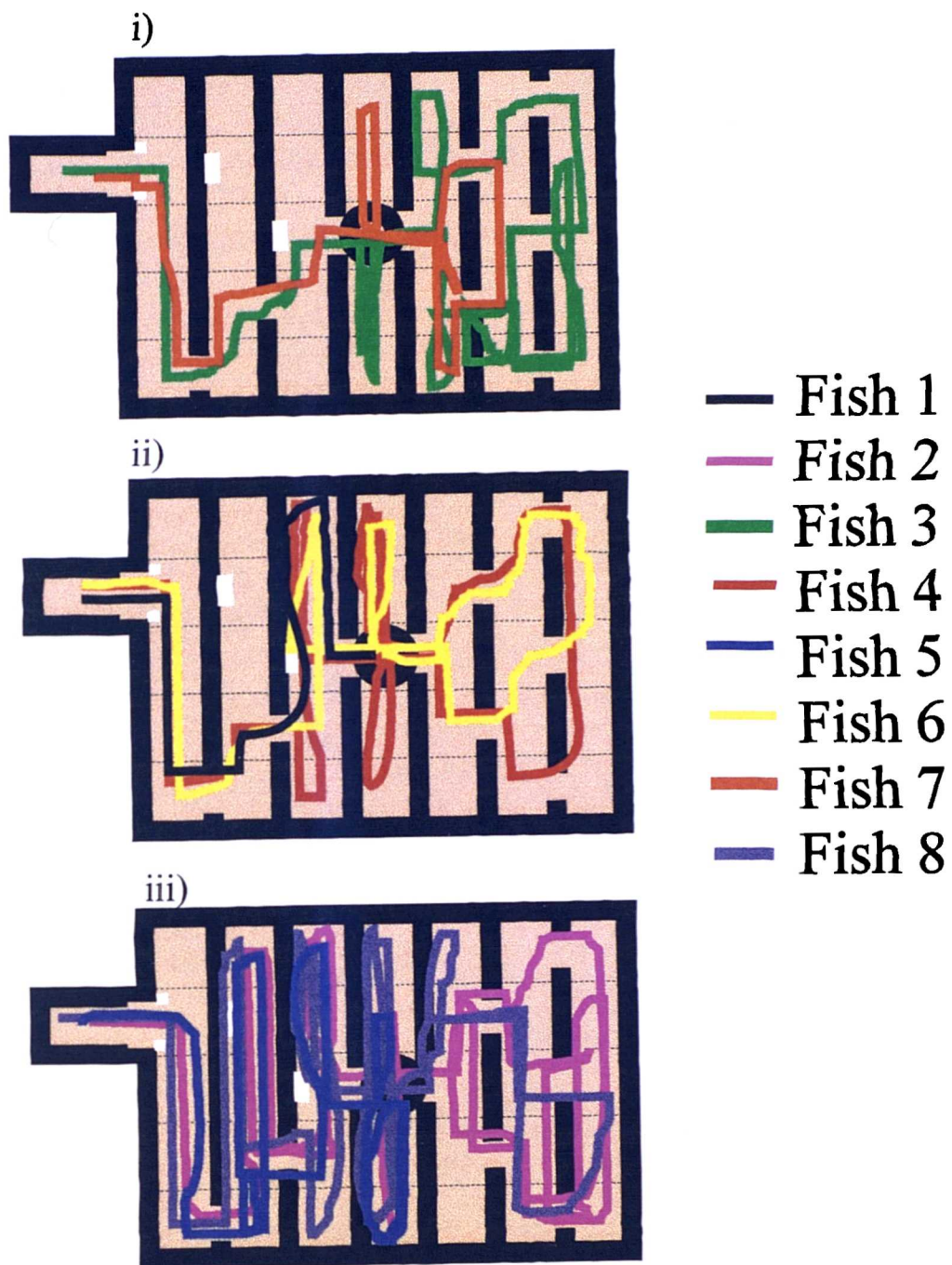
**Table 3.5** Results of Page's test for significant decrease in mean time to the reward box ( $n = 8$ ) for *L. Pholis* in each treatment, Group 1.

Treatment	Page's statistic	Critical value	
Pre <sub>1</sub>	714	723.921	NS
M&H180°	42	53.725	NS
R <sub>10</sub>	44	53.725	NS
- Olf	64	86.873	NS
Blocked	54	53.725	*
- Screen	496	578.421	NS
A to C	48	53.725	NS
R <sub>20</sub>	52	53.725	NS
C to B	39	53.725	NS

**Table 3.6** Results of Page's test for significant decrease in mean path length to the reward box ( $n = 8$ ) for *L. Pholis* in each treatment, Group 1.

Treatment	Page's statistic	Critical value	
Pre <sub>1</sub>	647	723.921	NS
M&H180°	40	53.725	NS
R <sub>10</sub>	39	53.725	NS
- Olf	51	86.873	NS
Blocked	39	53.725	NS
- Screen	464	578.421	NS
A to C	51	53.725	NS
R <sub>20</sub>	39	53.725	NS
C to B	39	53.725	NS

**Fig. 3.7** Paths followed by each individual *L. pholis* on the journey from the start box to the reward box on Day 36 at the start of Treatment 4 Group 1, immediately after the most efficient route to the reward was blocked, i) Fish 3 and 7, ii) Fish, 1, 4 and 6, iii) Fish 2, 5, and 8.



On Day 51 (immediately after the reward was moved from position A to position C, Treatment 6) Fish 2, 4 and 6 returned to the previous site of the reward ignoring the new position of the Lego™ towers (Appendix 1A). Fish 1, 3, 5, 7 and 9 followed a relatively direct path to the reward box (Appendix 1B). On first reaching Lego™ tower 1, seven out of eight fish (all except Fish 2) turned right (Appendices 1A and 1B).

On Day 88, (immediately after the reward box was moved from position C to B, Treatment 8) four out of eight *L. pholis* (Fish 2, 3, 4 and 5) chose the correct direction to exit the start box and followed a relatively direct route to the reward box (Appendix 2A). The remaining four individuals (Fish 1, 6, 7 and 8) returned to the previous site of the reward (Appendices 2B and 2C). Five out of eight *L. pholis* (Fish 1, 4, 5, 7 and 8) turned left at Lego™ tower number 1 during this treatment (Appendices 2A and 2B).

### 3.3.3 Effects of changing conditions, Group 2

There was a significant difference in path length to the reward only between treatments in Group 2 (Friedman's test,  $P = 0.045$ , d.f. = 9, Table 3.7, Figs 3.8 and 3.9) however no significant difference in path length was found between specific treatments and the Pre-treatment (Table 3.8 and Fig. 3.9).

There was no significant decreasing trend in time to completion or path length over several days in Group 2 (Figs 3.8 and 3.9, Tables 3.9 and 3.10).

### 3.3.4 Individual route variation and clue use, Group 2

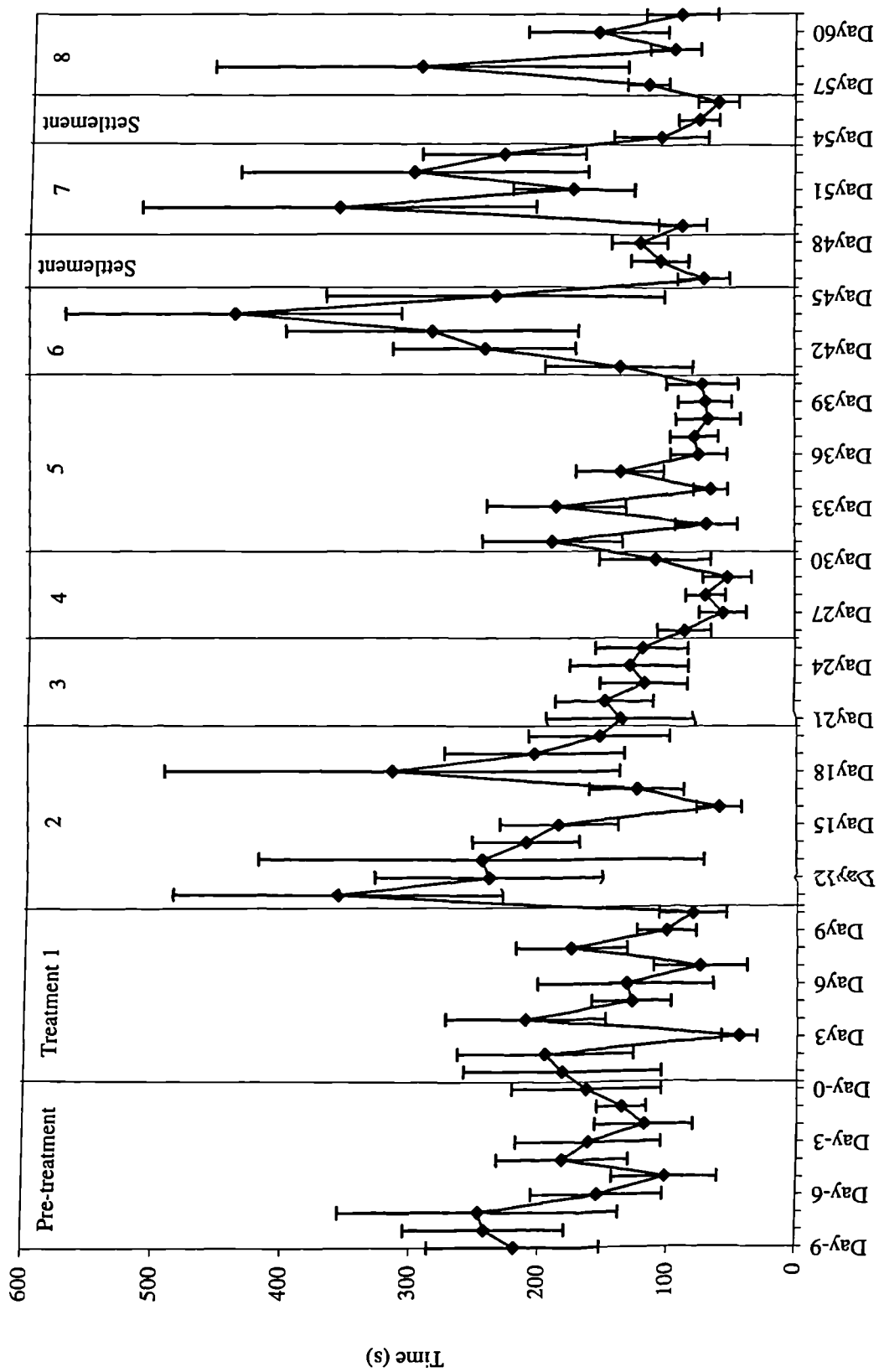
On Day 0 (immediately after the reward box was moved from position C to position D, Treatment 1) seven out of eight *L. pholis* (Fish 9, 10, 11, 13, 14, 15 and 16) visited site C before correcting their path and proceeding to site D (Appendices 3A and 3B). Fish 12 visited the wrong side of the maze initially but quickly corrected its mistake

**Table 3.7** Mean time ( $\pm$ SE) to reward box ( $n = 8$ ) and mean path length ( $\pm$ SE) to reward box ( $n = 8$ ) for *L. Pholis* in each treatment, Group 2.

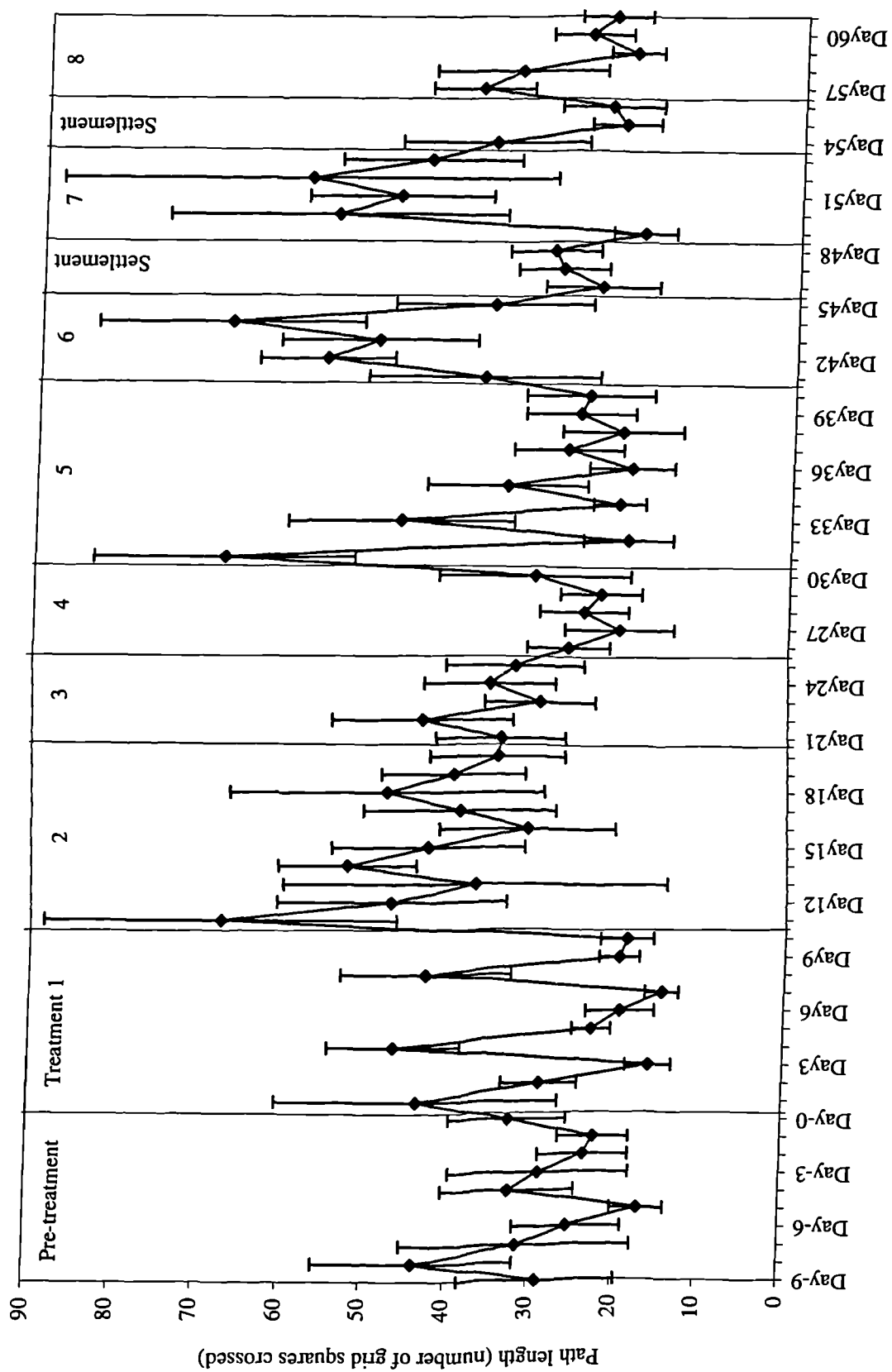
	Pre <sub>2</sub>	C to D	D to B	- Lego™1	- All Lego™	B to D	H180°E <sub>y</sub>	E <sub>y</sub>	H180°E <sub>x</sub>
Time to completion (s)	178.81 $\pm$ 95.55	140.09 $\pm$ 98.77	210.97 $\pm$ 178.25	121.03 $\pm$ 84.88	119.14 $\pm$ 96.32	115.30 $\pm$ 32.85	275.80 $\pm$ 174.74	228.46 $\pm$ 195.07	169.29 $\pm$ 150.46
Path length	27.81 $\pm$ 10.90	27.37 $\pm$ 9.25	42.14 $\pm$ 22.59	32.82 $\pm$ 10.51	24.37 $\pm$ 10.78	34.99 $\pm$ 5.47	50.87 $\pm$ 13.86	45.14 $\pm$ 21.97	30.96 $\pm$ 13.89

**Fig. 3.8** Mean time to the reward box for *L. pholis* in Treatment 1, reward box and Lego™ blocks moved from position C to position D, Treatment 2, reward box and Lego™ blocks moved from position D to position B, Treatment 3, Lego™ tower 1 removed, Treatment 4, all remaining Lego™ towers removed, Treatment 5, reward moved from position B to position D, Treatment 6, hide and the position of the experimenter rotated 180°, Treatment 7, hide rotated back to original position, experimenter entering from side y, Treatment 8, hide rotated 180° experimenter entering from side x, Group 2, days -9 to 62, n = 8. Error bars show the standard error of the mean and vertical lines indicate the beginning of a new treatment.





**Fig. 3.9** Mean path length to the reward box for *L. pholis* in Treatment 1, reward box and Lego<sup>TM</sup> blocks moved from position C to position D, Treatment 2, reward box and Lego<sup>TM</sup> blocks moved from position D to position B, Treatment 3, Lego<sup>TM</sup> tower 1 removed, Treatment 4, all remaining Lego<sup>TM</sup> towers removed, Treatment 5, reward moved from position B to position D, Treatment 6, hide and the position of the experimenter rotated 180°, Treatment 7, hide rotated back to original position, experimenter entering from side y, Treatment 8, hide rotated 180° experimenter entering from side x, Group 2, days -9 to 62, n = 8. Error bars show the standard error of the mean and the vertical lines indicate the beginning of a new treatment.



**Table 3.8** Results of Dunn's test comparing the mean path length to the reward box ( $n = 8$ ) for *L. pholis* in the Pre-treatment to mean path length in each treatment, Group 2,  $Q_{0.05} = 3.200$ ,  $k = 9$ .

	C to D	D to B	- Lego™1	- All Lego™	B to D	H180°E <sub>y</sub>	E <sub>y</sub>	H180°E <sub>x</sub>
Pre <sub>2</sub>	0.23 NS	1.39 NS	0.60 NS	0.32 NS	1.13 NS	2.78 NS	2.59 NS	0.02 NS

**Table 3.9** Results of Page's test for significant decrease in mean time to the reward box ( $n = 8$ ) for *L. Pholis* in each treatment, Group 2.

Treatment	Page's statistic	Critical value at 5%	
Pre <sub>2</sub>	339	723.921	NS
C to D	346	53.725	NS
D to B	51	53.725	NS
- Lego <sup>TM</sup> 1	44	86.873	NS
- All Lego <sup>TM</sup>	331	53.725	NS
B to D	41	578.421	NS
H180°Ex	42	53.725	NS
H180°E <sub>y</sub>	50	53.725	NS

**Table 3.10** Results of Page's test for significant decrease in mean path length to the reward box ( $n = 8$ ) for *L. Pholis* in each treatment, Group 2.

Treatment	Page's statistic	Critical value at 5%	
Pre <sub>2</sub>	336	723.921	NS
C to D	343	53.725	NS
D to B	48	53.725	NS
- Lego <sup>TM</sup> 1	42	86.873	NS
- All Lego <sup>TM</sup>	325	53.725	NS
B to D	46	578.421	NS
H180°Ex	42	53.725	NS
H180°E <sub>y</sub>	52	53.725	NS

and took a direct route from the centre of the maze to site D (Appendix 3C).

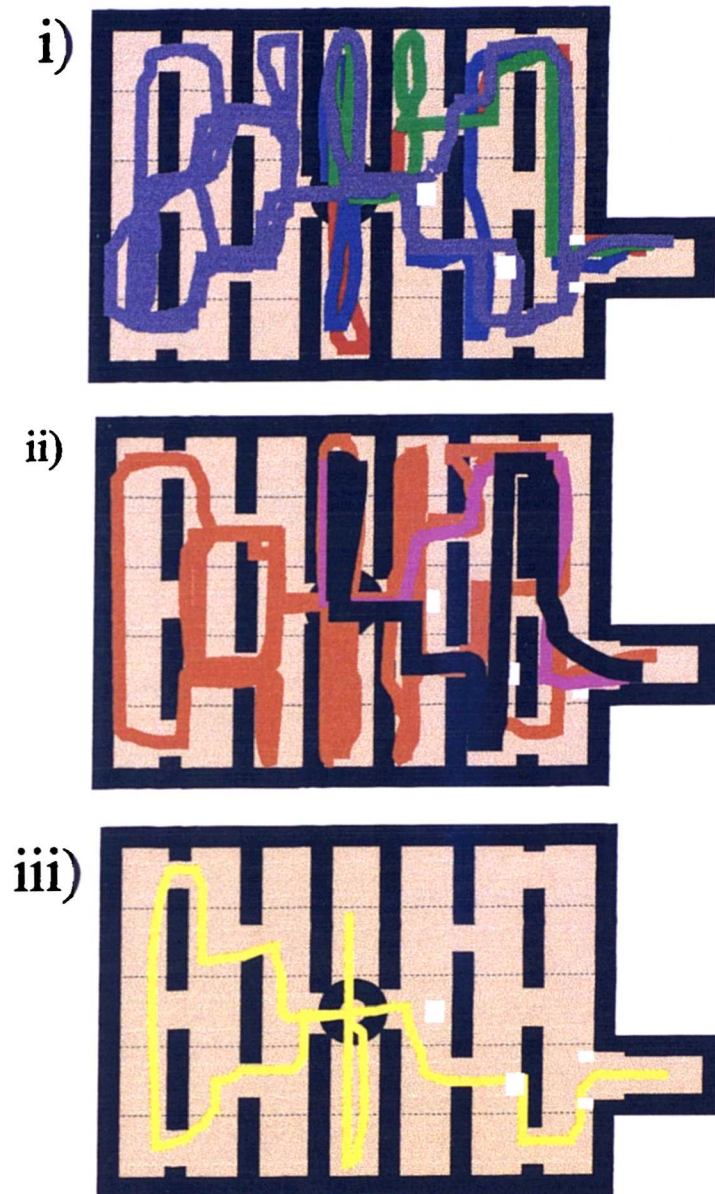
At the beginning of Treatment 2 (immediately after the reward box was moved from position D to position B, Day 11) five out of eight *L. pholis* (Fish 9, 10, 12, 15 and 16) visited site D before correcting their path and proceeding to site B (Figs 3.10A, 3.10B and 3.10C). The remaining three individuals (Fish 11, 13 and 14) followed relatively direct routes to the reward (Fig. 3.10C). Also, five out of eight fish (Fish 9, 10, 11, 14 and 16) turned right when they first encountered Lego™ tower 1 (Fig. 3.10D).

On Day 31 (immediately after the reward box was moved from position B to position D in the absence of Lego™ towers, Treatment 5) six out of seven fish (Fish 9, 10, 11, 14, 15 and 16) returned to site B before proceeding to site D (Appendices 4A and 4B). Fish 13 briefly visited the incorrect side of the maze and but then corrected its path and took a relatively direct route to the reward without first visiting site B (Appendix 4C).

On the first day of Treatment 6 (immediately following rotation of the hide, Day 41) five out of seven *L. pholis* (Fish 9, 11, 14, 15 and 16) travelled directly to the reward box (Appendix 5A), while Fish 10 and 13 visited the opposite side of the maze (Appendix 5B). On Day 42 (a further day into Treatment 6) all individuals visited the incorrect side of the maze before proceeding to the reward box by some roundabout route (Appendix 6).

On Day 49 (immediately after the hide was returned to its original orientation and the experimenter began entering from Position y, Treatment 7) 5 out of 7 individuals (fish 9, 10, 13, 14 and 16) proceeded directly to the reward box without first visiting the wrong side of the maze (Appendix 7A). Fish 11 and 15 initially entered a short distance into the incorrect side of the maze before correcting their paths (Appendix 7B). On Day 50 (a further day into Treatment 7) five out of seven fish (Fish 10, 11, 14, 15 and 16) visited the incorrect side of the maze before eventually reaching the reward box (Appendix 8A). Fish 9 and 13 followed relatively direct routes to the reward box

**Fig. 3.10** Paths travelled by each individual *L. pholis* on the journey from the start box to the reward box on Day 0 at the beginning of Treatment 1, Group 2, immediately after the reward and the Lego™ towers were moved from position D to position B, **i)** Fish 9, 10 and 11, **ii)** Fish 13 and 14, **iii)** Fish 9, **iv)** Fish 12 and 16.



- Fish 9
- Fish 10
- Fish 11
- Fish 12
- Fish 13
- Fish 14
- Fish 15
- Fish 16



(Appendix 8B).

### 3.4 DISCUSSION

The results showed that Treatment 4 (the most efficient route blocked) caused an increase in the path length to the reward and a significant decreasing trend in mean time to the reward in Group 1.

Many fish successfully completed the maze throughout these experiments, probably because from previous experience in the Pre-treatments all the fish had learnt that there was likely to be a food reward present in the maze and therefore were highly motivated to continue searching for it even when they did not find it immediately. Throughout the experiment the performance of each individual varied. This variation may be due to the fact that between one day and the next, fish forgot the most efficient route to the food. However, as they often performed well on one day, badly on the next and well again on the next, it is possible that even though they knew the position of the reward they “sampled” the rest of the maze at intervals to check they had not missed any other food patches. There was no evidence to suggest that this behaviour was rhythmic.

#### 3.4.1 Group 1

Rotating the maze and hide 180° (Treatment 1, Group 1) had no effect on performance and therefore *L. pholis* were not using geomagnetic clues to aid in their navigation of the maze.

Performance was not significantly different from the Pre-treatment following the retention periods of 10 days, 20 days and 30 days (Treatments 2, 7 and 9 respectively). These results suggest that *L. pholis* have a good memory for spatial locations and that they can store information about the location of the reward in the maze in their long-term memory, for at least 30 days.

The mean time to completion and mean path length in Treatment 3 (after removing the smell or taste emanating from the food reward by placing it behind glass) was not significantly different from those in the Pre-treatment, therefore *L. pholis* do not use

olfactory or gustatory clues when foraging in the hierarchical maze.

Path length in Treatment 4 (most efficient route to the food blocked) was significantly higher than that in the Pre-treatment. A slight increase in path length was to be expected in this treatment as the fish had to take a longer route to reach the reward box in order to by pass the blockades across the most efficient route. However, this result is significant, reflecting the confusion caused by this change in conditions. The paths followed by each individual during Day 36 (the first day of this treatment) showed that two out of eight fish chose the incorrect direction from the start box, but once they returned to the centre of the maze they followed the correct route, avoiding the blockades to the reward box. This result may have been due to chance or because these individuals favoured the route they followed, incidentally avoiding the blockades, and therefore did not notice them at all. The remaining fish attempted to use the most efficient route and, when they found it blocked, visited the wrong side of the maze and swam up and down the central corridor several times before eventually finding the reward. This increase in path length and confusion caused by blocking off the most efficient route to the reward box suggested that *L. pholis* do not possess a cognitive map of the maze according to the definition reported by Tolman (1948) and O'Keefe & Nadel (1978). If they had possessed a spatial map the subjects would have been able to correct their route immediately after the route was blocked and only a very small increase in path length would have resulted. Rather, they learned a specific path or "route" (O'Keefe & Nadel 1978) to the reward box by following the towers of Lego™ blocks.

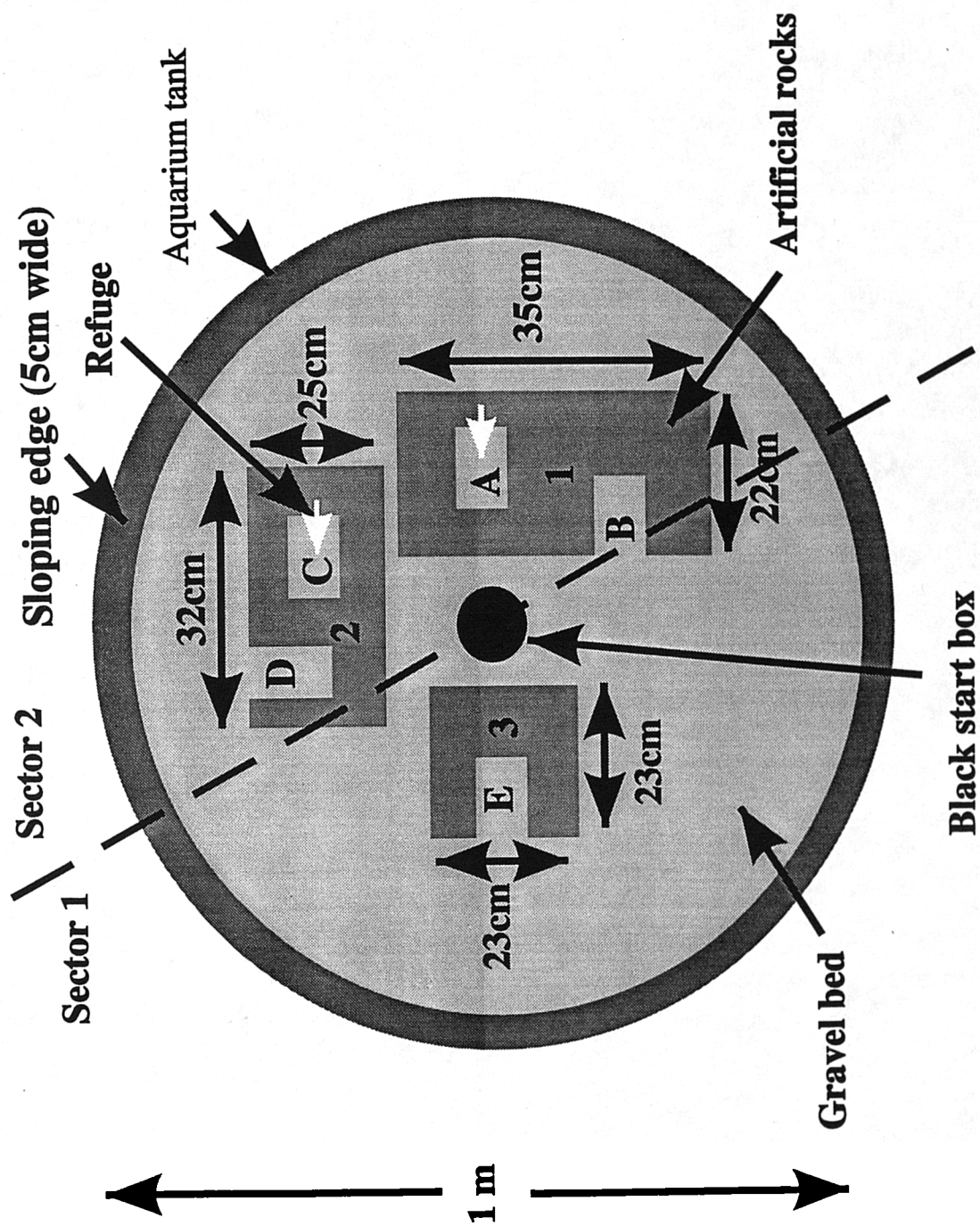
However, a significant decreasing trend in mean time to completion as treatments continued over several days occurred only in Treatment 4, after the most efficient route to the food was blocked. This results showed that *L. pholis* learned a new route to the food rapidly, and suggested that they are well adapted to conditions in the intertidal zone where routes may become blocked and landmarks may change during a storm at high tide.

Mean path length to the reward in Treatment 6 (following relocation of the reward box at position C) seemed to be higher than that in the Pre-treatment however did not prove to be significantly different using Dunn's test. A difference in path length was expected because the fish had to modify their route in 2 ways in order to reach the reward in its new position. Firstly they had to respond to the new position of the first local landmark by leaving the start box in a new direction, and secondly they had to reverse the sequence of left and right turns learned for the most efficient route to the previous reward box position. This lack of significant difference in path length following relocation of the reward box may have occurred because any change in efficiency of completing the task was concealed by individual variation or because *L. pholis* learned the new task so quickly that no overall difference in path length was detected. Rapid learning of the task would explain the absence of a statistically significant decreasing trend in mean path length to the reward in Treatment 6, however this result may also have been due to the fact that the experiment was not continued for long enough for a trend to become apparent.

On day 51 (immediately after the reward box was moved from position A to position C in Treatment 6) three out of eight *L. pholis* visited site A (the previous site of the reward), before correcting their path and eventually reaching the reward box at site C. Therefore these three individuals ignored the new position of the Lego™ towers and somehow remembered the route to the previous reward position. It is possible that they used other visual clues within the maze, or centrally stored recordings of their own movements or programmes of movement, a process known as idiothetic orientation (Papi 1992). The remaining five fish chose the correct direction from the start box and therefore responded correctly to the new position of Lego™ tower 1 by moving towards it. Seven out of eight fish turned right upon reaching Lego™ tower 2 even though this was not the most efficient turn towards the reward at this stage in the journey and their route had to be corrected at a later stage in order to reach position C. The mistake in turn direction at this point probably occurred because the fish remembered the sequence of left and right turns at various points along the most

efficient route to the reward from the previous treatment, Treatment 5 which included a right turn at Lego<sup>TM</sup> tower 1. Therefore the memory for a previous route was interfering with (or “overshadowing”, Mackintosh 1974) the performance following a change in conditions. These results suggested that local landmarks are very important to spatial memory in *L. pholis*; they are used as beacons (the fish move towards them when they mark a goal) and as a clue as to which direction a subject should turn at crucial points in its journey.

There was no apparent or statistically significant difference in path length between Treatment 8 (reward and Lego<sup>TM</sup> towers moved from position C to B) and the Pre-treatment. This may have been due to the fact that the fish had become accustomed to a change in conditions such as this during Treatment 6, and had learned simply to use the Lego<sup>TM</sup> towers as beacons marking the position of the reward box. However, this result may also have occurred because in Treatment 8 the route to reach the new reward box position (once the fish has passed out of the start box in the correct direction according to the position of the first landmark), incorporated the same number and sequence of left and right turns as in Treatment 7. Consequently, once each fish has passed out of the start box in the correct direction according to the position of Lego<sup>TM</sup> tower 1, it simply had to remember and repeat the sequence of left and right turns from the most efficient route in the previous treatment. The paths followed by each individual to reach the reward box on Day 88 (at the beginning of Treatment 8) showed that four out of eight individuals chose the correct direction to exit the start box and followed a relatively direct route to the reward box, while the remaining four fish returned to the previous site of the reward, ignoring the new position of the Lego<sup>TM</sup> towers. Five out of eight fish turned left at Lego<sup>TM</sup> tower number 1 as they would have done to travel directly to the reward in Treatment 7. This result suggested that the Lego<sup>TM</sup> towers were again being used as beacons and as a prompt as to which direction to turn at key points along the journey to the reward. There was no evidence to suggest that it was the same four individuals who always travelled directly to the reward box using the same route throughout this treatment. It cannot be concluded therefore that



reward box. This more immediate, successful performance suggested that these individuals relied more heavily on the visual clue provided by the Lego™ towers for navigation to the reward. However the same 3 individuals were not as efficient in their time or route followed to the reward box throughout the remainder of this treatment. Consequently, again there was no evidence to suggest that there was consistency in the way that individual *L. pholis* learned to navigate the hierarchical maze. Also on Day 11, five out of eight fish turned right at the Lego™ tower 1 as they would have done to proceed directly to the reward if it were in position D (in the previous treatment, Treatment 1). This constancy in turn direction at this point in the journey again suggested that Lego™ tower 1 was used as a clue for the correct direction to turn to reach the reward box by the most efficient route.

Mean time to completion and path length in Treatments 3 (Lego™ tower 1 removed) and 4 (all remaining Lego™ towers removed) were not significantly different from the Pre-treatment, therefore *L. pholis* were able to immediately employ some other method of navigation once the Lego™ towers were removed and it is possible that a hierarchy of clue use operated in these fish. Their readiness to follow local landmarks (Group 1 Treatment 8, Group 2 Treatment 1) suggested that these were the most important clue. However *L. pholis* continued to be able to navigate the maze efficiently when local landmarks were removed and therefore were apparently capable of using non experimental clues to complete this task. The fish may have been responding to some visual clue that had been overlooked up to this point, for example an inconspicuous mark on the wall of the hide, or the position at which the experimenter stood when the fish was placed in the start box. Alternatively, the subjects may have been using a directional clue such as the direction from which they were placed in the start box, or perhaps they used a different method of navigation altogether such as idiothetic orientation (Papi 1992).

Time and path length to the reward in Treatment 5 (reward moved from position B to position D in the absence of Lego™ towers) were not significantly different from those

in Pre-treatment. These results again suggested that the fish were using something other than the local landmarks to navigate the hierarchical maze efficiently. However, on Day 31 (the first day of Treatment 5), six out of seven fish visited site B before correcting their path and travelling to site D. Therefore path length increased immediately after the reward was moved in the absence of Lego™ towers. Nevertheless the fish learn the new spatial task so quickly that there was no significant effect on the treatment as a whole. Time to completion and path length had returned to levels similar to those in the Pre-treatment within 6 days, although this decrease was not significant, perhaps due to individual variation and the low number of replicates. In contrast, learning was relatively slow when the local clues were available. For example, in Treatment 2 re-learning took 9 days (again the decrease in time and path length to the reward was not significant probably due to individual variation and then low number of replicates). This contrast in learning rate between Treatments 2 (reward moved from position D to position B in the presence of Lego™ clues) and 5 (reward moved from position B to position D in the absence of Lego™ clues) suggested that the fewer clues present, the easier it is for *L. pholis* to overcome “overshadowing” (Warburton 1990) and learn a new route in the hierarchical maze. There is a potential for confusion if several clues are used particularly if the information supplied by one of these is changed and hence becomes contradictory to the other. In the wild, conditions are much more complicated and *L. pholis* are likely to have many different clues to refer to, for example, local and global visual clues, tide and current movement, temperature gradients, olfactory and gustatory clues. Consequently it is likely that a hierarchy of clue importance exists to avoid confusion and that *L. pholis* are able to adapt quickly when conditions change often in the intertidal zone.

How *L. pholis* continued to navigate the maze successfully in the absence of the Lego™ landmarks was investigated further in Treatments 6, 7 and 8. The path length during Treatment 6 (hide rotated 180°, experimenter entering the hide from Position y, in the absence of Lego™ clues) seemed higher than that in the Pre-treatment however was not found to be significantly different using Dunn’s test. This lack of a



statistically significant result may be because Treatment 6 was not continued for long enough or there were not enough replicates carried out for it to be apparent. However it appeared that rotating the hide and the direction from which the experimenter entered the maze by 180°, caused an increase in path length, which suggested that the fish were using something in the hide or the position of the experimenter as a visual clue to the position of the reward. Path length to the reward in Treatment 7 (hide rotated back to its original position, experimenter entering from Position y, in the absence of Lego™ clues) also appeared higher than that in the Pre-treatment, while path length in Treatment 8 (hide rotated 180° experimenter entering from Position x in the absence of Lego™ clues) did not. These results suggested that the fish were referring to their memory of the position of the experimenter as they were placed in the start box, just prior to the beginning of the experiment, in order to navigate the hierarchical maze efficiently in the absence of Lego™ clues.

The mean path length of and the paths followed by each individual on Day 49 and 57 the first day of Treatments 7 and 8 respectively, showed that at the beginning of each of these treatments a high proportion of fish followed a direct route to the reward box and therefore their path length was low. On Days 50 and 58 (the second day of each of these treatments) however, a higher proportion of fish visited the incorrect side of the maze before proceeding to the reward box and therefore their path length was higher. This difference between path lengths on the first and second day of Treatments 7 and 8 suggested that immediately following rotation of the hide the fish were using some other clue or some other method of orientation such as idiothetic orientation (Papi 1992) to navigate the maze efficiently. It is possible that this clue or alternative method may have been stored only in the short term memory, and therefore on the second day of each of these treatments the fish forgot their new method of navigation and reverted back to using the position of the experimenter as their clue. With hindsight, the equipment used in this experiment and the experimental protocol should have been designed to incorporate random direction of entry into the hide on consecutive days, therefore preventing the position of the experimenter just prior to

each experiment being used as an indirect clue as to the location of the reward.

There was no evidence to suggest that individuals were consistent in their response to similar changing conditions in either Group. However such a consistency in individual variation might have become apparent with a greater number of replicates.

### 3.5 CONCLUSION

*L. pholis* are capable of learning to navigate a relatively complex artificial habitat to reach a food reward more efficiently over time. Geomagnetic, olfactory / gustatory and current direction clues were all eliminated as the source of spatial information, as was the use of a cognitive map. Efficient navigation to the reward was accomplished by the use of visual clues to learn a specific route. Small local clues such as white Lego™ towers proved especially important and there was evidence to suggest that these prompted the fish to turn left or right at key points along their journey. The direction of entry to the experimental arena or the direction by which the experimenter left the arena just prior to a daily test were used by the fish as clues to the position of the reward box in the absence of Lego™ towers. Information was retained for a period of at least 30 days. These results suggested the possibility that *L. pholis* can use local clues such as rocks and clumps of algae in their home range to navigate to and from food patches in the wild. “Overshadowing” (Warburton 1990) influenced the learning of new spatial tasks, but *L. pholis* could be trained to solve new problems within 10 days, an ability which must prove useful in the dynamic environment of the rocky shore (Hughes *et al.* 1992).

## Chapter 4

**Acquisition of spatial information  
in *Lipophrys pholis*: exploration  
of a novel arena**

## 4.1 INTRODUCTION

Animals acquire information about their habitat by exploring. The best representation of exploratory behaviour is gained by placing an animal in a novel arena and observing the immediate changes in its behaviour and those which occur as it becomes habituated to its new environment. A few studies have investigated the exploratory behaviour of fishes. The first of these concerns the goldfish *Carassius auratus* which carries out a systematic locomotor exploration of a novel environment consisting of a grand tour of the arena followed by a successional area search (Kleerekoper *et al.* 1970). Kleerekoper *et al.* (1970) also reported a tendency of goldfish to revisit the same location several times in succession rather than to avoid it, as was observed in rats (Battig 1964). Exploration of a novel arena in rats has also revealed a correlation between velocity and exploratory behaviour, with velocity gradually increasing over several hours then decreasing sharply once the individual has finished exploring the arena (Glanzer 1961). This behaviour was not evident in *C. auratus* (Kleerekoper *et al.* 1970). Nevertheless, experiments carried out by Kleerekoper *et al.* (1970) designed to observe the exploratory behaviour of *C. auratus*, using light sensitive cells in the floor of a novel arena to record their movement, did reveal much information regarding the turning behaviour of these fish during exploration. Turning behaviour is considered to be an important aspect of exploration in many vertebrates. Spontaneous alternation of turns has been recorded in the exploratory behaviour of rats in T and Y mazes and in the open field (Tolman 1925, Dennis 1935), and is thought to be an expression of the tendency to acquire continuous novel stimulation of the central nervous system (Hebb 1955, Dember & Earl 1957, Glickman 1958, Zucker and Bindra 1961, Wimer & Sterns 1964). It was postulated that, if this assumption were correct, one would expect that the gradual decrease in novelty content accompanying exploratory behaviour would be reflected in the direction of consecutive turns in *C. auratus*, i.e. the fish would increasingly alternate the angle of turns the more familiar they became with their environment (Kleerekoper *et al.* 1970). No obvious relationship between novelty content of the environment and direction and average frequency of turns was found, at least up to 69 hours (Kleerekoper *et al.* 1970).

The distribution of turn angle between a population of New Jersey fish and a population of Texas fish were found to be significantly different, and were found to remain constant with time (Kleerekoper *et al.* 1970). It was suggested that there may be genetic difference in the handedness of different populations of *C. auratus*, a result which is in agreement with similar studies comparing the handedness of different populations of fishes (Herter 1930, 1948, Spencer 1939, Breder & Nigrelli 1938).

All but one out of eight *C. auratus* were found to carry out angle compensation (to maintain a nearly constant relationship between the magnitude of left and right turns) as first described by Kleerekoper *et al.* (1969). Random selection of turn angle was eliminated as a cause of this phenomena. Since studies have shown that spontaneous alternation decreases in rats with middle ear disease (which disrupts the vestibular system, Douglas 1966a, b), and maze learning in rats is dependent on normal function of semicircular canals (Watson 1907), Kleerekoper *et al.* (1969, 1970) concluded that the *C. auratus* perceives rotation while turning in the vestibular system, and uses the information collected to maintain a symmetry between cumulated left and right angles.

The edges of the arena were found to have a dramatic effect on the angle distribution and frequency of turning in *C. auratus* with the greatest effect occurring at 40 cm from the aquarium walls (Kleerekoper *et al.* 1970). It was suggested that this phenomenon simply supported earlier studies that reported the presence of negative accommodation, perspective vision and distance perception in fishes (Lee 1898, Scheuring 1920, Tamura 1957), and in goldfish specifically at a distance not less than 50 cm (Herter 1930, 1953).

In conclusion it was reported that the relationship between alternation and exploratory behaviour may be far less important than its association with orientation behaviour, of which angle compensation must be an integral part (Kleerekoper *et al.* 1970). The ability of *C. auratus* to maintain a constant relationship between cumulative angle of turn at a high level of efficiency has suggested that a direction of progression, once established, could be maintained by fishes in the absence of directional clues in the

environment. Moreover, this phenomenon could constitute an important inertial guidance system in long range orientation, once the general direction had been established by other means (Kleerekoper *et al.* 1969).

The blind cave fish *Anoptichthys jordani* markedly alters its behaviour in novel environments (Teyke 1985, 1989). Teyke (1989) suggested that the increased velocity of this species in unfamiliar surroundings maximises topographical information collected by the lateral line system, and aids in the formation of a cognitive map. This process, he concluded, takes between 6 and 30 hours as a period of anaesthesia following this interval does not produce any renewed increase in exploratory behaviour on return to the test arena, whereas exploration continues following a period of anaesthesia after less than 6 hours experience of the test arena.

Juvenile guppies similarly gradually decreased their swimming speed following introduction to a novel arena (Mikheev & Andreev 1993). A strong correlation between swimming speed and time spent swimming by the edge of the tank suggested that guppies explore a novel aquarium by swimming rapidly around the boundary wall. However, it is possible that the changes in swimming speed and path may be due to initial fear and subsequent habituation. A shorter period of fast swimming than that described for blind cave fish was recorded, and it was suggested that the guppies simultaneous use of vision and lateral line perception improved their efficiency of exploration. It is also possible that vision and lateral line perception may be used sequentially in exploratory behaviour and therefore that the transition from fast swimming to relatively slow swimming would not necessarily indicate the end of an exploration process. Alteration of surroundings during the second half of the active swimming phase, by insertion of a landmark, had no effect on swimming behaviour. However, alteration of major features such as position of the light source and the shape of the aquarium caused an increase in activity, although these alterations did not cause swimming speed to increase to the levels recorded just after release into the novel arena, or renewed swimming around the boundary wall. This difference in swimming speed following initial exposure to the novel arena and following a major change in the

arena later on can be attributed to difference in the degree of novelty of these two stimuli (Welker & Welker 1958).

When placed in a novel rock pool *L. pholis* explored from a series of refuges or “bases”, the first “base” being the first refuge that each individual entered at random (Almada *et al.* 1983). From this first “base”, the fish moved along a repeated path, actively inspecting other prospective refuges but often returning to the original “base”. Eventually they occupied a second “base” from which they carried out several more bouts of exploration until settling on a third “base” and so on until they occupied a favourite “base” according to a dominance hierarchy. It is possible that by exploring in this way and referring to the topography of the surroundings *L. pholis* form a map of the position of the refuges and use this information to enter the nearest refuge when threatened (Almada *et al.* 1983).

Many laboratory studies have shown that it is the socially dominant individual that has exclusive use of the shelter if there is only one provided in an arena (Breder 1950, 1954; Phillips 1971, Phillips & Swears 1979). However in the case of fishes one field study has shown the full importance of skill and agility in finding and utilising refuges (Phillip & Swears 1979). In that experiment, 7 out of 10 hooks baited with live striped blennies *Chasmodes bosquianus* and tethered to underwater pilings out of reach of shelter had caught toadfish the following morning. Only a single striped blenny managed to survive by sheltering in an oyster shell which had been overlooked during clearance of the area.

A study investigating the substrate preference of captive and wild *Coryphoblennius galerita*, *Gobius cobitis* and *L. pholis* showed that in monospecific groups in captivity *C. galerita* and *L. pholis* preferred predominantly rocky substrata while *G. cobitis* was found more frequently on open sand (Faria *et al.* in press). In the wild, *C. galerita* was significantly more abundant in rock pools containing rocky substrata when compared to an expected value, while *G. cobitis* and *L. pholis* were significantly more abundant in rock pools containing mixed substrata. It is suggested that this habitat preference



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arises from the suitability of available shelters to each species in each habitat.

Reputedly, only a single experience of swimming over a connected system of rock pools at high tide is sufficient to form a spatial map of the area by *Bathygobius soporator* (Aronson 1951, 1971). The behaviour of naive and experienced fish showed that information gained by the experienced fish while swimming over the rock pools during high tide caused a significant improvement in the jumping efficiency of this species (from one pool to another) when threatened. Also, blackeye gobies *Coryphopterus nicholsi* given 5 hours free range of a large tank containing a single burrow, found this refuge significantly faster (when attacked by a simulated predator) than individuals deprived of free access (Markel 1994).

The aims of this chapter were to observe the method by which *L. pholis* explore a novel habitat and the length of experience required for these fish to learn the position of refuges.

## 4.2 METHODS

### 4.2.1 Collection of study species

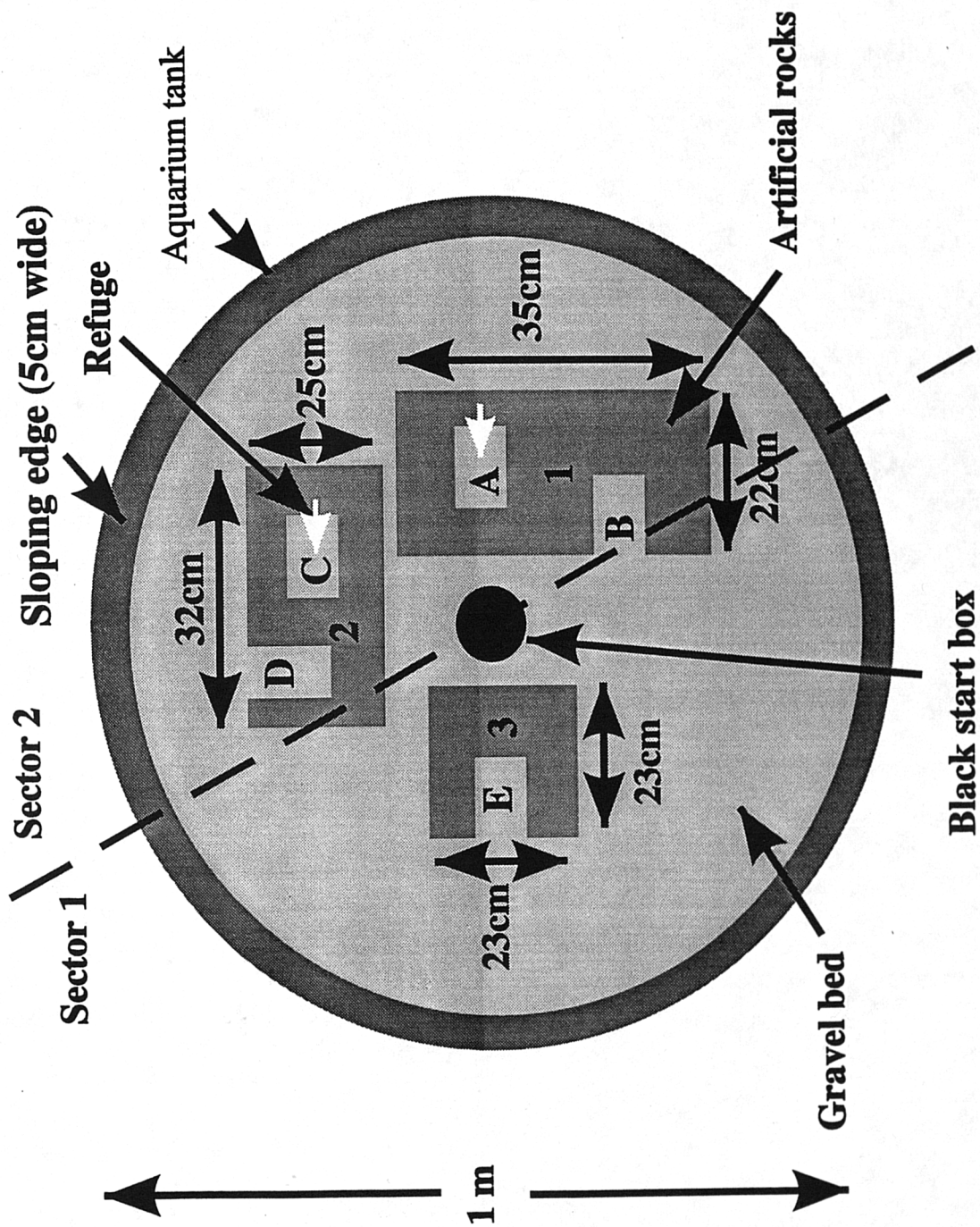
*Lipophrys pholis* were collected according to the method described in Chapter 2.

### 4.2.1 Equipment

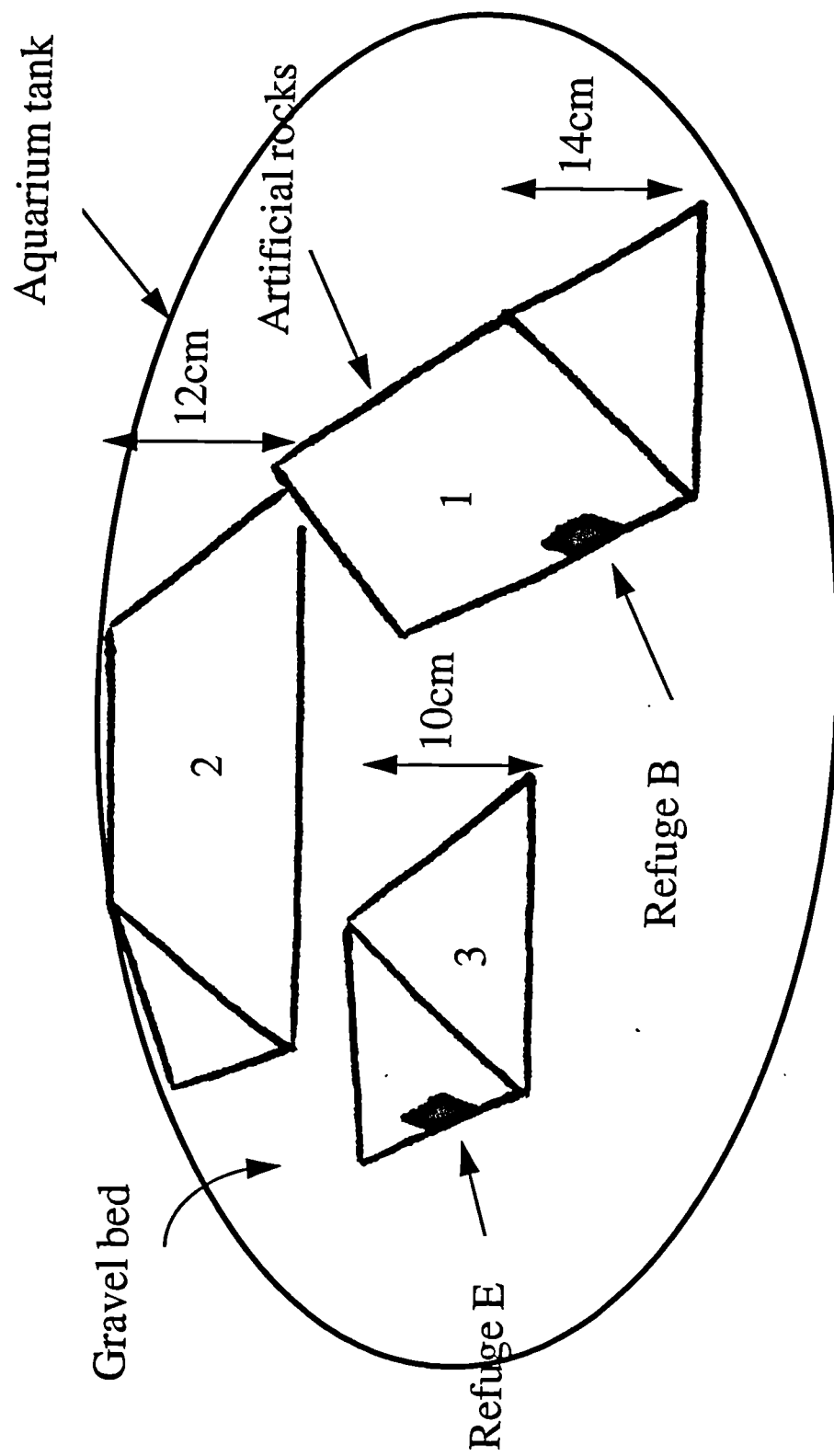
An artificial habitat for shannies was created by carving three artificial rocks, (numbered 1, 2 and 3 with base dimensions 35 cm x 22 cm, 25 cm x 32 cm and 23 cm x 23 cm and heights 14 cm, 12 cm and 10 cm respectively) from solid blocks of polystyrene, using a heated wire (Figs 4.1 and 4.2). Care was taken to fashion smooth, sloping sides to the artificial rocks in order to discourage the shannies from using the edges themselves as a refuge. Brown acrylic paint was used to impart a natural colour to the artificial rocks. Five refuges, labelled A, B, C, D and E all with dimensions 5 cm x 5 cm x 10 cm, were hollowed out of the artificial rocks. Two were made in Artificial Rocks 1 and 2, one at the level of the gravel (Refuges B and D respectively) and one 5 cm above the gravel level (Refuges A and C respectively) in each case, and one was made in Artificial Rock 3 (Refuge E) at the level of the gravel. The artificial rocks were placed in a sand coloured circular aquarium tank (diameter 1 m), the base of which was covered with gravel and filled with sea water to a depth of 10 cm so that the peaks of rocks 1 and 2 emerged from the water surface, and all refuges were completely covered with water. A sloping edge was also carved from polystyrene and placed at the perimeter of the tank to discourage *L. pholis* from using the angle created by the base and the wall as a refuge.

The arena was covered by a hide consisting of a Dexion™ frame lined with black plastic sheets and covered from above by a diffusing screen (Fig.4.3). Lighting was provided by two, 240 V, 500 W halogen lamps mounted on opposite corners of the frame. Movement of each individual could be observed through a viewing slit in the black sheet or via a TV monitor connected to a time code generator and a video camera, the output from which was video recorded for later analysis.

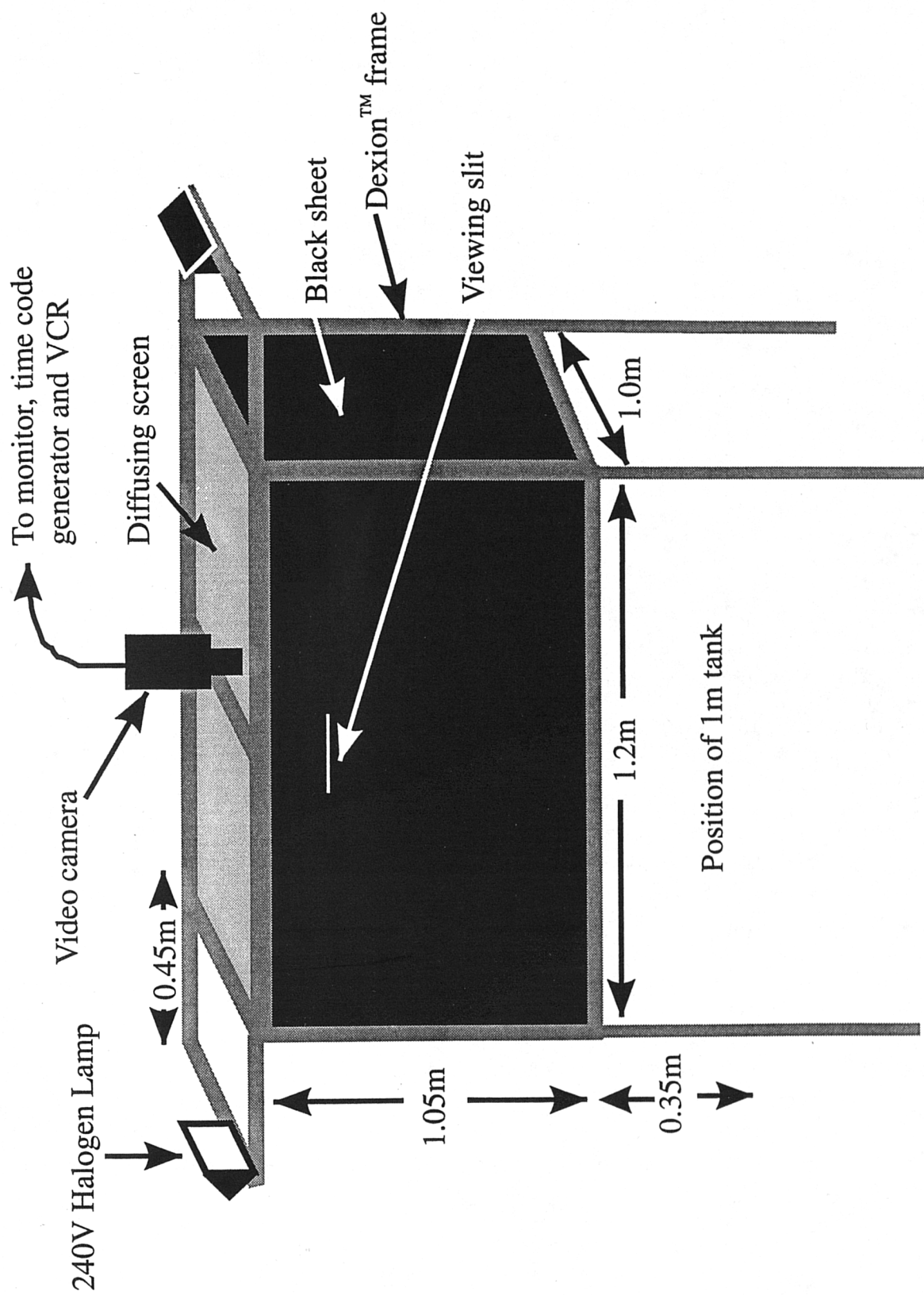
**Fig. 4.1** The novel arena used to study exploratory behaviour in *Lipophrys pholis*, showing the position of Artificial rocks 1-3 and Refuges A - E. The dashed line shows the division between Sectors 1 and 2, the white arrows indicate the position of the entrance to Refuges A and C.



**Fig. 4.2** 3D schematic of the novel arena showing the position and heights of Artificial Rocks 1, 2 and 3, and the position of Refuge B and E.



**Fig. 4.3** The hide used to cover the novel arena, showing position of the video camera and viewing slit.





### 4.2.2 Protocol for naive fish

Each individual from a group of 10 fish was released into the arena from a black, remotely operated, centrally placed start box, and their movement recorded for 6 hours. Each fish was fed with a small amount of chopped *Mytilus edulis* just prior to the experiment and the experiment was started at the same time everyday. Performance was measured as latency to movement (time elapsing between raising the start box and initial movement of the fish), time to enter first refuge (time elapsing between raising the start box and the fish entering the first refuge), the sequence of refuges entered, the number of times each refuge was entered and the residence time in each refuge (the time elapsing from when the fish entered a refuge to when it left it). A computer programme (M.T. Burrows *pers. comm.*) together with the video recording of movement, was used to plot the path travelled by each individual as it moved around the arena. The data were used to calculate the distance travelled to the first refuge, the angle of movement from the start position towards the first refuge entered, the total exploration time (the elapsed time each fish spent out of the refuge moving around the arena) and the exploration time spent in each sector of the arena. For the more active individuals the analysis of the path of movement was split into hourly intervals.

### 4.2.3 Protocol for experienced fish

Each of the 10 individuals was released into the arena again 24 hours later. The latency to first movement, the time to enter the first refuge, the distance travelled to the first refuge, the angle of movement from the start position to the first refuge and the path to, and the position of, the refuge chosen were recorded.

### 4.2.4 Analysis

The total residence time and the number of visits by each fish were compared among refuges using the Kruskal-Wallis test, and where this result was significant Dunn's test (Neave & Worthington 1988) was applied to identify significant refuge preference among individuals.

Latency to movement, time to enter the first refuge, distance travelled to the first refuge

and the angle of movement from the start position to the first refuge in Naive and Experienced groups were compared using the Wilcoxon signed rank test, in order to ascertain whether performance was improved by the 6 hour experience period. Mean exploration time spent in Sector 1 and Sector 2 of the arena were compared using the Wilcoxon signed rank test, to determine any preference for complex and simple areas of the arena among individuals.

Mean number of refuges visited in each hour and the mean speed of the most active fish (Fish 2, Fish 4, Fish 7 and Fish 9) in each hour were analysed for any decreasing trend, and the mean total refuge residence in each hour was analysed for any increasing trend using Page's test. These tests were carried out in order to learn whether there was any change in behaviour while the subjects were gaining experience of the arena and therefore whether there was a recognisable limit to the duration of exploration.

## 4.3 RESULTS

### 4.3.1 Behaviour immediately following release

When the start box was raised at the beginning of the Naive Treatment, all individuals swam quickly away from the start position in a random direction (angles of movement from the start position were uniformly distributed, Raleigh's test of distribution,  $z = 2.118$ ,  $z_{0.05, 10} = 2.919$ , therefore accept  $H_0$ , population is uniformly distributed) and entered a refuge (Figs 4.4 and 4.5). The residence time in this first refuge varied from several minutes to several hours. When moving around the arena, none of the individuals remained outside a refuge for longer than a few seconds. There was great variation in activity among individuals (Figs 4.6 and 4.7) and Fish 2, 4, 7 and 9 were the most active. In a few individuals (for example Fish 3), activity was low at first, then increased to a peak before decreasing towards the end of the 6 hour experimental period. There was a significant decrease in the mean number of refuges visited in each hour (Page's statistic = 90.000, critical value at 5% = 86.873, Fig. 4.8). After the peak of activity, many individuals entered a refuge and remained in it for several hours until they were removed from the tank. However there was no significant increase in refuge residence over time (Page's statistic = 82.000, critical value at 5% = 86.873, Fig. 4.9). There was no statistically significant trend in the mean swimming speed of the most active fish, Fish 2, 4, 7 and 9 (Page's statistic = 65.000, critical value at 5% = 86.873, Fig. 4.10). Individuals mainly moved along the walls of the arena or along the edge of one of the artificial rocks towards the beginning of the 6 hour trial period, but later, when they became more active, they appeared to move from refuge to refuge more directly, cutting across open space more often (Figs 4.11 and Appendices 9, 10 and 11). Movement was concentrated around Artificial Rocks 1 and 2, and in Sector 2 (mean total exploration time in Sector 1 =  $808 \pm 1619$ , Sector 2 =  $1980 \pm 3444$ , Fig. 4.12, Wilcoxon signed rank test, Median 150,  $P = 0.019$ ,  $n = 10$ ). On only one occasion was a fish disturbed out in the open during this experiment. When the black curtain was opened in order to remove Fish 1 from the tank after 6 hours, the fish was situated in the central part of the arena close to Artificial Rock 2 and swam directly to Refuge B.

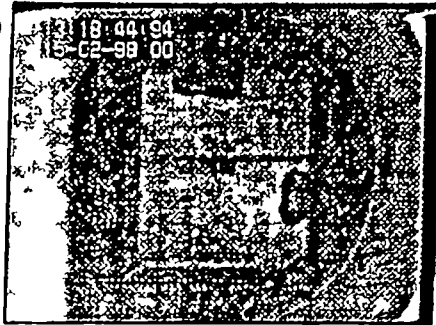
**Fig. 4.4** Screen print of computer programme for spatial analysis of fish behaviour (M.T. Burrows *pers. comm.*) showing **i)** photograph of the experimental arena, **ii)** the path travelled by each individual from the start box to the first refuge entered when Naive, **iii)** residence time of all individuals in each grid square of the arena (darker squares indicate longer residence time).

**Fig. 4.5** The angle of the trajectory of the initial movement from central area of arena, Naive treatment.

**Spatial Analysis of Fish Behaviour**

File Plot Fish Results

i)



First Time 30/01/98 09:52:30.28  
Last Time 17/02/98 09:53:36.00

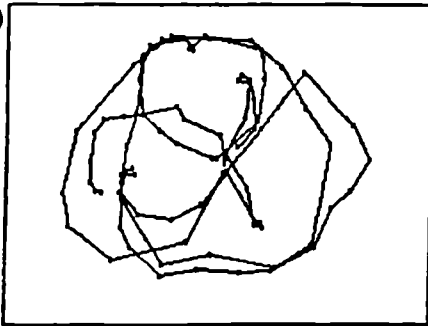
Files added Number of points 94

C:\SPEAF\FISHES.DAT

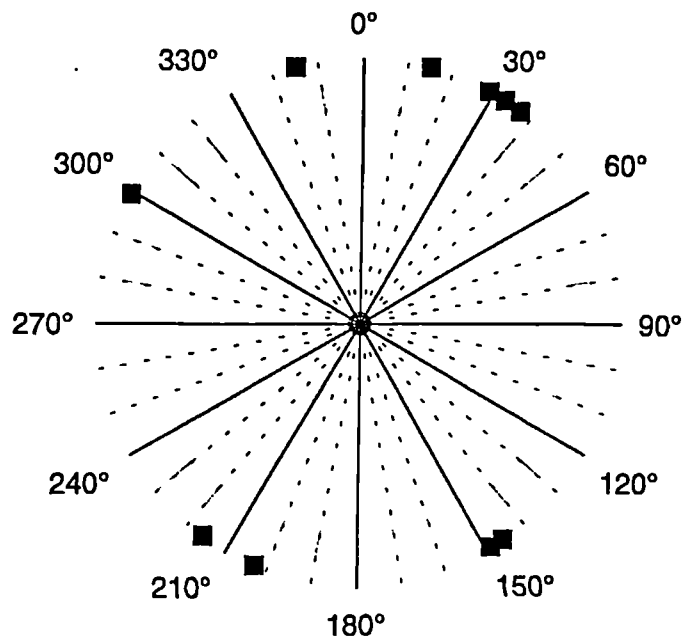
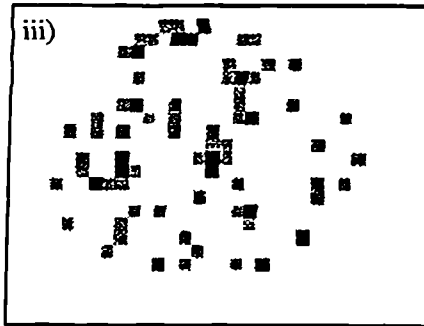
**Plot Options**

Hour elapsed  Swim Type ☒ Line ☒  
☐ Pelagic (off bottom)  
☐ Benthic (on bottom)  
 Cell size (pixels)  Both

ii)

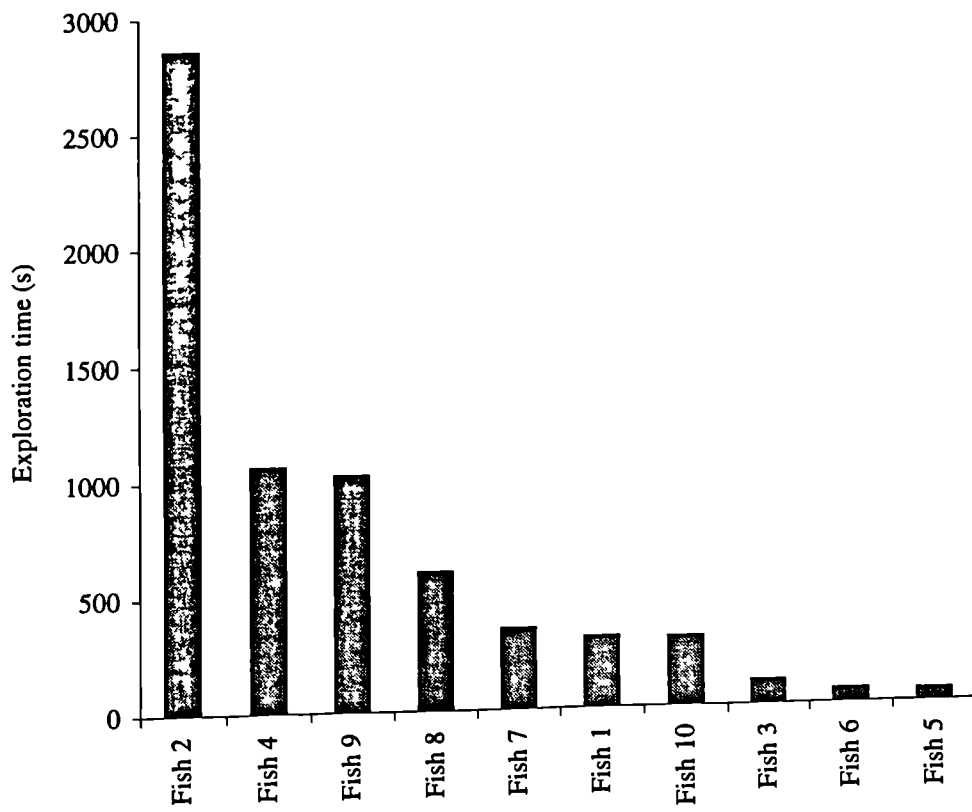
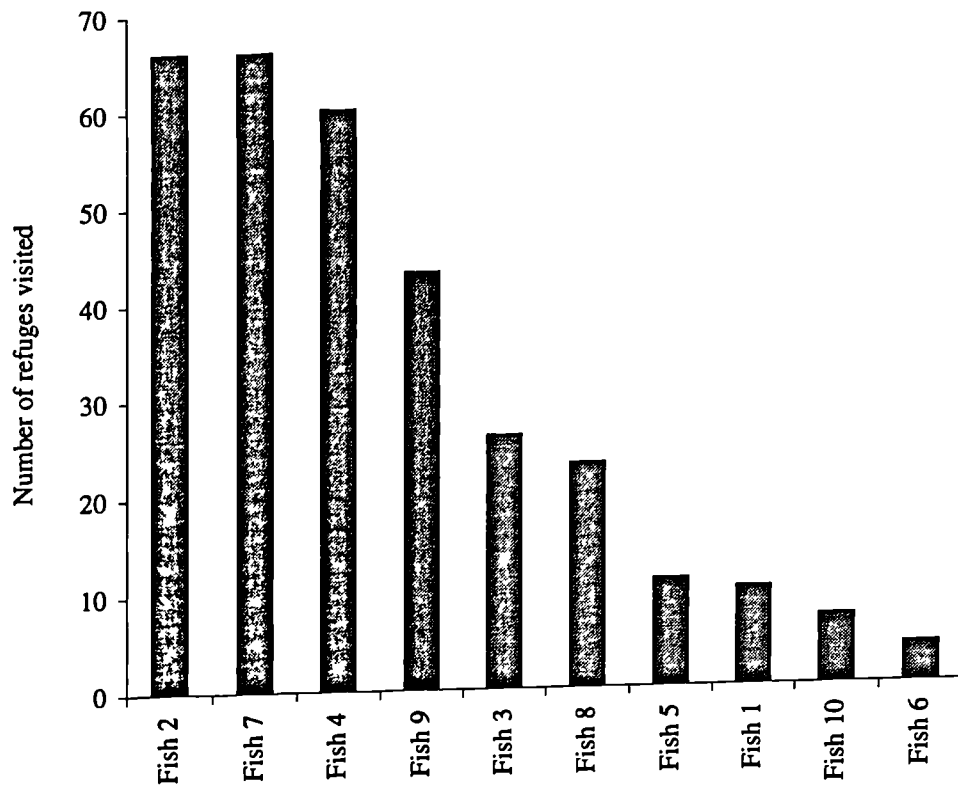


iii)



**Fig. 4.6** The number of refuges visited by each individual in the Naive treatment.

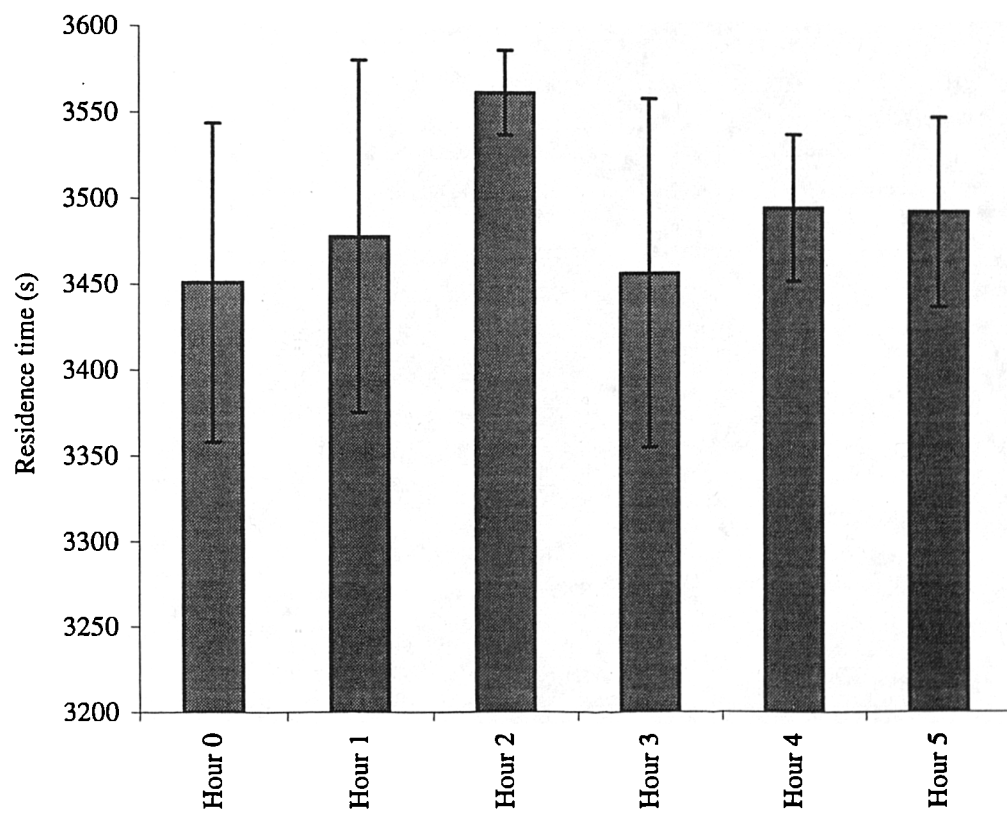
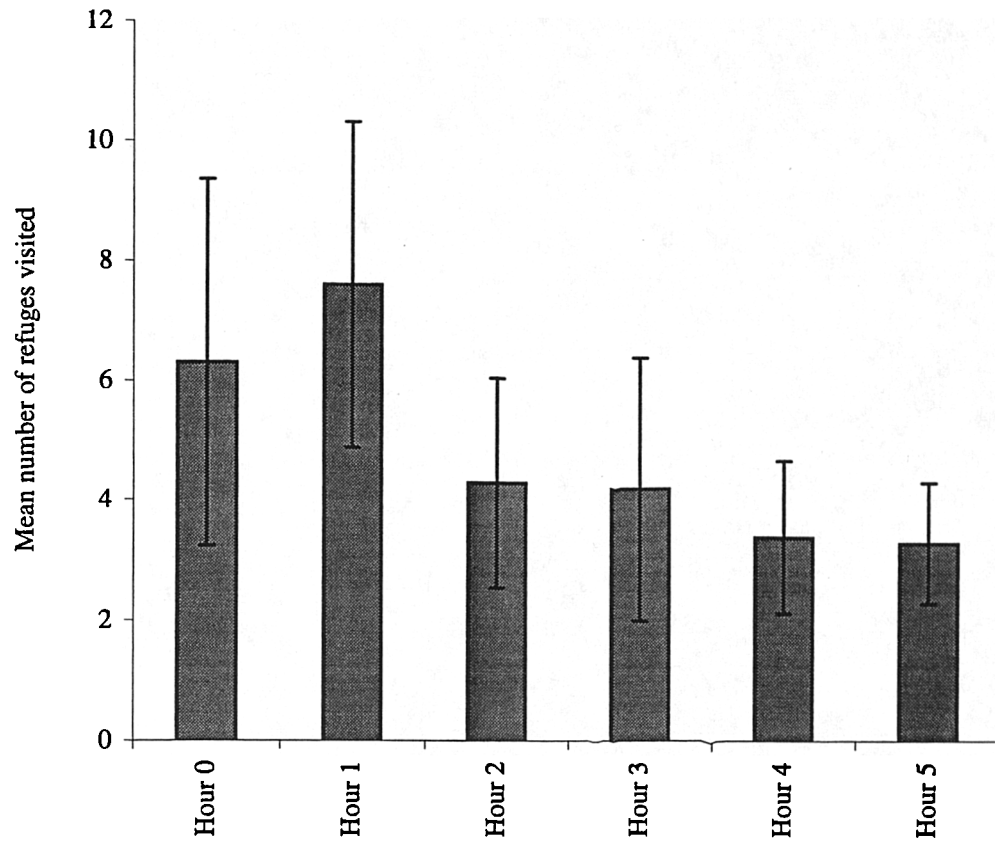
**Fig. 4.7** Exploration time (the total elapsed time spent outside the refuges moving around the arena) for each individual in the Naive treatment.



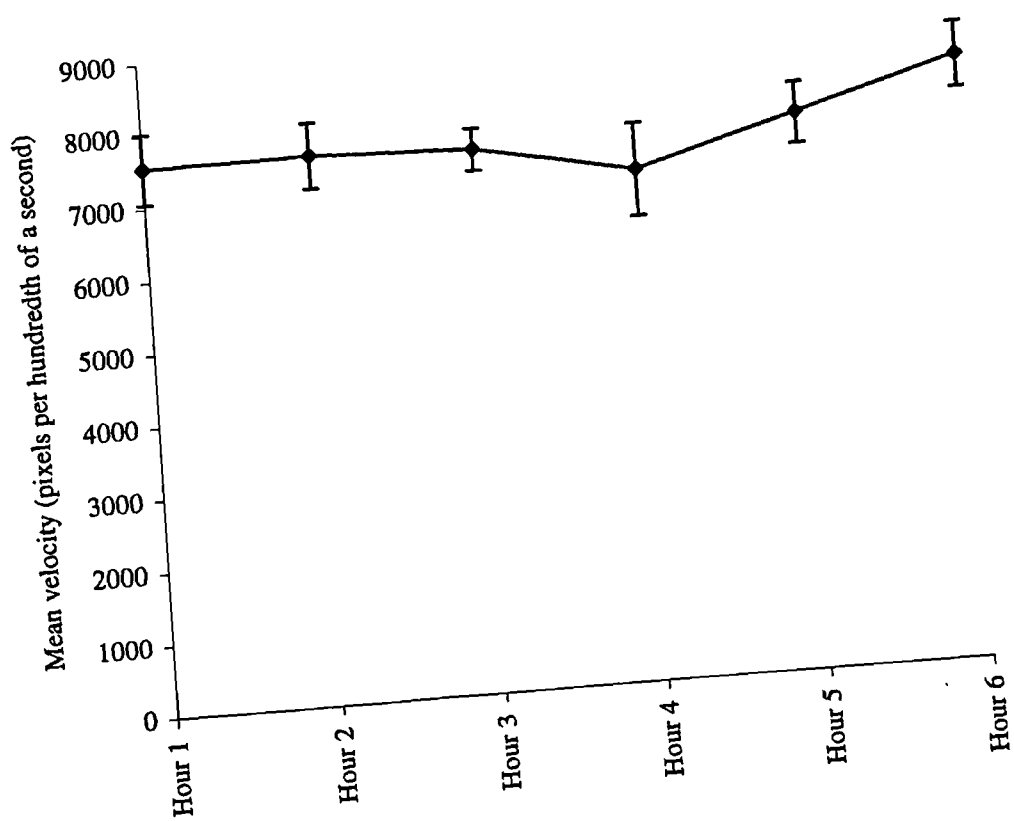
**Fig. 4.8** The mean number of refuges visited in each hour of the Naive treatment  $n = 10$ , error bars show standard error of the mean.

**Fig. 4.9** The mean total time spent sheltering in a refuge in each hour of the Naive treatment,  $n = 10$ , error bars show standard error of the mean.

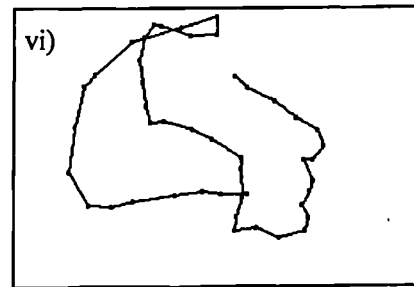
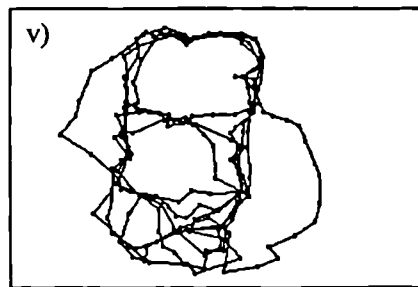
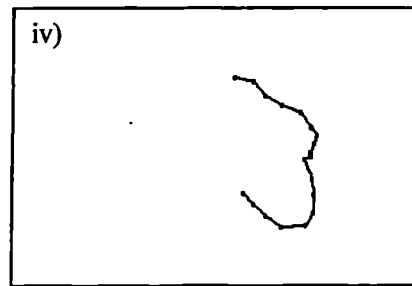
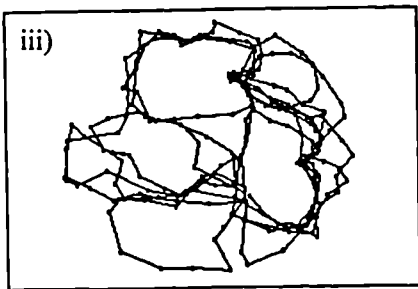
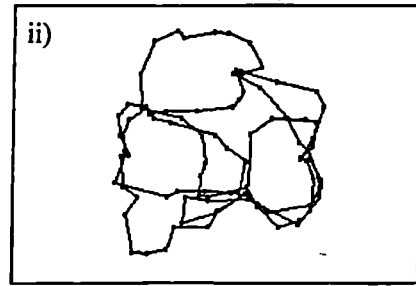
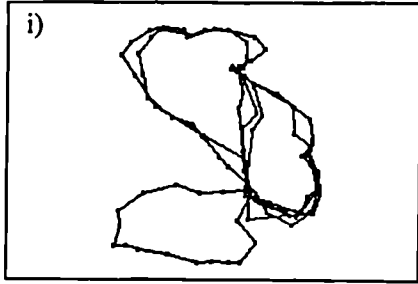




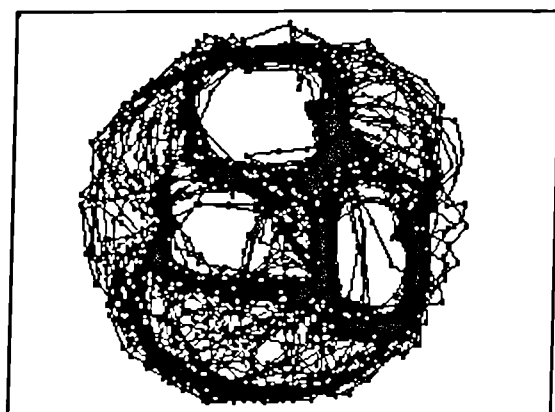
**Fig. 4.10** Mean speed in each hour of the most active fish, Fish 2, 4, 7 and 9,  $n = 4$ , error bars show standard error of the mean.



**Fig. 4.11** Path travelled by Fish 2 in each hour of the Naive treatment, **i)** 0-1 hours, **ii)** 1-2 hours, **iii)** 2-3 hours, **iv)** 3-4 hours, **v)** 4-5 hours, **vi)** 5-6 hours.



**Fig. 4.12** Total paths travelled around the artificial arena by all individuals during the 6 hour Naïve treatment.



### 4.3.2 Exploration

No clear pattern was seen in the sequence of refuges visited among all fish. However, activity in Fish 2 appeared to be concentrated around Refuge B (Fig. 4.11 and 4.13), as transitions towards this refuge accounted for 39% of the total (Fig. 4.14) and it continued to be regularly visited throughout every hour of the exploration period (Figs 4.11, 4.15 and 4.16). Activity of Fish 4 was concentrated around Refuge B overall (Appendix 12iii), as transitions towards this refuge accounted for 35% of the total (Appendix 13iii). Refuge B was regularly visited during hours 1, 2 and 3 of the experiment and then later Refuges A and C were visited more often (Appendices 9, 14 and 15iii and 16iii). Activity in Fish 7 was concentrated around Refuge A (Appendix 12vi), as transitions towards this refuge accounted for 24% of the total (Appendix 13vi). Activity in the first hour was concentrated around Refuge A and between this refuge and Refuges D and E, in the second hour activity was concentrated around Refuge B and in the third hour around Refuge D, after 3 hours there was no further movement by Fish 7 (Appendices 10, 15vi, 16vi and 17). Activity of Fish 9 was concentrated around Refuge B overall (Appendix 12viii) as transitions towards this refuge accounted for 33% of the total (Appendix 13viii). In the first hour activity of Fish 9 was concentrated around Refuge C, but later, activity was relatively low and more evenly distributed. In the second hour activity was evenly distributed between Refuges B, C and D, in the third hour Refuges A, B and C were all visited regularly. In the fourth hour activity was concentrated around Refuge B, in the fifth hour activity was evenly distributed between Refuges A and E and in the sixth hour Refuges A, B and C were all visited regularly (Appendices 11, 15viii, 16viii and 18).

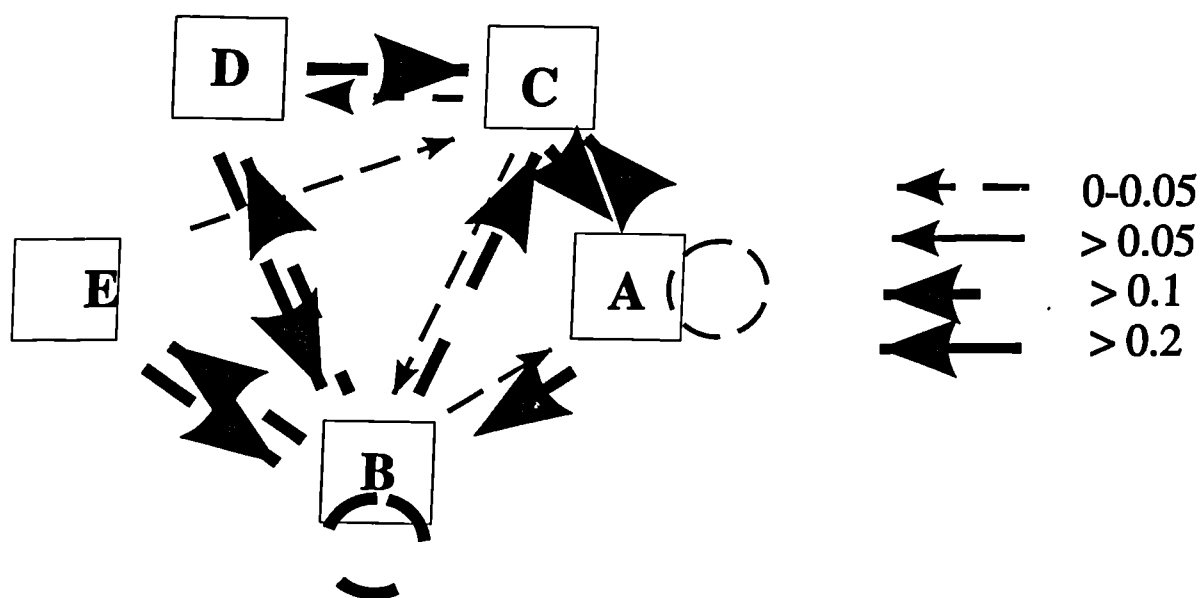
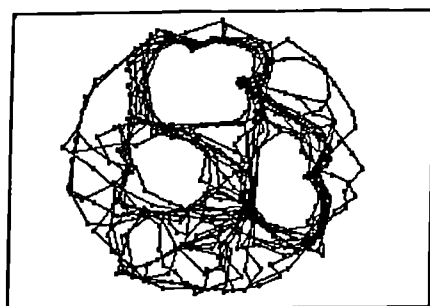
### 4.3.3 Refuge preference

Individual refuge preference in the Naive treatment varied considerably and in accordance with the preference criterion used (Table 4.1). Residence time differed significantly among refuges (Kruskal-Wallis test,  $P = 0.011$ ,  $n = 5$ , d.f. = 4) but the number of times each refuge was visited did not (Kruskal-Wallis test,  $P = 0.520$ ,  $n = 5$ , d.f. = 4, Figs 4.17 and 4.18). There was a significant difference in residence time of Refuges D and E and E and A only (Dunn's test,  $Q = 3.19$  and  $2.73$  respectively,

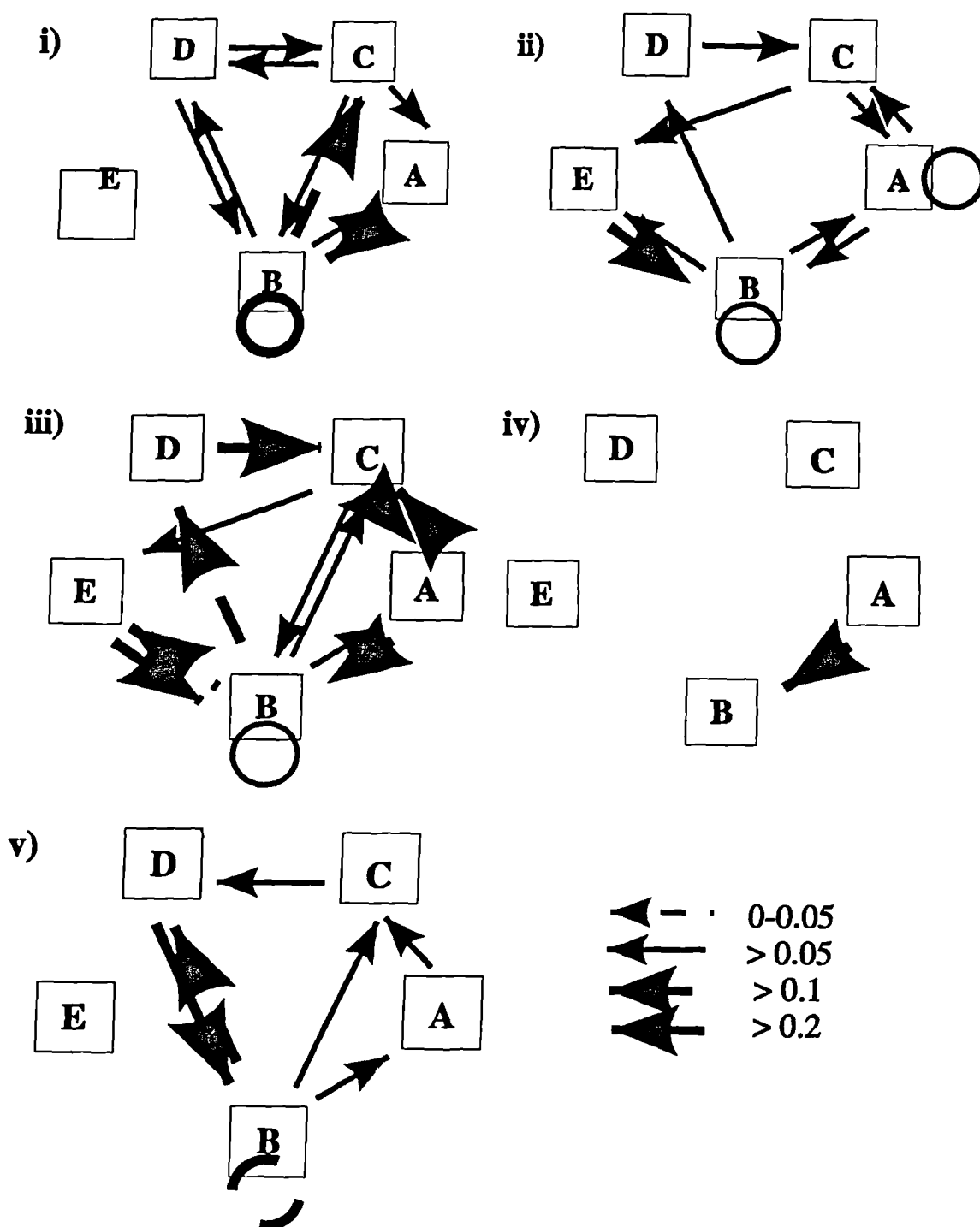


**Fig. 4.13** Path travelled by Fish 2 during 6 hour Naive treatment.

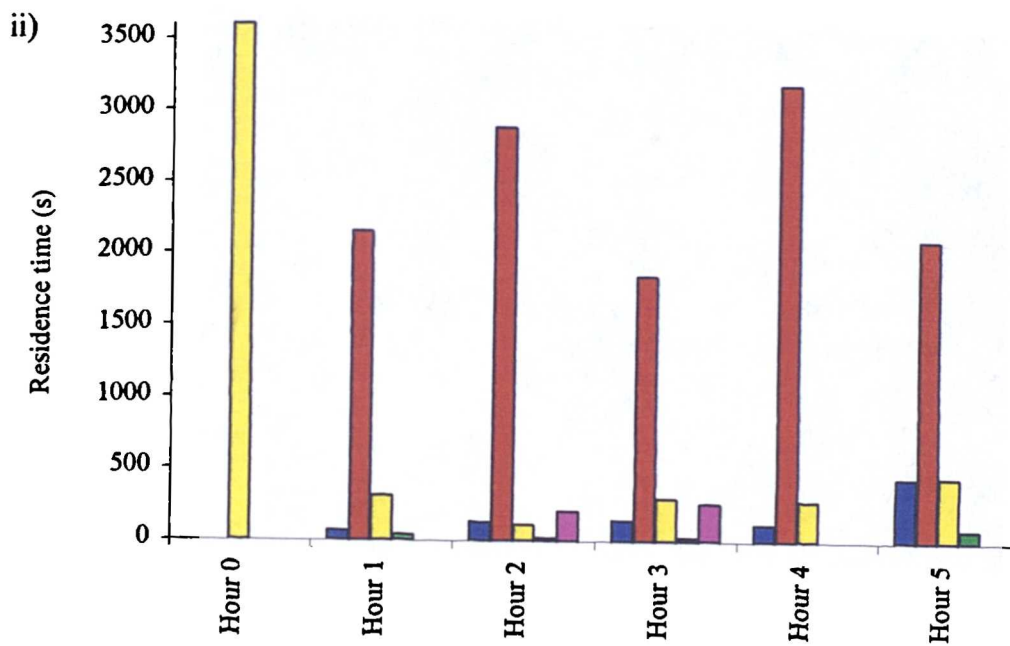
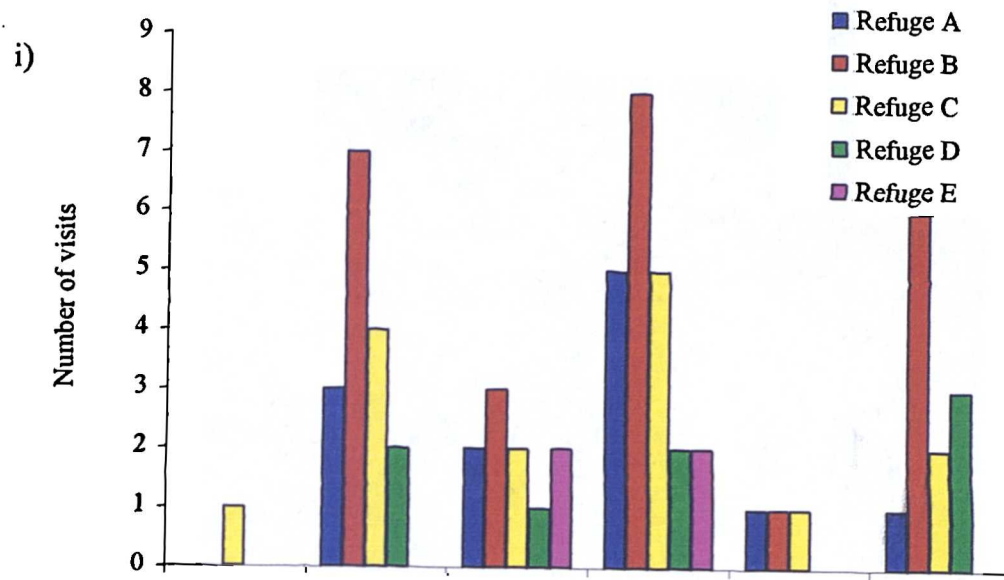
**Fig. 4.14** Transition diagram showing the proportion of total movements made between each pair of refuges for Fish 2 in the Naive treatment.



**Fig. 4.15** Transition diagram showing the proportion of total movements made between each pair of refuges for Fish 2 in each hour of Naive treatment, (no movement in hour 0-1) **i)** hour 0-1, **ii)** 1-2, **iii)** hour 2-3, **iv)** hour 3-4, **v)** hour 4-5 there was no further movement after 5 hours.



**Fig. 4.16** Refuge use over the 6 hour Naive Treatment by Fish 2, **i)** The number of visits to each refuge in each hour, **ii)** residence time in each refuge in each hour.



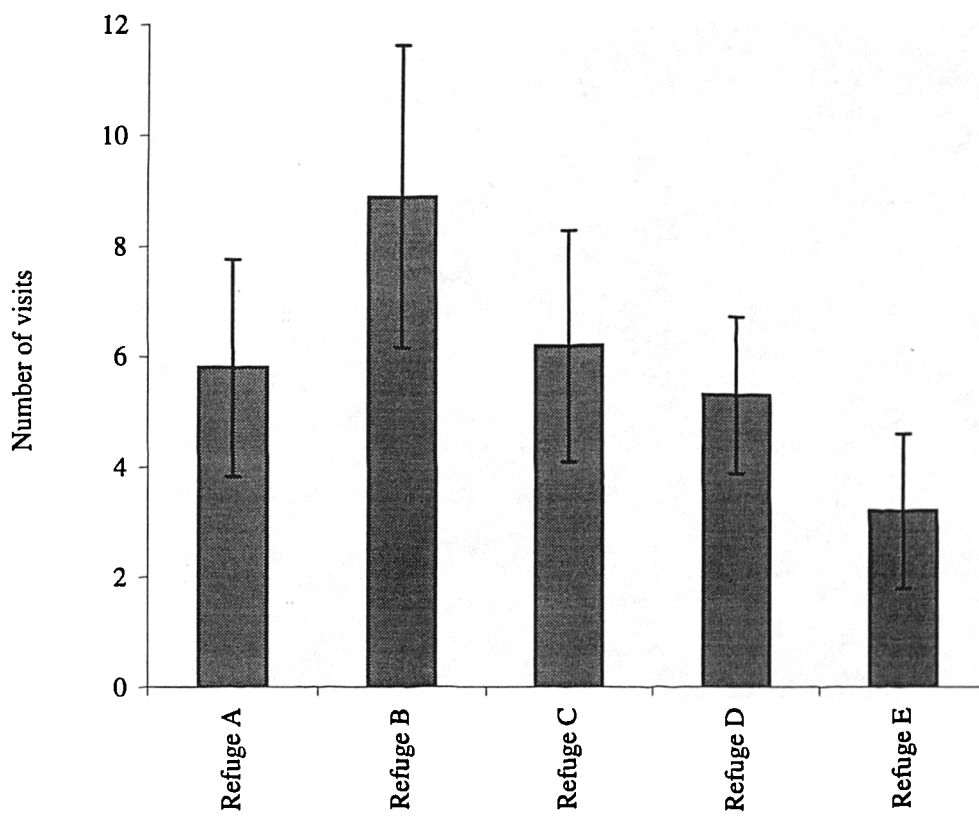
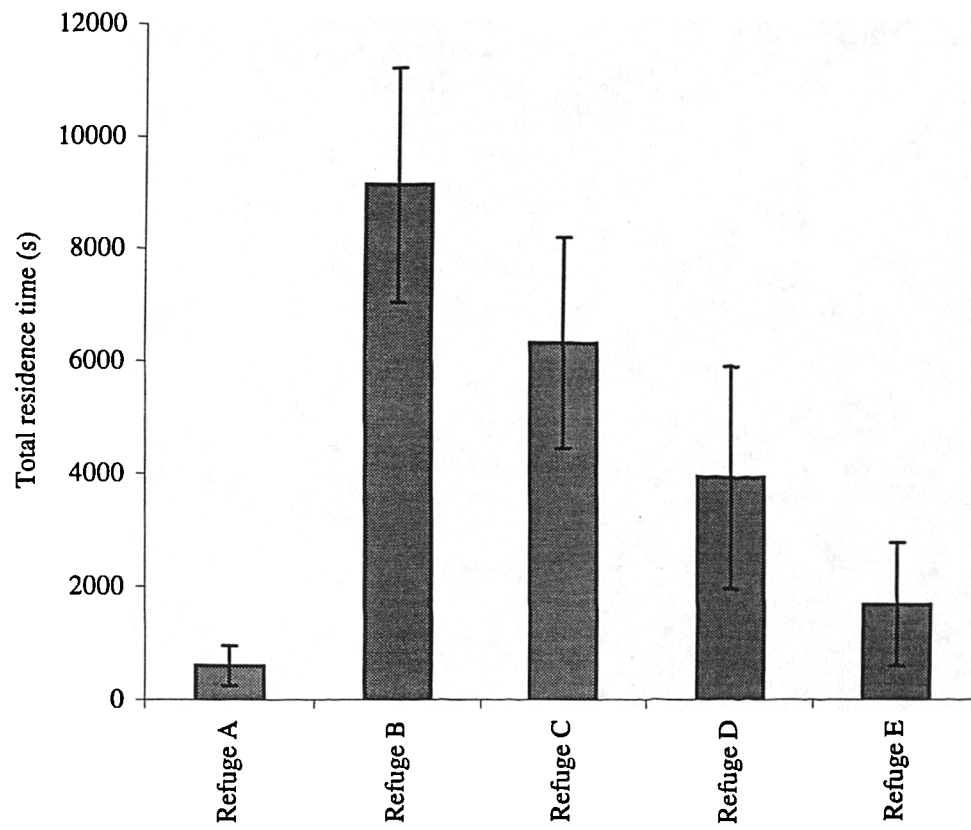
**Table 4.1** Total residence time and number of visits to each refuge by each individual, and the preferred refuge according to each of these criteria.

	Total residence time (s)						Number of visits to each refuge					
	Refuge A	Refuge B	Refuge C	Refuge D	Refuge E	Preferred refuge	Refuge A	Refuge B	Refuge C	Refuge D	Refuge E	Preferred refuge
Fish 1	20	4902	0	16907	703	D	1	3	0	4	2	D
Fish 2	573	15061	5111	174	407	B	14	25	15	8	4	B
Fish 3	83	7229	9726	0	0	C	1	1	2	0	0	C
Fish 4	3435	5814	10347	1698	11146	E	9	22	17	7	5	B
Fish 5	0	6194	14555	643	154	C	0	4	3	2	2	B
Fish 6	0	0	7475	14130	0	D	0	0	2	2	0	C/D
Fish 7	468	13258	1054	2142	828	B	17	10	9	15	15	A
Fish 8	103	16929	0	3558	412	B	6	7	0	8	2	D
Fish 9	944	2398	14907	179	3299	C	9	13	13	6	2	B/C
Fish 10	150	19632	168	35	0	B	1	4	1	1	0	B

**Fig. 4.17** The mean total residence time of each refuge during the 6 hour Naive treatment,  $n = 10$ , error bars show standard error of the mean.

**Fig. 4.18** The mean number of visits to each refuge during the 6 hour Naive treatment,  $n = 10$ , error bars show standard error of the mean.





$Q_{0.05} = 2.807$ ,  $k = 5$ , Table 4.2).

#### 4.3.4 Retention of spatial information

There was a significant difference between Naive and Experienced treatments in time to enter the first refuge only (mean latency to movement Naive =  $20.65 \pm 31.23$ , Experienced =  $4.42 \pm 2.08$ , mean time elapsed before entering the first refuge Naive =  $36.80 \pm 34.5$ , Experienced =  $12.15 \pm 8.89$ , mean distance travelled to the first refuge Naive =  $88.9 \pm 104.7$ , Experienced =  $78.8 \pm 46.1$ , Wilcoxon signed rank test, median = 25.4,  $p = 0.008$ ,  $n = 10$ , Table 4.3). The angles of trajectory from the start position to the first refuge were uniformly distributed in the Naive treatment (Raleigh's test of distribution,  $z = 2.118$ ,  $z_{0.05, 10} = 2.919$ , Figs 4.4 and 4.5), but were more directed towards Artificial Rocks 1 and 2 when Experienced (Raleigh's test of distribution,  $z = 0.071$ ,  $z_{0.05, 10} = 2.919$ , Figs 4.19 and 4.20). Also, the number of fish visiting each refuge first were randomly distributed in the Naive treatment, but, in the Experienced treatment, 4 out of 10 fish entered Refuge B first, and 5 out of 10 entered Refuge D first (Table 4.4, Fig. 4.21).

**Table 4.2** Comparison of residence time between refuges A-E using Dunn's test,  $Q_{0.05} = 2.807$ ,  $k = 5$ .

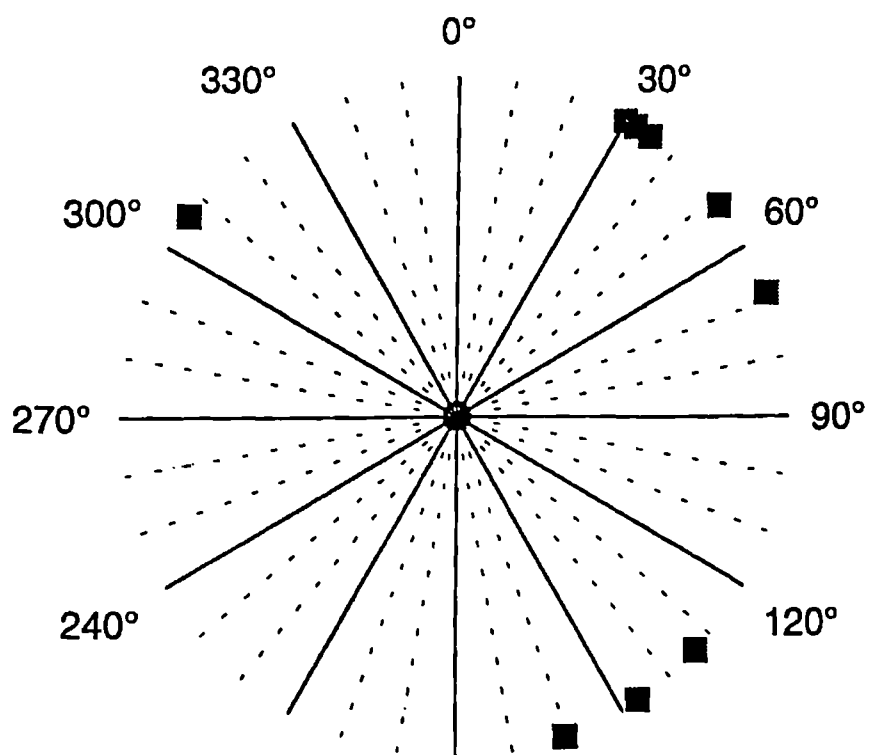
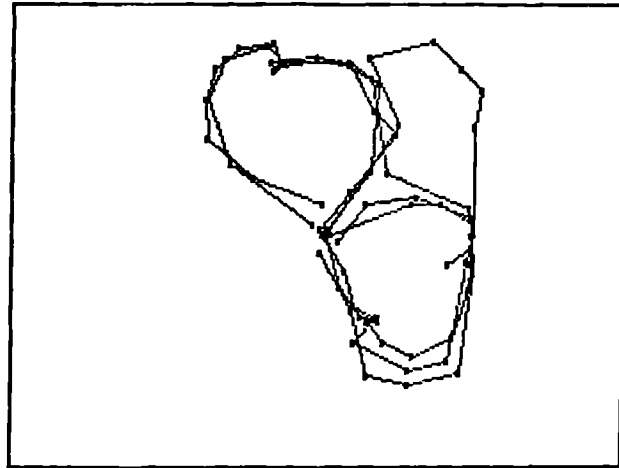
	Refuge A	Refuge B	Refuge C	Refuge D
Refuge B	1.66 NS			
Refuge C	0.98 NS	0.67 NS		
Refuge D	0.46 NS	2.12 NS	1.44 NS	
Refuge E	2.73 *	1.07 NS	1.75 NS	3.19 **

**Table 4.3** Comparison of performance in Naive and Experienced Treatments using a two tailed, Wilcoxon signed rank test, n = 10 in all cases.

Performance criteria	<i>P</i>	
Latency to movement	0.203	NS
Time to enter first refuge	0.008	**
Distance travelled to first refuge	0.067	NS
Angle of trajectory from the centre of arena	0.062	NS

**Fig. 4.19** The path travelled by each individual from the start box to the first refuge entered when Experienced.

**Fig. 4.20** The angle of the trajectory of movement of each fish from central area of arena, Experienced treatment.

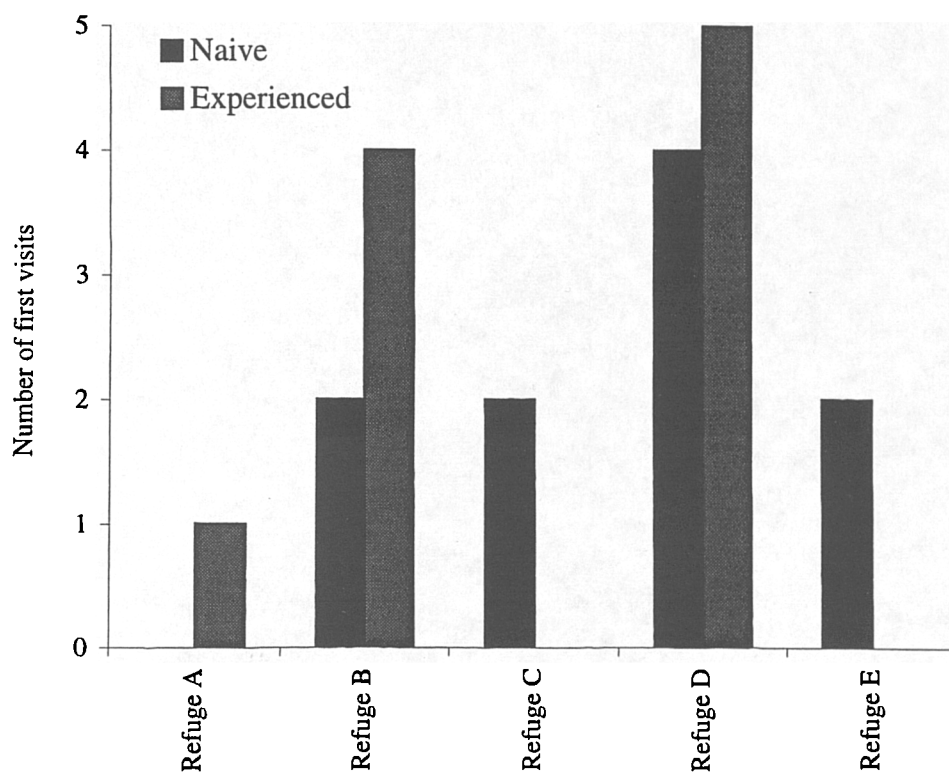


**Table 4.4** Refuge first entered by each individual on release from the start box in Naive and Experienced treatments.

Fish	Naive	Experienced
1	D	B
2	C	B
3	C	D
4	E	D
5	B	D
6	D	B
7	E	B
8	D	D
9	D	D
10	B	A

**Fig. 4.21** The number of *Lipophrys pholis* visiting each refuge first in the Naive and Experienced treatment.





#### 4.4 DISCUSSION

The results showed that there was great variation in the activity of individuals but that mean velocity remained constant throughout the experimental period. Also, *L. pholis* explored from several different “bases”, visited in sequence, until occupying one particular refuge towards the end of the experimental period. Residence time and the number of visits to each refuge showed that certain refuges were preferred over others. Time to enter the first refuge and distance travelled to the first refuge were not significantly different. However, differences in the path of movement to the first refuge when the fish were Naive and Experienced showed that *L. pholis* had learned the position of the refuges.

The fact that movement from the start position was random in the Naive treatment, and more directed in the Experienced treatment, suggested that the fish were simply moving away from the moving start box and the open space at the centre of the arena when they were not familiar with the arena. When the fish were more experienced, they were able to direct their movements towards the more complex area of the arena, Sector 2. Movement may have been directed towards, and concentrated in, Sector 2 because Artificial Rocks 1 and 2 were higher than Artificial Rock 1 and emerged from the water's surface, whereby they may have cast more shadow and afforded greater cover while *L. pholis* were moving around in the open. Also Sector 2 contained Artificial Rocks 1 and 2, which in turn contained a total of 4 refuges, while Sector 1 only contained Artificial Rock 1, which only contained one refuge. This uneven distribution of refuges in the two sectors obviated the need for fish to travel far in the open in Sector 2 before encountering the safety of another refuge.

It is possible that the fish increased their movements across open space when they became more active because they felt less threatened. The individuals moved along the edges of the tank and the artificial rocks probably because *L. pholis* are thigmotactic (Gibson 1982) and objects afford them some degree of cover during movement. Field observations of *L. pholis* on an intertidal cliff showed that they move predominantly along rock crevices, where not only are they protected from predators,

but they are able to avoid being dislodged by the surf (M.T. Burrows *pers. comm.*). It is possible also that the edges of rocks or crevices become learned thoroughfares between goals such as food patches and refuges in the wild. The fact that the mean speed of movement of the most active fish in this experiment (Fish 2, 4, 7 and 9) remained constant throughout the 6 hour observation period is contradictory to results reported by Kleerekoper *et al.* (1974), Andreev & Mikeev (1993) and Teyke (1985, 1989), who found that the swimming speed of goldfish, guppies and blind cave fish respectively decreased with the novelty of the arena in which they were placed. This difference in behaviour compared with that of *L. pholis* in the present experiment may be because *L. pholis* had not yet fully explored the arena after 6 hours. It may also be because goldfish, juvenile guppies and blind cave fish, being more demersal species, move around in the open and place less reliance on refuges during movement than *L. pholis*. It is possible that because *L. pholis* maximise time spent under cover, an increase in refuge residence time or a decrease in the mean number of refuges visited is a stronger indication of the end of exploration. For example, when an active individual has visited all the refuges in the arena, chooses one to remain in, and ceases to move around, this may be an indication that it has fully explored the arena. Therefore, the significant decreasing trend in the mean number of refuges visited in this experiment suggested that 6 hours is sufficient time for *L. pholis* to explore a novel arena of this size. However, an experiment of longer duration in which both criteria (increase in refuge residence time and decrease in the number of refuges visited over time) reached an asymptote would be desirable to accurately determine the length of exploratory behaviour in this species.

There was great variation in activity between fish. Some individuals, such as Fish 4 and Fish 9, began moving about the arena almost immediately and continued moving around between different refuges for almost the whole of the 6 hours. Others, such as Fish 6, 8 and 10, remained in the first refuge for long periods and did not visit all the refuges in the 6 hours allowed. This difference in the behaviour may have been due to a difference in the internal state of individuals, however, this variable was minimised by feeding each individual just prior to placing them in the novel arena and

beginning the experiment at the same time everyday, therefore it is more likely that the variation in behaviour was due to individual variation in risk assessment.

*L. pholis* in the present study generally explored from a “base” refuge, moving away, investigating and entering another refuge, but returning to the “base” on several occasions before taking up residence in a new refuge. This refuge then became the next “base” from which to investigate another refuge and so on. For example, Fish 2 returned consistently to Refuge B more than any other refuge and spent the longest time in this refuge throughout the experiment, therefore this seemed to be its permanent “base”. However activity and residence time were often contradictory when used as indicators of refuge preference or “base” use. For example, activity of Fish 4 was concentrated around Refuge B overall but this fish spent most time in Refuge E and therefore it was difficult to identify an overall “base” in this case. Also there was often a transition of “base” use throughout the 6 hour Naive treatment. For example, during the course of its exploration of the novel arena, Fish 4 repeatedly visited Refuge B and spent most of its time in Refuge C in the first 3 hours. Later, Fish 4 repeatedly visited refuges A and C and spent equal amounts of time in Refuges A and C. It is possible therefore that Fish 4 had two “bases” in the first and second half of its exploration, or that it was in the process of choosing a permanent “base”. The “base” was particularly difficult to identify when activity of an individual was low. For example Fish 1, 5, 6 and 10 did not visit all the refuges during the 6 hour Naive treatment period therefore these individuals could not have chosen the best possible refuge because they did not visit them all. The “base” or permanent refuge may have become more apparent were these fish given longer to explore. These results are in accordance with field data presented by Almada *et al.* (1983) for the exploration of a novel rock pool in *L. pholis*.

The mean number of refuges visited and the mean residence time in each refuge in each hour did not differ, indicating no overall trend in refuge use. This lack of any overall pattern may be due to individual variation and a pattern may have become apparent if a greater number of replicates had been performed.

Preference for any particular refuge was contradictory (depending whether total residence time or total number of visits to the refuge was used as the preference criterion), and showed great variation. However, the significantly greater mean total residence time in Refuge B, and, although not statistically significant, the greater number of visits to Refuge B, suggested that Refuge B was preferred. This refuge may have been more attractive because it was located centrally within the arena, it commanded a good view of the surrounding area, it was positioned at the level of the gravel and it was close to other refuges.

Mean time to enter the first refuge was significantly lower in the Experienced treatment than in the Naive treatment, and at the beginning of the Experienced treatment movement from the start box appeared much more directed than at the beginning of the Naive treatment. These results suggest that the fish learned the position of the refuges during the 6 hour Naive treatment, and remembered it 24 hours later in the Experienced treatment. Six out of ten fish (Fish 3, 4, 5, 8, 9 and 10) entered refuges facing outwards from the centre of the arena (Refuge A and D) in the Experienced treatment. This result implied that these fish were simply swimming out of the central area of the arena as quickly as possible, and then entering the first refuge they came across in order to avoid the moving start box. This may be true in the case for Fish 1, and 2 which travelled directly to Refuge B, and Fish 3 and 4 which travelled around Artificial Rock 2 directly to Refuge D without passing any other refuges. However, the fact that Fish 5 and 8 swam past Refuge C, and Fish 9 swam past Refuges B, A and C on route to Refuge D, while Fish 6 and 7 swam out of the central area, around the outside of Artificial Rock 1, and past Refuge A to reach Refuge B, suggested that these fish had learned the location of these refuges and that they were preferred (although this is not obvious from their residence time and number of visits to these refuges), as, despite being startled by the moving start box, these fish ignored other refuges in order to enter their preferred one.

Learning is not as apparent as expected in this experiment (the distance travelled to the food in the hierarchical maze (Chapter 2) had improved to asymptote after just 10 trials

(a maximum of 5 hours) in the presence of Lego™ towers). This result may be because there was insufficient incentive to learn this relatively simple task. There were so many refuges in such a small area that there was never a refuge far away when a fish was out in the open, therefore if it could not already see a refuge from its position it would merely have to swim a short distance and it would encounter one by chance. The fact that it is likely that any individual *L. pholis* can see a refuge from anywhere in the arena would explain how Fish 1 swam directly into Refuge B when it was disturbed out in the open at the end of the experimental period. Alternatively, the fish may have been too stressed, repetitive reinforcement of the stimulus (the raising of the start box to place the fish suddenly in the open) may be required for learning, or the Experience period was not sufficiently long enough for the fish to learn the task efficiently. The experimental design could therefore be improved by simply increasing the length of the exploration period, or by providing a single refuge only and concealing it from view.

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## 4.5 CONCLUSION

*L. pholis* explored from a central “base” refuge and between refuges. They moved along the edges of any available structures (as is their thigmotactic nature) to take advantage of any limited cover these provided during exploration, and only moved across open space when they were very active. Movement was concentrated in the areas of the arena containing the most complex topography. There was some evidence to suggest that *L. pholis* preferred a centrally located refuge that commanded a clear view of the surrounding area. There was also some evidence to suggest that *L. pholis* is capable of learning the position of a preferred refuge in a 6 hour experience period and of remembering this location 24 hours later.

## Chapter 5

### **Spatial memory for refuge position in *Lipophrys pholis***



## 5.1 INTRODUCTION

Refuges are an important spatial resource for all animals providing protection from predators and adverse weather conditions, and often a safe area in which eggs can be guarded. In the intertidal zone protection from wave turbulence and desiccation during emersion at low water are particularly important (R. N. Gibson 1967, 1982, 1993, Koppel 1988, Kotrschal 1988). The ability to learn the location of all the refuges in their home range and return to the nearest one when under threat would be a great selective advantage. *L. pholis* return to the same rock pool at low water and often to the same crevice in a rock pool the method by which they home is unknown however. Many authors have suggested that a topographical memory of a home range is formed with the aid of visual and olfactory clues (Gibson 1982, Almada *et al.* 1983).

Blackeye gobies (*Coryphopterus nicholsi*) given 5 hours free range of a large tank containing a single burrow found this refuge significantly faster, when attacked by a simulated predator, than individuals deprived of free access (Markel 1994). Moreover, the experienced fish took significantly longer to find the refuge when it was moved to a new location than did the inexperienced fish. This phenomenon of searching for an object in its previous position after it has been moved is known as “overshadowing” (Mackintosh 1974) and has also been recorded in goldfish searching for full and empty food patches (Warburton 1990).

The three spine stickleback (*Gasterosteus aculeatus*) learned to avoid dangerous feeding areas following a number of daily trials (Huntingford & Wright 1989). In this study different signs were hung over entrance doors to two different feeding habitats, one “safe”, and the other with constant, simulated predation threat. The signs were then reversed, such that the safe area was now marked with the dangerous sign and vice versa, while predation threat in the two areas remained unchanged. In the trial following reversal, 9 out of 11 fish all from a population subject to low predation risk in their wild habitat, swam directly to the previously safe patch ignoring the (now dangerous) sign over the door. The remaining 4 fish, originating from a high risk area,

swam to the previously safe patch, appeared to look at the sign, and then moved into the previously dangerous patch now bearing a “safe” sign. This led Huntingford & Wright (1989) to suggest that low risk population fish use primarily global clues for orientation, whereas high risk fish place greater emphasis on local clues.

The aim of this chapter was to ascertain whether *L. pholis* could learn the position of a single refuge in an arena, and if so, what clues were used to do so.

## 5.2 METHODS

### 5.2.1 Study animals

Animals were collected and held in captivity as described in Chapter 2.

### 5.2.2 Equipment, the refuge maze

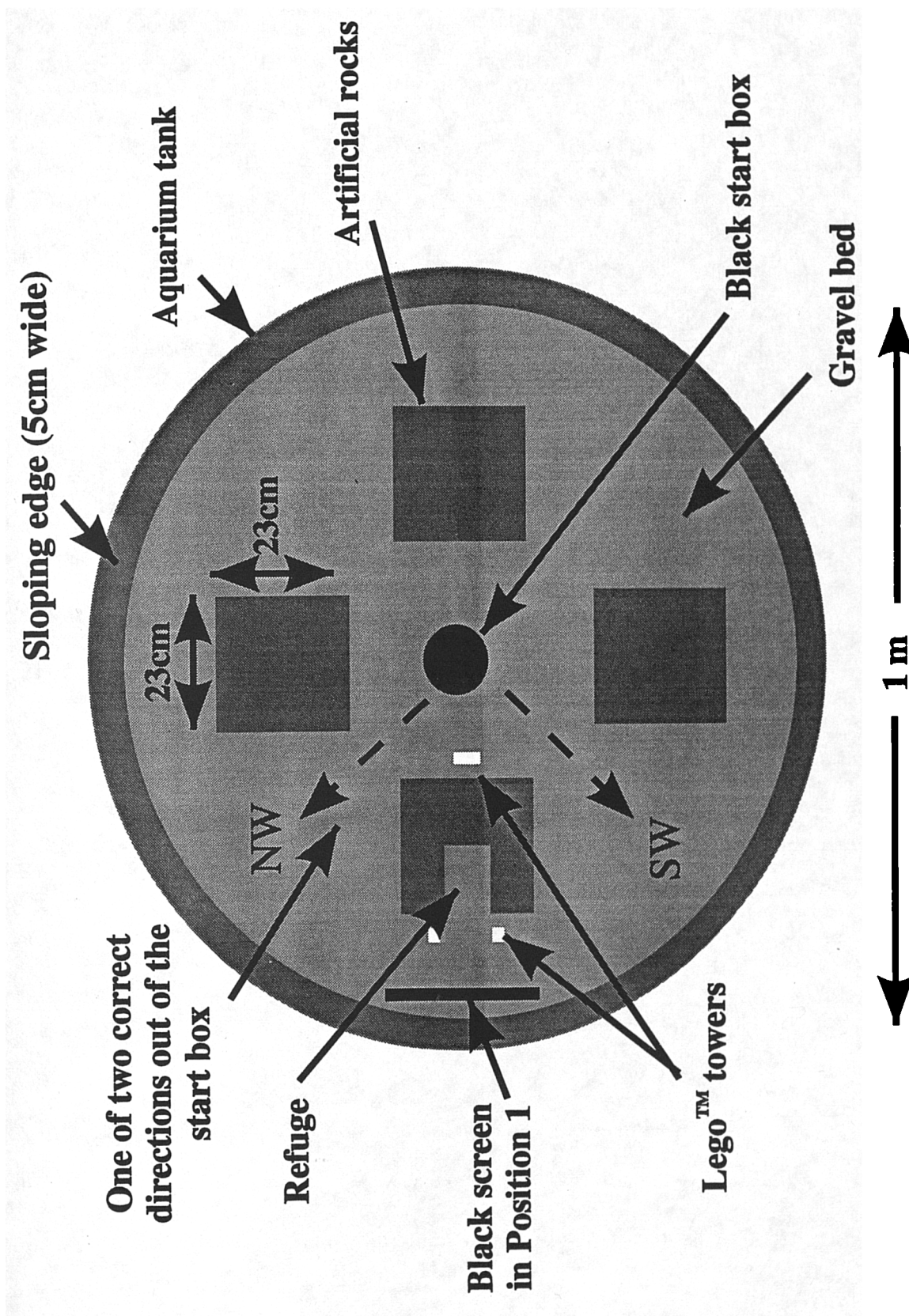
The refuge maze (Fig.5.1) consisted of four artificial rocks arranged in a cross. Each artificial rock was carved from a solid block of polystyrene (base dimensions 23 cm x 23 cm and height 12 cm), using a heated wire. Care was taken to fashion smooth, sloping sides to the artificial rocks in order to discourage *L. pholis* from using the outside of the structures as a refuge. A brown acrylic paint was used to colour the artificial rocks without detrimental effect to the fish. A hole (height 5 cm, width 5 cm and length 10 cm), was carved out of one of the artificial rocks to create a single refuge. The opening of this refuge faced the wall of the aquarium so that it was not visible from the start box position in the centre of the arena. The maze was placed in a sand coloured, circular aquarium of 1m diameter. The bottom of the aquarium was covered with a thin layer of gravel and filled to a depth of 8 cm with sea water, so that each artificial rock stood just clear of the water surface. A sloping edge was also carved from polystyrene, painted brown and placed at the perimeter of the tank to discourage the use of the angle between the base and side of the tank as a refuge.

The aquarium was covered by a hide consisting of a Dexion™ frame, the walls of which were lined with black sheets (Fig. 5.2). The roof of the Dexion™ frame was covered by a white, net, diffusing screen and lighting was provided by 2, 240V, 500W halogen lamps mounted on opposite corners of the frame. The performance of each fish could be observed through a viewing slit in the black curtain (Fig. 5.2) or via a TV monitor connected to a time code generator and a video camera the output from which was video recorded for later analysis.

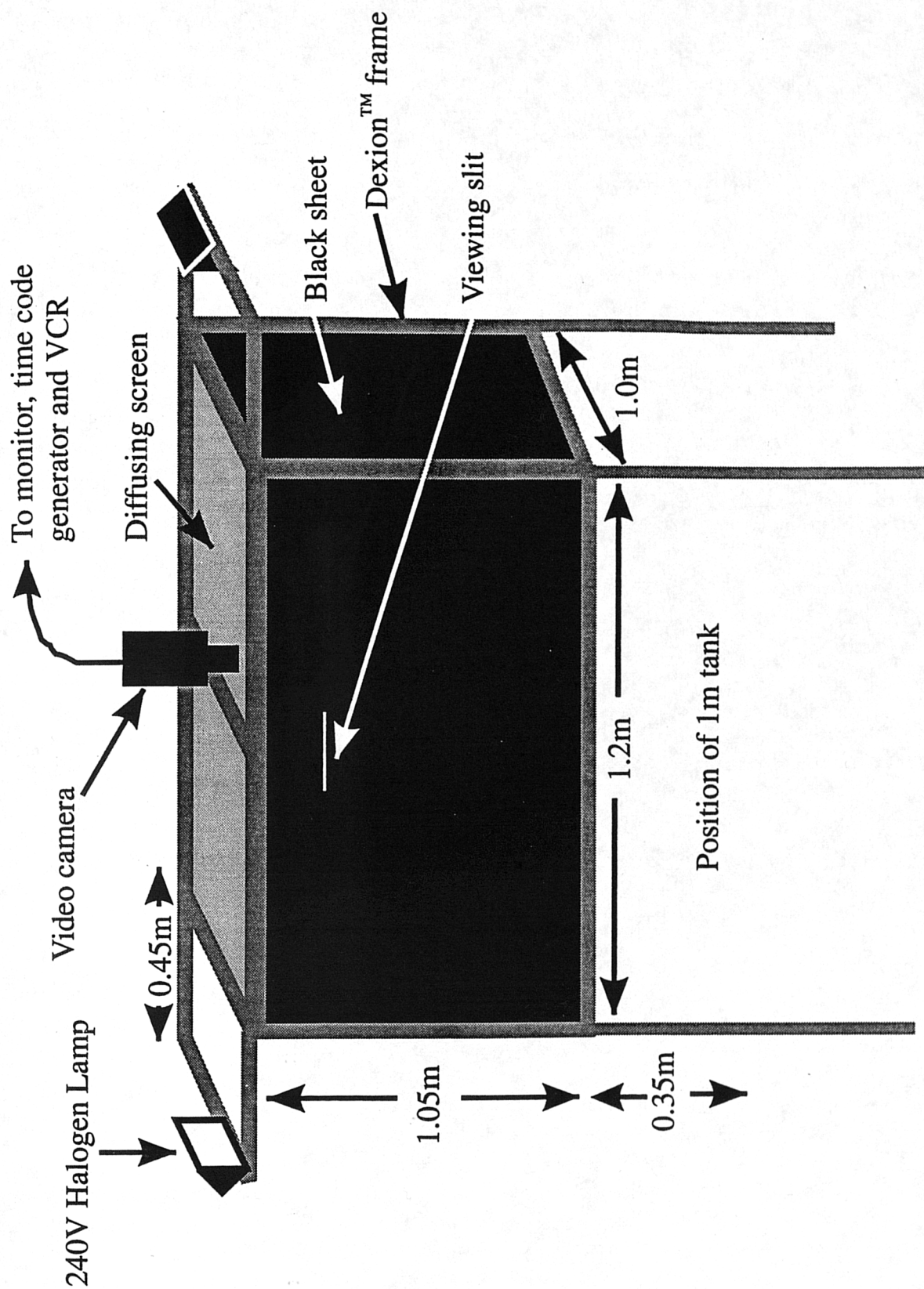
### 5.2.3 Protocol for Experiments 1, 2, and 3.

Each fish was placed in the start box at the centre of the maze and allowed to settle for

**Fig. 5.1** The refuge maze, Experiments 1-4 showing the position of the 4 artificial rocks, the refuge, the Lego™ towers and the black screen, and the two possible correct directions out of the start box.



**Fig. 5.2** The hide which covered the refuge maze showing the position of the video camera, the halogen lamps and the viewing slit.



5 min. It was then released by remotely raising the start box (by pulling on two lengths of fishing wire attached to the start box and looped over the frame of the hide), and allowed to explore the arena for a maximum of 30 min. If the fish entered the refuge within this time, it was removed from the tank, using a hand net, and replaced in its holding tank. Each fish was tested at the same time every day in order to avoid the effects of any endogenous rhythm on behaviour. Clean sea water was placed in the aquarium at the beginning of each day and the gravel was raked before each fish was placed in the start box.

The performance of four groups of eight fish (a total of 32) was tested daily for a period of 10 days under the influence of four different experiments (Table 5.1, Fig. 5.1).

The behaviour of fish was observed as they moved around in the maze and their performance was measured as latency to movement (the time taken from when the start box was raised to when the fish began to move), exploration time (time to enter the refuge minus latency to movement), path length (by using a computer programme that calculated the distance between points in the path taken by the fish from the start box to the refuge, M.T. Burrows *pers. comm.*) and the number of fish which chose the correct direction from the start box in order to travel directly to the refuge.

Mean latency to movement, mean exploration time, mean path length and the number of fish which chose the correct direction out of the start box in each experiment were compared between experiments using Friedman's test and, where this result was significant, using Dunn's test (Neave & Worthington 1988) in order to ascertain where performance differed. Mean latency to movement, mean exploration time to enter the refuge and mean distance travelled to the refuge were also tested for a decrease in magnitude over



**Table 5.1** Conditions of Experiments 1 to 5 designed to test the ability of *L. pholis* to learn the position of a single refuge in an artificial habitat under the influence of different visual clues.

Experiment	Days	Conditions	Hypothesis tested
1	1-10	No obvious visual clues	<i>L. pholis</i> cannot learn the position of the refuge in the absence of any obvious visual clues
2	1-10	Three towers of Lego™ blocks marking the position of the refuge	<i>L. pholis</i> cannot learn the position of the refuge using local, visual clues
3	1-10	White A4 size screen placed at Position 1 behind the refuge	<i>L. pholis</i> cannot learn the position of the refuge using a white distant / global clue
4	1-10	Black A4 size screen placed at Position 1 behind the refuge	<i>L. pholis</i> cannot learn the position of the refuge using a black distant / global clue
5	11-22	Black A4 size screen moved to Position 4	Moving the black distant / global clues will cause the efficiency of <i>L. pholis</i> to decrease

consecutive days, while the number of fish which chose the correct direction out of the start box was tested a priori for increase over consecutive days using Page's test, a distribution free test for ordered alternatives (Neave & Worthington, 1988). Page's test was carried out in order to determine whether the performance of the fish improved with experience over several days.

#### 5.2.4 Protocol for Experiment 4

Results were collected as described above for Experiments 1,2 and 3 with the addition of, the number of individuals visiting (physically touching) the black screen, and, the exploration time and distance travelled for each fish to complete this task. The mean exploration time before touching the black screen and distance travelled to the black screen were compared to the mean exploration time to the refuge and the mean distance travelled to the refuge using the Mann Whitney U test, to test for any difference in the completion of these two tasks. The number of fish visiting the black screen was tested for a decrease over consecutive days using Page's test, in order to ascertain whether fewer fish visited the black screen with increasing experience.

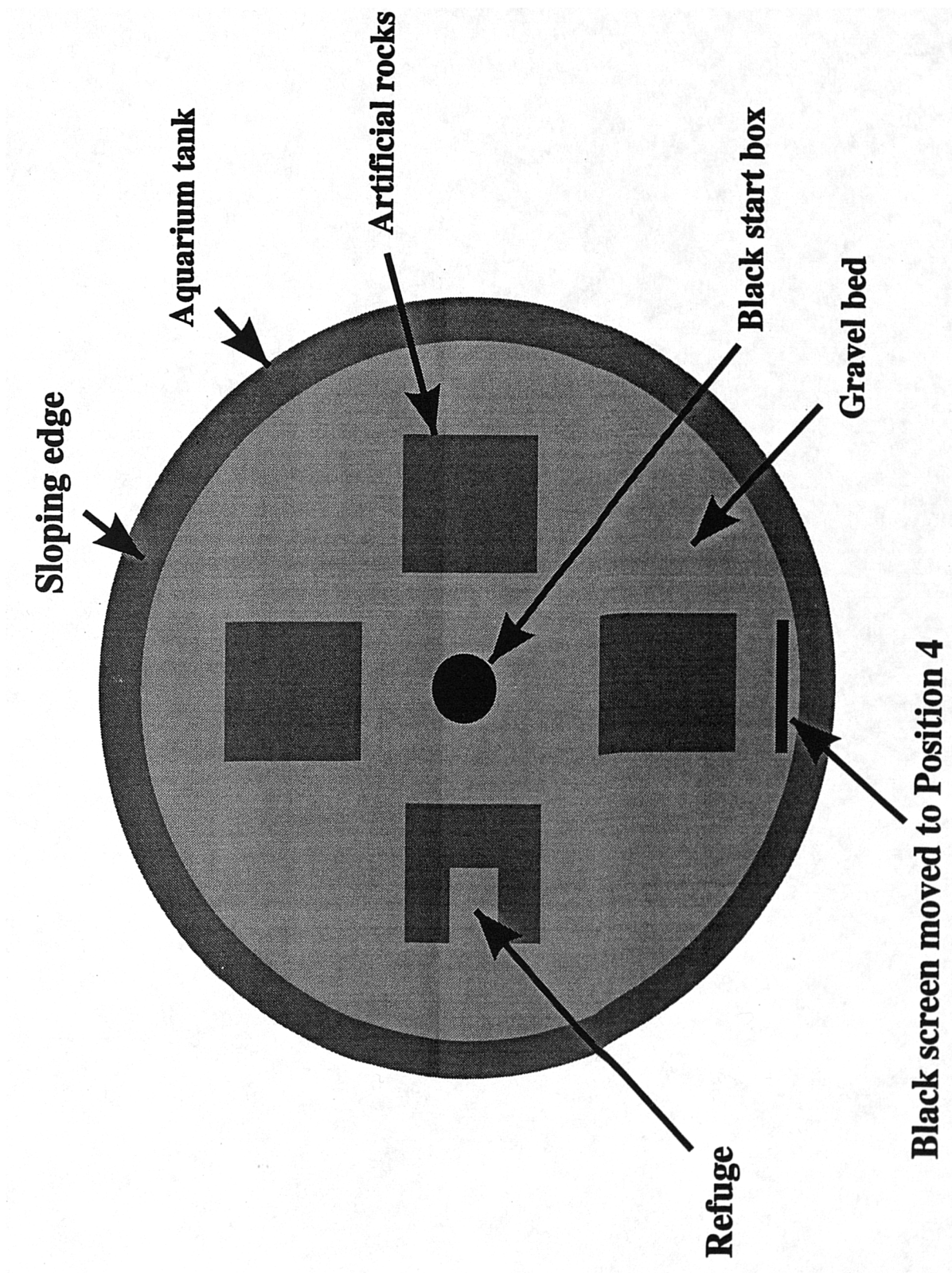
The ranks of mean latency to movement, mean exploration time to the refuge, mean distance travelled to the refuge and number of fish which chose the correct direction out of the start box on each day in each experiment, were also tested for concordance using Kendall's coefficient of concordance (Sokal & Rohlf, 1969), in order to ascertain whether there was a consistent hierarchy of clue use in *L. pholis*.

#### 5.2.5 Protocol for Experiment 5

Immediately following Experiment 4 this group of 8 fish was subjected to Experiment 5, in which the black screen was moved to Position 4 (Table 5.1, Fig. 5.3). This experiment was carried out every day for a period of 12 days using the protocol described above for Experiment 4.

Performance was analysed as described above for Experiment 4 with the addition of

**Fig. 5.3** The refuge maze, Experiment 5, showing the position of the 4 artificial rocks, the refuge, and the black screen.



a Mann Whitney U test comparing mean latency to movement, mean exploration time to refuge, mean distance to refuge, with mean exploration time to the black screen and mean distance to black screen in Experiment 4 and Experiment 5 (before and after the black screen was moved). These tests were carried out in order to ascertain the effect of moving the black screen on the spatial memory of the fish. Also, Page's test was used to test for a decrease in mean exploration time to the refuge, mean distance travelled to the refuge, mean exploration time before touching the black screen, mean distance travelled to the black screen and number of fish which visited the black screen before they entered the refuge over consecutive days. While latency to movement and number of fish which chose the correct direction from the start box were tested for an increasing trend over consecutive days, in order to determine whether the performance of the fish improved with experience over the course of the experiment.

## 5.3 RESULTS

### 5.3.1 Experiments 1-4

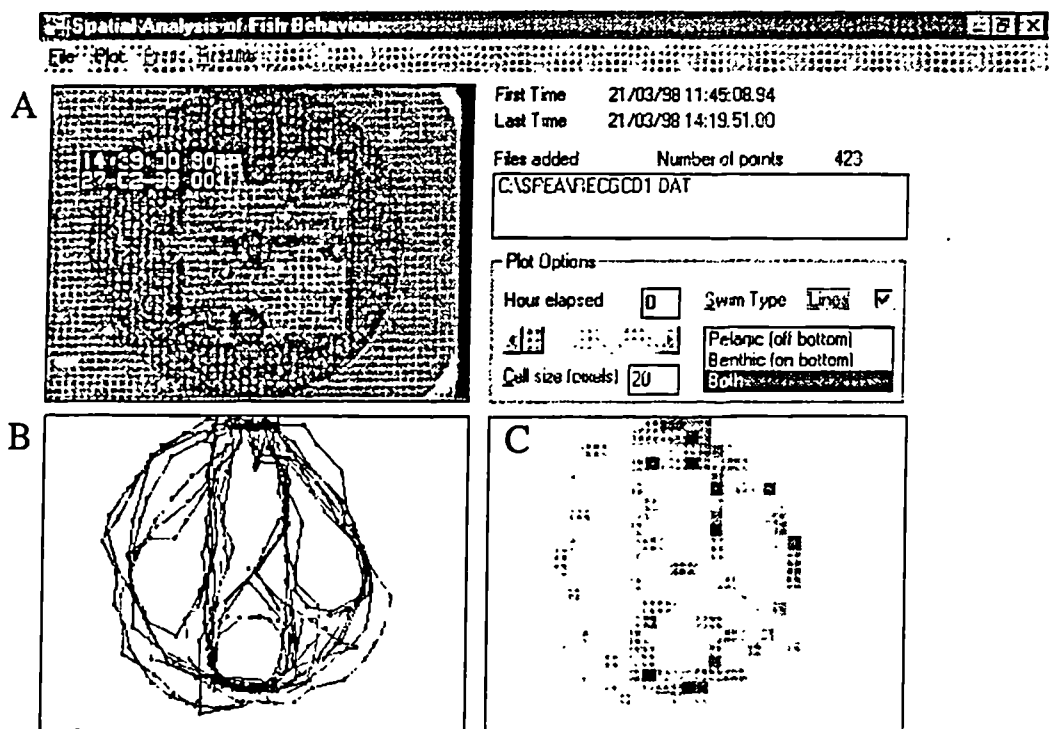
#### 5.3.1.1 Behaviour on release from the start box

On Days 1-3 many fish in Experiments 1, 2, and 3 exhibited a fright response on release from the start box, whereby they swam rapidly to the edge of the tank and continued to swim quickly around the edge until becoming stationary. After a few minutes they began to move again, apparently searching for a refuge. Later in the experiment, they remained stationary in the centre of the tank, until eventually beginning to move around. At the beginning of Experiment 4, all fish again exhibited a similar fright response. Three of them swam in a westerly direction out of the start box and then six of them swam rapidly towards the black screen, pressed themselves up against it and remained there a few minutes before beginning to move around again, often returning to the black screen (Figs 5.4B, 5.5A and 5.5B). Later in the experiment they often moved towards the black screen, but entered the refuge without touching it (Figs 5.5H and 5.5I). Fish in all experiment groups were successful in entering the refuge within the allotted time throughout the experiment and, once having entered would remain in the refuge until removed from the tank.

#### 5.3.1.2 The effects of different experiments

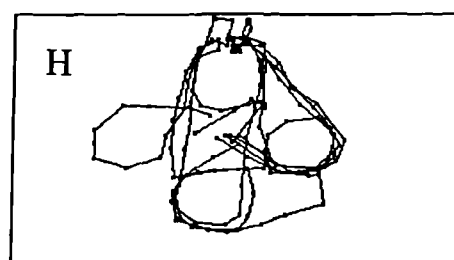
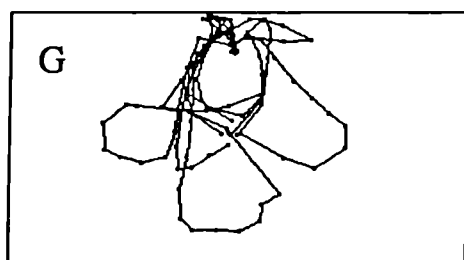
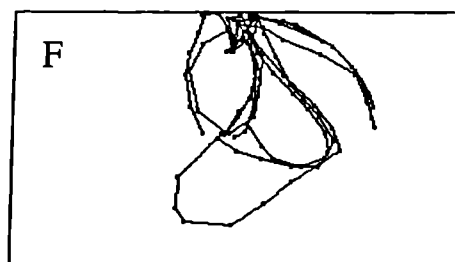
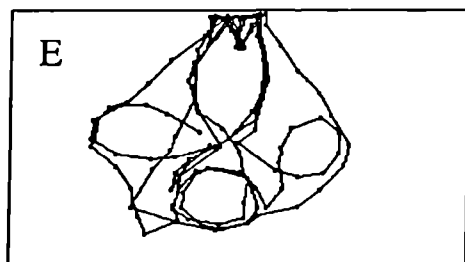
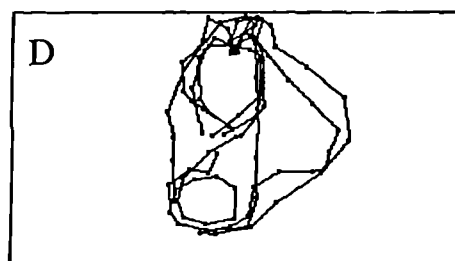
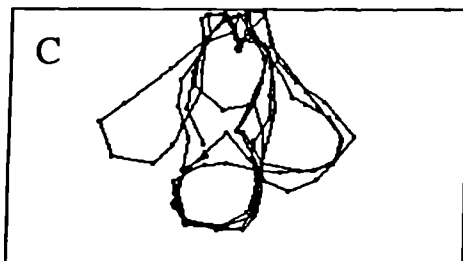
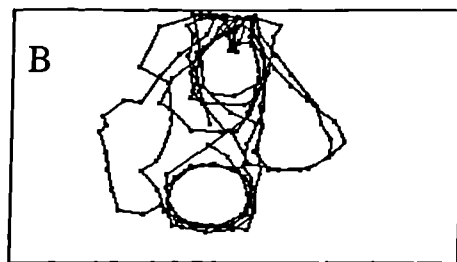
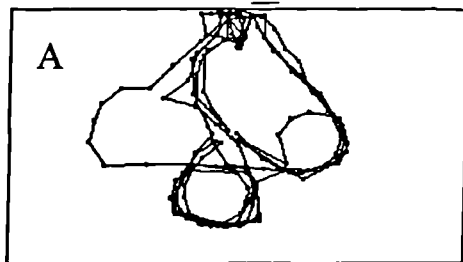
The mean latency to movement, mean exploration time to enter the refuge, mean distance travelled to the refuge appeared consistently lower and less variable in Experiment 4 than in all other experiments (Figs 5.6, 5.7 and 5.8). However, the mean exploration time to enter the refuge in Experiment 4 was only significantly lower than the mean exploration time to enter the refuge in Experiments 1 and 3 (Friedman's test,  $P = 0.008$ , d.f. = 3, Table 5.2, and Dunn's test,  $Q = 3.40$  and  $3.53$ , respectively,  $Q_{0.05} = 2.638$ ,  $k = 4$ , Table 5.3) and the mean distance travelled to the refuge in Experiment 4 was only significantly lower than the mean distance travelled to the refuge in Experiments 1 and 2 (Friedman's test,  $P = 0.022$ , d.f. = 3, Table 5.2, and Dunn's test,  $Q = 2.77$  and  $2.79$ , respectively,  $Q_{0.05} = 2.638$ ,  $k = 4$ , Table 5.4).

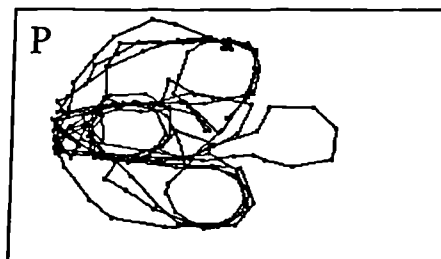
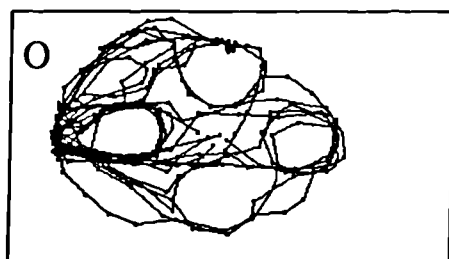
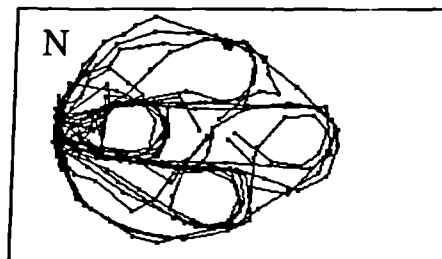
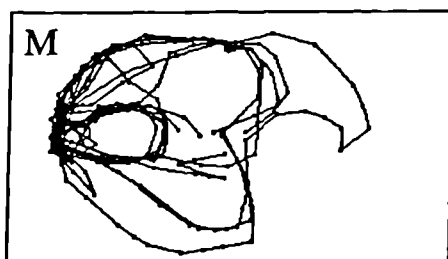
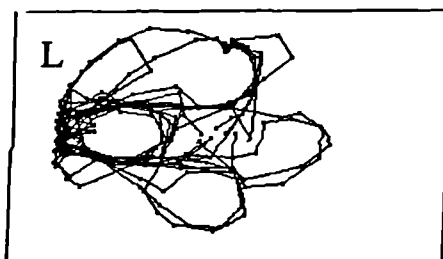
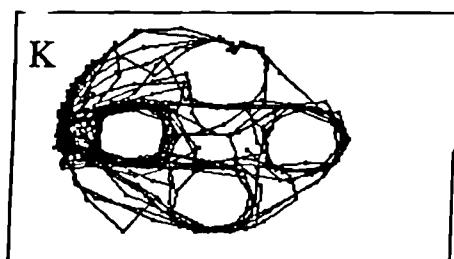
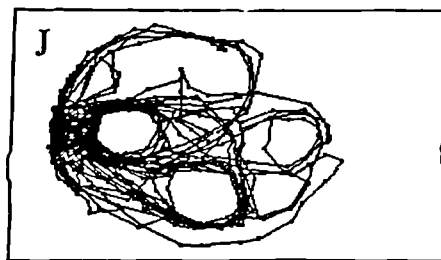
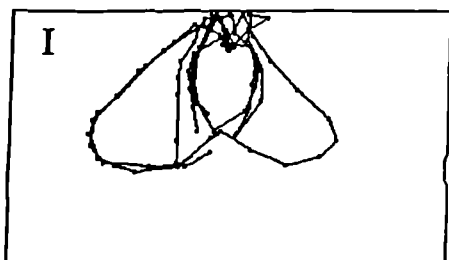
**Fig. 5.4** Screen print of the computer programme for spatial analysis of fish behaviour (M.T. Burrows *pers. comm.*), showing **A**: photograph of the experimental arena, **B**: the path followed by each individual from the start box to the refuge on Day 1, **C**: residence time of all individuals in each grid square of the arena (darker squares indicate longer residence time).

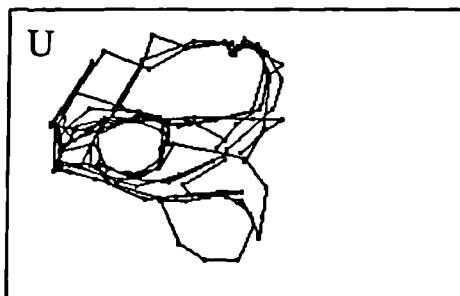
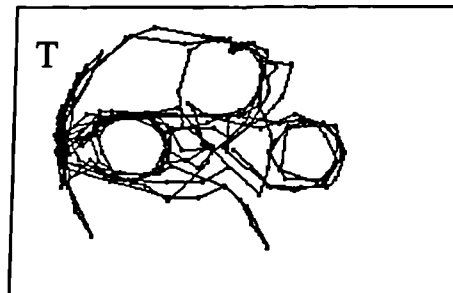
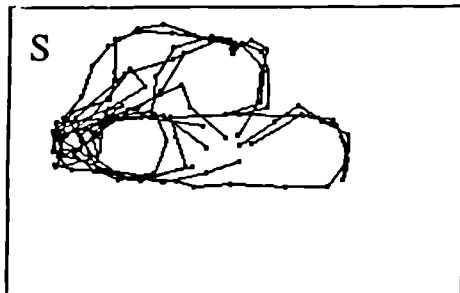
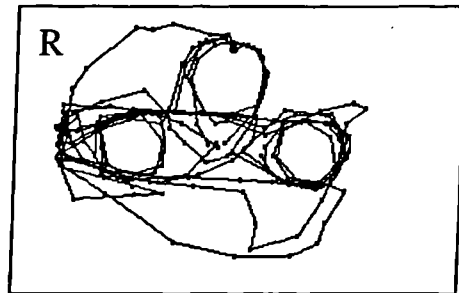
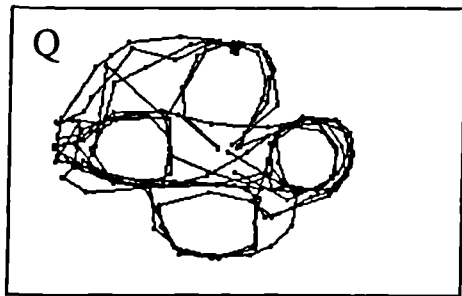




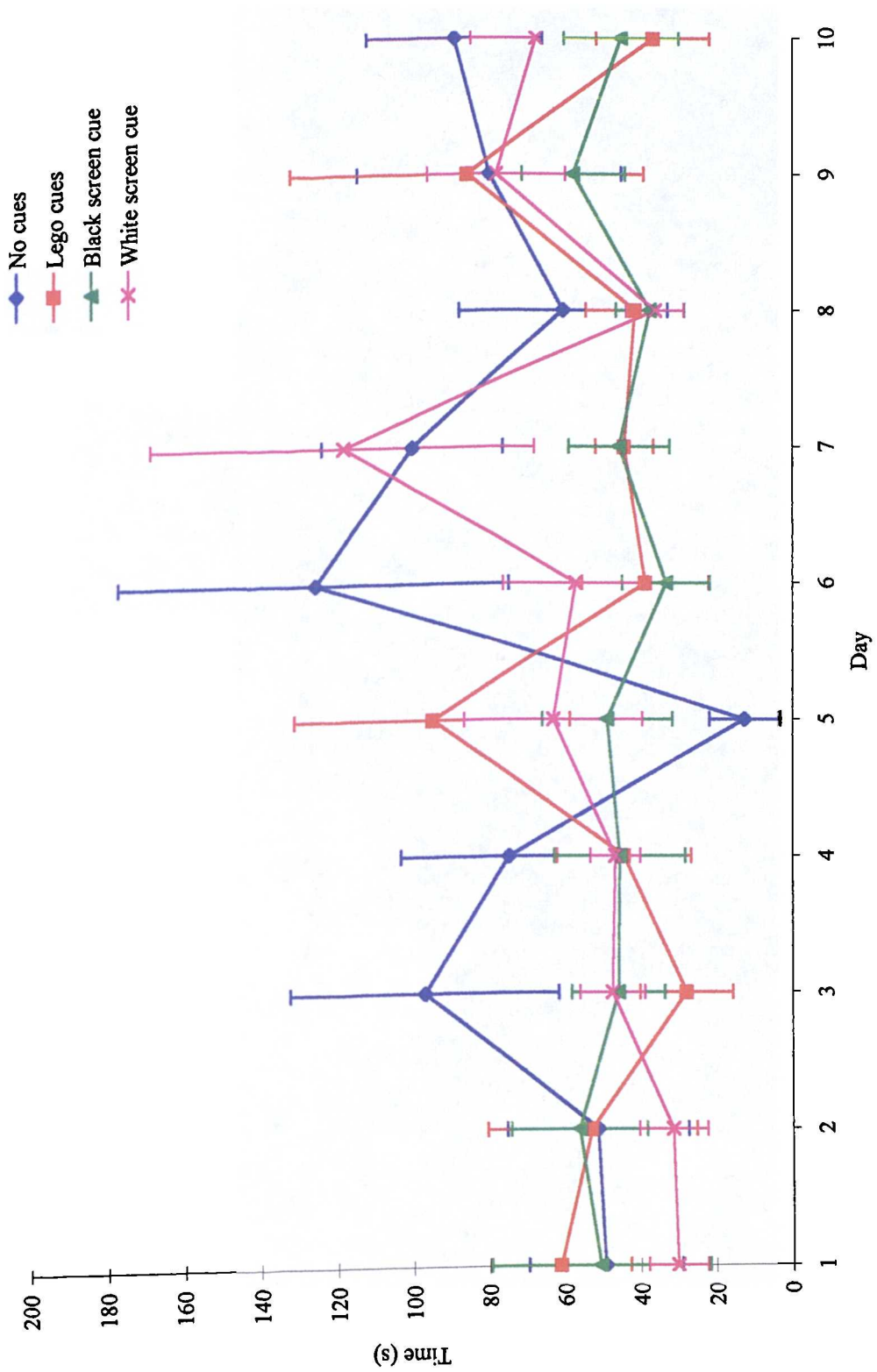
**Fig. 5.5** The paths followed by all fish in Experiment 4, **A:** Day 2, **B:** Day 3, **C:** Day 4, **D:** Day 5, **E:** Day 6, **F:** Day 7, **G:** Day 8, **H:** Day 9, **I:** Day 10, and Experiment 5, **J:** Day 11, **K:** Day 12, **L:** Day 13, **M:** Day 14, **N:** Day 15, **O:** Day 16, **P:** Day 17, **Q:** Day 18, **R:** Day 19, **S:** Day 20, **T:** Day 21, **U:** Day 22.



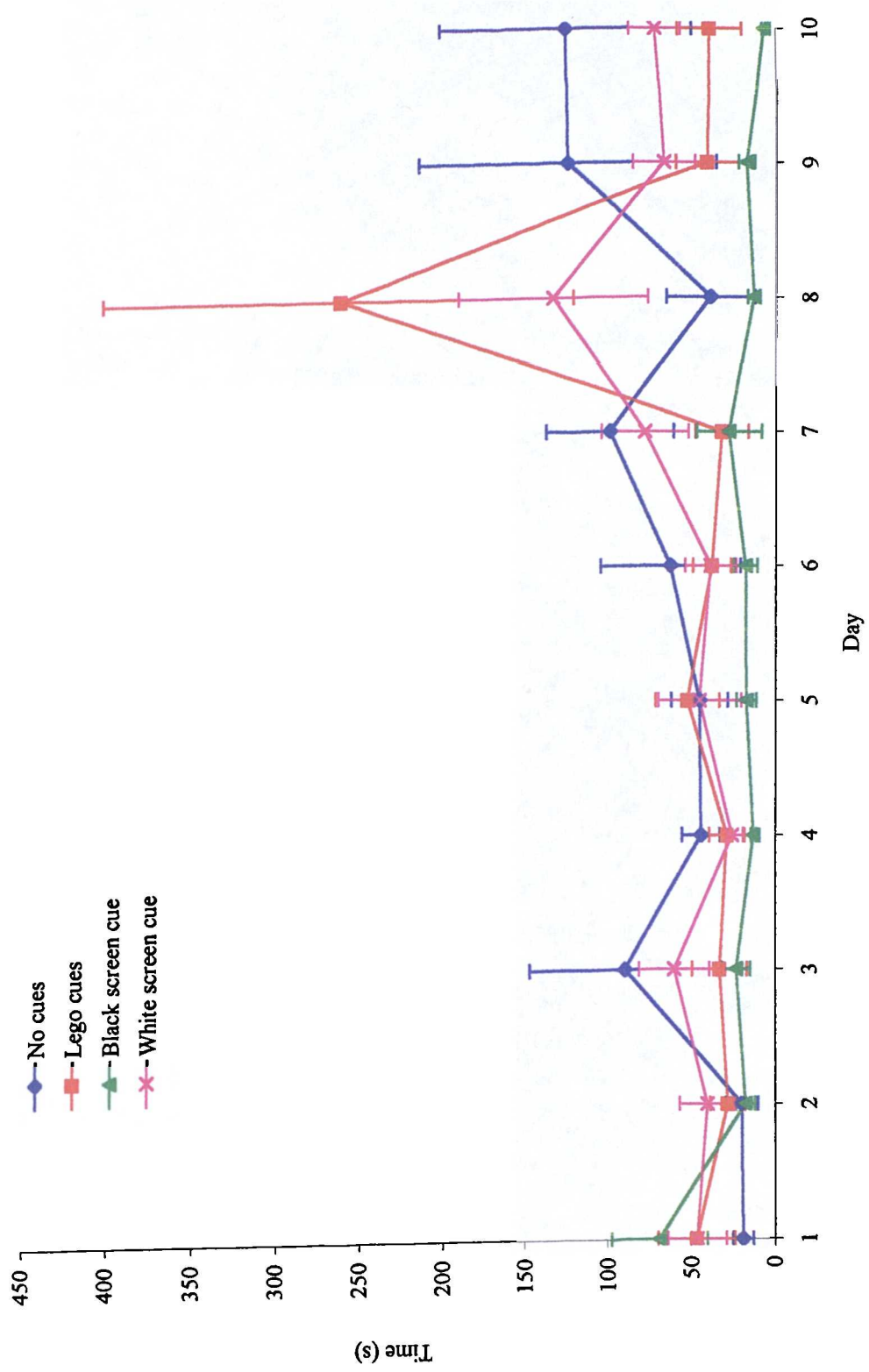




**Fig. 5.6** Mean latency to movement for *L. pholis* in the refuge maze in Experiment 1, no clues, Experiment 2, Lego™, Experiment 3, white screen, Experiment 4, black screen, n = 8, error bars show the standard error of the mean.

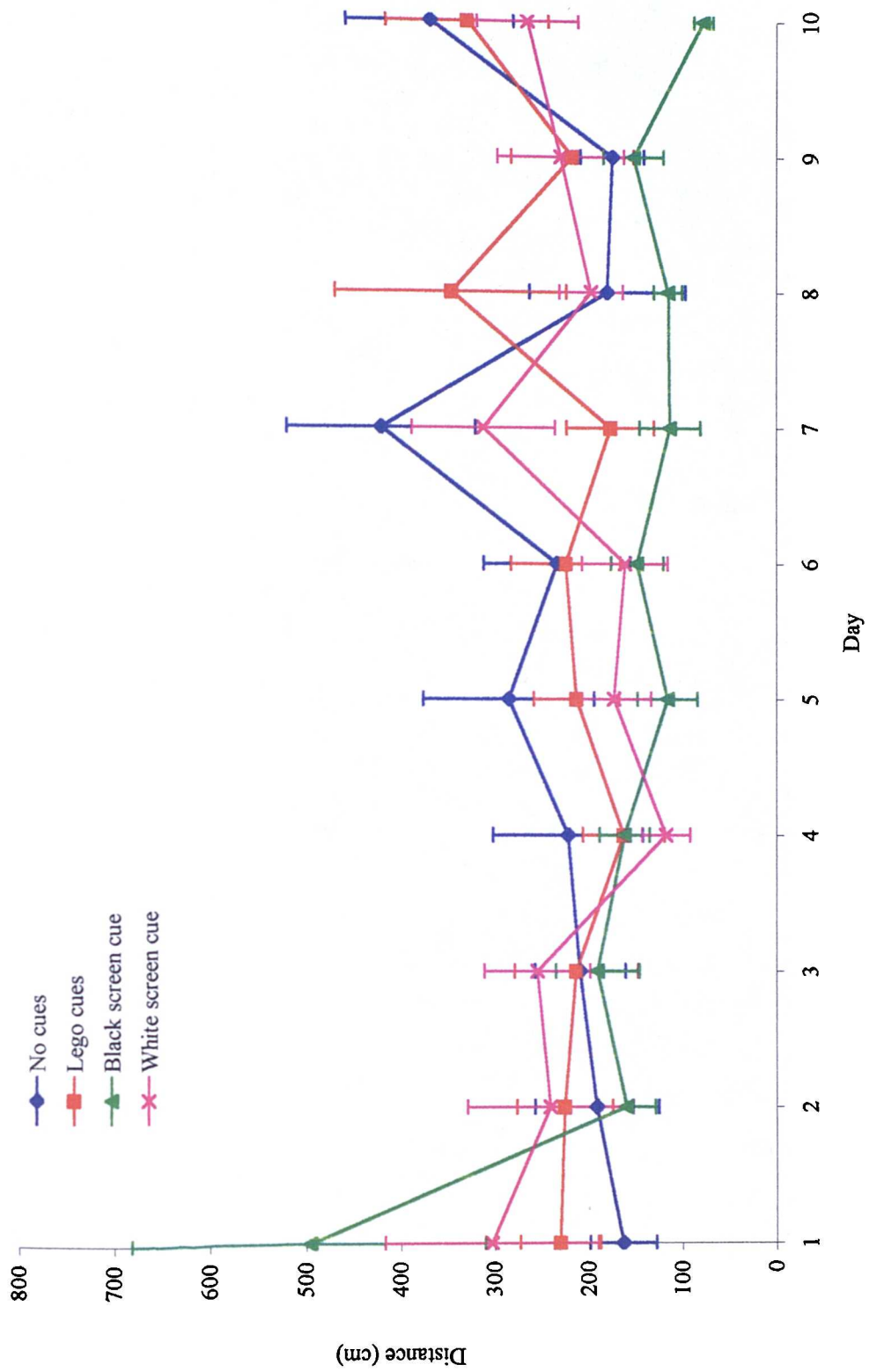


**Fig. 5.7** Mean exploration time to the refuge for *L. pholis* in the refuge maze. Experiment 1, no clues, Experiment 2, Lego™, Experiment 3, white screen, Experiment 4, black screen, n = 8, error bars show the standard error of the mean.





**Fig. 5.8** Mean distance travelled to the refuge for *L. pholis* in the refuge maze in Experiment 1, no clues, Experiment 2, Lego<sup>TM</sup>, Experiment 3, white screen, and Experiment 4, black screen, n = 8, error bars show the standard error of the mean.



**Table 5.2** Results of Friedman's test for a difference in latency to movement, exploration time, distance travelled to the refuge and the number of fish which chose the correct direction to the refuge for *L. pholis* among 4 experiments. Experiment 1, no clues, Experiment 2, Lego™, Experiment 3, white screen, and Experiment 4, black screen, d.f. = 3.

	Latency to movement	Exploration time before entering the refuge	Distance travelled to the refuge	Number of fish which chose the correct direction from the start position
<i>P</i> =	0.431	0.008	0.022	0.257
	NS	**	*	NS

**Table 5.3** Results of Dunn's test comparing the mean exploration time before entering refuge of *L. pholis* in each experiment. Experiment 1, no clues, Experiment 2, Lego™, Experiment 3, white screen, and Experiment 4, black screen, critical value  $Q_{0.05} = 2.638$ ,  $k = 4$ .

	Experiment 1	Experiment 2	Experiment 3
Experiment 2	0.91 NS		
Experiment 3	0.13 NS	1.04 NS	
Experiment 4	3.40 **	2.49 NS	3.53 **

**Table 5.4** Results of Dunn's test comparing the mean distance travelled to the refuge of *L. pholis* in each experiment. Experiment 1, no clues, Experiment 2, Lego™, Experiment 3, white screen, and Experiment 4, black screen,  $Q_{0.05} = 2.638$ ,  $k = 4$ .

	Experiment 1	Experiment 2	Experiment 3
Experiment 2	0.02 NS		
Experiment 3	0.15 NS	0.17 NS	
Experiment 4	2.77 *	2.79 *	2.62 NS

There was a significant decrease in the mean distance travelled to the refuge in Experiment 4 only (Page's test, Page's statistic = 356, critical value = 348 at 5%, d.f. = 9, Tables 5.5, 5.6 and 5.7). The number of fish which chose the correct direction out of the start box was highly variable in each experiment and showed no trend (Fig. 5.9). However, there was a significant increase in the number of fish which chose the correct direction out of the start box in Experiment 2 (Page's test, Page's statistic = 362, Critical value = 348 at 5%, d.f. = 7, Table 5.8).

Mean exploration time to the black screen and mean distance to the black screen were significantly lower than mean exploration time to the refuge and the mean distance travelled to the refuge respectively (Mann Whitney U test  $P = 0.011$ ,  $0.000$ , and  $0.020$  respectively, Table 5.9, Figs. 5.10 and 5.11 respectively).

The number of *L. pholis* which visited the black screen before they entered the refuge in Experiment 4 showed no significant decrease (Page's test, Page's statistic = 344, Critical value = 348 at 5%, d.f. = 7, Fig. 5.12).

There was no significant concordance in the ranks of mean latency to movement, mean exploration time to the refuge, mean distance travelled to the refuge or the number of fish which chose the correct direction out of the start box between experiments over the course of the 10 days (Kendall's coefficient of concordance,  $W = 0.034$ ,  $0.286$ ,  $0.324$ ,  $0.029$ ,  $X^2 = 1.022$ ,  $8.580$ ,  $9.720$ ,  $0.865$ ,  $\chi^2_{(0.05, 9)} = 16.92$ , d.f. = 9, Table 5.10).

### 5.3.3 Experiment 5

#### 5.3.3.1 Behaviour on release from the start box

On Day 11, the first day of Experiment 5 in which the black screen was moved to a different position, six out of eight fish moved in a south westerly direction out of the start box. Seven out of eight fish moved towards the black screen, touched it before investigating the refuge site D (situated opposite the new position of the black screen Fig. 5.3) and then continued to move around the arena in search of the refuge (Fig. 5.5J). Again, many fish often returned to the black screen after their initial visit (Figs

**Table 5.5** Results of analysis for a decreasing trend in mean latency to movement for *L. pholis* in the refuge maze using Page's test, critical value = 348 at 5%, d.f. = 9.

Experiment	Conditions	Page's statistic	
1	No clues	269	NS
2	Lego <sup>TM</sup>	243	NS
3	White screen	273	NS
4	Black screen	241	NS

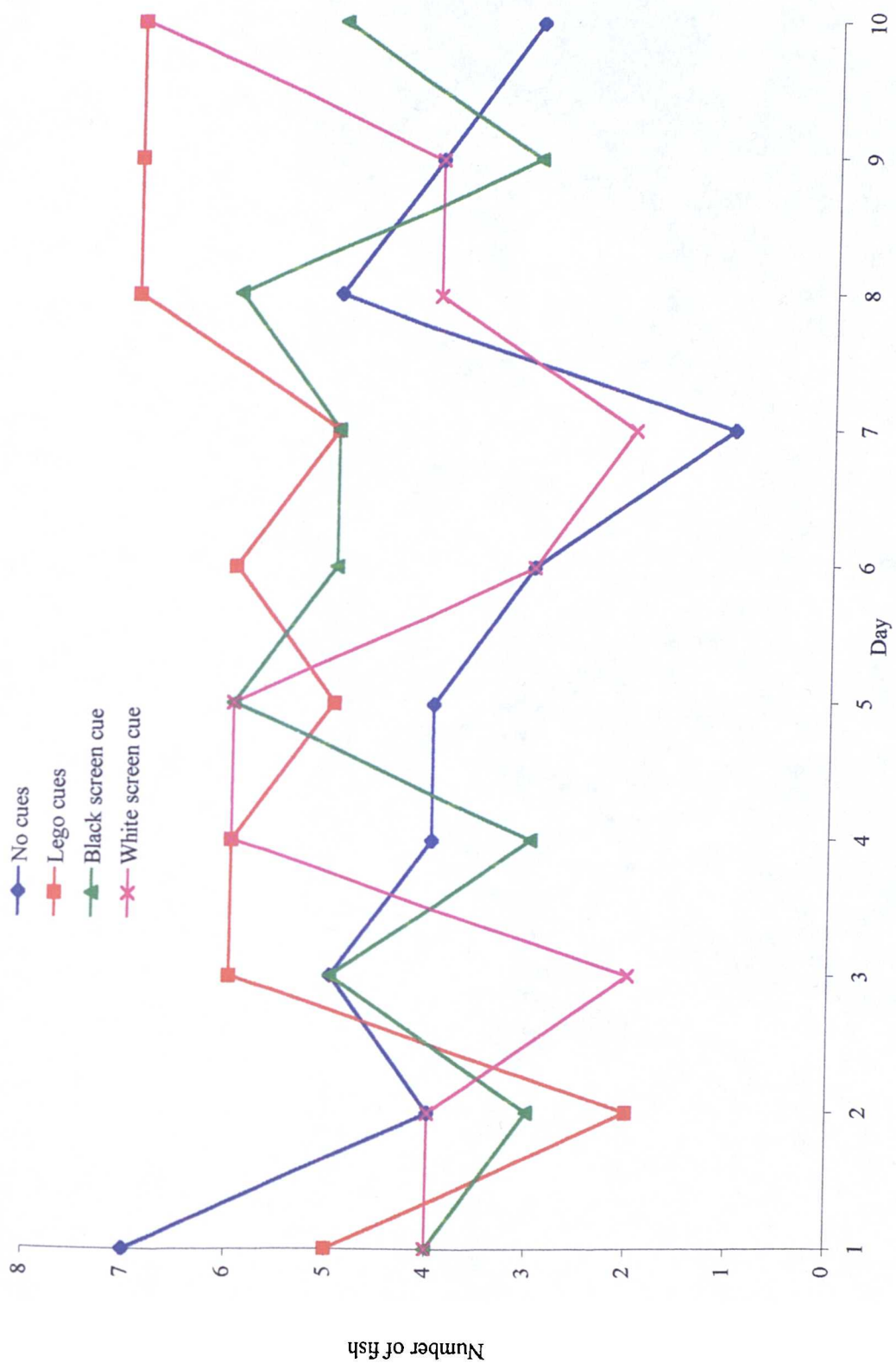
**Table 5.6** Results of analysis for a decreasing trend in mean exploration time for *L. pholis* in the refuge maze using Page's test, critical value = 348 at 5%, d.f. = 9.

Experiment	Conditions	Page's statistic	
1	No clues	241	NS
2	Lego <sup>TM</sup>	276	NS
3	White screen	246	NS
4	Black screen	338.5	NS

**Table 5.7** Results of analysis for a decreasing trend in mean distance travelled to the refuge for *L. pholis* in the refuge maze using Page's test, critical value = 348 at 5%, d.f. = 9.

Experiment	Conditions	Page's statistic	
1	No clues	273	NS
2	Lego <sup>TM</sup>	285	NS
3	White screen	303	NS
4	Black screen	356	*

**Fig. 5.9** The number of fish which chose the correct direction out of the start box for *L. pholis* in the refuge maze in Experiment 1, no clues, Experiment 2, Lego™, Experiment 3, white screen, Experiment 4, black screen.



**Table 5.8** Results of analysis for an increasing trend in the number of fish which chose the correct direction out of the start box for *L. pholis* in the refuge maze using Page's test, critical value = 348 at 5%, d.f. = 9.

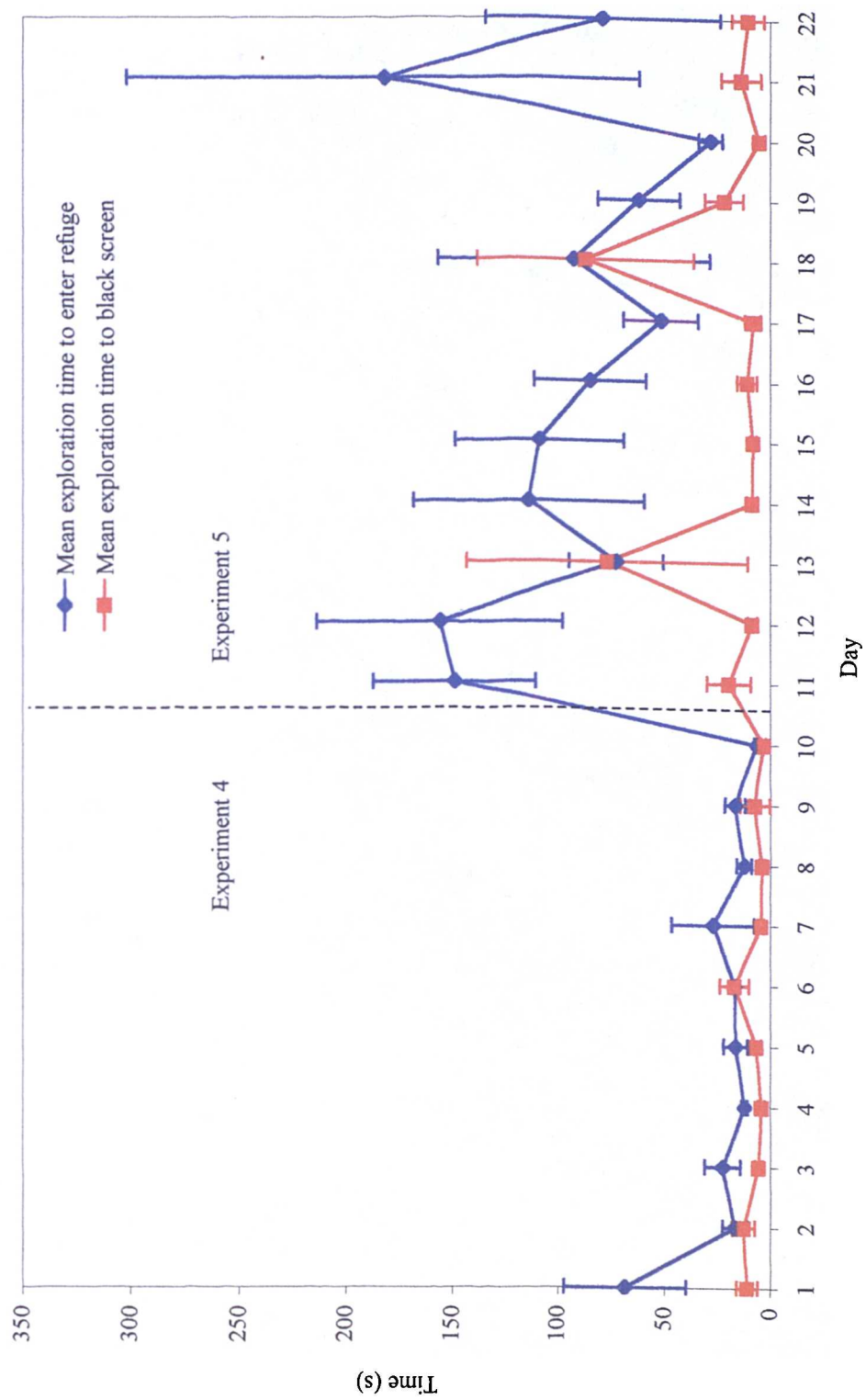
Experiment	Conditions	Page's statistic	
1	No clues	260.5	NS
2	Lego™	362	*
3	White screen	284	NS
4	Black screen	326.5	NS

**Table 5.9** Results of Mann Whitney U test comparing the mean performance to the refuge and the mean performance to the black screen for *L. pholis* in Experiment 4, d.f.= 10.

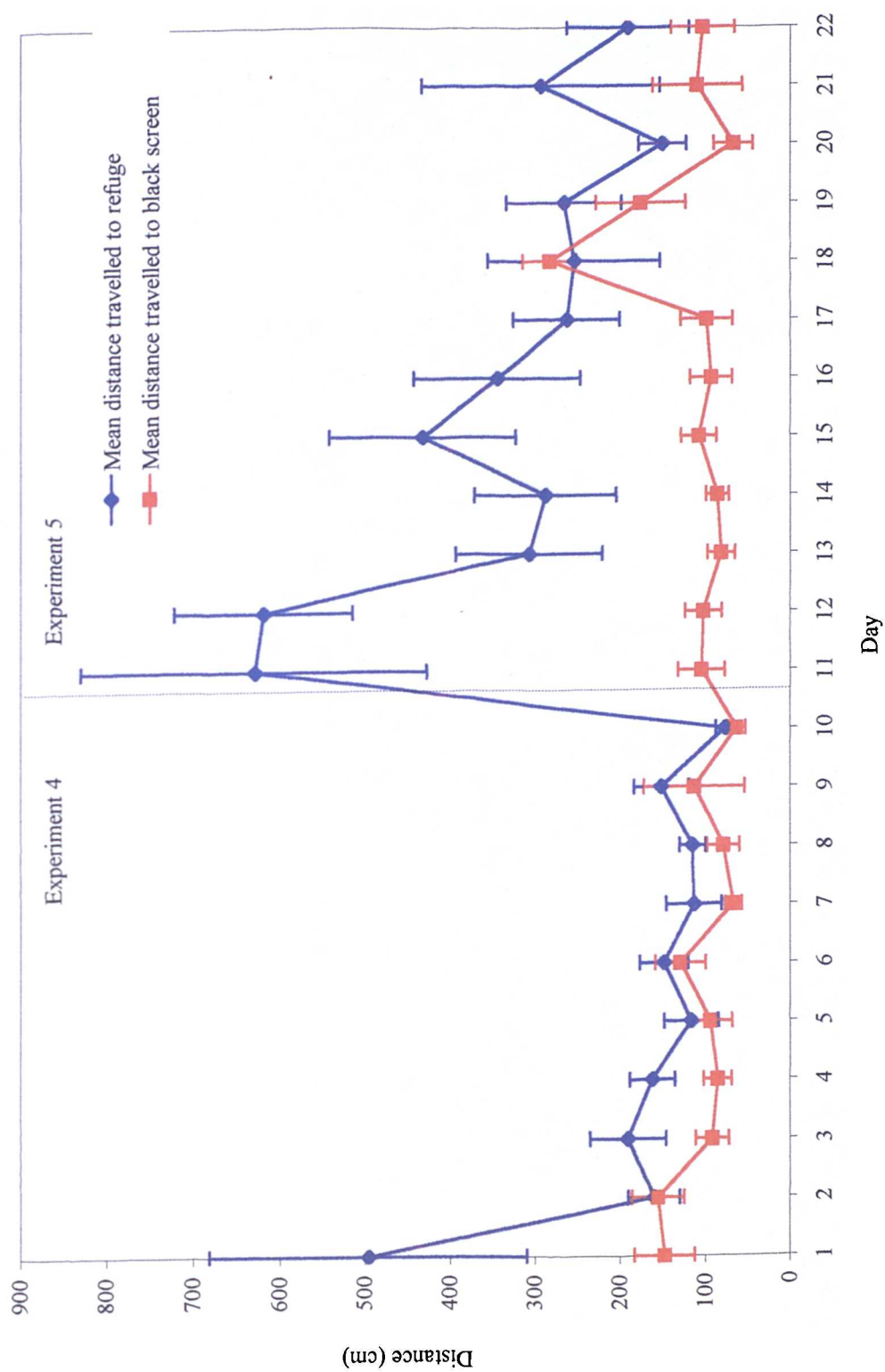
Performance criteria	<i>P</i>	
Mean exploration time to the refuge / the black screen	0.004	**
Mean distance travelled to the refuge / the black screen	0.001	***



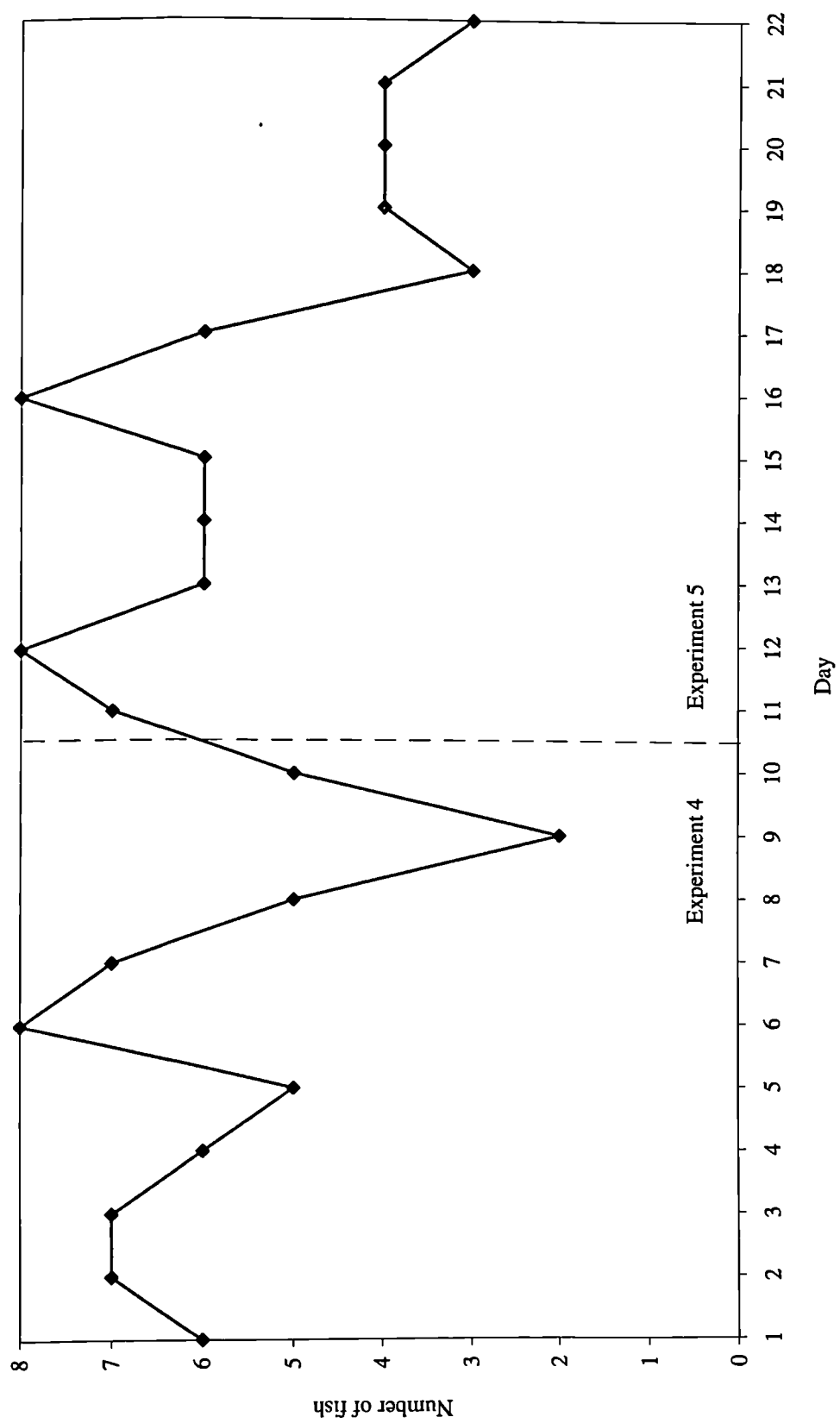
**Fig. 5.10** The mean exploration time to the refuge and to the black screen Experiment 4, black screen at Position 1, and Experiment 5, black screen moved from Position 1 to Position 4,  $n = 8$ , error bars show the standard error of the mean.



**Fig. 5.11** The mean distance travelled to the refuge and the black screen, Experiment 4, black screen at Position 1, and Experiment 5, black screen moved from Position 1 to Position 4,  $n = 8$ , error bars show the standard error of the mean.



**Fig. 5.12** The number of fish which visited the black screen before they entered the refuge, Experiment 4, black screen at Position 1, and Experiment 5, black screen moved from Position 1 to Position 4.



**Table 5.10** Kendall's coefficient of concordance to test for concordance in the mean performance to the refuge between Experiment 1, no clues, Experiment 2, Lego<sup>TM</sup>, Experiment 3, white screen, and Experiment 4, black screen,  $\chi^2_{(0.05, 9)} = 16.92$ , d.f. = 9.

Performance criteria	$W$	$\chi^2$	
Mean latency to movement	0.034	1.022	NS
Mean exploration time to the refuge	0.286	8.58	NS
Mean distance travelled to the refuge	0.324	9.72	NS
Number of fish which chose the correct direction out of the start box	0.029	0.865	NS

5.5J, 5.5K and 5.5L). This behaviour continued for several days until, later in the experiment, more fish moved more directly to the refuge without first visiting the black screen (Figs 5.5S, 5.5T and 5.5U).

### **5.3.3.2 The effects of moving the black screen**

Exploration time to the black screen and distance travelled to the black screen were significantly different to exploration time to the refuge and distance travelled to the refuge respectively during Experiment 5 (Mann Whitney U test  $P = 0.000$  and  $0.000$ , respectively,  $N = 12$ , Table 5.11, Figs 5.10 and 5.11).

Mean exploration time to the refuge, mean distance travelled to the refuge, mean exploration time to the black screen and the number of fish that visited the black screen before they entered the refuge were significantly lower in Experiment 4 than Experiment 5 (Mann Whitney U test  $P = 0.000$ ,  $0.000$ , and  $0.010$ ,  $N = 10$ , Table 5.12, Figs 5.10, 5.11 and 5.12).

There was a significant increasing trend in the number of fish which chose the correct direction out of the start box and the latency to movement in Experiment 5 (Page's test, Page's statistic = 595 and 584.5 respectively, Critical value = 578, d.f. = 7, Figs 5.13 and 5.14).

Only mean distance travelled to refuge and the number of fish which visited the black screen decreased significantly in Experiment 5 (Page's test, Page's statistic = 620 and 619.5 respectively, critical value = 578, d.f. = 7, Table 5.13, Figs. 5.11 and 5.12).



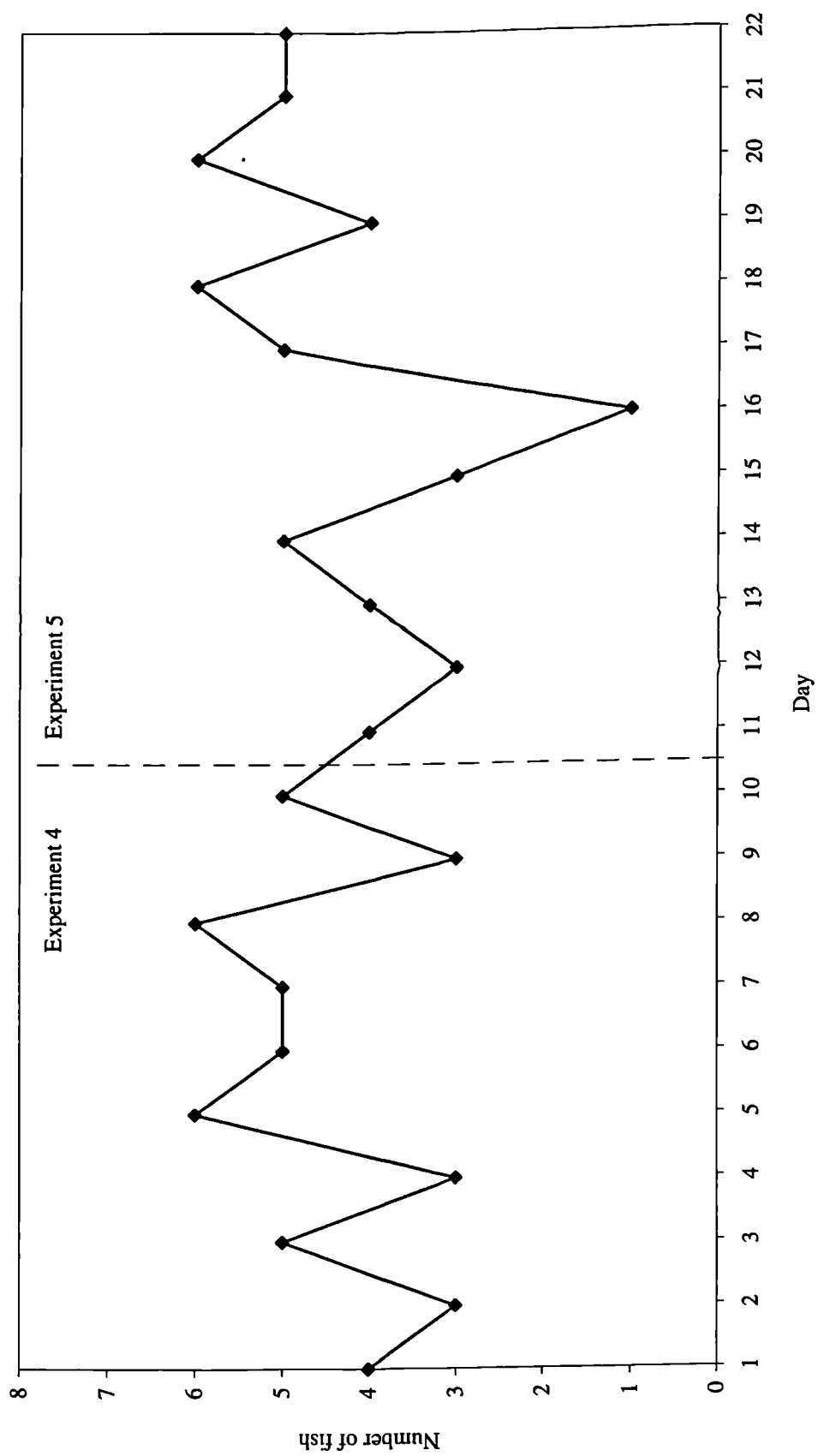
**Table 5.11** Results of Mann Whitney U test comparing the mean performance to the refuge and the mean performance to the black screen for *L. pholis* in Experiment 5, after the black screen was moved from Position 1 to Position 4 d.f. = 10.

Performance criteria	<i>P</i>	
Mean exploration time to the refuge / black screen	0.000	***
Mean distance travelled to the refuge / black screen	0.000	***

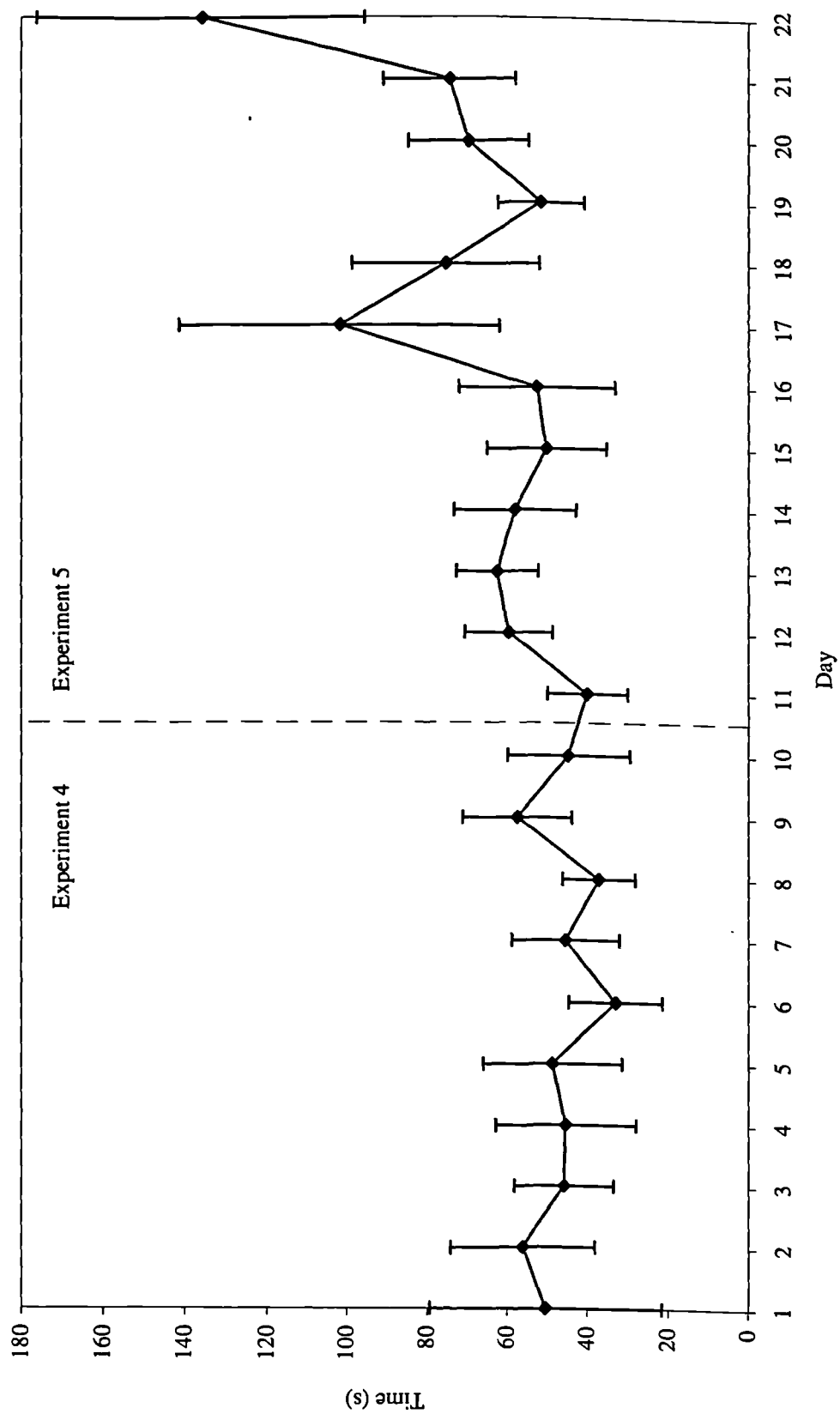
**Table 5.12** Results of Mann Whitney U test comparing mean performance of *L. pholis* in the refuge maze, Experiment 4, black screen at Position 1 and Experiment 5, black screen moved from Position 1 to Position 4, d.f. = 10.

Performance criteria	<i>P</i>	
Mean latency to movement	1.000	NS
Mean exploration time to the refuge	0.000	***
Mean distance travelled to the refuge	0.005	**
Mean exploration time to the black screen	0.028	**
Mean distance travelled to the black screen	0.623	NS
Number of fish which visited the black screen before they entered the refuge	0.009	**

**Fig. 5.13** The number of fish which chose the correct direction out of the start box, Experiment 4, black screen at Position 1, and Experiment 5, black screen moved from Position 1 to Position 4.



**Fig. 5.14** The mean latency to movement, Experiment 4, black screen at Position 1, and Experiment 5, black screen moved from Position 1 to Position 4,  $n = 8$ , error bars show the standard error of the mean.



**Table 5.13** Results of Page's test for significant decrease in mean performance to the refuge and to the black screen for *L. pholis* in Experiment 5, black screen moved from Position 1 to Position 4, critical value = 578, d.f. = 11.

Performance criteria	Page's statistic	
Mean exploration time to the refuge	559	NS
Mean distance travelled to the refuge	620	*
Mean exploration time to the black screen	517	NS
Mean distance travelled to the black screen	478	NS
Number of fish which visited the black screen they entered the refuge	619.5	*

## 5.4 DISCUSSION

The results showed that *L. pholis* learned the position of the refuge only in the presence of the black screen. They respond very positively to it, and often moved towards it and touched it on release from the start box before they entered the refuge. The fish continued to move towards the black screen in this way immediately after it is moved from the position behind the refuge in Experiment 5. However, over the course of the following 12 days of this experiment they became more efficient in navigating to the refuge once again.

All the fish in Experiment 1 initially showed a fright response when the start box was raised. This involved either swimming about quickly or remaining still, either in the centre of the arena or with part or all of the body in contact with the aquarium wall or the edge of one of the artificial rocks. However, all the fish entered the refuge within 30 min and remained there until replaced in their holding tank, indicating a *strong attraction for resting in the protection of complete cover*. Only fish in Experiment 4 showed any obvious interest in the clues placed in the experimental arena. In this experiment, most fish moved towards the black screen on their release from the start box, often pressing themselves up against it and then pausing in this position before beginning to move around the arena, apparently searching for a refuge. This behaviour was particularly apparent in the first few days of Experiment 4 and is reflected in the results of the Mann Whitney U tests and the paths of movement of the fish in this group. It is likely that this behaviour represents the natural instinct of *L. pholis*, when startled (in this case by the raising of the start box), to move towards the nearest or most obvious dark and shadowy place.

The mean exploration time to enter the refuge is lower in Experiment 4 than in Experiments 1, and 3, probably because most of the fish in this group moved directly to the black screen upon their release from the start box, and the refuge was only 15 cm from the black screen therefore was likely to be visible to *L. pholis* from this position. The journey from the black screen to the refuge therefore only took a very short time

once the fish had become acclimatised to the experimental protocol (in the first few trials they swam frantically around the tank after visiting the black screen and before they entered the refuge). It is possible that the lack of significant decrease in the mean exploration time to enter the refuge over 10 days was because this performance criterion reached asymptote after just one day.

The fish in Experiment 4 visited the black screen before they entered the refuge in the first few days of the experiment, and then later appeared to proceed more directly to the refuge. These results suggested that the fish learned that the refuge was a more satisfactory shelter than the black screen and that they learned to use the position of the black screen as a beacon or reference point to aid in navigation towards the refuge. However the number of fish visiting the black screen did not show a significant decrease in 10 days. It is possible that given more time, more fish in this group may have learned to use the black screen as a reference point rather than moving towards it and touching it before moving into the refuge.

The mean distance travelled to the refuge was significantly lower in Experiment 4 than in Experiments 1, and 2, and showed a significant decreasing trend over 10 days. These results implied that the black screen was the best visual clue, from among those provided, as to the position of the refuge. These results also implied that the fish were learning to use the black screen to direct *their movements* towards the refuge during the 10 day duration of Experiment 1. However, as the mean number of fish visiting the black screen before they entered the refuge did not show a significant decrease over 10 days, it is more likely that the fish were responding to the black screen simply because it was dark. When stressed, in this case by suddenly being placed in the open when the start box was raised, it may be the natural instinct of *L. pholis* to move towards the nearest dark place, in the present study the black screen, as it is likely that this will be a refuge. Therefore it was simply the fact that the refuge was close to, and visible from the black screen, that produced the significantly different and improving mean distance travelled to the refuge in this experiment.



The number of fish which chose the correct direction from the start position was not significantly different in any of the experiment groups. This result suggested that the fish did not learn the correct direction from the start to the refuge within 10 days, that none of the clues provided had a significant effect on performance, and that the fish were simply moving randomly from the start position until they reached the refuge. However, there was a significant increasing trend in the number of fish which chose the correct direction from the start position in Experiment 2 over 10 days. This result implied that the fish in Experiment 2 may have been using the Lego™ towers as beacons marking the position of the refuge. This use of the Lego™ towers is unlikely, however, as there was no improvement in the number of fish which chose the correct direction out of the start box in Experiment 4, the experiment in which there was most improvement in all other performance criteria, and there was no improvement in any of the other criteria for Experiment 2. It is more likely that this improvement shown by Experiment 2 was an artefact of the small group size and low number of replicates. It is possible, however, that given longer than 10 days the number of fish which chose the correct direction out of the start box would have increased in all of the experiments.

Mean latency to movement, mean exploration time to enter the refuge and mean distance travelled to the refuge were consistently lower in Experiment 4 than in Experiments 1, 2 and 3, therefore the black screen was the best clue as to the position of the refuge amongst those provided. However, there was no consistent, significant concordance in the ranks of mean latency to movement, mean exploration time to the refuge and mean distance travelled to the refuge in the remaining experiments, therefore there was no consistent hierarchy of clue use by *L. pholis* in the present study. Again this result may be the artefact of small group sizes and low numbers of replicates.

At the beginning of Experiment 5, a larger proportion of *L. pholis* moved toward the black screen and touched it before entering the refuge than in Experiment 4, even though it no longer marked the refuge position. Also mean exploration time to the black screen, and mean distance to the black screen in Experiment 4 were consistently

significantly lower than mean exploration time to the refuge and mean distance travelled to the refuge in Experiment 5. These results together with the significant increase in all performance criteria except latency to movement and distance travelled to the black screen after the black screen was moved, again showed the strong attraction of the black screen for *L. pholis* for initial shelter on release from the start box. However, the fish searched Site D (opposite the new position of the black screen) for the refuge at the beginning of Experiment 5, and over the course of this experiment the mean distance travelled and the number of fish which visited the black screen significantly decreased, while the number of fish which chose the correct direction out of the start box significantly increased. These results suggested that many of the fish had already learned to use the black screen as a visual clue to the position of the refuge in Experiment 4, and that they learned to use it as an indirect visual clue to aid in navigation to the refuge in Experiment 5.

The mean exploration time and the mean distance travelled to the black screen did not show any significant decreasing trend in Experiment 5, probably because a small number of fish always touched the black screen before entering the refuge. This result may have been due to individual variation in learning ability when under stress. It is possible that some individuals felt so stressed while out in the open that they continued to move immediately towards the most obvious “refuge”, the black screen, and were unable to learn the location of the actual refuge. Others, on the other hand, grew accustomed to the experimental protocol after a few days and became calm enough to remain still once the start box was lifted, note the position of the local landmarks (each artificial rock and the black screen) and move towards the refuge more directly. This possible change in refuge-search mechanism would account for the increase in mean latency to movement during the course of Experiment 5. It is probable also that once the black screen was moved further away from the refuge in Experiment 5, there was a greater incentive to omit movement between the black screen and the refuge from the total journey to the refuge, and rather to learn to use the black screen as an indirect reference to the refuge position. This change in cost and therefore incentive of learning would explain the fact that there was no significant decrease in the number of fish

which visited the black screen in Experiment 4, but that there was in Experiment 5.

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## 5.5 CONCLUSION

When *L. pholis* was placed in a novel arena, it moved towards the only large, dark object present, a black screen designed as a distant visual clue to the position of the refuge and visible from all over the arena. Performance in navigating towards the refuge was consistently better in the presence of the black screen than in the presence of Lego™ towers and a white screen clue, which were not approached in this way. When the black screen was moved to a new position, *L. pholis* learned to use it as an indirect clue to aid in navigating directly to the refuge. These results suggested that if they find themselves in a novel area in the wild, *L. pholis* move towards the nearest dark shadowy place (a crevice or rocky overhang) when threatened. Later, when they have explored the area, they are capable of using local visual clues to navigate back to a particular refuge more directly.

## **Chapter 6**

### **General discussion**

## 6.1 GENERAL DISCUSSION AND SUGGESTIONS FOR FUTURE WORK

The present study found that *L. pholis* is capable of learning a route to a food patch in a hierarchical maze by using Lego™ towers as beacons and as a prompt for which direction to turn at critical points in the journey. In a more open arena, *L. pholis* ignored the Lego™ towers and used a black screen, initially as a shelter, and then as an indirect clue to the position of the refuge. These results present the possibility that local clues are used in navigating towards food patches when *L. pholis* is not under threat, but that distant clues are used when the animal is, for example, under attack from a predator and therefore has to act quickly in order to find a refuge while stressed. This is conceivable, as one would expect the subject would have a lot of information to process when attacked by a predator, therefore it is likely that the most obvious clues visible from a wide area would be used.

*L. pholis* also demonstrated a hierarchy of clue use and an ability to overcome “overshadowing” (Macintosh 1974) and re-learn a spatial task very quickly when the conditions were changed throughout the present study. If this strategy were replicated in the behaviour of *L. pholis* in the wild it would be of great selective advantage in the intertidal zone, where local clues may change in appearance with each rising and receding tide, or may be destroyed completely during a severe storm.

A study of the jumping behaviour of *Bathygobius soporator* showed that subjects from a rocky area learned to jump more quickly and more accurately into adjacent pools than subjects from a more sandy area when threatened (Aronson 1951, 1971). This experiment suggested that the complexity of the habitat from which an organism originates affects its ability to learn spatial tasks. The original intention of the present work was to test this theory using rock gobies (*Gobius paganellus*) and sand gobies (*Pomatoschistus minutus*). However, a lack of rock gobies in the rock pools around Bangor and Oban, and the abundance of *L. pholis* encouraged the use of this species. Also, sand gobies would not move around the hierarchical maze. Once startled by the

opening of the start box they would bury in the sand and remain there for long periods of time. In contrast, *L. pholis* took very little time to settle after being placed in the hierarchical maze, began to move almost immediately once released from the start box and moved around readily, probably because the structure provided many corners for them to hide in during the journey from the start box to the reward. These results highlighted the interspecific differences in behaviour of *P. minutus* and *L. pholis* and how they are adapted to their natural habitat. For example, it is likely that *L. pholis* avoid predators in the wild by finding cover in the rocks and weed on the rocky shore, while *P. minutus* bury in the sand because there are few structures to use as shelter on the sandy shore. The interspecific differences in the behaviour of *P. minutus* and *L. pholis* suggested the use of two different experimental arenas to observe the learning ability of these species. Alternatively, experiments comparing the learning abilities of sand gobies and *L. pholis* could have been carried out in an arena incorporating elements from both simple and complex environments, therefore encouraging natural behaviour in both species. Unfortunately there was insufficient time to repeat the present experiments with a second species, or to construct an arena relevant to the behavioural strategies of subjects from both complex and simple habitats. This may prove to be an interesting area of study for future research. However, even when using an arena of mixed habitats, it would be advantageous to choose two species whose descent from a common ancestor could be assured. Therefore if a difference in learning ability was observed it could be inferred that it was due to the influence of the separate habitats on the behavioural phenotype following speciation. Then, after repeating the experiment with several of the “complex” and “simple” subjects from different populations it may be concluded that the complexity of the habitat from which an animal originates affects its ability to learn spatial tasks.

Experiments comparing the spatial learning ability of freshwater fish (the three-spined stickleback *Gasterosteus aculeatus*), from different habitat types have shown that subjects from more stable habitats are more dependent on visual landmarks for solving a maze problem than fish from less stable environments (J. Girvan *pers. comm.*). The

problem of whether a subject is pre-adapted to be a good learner because of genetic information passed on from its parents or whether the habitat affects its learning ability during ontogeny, remains to be addressed. The spatial abilities of fishes reared in stable or unstable habitats from the eggs of parents from stable or unstable habitats would provide an insight into this question. Again it would be advantageous to use fishes whose cladographic history is known in order to conclude that habitat influences behavioural phenotype during ontogeny, and to perform replicates with fishes from several populations in order for the ambiguities in population differences to be overcome.

The experiments presented in the present study showed that *L. pholis* is capable of using local and distant clues in completing a spatial task in an artificial habitat much smaller than their home range in the wild. This suggests that recognition of local and distant clues may be used on a wider scale in navigation back to home pool after feeding excursions at high tide (Gibson 1969, 1982). Further evidence of the finer detail of homing in *L. pholis*, for example a repeat of Gibson's (1967b) tag-recapture experiments or monitoring the path of individuals during their regular high water feeding excursions, and following displacement when sufficiently small electronic tags become available would be desirable. Also, experiments artificially recreating a home pool with a suitable refuge, surrounded by foraging habitat and subject to tidal conditions either outdoors or in the laboratory may lead to the further discoveries concerning the clues used in navigation. For example, the sun was eliminated as a clue from the experiments presented in the current study. Repeating learning trials in clear view of the sun's position and later inside under the influence of artificial lights, using protocols similar to those used by Hasler and his colleagues (Hasler *et al.* 1958, Hasler & Schwassman 1960), may reveal the use of sun compass navigation in *L. pholis*, particularly as *L. pholis* has already demonstrated the use of an endogenous clock (Gibson 1967a).

The present study showed that learned information was retained for at least 30 days.



Again there was insufficient time to study extended memory for learned spatial information, greater periods of retention need to be applied to *L. pholis* in order to ascertain the exact period for which spatial information endures in the absence of reinforcement.

The fact that *L. pholis* learned to use the black screen clue more quickly and efficiently as an indirect landmark when it was moved away from the refuge than when it was close to the refuge suggested that there is greater incentive to learn a spatial problem if resources would be wasted in the absence of learning. It is conceivable that learning itself requires energy, so to learn unnecessarily would also be wasteful. The cost of learning and the criteria animals use to decide whether to learn or not would also make interesting subjects for further study.

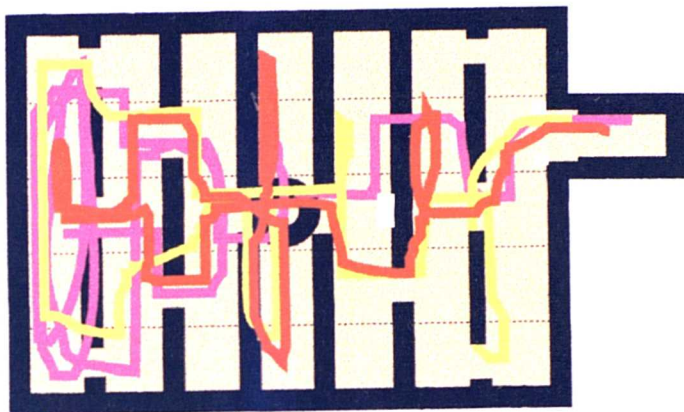
Consistent individual variation in learning ability was also suggested by the results of the experiments in the present study, but the low number of replicates dictated by the limited availability of subjects and time in which to carry out the experiments did not allow conclusions to be drawn. Larger numbers of replicates need to be carried out in order to determine if, in fact, some *L. pholis* do retain spatial information better than others.

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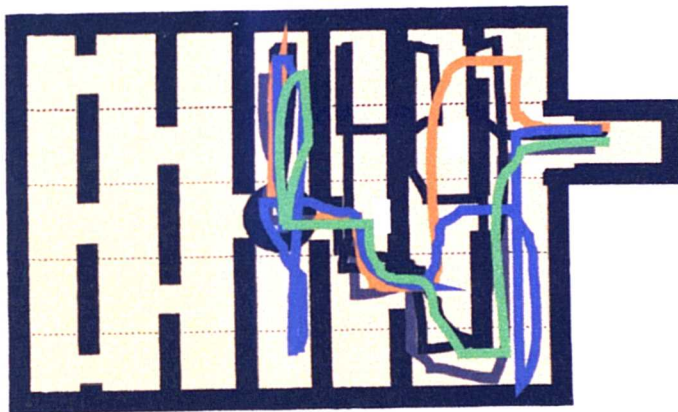
## **Appendices**

**Appendix 1** Paths travelled by each individual *L. pholis* on the journey from the start box to the reward box on Day 51 at the start of Treatment 6, Group 1, immediately after the reward and the Lego<sup>TM</sup> towers were moved from position A to position C, i) Fish 2, 4 and 6, ii) Fish 1, 3, 5, 7, and 9.

i)

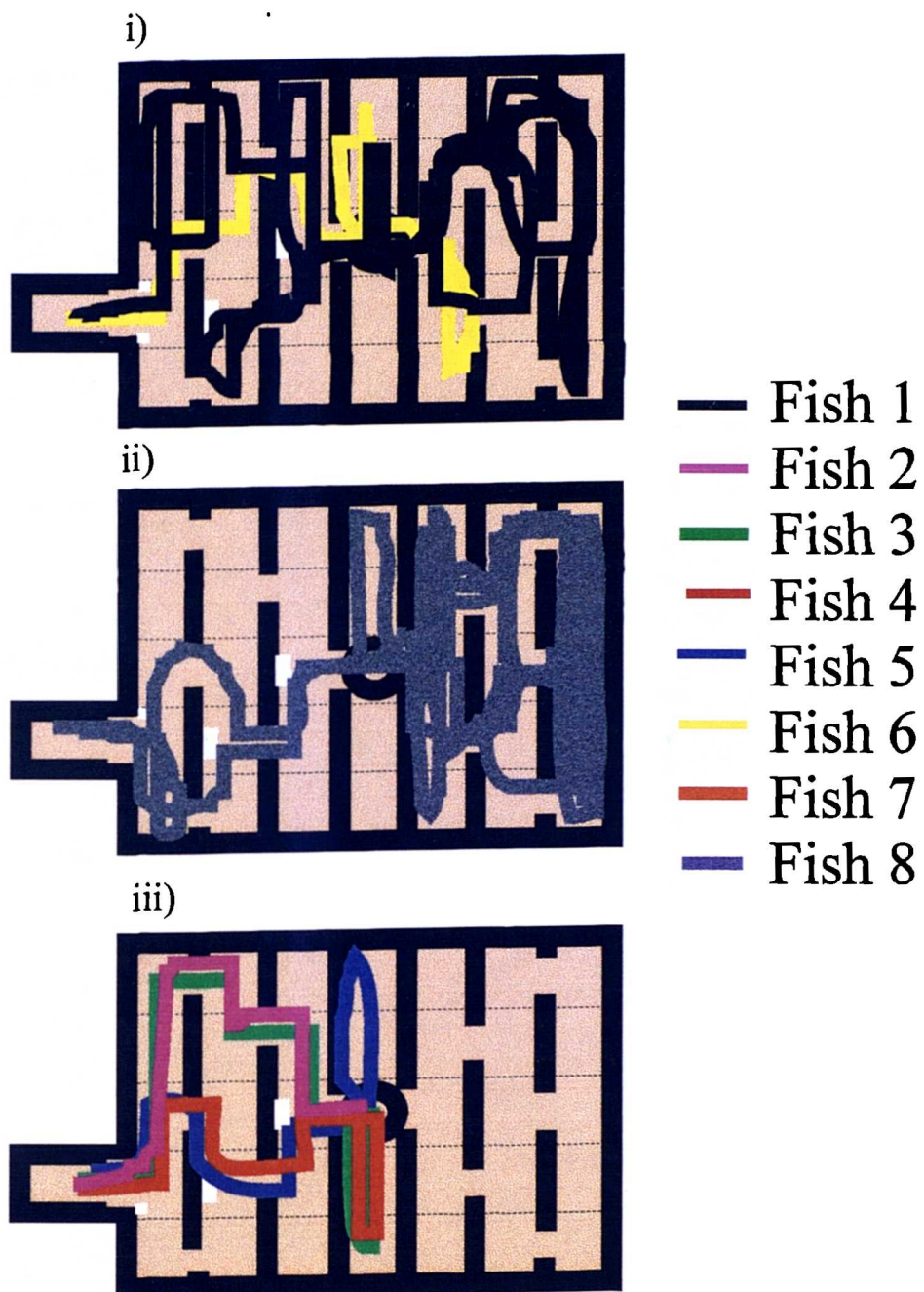


ii)

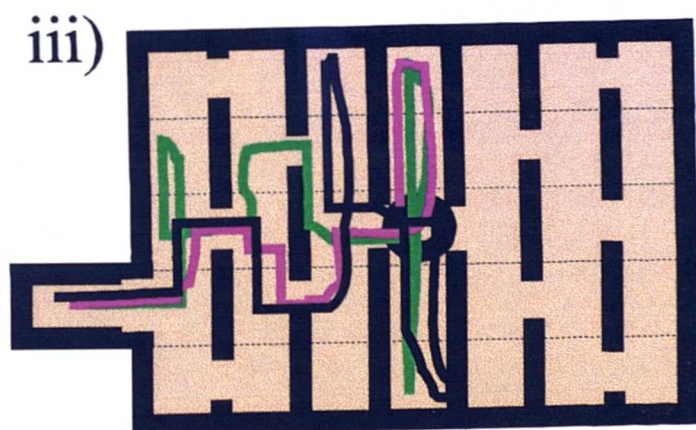
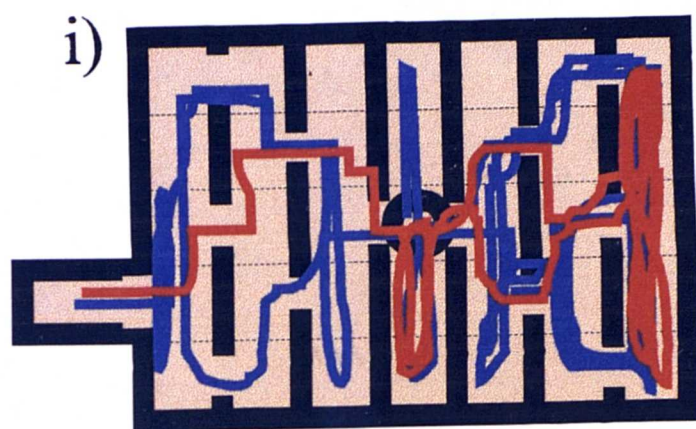


- Fish 1
- Fish 2
- Fish 3
- Fish 4
- Fish 5
- Fish 6
- Fish 7
- Fish 8

**Appendix 2** Paths travelled by each individual *L. pholis* on the journey from the start box to the reward box on Day 88 at the beginning of Treatment 8, Group 1, immediately after the reward box and the Lego<sup>TM</sup> towers were moved from position C to position B, **i)** Fish 2, 3, 4 and 5, **ii)** Fish 1 and 6, **iii)** Fish 7 and 8.



**Appendix 3** Paths followed by each individual *L. pholis* on the journey from the start box to the reward box on Day 11 at the beginning of Treatment 2, Group 2, immediately after the reward and the Lego™ towers were moved from position C to position D, **i)** Fish 9 and 10 **ii)** Fish 5 and 9, **iii)** Fish 10, **iv)** Fish 4, 6 and 8.

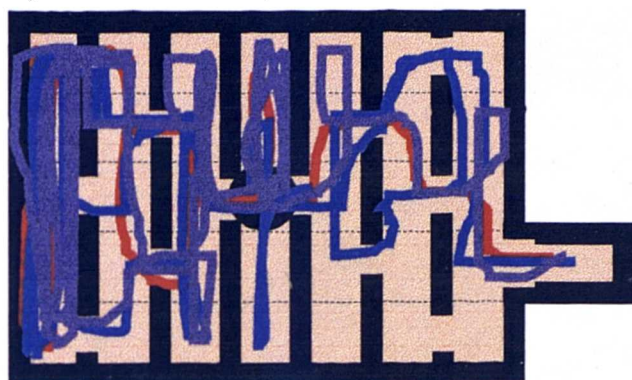


- Fish 9
- Fish 10
- Fish 11
- Fish 12
- Fish 13
- Fish 14
- Fish 15
- Fish 16

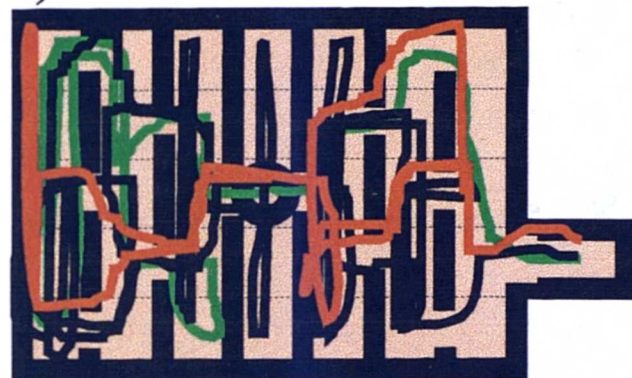


**Appendix 4** Paths travelled by each individual *L. pholis* on the journey from the start box to the reward box on Day 31 at the beginning of Treatment 5, Group 2, immediately after the reward was moved from position B to position D in the absence of Lego™ clues, **i)** Fish 9 and 10, **ii)** Fish 4 and 8, **iii)** Fish 6, **iv)** Fish 15 and 16.

i)

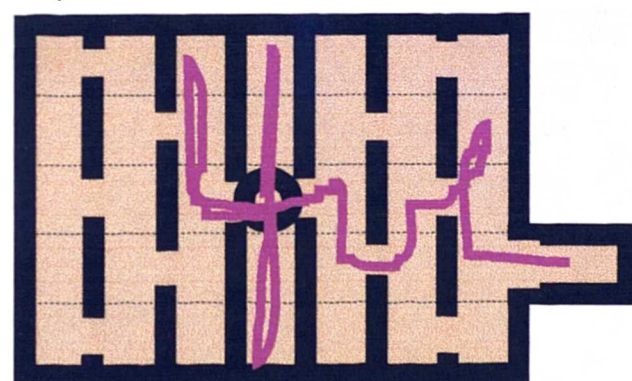


ii)



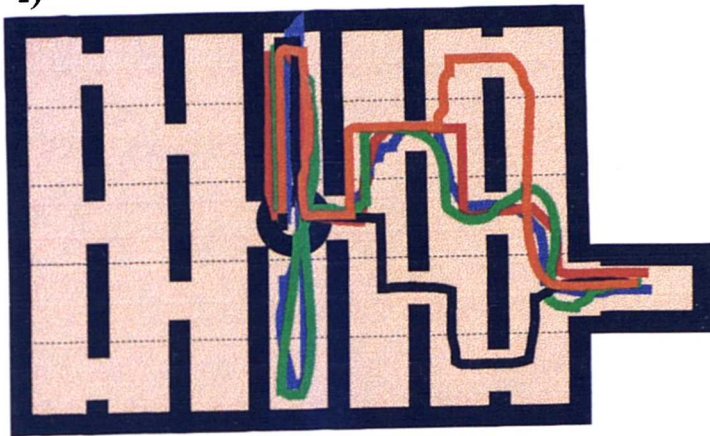
- Fish 9
- Fish 10
- Fish 11
- Fish 13
- Fish 14
- Fish 15
- Fish 16

iii)

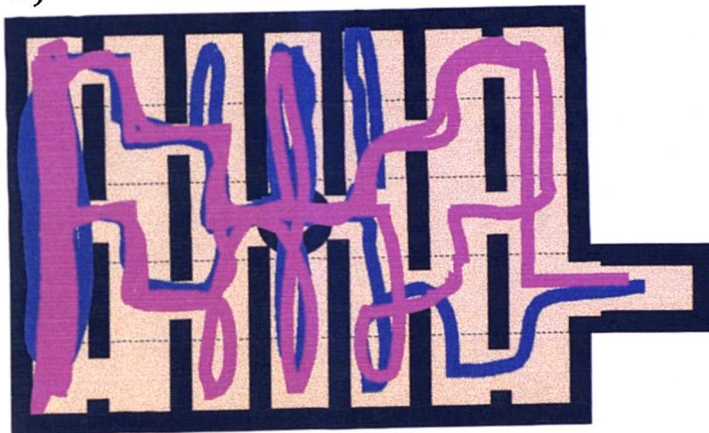


**Appendix 5** Paths travelled by each individual *L. pholis* on the journey from the start box to the reward box in Group 2, on Day 41 at the beginning of Treatment 6, immediately after the hide and the position of the experimenter was rotated 180°, **i)** Fish 9, 11, 14, 15, 16, **ii)** Fish 10 and 13.

i)



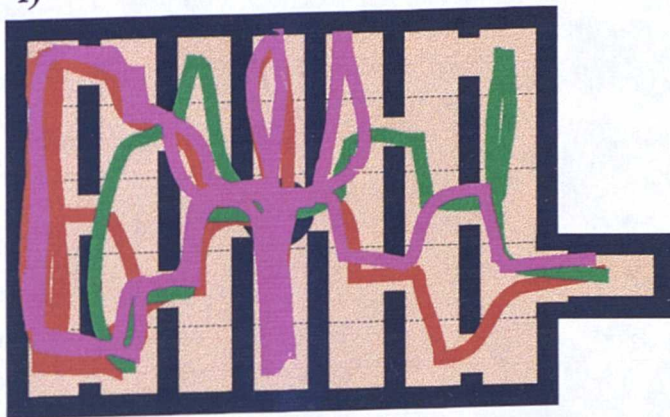
ii)



- Fish 9
- Fish 10
- Fish 11
- Fish 13
- Fish 14
- Fish 15
- Fish 16

**Appendix 6** Paths travelled by each individual *L. pholis* on the journey from the start box to the reward box in Group 2, on Day 42, Treatment 6, **i)** Fish 9, 11 and 13, **ii)** Fish 14, 15, 16.

i)



— Fish 9  
(Fish 10 did  
not complete  
the maze)

— Fish 11

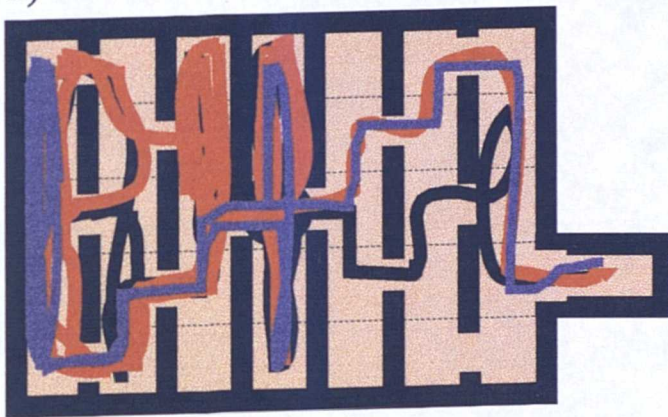
— Fish 13

— Fish 14

— Fish 15

— Fish 16

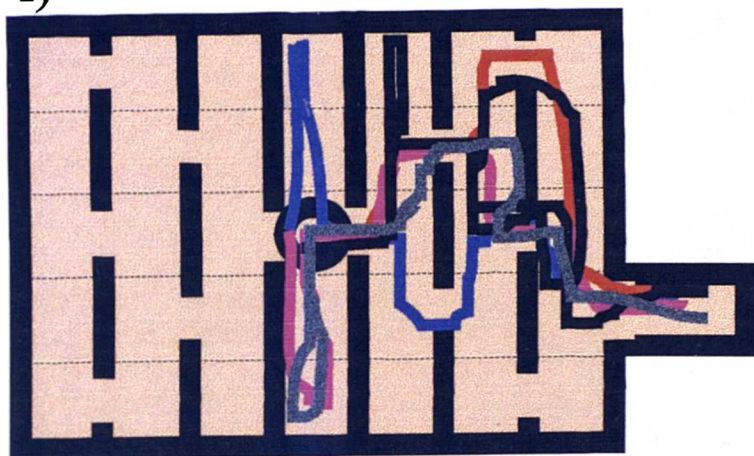
ii)



**Appendix 7** Paths travelled by each individual *L. pholis* on the journey from the start box to the reward box Group 2, on Day 49, Treatment 7, immediately after the hide was rotated back 180° and the experimenter began entering from side y, **i)** Fish 9,10 13 and 16, **ii)** Fish 11, 14 and 15.

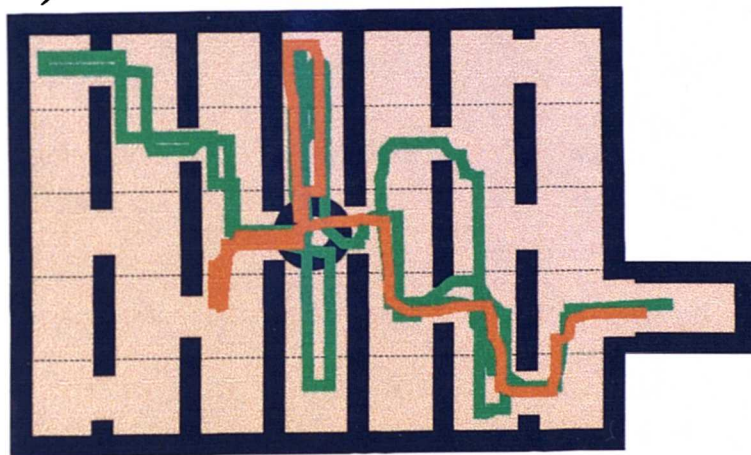


i)



- Fish 9
- Fish 10
- Fish 11
- Fish 13
- Fish 14
- Fish 15
- Fish 16

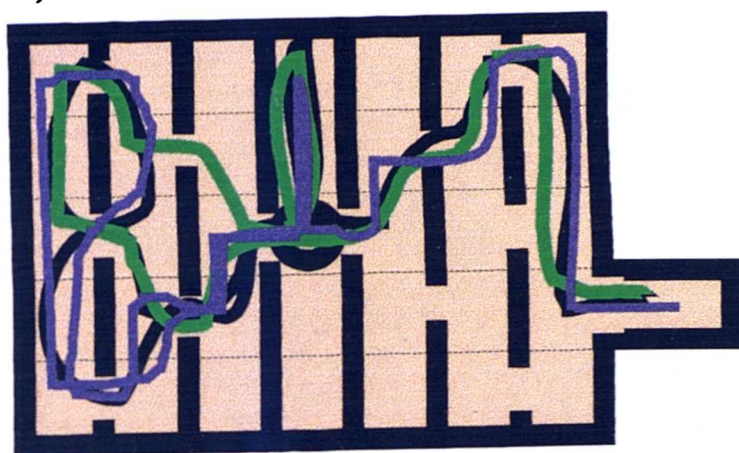
ii)





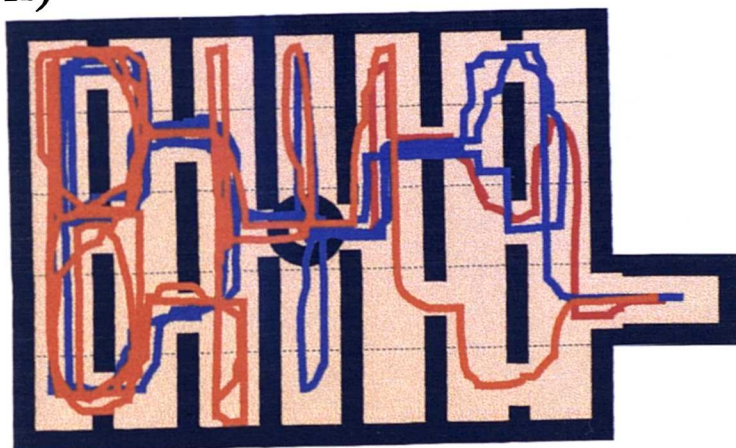
**Appendix 8** Paths travelled by each individual *L. pholis* on the journey from the start box to the reward box Group 2, on Day 50, Treatment 7, **i)** Fish 9, 11, 13, 14 and 16, **ii)** Fish 10 and 15.

i)

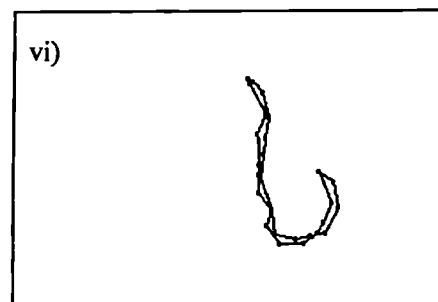
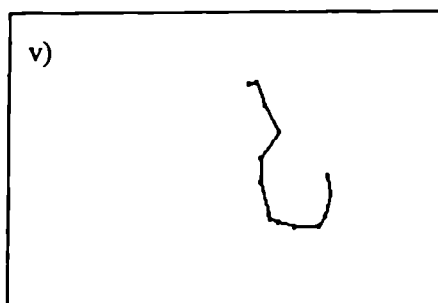
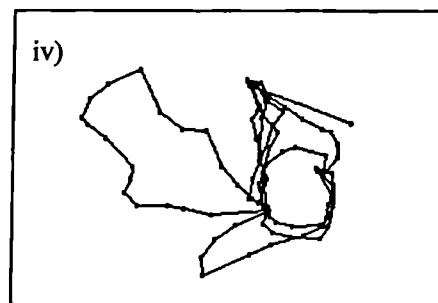
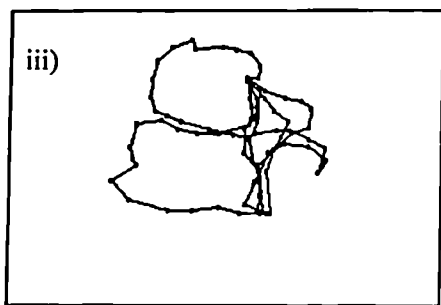
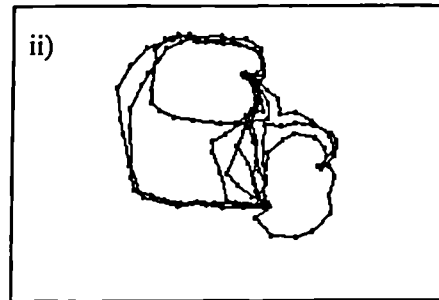
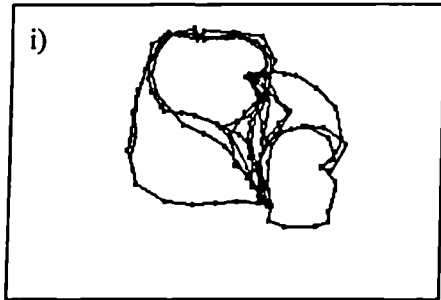


- Fish 9
- Fish 10
- Fish 11
- Fish 13
- Fish 14
- Fish 15
- Fish 16

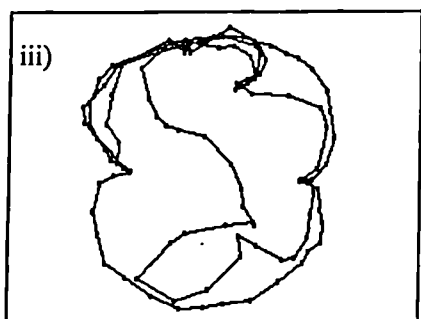
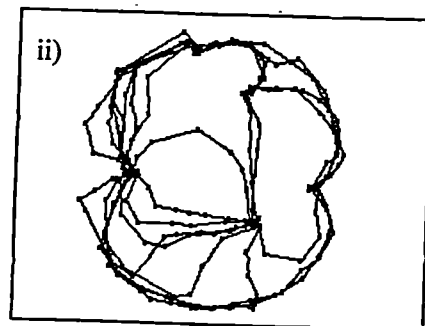
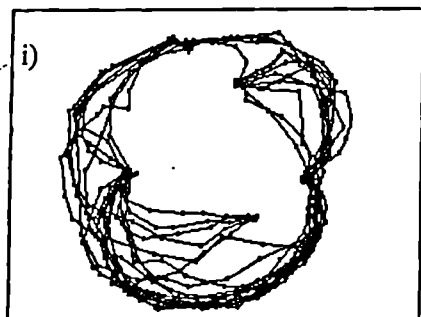
ii)



**Appendix 9** Path followed by Fish 4 in each hour of the Naive treatment, **i)** 0-1 hours, **ii)** 1-2 hours, **iii)** 2-3 hours, **iv)** 3-4 hours, **v)** 4-5 hours, **vi)** 5-6 hours.

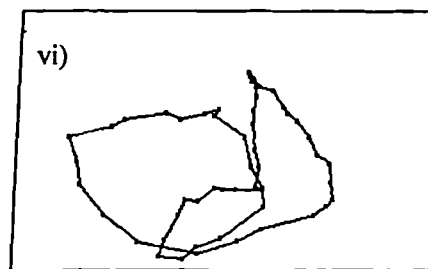
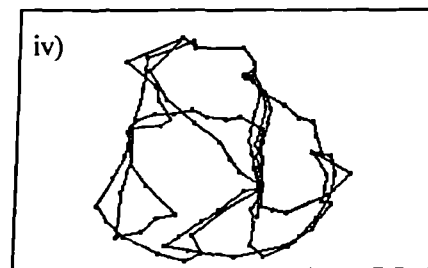
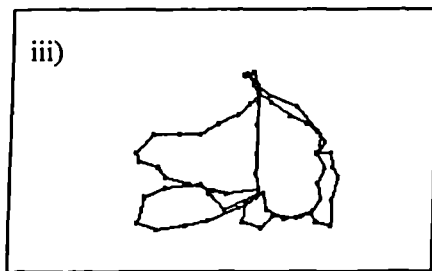
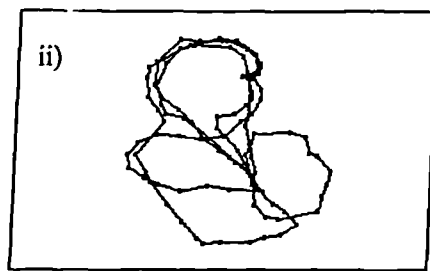
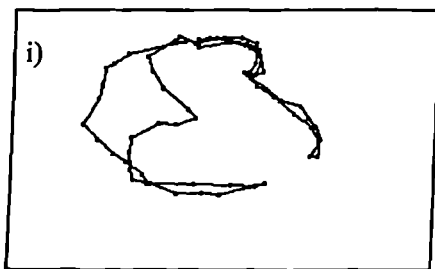


**Appendix 10** Path followed by Fish 7 in each hour of the Naive treatment, **i)** 0-1 hours, **ii)** 1-2 hours, **iii)** 2-3 hours, there was no movement after 3 hours.



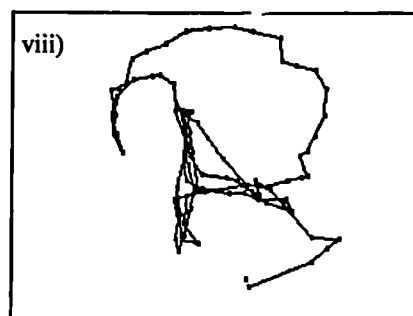
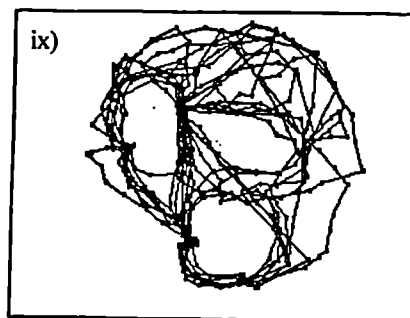
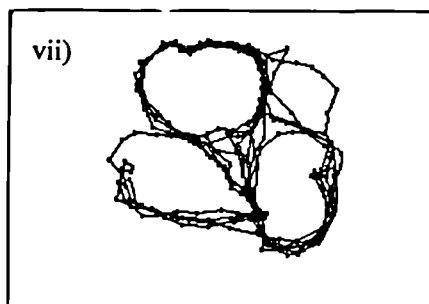
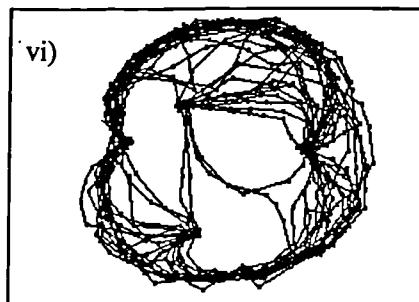
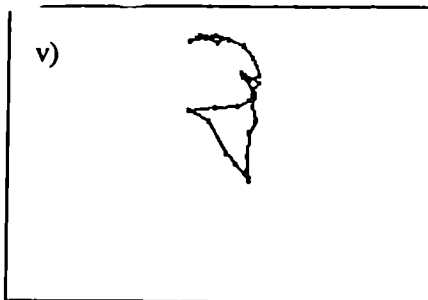
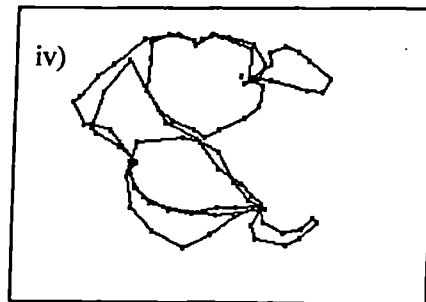
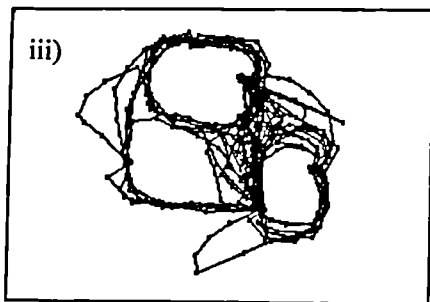
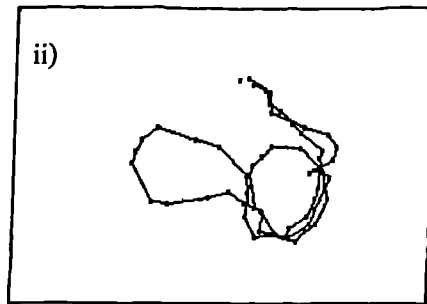
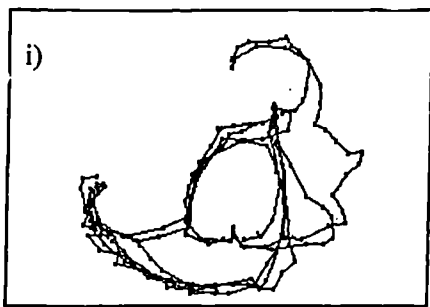
No further movement

**Appendix 11** Path followed by Fish 9 in each hour of the Naive treatment, **i)** 0-1 hours, **ii)** 1-2 hours, **iii)** 2-3 hours, **iv)** 3-4 hours, **v)** 4-5 hours, **vi)** 5-6 hours.



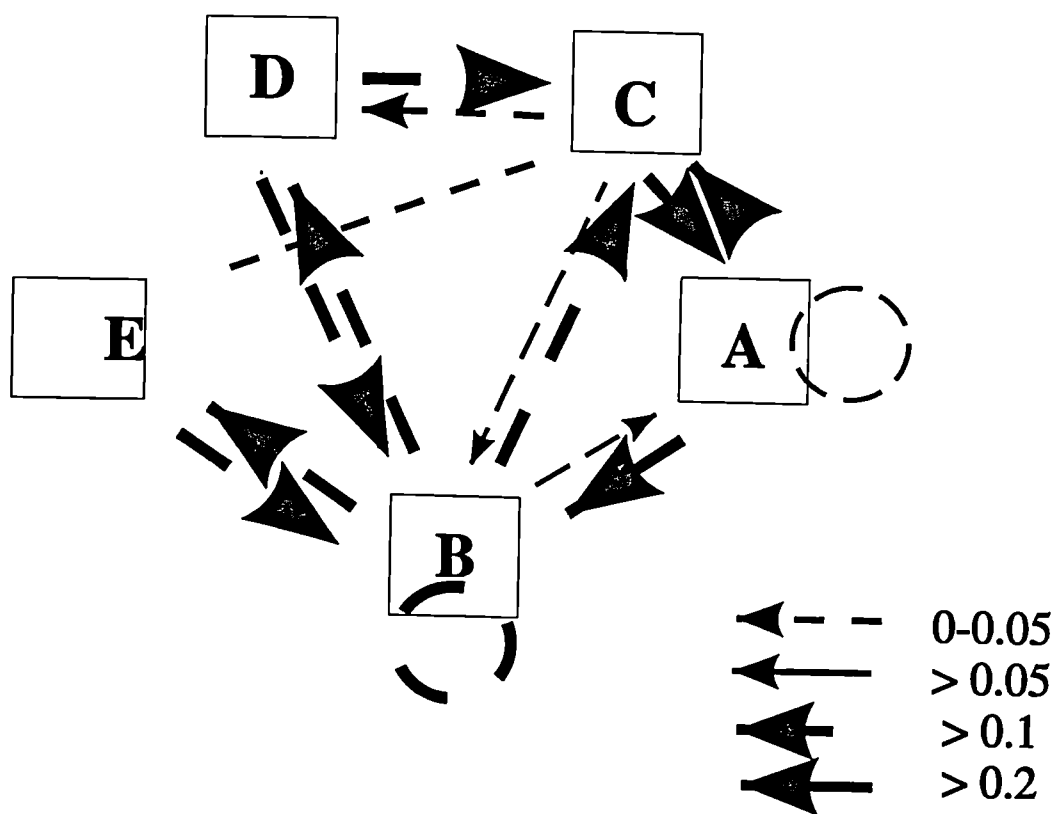


**Appendix 12** Path travelled by each Fish 1 and 3-10 during 6 hour Naive treatment, **i)** Fish 1, **ii)** Fish 3, **iii)** Fish 4, **iv)** Fish 5, **v)** Fish 6, **vi)** Fish 7, **vii)** Fish 8, **viii)** Fish 9, **ix)** Fish 10.

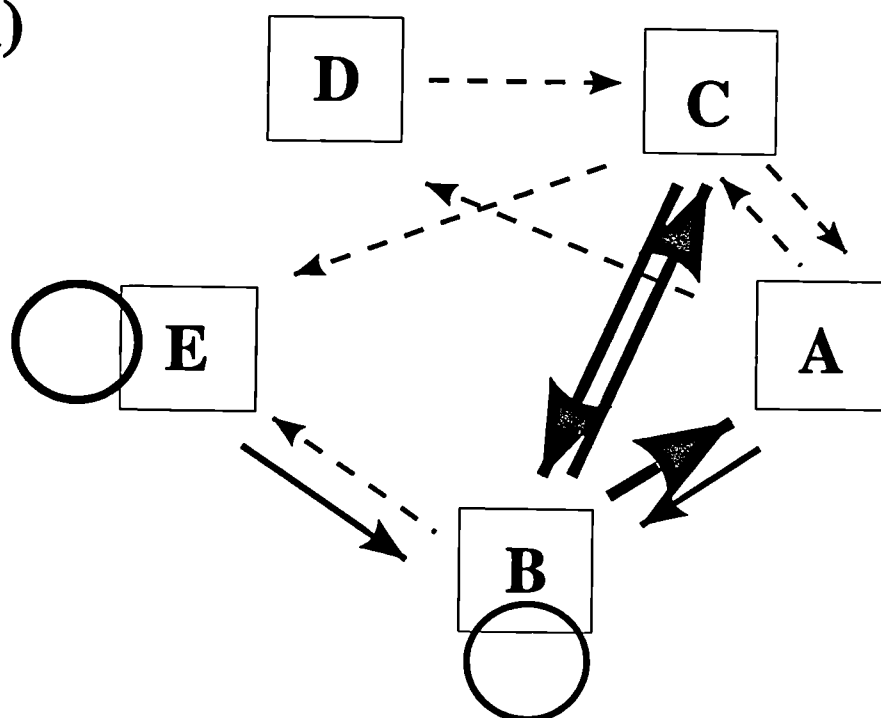


**Appendix 13** Transition diagram showing the proportion of total movements made between each pair of refuges for Fish 1 and 3-10 in Naive treatment, **i)** Fish 1, **ii)** Fish 3, **iii)** Fish 4, **iv)** Fish 5, **v)** Fish 6, **vi)** Fish 7, **vii)** Fish 8, **viii)** Fish 9, **ix)** Fish 10.

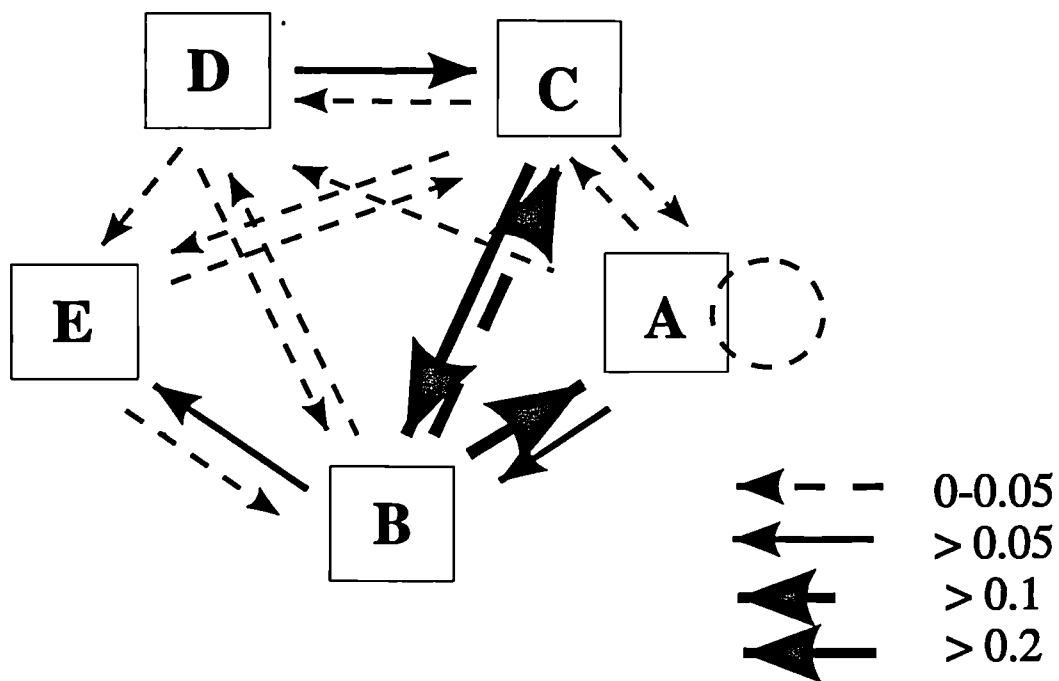
i)



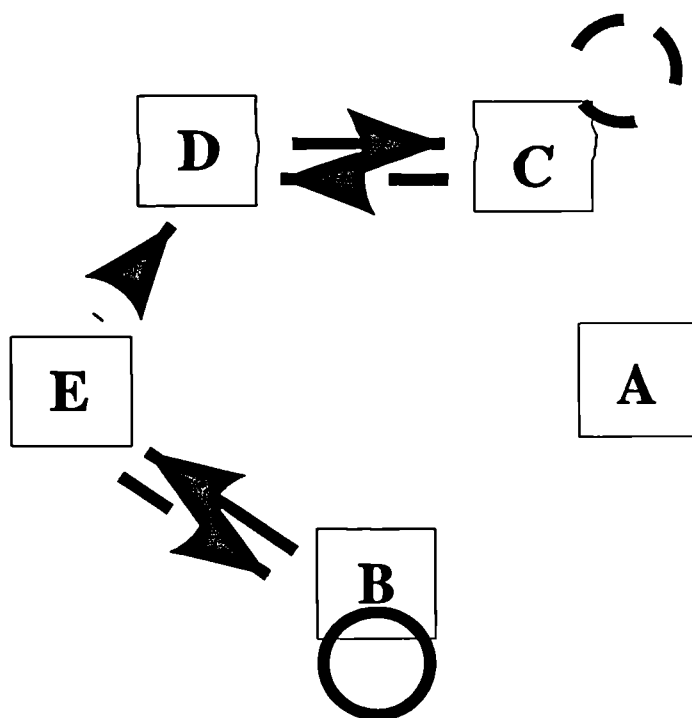
ii)



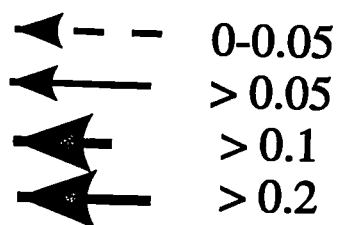
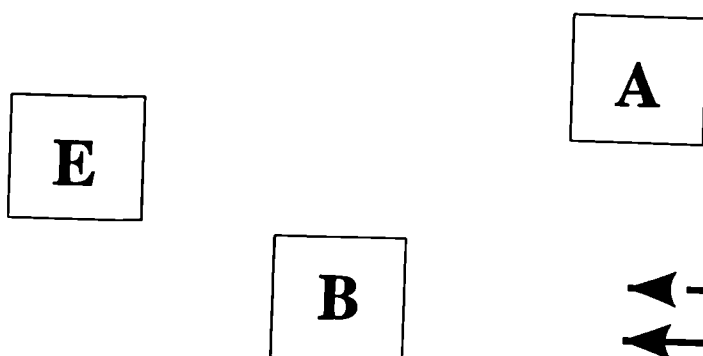
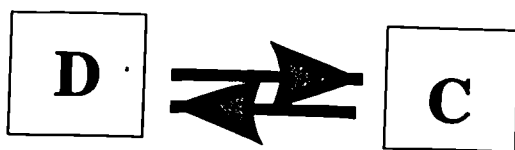
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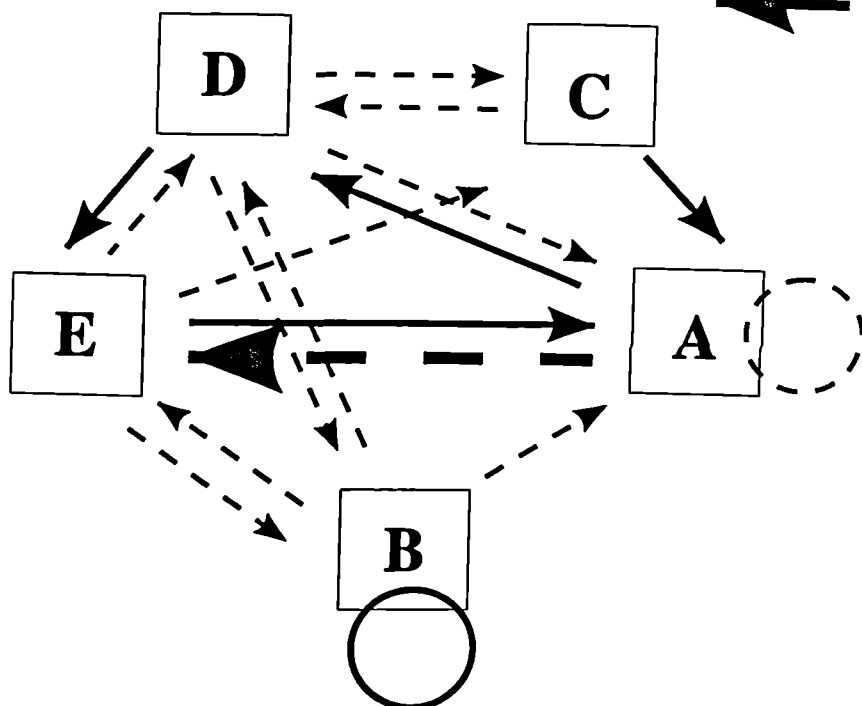
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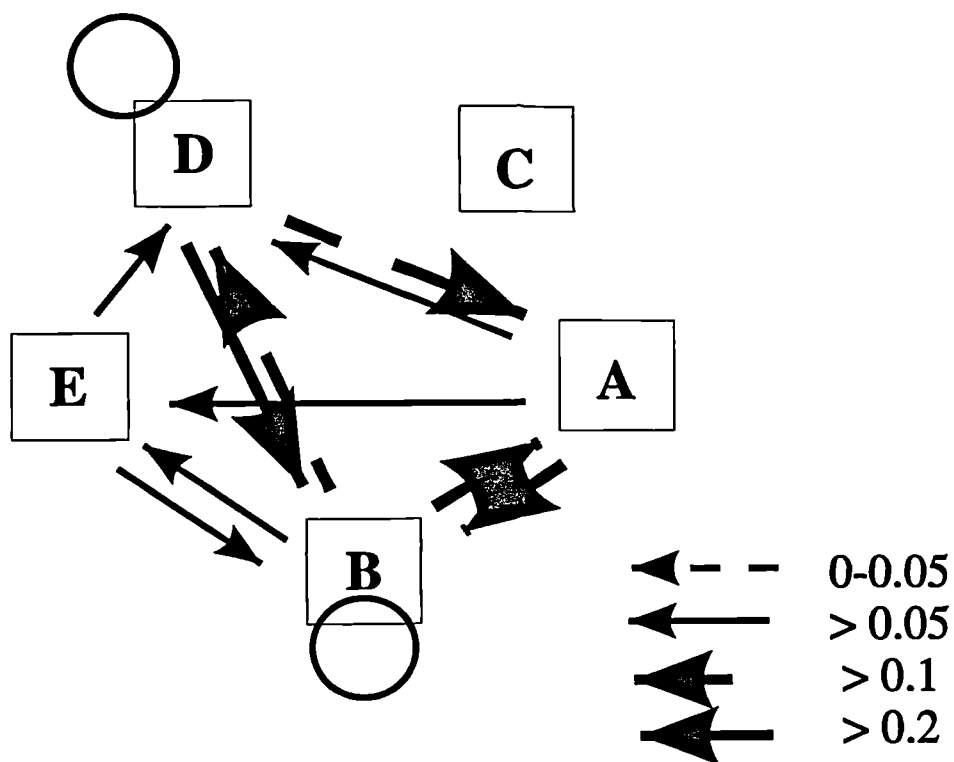
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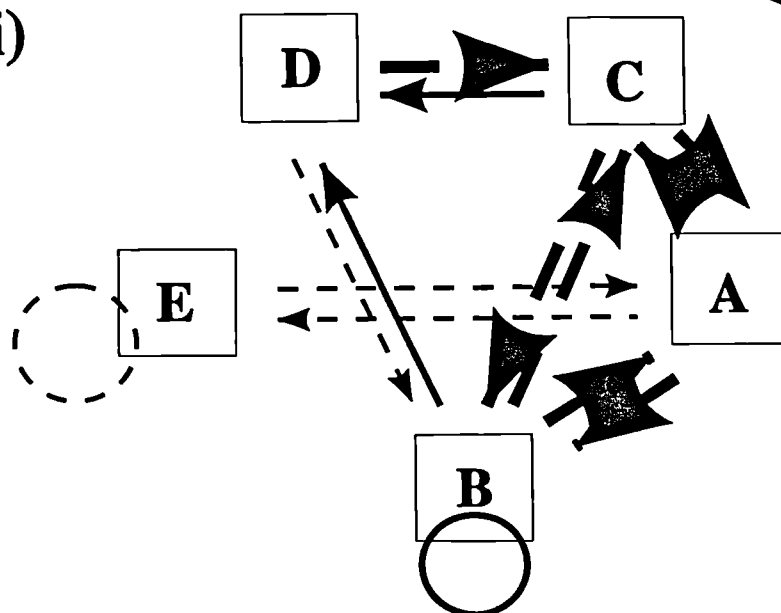
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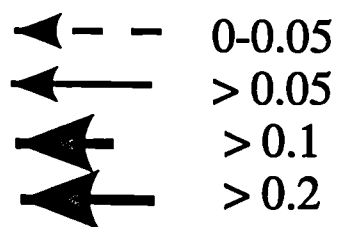
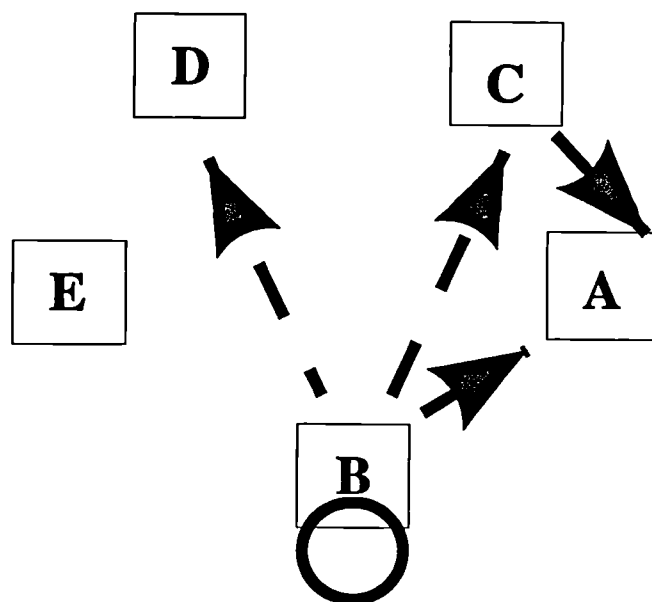
vii)



viii)

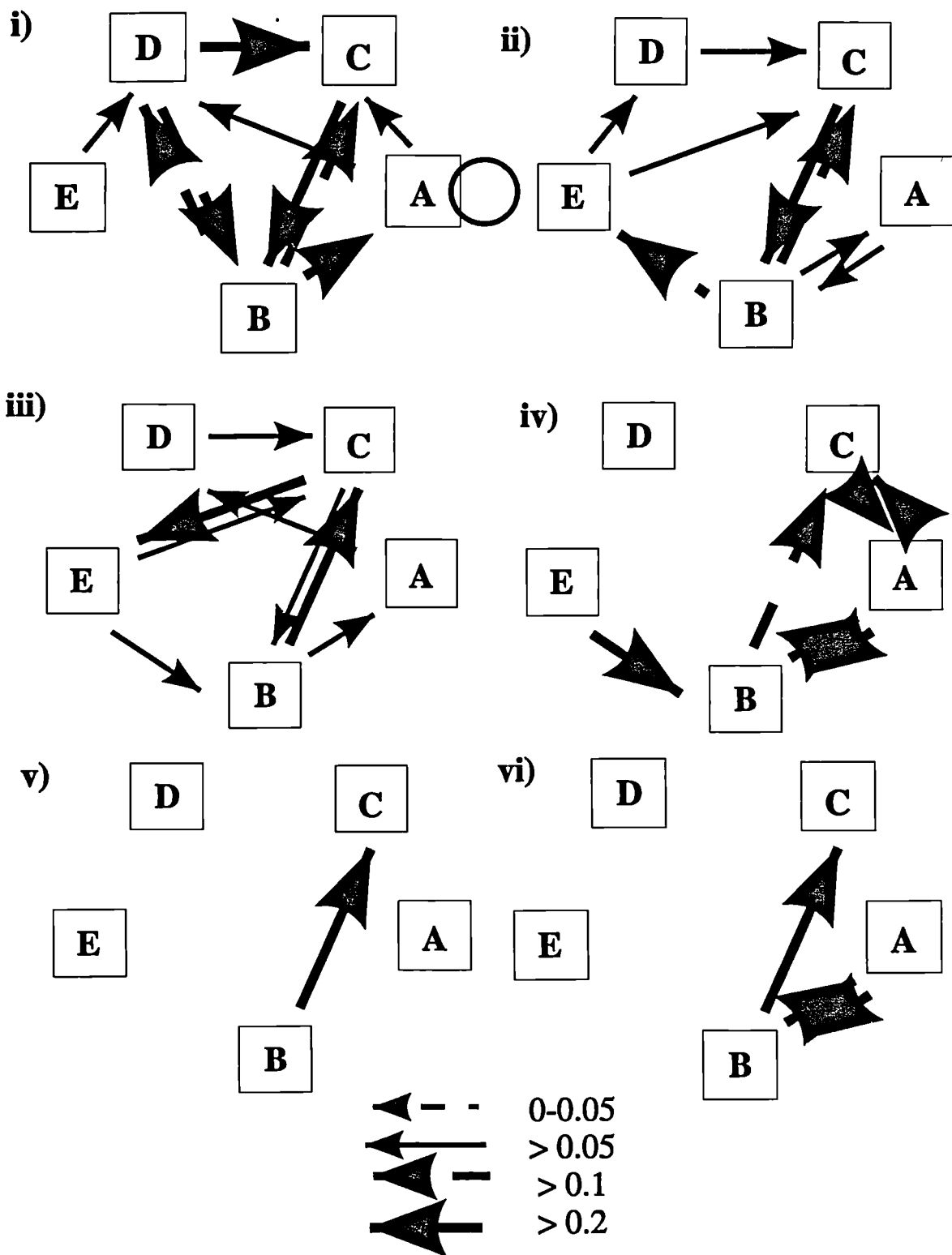


**ix)**

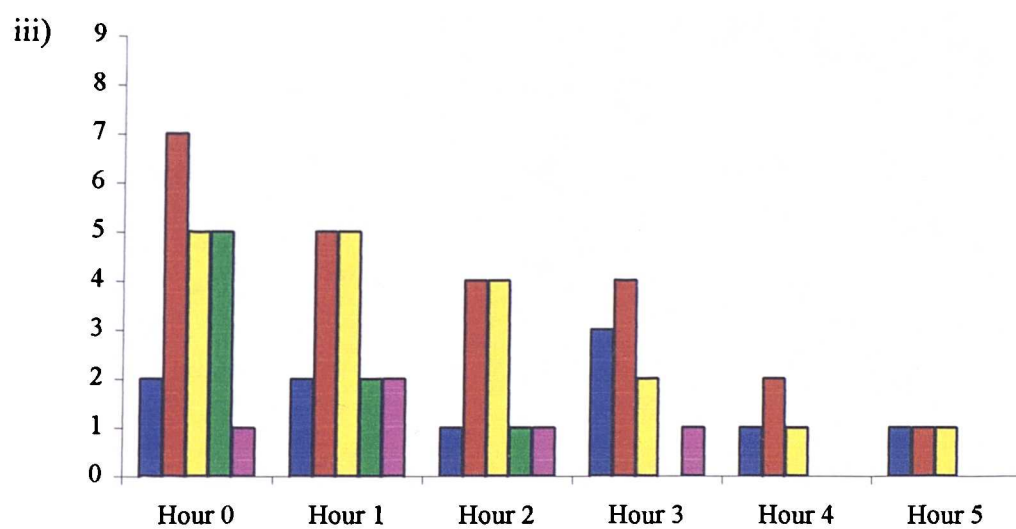
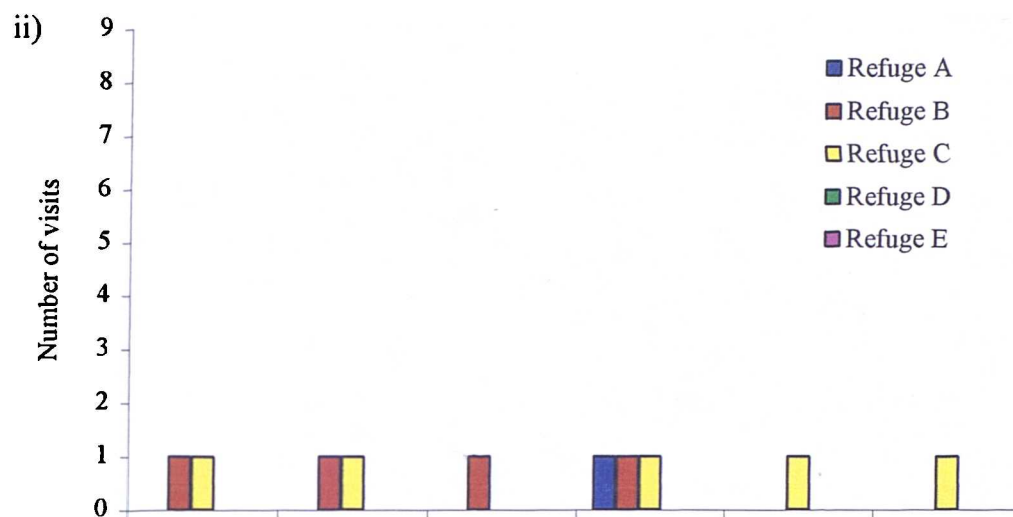
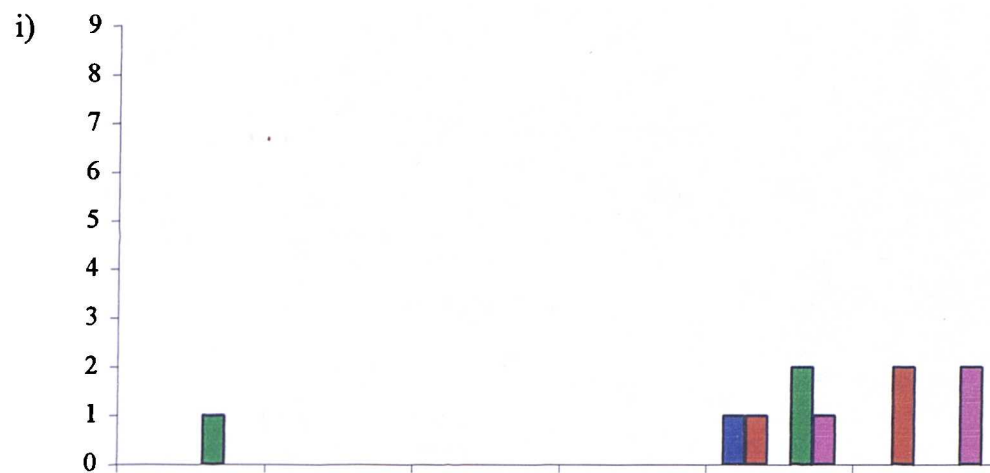


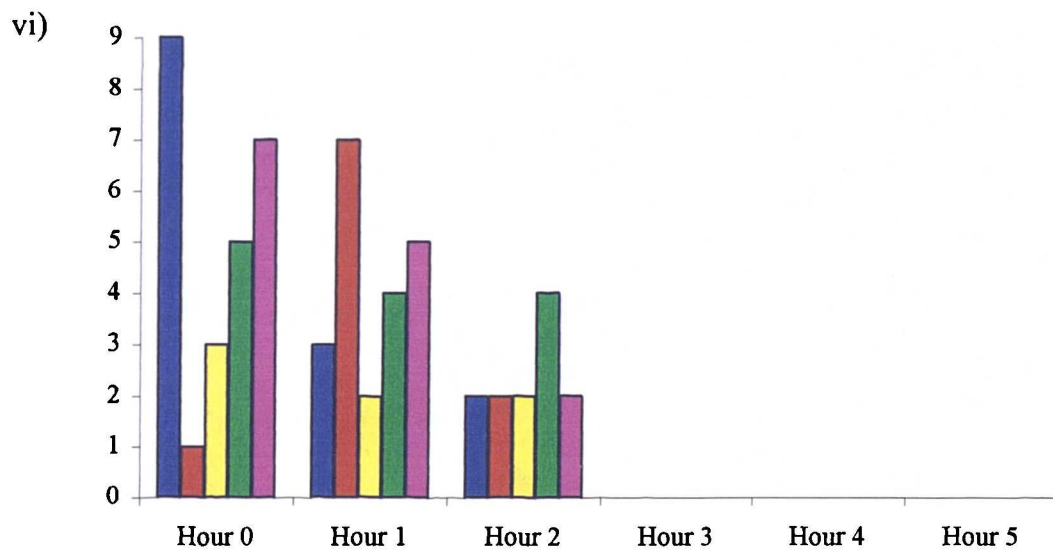
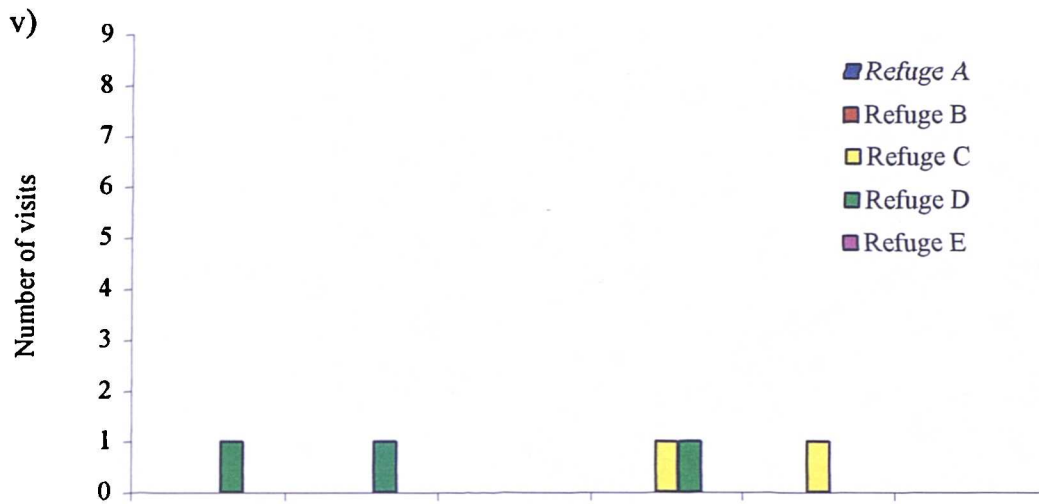
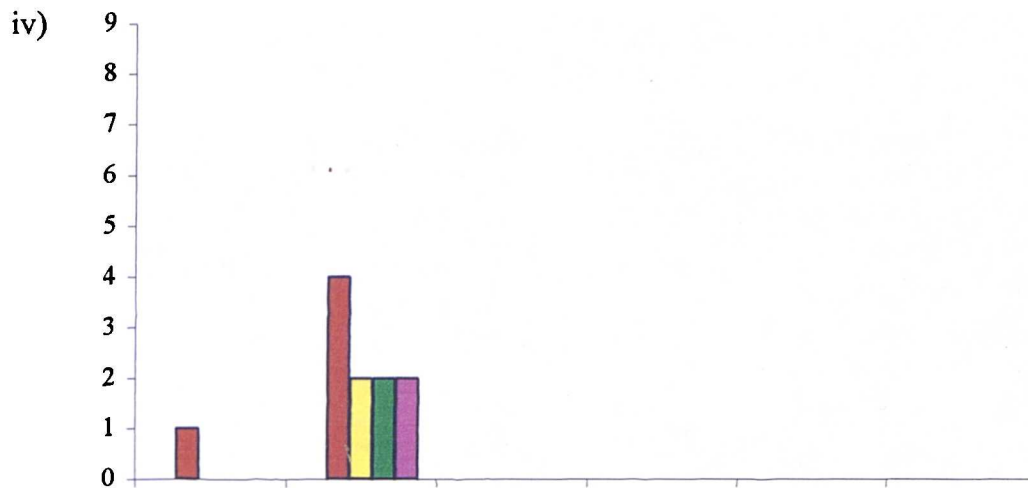


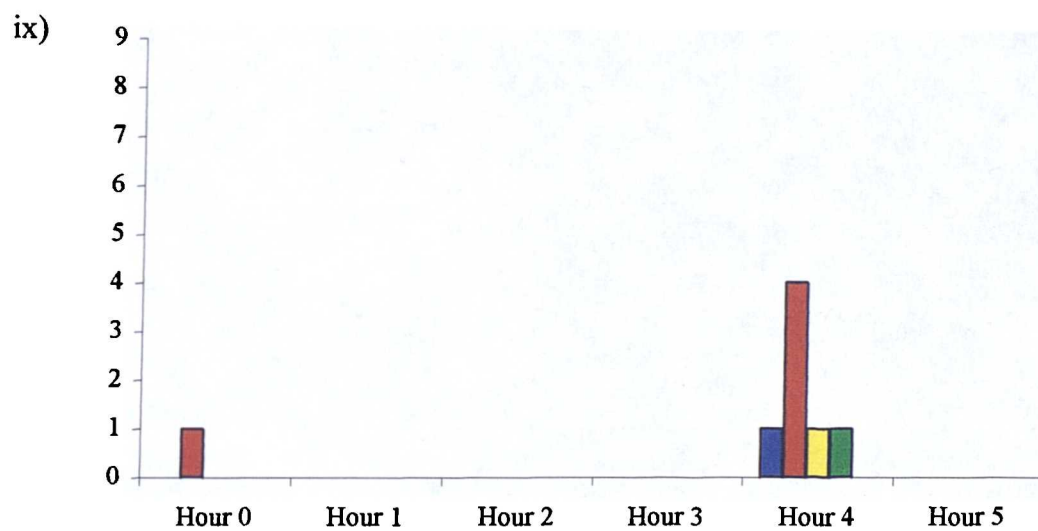
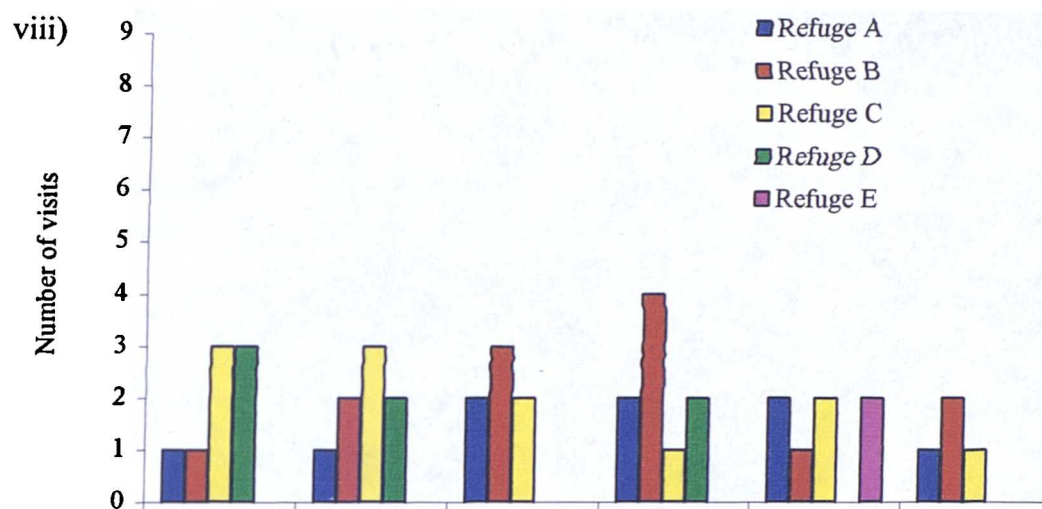
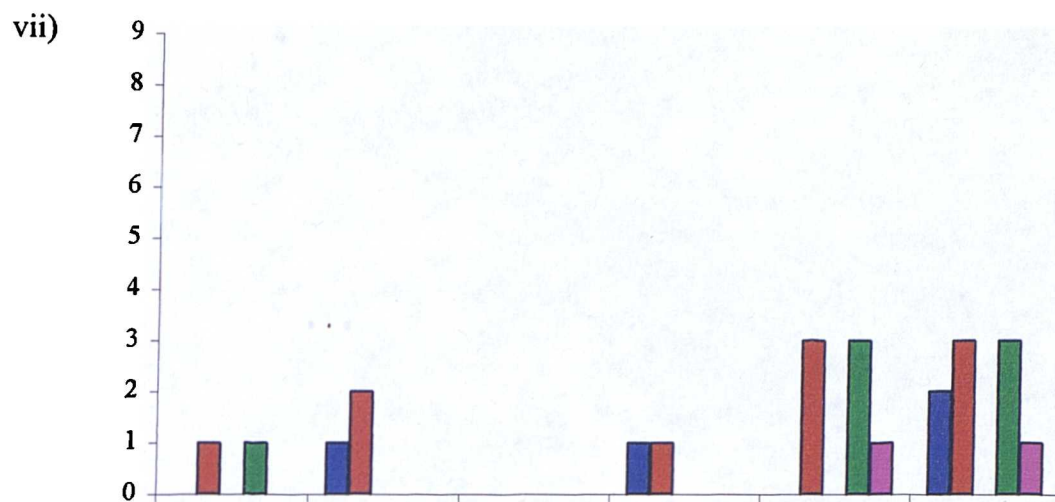
**Appendix 14** Transition diagram showing the proportion of total movements made between each pair of refuges for Fish 4 in each hour of Naive treatment, (no movement in hour 0-1), **i**) hour 0-1, **ii**) 1-2, **iii**) hour 2-3, **iv**) hour 3-4, **v**) hour 4-5, **vi**) hour 5-6.



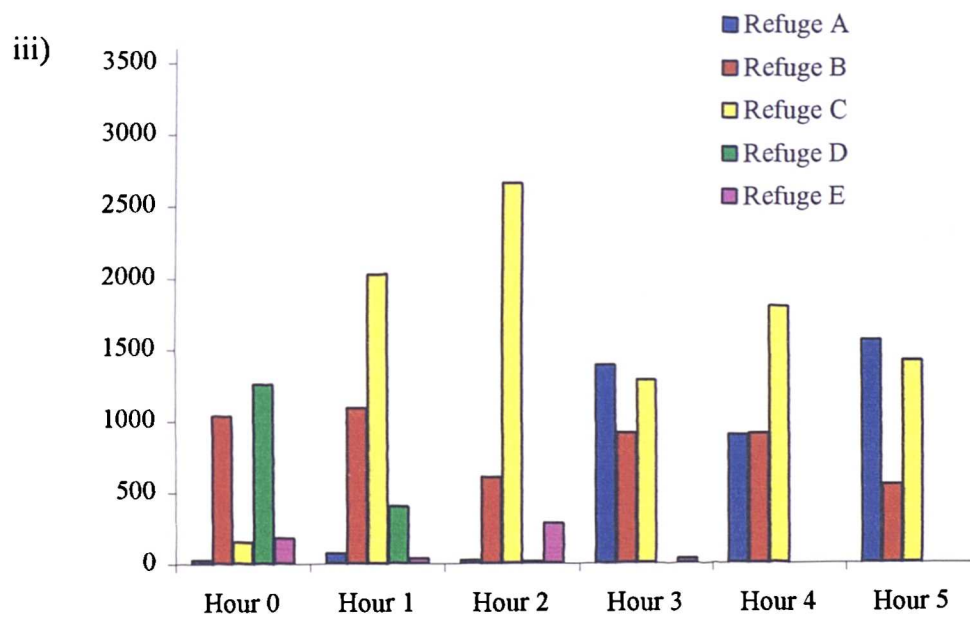
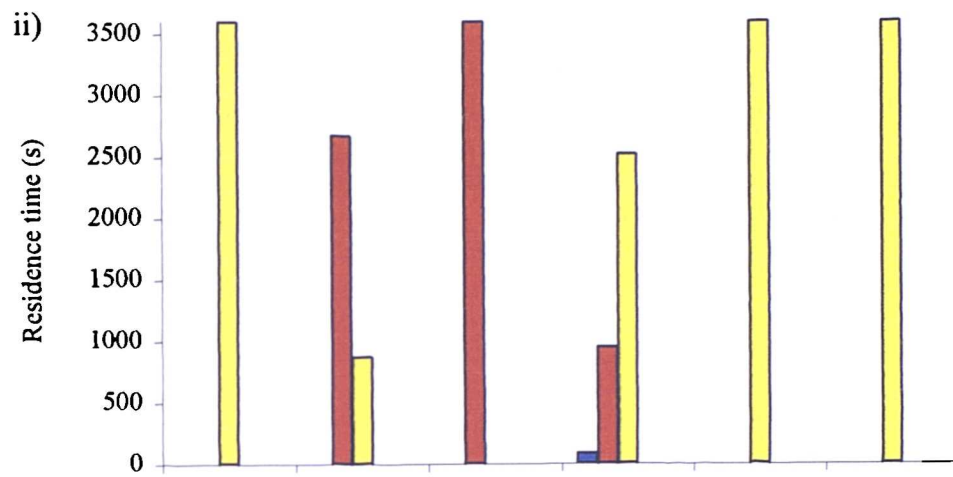
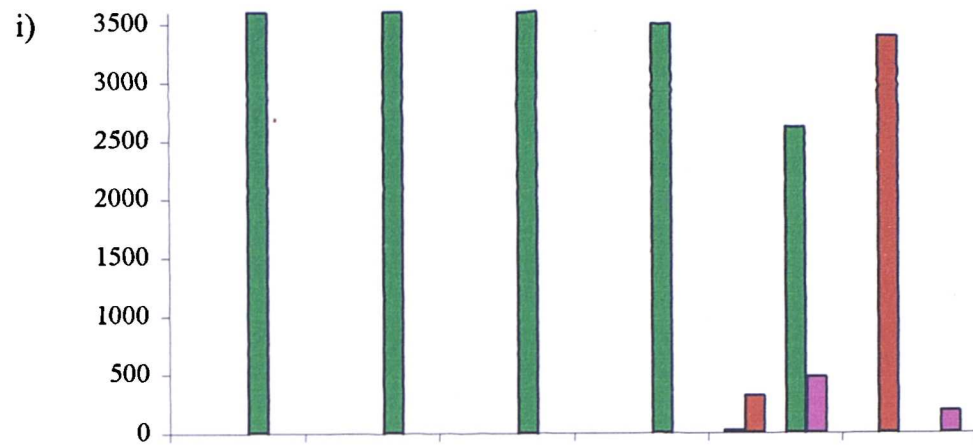
**Appendix 15** The number of visits to each refuge in each hour of the Naive treatment, **i)** Fish 1, **ii)** Fish 3, **iii)** Fish 4, **iv)** Fish 5, **v)** Fish 6, **vi)** Fish 7, **vii)** Fish 8, **viii)** Fish 9, **ix)** Fish 10.



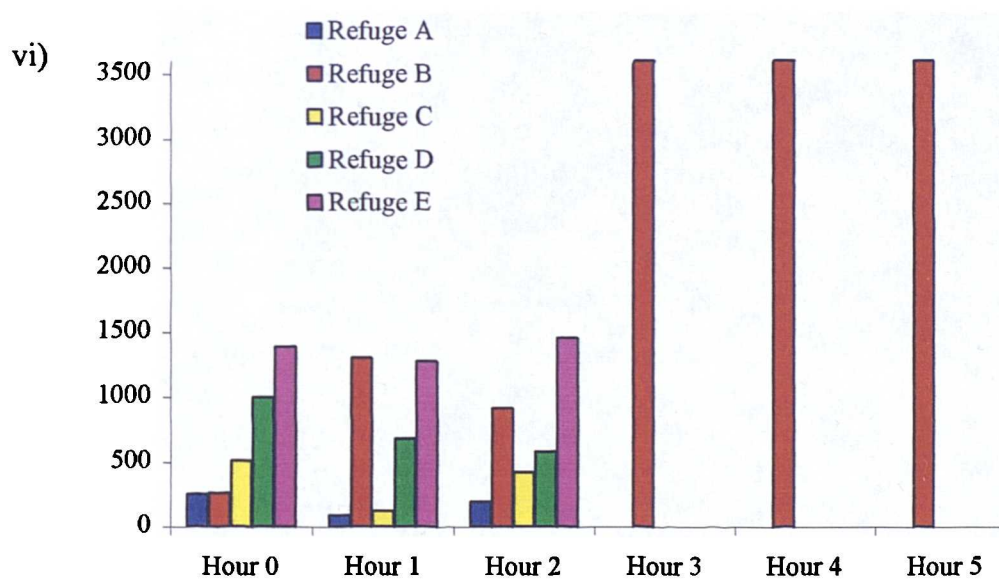
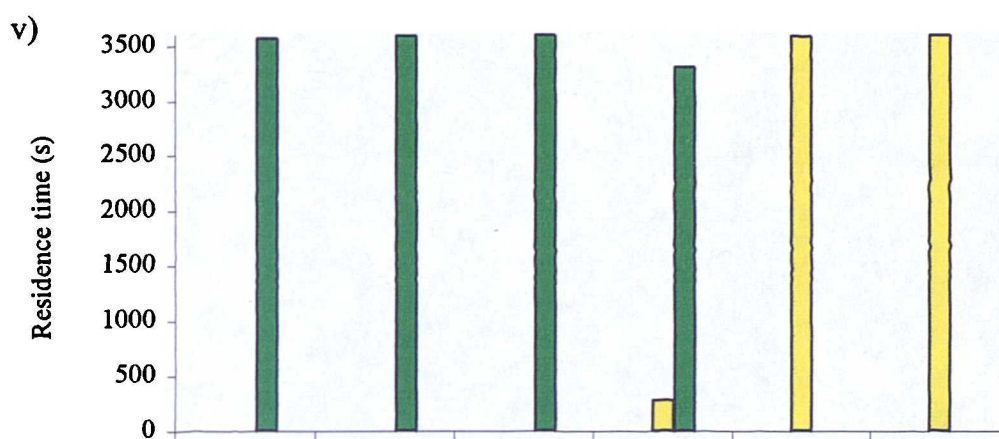
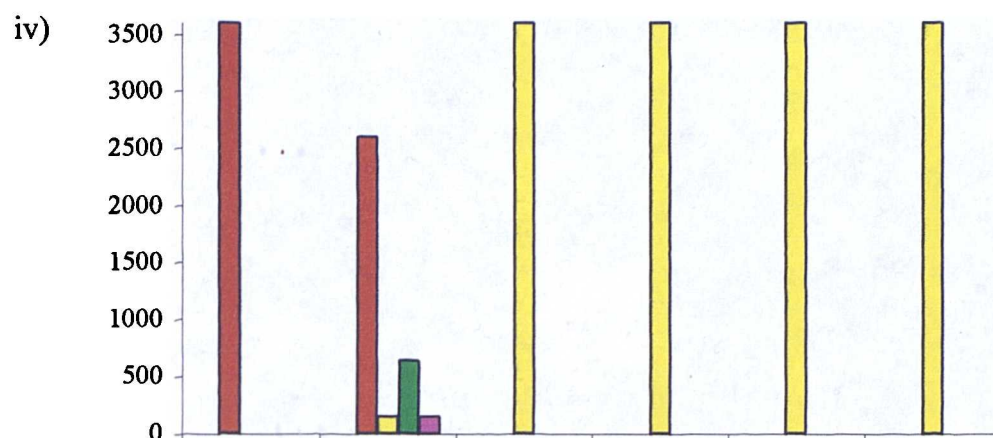


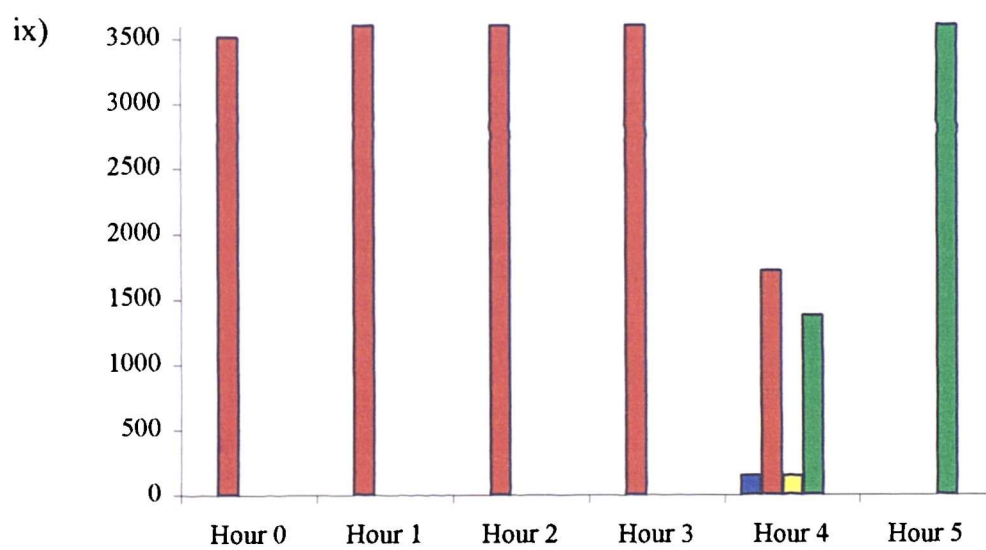
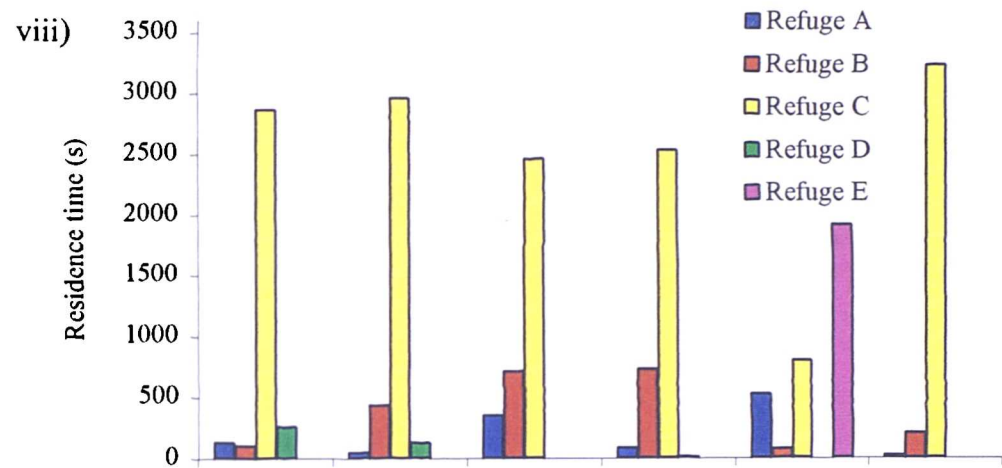
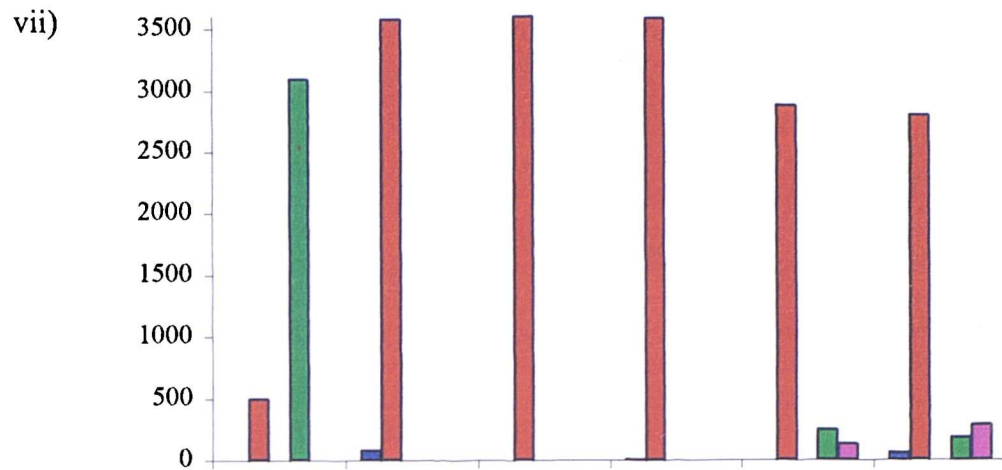


**Appendix 16** Residence time in each refuge in each hour of the Naive treatment **i** Fish 1, **ii**) Fish 3, **iii**) Fish 4, **iv**) Fish 5, **v**) Fish 6, **vi**): Fish 7, **vii**) Fish 8, **viii**) Fish 9 **ix**) Fish 10.

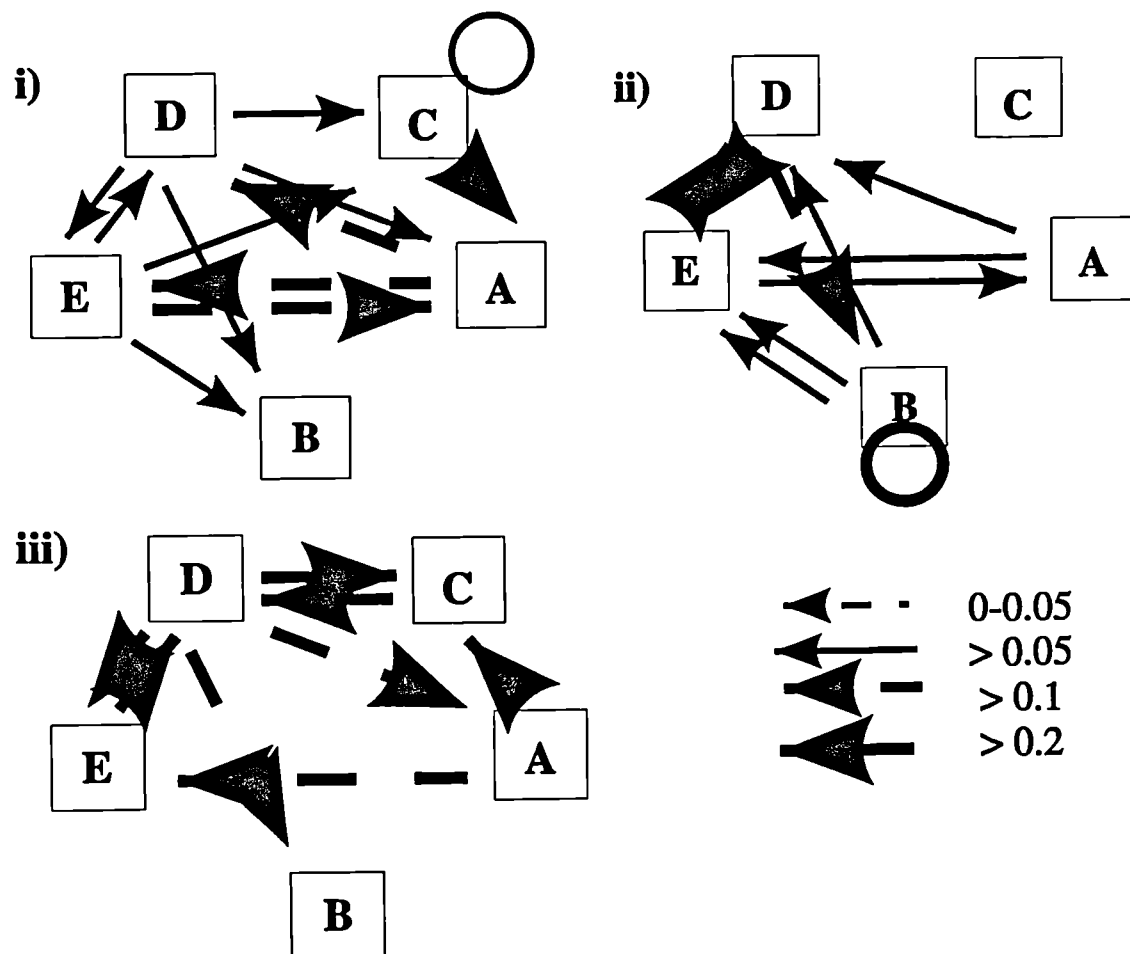




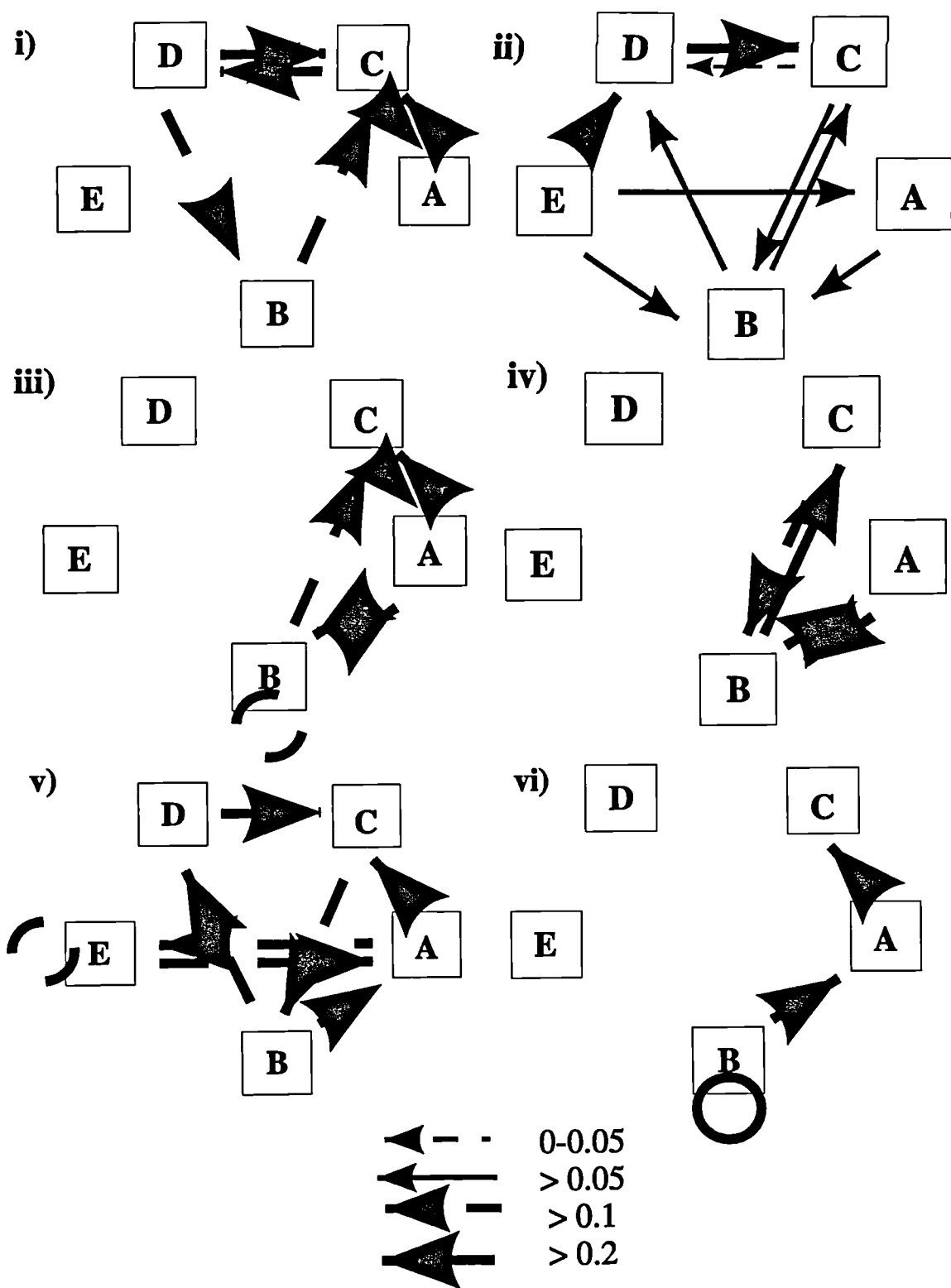




**Appendix 17** Transition diagram showing the proportion of total movements made between each pair of refuges for Fish 7 in each hour of Naïve treatment, (no movement in hour 0-1), **i)** hour 0-1, **ii)** 1-2, **iii)** hour 2-3, **iv)** hour 3-4, there was no movement after 3 hours.



**Appendix 18** Transition diagram showing the proportion of total movements made between each pair of refuges for Fish 9 in each hour of Naive treatment, (no movement in hour 0-1), **i)** hour 0-1, **ii)** 1-2, **iii)** hour 2-3, **iv)** hour 3-4, **v)** hour 4-5, **vi)** hour 5-6.



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