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A test of time-place learning in fish living in a public aquarium

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Abstract

This study presents the findings of a test of time-place learning in Atlantic Ocean fish in the National Marine Aquarium in Plymouth (a public aquarium). All the fish, which varied in sizes and species, were observed, excluding ocean predators. Observations of the fish's location and behaviour five weeks prior and during the three-week intervention period were carried out. The quantitative data does show major differences in the distribution of the fish, a 100% increase in the fish being evenly distributed across the tank. Results suggest that some fish are capable of exhibiting time-place learning.

Ethical Considerations
The University of Plymouth Animal Research Committee approved the design of this experiment. All procedures were carried out in accordance with the British Psychological Society guidelines. The National Marine Aquarium also approved this experiment. The design of this experiment did not require a license from the Animals (Scientific Procedures) Act 1986.

Due to the change in feeding routines, the large predatory animals in the aquarium may have posed a threat to the smaller fish as they learned to anticipate feeding time and location, as the predators may have also learnt to predict the aggregation of potential prey. If such problems arose, the study would have been ceased immediately.

The researchers and research apprentices have collected all data reported in this project. All the data collected has been shared with the researchers of this project.

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Introduction
Much research has been directed at the behaviour of fish in response to consistent feeding schedules. Fish specifically selected for use in laboratories have displayed behaviours associated with anticipation of food and time-place learning, which reflects their natural behaviour in the wild. Fish in public aquariums have also been found to display such anticipatory behaviours, but there is currently no research in this field.

The movements of the sun and the moon have created a temporal structure for the physiology and behaviour of organisms. It has been found that nearly all eukaryotic organisms show signs of a daily rhythm. This daily rhythm that has a span of approximately 24 hours and is synchronised to the solar day is called a circadian rhythm (Bolhuis & Giraldeau, 2009). Circadian rhythms are exposed to daily variations in temperature, light intensity, and humidity;

however, it has been found that animals kept in temporal isolation continue to display daily rhythms of behaviour (Reebs, 1994).

This finding suggests that animals not only use circadian rhythms to tell the time, but that they also have an endogenous timing device known as an internal clock, which allows them to work out what time of day it is without having to use the position of the sun or the moon, temperature, or light intensity. An internal clock usually has a periodicity of about 24 hours, which not only dictates wake and sleep onset but also enables fish to retrieve food at the appropriate time and avoid predators, both of which are crucial for survival. A model illustrated by Reebs and Lague (2000) called the "energetic hourglass" can be used to explain the daily food anticipatory activity (FAA) behaviour in fish. The model suggests that activity levels in fish could rise with hunger and the fish would manage their energy reserves so that they would only begin to feel hunger between 20 and 24 hours after their last meal, leading to the assumption that the internal clock is reset to zero at each specific daily activity such as being fed.

To test the presence of an internal clock in fish, Reebs (1994) kept Convict cichlids *(Cichlasoma nigrofasciatum)* in windowless rooms where the lights were turned off completely every evening without warning. The convict parents in the wild would normally begin to retrieve their young when night approaches and spit them into their pit so they can guard and defend against nocturnal predators after nightfall. In the study, the convict parents began to retrieve their young 15-20 minutes before the lights were turned off when there was no other cue to imply darkness was coming, thus displaying the presence and use of an internal clock.

It has been discovered that fish and other animals both in captivity and in the wild, display behaviours that are associated to the anticipation of being fed. Fish have various ways of showing their anticipation such as increasing their swimming activity, or they may come out of hiding (Reebs, 2001). Research by Davis and Bardach (1965) was the first serious study on anticipation and it was found that Atlantic tomcod *(Microgadus tomcod)* and Scup *(Stenotomus versicolor)* became noticeably more active two-six hours before feeding time and not at other times. The other interesting finding from this study was that even on days when food was withheld, the fish still displayed the same FAA at the time that they were used to being fed at, supporting the notion of food anticipation. However, pre-feeding activity may not be solely a direct result of anticipation to food delivery using circadian rhythms; it could also be in response to regular daily feeding which may be the consequence of conditioning the act of eating to an endogenous cue.

Following this study, further research has been directed towards FAA in fish. Spieler and Noeske (1984, as cited in Reebs, 2001) researched this behaviour and used Goldfish *(Carassius auratus)* in their experiment. It was found that the fish were only active around the hour at which they were usually fed, including the days when food was not given. This occurred at all usual feeding times whether it was during the day, night or near lights on or lights off. As food was not delivered on certain days, the increase in activity a few hours before normal feeding time could not be in response to presence of food. Interestingly, the goldfish decreased their activity after the normal feeding time had passed. This was not because they were no longer hungry as no food had been eaten, suggesting it was their internal clock telling them that feeding time had ceased.

Further research by Vera et al. (2007) presented findings that suggested that FAA in fish had beneficial outcomes. The fish used in their study were goldfish and they examined the presence of FAA and whether digestive enzymes were entrained by the periodic feeding in goldfish. The results from the study revealed that a scheduled feeding regime entrained certain physiological patterns in goldfish in which FAA enabled fish to prepare the digestive physiology in readiness for the arrival of food. The ability to anticipate the arrival of food allows fish to avoid foraging at times when they are unlikely to provide rewards.

As well as being able to anticipate the arrival of food, fish have also been found to display time-place learning. Time-place learning is the ability to associate places at specific times of the day. It has been suggested that time-place learning can be very advantageous for fish as it may serve to be an important function in survival if food availability if restricted, it would be very important for the fish to synchronise feeding activity with food availability to increase their chances of obtaining food (Barreto, Rodrigues, Luchiari & Delicio, 2006). With the absence of reliable cues such as sun height, temperature and light intensity, fish must use a continuously consulted internal clock to distinguish between various times of day.

In Reebs' 1996 study, the effects of changing food delivery was tested on eight Golden shiners *(Notemigonus crysoleucas)* that were kept in a windowless laboratory in one long tank divided into two sections that still allowed passage of fish from one side to the other but shielded most of the fish's view so they were unable to see activity on the other side of the tank. They were under a light-dark cycle with abrupt changes from 12 hours of light to 12 hours of darkness. The fish were fed for three weeks using automated feeders that delivered small flakes of food at the surface on the left side of the tank in the morning, and on the right side in the afternoon. After the three-week period, food was withheld for a day and the position of the fish was recorded for the whole day. It was found that most of the fish took the correct position in the tank at the appropriate time of the day, the left in the morning and the right in the afternoon. Furthermore, when a third time was introduced to the feeding regime, the fish continued to follow the pattern of where the food was to be delivered on test days when food was not presented.

Further research conducted by Chen and Tabata (2002) aimed their investigation towards the ability of individual rainbow trout (*Oncorhynchus mykiss*) to display time-place learning and whether time restricted feeding schedules and light-dark cycles had an influence on self-feeding activity. Their findings demonstrated that a single fish is capable of anticipating where and when food will be available and that the FAA was very short and precise in comparison to previous studies. The FAA of rainbow trout recorded in this study ranged from 11 minutes to 27 minutes prior to feeding, where as the FAA recorded in Davis and Bardach (1965) found that Atlantic tomcod and Scup became noticeably more active between two and six hours prior to feeding.

The findings from previous research suggest that fish are able to anticipate the arrival of food with the use of their internal clock or the circadian rhythm, or with the presence of reliable cues.

Previous research suggests that many species of fish show FAA and many are able to display time place learning, however do the findings suggest that all fish are able to learn where and when food will be present or is it just a select few that are able to learn and other fish follow them to where the food will be? Research by Reebs (2000) investigated the leadership of individual fish in shoals of golden shiners. To test the leadership, fish that had been trained to know where food would be presented were combined with a larger number of fish who were new to the tank, therefore they were untrained. The aim of the study was examine whether the trained fish were able to lead the untrained fish to the feeding area at the appropriate time. Evidence from this study revealed that it is possible for a minority of trained individual fish to lead a shoal of fish to where food would be available.

The welfare of animals kept in captivity has been widely studied. Behavioural activity that may be a direct result of time-place learning may have welfare implications on fish in captivity.

It is well known that fish and other animals such as primates have been used for research and recreation for many hundreds of years, but little is known about the effect living in captivity may have on fish, as this is mainly because they are viewed as non sentient beings (Soo & Todd, 2009).

Waitt and Buchanan-Smith (2001) conducted a study using primates, stump-tailed macaques (*Macaca arctoides*), and the findings can be applied to fish. This experiment aimed to assess how the anticipation of feeding times and the delays of feeding routines would affect the primates. The results from their study revealed that when the primates were waiting to be fed, there was an increase in self-directed, agnostic and abnormal behaviours that could all be the result of the animal being in a stressful and frustrating situation. From this it can be assumed that delaying a scheduled feeding time could be the cause for stress and social disruption for animals.

For fish living in the wild, the availability of food may vary during the day (Reebs, 1993). For example some fish feed on insects that are active only at specific hours of the day, or on phytoplankton that is more nutritious at specific times of the day (Zoufal & Taborsky, 1991, as cited in Reebs, 2001). With this in mind, would it be beneficial for fish in captivity to receive food at fixed times or at random times? As feeding is obviously essential to the physical well being of animals, the effects of predictability on psychological well-being is still being debated. Feeding animals at fixed times may provide security, but on the other hand it may lead to FAA, which is characterised by increased arousal and activity, that occurs on a day-to-day basis, several hours before feeding when food is delivered at the same time each day (Reebs & Lague, 2000).

Findings by Basset and Buchannan-Smith (2007) suggested that animals should be fed on unpredictable feeding schedules and unreliable signals related to feeding be eliminated such as the sound of food preparation. It was found that pigs that were constantly given unreliable feeding signals showed a significant increase in aggressive interactions after unexpected disturbances in their environment. It was suggested that the pigs were interpreting the unexpected noises as feeding signals, which lead to frustration and aggression, as food did not appear after the unexpected noise. This finding can be applied to fish in captivity.

Following this research, Sanchez, Lopez-Olmeda, Blanco-Vives and Sanchez-Vazques (2009) examined the effects of scheduled day time feeding compared to random feeding times in sea bream (*Sparus aurata*). It was found that the fish in the random daytime feed group maintained higher activity throughout the day suggesting that they were unable to predict the arrival of food which meant that the fish were constantly alert so as not to miss the arrival of food. This behaviour of the fish would be energetically demanding and uneconomical for the fish. Another finding from this research revealed the intense stress levels that the fish in the random daytime feeding group experienced. In comparison to the scheduled daytime feeding group, the random daytime feeding group showed 10-fold higher levels of cortisol indicating that the fish being fed at random times were under immense stress. Their findings suggest that the chaotic feeding regime of random feeding times could lead to the loss of temporal interrogation and could lead to undesirable negative effects.

The National Marine Aquarium in Plymouth found that the fish in their Atlantic Ocean tank would anticipate the arrival of food given on the right side of the tank long before food was available. As the fish spent so much of their time in one location of the tank, they were not engaging in behaviour that would occur in their natural environment such as foraging for food. With hundreds of fish gathering in one area of the tank, the wellbeing of some of the fish may have decreased with aggressive behaviour possibly present due to the competition to gain access to the food. The fish in the tank were a mixture of species including Sand tiger sharks *(Carcharius taurus)*, Nurse sharks *(Ginglymostoma cirratum),* Southern Stingrays *(Dasyatis americana)* Greater Barracudas *(Sphyraena barracuda)* and Tarpon *(Megalops atlanticus)*, which were not included in this experiment. It was in the interest of the National Marine Aquarium that the fish be more evenly distributed across the tank to increase the use of the whole tank and to reduce the risk of unwanted behaviour in the fish.

To increase the use of the whole tank, it was proposed that a second feeder be introduced to the tank on the left side, which could result in time-place learning. It was decided that the fish would be fed at the left side of the tank in the morning, and at the right side in the afternoon.

The aim of this study was to examine whether fish in a public aquarium were capable of exhibiting time-place learning and to generate evidence to support the claim that fish do have the ability to not only anticipate the arrival of food, but also where the food will be available and at what specific time. The consequence of the fish exhibiting time-place learning would be that the fish would be more evenly distributed across the tank as they travel from one feeder to another, which may in turn improve the welfare of the fish as there is less competition for resources and fish may engage in foraging behaviour.

Baseline observations of the fish's current behaviour with the original feeder on the right hand side were carried out. These observations took place for five weeks before the manipulation of the feeding commenced. The manipulation of the feeding areas was carried out for a three-week intervention period.

It was crucial that the fish were fed at the exact same time each day to maintain their daily rhythms and increase the likelihood of the fish's ability to exhibit time-place learning.

Based on the theory of time-place learning, the hypothesis is that the fish will learn which feeder will present food in the morning and which feeder will present food in the afternoon. Although the welfare of the fish was not directly measured, it can be assumed that with the fish being more distributed in the tank as a result of time-place learning, the welfare of the fish may have increased.

Method

Animals and Holding

Approximately 1000 Atlantic Ocean fish from the National Marine Aquarium in Plymouth were selected as study animals for this research. There were 40 species of fish living in this tank. All fish were studied except the ocean predators; Sand tiger sharks, Nurse sharks Southern Stingrays, Greater Barracudas and Tarpon.

The tank measured 10.5m (h) x 14m (w) x 24 (d), which is shown in Figure 1. It held three million litres of water and had a consistent filtration system and a light intensity cycle. The lights automatically turned on at 9:00a.m. and gradually reached the brightest light over a few hours. The lights would then gradually turn off from 4:45p.m. A lunar lantern was kept on throughout the whole night.

Feeding routines

During baseline observations (phase A), fish were fed twice daily on a consistent schedule once in the morning and once in the afternoon, both at the right hand side of the tank. Both the diets consisted of a 1.5kg mixture of frozen prawns, squid and muscles. Food was released from an automatic feeder at fixed times; 11:00a.m. and at 3:00p.m.

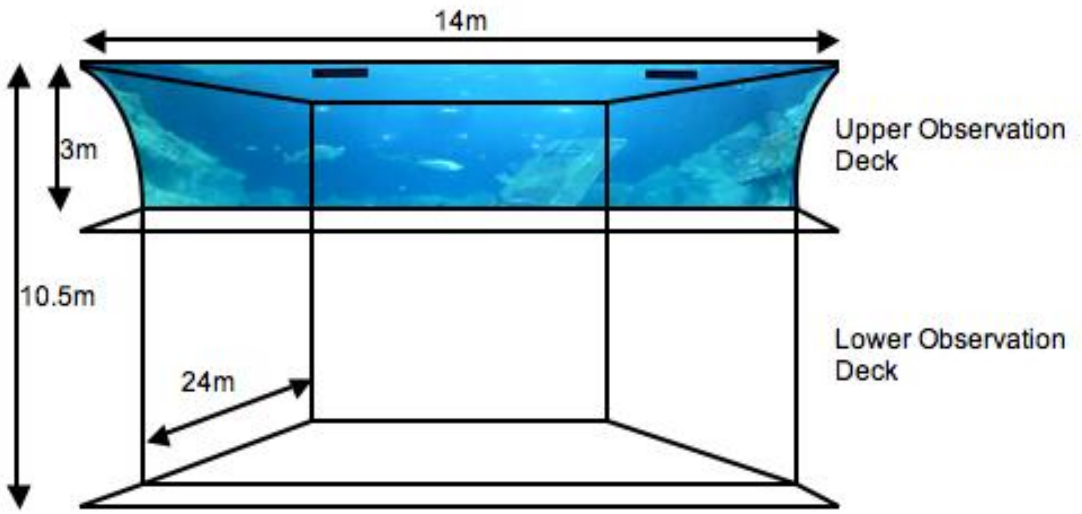

Figure 1: Diagram of the layout of the Atlantic Oceans tank, showing dimensions of the whole tank and the two observation decks

The automatic feeder was connected to a timer, which allowed water into the feeder at specific times which would slowly defrost the frozen food enabling it to flow into the tank, therefore the frozen food was placed into the feeder by the staff prior to the water being turned on. The pipe used to release the food into the tank measured 63mm in diameter. As the tank being studied was in a public aquarium, feeder schedules were already being used and had been used since September 2009.

The manipulations of the feeds were to come into action after baseline observations had taken place. During the intervention period (phase B), food was released from the automatic feeder on the left hand side of the tank in the morning, and then it was released from the automatic feeder on the right hand side of the tank in the afternoon, continuing with the same feeding times of 11:00a.m. and 3.00p.m. Figure 2 displays where the left and the right feeders were located in the tank.

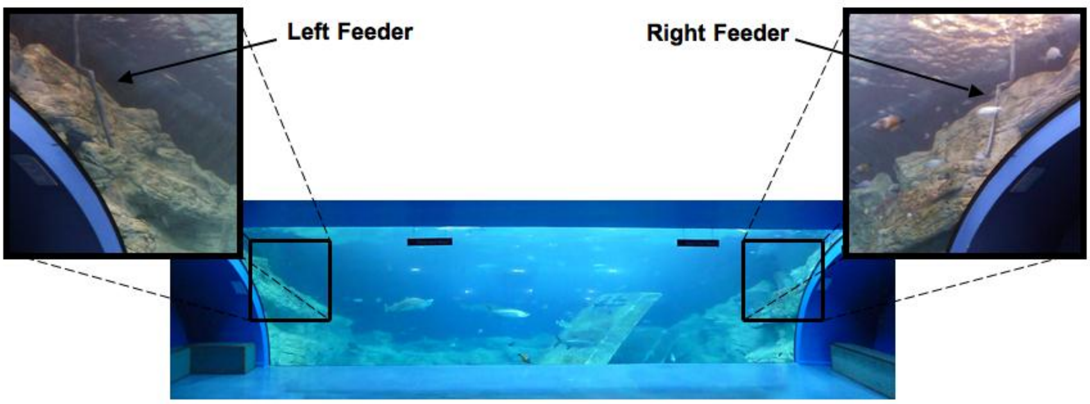

Figure 2: Image showing the left and right feeding pipes

Although the Sand tiger sharks were not included in this study it is important to note the feeding schedule of the sharks as this may have had an impact on the behaviour of the fish being studied. The Sand tiger sharks were pole-fed on Mondays, Wednesdays and Fridays, consisting of Mackerel, Squid and Octopus, this was to ensure that the sharks were completely full and did not consume the other smaller fish in the tank between being fed. They were fed at the surface of the water on the right hand side of the tank at different times to the feeding of the other fish to make sure the fish gained access to the food without the threat of the sand tiger sharks.

Materials

Observation check sheets and an explanation sheet were used by research apprentices and researchers to record the relevant information during baseline observations and during the intervention period where manipulation of the feeding took place.

Photographs of the tank and of the different classifications of behaviour were provided for the research apprentices to provide them with educated judgements when carrying out observations. A photograph of the feeding areas and the subsections was also provided.

An mp3 file was used to play a tone on each minute for 30 minutes, which was used by all researchers when recording data.

Procedure

This study was a single subject design where one tank was tested and observed for the duration of the experiment.

Research apprentices were recruited to assist with observations. They were Psychology undergraduates at the University of Plymouth. Appropriate training was provided to ensure that all data would be collected in a uniform manor to increase the likelihood of acquiring reliable data. Apprentices took part in a test run of observations to check they understood what was expected of them, and to rule out any misunderstandings of the data to be recorded.

Two observers, either researchers or research apprentices, observed at the same time, one observing the left side of the tank, and the other observing the right side of the tank. Observers were to stand in the same position in front of the tank at the upper observation deck, so that their perception of the tank was not compromised or altered throughout their observation period. The ‘mind your head’ signs in front of the tank marked the standing positions. The mp3 file would be played at the exact same time so that both observers recorded data at the same minute.

The tank was observed for one hour prior to feeding and one hour after feeding. Observers were required to complete thirty minutes of observations before taking a thirty-minute break, and then complete another thirty-minute observation slot. During the break, another pair of observers would take their place, so that the tank was under continuous observation. The morning observation slots were scheduled for 09:30a.m. - 11:00a.m. and at 10:00a.m. - 11:30 p.m. The afternoon observations were scheduled for 1:30p.m. - 3:00p.m. and at 2:00p.m. – 3:30p.m. It was decided that observers would conduct a maximum of thirty-minute observations at a time to reduce the risk of their concentration levels declining.

Due to external factors beyond our control, time was limited for observations to be carried out in both the morning and afternoon from Monday through to Friday, so compromises were made. Full day observations that consisted of both the morning and afternoon observations, took place only on a Monday, Wednesday, Thursday and Friday during the baseline observations period. During the intervention period, full day observations took place on a Monday, Wednesday, and Thursday. Tuesdays only had morning observations, and Fridays only had afternoon observations. The reason for this was to ensure an equal number of morning and afternoon data sets.

For the weeks during phase A, the usual feeding schedule was used, which involved the fish being fed by the staff at the aquarium from the right side of the tank in the morning (11:00a.m.) and the afternoon (3:00p.m.).

During the phase B, the fish were fed in the morning on the left side of the tank, and on the right side of the tank in the afternoon. The fish were fed by both the staff at the aquarium and by the researchers during this period.

Observations and analysis

Phase A observations took place for five weeks and phase B observations took place for three weeks. Observers were to make specific observations on the fish's behaviour; location/ distribution of the fish, amount of fish, and the busyness of the fish for two areas in the tank; the feeding areas and the tank as a whole.

Each feeding area was divided into three sections horizontally, and the tank as a whole was divided into 3 sections vertically shown in Figure 3.

Figure 3*:* Image to display the tank areas, and the feeding areas

A rating scale was used to measure the amount and busyness of the fish in the tank, whereas the location of the fish was recorded by judging where the majority of the fish were in specified areas.

Busyness is a subjective measure, which describes both the quantity and the activity of the fish in the tank throughout a whole minute. It was rated on a scale of zero to five, zero would be described as the fish being inactive and a rating of five would be described as fish swimming very fast or behaving in a very busy manner.

The amount of fish was measured on a rating of zero to five; zero being no fish present in feeding area and five being a lot of fish present. Figure 4 and Figure 5 provide an example of what an amount rating of zero would be and what a rating of five would be for the amount of fish.

Figure 4: Image to show what a rating of zero for the amount of fish would look like

Figure 5: Image to show what a rating of five for the amount of fish would look like

Results

Graphical representations of the data were the chosen method of analysis. Inferential statistical tests were not used. This decision was reached, as this experiment was a single subject design, where the aquarium as a whole was treated as one subject. Inferential statistical tests would not have been appropriate for this experiment design as they require more than one subject to complete the analysis. The advantages of using graphical representations of the data are that they allow for easy comparisons between phases and easy interpretation of the data that present certain trends in activity. The only statistical analysis tests used were to examine inter observer reliability between researchers and apprentices. This was to check the reliability of the data.

Observations were carried out for five weeks during phase A and for three weeks during phase B. However, not all the data collected was used as certain days were not appropriate for analysis as some data was incomplete. The data selected for analysis was the last full week of phase A and the last full week of phase B where complete days of data collection were in consecutive days. This allowed for a clear comparison between the baseline period and the intervention period.

Measures that were specifically formulated to record anticipation and time place learning were: amount of fish at feeders, busyness of fish at feeders and the area of the tank at which the fish are located. Some data that were not relevant, incomplete or inaccurate were not included in the analysis. The findings of the behaviour exhibited in the fish will not be presented as this data was not collected satisfactorily; behaviours were only recorded on the chance that an observer noticed a certain behaviour, therefore there were many gaps in the data where behaviours have not been recorded which would result in an inconsistent data set. The internal and external stimuli, which was also recorded will not be analysed as it was used as an explanation for anomalies shown in the patterns of busyness of the fish, the amount of fish, and the location of the fish.

To analyse the data, a mode of each ten-minute period was generated. For graphs that consisted of a weeks worth of data, a mean of the modes was used and standard deviations were calculated and presented on the graphs.

Inter-observer reliability

Inter-observer reliability between all researchers and apprentices was measured to ensure the data collected was reliable and of a satisfactory standard. A Spearman’s Rho correlation and Kappa values were calculated to examine the level of agreement between the observers. The statistical analysis of inter-observer reliability for the measure of the amount of fish at the feeders is shown in Table 1.

Table 1: Spearman's Rho correlations and Kappa values between observers

Amount of fish at feeders					
Observers	Correlation	Kappa	Observers	Correlation	Kappa
01 & 02	.40 (N: 10)	1.48 (N:10)	07 & 17	.52 (N: 11)	.42 (N: 11)
01 & 12	.25 (N: 10)	.22 (N: 12)	09 & 10	-.09 (N: 10)	-.06 (N: 11)
03 & 01	.41 (N: 13)	.12 (N: 13)	09 & 03	.67* (N: 10)	.62 (N: 10)
03 & 04	.67* (N: 11)	.62 (N: 11)	09 & 11	.75** (N: 30)	.32 (N: 31)
03 & 05	.60* (N: 12)	.21 (N: 12)	11 & 03	.47* (N: 20)	.38 (N: 20)
03 & 06	.72* (N: 11)	.35 (N: 11)	11 & 13	.57 (N: 10)	.35 (N: 12)
03 & 08	.44 (N: 10)	.22 (N: 12)	11 & 14	.27 (N: 10)	.49 (N: 12)
03 & 15	-.20 (N: 20)	-.08 (N: 21)	11 & 18	-.08 (N: 10)	.27 (N: 12)
03 & 11	.47* (N: 20)	.38 (N: 20)	19 & 20	.54** (N: 40)	.33 (N: 41)
03 & 16	.07 (N: 15)	.23 (N: 15)	19 & 05	.56* (N: 20)	.20 (N: 24)
07 & 08	-.04 (N: 10)	-.03 (N: 12)	21 & 13	.25 (N: 40)	.06 (N: 41)
07 & 01	.61 (N: 10)	.10 (N: 12)	21 & 05	.53* (N: 20)	.36 (N: 21)
07 & 04	.56 (N: 12)	.48 (N: 12)	21 & 22	-.06 (N: 20)	.10 (N: 23)
07 & 09	.85** (N: 40)	.68 (N: 41)			

Table 1 shows the correlation and kappa values between the different observers. Correlation values marked with a * are significant at the 0.05 level, and correlation values marked with ** are significant at the 0.1 level. Any kappa value that was above .4 was deemed acceptable.

The statistical analysis of inter-observer reliability for the measure of the busyness of the fish at the feeders is shown in Table 2.

Table 2: Spearman's Rho correlations and Kappa values between observers

Busyness of fish at feeders					
Observers	Correlation	Kappa	Observers	Correlation	Kappa
01 & 02	-.03 (N: 10)	1.00 (N: 10)	07 & 17	1 (N: 10)	1.00 (N: 10)
01 & 12	.19 (N: 11)	.18 (N: 11)	09 & 10	-.757* (N: 10)	-.13 (N: 10)
03 & 01	.58 (N: 13)	.22 (N: 13)	09 & 03	.272 (N: 10)	.14 (N: 10)
03 & 04	.52 (N: 11)	.42 (N: 11)	09 & 11	.823** (N: 30)	.49 (N: 30)
03 & 05	.29 (N: 11)	.15 (N: 11)	11 & 03	.607** (N: 20)	.20 (N: 21)
03 & 06	.73** (N: 12)	.25 (N: 12)	11 & 13	.272 (N: 10)	.32 (N: 10)
03 & 08	.00 (N:10)	.09 (N: 12)	11 & 14	.215 (N: 10)	.48 (N: 13)
03 & 15	-.22 (N: 20)	-.08 (N: 21)	11 & 18	.375 (N: 10)	.38 (N: 10)
03 & 11	.61**(N: 20)	.20 (N: 21)	19 & 20	.520** (N: 40)	.54 (N: 41)
03 & 16	.52 (N: 10)	.19 (N: 14)	19 & 05	.521* (N: 20)	.11 (N: 23)
07 & 08	.00 (N: 10)	.14 (N: 12)	21 & 13	.096 (N: 40)	.04 (N: 42)
07 & 01	.48 (N: 10)	.31 (N: 13)	21 & 05	.369 (N: 20)	.33 (N: 21)
07 & 04	.62* (N: 11)	.62 (N: 11)	21 & 22	-.070 (N: 20)	.08 (N: 23)
07 & 09	.79** (N: 40)	.35 (N: 41)			

Table 2 shows the correlation and kappa values between the different observers for the measure of the busyness of fish at the feeders. Correlation values marked with a * are significant at the 0.05 level, and correlation values marked with ** are significant at the 0.1 level. Any kappa value that was above .4 was deemed acceptable.

The statistical analysis of inter-observer reliability for the measure of the area of the tank is shown in Table 3.

Table 3: Kappa values for observers when measuring the area of the tank at which the fish were located

Area of tank			
Observers	Kappa	Observers	Kappa
01 & 02	.23 (N: 10)	07 & 17	.09 (N: 10)
01 & 12	.62 (N: 11)	09 & 10	-.43 (N: 10)
03 & 01	.21 (N: 11)	09 & 03	.79 (N: 12)
03 & 04	-.01 (N: 11)	09 & 11	.61 (N: 30)
03 & 05	.15 (N: 11)	11 & 03	.35 (N: 21)
03 & 06	.62 (N: 11)	11 & 13	.17 (N: 10)
03 & 08	.42 (N: 11)	11 & 14	.58 (N: 10)
03 & 15	-.14 (N: 20)	11 & 18	.76 (N: 12)
03 & 11	.35 (N: 21)	19 & 20	.11 (N: 41)
03 & 16	1.00 (N: 10)	19 & 05	.31 (N: 22)
07 & 08	.23 (N: 12)	21 & 13	.05 (N: 42)
07 & 01	.31 (N: 10)	21 & 05	.18 (N: 23)
07 & 04	.26 (N: 12)	21 & 22	.04 (N: 22)
07 & 09	.51 (N: 41)		

Table 3 shows kappa values between the different observers for the measure of which area of the tank the fish were. Correlation values marked with a * are significant at the 0.05 level, and correlation values marked with ** are significant at the 0.1 level.

Anticipation

Anticipatory activity was examined by the level of busyness demonstrated by the fish at each of the feeders an hour and 30 minutes prior to feeding. The data shown in Figure 6 represent the busyness of the fish, which was rated on a scale of zero to five (0= inactive, 5=very busy). The data used for this figure were the means of the modes for the last week of phase A and the last week of phase B. The results show a distinct change in behaviour in the morning observations from phase A and phase B. During phase B when the fish were fed at the left feeder, it can be seen that there is a steep increase in busyness at the left feeder starting thirty minutes before the average feeding time, suggesting anticipatory activity in the fish. There is a higher level of busyness at the left feeder in the afternoon during phase B compared to that at the left feeder in the afternoon during phase A.

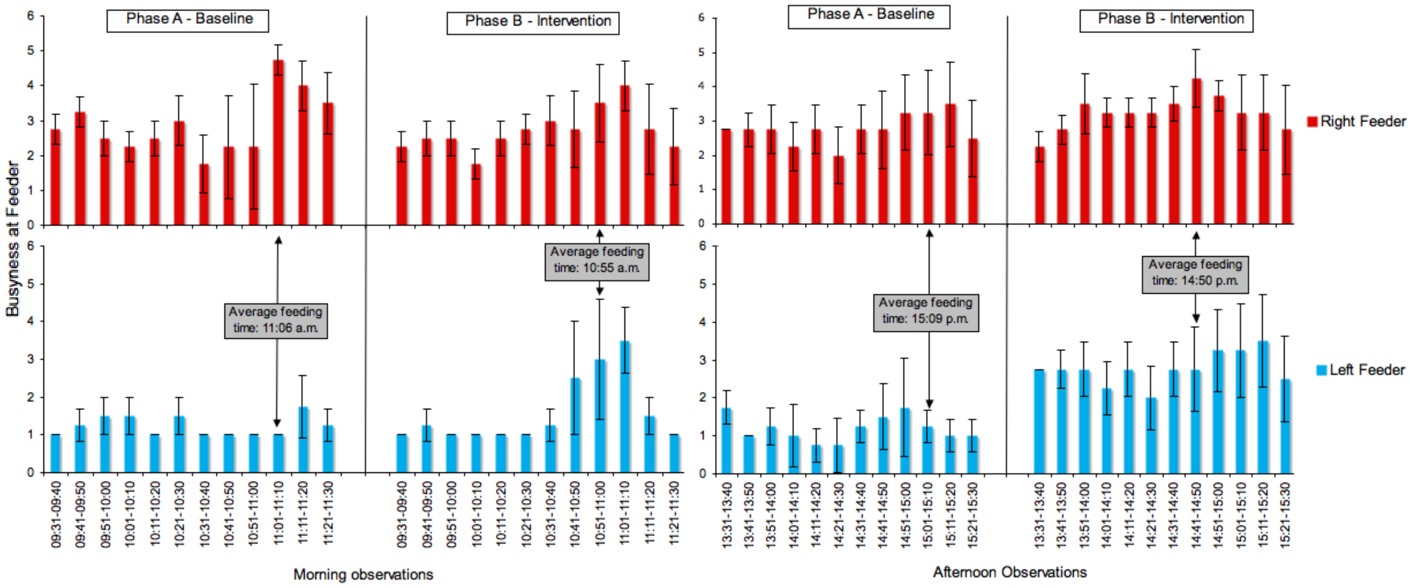

Figure 6*:* Bar graph showing the average busyness at left and right feeders in the morning and afternoon during phase A and phase B. Standard deviations are shown with error bars

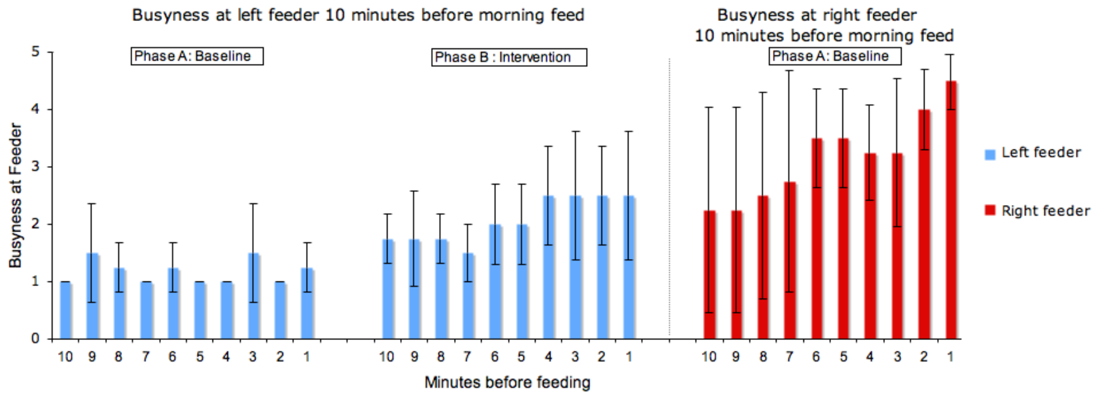

Figure 7: Bar graph showing the weekly average busyness of fish at feeders 10 min before feeding

The data used to create Figure 7 were the means of the modes for the last full week of phase A and the last full week of phase B. It can be seen that there is an increase in the busyness at the left feeder in the ten-minute lead up to the morning feed during phase B, where food was presented at the left feeder. The data showing the busyness at the right feeder is presented to provide a comparison between the ten-minute lead up to feeding in the morning between phase A and phase B.

Time place learning

The phenomenon of time place learning was measured by the amount of fish at each feeder during phase A and phase B for both morning and afternoon feeding periods.

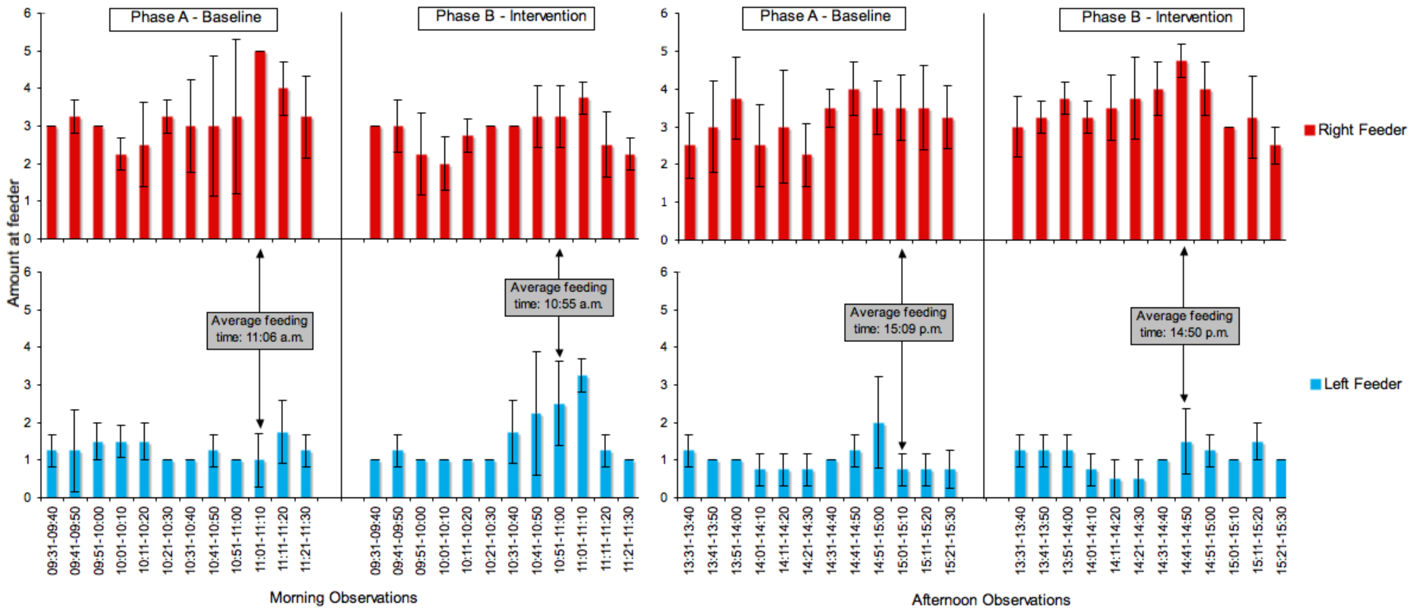

Figure 8: Bar graphs showing the average amount of fish at both feeders in the morning and afternoon during phase A and phase B. Standard deviations are displayed as error bars

The data shown in Figure 8 display a comparison of the amount of fish at the left and right feeders between the two phases of the experiment. The amount was rated on a scale of zero to five (0= no fish, 5=highest amount of fish). There is a peak in the amount of fish that is visible at the right feeder in the morning at the average feeding time of 11:06a.m, where in comparison, at the same time at the left feeder, there is no change in the amount of fish at the left feeder. The amount at the left feeder during phase B shows a gradual increase in the amount of fish before the average feeding time of 10:55a.m, at which there is also a slight increase at the right feeder also. The afternoon results show a high amount of fish at the right feeder during phase A and an equal amount of fish during phase B but with a slight increase leading to the average feeding time of 14:50p.m.

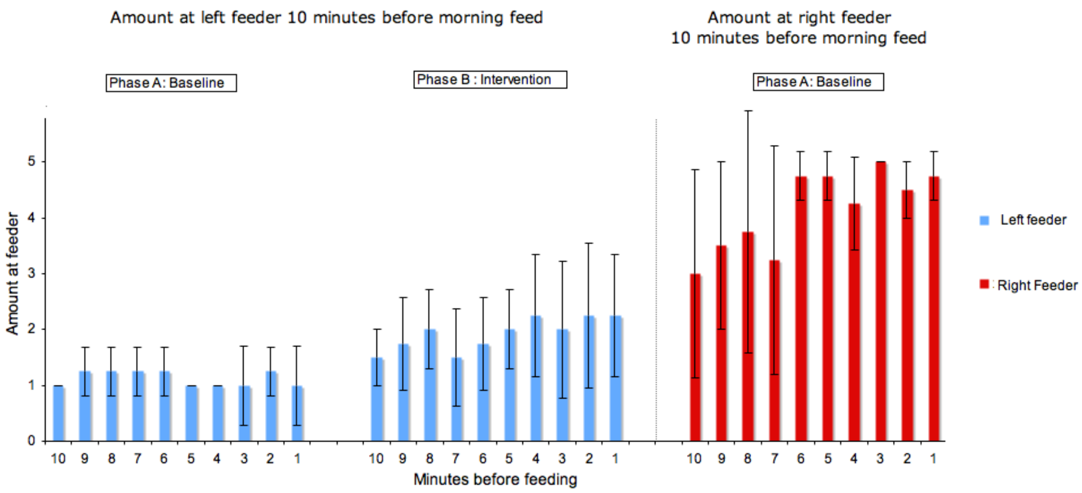

Figure 9: Bar graph showing the average amount of fish at feeders 10 minutes before feeding

The data shown in Figure 9 display the average amount of fish generated from the full week of phase A and the full week of phase B, ten minutes prior to feeding. It can be seen that there is an increase in the amount of fish at the left feeder during phase B in comparison to phase A. Although it is not as much as the amount of fish at the right feeder during phase A, there is a clear increase in the amount of fish at the left feeder during phase B.

Time place learning was also measured by the distribution of the fish in the tank throughout phase A and phase B. The descriptions for the distribution were: Area 1, Area 2, Area 3 and Evenly Spread. The three areas of the tank were vertical separations across the tank from left to right.

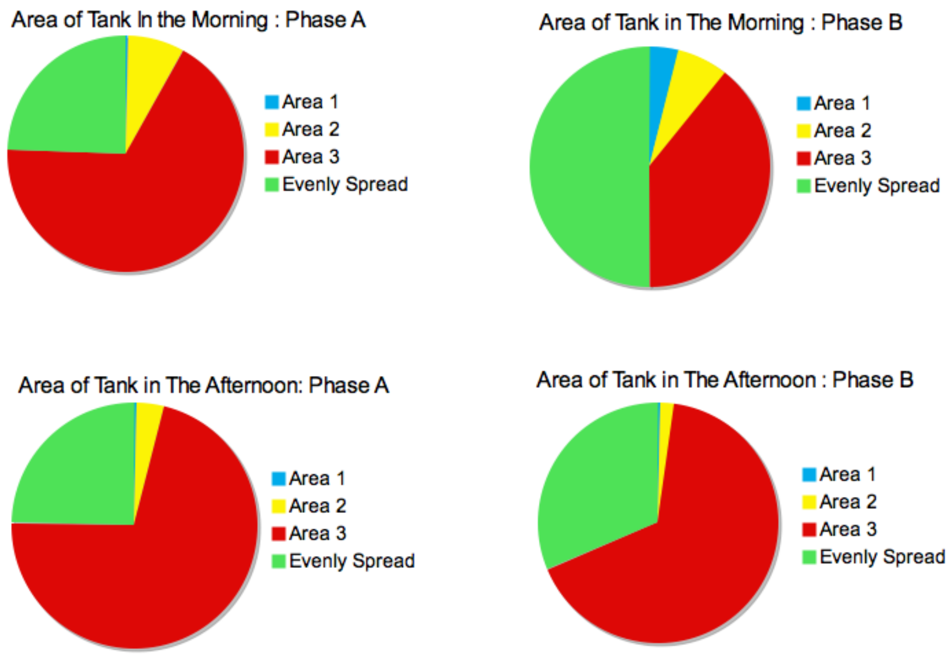

Figure 10*:* Pie charts displaying where the majority of the fish were in the tank in the morning and the afternoon during phase A and phase B

Each section of the pie charts in Figure 10 represent of the amount of time the fish spent in the different areas of the tank in the two hour morning and afternoon observation periods for the full week of phase A and the full week of phase B. This was calculated by totalling the number of minutes the fish spent in each area (no averages were calculated to generate this figure). The results for the morning observations show a 100% increase in the fish being evenly distributed across the whole tank. An increase is also seen in the afternoon, but to a lesser extent. The data used to generate Figure 11 were the total number of minutes the majority of the fish spent in each area of the tank for the ten-minute period leading to feeding time. The data does not include the minute that the food was presented. It can be seen that during phase A when the fish were fed from the right feeder, which is located in area 3, the majority of the fish are located in this area. No fish were located in area 1 where the left feeder is present.

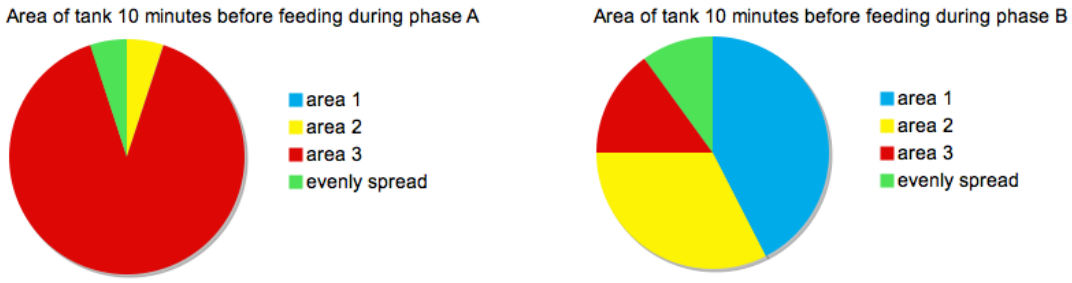

Figure 11: Where the majority of the fish were in the tank 10 minutes before food was presented in the morning (at the right feeder during phase A and at the left feeder during phase B)

During the 10 minutes before feeding during phase B where the fish were fed from the left feeder, which is located in area 1, it can be seen that the majority of the fish were located in area 1 and area 2. Comparing this data with the distribution of the fish during phase A, there is vast increase of fish present in area 1. There is also a slight increase in the even distribution of fish across the whole tank.

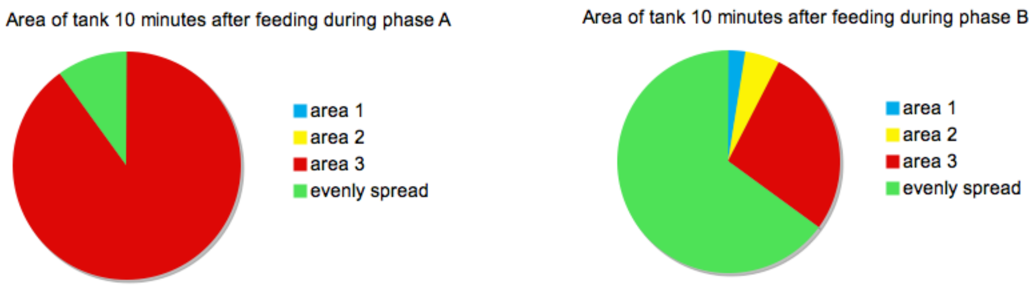

Figure 12: Pie charts showing where the majority of the fish were in the tank 10 minutes after feeding had finished in the morning (at the right feeder during phase A and at the left feeder during phase B)

The data used to create Figure 12 was the total number of minutes the majority of the fish spent in each area of the tank 10 minutes after feeding had ceased. The data does not include any minutes during which the fish were being fed. During phase A, the majority of fish were still located in area 3 where the right feeder was present 10 minutes after feeding had finished, whereas during phase B, when the fish were fed from the left feeder, located in area 1, the majority of the fish were evenly distributed across the whole tank. It can also be seen that after the morning feed during phase B, approximately 25% of the fish were located in area 3 even though food was presented at the left feeder in area 1.

Internal clock

Evidence for an internal present in fish was provided with the data collected from the day when food was inadvertently withheld one afternoon during phase B.

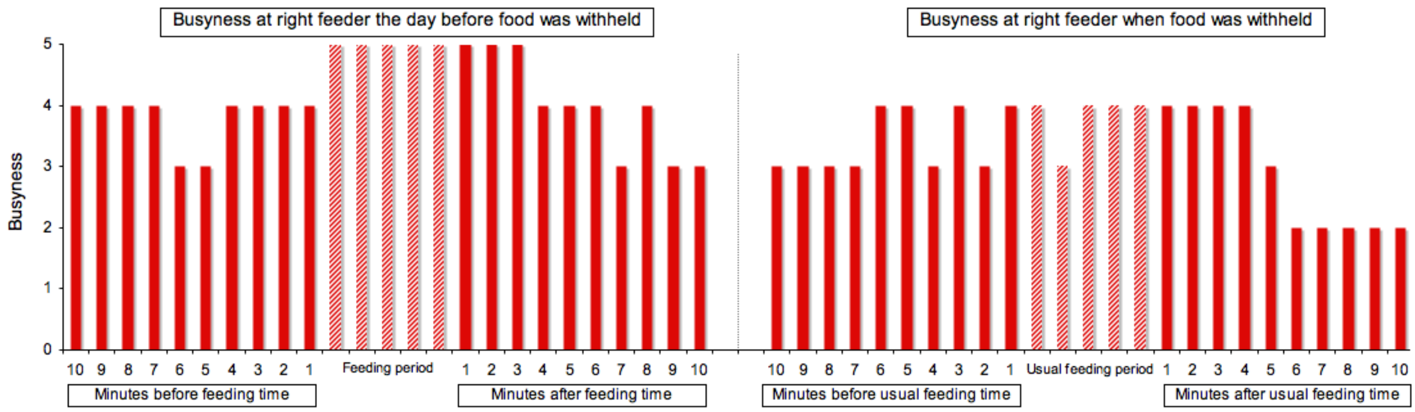

Figure 13: Bar graph to show the Comparisons between busyness of fish at the right feeder when food was present and when it was inadvertently withheld during phase B in the afternoon. Shaded bars represent the time during which food was presented and the usual feeding time

The data used in Figure 13 was the raw data collected during phase B on the day before and on the day that food was inadvertently withheld. The usual feeding period that is displayed for the day where food was withheld was calculated using an average of the feeding periods from the days leading up to the day when food was not presented. The fish were fed at the right feeder in the afternoon during phase B. The graph shows a similar pattern in the busyness of the fish for both days. There is an increase in the level of busyness leading up to the feeding period on the day before food was withheld which is also shown on the day where food was withheld. The only main difference between these days is that there is no peak in busyness during the feeding period as there is on the previous day when food was present. However, another similar pattern shown is the decrease in the level of busyness after the actual feeding period when food was presented and in the usual feeding period where food was not presented. The fish's busyness decreased on the fourth minute after feeding on the day food was presented, and busyness decreased on the fifth minute after the usual feeding period had finished.

The data presented in Figure 14 are from the day prior to the day when food was withheld, and from the day when food was withheld. No averages of the raw data had been calculated to produce this graph. The usual feeding period was created using an average of afternoon feeding periods during phase B. The graph shows a high amount of fish on both days during the 10 minute lead up to the feeding period. During the usual feeding period on the day when food was withheld, the amount of fish stays constant with the 10 minutes before the usual feeding period even though food was not presented. Both days show a decrease in the amount of fish as soon as the actual feeding period had finished and when the usual feeding period had finished even though no food was presented during the usual feeding period.

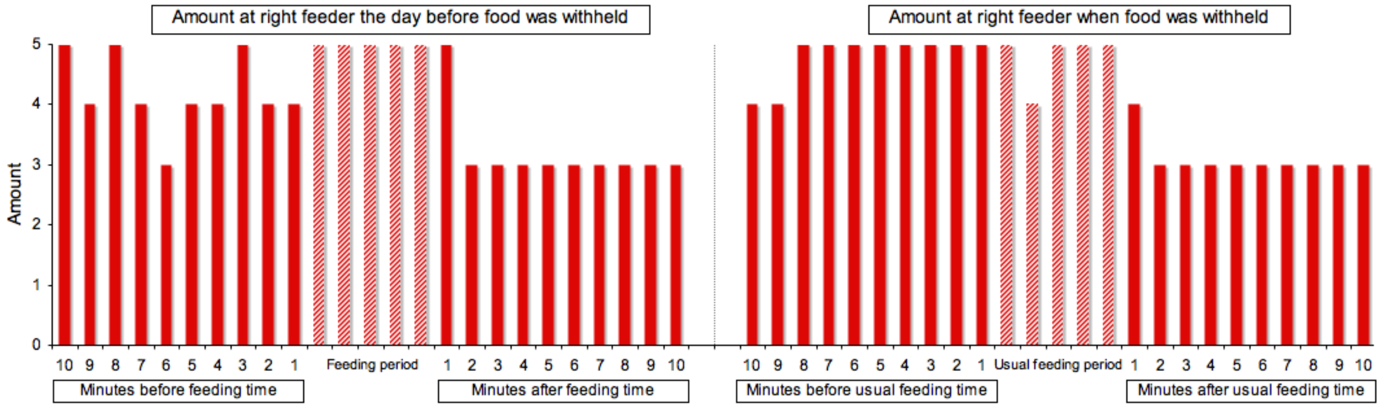

Figure 14: Bar graph showing the comparisons between the amount of fish at the right feeder when food was presented and when it was withheld during phase B. The shaded bars represent the time during which food was presented and the usual feeding time

Discussion

Overview of findings

The results reveal that the majority of the fish studied show clear signs of anticipation of the arrival of food during phase A of this experiment. In the hour prior to feeding, a gradual increase in both activity and the amount of fish is shown at the right feeder (see Figure 6 and Figure 8). During phase A, food was presented at the right feeder, which could explain the low level of busyness at the left feeder during that period (see Figure 7).

During phase B there are slight signs on anticipatory activity can also be seen at the left feeder prior to the morning feed and also prior to the afternoon feed also at the left feeder. The food was not presented at the left feeder in the afternoon during phase B, but there seems to be an increase in busyness at the left feeder, which suggests that the fish were anticipating food being presented at the feeder that they were fed from earlier in the day. Although the anticipatory activity did not occur at the correct feeders at all the feeding times during phase B, it does however suggest that the fish were able to learn that the food was delivered from a new feeder (the left feeder) but just did not learn the time (see Figure 6). This is shown in the activity displayed at the left feeder in the afternoon at which they had previously been fed at in the morning.

The amount of fish at the feeders was a measure of the fish's ability to exhibit time place learning. During phase B (see Figure 8), there is an increase in the amount of fish at the left feeder prior to feeding, which suggests that the first two weeks of the intervention period allowed time for the fish to learn which feeder would present food at what time and display this knowledge during the last week of phase B. Figure 9 clearly shows a gradual increase in the amount of fish at the left feeder in the 10 minute lead up to feeding. This suggests that either the fish had very precise timing or that only a minority of the fish had learned where the food would be available. There is no distinct increase in the amount of fish at the left feeder during phase B in the afternoon, which suggests that the fish had learnt that the food would not be presented at the left feeder in the afternoon, but that it would be presented at the right feeder instead. Although this experiment was a test of time place learning, the data suggests that the phenomenon of time place learning was not so robust as the amount of the fish at the right feeder does not seem to decrease when food was be presented at the left feeder, suggesting that some fish were still anticipating that food would be presented at the right feeder in the morning and not the left feeder.

However, taking into account the length of time the fish has been exposed to the previous feeding schedule it is not surprising that the fish did not display the behaviour of time place learning in such a great way as hypothesised.

The sharp increase in amount of fish shown at the left feeder in the morning during feeding time (see Figure 8) could be a result of the fish's response to the food being present in the tank. The study by Reebs (2000) could explain this behaviour, as it was found that some fish are able to learn and anticipate where food will be available and other fish simply follow, which could suggest that some fish were just following the informed fish to where the food was.

The results of the distribution of fish in the tank (Figure 10) suggest that the fish did exhibit time place learning, as there is a very noticeable change in the distribution from the morning of phase B to the afternoon of phase B. The fish were evenly distributed for half of the morning during phase B. During the afternoon the fish spent most of their time in area 3, which is where the food would have been presented. The results also show that fish had spent time in area 1 in the morning of phase B, when in comparison to phase A, no fish had spent any time in that area. This could be accounted for by the fact that the fish were in area 1 during phase B as a direct response to food being presented, so to investigate this further, an analysis of the minutes leading to feeding during phase B was carried out. The results presented in Figure 11 support the phenomenon of time place learning in fish as it can be seen that area 1 is where the fish spent most of their time in the 10 minutes prior to food being presented. As the data did not include any minutes that food was actually present, this rules out the possibility of fish being in area 1 as a direct response to food being present.

Figure 12 shows that even after feeding during phase A, the majority of the fish remain in the feeding area, but after feeding in phase B the fish are mainly evenly distributed. By positioning a new feeder at a different area this experiment has been successful in achieving better distribution of the fish in the tank and in the long term this could only improve the fish's welfare. However, these observations are only of the upper section of the tank that was visible from the upper observation deck. Therefore the distribution for the lower half of the tank cannot be accounted for.

An interesting finding that supports the notion of an internal clock is that when food was not presented at the usual feeding time one afternoon during phase B, the activity of the fish and the amount of fish both decreased after the usual feeding period had passed even though food had not been presented. This is shown in Figures 13 and 14. It might suggest that an internal clock that was used by fish for both the anticipation of food being present and time place learning.

Even though the fish did not show robust signs of time place learning activity, it was shown that by placing a new feeder at the opposite side of the tank, the fish were more evenly distributed, which was one of the desired results that the National Marine Aquarium Plymouth hoped for.

Theoretical Interpretation

Anticipation
The anticipatory activity shown in the fish during phase A and phase B has been shown in previous studies on anticipation in fish such as the study by Reebs and Lague (2000) who found that golden shiners displayed an obvious increase in activity before feeding.

Time place learning
The results from this experiment revealed that the fish showed a degree of time place learning. The reasons that the amount of fish at the left feeder during phase B did not seem to match the amount of fish there were at the right feeder during phase A could be explained through a comparison to research by Reebs (1993) where it was found that Convict cichlids also did not show evidence of time place learning. The fish instead learned which areas food would be presented in at any time of the day and would inspect each area in turn once the feeding signal had been given. This could also explain the increase in busyness at the left feeder in the afternoon during phase B in comparison to the busyness at the left feeder in the afternoon in phase A. The fish had been fed at the left feeder in the morning during phase B, so it is possible that the fish learned that the left feeder area presented food, but they did not learn what time the food would be presented at the feeder.

Internal clock
On the day that the food was inadvertently withheld, it was surprising to see a decrease in the amount and busyness of the fish (Figure 13 and Figure 14) after the usual feeding period had finished. This finding could well support the presence of an internal clock as shown by Spieler and Noeske (1984, as cited in Reebs, 2001) who deliberately withheld food in their study.

Limitations

Unreliable cues
Due to the fact that it was not possible to have complete control over the environment of the fish unlike previous experiments, it was difficult to control factors that may have had an effect on the behaviour of the fish. An example

of factors that could have been interpreted as cues for the arrival of food is the presence of staff at the surface of the tank which could happen at any time of the day and which would not be directly linked to feeding. The fish may have used the presence of staff as a cue for feeding as the feeder is at the surface of the tank near to where the staff were positioned. These unreliable cues may account for anomalies in behaviour where activity is randomly heightened at times where it could not be explained by the presence of food or the anticipation of food.

The feeding times of the fish were not always under the control of the researchers as other activities that the National Marine Aquarium were engaged in took priority over feeding such as divers being in the tank prevented the fish at the scheduled time as feeding could only be carried out when the divers were out of the tank. The delay and irregularities of the feeding times may have had an impact on the fish's ability to exhibit time place learning.

These problems would not have occurred if this experiment were carried out in a laboratory environment. The reason this experiment was not carried out in a laboratory was because the original question of how to evenly distribute the fish in the tank came from the National Marine Aquarium. This was requested to reduce the competition for access to the resources between feeding times, which would in turn reduce aggressive behaviour in the fish. The experiment tested time place learning in fish that had experienced a routine feeding schedule for a period of a year prior to the intervention of a new feeder. Other research such as Reebs (2000) gave the captured fish two weeks to habituate to the new living environment and feeding routines before they were tested. It could be that the lack of habituation time in our study in phase B is the reason why there was less anticipation observed for the arrival of food at the new position in our fish.

Problems with observations

The results from the inter-observer reliability tests suggest that the observers did not always agree with each other on certain measures. As all apprentices took part in a training session, further training or a top-up training session may have been beneficial to overcome the disagreements in the judgments for measures. To improve inter-observer reliability for future research, it may be constructive to have a test period of collecting data before the actual data for the experiment would be collected. After this test period, correlations and kappa values could be calculated to expose which observers require further training before the data collection for the experiment commences, to ensure that all research observers are trained to the same level to obtain reliable results.

Other reasons for the undesirable results from the correlation and kappa tests could be due to observers not measuring items on the same minute, and as fish can move very quickly from one place to another within seconds, it was crucial that observers played the timer at the same time to ensure they recorded the same minute. However, even if observers did follow this rule, there were many items of information to record all in one minute and as people work at different paces, it was possible that the observers did not record the same thing at the same time as the other observer.

Previous studies used different methods to measure activity in fish. A study by Davis & Bordach (1965) used an infrared beam to measure anticipatory activity in fish, which crossed the aquarium below the feeder and every time that a fish approached the feeder, the electrical circuit that linked the beam was broken. Another method used in their study was a network of rubber bands that were connected to an electrical switch that stretched across the aquarium. The electrical switch recorded the number of times fish would swim into the rubber band (Davis & Bordach, 1965). Although these methods have been successful in the recording of anticipatory activity in fish, it was impractical for this experiment as a public aquarium was being studied, so observational methods were used. A further way to overcome the problem of the difficulty of recording all the information within seconds could be to connect a camera to a timer that would take a photograph of the tank every minute. The photographs could then be analysed for the amount of fish at each feeder and the area of the tank at which the majority of the fish are located. However, this could be very time consuming and additional observers would be required to record the busyness of the fish as that needs to be assessed with live movement of the fish. If observers were trained to assess only the busyness of the fish, the inter-observer reliability test results may improve, as the observers could become more skilled and specialised in one aspect of behaviour.

One of the problems that arose when observing the fish and analysing the data is the problem with observing groups of fish. This can be a problem as it is difficult to ascertain exactly how many fish have learned where food will be presented and at what time it will be available. A study by Reebs (2000) found that it was possible for a minority of informed leaders to lead a shoal to the feeding area which was either through social facilitation of foraging movements or by obtaining following behaviour. This could provide an explanation for the small amount of fish at the left feeder prior to feeding (see Figure 8), which gradually increased as feeding time grew closer.

Sampling methods

Previous research that tested time place learning or FAA in fish studied the fish for longer periods before feeding than the periods studied in this experiment. The study by Reebs and Lague (2000) observed the fish three to six hours prior to the feeding time. It may have been beneficial to study the fish for a longer period to see if the anticipatory activity that had been well established at the right feeder began before an hour and 30 minutes before feeding. However, other research such as the study carried out by Chen and Tabata (2002) found that FAA was very short and precise. This could be due to the species of fish studied, and since there is a vast range of species that live in the aquarium, it may have been worthwhile to study individual species of fish in the tank at a time and compare the results between the species. This however would not have been appropriate for this study as the aim of the study was to examine how the distribution of the fish changed as a whole within the tank.

Future Research

This study took the form of an A-B design. The drawback of this design is that it is not correct to assume that the introduction of a new feeder changed the distribution of the fish as it was not tested further in an A-B-A design. The change in the distribution of the fish in the A-B design could be due to the time of year and a change in the breeding behaviour of the fish. Future research would benefit from conducting an A-B-A or A-B-A-B design in a public aquarium environment to test that the change in phases are the cause of the change in distribution of the fish.

Future research may also benefit from using randomisation tests in the analysis of the data; it was not possible for this study to use randomisation tests as not enough data was collected.

Applications & Implications

As this experiment was not repeated using a different aquarium, there is a threat to the external validity of this study. The results gained from this experiment would not be appropriate to use as a generalisation for any other aquarium as it was a single subject design. Using one subject does not allow generalisations to be made as the behaviour recorded have only been done so for one subject and it has not been replicated in another public aquarium. It would be beneficial for further research to carry out tests of time place learning in a similar environment to a public aquarium to establish whether the phenomenon of time place learning can be exhibited in fish living in a public aquarium.

Conclusion

In conclusion, fish showed strong FAA prior to feeding in phase A, where the fish continued to be fed at their usual feeding area. This suggests that the fish are capable of learning a single area at which food will be presented, and can correctly judge the time at which food will be presented.

The addition of another feeder at a new site resulted in the fish becoming more evenly distributed between feeding times. This should result in less competition for resources between feeding times, which should improve the welfare of the fish.

Time place learning was observed in most but not all of the fish, which suggests that only some fish are capable of learning a second time and location of a food source. A longer habituation period might produce a more marked time-place learning in the majority of the fish.

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