1 **Determining the effects of duration and recency of exposure to environmental enrichment**

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Abstract

Experience can help animals adapt their behaviour to fit the environment or conditions that they find themselves in. Understanding how and when experience affects behaviour is important for the animals we rear in captivity. This is particularly true when we rear animals with the intent of releasing them into the wild as part of population rehabilitation and conservation efforts. We investigated how exposure to a changing, more complex environment promotes behavioural development in juvenile trout. Four groups of fish were compared; (i) fish that were maintained without enrichment, (ii) fish that were exposed to an early period of enrichment, but were then returned to a plain environment, (iii) fish that were maintained in plain conditions, but were then exposed to enrichment towards the end of the rearing phase, (iv) a group that were kept in enriched conditions throughout the 12 week rearing period. We then assessed fish anxiety levels, their spatial learning ability, and the capacity of the fish to find their way through a barrier where different routes were presented across 4 different trials. Fish that experienced enriched conditions for the longest duration had superior spatial learning abilities, and they were better at finding the correct route to get past the barrier than fish from the remaining three treatments. Positive effects on behaviour were, however, also found in the fish that only experienced enrichment in the last part of the rearing period, compared to the control, or fish exposed to early enrichment. No effect of enrichment were found on levels of anxiety in any of the groups.

Keywords: Enrichment, behavioural development, behavioural flexibility, learning, captive rearing

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42 **1. Introduction**

43 Understanding how the environment fine-tunes and shapes behavior is particularly important for
44 those working in the area of captive rearing and release for population rehabilitation. Establishing
45 appropriate ways to rear animals so that they have the capacity to survive and thrive in a real
46 world environment remains a central goal of many applied research programs (Shier and Owings,
47 2006; Salvanes and Braithwaite 2006; Urbanek et al. 2010). Experience during early life can play
48 a significant role in the development of an animal's behavioural phenotype. However the
49 relatively unchanging nature of the captive environment can lead to animals with behavioural
50 deficits and diminished behavioural flexibility (Olla et al. 1994; Brown et al. 2003). The addition
51 of environmental enrichment, or the experience of variation within the environment has been
52 found to promote increased behavioural flexibility and improve aspects associated with cognition
53 (Leggio et al. 2005; Harburger et al. 2007; Strand et al. 2010; Salvanes et al. 2013). As such,
54 there is now growing interest in the effects of adding environmental enrichment and variability
55 into the captive environments in which animals are reared for later release (Rabin 2003;
56 Braithwaite and Salvanes 2005; Seddon et al. 2007).

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58 Although practices in terrestrial systems are extensively more developed than those in aquatic
59 systems (Brown and Day 2002), fisheries biology is a field that is actively exploring ways of
60 rearing animals that behave in ways that smooth the transition from captivity to life in the wild
61 (Heenan et al. 2009; Brockmark et al. 2010). Restocking in an attempt to restore or maintain
62 heavily exploited fish populations is a widespread management practice (Brown and Laland
63 2001). However, survival of hatchery fish is typically lower than for wild fish of the same size or
64 age class. The mortality levels immediately following release can be substantial and there is little

evidence that released fish survive long enough to effectively rehabilitate the struggling populations (Hilborn 1998, Salvanes and Braithwaite 2006).

From a stocking perspective, there appear to be benefits of rearing fish in more complex environments than the standard hatchery provide (Näslund and Johnsson 2014). However, despite this knowledge, enrichment is seldom used in hatcheries (Näslund et al. 2012, Näslund et al. 2013, Johnsson et al. 2014). Rearing fish with enrichment is more labour intensive than using barren tanks because enrichment makes it more difficult to clean, and there is concern that feed and waste may accumulate which might compromise the health of the animals within these systems (Braithwaite and Salvanes 2010). The addition of enrichment items into the hatchery environment may, however, be a more attractive prospect if the fish only needed a restricted period of exposure to the enrichment (Brown et al 2003).

In this study we investigate how the duration and recency of exposure to structural environmental enrichment affects the behavioural development in rainbow trout (*Oncorhynchus mykiss*), a commonly stocked species. Fish were exposed to three separate enrichment treatments differing in timing (early or late in the experiment) and duration (five or twelve weeks). The fish were then behaviourally screened, comparing three behaviours that are known to be positively influenced by enrichment (Salvanes et al. 2007, Spence et al. 2011, Salvanes et al. 2013); specifically we compared anxiety levels in a novel environment, spatial learning ability in a four armed maze and the ability of the fish to find novel paths to gain access to food (to assess behavioural flexibility). We hypothesized that the effects of enrichment would depend upon how long the fish were able to experience the enrichment, thus fish that had the longest exposure to enrichment would show the greatest benefits in terms of reduced anxiety and increased learning and flexibility. If, however, the effects depend on how recently the fish were exposed to enrichment, we would

expect that fish most recently exposed to the enrichment would outperform those that had no enrichment or that only experienced enrichment at the start of the rearing period only.

2. Material and Methods

2.1 Fish and treatments

Four groups of approximately 50 rainbow trout (*Oncorhynchus mykiss*) obtained from a local hatchery at 5 months of age were randomly distributed across 4 opaque, flow-through holding tanks (60x60x95 cm). The fish were fed once per day with commercially produced fish pellets, and were kept on a 12 hour light:dark schedule at a water temperature of 10°C. After one month of acclimation, four groups were established for the experiment, with four replicate treatment tanks (90x45x30 cm) in each of the groups and 10 fish in each tank (n=40 per treatment) randomly selected from the holding tanks. Enrichment, when provided, consisted of fine grain gravel, upturned, black plastic pots (10 cm in diameter) to create areas of shelter, plastic plants (12-30 cm tall) and novel objects (plastic plants of different colour, Ping-Pong balls, green bottle tops (3 cm diameter), grey pvc-pipes (3 cm in diameter). When enrichment was not available the tank contained only a filter and an airstone.

Group (i) fish experienced enrichment throughout the 12 week rearing phase of the experiment (12 full weeks – Full), Group (ii) were exposed to enrichment continuously for 5 weeks at the start of the experiment and then returned to regular holding tanks (5 weeks early – Early), Group (iii) were exposed to enrichment for the last 5 weeks of the experiment (5 weeks late – Late), and Group (iv), the control fish, were maintained for 3 months in treatment tanks without enrichment (Control). One complete control replicate tank had to be terminated prematurely owing to a fungal infection, none of these fish took part in the behavioural trials, hence there were only 30 individuals tested from the control groups. In addition, four other fish were lost in different tanks (1 Control, 2 Early and 1 Full) again these losses occurred prior to the behavioural assays. At the

end of the experiment, the fish were euthanized and length measurements (standard length) were taken to compare the sizes across the treatments.

2.2 Behavioural assays

Once the fish reached 9 months of age behavioural screening began. Anxiety, flexibility in habitat use and spatial learning were assessed. The same fish were used in the anxiety and the flexibility trials (n=76), because these two assays were not expected to interfere with each other owing to the very different nature of the tests. New fish, however, were used for the spatial learning task (n=71). Three days before the behavioural assays began, the fish were anaesthetised with buffered Tricaine-S (MS-222, Sigma) and marked to permit individual identification with 3 mm long UV-florescent elastomer tags under the skin of the lower jaw using a hypodermic syringe.

2.2.1 Assessing flexibility

To assess the flexibility in habitat use, a series of 4 tasks were given to the fish over 4 consecutive days (modified from Reader and Laland 2000). For each task, the fish had to find a route from one side of a tank to the other where they were able to access a feeder (Fig. 1). At the start of each trial, a fish was placed in one half of a tank with a dividing wall that had a single exit hole providing access to the other side of the tank. On each day of the test the exit was placed in a different position. Individual fish were transferred to the test tank and given a 30 minute settling period with an opaque partition blocking access to the partition wall. Trials began when the partition was removed, and the latency to enter the exit hole was noted. For tests 1 and 2, the exit was simply a hole on either the left or the right, and the trout were given 15 minutes to find it. For tests 3 and 4, the fish had to enter a tube to access the exit hole, as this was a more difficult task the fish were given 20 minute trials. The number of fish that found the route to the feeder was noted. These trials were run with n=20, 20, 21 and 15 for the Early, Late, Full and Control fish respectively.

2.2.2 Assessing spatial cognition

Spatial cognition, involving perceiving and interpreting spatial cues, as well as storing and retrieving this information (modified from Brockmark et al. 2010), was assessed using a maze with a central compartment (20x30x14 cm) and four arms at each corner leading to doors (Fig. 2) and contained nothing else. Three of the doors were blocked with transparent Perspex that created dead-ends, while one exit remained open. The whole maze was brightly lit and had a light grey interior. The maze was placed in a larger test tank (90x45x30 cm) containing a conspecific to motivate the test fish to exit the maze. Individual fish were tested once a day for 5 minutes, or until they found the maze exit. These trials were run for 13 consecutive days with n=20, 18, 20 and 15 for the Early, Late, Full and Control fish respectively. The number of false exits entered (errors) was recorded as the number of mistakes made, and the time it took to exit the maze was also noted.

2.2.3 Assessing anxiety

A novel tank diving test that is a three dimension version of the open field test, was used to measure anxiety (Cachat et al. 2010). Time spent in the center of the area and number of times fish left the tank floor to explore the upper water layers was taken as evidence of less anxious fish. The test tank measured 45x75x30 cm, with a water depth of 36 cm and contained nothing else. The tank was covered with black plastic on three sides. Individual fish were tested by releasing them from a dip net into the upper part of the tank in the same position to minimize bias. Across the treatments Early, Late, Full and Control we tested 21, 20, 20 and 15 fish respectively. Each fish was allowed to explore the tank for 5 minutes. The movement and behaviour of the fish was filmed from a camera positioned in front of the uncovered tank side. After the trials, the following were obtained from the video recordings; time spent motionless on

the bottom and horizontal and vertical position of the fish. During video analysis the tank was divided into three vertical zones (top, middle and bottom, each 12 cm deep) and the bottom vertical zone was further horizontally divided into middle and edge (edge reaching 12 cm from each tank wall). Every 15 seconds the horizontal zone in which the fish was observed was noted and the number of entries into any of the middle or top vertical zones and time spent immobile were continuously noted. More anxious fish are expected to spend more time motionless and around the edges of the tank and make fewer entries into higher water layers (see Cachat et al. 2010).

2.3 Ethical note

All behavioural screening was non-invasive. Individual fish were separated from the rest of their group for the duration of the tests only. If a fish showed any sign of illness or injury it was removed from experimental set up and euthanized with an overdose of buffered Tricane-S to prevent suffering. One of the control groups (10 fish) were prematurely eliminated from the experiment due to three fish developing fungal infections. The infected fish were euthanized with an overdose of buffered Tricane-S to prevent suffering and the entire group was excluded from the experiment as the social environment of the group had been changed, possibly affecting the behavioural development. Elastomer tagging was performed under anaesthesia and fish were allowed to recover for three days before screening. The long term effects of tagging such as pain, discomfort from anaesthesia or handling stress were considered minimal. There was no mortality associated to tagging, anaesthesia, handling or behavioural screening. When the behavioural screening was finished the fish were euthanized using an overdose of buffered Tricaine-S. As the fish were from a hatchery it was not possible to release them into local streams, hence the need for euthanasia after the completion of the experiment. All of the experiments were approved by the Penn State IACUC Committee, Protocol # 45265.

2.4 Data analysis

The data were analysed in StatView 4, R 3.1.1 (R Development Core Team, <http://www.r-project.org>) and Stata 13. Standard length data were compared in a one-way ANOVA with fish length as the dependent variable. In the flexibility tests the time to find the hole and reach the other side of the tank containing the feeder was analysed using survival analysis with Cox regression, where also censored observations are taken into account, followed by pairwise comparisons between treatments. Time was used as the dependent variable and treatment and task as the independent variables (factors). Based on the number of fish that completed the task, the probability of the fish finding their way to the other side of the tank was analysed using a mixed effect logistic regression model, with probability to exit (0/1) as the response variable and treatment and task number (1-4) as explanatory factors.

In the spatial learning assay, the number of fish that found the true exit was compared with a Chi Square-test on trial 1 and trial 13. Based on the number of false exits the fish entered before exiting the maze the average probability to exit the maze without making any mistakes was calculated using a geometrical probability model ($\text{logit}(p)=a+b*\text{trial}$). The model estimation was performed in Stata 13 with a maximum likelihood procedure where also censored observations are taken into account. To compare the exit-probability between treatments, the average p at trial 1 and trial 13 was compared using ANCOVA on logit-transformed p in order to fulfil normality, with length as a co-variate, followed by pairwise test. To compare the time it took for fish from the different treatments to exit the maze data was analysed using survival analysis with a Cox regression shared frailty model, where all observations from one specific fish shares the same frailty, corresponding to a random effect model at fish level. Explanatory variables are treatment, trial and length.

In the anxiety test the time spent motionless on the bottom of the tank and position on the tank floor (number of middle or edge registrations) were analysed using an ANOVA and Kruskal-Wallis test respectively. The proportion of fish that moved between the bottom of the tank and any of the surface water layers was compared with a Chi Square-test and the number of entries into higher water layers of the tank was analysed using Welch's test.

3. Results

3.1 Fish Size

All fish had their length measured when the behavioural trials were concluded. Differences in mean length was analysed separately for the flexibility and anxiety test and for the maze tests as two different groups of fish were used in these tests. Mean lengths $\pm$ SE for the flexibility and anxiety tests were 14.3 ± 0.4 cm for Early, 11.5 ± 0.3 cm for Late, 15.4 ± 0.3 cm for Full and 15.1 ± 0.5 cm for Control. For the maze test the mean lengths $\pm$ SE were 13.6 ± 0.3 cm for Early, 14.4 ± 0.4 cm for Late, 14.6 ± 0.3 cm for Full and 15.0 ± 0.5 cm for Control. The Early fish were significantly smaller than the other treatments ($F_{3,144} = 3.84$, $p=0.01$) in the maze group, hence length was incorporated into the maze analysis.

3.2 Flexibility

Based on the number of fish that completed the task the probability that a fish found the exit hole and moved to the other half of the test tank was consistently higher for the Full fish, followed by the Late treatment, the Early treatment and then the Control fish (Fig. 3), with a significant treatment effect (mixed effect logistic regression, Wald $\chi^2=37.67$, $p<0.03$). Post-hoc tests revealed that there were differences between Full and Early treatments (Fisher's LSD, $p=0.029$)

and between Full and Control fish (Fisher's LSD, $p=0.015$). The Full fish were also faster to find the exit hole to enter the side of the tank with the feeder than the Control fish (Cox regression with pairwise comparisons, Full vs. Control $p=0.01$).

3. Spatial cognition

There was no difference between treatments in the number of fish that managed to exit the maze on either trial 1 ($\text{Chi}^2=1.967$, $\text{df}=3$, $p=0.579$) or trial 13 ($\text{Chi}^2=3.098$, $\text{df}=3$, $p=0.377$). However, there was a significant difference between treatments in the probability to exit the maze without making any mistakes (trial 1: $F_{3,68}=4.79$, $p=0.004$, trial 13: $F_{3,68}=2.88$, $p=0.042$). The Full fish had higher mean probability to exit the maze without mistakes than Early and Control fish (Fig.4) on both trial 1 (Fisher's LSD, Full vs. Early $p=0.003$, Full vs. Control $p=0.018$,) and trial 13 (Fisher's LSD, Full vs. Early $p=0.028$, Control vs. Full $p=0.019$). Late fish had higher mean probability to exit the maze without mistakes than Early and Control fish on trial 1 (Fisher's LSD, Late vs. Early $F= p=0.008$, Late vs. Control $p=0.032$). There was no effect of length on probability to exit without mistakes (trial 1: $F_{3,68}=2.77$, $p=0.101$, trial 13: $F_{3,68}=1.40$, $p=0.241$). Fish significantly improved their time to exit across trials (Wald $\text{Chi}^2=136.24$, $p<0.001$, Fig. 5) and the Full and Late fish were also generally faster to exit the maze than the Control fish (Wald $\text{Chi}^2=136.24$, Full vs. Control $p=0.048$, Late vs. Control $p=0.014$). Length did not have a significant effect on time to exit (Wald $\text{Chi}^2=136.24$, $p=0.175$).

3.4 Anxiety assay

Anxiety was not affected by timing or duration of enrichment. There were no treatment differences in how long the fish froze, where they were positioned in the tank and how much they used the middle vertical part of the water column (Table 1). Only nine fish in total entered the upper most water layer hindering statistical analysis.

4. Discussion

Our results support earlier observations that exposing captive animals to physical enrichment positively affects behavioural development (Hunter et al., 2002; Kempermann et al., 2002; Bredy et al., 2003; Rabin, 2003; Braithwaite and Salvanes, 2005; Salvanes et al., 2013; Näslund et al., 2014). However, our results further refine these earlier observations by showing that timing and duration that the animals experience the enrichment can affect the strength of the response. Exposing juvenile rainbow trout to enrichment for three consecutive months produced fish that showed the most improvement in terms of their flexibility and in their learning ability, however we found no effect on anxiety-related behaviours. The next most competent group were the fish that had most recently experienced the enrichment, whereas the group that experienced enrichment in the first part of the experiment, but then returned to standard housing, behaved more similar to the control fish that never experienced any form of enrichment. Together, these results show that both the duration and the recency of which the fish are exposed to enrichment matter for their cognitive ability.

Being able to find alternative ways of tackling a problem (Giraldeau et al., 1994; Reader and MacDonald, 2003) likely improve survival in changeable, real-world environments (Salvanes and Braithwaite, 2006; Näslund et al., 2014; Johnson et al., 2014). Fish reared in an enriched hatchery environment have previously been shown to have a more context dependant shoaling behaviour (Salvanes et al., 2007) and shelter-seeking behaviour (Näslund et al., 2013). Physical enrichment further seems to improve flexibility of feeding behaviour as seen in the ability to rapidly switch to a new food source (Braithwaite and Salvanes, 2005). We tested this capacity to flexibly utilize the environment by determining how quickly fish could find their way into a part of the tank that contained a food reward. The first two tests were designed to be relatively simple for the fish, whereas the third and fourth tests were more difficult (Reader & Laland 2000). For each test, the

fish exposed to the enrichment for the longest, the Full group, were consistently better at solving these tasks. This group were not only faster at finding their way into the rewarded compartment, but the probability of a fish completing the tasks was also highest in this group. The proportion of fish that managed to find the exit decreased considerably across all treatments in the third and fourth assay when the fish now had to swim into and then orient themselves correctly within an opaque, dark tube which increased the difficulty of finding the way through to the other side of the tank (Reader & Laland 2000). The Full fish stand out in terms of both the speed with which they solved this problem and in terms of how many of the fish were able to reach the food reward successfully. The next best explorers were the group that had the enrichment most recently (the Late group). So both duration and recency effects appear to help the fish in terms of developing flexible behavioural responses.

As almost all animals need to find their way around and learn about their environment, tests of spatial learning can provide useful insight into an individual's learning and memory ability (Healy 1998). Spatial learning involves perceiving and interpreting spatial cues, as well as storing this information, and being able to retrieve the information to follow a specific route or find a location (Brown et al. 2008). Fish have shown increased learning abilities from environmental enrichment in a number of different foraging and spatial learning tasks (Spence et al., 2011; Braithwaite and Salvanes, 2005; Rodewald et al., 2011; Strand et al., 2010). For example, Atlantic salmon (*Salmo salar*) from enriched conditions were significantly faster to learn the correct exit in a maze, they made fewer mistakes and were faster to leave the maze, compared to fish from plain environments (Salvanes et al., 2013). The maze task we used to assess learning was relatively simple (Grassie et al. 2013), but the increasing probability to find the exit without entering any false exits and the decreased time it took to do so indicates learning, and there were clear differences in the ability to perform this task across the different treatment groups. Both the Full and the Late fish were faster to exit the maze than Control fish, but time to exit could be

315 affected by motivation and so may not necessarily a reliable indicator of learning. However, the
316 fact that this faster performance was also accompanied by more accurate choices is a stronger
317 indication that both the Full and the Late groups had superior learning and memory skills than
318 either of the Early and Control fish. At five months of age major selection has probably already
319 occurred in wild salmonids as early life stage mortality in the wild is very high (McNeil, 1991);
320 therefore there are likely critical periods for development and learning occurring before this age.
321 However, the present results indicate that learning abilities can still be manipulated after this
322 period, when fish are slightly older and less vulnerable to potential negative effects associated
323 with the structural enrichment, such as waste accumulation.

324
325 The novel tank diving test has previously been used to gauge levels of anxiety by testing how fish
326 respond to being isolated in a completely new environment (Cachat et al., 2010; Maximino et al.,
327 2010). Typically, fish dive to the bottom of the tank and remain there until they become
328 sufficiently comfortable to start exploring their surroundings. Fish that are less anxious tend to
329 move and explore the tank and swim up into the different parts of the water column earlier than
330 other fish. This assay is rather like a 3-dimensional version of the 2-dimensional open field arena
331 test where animals with lower levels of anxiety move away from the edges and into the middle
332 more quickly (Simon et al., 1994; Harris et al., 2009). Anxiety has previously been shown to
333 decrease in fish from environmentally enriched environments. Braithwaite and Salvanes (2005)
334 showed that cod from standard hatchery environment had significantly higher latency to enter a
335 new area with a stimulus fish compared to fish reared in an enriched environment, indicating that
336 enrichment promotes boldness. They further found that experience with a variable environment
337 produced cod that were faster to recover from a simulated predator attack. However, the results
338 from this study show that exposure to enrichment does not appear to affect anxiety in terms of
339 how often the fish left the floor of the test tank and how they moved in the water column. Even
340 though trout is not constrained to bottom dwelling behaviour in nature (e.g. Galbraith, 1967), the

novel tank diving test was originally designed to assess anxiety in zebrafish (Cachat et al., 2010), and it may be misleading when measuring anxiety in rainbow trout.

When any captive reared animal is released into the wild an entirely new world awaits it, and this puts considerable demand on the animal's ability to adjust and adapt. Stocked fish need to learn to recognize new food sources, how to find them and handle them, how to recognize and avoid predators and how to learn and interact appropriately with competitors (Johnsson et al. 2014, Jonsson and Jonsson 2014). Predator avoidance, possibly one of the most important skills to learn in most environments, is partly innate and partly dependant on experience (e.g. Vilhunen and Hirvonen 2003, Patton and Braithwaite 2015) and the ability to learn quickly will provide a survival advantage because there is seldom room for trial and error learning in predator encounters. Other than predation, however, variable and changing environments will naturally present different and varying challenges to animals. An ability to quickly adjust and be able to cope with changing conditions would add a further survival advantage (Näslund and Johnsson 2014). The flexibility test results indicate that the longer the fish experienced enrichment, the more flexible and the better they become at adjusting their behaviour to the current circumstances.

5. Conclusion

Together, the results of this study indicate that the duration and recency of exposure to environmental enrichment do influence trout behavioural robustness. The best improvements in terms of developing better learning and memory skills and higher flexibility were found in fish exposed to at least three months of enrichment. However, interestingly, positive effects were also seen in fish that had most recently experienced the enrichment, even though they had had less overall exposure to it. From an applied perspective, the longer hatcheries can provide environmental enrichment the better, but if for some reason long periods of enrichment are not

possible, then providing access to enrichment just before the fish are released might be the best alternative practice.

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Figure captions

Figure 1: Schematic diagram of the side of the test tank and four different wall arrangements for the innovation task. The fish had to find a route from one side of the tank to the other to gain access to a feeder. Each wall was opaque with a single rectangular hole on either the left side (Test 1), and or the right (Test 2). For Wall 3, the fish had to swim down and then up into an opaque tube to reach the hole in the divider. For Wall 4 the set up was the same, except the fish now had to swim down into the tube to reach the hole.

Figure 2: Schematic diagram of the four-armed maze used for the spatial learning assay. The fish is placed in a transparent cylinder in the middle of the maze before it is allowed to freely move around the maze to find the exit.

Figure 3: The predicted probability of a fish in each treatment group finding their way into the alternative half of the test tank and into the feeding area over each of the four flexibility trials shown as a fixed proportion.

Figure 4: The probability of exiting the maze without making a mistake for each treatment group over 13 maze trials.

Figure 5: The mean time fish took to exit the maze for each treatment group over all 13 maze trials.

Table 1. Summary of data and analyses for the novel tank test used to assess anxiety.

<i>Screened behaviour</i>	<i>Mean $\pm$ SE</i>	<i>Test used</i>	<i>Statistic result</i>
<i>Time spent motionless on the bottom of the tank (sec)</i>	<i>E=166 $\pm$16.3 L=176 $\pm$19.7 3M=183$\pm$20.8 C=182 $\pm$15.8</i>	<i>ANOVA</i>	<i>F_{3,72}=0.19 p=0.91</i>
<i>Number of observations on middle of tank floor</i>	<i>E=6.3$\pm$1.3 L=6.7$\pm$1.4 3M=8.5$\pm$1.5 C=5.9$\pm$1.3</i>	<i>Kruskal Wallis test</i>	<i>H=1.86 p=0.60 df=3</i>
<i>Number of observations on edges of tank floor</i>	<i>E=13.7$\pm$1.3 L=13.4$\pm$1.4 3M=11.5$\pm$1.5 C=14.1$\pm$1.3</i>	<i>Kruskal Wallis test</i>	<i>H=1.86 p=0.60 df=3</i>
<i>Number of entries into mid vertical zone of the tank.</i>	<i>E=2.1$\pm$0.3 L=1.7$\pm$0.4 3M=3.4$\pm$0.8 C=6$\pm$1.7</i>	<i>Welch's test</i>	<i>F_{3,9,15}=2.86 p=0.09</i>
<i>Proportion of fish leaving the tank floor</i>	<i>E=43% L=35% 3M=42% C=25%</i>	<i>Chi2-test</i>	<i>Chi2= 1.1, p=0.77</i>

Figure1

Wall 1

Wall 2

Wall 3

Wall 4

Figure2

Figure 3

Figure 4

Figure 5