



Article Behavioral Response of Multiple Rainbow Trout to Vertically Suspended Environmental Enrichment

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Abstract: Vertically suspended environmental enrichment improves the rearing performance of fish during aquaculture production, but the reason for this improvement is unclear. This study documented the behavior of groups of rainbow trout (*Oncorhynchus mykiss*), as indicated by their in-tank location preferences, in response to vertically suspended structure. The location of both experienced and naïve fish (with or without prior exposure to vertically suspended enrichment) in both barren and enriched tanks was recorded. In-tank locations were significantly different for experienced fish in both barren and enriched tanks but were similar for naïve fish. When observations were combined for both the presence and absence of enrichment, naïve fish locations were significantly different from experienced fish. Locations were also significantly different for all fish in enriched compared to barren tanks. These results indicate that trout location is influenced by the presence of vertically suspended environmental enrichment and that learning from prior experience with enrichment occurs.

Keywords: behavior; rainbow trout; Oncorhynchus mykiss; enrichment

1. Introduction

Enriching the rearing environment of typically sterile hatchery tanks has been investigated with many fish species [1–3]. This environmental enrichment has been shown to increase fish growth [4,5], increase post-stocking survival of hatchery-reared fish [6,7], promote fish learning [8,9], and decrease stress from the growth environment [3]. Not all results have been positive though. Tank enrichments have been shown to be a possible vector to trap feces and food while interfering with the hydraulic self-cleaning nature of circular tanks [10,11].

Kientz and Barnes [5] first described a form of environmental enrichment using intank structure vertically suspended from the top of a circular tank. They noted dramatic improvements in rainbow trout (*Oncorhynchus mykiss*) rearing performance in conjunction with no change in circular tank hydraulic self-cleaning. Numerous subsequent studies have confirmed these results [12–18].

The reason for the improvement in fish rearing performance is unknown. Vertically suspended enrichment alters the velocity profile of circular tanks, creating areas of increased and reduced flows within the same tank [19–21]. The fish may be using the variation of water velocities within the tank, and particularly the reduced velocities behind the suspended structure [21], to minimize energy expenditures.

Little is known about the behavior of fish exposed to vertically suspended environmental enrichment. Morris et al. [22] examined the behavior of individual fish and found similar responses of fish placed into tanks either with or without suspended structure. However, because fish behavior can be influenced by the presence of conspecifics [23], information based on single fish may not accurately reflect what is occurring in larger groups of fish used



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Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). in production aquaculture. Thus, the objective of this study was to evaluate the response of small groups of fish exposed to vertically suspended environmental enrichment.

2. Methods

This experiment was conducted at McNenny State Fish Hatchery, rural Spearfish, South Dakota, USA, using degassed and aerated well water (constant temperature 11 °C; total hardness as CaCO₃, 360 mg L⁻¹; alkalinity as CaCO₃, 210 mg L⁻¹; pH, 7.6; total dissolved solids, 390 mg L⁻¹) in 2000-L circular tanks (1.8 m diameter \times 0.8 m deep; 0.6 m water depth). The tanks were near fully covered [24] and were either barren (no environmental enrichment) or contained an array of four vertically suspended aluminum angles (2.5-cm wide on each angle side \times 57.15-cm long) suspended from the corrugated plastic cover as described by Krebs et al. [12] (Figure 1). The angles were arranged so that the angled portion faced into the direction of water flow.



Figure 1. Circular tank with suspended array of four aluminum angles, with the peak of the angle facing in the direction of the water flow.

At the start of the experiment, a group of five rainbow trout were randomly selected from a common pool (mean \pm SE, weight 38.7 \pm 3.9 g, length 147.8 \pm 4.4 mm) of either naïve (never exposed to enrichment) or experienced (reared with enrichment) fish. These fish had been reared in this manner for approximately 180 days post-hatch. Each group of five fish was then placed into a tank with the same configuration (i.e., fish reared with structure were placed into a tank with structure; fish reared in a barren tank were placed into a barren tank). After a 30-min acclimation period, the location of the fish in tank was recorded every hour for the next four hours. After this four-hour period, the group of fish was subjected to the alternative tank configuration. Vertically suspended enrichment was added to the barren tank, while suspended structure was removed from the enriched tank. A 30-min acclimation period was used again, followed by hourly observations over the next four hours. This procedure was repeated for a total of four times, each time with a new group of five naïve trout. It was also repeated for a total of four times, each with a new group of experienced trout (N = 4). In order to make observations, the experimental tank was split into quadrants by painted white lines on the tank walls and floor that were easily visible (Figure 2). Observations were recorded using Lorex LBV2531W security cameras (Lorex Technology Inc., Markham, ON, Canada) suspended from the tank covers (Figure 3). At each hourly observation period, a 15-s clip was recorded from the cameras, and the location of each fish was recorded.



Figure 2. Zones (quadrants A, B, C, and D) and camera (8 total cameras) locations in tank.



Figure 3. Image of fish behind vertically suspended enrichment in zone B (under the spray bar).

Data were analyzed using the SPSS (24.0) statistical program (IBM Corporation, Armonk, New York, NY, USA). A weighted cases chi-squared test was used with significance predetermined at p < 0.05.

3. Results

Experienced fish were in significantly different locations in tanks with and without enrichment ($\chi^2 = 19.169$, p = 0.001). Naïve fish locations did not differ with or without the presence of suspended environmental enrichment ($\chi^2 = 2.057$, p = 0.561; Table 1).

Table 1. Location frequencies for small groups of rainbow trout, either naïve and experienced with vertically suspended environmental enrichment, in tanks either barren or containing vertically suspended environmental enrichment (N = 4). Letter A is the location of the enrichment (if present), B is the spray bar, C is immediately after the spray bar, and D is immediately prior to the enrichment (if present).

	Enrichment	Α	В	С	D	x ²	p
Naïve	Yes	9	45	11	15	- 2.057	0.561
	No	10	42	17	11		
Experienced	Yes	25	36	8	11	- 19.169	0.001
	No	10	61	7	2		

4. Discussion

The results of this study indicate that the presence of vertically suspended environmental enrichment changes the in-tank locations of groups of juvenile rainbow trout, once they have become accustomed to it. This supports the hypothesis that some learning is required for the development of this behavior [25,26]. The area within the suspended structure is one of lower water velocity [19–21], suggesting that the observed improvements in rearing performance [5,13–18] may be because of reduced energy demands. This location, and the preference for the location immediately behind it (under the spray bar), also supports the hypothesis that it is more energetically profitable for fish to be in lower velocity microhabitats [27] only to briefly swim into areas of higher velocity to feed [28–30]. However, the relatively higher fish use of the area in front of the structure, in comparison to a barren tank, is contradictory. This suggests that minimizing energy expenditures in lower-velocity areas may not be the only reason for the improvements in fish growth, feed efficiency, or carrying capacity observed with the use of vertically suspended environmental enrichment [5,13-18]. While stress hormones were not tested in this experiment, the mere presence of environmental enrichment has been shown to reduce metabolic rates, which could increase growth parameters in a hatchery environment [31].

The results from this study with a group of fish are both similar to, and different from, those reported by Morris et al. [22]. Just as in this study, Morris et al. [22] reported a significant preference for the location under the tank spray bar. This is not surprising because fish in natural environments tend to prefer areas of turbulence or unsteady flows [32–37]. The area below the spray bar not only has reduced velocity from the structure in the preceding zone, it also contains the top water turbulence created from the incoming water.

The results from this study differ from the single-fish study of Morris et al. [20] because experienced groups of trout preferred different locations within the circular tank when structure was present or absent. When structure was present, the fish preferred locations in front of, and within, the structure and under the spray bar. When structure was absent, they overwhelmingly preferred to be under the spray bar. The difference between this study and Morris et al. [22] could be due to the effects of conspecifics on individual fish behavior [23,38,39]. For example, Schaerf et al. [40] showed that individual fish alter their individual behavior in relation to their conspecifics when an alarm of food stimuli is applied.

Similar to other behavioral studies, this experiment has obvious limitations. It is difficult to observe and quantify fish behavior for periods longer than an immediate reaction [41]. Making conclusions from a small group (five fish) is also problematic. While it would be beneficial to record observations of a tank of trout at production levels of thousands of fish, it would be extremely difficult.

5. Conclusions

In conclusion, this study indicates that fish react to vertically suspended environmental enrichment by altering their within-tank locations. In addition, there is likely an acclimation period, with learning occurring.

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Institutional Review Board Statement: This experiment was performed within the guidelines set out by the Aquatics Section Research Ethics Committee of the South Dakota Department of Game, Fish and Parks (approval code, SDGFPARC20222), and within the guidelines for the Use of Fishes in Research set by the American Fisheries Society.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to not having a public directory to share raw data.

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