An Education Platform for Observing the Group Swimming of Steelhead, a Migratory Salmonid Species Vulnerable to Climate Change

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Abstract
A migratory trout steelhead is an economically important cold water species whose habitat is shrinking due to environmental and climate changes. Recently, research on species conservation through aquaculture has sparked interest in group swimming in artificial environments. In this study, based on universally accessible two-dimensional image processing, we present a statistical framework of the effects of air sparging on group swimming and interaction of steelhead juveniles in a small aquaculture environment over time. The cross-correlation-based framework showed that steelhead learned to school in larger groups as time went by. The air sparger attracts fishes as oxygen suppler, but at the same time, it exists as a spatial barrier to interaction when juveniles are young. However, as growing older, they overcome this barrier and begin to interact with each other. These results indicate the importance of proper sparger design in helping steelhead learn to shoal in aquaria before release.

Keywords: Steelhead, Aquaculture, Shoaling, Juvenile, Statistical Framework

Introduction

Steelhead is an anadromous fish that migrate between the ocean and rivers. This cold-water species has seen its habitat shrink due to water pollution and rising ocean temperatures – it particularly works as an indicator of the effects of recent global warming (Wade, 2013; Winfree, 1998). Furthermore, climate change and overfishing are putting pressure on conservation efforts. Understanding fish ecology is the first step to conserve species, and their swimming patterns are a crucial part to consider, as well as feeding and breeding. Especially, group swimming, a group behavior characterized by schooling and shoaling, is a common behavioral pattern in many fishes (Fréon, 2000). Fish benefit from group swimming for reproduction, protection from predators, and other survival benefits (Partridge, 1982; Pitcher, 1982). In this sense, the study of steelhead swimming patterns is an important part of their ecology, which shows why it is currently being studied. Usually, fish learn to swim in groups as juveniles, influenced by the behavior of other fish around them. This process results in a collective swimming behavior. Steelheads also seem to go through a juvenile period when they prepare to learn this group pattern. As an anadromous fish, trout swimming patterns in dynamic fluid environments during the juvenile stage have been a topic of interest to several researchers (Liao, 2003; Harvey, 2022).

In response to the decline of steelhead habitat and populations, commercial steelhead culture, often through aquaculture at hatcheries, has been increasingly practiced in recent years. In particular, due to the nature of aquaculture, juvenile steelheads are often raised. However, unlike the natural environment, aquaculture is carried out in an artificial environment and a relatively narrow environment. For example, dissolved oxygen is a significant factor for fish, so in aquaculture, an air sparging module is used in a small cage space to supply the necessary oxygen to them. This environment provides the juveniles with stimuli different from their natural environment, which affects their ecology. For instance, studies have shown that hatchery-raised steelhead differ in 723 genes from wild ones and have a lower reproductive success rate when they return to the river (Araki, 2007; Christie, 2016). It is already known that the environment affects fish shoaling, including hatchery group size and familiarity. In fact, compared to a wild population of rainbowfish, captive-reared ones showed a lower shoaling preference for familiar individuals (Kydd, 2009). Therefore, as steelhead aquaculture increases in the future, studying the behavioral patterns of steelhead juveniles in artificial environments will be an important research topic, both economically and in terms of managing their adaptation in the wild when released. Yet, this research is still in its infancy and not many studies have been conducted.

A variety of complex sensors and analytical equipment are used to study fish behavior. Even so, two-dimensional images are the easiest to obtain and provide a wealth of data, and 2D image analysis is crucial to the study of fish in aquarium culture. However, there is a limitation that fish kinetics information is limited compared to three-dimensional information, and various methods for image analysis are needed. Therefore, this study presented a rationale and statistical framework to analyze behavioral patterns based on 2D images of steelhead fry in a small aquaculture. Observations on the grouping and shoaling of juvenile steelhead around the air sparging module were conducted, and statistical analysis was performed to understand the group behavior patterns of juveniles as they grow over time.

Materials and Methods

Design of the Aquarium for Steelhead

The small aquarium was made of glass. The length, width, and height were 20, 10, and 12 inches, respectively. The bubble sparger was placed in the center of the water bath, as described in Figure 1.

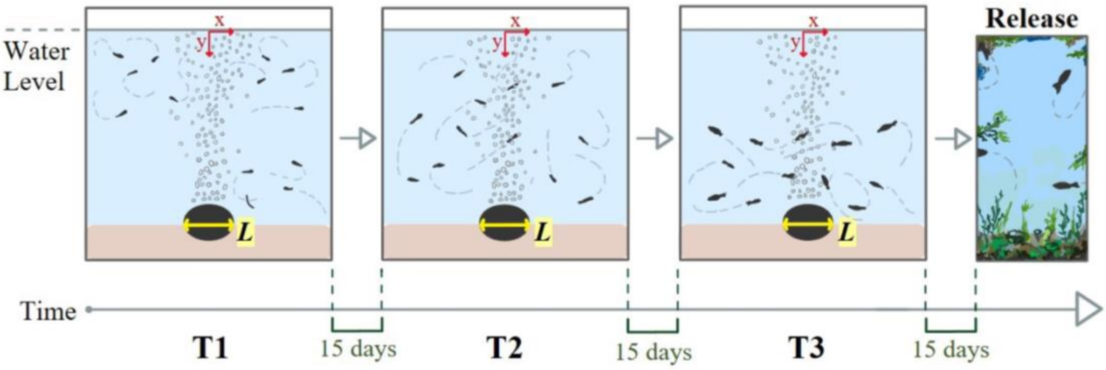

Figure 1: Construction of a Small Aquarium System for Juvenile Steelheads.

Capture of the Steelhead Motion

Steelhead fry movements were videotaped 45, 30, and 15 days prior to release. Filming was conducted from the same point at the front of the small aquarium. The free software, 'Video to GIF converter' supported by ezgif.com was used to extract time-serial images every 0.5 seconds.

Identification of Time-Dependent Position of Steelhead

The image was observed with ‘ALSee image viewer’ (ESTsoft Corp., Korea), and the center point of the bubbling sparger was set as the reference point of the horizontal axis, and the water surface was set as the reference point of the vertical axis. (Figure 1). The movement of each fish was tracked by the position of the fish's mouth over time. The position of each point was identified using the coordinates of the image consisting of 1280x720 pixels. To compare fish positions from different images, all position information was expressed as a dimensionless number. The method is shown in Eqn. 1. As shown in Figure 1, the horizontal length of the bubble sparger was set as the characteristic length (L), and all (x, y) positions from the reference point (0,0) of the fish were divided by L.

$$\text{Dimensionless } (X, Y) = (x/L, y/L) \qquad (1)$$

Identification of Movement Direction of Steelheads With Respect to Time

The movement direction of the fish was calculated using the position coordinates between two consecutive times t2 and t1. As shown in Figure 1, when the (x, y)=(0,0) reference point was set (Bubble sparger center, water surface), the sign [position (t2)-position (t1)] was used to determine whether the fish's movement direction was in the direction of the bubbling sparger or not. Since the movement of the fish is captured every 0.5 seconds, the number of movement directions calculated by the above method can be summed to find the time when the fish is facing the opposite direction of the bubbling sparger. A t-test with a 95%

confidence level was performed to determine whether there was a significance of preference for these two directions.

K-means Clustering

When the locations of the fish over time are plotted as a scatter plot of the dimensionless (x,y) coordinates of the image, the locations of the fish can show groupings. MATLAB's k-means clustering function was used to calculate the centroid of each of these groups.

Correlation Analysis

Pearson coefficient was used to determine the linear correlation between the swimming position tracks of two fish over the same time period (Eqn. 2).

$$\rho = \frac{\sum_{i=1}^{N}(X_i - \bar{X})(Y_i - \bar{Y})}{(\mathrm{N}-1)\sigma_X \sigma_Y} \quad (2)$$

where X, Y are the location coordinates of each fish, $\bar{X}$, $\bar{Y}$ are the means, and σ is the variance. In addition, cross-correlation was used to determine if the time-varying swimming movements of the two fish over the same time period were linearly correlated with a constant time lag (Eqn. 3).

$$\rho = \frac{\sum_{t=1}^{T-k}(X_t - \bar{X})(Y_{t+k} - \bar{Y})}{\mathrm{N}\,\sigma_X \sigma_Y} \quad (3)$$

Results

Statistical Framework to Assess Preference for Bubbling Area and Quantify Shoaling Behavior

In order to make a comparative analysis of time-dependent fish behavior, it was necessary to make the position of the fish in the image a dimensionless parameter. To do this, the horizontal and vertical distance of each fish's mouth from the reference point in Figure 1 was divided by the horizontal length of the bubbling sparger, L. Eventually, all positions become dimensionless parameters, and the position information of the images at three different times could be compared with each other. The orientation of the fish to the bubbling area was determined by a vector equal to the difference between the positions at two consecutive times. If the direction of the vector is toward the bubbling area, it is defined as a preference. Shoaling behavior was defined as whether the swimming path of one leader fish is followed by another fish within at most 2s time lag. For this purpose, a cross-correlation analysis was performed to check if there was a positive correlation between leader and follower fishes in both horizontal and vertical directions within at most 2s.

Validation of the Efficacy of the Suggested Statistical Framework Using the Well-Known Shoaling Behavior of Steelhead-Feeding

When feeding, fish interact with other individuals (Holbrook, 1992). In order to validate the efficacy of the suggested statistical framework for assessing shoaling performance of steelhead, the proposed statistical framework was applied to the shoaling behavior at T3 time, which was certainly observed when fishes were feeding. It has been observed that when a fish travels to the surface of the water to feed, another fish follows its path. Following the statistical framework, several pairs of fishes showed positive cross-correlations in both horizontal and vertical directions within at most 2s (Figure 2). The result implies that the statistical framework is suitable for assessing whether steelhead shoaling is occurring.

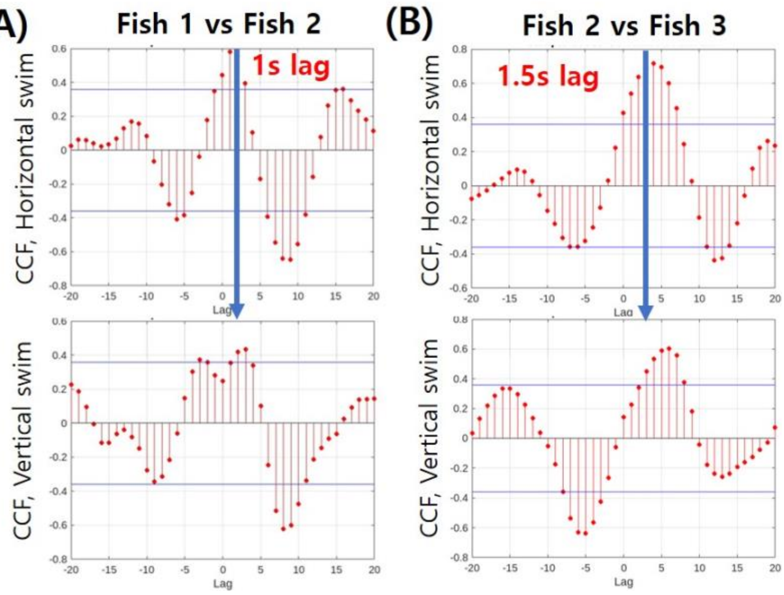

Figure 2: Validation of the Proposed Statistical Framework Through Cross-Correlation Analysis of Shoaling Behavior of Feeding Juvenile Steelheads.

Comparative Analysis of Group Behavior Patterns Over Time in the Aquarium

Figure 3(A) shows a scatter plot of the horizontal and vertical positions that each fish moved every 0.5 seconds. As shown in the results, the living space is organized into three groups. The size of the groups is such that most of them live close to the water surface and only a few are observed at depth. Even at the water surface, different groups form on both sides, starting from the bubbling area in the middle of the aquarium. The peculiarity is that fishes move mainly within their own group, with little interaction between them. Figure 3(B) shows the results of K-means clustering on the movements of all the fishes. The result shows the centroid of each group.

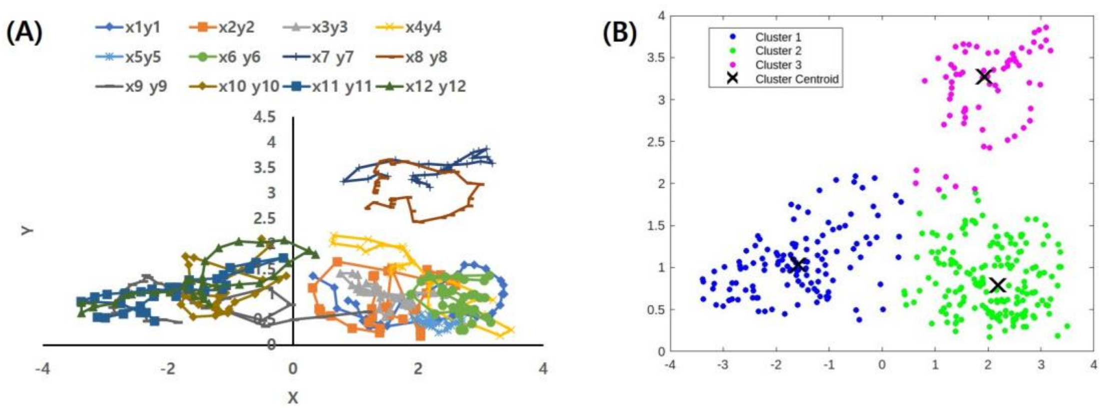

Figure 3: Scatter Plot and K-means Clustering of Horizontal and Vertical Swimming Positions of Juvenile Steelhead at Time T1. (A) Scatter Plot, (B) K-means Clustering.

Figure 4(A) shows a scatter plot of the horizontal and vertical positions that each fish moved every 0.5 seconds at T2, 15 days after T1. Compared to T1, the behavioral radius of the fishes increased with the number of fishes moving vertically from the water surface to deeper water. In addition, few fish crossed the bubbling area in T1, but some fish crossed it in T2. Therefore, although almost isolated groups were clearly distinguished in T1, the horizontal and vertical activity area became wider in T2, and the isolated group with centroid was not clear, even though 2 centroids were pointed out (Figure 4[A], 4[B]).

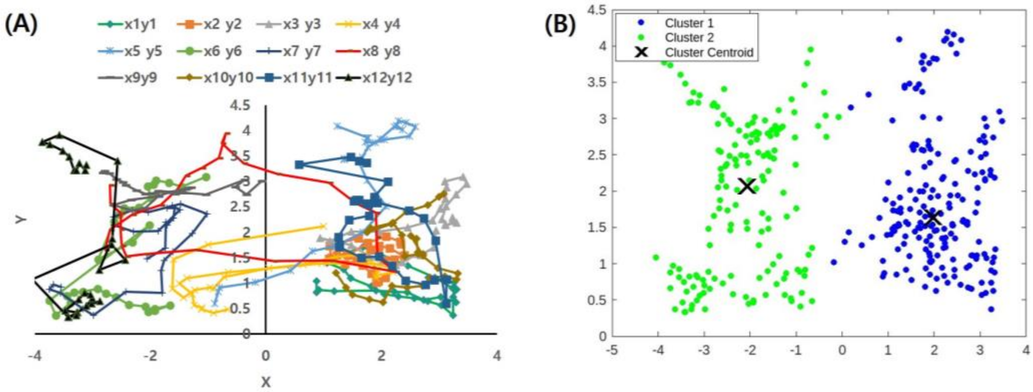

Figure 4: Scatter Plot and K-means Clustering of Horizontal and Vertical Swimming Positions of Juvenile Steelhead at Time T2. (A) Scatter Plot, (B) K-means Clustering.

The number of individuals crossing the bubbling area was higher than those at T2, and beyond the behavior of going deeper on the water surface in T2, almost all of the individuals were living at a significantly deeper depth compared to T1. As a result, home ranges became more similar and concentrated, as evidenced by the convergence into two groups with two centroids (Figure 5[A], 5[B]).

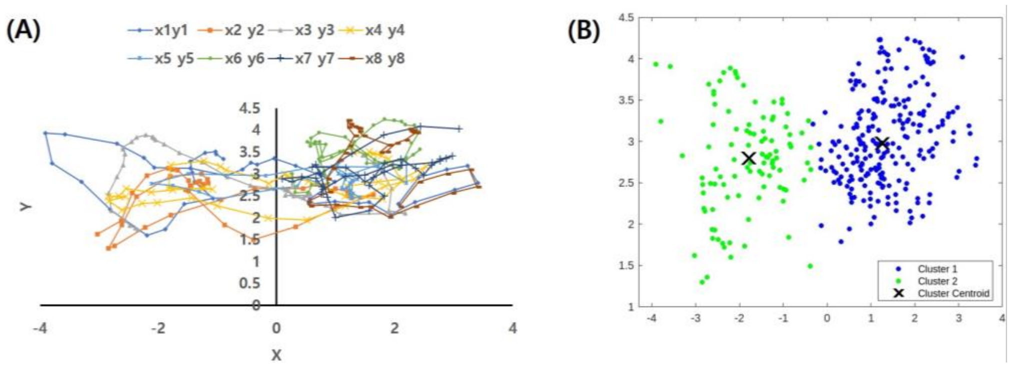

Figure 5: Scatter Plot and K-means Clustering of Horizontal and Vertical Swimming Positions of Juvenile Steelhead at Time T3. (A) Scatter Plot, (B) K-means Clustering.

Comparative Analyses of Distance Traveled and Movement to the Bubbling Area

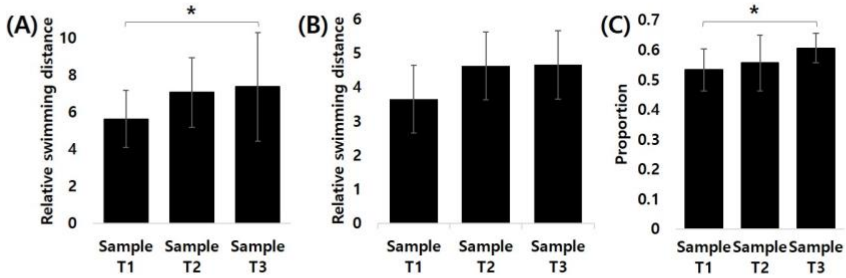

Figure 6: Horizontal and Vertical Swimming Distances and Proportion of Swimming Toward the Bubbling Area of Steelhead According to the Culture Time. (A) Horizontal Distance, (B) Vertical Distance, (C) Proportion of Swimming Toward the Bubbling Area.

As shown in Figure 6(A) and 6(B), horizontal and vertical swimming distances were compared during the observed time to determine the activity of the fishes. As a result, there was no significant difference in vertical swimming distance between T1, T2, and T3. On the other hand, the difference in horizontal swimming distance was significant for T1 and T3 and not significant for T2 and T3. In addition, the swimming preference ratio of T1, T2, and T3 fishes to the bubbling area is shown in Figure 6(C). The t-test showed a difference in preference between T1 and T3 at the 95% confidence level, but not significant between T2 and T3. These results indicate that there is no significant difference in the activity of T2 and T3 fish and their preference to swim to the bubbling area.

Comparative Analyses of Interactions of Fishes in a Group

A Spearman correlation analysis between fishes within each of the T1, T2, and T3 groups showed that although there were significant cases of correlation, the frequency was very low (data not shown). This suggests that even when fish are in the same group, they have individual swimming paths that make it difficult to find similarities in their movements at the same time.

	T1	T2	T3
Fishes crossing over the bubbling area (Number of positive cross-correlation within at least 2 second/Number of events)	-	4/20	7/25
Fishes not crossing over the bubbling area (Number of positive cross-correlation within at least 2 second/Number of events)	4/22	0/16	1/3

Table 1. Proportion of Fishes Expressing Shoaling Behavior According to Crossing Over the Bubbling Area.

On the other hand, when cross-correlation was examined for positive correlation cases in both horizontal and vertical directions within a maximum of 2 second lag (Table 1), 4 cases were found in T1 out of a total of 22 cases (Figure 7).

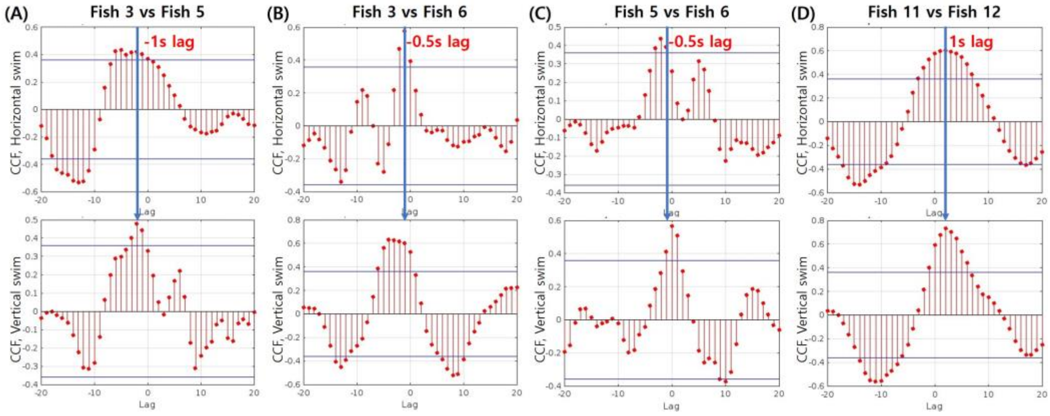

Figure 7: Cross-Correlation Analysis of Steelhead at Time T1. Positive Correlations Were Only Selected for Both Horizontal and Vertical Swimming Directions Within at Most 2 S Time Lag.

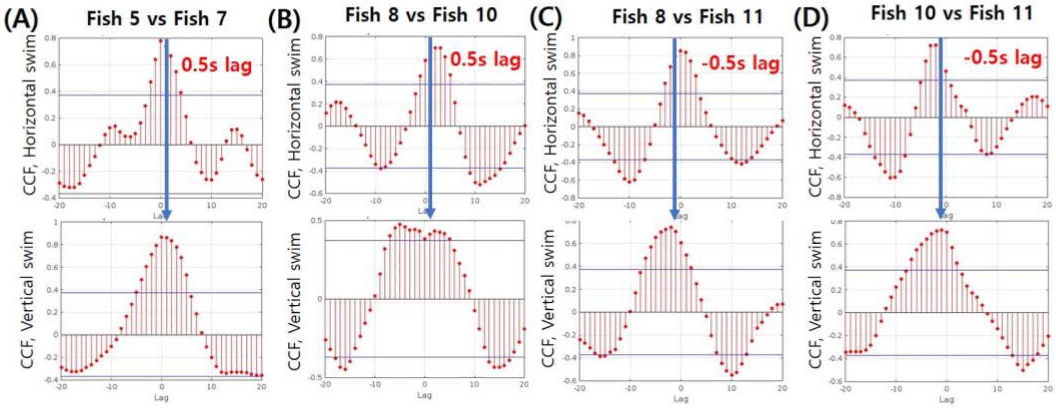

Figure 8: Cross-Correlation Analysis of Steelhead at Time T2. Positive Correlations Were Only Selected for Both Horizontal and Vertical Swimming Directions Within at Most 2 S Time Lag.

In T2, 4 cases were observed in which 3 fishes crossing the bubbling area showed positive correlation with other fishes, and 1 case was found for non-crossing fishes, out of a total number of 46 cases (Figure 8).

Finally, in T3, out of a total of 28 cases, there were 7 cases where the 5 crossing fishes made a correlation with another fish and 1 case where the non-crossing fish did (Figure 9). These results demonstrate that when crossing fish move in T2 and T3, their swimming paths are often positively correlated with the swimming paths of other fishes within a time lag of up to 2 seconds.

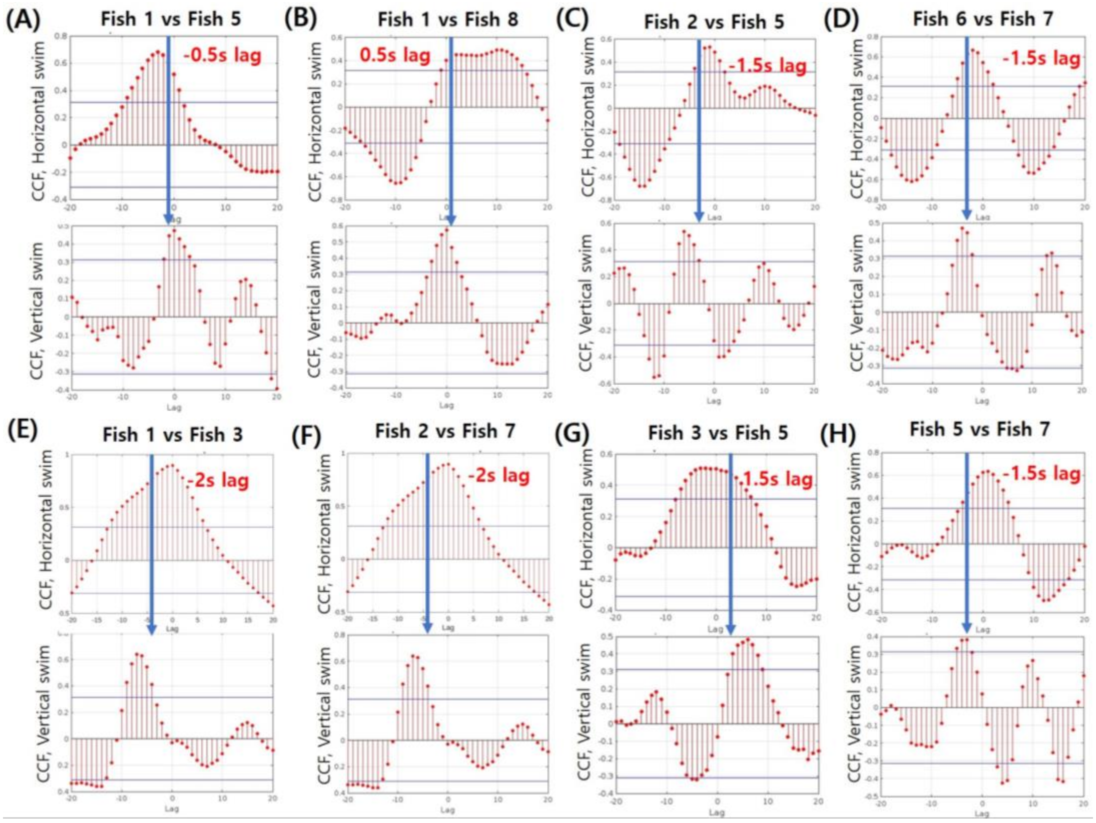

Figure 9: Cross-Correlation Analysis of Juvenile Steelhead at Time T3. Positive Correlations Were Only Selected for Both Horizontal and Vertical Swimming Directions Within at Most 2 Second Time Lag.

Discussion

As steelhead matured over time, they moved deeper in the water column, spent more time in the bubbling area behaviorally, and had a larger horizontal home range as they crossed the bubbling area. In terms of correlations with each fish, they are almost reconstructed as a single group, and as shown by the high similarity of the cross-correlation, there is a tendency for the time-series paths to be more similar, which means that as they move in the horizontal direction, there is a greater tendency for the other fish to follow the movements of one fish over time. However, this is a result. It's interesting to discuss what might have caused these results.

Assuming that the individual potentials for shoaling of T1, T2, and T3 fish are similar, the first effect of population density differences as the fish grow and their living space changes for physical reasons, such as gravity, can be considered. As the fish grows, the gravitational force becomes higher due to the increased mass, and it will eventually form a stable area deeper in the water column to the point where it equilibrates with the buoyancy force. At T1 time, the cross-correlation of swimming paths between fishes was about 18.2%, as the fishes mainly lived in a localized space close to the water surface and separated by a bubbling barrier. In T3, localized non-crossing fishes were also found to be cross-correlated, but there was no cross-correlation of non-crossing fishes in T2. In T2, as shown in Figure 2, the swimming distance in the vertical direction of the fishes is relatively long, which reduces the localization level of the living space. Therefore, it can be assumed that this population density affects the cross-correlation of swimming path. Familiarity is widely recognized as a reason for fish's shoaling preference (Sikkel, 2010; Thünken, 2016). Therefore, we can infer that shoaling in the isolated T1 in this experiment is a result of high familiarity and high fish density in a small space. However, unlike T1, there are many crossing fish in T3. And the behavioral patterns of these crossing fish may have affected the shoaling. Therefore, the cross-correlation of swimming paths in T1 and T3 cannot be explained by population density alone.

If so, it is worth examining the stark differences in the behavior of fish at T1 and T3. As shown in Figure 4, the proportion of both swimming toward the bubbling area increased at T3 compared to those at T1. In addition, the distance between the centroids of the two groups under the water surface at T3 was closer than the distance between the centroids of the two groups near the water surface at T1. This means that the fish at T3 spent more time near the bubbling area. In T1, the fishes were closer to the water surface. For this reason, the fish at T1 did not need to be near the bubbling area to get oxygen, which is one of the most important factors for fish, since they had plenty of opportunities to get it near the water surface. However, as they grew and lived deeper in the water, the oxygen concentration would decrease as they moved further away from the bubbling area, so the steelhead would need more oxygen at T3 than at T1. The difference in behavior is crossing the bubbling area. This crossing increases the horizontal activity radius of each fish. This is evidenced by the increased horizontal swim path compared to those at T1, as shown in Figure 5. Eventually, the interaction between the two groups, which were divided into left and right sides centered on the bubbling area, became active. The larger fish have more time to feel each other's presence near the high oxygen concentration near the bubbling area, and this behavior can be assumed to be the cause of the synchronization. In other words, the difference between T1 and T2 is that one fish encounters the behavior of another fish more frequently and the behavioral radius is larger, and it can be inferred that the individuals have more time and space to be influenced by each other's behavior and follow the pattern. Of course, it depends on the fish, but it is known that fishes have a strong preference for larger groups (Varmaa, 2020). It can be inferred that the increase in shoaling as the three isolated groups in T1 became one group while crossing at T3 is due to the preference for shoaling in larger groups.

The assertion that the increased horizontal swimming path through crossing increases the cross-correlation proportion is starkly demonstrated in T2. While no cross-correlation was found for the non-crossing fishes, the three crossing fishes exhibited cross-correlation despite the lower population density. These results at T2 demonstrate that not only population density, but also the opportunity to interact temporally and spatially as the swimming path of fishes increases in the horizontal direction, are important factors for steelhead shoaling. This assertion is supported by findings from other groups. According to the attraction rule theory, fishes become attracted to randomly chosen other fishes and eventually merge into larger groups as the time they continue this behavior increases (Hinz, 2017). This supports the argument that the increased horizontal swimming distance while crossing the bubbling area, as shown in our results, increases the opportunity for fishes to interact with each other, which may increase the influence on group swimming.

Conclusion

This study is a statistical analysis of how an air sparging module affects group swimming of Steelhead juveniles in a small cage. Observations were conducted at 15-day intervals for 45 days. Initially, the surface-dwelling juveniles showed cross-correlation in their swimming paths in localized high population density spaces, but failed to interact with each other by crossing the air sparging area. However, over time, as fishes moved deeper in the water column and lived closer to the bubbling area, they developed shoaling tendencies by crossing the bubbling area, increasing their interaction and horizontal swimming distance. In conclusion, we can say that horizontal swimming path distance plays as important a role in steelhead shoaling in small aquaculture as population density. From this, it can be inferred that it is possible to improve the problems of hatchery steelhead related to shoaling by designing the air sparging module for oxygen supply, which is most important for fishes, to favor the swimming shoaling of steelhead juveniles.

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