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Paul Flanagan & Mitchell Muske

Behavioral Response of Amano Shrimp with a Predatory Stimulus

Introduction:

This study observed the physical behavior of the crustacean *Caridina multidentata*, more commonly known as amano shrimp, and their response when introduced to a predation-like stimulus. Amano shrimp are natively found in the freshwater streams and swamps of Japan and Taiwan. The shrimp has a translucent body with brownish spots lining the sides of their bodies as well as two black eyes. Amano shrimp are commonly used in aquariums since their primary source of food is algae effectively cleaning its habitat.

Not much is known about the amano shrimp regarding predation other than the fact that they are often preyed upon by *Carassius auratus* (goldfish) in a short amount of time. This research sought to understand how amano shrimp are affected by the presence of fish and possible predation. This research carries significance as it could indicate whether the shrimp are affected by the water they're in, and if that affects their capabilities to carry out their main commercial purpose. While we don't specifically look at the shrimp's 'cleaning' ability in this study, the change in response based on the surrounding water may lead to further analysis of its efficiency.

When looking at the predator/prey relationship there are different ways in which prey can avoid predation. This study specifically looks at the amano shrimp and its ability to evade predation when exposed to a stimulus similar to that of a fish's suction to obtain food. When amano shrimp are attacked by fish, the fish sucks water in rapidly to bring the shrimp into its mouth. The shrimp avoids the predation primarily through the use of their entire abdomen to push itself away from the suction. This motion is conducted with the use of leg-like extensions called pleopods and a uropod. Pleopods protrude from the abdomen of the shrimp on the ventral side of their bodies and are forked to create more available surface area. These pleopods help shrimp move in water, but when used at once are very important in evading predation. The uropod is a conglomerate of fin-like appendages that create a larger fin structure perpendicular to the alignment of the body. When all these appendages are used at once, it creates a large stroke to move itself away from the predator that appears as a "jump." These jumps can reach a range of 8 cm for a 2 or 3 cm long shrimp putting a large distance between the predator fish and itself.

Our hypothesis states that amano shrimp will become more "jumpy" when introduced to a predatory stimulus while in the presence of fish. "Jumpy" is defined in multiple ways, firstly, jumpiness can be defined by distance traveled from the source of predation. It can also be defined as a quicker reaction time when first introduced to a predatory stimulus.

Methods and Materials:

This study was conducted on the Japanese Shrimp commonly purchased as household pets to clean tanks that contain fish. We purchased seven shrimp from a local pet store keeping them in clean spring water along with algae disks as their food source and a small bubbler providing oxygen. The trials were conducted in a plastic bin measuring 18 cm tall, 30 cm wide, and 42.5 cm long, with a tube inserted through its side. The tube was kept in place with epoxy to keep measurements of movement consistent. The tube had an inner diameter of 2 cm, or a round opening of 3.14 cm². Suction was created via a siphon mechanism to mimic fish predation. One half of the tube was submerged in the water while the other half of the tube hung below it allowing the siphon to function properly. The bottom end of the tube was plugged with one of our fingers, keeping the water stagnant, until the shrimp under analysis was in close enough proximity to the mouth of the tube. The plug was then released from the tube, initiating the suction, and the shrimp's response was recorded.

The shrimp's position from the tube was one variable measured and documented in this study. This was accomplished by taping all trials using a video camera that was stationed above the bin giving a bird's eye view of the entire display. Upon completion of each video the video was saved, slowed down, and watched to determine the shrimp's precise positions. From each video, we were able to distinguish each shrimp's specific starting and ending locations. Shrimp response was recorded in distance moved, in cm, from its original and response position on both x and y axis'. A piece of graph paper was placed underneath the bin, outlining distance markers, to determine distance moved. Each shrimp was placed in the bin, isolated from all others. The shrimp was prodded by the researchers to have each individual move into close enough proximity for testing. No shrimp was under testing conditions for longer than ten minutes or three separate trials. Upon completion of the trials, all shrimp were returned to a common resting place and given a buffer period of at least four hours before testing was conducted again.

The study focused on discerning the difference in responses of shrimp in spring water and fish contaminated spring water. The fish contaminated spring water was created by leaving four gallons of spring water with four goldfish undisturbed for three days. This was the water used for all the trials indicating the use of "fish water." Measurements were made and recorded for both the head and tail of each shrimp at its original position, response position, and final position. The shrimp's original position was where the shrimp was located when the plug was initially removed, creating the suction. The response position is where the shrimp was located when it responded to the suction. If the response and original position are identical, it indicates that there was no delay in the shrimp's response. If there is a recording for the shrimp's original position but no recording for its response position, it indicates the shrimp had no response to the suction. The final position of the shrimp is its position after responding to the suction, or after its "jump."

All results were then recorded on paper after viewing the videos, and transferred to a spreadsheet for analysis.

Results:



Figure 1.1 Outlines the percent of shrimp that responded and the various distances their heads were located from the center point of the tube. Both the control and fish water means are displayed, along with a p value of 0.0005, suggesting a significant difference between the two.



Figure 1.2 Shows the percent of shrimp that responded and the various distances their tails were at from the center point of the tube. The figure shows both control and fish water means and the p value which is 0.5476 suggesting the difference is not significant.



Figure 2.1 Outlines the distance the head moved from its original position to the center of the tube for shrimp who displayed a delayed response. The mean distances are provided along with a p value of 0.0002 suggesting a significant difference.



Figure 2.2 Outlines the distance the tail moved from its original position to the center of the tube for shrimp who displayed a delayed response. The mean distances are provided along with a p value of 0.0067 suggesting some significant difference.



Figure 3.1 Outlines the distance the head moved for each shrimp from its response position to its final position in relation to the center of the tube. The negative values indicate movement away from the tube. Both means are provided and a p value of 0.7233 suggests there's no significant difference.



Figure 3.2 Outlines the distance the tail moved for each shrimp from its response position to its final position in relation to the center of the tube. The negative values indicate movement away from the tube. Both means are provided and a p value of 0.0858 suggests there's no significant difference.



Figure 4.1 Gives a visual representation of the percent of shrimp that produced a response compared to the percent of shrimp that did not produce a response in clean spring water.



Figure 4.2 Gives a visual representation of the percent of shrimp that produced a response compared to the percent of shrimp that did not produce a response in fish contaminated water.



Figure 5.1 Gives a visual representation of the percent of responding shrimp that had a delayed reaction versus those that had an immediate reaction in clean spring water.



Figure 5.2 Gives a visual representation of the percent of responding shrimp that had a delayed reaction versus those that had an immediate reaction in fish contaminated water.



Figure 6.1 Gives a visual representation of the percent of shrimp that were sucked up in to the tube versus the percent of shrimp that were not sucked up in to the tube, whether they responded or not, in clean spring water.



Figure 6.2 Gives a visual representation of the percent of shrimp that were sucked up in to the tube versus the percent of shrimp that were not sucked up in to the tube, whether they responded or not, in fish contaminated water.

Discussion:

As stated in the methods section, our study was conducted on a series of seven shrimp ranging in size from 2-4 cm. Throughout the testing period, each shrimp was placed in the bin individually as to limit the amount of confounding variables. Each individual's response was categorized in one of four ways: no response (no movement), no response (sucked up), jump response (immediate), or a jump response (delayed). Our main focus was determining if there was a significant difference between the jump responses of those in clean spring water compared to those in fish water.

Figures 1.1 and 1.2 shows the percent of shrimp that responded from various distances from the center point of the tube. Both the heads and the tails of the shrimp were recorded in an attempt to get a more accurate idea of sensitivity throughout the shrimp's body. The center point of the tube was used as our reference point as it would be the point of the tube that displayed the strongest suction force. Figure 1.1 describes the data from the head of the shrimp when it responded to the suction created by the siphon. The mean distance away for the control group,

clean spring water, was 1.92 cm, while the mean distance away for the experimental group, fish water, was 2.39 cm. Using a standard t-test we were given a p value of 0.0005, suggesting that the difference between the two is significantly different. For Figure 1.2, the mean distance away for the control group was 3.65 cm, and the mean distance for the experimental group was 3.48 cm away. The t test gave us a p value of 0.5476 suggesting the difference between the two groups are not significantly different.

Figures 1.1 and 1.2 suggest that the most sensitive part of the shrimp is its head while its tail doesn't seem to be as important to its response. The figure also suggests that there is a significant difference between the shrimp submerged in clean spring water and the shrimp submerged in fish water. The significance suggests that the shrimp in fish water are more sensitive to the sucking motion, mimicking fish predation. This suggests that the shrimp have a greater sensitivity to the suction when in fish water, or a lower threshold for stimulating a response. The tail seems to hold no bearing on determining sensitivity in the shrimp as the p value is quite large for Figure 1.2. These findings line up with our initial hypothesis that the shrimp are more sensitive to a predatory response when in fish water.

Figures 2.1 and 2.2 plot the distance moved for each shrimp when a delayed response occurs for the control and experimental testing scenarios. The distance recorded is the distance between the original position and the response position with reference to the center point of the tube. Figure 2.1 is the shift seen in the heads of each shrimp. The mean for the control is 0.76 cm while the mean for the experimental is 0.26 cm, with a p value of 0.0002, suggesting a significant difference. This agrees with our first set of figures, that sensitivity is greater in the shrimp placed in the fish water. The shorter delay distance for the experimental test setting explains that their sensitivity to the predatory signal is higher, making them respond quicker to the stimulus.

Figures 3.1 and 3.2 show the results of the distance moved for each shrimp from its response position to its final position. For both plots, the mean values are comparable, with large p values, suggesting that the difference between the two is not significant. This would seem to disagree with our initial hypothesis, that the shrimp would have a greater response in the fish water as opposed to the clean spring water. Our new theory would be that when any shrimp makes the decision to "jump" away from its predator, whether it's in clean spring water or fish contaminated water, it's always at its max capacity.

For figures 4-6, pie charts were used to depict the data. There was a total of 60 trials for the control setting and 59 for the experimental setting. The depiction of data via pie charts is used as the shrimp were graded on a binomial account, were given either a 1 or a 0, for their response, or lack of. Figure 4.1 shows that of the 60 trials, 17 runs were awarded a 0 for no response and 43 were awarded a 1 for an escape response. Figure 4.2 shows that of the 59 trials, 9 were awarded a 0 for no response and 50 were awarded a 1 for an escape response. Figure 5.1 shows that of the 43 trials awarded a 1 in figure 4.1, 31 of them had a delayed response and 12 had an immediate response. Figure 5.2 shows that of the 50 awarded a 1 in figure 4.2, 14 of them had a delayed response and 36 of them had an immediate response. Figure 6 outlines the number of shrimp sucked up into the tube for both test settings. Figure 6.1 has a total of 7 shrimp sucked up out of 60, and Figure 6.2 has a total of 2 shrimp sucked up out of 59.

From these figures, the greatest difference seen is between the control and experimental settings of Figures 5.1 and 5.2. There was a much higher incidence of shrimp responding immediately to the stimulus in the experimental setting as opposed to the control setting.