2003

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Recommended Citation

Brown, Megan Thomas, "Aspects of Schooling Behavior in the Golden Shiner, Notemigonus Crysoleucas: Nearest Neighbor Distance, Angle of Sqimming, and Antiphasic Coupling" (2003). *Honors Theses*. 1957.
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ASPECTS OF SCHOOLING BEHAVIOR IN THE GOLDEN SHINER, *NOTEMIGONUS CRYSOLEUCAS*: NEAREST NEIGHBOR DISTANCE, ANGLE OF SWIMMING, AND ANTIPHASIC COUPLING

By
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A thesis submitted to the faculty of The University of Mississippi in partial fulfillment of the requirements of the McDonnell-Barksdale Honors College.

Oxford
May 2003

Approved by

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Reader: Dr. Tamar Goulet

Reader: Dr. Michael Mossing

ACKNOWLEDGEMENTS

"It is more pleasant to present great ideas than to engage in painstaking collection of data." - Tjeerd van Andel

Never have I found this statement more true than during my research on golden shiners. Study of behavior is key to our understanding of the world, but the innumerable variables sometimes create seemingly insurmountable obstacles. Many thanks to my advisor, Dr. Glenn Parsons, for his direction and insight when original plans failed, as well as the use of his equipment and laboratory. Also thanks to my outside readers, Dr. Tamar Goulet and Dr. Michael Mossing.

I appreciate the input of Dr. Denis Goulet, who assisted in polishing the manuscript. A special thanks goes to Dr. Gary Gaston, who graciously lent me books on schooling and fixed my precious broken video tape that contained hours of swimming. This research would have never been completed or compiled without the help of Mr. John Schuster, supervisor of the University of Mississippi Electronics Maintenance Group. Also, my parents, Mr. and Mrs. William Brown, have been very supportive throughout my research and have encouraged me, without pushing, to live up to my potential. To all of my friends who endured my griping, and especially my roommate, Brooke Crawford, who forego watching favorite television shows when I collected my data, I appreciate you!

ABSTRACT

MEGAN THOMAS BROWN: Aspects of schooling behavior in the golden shiner, *Notemigonus crysoleucas*: Nearest neighbor distance, angle of swimming, and antiphasic coupling behavior
(Under the direction of Dr. Glenn Parsons)

Many fish swim in schools at some point in their life. This behavior is beneficial for feeding and safety from predators, and may reduce energy expenditure during swimming. The hydrodynamic theory of schooling states that fish in a school take advantage of the wakes produced by other members of the school. This theory has been both supported and refuted in various studies. Using a swim tunnel, nearest neighbor distance, antiphasic coupling and the angle of swimming were studied in golden shiners (*Notemigonus crysoleucas*) while schooling. A plexiglass container placed inside the swim tunnel restricted fish to swimming in one plane in order to simplify behavioral observations. Swimming behavior was recorded using both still and video cameras. Infrared video was utilized for observations at night to eliminate any visual cues that might obscure interactions between fish. Nearest neighbor distance slightly increased as swimming speed increased, with average nearest neighbor distances of 4.07 cm at 0.20 m/s and 5.15 cm at 0.50 m/s. The average angle of swimming was 18.055 degrees at 0.10 m/s and 9.724 degrees at 0.40 m/s, with angle of swimming decreasing as swimming speed increased. At 0.60 m/s, adjacent fish swimming was timed and the proportion of antiphasic caudal movement was calculated and compared with non-adjacent fish. In the first five minutes of the 30-minute swimming bout, 53.9% of adjacent swimming and 33.8% of non-adjacent swimming was spent in antiphasic motion. In the last five minutes of swimming, 70.3% of adjacent swimming and 47.0% of non-adjacent swimming was spent in antiphasic motion. Adjacent fish did not swim in an antiphasic manner more frequently than non-adjacent fish. This suggests that schools are not making use of antiphasic behavior as an energy-saving mechanism. However, individuals spent more time within 0.5 body lengths of another fish at the end of the 30-minute swimming bout. Average length of coupling at the beginning and end of the swim was 2.6 seconds and 3.9 seconds. These results suggest that adjacent swimming may be beneficial in other ways.

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

Introduction:

The uniform behavior of certain animals has generated many theories and questions in biology. Determining why fishes swim in schools has intrigued scientists for centuries. Schooling occurs in over 50% of the world's known fishes, but this behavior is still somewhat mysterious (Shaw 1978). Various hypotheses have been proposed to explain why many fish school. One popular hypothesis is the "many eyes theory," which states that a group of fish will be more aware of approaching predators (Milinski 1993). Predators may also become confused by the varied color and movement of the schooling fish, which often diverge into groups when faced with danger (Levinton 1995). For whatever reason, schooling has been shown to reduce the probability of predation (Turner and Pitcher 1986; Magurran 1990).

A second hypothesis is that fish school to decrease energy expenditure (Breder 1965, Zuyev and Belyayev 1970; Weihs 1973, 1975). The mechanism whereby schooling fish increase efficiency is unclear. Fish must expend a certain amount of energy in order to propel themselves forward. Forces, such as drag, must be overcome, and drag (which is found to be the square of velocity) increases as the water velocity increases (Schmidt-Nelson 1972). Natural selection will select those fish that minimize the amount of energy spent swimming. This "saved" energy can then be used for feeding or reproduction, thus making these individuals more "fit," or more likely to propagate healthy young. Also, the ability to reduce energy expenditure may be rewarded in fish because of greater capacity to swim long periods of time or evade predators

(Tietjens 1957). If an animal expends most of its energy, the chance of death increases greatly (Priede 1985). Some energy efficient fish behaviors and body shapes are already known, such as streamlining and hiding in areas with low water velocity but high probability of food capture (Ware 1981; Fausch 1984).

To understand these hypotheses, one must understand how individual fish swim. The caudal fin is used by many fish for the majority of propulsion (Alexander 1982). This caudal movement can be classified into at least 5 types, depending on body shape. Anguilliform uses almost the entire body to propel itself. Subcarangiform moves at least half of the body along with the tail. The progression of reduced body movement and increased caudal movement continues with carangiform and thunniform. In ostraciiform, most of the body remains still while the tail and a small part of the body are oscillated (Prince 1981). Different species of fishes have developed different types of tail movement in order to best fit their needs.

A fish swimming causes vortices in the water because of the movement of its tail and body (Lighthill 1969). Vortex sheets can be thought of as small whirlpools in the otherwise homogenous flow of water. These sheets or wakes are present behind a swimming fish on the left and right, with each vortex spinning in the opposite direction and staggered from each other. For example, as the tail moves to the right, it creates a vortex to the right. The next vortex will be on the left a little farther up, as the tail oscillates in the same direction and propels the fish forward (Pradntl and Tietjens 1934; Hertel 1966).

Due to these vortex sheets, it has been suggested that a crystal lattice pattern while schooling is the most energy efficient. These vortices have a direction equal and opposite to the direction of the fish. Thus, in the area directly behind the fish, another fish will have to work much harder to swim against both the velocity of the water and the vortex created by the first fish's swimming. However, if a second fish swims diagonally to the first fish, less energy is needed to swim at the same speed. This phenomenon occurs because outside of the vortices, the water is moving in the direction of swimming, reducing the work the fish must do. Two fish swimming on a diagonal to the first fish and parallel to each other will avoid the vortex sheets and benefit from their wake (Weihs 1975). In theory, this type of swimming pattern would reduce by four to six times the amount of force the individuals in the second row need to swim (Weihs 1974).

Careful theoretical analysis of the forces produced by tail oscillation has suggested costs and benefits to fish that swim in the crystal lattice formation (Weihs 1975). Adjacent fish that are actively swimming may move the caudal fin in a phasic, antiphasic, or random manner. Antiphasic swimming occurs when an adjacent fish moves its tail opposite to its neighbor, an action that in effect pushes off of each other's sideways vortex of water to increase thrust. When the caudal fins are in synchrony, this is referred to as phasic behavior. Whether adjacent active swimmers are phasic or antiphasic, their coupled caudal oscillations create an induced flow that is in the direction of swimming, theoretically reducing work (Weihs 1975). This induced flow is greatest, though, for antiphasic swimmers.

Antiphasic swimming, however, is not without cost. This behavior will create waves that make locomotion difficult for the individuals behind them, depending on the position. However, if adjacent fish are swimming in a phasic manner, in which both caudal fins are moving in the same direction, they will create an induced lateral flow that would work against fish swimming in the position diagonal from the first two fish. This phasic induced lateral flow is more taxing to the fish than antiphasic swimming. Furthermore, this phasic flow will deter other fish from utilizing the position diagonal from the first two fish (Weihs 1975). Thus, if this hydrodynamic model of fish schooling is occurring, then antiphasic caudal movement must be observed in adjacent individuals.

The crystal lattice theory of hydrodynamic swimming has been difficult to verify. Research on jack (*Trachurus symmetricus)* demonstrated a general diamond shape (Breder 1976), while studies on saithe (*Pollachius virens*), herring (*Clupea*), and cod (*Gadus morhua*) showed no such pattern (Partridge and Pitcher 1979). Partridge and Pitcher uncovered supporting evidence for the idea of wakes behind fishes. However their data did not show that fish were positioning themselves in a definite diamond pattern or using certain tail oscillation patterns with neighboring fish.

The results of previous studies on hydrodynamic swimming are conflicting, with no clear indication of the role of crystal lattice swimming. These results beg the question of what parameters must be apparent for fish to utilize this type of behavior. Since the cost of swimming is the square of the speed of locomotion, metabolic output increases as swimming speed increases (Fry 1957;

Brett 1965; Tytler 1969), making energy efficient behaviors more important at higher speeds.

When Weihs proposed his hydrodynamic theory (1975), he stated that individuals would maintain the same nearest neighbor distance at all swimming speeds when utilizing the crystal lattice. However, since no clear pattern has been observed, perhaps nearest neighbor distance must reach a certain minimum before a hydrodynamic advantage is obtained. Changes in the average nearest neighbor distance could have several implications. If neighbors are not swimming close to each other, the vortices will have less effect on other fish. Also, at higher speeds, individual fish must expend more energy to propel themselves and thus may have a greater need to save energy by swimming in a crystal lattice formation or by participating in antiphasic caudal fin behavior.

Many active fish have evolved a streamlined shape that allows them to swim with the least amount of resistance (Prince 1981). As current increases, the possible resistance to fish swimming increases. To reduce resistance, we suspect that the angle of swimming to the current of water will decrease as the speed of the water flow increases. Changes in the angle of swimming may also reduce the likelihood that a school will orient itself in a crystal lattice swimming pattern. In his hydrodynamic theory, Weihs (1975) assumed that fish were swimming parallel to the water current at a zero degree angle. However, if the fish swam at an angle, then the vortices would be in different places, possibly rendering the most efficient shape of the school in a different form altogether.

The goal of this study is to identify the most likely scenario for schooling in crystal lattice, through clues in nearest neighbor distance and swimming angle of individuals. I hypothesize that as swimming speed increases, golden shiners (*Notemigonus crysoleucas*) have lower nearest neighbor distances and angles of swimming. This hypothesis supports the idea that the crystal lattice model of schooling will occur at higher swimming speeds. Furthermore, I plan to test for characteristic hydrodynamic behavior in schools at the most likely swimming speeds. If I demonstrate that antiphasic movement occurs more frequently than phasic movement, the crystal lattice theory will be supported. I hypothesize that antiphasic movement will occur more frequently than phasic movement when fish are coupled, and that the frequency of antiphasic movement will increase as the fish swim for long periods of time at high speeds. Furthermore, I expect to see adjacent fish coupling in phasic and antiphasic behavior for longer periods of time after swimming for long periods.

Materials and Methods:

Study animal:

Notemigonus crysoleucas, golden shiners, (Order Cypriniformes, Family Cyprinidae) were selected for this study for various reasons. These fish are known to school and are small enough for several individuals to comfortably swim in the swim tunnel at the same time. Furthermore, this fish utilizes its caudal for locomotion (Castro and Huber 2000). *N. crysoleucas* are also easily available during most of the year because of its popularity as a bait fish. Finally, fish are unaware of the infrared conditions because they do not detect that range of wavelength (>750 nm), permitting observations to be conducted in the dark (Levine and Macnichol 1979).

Experimental protocol:

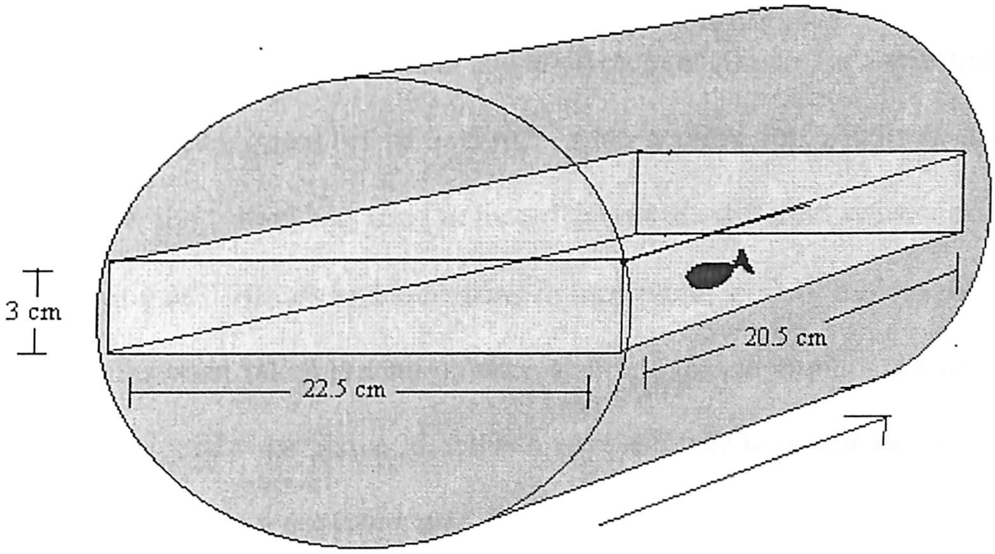

Figure 1: Plexiglass apparatus, covered with mesh on two ends of water flow, situated inside the swim tunnel.

Seven commercially obtained *N. crysoleucas* were placed into a Brett (1964) type swim tunnel with constant water temperature. Within the swim tunnel was placed a box with plexiglass sides (Figure 1). The front and rear were covered in netting small enough that the fish could not bite with their mouths. This prevented the fish from orally grasping the netting. The box had dimensions (3 cm, 20.5 cm, 22.5 cm) and was only deep enough for the golden shiners to swim parallel to each other. A flow meter was used to check that the architecture of the plexiglass box did not affect the velocity of the water. Velocity of the water was checked at both sides of the box and in the center of the box each time the speed was changed. The top and sides of the swim tunnel were covered in black plastic so that movement around the swim tunnel would not affect or disturb the fish's behavior.

Nearest neighbor distance:

The fish were chosen at random and allowed to acclimate for a minimum of two hours in the plexiglass box at 0.05 m/s. After acclimation, a digital camera positioned below the tunnel was used to record data at six different swimming speeds, beginning at 0.10 m/s and increasing in increments of 0.10 m/s to 0.60 m/s. Photographs were taken at random once a minute for ten minutes at each speed. After the speed was increased, the fish were allowed to acclimate for one minute before more data were collected.

Nearest neighbor distances were determined to the nearest 0.1 cm. This distance was measured from the head of one fish to the heads of each of its neighbors. At each speed, 9 or 10 images were obtained. In each image, all nearest neighbor distances were calculated and averaged, and the average of averages was taken within each speed.

Single factor analysis of variance (ANOVA) was used to examine differences in nearest neighbor distance among the different water velocities. A P-value of 0.05 was pre-determined as the minimum level of significance. When significance was detected, a two-sample t-test assuming unequal variances was used for post-hoc testing.

Angle of swimming:

The images that were collected at each speed were also used to determine if there was a correlation between speed and angle of swimming. Swimming parallel to the sides of the swim tunnel was designated zero degrees. The angle of the fish was determined by drawing a line down the fish image, disregarding the movement of the caudal fin. Individual angles were determined, and then all the data points were averaged for each image. Ten images were collected and the average of averages was graphed for each speed. A single factor ANOVA was used to examine differences in angle of swimming among the different water velocities.

Antiphasic caudal movement:

Data were collected using a video camera affixed beneath the swim tunnel. The conditions of the swim tank, fish selection, and acclimation time remained the same as previously described. Fish swimming was recorded in hour intervals at each speed from 0.10 m/s to 0.40 m/s. At 0.50 m/s and 0.60 m/s swimming was recorded for thirty minutes.

I also conducted caudal movement observations using infrared video taken at night. This technique was implemented to eliminate any visual cues that might obscure interactions between fish. Fish swimming was recorded for thirty minutes each at 0.10 m/s, 0.20 m/s, and at 0.30 m/s. Data collection continued for an hour at 0.40 m/s and thirty minutes each at 0.50 m/s and 0.60 m/s. Each time the water speed was changed, the fish were allowed a one-minute period to readjust before more data were collected.

Video recordings of day and night swimming were used to examine possible patterns of tail movement. One-second frames of video were selected for observation, six at each speed. The frames were selected at specific predetermined times to prevent bias. When the frame was first selected, the positions of the caudal fins were recorded. Changes in tail position were noted over the one-second period, and adjacent individuals actively oscillating the caudal fin were counted.

These recordings were also used to observe adjacent fish, using slow motion and pause functions to determine if antiphasic caudal movement in coupled fish occurred more often than phasic movement. Coupled fish were selected if they were within one-half body length of each other. This distance was chosen because tail movement would not affect an adjacent fish if the two fish were not close to each other. The amount of time that the coupled fish spent in phasic and antiphasic tail movement was recorded. If the fish moved apart, or if tail movement was obstructed, then data collection was halted.

Using the above method, data collection was attempted at 0.40 m/s, 0.50 m/s, and 0.60 m/s. Ten coupled fish were observed in the first five minutes of swimming at 0.60 m/s, and ten more observations were made in the last five minutes of the thirty-minute segment. The proportion of time spent in antiphasic caudal movement was calculated.

A control was used to account for randomness in tail movement. The two fish that were farthest apart were observed and the proportion of time in antiphasic movement was collected. In this control, ten samples were collected within the first five minutes of the swimming segment, and ten were collected in the last five minutes. ANOVA was performed to determine significance.

Duration of adjacent swimming:

As the proportion of adjacent swimming was collected, the overall duration of continuous adjacent swimming was noted. Ten samples of duration of coupling within the first five minutes of swimming were compared to ten samples in the last five minutes of swimming, using ANOVA. No control could be created for the time spent interacting.

Results:

Nearest neighbor distance:

There were significant differences among nearest neighbor distances at various swimming speeds (P=0.029, F= 2.73) (Table 1, Figure 2). The post-hoc tests (Table 2) indicated that 0.20 m/s (4.07 cm) was significantly different from 0.50 m/s (5.15 cm), and 0.40 m/s (3.87 cm) was statistically different from 0.30 m/s (4.70 cm) and 0.50 m/s (5.15 cm). Nearest neighbor distances at 0.10 m/s (4.38 cm) were not significant in comparison to any other speed. This was also the case for 0.60 m/s (4.59 cm), which was not statistically different from any other speed.

Table 1: Single factor ANOVA of nearest neighbor distance at different swimming speeds.

Swimming speed (M/s)	*Count*	*Sum*	*Average (cm)*	*Variance*
0.10	10	43.8	4.38	1.128
0.20	9	36.6	4.07	0.565
0.30	10	47.0	4.70	0.376
0.40	10	38.7	3.87	0.969
0.50	10	51.5	5.15	1.085
0.60	10	45.9	4.59	0.441

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	10.441	5	2.088	2.732	0.029	2.389
Within Groups	40.511	53	0.764			
Total	50.952	58				

Figure 2: Average nearest neighbor distance (cm) at different swimming speeds (m/s), ranging from 0.10 m/s to 0.60 m/s. Double error bars shown for each speed. Nearest neighbor distances denoted with letters indicate significance. If no letter is indicated, the nearest neighbor distance is not significant.

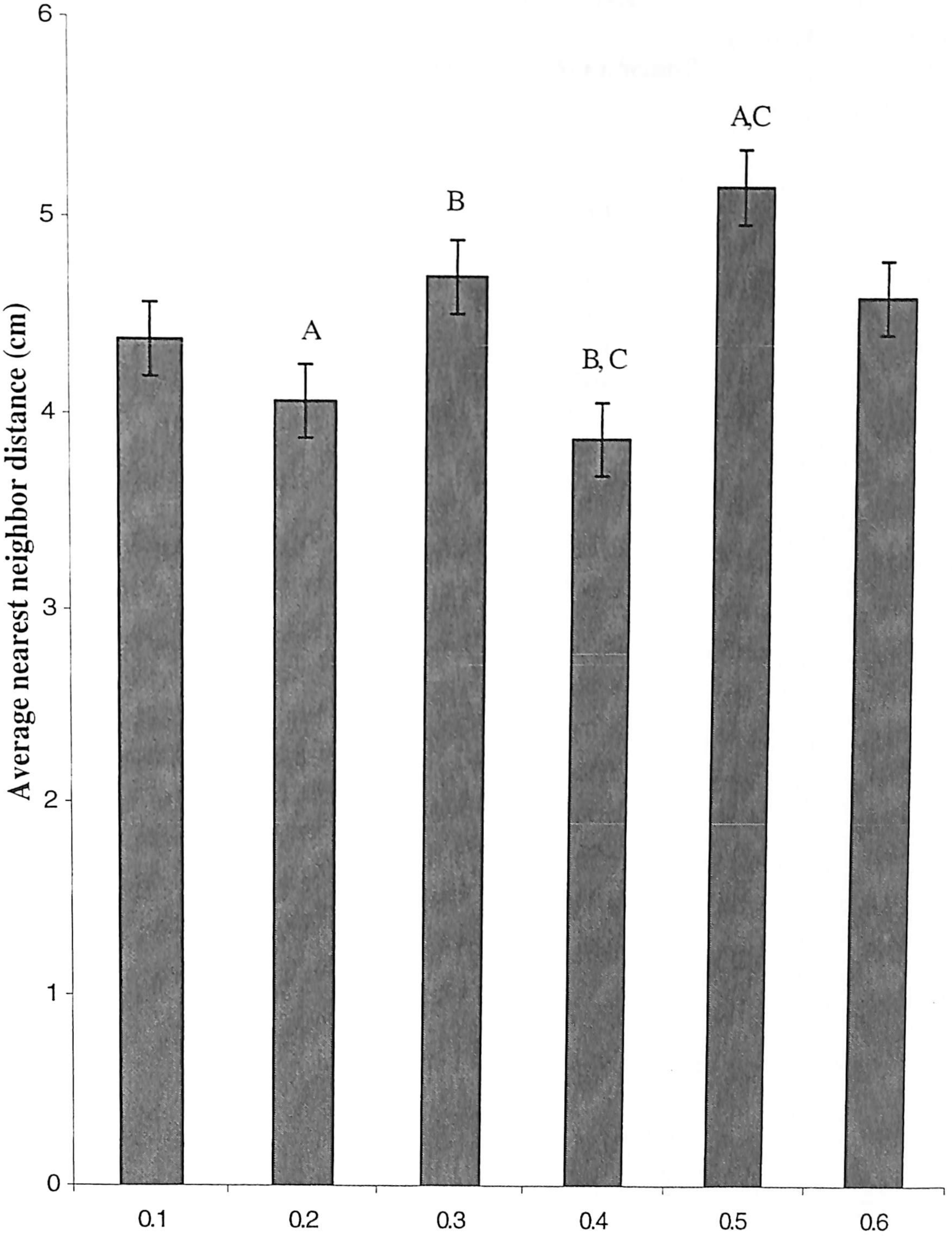
6
5
4
3
2
1
0
Average nearest neighbor distance (cm)
A
B
B, C
A,C
0.1
0.2
0.3
0.4
0.5
0.6
Swimming Speed (m/s)

Table 2: Two-sample t-tests assuming unequal variances for nearest neighbor distances at various swimming speeds.

T-test (M/s)	*P-value (two-tail)*	*Significant?*
0.10, 0.20	0.466	No
0.10, 0.30	0.423	No
0.10, 0.40	0.280	No
0.10, 0.50	0.119	No
0.10, 0.60	0.604	No
0.20, 0.30	0.063	No
0.20, 0.40	0.629	No
0.20, 0.50	0.019	Yes
0.20, 0.60	0.129	No
0.30, 0.40	0.039	Yes
0.30, 0.50	0.257	No
0.30, 0.60	0.705	No
0.40, 0.50	0.011	Yes
0.40, 0.60	0.073	No
0.50, 0.60	0.172	No

Skew was suspected because some of the images did not contain data for each individual in the school. Therefore, another ANOVA was performed (Table 3), using only data from those images that were clear enough to generate 85% of the nearest neighbor distances in the frame. The P-value between groups was statistically significant, with a value of 0.019.

Angle of swimming:

The average angle of swimming at 0.10 m/s was 18.055 degrees (Table 4, Figure 3). This angle decreased to 13.603 degrees and 14.83 degrees at 0.20 m/s and 0.30 m/s respectively, and further decreased to 9.724 degrees at 0.40 m/s. ANOVA analysis revealed a significant decrease in angle of swimming at different speeds (P=0.007, F= 4.722). Post hoc tests (Table 5) showed that swimming angles at 0.40 m/s were statistically different from the angles at 0.10 m/s, as well as at 0.30 m/s. All other speed comparisons were statistically insignificant.

Antiphasic caudal movement:

In the portion of the experiment where one-second portions of the video were observed for patterns in tail movement, several important features were discovered (Figure 4, Table 6). In the 6 frames of the 0.10 m/s video, only 7 incidents of caudal tail movement were seen, and none of the fish involved in tail movement were adjacent to another fish with caudal tail movement. In the 0.20

Table 3: Single factor ANOVA of nearest neighbor distance excluding images in which less than 85% of the NND's could be calculated.

Swimming speed (M/s)	*Count*	*Sum*	*Average (cm)*	*Variance*
0.10	6	26.7	4.450	1.163
0.20	4	15.7	3.925	0.203
0.30	4	19.1	4.775	0.069
0.40	7	25.8	3.686	0.468
0.50	8	42.0	5.250	1.303
0.60	10	45.9	4.590	0.441

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	10.860	5	2.172	3.182	0.019	2.503
Within Groups	22.528	33	0.683			
Total	33.388	38				

Table 4: Single factor ANOVA of angle of swimming (with swimming directly into the current denoted as zero degrees) related to different swimming speeds.

Swimming speed (M/s)	*Count*	*Sum*	*Average angle of swimming (degrees)*	*Variance*
0.10	10	180.55	18.055	15.079
0.20	10	136.03	13.603	50.238
0.30	10	148.30	14.830	26.114
0.40	10	97.24	9.724	8.982

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	355.625	3	118.542	4.722	0.007	2.866
Within Groups	903.712	36	25.103			
Total	1259.336	39				

Figure 3: Average angle of swimming (degrees) at different swimming speeds (m/s), ranging from 0.10 m/s to 0.40 m/s. Zero degrees indicates swimming parallel to flow of water. Double error bars given for each swimming speed. Values denoted with letters indicate significance.

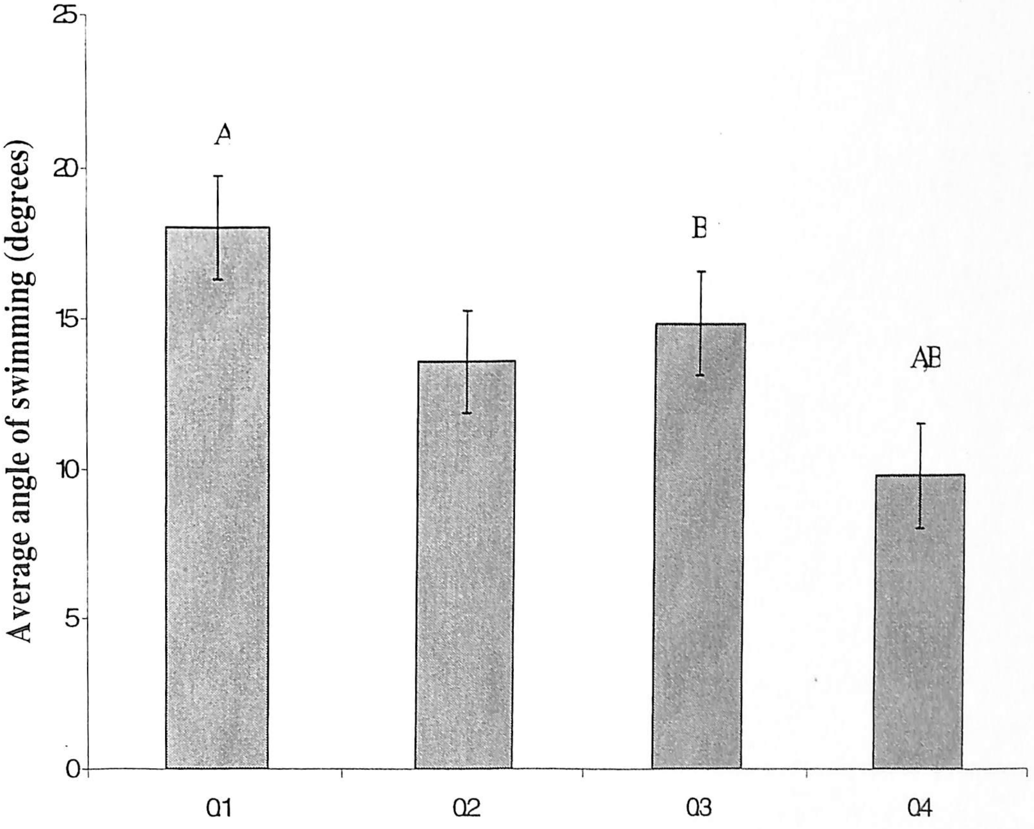
25
20
15
10
5
0
A
B
AB
0.1
0.2
0.3
0.4
Average angle of swimming (degrees)
Swimming Speeds (m/s)

Table 5: Two-sample t-tests assuming unequal variances for angle of swimming (with 0 degrees for swimming directly into the current) at various swimming speeds.

T-test (M/s)	*P-value (two-tail)*	*Significant?*
0.10, 0.20	0.103	No
0.10, 0.30	0.131	No
0.10, 0.40	5.08E-05	Yes
0.20, 0.30	0.663	No
0.20, 0.40	0.137	No
0.30, 0.40	0.0156	Yes

Figure 4: Number of coupled fish with phasic or antiphasic caudal movement at different swimming speeds, 0.10 m/s to 0.60 m/s.

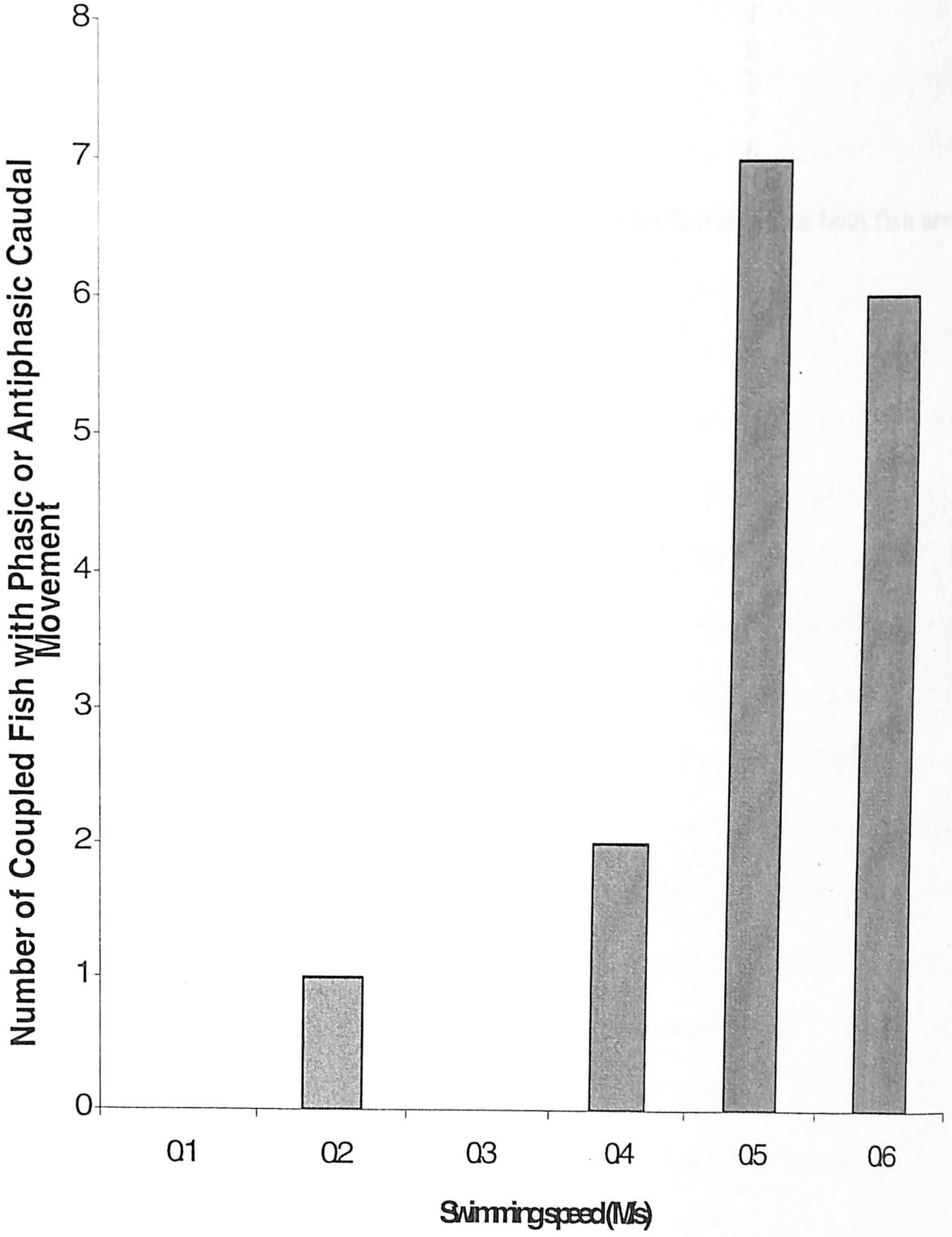
Number of Coupled Fish with Phasic or Antiphasic Caudal Movement
0
1
2
3
4
5
6
7
8
0.1
0.2
0.3
0.4
0.5
0.6
Swimming speed (M/s)

Table 6: Number of caudal movements and paired movements at different swimming speeds.

Swimming Speed (m/s)	# Caudal Tail Movements	# Co-occurrences *
0.1	7	0
0.2	11	1
0.3	13	0
0.4	13	2
0.5	20	7
0.6	14	6

*This refers to fish within 0.5 body length of another fish in which both fish are oscillating the caudal fin.

m/s video, 11 occurrences of tail oscillation occurred, and only once was a fish adjacent to another with tail movement. The 0.30 m/s video showed 13 caudal tail movement events, and none of these events were next to another event of tail movement. At the higher speeds, tail movement coupling became more apparent. At 0.40 m/s, 13 tail movement incidents were captured in the 6 seconds of video, and 2 groups of fish were found participating in tail movement adjacent to each other. At 0.50 m/s, there were 20 separate tail movement events, with 7 occurrences of 2 adjacent fish participating in caudal fin movement. The 0.60 m/s images had 14 tail movement incidents and 6 coupled occurrences. Due to the low proportion of coupled tail movement at the lower speeds (0.10 m/s, 0.20 m/s, and 0.30 m/s), further experimentation involving phasic and antiphasic caudal movement was limited to the higher speeds (0.40 m/s, 0.50 m/s, and 0.60 m/s).

Two-sample t-tests were performed on the data collected at the beginning and end of the 0.60 m/s infrared swimming segment (Table 7). The proportion of antiphasic movement was analyzed by using t-tests between the experimental and control groups. The proportion of antiphasic coupling in the first 5 minutes of the 0.60 M/s segment was compared to the control of antiphasic behavior between distant fish. There was no significant difference in antiphasic behavior between the experimental and control in the first 5 minutes of swimming ($P= 0.28$, $N= 10$) and the last 5 minutes of swimming ($P= 0.09$, $N= 10$). In the first 5 minutes, 53.9% of adjacent swimming and 33.8% of non-adjacent swimming was spent in antiphasic motion. In the last 5 minutes of swimming, 70.3% of adjacent swimming and 47% of non-adjacent swimming was spent in antiphasic behavior.

Table 7: Two-sample t-tests assuming unequal variance for the proportion of time two fish spend in antiphasic caudal movement.

	Point during 30 minute swim	*Number of Observations*	*Proportion of swimming spent in antiphasic caudal movement (%)*	*Variance*	*P-value (two-tail)*	*Significant?*
Exp. (adjacent fish)	First 5 minutes	10	53.9	0.1063	0.277	No
Control (non-adjacent fish)	First 5 minutes	10	33.8	0.1544		
Exp.	Last 5 minutes	10	70.3	0.085	0.086	No
Control	Last 5 minutes	10	47.0	0.0804		

In addition, no statistical difference was found between the proportion of phasic behavior at the beginning and end of the thirty-minute swim (P= 0.25, N= 10)

Duration of adjacent swimming:

A two-sample t-test of the duration of coupling (both phasic and antiphasic) was used to statistically analyze behavior at the beginning and end of the swimming segment (Table 8). There was statistical difference in duration of coupling at the beginning and end of swimming (P=0.038, N=10). The median length of fish coupling at the beginning of the swim was 2.6 seconds, and the median length at the end of the swim was 3.9 seconds.

Discussion:

The results of the study showed that nearest neighbor distance did change with the speed of swimming. A weak relationship between increases in swimming speed and increase in nearest neighbor distance was apparent. The nearest neighbor distance increased significantly from 0.20 m/s to 0.30 m/s and 0.50 m/s. Therefore, our hypothesis that nearest neighbor distance would decrease as swimming speed increased was not supported. The significance was slightly greater in the precautionary ANOVA that removed images with incomplete data. These results indicate that some skew may have occurred in the analysis of nearest neighbor distance, but not enough to change the overall validity of the study.

Pitcher and Partridge (1979) reported a decrease in nearest neighbor distance as speed increased. Another study of *N. crysoleucas* studied both horizontal and vertical nearest neighbor distance in three dimensions. Their

Table 8: Two-sample t-tests assuming unequal variance for the length of time two fish spend continuously swimming within 0.5 body length of each other.

Point during 30 min swim	*Number of Observations*	*Mean time spent in adjacent swimming (sec)*	*Variance*	*P-value*	*Significant?*
First 5 minute	10	2.6	0.933	0.0378	Yes
Last 5 minute	10	3.9	2.32		

results indicated that horizontal nearest neighbor distance did not change significantly as swimming speed increased from 0.10 m/s to 0.40 m/s (Boyd and Parsons 1998). Though these results also refute our experimental hypothesis that schools will compact as swimming speed increases, they show that nearest neighbor distance remains approximately the same over all speeds examined. This difference in results is not related to the increased range of swimming speed in this study, because in our study, the nearest neighbor distance at 0.30 m/s was significantly different from 0.40 m/s.

The results of change in angle of swimming had a clear relationship. The average angle at 0.10 m/s was significantly larger than the average angle at 0.40 m/s, which had the lowest average angle of swimming. This supports our hypothesis that the angle of swimming decreases as the swimming speed increases.

This trend in angle of swimming suggests a greater possibility of hydrodynamic swimming at greater swimming speeds. Since variance is low at the higher speeds, the fish are swimming more steadily, with fewer occurrences of darting about the swim tank and jockeying for position. The lower angle of swimming shows that the individual fish are making use of their streamlined shape to cut through the water as the resistance of the water is increasing. Both of these findings bode well for the possibility of detecting hydrodynamic swimming at higher swimming speeds. The fish are spending more time in steady forward motion, with less turning from side to side. Also, swimming directly into the water current should create vortices in the exact positions Weihs (1975) determined. So, if hydrodynamic swimming is occurring, the angle of swimming supports the idea that this type of energy-efficient motion will occur at higher swimming speeds.

The observations of the one-second swimming segments at various speeds, though not statistically significant, suggests hydrodynamic swimming is more likely to occur at higher swimming speeds, because of the greater frequency of adjacent caudal fin coupling. This observational evidence, along with support from the decreased angle of swimming at higher speeds, narrowed the testing field for antiphasic caudal oscillations, the lynch pin of the hydrodynamic theory.

The antiphasic coupling experiment did not support the hypothesis that adjacent fish would purposefully participate in antiphasic caudal behavior because of energy efficiency. Adjacent fish showed no more likelihood of swimming in this manner than fish that were not adjacent. Even after sustained swimming, at a constant high speed for 25 minutes, neighboring fish were no more likely to swim in antiphasic behavior than fish that were not close together. This behavior also did not significantly increase after the fish were swimming for an extended period of time. These results are supported by the work of Partridge and Pitcher (1979) in three other species of fish, indicating that these results may be applicable to species other than *Notemigonus crysoleucas.*

If antiphasic coupling were beneficial, then it should occur more frequently between pairs of fish as they deliberately align themselves in this manner. Since the crystal lattice theory seems to hinge upon antiphasic coupling between fishes, these results cast considerable doubt upon the probability that golden shiners use this hydrodynamic approach to reduce energy consumption.

Coupled fish remained adjacent to each other significantly longer at the end of the 0.60 m/s swimming segment than at the beginning, supporting our hypothesis that fish would utilize hydrodynamic swimming behavior as duration of swimming increased. Since the fish are not relying on antiphasic behavior, this could signify several things. This result may simply be caused by less jockeying amongst fish as more energy must be exerted to maintain the swimming speed. Another possibility is that adjacent fish derive some sort of energy reduction by adjacent swimming behavior (both antiphasic and phasic) and use it as energy levels are depleted.

Except in very shallow water, fish schools are typically three-dimensional, with fish swimming both within the same plane and in the planes above and below each other (Cullen et al. 1965; Pitcher and Partridge 1979). However, swimming in three dimensions is much harder to capture and quantify, especially when a fourth dimension, time, is added. Some studies have ignored time, collecting still frames of schools, but this approach limits the questions that can be asked (Graves 1977; Partridge et al. 1980; Koltes 1984). Other studies continuously recorded swimming, but experienced difficulty in capturing the entire school in video, as fish swam in and out of view (Partridge 1981).

Simply ignoring the fact of three-dimensionality does not necessarily give accurate data, especially information on nearest neighbor distance (Symons 1971b). This experiment attempted to eliminate the problem of three-dimensional data collection and analysis without compromising the validity of the research. This solution of limiting movement to one plane was first used by Aoki (1984) to study school structure.

Many researchers have resorted to computer simulations and motion analysis in order to learn more about three-dimensional schooling (Potel and Wassersug 1981). These simulations have programmed rules that are implemented on a group of computer-generated fish, and the fish react to each other in accordance to these rules (Reynolds 1987; Tu 1996). Though this research is helpful in generating theories, it may not accurately portray actual fish schooling. Inaccuracies arise when certain variables are not deemed important for inclusion in the artificial set-up. This pre-determination reduces time spent in creating the system, but may overlook important factors in fish swimming (Zaera et al. 1996).

Future work:

Observing fish in a laboratory setting has the potential of yielding results different from natural behavior. Observations in an unnatural environment, such as a two-dimensional school, may not apply to three-dimensional behavior. Future studies might use computer analytical tools in order to test schooling in a more natural context. To create a three-dimensional image, several cameras record simultaneously using pixel synchronous framegrabbing (Beyer 1990, 1992, 1993). One program, Motion Analysis VP310, automatically selects ten-second frames of swimming (Parrish and Turchin 1997). This system notes the outline of fish as well as the center of the body and tracks individuals' movement over time.

VP310 is ideal for tracking less than fifteen fish, and is best limited to ten-second intervals of swimming.

The advantage of this type of system is the relative ease of collecting large data sets. This system, though more sophisticated than our form of data collection, may not be flexible enough to answer questions about caudal movement. This technology remains in the development stages and does not eliminate error. Many of these computer-based programs use feature- or attribute-matching, in which shapes are automatically recognized (Forstner 1986). Though suitable for industrial projects where a standard shape is always projected, this method is difficult to implement when the silhouette of the object (i.e. fish) is constantly changing due to movement (Aloimonos and Rosenfeld 1991). As technology increases and cost decreases, behavioral studies may find computer programs such as Motion Analysis VP310 more appealing.

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