

SENSORY BASES OF SCHOOLING

IN FISH

SENSORY BASES OF SCHOOLING IN FISH, IN
THE ABSENCE OF VISUAL, TACTILE AND
CHEMICAL STIMULI

by

Gareth Vaughn Terry, B.A.

A Thesis
Submitted to the Faculty of Graduate Studies
in Partial Fulfilment of the Requirements
for the Degree
Master of Arts

McMaster University

August 1969

Master of Arts (1969)
Psychology

McMaster University
Hamilton, Ontario

TITLE: Sensory bases of schooling in fish, in the absence of
visual, tactile and chemical stimuli.

AUTHOR: Gareth Vaughn Terry, B.A. (Leeds University)

SUPERVISOR: Dr. B. G. Galef, Jr.

NUMBER OF PAGES: 39, vi

CONTENT: Several experimental situations were designed to
show that when all visual, tactile and chemical
stimuli are eliminated, fish are still capable of
attracting one another and orienting to one
another. Attempts to ascertain the nature of this
unknown sensory basis of schooling were unsuccessful.

ACKNOWLEDGEMENT

I should like to express my sincere thanks to Dr. B. G. Galef, Jr. for his help, guidance and encouragement throughout the research reported in this thesis.

I should also like to thank Mr. J. Turkell and Mr. R. Wilson for their technical assistance in conducting Experiment 5, and the Chesapeake Instrument Corp. who kindly furnished the hydrophone, free of charge.

Lastly I should like to thank my wife, Carol, for her patience in typing the innumerable drafts of this thesis.

TABLE OF CONTENTS

Introduction	1
Experiment 1	6
Experiment 2	16
Experiment 3	21
Experiment 4	24
Summary and Discussion	28
An attempt to clarify the nature of the unknown sensory basis of schooling	29
Experiment 5	31
Conclusion	33
Summary	36
Literature	37

FIGURES

- Figure 1 Diagram of partitioned tank used in
 experiment 1.
- Figure 2 Diagram showing method of locating subjects.
- Figure 3 Diagram showing method of locating subjects
 in experiment 2.
- Figure 4 Circular apparatus used in experiment 3.
- Figure 5 Diagram showing positioning of bags in
 aquarium used in experiment 4.

TABLES

- 1 Histograms showing the mean number of observations of the conspecific and non-conspecific fishes, in the various possible positions.
- 2 Mean number of times the stimulus fishes were observed in the two possible positions.
- 3 A comparison of the conspecific and non-conspecific subjects, on the basis of movement scores and number of times observed in the "1" position.
- 4 A comparison of the original and first control group giving the mean number of times fishes were observed in the possible positions.
- 5 A comparison of the original and second control group giving the mean number of times the fishes were observed in the possible positions.
- 6 A comparison of the original and replication data, giving the mean number of times the fishes were observed in the various possible positions.
- 7 A comparison of the conspecific and non-conspecific subjects (used in the replication), on the basis of movement scores and number of times observed in the "1" position.
- 8 Interval scores obtained in Experiment 2.
- 9 Distribution around the tank of the experimental and control groups.
- 10 Graph illustrating the differences in distribution around the tank of the experimental and control groups.
- 11 Cumulative distribution of low interval scores for the ten subject pairs used in Experiment 3.
- 12 Mean % time spent by the four groups of subjects from Experiment 4, in the stimulus half of the tank.
- 13 Total number of center crossings made by the four groups of subjects from Experiment 4.

Introduction

There is a widespread tendency for living things of the same species to aggregate, more often than not with mutual advantage. Fish schooling is a typical aggregatory behaviour, affording an excellent opportunity for the study of aggregations in the laboratory.

Allee (1931) classified aggregations of animals into two distinct types; 1) "associations" which he described as loosely integrated, unstable and dependent on the reactions of individuals to environmental stimuli; 2) "societies" stable and permanent systems dependent on the reactions of individuals to each other. He argued that fish schools of such species as trout and herring be classified as societies, because these species form permanent schools in which the individual members are uniformly spaced, orient in the same direction and swim at the same speed (Gudger, 1949). He goes on to argue that schools of fish which do not show these characteristics, should be classified as associations.

These categories, however, ignore the majority of species of fish, which form loosely assembled and impermanent aggregations and yet do so through the reactions of individuals to each other. Allee's binary classification cannot incorporate this majority.

Breder and Halpern (1946) took issue with Allee, proposing that the highly uniform and permanent school was only a special case of schooling and could not be discretely separated from other types of schooling behavior. Later, however, Breder (1959) described four

distinct groupings:

- 1) "pods" in which the fish maintain actual physical contact (e.g. young catfish).
- 2) "schools" in which the fish remain at a fixed distance from each other and are lined up in parallel order (e.g. trout).
- 3) "aggregations" in which the fish are loosely assembled with random orientation (e.g. zebra danios).
- 4) "solitary fish" which are only found together as a result of preferences for certain environments (e.g. pike).

Keenleyside (1955, page 185) proposed an operational definition, "...the school will be considered an aggregation formed when one fish reacts to one or more other fish by staying near them." He went on to exclude fighting between territory-holding males and temporary pair formation during spawning from consideration as schooling behavior. Both Breder and Keenleyside point to the basic criteria of a school. The members must react to each other and not to environmental stimuli, and animals which aggregate due to reproductive or aggressive drives must be excluded.

In this paper Keenleyside's definition of a school will be adopted and the term "aggregation" will be reserved to describe any group of fish, regardless of the reasons for their being together. Breder's first three categories will be combined and schooling viewed as a continuum along dimensions of integration and permanence. This approach to schooling seems desirable because it is not always possible to assign a species' aggregatory behavior to one of Breder's categories, and because his use of the word "aggregation" is somewhat confusing.

It appears preferable to use the term "school" to refer to all groups in which the individuals react to each other and to consider such groups as ranging along a continuum from loosely integrated and impermanent to spatially ordered and permanent.

In order to gain some understanding of fish schools, one of the first questions which comes to mind is "How is the school initially formed (i.e. what attracts one fish to another in the first place) and how is the school maintained?" Most investigators of schooling have turned their attention to ascertaining which sense modalities are involved in the instigation and maintenance of fish schools. Nearly all of these investigators have found vision to be a sufficient sensory basis for the instigation and maintenance of a school. For example, Verheijer (1956) placed groups of fish of the same species in two adjacent aquaria, whereupon both groups moved to that side of their aquarium adjacent to the other aquarium, forming one cohesive group separated by two glass walls. When an opaque card was placed between the aquaria, the fish moved into the center of their respective tanks. Mackerel have been shown to rely mainly on vision for schooling by Parr (1927) and Shlaifer (1942); mullett and golden rudd by Boulenger (1929); sunfish and goldfish by Breder and Nigrelli (1935); characin by Breder and Rasquin (1943) and silversides by Shaw (1960, 1961). Bowen (1931, 1932) has shown that even adult bullheads (*Ameiurus melas*), which have a very poorly developed optical system, rely mainly on vision for the maintenance of schools.

Other sensory systems have been suggested as important in schooling. Harris and van Bergeijk (1962) have shown that water displacements can be detected by fish. They created a water disturbance with a

metallic ball and recorded activity from an electrode placed in the nasal section of the lateral-line canal of the killifish (*Fundulus heteroclitus*). They conclude their paper by suggesting that the lateral-line system may be responsible for the spacial ordering of some schools. Dijkgraaf (1962) using conditioning techniques, was able to show that blinded fish could identify the locations of disturbances made by solid objects in the water. Hemmings (1966) proposed that vision is the modality which keeps fish together in a school, while the lateral-line sense keeps them a certain distance apart. Hence there is a balance between an attractive and a repulsive force in school formation and the maintenance of individual distance.

Von Frisch's studies (1941) on the chemical senses of fish led him to propose that chemical cues might be used in schooling. Göz (1941) was able to condition minnows to distinguish not only between different species but between individuals of their own species as well, with the sole aid of chemical cues. Breder and Rasquin (1943) showed that the schooling behavior of cave fish was largely unaffected by blinding and they concluded that these fish were able to use chemical cues to maintain the school. Hemmings (1966) found an attractive species-specific odor in Rudd and proposed that this modality is used to keep schools together at night when vision can no longer be operative.

However apart from Hemmings who used a situation in which the fish producing the chemical odor to attract the test fish was removed, before the test fish was placed in the tank, no other investigator has controlled for the possible effect of species-specific auditory stimuli producing attraction and schooling behavior.

Moulton (1960) proposed that fish may use auditory stimuli for both the purposes of an individual finding a school and the maintenance of schools, after recording noises made by *Anchoviella choerostoma* and observing behavior correlated with such noises. He consequently tried blinding one fish and observing its behavior in a school. He found that the blinded fish was incapable of orienting to the others, except at times when the rest of the school were startled or alarmed. He concluded from this that auditory stimuli were in fact used in schooling behavior. However, he took no precautions to rule out the possibility that chemical stimuli were being used. In the light of the work done by von Frisch (1936, 1941), Verheijer (1956) and Pfeiffer (1962, 1963) on Schreckstoff, a repellent odor given off by wounded or dying fish, it is possible that certain chemical odors are given off by fish in a state of alarm and that these were responsible for Moulton's results.

Nevertheless observations in the field by Moulton (1960), Myrberg (1969) and Tavalga (1958) indicate that the playback of fish noises does lead to approach reactions by conspecifics of the recorded fish and also by their natural predators. In view of these findings and because water is an excellent medium for sound transmission and most species of fish are physically capable of both producing and receiving sounds, it seems most likely that sound is yet another sensory basis of schooling.

The following series of experiments were undertaken in order to ascertain whether or not fish are attracted by some sensory modality other than visual, tactile or chemical. It was felt that if this were shown to be so, then that other modality would in all likelihood be auditory.

EXPERIMENT 1

The first experiment was designed to determine whether or not a fish will be attracted by another of the same species, when all visual, tactile and chemical stimuli are absent. Since most species of fish form species-specific schools, it is generally assumed that the sensory bases of schooling contain species-specific elements. It was therefore decided to look at the reactions between different species as well as between conspecifics. The significance of the attraction between conspecifics can be ascertained by comparing their behavior with that of fish of a different species or with the behavior of isolated fish. If a significant attraction between conspecifics can be shown, then the basis of that attraction can be said to be sufficient for the instigation of schooling behavior.

Subjects

Subjects were 18 barbs (6 *Barbus partipentazona*; 6 *Barbus terio* and 6 *Barbus titteya*) approximately 1.5" long, and 12 zebra danios (*Brachydanio rerio*) approximately 2" long. These four species are members of the Cyprinidae family, which are not noted for forming schools of great stability or spatial ordering, but nevertheless remain in close-knit groups for considerable periods of time. Cyprinids are also considered to have highly developed and acute hearing ability (von Frisch, 1936), which would increase the likelihood of their using sonic communication in schooling.

The subjects were maintained in a large holding tank and fed on a commercial dried food ("Perfect Fish Food"). Aged water was used in both the holding and experimental tanks and the water temperature was kept at 75°F.

Apparatus

Aquaria measuring 11.5" x 6" x 8" deep were each partitioned into three sections, giving two sections measuring 9" x 3" and one of 6" x 2.5" (see Fig. 1.). The partitions were made of black perspex 0.1" thick and the outer sides of the aquaria were painted black, so that from the inside all four walls appeared identical. All sections were thus

Figure 1 about here

opaque and independently water-tight, so that no visual, tactile or chemical cues could pass through the partitions. Each tank was placed over a white sheet of paper divided into a grid of squares 3" x 2" (see Fig. 2).

Figure 2 about here

Procedure

One zebra danio was placed in section A and another zebra danio in section C, whilst a barb was placed in section B of the apparatus (see Fig. 2). The fish were allowed five minutes at the beginning of the experiment, in which to acclimatise themselves to the apparatus. E then observed the location of the three fish at ten second intervals. A total

of one hundred observations were taken of each subject and twelve series of three subjects run.

Two control experiments were also run.

Control 1 One zebra danio was placed in section A and another in section C, whilst section B was left empty. The fish were allowed 5 minutes to acclimatise themselves to the experimental tank. One hundred observations of each subject's position were taken as before and six pairs of subjects were run.

Control 2 One zebra danio was placed in section C and all other sections left empty. Five minutes of acclimatisation time was allowed and then one hundred observations were taken of the subject's position at ten second intervals. Ten subjects were run.

In all the above experiments, fish of the appropriate species were selected at random from the holding tank.

Results

Let the fish in section A be called the conspecific, the fish in section B the non-conspecific, and the fish in section C the stimulus fish. If cues other than visual, tactile or chemical are sufficient for the formation of schools, then the conspecific will be attracted to the end of the section adjacent to the stimulus fish significantly more often than the non-conspecific. Similarly the stimulus fish should be observed in the position adjacent to the conspecific significantly more often than in the other position.

Table 1 shows two histograms, giving the mean number of observations of the conspecific and non-conspecific fishes, in the various possible positions. It is clear that the conspecific fishes spent a large

proportion of their time in the "1" position, adjacent to the stimulus fish. A Mann Whitney U Test was used to compare the two groups with respect to the number of times they were observed in the "1" position. This revealed a U value of 101 with $p=.048$, showing that the conspecific subjects did indeed spend a significantly large amount of time in the position nearest to the stimulus fish, when compared with the non-conspecific subjects. A chi square comparison of the two groups, using the total number of observations in each position, revealed a value of 214.74 with $df=2$ and $p<.001$.

An examination of the mean number of observations of the stimulus fishes in each position (see Table 2), reveals a distinct attraction to the side adjacent to the conspecific (i.e. position "L"). A binomial test revealed a Z score of 17.35 with $p<.001$, showing that the stimulus fishes spent a significantly greater amount of time in position L.

Table 1 Histograms showing the mean number of observations of the conspecific & non-conspecific fishes, in the various possible positions.

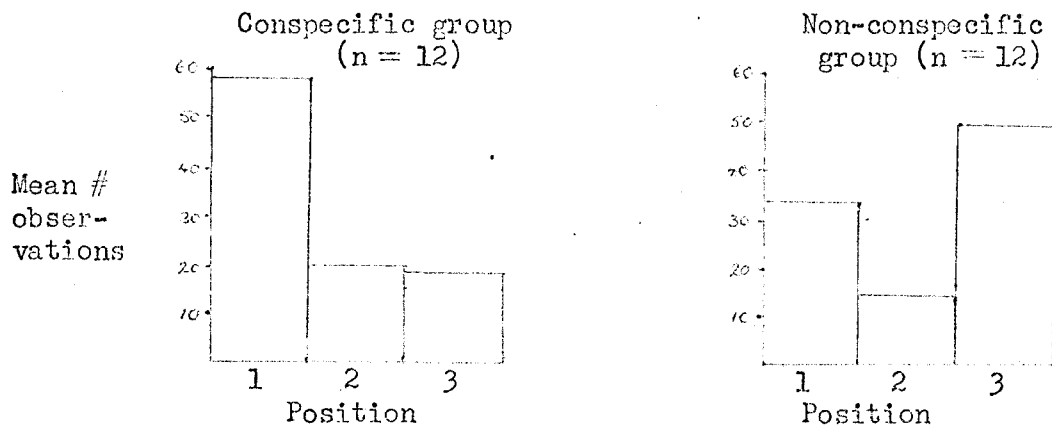


Table 2 Mean number of times the stimulus fishes were observed in the two possible positions.

Mean number of observations (n = 12)	Stimulus Fish in position	
	L	R
	75.08	24.92

A further measure was made of the amount of movement made by the conspecific and non-conspecific subjects. This was done by calculating the number of changes in position during a series of one hundred observations.

Table 3 A comparison of the conspecific & non-conspecific subjects on the basis of movement scores & number of times observed in the "1" position.

	Conspecific Subjects											
	1	2	3	4	5	6	7	8	9	10	11	12
# changes in position	54	52	0	0	62	65	6	58	7	63	1	0
# of "1" positions	23	12	100	100	25	19	50	79	80	47	59	100

	Non-conspecific Subjects											
	1	2	3	4	5	6	7	8	9	10	11	12
# changes in position	19	22	59	12	15	39	24	28	35	25	13	2
# of "1" positions	9	21	32	0	5	25	66	29	95	75	59	0

	Conspecifics		Non-conspecifics	
	\bar{x}	σ	\bar{x}	σ
Changes in position	30.67	28.61	24.42	14.27
"1" positions	57.83	32.09	34.67	30.42

A comparison of the movement indices for the conspecific and non-conspecific groups revealed close means, but enormous differences in variance. It is clear from the actual data (see Table 3) that the conspecific fish scores produced a bimodal distribution. A Moses Test of Extreme Reactions performed on the two groups revealed a probability of less than .001 that the two distributions came from the same population. Comparing the movement indices with the frequency of 1 positions, for individual conspecific fish, the two sets of figures appeared to vary inversely. A Spearman Rank Correlation Coefficient produced an r_s of $-.74$ with $p < .01$.

The first control experiment was used to ascertain whether the presence of the non-conspecific fish had in any way affected the reactions of the conspecific fish in the original experiment. The mean number of observations of both the conspecific and stimulus fishes, in the various positions, were compared with the respective original means (see Table 4).

A Mann Whitney U comparison of the two groups of stimulus fishes (on the basis of individual subject "L" scores) gave a U value of 35 with $p > .05$, which showed no significant difference between the two groups. Likewise a comparison of the two groups of conspecific fishes showed no significant difference. A Mann Whitney U value of 29 was obtained with $p > .05$.

Table 4 A comparison of the original and first control group giving the mean number of times fishes were observed in the various possible positions.

	Stimulus fish in position		Conspecific fish in position		
	L	R	1	2	3
Original means (n = 12)	75.08	24.92	57.83	21.50	20.67
Control means (n = 6)	82.00	18.00	70.67	17.67	11.66

The second control experiment was used to check the validity of the assumption that a fish would spend equal amounts of time in each possible position, when no other fish were present in adjoining sections. The assumption was in fact borne out, when a Binomial Test performed on the control observations revealed a Z score of 1.297 with $p = .099$. A Mann Whitney U test was used to compare the control subjects with the stimulus fishes in the original experiment. This revealed a U value of 20 with $p < .01$, showing once again that the presence of a conspecific in an adjoining section of the tank makes a crucial difference to the positioning of the stimulus fish (see Table 5).

Table 5 A comparison of the original and second control group, giving the mean number of times the fishes were observed in the possible positions.

		Stimulus fish in position	
		L	R
Original means	(n = 12)	75.08	24.92
Second control means	(n = 10)	52.10	47.90

In view of the interesting results obtained, it was decided to attempt a replication of the original experiment. Using exactly the same procedures, very similar results were obtained (see Table 6).

A chi square comparison of the conspecific and non-conspecific distributions revealed a value of 76.10 with $df = 2$ and $p < .001$. A comparison of the conspecific and non-conspecific subjects was also made on the basis of frequency of observations in the "1" position. Using a

Mann Whitney U Test, a U value of 110.5 was obtained with $p = .013$. As seen in the original experiment, the conspecific fishes spent a significantly greater proportion of their time in the position adjacent to the stimulus fishes, than did the non-conspecifics. The stimulus fishes were observed significantly more often in the "L" position (the Binomial Test gave a Z score of 13.25 with $p < .001$).

A Mann Whitney U comparison of the original and replication data, with respect to the number of times stimulus fishes were observed in the "L" position, revealed a U value of 84.5 with $p = .236$. A comparison with respect to the number of times the conspecifics were observed in the "1" position revealed a U value of 81.5 with $p = .291$.

Table 6 A comparison of the original and replication data, giving the mean number of times the fishes were observed in the various possible positions.

	Stimulus fish in position		Conspecific in position		
	L	R	1	2	3
Original means	75.08	24.92	57.83	21.50	20.67
Replication means	69.16	30.84	49.33	21.25	29.42

	Non-conspecific in position		
	1	2	3
Original means	34.67	15.50	49.83
Replication means	32.83	33.50	33.67

When movement indices were drawn up for the conspecific and non-conspecific groups (see Table 7), they did not appear to follow the same pattern as the original data. One of the more interesting aspects of the original data was the negative correlation between movement and frequency of observations of the conspecifics in the 1 position. The same correlation performed on the replication data, using a Spearman Rank Correlation Coefficient, produced an r_s of $-.49$ with $.10 > p > .05$.

Table 7 A comparison of the conspecific & non-conspecific subjects (used in the replication), on the basis of movement scores & number of times observed in the "1" position.

	Conspecific Subjects											
	1	2	3	4	5	6	7	8	9	10	11	12
# changes in position	40	20	39	35	37	42	46	50	29	47	39	41
# of "1" positions	50	81	21	69	31	28	51	43	57	39	78	44

	Non-conspecific Subjects											
	1	2	3	4	5	6	7	8	9	10	11	12
# changes in position	33	50	23	16	1	3	47	43	38	5	17	29
# of "1" positions	70	25	38	25	31	0	30	18	27	83	11	36

	Conspecifics		Non-conspecifics	
	\bar{x}	σ	\bar{x}	σ
Changes in position	38.75	8.14	25.42	17.29
"1" positions	49.33	19.17	32.83	23.12

Discussion

It is clear from the original experiment and the replication that the conspecific subjects spend a significantly greater amount of time than the non-conspecific subjects in the position nearest to the stimulus fish. It is also clear that the stimulus fishes are significantly attracted to the position adjacent to that containing the conspecific subject. The first control experiment shows that the presence of the non-conspecific subject does not make a significant difference to the results. The second control shows that when no other fishes are present in the adjoining section, a subject alone in the apparatus will not be observed in any one position significantly more often than in any other. This is what we would expect by chance alone. Therefore there can be no doubt that conspecifics are attracted to one another by some means other than by visual, tactile or chemical cues.

If we accept Tinbergen's proposition (1948) that approach behavior is an appetitive action in fish which form schools, then since the conspecific fish in the experiment can never achieve the desired state of finding a conspecific and swimming along side of it (i.e. consummating the appetitive behavior), we could predict that the conspecific will display more movement than the non-conspecific fish.

Looking at the means of the movement indices for both the original experiment and the replication, we find that the conspecific fishes did display more movement than the non-conspecifics. However, the unusually large differences in standard deviation, precluded any useful comparison of means, but did however clearly reveal the inverse relationship between amount of movement and number of "1" positions, in the case of the original

conspecific subjects. This inverse relationship shows that the conspecifics either remain still nearest the stimulus fish or display a great deal of movement. Unfortunately the replication did not reproduce a bimodal distribution in the conspecific movement indices nor a significant negative correlation.

EXPERIMENT 2

Having established that fish are attracted by some sensory modality other than visual, tactile or chemical, the following experiment was set up in order to ascertain whether or not conspecifics are capable of orienting to one another without the benefit of visual, tactile or chemical cues. If the subjects in the following experiment are capable of orienting to each other, then we can conclude that the unknown sensory basis is sufficient for both the initial formation and the maintenance of schooling.

Subjects

The 12 zebra danios from Experiment 1 were used and kept in exactly the same conditions as reported in Experiment 1.

Apparatus

A partitioned tank from Experiment 1 was used and placed over a white sheet of paper, which marked off each of the two long sections of the tank (A and B) into seven segments (see Fig. 3).

Figure 3 about here

Procedure

One zebra danio was placed in section A and another zebra danio in section B. E observed the location of the two fish at ten second intervals and a total of fifty observations were taken. Six pairs of subjects were run altogether.

Results

Interval scores were computed for each pair of subjects, such that if the fish in A was observed in position 6 at the same time as the fish in B was observed in position 2, this was classified as an interval score of 4. Thus an interval score of 0 represents the two fish opposite one another, at their respective sides of the partition, whilst a score of 6 represents the two fish as far apart as possible, at opposite ends of the tank. If the fish are orienting to one another, one would predict that there be a greater than chance number of low interval scores. Interval scores of 2 and below were chosen as representative of one fish orienting to the other. Inclusion of scores other than 0 is necessary, since it is clear that even if the fish could use all their sensory modalities and had a tendency to remain together, they would be unlikely to remain exactly opposite one another, unless they remained stationary throughout the experiment. The cut-off point is entirely arbitrary, but the closer it is to 0, and if we are still able to obtain a significantly large number of the included scores, then the more indicative are the results.

However the actual data showed a significantly large number of 0, 4 and 6 scores, when the frequency of interval scores was summed over the six pairs of subjects (see Table 8). A chi square comparison of the interval

scores with a chance distribution, revealed a value of 55.93 with $df = 6$ and $p < .001$. Partitioning of chi square showed that most of the significance could be attributed to the inflated number of 0, 4 and 6 interval scores.

A closer look at the actual number of observations of the individual fish in the seven possible positions (see Table 9) revealed that all the subjects spent a greater than expected amount of time at the extreme ends of the tank. A chi square comparison of the actual number of observations in the seven positions with what would be expected by chance alone, gave a value of 355.83 with $df = 6$ and $p < .0001$. Partitioning of chi square showed that most of the significance was due to the inflated extremes of the distribution (i.e. a preference for the ends of the tank).

Table 8 Interval scores obtained in Experiment 2.

	Interval						
	0	1	2	3	4	5	6
# of scores summed over all pairs.	65	39	20	29	71	25	51

Table 9 Distribution around the tank of the original and control groups in Experiment 2.

	Position in Tank						
	1	2	3	4	5	6	7
# of observations summed over all individual fish.	Experimental:						
	150	51	103	28	27	32	209
	Control:						
	111	33	20	25	39	79	293

To ascertain whether or not the preference for the ends of the tank was independent of any possible contact between two subjects, a control experiment was undertaken using only one fish at a time. Twelve individual fish were run and fifty observations taken of each subject's position. In all other respects, conditions were identical to the original experiment.

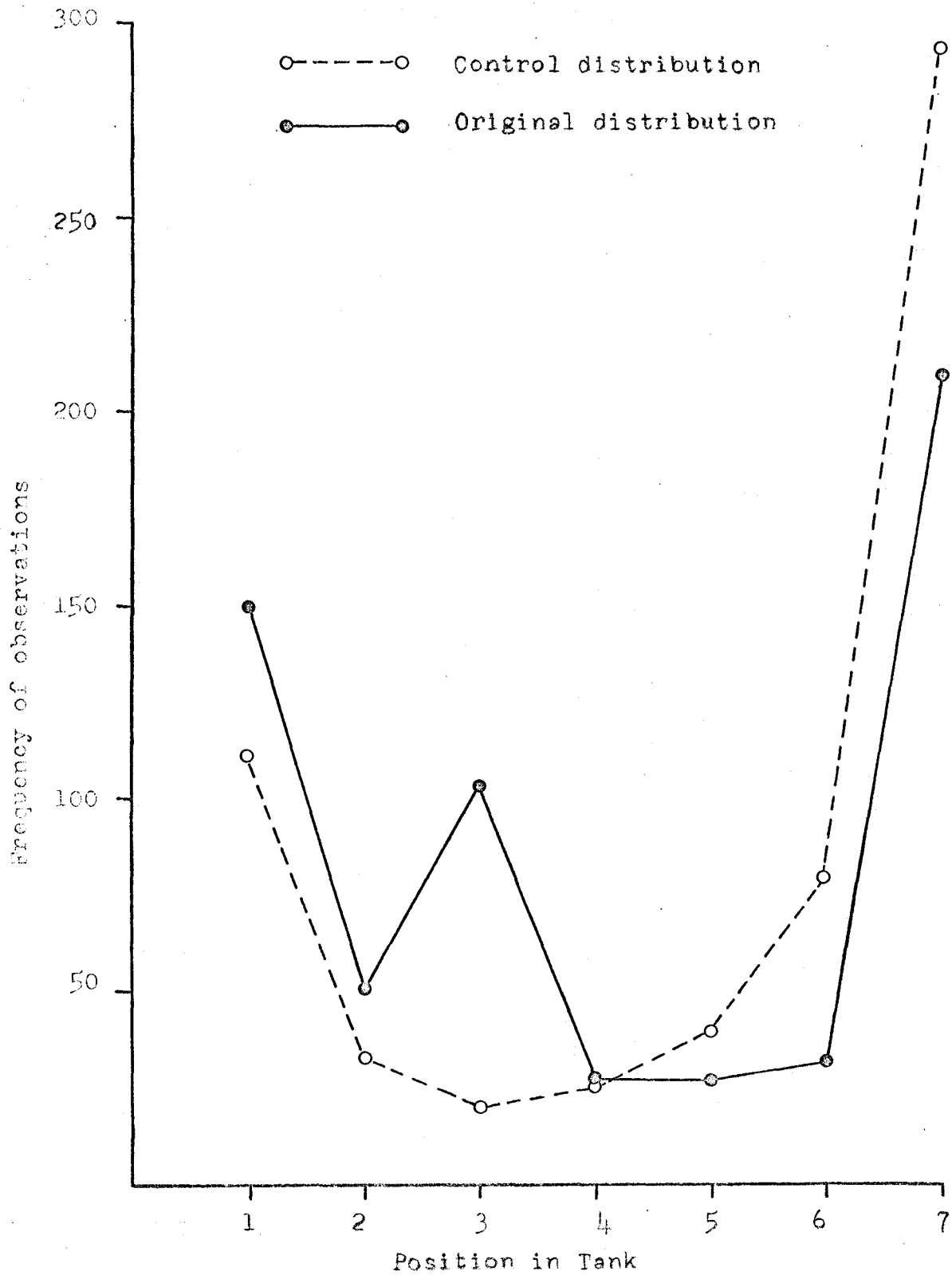
The results of the control experiment (see Table 9) clearly show a very marked preference for the ends of the tank. A comparison of this distribution with a chance distribution, using chi square, revealed a value of 660.68 with $df = 6$ and $p < .001$. If these results are compared with the original results (see Table 10), it would appear that the presence of another fish in the adjoining section, distorts the preference for the ends of a tank. A chi square comparison of the two distributions gave a value of 101.98 with $df = 6$ and $p < .001$.

Discussion

It appears that given no external or internal stimuli, fish prefer the ends of a tank. This preference was so marked in the above experiment that it was impossible to draw any conclusions regarding the orientation of one fish to another of the same species. However it seems likely that the fish are aware of each other in the experimental situation, since this is the only factor which could account for the distortion of the end-preference pattern, so clearly revealed by the control experiment.

Table 10

Graph showing difference in distribution around the tank, of the original & control groups in Experiment 2.



EXPERIMENT 3

The following experiment was a further attempt to ascertain whether two conspecific fish are capable of orienting to one another, when all visual, tactile and chemical cues are absent. The experiment was designed in such a way so as to avoid the preference for ends of a tank and to employ a more sensitive method of locating the subjects. Video tape recordings were used as a more effective method of observation and also to eliminate any effects due to the presence of an observer.

Subjects

Subjects were 20 zebra danios (*Brachydanio rerio*) approximately 1.5" long and were maintained in the same way as those subjects used in the previous experiments.

Apparatus

The apparatus consisted of two circular plastic bowls, measuring 10" dia x 3.5" deep and 6" dia. x 2.5" deep. The sides of the bowls were painted black to eliminate all outside visual cues. The smaller bowl was then placed in the center of the larger one, and water placed in the bowls to a depth of 2". The apparatus was then placed over a white sheet of paper, marked off into sixteen wedge-shaped segments (see Fig. 4).

In preliminary tests, three graded sizes of circular bowls were used, so that the subjects placed in the two outer bowls would be moving round similar circular pathways. However it soon became apparent that the pathways were too narrow for the subjects to turn round easily, and hence obviously restricted them from orienting to each other. As a result, the medium-sized bowl was discarded, and the apparatus first described was used for the actual experiment.

Figure 4 about here

Procedure

Two zebra danios were taken at random from the holding tank and one placed in each section of the apparatus. After a ten minute acclimatisation period, a video recording was made for ten minutes. Using the monitoring screen, E observed the positions of the two subjects, at five second intervals, and made 120 such observations on each pair of subjects. Ten subjects were run in all.

Results

Scores for each pair of subjects, were based on interval positions. These were calculated in the same manner as in the last experiment, such that if the fish in the outer section was observed in segment 1 at the same time as the fish in the inner section was observed in segment 3, this was classified as an interval position of 2. In this way a frequency distribution over interval positions was drawn up for each pair of subjects. Since we were only interested in interval scores which represented the subjects orienting to one another (i.e. the small intervals), an arbitrary cut-off point was made, such that all interval scores higher than 3 were classified as representing no orientation ("NO" scores).

If the fish are orienting to one another and thus using some sensory communication other than visual, tactile or chemical, we should expect subject pairs to have a greater than chance number of interval scores of 3 and under. We should also expect a greater than chance number of subject pairs to have such a distribution of their interval scores.

Nine out of ten subject pairs had a greater than chance number of interval scores of 3 and under and the Sign Test showed the probability of such an outcome to be less than 0.001. When the arbitrary cut-off point was reduced to 2, the results were still found to have a probability of less than 0.001 (see Table 11).

Table 11 Cumulative distribution of low interval scores for the 10 subject pairs used in Experiment 3.

Subject pairs:	Interval Scores				
	0	01	012	0123	"NO"
A	20	31	56	79	41
B	13	28	47	62	58
C	12	30	47	63	57
D	15	30	48	70	50
E	6	19	38	64	56
F	13	20	50	63	57
G	5	12	26	46	74
H	8	26	41	60	60
I	14	41	60	80	40
J	10	34	59	77	43
Expected scores:	7.5	22.5	37.5	52.8	67.2

Discussion

There is little doubt whatsoever that the subjects were capable of finding and orienting to one another in this experiment. The added precautions taken to rule out any observer effects and to rule out all positional cues by using circular sections, make it exceedingly difficult to find any other explanation for the results other than that the subjects were using some communication channel other than visual, tactile or chemical ones.

EXPERIMENT 4

The following experiment was designed to provide a situation most like that of the home tank of the subjects, in order to make sure that the unknown sensory modality discovered, would be used in a fairly normal environment and not just in a state of relative sensory deprivation. Working on the assumption that sound is the modality being used by the fish, it was decided to use two fish as the stimulus, to increase the likelihood of sounds being produced which would attract a test subject (Moulton, 1960). Polyethylene was used to separate subjects, in order to see whether a thinner dividing material would affect the results. George (1960) reasoned that a thin plastic membrane should most effectively cut out all visual, tactile and chemical cues while allowing the passage of auditory stimuli.

Subjects

Thirty zebra danios (*Brachydanio rerio*) and 10 Cherry Barbs (*Barbus titteya*) were used as subjects and were kept in a holding tank under the same conditions as all previous subjects.

Apparatus

An aquarium 15.5" x 8" x 10" deep, containing gravel and weeds, was filled with aged water to a depth of 9" and two green opaque polyethylene conical-shaped bags, measuring 6" deep x 4" dia. were suspended in the water at either end of the aquarium (see Fig. 5). Two zebra danios were placed in one of the bags and the other was left empty. A masking box was erected around the aquarium and observations made through a peep-hole set to view the aquarium sideways on.

Figure 5 about here

Procedure

One zebra danio was selected at random from the holding tank, placed in the experimental tank and allowed ten minutes to acclimatise to the new environment. E then observed whether the subject was in the half of the tank containing the stimulus fishes or not. One hundred such observations were made on each subject and twenty subjects were run.

A slight variation was then introduced by using a group of six fishes instead of one individual subject. Observations were taken as above and a record kept of any departures from the group by one or more of the six fishes. Ten groups of six were run altogether.

Two control experiments were also designed. The first control was used to demonstrate the effectiveness of visual stimuli on schooling, by substituting transparent bags for the opaque ones. Ten individual subjects were run and observations were taken in the manner described above.

In the second control, opaque bags were used again, one of them containing two zebra danios, but individual barbs were used as subjects. Observations were made as before and ten subjects were run.

Results

In the second experimental situation, very few departures of individuals from the group were noted, and so each group of six was treated as a single subject unit. Means of the number of observations of the subjects in the stimulus half of the tank were taken for each of the four conditions. This gave a reasonably accurate measure of the percentage of time spent by subjects in the stimulus half of the tank. As can be seen from Table 12, the only condition in which subjects spent a significantly greater amount of time in the stimulus half as opposed to the other half of the tank, was the first control involving the use of transparent bags.

Finally indices of movement were obtained for each subject, by taking the number of times the subject crossed the center of the tank. Totals of movement indices were taken for each of the four conditions and a chi square comparison showed no significant differences between conditions.

Table 12

Mean % time spent by the four groups of subjects in the stimulus half of the tank.

	Single subject (z. danio)	Group of Six	Bags transp.	Single Subject (barb)
Mean % time spent in stim. half	46.9	42.0	85.1	51.7
n =	20	10	10	10

Discussion

As might be expected, when the subjects can see the conspecifics in the transparent bag, they spend a significant amount of time in the half of the tank containing the conspecifics. Although no statistical tests were employed, it is quite obvious that under the other three conditions, subjects spent more or less equal amounts of time in either half of the tank. This of course is in direct contradiction to the evidence of the previous experiments and it was decided to see if some confounding factor were in operation.

Chance results were obtained in all cases where opaque bags had been used and in all cases the movement indices were fairly high (see Table 13). Therefore it seemed possible that the opaque bags were aversive to the subjects. For if this were so, we might expect the fish to avoid either end of the tank and swim back and forth in the middle of the tank.

When placed in the tank with only one opaque bag at one end, fish did indeed display a very noticeable aversion to the side containing the bag. No experiments were conducted to ascertain the aversive qualities of the bag,

but it seems likely that a conical dark shape may represent some large predator, which would naturally produce fright and aversion reactions.

Table 13

	1 subject (z. danio)	Group of Six	Bags transp.	1 subject (barb)
Total # of center crossings	861 n = 20	398 n = 10	389 n = 10	426 n = 10

Summary and Discussion

If we accept the idea that the opaque bags in Experiment 4 were aversive, to explain the negative results of that experiment, then we are left with a considerable amount of evidence for a sensory basis of schooling other than visual, tactile or chemical. The main problem at this stage is to ascertain whether or not that sensory basis is auditory. It cannot be assumed that the results obtained were due to auditory communication, just because there would appear to be very little else to which they could be attributed. Moulton (1960) had great difficulty in getting Anchovies to emit sounds when isolated, thus it cannot be assumed that the fish were making any sounds during the experiments.

The only conclusive way of showing that sonic communication was responsible for the results obtained, was to monitor subjects for sound

production while making observations of their movements in an experimental situation such as the one used in Experiment 3. It was decided, therefore, to obtain a hydrophone and do such an experiment.

AN ATTEMPT TO CLARIFY THE NATURE OF THE UNKNOWN

SENSORY BASIS OF SCHOOLING.

To date, there are very few reports of auditory recordings from Cyprinids. Winn and Stout (1960) were able to associate a knocking sound with aggressive behavior in the Satinfish Shiner. They reported that this sound contained frequencies ranging from 85 cy/sec. to at least 11,000 cy/sec. Apart from this there have been several recordings of mechanical noises such as chewing and fin scraping on the sides of aquaria. Whether through lack of interest or abysmal failure, there have been no other reports of biologically significant sounds produced by Cyprinids.

Fish (1954) has classified sounds made by fish as either "mechanical" or "biological". Under the heading of "mechanical" come such sounds as the chewing and fin scraping noises mentioned above (ie. unintentional noises produced by the fish). "Biological" is used to describe sounds produced by some anatomical structure of the fish, specifically designed for the purpose of sound production. However, Winn

and Stout (1960), Tavalga (1960) and Myrberg (1965) are dubious about the validity of these two classifications as discrete entities, since they frequently grade into each other. For our purposes, it is largely irrelevant which type of sound is being produced, if it is being used as a sensory basis for schooling.

It was decided to monitor sounds produced by the fish in the apparatus used in Experiment 3. Hopefully the movements of a pair of conspecifics could be recorded on video tape whilst any sounds being made were tape recorded, so that any correlation between movement and sound could clearly be seen.

However, to start with, it was felt advisable to ascertain what sounds could be picked up from the fish with the aid of a hydrophone, before using a proper experimental situation. The hydrophone used was a spherical model (# SB 154B) from Chesapeake Instrument Corporation, having a frequency range of from 10 cy/sec. to 6,000 cy/sec. and a sensitivity of -90 db. re 1v/ubar.

Several zebra danios were placed in a polythene bucket and the hydrophone suspended in the water. The hydrophone was then connected to a preamplifier and oscilloscope, which was monitored for several hours. However nothing was seen, apart from 60 cycle noise, presumably emanating from the power source.

EXPERIMENT 5

Despite the discouraging start, it was decided to continue monitoring for signals in an experimental situation and attempt to cut out some if not all of the 60 cycle noise.

Subjects

The zebra danios from Experiment 4 were used as subjects.

Apparatus

The two circular bowls, described in Experiment 3 were used to hold the subjects. The hydrophone, as described above, was connected through a Grass DP9 Preamplifier with band pass set from 10 cy/sec. to 10,000 cy/sec., to a Tektronix 502A dual-beam oscilloscope, which in turn was connected to a Schmitt Trigger providing an audible click for signal voltage above a preset level. The bowls containing the subjects were placed inside a 60 cycle screening cage.

Procedure

The hydrophone was suspended in the center of the smaller bowl and a zebra danio placed in each of the sections of the apparatus. After a ten minute acclimatisation period, the oscilloscope was monitored for two hours, after which the subjects were replaced with a new pair of subjects

and the oscilloscope monitored for another two hours. Six pairs of subjects were monitored altogether.

To find out what types of signal if any were being picked up without the presence of the subjects, the oscilloscope was monitored for an hour with the bowls empty except for water.

Results

60 cycle noise was continuously present when the fish were absent and present. A small signal riding on top of the 60 cycle signal was always present when the fishes were in the bowls. This may have been due to the movement of the subjects in the water. Since there was no readily available means of keeping the subjects perfectly still, the cause of this small non-patterned signal could not be determined. Apart from this, nothing of any significance was seen or heard on the equipment.

Discussion

The complete absence of any clear patterned signal was highly surprising in the light of the results already obtained. The data in this paper supports the idea of a fourth communication channel and basis for schooling; the work of Moulton and Tavolga indicates that sound is the most likely sense being used; Cyprinids have been shown capable of hearing a very wide spectrum of sound (von Frisch, 1936). It therefore seems very strange that zebra danios do not produce any clear sounds.

It is possible that the non-patterned sound seen on top of the 60 cycle noise may have been used by the subjects to orient to one another, but there appeared no way of identifying this signal, and therefore no

conclusions can be drawn concerning its function.

Thus so far, no definite or even tentative statement can be made about the nature of the fourth sensory basis of schooling. The above experiment in no way rules out sound as the modality being used.

Conclusion

Strong behavioral evidence has been found to support the idea of a species-specific sensory basis of schooling which is neither visual, tactile nor chemical. The failure to pick up any significant sounds from the subjects, precludes any conclusion that the sensory basis is auditory. However it cannot be claimed with any certainty that certain Cyprinids do not produce any sounds or use sonic communication.

Dutch and German zoologists have shown that some species of minnow are capable of hearing frequencies well above 7,000 cy/sec. (see Winn and Stout, 1960), which gives rise to the possibility that other members of the Cyprinidae family are also capable of hearing very high frequency sounds and hence may be able to produce such sounds for the very purpose of communication. Taking this into consideration, it may be necessary to use far more sensitive pick-up and recording devices, capable of receiving very high frequencies, before members of the Cyprinidae family are written off as non-sound producing fish.

There is some argument over the capability of fishes to localise sound (Tavolga, 1964). Kleerekoper and Chagnon (1954) claim behavioral proof of localisation, whereas von Frisch and Dijkgraaf (1935) conclude from their experiments that fishes are incapable of localising sound sources,

except when they are within the near field¹ of an intense sound. Van Bergeijk (see Tavalga, 1964) states that most species of fish, having only a single pressure receptor, are physically incapable of localising sound. However, he does allow, that since fish can detect the presence of a sound, they can find a sound source by swimming around in random patterns until they enter the near field of the sound source, where the lateral-line² system is capable of locating the source.

If this is so, then there may be some question about the likelihood of fishes using sound to locate a school, when it is more than a few centimeters away. However this will not weaken the assumption that sound may be used to keep a school together, once formed; nor is localisation ability in question in the experiments reported in this paper, since subjects were never at any great distance from one another in the experimental apparatus.

There are other possibilities of communication, as yet little explored. Lissman (1958, 1965) has found that non-electric catfish and eels put out electrical signals and he has proposed that such fish are a link in the evolutionary chain, between strictly non-electric fish and the highly specialised electric fish which use electrical charges to stun prey, ward off predators or navigate the murky depths of the oceans.

1 The dimensions of the near field vary in size and shape, depending on the size and frequency of the sound source. In the case of most small fishes similar in size and shape to the zebra danio, the near field will tend to be spherical with an approximate radius of 8 cms.

2 The lateral-line system is most responsive to pressure sources in the near field.

Electrical signals have also been recorded in the Sea Lamprey by Kleerekoper and Sibakin (1956). Barham et al. (1969) were able to record signals from catfish, crappie, sunfish and stingray.

So far, well over a hundred species of so-called non-electric fish have been shown to produce electrical signals. Size, shape and habitat of a fish appear to have no relevance to whether or not a fish is capable of putting out electrical signals, since nearly every size, shape and habitat is represented by the fishes so far discovered to be electrical.

Few attempts have yet been made to show that these signals are used for any meaningful purposes. Lissman (1965) has shown that *Gymnarchus niloticus* uses a weak electric field for the purpose of sensing its environment. Agalides, Bernardini and Zinsmeister (1964) have shown that *Sternarchus albifrons* is capable of using electrical signals for communicating fear and presence of food.

Agalides et al. (1964) state that noise in the environment is no problem in the use of electrical signals for communication purposes, since many electric fishes have a very sophisticated way of encoding signals. *Electrophorus electricus* uses a tridimensional encoding system, varying the position of the pulse of the signal, its duration and its amplitude.

There appear to be no valid reasons why fishes capable of producing electrical signals, could not use those signals as a sensory basis for schooling. If it could be shown that zebra danios put out small electric signals, the results reported in this paper could be attributed to either sonic or electrical communication or a combination of both.

One important point must be remembered in this type of enquiry, and that is the lack of generalisation possible. It cannot be assumed that a fish will use the same sense modalities in an experimental situation as in a natural situation. Most minnows and barbs live very close to the surface, in fairly clear waters, and hence their visual sense is highly developed. It is possible, therefore, that they normally use vision in all their activities, largely to the exclusion of other sense modalities. (We also rely, to a large extent, on vision to the exclusion of our other senses.) This does not mean that they are not capable of employing other sense modalities and may in fact do so under abnormal circumstances, such as exist in most experimental situations.

Summary

Several experimental situations were designed to show that when all visual, tactile and chemical stimuli are eliminated, fish are still capable of attracting and orienting to conspecifics. Monitoring the subjects with a hydrophone revealed that zebra danios do not make any meaningful or frequent sounds in the range of 60 to 6,000 cy/sec. It is suggested that the use of electrical signals or very high frequency sounds or a combination of both might be responsible for the results obtained in the experiments reported.

LITERATURE

- Agalides, E., Bernardini, J., & Zinsmeister, R. Paper presented at 1964 Rochester Conference on Data Acquisition and Processing in Biology and Medicine.
- Allee, W. C. Animal Aggregations. Univ. Chicago Press.
- Barham, E. G., Huckabay, W. B., Gowdy, R. & Burns, B. Microvolt electric signals from fishes and the environment. Science, 1969, 164, 965-968.
- Boulenger, E. G. Observations on the nocturnal behavior of certain inhabitants of the Society's aquarium. Proc. Zool. Soc. London, 1929, 2, 359-362.
- Bowen, E. S. Studies in the aggregating behavior of *Ameiurus melas*. Ecol. Monogr., 1931, 1, 1-35.
- Bowen, E. S. Further studies of *Ameiurus melas*. Biol. Bull., 1932, 63, 252-270.
- Breder, C. M., Jr., & Halpern, F. Innate and acquired behavior affecting the aggregation of fishes. Physiol. Zool., 1946, 19, 154-190.
- Breder, C. M., Jr., & Nigrelli, R. F. Winter aggregations of sunfish. Ecology, 1935, 16, 33-47.
- Breder, C. M., Jr., & Rasquin, P. Chemical sensory reactions in Mexican blind characins. Zoologica, 1943, 28, 169-200.
- Breder, C. M., Jr. Studies in social groupings of fish. Bull. Amer. Mus. Nat. Hist., 1959, 117, 393-482.
- Dijkgraaf, S. The function of the lateral-line organ. Biol. Rev., 1963, 38, 51-105.
- Fish, M. P. The character and significance of sound production among fishes of the western North Atlantic. Bull. Bingham Oceanogr. Coll., 1954, 14, 1-109.
- von Frisch, K. Über den Gehörsinn der Fische. Biol. Rev., 1936, 11, 210-245.
- von Frisch, K. Über einen Schreckstoff der Fischhaut and seine biologische Bedeutung. Z. vergl. Physiol., 1941, 29, 46-145.

- von Frisch, K., & Dijkgraaf, S. Können Fische die Schallrichtung wahrnehmen? Z. vergl. Physiol., 1935, 22, 641-655.
- George, C. J. W. Behavioral interaction of the pickerel and the mosquitoefish. Unpublished Ph.D. thesis, May 1960. Dept. of Biology, Harvard University.
- Göz, H. Über der Art- und Individualgeruch bei Fischen. Z. vergl. Physiol., 1941, 29, 1-45.
- Gudger, E. W. Fishes that rank themselves like soldiers on parade. Zoologica, 1949, 34, 99-102.
- Harris, G. C. & van Bergijk, W. A. Evidence that the lateral-line organ responds to near field displacements of sound sources in water. J. Acoust. Soc. Amer., 1962, 34, 1831-1841.
- Hemmings, C. C. Olfaction and vision in fish schooling. J. Expt. Biol., 1966, 45, 449-474.
- Keenleyside, M. H. A. Some aspects of the schooling behavior of fish. Behavior, 1955, 8, 183-248.
- Kleerekoper, H. & Chagnon, E. C. Hearing in fish. J. Fish. Res. Board Canada, 1954, 11, 130-152.
- Kleerekoper, H. & Sibakin, K. Electric potentials in the sea lamprey. J. Fish. Res. Board Canada, 1956, 13.
- Lissman, H. W. On the function and evolution of electric organs in fish. J. Expt. Biol., 1958, 35, 156-191.
- Lissman, H. W. Electric location by fishes. Sci. Amer., 1965, 208, 50-59.
- Moulton, J. M. Swimming sounds and the schooling of fishes. Biol. Bull., 1960, 119, 210-223.
- Myrberg, A. A., Jr., Kramer, E. & Heinecke, P. Sound production by Cichlid fishes. Science, 1965, 149, 555-558.
- Myrberg, A. A., Jr., The Shark Caller. Article in Time, June 1969.
- Parr, A. E. A contribution to the theoretical analysis of schooling behavior in fish. Occ. Pap. Bingham Oceanogr. Coll., 1927, 1, 1-32.
- Pfeiffer, W. The fright reactions of fish. Biol. Rev., 1962, 37, 495-512.

- Pfeiffer, W. Alarm substances. Experientia, 1963, 19, 113-123.
- Shlaifer, A. Schooling behavior of the mackerel. Zoologica, 1942, 27, 75-80.
- Tavolga, W. N. Significance of underwater sounds. Physiol. Zool., 1958, 31, 259-271.
- Tavolga, W. N. Marine Bio-Acoustics. Macmillan, 1964.
- Verheijer, F. J. Transmission of flight reactions in schools. Experientia, 1956, 12, 202-204.
- Winn, H. E. & Stout, J. F. Sound production by the Satisfin Shiner, *Notropis analostanus*, and related fishes. Science, 1960, 132, 222-223.

Figure 1: Diagram of partitioned tank used in Experiment 1.

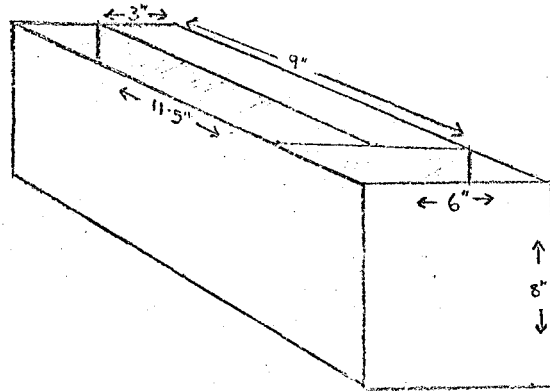


Figure 2: Diagram showing method of locating subjects.

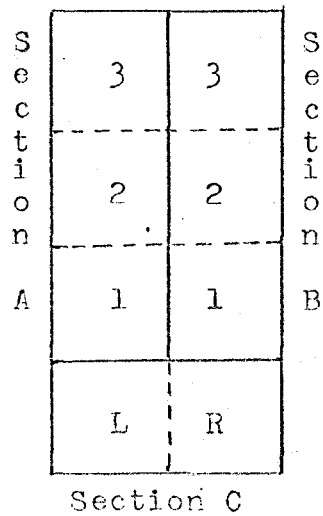


Figure 3: Diagram showing method of locating subjects in Experiment 2.

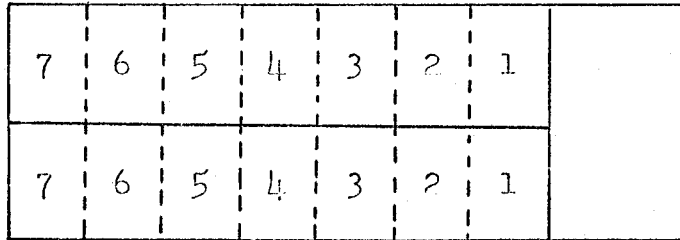


Figure 4: Circular apparatus used in Experiment 3.

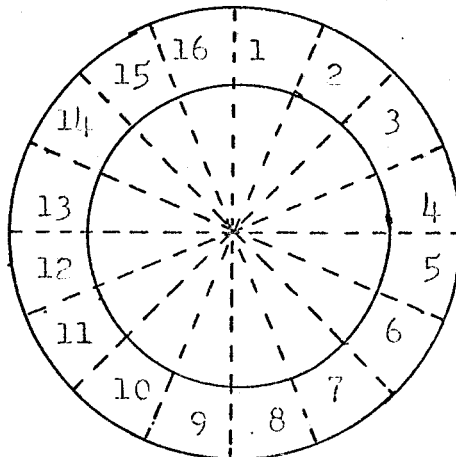


Figure 5: Diagram showing positioning of bags in aquarium used in Experiment 4.

