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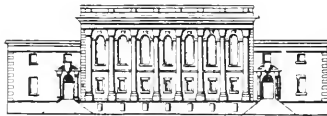


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A TEST OF INDIVIDUAL RECOGNITION
IN BLUEGILL SUNFISH USING OPERANT PROCEDURES

by

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CHAPTER I
THE PROBLEM

A considerable array of interactions occur among fish as members of a social organization. Behavior associated with territoriality, dominance, mutual stimulation and leadership are major parts of their social system (Klopfer & Hailman, 1967). According to Wynne-Edwards (1960), a social organization presupposes communication among its members. The most basic exchange of information is mutual or individual recognition. An individual's identity is established via a two way process of transmitting and receiving signals that involve one or more of the senses (Wynne-Edwards, 1960).

The study of signal communication has value in determining not only what patterns of stimulation are likely to cause what responses, but also what patterns the organism is capable of discriminating.

Since fish are classified as lower vertebrates, the question arises as to whether they are capable of communicating on this level, particularly in an aquatic medium. Schneirla (1953) and Tavolga (1968) discuss this problem, and include fish in the higher levels of animal communication hierarchies.

The channels of animal sensory interactions can be visual, tactual and acoustic, gustatory and olfactory, and/or electrical (for a very few species of fish). The tactual and acoustic communicative abilities of fish are well developed (Bardach, Todd, & Crickmer, 1967; Scott, 1958). Studies by

Todd et al.(1967) show that yellow bullheads have olfactory means for discriminating odors of individual fish, while the results of Hasler and Wisby (1951) and Hasler (1966) indicate that fish can also discriminate stream odors. Also, pheromones have been shown to be important for fish in making schooling discriminations (McFarland & Mess, 1967). Although the chemoreception and the tactile senses are of evident importance to fish in discriminating individuals, it would seem that the development of the visual sense would be equally if not more effective in such discriminations. It has been observed by Lorenz (1963, 1970) that visual signals contribute to individual recognition behavior in birds and fish. Discriminations based on visual characteristics can be instantly perceived, and from a greater distance. The ability to recognize or discriminate among individuals would seem of considerable adaptive significance in the social organization of fish. That fish discriminate conspecifics as individuals and not merely as members of a particular social or sexual class would allow one individual meeting another to respond immediately and appropriately. This ability to instantly perceive another's identity would be of value in a variety of interactions. A fish could discriminate between a stranger and a familiar fish, a potential or actual mate, or a dominant or submissive individual. This would presumably occur within male-female, juvenile-adult, conspecific-contraspecific, inferior-superior class framework (Johnson, 1968). It has been well-established that a variety of fish can be conditioned to discriminate among

visual cues employed in laboratory learning experiments (Sutherland, 1968; Matthews, 1964). Most of these discriminations were based on geometric stimuli. Few attempts have been made to demonstrate or quantify recognition capabilities using conspecific fish as discriminative stimuli.

The purpose of this experiment was to attempt to demonstrate individual recognition in sunfish using an operantly conditioned "nose bump" response in a two-choice successive discrimination learning problem. Conspecific fish varying only in the visual dimension served as the discriminanda. The basic question to be answered in this study was, "can fish (bluegill sunfish) learn to respond in the presence of one conspecific individual (S^D) and not to respond in the presence of another conspecific (S^A)?"

CHAPTER II
REVIEW OF LITERATURE

Laboratory Studies

Fish have been useful subjects in learning research. The majority of the published investigations have dealt with the ability of fish to discriminate among visual signals. Using classical and instrumental techniques, it has been shown that fish can be conditioned to make a number of different responses under several stimulus dimensions. Goldfish and African mouth-breeders were conditioned to make discriminations of hue, brightness, size and form by Bitterman and his collaborators (Wallinsky & Bitterman, 1959, 1960; Horner, Longo & Bitterman, 1961; Gonzales, Milstein & Bitterman, 1961; Gonzales, Longo & Bitterman, 1961). The operant responses included shuttle box swimming, maze swimming, and an operant "strike" or "nose bump" to an underwater manipulandum. Other studies have demonstrated that goldfish as well as several species of tropical fish can discriminate shapes and forms (Matthews, 1964; Mackintosh & Sutherland, 1963). Goldfish detect the presence of points in a shape, appear to recognize differences in the relative number of points present in two shapes, and rely more heavily on points than on orientation of contours. Also, goldfish tend to recognize figures on the basis of their upper halves (Sutherland, 1968). It has been established that various fish can distinguish colors. The most representative study is by Yager (1968), which examines tri-color wavelength discrimination abilities in goldfish.

Field Studies

Although there is an impressive body of experimentation concerned with discrimination learning in the laboratory, it seems important to examine field studies for confirmations. There is evidence in a study by Nelson (1964) that individual recognition is of adaptive significance in the establishment of dominance hierarchies and courtship preferences. The species studied were members of the Characidae family. Behavior of Corynopoma males and groups of Glandulocauda and C. microlepis indicated that they could readily differentiate one another. Consistent differences in behavior toward different fish were observed in altered groups of C. microlepis. When offered a choice of females to court, males showed a preference for certain females who were not necessarily the least aggressive nor the largest. This relationship which lasted several months implied a strong mate preference approaching a true pair bond. Nelson suggested that the restriction of a male's attention to one particular mature female would help insure that courtship would be continuous and result in successful fertilization of females who might otherwise not be persuaded to mate.

In a study by Noble & Curtis (1939), visual stimuli from the head were found to be critical cues for male jewel fish in recognizing their mates. When confronted with a mate who had been painted differently on both sides of her head, the male would recognize her from one side and attack her from the other. When his mate was not present, he would reject other

conspecific females presented. A mate and a similar female were placed in neighboring tanks, and the male would approach only his mate. It was noted that an approaching group of fish of which a mate was a member was attacked, while the mate was left unharmed.

The importance of visual cues for schooling fish has been demonstrated by Keenleyside (1955). It was found that the chief factor common to all schools was a definite mutual attraction among individuals. The Pristella riddlei used by Keenleyside were able to distinguish between two highly similar groups of fish. Each group of conspecifics was placed in a jar, which was lowered into each end of an aquarium. A fish of one species was placed in the middle of the tank, equidistant between the two jars. The experimental animal swam to its conspecifics, apparently making the recognition on the basis of visual cues.

Evidence for individual recognition has been observed in garibaldis (Clark, 1970). In the establishment of individual territories, a neighboring fish was allowed to intrude without a challenge if it was chasing another fish. However, non-neighbors chasing fish were attacked if they crossed territorial boundaries.

Visual recognition within most species of fish seems to be enhanced by conspicuous displays of bright colors and patterns. The development of visual recognition marks is most apparent for species living in shallow areas. Light reaches a high intensity in transparent water, making color patterns

easily visible from a distance (Wynne-Edwards, 1960).

Konrad Lorenz (1963) has noted the importance of bright color patterns for coral reef fish in determining territoriality. Taking some of these fish into the laboratory, Lorenz observed that fish with "flag colors" were more readily identified by their conspecifics than were blandly colored fish.

Numerous species of sunfish in the North American fresh water family Centrarchidae are brightly colored and easily recognizable by a special ear flap on the operculum. Their well-developed social interactions have been observed by Miller (1962) and Witt & Marzolf (1954), suggesting that they would be useful subjects for experimentation concerned with individual recognition abilities.

CHAPTER III

METHOD

Subjects

Twelve bluegill sunfish (*Lepomis macrochirus*) were caught by hook and line in Sweet Briar lakes. They were kept in common aquaria until three emerged as dominant individuals. These three fish served as the experimental subjects. It was felt that the most aggressive fish would be most likely to exhibit the highest response rate to the discriminative stimuli.

The fish were from 15 to 17 cm long and weighed approximately 110 grams each.

Each subject was housed in separate 32 or 64 liter glass aquaria. Heavy brown paper masked the sides of the tank to accomplish visual isolation and prevent possible learning from observation of other fish. Subjects were maintained on a 24 hour deprivation schedule. They were fed Noyes 20mg reinforcement pellets which were earned during each discrimination trial each day. On those occasions when an experimental session was not held, the fish were given size four Purina Trout Chow once a day for 30 minutes.

Toward the middle of the study it was discovered that one subject, S₃, was infested with tapeworm and ascaris roundworm. It was assumed that this condition contributed to the animal's slow rate of responding.

Apparatus

The basic device for conditioning instrumental responses was a Foringer pellet dispenser which released a single reinforcement pellet whenever S struck or "nose bumped" the manipulandum. The manipulandum was a 2.7 cm brass disc soldered to a 23 cm length of clothes hanger wire. An inch above the disc the wire pivoted around a nail mounted horizontally between two plexiglass struts. A flat rectangular brass shutter was attached ventrally to the top of the wire. When the manipulandum was struck and thus pushed backward, the fulcrum action of the wire and nail moved the shutter forward. Below the shutter was a photoelectric cell with a 1.5v light positioned 2 cm above it. The photo cell served to activate a relay which in turn controlled the feeder. When the shutter was at rest, the light beamed directly into the cell. With each strike, the shutter advanced so as to prevent the light beam from hitting the cell. This interruption allowed current to flow to the feeder, which dropped a food pellet into the water directly above the manipulandum. The entire mechanism was mounted on plexiglass and situated above one end of the aquarium. A switch operated by E prevented reinforcement for responses made when the S^{Δ} was presented. Prior to each discrimination learning session, a fitted projection screen was attached to one end of the subject's tank. The screen was made of cardboard sandwiched between two pieces of black construction paper. A 10.2 cm by 5.4 cm area was cut out of the middle and replaced with translucent colorless plastic.

When in position, the manipulandum extended 5 cm below the water level, which placed it directly above the head of the stimulus fish projected onto the plastic. It was constructed in this manner so that the stimulus fish would always be within visual range when a strike response was made.

Stimulus Material

Thirty-five mm color slides of two stimulus fish served as discriminative stimuli. The use of live subjects as stimulus material was considered too complicated for this stage of the investigation. Possible artifacts due to postural effects and individualized movements might have introduced variables unnecessary to contend with at this time. A reasonable amount of control was obtained by equating the two fish along dimensions of size, general coloration and fin contour. The only prominent difference between the two was a bright irridescent blue stripe along the gill flap and jaw of one fish.

Ten slides each of the S^D and S^A were arranged in Gellerman order (Gellerman, 1933) to prevent establishment of possible response sequences.

Procedure

The experiment was designed to proceed in three steps: Familiarization, Operant Training and Discrimination Training.

Familiarization Stage

This initial stage allowed each experimental fish to become acquainted with the introduction of the manipulandum

into his home tank. During each S's feeding period each day, the operant apparatus was set in place in the tank. Worms and reinforcement pellets were dropped by hand into the area of the manipulandum so that the fish were required to approach it in order to eat. At first, the Noyes pellets were immediately spit out; however, they were later readily accepted when worms were no longer offered. Eventually, the subjects swam to the experimental end of the tank whenever E entered the room. This stage involved approximately 10 hours of training. It also served to insure that the animals could be maintained under these conditions before operant training began.

Operant Training

Each fish was shaped by successive approximations to "strike" or "nose bump" the manipulandum. The first manipulandum used was a 7.6 cm diameter circle of thin electrical wire connected to the lever of a microswitch. S was reinforced for putting his nose through the loop, which was replaced by a smaller 2.7 cm loop. Reinforcement was given under the same contingency as before. Eventually a brass disc was connected to the lever, and the fish was reinforced for touching it with his nose. A reliable response began to emerge, though initially the strike was not made with sufficient force to operate the switch automatically. Because of the frequency of these "light" responses, a large number of trials were not being reinforced. The apparatus was modified using the photoelectric cell and shutter so that the most delicate

strike would be reinforced automatically.

There was some degree of individual difference in the manner in which each fish learned the desired response. Subject one acquired the appropriate response pattern by the usual method of approximations. Subject two maintained a highly reliable operant response of swimming to within less than an inch of the disc, although he would repeatedly approach it from the rear or from the side. The procedure was then modified to insure a head on nose bump by baiting the disc with food. A thin length of wire with a piece of worm on the tip was lowered into the water until it hung just on the top face of the disc. Under these arrangements, the fish had to strike the worm from the front in order to remove it from the wire. At the same time the wire was struck, the fish made contact with the disc. The wire was removed after several sessions of this baiting procedure. After the appropriate orienting behavior was established, the correct response emerged.

Subject three did not exhibit his first nose bump response until 7 sessions (8-10 reinforcements per session) after the other fish had learned the response reliably. The baiting procedure had to be used to make the disc more attractive, rather than waiting indefinitely for the correct response to emerge.

Discrimination Training

During this phase, the screen was taped to the aquarium

and the operant feeder apparatus placed across the end of the tank. The stimulus material was presented in a successive sequence to each S by a carousel projector positioned 83 cm from the screen. Slides were changed by a remote control device operated by E.

Each S was given the opportunity to respond to the stimulus during a 60 second trial period. A correct response consisted of "nose bumping" in the presence of the S^D . Nose bumps made in the presence of the S^A were never reinforced. If S did not respond to the stimulus presented within the 60 second period, it was counted as a non-response. Each fish was run daily for 30 minutes.

Changes in Procedure

Several modifications were made in an effort to improve procedural design. Under the assumption that the visual differences in stimulus fish were too subtle or too difficult to discriminate, slides of the positive fish were retouched with acetate ink pens. In order to accentuate an already existing color pattern, the gill covering was painted a brighter blue, while the pectoral fin region was tinted yellow.

There was also the possibility that the fish were not attending properly to the stimuli. The manipulandum was repositioned so that from the S's position inside the tank, the brass disc would appear to just barely contact the head of each stimulus fish. It was assumed that the subjects would be more likely to attend to the appropriate cues if they were

more conspicuously placed in relation to the manipulandum.

After these modifications it was noted that S₁ began to respond within 15-20 seconds after the stimuli were presented. This finding would indicate that some of the difficulty in forming discriminations could be an artifact of the presentation rate. The stimuli were presented in a successive rather than a simultaneous order. Because of this arrangement, subtle differences between stimuli could have been lost if the period between presentations was too long for information to be retained in memory. Consequently, the period between trials was shortened to 30 seconds for this fish.

CHAPTER IV

RESULTS

It became apparent late in the experiment that all animals were responding far more to the S^D than the S^A . None of the experimental animals reached a traditional learning criterion before the termination of the experiment. Consequently, the data are presented in a purely descriptive manner.

The total number of responses to the S^D and S^A were obtained for each subject. These totals are presented in Table 1. Table 1 also shows the total responses summed across the three experimental S_s .

TABLE 1
Total Responses and Non-Responses
to S^D and S^A

Subjects	S^D		S^A	
	Responses	Non Responses	Responses	Non Responses
1	444	50	322	154
2	454	67	343	148
3	279	39	189	107
TOTAL	1177	156	854	409

NOTE: Figures in the non-response column represent the number of stimulus presentations to which S_s did not respond within the allotted time period.

In addition, it was decided to arrange these data into blocks of trials in an attempt to determine the shape of the learning curve up to the point at which trials were terminated. For each S the total number of trials for both the S^D and S^A was determined. This total was then divided into 10 equal blocks. Within each of the resulting 10 blocks, a discrimination ratio was computed by dividing the total responses to the S^A by the total responses to the S^D . An assumption of no learning would predict a discrimination ratio of 1.0 at the beginning of training. Complete learning would reduce the discrimination ratio to zero. These discrimination ratios are presented as learning curves in Figure 1. It should be pointed out that the first four blocks of trials presented in Figure 1 differ from the others in that these sessions were approximately 60 minutes in duration as opposed to the 30 minute duration of the remaining trials. Also, during the initial four blocks of trials each stimulus was presented on an average of two minutes as opposed to one minute in later trials. These initial four blocks served in part to enable E to determine an optimal presentation rate and to refine procedures. Even though there were procedural differences between these trials and the later ones, it seemed essential to include them in Figure 1 since they indicate through the discrimination ratios that a considerable amount of learning occurred during this phase.

The discrimination ratio curve for S_3 shows discrimination ratios estimated for blocks one and four. The actual responses by this animal during these blocks were too few to yield a meaningful discrimination ratio.

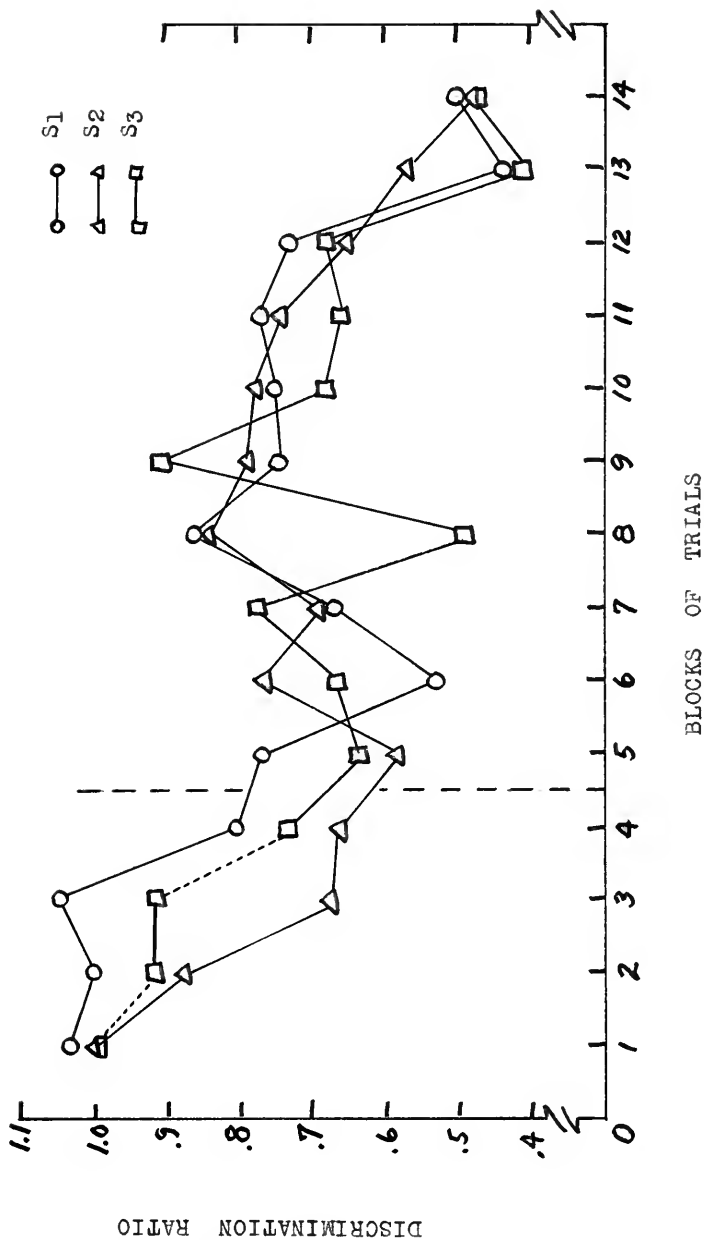


Fig. 1. Discrimination ratios as a function of blocks of trials.
 Note: Vertical broken line separates initial four blocks of trials from remaining trials. This point represents a procedural change.

CHAPTER V
DISCUSSION

Even though the Ss in this experiment failed to reach a typical discrimination learning criterion, the results obtained from the discrimination ratio curves would indicate that a considerable amount of learning had taken place. At the beginning of the experiment, all subjects were responding virtually equally to the S^D and S^A , having discrimination ratios of approximately 1.0. At the termination of the experiment, the discrimination ratios for all animals had reached approximately .5. This finding seems to provide strong support for the fact that a considerable response preference for the S^D over the S^A had been developed.

It is not uncommon to find in the published literature that even "higher" animals require several hundred trials to reach criterion in tasks similar to that used in this study. Of the several levels of animal subjects Bitterman has worked with so far, he cites fish as the least intelligent (Bitterman, 1967). Considering these indications, it seems highly probable that the animals in this study were well on the way to complete mastery of the problem. E is of the opinion that given sufficient time, these animals are capable of learning this sort of discrimination. The reader should also note that the task involved in this investigation was a successive discrimination task, which has consistently proven to be more difficult than one involving simultaneous discriminations of discriminative

stimuli. Furthermore, since there is almost no published research on learning in this and highly similar species, it is possible that such factors as presentation rate, size of stimulus fish, etc. were not optimum.

Given these results, which are highly suggestive of this organism's ability to make this particular discrimination, the visual recognition hypothesis seems even more plausible than before. What is needed to further substantiate individual recognition in fish are more extensive experiments of this sort in which finer and more subtle differences in the discriminative stimuli could be manipulated to determine more precisely the discriminative ability of this and other species of fish.

CHAPTER VI
SUMMARY AND CONCLUSIONS

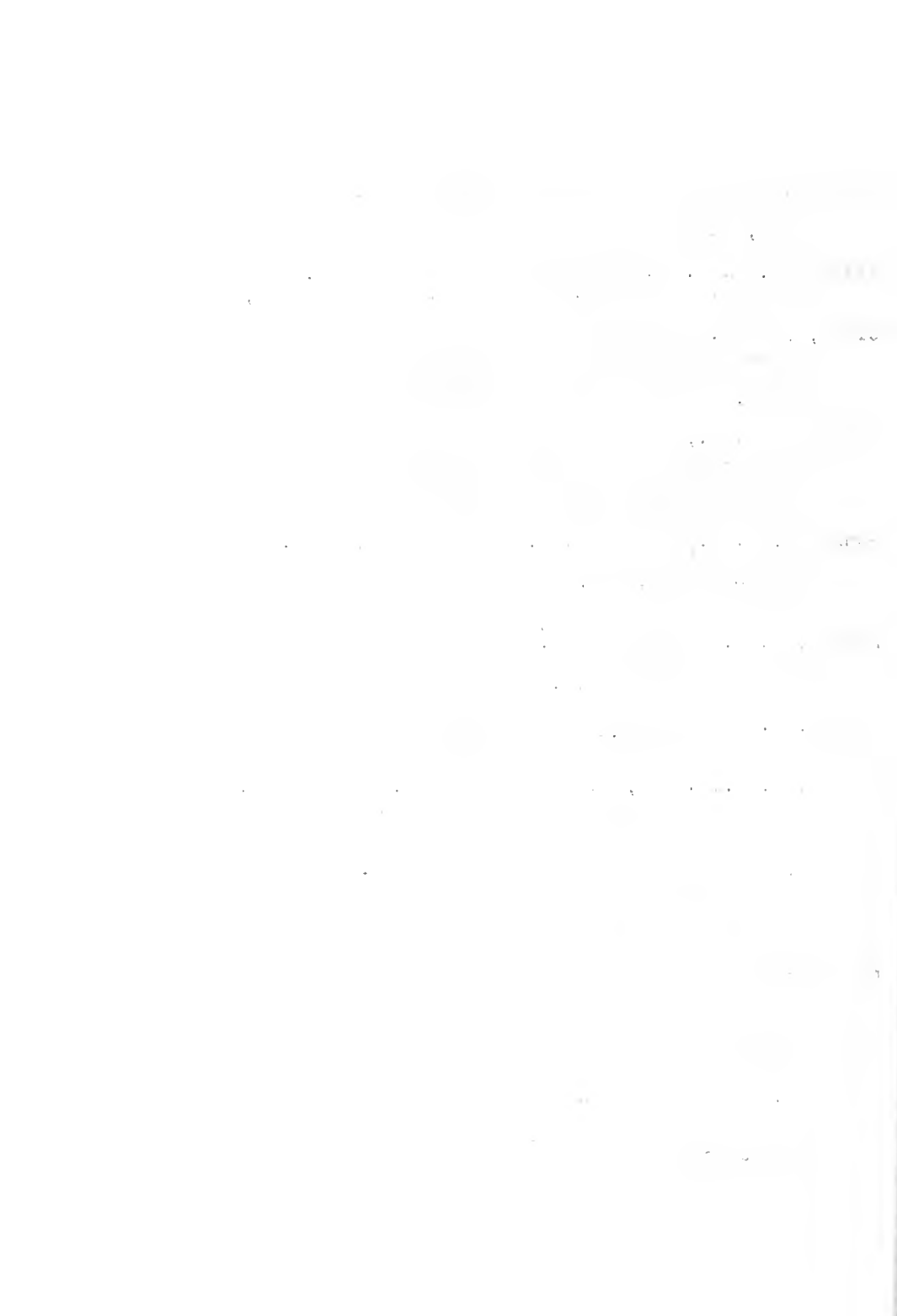
In a study designed to substantiate the assumption of individual recognition frequently occurring in theories of animal social behavior, three bluegill sunfish, Lepomis macrochirus, were operantly trained to strike a submerged manipulandum to obtain food reinforcement. The same animals were then introduced to a discrimination learning task intended to appraise the ability of this species to discriminate among its conspecifics on an individual basis. The two-choice, successive, discrimination task employed back projected color slides of conspecific fish as the discriminanda.

The results were not conclusive in that none of the three subjects had yet attained a typical discrimination learning criterion. However, the data reveal significant trends in the predicted direction. In view of similar studies which often report that very large numbers of trials are required for lower vertebrates to reach standard learning criteria, it seems entirely possible that these subjects have attained some intermediate point on a learning curve which would eventually reach criterion levels.

That these subjects were capable of discriminating between conspecific stimuli as indicated offers plausibility to the individual recognition abilities of bluegill sunfish.

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