VISUAL ASPECTS OF EGG CARE BEHAVIOR IN CICHLASOMA NIGROFASCIATUM (GUNTHER)

Thesis for the Degree of Ph. D.
MICHIGAN STATE UNIVERSITY
PETER G. WEBER
1968

THESIS

This is to certify that the

thesis entitled

VISUAL ASPECTS OF EGG CARE BEHAVIOR

IN <u>CICHLASOMA</u> <u>NIGROFASCIATUM</u>

presented by

Peter G Weber

has been accepted towards fulfillment
of the requirements for

PhD degree in Zoology

Date 6-1

O-169

ABSTRACT

VISUAL ASPECTS OF EGG CARE BEHAVIOR IN *CICHLASOMA NIGROFASCIATUM* (GÜNTHER)

By Peter G. Weber

The egg care behavior of *Cichlasoma nigrofasciatum* (Günther) was studied with regard to the role of the spawn as stimuli. It was postulated that egg size is an important aspect of the spawn. Egg size was manipulated by the use of artificial "eggs" while maintaining all other visual aspects of the spawn as constant as possible. Two sets of experiments were performed. In the first, the artificial "eggs" were exchanged for each female's own, and several parameters of egg care behavior towards these were recorded. Egg care behavior was compared under two experimental and three control conditions. The experimental conditions consisted of presenting larger and smaller "eggs" than the fishes' own. The control groups included a baseline recording of egg care towards their own eggs, a situation with no eggs, and one with artificial "eggs" similar in size to natural eggs. The results of this non-choice experiment showed that "eggs" larger or smaller than the natural were

discriminated, since a considerable decrement in several parameters of fanning occurred when these results were compared either to the baseline or to the "egg" groups similar in size to the natural eggs. The total absence of eggs resulted in the greatest decrement in fanning. Under this circumstance fanning was not completely lost.

A second experiment allowed each female to choose between her own eggs and artificial "eggs" similar to these in size. This was a test for discrimination and/or preference. It was found that females were unable to distinguish simulated from natural "eggs" early in the egg care cycle. At this stage the two types are visually similar. As development proceeded, the two egg types became distinct visually and discrimination was noted in favor of the natural eggs. This was indicated by fanning which was directed more often at these than at the artificial eggs. From the results of these two situations it appears that egg size is indeed one of the factors influencing egg care behavior in _C. nigrofasciatum_.

The presence of simulated "eggs" which did not hatch extended the egg care period appreciably. It was supposed that the inter-spawn interval would also be extended. The results, although they suggested this, were not significant.

Several aspects of the structure of fanning were also recorded and described. These included the possible relationships between tempo and beats, tempo and bouts, intervals preceding and following bouts and the duration of a bout, and beats and bouts. The only correlation established was that between beats and bouts. This was inverse early in the fanning cycle and direct later on. These results are discussed in detail.

The presence of simulated "eggs" when a female was ready to spawn appeared to cause her to deposit her eggs among them. This may be a mechanism restricting the females' choice of egg deposition sites.

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By

Peter G. Weber

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Zoology

1968

ACKNOWLEDGMENTS

For meticulously reading and correcting the manuscript, his encouragement, and most of all, for his patience under fire, I am indebted to Dr. James C. Braddock.

Special thanks go to Dr. H. M. Slatis. He provided useful advice, especially relative to the statistical methods used, as well as critically reading the manuscript and offering suggestions as to its improvement.

Drs. J. A. King, H. Band, and W. D. Collings, members of my committe, read earlier drafts of the manuscript and offered helpful suggestions.

Dr. W. Cooper provided helpful suggestions with regard to the statistics, as well as allowing me the use of the Olivetti-Underwood Programma 101. I thank him for this.

It would be difficult to acknowledge all of the students who have in one way or another contributed to or listened to my ideas during the execution of this project. I must single out Miss S. E. Anderson who listened with patience and made helpful suggestions in reading portions of the manuscript.

Charles Bursey and Alan Bancroft contributed in the photography; Miss Rosalind Price made a number of the drawings.

Finally, I thank Mrs. Bernadette Henderson for her many little favors.

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INTRODUCTION

Parental care in fishes varies from none to rather elaborate nest building and nurturing of the young. All cichlids, as far as is known, undertake parental care to some degree (Breder and Rosen, 1966). This study concerns one aspect of the parental care in *Cichlasoma nigrofasciatum* (Günther).

Reciprocal spawn exchanges between various cichlid species have emphasized the importance of visual, rather than tactile or olfactory stimuli in mediating these fishes' ability to discriminate their own from other species' eggs (Greenberg, 1961, 1963 a, b, Myrberg, 1961, 1964, 1966, and Collins, 1965). Collins (1965) was unable to achieve acceptance of eggs in exchanges between *Tilapia sparrmani* and *Aequidens latifrons*, and he attributed this to the obvious difference in shape, size, and color of the eggs of these species. Collins not only mentioned these differences but also supplied egg measurements of *T. sparrmani*, *A. latifrons*, and *Hemichromis bimaculatus* as part of his evidence (see Figures 1 and 2 of Appendix). From this work, however, it is not possible to determine which precise features of the eggs the fish were discriminating. Kühme (1963,1964 a,b) demonstrated that *H. bimaculatus* parents are able to make fine discriminations between water which had contained their own larvae and free-swimming young and that which had contained those of conspecifics, as well as of other species, e.g., *H. fasciatus*. However, these fish gave no response to water which had contained eggs, and made no discrimination between this and the filtered aquarium water control. Neither olfaction, gustation, or tactile information plays a major role in the egg care behavior of *C. nigrofasciatum* (Mertz, 1967). Vision, on the other hand, is important (Mertz, 1967). All of this evidence suggests that visual stimuli are more important than chemical stimuli in eliciting parental care of cichlid eggs. The precise visual aspects of the spawn which produce parental response were not determined by these studies.

In the substrate spawning cichlids such as *C. nigrofasciatum*, the substrate on which eggs have been deposited, and cared for may conveniently be exchanged for an identical one with dummy eggs. One may then systematically vary egg characteristics such as size, shape, and color. In addition, the density of the eggs in the spawn and the spawn configuration can be manipulated. This technique was adopted for the present study.

Size and shape of the eggs varies from species to species. Color varies among species and within a given spawn as development proceeds. Thus, *C. nigrofasciatum* eggs are light grey and transluscent upon deposition and measure

1.6 mm^2 in sectional area ($L \times W \times \frac{\pi}{4}$) (Figure 7). As they approach hatching, the eggs become a darker grey due to the development of pigments in the embryo. These are especially concentrated in portions of the yolk sac. Overall shape of the spawn (spawn configuration) appears to vary among species and also varies considerably among individuals and for the same individual with successive spawns. *C. nigrofasciatum* deposits an irregularily shaped spawn whose shape cannot be predicted prior to spawning. In contrast, *H. bimaculatus* consistently deposits an oval shaped spawn (Figure 1).

The purpose of this study was to determine the effects of egg size upon egg care behavior in *C. nigrofasciatum*. It was hypothesized that the size of the eggs determines the frequency, duration and rate of fanning, guarding and nipping the eggs (egg care behavior). It was further hypothesized that the size of the eggs could be altered sufficiently to eliminate egg care behavior.

Synopsis of the Reproductive Sequence in Cichlids with Special Reference to *Cichlasoma nigrofasciatum* (Günther)

Cichlids are known for their characteristic reproductive behavior, which has been described for a number of different species (see Review of Literature). Of the papers listed, the most relevant to the work reported here are: Breder (1934), Peters (1941), Aronson (1949), Baerends and

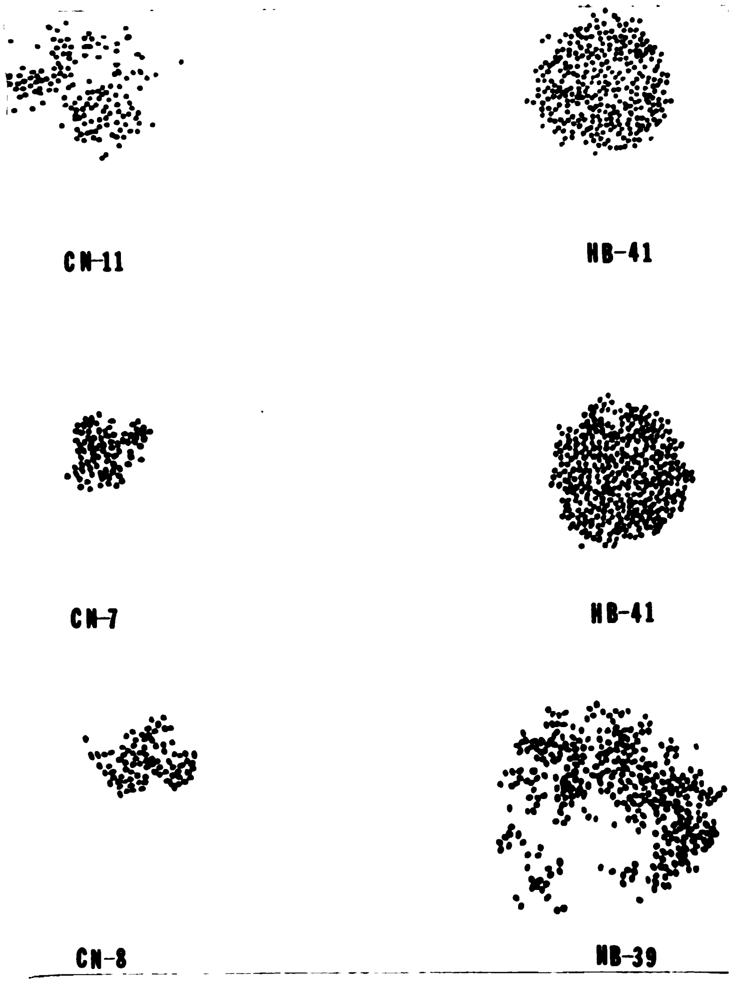
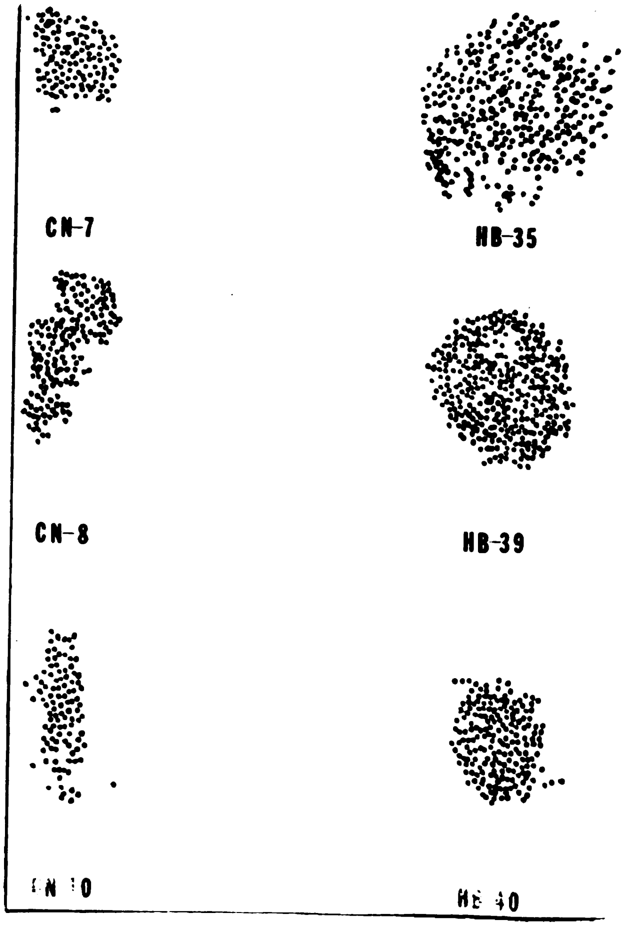


Figure 1. Spawn configuration diagram. Spawns taken from four *C. nigrofasciatum* (CN-) and four *H. bimaculatus* (HB-) females. Illustrates differences in spawn configuration between the two species.



Baerends von Roon (1950), Küenzer (1962), Collins (1965), Greenberg et al. (1965), Myrberg (1965), and Mertz (1967). Cichlid fishes can be classifed as two groups on the basis of their brooding behavior. These are the substrate spawners and the oral incubators (Baerends and Baerends von Roon, 1950). In the latter, one of the parents picks up the eggs either prior to or after fertilization, and then incubates them in the mouth cavity (Wickler, 1962). In certain species the male does this; in others, the female.

The substrate spawning group deposits the adhesive eggs on a surface after which they are cared for by one or both of the parents. The following is a description of the behavior associated with pair formation, spawning, and care of the eggs and young in C. nigrofasciatum. This account is similar to that of other workers, many of whom worked with other species, since the behavior associated with reproduction is qualitatively similar among cichlids (Baerends and Baerends von Roon, 1950).

The cichlid reproductive cycle may be broken down into several phases: pre-spawning or courtship phase, spawning, and post-spawning or parental phase. The latter involves egg care (incubation), wriggler tending, and care of free-swimming young.

Synchronization of reproductive behavior in fishes, as has been demonstrated for most vertebrates, is hormonally as well as neurally controlled (Noble and Kümpf, 1936, Aronson, 1948, Fiedler, 1962, Blüm and Fiedler, 1964, 1965, Wai and Hoar, 1963, and Smith and Hoar, 1967).

Different hormones probably affect the predominant behavioral patterns during various phases of the reproductive cycle. For instance, the fanning and decreased aggression associated with the incubation period is strongly influenced by prolactin (Fiedler, 1962, Blüm and Fiedler, 1964, 1965).

Male and female C. nigrofasciatum form pair bonds. In most species the male initiates this cycle by choosing an area and defending it against other individuals, and these include females and members of certain other species (Collins, 1965, Mertz, 1967). A ripe female may eventually enter the male's territory, persist despite his attacks and finally pair with him. Both members of the pair then jointly defend the territory and prepare for eventual spawning through a period of mutual displaying. The pre-spawning cycle lasted from one to seven days.

In this study, the same pair bond continued for several successive spawning cycles. This was partly a consequence of the same individuals being confined together continuously. This extended pair bond made it possible to use the same pairs throughout the study. How long pair bonds are maintained for extended periods under natural conditions is not known.

During initial encounters between prospective mates, agonistic behavior predominates. This consists of mouth fighting, chasing, butting, and biting at the flanks. This is gradually replaced by courtship displays with their characteristic cichlid behavior patterns such as brief frontal displays involving spreading of the gill covers, head shakes, and repeated rushes at the mate. Lateral displays, which are stiff undulating side-to-side weaving movements with the median fins extended, also occur. Such displays may end with vigorous tail beating. At times, one member of a pair may demonstrate appeasement behavior, which involves a lateral display with the head up and pectoral fins folded. This occurs when an individual is threatened by a more aggressive mate. Certain other behavioral patterns are associated with the pre-spawning period. These include picking up of objects such as gravel, chafing, skimming, jerking, quivering, nipping off, and digging (Greenberg et al., 1965). During the initial courtship phase the female appears in the more dominant role. As the pair approaches spawning condition, these activities increase in frequency. Differences between the sexes are largely of a quantitative nature (Aronson and Holz-Tucker, 1949, Greenberg et al., 1965). An intensification of color accompanies these pre-spawning behavioral changes. This has been noted by numerous workers such as Kramer (1960), Neil (1964), and Collins (1965). In _C_. _nigrofasciatum_ this chiefly involves a heightening of the contrast in the vertically striped pattern, and, in the female, a brightening of an orange plaque on the flanks and of the iridescence in the fins and opercular regions. This lasts until the end of the egg-incubation phase.

A more or less secluded spot is usually selected as a laying site. *C. nigrofasciatum* and *H. bimaculatus*, as observed in our laboratory, prefer an enclosed location such as the underside of a flowerpot, the area under a slate, or a hole in a brick. The behavioral patterns noted were nipping off, skimming, and displaying at the chosen place. At this time the female spent most of her time in cleaning off the prospective site by means of vigorous nipping at the substrate. This preference for a secluded spot made it possible to induce the pairs to spawn in a place selected by the observer.

From a few hours to a day prior to spawning, the members of each sex protrude their genital tubercles, and the frequency of skimming movements at the spawning site increases. Skimming is very similar to fanning, but in the former the frequency of fin beats is higher and the belly and ovipositor are dragged over the substratum. Once a spawning spot has been chosen, the area is cleaned by nipping and skimming. Displaying becomes largely restricted to the area. Immediately prior to and during egg deposition, aggression between the mates decreases. Egg deposition is accompanied by behavior very similar to skimming but involves a smoother gliding movement with the head up and the ovipositor pressed against the substrate. The female makes a run which consists of gliding over the substrate by means of rapid coordinated beats of the pectoral, dorsal, and caudal fins. During this run she deposits a row of five to twenty eggs. The male typically follows close behind, and exhibits similar behavior while emitting sperm over the eggs. Spawning continues for one-half to one and one-half hours. This depends upon the size of the spawn which largely reflects the size of the female (Noble and Curtis, 1939, Collins, 1965). There was no apparent pattern during these experiments as to time of the photoperiod when _C_. _nigrofasciatum_ spawned. In contrast, _H_. _bimaculatus_ demonstrated a propensity to spawn in the late afternoon (3-6 p.m.).

The parental phase of the reproductive cycle begins immediately after egg laying. Although the female is by far the more dominant member, both partners take part in egg care, which primarily consists of fanning, nipping, and guarding the spawn. It is this stage of the reproductive cycle with which this study was concerned.

At the termination of spawning the aggressiveness of the female towards the male increases considerably. She will often display vigorously and attack her mate, especially threatening him with repeated rushes of frontal displays.

During the parental phase individuals of both sexes attacked and displayed frequently to others in the adjacent compartments as well as towards one another. During the incubation period the pair dig pits in the gravel substrate, and the frequency of this behavior increases toward the end of this period (Greenberg et al., 1965). Pit digging, performed by either parent, involves picking up gravel in the mouth and spitting it out away from the area intended to be the pit nest. This behavior continues until a depression is created in the gravel substrate. When the eggs hatch into wrigglers they are immediately picked up by the parents and placed in the previously dug pits or onto cleared areas of the bottom (if no gravel is present). The parents do not continue to fan the wrigglers, as in other cichlid species, but they do occasionally mouth and move them from one pit to another until they become free-swimming larvae. In our laboratory the transition from prolarval wrigglers to free swimmers occurred at approximately four days post-hatching. The free-swimming young are then herded into a dense school and are guarded by the parents until a new spawn is imminent. As they become increasingly more independent, the free-swimming young exhibit a progressive decrease in responses to the parental signals.

In our laboratory the mean interval between spawnings was ten days. This is variable because duration of the egg and wriggler stages are temperature dependent (Peters, 1941, Cridland, 1962, and Greenberg, 1963). In contrast, the transition from free-swimming larva to juvenile stage is partly temperature dependent and is partly the result of feeding rate.

METHODS AND MATERIALS

Stocks

Cichlasoma nigrofasciatum (Günther) is a native Central American substrate-spawning cichlid. It was chosen for this study, in part, because the species was known to be a prolific breeder in captivity, and to be sufficiently unaggressive to permit pair compatibility through a sequence of spawns. In this respect it was a better choice than the more aggressive Hemichromis bimaculatus (Gill), which had been used in earlier pilot studies. C. nigrofasciatum has also been used for studies of parental behavior (Myrberg, 1964, Mertz, 1966, 1967).

The original stock of C. nigrofasciatum was obtained from dealers in Lansing and Ann Arbor. The genetic history of these specimens was not known. This species occurs in two distinct color varieties. One is dark and vertically striped, and is known to dealers in this region as a "convict" or "zebra" cichlid. The other is a white phase sold commercially as a "golden" cichlid. The experimental fish were first generation descendants produced by a series of white male x dark female matings. All of these were dark in color. Presumably the offspring were not as inbred as the parental populations. This was desirable because they might be expected to exhibit behavior more closely approximating that of the wild population. None of the pairs used in the study had ever spawned. They were removed from the stock tanks at sexual maturity. This was judged by size, behavior and (in the case of the females) color. Thirteen individuals were used throughout the study; three of these died and were replaced by other individuals from the stock tanks.*

Experimental Arrangement

Seven 120 liter (76 × 42 × 40 cm.) and two 180 liter (121 × 56 × 34 cm.) rectangular aquaria with slate sides and floors were used for observation tanks. These were divided transversely into compartments by opaque green plexiglass partitions (Figure 2). There were three compartments in the 120 l. and five in the 180 l. tanks. Each compartment held a test pair. A sliding glass door was located at the bottom of each partition. The dimensions of these doors were 10 × 10 cm. This allowed visual contact between pairs. In addition, seven 120 l., two 180 l., and six 60 l., metal-framed aquaria were used as stock and rearing tanks.

The substrate consisted of white aquarium gravel approximately 2-6 cm. in depth. No vegetation was present.

Each test compartment contained a red clay brick (21 × 9 × 6 cm.) with two rows of five holes (approximately

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*In the non choice situation five spawns from each pair were used.

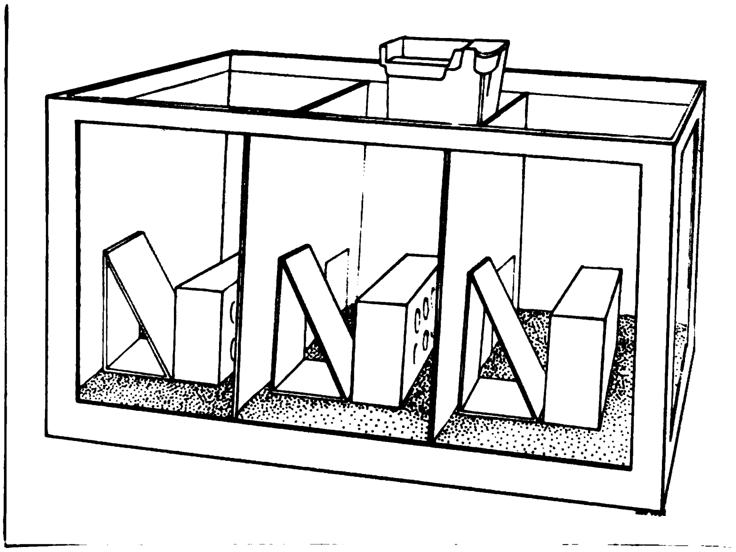

Figure 2. General observational set-up, showing 120 l. tank divided into three compartments, each containing a lean-to supported by a brick.

2 × 2.5 cm.) and a plexiglass lean-to (Figure 2). The lean-to was composed of three pieces of green opaque plexiglass 3 mm. thick. The vertical section and diagonal was 8 × 20 cm., while the horizontal was 8 × 8 cm. The angle between the vertical section and the diagonal was approximately 20-25°. The lean-tos functioned as spawning places, and spawning occurred on any of the three sections. Each of the individual plexiglass sections will henceforth be called a "spawning slate." Eighty-three percent of the spawnings occurred within the lean-tos. Any side of a lean-to could be conveniently replaced either with an identical piece for control purposes or with a section carrying a simulated spawn. The bricks were useful in two ways. First, they prevented collapse of the lean-tos resulting from disturbances caused by display activities inside. Secondly, the holes in the brick served as places of shelter when one pair-mate became overly aggressive. The males were often especially aggressive after the failure or the removal of a spawn. In such instances the females did not always terminate this male aggressiveness by appropriate submissive behavior. In such instances the females could escape, since they were small enough to enter the holes, while the larger males could not.

Each experimental aquarium was supplied with one Le Bern outside gravity flow filter containing glass wool, filter floss, and charcoal. The water intake was located

2-3 cm. above the tank floor. Air to drive the filters and air stones was supplied to the entire laboratory via an air compression unit. Each test compartment contained one air stone.

The water temperature was generally maintained at 27° ± 1 supplementing the heating unit by an air conditioning unit servicing the entire laboratory. Illumination was provided by overhead 40 W fluorescent units supplying the laboratory. The light source for the stock tanks consisted of 15 and 30 W tubes located approximately 60 cm. above each tank. A twelve-hour photoperiod (on and off at eight) was maintained by time switches. Some diffuse light entered through the covered laboratory windows.

Pilot studies were started on 12-10-65, and until 6-8-66 all of the water in the tanks was aged city tap water supplemented by distilled water to maintain the water level. Beginning at the latter date, water in all tanks was replaced with a mixture of distilled water and Utility Seven-Seas marine salt mix (5 g/l).* With this change of medium a definite increase in spawning rate and success of brood rearing occurred. There was also a marked improvement in the general health of the fish and a decrease in the amount of cannibalism by the parents of their own eggs and young.

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*Thanks are due to Dr. G. W. Barlow for suggesting this medium.

No attempt was made to control pH, which ranged from 6-7 as determined periodically with short-range alkacid paper.

Free-swimming young were fed brine shrimp (Artemia) nauplii once per day. Adult fish were fed a mixture developed by Collins (1965). This consisted of ground shrimp, oatmeal, and Wardley's Supremix (mixed approximately 1 : 1 : 1) frozen into patty form. Dried commercial preparations (e.g., TetraMin) were given as supplemental food. Test pairs from which spawns were needed were fed primarily ground shrimp or frozen brine shrimp. All fish were fed once per day between 11 a.m. and 1 p.m.

Pairing of test fish was accomplished by removing apparently healthy males and females from the stock tanks and placing them together in a closed compartment of an experimental tank. C. nigrofasciatum is markedly dimorphic. The females are characterized by an orange coloration of the flanks, blue in the head region (e.g., operculum, mouth), and iridescent turquoise blue or green on the anal, dorsal, and pelvic fins. The males which are larger, lack this coloration and have a larger, more pointed dorsal fin. Pairs usually spawned within a week after being placed together. Attempts were always made to assemble pairs where the male was slightly larger than the female by approximately 25% since this size difference was more likely to insure breeding success (Barlow, 1962, 1965).

Recording and Experimental Procedure

Spawn Exchange: The Non-Choice Situation

Thirteen pairs were used in the course of the study. Pilot investigations indicated that the males usually did not contribute significantly to spawn care, but could be a factor in increasing variability in the recordings. Therefore, males were removed by netting after spawning and were reintroduced when the eggs hatched and observations were terminated. For each pairing it was determined by a coin flip whether the spawn was to be exchanged for a control or experimental (simulated spawn) slate or used for baseline observations. That short (10-15 minute) observation periods, taken at various times of the photoperiod, accurately represent the real changes in temporal organization of fanning during the course of the egg-care cycle in _C_. _nigrofasciatum_, has been demonstrated by Mertz (1967). On the basis of this, as well as preliminary studies, it was felt that recordings taken for ten minute observation periods three times per day would yield reasonably accurate data. These occurred at 9 a.m., 12 noon and 3 p.m.. Each started one-half to one hour after completion of spawning after which interval it was certain that spawning behavior had ceased. Where exchanges were made, the parents were first observed for a ten minute observation period to insure that they were performing within limits comparable to those of the baseline group. The exchanges were then made as follows. The slate containing the pair's own eggs was removed and replaced either with an identical slate with no eggs ("control") or with an identical slate containing a simulated spawn (experimental). One-half hour after each such exchange, the first subsequent observation was taken and was followed by others as outlined above. These recordings were terminated for each pair when all eggs hatched. In the *case* of pairs whose eggs had been removed, the time of hatching was established by rearing the spawns in one liter cylindrical battery jars in which the parental water was agitated by means of an air stone. When these "artificially incubated" eggs hatched into prolarvae, recording of the parental behavior was terminated. In the cases of the control pairs parental behavior generally ceased before the artificially incubated eggs hatched. In spawn exchanges involving simulated eggs, which obviously did not hatch, fanning often continued beyond the time when the "artificially incubated" eggs hatched. In such instances recording was continued for fifteen days, which exceeded the average interval between spawns.

Spawn Exchange: The Choice Situation

One-half to one hour after the completion of spawning the male was removed, and fifteen minutes to one-half hour later the spawn was also removed. The latter was duplicated "artificially" which generally took from 20-45 minutes depending upon the size of the spawn and then both the simulated and the pair's own natural spawn were returned. The simulated "eggs" were placed where the actual eggs had been deposited, and the slate with the natural eggs was randomly placed in one of the two remaining positions under the lean-to (Table 12). The same recording procedures, schedule, and length of observation periods, as well as the same individuals were used as in the previous experiment. In this situation, however, fans toward the natural and simulated eggs were simultaneously recorded on two different channels of the event recorder.

Apparatus

The initial recordings were made with a Gerbrand six-channel multiple-event recorder (Figure 3). The recorder pens were activated by means of a manual keyboard; the chart speed was 2 mm/sec. The majority of the recordings were made with a Rustrack four-channel multiple-event recorder (model 92) at a chart speed of 2 mm/sec. For the non-spawn control group, measurements were begun the day after the wrigglers became free-swimming. Young were always removed from the parents. In the cases where no exchanges were made, this was done on the day they became free-swimming.

Representative records of fanning (top channel), departures (middle channel), and nips (bottom channel) for female No. 37 which had its own spawn removed in exchange for a blank "control" slate. Recordings were made with a Gerbrand multiple channel event recorder at a chart speed of 2 mm ps. The numbers below a bout of fanning give the number of beats (top) and duration (bottom).

A. First 10 minute recording, taken 1/2 hour after termination of spawn deposition. This is a baseline recording of behavior toward own spawn (7-23-67; 10:15 a.m.; 10' record: 957 beats/12 bouts/449 duration/2.131 tempo; 0 nips).

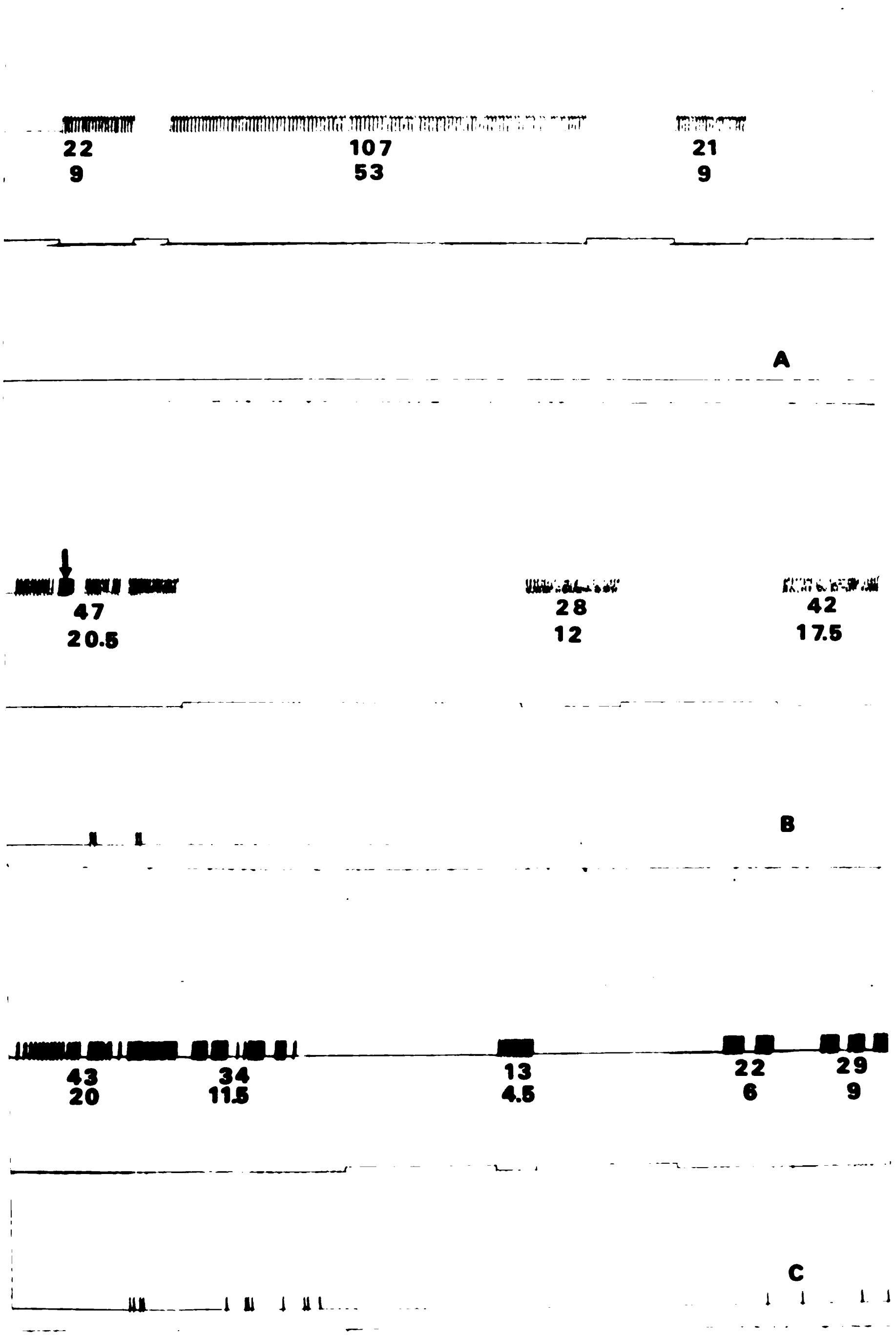
B. Measurements taken at 24 hours after first recording above; this shows behavior recorded toward blank "control" slate (arrow indicates incipient "displacement fanning") (7-24-67; 9:45 a.m.; 10' record: 534 beats/18 bouts/239 duration/2.234 tempo; 22 nips).

C. Measurements taken at 72 hours after initial recordings above; of behavior towards control slate. Most of the fanning bouts shown are "displacement fanning" and only the left end of the recording shows any "normal" fanning. Displacement nipping does not show up on the recording as does the displacement fanning (7-25-67; 9:45 a.m.; 10' record: 619 beats/20 bouts/202 duration/3.064 tempo; 41 nips).

Figure 3. A portion of the fanning record for female 37.

Legend: tempo: fanning beats/duration departures: the frequency with which the female leaves the lean-to; not used in the analysis of egg care behavior.

See pages 25, 27, and 28 for detailed explanation of the behavioral terms used.


22
9
107
53
21
9
A
47
20.5
28
12
42
17.5
B
43
20
34
11.5
13
4.5
22
6
29
9
C

It was noted in certain pilot studies that both H. bimaculatus and C. nigrofasciatum would tend rather unrealistic spawns of Indian beads for periods as long as 24 hours. Where "artificial" spawns were constructed of glass beads, however, these proved to be ineffective as egg-substitutes for more than one day probably because of the reflectance and refractance of glass, as well as lack of appropriate texture and color. A technique was then developed for making simulated cichlid "eggs." This consisted of dipping a glass rod, which had been drawn into a point, into melted paraffin and allowing the paraffin to drop off the point. This formed a ball. Size could be controlled by the use of rods drawn into variously sized points, as well as by controlling the amount of paraffin that collected on the point before dropping off. Shape could be controlled by varying the shape of the needle point and by manipulating the rod as the paraffin dropped off. Color similarity to that of actual eggs was achieved by addition of powdered charcoal to the melted paraffin. The paraffin used was commercial "Parowax" (Standard Oil Co.).

As soon as a pair finished spawning, a quick sketch was made indicating the position of the eggs on the plastic slate and the general spawn configuration. This was used as a model in constructing the simulated spawn which was made to duplicate the real spawn as closely as possible.

Experimental Design

The following is a description of the independent variables. There were two experimental groups as follows:

1. Larger than natural eggs: this involved the exchange of a female's own spawn for a simulated spawn similar in all aspects except that the "eggs" were larger than the "Own" ($\bar{\bar{x}}$ sectional area = 7.20 mm^2).

2. Smaller than natural eggs: similar to the above group except the exchanged "eggs" were smaller ($\bar{\bar{x}}$ sectional area = 0.733 mm^2).

The control groups were as follows:

1. Same size as natural eggs: this, again, was similar to the above except that the own were exchanged for simulated "eggs" as close as possible to the female's own in size.

2. Baseline: this involved the female's own spawn. No exchanges were made.

3. Blank slate: in this group the female's own spawn was exchanged for an identical slate without real or simulated eggs.

Dependent variables: various parental motor acts were quantified. For the most part, these were established by other workers (van Iersel, 1953, Morris, 1955, 1958, Sevester, 1961, Barlow, 1965, Mertz and Barlow, 1966, Mertz, 1967) and are merely reviewed here. In comparing parental behavior under different spawn conditions as well as "control" conditions, aspects of three behavior patterns were analyzed:

1. Fanning is a motor act associated with care of eggs and larvae in nearly all substrate spawning cichlids. This behavior is very similar to swimming but lacks forward movement. The caudad movement of the pectoral fins is counteracted by movement of the caudal and dorsal fins The beginning and end of fanning were at times difficult to determine. Fanning was occasionally confused with swimming or hovering to which it was similar. The latter two, however, were not directed at the spawn. In practice, such movements were labelled fanning only when oriented toward the spawn, and these judgements were sometimes subjective. Positioning of the parent with respect to the spawn was generally as indicated in Figure 4, although virtually any orientation of close proximity to the clutch was adopted.

2. A beat was defined as a complete undulatory cycle of the pectoral fins. These alternated in their movement, but in recording fanning only the movement of one fin was counted. Occasionally a fish would place itself in a position such that movement of the pectoral fins was difficult to observe; and, since the undulations of the caudal and dorsal fins are in rhythm with those of the pectorals, their movements were counted instead. Actually, the overall picture of a parent fanning suggests a pumping movement

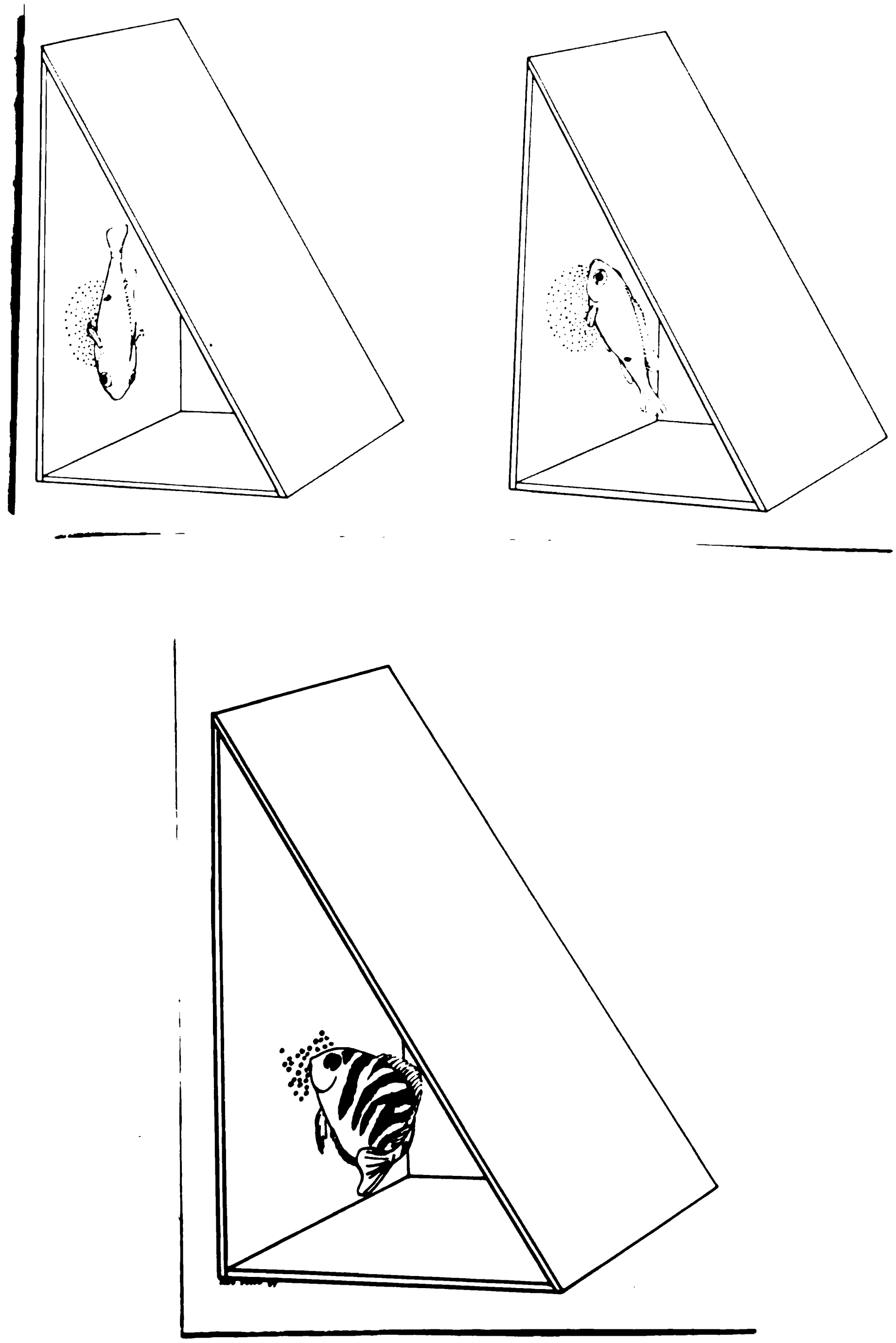


Figure 4. The more common positions assumed under the lean-to during fanning of the eggs. Upper drawing is of *Hemichromis*, lower is of *C. nigrofasciatum*.

with the motion of the entire body reflecting the tempo of fanning.

2. The digital fanning tended to occur in distinct units which were termed bouts. On the basis of preliminary studies, and the work of others (Barlow, 1964, Mertz and Barlow, 1966, and Mertz, 1967) a bout was considered as a group of beats separated from the preceding and succeeding groups by intervals of at least two seconds.

Six parameters of fanning were either measured or derived:

Measured

a. Bouts: the number of fanning bouts per ten minutes.

b. Beats: the number of fanning beats observed per ten minute observation.

c. Fanning duration: the total time (in seconds) spent fanning per ten minute observation period. This was obtained by summing individual times for all bouts and was measured to the nearest half-second.

Derived

a. Tempo: the average number of beats per second for all fanning per ten minute observation period. This measurement was obtained by dividing the number of beats by fanning duration.

b. Beats per bout: the average number of beats per bout for all fanning during ten minutes. This was obtained by dividing beats by the number of bouts.

c. Bout duration: the average duration in seconds of all bouts within the ten minute observation period. This was obtained by dividing fanning duration by the number of bouts.

In addition to the various aspects of fanning, the frequencies of two other behavior patterns were recorded:

1. Nips: throughout the duration of egg care, the parents nip at the eggs and eat moldy-looking, and occasionally, healthy eggs. All the nips directed at, or within 1-2 cm. on either side of the spawn were counted.

2. Departures: were the number of times a fish left the lean-to area during the ten minute observation period.

Records were also kept of the time and place of all spawns, the time of hatching, and the time the wrigglers became free-swimming larvae.

Spawn-Position Preference

The locations where successive spawns were deposited were recorded. When simulated "eggs" were allowed to remain with the pair until the next spawn, the pair often chose to spawn among or near these "eggs." Figure 5 shows the location of the natural eggs with respect to the simulated eggs on the spawning slate. These diagrams were made after

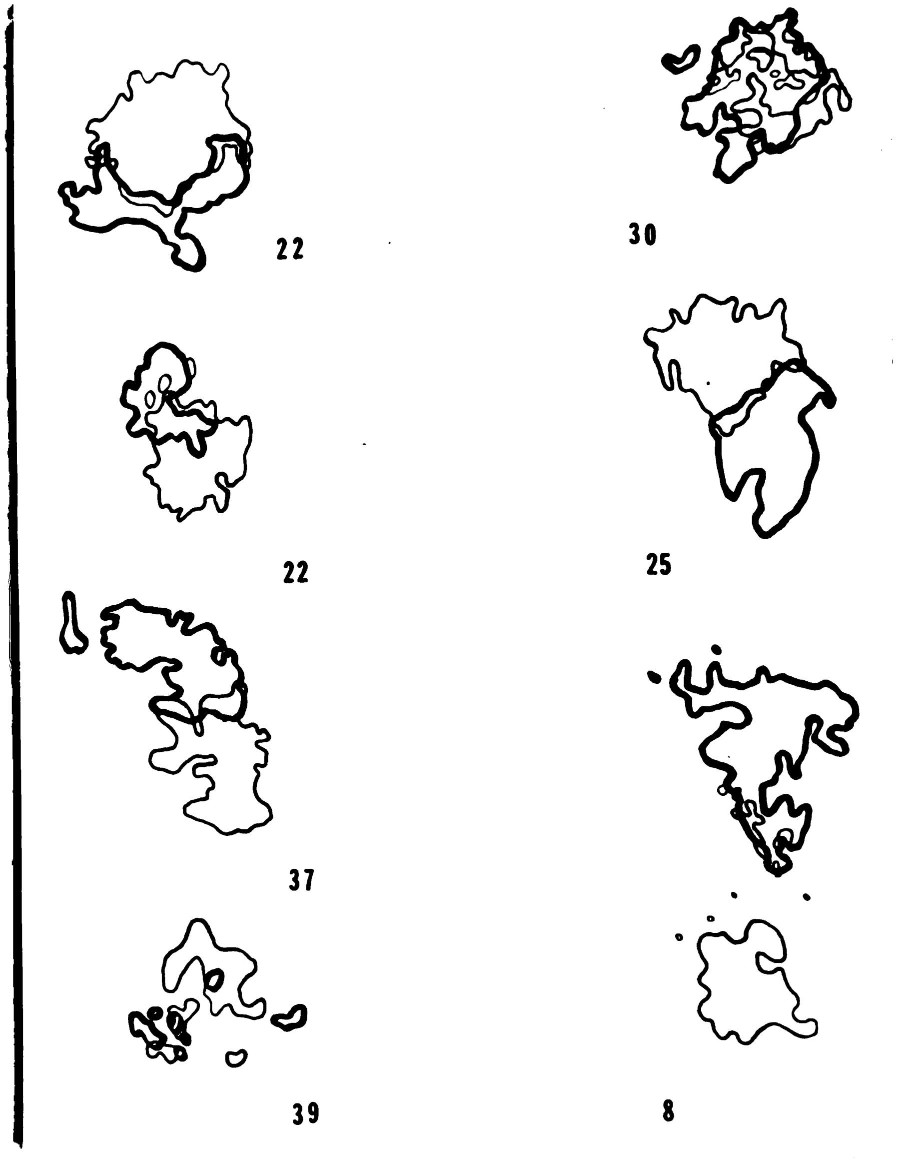


Figure 5. Position of "own" spawn (thin line) in relation to location of the already present simulated spawn (heavy line). Spawns from several different individuals are represented. The number alongside the spawn diagram is the pair number.

egg-hatching when the spawning slates had been removed from the tanks. The scars left by the natural eggs were filled in with India ink and their outlines, as well as those of the simulated eggs were then traced.

Statistical Analysis

Wherever feasible a t-test or an analysis of variance was performed. The results of both the choice and non-choice situations fall into a time sequence that does not lend itself to an overall statistical analysis. However, the standard error was calculated for each point in time, and graphs (e.g., Figures 9 to 12) have been drawn showing the means and one standard error on each side of each mean. Where two series of observations were so divergent that their standard errors did not overlap, it is assumed that they represented statistically significant differences.

RESULTS

The main portion of the results is concerned with the effects of presenting various sized "eggs" on egg care behavior. The relationship between egg care and the egg stimulus was investigated by two techniques: (1) a non-choice situation where the real eggs were removed and simulated "eggs" put in their place, and (2) a choice situation where real eggs and simulated "eggs" were presented simultaneously. Most of the data presented here concerns the former condition, since this did not involve the confounding effect of real eggs visually changing with time; the purpose of the choice situation was to test for discrimination and/or preference.

The Non-choice Situation.--The exchange of natural eggs for simulated "eggs" of the same size resulted in no appreciable change from the baseline in the fanning parameters measured; exchange for "eggs" either larger or smaller in size than the natural resulted in a decrease in all of the fanning parameters; removal of the eggs altogether resulted in the most rapid decrement of fanning but not in its total extinction.

The Choice Situation.--Simultaneous presentation of natural eggs and simulated "eggs" of the same size resulted in no preference between the two during the very early periods of the egg care cycle. As development proceeded, the natural eggs become visually distinct from the simulated ones. The female at this time discriminated in favor of the natural eggs by fanning more at these than at the simulated "eggs."

The presentation of simulated "eggs" of various sizes did not appreciably alter the temporal organization fanning. The trends in this organization in the exchange groups, as well as the baseline, confirmed those found by Mertz (1967): Changes in number of beats and duration corresponded closely; also, changes in beats per bout duration paralleled one another. In all groups these four measures showed high values during the early period (first twelve hours), and declined to a lower level which was maintained until just before hatching. At hatching there was again a decline, and finally, cessation. Tempo differed from all of the other fanning parameters in its relative stability during the entire fanning phase. It showed neither the early decline or the rapid decrement at hatching.

Parameters Relevant to Spawning

The three sections which follow are intended to present background data relevant to the egg exchange experiments.

Choice of Spawn Site

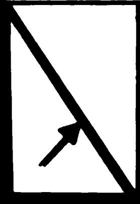
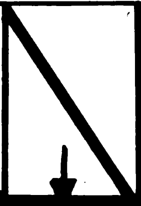
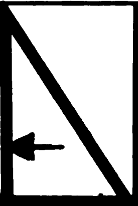
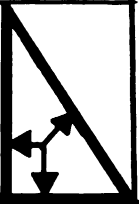
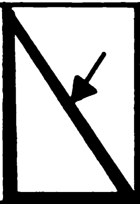
Table 1 shows where the females chose to spawn during the course of the study and the preceeding pilot study. Eighty-three percent deposited their eggs under the lean-to and this indicates its effectiveness as a spawning site.

Egg Measurements

Simulated "eggs" used in the exchanges were made by hand as previously described. Their sizes and shapes were visually determined. It was necessary to make them rapidly, since egg exchanges were made within a few hours of spawn deposition, and irregular configurations had to be duplicated. Because of this time factor the artificial "eggs" could not be made to resemble a fish's own in size, shape, and homogeneity as closely as would have been desired. Subsequently, an analysis was made to describe accurately the size (area) and shape (shape index) of both the real and simulated "eggs."

Length and width were measured to the nearest 0.5 mm. with an ocular micrometer (Collins, 1965). Six samples, each consisting of eggs taken from one pair, or artificial "eggs" from one simulated spawn, were used. Thirty eggs were measured in each sample as observed from the top. These were chosen by haphazardly moving the spawning slate to which they were attached. The egg to be measured was

Table 1.--Choice of Spawn Site

	Inside the Lean-to				Total	Outside the Lean-to								Total
Spawn Location						Brick		Divider	Slate Sides		Glass Sides	Divid- er Door	Slate Bottom	
Number of Spawnings (N)	30	27	26	6	89	5	4	4	2	2	1	0	0	18
Percent	28.0	25.2	24.3	5.6	83.0	4.7	3.7	3.7	1.9	1.9	0.9	0.0	0.0	17.0

the one closest to the micrometer. From the length and width measurements, sectional area and shape index (Width/Length) could be calculated. The sectional area of each egg was computed as the area of an oval (Length × Width × η/4). The latter was an indication of deviation from roundness.

Shape Index.--An hierarchial Analysis of Variance was performed to determine homogeneity among egg types. Table 2 gives a summary of this analysis. Figure 6 shows that the simulated eggs approximated roundness ($\bar{\bar{X}} = 0.901$, 0.937, and 0.947) much more closely than did the fishes' own eggs ($\bar{\bar{X}} = 0.721$). As is also evident from Table 2, there was a highly significant difference among egg types *per se* ($p << 0.005$) and samples ($p << 0.005$). The various simulated "egg" types were homogeneous with respect to shape index ($p > 0.05$). The variability within samples also indicated significant heterogeneity ($p < 0.005$).

Sectional Area (Length × Width × η/4).--Again, an hierarchial Analysis of Variance was performed and both samples ($p << 0.005$) and egg types ($p << 0.005$) were signigicantly heterogeneous. Table 3 presents the results of this analysis. Significant differences among both samples and egg types are quite evident from Figure 7, since larger artificial "eggs" have a much greater sectional area ($\bar{\bar{X}} = 7.206$ mm.^{2}) than the similar to natural "eggs." The analysis was applied to demonstrate

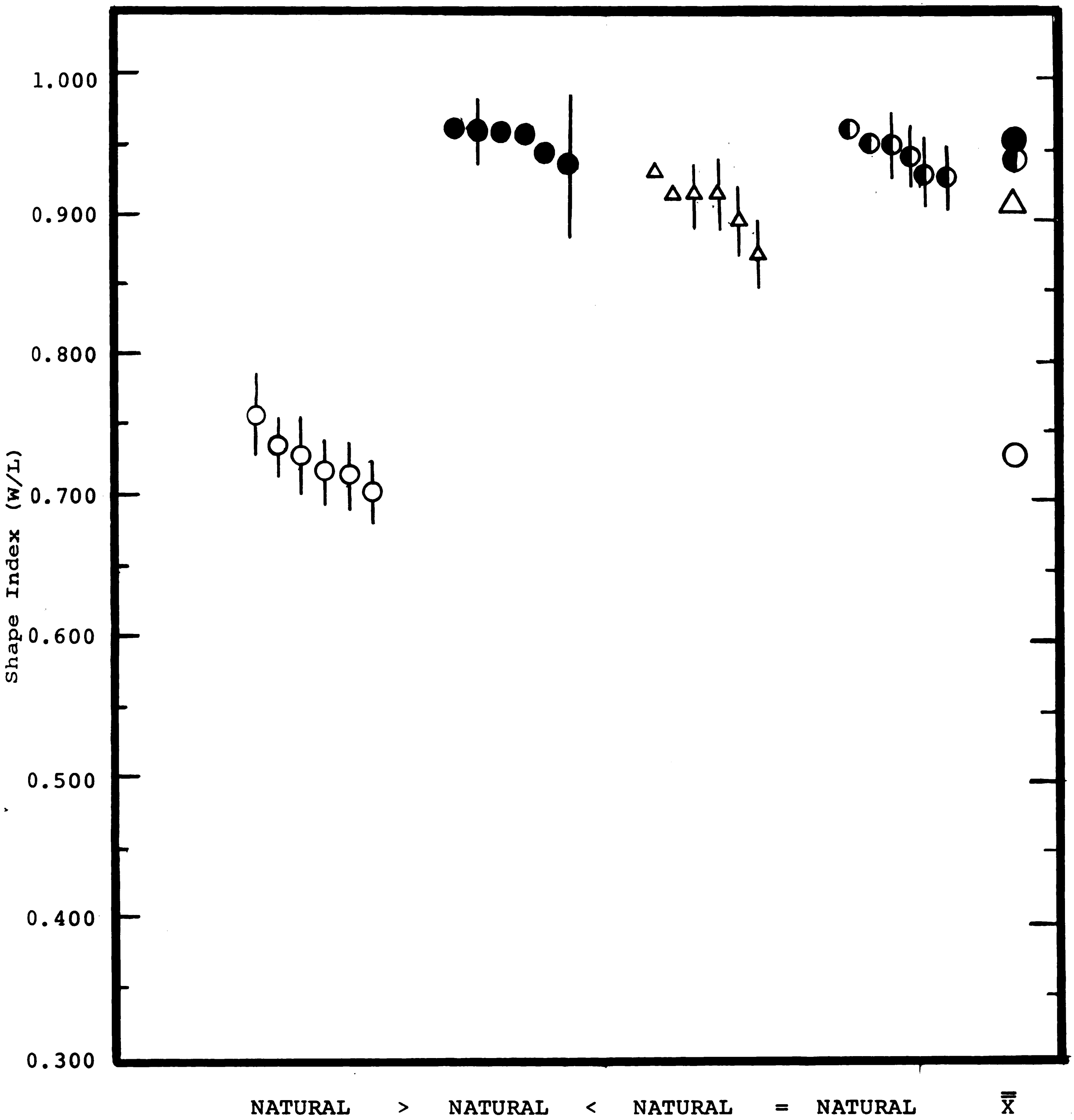


Figure 6.--The mean shape index of natural eggs, and of simulated "eggs" of the same size, smaller, and larger than natural eggs. Vertical bars indicate two standard errors. The grand mean for each egg category is given on the right side of the figure.

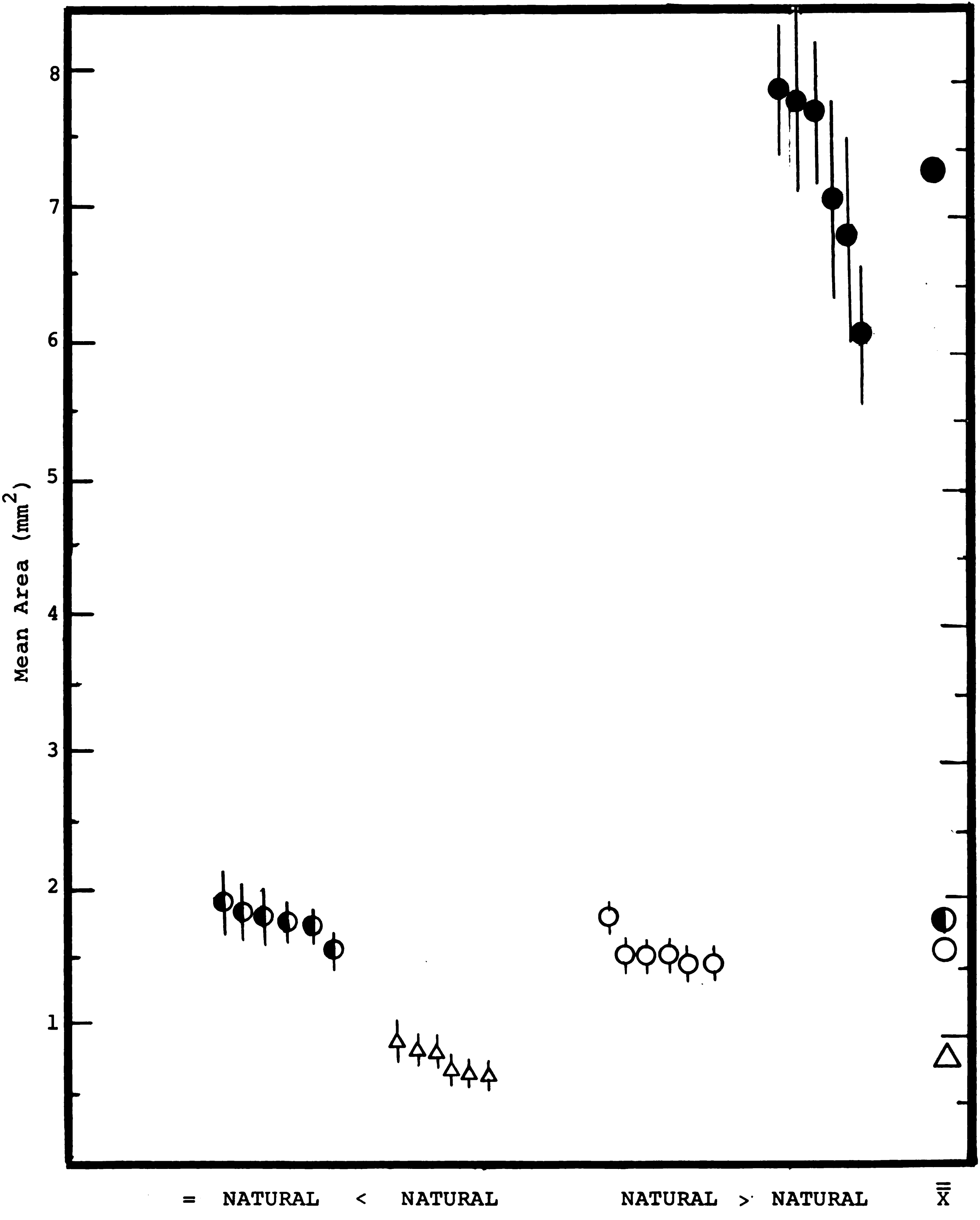


Figure 7.--The mean sectional area (mm^2) of natural eggs, and of simulated "eggs" of the same size, smaller, and larger than natural eggs. Vertical bars indicate two standard errors. The grand mean for each egg category is given on the right side of the figure.

Table 2.--The results of an hierarchial Analysis of Variance of shape index for natural eggs, and simulated "eggs" of the same size, smaller, and larger than natural.

Shape Index

Natural vs. Similar to Natural vs. Smaller vs. Larger

Source	SS.	MS.	df.	F	p
Egg Type	6.038	2.013	3/20	80.520	$<<$ 0.005
Samples	0.498	0.025	20/696	6.250	$<<$ 0.005

Similar to Natural vs. Larger vs. Smaller

Source	SS.	MS.	df.	F	p
Egg Type	0.200	0.100	2/15	1.176	$>$ 0.050
Samples	0.128	0.085	15/522	18.889	$<<$ 0.005

heterogeneity among all of the eggs except those which were larger than the natural ones. Figure 7 shows that "eggs" smaller than the natural ones were significantly smaller ($\bar{\bar{X}}$ = 0.733 mm.^{2}); the Analysis of Variance between these eggs (natural, smaller than natural, and equal to natural) showed a high significance for heterogeneity among egg types ($p << 0.005$) and samples ($p << 0.005$). An Analysis of Variance was also done for natural vs. similar to natural "eggs;" and, although these were roughly the same in sectional area (natural $\bar{\bar{X}}$ = 1.555 mm.^{2}, similar to natural $\bar{\bar{X}}$ = 1.766 mm.^{2}), both egg types ($p < 0.025$) and samples $p << 0.005$ showed significant heterogeneity.

Table 3.--The results of an hierarchial Analysis of Variance of sectional egg area (mm.^{2}) for natural eggs, and simulated "eggs" of the same size, smaller, and larger than natural.

Sectional Area

Natural vs. Similar to Natural vs. Smaller vs. Larger

Source	SS.	MS.	df.	F	p
Egg Type	4733.92	1578	0/20	39.450	<< 0.005
Samples	79.79	40	20/696	52.840	<< 0.005

Natural vs. Similar to Natural vs. Smaller

Source	SS.	MS.	df.	F	p
Egg Type	107.121	53.561	2/15	123.129	<< 0.005
Samples	6.530	0.435	15/22	4.860	<< 0.005

Natural vs. Similar to Natural

Source	SS.	MS.	df.	F	p
Egg Type	4.001	4.001	1/10	8.951	<< 0.025
Samples	4.471	0.447	10/348	4.382	<< 0.005

Spawn Hatch Time: Parentally Attended vs. Artificially Raised Eggs

Hatching appears to be a critical event bringing about change in parental behavior. Whenever a spawn exchange was undertaken (i.e., own spawn exchanged for a simulated spawn or blank slate), the real spawn was placed within a beaker of water from the parents' tank which was agitated with an air stone. The clutch was thus raised without the parents. Time to hatching was recorded for spawns with and without parental care (artificially raised). This was begun when most of the eggs had been deposited on the slate and was terminated when the majority had hatched into wrigglers. The time when fifty percent had hatched was compared between parentally reared and artificially raised eggs (Table 4). A two-tailed t-test indicated that there was no significant difference between the means of the two rearing conditions ($t = 1.784$, 64 df., $p > 0.05$). Values from the two conditions were then combined, and the cumulative percent hatch calculated. A cumulative percent hatch curve is plotted along with the egg care parameters in Figures 9-12.

The mean hatching time for all eggs was 69 hours. The variability was great in that the earliest time of hatching was 39, the latest, 93 hours.

Table 4.--Basic statistics for the time to hatch for parentally cared vs. artificially cared spawns; x = time (hours) when most (one-half) of the spawn hatched.

	xf	x^2f	S^2x	Sx	SE	$\overline{X}$	N
Parentally Attended Eggs	1710.00	123786.00	84.717	3.530	1.879	71.250	24
Artificially Raised Eggs	2793.00	190719.00	121.573	2.895	1.701	66.500	42

The Effects of Egg Size Upon Egg Care Behavior

Duration of Egg Care.--Egg care was considered terminated when the female no longer fanned the eggs. This behavior ceased at different times depending upon the spawn condition presented to the female (Figure 8).

Females presented with a blank slate were the first to terminate egg care ($\bar{X}$ = 52 days). The baseline group was second ($\bar{X} \cong 69$ days). Egg care ceased earlier when females were presented with artificial "eggs" larger than the natural ($\bar{X} \cong 82$ days) than when given smaller than natural ones ($\bar{X} \cong 124$ days) in exchange for their own. Finally, the group which persisted the longest, in terms of when the first and last individuals stopped fanning was the one given natural sized "eggs" ($\bar{X} \cong 163$ days). Table 5 gives the basic statistical data.

Table 5.--Basic statistics for the duration of egg care for the five groups; x = the time (hours) when a female ceased fanning.

	Baseline	Blank Slate "Control"	= Natural	< Natural	> Natural
Σxf	901	676	2121	1615	1072
$\Sigma x^2 f$	63185	38612	353715	208025	103106
$\bar{x}$	69.308	52.000	163.154	124.231	82.462
$s_x{}^2$	61.564	288.333	638.808	616.026	1225.603
s_x	4.736	22.179	49.139	47.387	94.277
SE	2.176	4.710	7.010	6.884	9.710
N	13	13	13	13	13

Figure 8.--The cumulative number of females which stopped fanning per duration of egg care (in hours). Horizontal lines are one standard error on either side of the mean.

Legend:

○ = baseline group

▲ = blank slate "control" group

● = > natural "egg" group

◐ = natural "egg" group

△ = < natural "egg" group

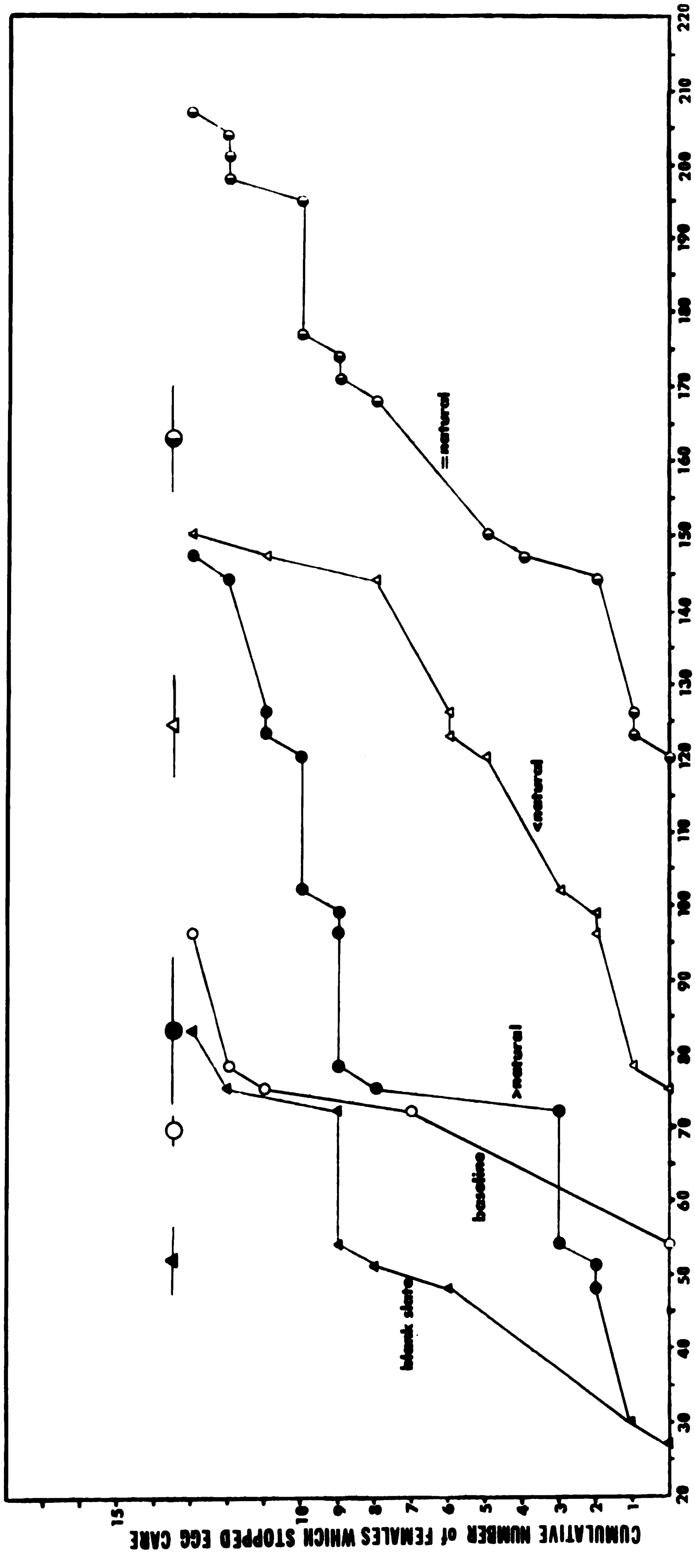

CUMULATIVE NUMBER of FEMALES WHICH STOPPED EGG CARE
DURATION of EGG CARE (hours)
1
2
3
4
5
6
7
8
9
10
15
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
210
220
blank slate
baseline
>natural
<natural
=natural

Figure 8 shows egg care duration within each group. Curves for both blank slate and baseline were comparatively steep, indicating that the majority of females stopped fanning within a short time period. Curves for the simulated condition were less steep, showing that the individuals exposed to these "eggs" terminated egg care over a longer period of time.

A one-way Analysis of Variance of the egg care duration means showed that these were significantly heterogeneous ($p < 0.005$, Table 6).

Table 6.--The results of a one-way Analysis of Variance of the egg care duration means under the baseline and four exchange conditions.

Source	SS.	MS.	df.	F	p
Main Effect	33964	8491	4	4.911	< 0.005
Deviations	105476	1729	61		
Total	139440		65		

This analysis was further extended by means of a series of Tukey T-tests to determine which groups differed from one another (Scheffé, 1959). The results are presented in Table 7.

Significant differences (at the 0.05 and 0.01 levels) in the duration of egg care were found between the baseline, similar to natural, and smaller than natural

Table 7.--The results of Tukey's T-test, testing all possible pairs of means of the duration of egg care.

	Blank Slate "Control"	= Natural	< Natural	> Natural
Baseline	1.060 N.S.	5.750**	3.020*	0.833 N.S.
Blank Slate "Control"		6.815**	4.430**	1.870 N.S.
= Natural			2.390 N.S.	4.950**
> Natural				1.950 N.S.

N.S. denotes No Significance

*denotes $p < 0.05$

**denotes $p << 0.01$

groups; between the blank slate"control," similar to natural, and smaller than natural groups and between the similar to natural and larger than natural groups.

Egg care Measurements: Non-choice Situation

Fanning, by virtue of its predominance in egg care, is here considered to be the most important aspect of clutch-oriented behavior. The sections to follow present the effect different egg sizes had upon the various facets of fanning.

The Number of Fanning Beats.--The mean numbers of beats per 10 minute observation period did not differ significantly in magnitude, and showed essentially the same temporal organization whether females were presented with their own eggs or artificial "eggs" similar in size. Figure 9 shows the temporal organization in the baseline and natural "egg" groups. This involves a high magnitude of beats early, a leveling off at about six hours, and then a dorp at hatching. The two groups were similar up to the time of hatching, at which time differences occurred due to the rapid decrement in the baseline beats. Fanning was maintained, though at a gradually decreasing level in the simulated "eggs," which did not hatch. In the baseline group the rapid drop in beats at hatching agrees with what has already been found for this species (Mertz, 1967). It differs from what has been reported for Gaserosteus (van Iersel, 1953 and Sevester, 1961), Cottus (Morris, 1955), or Badis (Barlow, 1964). No such rapid decrement in beats occurred in any of the simulated "egg" groups at hatching time.

The exchange of the females' own eggs for either a blank slate or a slate with large "eggs" resulted in the most rapid fanning decrement. There were no significant differences between these two groups at any of the points measured. The temporal pattern of fanning was, nevertheless, maintained in both. It is possible that in the period immediately prior to hatching fanning towards the natural eggs may be less than towards simulated "eggs" of appropriate size. After three hours the level of fanning in the blank slate groups was significantly lower than in either of the others. It remained low and extinguished early (51 hours).

The introduction of "eggs" smaller than the natural resulted in no significant differences from the baseline condition until approximately half way through the fanning cycle. At this time fanning beats toward the smaller "eggs" decreased to a level similar to that of the blank slate and large "egg" groups. The females maintained levels of fanning toward the small "egg" groups which were similar to the baseline level until about two-thirds through the egg care period. At this time fanning beats gradually began to decline, their magnitude becoming intermediate between the blank slate "control" and baseline groups.

After the time of hatching the three simulated groups still maintained fanning, though at a progressively decreasing level. The similar to natural group was higher than the small or large "egg" groups. The latter two did not differ in beat magnitude from one another, which remained low until the end of the recording period.

Superficially, these data appear to be of the type that would be analyzed by an Analysis of Variance or of Convariance. However, there are several reasons why such analyses are inapplicable to the data. (1) The observations at each point are correlated with those at the other points (e.g., a fish that beats at a high level at

Figure 9.--The mean number of fanning beats per ten minute observation period taken three times per day for all groups. Horizontal lines are one standard error on either side of the mean. Arrow indicates time of spawn exchange; stippled region indicates the ten minute baseline observation prior to spawn exchange.

Legend:

○ ——— baseline

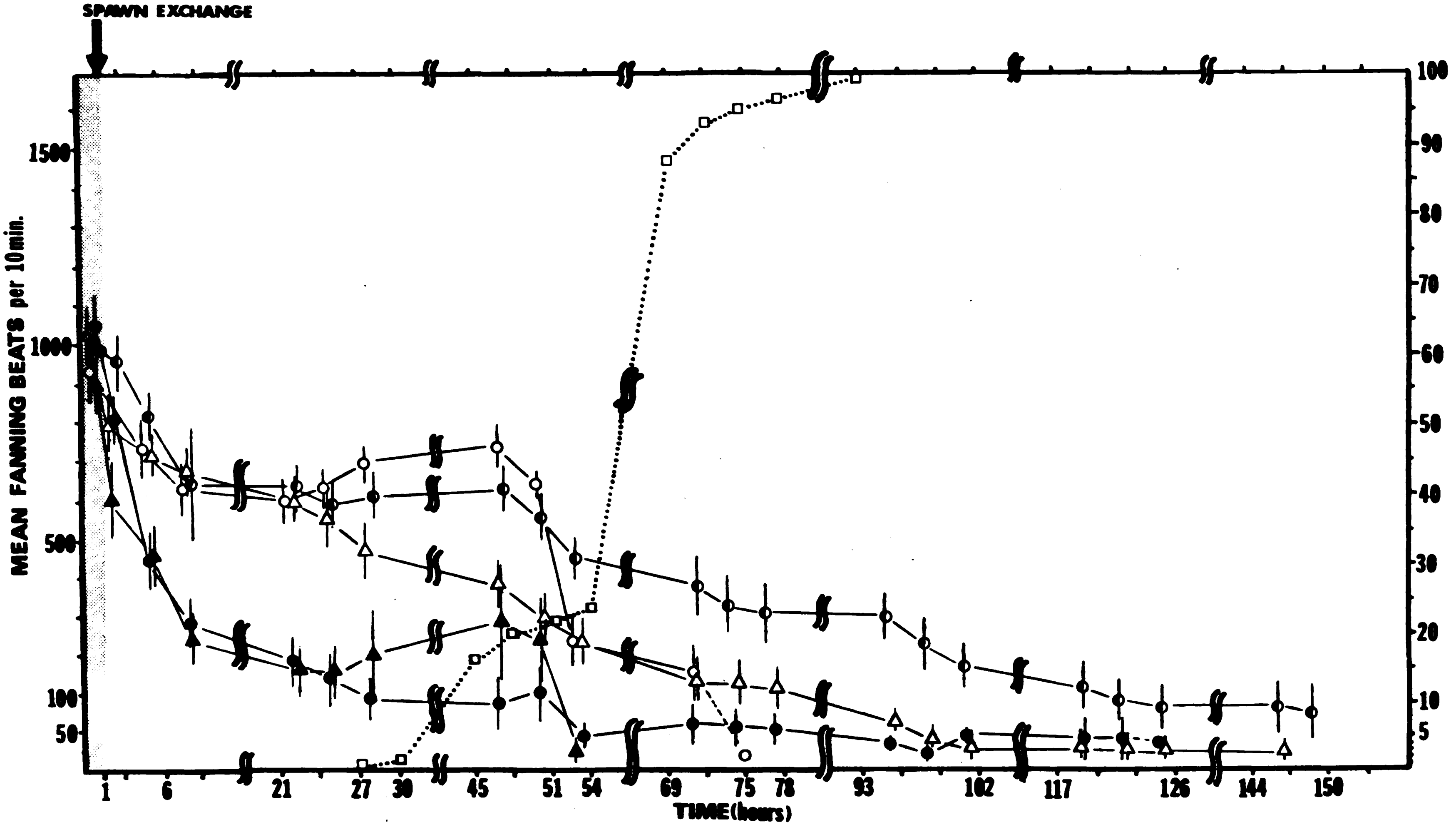
● ——— > natural "egg" group

◐ ——— = natural "egg" group

△ ——— < natural "egg" group

▲ ——— blank slate "control" group

□ ...... cumulative percent hatch


SPAWN EXCHANGE
MEAN FANNING BEATS per 10min.
1500
1000
500
100
50
CUMULATIVE PERCENT HATCH
100
90
80
70
60
50
40
30
20
10
5
1
6
21
27
30
45
51
54
69
75
78
93
102
117
126
144
150
TIME(hours)

one time will generally also beat at a high level at a later time). (2) The general slope of the lines is downward, but there is no reason to believe that this slope should follow any given pattern, so that the change from point to point is not predictable or testable. (3) Two of the five groups (baseline and blank slate "control") stopped maternal care at an early stage relative to the other three groups, and this causes a severe change in the variance of these observations, while the tests assume that the variance components remain constant. (4) Many of the tests that might be performed (at points where the results appear to differ enough to "warrant testing") are chosen on an *a posteriori* basis, rather than on an *a priori* basis. For all of these reasons, it seems wise not to place emphasis on complex tests of significance.

Table 8.--The results of a one-way Analysis of Variance of fanning beats of the baseline, blank slate "control," similar to the natural, smaller, and larger than the natural "egg" groups at twenty-four hours.

Source	SS.	MS.	df.	F	p
Main Effect	3954368	988592	4		
Deviations	2540845	41653	61	23.734	<< 0.005
Total	6495213		65		

To show that complex tests of significance can be applied to parts of the data, a single example was chosen. This example was the beat data for the five groups at twenty-four hours (Table 9 and Figure 9). It was chosen because there were definite differences between the low fanning and high fanning groups and it was of interest to see if the differences were significantly different. A one-way Analysis of Variance showed that these five groups were significantly heterogeneous ($p << 0.005$, Table 8) with respect to fanning beats. This analysis was extended by Scheffe's test for selected comparison (Scheffe, 1959). This showed significant differences between the groups chosen for comparison (large "egg" and blank slate vs. baseline, similar, and small "egg" groups) at the 0.005 level.

Table 9.--Results of a Scheffe test for multiple comparison. Means which did not differ at the 0.005 level are underlined.

	Blank Slate "Control"	>Natural	<Natural	=Natural	Base-line
$\bar{X}$ Fanning Beats at 24 Hours	266	289	692	572	734

Bouts.--Fanning is not continuous, but occurs in groups of beats, each of which is termed a bout.

Figure 10.--The mean number of bouts per ten minute observation period taken three times per day for all groups. Horizontal lines are one standard error on either side of the mean. Arrow indicates time of spawn exchange; stippled region indicates the ten minute baseline observation prior to spawn exchange.

Legend:

○ ———— baseline

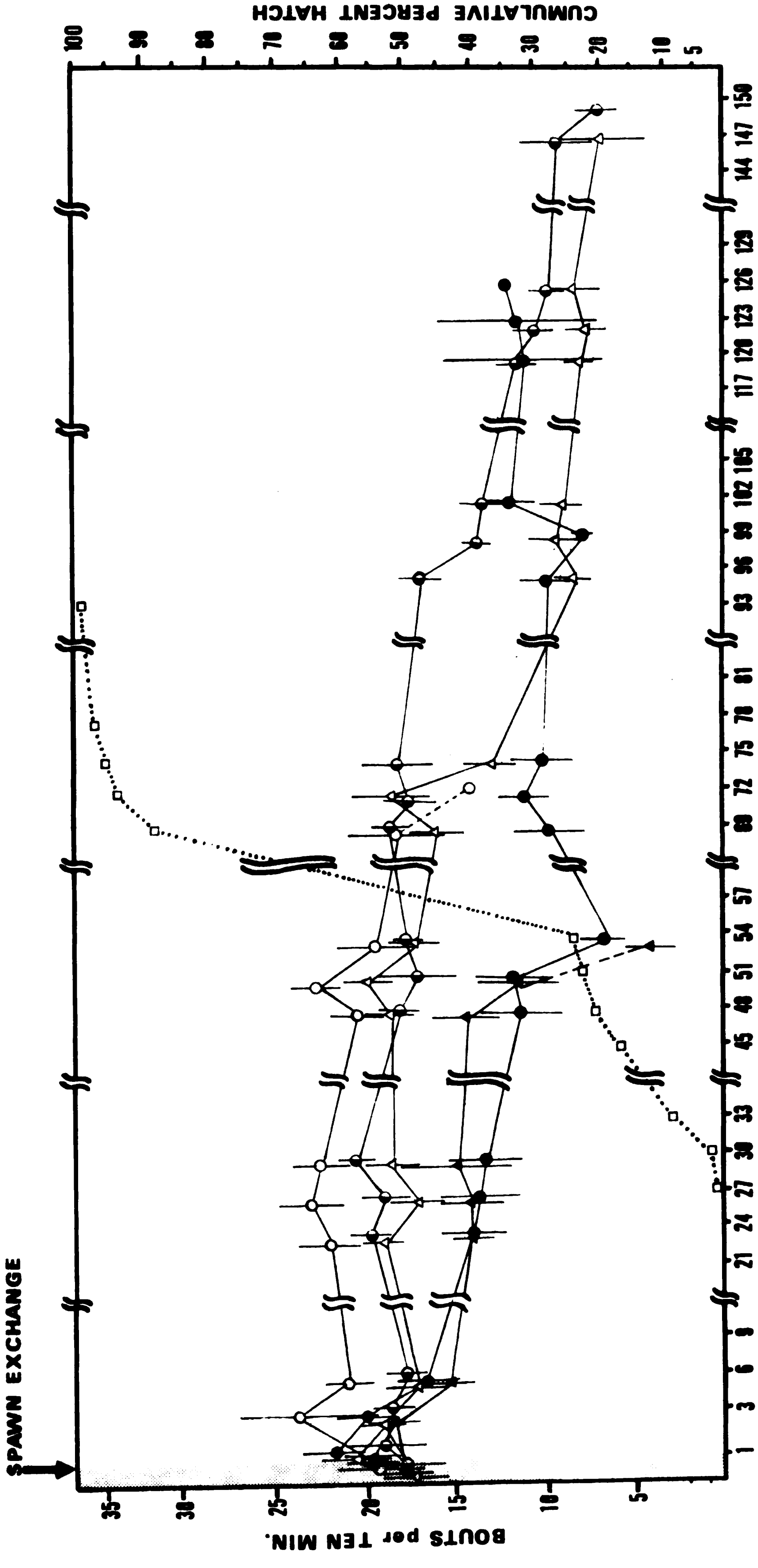
● ———— > natural "egg" group

◐ ———— = natural "egg" group

△ ———— < natural "egg" group

▲ ———— blank slate "control" group

□ .... cumulative percent hatch


SPAWN EXCHANGE
BOUTS per TEN MIN.
35
30
25
20
15
10
5
CUMULATIVE PERCENT HATCH
100
90
80
70
60
50
40
30
20
10
5
TIME (hours)
1
3
6
9
21
24
27
30
33
45
48
51
54
57
69
72
75
78
81
93
96
99
102
105
117
120
123
126
129
144
147
150

The overall temporal pattern of bouts for all groups differed from that of the beat pattern in that there was only a slow decrease of bout frequency over time, except in the "control" group at hatching. This was in contrast to the pattern of initial decrease, leveling off, and drop at hatching evidenced in the beat data for all of the groups. The baseline "control" groups, however, stopped fanning and therefore all bouts ceased at hatching time.

Clear-cut differences between the group means at the various observation times did not occur as they had in the beat data. Immediately prior to hatching, however, the order of magnitude of curves for the different groups was similar to that of the beats. The baseline group had the highest bout frequency which was not significantly different from the lower natural or the small "egg" groups. The blank slate and large "egg" groups had the lowest bout frequency, often differing in their means from the baseline (Figure 10).

Duration.--The mean length of time females fanned per 10 minutes followed the shape of the fanning beat curves (Figure 11). The order in which the five groups occurred at the different observation periods also followed that for the beats (Figure 11).

Tempo (Beats per second).--The overall pattern of beat rate is similar to that of the bouts (Figure 12),

Figure 11.--The mean fanning duration per ten minute observation period taken three times per day for all groups. Horizontal lines are one standard error on either side of the mean. Arrow indicates time of spawn exchange; stippled region indicates the ten minute baseline observation prior to spawn exchange.

Legend:

○ ——— baseline

● ——— > natural "egg" group

◐ ——— = natural "egg" group

△ ——— < natural "egg" group

▲ ——— blank slate "control" group

□ .... cumulative percent hatch

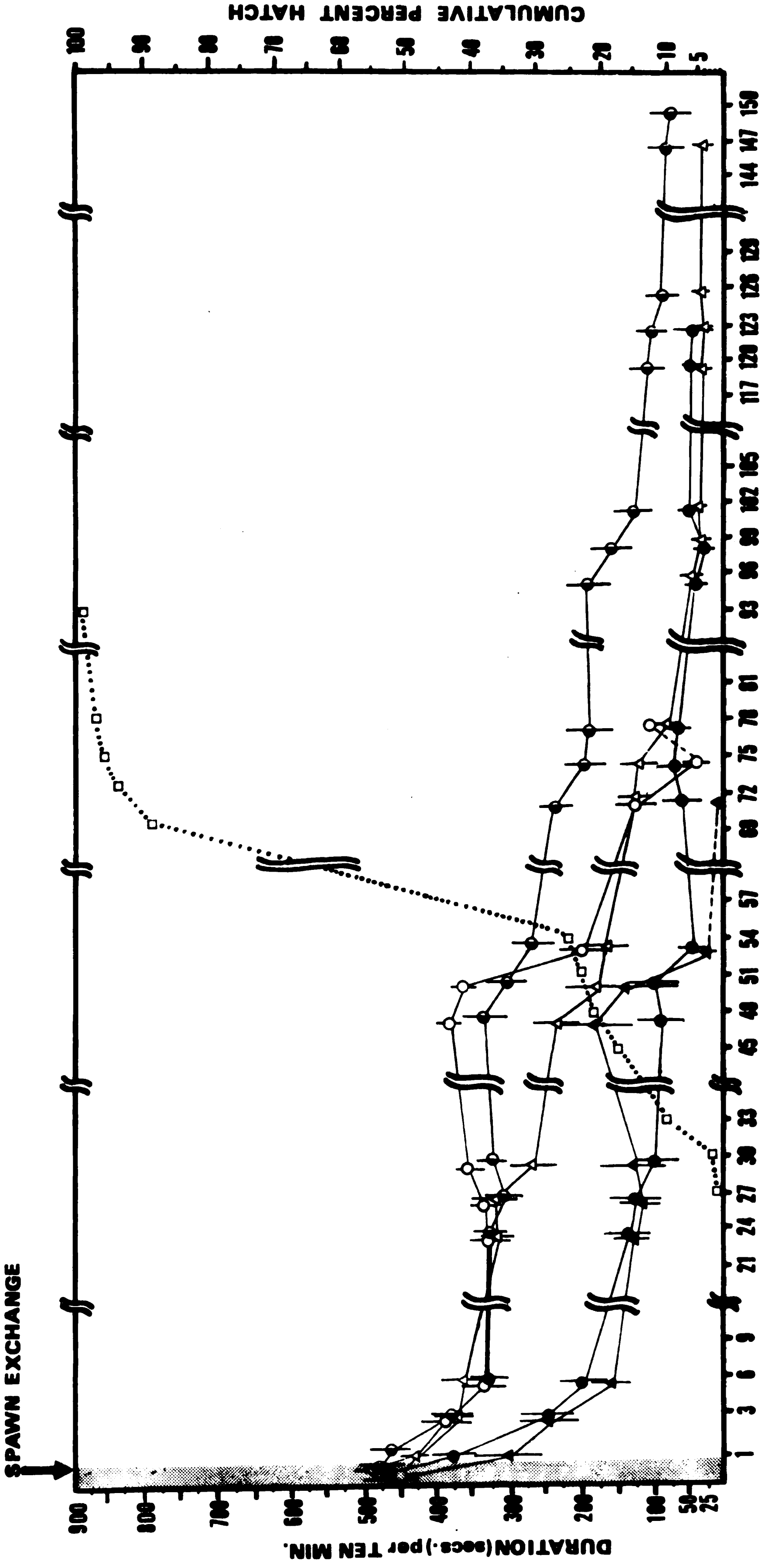

SPAWN EXCHANGE
DURATION(secs.) per TEN MIN.
900
800
700
600
500
400
300
200
100
50
25
CUMULATIVE PERCENT HATCH
100
90
80
70
60
50
40
30
20
10
5
TIME(hours)
1
3
6
9
21
24
27
30
33
45
48
51
54
57
69
72
75
78
81
93
96
99
102
105
117
120
123
126
129
144
147
150

Figure 12.--The mean tempo (F/D) per ten minute observation period taken three times per day for all groups. Horizontal lines are one standard error on either side of the mean. Arrow indicates time of spawn exchange; stippled region indicates the ten minute baseline observation prior to spawn exchange.

Legend:

○ ——— baseline

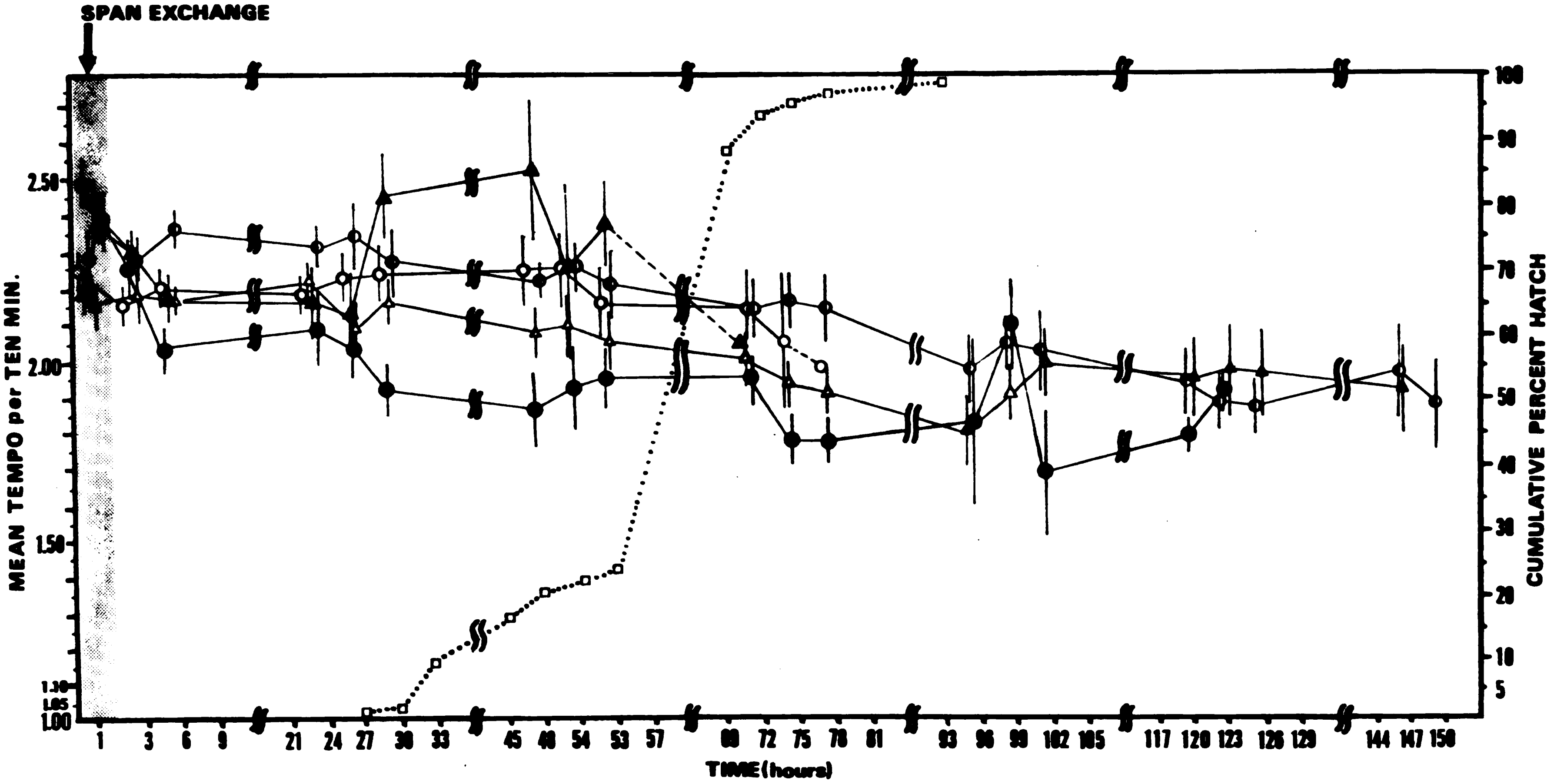
● ——— > natural "egg" group

◐ ——— = natural "egg" group

△ ——— < natural "egg" group

▲ ——— blank slate "control" group

□ .... cumulative percent hatch


SPAN EXCHANGE
MEAN TEMPO per TEN MIN.
1.00
1.05
1.10
1.50
2.00
2.50
CUMULATIVE PERCENT HATCH
5
10
20
30
40
50
60
70
80
90
100
TIME(hours)
1
3
6
9
21
24
27
30
33
45
48
54
53
57
69
72
75
78
81
93
96
99
102
105
117
120
123
126
129
144
147
150

in contrast to the beat and duration measurements. This constancy of tempo throughout the period was not affected by the absence of eggs or by "eggs" of different sizes. The rapid drop in performance (characteristic of the other fanning measurements) during the initial hours of egg care (0-6 hours) and at hatching did not occur here in any of the groups.

Beats per Bout (B/B) and Duration per Bout (D/B).--B/B and D/B were very similar to one another in both temporal organization and the mean level of performance at any given point of obseration. This similarity is due to the fact that tempo (B/D) does not change measurably with time over the fanning cycle (Figure 12). Both the temporal pattern and the position of the different groups in relation to one another were similar to that of the beat and duration data. Thus, these two parameters added no information to the latter two aspects of fanning. The basic statistics and figures are given in Appendices 4-12.

Choice Situation: Real vs. Simulated "Eggs" Presented Simultaneously

The preceding sections have been concerned with fanning behavior toward eggs which were either real (baseline) or simulations of the real eggs which had been exchanged for the female's own eggs. In this situation there was no opportunity for a comparison between the effects of real and simulated "eggs." The results obtained by simultaneously presenting natural and simulated "eggs" of the same size follow. The purpose of this procedure was to test for the effects of visual changes which occurred in the natural eggs over time.

C. nigrofasciatum females, when simultaneously presented with simulated "eggs" of approximately natural size and with natural eggs, made no apparent discrimination between these until after approximately one day of incubation. Although the variability was quite great in all of the measurements at any point in time, the pattern which appeared was one in which the fanning beats toward the simulated natural size "eggs" started with a high frequency and decreased over time. Fanning beats toward the females' own eggs started at a significantly lower frequency, increased by the beginning of the third day to a level significantly greater than the beat level toward the simulated "eggs," and then dropped at hatching (Figure 13). The females appeared to have been discriminating between the real and natural size simulated "eggs." The above pattern is clearest in the beat data but is also evident in the duration, and, to a lesser extent, in the tempo measurements (Figures 14 and 15).

In the choice situation the females stopped caring for the simulated spawn earlier than the natural spawn ($\bar{X}$ length of time fanning was about 51 hours for the

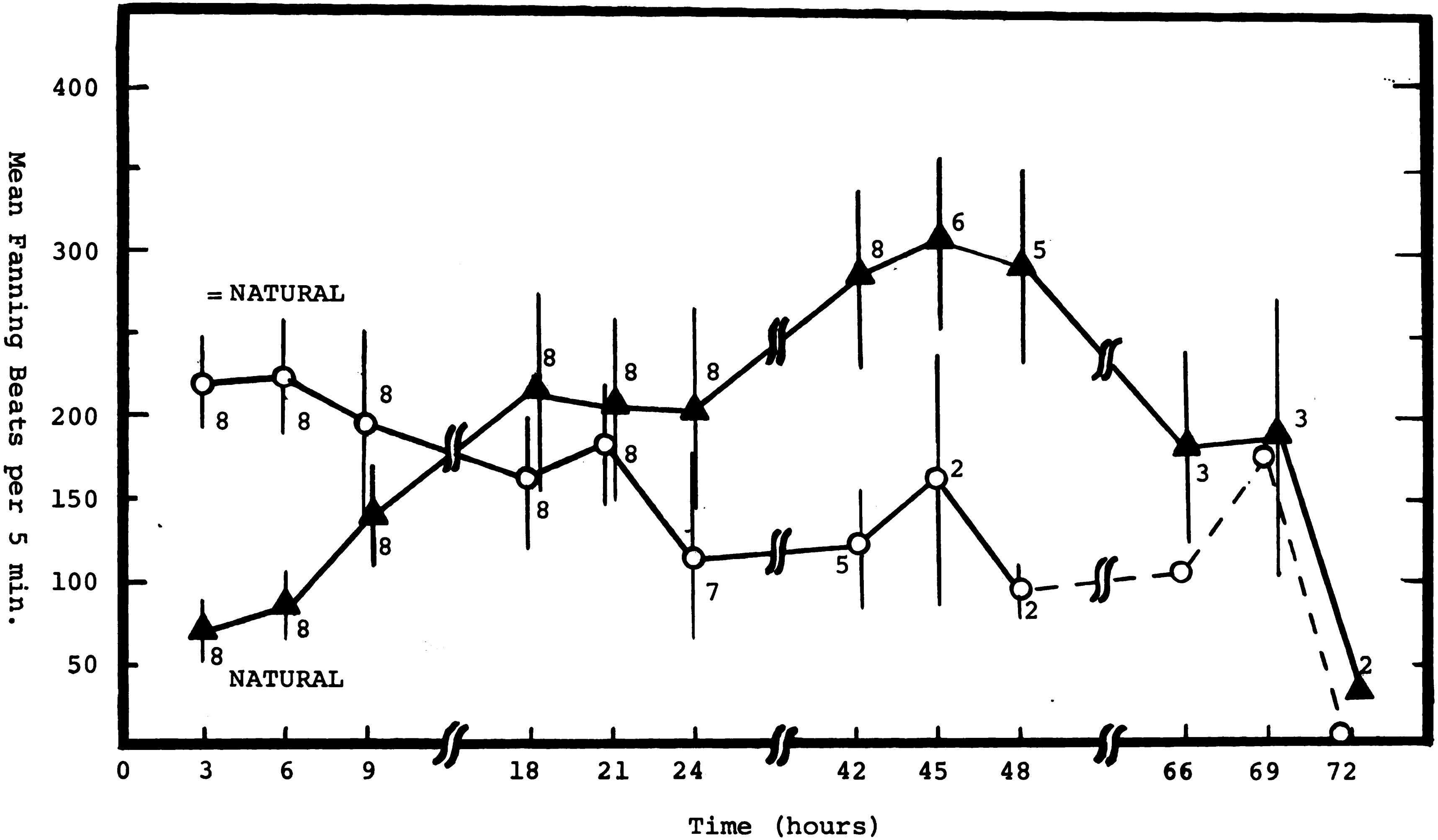


Figure 13.--The mean number of fanning beats recorded simultaneously toward own spawn and an identical simulated spawn (closed triangles = own spawn, open circles = simulated spawn similar to the natural). Each point represents a five minute observation recorded three times per day. Horizontal lines denote one standard error on either side of the mean; numbers along means are the sample size (N).

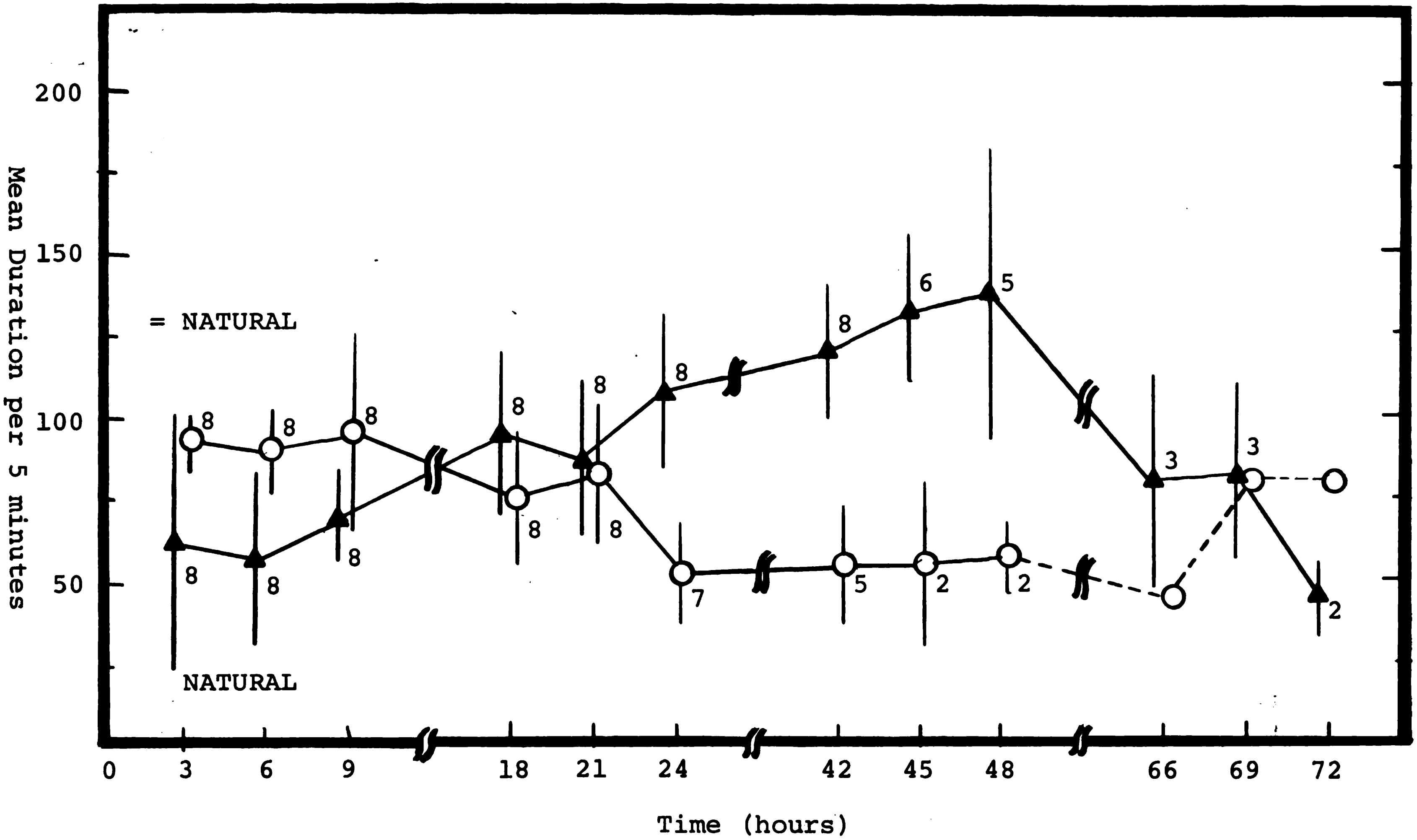


Figure 14.--The mean duration recorded simulatenously toward own spawn and an identical simulated spawn (closed triangles = own spawn, open circles = simulated spawn similar to the natural). Each point represents a five minute observation recorded three times per day. Horizontal lines denote one standard error on either side of the mean; numbers along means are the sample size (N).

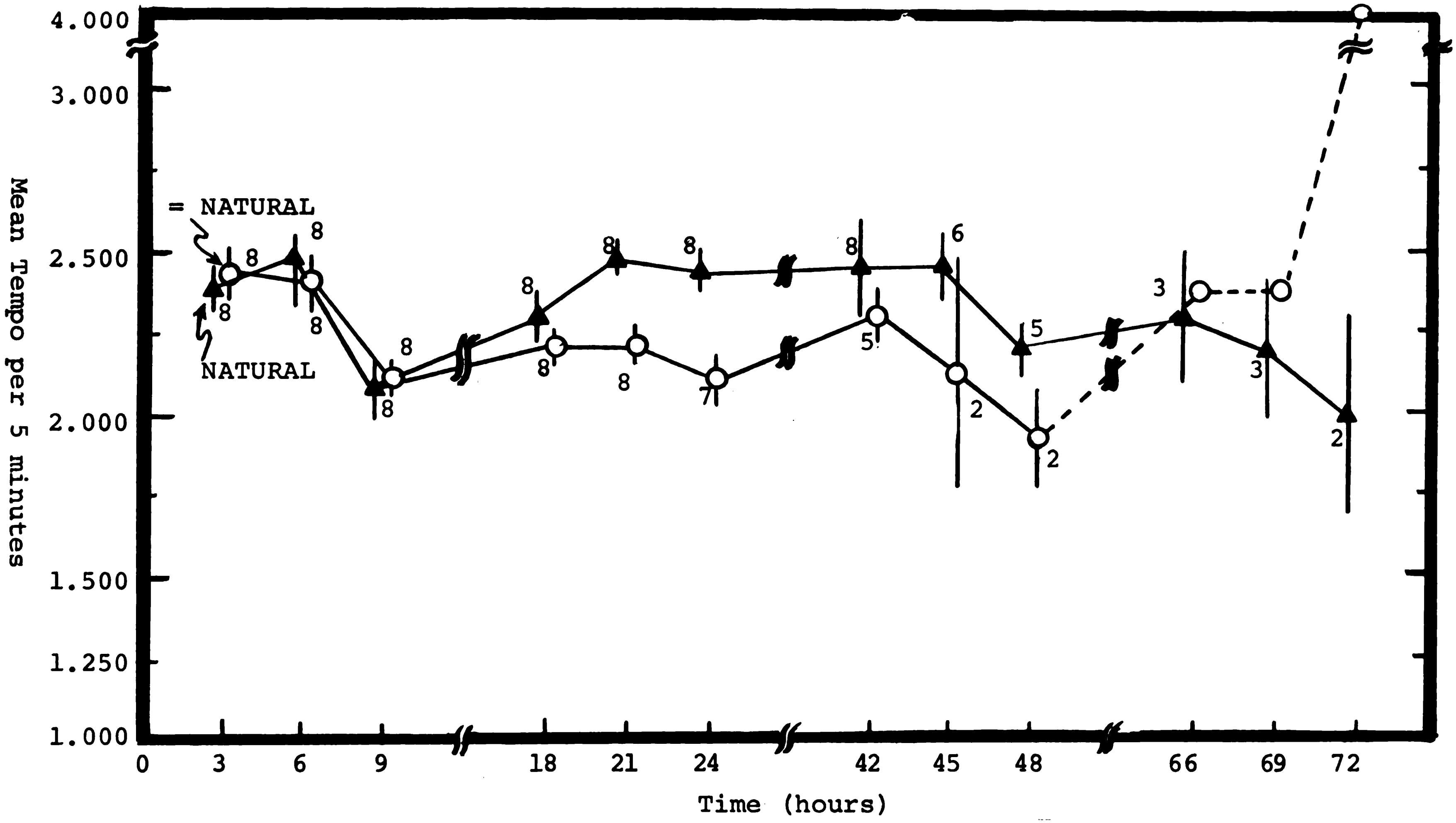


Figure 15.--The mean tempo recorded simultaneously toward own spawn and an identical simulated spawn (closed triangles = own spawn, open circles = simulated spawn similar to the natural). Each point represents a five minute observation recorded three times per day. Horizontal lines denote one standard error on either side of the mean; numbers along means are the sample size (N).

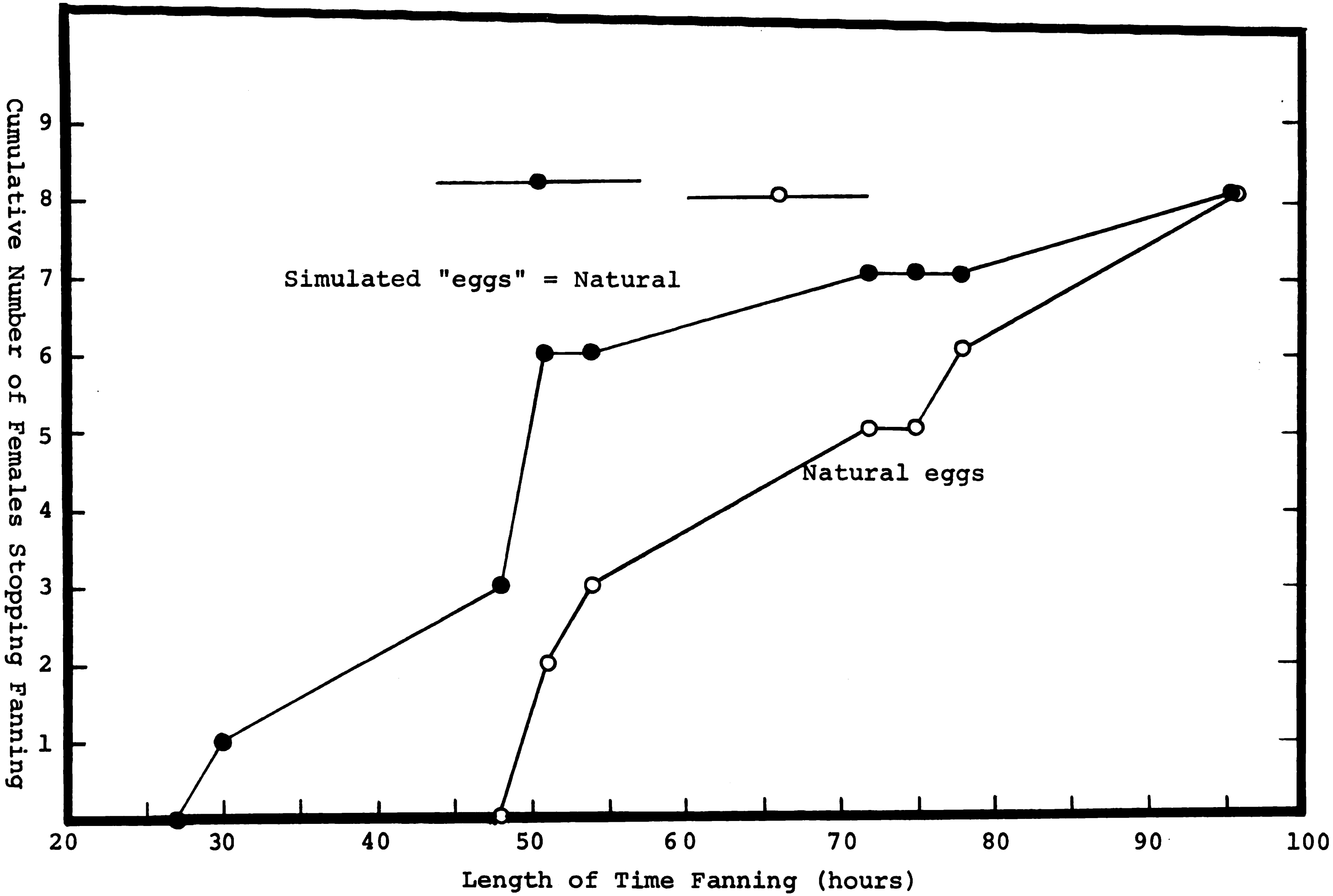


Figure 16.--The cumulative number of females which stopped fanning as a function of the length of time fanning (in hours) toward own (closed) and a simulated spawn (open circles) presented simultaneously. Means for each group are given at the top of the figure. Horizontal lines indicate one standard error on either side of the mean.

simulated and about 66 hours for the natural spawn). Egg care dropped off faster and earlier towards the simulated than towards the natural spawn (Figure 16). However, a one-way Analysis of Variance of the length of time spent caring for the spawn (Table 10) demonstrated that there was no heterogeneity between the natural and simulated "egg" groups ($p > 0.05$).

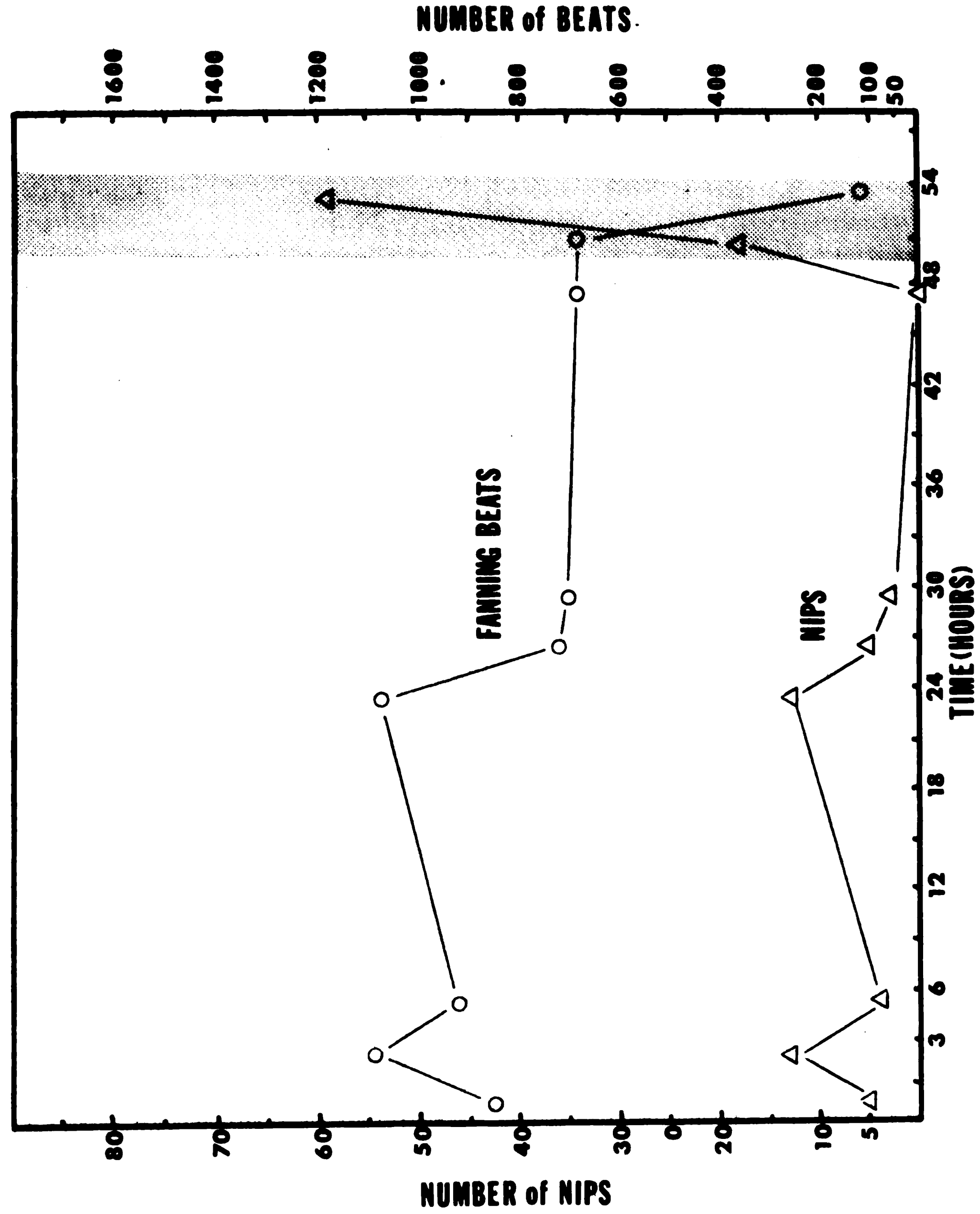
Table 10.--The results of a one-way Analysis of Variance of the egg care duration means for natural eggs and simulated "eggs" presented simultaneously.

Source	SS.	MS.	df.	F	p
Main Effect	946	946	1	3.625	> 0.05
Deviations	3916	261	15		
Total	4862		16		

Nips.--Nipping at the clutch was absent or occurred at a low frequency except when the natural eggs were hatching. Nipping frequency had a very low level at all times in the egg exchange groups.

Figure 17 shows beat and nipping curves for a representative baseline female, and was generally characteristic of most individuals. Thus, there were no appreciable changes in nips until hatching. At this time nipping frequency increased several fold. Concomitant with this increase, fanning beats toward the eggs decreased abruptly.

Figure 17.--Curves for nipping and fanning beats for a representative individual (female 31) under baseline conditions. Approximate time of hatching is indicated by shaded ordinate.


NUMBER of BEATS
1600
1400
1200
1000
800
600
400
200
100
50
FANNING BEATS
NIPS
TIME (HOURS)
3
6
12
18
24
30
36
42
48
54
NUMBER of NIPS
80
70
60
50
40
30
0
20
10
5

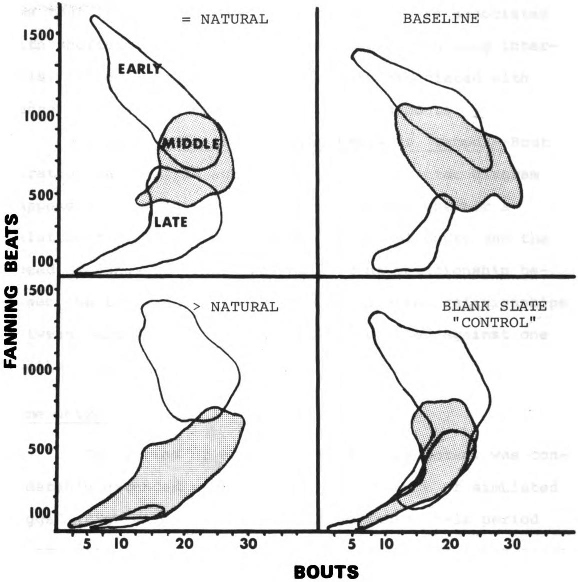
Other Aspects of the Structure of Fanning

While the purpose of this study was to determine the effects of egg size upon egg care behavior, it was also possible to obtain data relevant to certain aspects of the structure of fanning. The sections to follow present these results.

Fanning Beats vs. Bouts.--Fanning beats were plotted as a function of bouts for each of the five conditions. Figure 18 gives the results for four of these. The general pattern, which appeared consistently in all groups, was one of an inverse relationship early in the egg care cycle and a direct relationship during the later phase. The correlation between beats and bouts was inverse when fanning frequency was low and direct when fanning frequency was high. The change in this relationship occurred at approximately 200-300 beats per 10 minutes.

Interval Preceding and Interval Following vs. Duration of One Bout.--The inter-bout interval does not appear to indicate a work and rest relationship with fanning beats. The intervals preceding (IPB) and the interval following (IFB) a bout were plotted against duration of bouts on a scatter diagram for seven different females. Figures are given (Appendices 13-17) for three representative individuals of these seven. None of these demonstrated correlations. This held true for either individuals of long or

Figure 18.--Fanning beats as a function of bouts for the baseline and three of the four exchange conditions. The outlined areas show the highest density of points at each of three times during the fanning cycle. These times are early (0-24 hours) middle (stippled; 25-72 hours), and late (beyond 73 hours).


= NATURAL
BASELINE
> NATURAL
BLANK SLATE
"CONTROL"
EARLY
MIDDLE
LATE
FANNING BEATS
1500
1000
500
100
1500
1000
500
100
BOUTS
5
10
20
30
5
10
20
30

short fanning duration. No correlation was noted with either IPB or IFB and duration. Also, there were no evident correlations among early, intermediate, or late points during the egg care period. The only pattern which appeared was that longer durations, by and large, were associated with shorter IPB and IFB, and less often with long intervals. Thus the shorter durations were associated with longer intervals preceding and following bouts.

Bout Duration and Fanning Beats vs. Tempo.--Bout duration was plotted against tempo on a scatter diagram (Appendix 18). This was done to determine whether a relationship existed between the length of bouts and the speed of fanning. There was no evident relationship between the two. Also, there were no apparent relationships between fanning beats and tempo when plotted against one another (Appendix 19).

Time Between Spawns

The period of egg care, as already noted, was considerably extended by the continual presence of simulated "eggs" (Figure 9). Females that extended their period of egg care appear also to have extended their inter-spawn interval. The time of spawn deposition, hatching, and the time wrigglers became free-swimming was recorded for all individuals which spawned. A spawn was considered to be free-swimming when the majority of the young were able to propel themselves above the substrate.

Figure 19.--The number of days fanning above 200 beats per ten minutes plotted as a function of the number of days between spawns. Solid circles are means, the accompanying numbers in brackets the N, for the situation where simulated "eggs" similar to the natural (11), smaller than the natural (6), and larger than the natural (5) were left in place.

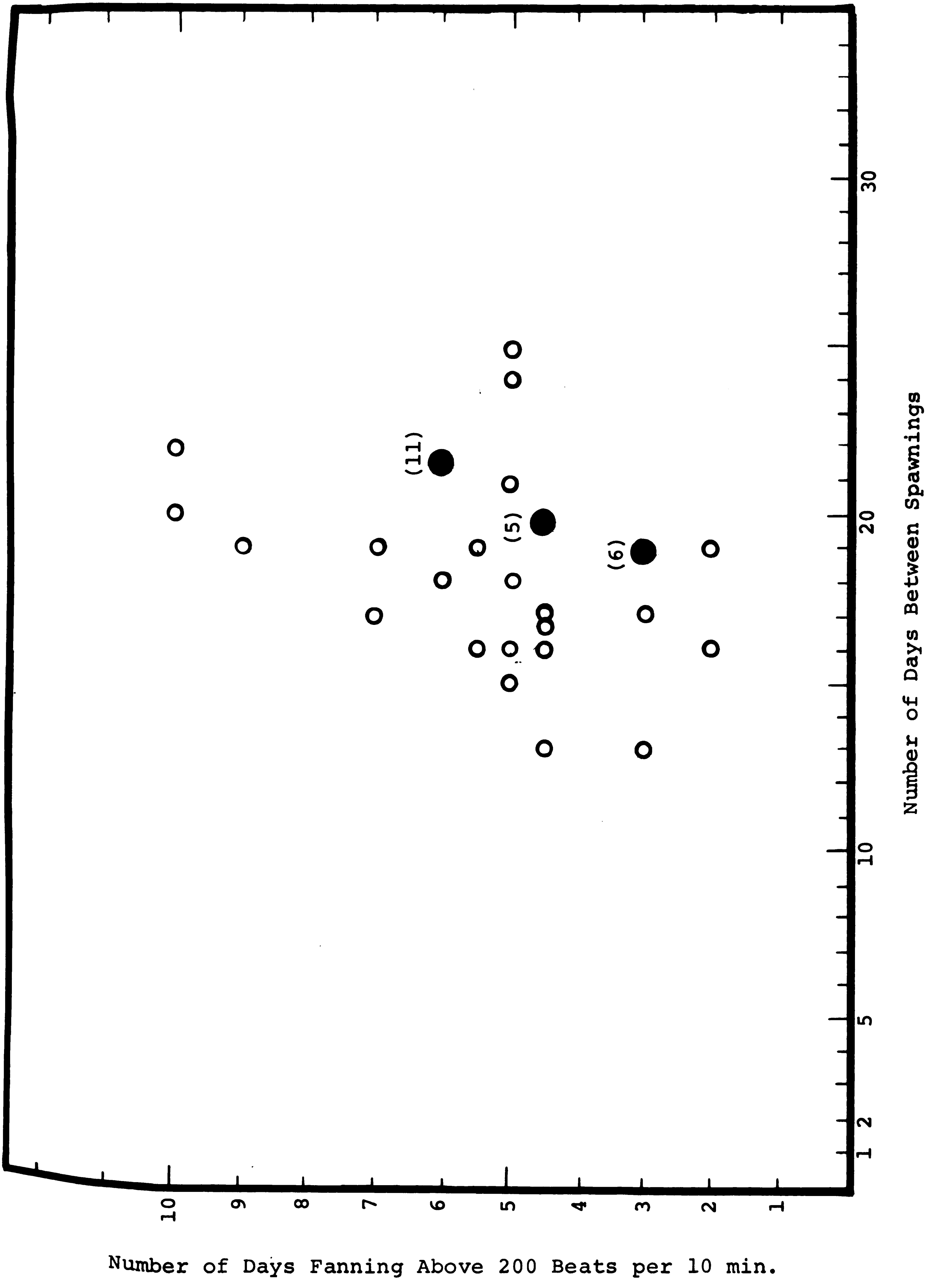

Number of Days Between Spawnings
1
2
5
10
20
30
(11)
(5)
(6)
10
9
8
7
6
5
4
3
2
1
Number of Days Fanning Above 200 Beats per 10 min.

Table 11.--Basic Statistics for time (days) between spawnings (x) when the natural spawn was left with the parents and when it was exchanged for either a blank slate or simulated "eggs."

No Egg Exchange

	Σ_x	Σ_x^2	S_x^2	$S_{\bar{x}}^2$	$S_{\bar{x}}$	$\bar{x}$	N
Spawn Either Removed as Pro-larvae or First Day Larvae, or Eaten as Eggs by Female	740	14004	16.198	0.395	0.628	18.049	41

Egg Exchange

	Σ_x	Σ_x^2	S_x^2	$S_{\bar{x}}^2$	$S_{\bar{x}}$	$\bar{x}$	N
Blank Slate "Control"	277	5025	31.427	1.849	1.360	16.310	17
Simulated "Eggs"	406	7796	14.450	0.657	0.810	18.455	22

The time between spawns was recorded for three categories. The first was a blank slate "control" group, which has been presented under this same heading in previous sections (see page 24 of Methods and Materials). Here, the natural eggs were removed one to two hours after the completion of spawning. The second category was a simulated group which included all the cases in which artificial "eggs" had been exchanged for the female's own. The third was a baseline group where the spawn was left with the female until the first day of free-swimming at which time the larvae were removed. Table 11 summarizes these conditions.

Table 12.--Basic Statistics for the number of days spent fanning above 200 beats per 10 minutes and the number of days between spawnings when the natural spawn was exchanged for one of simulated "eggs."

Number of Days Spent Fanning
Above 200 Beats per 10 minutes

	S_x^2	$S_{\bar{x}}^2$	$S_{\bar{x}}$	$\bar{x}$	N
Simulated "Eggs" Equal to Natural	3.835	0.274	0.523	6.786	14
Simulated "Eggs" Larger than Natural	0.2617	0.044	0.210	4.333	6
Simulted "Eggs" Smaller than Natural	1.143	0.163	0.404	3.143	7

Days Between Spawns

	S_x^2	$S_{\bar{x}}^2$	$S_{\bar{x}}$	$\bar{x}$	N
Simulated "Eggs" Equal to Natural	30.423	2.173	1.474	21.500	14
Simulated "Eggs" Larger than Natural	55.367	9.228	3.038	19.833	6
Simulated "Eggs" Smaller than Natural	34.333	4.905	2.215	19.00	7

When the means of the three groups (baseline, blank slate "control," and simulated) were tested against one another by a series of paired t-tests, it was found that the differences among these means were as expected, though not statistically different (Table 13). The presence of simulated "eggs" tended to increase the time between spawns, while the removal of eggs soon after spawning tended to decrease the time (Table 12). The effect of leaving the spawn with the female appears to have been comparable to leaving the simulated "eggs" until broodiness was lost.

Table 13.--The results of t-tests comparing the mean interspawn interval under three conditions (see text).

	df	t	p
Baseline vs. Simulated	61	0.123	> 0.9
Baseline vs. Blank Slate "Control"	56	0.423	> 0.6
Blank Slate Control vs. Simulated	37	1.423	0.2-0.1

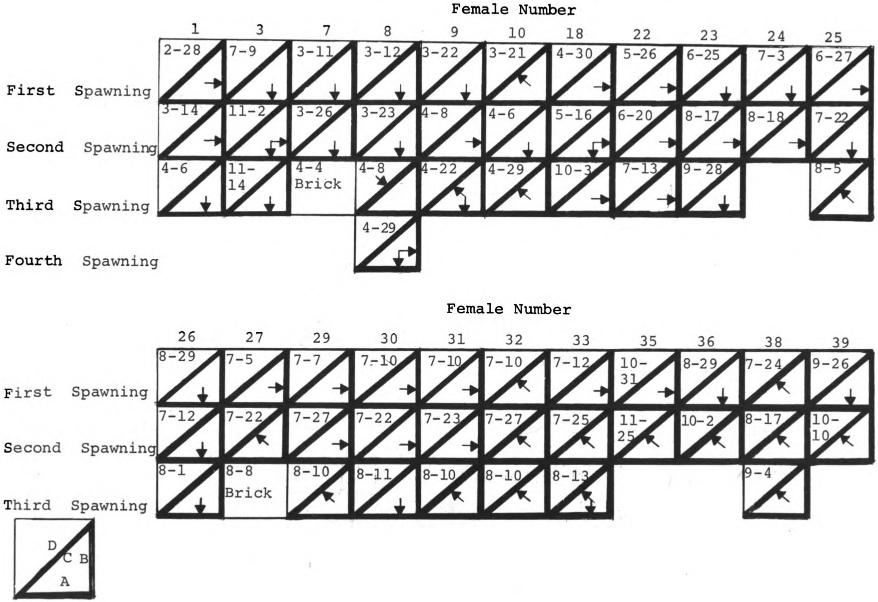
Fanning decreased with time (Figure 9). At very low beat frequencies it is difficult to be certain of the broody condition of a particular individual. Thus a cutoff point at 200 fanning beats per 10 minutes was chosen as a criterion of broodiness toward the eggs. Days fanning above 200 beats per 10 minutes was plotted against days between spawnings (Figure 20). A cutoff point was established at 28 days, since no females fanned above 200 beats between 25 and 29 days. Thus no data after 28 days were included in the analysis. For unknown reasons some pairs ceased spawning for considerable periods of time. Later, some of these resumed spawning. This seemed to have occurred independently of the conditions noted for their previous spawns. Thus, when more than 28 days elapsed it was difficult to determine whether the extended period of time was due to a particular treatment given during the previous spawning phase, or to factors still unknown. Figure 20 shows a trend which is not quite significant (correlation coefficient = 0.3895, $p > 0.05$). This trend, a direct relationship between interspawn interval and time spent caring for eggs, is due to a few low and high points and appears to be unimportant within the center of the range.

Spawn Site Preference

Most spawns were deposited within the lean-to (Table 1). These could have been deposited as follows: on the horizontal section within the lean-to (position A), on the vertical wall of the lean-to (position B), or on the under surface of the diagonal slate (position C) (Table 14). The outer surface of the diagonal and sites away from the lean-to were infrequently chosen, although they made up most of the surface area available for spawning. There apparently was no preferred site for deposition of eggs within the lean-to. In addition, females showed little if

Table 14.--Order and position of successive spawnings. There were 22 spawnings in position A, 20 in position B, and 17 in position C of the lean-to. (These positions are shown in the cell in the lower left corner of the table.) The expected frequency of successive spawns in similar positions was calculated on the assumption that spawns by a given female are randomly placed, but have the observed frequencies of 0.373, 0.339, and 0.288 in positions A, B, and C respsectively. The probability of a similar position pair is $(0.373)^2 + (0.339)^2 + (0.288)^2 = 0.337$. Of the 39 pairs, the expectation was that 13 would be in similar positions and 16.5 were observed, which is not significantly different from the expectation ($X^2 = 1.328$, ldf, $p > 0.05$).

Order and Position of Successive Spawnings



any preference for using the same position for two successive spawnings. However, if simulated "eggs" were present, they tended to lay their eggs among them.

Of the 59 spawns deposited within the lean-to, 37 percent were on the horizontal section, 33 percent were on the vertical wall, and 28 percent were on the undersurface. This is not significantly different from random ($X^2 = 4.31$, 2 df, $p > 0.05$). In the analysis that follows, the observed frequency of spawning at each site is used to predict the probability that a fish will spawn at that site.

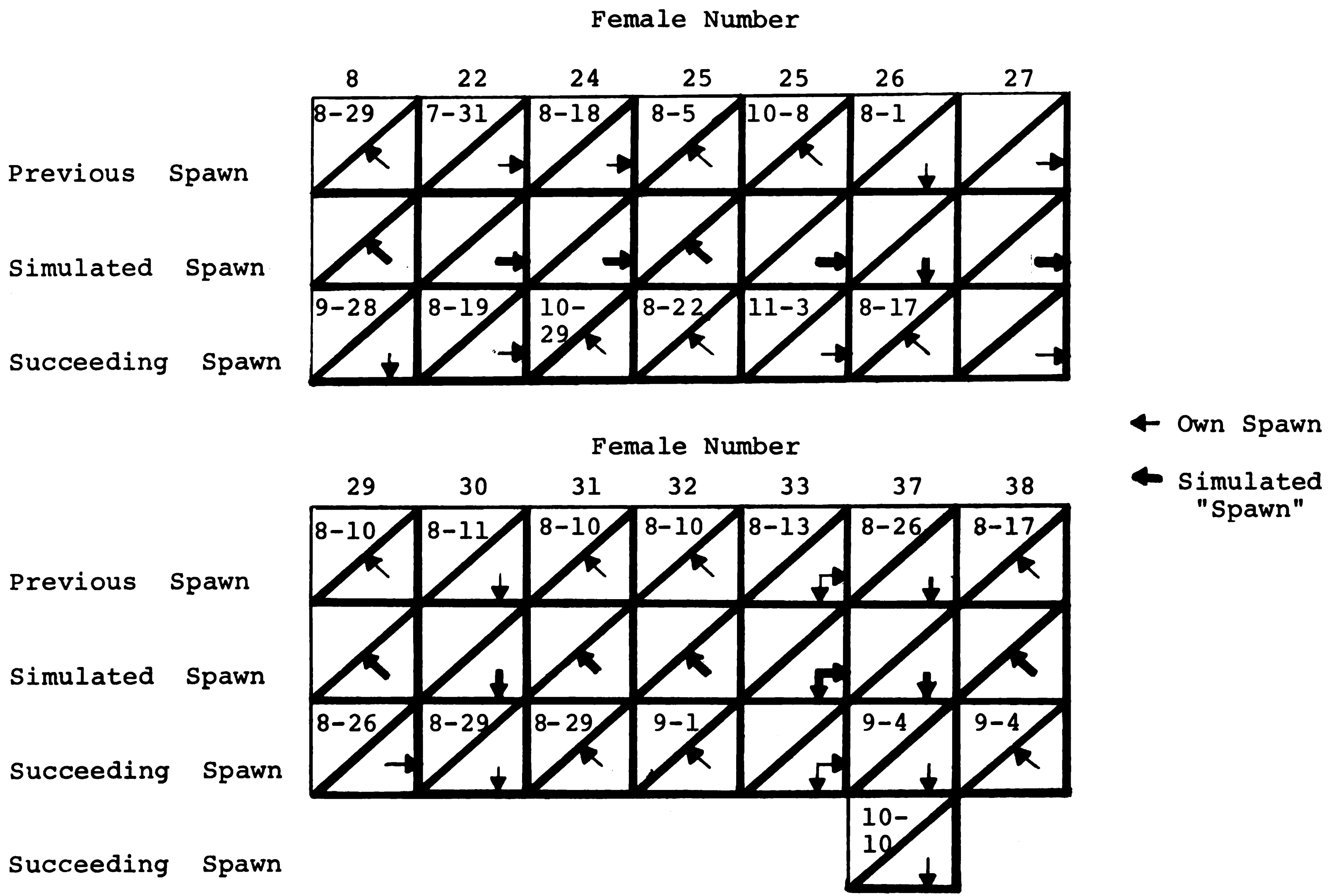
Wherever two successive spawns of a given female occurred on the same section of the lean-to, they were considered to constitute a "similar" pair. Successive spawns on different sections of the lean-to were considered as "dissimilar" pairs. There were 16.5 "similar" and 22.5 "dissimilar" pairs. (Occasionally eggs of a single clutch were deposited on two different slates. If one of these sites was concordant with that of a previous egg deposition, the spawning was classified as being 0.5 "similar" and 0.5 "dissimilar.") From the distribution of site choices 0.337 is the expected frequency of choosing the same site on two successive occasions (Table 14). There is no significant evidence that one spawn was followed by another in the same position of the lean-to ($X^2 = 1.328$, 1 df, $p > 0.05$).

During the latter portion of this study, simulated "eggs" were left under the lean-to for a time longer than the usual fifteen days. A female when spawning again usually deposited her eggs on the slate with the simulated "eggs," often right among them (Figure 5). She did this even when the "eggs" were quite moldy and misshapen, which they frequently became after remaining in the tank for a long time. Whether or not the presence of eggs (artificial in this case) tended to significantly increase the bias to spawn on a special lean-to segment was tested by comparing the frequency of "similar" vs. "dissimilar" pairs at the various spawning sites for three conditions: (1) successive spawns in a series of two or more spawns without the presence of artificial "eggs"; (2) successive spawns in a series of two or more spawns where simulated "eggs" of the same size as the natural were present; and (3) successive spawns where simulated "eggs" larger than natural were present.

There appeared to be a strong tendency for females to spawn on the same slate that contained simulated "eggs" of natural size. The distribution of sites at which the females spawned was tested on the assumption that this distribtuion should have the same frequencies that were observed for spawns deposited when simulated "eggs" were not present (Table 15). According to this assumption, 0.337 of the spawns should have been in a "similar" position to the eggs, but 11.5 of the spawns actually were "similar" and only 4.5 were "dissimilar." This is a highly

Table 15.--Order and position of successive spawns where simulated "eggs" of the same size as the natural were present. There were 11.5 "similar" and 4.5 "dissimilar" pairs which is a significant difference from expectation based on the distribution of the 59 spawns in Table 12 (X^2 = 9.891, 1 df, $p < 0.005$). When "similar" and "dissimilar" pairs were this condition were tested against the baseline, there was no significant difference (X^2 = 2.874, 1 df, $p > 0.05$).

Order and Position of Successive Spawns



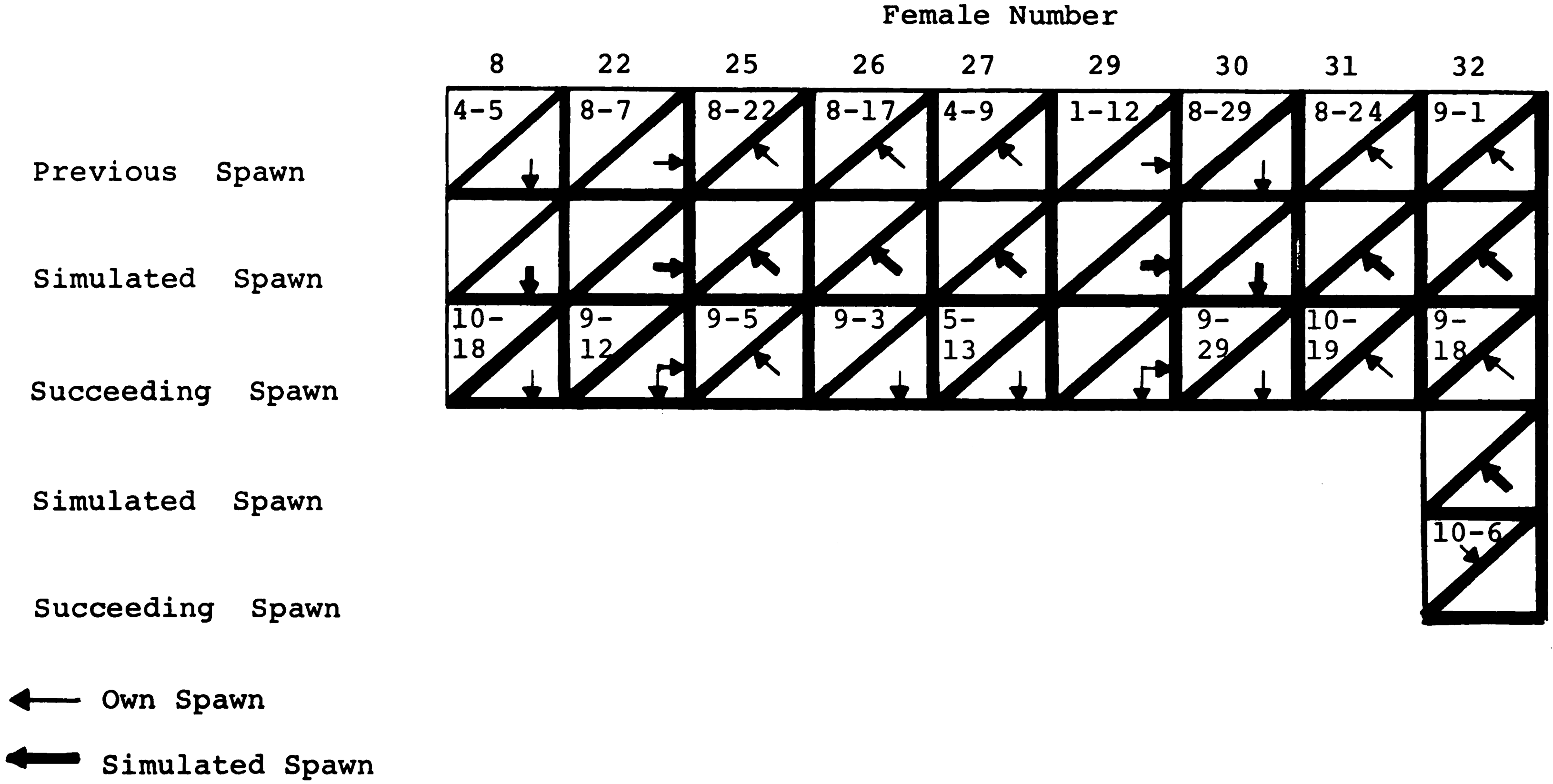
significant difference from random ($X^2 = 9.891$, 1 df, $p < 0.005$), and indicates that females place their eggs on a slate bearing "eggs" with a much greater frequency than expected by chance alone. It was found that the frequency of "similar" pairs was not significantly higher than the "dissimilar" when simulated "eggs" were present than under the baseline conditions ($X^2 = 2.874$, 1 df, $p > 0.05$).

A similar test was given where "eggs" larger than the natural had been left under the lean-to (Table 16). The sample was small in that it consisted of only 6 "similar" and 4 "dissimilar" pairs. Spawning did not occur significantly more frequently with "similar" than with "dissimilar" pairs ($p > 0.05$). When compared to previous spawns, the presence of larger than natural "eggs" on the slate did not significantly bias the placement of a succeeding spawn on that slate ($X^2 = 0.417$, 1 df, $p > 0.05$). Further, there was no significant difference when the frequency of occurrence was compared relative to the natural size "eggs" ($X^2 = 0.393$, 1 df, $p > 0.05$).

Insufficient data prevented a test for simulated "eggs" smaller than natural or for instances where the spawn was consistently placed on the outside of the lean-to.

Table 16.--Order and position of successive spawnings where simulated "eggs" larger in size than the natural were present. There were 6 "similar" and 4 "dissimilar" pairs which are not significantly different from expectation based on the distribution of the 59 spawns from Table 12 ($X^2 = 3.152$, 1 df, $p > 0.05$). When "similar" and "dissimilar" pairs under this condition were tested against the baseline, there was no significant difference ($X^2 = 0.417$, 1 df, $p > 0.05$); when tested against the frequency of "similar" and "dissimilar" pairs when natural sized "eggs" were present there also was no significant difference ($X^2 = 0.393$, 1 df, $p > 0.05$).

Order and Position of Successive Spawns



DISCUSSION

This study was concerned with an analysis of the relationship between an environmental stimulus, eggs, and the care behavior associated with this stimulus. Of the measurements taken, egg care was best defined in terms of fanning, a multidimensional behavior which circulates water over the spawn as a result of the beating of the parent's pectoral fins.

The work of Kühme (1964) with Hemichromis and Mertz (1967) with C. nigrofasciatum, along with the egg exchange results of Greenberg (1961, 1963), Collins (1965), and Myrberg (1965) with several cichlid species established that vision, not olfaction, is most likely the major stimulus involved in egg care behavior. This is a question to which Noble and Curtis (1939) had directed their attention some twenty-five years earlier (in Hemichromis). They reached the same conclusion. Accepting the hypothesis that vision is primarily involved, it was inferred that exact, or close, visual replication of a species' spawn should not appreciably alter that species' behavior when presented with this simulated spawn. Furthermore, it was believed that changes in various critical aspects of the spawn should result in quantitative, and possibly qualitative, behavioral changes in the parent's responses.

It was further hypothesized that egg size might be one of the more important variables in influencing egg care behavior. Therefore, this was the only variable purposefully manipulated.

According to the data presented here, fanning frequency decreases with time regardless of the size of the eggs presented to the female. The overall temporal pattern is identical to that which Mertz (1967) found for this species. This pattern across egg groups implies some sort of internal, possibly hormonal effect, and/or some sort of stimulus satiation over time. Complete removal of the spawn resulted in the most rapid decrease. However, a low level of fanning was maintained and lasted for as long as three days in some females. This was directed toward the slate which previously held the spawn. The magnitude of fanning after the eggs were removed is thought to be the level of fanning under hormonal control. The difference between this and the level when the eggs were present is possibly due to an additive effect of visual stimulation by the eggs. In other words, the magnitude of fanning in the absence of eggs should be similar to the level obtained by injecting non-broody C. nigrofasciatum with a physiological dose of prolactin to induce fanning, a procedure reported by Blüm and Fiedler (1965) and Fiedler (1967).

This interpretation differs from that of Mertz (1967) who offered two explanations for the persistance of fanning in *C. nigrofasciatum* after removal of all visual cues associated with the clutch. First, fanning "motivation" might be sufficient to cause its expression even in the absence of an adequate external stimulus. Secondly, the location of the spawn might serve as an effective stimulus for fanning. Mertz emphasized the importance of "motivation" as a partial explanation. The basis for this explanation was his finding that fanning tended to "overshoot" the normal level when the appropriate stimulus again became available. Both of the above factors may contribute to the persistence of fanning. However, they do not adequately explain its two to three day persistence in the absence of the appropriate stimulus which was found here for some females. A slow hormonal change underlying the fanning decrement might be a more parsimonious explanation.

The non-choice situation was designed to avoid the confounding effects of a temporal visual change in the natural eggs. This occurred in the choice situation where natural eggs were left in view at the same time with the simulated "eggs." The data seem to support the conclusion that egg size is one of the more important factors which influence the egg care behavior of *C. nigrofasciatum* females. This is emphasized even more by the fact that the artificial "eggs" differed significantly in shape from the natural ones in that they approached roundness much more closely (Figure 6). As predicted, the sight of "eggs" similar in size to the natural did not appreciably alter the organization or magnitude of fanning. There was no decrease in fanning toward natural sized simulated "eggs" (compared with the real) despite shape differences. It did, however, result in the absence of the beat and duration decrement at hatching and in a prolongation of fanning. "Eggs," either larger or smaller, were disciminated. Their exchange for the real eggs resulted in a decrease in all of the fanning measurements except tempo. This decrease was such that the counts in these groups approached similarity to those for the blank slate "control," where no eggs were present.

Larger "eggs" caused a more marked decrease in fanning than did smaller "eggs." This most likely was due to the fact that the larger "eggs" were larger than the natural by a factor of about five, whereas the smaller "eggs" were smaller only by a factor of one-half. Removal of the clutch and its replacement with an identical slate minus real or artificial spawn shortly after spawning resulted in the most rapid decrement in fanning. These results are compatible with those of Collins (1965) who found "Total rejection of heterospecific eggs . . ." when eggs of *Tilapia sparrmani* were exchanged for those of *Aequidens latifrons* during any stage of the fanning phase of either species.

The eggs of these two species, as described by Collins, are vastly different. Not only do they differ in size and shape (see Appendices 1 and 2), but in color as well. The data are also in accord with the reciprocal spawn exchange performed by Myrberg (1964) and Greenberg (1961, 63) with several cichlid species. These workers achieved acceptance only between species whose eggs are visually quite similar. Collins (op. cit.) further stated that the only instance of egg acceptance he obtained was in two cases where Aequidens were given Hemichromis eggs. Again, of the three species tested these two have eggs approximately similar in shape and size.

It should be mentioned that other workers (e.g., Myrberg, 1966) have cited spawn configurational differences as possibly influencing spawn recognition. Collins (1965) remarked that ". . . no such differences were noted in the three species studied. . . ." As already stated, Figure 1 shows differences in spawn configuration between Hemichromis bimaculatus and C. nigrofasciatum. Although it is unlikely that this contributes a major effect in the control of fanning in most species, it is still a possible minor factor in spawn recognition.

Natural eggs (at 26°C) hatch after about 72 hours. This event, alone, or in combination with visual and/or chemical changes in the eggs prior to hatching, has an effect upon egg care behavior. At the time of hatching, fanning decreases abruptly, while nipping increases just as abruptly (Figure 17). Neither of these changes was observed in the situations where artificial eggs were used. Instead there was a gradual decline and disappearance of fanning. No increase in nipping frequency was observed. These differences imply some sort of influence of a change in the natural eggs upon the egg care behavior. The major change which occurred involved darkening due to the development of chromatophores in the embryo as hatching approached. Concomitant with this change is a possible increase in embryonic movements. It could be hypothesized that some aspect of both or either of these changes reaches a threshold level and releases the high frequency of nipping at hatching. A simultaneous inhibition of fanning might also be brought about. Another possible factor is a chemical change postulated to be associated with the breakdown of the egg membranes (Myrberg cited by Mertz, 1967). Nipping itself may be a behavioral adaptation aiding the larvae to emerge from the chorionic membranes.

The results also indicate that the presence of simulated "eggs" not only causes _C_. _nigrofasciatum_ considerably to extend its period of egg care but also appears to lengthen the time between spawns. Thus, the continued presence of eggs which neither hatch, nor present a temporal visual change, appears to maintain the female in an extended egg care phase of parental behavior. Such lability, although not as extreme, was noted in the fanning phases of T. sparrmani and A. latifrons by Collins (1965). One might postulate a mechanism by which the visual presence of eggs or larvae inhibits the spawning readiness of the female. Unfortunately the data are not sufficiently extensive to carry such an hypothesis very far.

The data from the spawn site choice situation suggest two conclusions. First, they mitigate against the suspicion that the simulated "eggs" were not treated as eggs, but rather, as artifacts. If treated as artifacts they would most likely have been removed by the females. This would occur when a pair becomes ready to spawn. Secondly, these data indicate that prior presence of eggs influences a female to deposit her eggs in the same place. This might possibly suggest a mechanism which limits substrate spawning cichlids to depositing eggs at a particular spot rather than over a diffuse area. Further work is indicated regarding this point. For example, one question concerns the minimum number of eggs which could bias the female's choice of depositing more eggs in a specific spot.

When several dimensions of fanning and their possible interactions are examined, no relationship was noted between either the interval preceding or the interval following a bout and the duration of that bout. This is identifical with the findings of Mertz (1967). Thus, absence of stimulation by the eggs, when the females left the lean-to, did not result in an increase or decrease in fanning upon re-exposure to the eggs. "Motivation" for fanning does not build up during inter-bout intervals. It is possible, however, that some other dimension of fanning is affected by the duration of the inter-bout intervals. For example, either the strength of the fanning beat or the tempo might be correlated with the duration of either the interval preceding or succeeding a bout. Of these, the former was not measured, and the latter was not measured precisely enough. There were no apparent relationships between duration of bouts and the speed of fanning, in that females with a propensity to fan for a long time did not fan any faster than females exhibiting low measures of fanning beats and short bout durations. In examining fanning beats as a function of bouts, however, an unexpected relationship was found. Whereas one might expect bouts and fans to be directly proportional, this was only partially the case. Bouts and fanning beats were inversely proportional at the high beat levels, but directly related at low levels. The change in this relationship occurred at about 200 beats per 10 minutes across all egg groups. As Figure 18 shows, the two types of relationships occurred at different stages of the egg care cycle. The inverse relationship occurred during the early stages; the direct correlation during the later stages. In Badis, Barlow (1964) found a general negative correlation between bout duration and tempo. The exception to this was the results for day one. On day one he found that above 4 beats per second, duration and bouts were inversely related, while below this tempo the relationship was reversed. A possible reason for this observation is as follows. During the first twenty-four hours after egg deposition the motivation for egg care is high. In fact, some females fan almost continuously during this time. To get a high number of bouts, there must be intervals. These represent time taken away from fanning. Later in the cycle the female spends less and less time fanning, and the intervals are far longer than the bouts. Therefore each bout represents some beats added to the basic nothing: more bouts, more beats.

On the Function of Fanning

Baerends and Baerends van Roon (1950) pointed out that cichlid eggs succumb to fungal infection if removed from the parents. This appears to have been demonstrated in the case of mouth brooding cichlids (Shaw and Aronson, 1950). Baerends and Baerends van Roon, however, concluded that fanning prevents fungal spores from settling on the clutch. This may well be a partial function of fanning in cichlids. The high degree of success with regard to the development of the artificially reared eggs, however, appears to somewhat mitigate against this hypothesis at least in the case of _C_. _nigrofasciatum_. Also, as cited by

Mertz (1967), and confirmed by this study, some of the eggs developed fungal infections while receiving parental tending. He further found (op. cit.) that C. nigrofasciatum deposits eggs preferentially on a vertical surface. He states ". . . it is difficult to envision sedimentation as a factor critical to the survival of the young when clutches are preferentially deposited on a vertical surface." In this study, the lean-to presented three surfaces (excluding the outside) for egg deposition (Table 12, page 75). One of these was vertical, another horizontal, and one was an angle between the two others. The latter was so arranged that in order to spawn on it the female had to deposit her clutch nearly in an upside down position. Spawnings occurred with nearly equal frequency on all of these surfaces. This contradicts the findings of Mertz. It should be further mentioned that fungae are only secondary invaders at least on the eggs of mouth-brooding Tilapia raised extra-orally (Shaw and Aronson, 1950). The initial cause of egg decay is bacterial infection. It might be more parsimonious, in support of sedimentation, to hypothesize that fanning in C. nigrofasciatum functions to maintain a more or less continuous water current over the clutch and that this prevents the settling of an excessive bacterial population.

In fishes whose parental repertoire includes fanning one of the major functions suggested is facilitation of gaseous exchange between the embryo and its environment. The total metabolic activity of the brood increases with the approach of hatching. This results in an increase in fanning which presumably removes metabolites and supplies the clutch with an increased oxygen source (van Iersel, 1953, Morris, 1954, 1958, Sevester, 1961, Barlow, 1964, and Mertz and Barlow, 1966). The species tested included *Gasterosteus aculeatus*, *Pungitus pungitus*, *Badis badis*, *Florinella japonica*, and *Cottus gobi*. The shape of the baseline as well as the simulated "egg" fanning curves, suggests that fanning in *C. nigrofasciatum* is not regulated in accordance with the metabolic requirements or output of the young. This supported the findings of Mertz (1967) where the temporal structure of fanning did not correlate with the supposed temporal changes needed for gaseous exchange. In fact, fanning dropped rapidly at hatching when it presumably is needed the most. Furthermore, according to Mertz (1967), when olfactory cues are removed from the natural eggs, fanning is not affected. This was confirmed here by the behavior towards the simulated group of "eggs" similar to the natural. It therefore appears unlikely that fanning in most cichlid species serves the function of "aerating" the clutch.

It seems probable that fanning contributes to the survival of the young. It has yet to be demonstrated, however, even on a statistical basis, that fanners produce more surviving young than non-fanners. This could easily be tested with eggs raised under various artificially tended conditions. These could then be compared with a baseline of parentally raised eggs. This would provide little, if any, information regarding the immediate functions of fanning which might be subtle. For example, a slight selective advantage might be accorded to fanners with regard to the prevention of sedimentation of detrimental bacteria or protozoa which are a portion of the microecology of this species' habitat.

The egg care system of _C_. _nigrofasciatum_ is extremely complex. Understanding complex systems such as the behavior of whole organisms is best handled by dissecting the components into simply analyzable parts. One can then resynthesize these into a meaningful whole. This philosophy was the one underlying the above study. In conclusion, it might be added that, at present, the findings are at best a partial picture. To gain a more complete understanding of the multivariate egg care interaction with the spawn stimuli, one needs to look at more of the critical spawn parameters. One of these might be color. Within the genus _Tilapia_ alone there is great variability in egg color. For example, _T_. _macrocephala_ eggs are yellow-orange, _T_. _nilotica_ light yellow to yellow, _T_. _galilaea_ olive green. The species _T_. _tholloni_, _T_. _zillii_, and T. *guinensis*, all substrate spawners, range from green to brown (Dambach, 1963). Finally, configurational differences as already mentioned plus egg shape, density, and texture are factors which could take part in shaping the egg care system in C. *nigrofasciatum*, especially with regard to fanning. Also, the results presented here indicate that egg care behavior is influenced by internal factors expressed in the presence of appropriate stimuli of the spawn. These are visual. The causal factors underlying the expression of these behaviors are not known as yet. It is strongly suggested that these may be, at least in part, hormonal in nature.

SUMMARY

1. This study was designed to test the hypothesis that egg size influences various parameters of parental (egg care) behavior in Cichlasoma nigrofasciatum either by increasing, decreasing, or extinguishing them.

2. The parameters examined were fanning, nipping, and duration of egg care.

3. The effects of different egg sizes upon these parameters were recorded under two experimental and three control conditions. The former involved presenting a female with artificial spawns as similar to the natural as possible except in egg size in exchange for her own spawn. Three groups of egg sizes were presented: similar, larger, and smaller than natural. Here females had no opportunity for making a choice.

4. It was found that "eggs" larger or smaller than natural, when substituted for the natural spawn, specifically caused an appreciable decrease in several parameters of fanning--duration and beats. Removal of the spawn resulted in the most profound decrement in fanning. No difference was noted between artificial "eggs" similar to the natural and the natural in terms of the fanning parameters measured. This was as expected. This supports the

hypothesis that egg size influences egg care behavior in *C. nigrofasciatum*.

5. When the natural eggs and simulated "eggs" of the same size were presented simultaneously (choice situation), the females were unable to discriminate between the two until after approximately one day of the fanning cycle.

6. Certain details of the structure of fanning were also determined. It was found that the relationship between fanning beats and bouts was inverse early in the fanning cycle and became direct later on.

7. The presence of artificial "eggs" (which do not hatch) was expected to prolong the interspawn interval. The results appeared to support this, but were not statisticially significant.

8. Spawns were usually deposited under the lean-to and were randomly placed with regard to the three available possibilities. The presence of artificial "eggs" on a particular section of the lean-to increased the probability that the succeeding spawn would occur in the same place.

9. These results are related to factors influencing organization, and functions of egg care behavior in *C. nigrofasciatum*.

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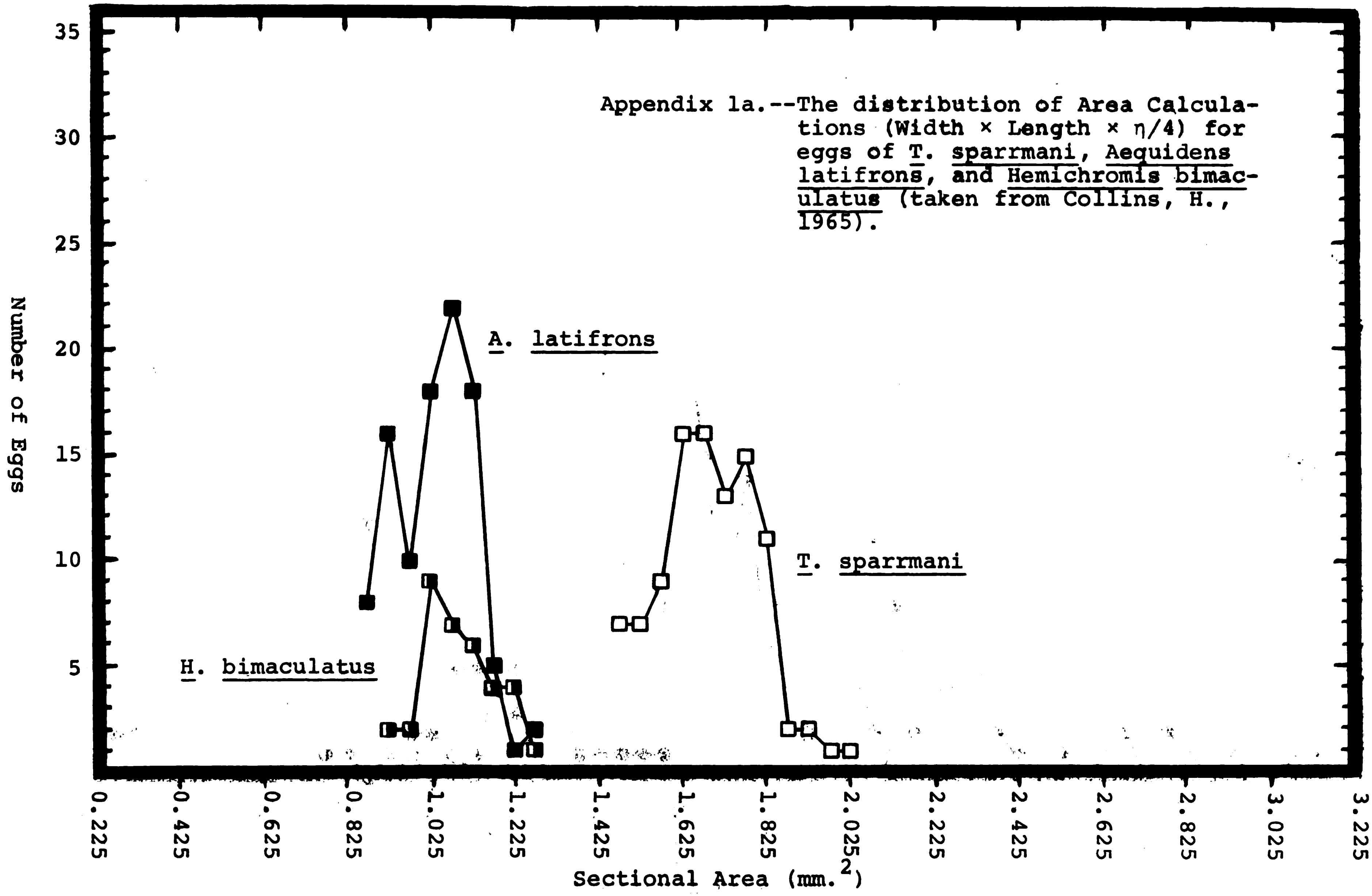
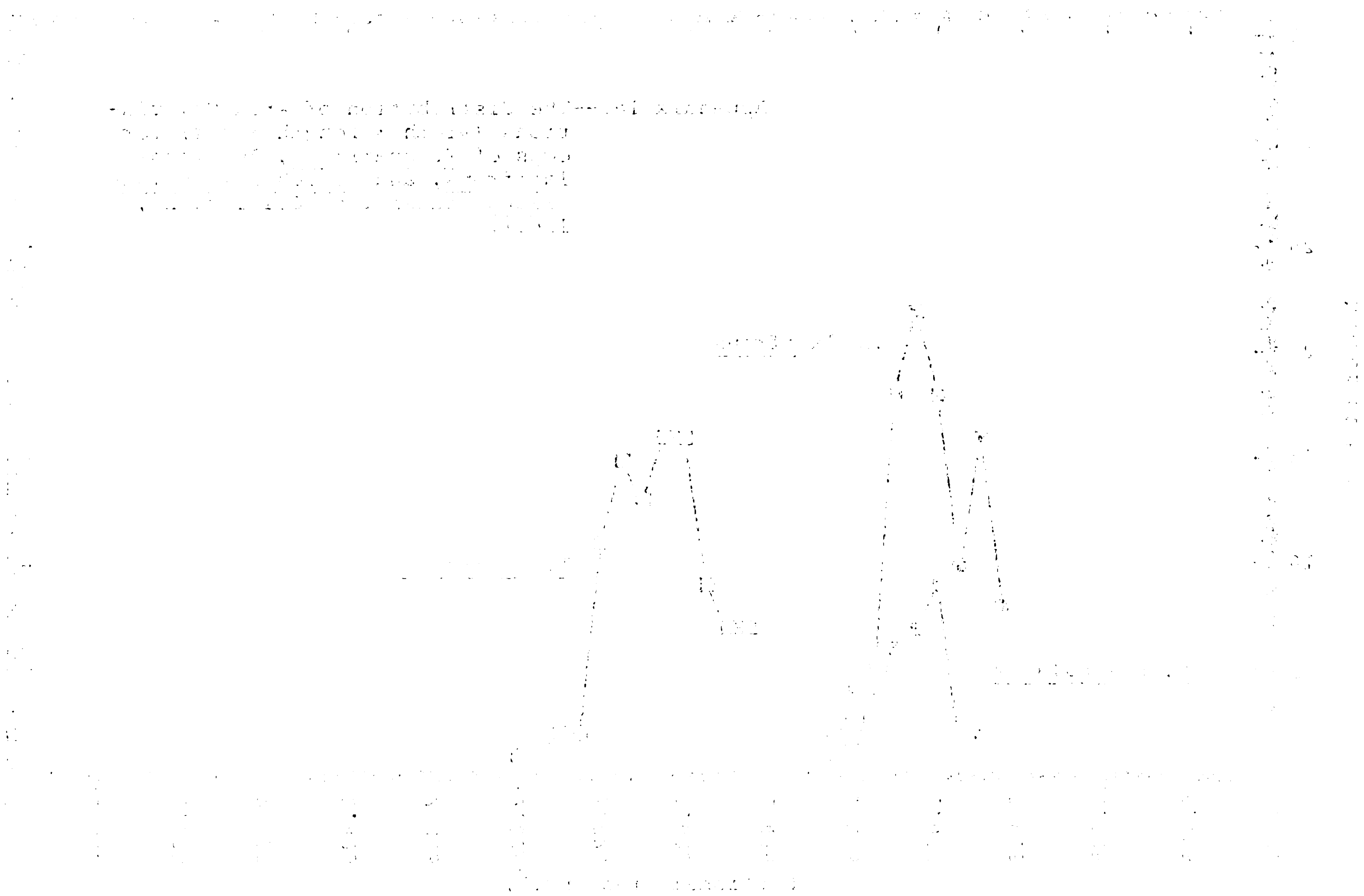
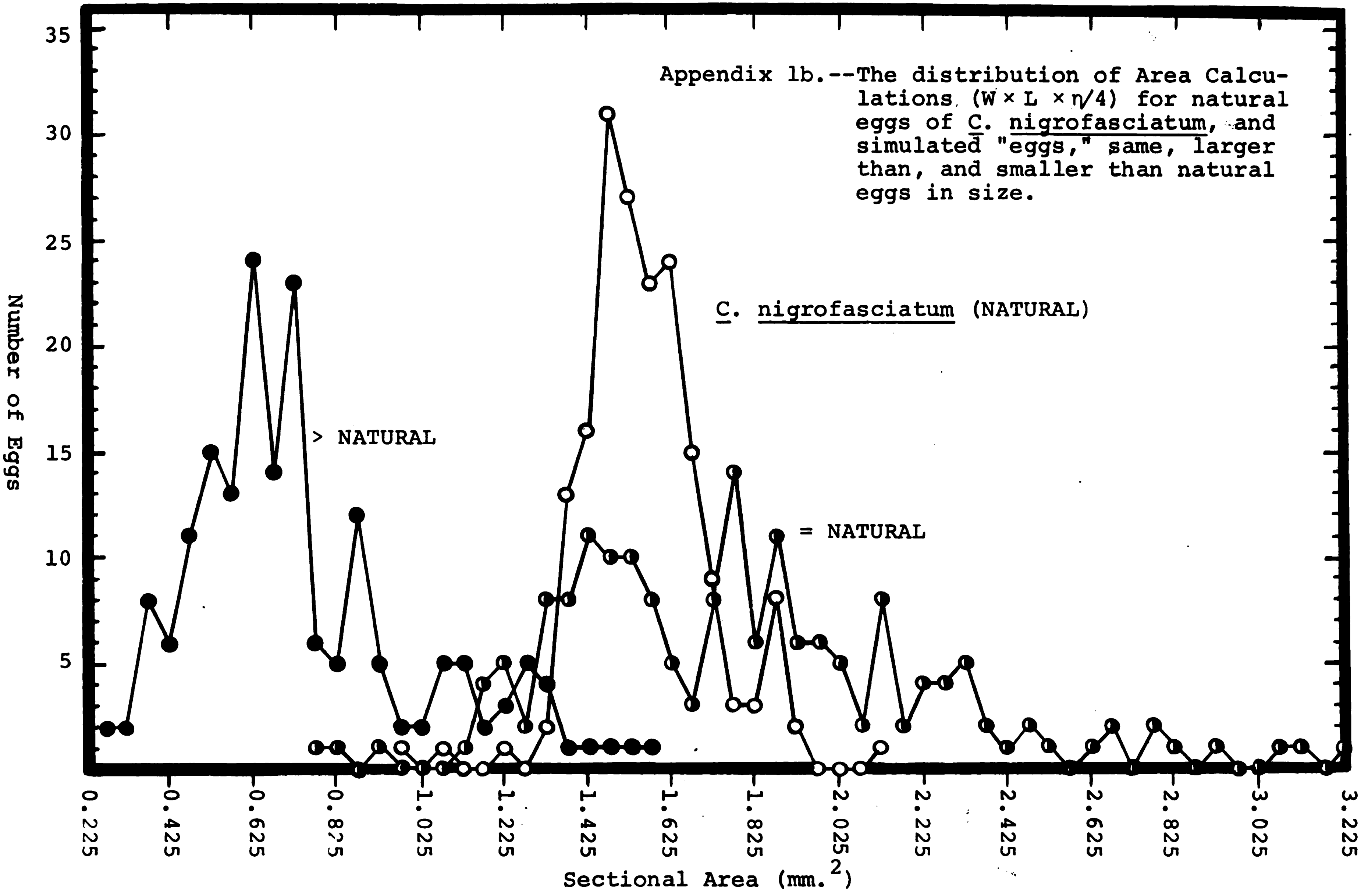
APPENDICES

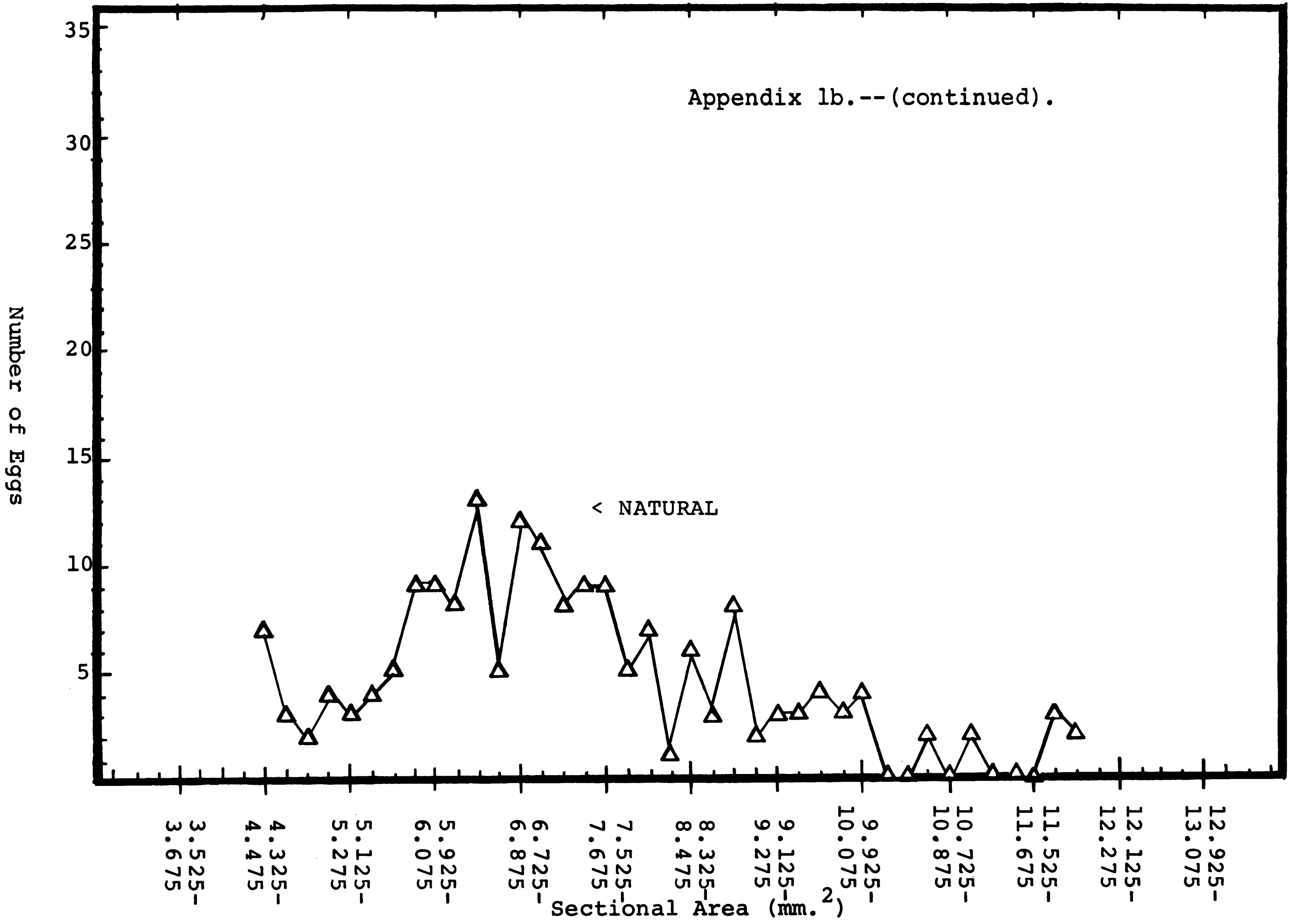


Appendix 1a.--The distribution of Area Calculations (Width × Length × η/4) for eggs of T. sparrmani, Aequidens latifrons, and Hemichromis bimaculatus (taken from Collins, H., 1965).

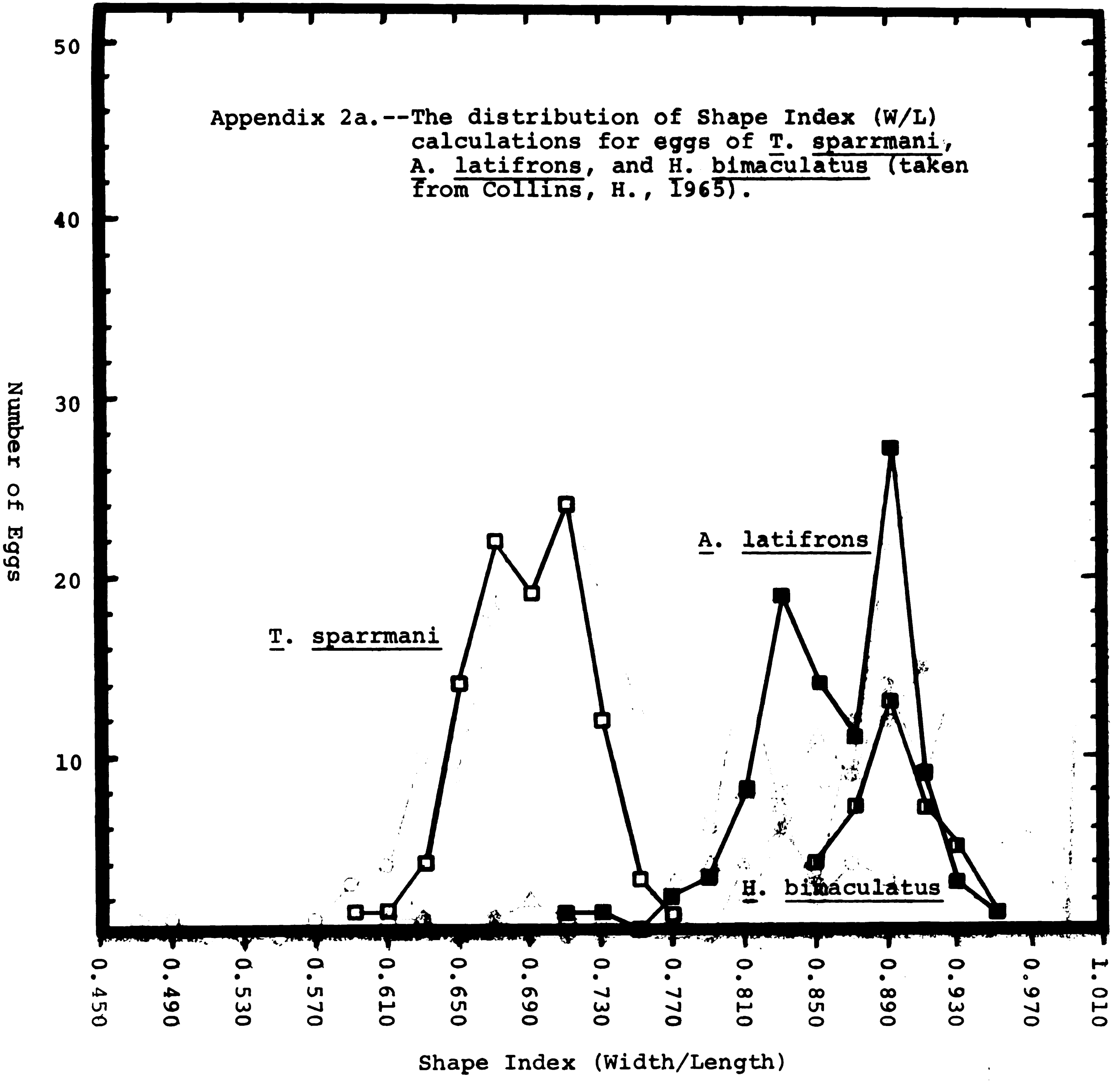




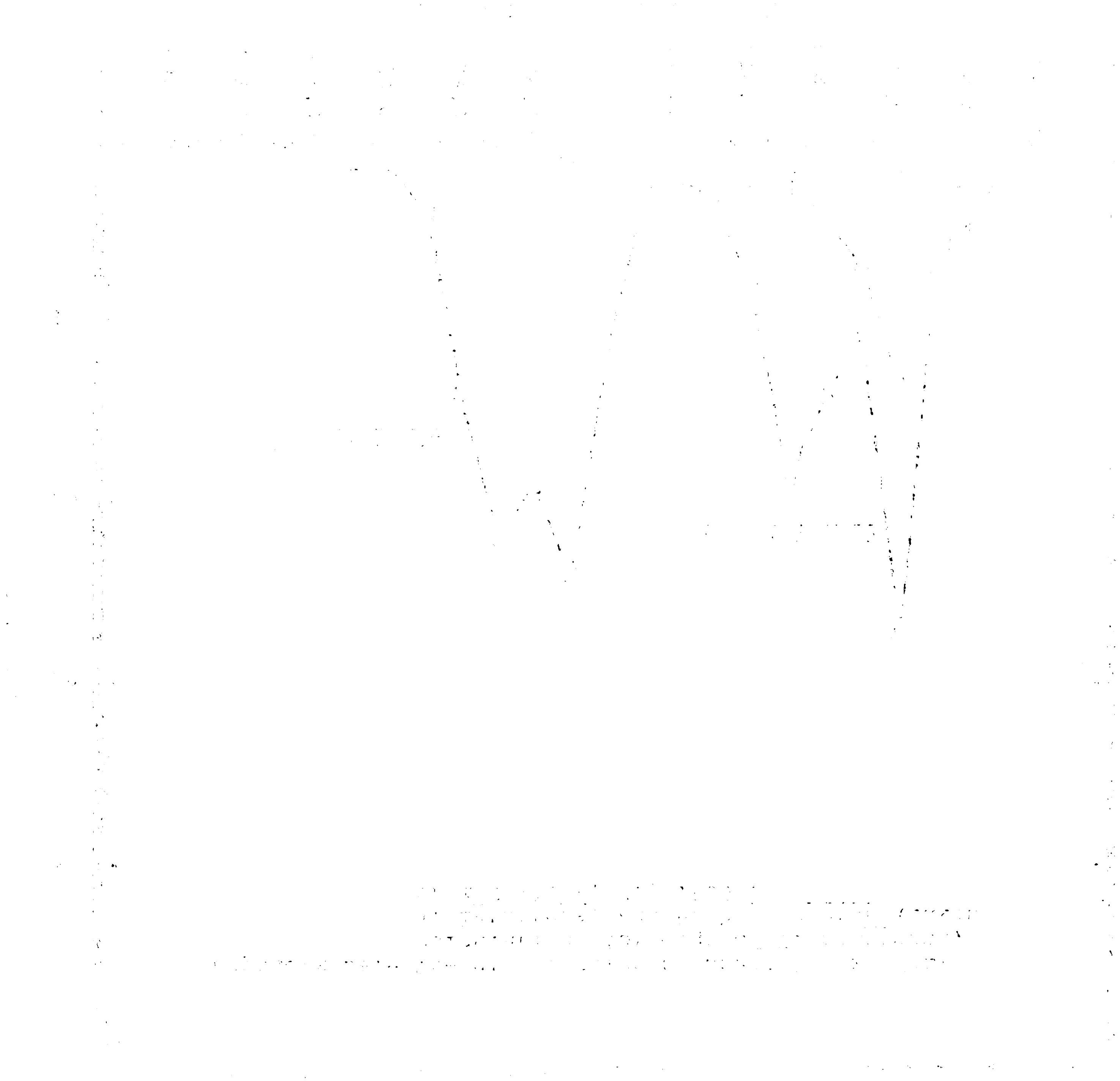
Appendix 1b.--The distribution of Area Calculations (W × L × π/4) for natural eggs of *C. nigrofasciatum*, and simulated "eggs," same, larger than, and smaller than natural eggs in size.



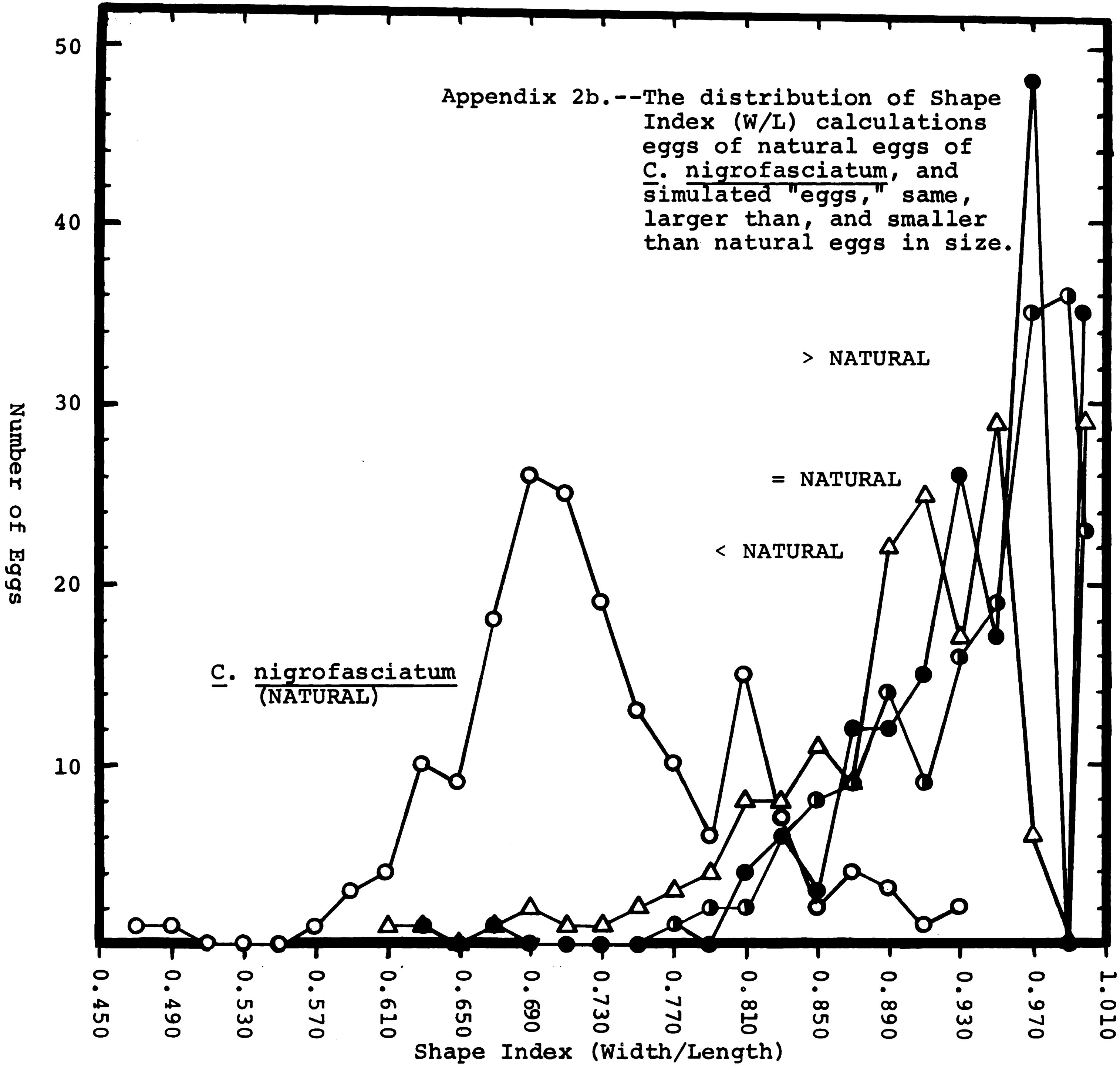
Appendix 1b.--(continued).



Appendix 2a.--The distribution of Shape Index (W/L) calculations for eggs of T. sparrmani, A. latifrons, and H. bimaculatus (taken from Collins, H., 1965).



Appendix 2b.--The distribution of shape



Appendix 2b.--The distribution of Shape Index (W/L) calculations eggs of natural eggs of <u>C. nigrofasciatum</u>, and <u>simulated</u> "eggs," same, larger than, and smaller than natural eggs in size.

Appendix 3.--Basic statistics for egg size (Sectional Area in mm^2) and shape (Shape Index) for each of the egg groups.

Female No.		Natural Eggs							"Eggs" Same Size as Natural						
		Σx	Σx^2	s_x^2	s_x	SE	$\bar{x}$	N	Σx	Σx^2	s_x^2	s_x	SE	$\bar{x}$	N
8	A[a]	45.38	69.37	0.025	0.001	0.028	1.51	30	53.16	101.91	0.230	0.008	0.087	1.78	30
	SI[b]	21.32	15.31	0.005	0.001	0.010	0.71	30	28.70	27.47	0.001	0.000	0.000	0.96	30
30	A	45.08	68.28	0.018	0.000	0.024	1.50	30	45.80	74.62	0.162	0.005	0.073	1.53	30
	SI	20.95	14.76	0.004	0.001	0.010	0.70	30	28.03	26.28	0.003	0.000	0.010	0.93	30
22	A	45.61	69.57	0.008	0.000	0.014	1.52	30	55.13	109.43	0.280	0.009	0.096	1.84	30
	SI	22.51	17.08	0.007	0.000	0.014	0.75	30	28.38	26.98	0.005	0.000	0.010	0.95	30
29	A	53.56	96.06	0.016	0.000	0.022	1.79	30	57.15	115.78	0.238	0.008	0.089	1.91	30
	SI	21.68	15.97	0.010	0.001	0.017	0.72	30	27.64	25.55	0.003	0.000	0.010	0.92	30
32	A	45.96	70.65	0.008	0.000	0.011	1.53	30	52.27	93.28	0.076	0.003	0.050	1.74	30
	SI	21.90	16.09	0.004	0.000	0.010	0.73	30	28.38	26.92	0.002	0.000	0.000	0.95	30
38	A	44.32	65.62	0.005	0.000	0.010	1.48	30	54.07	102.01	0.159	0.005	0.072	1.80	30
	SI	21.39	15.35	0.004	0.000	0.010	0.71	30	27.61	25.57	1.005	0.000	0.010	0.92	30
	$\bar{\bar{X}}_A$						1.56							1.77	
	SE_A					0.046							0.052		
	$\bar{\bar{X}}_{SI}$						0.72							0.94	
	SE_{SI}					0.000							0.000		

Appendix 3--Continued.

Female No.		"Eggs" Larger than Natural							"Eggs" Smaller than Natural						
		Σx	Σx^2	S_x^2	S_x	SE	$\bar{x}$	N	Σx	Σx^2	S_x^2	S_x	SE	$\bar{x}$	N
8	A	234.84	1887.41	1.691	0.056	0.237	7.83	30	26.74	26.61	0.095	0.003	0.054	0.890	30
	SI	28.51	27.17	0.003	0.000	0.000	0.95	30	27.82	25.90	0.003	0.000	0.000	0.927	30
30	A	203.77	1507.93	4.270	0.142	0.377	6.79	30	24.26	22.90	0.113	0.003	0.054	0.808	30
	SI	28.74	27.60	0.002	0.000	0.000	0.96	30	27.24	24.91	0.006	0.000	0.000	0.907	30
22	A	211.94	1615.09	4.063	0.135	0.368	7.07	30	24.24	22.12	0.087	0.003	0.054	0.808	30
	SI	28.58	27.30	0.002	0.000	0.000	0.95	30	26.01	22.84	0.010	0.000	0.017	0.867	30
29	A	233.99	1902.57	2.781	0.093	0.304	7.80	30	18.62	12.64	0.038	0.001	0.035	0.621	30
	SI	28.65	27.39	0.001	0.000	0.000	0.96	30	27.26	24.93	0.006	0.000	0.010	0.909	30
32	A	230.58	1833.11	2.100	0.070	0.265	7.69	30	18.50	12.13	0.025	0.001	0.028	0.616	30
	SI	27.85	26.30	0.016	0.001	0.022	0.93	30	26.60	23.79	0.007	0.000	0.014	0.887	30
38	A	182.12	1153.27	1.644	0.055	0.234	6.07	30	19.67	13.75	0.029	0.001	0.030	0.656	30
	SI	28.16	26.50	0.002	0.000	0.000	0.94	30	27.29	24.92	0.003	0.000	0.010	0.910	30
	$\bar{\bar{X}}_A$						7.21							0.730	
	SE_A					0.285							0.047		
	$\bar{\bar{X}}_{SI}$						0.95							0.900	
	SE_{SI}					0.000							0.000		

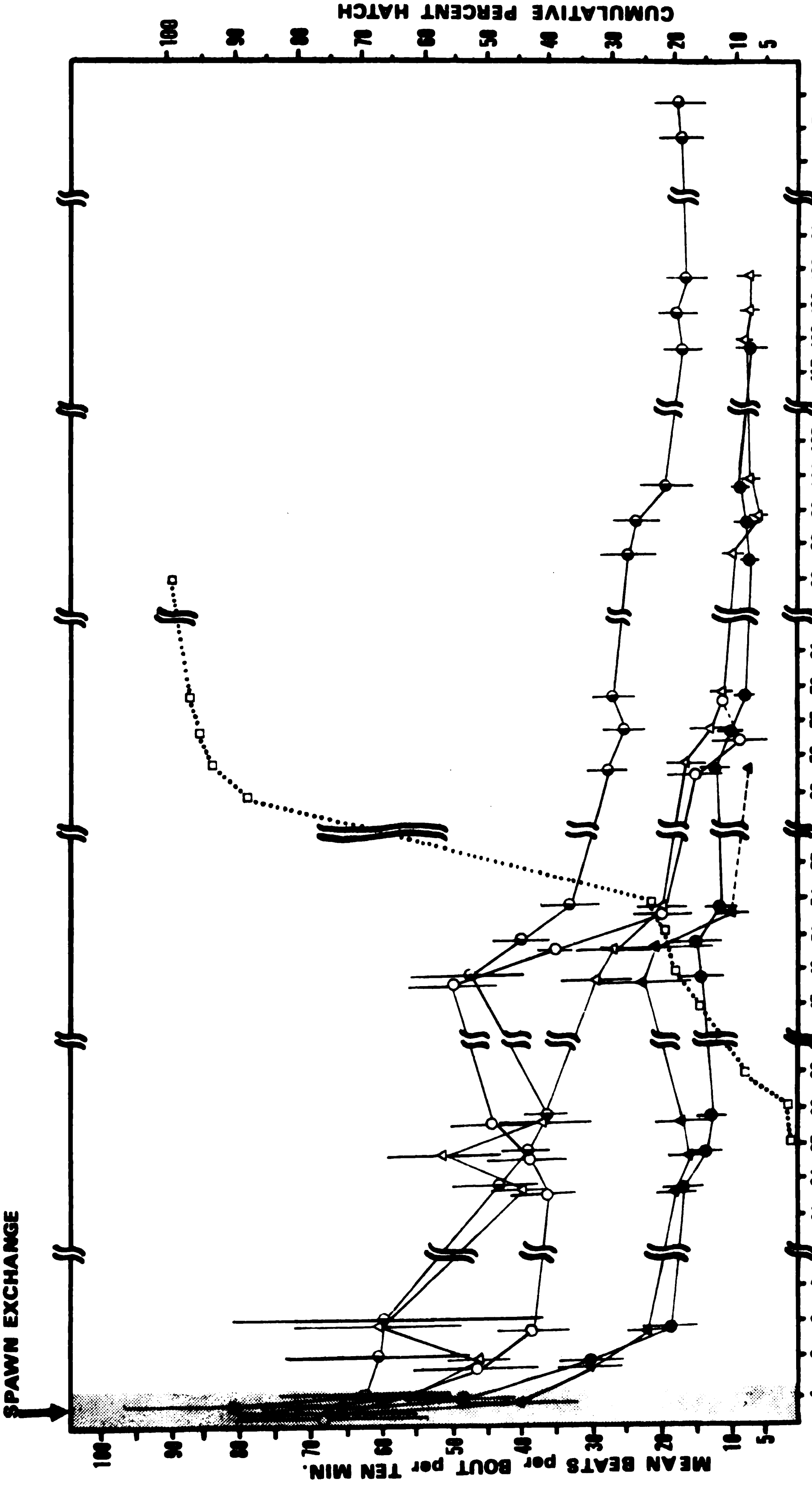
[a] Sectional Area.

[b] Shape Index.

Appendix 4.--The mean number of fanning beats per bout per ten minute observation period taken three times per day for all groups. Horizontal lines are one standard error on either side of the mean. Arrow indicates time of spawn exchange; stippled region indicates the ten minute baseline observation prior to spawn exchange.

Legend:

○ ———— baseline
● ———— > natural "egg" group
◐ ———— = natural "egg" group
△ ———— < natural "egg" group
▲ ———— blank slate "control" group
□ .... cumulative percent hatch


SPAWN EXCHANGE
MEAN BEATS per BOUT per TEN MIN.
100
90
80
70
60
50
40
30
20
10
5
CUMULATIVE PERCENT HATCH
100
90
80
70
60
50
40
30
20
10
5
TIME (hours)
1
3
6
9
21
24
27
30
33
45
48
51
54
57
69
72
75
78
81
93
96
99
102
105
117
120
123
126
129
144
147
150

Appendix 5.--The mean duration of fanning per bout per ten minute observation period taken three times per day for all groups. Horizontal lines are one standard error on either side of the mean. Arrow indicates time of spawn exchange; stippled region indicates the ten minute baseline observation prior to spawn exchange.

Legend:

○ ——— baseline

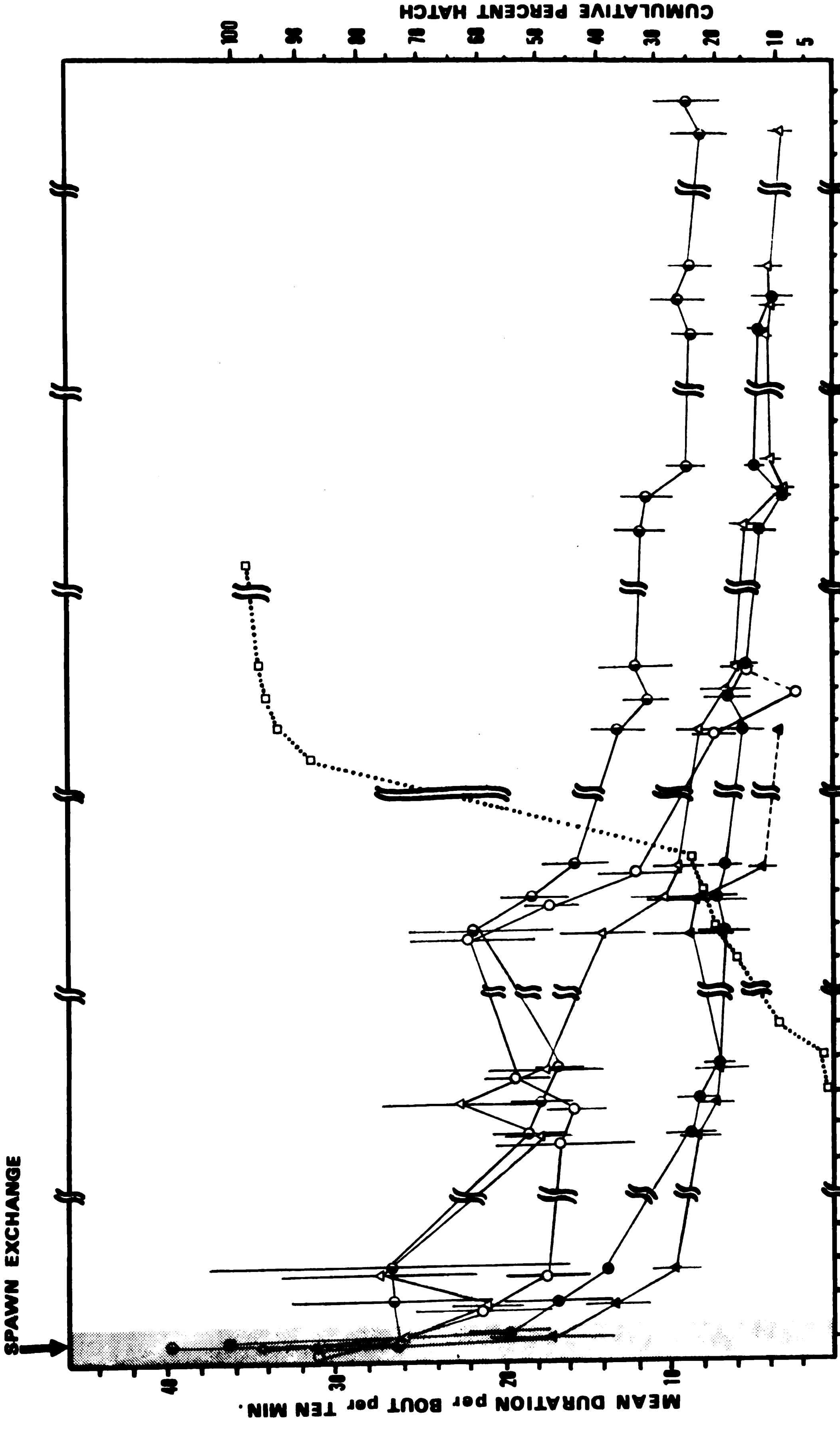
● ——— > natural "egg" group

◐ ——— = natural "egg" group

△ ——— < natural "egg" group

▲ ——— blank slate "control" group

□ .... cumulative percent hatch


SPAWN EXCHANGE
MEAN DURATION per BOUT per TEN MIN.
10
20
30
40
CUMULATIVE PERCENT HATCH
5
10
20
30
40
50
60
70
80
90
100
TIME (hours)
1
3
6
9
21
24
27
30
33
45
48
51
54
57
69
72
75
78
81
93
96
99
102
105
117
120
123
126
129
144
147
150

Appendix 6.--Basic statistics for fanning beats. Each set of data represents the result of a ten minute observation taken three times per day. Sample size, mean, variance, standard deviation, and standard errors are given for each of the five groups.

Fanning beats per 10 minutes									
Baseline					Blank Slate "Control"				
N	$\bar{x}$	S_x^2	S_x	SE	N	$\bar{x}$	S_x^2	S_x	SE
13	1032	72960	270.112	74.917	13	1009	77595	278.559	77.256
13	825	77153	277.763	77.039	13	699	150539	387.994	107.612
13	720	55976	236.592	65.620	13	556	109463	330.853	91.763
13	710	49205	221.822	61.523	13	342	61105	247.195	68.561
13	734	42171	205.357	56.957	13	266	39111	197.766	54.851
13	796	36013	189.770	52.634	12	261	54470	1100.632	67.373
13	849	23951	154.763	42.924	7	303	94706	307.744	116.318
13	748	6750	82.160	22.787	5	387	112795	335.849	150.201
13	340	4876	220.826	61.247	4	347	174359	417.563	208.782
6	258	29034	170.394	69.566	4	50	4121	64.194	32.097
2	89	1568	784.000	28.000	1	22	000000	0000.000	000.000
1	193	00000	000.000	00.000	0				
0									

Appendix 6.--Continued.

Same Size as Natural					Larger than Natural					Smaller than Natural				
N	$\bar{x}$	S_x^2	S_x	SE	N	$\bar{x}$	S_x^2	S_x	SE	N	$\bar{x}$	S_x^2	S_x	SE
13	1080	68736	262.176	72.716	13	1160	49541	3810.857	61.732	13	1058	23241	1787.737	42.281
13	1054	95670	309.306	85.787	13	923	73237	5633.591	75.057	13	908	43215	3324.191	57.655
13	866	76135	275.925	76.529	13	550	72416	5570.455	74.635	13	803	37266	2866.583	53.540
13	739	328599	573.235	158.989	13	394	52752	4057.878	63.701	13	765	45974	3536.474	59.468
13	735	46160	214.848	59.589	13	289	64033	4925.634	70.183	13	692	35763	2751.021	52.450
13	689	46659	216.006	59.910	12	249	58316	4859.653	69.711	13	653	81009	6231.481	78.940
13	712	38901	197.233	54.703	11	190	29957	2723.383	52.186	13	561	64086	4929.665	70.213
13	731	59176	243.361	64.412	11	168	40985	3725.942	61.040	13	480	52942	4072.476	63.816
13	661	53934	232.237	60.117	10	203	58867	5886.712	76.725	13	385	64679	4975.322	70.536
13	563	46982	216.753	49.548	10	93	9767	976.646	31.251	13	329	50727	3902.077	62.467
13	489	31914	178.644	71.052	5	119	10804	2160.800	46.484	13	248	31353	2411.736	49.109
13	431	65627	256.177	73.758	4	110	6688	1672.000	40.890	13	227	37200	2861.508	53.493
13	414	23739	265.936	75.659	4	108	3642	910.396	30.173	12	216	4183	3484.381	59.029
13	395	74414	272.789	55.636	4	70	450	112.417	10.603	11	129	4488	407.972	20.198
13	316	40239	200.596	65.492	3	47	163	54.333	7.371	11	82	2357	214.299	14.639
13	265	55591	236.130	53.341	3	74	1814	604.778	24.592	10	55	1342	134.201	11.585
12	205	133377	184.780	53.341	2	86	18	9.000	3.000	8	61	492	61.480	7.841
12	189	27540	165.953	47.906	2	89	6845	3422.250	58.500	7	59	203	29.000	5.385
11	164	31038	176.177	53.120	1	76	00000	0000.000	00.000	7	54	815	116.401	10.789
9	162	409848	202.447	67.482	0					5	61	1637	327.340	18.093
8	144	4142	203.530	71.959						2	36	162	81.000	9.000
5	197	101102	229.326	102.561						0				
4	187	25547	6386.750	79.917										
4	172	25385	6346.187	79.662										
3	238	16914	5638.222	75.088										
3	220	84508	28169.333	167.837										
1	120	000000	0000.000	000.000										
1	64	000000	0000.000	000.000										
1	48	000000	0000.000	000.000										

Appendix 7.--Basic statistics for bouts per ten minute observation period. Each set of data represents the result of a ten minute observation recorded three times per day. Sample size, mean, variance, standard deviation, and standard errors are given for each of the five groups.

Bouts per 10 minutes									
Baseline					Blank Slate "Control"				
N	$\bar{x}$	S_x^2	S_x	SE	N	$\bar{x}$	S_x^2	S_x	SE
13	19.154	72.643	8.523	2.364	13	17.769	28.860	5.372	1.490
13	23.615	129.427	11.377	3.155	13	19.462	52.104	7.218	2.002
13	20.692	20.564	4.535	1.258	13	18.231	30.528	5.525	1.532
13	21.615	32.760	5.724	1.587	13	15.154	18.976	4.356	1.208
13	22.615	43.094	6.565	1.821	13	13.923	13.413	3.662	1.016
13	20.154	28.310	5.321	1.476	12	14.083	32.084	5.664	1.635
13	20.385	49.257	7.018	1.947	7	14.857	62.478	7.904	2.988
13	22.615	23.094	4.806	1.333	5	14.400	89.800	9.476	4.238
13	19.308	52.401	7.239	2.008	4	11.500	121.000	11.000	5.500
6	18.167	39.370	6.275	2.562	4	4.250	16.250	4.031	2.016
2	13.500	24.500	12.250	3.500	1	3.000	000.000	00.000	0.000
1	18.000	000.000	00.000	0.000	0				
0									

Appendix 7.--Continued.

Same Size as Natural					Larger than Natural					Smaller than Natural				
N	$\bar{x}$	s_x^2	s_x	SE	N	$\bar{x}$	s_x^2	s_x	SE	N	$\bar{x}$	s_x^2	s_x	SE
13	17.769	77.1935	8.786	2.437	13	19.308	23.897	1.838	1.356	13	17.077	40.077	3.083	1.756
13	18.769	99.6936	9.934	2.755	13	21.345	47.756	3.674	1.917	13	20.308	56.564	4.351	2.086
13	18.077	40.4111	6.357	1.763	13	19.769	34.692	2.669	1.634	13	18.846	20.641	1.588	1.260
13	14.462	29.2706	5.410	1.501	13	16.385	28.090	2.161	1.470	13	16.923	47.577	3.660	1.913
13	19.385	32.2570	5.680	1.575	13	13.769	50.359	3.874	1.968	13	18.692	16.397	1.261	1.123
13	18.692	16.0644	4.008	1.112	12	13.250	58.932	4.911	2.216	13	16.923	33.577	2.583	1.607
13	20.308	11.5681	3.401	0.943	11	12.909	49.091	4.463	2.113	13	18.308	32.231	2.479	1.575
13	18.154	33.8095	5.815	1.613	11	11.273	57.018	5.183	2.277	13	18.538	24.936	1.918	1.385
13	17.077	13.7443	3.707	1.028	10	11.700	43.122	4.312	2.077	13	19.769	21.192	1.630	1.277
13	17.538	11.9382	3.455	0.958	10	6.700	12.678	1.268	1.126	13	19.231	28.526	2.194	1.481
13	18.615	9.9265	3.151	0.874	5	9.800	19.200	3.840	1.960	13	16.000	29.833	2.295	1.515
13	17.154	26.8094	5.178	1.436	4	9.000	16.667	4.167	2.041	12	18.000	53.293	4.439	2.107
13	17.846	52.3098	7.233	2.006	4	10.750	7.583	1.896	1.377	11	12.545	22.073	2.007	1.417
13	16.769	22.3601	4.729	1.311	4	9.750	10.917	2.729	1.652	11	8.273	10.818	0.983	0.992
13	13.385	10.5901	3.254	0.903	3	7.667	0.333	0.111	0.333	10	9.200	15.511	1.551	1.245
13	13.077	17.7442	4.212	1.168	3	11.667	6.333	2.111	1.453	9	18.778	10.694	1.188	1.090
12	11.333	24.0615	4.905	1.416	2	11.000	2.000	1.000	4.500	7	9.857	4.476	0.639	0.800
12	10.167	11.9712	3.460	0.999	2	11.500	40.500	20.250	4.500	7	7.571	8.286	1.184	1.088
11	9.636	15.0559	3.982	1.201	1	12.000	00.000	00.000	0.000	5	8.200	13.700	2.740	1.655
9	9.111	49.3612	7.023	2.342	0					2	6.500	12.500	6.250	2.500
8	6.750	11.0714	3.327	1.176						0				
5	9.200	48.7000	6.979	3.121										
4	11.000	53.5000	13.375	3.657										
4	12.500	36.7500	9.187	3.031										
3	15.667	29.5550	9.851	3.138										
3	12.333	108.3330	36.111	6.009										
1	10.000	000.0000	00.000	0.000										
1	8.000	000.0000	00.000	0.000										
1	6.000	000.0000	00.000	0.000										

Appendix 8.--Basic statistics for fanning duration. Each set of data represents the result of a ten minute observation taken three times per day. Sample size, mean, variance, standard deviation, and standard error are given for each of the five groups.

Duration per bout per 10 minutes									
Baseline					Blank Slate "Control"				
N	$\bar{x}$	s_x^2	s_x	SE	N	$\bar{x}$	s_x^2	s_x	SE
13	31.554	523.875	22.888	6.348	13	31.153	409.286	20.231	5.611
13	21.355	214.471	14.645	4.062	13	16.924	164.969	12.844	3.562
13	17.284	75.116	8.667	2.404	13	13.079	46.240	6.800	1.886
13	16.289	226.658	15.055	4.176	13	9.570	20.975	4.580	1.270
13	15.450	44.105	6.641	1.842	13	8.083	12.736	3.569	0.990
13	19.069	52.410	7.239	2.008	12	7.100	7.548	2.747	0.793
13	21.967	175.254	13.238	3.672	7	6.795	16.169	4.021	1.520
13	16.846	26.487	5.147	1.427	5	8.360	26.855	5.182	2.318
13	11.851	83.169	9.120	2.529	4	8.143	34.220	5.850	2.925
6	6.674	11.714	3.422	1.398	4	4.000	1.167	1.080	0.540
2	2.070	0.058	0.029	0.169	1	3.000	000.000	00.000	0.000
1	5.444	000.000	00.000	0.000	0				
0									

Appendix 8.--Continued.

Same Size as Natural					Larger than Natural					Smaller than Natural				
N	$\bar{x}$	S_x^2	S_x	SE	N	$\bar{x}$	S_x^2	S_x	SE	N	$\bar{x}$	S_x^2	S_x	SE
13	39.924	495.559	22.261	6.174	13	41.290	3045.596	234.277	15.306	13	34.330	438.780	33.752	5.810
13	26.459	263.105	16.220	4.499	13	19.495	75.195	5.784	2.405	13	25.772	283.705	21.823	4.672
13	26.543	475.001	21.795	6.045	13	16.459	133.287	10.253	3.202	13	20.785	57.320	4.409	2.100
13	26.763	1398.900	37.402	10.374	13	13.463	144.931	11.149	3.339	13	27.249	455.179	35.014	5.917
13	18.224	610.964	7.816	2.168	13	8.502	27.758	2.135	1.461	13	17.662	48.602	3.739	1.934
13	17.459	419.859	6.480	1.797	12	8.071	17.731	1.478	1.215	13	22.491	281.838	21.680	4.656
13	16.253	21.161	4.600	1.276	11	6.747	6.990	0.635	0.797	13	17.120	158.463	12.189	3.491
13	21.469	225.138	15.005	4.161	11	6.471	7.867	0.715	0.846	13	13.831	80.203	6.169	2.484
13	18.123	51.915	7.205	1.998	10	6.860	19.183	1.918	1.385	13	10.039	60.589	4.661	2.159
13	15.608	46.920	6.850	1.900	10	6.320	8.673	0.867	0.931	13	9.153	28.216	2.170	1.473
13	12.711	25.899	5.089	1.414	5	5.421	7.779	1.556	1.247	13	7.854	22.577	1.737	1.318
13	11.322	17.955	4.238	1.175	4	6.217	6.742	1.686	1.298	12	6.315	26.829	2.236	1.495
13	11.804	65.014	8.063	2.236	4	5.234	1.359	0.340	0.583	11	5.787	6.884	0.626	0.791
13	11.548	29.714	5.451	1.512	4	4.292	3.086	0.771	0.878	11	5.344	5.586	0.508	0.713
13	11.047	25.829	5.082	1.410	3	2.899	0.178	0.059	0.244	10	3.036	2.179	0.218	0.467
13	8.753	22.498	4.743	1.316	3	3.564	0.722	0.241	0.491	8	3.506	1.220	0.153	0.391
12	8.407	13.920	3.731	1.077	2	4.409	0.483	0.242	0.491	7	3.968	0.630	0.090	0.300
12	9.329	25.714	5.071	1.464	2	3.487	2.885	1.442	1.201	7	3.744	2.347	0.335	0.579
11	8.532	25.346	5.035	1.518	1	3.000	0000.000	000.000	0.000	5	3.757	3.004	0.601	0.775
9	7.956	25.544	5.055	1.685	0					2	3.097	0.853	0.426	0.653
8	8.663	34.447	5.870	2.075						0				
5	10.006	8.500	2.916	1.304										
4	8.081	8.092												
4	5.813	9.604												
3	7.818	6.158												
3	6.087	10.683	3.561	1.887										
1	7.300	000.000	00.000	00.000										
1	3.500	000.000	00.000	00.000										
1	4.333	000.000	00.000	00.000										

Appendix 9.--Basic statistics for tempo per ten minute observation period. Each set of data represents the result of a ten minute observation recorded three times per day. Sample size, mean, variance, standard deviation, and standard errors are given for each of the five groups.

Tempo per 10 minutes									
Baseline					Blank Slate "Control"				
N	$\bar{x}$	S_x^2	S_x	SE	N	$\bar{x}$	S_x^2	S_x	SE
13	2.250	0.038	0.195	0.054	13	2.183	0.046	0.215	0.060
13	2.153	0.034	0.185	0.051	13	2.380	0.093	0.305	0.085
13	2.202	0.039	0.197	0.055	13	2.304	0.031	0.176	0.049
13	2.173	0.044	0.210	0.058	13	2.168	0.046	0.215	0.060
13	2.227	0.060	0.244	0.068	13	2.155	0.098	0.314	0.087
13	2.231	0.064	0.253	0.070	12	2.124	0.098	0.314	0.091
13	2.249	0.132	0.363	0.101	7	2.450	0.091	0.301	0.114
13	2.251	0.166	0.407	0.113	5	2.522	0.192	0.438	0.196
13	2.154	0.150	0.387	0.107	4	2.255	0.225	0.475	0.237
6	2.134	0.139	0.373	0.152	4	2.371	0.054	0.232	0.116
2	2.046	0.065	0.033	0.180	1	2.444	0.000	0.000	0.000
1	1.969	0.000	0.000	0.000	0				
0									

Appendix 9.--Continued.

Same Size as Natural					Larger than Natural					Smaller than Natural				
N	$\bar{x}$	S_x^2	S_x	SE	N	$\bar{x}$	S_x^2	S_x	SE	N	$\bar{x}$	S_x^2	S_x	SE
13	2.284	0.042	0.204	0.057	13	2.500	0.049	0.004	0.062	13	2.183	0.019	0.001	0.037
13	2.395	0.051	0.227	0.063	13	2.438	0.054	0.004	0.064	13	2.158	0.041	0.003	0.056
13	2.283	0.036	0.191	0.053	13	2.255	0.088	0.007	0.082	13	2.188	0.045	0.003	0.058
13	2.361	0.028	0.169	0.047	13	2.029	0.047	0.004	0.059	13	2.178	0.024	0.002	0.042
13	2.307	0.030	0.172	0.048	13	2.137	0.161	0.012	0.111	13	2.215	0.029	0.002	0.047
13	2.347	0.039	0.199	0.091	12	2.019	0.063	0.005	0.072	13	2.092	0.053	0.004	0.063
13	2.273	0.009	0.294	0.090	11	1.917	0.061	0.006	0.074	13	2.155	0.042	0.003	0.057
13	2.204	0.024	0.155	0.043	11	1.852	0.047	0.004	0.066	13	2.084	0.055	0.004	0.065
13	2.258	0.055	0.236	0.065	10	1.924	0.104	0.010	0.101	13	2.098	0.091	0.007	0.083
13	2.203	0.090	0.300	0.093	10	1.953	0.099	0.010	0.099	13	2.045	0.090	0.007	0.083
13	2.140	0.070	0.253	0.070	5	1.951	0.029	0.006	0.075	13	2.012	0.073	0.006	0.075
13	2.163	0.073	0.274	0.076	4	1.780	0.016	0.004	0.063	12	2.008	0.080	0.006	0.077
13	2.149	0.820	0.290	0.091	4	1.771	0.051	0.013	0.112	11	1.907	0.036	0.003	0.057
13	1.981	0.100	0.314	0.097	4	1.821	0.166	0.041	0.203	11	1.801	0.116	0.011	0.102
13	2.047	0.049	0.222	0.062	3	2.085	0.051	0.017	0.131	10	1.909	0.204	0.020	0.143
13	2.025	0.170	0.414	0.115	3	1.684	0.094	0.031	0.177	8	1.991	0.107	0.013	0.115
12	1.940	0.108	0.329	0.095	2	1.794	0.020	0.010	1.000	7	1.951	0.057	0.008	0.090
12	1.875	0.070	0.261	0.075	2	1.918	0.004	0.002	0.042	7	1.971	0.099	0.014	0.119
11	1.870	0.066	0.258	0.078	1	2.111	0.000	0.000	0.000	5	1.968	0.069	0.014	0.117
9	1.966	0.164	0.405	0.135	0					2	1.923	0.030	0.015	0.123
8	1.877	0.107	0.340	0.120						0				
5	1.807	0.086	0.017	0.130										
4	2.019	0.098	0.024	0.154										
4	1.966	0.059	0.014	0.118										
3	1.894	0.058	0.019	0.137										
3	1.935	0.087	0.029	0.170										
1	1.644	0.000	0.000	0.000										
1	2.286	0.000	0.000	0.000										
1	1.846	0.000	0.000	0.000										

Appendix 10.—Basic statistics for fanning beats per bout per ten minute observation period. Each set of data represents the result of a ten minute observation taken three times per day. Sample size, mean, variance, standard deviation, and standard errors are given for each of the five groups.

Beats per bout per 10 minutes									
Baseline					Blank Slate "Control"				
N	$\bar{x}$	S_x^2	S_x	SE	N	$\bar{x}$	S_x^2	S_x	SE
13	67.689	2771.062	52.641	14.600	13	67.361	1790.413	42.313	11.736
13	45.854	1095.065	33.092	9.178	13	39.935	872.407	29.537	8.192
13	37.683	336.153	18.334	5.085	13	29.815	273.668	16.543	4.588
13	35.224	232.274	15.241	4.227	13	21.314	133.592	11.558	3.206
13	37.564	417.685	20.437	5.668	13	17.396	77.621	8.810	2.444
13	43.554	420.037	20.495	5.684	12	15.245	63.228	7.952	2.295
13	48.422	589.545	24.281	6.734	7	16.526	94.979	9.746	3.684
13	34.526	74.645	8.640	2.396	5	22.384	252.663	15.895	7.109
13	19.336	242.705	15.579	4.321	4	20.000	265.000	16.279	8.139
6	14.388	407.196	20.179	8.238	4	9.313	14.057	3.749	1.875
2	7.644	32.902	16.451	4.056	1	7.333	0000.000	00.000	00.000
1	10.722	0000.000	00.000	00.000	0				

Appendix 10.--Continued.

Same Size as Natural					Larger than Natural					Smaller than Natural				
N	$\bar{x}$	s_x^2	s_x	SE	N	$\bar{x}$	s_x^2	s_x	SE	N	$\bar{x}$	s_x^2	s_x	SE
13	81.104	3043.4163	55.167	15.300	13	64.701	546.427	42.033	6.483	13	75.351	1898.596	146.046	12.085
13	62.490	1838.9767	42.883	11.894	13	47.560	493.945	37.996	6.164	13	55.563	1411.107	108.547	10.419
13	60.330	2321.3537	48.180	13.363	13	29.577	299.527	23.041	4.800	13	45.256	268.848	20.681	4.548
13	59.230	6278.5963	79.238	21.977	13	26.647	447.333	34.410	5.866	13	59.674	2070.373	159.259	12.620
13	42.799	582.3976	24.133	6.693	13	18.553	165.741	12.749	3.571	13	39.027	225.566	17.351	4.165
13	38.540	186.1160	13.642	3.784	12	16.131	75.550	6.296	2.509	13	50.818	850.368	65.413	8.088
13	36.081	117.4587	10.838	3.006	11	12.996	29.541	2.686	1.639	13	36.119	682.501	52.500	7.246
13	46.901	1094.9295	33.090	9.178	11	12.068	38.150	3.468	1.862	13	28.461	318.989	24.538	4.954
13	39.813	242.9025	15.585	4.323	10	13.805	107.536	10.754	3.279	13	26.351	453.627	34.894	5.907
13	33.317	199.0758	14.109	3.913	10	14.614	125.686	12.569	3.545	13	19.215	180.862	13.912	3.730
13	26.771	94.1579	9.703	2.691	5	11.407	24.503	4.901	2.214	13	15.850	123.963	9.536	3.088
13	24.796	112.1622	10.591	2.937	4	10.937	16.159	4.040	2.010	12	12.300	82.488	6.874	2.622
13	25.570	423.8022	20.586	5.710	4	9.436	9.762	2.440	1.562	11	10.789	24.592	2.236	1.495
13	20.033	228.1874	15.106	4.190	4	7.303	1.693	0.423	0.650	11	9.988	18.795	1.709	1.307
13	23.134	161.1029	12.693	3.520	3	6.071	1.632	0.544	0.737	10	5.791	8.968	0.897	0.947
13	18.671	176.1663	13.273	3.681	3	6.080	5.304	1.768	1.330	8	6.934	4.869	0.609	0.780
12	16.416	116.0400	10.772	3.110	2	7.900	0.320	0.160	0.400	7	7.667	2.021	0.289	0.587
12	17.247	96.3584	9.816	2.834	2	6.737	12.010	6.005	2.450	7	7.184	8.154	1.165	1.079
11	16.080	118.0158	10.864	3.275	1	6.333	000.000	00.000	0.000	5	7.168	10.588	2.118	1.455
9	16.420	166.0189	12.885	4.295	0					2	5.875	1.531	0.766	0.875
8	16.882	176.3921	13.281	4.696						0				
5	18.523	58.7433	7.664	3.428										
4	15.050	39.4922	6.284	3.142										
4	11.513	49.4674	7.033	3.517										
3	15.368	30.8580	10.286	3.207										
3	12.424	73.5170	24.506	4.950										
1	12.000	0000.0000	00.000	00.000										
1	8.000	0000.0000	00.000	00.000										
1	8.000	0000.0000	00.000	00.000										

Appendix 11.--Basic statistics for fanning duration per bout per ten minute observation period. Each set of data represents the result of a ten minute observation taken three times per day. Sample size, mean, variance, standard deviation, and standard errors are given for each of the five groups.

Duration per 10 minutes									
Baseline					Blank Slate "Control"				
N	$\bar{x}$	S_x^2	S_x	SE	N	$\bar{x}$	S_x^2	S_x	SE
3	449.539	12874	113.465	31.470	13	462.077	14635	120.974	33.5524
3	386.077	16305	127.689	35.415	13	294.615	25915	160.981	44.6486
3	331.615	14757	121.478	33.692	13	242.923	19153	138.393	38.3830
3	327.846	10127	100.635	27.911	13	153.000	10221	101.098	28.0400
3	331.385	8356	91.410	25.353	13	118.923	6158	78.476	21.7660
3	354.231	3592	59.937	16.640	12	112.750	8226	90.699	26.1830
3	375.154	4410	66.409	18.419	7	125.143	16076	126.793	47.9240
3	361.385	2222	47.141	13.075	5	140.200	13498	116.182	51.9600
3	195.385	18326	135.375	37.547	4	132.750	25624	160.078	80.0390
6	119.500	4739	68.838	28.104	4	20.000	592	24.324	12.1620
2	28.500	180	90.250	9.500	1	9.000	00000	000.000	00.0000
1	98.000	00000	000.000	00.000	0				
0									

Appendix 11.--Continued.

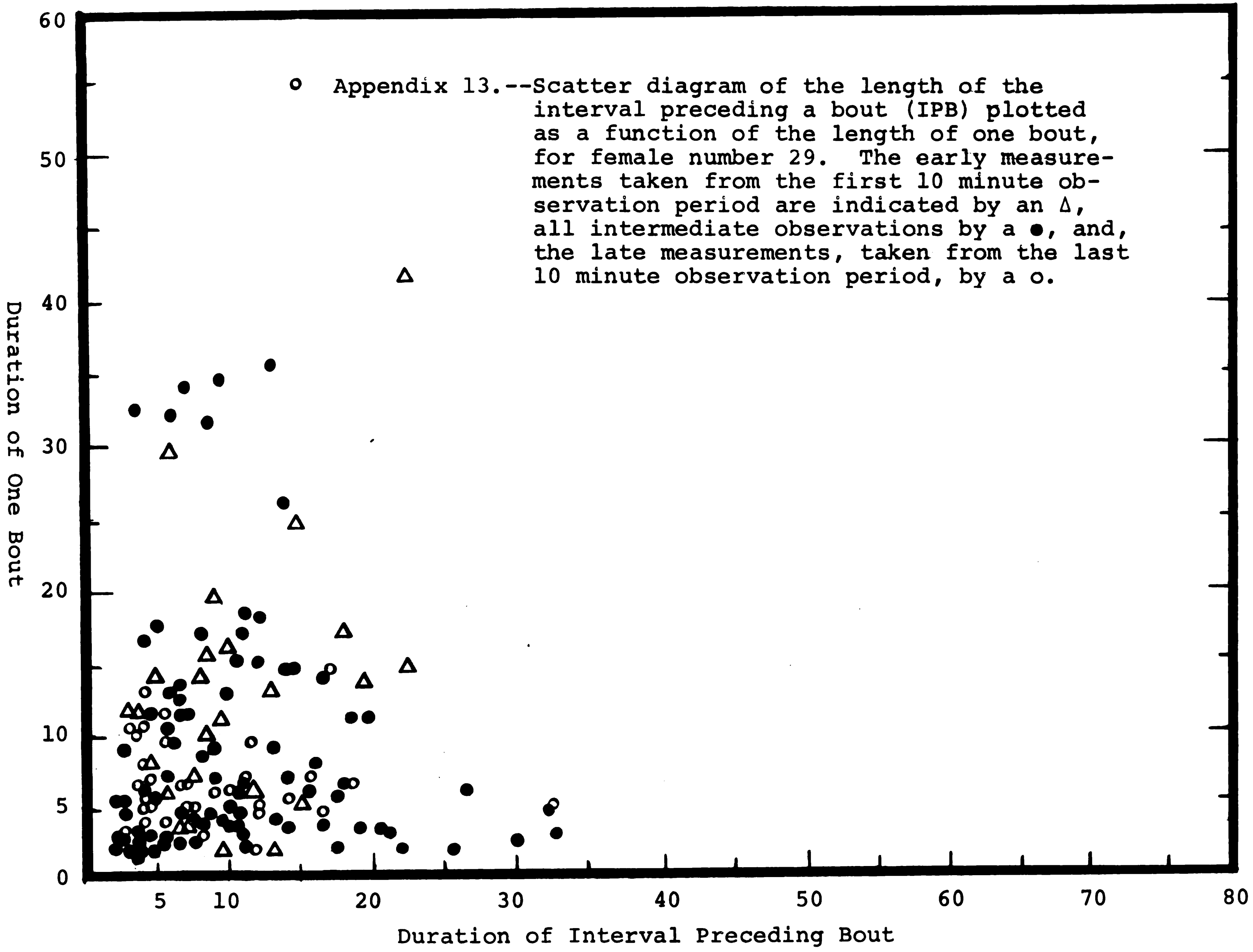
Same Size as Natural					Larger than Natural					Smaller than Natural				
N	$\bar{x}$	S_x^2	S_x	SE	N	$\bar{x}$	S_x^2	S_x	SE	N	$\bar{x}$	S_x^2	S_x	SE
13	474.308	12179	110.361	30.609	13	463.538	7181	552.354	23.502	13	486.846	6386	491.203	22.163
13	457.615	9521	97.578	27.064	13	375.308	10960	843.069	29.036	13	421.846	8905	685.972	26.172
13	377.846	13509	116.228	32.236	13	244.000	14572	1120.910	33.480	13	368.231	7436	572.105	23.917
13	326.231	13333	115.400	32.007	13	198.231	16419	1263.015	35.539	13	354.308	11006	846.620	29.097
13	327.385	7029	83.841	23.254	13	131.231	12509	962.194	31.019	13	311.154	5956	458.152	21.404
13	307.769	7898	88.871	24.649	12	124.583	13145	1095.386	33.097	13	313.000	20072	1543.962	39.293
13	319.539	5753	75.849	21.037	11	98.455	7507	682.443	26.124	13	263.846	14442	1110.921	33.330
13	332.154	12182	110.371	30.612	11	85.636	8559	778.096	27.894	13	229.000	10363	797.115	28.233
13	297.231	9189	95.858	26.587	10	98.300	11845	1184.534	34.417	13	172.385	8263	635.635	25.212
13	262.692	10334	101.656	28.195	10	47.200	2489	248.907	15.777	13	158.692	8554	657.966	25.651
13	231.385	7803	88.334	24.500	5	61.400	3047	609.360	24.685	13	122.154	5774	444.139	21.075
13	194.692	10459	102.267	28.364	4	63.000	2481	620.167	24.903	12	110.083	7381	615.037	24.800
13	193.462	14304	119.599	33.171	4	58.500	698	174.417	13.207	11	70.909	1874	170.372	13.053
13	192.000	11821	108.724	30.155	4	38.250	50	12.396	3.521	11	43.727	639	58.111	7.623
13	151.462	7644	87.430	24.249	3	22.333	20	6.778	2.603	10	28.200	283	28.307	5.320
13	122.769	8192	90.509	25.103	3	43.000	343	114.333	10.693	8	31.000	127	151.893	3.987
12	104.750	6926	83.225	24.025	2	48.000	2	1.000	1.000	7	30.571	66	9.374	3.062
12	98.083	4288	65.479	18.902	2	45.500	1741	870.250	29.500	7	27.429	144	20.565	4.535
11	84.000	5323	72.956	21.997	1	36.000	00000	0000.000	00.000	5	31.600	427	85.460	9.244
9	78.889	7552	86.900	28.967	0					2	13.000	8	4.000	2.000
8	72.250	8442	91.882	32.485						0				
5	99.800	10585	102.884	46.013										
4	88.500	3539	884.812	29.745										
4	86.500	5307	1326.687	36.423										
3	120.000	2749	916.222	30.269										
3	100.667	15664	5221.444	72.260										
1	73.000	00000	000.000	00.000										
1	28.000	00000	000.000	00.000										
1	26.000	00000	000.000	00.000										

Appendix 12.--Basic statistics for the frequency of nipping per ten minute observation period. Each set of data represents the result of a ten minute observation taken three times per day. Sample size, mean, variance, standard deviation, and standard errors give for each of the five groups.

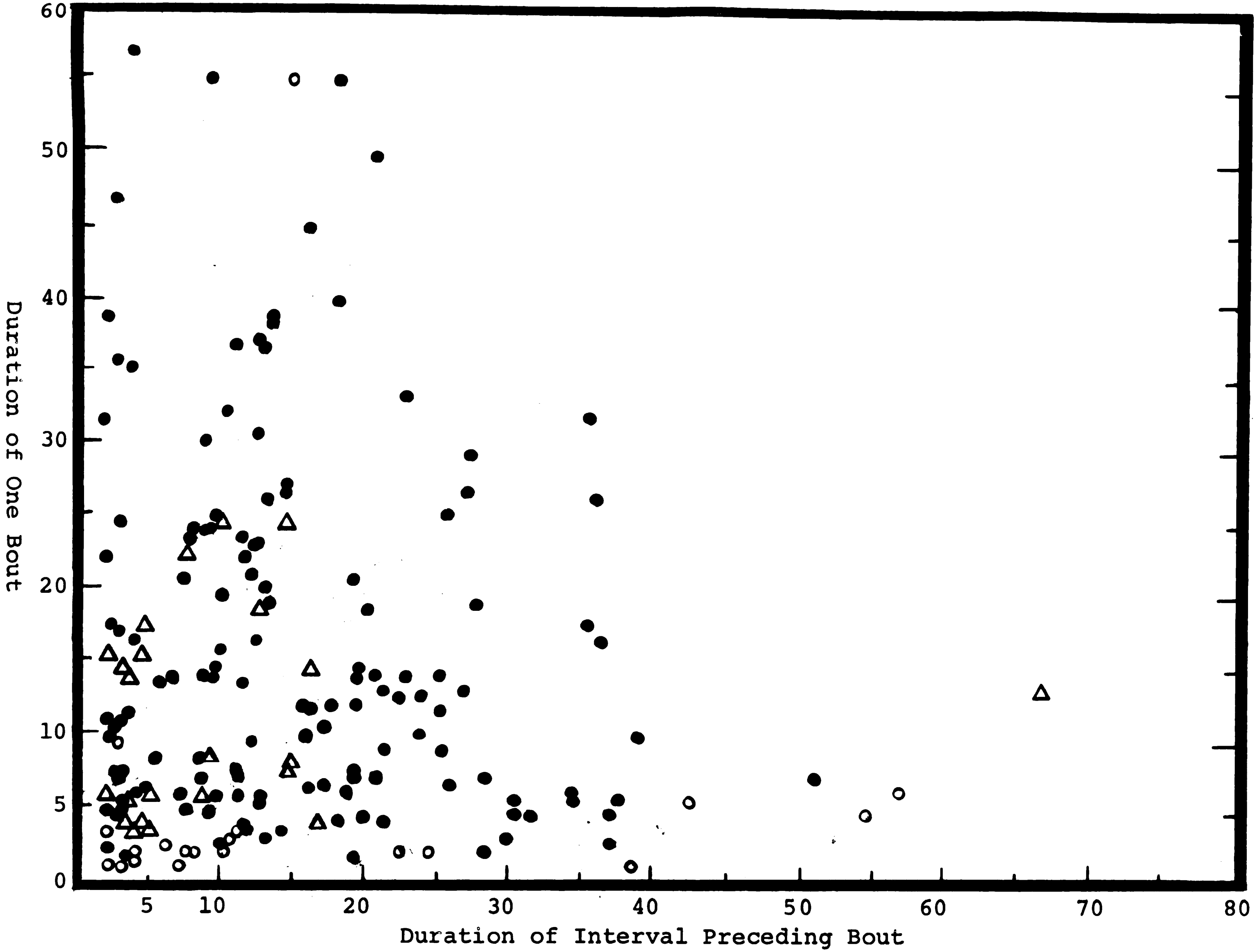
Nips per 10 minutes									
		Baseline					Blank Slate "Control"		
N	$\bar{x}$	s_x^2	s_x	SE	N	$\bar{x}$	s_x^2	s_x	SE
9	4.444	12.5280	3.540	1.1800	11	7.909	64.893	8.056	2.429
11	8.273	38.2190	6.182	1.8640	12	9.917	56.267	7.501	2.165
8	11.875	93.8390	9.687	3.4250	12	5.917	12.266	3.502	1.011
11	11.454	49.0740	7.005	2.1120	18	8.250	32.500	5.701	2.016
10	5.000	28.6670	5.354	1.6930	6	7.167	70.168	8.377	3.420
9	4.778	12.1950	3.492	1.1640	7	11.571	229.620	15.153	5.727
11	7.818	62.1650	7.884	2.3770	6	9.167	138.168	11.754	4.799
12	10.417	145.9030	12.079	3.4870	6	16.333	245.468	15.667	6.396
13	30.462	721.6051	26.863	7.4500	3	15.333	24.335	4.933	2.848
6	39.000	1380.0000	37.148	15.1662	4	6.250	29.583	5.439	2.720
2	15.500	84.5000	42.250	6.5000	1	2.000	000.000	00.000	0.000
1	52.000	0000.0000	00.000	00.0000	0				

Appendix 12.--Continued.

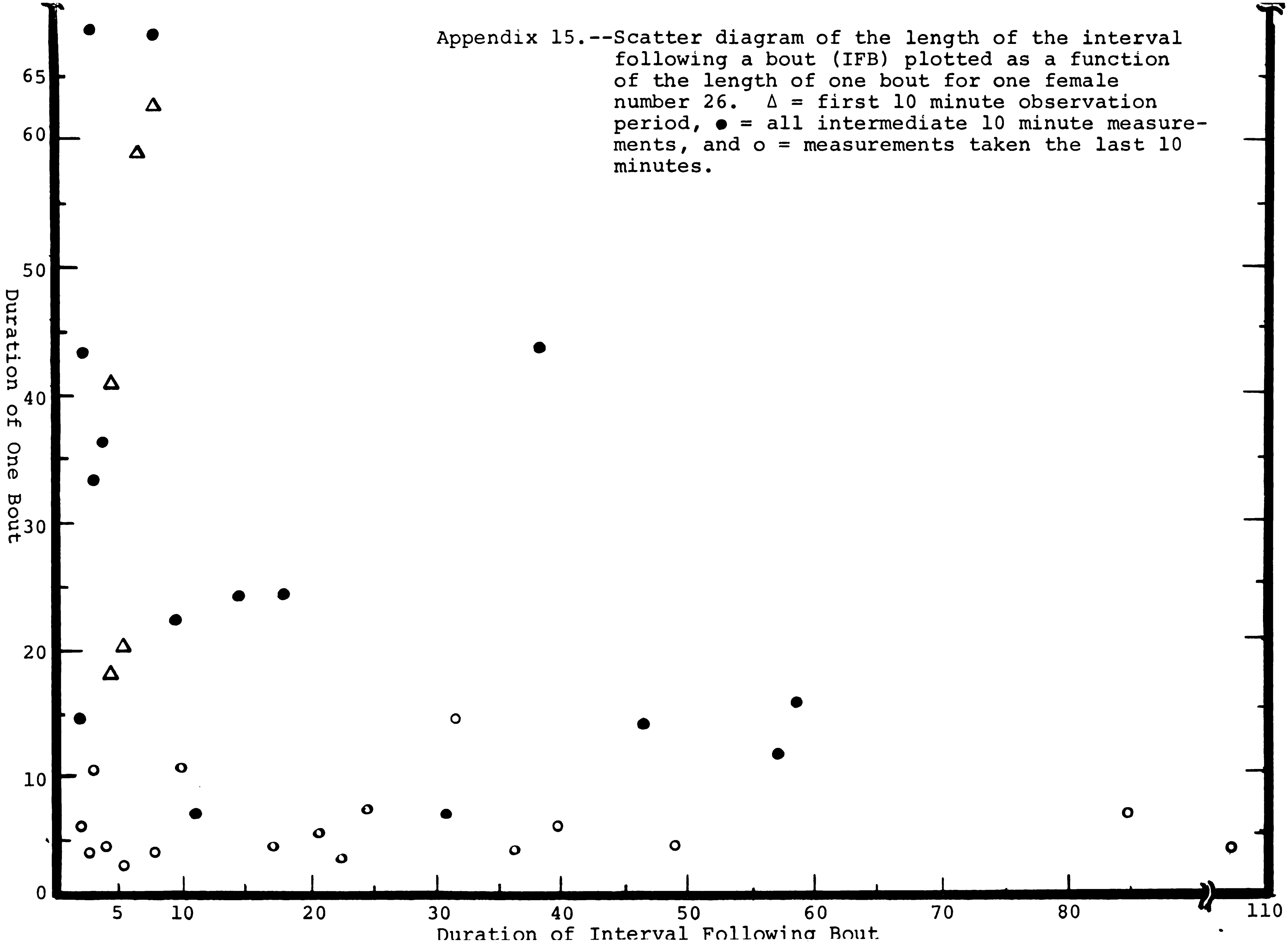
Same Size as Natural					Larger than Natural					Smaller than Natural				
N	$\bar{x}$	S_x^2	S_x	SE	N	$\bar{x}$	S_x^2	S_x	SE	N	$\bar{x}$	S_x^2	S_x	SE
11	4.636	35.800	5.988	1.805	9	4.667	29.000	3.222	1.795	7	3.286	6.571	0.939	0.969
12	4.500	35.727	5.977	1.725	13	8.462	68.269	5.251	2.292	11	6.182	14.964	1.361	1.166
12	3.600	9.818	5.133	0.905	10	4.300	15.567	1.557	1.248	9	4.333	45.500	5.056	2.248
10	4.600	11.556	3.406	1.077	7	3.857	14.143	2.020	1.421	7	2.256	3.571	0.510	0.714
11	3.455	9.300	3.045	0.918	7	4.000	24.000	3.429	1.852	10	2.500	5.167	0.517	0.719
6	6.500	27.000	5.206	2.125	7	1.857	1.476	0.211	0.459	10	2.300	3.344	0.334	0.578
10	4.400	29.333	5.420	1.714	6	2.500	1.900	0.317	0.563	9	4.000	36.250	4.028	2.007
10	3.300	8.222	2.869	0.907	6	1.833	1.767	0.294	0.543	10	2.700	19.122	1.912	1.383
8	3.875	24.143	4.912	1.737	6	2.167	2.167	0.601	0.601	9	2.000	3.250	0.361	0.601
6	3.333	4.600	2.160	0.882	7	2.857	6.476	0.925	0.962	10	2.100	1.656	0.166	0.407
7	3.286	7.167	2.690	1.017	5	1.600	0.800	0.160	0.400	9	2.444	5.528	0.614	0.784
7	2.000	1.000	1.000	0.378	5	2.000	5.000	1.000	1.000	7	3.571	9.619	1.374	1.172
8	6.250	28.714	5.365	1.900	3	1.000	00.000	0.000	0.000	8	1.750	1.926	0.241	0.491
9	2.333	2.500	1.581	0.527	0	0.000	00.000	0.000	0.000	6	1.000	0.000	0.000	0.000
10	3.500	6.000	2.461	0.778	2	1.000	00.000	0.000	0.000	7	1.143	0.143	0.020	0.143
5	1.600	0.250	0.548	0.245	1	1.000	00.000	0.000	0.000	7	1.286	0.238	0.034	0.184
7	1.857	0.833	0.900	0.340	1	2.000	00.000	0.000	0.000	5	1.600	0.800	0.160	0.400
7	3.857	5.500	2.340	0.884	1	1.000	00.000	0.000	0.000	6	2.167	4.167	0.694	0.833
8	2.000	1.714	1.309	0.463	1	1.000	00.000	0.000	0.000	6	1.667	0.667	0.111	0.333
6	2.667	7.000	2.658	1.085	0					4	1.750	0.917	0.229	0.479
5	1.800	1.250	1.095	0.490						5	1.200	0.200	0.040	0.200
2	1.500	0.250	0.125	0.353						0				
2	4.500	2.250	1.125	1.060										
4	6.500	4.750	1.187	1.089										
2	3.500	6.250	1.767	3.500										
2	1.000	00.000	0.000	0.000										
1	1.000	00.000	0.000	0.000										
2	1.500	0.500	0.250	0.500										
1	4.000	00.000	0.000	0.000										



Appendix 13.--Scatter diagram of the length of the interval preceding a bout (IPB) plotted as a function of the length of one bout, for female number 29. The early measurements taken from the first 10 minute observation period are indicated by an Δ, all intermediate observations by a ●, and, the late measurements, taken from the last 10 minute observation period, by a o.

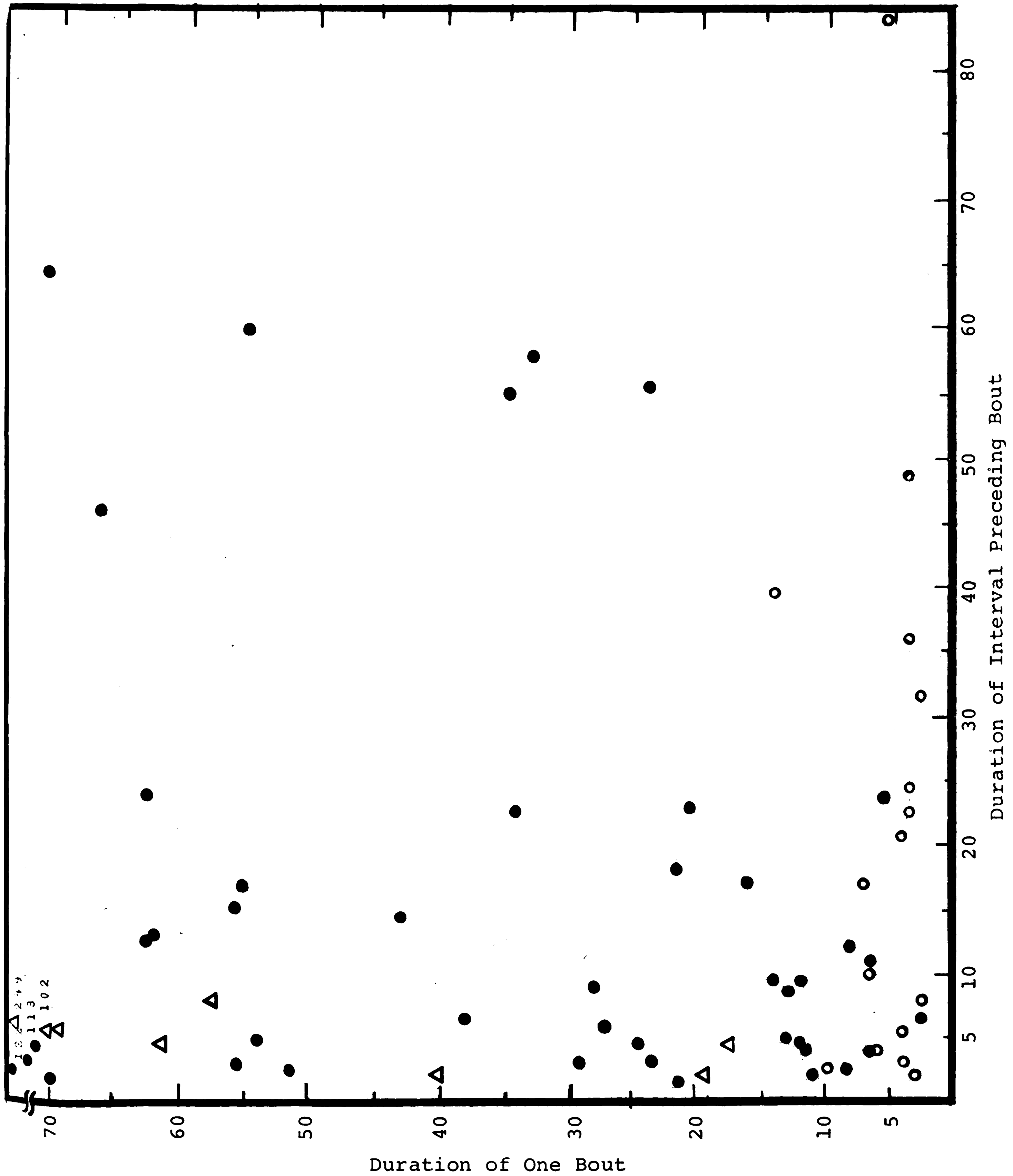


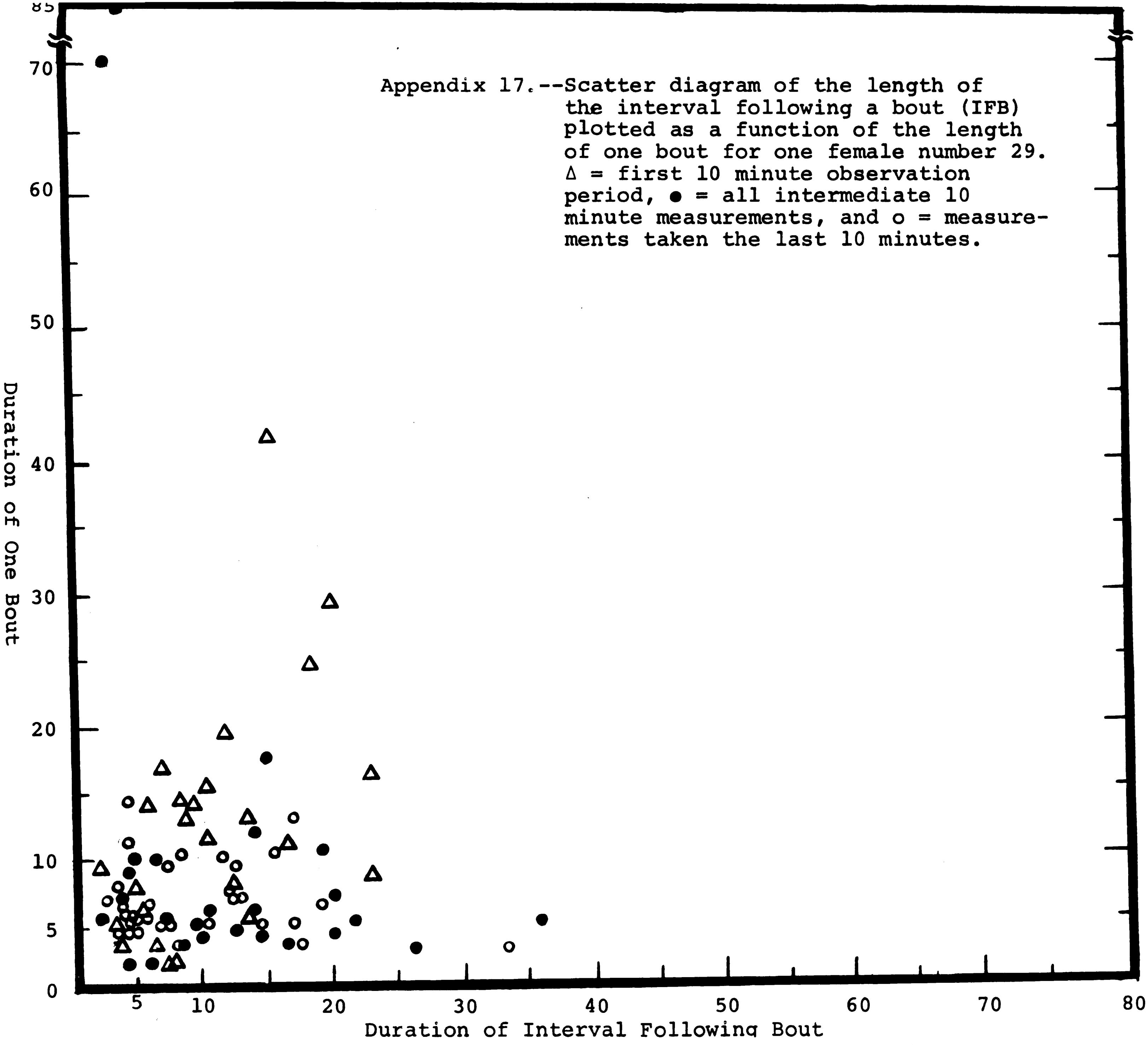
Appendix 14.--Scatter diagram of the length of the interval following a bout (IFB) plotted as a function of the length of one bout for one female number 20. Δ = first 10 minute observation period, ● = all intermediate 10 minute measurements, and o = measurements taken the last 10 minutes.



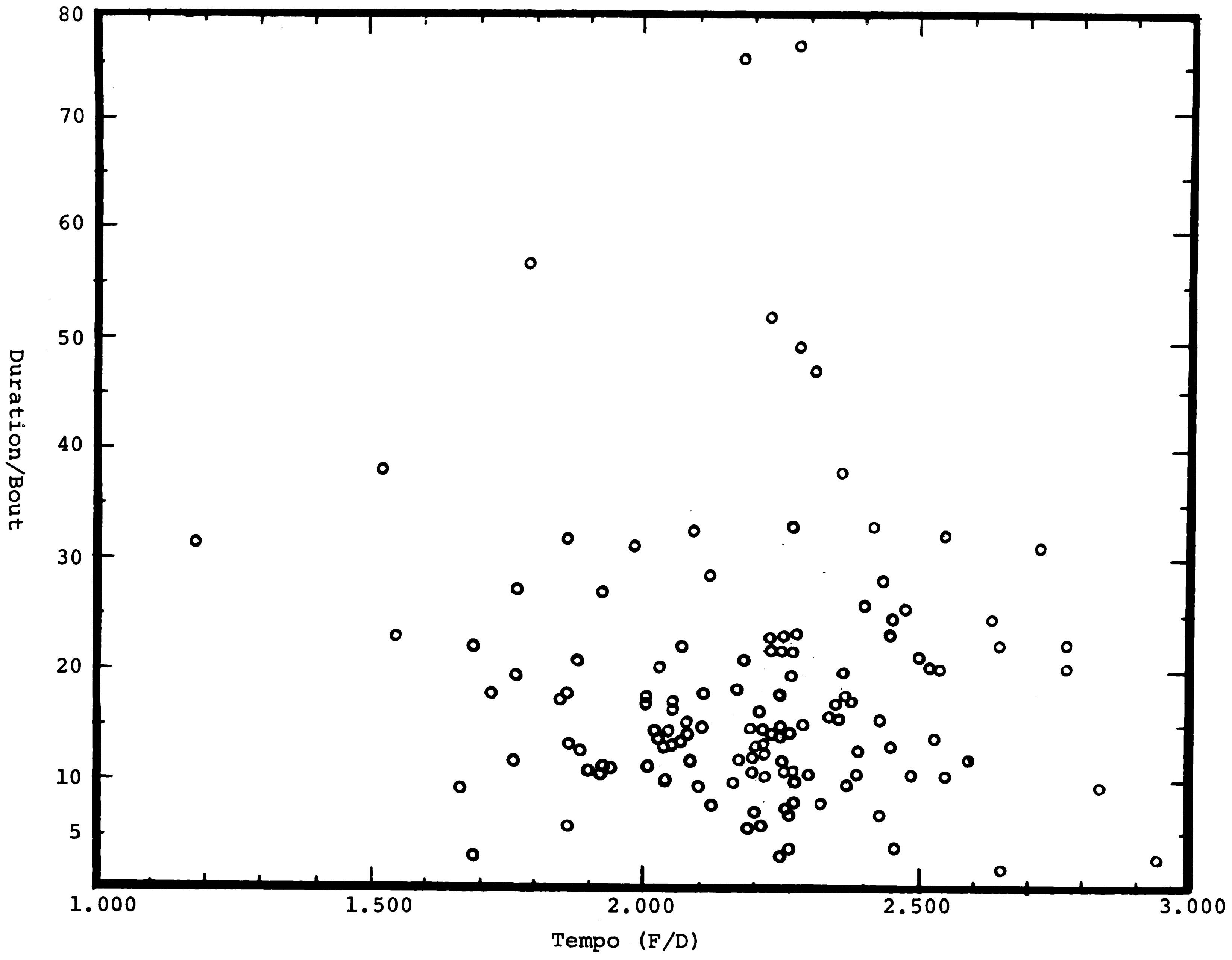
Appendix 15.--Scatter diagram of the length of the interval following a bout (IFB) plotted as a function of the length of one bout for one female number 26. Δ = first 10 minute observation period, ● = all intermediate 10 minute measurements, and o = measurements taken the last 10 minutes.

Appendix 16.--Scatter diagram of the length of the interval preceding a bout (IPB) plotted as a function of the length of one bout, for female number 26. The early measurements taken from the first 10 minute observation period are indicated by an Δ, all intermediate observations by a ●, and, the late measurements, taken from the last 10 minute observation period, by a o.

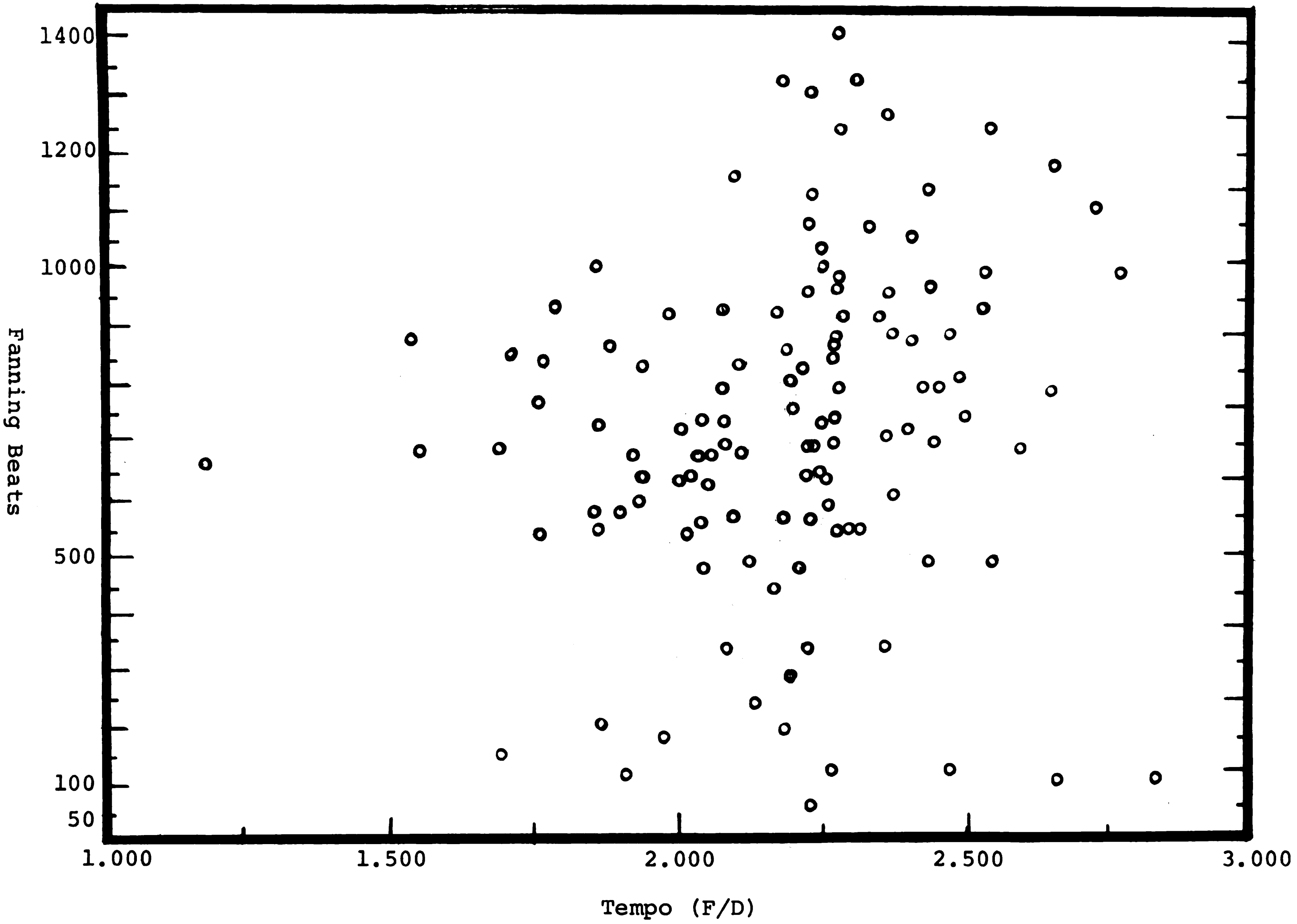

Duration of Interval Preceding Bout
Duration of One Bout
80
70
60
50
40
30
20
10
5
70
60
50
40
30
20
10
5
122
249
113
102



Appendix 17.--Scatter diagram of the length of the interval following a bout (IFB) plotted as a function of the length of one bout for one female number 29. Δ = first 10 minute observation period, ● = all intermediate 10 minute measurements, and o = measurements taken the last 10 minutes.



Appendix 18.--Scatter diagram of fanning duration per bout as a function of tempo. Data taken from baseline measurements.



Appendix 19.--Scatter diagram of fanning beats as a function of tempo. Data taken from baseline measurements.

LITERATURE REVIEW

Noble and Curtis (1939) observed the responses of parent Jewel fish to their eggs and established that visual cues were used in egg recognition. They found that *Hemichromis bimaculatus* could not discriminate their own eggs from those of certain other species. Reciprocal spawn exchanges between several different cichlid species in which eggs were accepted and the young raised demonstrated that some cichlids are unable to distinguish between eggs of their own and other species' (Greenberg, 1961, 1964 a, b, and Myrberg, 1964). The eggs of these species were not visually distinct to these observers. When reciprocal exchanges were performed between certain other species, however, the eggs were immediately eaten. Eggs of these species differed in size, shape, and color (Collins, 1962, 1965).

The egg exchange studies cited implied that chemical stimulation was not of great importance in egg recognition. This was further suggested since *Hemichromis sp*. parents, when presented with a choice between tap water and water which had flowed over their own eggs, were unable to discriminate. "Fine" discriminations, however, were made between tap water and water which had come in contact with either prolarvae or free-swimming larvae (Kühme, 1963, 1965). Furthermore, it is possible selectively to vary a female's exposure to olfactory and visual stimulation provided by the eggs. When this was done, there was no difference in either the magnitude or temporal structure of fanning in cases where _C_. _nigrofasciatum_ was presented with visual but no olfactory access, as opposed to both visual and olfactory stimulation by their eggs (Mertz, 1967). The conclusion reached was that parental fanning is caused at least, in part, by visual stimulation by the spawn.

Fanning is not completely eliminated in the absence of all stimulation from the clutch (Mertz, 1967). Thus, it is possible to obtain fanning to a fixed point from non broody cichlids by injecting them with prolactin (Blüm and Fiedler, 1962). This suggests that there are also internal factors which contribute to the expression of egg care behavior.