

# ORGANIZATION OF PARENTAL CARE BEHAVIOR IN CICHLASOMA NIGROFASCIATUM (GUNTHER)

Dissertation for the Degree of Ph. D. MICHIGAN STATE UNIVERSITY JOAN W. BERNSTEIN 1976





# This is to certify that the

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Joan W Bernstein

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#### ABSTRACT

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#### ORGANIZATION OF PARENTAL CARE BEHAVIOR IN CICHLASOMA NIGROFASCIATUM (GÜNTHER)

By Joan W. Bernstein

This research was designed to test the hypothesis that cichlid parents possess distinct behavioral STATES corresponding to natural developmental stages of their young and are thus incapable of accepting young whose developmental stage does not match the state of the parent. Experiments were designed to resolve the question of whether age and stage differences between a parents' own and foreign young in exchange experiments contribute to rejection of the foreign young. This rejection has been the basis for proof of imprinting in other studies. Parents were first allowed to tend their own young. Then a short time after removal of the parents' young, non-filial young, different in age and stage from the parents' own and of the same species, were introduced. After a series of observation periods, these non-filial young were removed, and the parents' own young were returned. The results were assessed with regard to four different criteria. In no case did parents eat live young. However, rejection

Joan W. Bernstein

did occur in the form of abnormal behavior, i.e. behavior not normally exhibited to young of that stage during the parental care cycle. Approximately two thirds of the parents did not exhibit normal care behavior. Those that did exhibit normal care behavior, did so only after a considerable latency period. In addition, only during the final treatment observation period was the level of normal care behavior non-significantly different from that exhibited towards their own. Thus, three out of four criteria demonstrated that parents do not fully accept young of a different age and developmental stage. It therefore appears that <u>Cichlasoma nigrofasciatum</u> parents possess behavioral states.

#### ORGANIZATION OF PARENTAL CARE BEHAVIOR IN

### CICHLASOMA NIGROFASCIATUM (GÜNTHER)

By

Joan W. Bernstein

#### A DISSERTATION

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

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#### TABLE OF CONTENTS

Pa	age
ACKNOWLEDGEMENTS	ii
LIST OF TABLES	iv
LIST OF FIGURES	v
INTRODUCTION	1
Synopsis of the Parental Care Cycle.	1
Literature Review	2
MATERIALS AND METHODS	14
Stocks, Maintenance, and Pair-Selection.	14
Criteria of Parental Behavior Patterns	18
Fanning of Eggs	18
Nipping of Eggs	19
Calling of Free-Swimmers	19
Retrieving of Free-Swimmers	21
Experimental Design	22
RESULTS	25
Criterion I	25
Criterion II	27
Criterion III	28
Criterion IV	30
Parental Behavior Towards Young of a Different	
Stage	35
Free-Swimmers	35
Eggs	35
Effect of Male's Presence on Female Parental Care	
Behavior Towards Young of a Different Stage	36
DISCUSSION	39
Behavior Towards Foreign Young	44
LITERATURE CITED	46

#### LIST OF TABLES

Table		Pa	ige
1.	Intraspecific Exchanges and Their Results	•	12
2.	Outline of Experimental Design		23
3.	Results of all Exchanges. Response of Parents for all Criteria	•	26
4.	Parental Response to Young of Different Stages. Criterion II, Exhibiting Normal Care Behavior	•	29
5.	Parental Response to Eggs. Criterion II, Ex- hibiting Normal Care Behavior	•	29
6.	Comparative Analysis of Female Parental Care Behavior Before (pretreatment) and After (Post Treatment) Treatment	•	31
	a. Outline		
	b. Analysis of Variance (Duration)		
7.	Paired Comparison T-Test. Criterion IV: Com- parison of Normal Care Behavior During Pre- treatment and Final Treatment Observation Periods		34
8.	Response of Parents in the Free-Swimming State to Foreign Eggs. Acceptance Criteria: Appro priate Behavior and No Eating of Live Young.	-	38

### LIST OF FIGURES

Figur	e	Pa	age
1.	General experimental tank set-up	•	15
2.	$\frac{\texttt{Cichlasoma}}{\texttt{position}} \; \frac{\texttt{nigrofasciatum}}{\texttt{fanning eggs}} \; \texttt{shown in common}$	•	20
3.	Response of parents in the egg state towards free-swimming young	•	32
4.	Response of parents in the free-swimming state towards eggs		33

v

#### INTRODUCTION

#### Synopsis of the Parental Care Cycle

Although considerable information exists concerning the experimental analysis of parent-offspring responses among mammals (see review by Harper, 1970), little is known about comparable relationships in fishes. While parental care is known to exist in many families of fish, it is particularly elaborate in the family Cichlidae (Cole and Ward, 1970). The cichlid family is often divided into two groups based on whether they are substrate-spawners or oral incubators (Baerends and Baerends von Roon, 1950).

The parental care cycle in substrate-spawners contains several recognizable components: the egg stage, the wriggler stage and the free-swimmer stage. These three stages reflect distinct developmental stages of the young. In addition, parents exhibit distinct care behaviors toward young that are in each of these developmental stages. The female parent fans and nips at the eggs during the egg stage which lasts about three and one-half to four days. When eggs hatch into wrigglers, parents mouth and move them from one previously-dug pit to another. The transition from wrigglers to free-swimmers occurs at approximately four days post-hatching. Free-swimming is

the third and final stage. During this period, parents remain close and keep the free-swimming young in a compact group, both by retrieving them in their mouths and by performing calling movements. The young follow the parents around the tank.

# Literature Review

Most of the literature has been concerned with recognition studies of parents by the young (Noble and Curtis, 1939; Baerends and Baerends von Roon, 1950; Küenzer, 1966, 1968; Cole and Ward, 1970), recognition of young by the parents (Noble and Curtis, 1939; Lorenz, 1950; Collins and Braddock, 1962; Greenberg, 1963a,b; Kühme, 1963; Myrberg, 1964), and studies on the development of juvenile behaviors (Noakes, 1971; Noakes and Barlow, 1973). Patterns of recognition of young by parents have generally been investigated through exchanges of the parents own young with those of different species. Substrate-spawners have been used in most parent-young response investigations in order to facilitate exchanges of parents or young. Survival of the young, especially during early stages of development, depends on the synchronized interaction of parents and young. Consequently, it is important that both parents and young recognize and appropriately respond to each other.

Cichlid parental behavior, especially recognition of young, has thus been the subject of much research;

however, questions remain concerning the extent of this recognition. Noble and Curtis (1939) were first to provide evidence that cichlid parents recognize their young. They found that inter-specific recognition (eating), occurs in experienced cichlids only during the free-swimming stage. They concluded that imprinting of parents to their own young takes place, by experienced parents, during the free-swimming stage. Baerends and Baerends von Roon (1950), showed that recognition might be possible even earlier in the parental care cycle (i.e. during the egg stage). This partially contradicted Noble and Curtis (1939), who found foreign eggs and wrigglers, but not freeswimmers, to be accepted during exchanges between species. The concept of imprinting in cichlid fishes thus was accepted in the literature and was incorporated into general texts by Tinbergen (1951) and Thorpe (1956). Myrberg (1961) found further evidence of recognition and parental imprinting in cichlid fish. However, at the same time, Greenberg (1961) reported that his exchanges of eggs and wrigglers resulted in acceptance, and that "...experienced and inexperienced pairs raised foreign spawn successfully in alternation with their own or were induced to brood mixed schools." He concluded that the two cichlid species (Hemichromis bimaculatus, Aeguidens portalegrensis) used did not imprint on species characteristics of their first or subsequent young.

Further studies served to increase this dichotomy and generate new explanations. Collins and Braddock (1962), Greenberg (1963a,b) and Collins (1965), found no evidence of parental imprinting. Myrberg (1964), however, working on <u>H</u>. <u>bimaculatus</u>, <u>Cichlasoma</u> <u>nigrofasciatum</u>, and <u>C</u>. <u>biocellatum</u>, further supported the existence of imprinting in cichlid fish, and in addition, suggested that possible chemical cues associated with arrangement of pairs in tanks might be contributing to these divergent results.

Greenberg (1963a) hypothesized that instead of imprinting, there is an internal mechanism which regulates the duration of the phases of the parental care cycle, and limits the responsiveness of the parents to particular stages of young. This implies that eggs, wrigglers, or free-swimming young, regardless of species, would be acceptable only in those instances where their appearance and behavior matched the parents' brooding phase.

When these studies are viewed together, three main areas, taken alone or in combination, serve to explain the imprinting controversy: methodology, species differences, and Greenberg's hypothesis. Methodology includes procedural differences in exchanges (e.g. whether a species' own young are present at the time of introduction of foreign young), and the manner in which foreign young are introduced. This however, does not appear to be very critical (Myrberg, 1964). Duration of the study and

arrangement of fish in tanks may also be important. Also, criteria utilized for determination of acceptance or rejection have not always been clear or uniform. Second, imprinting may exist but species differences may account for the divergent conclusions of these investigators. This may occur in two possible ways. One, the stimulus pattern that parents respond to may be general enough to include young of certain species but not others. Or two, development of a strong parent-young bond may occur at a different time (or rate) during the parental care cycle in different species. Although there is some overlap, most of the investigators have worked with different species.

Myrberg's (1964) study involved exchanges between <u>H. bimaculatus, C. biocellatum</u>, and <u>C. nigrofasciatum</u> and resulted in rejection of young by parents. Greenberg's exchanges (1961, 1963a,b) were between <u>H. bimaculatus</u> and <u>Aequidens portalegrensis</u>, and <u>C. biocellatum</u> exchanged with <u>Aequidens portalegrensis</u>, and resulted in acceptance. An exception in Myrberg's (1964) study, where foreign young were accepted, involved an exchange with the genus <u>Aequidens</u>. <u>Aequidens</u>, in other studies, is also found to be associated with other acceptances (Collins, 1965; Collins and Braddock, 1962). Lastly, Greenberg's (1963a) hypothesis might explain the divergent results. If Greenberg is correct, the existence of these distinct behavioral

stages would confound an experiment unless careful controls and definitions were presented. This work was designed to resolve the question of whether Greenberg's hypothesis contributes an important explanation to the imprinting controversy.

Specifically, this research was designed to test the hypothesis that cichlid parents possess distinct behavioral STATES corresponding to natural development of their young, and are thus incapable of accepting young whose developmental stage does not match the state of the parent.

If parental states do not exist, then parents should accept different aged young regardless of the "state" the parent is in. One such experiment in support of this (Myrberg, 1964, p. 60), was a series of 18 intraspecific exchanges between parents and young in the substrate brooding <u>Cichlasoma nigrofasciatum</u>. Only two exchanges resulted in non-filial young being eaten. Young which were eaten were 17 and 70 days older respectively than the parents' own young and were either eaten immediately or within two minutes after introduction. Control young (the pair's own young) were accepted in all cases. In the remaining 16 tests, non-filial young were accepted, even though differing in age by as much as 15 days from the pair's own young. The design of this experiment involved a series of different exchanges between parents and

young covering a range of age differences from 0 to 70 days. Myrberg concluded that adult <u>C</u>. <u>nigrofasciatum</u> apparently do not discriminate between young of their own species. In addition, he reached the same conclusion for intraspecific exchanges in <u>C</u>. <u>biocellatum</u> and <u>H</u>. <u>bimaculatus</u>, in a similar series of tests.

Myrberg's (1964, p. 60) study, however, is not a critical test of Greenberg's hypothesis although it involves intraspecific exchanges of young. The age differences were based upon a chronological, not a behavioral, time scale. The actual number of days may not be as important as where an age difference interval lies within the parental care cycle. For example, a four day difference in age could still place individuals within a developmental stage (egg, wriggler, free-swimmer), or between developmental stages (egg-wriggler, wriggler-freeswimmer). Thus results could differ depending upon where this age difference occurred. Myrberg's study did not cover representative exchanges over all of the parental care cycle. Thirteen of the 18 exchanges were within the same developmental stage (two during the end of the egg stage, two in the wriggler stage, and nine during the freeswimming stage). One of the latter free-swimming exchanges involved a 70 day age difference, and resulted in rejection. All of the rest of the exchanges resulted in acceptance of young by the parents. Two additional exchanges

involved eggs which were hatching and wrigglers respectively. These could be viewed as belonging to the beginning and end of the wriggler stage and thus also within the same developmental stage. These also resulted in acceptance. Only three exchanges involved age differences between developmental stages, two with free-swimming parents given eggs or wrigglers (resulting in acceptance), and one involving parents whose own young were hatching and were given 17 day old free-swimmers, (resulting in rejection). As seen from these data, most exchanges were within, not between, developmental stages. Thus an age difference by itself may not be the key to understanding the parental care phenomenon. If parents do possess behavioral states, and if these states correspond to the developmental stages of the young, then parents would be expected to accept different aged young of the same developmental stage and conversely to reject those which differed in stage from their own young. Myrberg did not do a sufficient number of exchanges between developmental stages to constitute an appropriate test of Greenberg's hypothesis.

Additional evidence exists showing that parents given young which are not their own but the same age and species will accept them. The series of exchanges conducted by Myrberg (1964) and cited earlier is one example. All intraspecific exchanges involving non-filial young of

the same age as the parents' own, resulted in acceptance. Additional intraspecific exchanges in <u>C</u>. <u>nigrofasciatum</u> done only during the egg stage showed that in all cases eggs were accepted (Myrberg, 1964). This was true whether the pairs' own eggs were present or not. Similar tests involving <u>C</u>. <u>biocellatum</u> and <u>H</u>. <u>bimaculatus</u> also resulted in acceptance whenever non-filial young matched the age of the parents' own young. Eight out of nine tests with freeswimming young involving <u>C</u>. <u>biocellatum</u>, resulted in acceptance. There was an age difference of four days in the rejection case. Four of five tests involving intraspecific exchanges of free-swimming <u>H</u>. <u>bimaculatus</u>, resulted in acceptances. There was an age difference of 14 days in the rejection case.

Noble and Curtis (1939) found that pairs do not discriminate between their own and other eggs of the same species (<u>H</u>. <u>bimaculatus</u>, <u>C</u>. <u>bimaculatum</u>, <u>C</u>. <u>cutteri</u> and <u>C</u>. <u>biocellatum</u>). In addition Noble and Curtis (1939) found that "...a jewel fish will readily adopt young from other broods of jewel fish if these are approximately the same age." Myrberg (1964) and Noble and Curtis (1939) differed in their assessment of exactly how great an age difference in young will be tolerated by parents. Yet they do agree that parents will accept non-filial young that are the same age, the same stage, and the same species as their own.

In another study involving the cichlid species Tilapia sparrmani and Aeguidens latifrons Collins (1965) found that 17 intraspecific exchanges of eggs resulted in acceptance even when age differentials within the developmental stage were great. In seven egg exchanges using A. latifrons, all were accepted. In ten egg exchanges using T. sparrmani, all were accepted. In addition, 14 out of 15 intraspecific exchanges of wrigglers in T. sparrmani resulted in acceptance, even when age differentials were present. However, wriggler exchanges did not always result in acceptance in A. latifrons. Six out of 23 exchanges resulted in rejection. All of the above rejections included wrigglers who differed in age from the parents' own young. Young the same age as the parents' own were always accepted. In every case the parents' own young were returned and immediately accepted.

Thus, regardless of different experimenters, different species, and slight differences in techniques, intraspecific exchanges always resulted in acceptance when young were matched in age and stage with the parents' own young. In no instance during intraspecific exchanges of non-filial young of the same developmental stage for eggs and the same age for wrigglers or free swimmers, is rejection reported in <u>C</u>. <u>nigrofasciatum</u> and many other closely related species. Whether, and how much of, an age difference within the wriggler stage is tolerated, depends on the species. <u>Aequidens latifrons</u> shows the least tolerance to age differences at this time. In other species an age difference appears to have no effect within the wriggler stage. Rejections also begin to occur in exchanges during the older free-swimming stage. Table 1 presents a summary of these results.

It is conceivable that a different mechanism is involved in rejections occurring later in the parental care cycle. One possible explanation is the development of a bond between parents and free-swimmers as they mature (Noakes and Barlow, 1973). It may start earlier in some species, i.e. <u>Aequidens</u>. Thus, it may be that parents distinguish different stages within free-swimming young.

The problem can be restated as follows: if parental states do exist, then parents should not accept different-aged young (assuming the age differences also include stage differences) because these young do not match the state of the parents. The parental care cycle can be visualized as a continuum of parental care behaviors synchronized with the young as they progress through their developmental stages. Therefore it is necessary to concentrate on points within the cycle where parental behaviors are discrete and predictable. Such points are day one of the egg stage and day one of the free-swimming stage. Parents at these points in the cycle

Exchange C Age	Condition Stage	Pstate	Y <sub>stage</sub>	Results	Authors
Same	Same	Pe	Чe	Accept	Myrberg, 1964 Noble and Curtis, 1939
		Pw	Y <sub>w</sub>	Accept	Same as above
		<sup>p</sup> fs	Yfs	Accept	Same as above
Different	Same	Pe	Чe	Accept	Collins, 1965
		Pw	Y <sub>w</sub>	Accept	Collins, 1965
				Mixed <sup>a</sup>	Myrberg, 1964 Collins, 1965
		Pfs	Y <sub>fs</sub>	Mixed <sup>a</sup>	Myrberg, 1964 Noakes and Barlow, 1973
Different	Different	Pe	Y <sub>fs</sub>	Mixed <sup>a</sup>	This study
		Pfs	Чe	Mixed <sup>a</sup>	This study

Table 1.--Intraspecific Exchanges and Their Results.

<sup>a</sup>Includes examples of both acceptance and rejection.

#### Terminology:

Pstate	=	Pe	or	P <sub>fs</sub>	=	Parents in the egg state or parents in the free-swimming state. This is determined by the age of the parents' own young. For ex-
						ample, if the parents' own young are eggs, these parents are in the egg state (P $_{\rm e}$ ).
Y <sub>stage</sub>	=	Y <sub>e</sub>	or	Y <sub>fs</sub>	=	Young which are eggs and thus in the egg stage or young which are free swimmers and thus in the free-swimming stage

exhibit discrete and different parental behaviors (fanning and calling respectively). These points are based on biological, not chronological, time. Each parent fish is thus comparable to the others in terms of experience with their own young. In addition, these points are far enough apart so that if behavioral states do exist, their effect(s) should be evident. The question can now be tested by interchanging parents and young given appropriate matching of behavioral ages. Parents at day-one egg stage should be given young who are day one free-swimmers and parents at day one free-swimming should be given young who are one day old eggs.

<u>Cichlasoma nigrofasciatum</u> (Günther) is a native Central American substrate-spawning cichlid. It was chosen for the following reasons. (1) It is easy to rear and breed under normal laboratory conditions. (2) Certain of its parental care behaviors have previously been studied (Mertz, 1967; Weber, 1967). (3) It is one of the species involved in the imprinting controversy (Myrberg, 1964).

#### MATERIALS AND METHODS

#### Stocks, Maintenance, and Pair-Selection

The 250 fish used in this study were obtained from dealers in Lansing and Ann Arbor. The white morph called a "golden" cichlid was used. These fish were reared in the laboratory to provide complete reproductive histories. Individuals whose spawning histories were not known, or controlled, were used only as donors.

Ten 90 liter and one 180 liter aquaria were used as experimental tanks. Fish were screened from experimenter contact by a wooden frame with a horizontal slit viewer in front of each tank. Tanks were divided into 45 liter compartments by dark green plexiglass partitions (two compartments in the 90 liter tanks, and four compartments in the 180 liter tank), thus providing 24 compartments holding one pair each. There was no visual contact between pairs.

Each 45 liter compartment was equipped with an outside Le Bern filter with glass wool as the filtering medium. An air stone supplying oxygen, a green plexiglass shelter and a 15 hole brick were present in each compartment (Figure 1). The sides of the triangular shelter provided spawning sites. The holes in the brick provided



Figure 1.--General experimental tank set-up.

refuge when one pair-mate became overly aggressive towards the other, or towards the young. Each compartment held only one pair plus a dither fish (Armitage, 1960; Wallach, 1970) isolated from the pair by a clear plexiglass partition. Hostility between members of a pair is redirected from pair-mates in the presence of a dither fish.

The substrate used in all tanks was white aquarium gravel approximately two inches deep. No vegetation was present. Water temperature was maintained at  $26^{\circ} \pm 1^{\circ}$ C. A sixteen-hour on, eight-hour off, photoperiod was used. Fish were fed dry tetramin mix and newly-hatched brine shrimp.

Fish were kept in high density tanks to inhibit spawning and fighting. Additional aquaria of assorted dimensions were used for housing adult donor pairs and young fish.

Mature individuals (based on size, behavior, and color) were placed in less densely populated tanks for establishment of pair bonds. As soon as courtship or close compatibility was observed between any two individuals (defense of a territory) they were transferred to an experimental compartment. Individuals were thus allowed to choose their own mates from a limited selection. A total of 74 pairs were allowed to spawn once and raise young to the free-swimming stage. Some established pairs

later became incompatible although they had tended a previous brood.

A test pair was separated from their free-swimmers by an opaque plexiglass partition. Young were then exchanged. The partition was removed after 30 minutes. This was found to be an adequate time period to allow parents to return to a reduced activity level. Since eggs were deposited on shelters, exchange of eggs involved removal of the shelter and replacement with an identical shelter without eggs. Free-swimmers were then placed inside the shelter. Exchanged young were placed on the site of the original brood. The exchange itself plus time for the free-swimmers to begin behaving normally took 30 minutes.

All exchanges were limited to young of specified ages during each stage. One day old eggs were exchanged with young who had been free-swimming for one day, and vice versa. Measurements were taken approximately six hours after spawning in the case of eggs, or six hours after more than 50% attained a height greater than two inches above the substrate in the case of free-swimmers. Mertz (1967) and Weber (1967) studying fanning behavior in <u>C. nigrofasciatum</u> found that females performed fanning at a fairly constant rate throughout the day. Limiting young to specified ages during each stage allowed comparison of parental behavioral differences between stages rather than



confounding differences within stages. In order to correctly match ages of young (filial and non-filial), approximately five pairs were used per experiment. This was necessary because spawning times could not be adequately predicted.

### Criteria of Parental Behavior Patterns

Behavior patterns were recorded on a ten-channel Esterline Angus multi-event recorder. Recorder pens were activated by means of a manual keyboard, (one channel for each recorded behavior pattern). The chart speed was 3/4 inch/minute. Only the female's behavior was recorded. Pilot studies indicated that the females exhibited more of the parental care behavior and were fully capable of raising young without the male present. This agrees with the findings presented by Weber (1967). In the first series of experiments the male was allowed to remain in the tank with the female, both free ranging. In the second series, males were isolated behind a transparent partition, while the female, being smaller, was able to swim around it and visit the male. The following behavioral characteristics were recorded. (Terminology follows Barlow, 1964; Weber, 1967; Williams, 1972.)

### Fanning of Eggs

The fish is positioned near and oriented towards the eggs. 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 tail is level with or lower than the head when the eggs are deposited on a horizontal surface. The body is slightly curved in a C, the tail and tail-fin are more curved (Figure 2). The tail and tail-fin beat at low amplitude as do the pectoral, soft dorsal and soft anal fins. The spinous dorsal and ventral fins are usually folded. When the eggs are on a vertical surface, the constant feature is the body curvature.

### Nipping of Eggs

The fish nip at the eggs to "keep them clean" (Weber, 1967). Eggs that turn partially or totally milky white and opaque are dead and are removed by the parents. All nips directed at the eggs were counted.

## Calling of Free-Swimmers

This is sometimes called herding or leading the young. The parent is present within two body-lengths of the swarm. The female is often found more centrally with the swarm than the male, who often is at the periphery. In short bursts the fish alternately propels itself backwards then forward with the pectoral fins; in so doing it slowly rotates in a circular direction to one side and then the other. When the fish is moving backwards, the spinous dorsal fin may be spread or folded, while the



Figure 2.--Cichlasoma nigrofasciatum shown in common position fanning eggs.

spinous anal and ventral fins are folded. The fish arcs upwards when moving backwards. The fish then remains motionless before repeating this behavior.

### Retrieving of Free-Swimmers

A sequence of swimming to displaced free-swimming young, looking-at-young, biting-at-young, carrying-young in the mouth and then spitting-young into a specified area. The parent may swim with up to three young held in the mouth at one time. If the free-swimmer moves quickly enough back to the swarm, behavior of the retrieving parent is truncated at that point.

Fanning and calling occur in distinct units which were termed bouts. On the basis of preliminary studies (see also: Barlow, 1964; Mertz and Barlow, 1966; Mertz, 1967; Weber, 1967) a bout is defined as a period of fanning or calling separated from preceding and succeeding periods of fanning or calling by at least two seconds. Parameters of fanning and calling were recorded as: (1) Duration; the percent of time (in seconds) spent exhibiting the behavior per observation period (obtained by summing individual times for all bouts and dividing by the total observation time in seconds), and (2) bouts; the number of bouts per observation period divided by length of observation period (in seconds). (3) Average bout length was derived from (1) and (2) above by summing the

duration of all bouts during the observation period and dividing by the number of bouts.

In addition to the major behavioral patterns above, the following patterns are differentially exhibited throughout the parental cycle. (1) Vicinity: the fish was within two body lengths of the young but not looking, nor oriented, towards the young. (2) Looking-at-young: the eyes fix on and the body is turned toward the young. The ventral fins are almost always folded and the spinous dorsal fin is usually more than half-folded. When the young are on the bottom the parent tilts downward to an angle of about 40° with lowered head and at a distance of up to half a body length from the bottom. There is little or no movement. (3) Digging: the substrate was removed from an area (creating a depression) by a sequence of movements. The fish looked-at-bottom, bit-at-sand, carriedsand, spit-sand and them swam back to the initial area.

#### Experimental Design

Experimental procedures were conducted under two conditions: (1) when both parents were free-ranging in the tank and (2) when only the female was free-ranging (Table 2). Twenty minute pretreatment observations were conducted to determine whether parents were exhibiting normal parental care behavior toward their own young.

The pretreatment period was followed by five successive treatment observation periods. The first one

Observation Periods		Parental State	Stage of Young	Young Own/non-	Appropriate Care	
NO.	licie	State	Stage (b)	filial	Behavior	
		PROCED	JRE I			
1	Pretreatment Baseline	Pe	Y <sub>e(e)</sub>	Own	Fanning	
2-6	Treatment	Pe	Y <sub>fs(e)</sub>	Non-filial	Calling (Fanning)	
7	Post treatment	Pe	Y <sub>e(e)</sub>	Own	Fanning	
		PROCEDU	JRE II			
1	Pretreatment	Pfs	Y <sub>fs(fs)</sub>	Own	Calling	
2-6	Treatment	Pfs	Y <sub>e(fs)</sub>	Non-filial	Fanning (Calling)	
7	Post treatment	Pfs	Y <sub>fs(fs)</sub>	Own	Calling	

Table 2.--Outline of Experimental Design.<sup>a</sup>

Fe Table

<sup>b</sup>Literature Data: Myrberg, 1964.

began immediately after an exchange and lasted twenty minutes. If at the end of this period the non-filial brood had been accepted, 20 minutes were allowed to elapse followed by a series of four, ten minute observation periods at one-half four intervals. Parents were observed during these times, for response to non-filial young of the same species, but different in age and stage from their own. The criterion for response was normal care behavior in relation to the stage of the young.

Post-treatment observations consisted of returning parents' own young to determine whether exposure to non-filial young had influenced the pair's ability to accept and care for their own young. A 20 minute continuous recording of parental responses toward their own young was taken.

## RESULTS

A total of 17 exchanges was conducted. Parents were first observed with their own young; then the exchanges were performed. When the treatment observations were over, non-filial young were removed and the parents' own young returned. Eleven of the exchanges involved parents in the free-swimming state whose own young were removed and replaced with non-filial eggs of the same species. Six exchanges involved parents in the egg state whose young were replaced with non-filial free-swimmers of the same species. Results of these exchanges are shown in Table 3.

The criterion in the literature for the acceptance/ rejection of foreign young is either normal care of young or eating the live young. From the experimental results it became apparent that this criterion was not meaningful, since it combines two criteria rather than one: i.e. whether live young are eaten or not and whether or not normal parental care is exhibited. Therefore, in analysing the following results, these criteria have been separated.

# Criterion I

The first criterion used is whether or not parents eat live young. All categories of young were accepted

			CATEGORY O	F YOUNG		
PARENTAL RESPONSE CRITERIA	PARENTAL STATE	SAME AGE (Lit. data non-filial)	SAME AGE (Pretrt. filial)	DIFF. AGE AND STAGE (Trt. non- filial)	SAME AGE (Post-trt. filial)	
		Y <sub>fs</sub>	Y <sub>fs</sub>	Yee	Yfs	
		<sup>Y</sup> е	Чe	Yfs	Че	
I. Eating	Pfs	0 <sup>a</sup> n=8	0 n=11	0 n=11	0 n=11	
Live Young	Pe	0 <sup>b</sup> n=8	0 n=6	0 n=6	0 n=6	
II. Exhibiting Normal Behavior	P fs	8 <sup>a</sup> n=8	ll n=ll	4 n=11	ll n=ll	
	 Р е	8 <sup>b</sup> n=8	6 n=6	2 n=6	6 n=6	
III. Latency to all	Pfs	-	45sec n=4	160 min n=4	13 min n=4	
showing Normal Behavior	 Р е	-	30sec n=2	160 min n=2	4 min n=2	
IV. "evel of Normal Behavior <sup>C</sup>	Pfs	- #Bouts: DUR: MBL:	1.70 ± .51 .59 ± .07 .47 ± .13 n=	at 160 min 1.27 ± .14 .61 ± .10 .53 ± .13 4 n=4	.82 ± .29 .25 ± .11 .34 ± .12 n=	
	Pe	_ #Bouts: DUR: MBL:	1.63 ± 1.15 .70 ± .26 1.09 ± .93 n:	at 160 min 1.81 ± .55 .79 ± .07 .50 ± .19 =2 n=2	1.85 ± .81 .23 ± .08 .13 ± .01 n	

Table 3.--Results of all Exchanges. Response of Parents for all Criteria.

<sup>a</sup>Myrberg, 1964 (own young present during exchange)

<sup>b</sup>Myrberg, 1964 (own young absent during exchange; same procedure used in this study)

<sup>C</sup>Mean + standard error of the mean

(Table 3). Pertinent literature studies are included for comparative purposes. Young of the same stage as the parents' own, were accepted whether they were the parents' own or non-filial. Parents also accepted young whether they were the same age and stage or a different age and stage from their own. There was no difference in responses between parents in the egg state and parents in the freeswimming state across all categories. Thus, no discrimination against young took place when the first criterion was applied.

## Criterion II

The second criterion was whether or not parents exhibited normal parental care behavior towards young. For example, fanning is normally exhibited towards eggs during the parental egg stage. Calling is normally exhibited towards free-swimmers during the free-swimming stage.

Parents accepted all young of the same age and stage, whether they were filial or non-filial (Table 3). However, parents did not always accept young of a different stage and age. In a group of 11 parents in the freeswimming state, whose young were exchanged for eggs, there were four acceptances and seven rejections. Six parents in the egg state, whose young were exchanged for freeswimming young, exhibited two acceptances and four rejections. The two groups of parents ( $P_e$ ,  $P_{fs}$ ) differed in

the manner in which they rejected young of different age and stage from their own young. This will be discussed in greater detail later.

A significant difference existed between parental response to non-filial young the same and different stage and age as their own (Table 4). A significant difference also existed between parents in their response to eggs. Parents in the egg state and parents in the free-swimming state differed significantly in their response to eggs (Table 5).

# Criterion III

The third criterion involved measuring the time it took for all parents to exhibit normal behavior. No information on this or the next criterion (IV) is available in the literature for comparative purposes. Parents exhibited a latency period of less than one minute with their own young. When given young of a different stage, these same parents took up to 170 minutes before all were exhibiting normal behavior. When their own young were returned, latency was reduced to 13 minutes for parents in the free-swimming state and four minutes for those in the egg state. A summary of these results is contained in Table 3.

Table 4.--Parental Response to Young of Different Stages. Criterion II, Exhibiting Normal Care Behavior.

F	isher Exact 1	Probability	Test
	PARENTAL	L RESPONSE	
	Accept	Reject	
Same Stage <sup>a</sup>	8	0	
Different Stage	6	11	
			p = .003

<sup>a</sup>Literature data; Myrberg, 1964.

Table 5.--Parental Response to Eggs. Criterion II, Exhibiting Normal Care Behavior.

	Fisher Exact Probability Test PARENTAL RESPONSE TO EGGS								
	Accept	Reject							
P a e	8	0							
P <sub>fs</sub>	4	7							
			p = .007						

<sup>a</sup>Literature data; Myrberg, 1964.

### Criterion IV

The level of normal care behavior exhibited by parents is compared in Table 3. An analysis of variance with repeated measures was used to compare the level of female parental care behavior towards their own young, before (pretreatment) and after (post-treatment), being given young of a different stage (Table 6a). Females exhibited a significantly higher duration of parental care behavior during the pretreatment observation period than they did during the post-treatment period (Table 6b). This was probably due to the longer latency period during posttreatment. Number of bouts and mean bout length, did not show any significant differences. There was no significant interaction effect between pre- and post levels of normal care behavior and stages.

Figures 3 and 4 show the amount of normal parental care exhibited throughout the entire experiment. During the treatment observation periods and once parents begin to exhibit normal care towards young of a different stage, this behavior continues to increase.

The level of normal care behavior exhibited during the last treatment observation period was compared to the pretreatment observation period. Data from the two groups of parents ( $P_e$ ,  $P_{fs}$ ) was combined (Table 7). None of the variables were significant at the  $\alpha = .01$  level. Mean bout length did not differ significantly between

a. Outline:							
		(	GROUPS				
Measures		Egg Stage (PeYe)	Free-Swimmi <sup>(P</sup> fs <sup>Y</sup> f	ing Stage Es <sup>)</sup>			
Before (pretreatm	nent)	N = 6	N = 8	3			
After (Post-trea	tment	z) N = 6	N = 8	3			
	b. /	Analysis of Variand	ce. (Duration)				
Source	DF	Sums of Squares	Mean Squares	F			
Groups (Egg/FS)	1	.00533	.00533	.127			
SUBJ-G	12	.50278	.04189				
Rep. Meas (Before/ After)	1	.32164	.32164	13.193**			
RM-G	1	.06602	.06602	2.708			
Total	27	1.18833					

Table 6.--Comparative Analysis of Female Parental Care Behavior Before (pretreatment) and After (posttreatment) Treatment.  $\alpha = .01$ 

\*\*Significant at the  $\alpha$  = .01 level









Table	7Paired Comparison T-Test. Criterion IV:	
	Comparison of Normal Care Behavior During	Pre-
	Treatment and Final Treatment Observation	Periods.
	df = 5; two tailed $\alpha$ = .01	

	df	t	Р
Number of Bouts	5	2.3167	< .025
Mean Bout Length	5	1.6090	< .05
Duration	5	2.6670	< .01

pretreatment and treatment observation periods. However, although not significant, number of bouts and duration were close to significance. This appeared to be due to a high amount of within group variability.

# Parental Behavior Towards Young of A Different Stage

### Free-Swimmers

Two females in the egg state appeared to retain a memory for their eggs and/or site. At first they retrieved and kept the free-swimmers at the egg site and on the bottom, not allowing them to rise off the bottom, which the young persistently attempted to do. Given time, these females modified their behavior, allowing the young to rise off the bottom and eventually exhibited calling behavior while the young schooled. Four females rejected young and did not exhibit calling behavior. Their behavior, however, was similar to the group accepting young. They exhibited the same intermediary behaviors described above.

### Eggs

Females in the free-swimming state did not appear as attached to a site. This concurs with normal freeswimming care behavior where the school moves around the tank and does not remain in one area. When given nonfilial eggs, parent females first looked at the eggs but

then wandered around the tank. The four that demonstrated normal care towards the eggs, did so by progressively spending more time exhibiting normal care. The seven females which rejected non-filial eggs spent progressively less and less time in the vicinity of the eggs, effectively ignoring them. This resulted in many of the eggs dying.

## Effect of Male's Presence on Female Parental Care Behavior Towards Young of a Different Stage

The amount of aggressive behavior parents exhibited differed among pairs, but was not enough to terminate parental care towards their own young. In several cases intraparental aggression became obvious when young were exchanged. Parents in the free-swimming state, when given non-filial eqgs, often exhibited aggressive behavior over all other types of behavior. In cases where the eggs were ignored it was difficult to determine whether parents were capable of noticing the foreign eggs at all. Female aggressiveness is normally high during the parental phases compared to the non-parental phases of the reproductive It thus appeared feasible to test the females in period. a situation where this aggression was reduced, to see whether their rejection of eggs was related to aggression within the pair. In a second series of experiments the male was isolated behind a transparent partition from which he could not leave while the female, being smaller, was able to swim around this barrier and visit the male.

Non-filial young (eggs) were then offered to the female and her responses recorded. Whether the male was freeranging or not did not appear to affect the outcome of these experimental exchanges. The two series showed the same results in terms of acceptance and rejection of eggs (Table 8). It appeared that females in the free-swimming state will not always accept non-filial eggs and exhibit appropriate (fanning) parental care, whether the male is free-ranging or not. In cases where females ignored introduced eggs, and the male was free-ranging, the females spent their time interacting (aggressive behavior) with the male. It appears therefore, that rejection of the eggs occurs, and then the female spends her time interacting with the male. Females ignoring eggs when males were not free-ranging, remained far from the young, investigating and slowly moving around the tank.

Table 8.--Response of Parents in the Free-Swimming State to Foreign Eggs. Acceptance Criteria: Appropriate Behavior and No Eating of Live Young.

Parental Response	Male	Number of Subjects
Appropriate behavior (Fanning) and no eating of live young	Free-Ranging	2
	Behind Partition	2
Inappropriate be- havior (anything other than fanning behavior)	Free-Ranging	3
	Behind Partition	4

## DISCUSSION

This research was designed to test the hypothesis that cichlid parents possess distinct behavioral states corresponding to the natural development of their young. If these states do not exist, it was predicted that parents would accept young whose developmental stage did not match the state of the parents. When parents were given young of a different stage, the results were assessed with regard to four different criteria. The rejections that occurred were not as extreme as those reported in the literature; in no case did parents eat live young. However rejection did occur in the form of abnormal behavior, i.e. behavior not normally exhibited to young of that stage during the parental care cycle. Approximately two thirds of the parents did not exhibit normal care behavior. In addition, those that did exhibit normal care behavior, did so after a considerable latency period. Only at the end of this latency period did parents exhibit normal care behavior at a level that was not significantly different than towards their own. Thus, three out of four criteria demonstrated that parents do not accept young of a different developmental stage. It therefore appears that

<u>Cichlasoma</u> <u>nigrofasciatum</u> parents possess behavioral states. This study has demonstrated that not matching the stage of the young with that of the parents' own young, generally results in parental rejection, thus supporting Greenberg's hypothesis.

I expected that young differing in stage from the parents' own young would elicit either universal acceptance or universal rejection. Variation in results obtained may indicate that the limit in parental ability to extend parental care to different aged young had been reached. This may have been the case in the group of parents in the freeswimming state given eggs. They either showed normal care or ignored the eggs. However, the group of parents in the egg state given free-swimmers all interacted with the free swimmers. This group did not appear to be as limited in their ability to extend care since they all interacted with the young. All showed a progression of behaviors. It is possible that more parents would have shown the normal care behavior, if the observation time had been extended.

In addition, the stimuli offered by the young differed. Studies investigating mechanisms of parental recognition of young show that visual and chemical cues are important. These studies on the whole, compare parental preferential response to their own young and those of other species (Kühme, 1963; Myrberg, 1966; Noble and

Curtis, 1939). Noble and Curtis (1939) concluded that vision played the primary role in preferential care of free-swimming young by parents of their test species. Preferential care of wrigglers, however, may be mediated by chemical cues (Myrberg, 1966). Myrberg (1966) concluded that optical cues alone are insufficient for preferential care of wriggling young. Other studies on cichlids (including <u>C</u>. <u>nigrofasciatum</u>) have shown that visual cues are also the primary modality used during the egg stage (Kühme, 1963; Mertz, 1967; Myrberg, 1966; Noble and Curtis, 1939).

Studies investigating mechanisms of recognition of parents by free-swimming young have also shown visual and chemical cues to be important. Kühme (1963) found chemicals from parents in the water played a role in recognition in <u>H</u>. <u>bimaculatus</u>. Cole and Ward (1970) showed vision to be the primary modality in <u>Etroplus maculatus</u>. It is possible that chemical sensitivity varies depending upon the species studied. Cole and Ward (1970) further showed that free-swimmers selected their parents in preference to fish of a different species. The flickering of the pelvic fins was found to be the visual cue used by the young. Calling behavior is similar, but not the same in <u>E</u>. <u>maculatus</u> and <u>C</u>. <u>nigrofasciatum</u>. Thus vision, in some species, may be the primary modality for recognition of

parents by free-swimmers, while in other species chemical cues may also be important.

The literature indicates that the parent-young relationship depends upon appropriate responses by <u>both</u> parents and young. In this study, all parents given nonfilial free-swimmers interacted with the young. However, this was not the case for parents given non-filial eggs. It is possible that active free-swimmers were better able to elicit care from parents than passive eggs.

Parents did not eat live young. Initially, I expected rejected young to be eaten, as reported for other rejections cited in the literature. Since the reported rejections are due to two factors (age differences and species differences), this may account for the results presented in this study and those reported in the literature. Young exchanged in this study belonged to the same species, and they may not have differed sufficiently to elicit an eating response on the part of the parents.

The testing of Greenberg's hypothesis was undertaken to resolve the question of whether this hypothesis contributes an explanation to the imprinting controversy. Although my results support Greenberg's hypothesis, the evidence indicates the possible presence of other factors. It is probable that this hypothesis, although important, may not be the sole factor contributing to this controversy. The fact that parents did not eat rejected young

supports this. Other areas of explanation include species differences and methodology. Since this work did not involve different species no definite statement concerning this area can be made. However some of the results pertain to the area of methodology. First, I used four criteria to assess the results of this study. This was necessary in order fully to interpret the results. The literature criteria were found to be superficial in that they overlooked areas where differences existed among The criteria, latency to exhibit normal behavior groups. and measuring the level of normal behavior, contribute towards understanding exchange studies. For example, a long latency period was found to influence the survival of the young in the case of eggs. Parents eating dead eggs (a normal care behavior during the parental care cycle) might be misjudged as discriminating against eggs via an eating criteria. It then becomes a question of whether ignoring eggs is the same as discriminating against them. This question is unimportant. The real area of interest is the interaction between parents and young. For example, how young are capable at different ages of surviving when parents do not exhibit normal care behavior. Measuring the level of normal behavior is also important. It may take a specific level to maintain survival of young, depending on the age of the young, so that although parents may be exhibiting normal care behavior,

it may not be sufficient for the young. Eggs not receiving sufficient care may die. Thus the criteria for acceptance and rejection should be expanded to include the four used in this study. In this way, some of the divergent conclusions of investigators may become more understandable.

## Behavior Towards Foreign Young

Parents in the egg state that exhibited normal care behavior towards free-swimmers, appeared to go through a distinct series of behavioral transitions very similar to those behaviors seen during the normal parental care cycle. At first parents appeared to treat the free-swimmers as if they were eggs, keeping them on the bottom where the eggs has been, then like wrigglers, hovering over them and spitting them back down onto the bottom and in some cases digging a pit and placing the young inside, and lastly as free-swimming young, allowing them to school and leading them around the tank.

Parents in the free-swimming state, on the other hand, did not go through a series of different behaviors, but rather, beginning with short periods, spent progressively more time exhibiting the normal care behavior.

Only the first groung of parents  $(P_e)$ , given older young  $(Y_{fs})$ , exhibited a similarity to the normal progression of parental care behaviors. The other group  $(P_{fs})$ given younger young  $(Y_e)$ , did not. In the latter

situation, modification of female parental care behavior did not proceed in a reverse stepwise order of the normal parental cycle behaviors. Instead, females who accepted non-filial eggs began slowly to exhibit the appropriate behavior for longer periods of time. This may imply that to go in the normal forward direction, females must first experience the intermediate steps.



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