NOTES ON THE BREEDING BEHAVIOR, EMBRYOLOGY AND LARVAL DEVELOPMENT OF *CYPRINODON VARIEGATUS* LACÉPÈDE IN AQUARIA[1]

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ABSTRACT

Several adult *Cyprinodon variegatus* were placed in aquaria, maintained at 23°C, where reproductive behavior was observed. After each spawning the eggs were collected and observed to record the developmental stages. They were 1.4 - 1.6 mm in diameter, spherical, and covered with semi-adhesive threads. Embryological development proceeded rapidly at first but slowed in the later stages. Hatching was first observed between 120-125 hours after fertilization. Newly hatched prolarvae were 3.0 mm in total length, had 24 myomeres, black eyes and an apparently fully developed mouth. The heart was visible and the heart rate averaged 124 beats per minute. A fin fold was present that contained seven or eight caudal but no dorsal or anal fin primordia. One or two fin ray primordia were present in each pectoral fin. The vertical fins were developed and the fin fold had disappeared on individuals over 8.2 mm standard length. Pelvic fin buds began to develop last (at about 8.1 mm SL) and all rays were present at SL 9.0 mm. All individuals possessed most adult characteristics at 12-13 mm SL.

INTRODUCTION

Various aspects of the early life history of *Cyprinodon variegatus* have been reported by several investigators; however, none have given a complete description of the sequence of events beginning with the spawning behavior and extending to the development of juvenile individuals. Newman (1907) presented the first data on the spawning activities of *Cyprinodon variegatus* in aquaria. His observations were later supplemented by Raney, et al. (1953) based on observations in south Florida lagoons. Kuntz (1916) gave the most complete account of the embryology of the *Cyprinodon variegatus* but emphasized information that would aid in the ready identification of the eggs and larvae rather than details of its embryology and development. Hildebrand (1917), Hildebrand and Schroeder (1928), Nichols and Breder (1927) and Simpson and Gunter (1956) reported on various aspects concerning the life history of *C. variegatus* along the Atlantic and Gulf coasts. Most of these data were summarized by Breder and Rosen (1966). The two most recent comprehensive studies on estuarine larval fishes are those by Lippson and Moran (1974) and Scotten, et al. in 1973. The data presented on *C. variegatus* in those reports, however, is essentially a summary of earlier studies.

The purpose of this report is to give a complete account of the reproductive sequence of *Cyprinodon variegatus* including spawning behavior, embryology and larval development. Where possible, our observations and results are compared with those of earlier studies.

MATERIALS AND METHODS

Specimens of *Cyprinodon variegatus* were collected from a tide pool to Mobile Bay, Mobile County, Alabama on 7 April 1974. They were placed in a styrofoam container and transported to the laboratory at the University of Alabama and several males and females were placed in each of two 40-L aquaria filled with freshwater and a clean sandy substrate. Water temperature was

maintained at 23°C ±1°. Fishes were fed brine shrimp *(Artemia)* nauplii each day. The room that housed the aquaria was maintained on a 14-hour light/10-hour dark photoperiod using banks of fluorescent lights controlled by an automatic timer. This lighting was augmented with daylight.

After each observed spawning, the eggs were removed from the aquarium and placed in small culture dishes. Eggs obtained from unobserved spawns were sorted into separate dishes based on their stage of embryonic development. In the initial stages of the study, observations on eggs were made continuously in order to record all embryological changes, however, after the development of several groups had been thoroughly monitored and a schedule of the principal embryonic stages had been devised, eggs were checked only periodically. A written and photographic record of the developmental process was kept throughout the study. Terminology employed in the descriptions of eggs, embryological stages and larvae is similar to that used by Hubbs (1943), Rugh (1962), and Blaxter (1969). Eggs were photographed with a 35 mm single lens reflex camera through a dissection microscope using tungsten light.

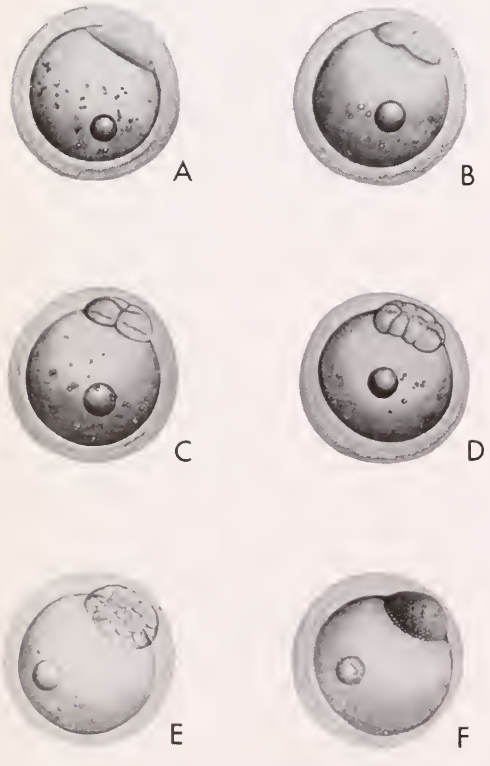

Figure 1. Developing eggs of *Cyprinodon variegatus*. A. One-celled stage; B. Two-celled stage; C. Four-celled stage; D. Eight-celled stage; E. Early blastula; F. Late blastula.

On about the third day after hatching, the prolarvae were fed freshly hatched brine shrimp *(Artemia)* nauplii. This food supply was maintained throughout the study. After each feeding period, uneaten nauplii were removed from the culture dishes to avoid problems of fungus growth associated with water spoilage and disease.

During the period after hatching, one or more individuals were periodically preserved in a 5% formalin solution for observation and measurement. The schedule for preservation of specimens was irregular and dependent on the amount of morphological change that occurred with increased age. As the larvae grew older and assumed adult characteristics, fewer specimens were required for preservation.

During the study 89 larvae were preserved which ranged in age from one to 160 days. Observations on changes in body shape, fin development and coloration were made on each specimen. The following morphological measurements were made with a vernier stage micrometer attached to a dissecting microscope.

STANDARD LENGTH (SL) – length from the tip of the snout to the posterior end of the notochord or the hypural plate.

HEAD LENGTH (HL) – length from the tip of the snout to the rear edge of the gill cover.

MAXIMUM DEPTH (MAXD) – greatest body depth.

SNOUT-TO-DORSAL-FIN-ORIGIN LENGTH (SNTDO) – length from the tip of the snout to the anterior base of the dorsal fin.

SNOUT-TO-VENT LENGTH (SNTVT) – length from the tip of the snout to the anterior edge of the vent.

The snout-to-vent length as a percentage of standard length (SNTVT/SL x 100) was also calculated to determine relative proportional growth. Morphometric data on the larvae were punched on computer cards and analyzed on an IBM 260 computer to obtain mean data for each age group. Product moment correlation values (r) were also computed and plotted for various pairs of these data.

BEHAVIOR

Within a period of 7 to 10 days after the fishes were placed in the aquaria, one of the males assumed a dominant role and established a territory, that occupied approximately one-half of the total area of the tank and extended from the top to the bottom. Any individual that approached the territory was quickly confronted by the male and ultimately sent fleeing to the opposite end of the aquarium. Eventually, one of the females was allowed to enter the territory unmolested.

Once together, however, the male and female did not spawn immediately. The male seemed unattentive to the female and concentrated principally on maintaining his territorial perimeter. Meanwhile the female swam about the area in a seemingly nonchalant manner. Periodically, she proceeded to the bottom of the aquarium and scooped up a mouthful of sand. She held it in her mouth for an instant as if to test it, then spit it out and continued swimming about the

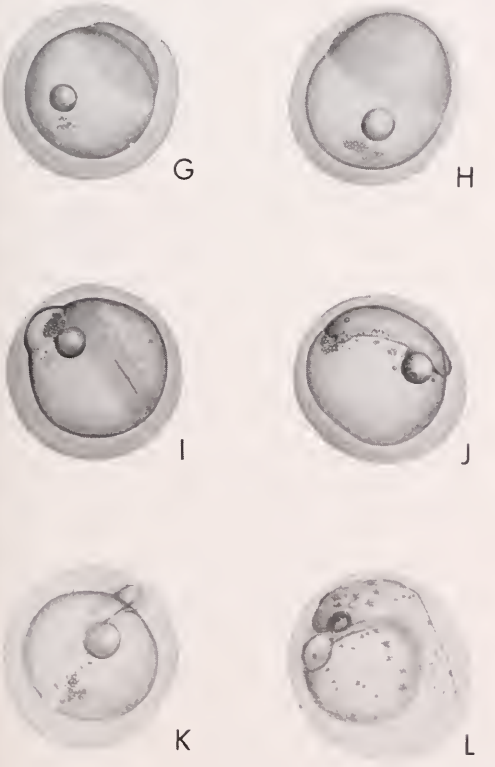

Figure 2. Developing eggs of *Cyprinodon variegatus*. G. Early neurula; H. Mid neurula; I. Late neurula; J. Six-somite stage; K. Ten-somite stage; L. Free-tail prolarva.

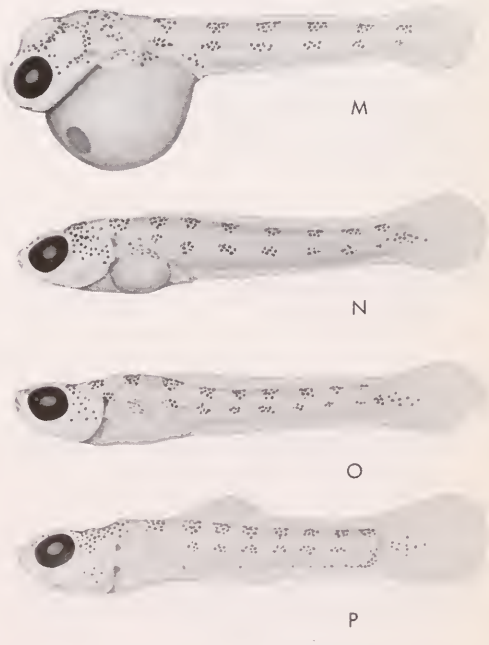

Figure 3. Larvae of *Cyprinodon variegatus*. M. Newly hatched prolarva (2.7 mm SL); N. 3-day old larva (3.5 mm SL); O. 8-day old larva (4.7 mm SL); P. 12-day old larva (5.6 mm SL).

territory. This behavior was repeated several times. After spitting out one of these mouthfuls, the female shoved her snout into the sand at the same spot and threw her head sideways, thereby raising a small cloud of sand particles up off the bottom.

Once he observed this behavior, the male's attentions changed immediately. He no longer seemed interested in defending his territory; instead he positioned himself by the side of the female and began to swim in unison with her. During this time, his body and fin coloration intensified tremendously. The dorsal part of his body and head turned almost black. On each shoulder there was an iridescent blue patch that measured approximately 3 to 5 mm wide and 8 to 12 mm long. The entire ventral surface turned a bright, brassy orange. The dorsal and caudal fins were moderate to bright yellow and each had a distinct black margin. The anal and pelvic fins were yellowish orange. The pectoral fins remained essentially clear. The color of the female changed very little during this time. The dorsal part of her body became slightly darker and her venter assumed a pale yellow while her pectoral, caudal and dorsal fins remained essentially clear. The black spot in her dorsal fin intensified only slightly.

After a brief period of swimming side by side, the pair settled to the bottom where the female had previously nosed the substrate. Suddenly, both fishes began to tremble violently. Simultaneously, they waved their caudal fins rapidly from side to side, creating a small cloud of sand particles in the water. At this time, the eggs and sperm must have been released and covered with sand, but the process occurred so rapidly that it was not actually observed. After the spawning act was completed, the male and female swam away from the area and any eggs that remained uncovered were quickly eaten by the other fishes that had congretaged around them.

Two activities that have been observed in the aquaria have not otherwise been reported in the literature. They appear, however, to be important aspects of the spawning ritual, because of the unfailing frequency with which they occurred. The first activity concerns the apparent control of the spawning time and place by the female. During each pairing, the male indicated no intent to spawn with the female until after she had "signaled" him by running her snout into the sand. Secondly, Newman (1907) indicated that the male almost forced the female to spawn by trapping her either in a corner of the aquarium or at the bottom. After he had captured her, he then "held the female just forward of her caudal fin, using chiefly his very strong dorsal fin." Neither the present authors nor Raney et al. (1953) ever observed the male to hold the female, even during the height of the spawning act.

Repeated observations of the substrate testing by the female followed by egg burial led to the conclusion that these two activi-

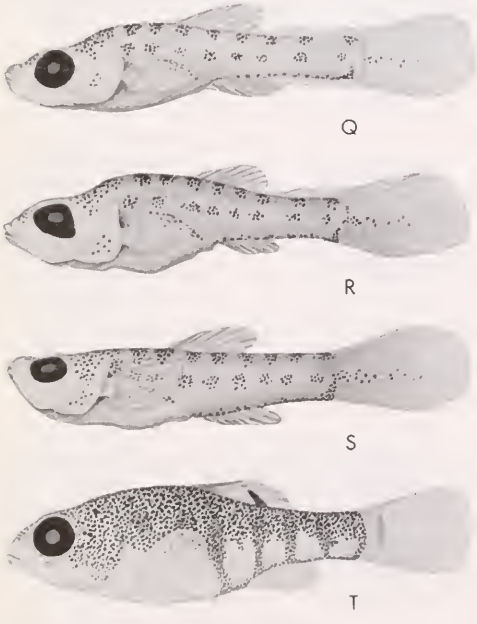

Figure 4. Larvae of *Cyprinodon variegatus*. Q. 16-day old larva (6.5 mm SL); R. 26-day old larva (7.5 mm SL); S. 40-day old larva (8.1 mm SL); T. 98-day old larva (14.4 mm SL).

ties were closely related and that they may represent an ecological adaptation to the heavy predation and/or other environmental conditions in the natural habitat of *Cyprinodon variegatus*. The same result was probably obtained by the nest construction followed by spawning that has been described by Nichols and Breder (1927) and Raney et al. (1953). Both of those reports described how a nest consisting of a clean shallow depression was constructed by a fish, usually a male, during which time he vigorously wiggled his venter against the substrate. The spawning act that followed invariably disturbed bottom sediments and debris which, upon settling, probably covered the eggs. The end result of this activity was that the eggs were out of sight and predation on them was probably reduced.

The spawning of *Cyprinodon variegatus* was observed on four different occasions during this study: 10:15 a.m. (21 April), 11:45 a.m. (1 May), 8:00 p.m. (15 April) and 9:45 p.m. (22 April). Raney et al. (1953) found that spawning activity of *C. variegatus* diminished in the late afternoon and ceased after dark. Nocturnal spawning of the fishes in the present study probably was due to the lengthy light period maintained by fluorescent light banks that masked the normal effects of dusk and darkness.

Spawning did not occur as a single act but consisted of a period of minutes during which a male and female spawned as many as six or seven times. Similar observations were made by Raney et al. (1953) in Florida.

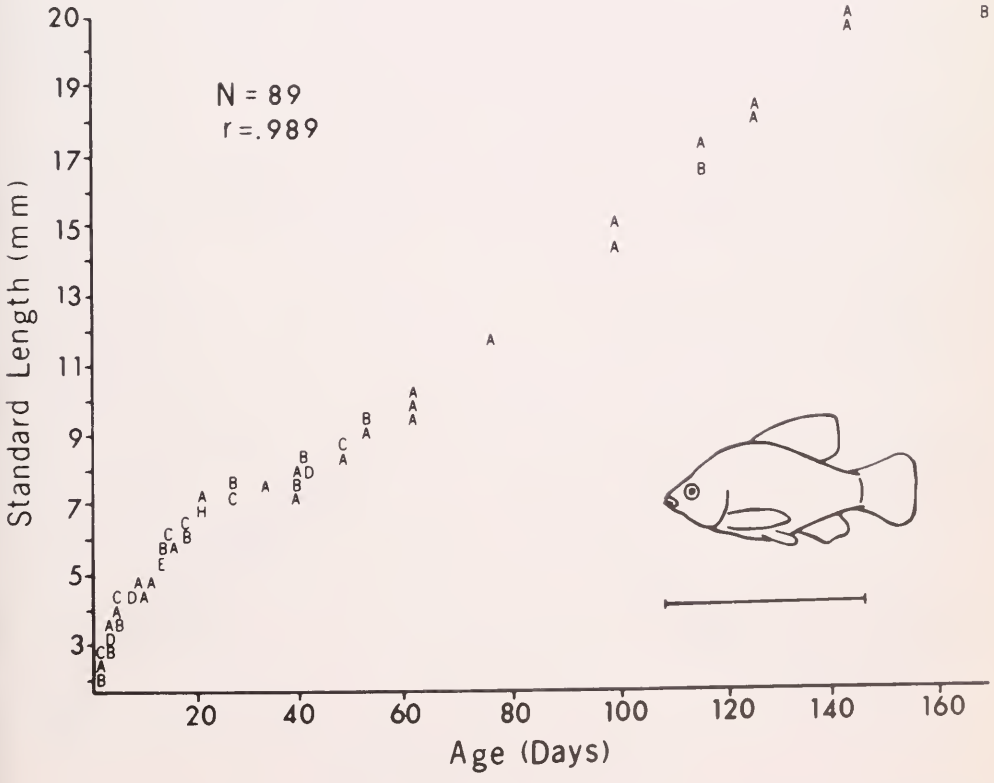

Figure 5. Growth of young *Cyprinodon variegatus* expressed as standard length. A= one observation, B= two observations, etc.

The number of eggs deposited during any one pairing could not be determined because of the rapidity with which the act occurred and their being covered. Moreover, it was not uncommon for the eggs of one spawning to be placed either close to or on top of the eggs from a previous one deposited during the same spawning period. Consequently, when the eggs were collected, they could not be assigned to one spawn or the other. Best estimates indicate that between 8 to 10 eggs were deposited per spawn.

EMBRYOLOGY

Two hundred and twenty-four eggs of *Cyprinodon variegatus* were collected and observed during the study. The newly laid eggs averaged 1.1 to 1.3 mm in diameter, were spherical, yellowish in color and demersal. Each egg contained a yolk that averaged 0.9 mm in diameter and each yolk contained one large, round oil droplet (0.2 mm average diameter) and numerous smaller ones of various sizes randomly distributed throughout it. The surface of each egg was covered with numerous tiny adhesive threads like those noted by Kuntz (1916). The threads caused eggs to stick to each other and any objects that they touched.

The following is a composite description of the various embryological stages that were observed during the study. The letter assigned to each stage corresponds to the drawings in Figures 1 and 2.

A. One-celled stage (45 minutes after fertilization)

Each egg has swelled slightly due to the infusion of water so that the average

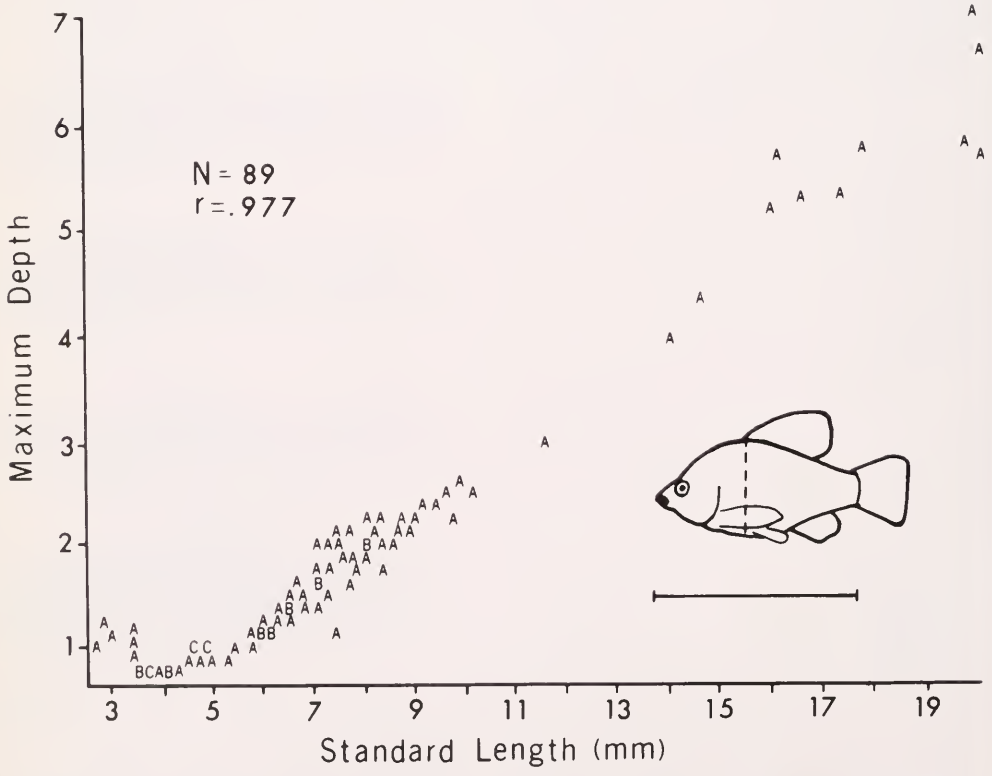

Figure 6. Proportions of young *Cyprinodon variegatus* expressed as maximum depth vs standard length. Legend as in Fig. 5.

diameter is 1.5 mm. A distinct perivitelline space is visible around each yolk. A prominent crescent-shaped structure, the blastodisk, is present on one side of the yolk.

B. Two-celled stage (75 minutes)

As a result of the first mitotic division, two blastomeres of equal size are formed. The chorion has become taut so that it is difficult to press downward on the egg without it being deflected to one side. The area immediately below the blastomeres now contains only a few oil droplets.

C. Four-celled state (2 hours)

The second cleavage occurs at right angles to the first and the resulting four blastomeres are equal in size and approximately one-quarter the dimensions of the previous two. Approximately one-fourth of the yolk below the blastomere is essentially clear of oil droplets.

D. Eight-celled stage (2½ to 3 hours)

The third cleavage occurs at right angles to the second and forms two rows with four blastomeres in each row. The cells appear to be slightly unequal in size. Approximately one-third of the yolk is free of oil droplets.

E. Early blastula (4 hours)

The early blastula stage is formed as the result of continuous asynchronous cell division at several planes. With each succeeding division, cell size has decreased.

F. Late blastula (6 to 7 hours)

Cell division has continued and a prominent berry-like structure called the morula is present on the side of the yolk.

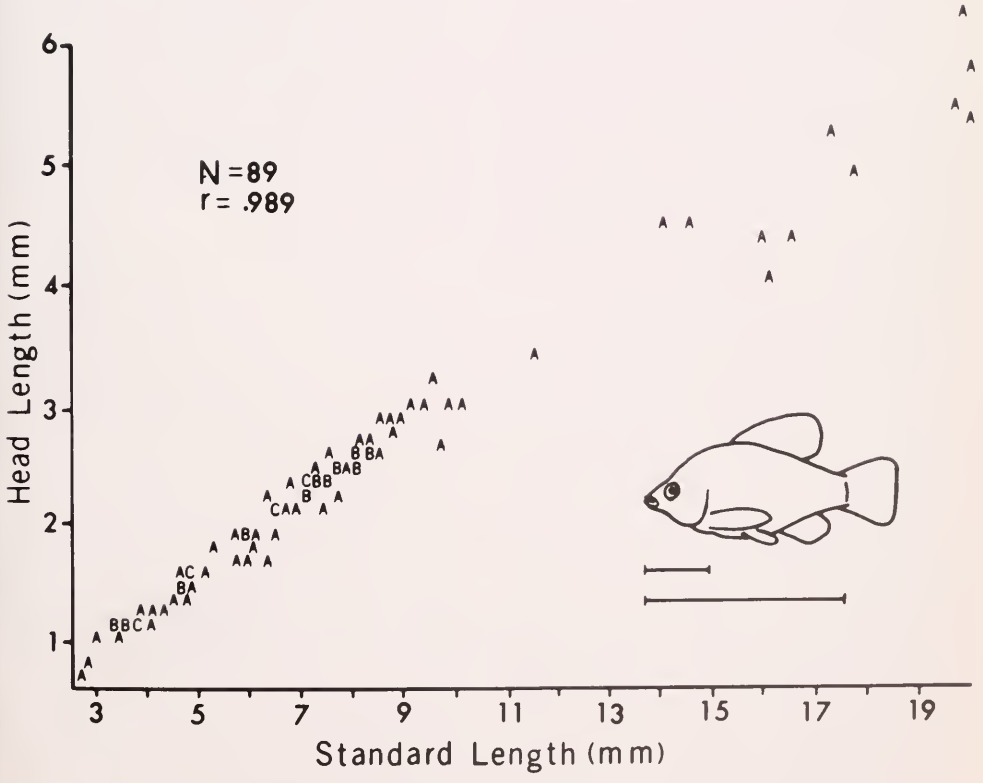

Figure 7. Proportions of young *Cyprinodon variegatus* expressed as head length vs standard length. Legend as in Fig. 5.

G. Early neurula (10 hours)

At the beginning of this stage, the morula becomes flattened on top and its base starts to spread out evenly over the surface of the yolk. Soon, the leading edge of this layer of cells (the germ ring) will thicken and slightly constrict the yolk.

H. Mid neurula (15 hours)

The blastoderm has migrated over approximately one-half of the yolk. The constriction of the yolk by the germ ring is more apparent.

I. Late neurula (22 to 23 hours)

The uncovered portion of the yolk is seen as a prominent bulge. The embryonic shield, which indicates the future longitudinal axis of the embryo, is visible as a thickened area on the blastoderm. The neural keel is present on the embryonic shield.

J. Six-somite stage (36 hours)

Six pairs of somites have developed at about the middle of the embryo. Its anterior end may be recognized by the optic placodes on either side of the head. There is a distinct dome on top of the head in the region of the midbrain. The large oil droplet is now positioned against the ventral side of the yolk opposite the developing embryo.

K. Ten-somite stage (48 to 50 hours)

The optic placodes are more circular around their edges and for the first time a faint outline of a pupil is present. The area over the midbrain has increased in size and there is also a slight bulge over the area of the hind brain. The first melanophores have developed on the dorsolateral portions of the yolk and each adjacent side of the body. The yolk has decreased slightly in size. Immediately anterior to it is a small cavity that contains the structure that will become the heart. The heart is not beating at this time. The notochord is visible as a rod that extends from the rear edge of the brain posteriorly to the end of the body.

L. Free-tail prolarva (90 hours)

The ventral edge of the posterior end of the body has completely separated from the yolk, and this forms the basis for the name of this stage. The tail has oriented to one side of the body because of the occasional wriggling of the prolarva inside the egg. The eyes appear to be fully formed and the iris of each is turning black. A small pectoral fin bud is present on either side of the body immediately posterior to the head and dorsal to the yolk. Melanophores are present on the snout and the dorsal and lateral sides of the body. Three or four concentrations of melanophores on the back form dorsal saddles. The heart is pumping at 104 to 106 beats per minute. The blood is light pink in color and the circulatory pathway throughout the head and body is visible. Twenty to 22 somites are present in the posterior three-fourths of the body, many of which occur as myomeres of muscle. A continuous fin fold is present; it arises on the dorsal midline above the yolk, extends posteriorly around the tail and anteriorly on the ventral midline to the vent. The first indications of two or three caudal fin rays are present in the fin fold.

THE HATCHING PROCESS

The earliest time at which hatching occurred was between 120-125 hours after fertilization, the temperature having been 23°C ±1°. The majority of the prolarvae had hatched by 150 hours but a few required as long as 160 to 170 hours.

Prior to hatching, the movements of each prolarva became more frequent and violent inside the egg. During one of these periods, the chorion ripped and this freed the posterior end of the fish. After a brief rest, the prolarva vigorously shook its head from side to side and eventually managed to free itself from the egg.

For the first few hours, the prolarvae remained motionless, lying on their sides on the bottom. Periodically, one or two individuals darted ahead for a short distance along the bottom or swam upward in the

water column in a short burst of speed. As quickly as they started, however, they stopped and drifted back to the bottom.

DESCRIPTION OF THE PROLARVA

The newly hatched prolarva of *Cyprinodon variegatus* was tear-drop in shape except for the large round yolk on its ventral side (Figure 3,M). Total length averages 3.0 mm and standard length 2.7 mm (Table 1). The eyes were black with a gray iris. Kuntz (1916) noted that the head of *Cyprinodon variegatus* was not deflected; however, it was deflected on all of the newly hatched prolarvae that were observed in the present study. It is not certain if the references to the non-deflected head by Lippson and Moran (1974) and Scotton et al. (1973) were based on Kuntz (1916) or on original observation. A large bump occurred on top of the head over the enlarging brain. A small, incompletely formed mouth was visible in a terminal position on the snout. The rear edges of the opercles were free and moved as the prolarva breathed.

The body of the prolarva was translucent pale yellow and the dorsal and lateral portions of it and the head were covered with randomly distributed melanophores. Seven or eight irregular saddles of melanophore concentrations appeared on the back. The total number of myomeres in the body varied from 23 to 26. Postanal myomeres ranged from 16 to 19 but averaged 17 or 18. Inside the yolk and located along its anterio-ventral wall was a small but distinct oil

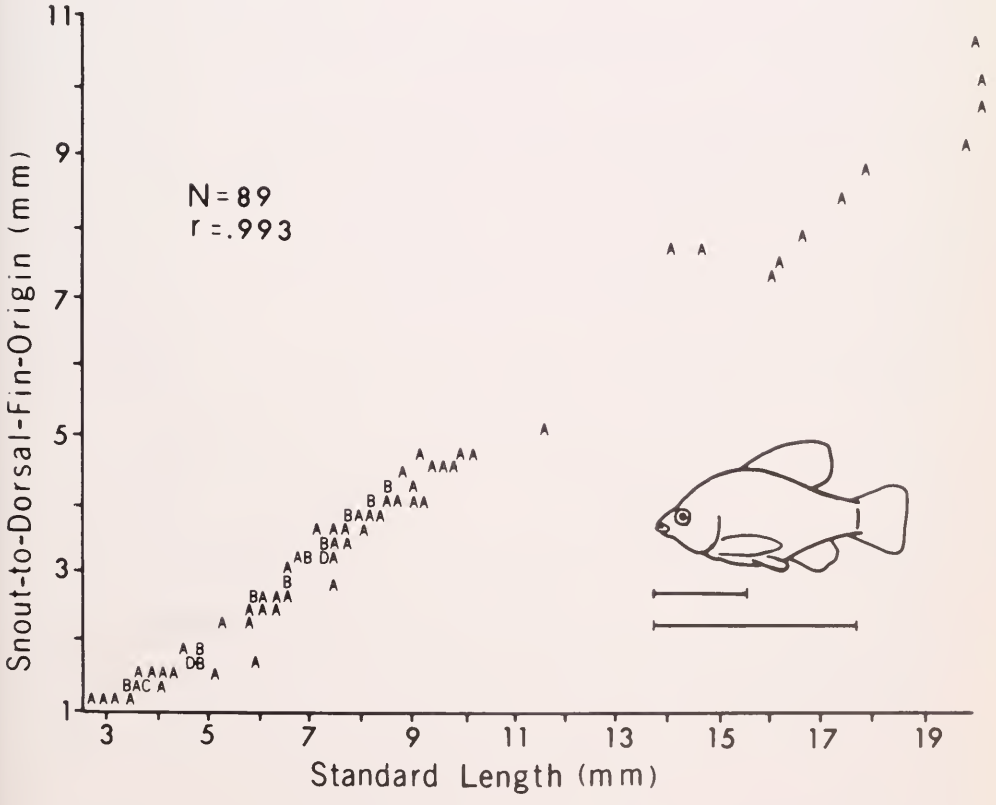

Figure 8. Proportions of young *Cyprinodon variegatus* expressed as snout-to-dorsal-fin-origin length vs standard length. Legend as in Fig. 5.

droplet. The heart was easily seen inside the pericardial cavity which was located immediately anterior to the yolk in the area of the isthmus. The heart beat averaged 124-125 beats per minute. The blood was light pink, the erythrocytes were distinguishable and their pathway throughout most of the body was easily observed.

A continuous finfold was present on the dorsal midline, slightly behind the head. It extended around the posterior end of the body and continued anteriorly on the ventral midline to the vent. Six or seven caudal fin ray primordia were present but no dorsal or anal primordia could be seen. A small pectoral fin bud that contained no rays was present on either side of the body immediately posterior to the opercle. No pelvic fin buds were observed on the newly hatched prolarvae.

LARVAL DEVELOPMENT

Growth of *Cyprinodon variegatus* larvae was rapid. Age vs standard length is presented in Table 1 and Fig. 5; various morphological measurements in relation to standard length are presented in Table 1 and Figs. 6-9. Figures 3 and 4 depict significant morphological stages during larval development.

YOLK: Shortly after hatching, the yolk began to decrease noticeably in size. On day 3 (Figure 3,N), only a small portion was left and the oil droplet was not discernible. By day 8 (Figure 3,O), the yolk had completely disappeared.

FINS: Dorsal fin ray primordia were first apparent on 9- and 10-day old larvae. Twelve-day old larvae (Figure 3,P) possessed

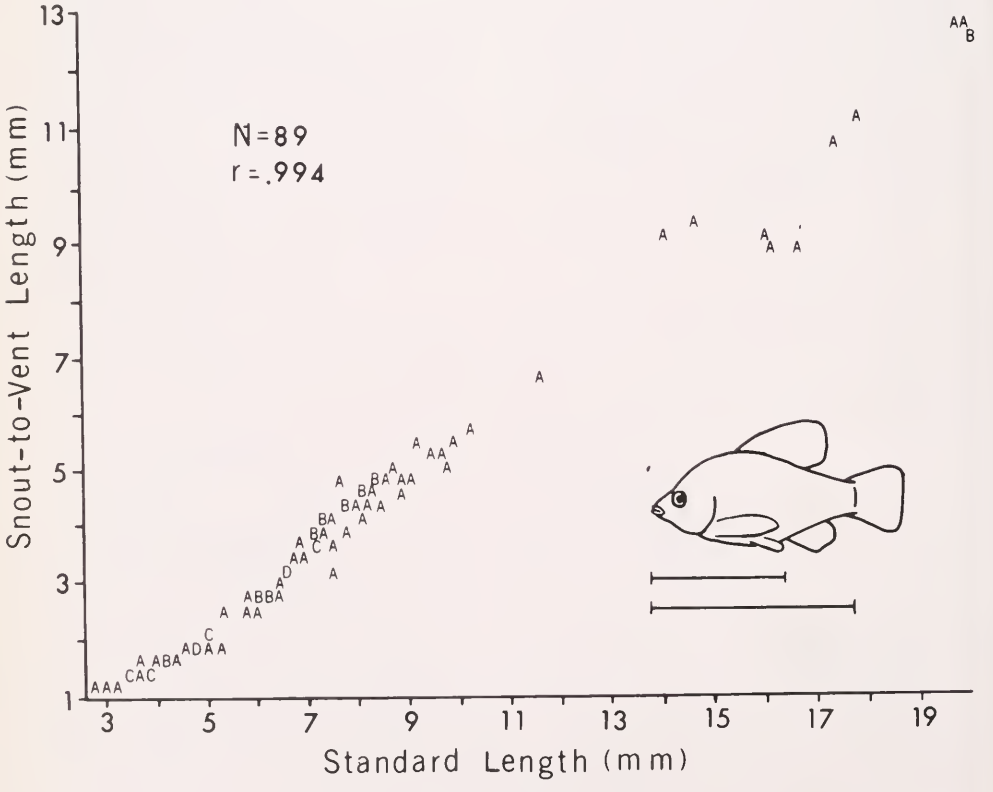

Figure 9. Proportions of young *Cyprinodon variegatus* expressed as snout-to-vent length vs standard length. Legend as in Fig. 5.

Table 1. MEAN DATA FOR MORPHOMETRIC MEASUREMENTS MADE ON 139 AQUARIUM-RAISED SPECIMENS OF CYPRINODON VARIEGATUS: N = Number; SL = Standard length; HL = Head length; SNTL = Snout length; EL - Maximum eye width; SNTDO = Snout to dorsal fin origin length; SNTVT = Snout to vent length; MAXD = Maximum depth; and SNTVT/SL x 100 = Snout to vent length divided by standard length x 100.

Age	N	SL	HL	SNTL	EL	SNTDO	SNTVT	MAXD	SNTVT/SL x 100
1*	53	2.7	0.6	0.1	0.2	1.1	1.3	1.1	47.0
2	3	3.3	0.9	0.1	0.3	1.3	1.5	1.0	45.0
3	7	3.6	1.0	0.2	0.3	1.4	1.7	0.8	45.6
4	2	4.0	1.1	0.2	0.4	1.5	1.8	0.7	43.8
5	4	4.6	1.3	0.2	0.5	1.8	2.0	1.0	44.4
8	4	4.7	1.4	0.3	0.5	1.9	2.9	1.0	46.1
9	2	4.9	1.4	0.3	0.5	1.7	2.1	1.0	41.8
10	1	5.2	1.7	0.3	0.6	2.3	2.6	1.0	50.0
13	7	5.8	1.7	0.4	0.6	2.5	3.0	1.1	49.6
15	4	6.4	2.0	0.4	0.7	2.8	3.2	1.4	50.4
18	5	6.6	2.1	0.5	0.7	3.2	3.6	1.5	53.9
21	9	7.1	2.3	0.4	0.8	3.4	4.0	1.7	55.5
26	5	7.5	2.3	0.5	0.8	3.8	4.3	2.0	57.0
32	1	7.8	2.4	0.6	0.8	3.9	4.5	1.8	57.7
38	4	7.8	2.5	0.6	0.9	3.9	4.7	2.0	59.5
40	6	8.2	2.6	0.6	0.9	4.2	4.8	2.0	58.2
47	4	8.8	2.9	0.7	1.0	4.3	5.0	2.2	56.6
51	3	9.4	3.0	0.7	1.1	4.7	5.4	2.4	57.3
60	3	9.8	3.1	0.7	1.2	4.8	5.7	2.6	57.9
75	1	11.5	3.5	0.9	1.3	5.2	6.9	3.1	60.0
98	2	14.3	4.8	1.2	1.5	8.0	9.5	4.3	66.1
115	3	16.2	4.5	1.1	1.5	7.8	9.2	5.5	56.4
125	2	17.6	5.5	1.4	2.0	9.0	11.2	5.8	63.5
143	2	20.0	5.9	1.6	1.8	9.9	12.9	5.9	64.5
170	2	20.1	6.6	1.6	2.2	10.9	12.9	7.1	64.1

*Day 1 measurements were made on 50 live and 3 preserved individuals.

about the same numbers of dorsal and anal fin primordia. On day 16 (Figure 4,Q), segmentation was visible at the bases of several of the dorsal and anal rays which had increased in length. The ventral fin fold and part of the dorsal fin fold had disappeared by day 26 (Figure 4,R). At 40 days after hatching (Figure 4,S), all remnants of the fin fold had disappeared and for the first time, tiny pelvic fin buds were present on either side of the mid-ventral line. Several rays were present in each of the pectoral fin buds. At the juvenile stage (Figure 4,T) all fins were completely developed.

THE HEAD. Lippson and Moran (1974) indicated that the mouth on larval *Cyprinodon variegatus* was superior. In our specimens, however, it occupied a more or less terminal position. As the yolk disappeared, the head assumed a more horizontal

position. The eyes were large, even in newly hatched prolarvae, their diameter averaging one-third the head length. An otolith was present in a position immediately posterior to each eye. The dorsal and lateral sides of the head contained scattered melanophores, the number of which increased with age so that the head of a 98-day old juvenile (Figure 4,T) was completely covered except for the ventral surface.

THE BODY: The body of the youngest larva was fairly elongated and compressed. Within three to four weeks after hatching, it began to assume the more diamond-shaped appearance characteristic of adults. The notochord was easily visible as a rod extending almost the entire length of the body, but it gradually became indistinguishable as the body became more opaque and the vertebral column developed at approximately days 20 and 24. Although the number of body melanophores increased, their distribution remained unchanged until about 60 days when they began to appear over most of the back and sides. By 98 days (Figure 4,T), they covered most of the body except the ventral region anterior to the vent and several irregularly shaped patches on either side above the anal fin and on the caudal penducle.

GROWTH: Figure 5 indicates the direct relationship between age and standard length ($r = .989$) in the young *Cyprinodon variegatus*. The decrease in maximum depth in Figure 6 is due to yolk absorption. Shortly thereafter, the maximum depth began to increase proportionately with standard length ($r = .977$) as the larvae began to actively feed on the *Artemia* nauplii. The small, abrupt increase in head length at 3 to 3.5 mm standard length in Figure 7 is due to yolk absorption and the subsequent upturn of the head, especially the snout, to assume a more horizontal position. The relationship between snout-to-dorsal-fin-origin and standard length (Figure 8) is highly correlated, with an r value of .993. Snout-to-vent length versus standard length are also highly correlated ($r = .994$) in Figure 9.

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