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Egg, Larval and Juvenile Development of Longnose Dace, *Rhinichthys cataractae*, and River Chub, *Nocomis micropogon*, with Notes on Their Hybridization

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Artificially spawned eggs of longnose dace and naturally spawned eggs of river chub were incubated at 21 ± 3 C through hatching and the larvae reared through the juvenile phase. Development was similar in each species but rates differed. Opercles, mouth, heart and blood circulation developed earlier in longnose dace; fin ray and scale development occurred at a faster rate in river chub.

Mean total length of longnose dace at hatching ranged from 4.5 to 5.9 mm and differed significantly between two streams: the smaller larvae hatched from significantly smaller eggs produced by smaller females. No significant differences were found in egg size or mean hatching length in river chub which hatched at a mean total length of 5.9 mm. Larval development was complete at 110 days in longnose dace and 57 days in river chub.

Collections in river chub nests contained eggs of longnose dace, river chub, common shiner, creek chub and rosyface shiner in various combinations. Longnose dace and river chub eggs occurred together in each stream sampled and hybrid longnose dace × river chub have been reported from three of the five streams sampled. The use of river chub nests by longnose dace for spawning may be due to a lack of spawning habitat for dace elsewhere in the stream or to a preference for stone nests and may be a primary cause for hybridization between longnose dace and river chub.

LONGNOSE dace (*Rhinichthys cataractae*) and river chub (*Nocomis micropogon*) are common fishes of eastern United States but their egg and larval development has not been described in detail. Fish (1932) described a single specimen of each species from Lake Erie. Identifying characteristics of longnose and blacknose dace larvae have been reported by Bartnik (1970) and Fuiman and Loos (1977) but neither study described development in detail.

This study describes the egg, larval and juvenile development of the longnose dace and river chub and speculates on a possible origin of *Rhinichthys bowersi*, a reported hybrid between these two species (Raney, 1940).

Goldsborough and Clark (1908) described *Rhinichthys bowersi* from the Cheat River System, West Virginia, as a new species but recognized that it exhibited an intermediacy of characters between longnose dace and river chub. Raney (1940) redescribed the cotype and specimens he collected as longnose dace × river chub hybrids. Further collections of the hybrid have been made in New York (by Smith and Anderson in 1949), Pennsylvania (E. L. Cooper, Pennsylvania State University, pers. comm.), Maryland and West Virginia (Stauffer et al., 1979) and Ohio (Ross and Cavender 1977). All reported collections of the hybrid fall within the overlap of distribution of the putative parent species. A summary of the putative parent species is given by Scott and Crossman (1973).

MATERIALS AND METHODS

Ripe adults were collected in West Virginia in Shavers Fork at Cheat Bridge, Randolph Co., and in Snowy Creek below Terra Alta Lake, Preston Co.; in Flaugherty Creek, Somerset Co., Pennsylvania; and in Wills Creek, Allegany Co., Maryland. Collections were made with electroshocking gear and seines from May through July, 1977 and 1978. The fish were segregated by sex using the presence and pattern of tubercles and spawning coloration and transported to the laboratory with constant aeration.

Artificial stripping of eggs and sperm was unsuccessful with river chub even though natural spawning was occurring at the collection

site. Developmental observations of river chub eggs were made on naturally spawned eggs collected from river chub nests in Wills Creek. Water temperature at the time of collection was 21 C. Fertilized eggs in the one-cell stage were observed at the collection site through the blastula stage and then transported to the laboratory (25 min travel time) for further observation.

Pairs of longnose dace were artificially stripped of eggs and sperm into one-liter glass aquaria containing enough water to cover the eggs. The products were gently mixed by swirling the water and subsequently were not moved for 15 min to allow the eggs to water harden.

Observations of developing eggs of both species were made according to the following schedule: every ten min through the blastula stage; every two hours through the tail-bud stage; and every six hours until hatching. Eggs were observed and described prior to preservation since the refractive properties of the chorion are often changed with preservation.

The larvae were fed brine shrimp (*Artemia* sp.) nauplii and copepodites from cultures maintained in the laboratory. Brine shrimp were added to each aquarium twice per day for two weeks, then reduced to once per day.

To determine river chub nest utilization by other fish species, egg collections were made in river club nests in the same locations as for adult collections with the addition of the Savage River, Garrett Co., Maryland. A total of 30 nests was sampled. Collections were made by manually digging through the nest, allowing the eggs to drift downstream into a dip-net. Eggs from 16 nests were transferred to one-liter glass aquaria and incubated to the juvenile phase at 21 ± 3 C. The remaining collections were preserved. Feeding followed the schedule for the other larvae.

Temperature measurements for all studies were made twice daily with a laboratory thermometer prior to each feeding. Photoperiod was controlled with a 24-h commercial timer wired to three 14-watt "vita-light" fluorescent tubes equally spaced from each aquarium. One fluorescent tube was used to light four aquaria. Photoperiod followed that observed naturally.

Preserved larvae were rinsed in distilled water and then soaked in a solution of 3% alizarin red dye-distilled water for two minutes to highlight myomeres, fin structure, internal development and scale development. Measurements were made with an ocular micrometer read to the nearest 0.1 mm. All body lengths given are total lengths. Terms used for descriptions are from Koenig and Livingston (1976) and Mansueti and Hardy (1967). Myomere counts were made according to Fuiman and Loos (1977). Terms designating larval development phases are from Snyder et al. (1977). Illustrations were done freehand at 10× magnification and each is to scale based on the respective recently hatched larva.

Longnose Dace

Embryogenesis.—Unfertilized eggs had a smooth chorion, granular yellow yolk, and are generally spherical (1.0 to 1.5 mm diam). During fertilization, the granular appearance disappeared as a wave beginning at the micropyle. The average diameter increased to 2.1 mm as water was absorbed. Chronological development of eggs from Shavers Fork is summarized in Table 1.

Four pairs of somites formed 26 h after fertilization. The rate at which pairs of somites were added decreased from 4 pairs per h at 28 h to less than 1 pair per h at 48 h. At 48 h, 33 pairs of somites were present. The somites became functional myomeres between 55 and 60 h and limited flexure of the tail was observed. There were 23 to 24 preanal myomeres and 10 postanal myomeres after 72 h (Fig. 1).

The rudimentary heart formed anteriorly to the otic placodes below the midbrain and had a slight, irregular beat at 62 h. Circulation in the major vessels became apparent at 72 h and formed two circular patterns in the anterior part of the yolk sac: each flowed toward the other creating one clockwise and one counterclockwise pattern. Circulation along the spinal cord and ventral edge of the tail was observed after 80 h.

Separation of the tail from the yolk sac at 34 h began a series of changes in the shape of the yolk sac. A deep cleft was formed which increased as the tail continued to grow. After 85 h, the yolk was divided: anteriorly, it was nearly spherical; posteriorly, it was elongated from the twelfth myomere to the vent.

Protolarvae.—Protolarvae hatched between 3 and 4 days after fertilization at an average length of 5.9 mm (range = 5.5 to 6.0 mm; N = 10). No body pigment was present except on the margin of the eyes (Fig. 2A). Two-thirds of the surface of the eyes were pigmented after

TABLE 1. CHRONOLOGICAL DEVELOPMENT OF LONGNOSE DACE AND RIVER CHUB EGGS AT 21 ± 3 C AND THEIR CORRESPONDING FIGURES.

Developmental features	Longnose dace		River chub	
	Hours from fertilization	Figure 1	Hours from fertilization	Figure 4
Fertilization, blastodisc formation	0.0–0.5	A	0.0–0.5	—
One cell	0.5–1.0	B	0.5–1.0	A
Two cells	1.0–1.3	C	1.0–1.5	B
Four cells	1.3–1.6	D	1.5–2.0	C
Eight cells	1.6–2.1	E	2.0–2.5	D
Sixteen cells	2.1–2.6	F	2.5–3.0	E
Thirty-two cells	2.6–3.1	G	3.0–3.5	F
Formation of upper and lower blastomeres	3.1–3.6	H	3.5–4.0	G
Start of epiboly	3.6–5.1	I	6.0–8.0	H
Germ ring formation	6–11	J	10–12.5	
Embryonic shield	13–15		14–16	I
Embryonic keel	18–24	K	18–20	J
Somite development	24–25	L	20–22	K
Closure of blastopore	26–27	M	20–22	
Optic vesicle formation, tail separates from yolk	30–34	N	24–25	L
Otic vesicles, optic placodes form	42–48		36–44	M, N
Flexure of tail, brain differentiation	50–60	O	47–58	O
L. dace heart forms	60–65	P	—	
Elongation of yolk sac, urogenital duct forms	70–85		58–65	P
R. chub heart forms	—		85–95	
Blood circulation, hatching	72–96	P	99–144	

6 days (Fig. 2B) and complete pigmentation was present 7 days after fertilization. Body pigmentation formed in three rows 6 days after fertilization: a dorsal series of paired melanophores at nearly every somite (Fig. 2C); a series along the horizontal septum to the tail; and a series which followed the dorsal surface of the yolk sac to the vent and posteriorly along the ventral edge of the tail. An elliptical series of melanophores was present on the dorsal surface of the head.

Eight days after fertilization, the anlage of the hypural plate was formed and the olfactory placodes, jaws and gill arches with rakers were present (Figs. 2D, E). The first lobe of the gas bladder was filled and its dorsal surface was pigmented. The external nares were formed.

Mesolarvae.—The basal elements of the hypural plate were formed at 9.5 days and development was complete after 14 to 19 days. The anlage of the dorsal and anal fins and six incipient caudal rays were present 9.5 days after fertilization. Ten incipient caudal rays were present at 12 days (Fig. 3A). The caudal rays were segmented after 14 to 19 days. At this time, incipient rays had formed in the dorsal fin, and along with the caudal rays, were occasionally pigmented at their tips.

At 10 days, external pigmentation extended anteriorly around the yolk sac and internally from the gas bladder toward the head. An internal V-shaped melanophore series was present on the isthmus. Two wide rows of pigment were formed dorsally (Fig. 3B) and pigment was concentrated on the head. Between 14 and 19 days, pigment became concentrated on the hypural plate forming a distinct spot. Melanophores formed across the snout in 9.6 mm mesolarvae (Fig. 3C). The yolk was absorbed between 10 and 11 days and mesolarvae ranged from 9.0 to 9.5 mm in length.

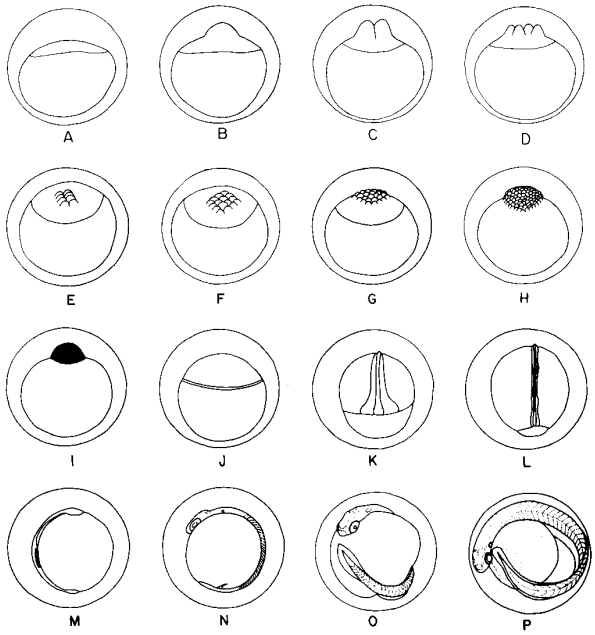

Fig. 1. Developing eggs of the longnose dace. See Table 1 for explanation of developmental changes.

Metalarvae.—Incipient rays in the anal fin were present and the pelvic fin buds appeared between 21 and 37 days after fertilization. The pelvic fin buds were located just posterior to the gas bladder (Fig. 3D). The incipient rays of the pelvic and pectoral fins were present and the anal fin rays were segmented between 42 and 77 days (Figs. 3E, F). Fin ray development was complete at 96 days (Fig. 3G).

The division of the gas bladder from one to two lobes was complete between 21 and 37 days. The anterior lobe formed last and both lobes were equal in size at 96 days. Other structural changes during this period were the reduction of the choroid fissure and elongation of the snout over the lower jaw. The external nares were separated by a prominent flap between 42 and 77 days. Metalarvae averaged 10.8 mm at 37 days, 13.7 mm between 42 and 77 days, and 16.5 mm between 83 and 100 days.

Pigmentation increased internally on the dorsal surface of the gas bladder at the start of the metalarval period. Pigment extended internally under the otoliths to the eyes and was concentrated externally at the bases of the dorsal, anal, and caudal fins. Dorsal pigmentation was more diffuse (Fig. 3H) than in earlier phases. Between 42 and 77 days, melanophores developed along the rays of the dorsal and caudal fins, guanin deposits were present along the gut, and green chromatophores developed on the opercles. A distinct internal concentration of pigment developed ventrally between the opercles.

Juveniles.—The preanal finfold was absorbed at 110 days marking the transition from metalarva to juvenile. Barbel formation occurred between 110 and 115 days (23 to 25 mm fish length).

Scale development began on the caudal peduncle when fish were between 19 and 21 mm in length. No scales were found on specimens 18 mm or less. Between 24 and 26 mm, scales had formed along the horizontal septum to just posterior to the opercles. Complete squamation existed on the tail after 118 to 123 days (fish lengths of 27 to 28 mm). Scale formation was complete between 135 and 145 days at fish lengths of 39 to 40 mm.

River Chub

Embryogenesis.—Unfertilized eggs had a smooth chorion, granular yellow yolk, and ranged from

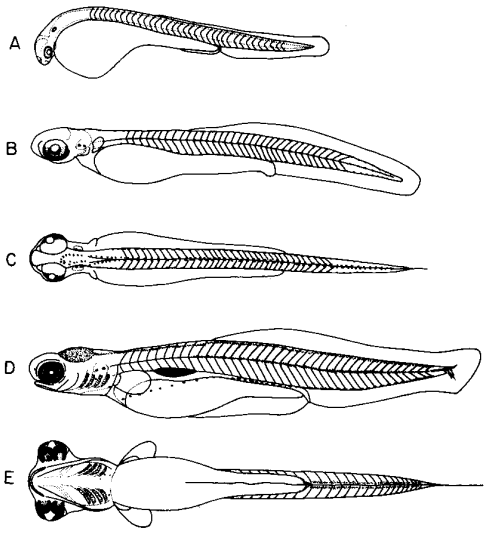

Fig. 2. Developing larvae of the longnose dace. A) protolarva, recently hatched, 5.5 mm (3–4 days); B) protolarva, 7.4 mm (5–6 days); C) protolarva, 7.4 mm, dorsal view; D) protolarva, 8.5 mm (7–8 days); E) protolarva, 8.5 mm, ventral view.

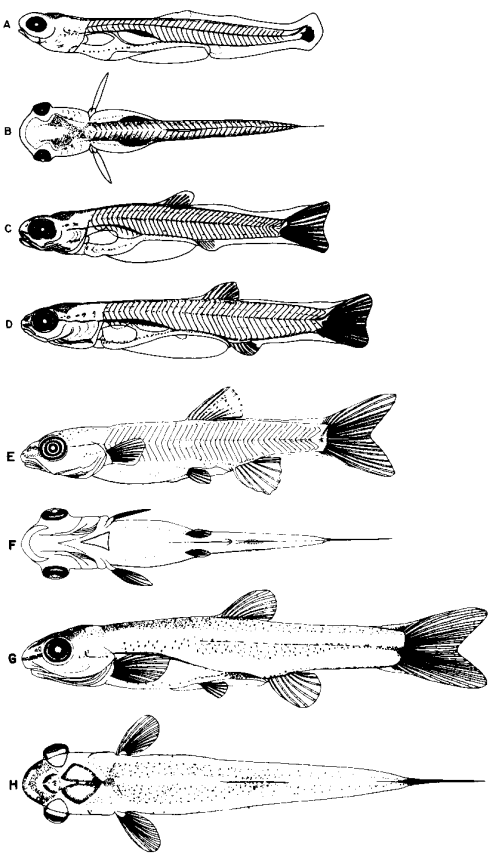

Fig. 3. Developing larvae of the longnose dace. A) mesolarva, 9.0 mm (9.5–10 days); B) mesolarva, 9.0 mm, dorsal view; C) mesolarva, 9.6 mm (14–19 days); D) metalarva, 10.8 mm (21–37 days); E) metalarva, 13.7 mm (42–77 days); F) metalarva, 13.7 mm ventral view; G) metalarva, 16.9 mm (96 days); H) metalarva, 16.9 mm, dorsal view.

1.0 to 1.5 mm in diameter (N = 50). One h after fertilization, the average diameter was 2.7 mm due to water absorption. Chronological development of eggs from Wills Creek is summarized in Table 1.

Somite formation began 20 h after fertilization. Five pairs of somites were formed at 22 h; 13 at 25 h; 18 at 32 h; and 25 at 44 h. The tail budded away from the yolk at 44 h and, as growth continued, part of the yolk was drawn away from the main sphere. Functional myomeres were formed at 46 h and occasional flexure of the tail was observed at 47 h (Fig. 4).

Prior to hatching, the brain had differentiated into the fore, mid, and hind sections. The head was elongated and remained flat on the yolk surface. Heart formation and blood circulation were present at 85 to 99 h.

Protolarvae.—Hatching occurred between 5 and 6 days after fertilization (Fig. 5A). Recently hatched protolarvae averaged 5.9 mm in length (range = 5.7 to 6.1 mm; N = 10) and were without pigmentation. The eyes became pigmented at their margins in the middle of the sixth day and were completely pigmented between 8 and 10 days. Two rows of melanophores were present along the dorsal body surface, and single rows were present on each side of the body along the horizontal septum and yolk sac.

On the seventh day, bright red blood flowed posteriorly in the dorsal surface of the body and returned toward the heart along the ventral surface of the tail and yolk sac. The yolk sac was large, accounting for 36 to 40% of the total length (Fig. 5B).

The pectoral fins budded on the dorsal surface of the yolk sac, posterior to the head, when protolarvae were 8.0 mm in length (Fig. 5C). Between 8 and 10 days, the urostyle turned

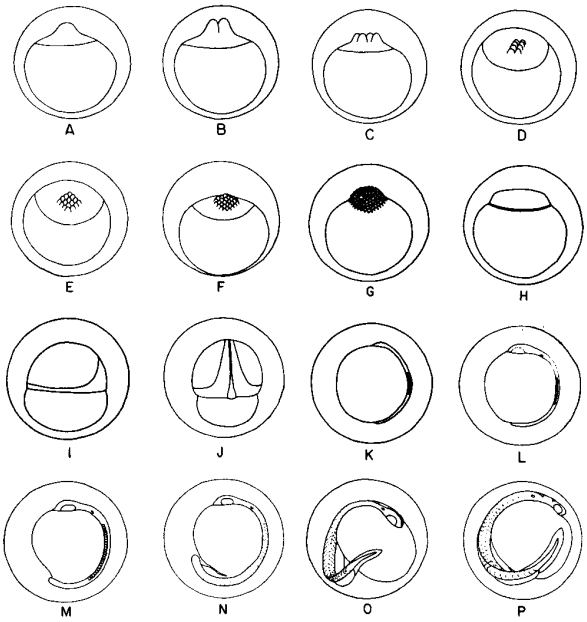

Fig. 4. Developing eggs of the river chub. See Table 1 for explanation of developmental changes.

upward and the hypural plate anlage was evident. The mouth, intestine, and vent were functional and two gill arches with rakers had formed (Fig. 5D). Otoliths and olfactory placodes were evident (Fig. 5E). There were 26 preanal myomeres and 15 postanal myomeres. Protolarvae were 9.5 mm in length after 9 days.

Mesolarvae.—Development of the hypural plate was complete after 12.5 days and incipient rays were present in the caudal fin (Fig. 6A). Caudal fin rays became segmented at 14 days. Incipient rays were present in the anal and dorsal fins at 14 days and had 7 and 9 rays, respectively, at 17 days.

The gas bladder was filled at 13 days and was divided into two lobes at 14 days. The urogenital duct was evident above the intestine. Yolk absorption was complete at 17 days at fish lengths averaging 10.8 mm.

Light pigmentation covered the dorsal surface of the gas bladder, and, after division of the gas bladder was complete, became dense on the posterior lobe. Large melanophores were also present along the dorsal body surface (Fig. 6B); the horizontal septum; and body-gut juncture. Concentrated melanophores formed a spot at the base of the caudal fin (Fig. 6C) and internal pigmentation was present on the spinal cord. Mesolarvae averaged 10.3 mm at 14 days and 10.8 mm at 17 days.

Metalarvae.—The pelvic fin buds appeared 21 days after fertilization (11.6 mm fish length) marking the transition from mesolarva to metalarva (Fig. 6D). Anal fin ray formation was complete at 25 days and the dorsal fin rays were segmented at fish lengths ranging from 13.5 to 14.0 mm (Fig. 6E). Incipient rays appeared in the pelvic and pectoral fins 27 days after fertilization (Fig. 6F) and were segmented at 39 days (18 mm fish length).

The horizontal septum pigmentation increased in width after 40 days especially on the caudal peduncle. The caudal spot was prominent and extended onto the caudal fin (Fig. 6G).

Juveniles.—The preanal finfold was absorbed at 57 days (19 mm fish length). Barbels were present at 70 days when fish averaged 20 mm in length.

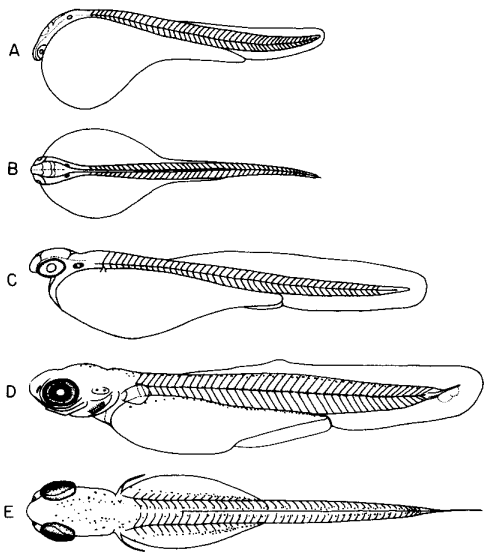

Fig. 5. Developing larvae of the river chub. A) protolarva, recently hatched, 5.7 mm (5–6 days); B) protolarva, 5.7 mm, dorsal view; C) protolarva, 8.0 mm (6 days); D) protolarva, 9.5 mm (8–10 days); E) protolarva, 9.5 mm, dorsal view.

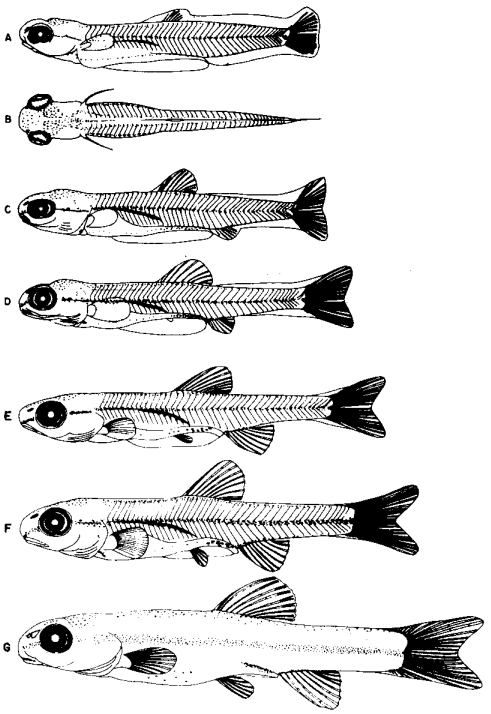

Fig. 6. Developing larvae of the river chub. A) mesolarva, 10.5 mm (14 days); B) mesolarva, 10.5 mm, dorsal view; C) mesolarva, 10.8 mm (17 days); D) metalarva, 11.6 mm (21 days); E) metalarva, 14.5 mm (27 days); F) metalarva, 15.5 mm (31 days); G) metalarva, 18.0 mm (39 days).

Pigmentation was present on the margin of the upper jaw and the horizontal band was connected to the dorsal melanophore series by melanophores along each myoseptum at 85 days (26 mm fish length).

Scale formation began on the caudal peduncle in fish 17 mm in length at 45 days. Between 18 and 19 mm (50 to 57 days), imbricated scales were present along the horizontal septum and covered most of the peduncle. Developing scales extended along the horizontal septum beyond the dorsal fin origin, radiating posteriorly. At 24 mm (78 days), imbricated scales covered most of the body. Fish were completely scaled between 29 and 30 mm in length (100 to 106 days).

River chub nest utilization by cyprinids.—Egg collections in river chub nests contained three cyprinid species in addition to longnose dace and river chub (Table 2). Longnose dace and river chub eggs were found in the same nest at least once in each stream sampled. Longnose dace × river chub hybrids have been reported for each of these areas except Wills Creek and Savage River. More species were collected together in Wills Creek than any other area. In one nest, all five species were present: differences in developmental stage indicated that spawning occurred over some length of time. Eggs of the stoneroller were not found in any of the nests sampled but adults were present in the streams.

Discussion

Development of the longnose dace and river chub was similar and followed nearly the same time sequence through the first 60 h. After this, longnose dace developed at a faster rate except for fin ray formation. Longnose dace hatched one to two days earlier than river chub and were more advanced in eye pigmentation. Development of gill covers, mouth, heart, and

TABLE 2. LARVAL CYPRINIDS REARED FROM EGGS TAKEN FROM 16 RIVER CHUB NESTS IN 1978. Each horizontal group represents one nest. Species designations, and, in parentheses, the number of times each species was found: LD = longnose dace (11); RC = river chub (10); CC = creek chub (4); RS = rosyface shiner (3); CS = common shiner (3).

Sampling date	Collection site				
	Wills Creek	Snowy Creek	Flaugherty Creek	Shavers Fork	Savage River
1 June	LD				
5 June		RC			
6 June		LD, RC, CC			
7 June		LD			
8 June		LD			
13 June		LD, RC			
17 June	LD, CC, RS, CS, RC				
	CC, RS				
	RS, RC				
	LD, RC				
	RC				
19 June			LD, RC		
20 June					CS
23 June					LD, CC, RC
25 June					LD, RC
26 June				LD, RC	

blood circulation occurred two to five days earlier in longnose dace.

Formation of caudal fin rays occurred earlier in longnose dace; dorsal ray formation was nearly equal in time between the two species; and anal, pelvic, and pectoral fin rays were formed earlier in river chub. Segmentation of fin rays occurred first in river chub which reached the juvenile phase in nearly half the time required by longnose dace.

A significant difference ($P < .05$) was found for the mean egg diameter of longnose dace between Shavers Fork and Flaugherty Creek. Mean egg diameter (N = 25) from Shavers Fork was 2.4 mm spawned from an 80 mm standard length female and 1.6 mm (N = 25) from Flaugherty Creek from a female 71 mm standard length. Hatching length was significantly ($P < .05$) larger at Shavers Fork (5.9 mm) than at Flaugherty Creek (4.5 mm). No significant differences were found in egg size or hatching length for river chub from any area.

Development of longnose dace in this study was similar to that found by Fuiman and Loos (1977) except for egg size and myomere counts. The smallest mean egg diameter in the present study was 1.6 mm, considerably smaller than the 2.5 mm average reported by Fuiman and Loos (1977). The larger mean size of longnose dace eggs was used to separate them from blacknose dace in their study. The application of this characteristic may only be useful in small geographical areas.

Three to four fewer preanal myomeres were counted in longnose dace specimens in the present study, similar to counts given by Fuiman and Loos (1977) for blacknose dace. These authors did not use myomere counts for identification because of variations in counts.

Longnose dace are less advanced than blacknose dace (Fuiman and Loos, 1977) at hatching in that the pectoral buds have not formed. The developmental changes following the protolarval phase are similar in both species.

River chub development was similar to that of the fallfish (*Semotilus corporalis*: Reed, 1971) although river chub were unpigmented at hatching. River chub hatched at a smaller size than fallfish (6.8 mm: Reed, 1971) and possessed a larger, more spherical yolk. Pigmentation patterns were similar between the two species.

Scale formation occurred at smaller lengths in the river chub compared to longnose dace and fallfish (Reed, 1971), but at greater lengths than in blacknose dace (Noble, 1965). At approximately 24 mm, scale formation was nearly complete in river chub but was present only on

the caudal peduncle and horizontal septum in longnose dace and fallfish. Blacknose dace and fallfish developed scales at smaller lengths than did longnose dace.

Hybridization.—Raney (1940) postulated that chance fertilization over a river chub nest was responsible for the longnose dace × river chub hybrids he collected. This theory is supported by observations made in the present study. Longnose dace were observed spawning in a river chub nest in Snowy Creek and subsequent collections in the nest revealed longnose dace and river chub eggs. Eggs of both species were close in development indicating that spawning had occurred at close intervals. Simultaneous spawning of both species was not observed. Eggs collected in other streams varied in developmental stage.

The use of river chub nests by longnose dace may be attributed to the lack of suitable spawning habitat for the dace. In each stream sampled, gravel areas were not numerous—the collections of stones in the river chub nest provide a suitable spawning area for longnose dace as well as other species. Tsai and Zeisel (1969) attributed the concentration of common shiner, rosyface shiner and rosyside dace (*Clinostomus funduloides*) over river chub nests to a lack of spawning habitat in the Little Patuxent River, Maryland. They reported several hybrids between these species from that area due to spawning over the nest of the river chub.

Hankinson (1931) observed common shiners spawning over nests of the creek chub and stated that common shiners may prefer the stone nests of other fishes as spawning sites. Although no attempt was made in the present study to determine spawning preferences, longnose dace may prefer stone nests of other species for spawning.

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