Identification of Eggs, Larvae, and Juveniles of the Rainbow Smelt, *Osmerus mordax*, with Comparisons to Larval Alewife, *Alosa pseudoharengus*, and Gizzard Shad, *Dorosoma cepedianum*

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ABSTRACT

Eggs and larvae of the rainbow smelt, *Osmerus mordax*, were reared in the laboratory to 7.4 mm total length. Larger sizes were collected by plankton net from Lake Erie, Lake Ontario, and Cayuga Lake, New York. Manually stripped eggs were adhesive and demersal with a granular, yellow yolk. Egg diameter after fertilization and water hardening was 1.0 mm. The majority of eggs hatched between 183 and 195 h after fertilization at an average water temperature of 16.5 C. Average length of larvae at 195 h was 5.6 mm. Yolk absorption was complete at 6.4 mm. Pectoral fin development began before hatching. The dorsal, anal, and caudal fin rays began development at approximately 14 mm. The pelvic fins budded between 17 and 20 mm. All fin rays were complete at 36 mm. The adipose fin was complete at 29 mm. Separation of rainbow smelt from alewife (*Alosa pseudoharengus*) and gizzard shad (*Dorosoma cepedianum*) was accomplished using yolk sac position, preanal and postanal myomeres, and snout-to-vent length as a percentage of total length.

Few studies of egg and larval development of the rainbow smelt, *Osmerus mordax*, have provided specific characteristics useful for identification. Nelson and Cole (1975) presented several characteristics but no illustrations of eggs or larvae. Marcotte and Tremblay (1948) photographed egg and larval stages but did not give specific descriptions useful for identification. Kendall (1927) compiled information and illustrations of the European smelt, *O. eperlanus*. He also made reference to the rainbow smelt but without specific descriptions or illustrations.

Rainbow smelt larvae are similar to several clupeid larvae and could be mistakenly identified as clupeids. The purpose of this study was twofold: to describe changes in development of rainbow smelt eggs and larvae for identification purposes; and to present characteristics that would separate larval smelt from larval alewife (*Alosa pseudoharengus*) and larval gizzard shad (*Dorosoma cepedianum*).

METHODS

Ripe rainbow smelt were collected by dip net at the mouth of Trout Run, a stream tributary to Lake Erie, Erie County, Pennsylvania, on 27 April 1975. The fish were segregated by sex according to the presence or absence of nuptial tubercles (Scott and Crossman 1973), transported to the laboratory, and artificially stripped of eggs or milt into three shallow, floating hatching boxes. Approximately 200 eggs were placed in each box. The hatching boxes were constructed of nylon hosiery suspended from wood frames and kept in a 40-liter aquarium filled with Trout Run water. The water was circulated into a separate tank, filtered with a diatom filter, and returned to the aquarium. Filtering was discontinued after the first larva hatched.

The aquarium was loosely fitted with a styrofoam top and sides to provide some temperature stabilization. No other type of temperature control was attempted. The styrofoam was not sealed at the ends and did not exclude all light from the aquarium. Temperature measurements were made at each sampling and are given as means in Table 1.

Egg samples were taken before, immediately after, and 15 minutes after fertilization. Subsequent egg samples were taken twice daily; morning and evening, until the first larva hatched. Dead eggs were removed at each sampling. Larval samples were taken at unequal intervals usually not more than 24 h apart. Three or more specimens were examined for developmental changes

TABLE 1.—*Daily mean aquarium water temperatures.*

Date	Cumulative hours	Temperature C
April 27	0	9.5
28	10	13.0
29	33	16.3
30	57	18.0
May 1	80	18.5
2	103	16.0
3	127	16.4
4	151	15.0
5	183	15.3
6	195	17.9
7	205	17.0
8	225	18.0
9	238	18.0
11	265	18.5
12	279	20.1
13	288	17.6
14	300	21.9

prior to preservation in 4% buffered formalin. Measurements and counts were made after preservation.

Mortality increased following the 265-h sampling and all larvae were dead at 300 h. Fungal infections were difficult to control although several formalin baths were given.

TABLE 2.—*Measurements (mm) of larval rainbow smelt. Each triplet gives the mean, one standard deviation (in parentheses), and the percent of total length.*

Size range (Total length)	N	Total length	Standard length	Head length	Diameter of eye	Snout-vent length
5.00–6.50	18	5.8	5.7	0.8	0.3	4.2
		(0.4)	(0.3)	(0.1)	(0)	(0.3)
			96.9	13.5	4.4	72.1
6.51–7.50	5	7.0	6.8	1.0	0.3	5.0
		(0.3)	(0.2)	(0.1)	(0)	(0.2)
			96.9	14.6	4.0	71.9
7.51–9.50	6	8.6	8.3	1.2	0.3	6.4
		(0.6)	(0.7)	(0.1)	(0.1)	(0.6)
			96.6	13.7	3.6	74.8
9.51–15.50	9	14.3	13.4	2.0	0.4	10.5
		(1.0)	(0.8)	(0.2)	(0)	(0.9)
			93.6	14.1	2.8	73.2
15.51–22.50	6	17.9	~~13.6~~ 16.5	2.7	0.7	12.8
		(2.0)	(1.6)	(0.5)	(0.1)	(1.3)
			~~76.0~~ 92.1	15.4	3.8	71.4
22.51–28.50	10	26.2	23.4	4.5	1.0	18.1
		(1.6)	(1.1)	(0.6)	(0.1)	(1.0)
			89.2	17.3	3.9	68.8
28.51–32.50	8	31.1	27.5	5.6	1.3	21.1
		(0.7)	(0.5)	(0.2)	(0)	(0.5)
			89.0	18.1	4.1	68.0
32.51–38.00	7	35.1	30.5	6.1	1.3	22.8
		(1.5)	(1.3)	(0.8)	(0.1)	(1.1)
			86.9	17.5	3.8	64.9

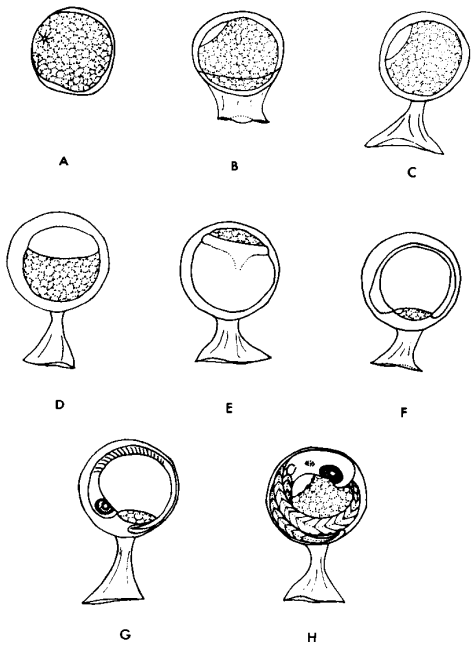

FIGURE 1.—*Developing eggs of the rainbow smelt. (A) Unfertilized, the membrane has been released; (B) separation of the adhesive outer layer of the chorion; (C) 15 minutes after fertilization; (D) early blastula (16 h); (E) early gastrula (33 h); (F) early embryonic development (57 h); (G) tail-free embryo (92 h); (H) late embryo just before hatching (162 h).*

The fungus may have contributed to the mortality although starvation was probably the major cause. Frozen brine shrimp had been added at 250 h but no evidence of feeding was found.

Preserved larvae were rinsed in distilled water and then soaked in a 3% solution of alizarin red S-distilled water for five minutes to highlight myomeres and fin structure. Measurements were made with an ocular micrometer read to the nearest 0.1 mm. All body lengths are total lengths. Morphometric and meristic data are summarized in Tables 2 and 3. Terms used for descriptions and measurements were from Mansueti and Hardy (1967); those terms designating developmental phases were from Snyder et al. (1977).

Descriptions of larvae up to 7.4 mm were based on reared stock. Larger larvae were

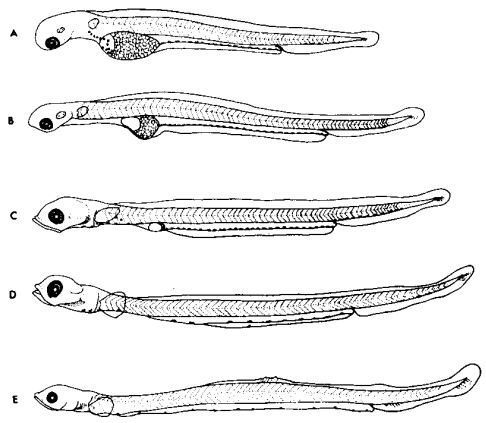

FIGURE 2.—*Developing rainbow smelt larvae. (A) Protolarva, recently hatched, 5.0 mm; (B) protolarva, 5.6 mm; (C) protolarva, 6.3 mm; (D) protolarva, 9.3 mm; (E) mesolarva, 14 mm.*

from plankton collections made in Lake Erie, Lake Ontario, and Cayuga Lake, New York, in 1975. Alewife and gizzard shad used for comparison were collected in the Potomac River in 1974 and in Lake Erie in 1975 and 1976. Collected larvae were identified by using the characteristics presented.

RESULTS

Embryogenesis

Manually stripped unfertilized eggs were extruded in clumps and readily adhered to the first surface encountered. Unfertilized eggs were demersal with a granular yellow yolk that contained numerous oil globules. The chorion was smooth and strong. There was no perivitelline space; however, the membrane had been released in some unfertilized eggs (Fig. 1A). Unfertilized eggs ranged from 0.8 to 0.9 mm in diameter and were irregular in shape.

Agitation of the eggs caused the adhesive outer membrane of the chorion to separate opposite the micropile. The outer membrane turned inside out and became attached to the substrate (Figs. 1B, C), suspending the egg above the substrate. Fertilization of the egg was not necessary for this to occur. The outer layer was very elastic and remained elastic and adhesive after preservation.

Sampling of fertilized eggs began 15 minutes after the milt and eggs were combined. In the first sample (Fig. 1C, egg diam. 1.0 mm), protoplasm had begun to accumulate at the animal pole. The blastoderm had covered nearly one third of the yolk surface after 10 h and individual blastomeres could not be seen. At 10 h, the average perivitelline space was 0.1 mm, average yolk diameter was 0.7 mm, and egg diameter was 1.0 mm. Eggs became spherical after fertilization.

The blastula began to form at 16 h (Fig. 1D). There were no dimensional changes in egg or yolk diameter. The chorion was very rigid. Germ ring formation (Fig. 1E) was detected at 33 h by varying the light direction from a microscope illuminator. Development of the gastrula after 33 h may have been accelerated due to a 4-C increase in water temperature (from 14.5 to 18.5 C). The temperature increase occurred over an 11-h period during an unusually warm period. This condition continued for an additional 32 h. From 8 to 14 May, water temperature in the aquarium fluctuated between 18 and 21.9 C.

The developing neural fold was partially visible at 57 h. The tail region was slightly thicker than the portion preceding it (Fig. 1F). There was no development detected in the head region other than an enlargement.

Somites, auditory vesicles, and pigmented eyes were visible at 92 h and the tail was free (Fig. 1G). Egg diameter still was 1.0 mm and the perivitelline space 0.1 mm. The chorion had become very flexible. No pigmentation was present.

At 151 h, the embryo was quite active when light was directed toward it. The heart was visible and light-red blood was circulating in the major vessels. A large oil globule was visible in the anterior part of the yolk sac and the pectoral fin buds were present. A single line of melanophores was present on the ventral edge of the tail.

Protolarvae

The first egg hatched at 162 h after fertilization at a length of 5.0 mm (Figs. 1H, 2A). The egg diameter prior to hatching was 1.0 mm. Measurements of the larva taken after preservation were: horizontal axis of eye 0.3

mm; snout-to-vent length 3.3 mm; yolk-sac length 0.8 mm; and oil globule diameter 0.3 mm. Pigmentation consisted of a series of black melanophores encircling the ventral margin of the yolk sac continuing anteriorly below the pectoral fin buds and posteriorly along the ventral margin of the gut. A single line of melanophores was occasionally present on the ventral edge of the tail, similar to that found in 151-h embryos. The majority of eggs hatched between 183 and 205 h. The protolarvae averaged 5.6 mm at 195 h (Fig. 2B). The average length of the yolk sac was 0.4 mm and the oil globule diameter was 0.2 mm. The yolk sac had moved posteriorly one-third of the length of the body. Pigmentation was similar to that shown in Figure 2A.

The raised larvae reached a length of 6.0 mm after 225 h. The mouth was well formed. The yolk-sac length decreased to 0.3 mm and the oil globule diameter remained 0.2 mm. Two ventral series of melanophores had developed posterior to the cleithrum extending to the yolk sac. A single row was present in some larvae along the ventral edge of the gut and tail. The yolk was nearly absorbed in larvae 6.3 mm in length (Fig. 2C, 265 h) and was complete in 6.4-mm larvae (288 h).

The jaws were well formed in 9.3-mm larvae (Fig. 2D) but no teeth were present. The pectoral fins had increased in size to 0.5 mm at their greatest length and were not rayed. The gut was thickened over much of its length. Field-collected protolarvae had very little pigmentation. A few melanophores were present along the ventral edge of the gut and tail. The ventral melanophore series behind the head as seen in the raised larvae was not prominent in many field-collected larvae.

Mesolarvae

At 14 mm (Fig. 2E), fin-ray development had begun in the dorsal, anal, and caudal fins. The head was more pointed than in the 9.3-mm larvae. The finfold along the gut had disappeared and an indication of the adipose fin was detectable in the dorsal finfold. Pigmentation was present only along the gut as single, scattered melanophores.

By 17 mm (Fig. 3A), the dorsal and anal fin rays were well developed. The caudal fin rays were nearly complete and the adipose fin was partially formed. The pelvic fin buds were not apparent. Teeth were present in the upper jaw. The jaws were flexed upward in all larvae examined at this size but it is not known if this is a characteristic or an artifact of preservation. The posterior half of the gut was thickened and the urostyle had turned upward. Single melanophores were found on the ventral margin of the body and ventral posterior half of the gut. No internal pigment was found.

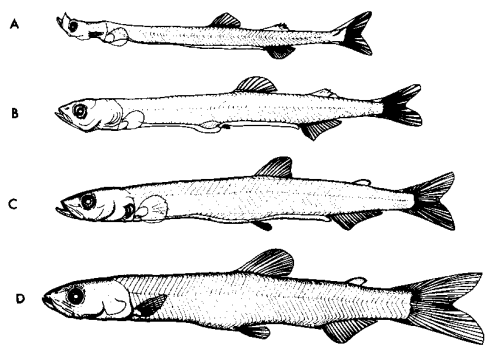

FIGURE 3.—*Developing rainbow smelt. (A) Mesolarva, 17 mm; (B) mesolarva, 22 mm; (C) metalarva, 30 mm; (D) juvenile, 36 mm.*

At 22 mm (Fig. 3B), the anal and caudal fin rays were complete and rudimentary fin rays were present in the dorsal fin. The pelvic fin buds were present. The gas bladder was distinct and forced the gut downward anterior to the pelvic fin buds. The dorsal surface of the gas bladder had numerous melanophores. Single melanophores were found along the ventral margin of the body.

Metalarvae

All fin rays were complete, except the pectoral rays, at 30 mm (Fig. 3C). The adipose fin was developed. The head shape was similar to the adult and teeth were present in both jaws and on the tongue. The teeth could be seen only after staining. The ventral paired row of melanophores behind the head was not always present in larvae of this size. Single melanophores in the caudal fin were arranged in lines following the individual rays.

TABLE 3.—*Meristic counts of larval rainbow smelt. Each couplet gives the mean and one standard deviation (in parentheses).*

Size range (Total length)	N	Branchiostegal rays	Fin rays					Gill rakers	Myomeres		
			Pectoral	Pelvic	Dorsal	Anal	Caudal		Preanal	Postanal	Total
7.51–9.50	6								45.0 (1.1)	14.3 (0.8)	59.3 (1.6)
9.51–15.50	9				9.7 (0.5)	12.9 (2.8)	18.3 (3.4)		44.8 (1.4)	15.0 (1.1)	59.9 (1.5)
15.51–22.50	6	4.0 (0)		5.5 (0.7)	9.8 (0.4)	15.3 (0.8)	19.2 (0.7)	11.0 (0)	45.2 (0.4)	14.7 (1.4)	59.8 (1.0)
22.51–28.50	10	6.2 (1.0)	8.6 (1.9)	6.7 (0.7)	10.1 (0.9)	15.7 (0.7)	19.4 (0.8)	16.0 (0)	45.3 (1.3)	14.6 (1.2)	59.9 (1.1)
28.51–32.50	8	7.0 (0)	9.8 (3.1)	7.5 (0.5)	10.6 (0.5)	16.6 (0.5)	19.5 (0.7)	19.0 (0)	45.1 (1.1)	14.8 (0.7)	59.9 (1.2)
32.51–38.00	7	7.0 (0)	10.0 (1.0)	7.9 (0.4)	11.0 (0)	16.9 (0.7)	19.9 (0.4)	20.0 (0)	45.4 (1.1)	15.1 (0.7)	60.6 (1.0)

Juveniles

All fin rays were complete at 36 mm (Fig. 3D). Scale formation (not shown) began on the tail and extended halfway to the vent. Pigmentation was variable: only one melanophore was present in some specimens, located at the posterior edge of the opercle; other specimens had scattered melanophores along the tail and on the caudal fin rays. Juveniles 40 mm and larger had considerable numbers of melanophores on the caudal fin which gave it a dusky appearance.

DISCUSSION

Unfertilized eggs ranged from 0.8 to 0.9 mm which compared favorably with 0.8 mm reported by Langlois (1935) and 0.9 mm by Charles F. Cole (University of Massachusetts, personal communication). In my study, the egg diameter after fertilization and water hardening was 1.0 mm. Bigelow and Welsh (1924) list fertilized egg size from the Gulf of Maine as 1.2 mm. Fertilized eggs were 1.1 mm in diameter from the Weweantic River and 1.2 mm in diameter from the Parker River, Massachusetts (C. F. Cole, personal communication). The differences in size may indicate a geographical variation or a difference in size of spawning females.

Fertilized eggs in my study hatched in 8 days at 9 to 21 C (mean, 16.5 C), similar to observations by Hoover (1936). Hale (1960) found hatching at 19 to 20 days with a mean temperature range of 5 to 8 C. McKenzie (1964) listed several hatching periods at various temperatures: 29 days at 6 to 7 C; 25 days at 7.1 to 8 C; and 19 days at 9 to 10 C. Rupp (1965) reported hatching in 24 days at 4.4 to 10 C. The higher mean water temperature in my study would account for the shorter hatching time.

The recently hatched larva in my study differed from that illustrated in Marcotte and Tremblay (1948). My study shows the yolk sac closer to the head and the head more rounded. Larvae at 5.6 mm in my study were quite similar to the recently hatched larva in Marcotte and Tremblay. This may indicate that the first larva to hatch in my study was premature. Recently hatched larvae from the Parker River, Massachusetts, were 6.0 mm in length (C. F. Cole, personal communication). Average hatching size would probably be between 5.5 and 6.0 mm. Environmental variations as well as the size of the egg would affect hatching size.

The location of the pelvic fins changed during development, moving from anterior (Fig. 3B) to posterior (Fig. 3D) of the dorsal fin origin. Bigelow and Welsh (1924) illustrate a larval smelt (26 mm) that shows the pelvic fin position intermediate between those illustrated in the present study.

Ventral pigmentation varied from a few scattered melanophores to several rows in the larvae examined in this study. Ventral views of larval smelt were not illustrated due to this variability. Many larvae possessed the paired row of melanophores from the

pectoral fin bases to the pelvic fin bases as described by Dorr et al. (1976), but showed extreme variation in the patterns posterior to the pelvic fin bases. Two paired rows of melanophores were often present, similar to those described above. The posterior rows were occasionally divided by a central row on the most ventral aspect of the gut. The single ventral row described by Nelson and Cole (1975) and by Dorr et al. (1976) was probably the central row seen in the present study. None of the patterns were consistently present in specimens examined in my study. Dorr et al. also describe several elongate ventral melanophores posterior to the anal fin, and although several melanophores were present on some specimens in my study, the pattern was not consistent. Bigelow and Welsh (1924) illustrated a larval smelt with possibly a paired row of ventral melanophores but no description of pigmentation was made.

Comparisons with Alewife and Gizzard Shad

The occurrence of alewife and gizzard shad in the Great Lakes compounds the problem of identifying rainbow smelt larvae. In several phases of development, all three species are similar in shape and some meristic characteristics overlap. These species can be distinguished using the following characteristics.

The position of the yolk sac with respect to the head will separate rainbow smelt from gizzard shad at 6.0 mm. The origin of the yolk sac is directly behind the head in gizzard shad (Mansueti and Hardy 1967) and more than one head length posterior to the head in rainbow smelt. The yolk sac in 6.0-mm alewife will have been absorbed (Norden 1967). The oil globule is posterior in the yolk sac of gizzard shad, anterior in rainbow smelt yolk sacs, and not present in alewives (Mansueti and Hardy 1967). Illustrations of these characteristics are given in Mansueti and Hardy (1967) and Norden (1967). Other distinguishing characteristics are summarized in Table 4.

Myomere counts in alewife are useful until anterior migration of the vent occurs at approximately 14 mm (Chambers et al. 1976). This may also be true for gizzard shad although the size at which migration occurs is unreported. Ranges of myomere counts may overlap between species and may vary from the values given in Table 4. Mansueti and Hardy (1967) give a range of 39 to 44 preanal myomeres for gizzard shad. These values differed from that found in Lake Erie gizzard shad used in my study, but a larger sample size may have contained some larvae with lower counts. Geographic location and environmental variations will affect counts and this should be recognized when comparing new counts to those listed in Table 4.

TABLE 4.—*Comparative characteristics of larval rainbow smelt, alewife, and gizzard shad.*

	Rainbow smelt	Alewife	Gizzard shad
Hatching length (mm)	5.5–6.0	3.5–5.0[a]	3.25[a]
Preanal myomeres			
range	42–48	37–43[b]	42–46[c]
mean	45.1	38.3[b]	43.3[c]
Postanal myomeres			
range	13–17	5–14[b]	4–6[c]
mean	14.7	8.3[b]	4.8[c]
Snout-vent length as percent total length	65–75	77–81[d]	82–85[d]

[a] Mansueti and Hardy (1967).
[b] Norden (1967).
[c] Author, unpublished data.
[d] Nelson and Cole (1975).

Pigmentation was not found to be a reliable characteristic for separating larval smelt from larval alewife and gizzard shad. In addition to the variability described in smelt, pigmentation was not always found in alewives immediately after hatching and often not in recently hatched smelt (Dorr et al. 1976). Pigmentation was not considered to be a reliable primary identification characteristic by Dorr et al. (1976) and I agree, based on specimens used in the present study.

Reliable characteristics for separating larval smelt from larval alewife and gizzard shad were: presence and position of the yolk sac relative to the head; presence and position of the oil globule; snout-to-vent length as a percentage of total length; and mean counts of preanal and postanal myomeres.

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