EHRLICH, K. F., J. H. S. BLAXTER, AND R. PEMBERTON.
1976. Morphological and histological changes during the growth and starvation of herring and plaice larvae. Mar. Biol. (Berl.) 35:105-118.

HARTREE, E. F.
1972. Determination of protein: A modification of the Lowry method that gives a linear photometric response. Anal. Biochem. 48:422-427.

HINEGARDNER, R. T.
1971. An improved fluorometric assay for DNA. Anal. Biochem. 39:197-201.

HOLM-HANSEN, O., W. H. SUTCLIFFE, JR., AND J. SHARP.
1968. Measurement of deoxyribonucleic acid in the ocean and its ecological significance. Limnol. Oceanogr. 13:507-514.

LAURENCE, G. C.
1975. Laboratory growth and metabolism of winter flounder *Pseudopleuronectes americanus* from hatching through metamorphosis at three temperatures. Mar. Biol. (Berl.) 32:223-229.

LAURENCE, G. C., T. A. HALAVIK, B. R. BURNS, AND A. S. SMIGIELSKI.
1979. An environmental chamber for monitoring "in situ" growth and survival of larval fishes. Trans. Am. Fish. Soc. 108:197-203.

MUNRO, H. N., AND A. FLECK.
1966. The determination of nucleic acids. Methods Biochem. Anal. 14:113-176.

NEYFAKH, A. A., AND N. B. ABRAMOVA.
1974. Biochemical embryology of fishes. *In* M. Florkin and B. T. Scheer (editors), Chemical zoology, Vol. VIII, p. 261-286. Acad. Press, N.Y.

O'CONNELL, C. P.
1976. Histological criteria for diagnosing the starving condition in early post yolk sac larvae of the northern anchovy, *Engraulis mordax* Girard. J. Exp. Mar. Biol. Ecol. 25:285-312.

REGNAULT, M., AND P. LUQUET.
1974. Study by evolution of nucleic acid content of prepuberal growth in the shrimp *Crangon vulgaris*. Mar. Biol. (Berl.) 25:291-298.

SMIGIELSKI, A. S.
1975. Hormonal-induced ovulation of the winter flounder, *Pseudopleuronectes americanus*. Fish. Bull., U.S. 73:431-438.

SUTCLIFFE, W. H., JR.
1965. Growth estimates from ribonucleic acid content in some small organisms. Limnol. Oceanogr. 10:R253-R258.
1970. Relationship between growth rate and ribonucleic acid concentration in some invertebrates. J. Fish. Res. Board Can. 27:606-609.

THEILACKER, G. H.
1978. Effect of starvation on the histological and morphological characteristics of jack mackerel, *Trachurus symmetricus*, larvae. Fish. Bull., U.S. 76:403-414.

L. J. BUCKLEY

*Northeast Fisheries Center Narragansett Laboratory*
*National Marine Fisheries Service, NOAA*
*R.R. 7A, Box 522A*
*Narragansett, RI 02882*

# EGGS AND EARLY LARVAE OF SMALLMOUTH FLOUNDER, *ETROPUS MICROSTOMUS*

Smallmouth flounder, *Etropus microstomus* (Gill), ranging from early postlarvae to adult were described and illustrated in detail by Richardson and Joseph (1973). Eggs and larvae through yolk-sac absorption had yet to be identified.

During a 1975-76 ichthyoplankton survey of Block Island Sound conducted by Marine Research, Inc. small unidentified planktonic fish eggs were taken. Through subsequent rearing of a number of these eggs and completion of a length series with larger, known larvae, we identified the specimens as *E. microstomus* eggs. Our descriptions of eggs and yolk-sac larvae together with the work of Richardson and Joseph (1973) provide a complete developmental series for identification of this species.

## Methods

Sampling was conducted in Block Island Sound at five stations along each of three transects running from Charlestown and East Beach, R.I., to Block Island, a distance of approximately 14.8 km. Collections were made with 60 cm, 0.505 mm mesh, bongo nets. All tows were made obliquely, bottom to surface at approximately 2.5 kn for about 5 min. Digital flowmeters provided volume estimates and quantitative density estimates. Periodically, a 30 cm, 0.505 mm mesh, plankton net was fixed above the bongo net to collect samples of live eggs. These were returned to the laboratory in aerated 4 l thermos jugs and incubated at 20°-21° C. *Etropus microstomus* eggs and larvae were stored in 3-5% buffered Formalin[1] solutions before examination.

## Descriptions of the Egg

*Etropus microstomus* eggs (Figure 1, Table 1) are small, 0.561-0.740 mm in diameter ($\bar{x}$ = 0.64) with a single small oil globule, 0.051-0.165 mm ($\bar{x}$ = 0.12). The egg is spherical with a transparent, unsculptured chorion. The oil globule is also spherical. Occasonally two oil globules were noted or a single one with several surrounding oil particles were found. This condition has commonly been noted for other species (Ahlstrom and Ball

[1]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.



FISHERY BULLETIN: VOL. 77, NO. 3, 1980.

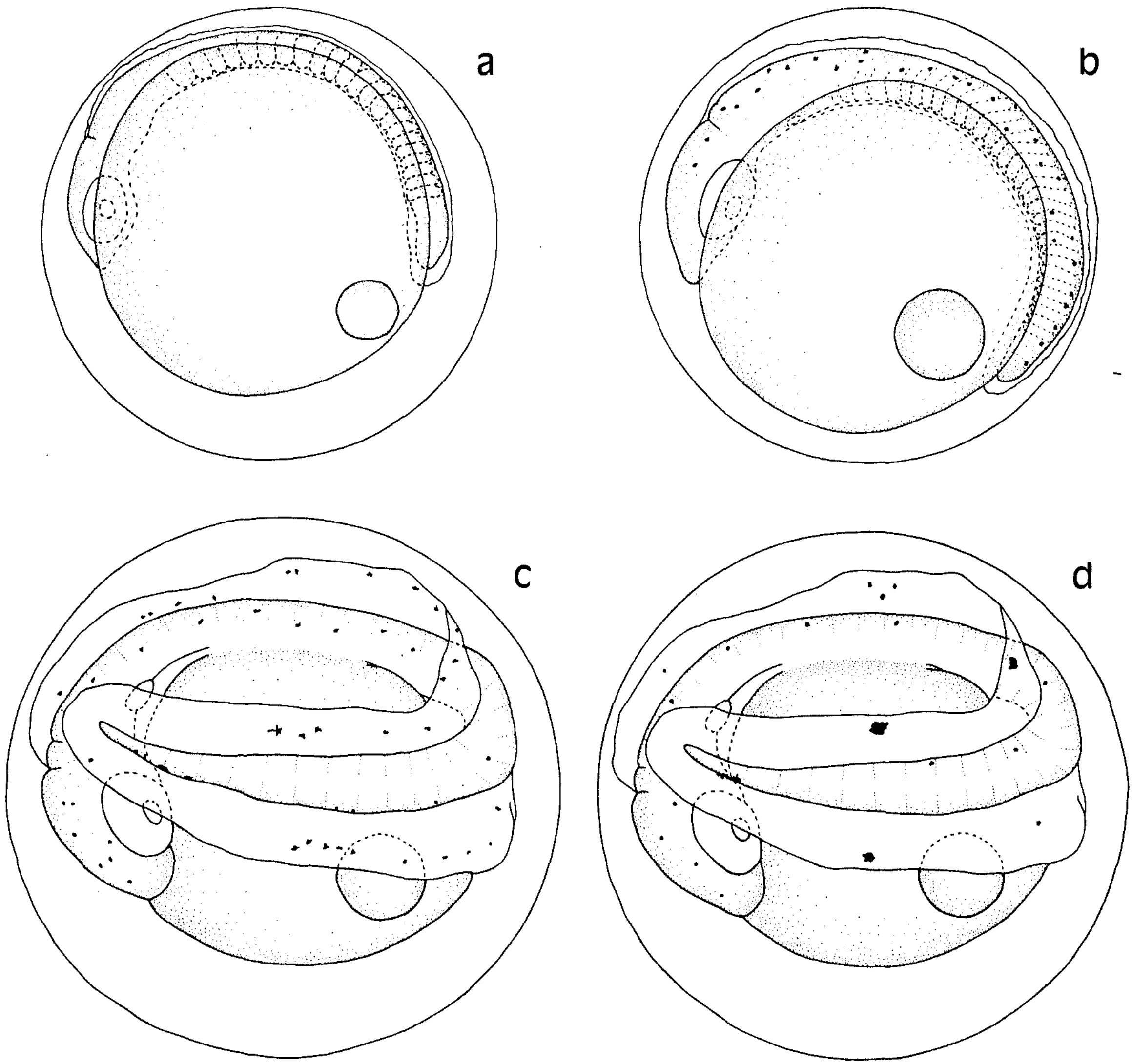


FIGURE 1.—*Etropus microstomus* eggs; mean diameter = 0.64 mm: a) middle stage; b-d) development of pigmentation during late stage.

TABLE 1.—Egg, yolk, and oil globule diameters (millimeters) for *Etropus microstomus* eggs taken in Block Island Sound, 1975.

| | Egg diameter | | | | Oil globule diameter | | | | Yolk diameter | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Stage | *n* | Mean | SD | Range | *n*[1] | Mean | SD | Range | *n*[1] | Mean | SD | Range |
| Early | 449 | 0.64 | 0.02 | 0.59-0.73 | 435 | 0.12 | 0.01 | 0.08-0.17 | 111 | 0.52 | 0.03 | 0.43-0.59 |
| Middle | 261 | 0.65 | 0.02 | 0.59-0.71 | 257 | 0.11 | 0.01 | 0.05-0.13 | 239 | 0.55 | 0.03 | 0.49-0.61 |
| Late | 111 | 0.65 | 0.03 | 0.56-0.74 | 102 | 0.11 | 0.02 | 0.08-0.15 | 103 | 0.56 | 0.05 | 0.49-0.69 |
| Total | 821 | 0.64 | 0.02 | 0.56-0.74 | 794 | 0.12 | 0.02 | 0.05-0.17 | 453 | 0.54 | 0.04 | 0.43-0.69 |

[1]Discrepancies in sample sizes resulted from shattered oil globules and yolks which were not measured.

1954; Smith and Fahay 1970; Berrien 1975) and is generally believed to result from shattering during collection or preservation. About 75% of the early stage eggs in our preserved samples also contained broken yolks which could not be accurately measured. To facilitate descriptions, eggs were separated into three stages following Ahlstrom and Ball (1954): early (fertilization to closure of the blastopore), middle (blastopore closure to tail separation), and late (tail separation to hatching).

### Early Stage

During this stage, eggs were distinguishable by measurement of egg and oil globule diameters. The yolk occupied about 81% of the egg diameter. It appeared translucent and yellow-to-amber in color with transmitted, incandescent light. With closure of the blastopore the embryo encompassed about half the circumference of the yolk.

### Middle Stage

Faint melanophores began to appear on the dorsal surface of the embryo (Figure 1a). They were widely spaced, appeared randomly distributed, and were easily overlooked at magnifications under 50 ×. No pigment was noted on either the yolk or oil globule. Myomeres (12-22) became visible but were difficult to count with any accuracy. The optic vesicles became clearly visible but lacked pigment. By the end of this stage the number of melanophores increased although they were still present only on the dorsum. In some eggs they began to appear more numerous just behind the head while a few developed on the occiput. As the tail developed free of the yolk material, traces of finfold became visible on the posterior edge of the embryo.

### Late Stage

Melanophores enlarged so they became clearly visible (Figure 1b). Some developed along the sides and in some cases a few were noted on the yolk near the embryo. Melanophores along the dorsum commonly migrated into a more or less straight middorsal row extending from the nape to the tip of the tail. As the embryo developed, the portion of this line of pigment posterior to the vent migrated into the dorsal finfold while the lateral melanophores migrated into the ventral finfold (Figure 1c). As this occurred, little pigment remained on the trunk except for the anterior portion of the middorsal row. Numerous small dots persisted on the nape and dorsal surface of the head. Once melanophores had migrated into the finfold they began to coalesce into four distinct spots—two in the dorsal and two in the ventral finfolds; the dorsal pair aligned above the ventral pair. An additional group of melanophores aggregated ventrally near the tip of the notochord (Figure 1d). Much of this pigment spot appeared to be in the finfold but it was always in contact with the trunk and often extended upon it. Some of the small melanophores remaining on the anterior dorsum coalesced and moved into the dorsal finfold approximately midway between the vent and head. In some embryos a portion of the finfold melanophores became dendritic before they coalesced.

The oil globule was located posteriorly in the yolk near the developing vent where it remained through hatching. In some advanced, late stage eggs, one or two melanophores formed on the surface of the oil globule. No additional pigmentation developed on the yolk. Shortly before hatching the embryo encircled the yolk with the tip of the tail almost reaching the snout.

## Description of Early Larvae

Two recently hatched larvae measured 1.4 mm NL (notochord length) and were essentially identical to advanced late stage embryos. Three dark spots were present near the margin in the dorsal finfold, two near the margin in the ventral finfold, and one along the ventral body margin near the tip of the notochord. Small melanophores were scattered over the dorsum from the occiput to a point about halfway to the tip of the tail. The eyes remained unpigmented. The oil globule was located at the posterior edge of the yolk sac.

By 2.0 mm NL (Figure 2a) no change in pigmentation had occurred. The yolk was reduced in size by about 50% and the gut and vent more clearly defined.

Specimens >2.0 mm NL were obtained from preserved plankton samples where the finfold and its pigmentation were frequently lost. Between 2.1 and 2.3 mm NL (Figure 2b) the yolk sac was fully resorbed, eye pigmentation developed, and larvae developed many of the characteristics described by Richardson and Joseph (1973) for 2.3-2.5 mm larvae. Melanophores developed along the ventral body margin from the gut to the pronounced spot near the tip of the notochord. As these melanophores developed, most or all of the pigmentation on the dorsum was lost. The distinctive markings in the dorsal and ventral finfolds of yolk-sac larvae remained with the exception of the posterior dorsal spot. This spot was either lost as the caudal band, described by Richardson and

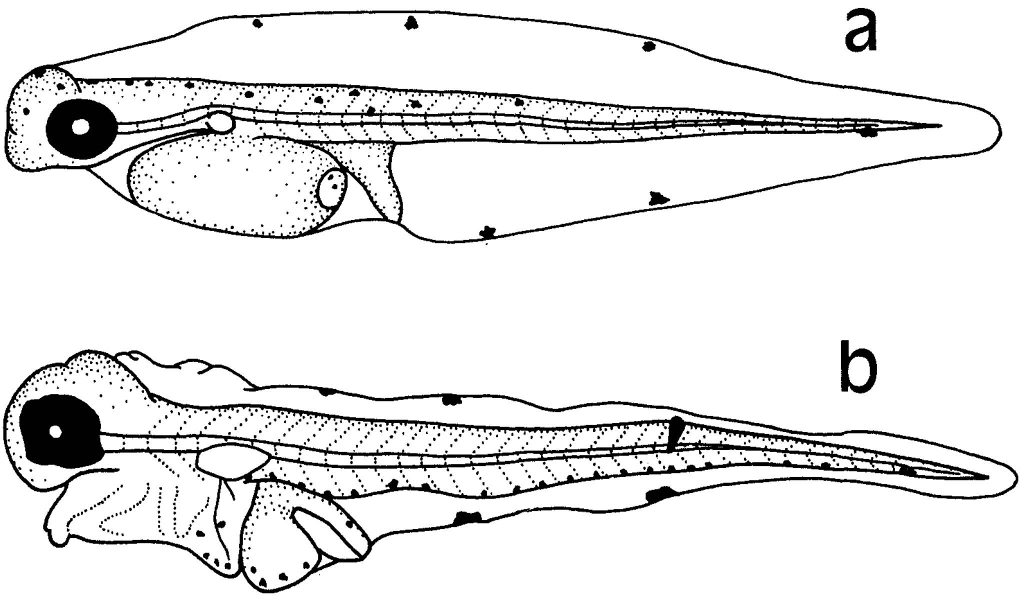


FIGURE 2.—*Etropus microstomus* early larvae: a) 2.0 mm NL; b) 2.2 mm NL.

Joseph, formed or the melanophores migrated ventrally to form all or part of the band. The midcaudal band was found in larvae as early as 2.1 mm in our collections; it was never observed in larvae still displaying the posterior dorsal finfold spot. The finfold markings appear to have been lost in the 2.5 mm SL (standard length) specimen illustrated by Richardson and Joseph due to finfold mutilation but do appear in their illustrations of 3.5 and 4.5 mm specimens.

The smallest specimen containing pigmentation on the gas bladder in our collections was 2.4 mm. Preopercular spines were first observed at about 2.3 mm. Gas bladder pigmentation and preopercular spines were described by Richardson and Joseph (1973) for their smallest specimen (2.5 mm).

### Occurrence

*Etropus microstomus* eggs were found in our Block Island Sound samples from 11 June until 10 September 1975; sampling was weekly through August, monthly thereafter. Samples taken again on 14 October 1975 did not contain *E. microstomus* eggs. Surface water temperatures during this period ranged from a low of 15.3° C in June to a high of 22.3° C in early August. Larvae were taken from 9 July to 14 October 1975 at which time water temperatures were 15.6° C.

In our weekly 1976 collections, eggs were taken beginning 1 June, larvae beginning 17 June. Both eggs and larvae were found regularly until 26 August when sampling ended. Surface water temperatures averaged 11.9° C on 1 June, 13.2° C on 17 June, and 20.0° C on 26 August.

### Similar Species

Prior to formation of the distinctively pigmented embryo in *E. microstomus*, some confusion may occur in separating similar stage eggs of the fourbeard rockling, *Enchelyopus cimbrius*; hakes, *Urophycis* spp.; and butterfish, *Peprilus triacanthus*. According to Scotton et al. (1973), *E. cimbrius* spawns from Nova Scotia to Block Island and *Urophycis* spp. spawn from Nova Scotia to South Carolina, depending upon the species. *Peprilus triacanthus* spawns from Nova Scotia (Scotton et al. 1973) to Chesapeake Bay (Pearson 1941). We regularly collected eggs and larvae of these species in Block Island Sound at the same time that *Etropus microstomus* eggs were taken. Most early and middle stage *E. microstomus* eggs may be distinguished on the basis of their smaller egg and oil globule diameters. Although ranges overlap to some extent (Table 2) mean values for egg and oil globule diameters are fairly distinctive. Only 2% of the 794 eggs we measured contained oil globule diameters $\geq 0.13$ mm, the smallest oil

TABLE 2.—Egg and oil globule diameters (millimeters) as reported in the literature for species which might be confused with *Etropus microstomus* eggs. References represent only a portion of those available. Recent literature summaries may be found in Hardy (1978) and Martin and Drewry (1978).

| Species | Egg | Oil | Source |
|---|---|---|---|
| *Enchelyopus cimbrius* | 0.65-0.75 | 0.13-0.15 | Battle (1929) |
| | 0.74-0.89 ($\bar{x}$ = 0.82) | 0.13-0.20 ($\bar{x}$ = 0.16) | Colton and Marak[1] |
| *Urophycis chuss* | 0.72-0.76 ($\bar{x}$ = 0.74) | 0.15-0.17 | Bigelow and Welsh (1925) |
| | 0.62-0.97 ($\bar{x}$ = 0.76) | 0.15-0.22 ($\bar{x}$ = 0.19) | Colton and Marak[1] |
| *Urophycis regius* | 0.67-0.81 ($\bar{x}$ = 0.73) | 0.14-0.22 ($\bar{x}$ = 0.18) | Barans and Barans (1972) |
| *Peprilus triacanthus* | 0.69-0.80 ($\bar{x}$ = 0.75) | 0.14-0.22 ($\bar{x}$ = 0.18) | Wheatland (1956) |
| | 0.75-0.79 ($\bar{x}$ = 0.77) | 0.17-0.21 ($\bar{x}$ = 0.20) | Colton and Honey (1963) |

[1]Colton, J. B., Jr., and R. R. Marak. 1969. Guide for identification of the common planktonic fish eggs and larvae of continental shelf waters, Cape Sable to Block Island. Bur. Commer. Fish. Lab. Ref. 69-9, Woods Hole Biol. Lab., 43 p.

globule diameter reported for the other species. Once pigmentation appears on the embryo, distinction is greatly facilitated. *Enchelyopus cimbrius* and *Urophycis* spp. are easily separated by their heavier and more numerous melanophores. Heavy mealonophores are always scattered on the yolk and oil globule of *Urophycis* spp. while *E. cimbrius* have pigmented oil globules and occasionally pigmented yolk. *Peprilus triacanthus* are somewhat more difficult to separate because, like *Etropus microstomus* they have fine melanophores located on the dorsum. However, this pigment generally forms two distinct rows from the eyes to the tail in *P. triacanthus* and does not migrate into the finfold as it does in *E. microstomus.*

Eggs of Gulfstream flounder, *Citharichthys arctifrons*, may resemble *E. microstomus* eggs since the early larvae are quite similar (Richardson and Joseph 1973). Diameters of unfertilized eggs given by Richardson and Joseph ranged from 0.70 to 0.82 mm ($\bar{x}$ = 0.74) which is considerably larger than *E. microstomus*. Presumably water hardening would increase this diameter even further.


## Acknowledgments

We are grateful to Masahiro Dojiri for the illustrations.


## Literature Cited


AHLSTROM, E. H., AND O. P. BALL.
1954. Description of eggs and larvae of jack mackerel (*Trachurus symmetricus*) and distribution and abundance of larvae in 1950 and 1951. U.S. Fish Wildl. Serv., Fish. Bull. 56:209-245.

BARANS, C. A., AND A. C. BARANS.
1972. Eggs and early larval stages of the spotted hake, *Urophycis regius*. Copeia 1972:188-190.

BATTLE, H. I.
1929. Effects of extreme temperatures and salinities on the development of *Enchelyopus cimbrius* (L.). Contrib. Can. Biol. Fish., New Ser. 5:109-192.

BERRIEN, P. L.
1975. A description of Atlantic mackerel, *Scomber scombrus*, eggs and early larvae. Fish. Bull., U.S. 73:186-192.

BIGELOW, H. B., AND W. W. WELSH.
1925. Fishes of the Gulf of Maine. Bull. U.S. Bur. Fish. 40(1), 567 p. (Doc. 965.)

COLTON, J. B., JR., AND K. A. HONEY.
1963. The eggs and larval stages of the butterfish, *Poronotus triacanthus*. Copeia 1963:447-450.

HARDY, J. D., JR.
1978. Development of fishes of the Mid-Atlantic Bight. An atlas of egg, larval, and juvenile stages. Vol. II. Anguillidae through Syngnathidae. U.S. Fish Wildl. Serv., Biol. Serv. Program, 458 p.

MARTIN, F. D., AND G. E. DREWRY.
1978. Development of fishes of the Mid-Atlantic Bight. An atlas of egg, larval, and juvenile stages. Vol. VI. Stromateidae through Ogcocephalidae. U.S. Fish Wildl. Serv., Biol. Serv. Program, 416 p.

PEARSON, J. C.
1941. The young of some marine fishes taken in lower Chesapeake Bay, Virginia, with special reference to the gray sea trout, *Cynoscion regalis* (Bloch). U.S. Fish Wildl. Serv., Fish. Bull. 50:79-102.

RICHARDSON, S. L., AND E. B. JOSEPH.
1973. Larvae and young of western north Atlantic bothid flatfishes *Etropus microstomus* and *Citharichthys arctifrons* in the Chesapeake Bight. Fish. Bull., U.S. 71:735-767.

SCOTTON, L. N., R. E. SMITH, N. S. SMITH, K. S. PRICE, AND D. P. DE SYLVA.
1973. Pictoral guide to the fish larvae of Delaware Bay with information and bibliographies useful for the study of fish larvae. Delaware Bay Rep. Ser. 7, Coll. Mar. Stud., Univ. Del., 206 p.

SMITH, W. G., AND M. P. FAHAY.
1970. Description of eggs and larvae of the summer flounder, *Paralichthys dentatus*. U.S. Fish Wildl. Serv., Res. Rep. 75, 21 p.

WHEATLAND, S. B.
1956. Oceanography of Long Island Sound, 1952-1954. VII. Pelagic fish eggs and larvae. Bull. Bingham Oceanogr. Collect., Yale Univ. 15:234-314.



MICHAEL D. SCHERER
DONALD W. BOURNE

*Marine Research, Inc.*
*141 Falmouth Heights Road*
*Falmouth, MA 02540*