

# OBSERVATIONS ON THE BIOLOGY OF THE VARIEGATED DARTER, *ETHEOSTOMA VARIATUM* (KIRTLAND)<sup>1, 2</sup>

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## ABSTRACT

Between June, 1964, and October, 1965, field and laboratory studies were conducted on the reproductive biology and larval development of variegated darters inhabiting Big Darby Creek, Pickaway County, approximately 25 miles southwest of Columbus, Ohio. These studies lead to the following conclusions. Prior to spawning, the darters migrate upstream from their wintering areas in the pools to the spawning riffles. The majority of the males become ripe when the water temperature reaches 40°F, whereas most females do not ripen until water temperature approaches 50°F. Spawning activity is confined mainly to the period from April 15 to May 10 when the water temperature is between 50°F and 70°F. Integration of field and laboratory data indicates that the eggs are deposited in the sand that accumulates behind rocks and boulders in the upper parts of riffles.

Males, in a laboratory environment, exhibit both intra- and inter-specific territorial defense. The spawning behavior includes the typical darter ritual of following, nosing, and quivering movements, with the female choosing the spawning site. Eggs deposited in sand behind and between rocks and in fine gravel hatch in approximately 14 days at the prevailing temperatures. Fry, fed a diet of brine shrimp larvae, attain the juvenile stage within six weeks after hatching.

## INTRODUCTION

Percid fishes of the genus *Etheostoma* form an important part of the freshwater fish fauna of eastern North America (Winn, 1958, p. 156). In spite of this, comparatively little is known about the life histories of the vast majority of these species. This paper deals with the reproductive behavior and larval development of the variegated darter, *Etheostoma variatum* (Kirtland). Previous work on this species consisted largely of studies on taxonomy, range, habitat, age and growth, and food habits.

*Etheostoma variatum* was described by Kirtland (1838, p. 21) from specimens taken from the Mahoning River, Mahoning County, Ohio. Hubbs and Black (1940, p. 2) placed, in the *Etheostoma variatum* group, the species *E. osbourni*, *E. tetrazonus*, *E. variatum*, *E. blennis*, *E. euzonus*, and *E. kanawhae*, all of which are characterized by having four or five conspicuous dark crossbars over a more or less greenish-olive background above the lateral line. Hubbs and Black (1940, p. 7) gave the range of the species *E. variatum* as the Ohio River drainage basin in New York, Pennsylvania, West Virginia, Ohio, Indiana, and Kentucky, exclusive of the Upper Kanawha and the Wabash, Kentucky, and Tennessee River systems. Trautman (1957, p. 579) noted that in Ohio this species was most abundant in those riffles of moderate- or large-sized streams having a rapid current flowing over a bottom of clean, glacial rubble and boulders. Lachner, Westlake, and Handwerk (1950, p. 104) observed that males grow faster than females, that both sexes mature and spawn at age II, and that four years is the maximum life span. Turner (1922, p. 53) reported that the food of variegated darters consists primarily of aquatic insect larvae.

## STUDY AREA

Field observations were conducted between June, 1964, and October, 1965, in the lower 20 miles of Big Darby Creek, located in Pickaway County, approxi-

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mately 25 miles southwest of Columbus, Ohio. The two soil groups found most commonly in the Darby watershed are developed in Wisconsin glacial drift. The till is chiefly from limestone, though small amounts of igneous and shale material are also present. The bottoms of the larger riffles are characterized by glacial rubble and boulders. The gradient in the study section averages 5.6 feet per mile and the stream consists of a series of alternating pools and riffles with a median flow of 84 million gallons per day.

#### METHODS AND EQUIPMENT

Darters in the laboratory were held in a tank similar to one described by Mount (1959, p. 240), which was three feet wide, four feet long, and 18 inches deep. A plate-glass window was installed in each of the longer sides. An input, shaded pole motor, generating 1/70 horsepower, supplied power to circulate the water. Sand, gravel, and several large rocks were scattered on the bottom. The darters introduced into the tank were fed live Ephemeroptera larvae twice daily.

The larval development was studied from specimens that hatched from eggs spawned in the tank. Immediately after being spawned, the fertilized eggs were transferred from the tank into a 15-gallon aquarium which contained aquatic vegetation, planaria, snails, daphnia, and tadpoles. The larval fishes were fed a diet of brine shrimp larvae.

Sketches were drawn of four larval stages with the aid of a microprojector and a binocular microscope. Each drawing was made from a single specimen which had been preserved in a four percent solution of formalin. The measurements recorded pertain only to the individual from which the sketch was made. Age was computed from the time of hatching and the terminology was taken from Hubbs (1943, p. 260). The age of adult fish was determined by counting the number of annuli, or year marks, on scales taken from between the lateral line and spinous dorsal, mounted in a glycerine medium, and examined with a microprojector.

A total of 1,133 variegated darters taken from three riffles during the fall of 1964 were finclipped to facilitate the study of their seasonal movements. Darters collected from the upstream riffle, 16.8 miles from the mouth of Big Darby, had the right pelvic fin clipped; those collected from the intermediate riffle, 14.1 miles from the mouth, had the anal fin clipped; and those collected from the downstream riffle, 3.4 miles from the mouth, had the left pelvic fin clipped. These three riffles are designated as the marking riffles in the text. After marking was completed, riffles were seined systematically every three or four weeks from November, 1964, through May, 1965. The seine that was used was four feet deep and six feet long, with a one-quarter-inch mesh, and was easily handled by one person. A glass-bottomed box was employed to observe the fishes on the riffle bottom.

The field observations provided information on seasonal movements, spawning period, and spawning site in the stream, whereas the laboratory study was concerned mainly with spawning behavior, territoriality, and larval development.

#### FIELD OBSERVATIONS ON REPRODUCTION

##### *Migration*

The riffle areas in Big Darby Creek were reduced considerably during the late summer and fall of 1964, when the stream flow lessened. This reduction in riffle habitat appeared to be instrumental in influencing a downstream movement of variegated darters by increasing competition for food and space. During this period, the numbers of *Etheostoma variatum* collected from most riffles fluctuated widely between sampling dates, indicating that large numbers of darters were migrating from riffle to riffle. Four individuals collected in December had moved nearly three miles downstream from the riffle where they had been finclipped. On the other hand, a recapture rate of over 40 percent on the marking riffles in

November and December revealed that a considerable part of the population did not move from the riffle on which they had been marked. Later in the winter there was a tendency for the darters to move into the lower ends of the riffles, and into the pools immediately below these riffles, when water temperatures dropped below 35°F.

When temperature and stream flow increased in the spring, the darters moved back into the riffles. The first upstream movement was noted on February 20, after a heavy rain had raised the stream level almost two feet. On this date, individuals finclipped the previous fall were captured approximately 400 yards upstream from the point of marking, and darters were present on small riffles from which they had been absent during the winter. During March and April, between 15 and 38 percent of the fish collected on the marking riffles were finclipped returns. Fewer fish were collected on the riffles in the spring than in the fall because high water during the spring precluded efficient sampling.

The above data indicates that a part of the *E. variatum* population migrates in the fall and spring, whereas another part remains on or near a particular riffle throughout the year. This segregation into a mobile group and a sedentary group substantiates Funk's (1955, p. 56) observations on other stream fish. He noted that competition for food and space appear to be important factors inducing migrations, because competition forces individuals to defend a suitable territory vigorously enough to maintain it, or to move in search of less crowded conditions.

#### *Spawning Period*

Gonadal development and color intensity become pronounced in March, at which time males exhibit diverse shades of reds, yellows, greens, and blues, whereas colors of the females are mostly olives and brownish-greens. An excellent description of breeding coloration was given by Trautman (1957, p. 578). The majority of the males are ripe by March 19, when water temperature approaches 40°F, while the majority of females do not ripen until water temperature reaches 50°F, as it did on April 17. All spawning must have occurred between that date and May 10, for all individuals which were collected after May 10 were spent.

#### *Spawning Site*

During the spawning period, large ripe males were usually found near the center of the stream behind rocks and boulders in the upper portions of the riffles. These sections of the riffles apparently contained the spawning sites, because it was the only area where ripe males and females were captured together with any degree of regularity. The unripe or partially spent females were most frequently taken in the lower portions of the riffles. Presumably, the females when ready to spawn, move into the upper part of the riffles, where the mature males are residing. High, turbid waters precluded the observation of any redds, but laboratory observations of spawning fish indicated that the eggs are deposited in the sand and small gravel that accumulates behind large rocks.

#### LABORATORY OBSERVATIONS ON REPRODUCTION

On March 23, 1965, two ripe males, both age III, and two ripe females, one age II and one age III, were taken from Big Darby Creek and transported to the tank described previously. These fish were observed spawning on March 26, 1965; on March 28, 1965; and on April 1, 2, and 3, 1965. During this period, the water temperatures in the tank varied between 50°F and 60°F.

#### *Territoriality*

Throughout the period from March 23 to April 8, each of the two males usually remained under or near a specific rock in the tank. A stylized type of conflict described below took place when one male approached within six to ten inches

of the other's rock. The area defended was irregular in outline; its dimensions appeared to be determined by the shape and position of the center rock, probably because this largely circumscribed the vision of the resident male. A similar type of conflict was noted when the males were pursuing a female. In each circumstance, the pattern of conflicts was almost identical, and resembled that described for *Etheostoma nigrum* and *E. flabellare* by Winn (1958, p. 169).

When the two males confronted each other, their respiration rates visibly increased and their coloration intensified, particularly in the red bar posterior to the pectoral fins. The dorsal, anal, caudal, and pectoral fins became erected, the latter pair being almost at right angles to the body axis. During these displays, the males often positioned themselves in one of two ways: (1) so that their bodies were at right angles to each other, with the snout of one male close to the pectoral region of the other; or (2) in the more conventional lateral display, with the longitudinal axes of their bodies assuming a sigmoid shape. This was followed by a brief flurry of action in which each male attempted to nip his opponent's fins. Although no damage was done to the fins, these attacks usually resulted in one male being chased from the conflict area.

One male, termed A, was usually victorious in the early encounters. After A appeared to have established dominance over Male B, a brief display sufficed to induce the retreat of Male B whenever B infringed on the territory of A. On the other hand, the conflicts occurring when the males were following a ripe female continued to involve several series of displays and attacks before Male B was defeated.

Male *Etheostoma caeruleum* introduced into the tank were challenged when they infringed on the territory of an *E. variatum* male. The emergence of an *E. variatum* male from under his rock, followed by a brief display and a short dash, was usually sufficient to induce the departure of the intruding male *E. caeruleum*. No physical contact was noted between the two species during these conflicts, mainly because male *E. caeruleum* did not challenge the *E. variatum* male. These observations suggest that male *E. variatum* defend their territories against other species of darters.

#### *Spawning Behavior*

Spawning activity appeared to be initiated by a ripe female and resembled the pattern described for *Etheostoma camurum* by Mount (1954, p. 242). At first, the female moved throughout the tank in short darts, pausing in the open instead of near or under rocks, frequently swimming to the water's surface, only to sink back again. This type of behavior appeared to attract the males and they quickly began to follow her movements. The trailing of the female was interrupted occasionally by mock battles between the males as they vied for the opportunity to spawn.

The above behavior lasted 15 to 90 minutes before the female was sufficiently stimulated to spawn. At this time she usually selected a sandy location between some rocks or gravel, orienting the long axis of her body at approximately a 45-degree angle to the substrate, and then, with several vigorous vibrations of her tail, buried her snout and head into the sand. This nosing motion appeared to be the stimulus that released the mounting behavior of males, because the male moved close to the nosing female and mounted her while she was settling into a horizontal position. In this position, the male's snout was anterior to the eye region of the female, his pelvic fins appeared to clasp her anterior to her spinous dorsal, and his body, posterior to the abdomen, was juxtaposed along her side. Immediately after the female had been mounted, the paired fish vibrated vigorously for as long as 30 seconds. When these vibrations ceased, both lay motionless one to three minutes, after which the two fish separated and the female again began to move throughout the tank in short darts, leading to a repeat of the ritual. One female was observed to spawn four times in two and one-half hours.

The release of sexual products during vibrations was verified by the finding of fertilized eggs buried in the sand where the vibrations had occurred. When first released, the eggs adhered to each other and to adjacent sand particles. Six of the eight clusters removed from the tank consisted of 20 to 40 eggs; one contained nearly 70. Because ovaries of age-II and age-III fish contain 700 to 1,000 ripe or nearly ripe eggs at the onset of spawning, each female must spawn 25 to 50 times if all of these eggs are to be released.

#### *Sexual Selection*

Male A was observed to engage in ten successful vibrations, Male B in only two. Ten of the twelve spawnings occurred in and around the territory of Male A, nine of which involved Male A, and the two other spawnings, one by each of the two males, took place in a pile of coarse gravel and sand adjacent to the territory of Male B. These two areas utilized for spawning were the only places in the tank where the current had piled sand higher than one-half inch. It is possible that the swifter current over the territory of Male A made it more desirable as a spawning site than the other area.

Because the males followed the females, it appears that females select a spawning site suitable for deposition of eggs, rather than being attracted by the appearance and/or behavior of the male occupying the area. Thus, the selection by males of territories attractive to females as spawning sites appears to be an important factor in determining the spawning success of males. This is in accord with the findings of Winn (1958, p. 185), who concluded that females mated with the larger male darters residing in the centers of riffles, because the females, when ready to spawn, tended to move into the central portions of the upper parts of riffles.

#### LARVAL DEVELOPMENT

The incubation period for the eggs fertilized naturally in the tank varied between 13 and 14 days. During this period, the water temperature varied between 50° and 60°F for the first ten days following fertilization and remained within 3 degrees of 70°F for the duration of the study. Almost 90 percent of the eggs removed from the tank were viable, and hatched. Descriptions of the larvae at different ages follow.

#### *Prolarva less than one day old (Fig. 1).*

Total length, 6.9 mm; distance to anus, 3.8 mm; length of yolk sac, 2.3 mm; diameter of oil globule, 0.9 mm; myomeres 18 to anus, 22 behind anus.

This larva is characterized by a large oil globule which occupies the anterior end of a very large yolk sac, by distinct auditory placodes, by large eyes, and by a distinct notochord. The dorsal and ventral parts of the median fin fold are

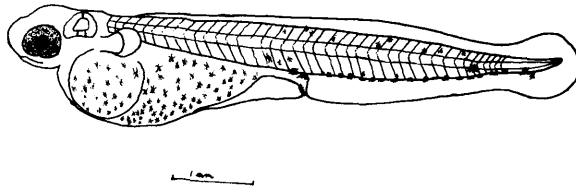


FIGURE 1. Newly hatched prolarval *Etheostoma variatum*.

continuous with the caudal fin, and a comparatively small pectoral fin bud has developed. Large stellate melanophores are scattered about the oil globule and the yolk sac, near the dorsal edges of somites behind the anus, and in a row on the ventral edges of somites behind the anus. At this stage, the larvae usually lie quietly on the bottom, but occasionally swim erratically by short, rapid, jerky movements.

*Prolarva three days old (Fig. 2)*

Total length, 8.0 mm; distance to anus, 4.3 mm; myomeres 18 to anus, 22 behind anus.

The volumes of the oil globule and yolk sac are considerably reduced, the auditory placodes and the notochord are both still quite distinct, and the median fin folds are large, with ray formation noticeable in the caudal and pectoral fins. Large stellate melanophores are evident in the auditory placodes, on the base of

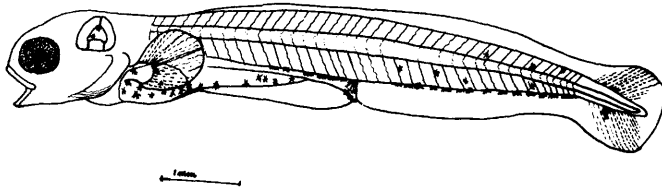


FIGURE 2. Three-day larval *Etheostoma variatum*.

the pectoral fin, on the surface of the oil globule and yolk sac, in a row on the posterior part of the alimentary canal, on myomeres above and below the notochord behind the anus, in a row on the ventral edge of somites behind the anus, and on the lower base of the caudal fin. At this stage, the larvae are more active than one-day-old fish, but still swim by short, jerky movements.

*Postlarva ten days old (Fig. 3)*

Total length, 8.1 mm; distance to anus, 4.3 mm; myomeres 18 to anus, 22 behind anus.

By this stage, the yolk sac and oil globule have disappeared. The auditory placode is still obvious, and ray formation is proceeding in the caudal and pectoral fins. Rows of melanophores are prominent on the ventral edge of somites be-

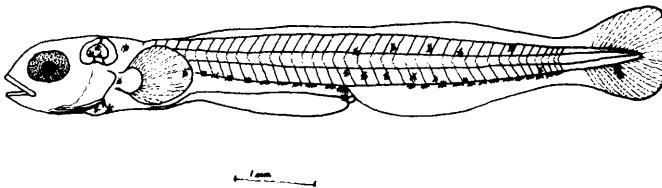


FIGURE 3. Ten-day larval *Etheostoma variatum*.

hind the vent, and on the dorsal surface of the alimentary canal. Large branching melanophores are evident on and immediately behind the auditory placode, on the base of the pectoral fin, on the breast below the operculum, in a row above and below the notochord behind the anus, and in a group at the base of the lower caudal rays. At this stage, the larval fish are quite active, usually swimming immediately below the water surface. They have begun to consume brine shrimp larvae.

*Postlarva 15 days old (Fig. 4).*

Total length 9.3 mm; distance to anus, 4.3 mm; myomeres 18 to anus, 22 behind anus.

The auditory placodes and eyes are prominent. Rays are beginning to form in the second dorsal and anal fins and the caudal fin is almost distinct from the median fin fold. Rows of melanophores are present on the dorsal surface of the alimentary canal and along the lower edge of the myomeres behind the anus.

Large branching melanophores are evident on the pre-opercle region of the head, posterior to the auditory placode, on the base of the pectoral fin, on the ventral surface of the breast, on the myomeres above the notochord, and along the basal part of the lower caudal rays. These fish swim near the surface of the water and feed voraciously on brine shrimp larva.

Six weeks after hatching, the fins are completely developed and the fish may be classified as juveniles. At this stage, the external morphology of these small fishes resemble the adult, except that they are more slender and lack adult pigmentation.

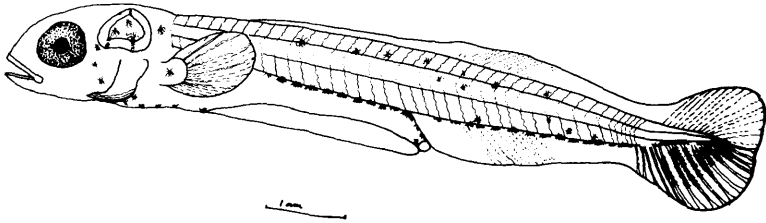


FIGURE 4. Fifteen-day larval *Etheostoma variatum*.

The adult morphology develops concurrently with obvious changes in behavior. For the first four weeks following hatching, the larval fish are nektonic and feed near the surface. In contrast to this, six weeks after hatching, the fish have developed benthic habits, characteristic of adult fishes, and capture brine shrimp larvae after they have drifted to the bottom.

#### SUMMARY

The reproductive behavior and larval development of the percid fish, *Etheostoma variatum*, were studied both in the field in the Columbus, Ohio, area and in the laboratory.

Prior to spawning, *E. variatum* migrates from wintering areas in pools to the larger riffles, which have a substrate of sand, gravel, and glacial rubble and boulders. The ripe males and females occur together frequently in the upper parts of these riffles, whereas the unripe or partially spent females, along with immature males, normally occupy the lower sections. The integration of field and laboratory data indicates that mature males establish territories toward the center of the stream behind rocks and boulders in the upper portions of the riffles. When the females are ready to spawn, they move into this part of the riffle. Following a brief courtship display, the eggs are deposited in the sand and fine gravel that has accumulated behind the glacial rubble and boulders. The establishment of territories by the larger, ripe males in the upper portions of the riffles insures spawning with the females as they move into this area. The majority of the spawning in 1965 occurred from April 15 to May 10, when water temperatures climbed from 50°F to 70°F.

Laboratory observations indicate that erratic swimming by a female stimulates the males to follow her movements as she moves through their territories. Mock battles occur between the males as they compete to spawn with the female. When sufficiently stimulated, the female noses into the sand and small gravel behind a rock or boulder at about a 45-degree angle, a position which releases the mounting behavior of the male. She is mounted by the resident male as she settles to a horizontal position. During the ensuing vibrations, the sexual products are deposited in the sand. After a brief rest, the two fish separate and the female resumes her erratic swimming, following which the ritual is repeated.

The eggs fertilized naturally in the laboratory hatch in approximately 14 days

at prevailing temperatures. Fry raised on a diet of brine shrimp larvae attain the juvenile state of development within six weeks after hatching.

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