ANOMALIES, SOCIAL BEHAVIOR, DEVELOPMENT AND HYBRIDIZATION OF PERCID FISHES, ETHEOSTOMA, BRED BY NATURAL MEANS IN THE LABORATORY

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1951

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1952

Submitted to the faculty of the Graduate
School of the Oklahoma Agricultural
and Mechanical College in partial
fulfillment of the requirements
for the degree of
DOCTOR OF PHILOSOPHY
May, 1956

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PREFACE

Fish hybridization has interested biologists since about the beginning of the twentieth century. The earlier workers were cytologists interested in the action of chromosomes and, therefore, studied only the early cleavage stages and the early development of fish hybrids. They crossed animals which were very distantly related, such as members of different families or even different orders. More recent studies of fish hybrids have been carried out by geneticists and taxonomists interested in how morphological characters are expressed in the hybrid offspring. The present paper discusses experiments in which two distinctly different species were crossed in the laboratory in an attempt to verify the identification of a natural fish hybrid, to determine how characters are expressed by the hybrid offspring and also to try to determine why these distinctly different sympatric forms cross in nature.

I am indebted to Drs. R. W. Jones, I. E. Wallen, D. Chambers and I. V. Holt for carefully evaluating this thesis and to Dr. G. A. Moore for his assistance and direction in carrying out the research and in writing the paper. I wish to thank A. P. Juhlin of the Oklahoma

Agricultural and Mechanical College Library for obtaining a considerable number of publications which were not available at the library, D. R. Miller of the Carlsbad, New Mexico Public Schools and John Preston of the Utah Game and Fish Department for assistance in the field. I also wish to acknowledge the financial support supplied by the American Association for the Advancement of Science through the Oklahoma Academy of Science.

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INTRODUCTION

It has become well established that distinct species and even genera of fishes hybridize successfully in nature. Although some species of most freshwater fish families have been known to hybridize, it has been reported most frequently among the members of the Centrarchidae and Cyprinidae. Many examples have also been reported in the Catostomidae, Salmonidae, Cyprinodontidae and Cyprinidae. Descriptions of fishes presumed to be new species have even been based on specimens later diagnosed as hybrids (Hubbs, 1926, 1945 and 1951; Hubbs and Brown, 1929; Hubbs and Hubbs, 1932a; Raney, 1940; Carlander, 1941; Hubbs and Bailey, 1952). Linder (1955) appears to have published the first detailed description of an etheostomid hybrid in describing Etheostoma radiosum cyanorum (Moore and Rigney) X Etheostoma spectabile (Agassiz) from Blue River in south-central Oklahoma. This hybrid combination (2 specimens) was described by using the hybrid index method proposed by Hubbs and Kuronuma (1942) and explained by Hubbs, Hubbs, and Johnson (1943). Linder (1955) suggested that this hybrid combination should be experimentally produced in order to determine the mode of inheritance of characters in offspring produced by crossing these two species. The production of this hybrid combination was initiated in the fall of 1953 with the following purposes in mind: to verify the identification of the natural hybrid, to test in the laboratory the present method of describing natural putative hybrids, to determine if reciprocal etheostomid crosses give identical results and to determine any similarities in the habits of these fishes which would help to explain the crossing of species lines.

REVIEW OF LITERATURE

Kammerer (1907), apparently the first to study natural fish hybrids in detail, reported two percid hybrids as Perca fluviatilis (Linnaeus) × Acerina cernua (Linnaeus). He found that in a number of respects the hybrids appeared to be intermediate between these two species. The correctness of his diagnosis was tested by crossing the two species in the laboratory, with the result that vigorous offspring were obtained. These hybrids were reared to an age of six months and, at this age, showed characters which resembled the hybrids collected in nature. Kammerer presented considerable data which tended to show that, in practically all characters examined, the hybrids reared in captivity were intermediate between the parental species. This was true of the number of scales in a row, number of fin rays, number of teeth and other integral variates. Characters involving dimensions showed a similar blending inheritance between the two species. He made no mention of any type of inheritance suggestive of Mendelian dominance. Radcliffe (1914) working with natural sunfish hybrids showed that, in some characters, the hybrid tended to resemble one or the other parent and, in others, tended to be intermediate. Hubbs (1920) also identified some sunfish hybrids from nature and concluded that characters of form, structure and position of the mouth, pharyngeal arch and teeth and some color characters tended to be intermediate. The tendency for these naturally produced sunfish hybrids to be intermediate prompted Hubbs and Hubbs (1933, 1932a) to produce experimentally the various centrarchid hybrid combinations collected in nature. As a result of these experiments they found that, in sunfish hybrids, taxonomic characters were intermediate between the parental species. Bailey and Lagler (1938) experimentally verified the identification of some natural sunfish hybrids and were in complete agreement with the above writers in that the taxonomic characters were intermediate in the hybrids. Hubbs and Hubbs (1932b) reported the intermediacy of offspring produced by crossing different species of poeciliids. Lagler and Bailey (1947) crossed two subspecies of Boleosoma nigrum (Rafinesque) and found that the hybrids were intermediate. They concluded that the genetic differences between the two forms could not be explained by simple dominance. Linder (1955) found that the hybrids (one male and one female) from Blue River tended to be intermediate when compared with specimens of the presumed parental species. An average hybrid index value of 57.5 was obtained when comparing the male hybrid with male specimens of the parental species and an average value of 45.2 was obtained when comparing the female specimen with females of the parental species. When both hybrids were compared with male and female specimens of the parental species an average value of 46.7 was obtained.

All the workers cited above have indicated that the laboratory-reared hybrids were intermediate between the parental species.

Since the time of the experiments of Hubbs and Hubbs (1933, 1932a), there have been numerous hybrid combinations listed in the literature. Although most are not accompanied by a detailed description, the writers have indicated that the hybrids are intermediate in most respects between the parental species. Linder (1955) found that the hybrids between *Etheostoma radiosum* and *E. spectabile* were not entirely intermediate, but had some characters identical with those of one parent, some in various degrees of intermediacy between those of the parental species, some identical with those of the other parent and still others beyond the character average of either parent. This indicated that the hybrids of these fishes may present a somewhat different problem than that of centrarchids, poeciliids and the other percids.

Since prominent sexual dimorphism exists in the presumed parental species of the natural hybrid combination, Linder (1955) made a comparison of male *spectabile* and female *radiosum* with the two hybrids and also the reciprocal cross, male *radiosum* and female *spectabile*, to determine if identical results would be obtained. Hubbs and Hubbs (1932a), when verifying the intermediacy of fish hybrids with laboratory production of several sunfish hybrids, showed that reciprocal crosses in the family Centrarchidae produced identical results. Similar results were obtained by Hubbs (1940), working with reciprocal peociliid crosses. The comparison of female *radiosum* and male *spectabile* with the two natural hybrids showed ten characters, five of them scale counts, which yielded hybrid index values between 0 and 100 with an average value of 40.7.

The reciprocal cross, using male *radiosum* and female *spectabile*, produced 26 characters which yielded values between 0 and 100, with an average hybrid index value of 61.6. When individual characters were considered, there was sometimes a lack of expression in one sex, suggesting the possibility that the character was of a sex-linked or sex-influenced type. For example, the "humeral scale" was found to be well developed in *radiosum* but poorly developed in *spectabile*. The male hybrid resembled *radiosum* in this character while the female did not. It seemed that in this cross between the two species the "humeral scale" went only to the male, whereas in normal matings, it was either well expressed or not evident. Squamation was similarly transmitted, the *radiosum* head being quite regularly scaled and the *spectabile* head naked. The hybrid male had scaly cheeks and opercles, whereas, the female had these areas naked. It was concluded (Linder, 1955) that, without experimental data, it would be impossible to predict the appearance of the hybrids between the two possible reciprocal crosses. If darter characters behave in the same manner as those of their relatives, the centrarchids, or as those of the poeciliids (Hubbs, 1940), the data obtained, by comparing the two possible parental combinations, should have been closely similar. Offspring of reciprocal crosses between different species of birds (pheasants and chickens), which express prominent sexual dimorphism, have been reported to have a different appearance (Phillips, 1921; Davenport, 1906).

The fact that eggs of one species can be fertilized by the sperm of very distantly related forms has been established by many cytologists

(Appellof, 1894; Moenkhaus, 1894 and 1911; Newman, 1915 and 1923; and Pinney, 1918 and 1922). These workers found that crosses between different genera, different families and even different orders of fishes were successful in varying degrees. Newman (1915) found that the success of the crosses was not correlated with the nearness of relationship of the species used, but that certain specific characters of the egg determined the success or failure of hybrid development. He found that some crosses developed as far as hatching and he indicated that, with more care, they may have been cultured through the hatching stage.

MATERIALS AND METHODS

Collection of Fishes

Specimens to be used as breeding stock (*E. radiosum* and *E. spectabile*) were first collected in October 1953, from the same locality as that from which the original hybrid combination came (Linder, 1955). It was discovered, however, that although the males of both species and the large female *radiosum* were readily identifiable, it was impossible to separate, in life, small female *radiosum* from female *spectabile*. Faced with this difficulty it was decided that, in order to be certain of the identity of the fish, the specimens of *spectabile* should be obtained from a locality where *radiosum* is not known to occur. All specimens of *spectabile* and the indistinguishable females from Blue River were preserved.

New specimens of *spectabile* were obtained from Dog Creek 7.6 miles east and 3 miles north of Foraker, Osage County, Oklahoma. Because of drouth conditions, the creeks in the Foraker area were dry, except for isolated pools. Many *spectabile* were collected from one such pool which was about 30 feet long, 10 feet wide and three feet deep. One shore consisted of mud and the other of rocks. The water was very turbid and it did not appear to be a logical place to expect to find riffle-inhabiting darters, but specimens of *spectabile* were collected along the rocky shore. The specimens were very gaunt and apparently stunted. The collecting of such a hardy race may explain the ease with which they adapted themselves to life in aquaria. The specimens of radiosum from Blue River and of spectabile from Dog Creek were used in the experiments carried out in the school year of 1953-54. Electrical failure, to be discussed later, caused the loss of these fishes, and all but nine of their offspring, in April 1954.

New breeding stock was obtained in May 1954. The specimens of radiosum came from Jack Fork Creek at the bridge on Highway 99 south of Ada, Oklahoma and those of spectabile from Spring Creek, 13 miles north of Tishomingo, Oklahoma. These specimens were kept until August 1954 when they were preserved.

Additional specimens of radiosum and spectabile were collected from Blue River in August 1954. These fish refused to spawn and, since there was again some doubt as to the identification of some of the female specimens, were preserved in November 1954.

New breeding experiments were undertaken in November 1954, in an effort to replace specimens lost in the 1953-54 experiments. Specimens of radiosum were again collected from Blue River. Specimens of spectabile were collected from Mill Creek 1 mile west of Mill Creek, Oklahoma and from Dog Creek near Foraker, Oklahoma. These fishes were used as breeding stock until May 1955, when the breeding experiments were terminated.

Transportation of Fishes

The fishes, used in this study, were transported easily from the field to the laboratory in a container large enough to hold about two gallons of water without loss caused by splashing. Canvas minnow buckets proved to be the most satisfactory container since evaporation of water from the walls of the bucket helped to maintain a lower temperature and their flexibility kept splashing to a minimum. The water was aerated by means of an air breaker attached by a long plastic hose to an inflated inner tube. It was found that as many as 30 darters could be transported over one hunbred miles, in this manner, with few fatalities. Upon arriving at the laboratory the fish were placed in aged tap water of the same temperature as that in which they were transported.

Laboratory Care of Fishes

Breeding tanks.--The aquaria in which the breeding fish were kept were six inches wide, twelve inches long and seven inches deep. Small rocks and gravel were placed at one end of the tank and the water level was maintained at about a three-inch depth. Aquatic plants were at first maintained in the tanks, but removed later to facilitate cleaning of the tanks and collecting of eggs. Snails (*Heliosoma*) were kept in aquaria to aid in removal of decaying organic matter. Tanks were set up with the following matings: *spectabile* males with *spectabile* females, *spectabile* males with *radiosum* females, *radiosum* males with *spectabile* females and *radiosum* males with *radiosum* females. Several tanks of each combination were set up with one or two fish of each sex in each tank. When fishes refused to spawn, or if the eggs produced failed to develop, new specimens were placed in the tanks. The tanks were aerated with an air pump which was capable of aerating about twenty-five tanks.

Foods and Feeding.--The feeding of these fishes at first posed a very difficult problem. When first brought into the laboratory the fishes were offered shredded shrimp, ground beef steak, and raw liver. Although some specimens appeared to take this food, the feeding method was discontinued since much of the food remained uneaten and the tanks became foul and murky from excessive organic decay. Chopped earthworms and June-bug larvae were used early in the experiments and eaten by the fish until live foods were made available. Small earthworms were readily eaten by all the fish and occasionally worms of such a large size were taken that it required over twenty-four hours for the fish to ingest an entire worm.

An attempt was made to culture *Drosophila* larvae for fish food. Use of culture media suggested by Demerec (1950) was attempted, but no practical method of separating the larvae from the media was developed.

Dwarf white worms (small oligochaets) proved to be the most satisfactory food with respect to ease of rearing and handling, acceptance by the fish and maintenance of uncontaminated aquaria. These worms were cultured in covered containers of various sizes (refrigerator dishes) filled with sterilized soil to about an inch from the top (Galtsoff et al, 1937). After innoculating the soil with white worms, a mixture of precooked baby cereal, brewer's yeast, soybean flour and water was prepared in paste form and placed on a piece of glass cut small enough to fit inside of the container. The glass was then placed, food downward, on the moist soil and the dish covered. The worms were harvested every two or three days. The yield of worms varied with the size of the culture dish used.

Mosquito larvae were preferred by the fish. When both white worms and mosquito larvae were available to the fish, the worms were ignored. No attempt was made to rear mosquito larvae in the laboratory since they were usually available from nearby stagnant ponds. On occasions they were collected in a fine-mesh net in mid-winter, after breaking the ice cover.

Cladocerans and copepods were readily accepted by the smaller fish, but the larger ones seemed to ignore them. These crustaceans were collected from a small lake (Theta Pond) on the Oklahoma Agricultural and Mechanical College campus. *Daphnia* was reared in a culturing tank made of an enameled ice box liner filled with water. Slices of potatoes, replaced every 48 hours, and commercial infusoria tablets were used as food for this tiny Cladoceran. *Daphnia* was also propagated with some success, by adding dry baker's yeast to the water twice a week. The use of barnyard manure, as suggested by Galtsoff et al (1937), was tried but did not prove to be satisfactory. Although *Daphnia* was easily reared at an optimum temperature of about 80° F., it was quite cyclic with frequency of harvest varying considerably.

Microworms (small nematodes), used as food for the young fishes, were easily reared in the laboratory. Precooked cereal, baker's yeast and water were mixed to form a paste which was placed in a petri dish along with some of the nematodes. In two or three days the worms began crawling up on the lid of the dish and were harvested by washing them from the lid with water (Emmons, 1953).

Although some of the fishes ate pieces of earthworms and shredded shrimp, when they were first brought to the laboratory, they later refused to eat anything that did not move, apparently preferring living foods. Live foods comprise the greater part of the diet of related forms in nature (Hankinson, 1908; Petravicz, 1936; Petravicz, 1938; Raney and Lachner, 1939; Winn, 1953; Fahy, 1954).

Temperature.--Since these fishes are typically found in the swifter, colder streams of the state, it was believed that it would be necessary to subject them to low temperatures during the summer months. This belief was strengthened in the fall of 1953 when fish, in a tank placed near a steam line, died after the steam was turned on during the night. In the morning, when the kill was noted, the tank in question was about 3.5° C. higher than the neighboring tanks. When the temperature of the water in the tank was lowered by adding cold water, the few remaining fish appeared to be relieved and no more were lost at that time. The fish that survived were primarily the very small ones. With this experience in mind, a refrigerator was obtained to reduce the temperature of the tanks during the hot, summer months. Most of the aquaria were placed in the refrigerator, but some were left out to determine the effect of high temperature on the fish. During the months of June, July and August, 1954, and temperature in Stillwater was abnormally high. The water temperature in the unrefrigerated aquaria remained at 30° C. or more for 70 days. No ill effects were noted and the fishes without refrigeration did almost as well as the refrigerated ones, although high temperatures were tolerated only when the water was well aerated. Apparently a slow rise in temperature is not detrimental to these darters, but a rapid rise is fatal. The range of tolerance for temperature changes seems to be greater at lower temperatures than at higher ones. On one occasion a small female radiosum, transferred from a tank in which the water temperature was 14.5° C. to one in which the temperature was 20° C., showed no ill effects in response to the sudden 5.5° C. change in water temperature.

Care of eggs.--The breeding tanks were examined for eggs once or twice a day and all eggs found were removed. The eggs were adhesive and were easily removed from the tanks by picking out the stones to which they were attached. They were first placed in finger bowls containing water taken from the breeding tanks and later were placed in aged tap water. The eggs often became infected with a fungus when left in the water from the aquaria. The dead eggs were removed as soon as they were found because invariably they were infected with a fungus which seemed to spread to other eggs. The eggs were retained in the finger bowls throughout the incubation period.

Care of young.--The care of the young in the laboratory did not present a very difficult problem. They were kept in fingerbowls, until the yolk sac disappeared, and were then placed in aquaria containing about one inch of water. During the first two or three months the depth of water is apparently a critical factor. The fishes showed signs of distress when the water depth was increased, but recovered when the water level was again lowered to about an inch. The first groups of young were fed infusoria for about two weeks and were then fed microworms. It was later found that these larvae were able to take microworms as soon as they were able to start feeding.

Daphnia and dwarf white worms were fed when the fish attained a length of 15-20 mm.

Diseases.--The fishes studied did not seem to be susceptible to many of the numerous diseases reported for exotic aquarium fishes (Innes, 1953). The pathogen most commonly encountered was a fungus which appeared as a velvety growth along the back and on the caudal peduncle. This infection was corrected by bathing the fish in a dilute salt solution (3% NaCl). It was found that if the aquaria were kept free of decaying organic matter the disease could be eliminated completely.

On several occasions some fishes became emaciated, refused food, soon lost their sense of equilibrium and died, unless placed in clean, aged water. Usually no visible abnormalities were seen, but on some occasions a concentration of blood along the lateral line and the myomeres below the dorsal fins was noted. On each occasion examination of the water revealed a heavy concentration of ciliates. Salt and formalin solutions, in concentrations tolerated by the fishes, failed to control these protozoans. The only way found to keep them in check was to replace the water in the aquaria with fresh water every three or four days. It is not known if these organisms are parasitic or if they merely thrived on fish excrement, but if a tank with a heavy population of these protozoans was permitted to stand, with the fish removed, the ciliates disappeared. No other cause could be ascribed for the death of the fish. Dr. Nelson Cooley, of the Department of Zoology, identified the ciliate as a member of the family Frontoniidae, order Holotricha.

Counts and Measurements

All fish specimens used in this study are deposited in the Museum of Zoology of the Oklahoma Agricultural and Mechanical College. Only adult specimens of the parental species (20 spectabile from Dog Creek and 20 radiosum from Blue River) were used for the counts and measurements. All counts and measurements were taken according to the methods of Hubbs and Lagler (1947), except some (explained below) which deviate from or are not mentioned by those writers.

Because the scales at the origin of the spinous dorsal are embedded and difficult to see, the count above the lateral line was made below the origin of the soft dorsal fin.

Since the lateral canal is absent in the laboratory-reared hybrids, the lateral-line scale count was made along the row of scales where the canal would be, if present.

Scales above and below the lateral line were enumerated, in the hybrids, from the origin of the soft dorsal and anal fins, respectively, to the scale row upon which the lateral line normally would occur.

The measurement of the distance from the origin of the dorsal fin to the occiput was made from the structural base of the first dorsal spine to the occiput.

The distance from the union of the gill membranes to the mandible was measured from the apex of the angle, formed by the union of the gill membranes, to the mandible tip.

The distance from the union of the gill membranes to the pelvics was measured from the apex of the angle, formed by the union of the gill membranes, to the insertion of the pelvic spine.

The distance from the mandible to the pelvics was made from the tip of the mandible to the insertion of the pelvic spine.

All scale counts and all measurements were made on the left side of the fish. Measurements were recorded to the nearest tenth of a millimeter and computed by machine into thousandths of standard, head, soft dorsal or caudal peduncle lengths.

Hybrid Index

The hybrid index method of describing fish hybrids is designed to express the relationship of the traits of the hybrid to those of the presumed parental species. In the comparison of the supposed hybrid with the parental species, each individual trait is expressed as a figure on a scale of 0 to 100, in which the average value for one parental type is set at 0 and that of the other parental type at 100. By using the formula:

$$P \text{ (position of hybrid)} = \frac{V_H - M_1}{M_2 - M_1}$$

where V_H represents the value of a character of the hybrid and M_1 the value for one parent and M_2 the value for the other parent, it is possible to determine the relationship of the hybrid to the parents. The hybrid index value is obtained by multiplying P times 100. A hybrid index value of 50 would indicate exact intermediacy between the two parental types while a value of 25 would indicate a tendency toward the parental type assigned the value of 0 and a value of 75 would indicate a tendency toward the parental type assigned the value of 100.

An average of all individual hybrid indexes should give a value near 50 or approaching 50. If the assumed hybrids were merely selected specimens of the two species, the individual indexes when combined would show a bimodal frequency distribution with modes close to 0 and 100 on the above-mentioned scale. A unimodal curve with a peak near 50 would confirm the interpretation of the specimens as hybrids, and would indicate a multiple type of inheritance for systematic characters or merely lack of dominance.

Preparation of Illustrations

Photomicrographs and drawings were made of the eggs at various stages of development (Figures 1 to 16). The photomicrographs were taken, with a Leica camera (Model IIIf, 35 mm.), through a compound microscope using a 10X ocular and three different types of objectives (40 mm., 48 mm., and 120 mm.): the condenser was lowered and its top lens removed; the flat side of the mirror was used to reflect the light produced by a Bausch and Lomb arc lamp. The film used (Panatomic X) was made by Eastman Kodak Company. Various exposure times were used, best results being at 1/30 second. The prints were processed by a commercial studio.

Larvae were fixed in formalin at regular intervals and drawings (Plates I, VI, VII) of representative stages were made by the use of a camera lucida mounted on a compound microscope.

OBSERVATIONS AND EXPERIMENTS

General Behavior of Laboratory-kept Darters

Since Etheostoma lacks an air bladder and usually inhabits swift waters, it spends most of the time, in nature, lying on the stream bottom under the protection of rocks and aquatic vegetation. In the laboratory, the species studied are very inactive in that they remain on the bottom of the aquaria most of the time, resting on the pelvic and caudal or anal fins. Movement is in the form of short, sudden dashes or darts, a few inches at a time, accomplished by rapid vibration of the caudal fin.

These fish adapt themselves to life in aquaria quite readily, most individuals taking food a day or two after being brought to the laboratory. After the first week spectabile became quite tame and would sit in the open when the aquaria were approached. The radiosum continued to dart for cover under or behind the rocks and some, especially the large males, never did become accustomed to human intrusion. The half-starved and stunted spectabile, which were collected near Foraker, after the first few days actually sat around apparently begging for food. When a pipetteful of white worms was placed in the tank, the fish swam to meet the stream of worms and began eating them almost as soon as they left the pipette. Most frequently the worms were eaten from the bottom of the tank. The fish sat with the head bent to one side as if watching the worms and then with a sudden dart, snapped the worms from the bottom by raising the body and tail slightly so that the long axis of the body formed about a 30° angle with the tank bottom. Mosquito larvae, apparently preferred to other foods, were usually eaten from the bottom of the tank, but frequently the fish darted to the surface to feed on them. The fish captured *Daphnia* by darting after them as they swam through the water. These fishes usually refused to feed on non-motile foods or prepared dry foods. Similar behavior was reported by Fahy (1945) in *E. blennioides* Rafinesque and Petravicz (1936) in *Microperca punctulata* Putnam (= *E. microperca* Jordan and Gilbert).

When *spectabile* was first placed in the breeding tanks with *radiosum* there was a considerable amount of fighting, with both male and female *radiosum* resenting the intrusion of *spectabile*. This subsided in most tanks after a few days, stopping first in the tanks with female *radiosum* and later in the ones with male *radiosum*. In one tank which contained a very large male *radiosum* and a relatively small female *spectabile* the chasing did not cease until the female died. The acceptance of *spectabile* in the tanks by *radiosum* varied considerably with different individuals depending on the size of the respective fishes. It was found that by placing similar-sized specimens of the two species in the tank there was much less antagonism.

Reproduction

Time of spawning.--Sexual maturity of fishes in the laboratory was reached in approximately one year. Two male specimens (one *radiosum* and one *spectabile*) and one female (*radiosum*), collected from nature as ten-millimeter fry in July 1954, were observed to spawn in the spring of 1955. Many female *spectabile*, collected from Dog Creek in March 1955, of a size comparable to the above specimens, spawned in the laboratory shortly after being collected. It has been reported that *E. f. flabellare* Rafinesque (Lake, 1936), *E. blennioides* (Fahy, 1954) and *E. nigrum* (Raney and Lachner, 1943) also spawn when one year old. Lachner, Westlake and Handwerk (1950) also found that some *E. zonale* (Cope) spawn as yearlings, but they reported that *E. variatum* Kirtland spawns at two years, an observation in agreement with that of Raney and Lachner (1939) in *E. maculatum* Kirtland.

These fishes apparently begin to spawn early in the spring. Females collected from Dog Creek in March 1955 were heavy with eggs and spawned two days after being put in aquaria. In both the 1953-54 and 1954-55 experiments, the fishes collected in the fall began spawning in December and continued spawning until April, indicating that in the laboratory, spawning extends over a considerable period. In an effort to initiate early spawning the fish, used in 1953-54, were subjected to increased length of day by artificial lighting. This apparently had no effect since the fish used in 1954-55, not subjected to increased length of day, spawned just as early as those used in 1953-54. In another attempt to initiate spawning, several fishes collected in August 1954 were refrigerated at 5° C. for six weeks and then some were subjected to a gradual rise in temperature and others to a sudden rise in temperature, but this also failed to initiate spawning. Harrington (1950) induced early spawning of <u>Notropis bifrenatus</u> (Cope) by means of controlled light and temperature.

There seemed to be no correlation between water temperature and spawning activity. Both species were observed to spawn at temperatures varying from 17° to 26° C. The fish generally spawned following a sudden change in the temperature, but they also spawned when the temperature remained relatively constant. Fahy (1954) indicated that a temperature of 51° F. (10.5° C.) had to be maintained for a few days to initiate spawning in the greenside darter.

<u>Spawning site</u>.--The fishes occasionally used the slate bottom of the aquaria, but most frequently the eggs were buried in the gravel and sand which covered about one-half of the aquaria bottoms. The female formed a small depression by pushing her head through the gravel and sand with rapid movements of the caudal fin while holding her body at an angle of about 45°. So rapid and intense were the vibrations of the tail that, when it occurred in shallow water, the walls of the tank were splattered with water. She then sat in the depression where the spawning act took place. There was also a tendency for the fish to settle into the gravel and sand while spawning and the eggs were thereby further buried. There appeared to be no tendency to concentrate the eggs in any one part of the tank or to form a redd. Darters are known

to deposit their eggs in a great variety of places. *E. f. flabellare* (Lake, 1936) and *E. nigrum olmstedi* (Storer) Atz, 1940 and Seal, 1892) deposit their eggs on the underside of stones. *Hadropterus maculatus* (Girard) (Petravicz, 1938) and *H. copelandi* (Jordan) (Winn, 1953) lay their eggs in the gravel and sand. *E. microperca* (Petravicz, 1936) deposits its eggs on submerged vegetation, objects in the water or even on the sides of aquaria.

Spawning act.--The first unusual activity noted among the fishes was the establishment of a territory by one of the larger female *radiosum*. This fish had taken over an area under a large rock and refused to permit any of the other fish near her. When the female was placed in a breeding tank with a large male *radiosum*, he chased and nipped her, but spawning was never observed and no eggs were collected.

In late November 1953 it was noted that the fish began taking on a different appearance. Some of the females became plump, as if eggs were being developed in the ovary, and the males of both species took on a much brighter coloration. In male *spectabile* the caudal peduncle became barred with green and black and the anal fin changed from a dull green to a dark, emerald green. In male *radiosum* coloration of the body became darker and the dorsal and anal fins became more intensely colored.

Spawning was observed in the laboratory, between male *spectabile* and female *radiosum*, on December 21, 1953, 56 days after the fish were placed in the breeding tanks. Spawning was observed between the two reciprocal crosses on numerous other occasions and detailed notes of these observations were made. These notes indicate that the relationship between the two sexes follow the same general pattern prior to and during the spawning act. The notes taken on December 21, 1953 are given below, with minor grammatical corrections, to demonstrate the activities usually observed when spawning occurred.

> December 21, 7:30 AM - Unusual activity in Tank V (male *spectabile* and female *radiosum*). Male follows female around quite closely and when she stops he places his head over her back; then he pokes at her with his nose and swims away to the back of the tank. He has repeated this act several times during the ten minutes I have been watching them.
>
> 7:40 AM - Male has ceased poking or nipping at female, but still pays strict attention to her and follows her everywhere she goes.
>
> 7:45 AM - Male again places his head on the female, darts to back of tank and then return, nips at her and chases her to the back of tank; then he sits next to her in that area.
>
> 7:55 AM - Both fishes are under a rock, male has dorsal fins erect and occasionally vibrates the pectorals. Now the male tries to place his head over female but the overhanging rock prohibits this.
>
> 8:00 AM - Male again attempts to place head over female but rock is again in the way. Now the female leaves rock and is in front of tank. Male chases and nips at her until she returns under rock. Female leaves rock and male begins to chase and nip at her again. Both are now sitting behind rock.

Male erects and lowers dorsals and vibrates pectorals. Female swims to front of tank and male begins to annoy her until she returns to the rock. Male continues to chase female whenever she leaves area behind or under the rock.

8:30 AM - Male appears to have lost interest in female. Occasionally he swims to front of tank where female is sitting and nips at her, but then he swims away.

9:00 AM - Now the female is sitting by the rock. She seems to be wiggling herself into the sand. She is vibrating her tail and now the male takes position beside her. The male has mounted her and both fish are rapidly vibrating their bodies. The male has the female clasped with his pelvic fins and has his tail closely pressed to hers. The female seems to be bending her head and caudal fin upward while the male has his caudal fin bent downward. The spawning act lasted only for about two seconds. Both fish, for a brief period, remain motionless except for rapidly moving gill covers. Now female moves away from the rock to an open area and again settles into the sand. The male comes over to mount her again and the spawning act is repeated. Their bodies vibrate so rapidly that gravel is sprayed against the tank wall. The female swims away with the male in pursuit. The spawning act is repeated in still another area. No eggs can be seen where spawning occurred.

9:15 AM - Male appears to have lost interest in female, he occasionally darts at her and swims away. If she is sitting quietly in the gravel he will attempt to mount her, but then she swims away. It appears that the actual spawning act is initiated by the female.

10:15 AM - Male has dorsals erected, so that the bright colors are very noticeable, and is swimming around female.

11:00 AM - No additional spawning observed. Male continues to irritate female by dashing at her, but not as frequently as before.

11:45 AM - Male no longer attacks female. No more spawning acts observed.

The preceding activities occurred when the water temperature was about 19° C. but, on the night of December 22, 1954, a cold wave passed through Stillwater and the water temperature dropped to 11° C. The temperature remained low until December 24 when it returned to 20° C. No more spawning activity was noted until January 2, 1955, but on December 28 an egg in the early stages of development was found in the tank.

Although eggs were obtained from female _spectabile_ mated with male _radiosum_ on January 4, 1954, spawning was not observed between them until January 11. The act was similar to that of the reciprocal cross although there did not seem to be as much forcefulness and display on the part of the male.

Viable eggs were obtained from normal matings in both species, but the actual spawning act has escaped notice. The first eggs from normal spectabile matings were obtained on January 29 and the first eggs from normal radiosum matings on February 22, 1954.

Relation of sexes during spawning.--Most breeding tanks were supplied with two or three specimens. In tanks containing two females, the female not participating in spawning did not seem to be interested in the spawners or in the eggs produced, but in tanks which had an extra male there was continuous fighting between them. One male, not always the larger but generally the most brightly colored, seemed to dominate the other. He occasionally darted at the other fish and chased him to the other end of the tank.

On one occasion two males were observed to spawn simultaneously with a single female. The dominating male had mounted the female and during the spawning act the unaccepted male swam along side the female and began vibrating his body. At the end of the spawning act all three fish remained motionless for a short period and then the dominating male chased the other male away and began attending the female as before.

Breeding Experiments

Experiments of 1953-54.--The fishes collected from Dog Creek and Blue River in October 1953 began spawning in December 1953. A total of 339 eggs were produced by female radiosum mated with male spectabile. Seventy of these hatched with 51 developing to the postlarval stage. Female *spectabile* produced 528 eggs, when mated with male *radiosum*, but only twelve of these developed to hatching and six lived to the postlarval stage. None of the 349 eggs produced by normal *radiosum* matings and only 20 of 380 eggs produced by normal *spectabile* matings hatched.

Since these fishes typically live in the colder streams of the state, it was deemed necessary to maintain low summer temperatures. Early in April all but nine of the above specimens were placed in a refrigerator at 26° C. Nocturnal electrical failure in late April resulted in the loss of all of the above specimens, except the nine laboratory-reared hybrids, *E.* (*spectabile* × *radiosum*), kept at room temperature.

The specimens of *radiosum* collected from Jack Fork Creek and *spectabile* collected from Spring Creek in May 1954 failed to produce any hatchlings. The female *radiosum*, mated with a male *spectabile*, laid only 15 eggs and the reciprocal combination produced 72 eggs, but the normal matings failed to yield any eggs. All the above eggs died in the early stages of development.

The specimens of *radiosum* and *spectabile* collected from Blue River in August 1954 did not spawn.

Experiments of 1954-55.--In November of 1954 specimens of *radiosum* were again obtained from Blue River, and specimens of *spectabile* were taken from Mill Creek. The female *spectabile* mated with male *radiosum*, produced 652 eggs of which 165 hatched; 60 were reared to the postlarval stage. The female *radiosum* mated with male *spectabile* laid only 184 eggs, 15 of which hatched. Two of these (both abnormal) were reared to the postlarval stage of development. The normal *radiosum* matings failed to produce any eggs while the normal *spectabile* matings produced 67 eggs, five of which hatched.

Since the reciprocal matings between the Mill Creek *spectabile* and *radiosum* gave different results than the crosses between *radiosum* and Dog Creek *spectabile*, it was thought that it would be desirable to test the success of crosses between the two different populations of *spectabile*. Therefore, in March 1955 some *spectabile* were collected from Dog Creek. These specimens were mated with Mill Creek *spectabile* as well as with *radiosum*.

The matings with *radiosum* gave results similar to those obtained in 1953-54. Female *radiosum* mated with male Dog Creek *spectabile* produced 188 eggs with 44 hatching and 30 reaching the postlarval stage of development. The female *spectabile* mated with male *radiosum* laid only 97 eggs, nine of which hatched and seven reached the postlarval stage. Female *spectabile* from Mill Creek mated with males of the same species from Dog Creek produced 227 eggs, of which 64 hatched. Females from Dog Creek mated with Mill Creek males produced 359 eggs, 79 of which hatched.

Spawning Success and Hatchability of Eggs

A comparison of the spawning success of the various matings attempted between *radiosum* and *spectabile*, and also the hatchability of the eggs produced, was made and is shown in Table 1. This table shows that, in general, spawning was quite successful in all combinations listed, but that there were considerable differences in the number of hatchlings. The normal matings and some species crosses had a very low rate of hatchability, whereas other species crosses and the crosses between the two populations of spectabile had a relatively high rate. Male radiosum mated with spectabile from Dog Creek produced few hatchlings (3.3%). In contrast, the reciprocal cross had relatively high hatching success (21.6%). These results are directly opposite to those obtained from Mill Creek spectabile mated with radiosum, in that Mill Creek spectabile females were relatively successful (25.3%) with male radiosum. The reciprocal cross was the unsuccessful one (8.2%). Table 1 shows that the reciprocal crosses between the two populations of spectabile were equally successful.

The number of eggs spawned in a twenty-four hour period varied considerably. As many as 190 eggs were obtained from one female spectabile in one day. This same female produced 349 eggs in seven days. On one occasion a single female radiosum was observed to spawn eleven times in 75 minutes; a total of 71 eggs were obtained from these spawnings. Atz (1940) observed E. nigrum olmstedi to spawn 50 times in two hours. The highest number of eggs obtained from one female radiosum in one day was 76. The largest total number of eggs obtained from a female radiosum was 272 over a period of 82 days. However, it is highly doubtful that all eggs from these spawnings were collected.

TABLE I

Spawning success and hatchability of the eggs of the different combinations of Blue River *E. radiosum* and *E. spectabile* (Number of eggs spawned followed by number hatched with percent hatched in parentheses).

	radiosum (male)	spectabile (Dog Cr. male)	spectabile (Mill Cr. male)
radiosum (female)	349;0 (00.0)	527;114 (21.6)	184;15 (8.2)
spectabile (Dog Cr. female)	625;21 (3.3)	380;20 (5.3)	359;79 (22.0)
spectabile (Mill Cr. female)	652;165 (25.3)	227;64 (28.2)	67;5 (7.4)

The number of eggs listed in Table 1 is not the total number produced by the fish since they were observed eating their eggs and also since all eggs produced could not be collected from the tanks.

Embryology

The embryology of the normally-produced spectabile and the hybrids showed no apparent differences. The eggs appeared to follow the same pattern of development and the time of hatching did not seem to vary in the different combinations. The time required for hatching varied with temperature in that eggs kept in an icebox set at 18° C. hatched in eight days while eggs left at room temperature varying between 21° C. and 25° C. hatched in six or seven days.

The following discussion applied specifically to the eggs of radiosum fertilized by spectabile sperm. None of the eggs of normal radiosum matings underwent complete development and since so few of the eggs of pure-bred spectabile and of spectabile females fertilized by radiosum males developed during the 1953-54 experiments, it was not deemed desirable to sacrifice any of these eggs for detailed study, although observations were made in life.

Description of eggs.--The eggs of these two species appear to be very similar and are on the average about the same size. The fertilized eggs are spherical, transparent, demersal and adhesive. A prominent median oil globule, often surrounded by many smaller ones, is present. Eggs are laid singly or in clusters of up to eight or ten. At the place of adherence, the egg capsule is flattened and, since these darters typically bury their eggs in the sand and gravel, it is possible to pick up one piece of gravel with forceps and have several other pieces attached to the eggs. Immediately after the eggs are laid they adhere to the forceps or pipette, but after several hours the adhesiveness decreases, although they still cling to objects to which they were originally attached.

The following measurements (average, with extremes in parentheses) of 50 eggs of each species were obtained by means of a filar micrometer and a compound microscope: *radiosum*, 1.36 (1.02-1.50) mm.; *spectabile*, 1.35 (1.20-1.47) mm. Reeves (1892) found that the eggs of *spectabile* from Michigan measured 1.5 mm. in diameter. The average diameters of the eggs of several other darter species are as follows: *E. n. olmstedi* 1.5 mm. (Atz, 1940), *E. flabellare* 2.3 mm. (Lake, 1936), *H. maculatus* 2.0 mm. (Petravicz, 1938), *E. microperca* 0.7 mm. (Petravicz, 1936), and *H. copelandi* 1.4 mm. (Winn, 1953).

The live eggs are easily distinguished from the dead ones since the latter take on an opaque appearance which begins as a small white spot on the surface of the yolk and spreads over the entire egg.

Cleavage stages.--In the following discussion the developmental stages are given as observed in the laboratory with water temperatures varying from 21° C. to 25° C. Drawings made through a compound microscope with the aid of a camera lucida are shown in Plate I (Appendix). The figures in Plates II-V (Appendix) are photomicrographs showing various stages of development. Figure 6 shows a newly spawned egg with the large median oil droplet and several smaller, associated ones. The adhesiveness of the eggs is well exemplified by the adherent stones. The blastodisc can not be seen in this figure. Figure 1 is a drawing of a two-cell stage, about 30 minutes after fertilization. The four-cell stage was reached in an additional ten minutes. These observations are in general agreement with those of Fahy (1954) who observed the two-cell stage of E. blennioides at 65 minutes after fertilization and the four-cell stage in an additional 30 minutes at temperatures of 55°-58° F. (13°-14.5° C.). In Figure 7, an egg five hours after fertilization, the blastoderm, with cell structure, can be seen at the upper left of the egg. At 14 hours (Fig. 2 and 8) the blastodisc is in an elevated position surmounting the yolk mass. Figure 3 represents the 15-hour embryo with the continued expansion of the blastodisc over the yolk mass. The 17-hour stage shows a continued growth of the blastodisc by epiboly down around the yolk mass (Figs. 4 and 9), presenting an "hour-glass" appearance. Figure 10 shows that epiboly is continuing and at 20 hours (Fig. 11) the blastodisc has almost completely surrounded the yolk mass to form the yolk-plug stage.

Embryo formation.--At twenty-five hours the embryonic shield can be seen (Fig. 12), a stage reached by E. blennioides in 39 hours at 55°-58° F. according to Fahy (1954). Figure 5 represents the newly-formed embryo (about 47 hours) commonly referred to as the

"C" stage. The tail is attached to the yolk mass, the eyes are poorly developed and the yolk sac with its large oil droplet is quite prominent. The embryo, at 74 hours (Fig. 13), is completely formed with recognizable head, trunk and tail regions, the latter free from the yolk sac. The optic cups are clearly visible and, although the pigmentation is not yet complete, melanophores are seen on the body and yolk sac. The heart beat and other embryonic movements were observed at this stage. This again is in relative agreement with Fahy (1954) who observed the heart beat of blennioides at 90 hours. Figure 14, a dorsal view of the head of the embryo at 120 hours, shows that the yolk mass and oil droplets are quite large and prominent, the optic cups are very conspicuous and intensely black. Figure 15 shows an embryo, with the yolk mass much reduced, about 24 hours prior to hatching.

Hatching.--For about a day before hatching there was very vigorous and almost continuous movement by the embryo. During this period there seemed to be a change in the consistancy of the shell since it appeared to flake off. If the egg was attached to a stone, it usually dropped free. These fishes, as in blennioides (Fahy, 1954), emerged tail first. For several hours during hatching they were observed moving vigorously to free the head and pectoral fins, still encased in the shell. After freeing themselves from the egg capsule, the larvae (Fig. 16) remained motionless on the bottom of the finger bowl. When disturbed they swam vigorously around the edge of the container.

Abnormalities.--Many abnormalities were observed in both the normally-produced embryos and the hybrids. Frequently the optic cup failed to develop and large patches of pigment appeared on the body. Occasionally only one optic cup formed as a black blotch of pigment at various positions on the head. Often the tail and sometimes both the body and the tail failed to develop.

Development and Nurture of Young Fish

The nomenclature used to identify the various larval stages in the following discussion is that recommended by Hubbs (1943). Under this terminology the term "embryo" applies to the fish during the developmental stage to the moment of hatching. From hatching until they reach a stage in which they resemble the adults, they are called "larvae". The larval stage is subdivided into a "prolarval" stage and a "postlarval" stage. The former applies as long as the larva is still bearing yolk and the latter applies from the time the yolk is absorbed until the larva resembles the adult. The term "juvenile" applies from the end of the postlarval stage until adulthood is reached.

Specimens of the hybrid, E. (spectabile × radiosum), were fixed in formalin at regular intervals from the time of hatching until the juvenile stage was reached. Specimens of the reciprocal cross and the intraspecifically-bred fishes were either lacking or not available in sufficient numbers to permit fixing them at regular intervals, but observations made on the living specimens indicated that the stages of development were, in general, the same as that found in *E.* (*spectabile* x *radiosum*).

The following description applies specifically to the young produced by *radiosum* eggs fertilized by *spectabile* sperm. All measurements are given as total lengths.

Prolarva.--The newly-hatched prolarva measures 3.7 mm. (Fig. 17) and is similar, in size, to those of some other species of this subfamily: *E. iowae* Jordan and Meek = *E. exile* (Girard) 3.4 mm., Jaffa (1917); *H. maculatus* 5.75 mm., Petravicz (1938); *E. microperca* 3.0 mm., Petravicz (1936) and *E. maculatum* 5.0-6.0 mm., Raney and Lachner (1939). The yolk sac with its anteriorly-placed oil droplet is quite large in the newly-hatched prolarva. The short intestine terminates just behind the yolk sac at the margin of the embryonic limb fold, but does not yet open from the body. The embryonic marginal limb fold originates behind the head and continues posteriad around the body to the yolk sac on the ventral side, with a small indentation at the anus. The limb fold is low and even and the tail is lophocercal. Several melanophores are seen on the head and nape and usually on the yolk sac. The specimen in Figure 17 does not show as many melanophores as were observed in some newly-hatched specimens. The pectoral fins are prominent, but the other fins are mere folds. The newly-hatched fish has 11 myomeres before the anus and 21 countable ones behind.

In the prolarva measuring 4.5 mm. the yolk sac and oil droplet are much reduced (Fig. 18). The intestine is still quite short, but apparently a vent has been formed since there is a deep indentation in the embryonic limb fold. The embryonic limb fold is still complete, but not as even as in Figure 17. The tail is lophocercal. Melanophores, more widely distributed and larger than in the previous stage, are observable on the opercles, cheeks, belly and back. The pectoral fins are very prominent, with the rays apparently being formed, and extending past the middle of the yolk sac. The mouth is quite large and inferior and extends to below the eye which is centrally placed on the head.

Postlarva.--Under the terminology of Hubbs (1943) the 6.2 mm. fish is a postlarva because the yolk sac and oil droplet apparently are absorbed (Fig. 19). The intestine is no longer visible through the body wall and the embryonic limb fold, much reduced, is still present. Many melanophores are present on the head and belly. The pectoral fins are very prominent with observable rays and the pelvic, dorsal and anal fin anlagen are visible. The caudal fin, with visible rays, makes its appearance on the ventral side of the now heterocercal tail. There are 12 myomeres before the vent and 23 behind, in this specimen. The large mouth, terminating below the eye, exhibits prominent teeth on the lower jaw which is included in the upper. The dark eye, high on the head has a light colored lens.

The embryonic limb fold is much reduced in the 7.4 mm. stage, remaining only between the dorsals, the soft dorsal and caudal and the caudal and anal fins (Fig. 20). The tail has become homocercal with the caudal fin almost complete. The dorsals, anal, pectoral and caudal fins possess definite rays, but the pelvics are merely small projections ventrad to the pectorals. There are many small melanophores along the dorsum and venter, with large ones on the head. The mouth is large, terminal and oblique. The head is large with prominent, dark, dorsally-placed eyes. Myomeres number 13 to the vent and 19 behind. There are six spines and ten soft rays in the incompletely-formed dorsal fin and two spines and eight soft rays in the anal fin.

At the 10.0 mm. stage (Fig. 21) the embryonic limb fold is absent and all fins are formed with countable rays: dorsal IX-11; anal II,8; pelvics I,5. The tail remains homocercal. Pigmentation is well developed on the body, with a tendency to form bars posteriad. A preocular stripe extends over the snout and melanophores are present on the dorsal, caudal and anal fins. The mouth is large and extends to below the large, dorsally-placed eyes.

Juvenile.--This half grown, 18.3 mm., specimen apparently has completed its development (Fig. 22). The eye is very prominent, dorsally placed and slightly anteriad on the head. There are ten large, prominent blotches along the sides which tend to form bars on the caudal peduncle. Pigment is scattered over the dorsal, anal, pectoral and caudal fins. The fin ray counts follow: dorsals X-11; anal II,9; pelvics I,5; pectorals 11; caudal 23. The mouth is terminal and oblique.

Abnormalities.--The most frequent abnormality observed in the larvae was the presence of an excessively large pericardial sac.

This abnormality was observed in the hybrids as well as in normal *spectabile*. All specimens, possessing this abnormality, died soon after hatching. Another abnormality observed in the larvae was the presence of a mouth which was permanently open. These specimens lived for several days. The soft dorsal, caudal and anal fins were frequently continuous. This abnormality was seen in all specimens of (*radiosum* X *spectabile*) (Fig. 23) and in occasional specimens of (*spectabile* X *radiosum*) but the fins were usually normal in the latter cross (Fig. 22) and in normal *spectabile*. Another abnormality encountered in both the hybrids and normal *spectabile* was the absence of most of the lateral-line system. There were no pored lateral-line scales in any laboratory-reared young and most of the lateral-line system of the head was lacking. The pelvic fins were often abnormal in that the number of fin rays was reduced and the fins reduced in size. One specimen of (*radiosum* X *spectabile*), raised to the post-larval stage, had only one eye which was in a normal position, but the site where the other would normally be, merely had an indentation with no observable eye structure.

Feeding of young.--The prolarvae began eating paramecia and microworms the day after hatching and accepted small white worms at about the fifteenth day. Fahy (1954) stated that *blennioides* did not feed during the prolarval stage or for the first twenty-four hours after the yolk had been absorbed.

Study of Laboratory-reared Young

The present discussion is limited to a study of nine specimens of E. (spectabile × radiosum) hatched in the 1953-54 experiments and reared to maturity. Brief mention will be made of the small normal spectabile, produced in the 1953-54 experiments, which died in the spring of 1954 and were preserved before putrefaction had set in. Since specimens of laboratory-reared parental species were unavailable, the laboratory-reared hybrids were compared with field-collected specimens of the parental species, some of which were the experimental breeders.

Comparison of laboratory hybrids with natural female hybrid.--As indicated in the introduction, the primary purpose of this study was to verify, by laboratory methods, the identification of a percid hybrid combination, E. (radiosum × spectabile), collected in nature. Therefore, a comparison of the laboratory-reared hybrids (9 females, 30.5-38.5 mm. standard length) with the female hybrid (54.5 mm. standard length) collected in nature, is pertinent. As is shown in Table 2, the proportional measurements of the natural hybrid usually fall within the range of those of the laboratory-reared hybrids. In three measurements (orbit length, pectoral fin length and distance from mandible to pelvic fin) there are small, but insignificant, differences. Large differences, in the two measurements associated with the union of the gill membranes, probably can be accounted for by the abnormalities observed in the branchiostegal rays of some of

TABLE II

Specimens of *E.* (*spectabile* × *radiosum*), reared in laboratory,
compared with a natural hybrid presumed to have been
produced by the same parental species
(averages with ranges in
parentheses).

Character	natural hybrid (1 female)	laboratory hybrid (9 females)
Counts		
Fin rays		
Dorsal spines	10	10.2(10-11)
Dorsal soft rays	14	15.1(14-16)
Anal soft rays	8	10.7(7-14)
Scales		
Lateral-line	52	47.3(44-50)
Above lateral-line	6	4.8(4-5)
Below lateral-line	8	6.3(6-7)
Around caudal peduncle	23	18.0(16-20)
Proportions		
Standard length in mm.	54.5	35.3(30.5-38.5)
Thousandths of standard length		
Snout length	70	65(60-69)
Upper jaw length	84	85(81-92)
Orbit length	64	73(68-79)
Head length	281	289(267-310)
Caudal peduncle length	224	187(146-229)
Distance from occiput to dorsal fin origin	154	140(131-156)
Soft dorsal fin length	303	302(284-324)
Anal fin length	227	219(208-239)
Pectoral fin length	224	259(238-279)
Pelvic fin length	206	201(181-216)
Head depth	176	179(169-190)
Caudal peduncle depth	105	106(102-111)
Distance from UGM* to mandible	119	159(148-181)
Distance from UGM* to pelvic fin base	176	145(133-151)
Distance from mandible to pelvic fin base	295	304(297-317)

*UGM-Union of gill membranes

the hybrids. All scale counts of the natural hybrid as well as the average scale counts of the parental species are greater than those of the laboratory-reared hybrids. The scales of the laboratory-reared fish are much larger and less regularly arranged than in the parental species. The number of segmented rays in the dorsal and anal fins of the natural hybrid falls within the range of the laboratory-reared specimens, but the average number found in the latter is higher than the averages of the parental species.

Probably the most prominent difference between the laboratory-reared hybrids and the ones collected in nature is the absence of much of the lateral-line system in the former. All the laboratory-reared fish (hybrids and nonhybrids) have the lateralis canal entirely lacking and the supratemporal, infraorbital and preoperculo-mandibular canals much reduced or absent. The latter, when present, is found only in the region ventral to the eye.

The squamation of the head in laboratory-reared hybrids is in agreement with that of the natural hybrid in that the cheeks, opercles and breast are naked and the nape is scaly.

<u>Color description of laboratory-reared hybrids</u>.--The laboratory-reared hybrids show that some color characters associated with sex did not behave as they do in normal matings. The following color description is a composite taken from the nine, fourteen-month-old, adult, female hybrids after fixation in weak formalin.

The iris is dusky with gold near the pupil. The snout is a pale brownish with a few black blotches. The interorbital space and the parietal region of the head are dark with black mottlings. The preocular and subocular bars form a crescent bending postero-ventrad to and beyond the angle of the mouth. The postocular bar extends from the postero-ventral quadrant of the eye upward to the level of the pupil center and then backward on the upper part of the operculum. The cheeks are finely dotted with brownish-black pigment. The opercles are similar to the cheeks, but have an indistinct blotch of black. The branchiostegal rays and membranes are tinted with a brownish-orange.

The body is dorsally dark, from pectorals upward, and ventrally white. Most scales on sides and back bear a concentration of melanophores producing several rows of spots. The bluish-black postcleithrum is not as large and deeply pigmented as in radiosum. There are blotches of black pigment at the pectoral base.

The spinous dorsal has a very narrow border devoid of pigment and followed proximally by a greenish-black band about one-third the depth of the fin. Below the latter band is a narrow brick-red band running parallel to the tips of the spines and separated from the greenish-black band by a thin band devoid of pigment. Basally the spinous dorsal has fine black dots tending to be concentrated on the interradial membranes.

The soft dorsal fin has a distal blue-black band extending basad on each interradial membrane. There is a brick-red band in the middle of the fin, confined to the membranes and extending parallel to the fin margin.

The caudal fin has immaculate interradial membranes, but its rays, with alternating black and light bars, form a checkerboard pattern. There are two pale brownish areas, one above the other, at the caudal base.

The anal fin has a suggestion of a greenish tinge on the spines and the first interradial membrane. The posterior five interradial membranes have a concentration of melanophores.

The pectoral fin rays are tinged with brown and outlined with tiny black pigment cells.

The pelvic fins are usually immaculate but sometimes tinged with brown.

The breast and belly are white with scattered black dots.

The sides of the caudal peduncle are marked with six black bars overlaid with a greenish tinge. Inconspicuous bars with brownish interspaces appear below the bases of the dorsal fins.

The above color description is unusual in that these female hybrids have expressed certain color characters which, in normal matings, appear only in males. The females of both radiosum and spectabile are very drab and devoid of bright colors except for a thin pencil-line of red in the dorsal fins.

Comparisons of laboratory-reared hybrids with the parental species.--The second purpose of this experimental hybridization study was to test, in the laboratory, the present method of describing natural fish hybrids. The laboratory-reared hybrids (of known parentage) were compared with the two possible reciprocal parental combinations, with only females of the two parental species and also with males and females of the two parental species (Tables 3-6).

Some characters studied exhibited such small differences, between the parental species, that they were considered to be of little value in this study; and therefore, only those counts and proportions, which show more than four percent difference between the parental species, were used. Other characters exhibited values, in the hybrids, beyond the range of either parent. Hubbs and Kuronuma (1942) ascribed certain extreme characters, in hybrid flounders, to heterosis. They also indicated that, if there is a genetic tendency in both parental species to reduce or to increase a certain character, hybridization will cause an additive effect and produce an extremely reduced or increased character in the hybrid.

Several characters exhibited by the hybrids were identical with those of one or the other parental species, indicating a possible dominant influence. The aggregate of hybrid index values in each instance gave an average value near exact intermediacy, but the individual character values tended to range from 0 to 100.

Although the range of some of the characters of the parental species overlap, it was believed that if a random sample of the parental species was taken the average value would be of more significance than the range. This overlapping of characters was found to be common among some species of this large genus (Hubbs, 1943; Moore and Rigney, 1953).

Publications of recent work on fish hybridization show an emphasis on the more or less intermediacy of hybrids (Hubbs and Kuronuma, 1942; Hubbs, Hubbs, and Johnson, 1943; Hubbs, Walker, and Johnson, 1943; Hubbs and Hubbs, 1947; Trautman, 1948; Hubbs and Miller, 1953). These workers also indicated that all hybrid index values should give an average of nearly exact intermediacy.

The hybrid index method, discussed in the introduction of this paper, was used in comparing the laboratory-reared hybrids with the parental species. The value of 0 (M_1) was assigned to spectabile and 100 (M_2) to radiosum.

The hybrid combination, herein treated, tends to be intermediate in that, when compared with the parental species which produced them (male spectabile and female radiosum), the fifteen characters shown in Table 3 gave an average hybrid index value of 49.5. Six of these characters show a tendency toward one parent and nine show a tendency toward the other. The hybrids show their most direct intermediacy in the following measurements: distance from the union of the gill membranes to the mandible, expressed in thousandths of standard length; distance from occiput to the origin of the dorsal fin, distance from the union of gill membranes to mandible and caudal peduncle depth, expressed in thousandths of head length; and in the distance from the union of the gill membranes to mandible and pectoral fin length, expressed in thousandths of the soft dorsal fin length. These six characters yielded values between 35 and 65 and are an indication of intermediacy of the hybrids.

TABLE III

Comparison of laboratory-reared hybrids, E. (spectabile X radiosum), with male specimens of spectabile and female specimens of radiosum.

Character	spectabile	Hybrids		radiosum
	Average (Range)	Average (Range)	Hybrid Index	Average (Range)
Proportions				
Standard length in mm.	36.1(32.9-44.3)	35.3(30.5-38.5)	....	54.0(51.5-59.8)
Thousandths of standard length				
Head length	307(294-320)	289(268-310)	81.8	285(274-303)
Distance from occiput to dorsal fin origin	139(131-155)	140(131-156)	11.1	148(139-159)
Pectoral fin length	271(249-295)	259(238-279)	25.5	224(195-254)
Pelvic fin length	230(206-240)	201(181-216)	96.6	200(169-213)
Distance from UGM* to mandible	144(125-167)	159(148-181)	71.4	165(150-179)
Distance from mandible to pelvic fin	296(276-247)	304(285-317)	47.0	313(301-324)
Head length in mm.	11.1(10.0-13.8)	10.1(9.1-11.1)	....	15.4(14.1-18.0)
Thousandths of head length				
Distance from occiput to dorsal fin origin	450(433-505)	495(440-541)	65.2	519(461-582)
Caudal peduncle depth	342(327-362)	366(330-404)	39.3	403(374-434)
Distance from UGM* to mandible	468(409-529)	554(509-588)	78.1	578(541-625)
Distance from UGM* to pelvic fin	495(474-590)	505(437-545)	41.7	519(467-552)
Soft dorsal fin length in mm.	10.6(9.4-14.1)	10.6(8.8-11.8)	....	15.5(14.0-17.7)
Thousandths of soft dorsal fin length				
Orbit length	245(213-274)	245(212-273)	00.0	232(220-250)

TABLE III (continued)

Character	spectabile	Hybrids		radiosum
	Average (Range)	Average (Range)	Hybrid Index	Average (Range)
Thousandths of soft dorsal fin length (cont.)				
Distance from occiput to dorsal fin origin	472(426-505)	472(426-522)	00.0	516(454-562)
Pectoral fin length	925(863-1020)	858(737-913)	46.5	781(667-849)
Pelvic fin length	783(714-842)	670(591-750)	90.4	658(591-712)
Distance from UGM* to pelvic fin	491(422-585)	528(470-568)	44.6	574(527-614)
AVERAGE HYBRID INDEX	....	....	49.5	....

*UGM-Union of gill membranes

Index values higher than 80 were obtained for head length, expressed in thousandths of standard length; and pelvic fin length, expressed in thousandths of head and soft dorsal fin lengths. In contrast, index values below 20 were obtained for: the distance from the occiput to origin of dorsal fin, expressed in thousandths of standard length; and orbit length and distance from occiput to origin of dorsal fin, expressed in thousandths of soft dorsal fin length.

When compared with females of the parental species, these female hybrids show their most direct intermediacy in the caudal peduncle length, expressed in thousandths of standard length; anal fin length, expressed in thousandths of head length; and in pectoral length, expressed in thousandths of soft dorsal fin length. In this comparison (Table 4) there are ten characters which show intermediacy, giving an average hybrid index value of 58.3.

The laboratory-reared hybrids (9 females), when compared with both sexes (10 males and 10 females) of the parental species, have 15 intermediate characters with an average hybrid index value of 41.8 (Table 5). Seven of these characters lean toward one parent, whereas eight tend toward the other.

The results of these comparisons are in general agreement with those of other workers. Hubbs, Walker, and Johnson (1943), working with natural cyprinodont hybrids, obtained average hybrid index values varying from 42-66; Hubbs, Hubbs and

TABLE IV

Comparison of laboratory-reared hybrid, E. (spectabile X radiosum), with female specimens of the parental species.

Character	spectabile	Hybrids		radiosum
	Average (Range)	Average (Range)	Hybrid Index	Average (Range)
Proportions				
Standard length in mm.	36.9(29.0-44.5)	35.3(30.5-38.5)	....	54.0(51.5-59.8)
Thousandths of standard length				
Anal fin length	201(194-216)	219(208-239)	72.0	226(210-251)
Caudal peduncle depth	100(84-112)	106(102-111)	40.0	115(107-127)
Distance from UGM* to mandible	141(121-161)	159(148-181)	75.0	165(150-179)
Distance from mandible to pelvic fin	301(268-324)	304(285-317)	25.0	313(301-324)
Head length in mm.	10.5(8.2-12.5)	10.1(9.1-11.1)	....	15.4(14.1-18.0)
Thousandths of head length				
Anal fin length	705(676-761)	762(708-850)	65.5	792(743-834)
Caudal peduncle depth	352(293-392)	366(330-404)	27.5	403(374-434)
Distance from UGM* to mandible	495(427-568)	554(509-588)	72.3	578(541-625)
Soft dorsal fin length in mm.	9.4(7.6-11.5)	10.6(8.8-11.8)	....	15.5(14.0-17.7)
Thousandths of soft dorsal fin length				
Orbit length	277(246-304)	245(212-273)	71.1	232(220-250)
Pectoral fin length	926(833-1040)	858(737-913)	46.9	781(667-849)
Pelvic fin length	755(658-815)	670(591-750)	87.6	658(591-712)
AVERAGE HYBRID INDEX	....	....	58.3	....

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*UGM-Union of gill membranes

TABLE V

Comparison of laboratory-reared hybrids, *E.* (*spectabile* × *radiosum*), with male and female specimens of the parental species.

Character	spectabile	Hybrids		radiosum
	Average (Range)	Average (Range)	Hybrid Index	Average (Range)
Proportions				
Standard length in mm.	36.5(29.0-44.5)	35.3(30.5-38.5)	....	57.3(51.5-66.4)
Thousandths of standard length				
Soft dorsal fin length	275(249-323)	302(284-324)	75.0	311(271-354)
Pectoral fin length	264(212-295)	259(238-279)	12.8	225(197-254)
Pelvic fin length	211(172-240)	201(181-216)	62.5	195(169-213)
Caudal peduncle depth	100(84-112)	106(102-111)	22.2	121(107-135)
Distance from UGM* to mandible	143(121-167)	159(148-181)	72.2	165(148-181)
Distance from mandible to pelvic fin	299(268-347)	304(285-317)	27.8	317(301-344)
Head length in mm.				
Thousandths of head length				
Soft dorsal fin length	925(868-1022)	1050(942-1157)	83.9	1074(923-1206)
Anal fin length	758(676-862)	762(788-850)	4.2	854(743-1017)
Head depth	616(573-660)	624(544-690)	22.9	651(593-722)
Caudal peduncle depth	347(293-392)	366(330-404)	26.4	419(374-477)
Distance from UGM* to mandible	482(409-568)	554(509-588)	80.0	572(492-625)
Soft dorsal fin length in mm.	10.0(7.6-14.1)	10.6(8.8-11.8)	....	17.9(14.0-23.0)
Thousandths of soft dorsal fin length				
Snout length	250(216-266)	218(186-240)	100.0	218(190-252)

TABLE V (continued)

Character	spectabile	Hybrids		radiosum
	Average (Range)	Average (Range)	Hybrid Index	Average (Range)
Thousandths of soft dorsal fin length (continued)				
Orbit length	261(213-304)	245(212-273)	33.3	213(175-250)
Pectoral fin length	926(833-1040)	858(737-913)	34.3	727(632-849)
Pelvic fin length	769(658-842)	670(591-750)	70.4	628(522-712)
AVERAGE HYBRID INDEX	....	....	41.8	....

*UGM-Union of gill membranes

Johnson (1943) obtained an average hybrid index value of 49 for some catostomid hybrids; and Trautman (1948) obtained an average hybrid index value of 52.43 for an ameiurid hybrid.

A comparison of the laboratory-reared hybrids was also made with specimens of the reciprocal (male radiosum and female spectabile) of the cross which actually produced them (Table 6). In this comparison twenty characters were found to be intermediate, with an average hybrid index value of 49.3. Most characters, indicating intermediacy in Table 3, showed intermediacy in this and other comparisons. Linder (1955), working with a natural hybrid between these two species, also found that this combination produced a greater number of intermediate characters than did the reciprocal. These results are unusual since Hubbs (1940) indicated that reciprocal crosses in each of the families, Centrarchidae and Poeciliidae, gave identical results. If these darters behave in the same manner as centrarchids and poeciliids, the results obtained in Tables 3 and 6 should have been closely similar.

TABLE VI

Comparison of laboratory-reared hybrids, *E.* (*spectabile* X *radiosum*), with female specimens of *spectabile* and *male* specimens of *radiosum*.

Character	*spectabile*	Hybrids		*radiosum*
	Average (Range)	Average (Range)	Hybrid Index	Average (Range)
Proportions				
Standard length in mm.	36.9(29.0-44.5)	35.3(30.5-38.5)	....	60.5(52.5-66.4)
Thousandths of standard length				
Soft dorsal fin length	255(249-271)	302(284-324)	29.4	334(306-354)
Anal fin length	201(194-216)	219(208-239)	73.1	268(237-290)
Head depth	176(167-189)	179(169-196)	85.7	197(195-206)
Caudal peduncle depth	100(84-112)	106(102-111)	74.1	127(120-135)
Distance from UGM* to mandible	141(121-161)	159(148-181)	25.0	165(148-181)
Distance from mandible to pelvic fin	301(268-324)	304(285-317)	85.0	321(305-344)
Head length in mm.	10.5(8.2-12.5)	10.1(9.1-11.1)	....	17.7(14.9-19.7)
Thousandths of head length				
Soft dorsal fin length	895(868-974)	1050(942-1157)	37.0	1141(1074-1206)
Anal fin length	705(676-761)	762(708-850)	72.9	915(831-1017)
Head depth	619(587-660)	624(544-690)	90.6	672(631-722)
Caudal peduncle depth	352(293-392)	366(330-404)	83.1	435(406-477)
Distance from UGM* to mandible	495(427-568)	554(509-588)	15.7	565(492-602)
Soft dorsal fin length in mm.	9.4(7.6-11.5)	10.6(8.8-11.8)	....	20.2(17.5-23.0)
Thousandths of soft dorsal fin length				
Snout length	255(226-263)	218(186-240)	28.8	203(195-232)
Upper jaw length	340(307-370)	283(252-306)	9.5	277(269-308)

TABLE VI (continued)

Character	spectabile	Hybrids		radiosum
	Average (Range)	Average (Range)	Hybrid Index	Average (Range)
Thousandths of soft dorsal fin length (continued)				
Orbit length	277(246-304)	245(212-273)	61.9	193(175-211)
Distance from occiput to dorsal fin origin	596(518-661)	472(426-522)	22.5	436(400-469)
Head depth	691(645-761)	594(522-640)	4.9	589(557-650)
Pectoral fin length	926(833-1040)	858(737-913)	73.9	673(632-743)
Pelvic fin length	755(658-815)	670(591-750)	45.5	599(522-649)
Distance from UGM* to mandible	553(470-625)	528(470-568)	56.9	495(457-537)
Distance from UGM* to pelvic fin	628(560-674)	481(435-523)	9.8	465(432-498)
AVERAGE HYBRID INDEX	....	....	49.3	....

*UGM-Union of gill membranes

DISCUSSION

It has been shown that the two species used in this study (E. radiosum cyanorum and E. spectabile) will hybridize in the laboratory. This fact in itself is not unusual since many species which do not interbreed in nature will readily cross when placed in captivity, and forms which readily cross in the wild refuse to mate in the laboratory (Mayr, 1942; Bates, 1950). The fact that the laboratory-reared hybrids are in agreement in most characters with those of a specimen collected from nature and identified as a hybrid between these two species, indicates that these darters also cross in the wild.

The scale counts of the laboratory-reared hybrids are lower than those found in the hybrid from nature. Mottley (1931) has shown, in salmonids, that eggs developing under high temperatures produce fish with a lower scale count than those developed at lower temperatures. Since these laboratory-reared etheostomatid hybrids developed at a temperature much higher than that normally found in their natural habitat, it is probable that they were influenced by this factor in relation to scale counts. The effect of temperature may in part explain the variation in scale counts which occurs in natural populations of these species. The present study has shown that the spawning period extends from very early spring, when temperatures are relatively low, until late spring and early summer when temperatures are quite high. Those fishes which develop from eggs spawned early in the breeding season could be expected to have a higher scale count than those hatching later.

Some of the hybrids had unusually high numbers of anal soft-rays. Jordan (1922) stated that in fishes the number of vertebrae and also fin rays are fewer in warm than in cold climates. Hubbs (1921a) reported that in Notemigonus crysoluecas Mitchell the number of fin rays increases from north to south, but Hubbs (1921b) showed that the fin rays of Leptocottus armatus Girard gradually increase in number as one progresses northward through its range. The normal spectabile reared in the laboratory had the normal number of fin rays, indicating that the development of excessive fin rays in the hybrids was a result of hybridization and not a response to ecological factors. Some small, partially-decayed specimens of (radiosum X spectabile), salvaged after refrigeration failure, all had unusually high numbers of anal and soft dorsal rays (Fig. 23). Hubbs and Hubbs (1947) attributed the abnormally large fins of a sucker hybrid combination to heterosis. The same interpretation was offered by Hubbs and Kuronuma (1942), working with flounder hybrids. They indicated that if there was a genetic tendency in both parental forms to increase the number of fin rays, the hybrids could be expected to have an excessively high fin ray count attributable to an additive effect.

The laboratory-reared hybrids differed from the natural hybrid in that most of the lateral-line system was absent in the former. This was at first considered as a result of hybridization, but pure laboratory-reared spectabile also showed lateral-line deficiency. The incomplete development of this system apparently was brought about by a change of conditions under which embryonic development took place. The system was well developed in all adult specimens taken from nature for use in this study and also in three specimens (2 radiosum and 1 spectabile) collected as fry in August 1954 and reared to maturity in the laboratory. The literature has revealed no mention of such a condition arising in laboratory-reared fish. The extent of development of the lateral-line system has been considered by fish taxonomists to be a genetically fixed character and has been used, in part, to distinguish between different species of fishes. Etheostoma microperca is distinguished from E. proeliare (Hay) primarily in lacking the lateralis canal (sometimes with a single pore), whereas, the latter species has about seven pored lateral-line scales. E. nigrum and E. chlorosomum (Hay) are also recognized, in part, by the extent of development of this system. The present work indicates that the extent of development of the lateral-line system can be modified by the environment and, therefore, throws some doubt on the taxonomic value of this character.

Although ecological data are incomplete, museum records indicate that *Etheostoma nigrum*, with a more complete lateral-line system is taken in streams with considerable gradient, whereas, *E. chlorosomum* seems to prefer oxbow lakes and swamps. *Etheostoma microperca*, according to Hubbs and Lagler (1947), is an inhabitant of quiet waters and, from personal observation, *E. proeliare*, although often found in oxbow lakes, is most abundant in streams. Some species such as the pirate perch, *Aphredoderus sayanus* which inhabits sluggish waters, have extremely prominent lateral-line systems possibly developed in response to lightless habitats (Moore and Burris, 1956). Evidently fishes are highly plastic animals which readily respond to environmental change and exhibit extensive adaptive radiation (Hubbs, 1940).

Another unusual condition found to occur in these fish hybrids was the apparent parthenogenetic development of their eggs. On November 5, 1954 ten eggs were obtained from the tank in which the hybrid fish were kept. These eggs developed to about the yolk plug stage and died. The presence of developing eggs in the tank containing the hybrids, did not seem unusual at the time, but in April 1955 when these fish were killed, it was found that all nine specimens were females. I was unable to tell the sex of these hybrids in life; plumpness of the abdomen suggested femaleness, but the greenish hue on the caudal peduncle and anal fin indicated maleness. These color characters are reminiscent of *spectabile* males which have bright green in these areas, whereas, males of *radiosum* have only a thin band of green on the anal fin. Both *radiosum* and *spectabile* males have green in the dorsal fins. Davenport (1906) reported that, in poultry, certain characteristics of sex may be transferred to the other sex by hybridization, owing to the lack of complete correlation between primary and secondary sex characters.

The hybrids were mated with both males and females of the parental species. On one occasion eggs were found in a tank containing a hybrid (female) and a female *spectabile*, but they died in the early stages of development. Another hybrid and a female *spectabile* also produced eggs; but examination of the female *spectabile* showed that the eggs had ruptured through her body wall, possibly due to pressure exerted by maturing eggs which she could not or would not release in the absence of a male. The spawning act was observed to take place twelve times in one hour between a hybrid and a male *spectabile* but careful examination of the gravel and sand in the tank failed to reveal any eggs. No eggs were ever obtained from tanks containing males of the parental species and hybrids. The males were frequently observed chasing the hybrids and on one occasion this continued for several days before the hybrid was found dead. Kammerer (1907) stated that the hybrids of (*Perca fluviatilis* X *Acerina cernua*) could not spawn their eggs naturally; stripping was necessary to obtain eggs. This may be the case with these darters even though 80 eggs have been obtained from the tank containing only hybrids. The small number of eggs obtained from these very gravid females

(9 specimens) may in part be explained by the inability to release the eggs naturally. The few eggs obtained may have been forced from the ovary by a combination of the pressure of other eggs undergoing development and the vigorous activity of the fish when disturbed.

The literature contains many references to parthenogenetic development of vertebrate eggs. Harman (1935) and Loeb (1930, 1932) reported eggs developing, by parthenogenesis, as far as the morula stage in the ovaries of guinea pigs. Pincus and Shapiro (1940) reported parthenogenetic development of rabbit eggs subjected to low temperatures. Gage (1928) reported the development of lamprey eggs to the morula stage without fertilization. Melander and Monten (1950) found that about 15 percent of the unfertilized eggs of Coregonus lavaretus, put in a hatching jar, contained well-developed embryos after one month. Trifonowa (1934) reported that the eggs of the fish species Acerina cernua, Perca fluviatilis, Abramis brama Linnaeus and Rutilus rutilus underwent parthenogenetic development. He found that in all cases cleavage was abnormal and development usually stopped before the yolk plug stage. The percent of eggs which began parthenogenetic development increased under the influences of distilled water and higher temperatures. He indicated that, in two eggs, embryos were formed before death and one egg developed to, but did not survive hatching. All embryos formed were about half normal size and exhibited abnormal organogenesis.

Supported by these examples, it was concluded that the eggs of Etheostoma hybrids developed parthenogenetically. The possibility of a hermaphroditic fish as reported by Lagler and Chin (1951) for E. nigrum was at first considered. Gross and microscopic examination of the reproductive organs prompted rejection of this possibility. This type of parthenogenesis, halting of development in the early stages, was classified by Vandel (1931) as rudimentary parthenogenesis. Suomalainen (1950) stated that the unfertilized eggs of many normally bisexual species begin to develop, although the development as a rule sooner or later came to a standstill. Harrison (1928) and Peacock and Harrison (1926) in their study of an insect (Lepidoptera) cross found that the first generation hybrids reproduced parthenogenetically although neither parental species had been known to employ that method. They concluded that parthenogenesis was a function of hybridization. Parmenter (1940), in a study of parthenogenetically developing frog eggs, development of which was initiated by pricking, concluded that one of the possible means by which development occurred was by the retention of the second polar body. He indicated that the polar body united with the haploid nucleus of the pricked egg during first cleavage. This was also suggested as a cause for some cases of parthenogenesis by White (1945). It is possible that the maturation divisions took place in the eggs herein discussed and a polar body united with the egg nucleus, thereby permitting early stages of development.

It is also possible that the eggs underwent early development although haploid in nature. It is probable that conditions, such as unusual temperature, pH or other physico-chemical conditions, unknowingly produced in the laboratory, initiated development.

Dobzhansky (1937a) indicated that offspring, produced by crossing distinct species, are usually sterile in that they are incapable of producing gametes or, if gametes are produced, they are abnormal and not viable. He also stated that the cause of sterility is abnormal meiosis. Hubbs (1955) stated that the testes of hybrid sunfishes and sperm stripped from them were abnormal. The percid hybrids in the present study, are capable of producing apparently normal eggs but, since only females were available, their capability of producing normal sperm remains unknown. The viability of the eggs has not been thoroughly tested, since the hybrids did not spawn with either parent species.

The predominance of one sex in hybrids has been reported. Guyer (1909) found a predominance of males in a guinea hen and chicken cross. Goodnight (1914) reported the absence of males in the hybrids of bison and cattle. Hubbs and Hubbs (1933) showed that centrarchid hybrids are predominantly males and are sterile. The percid fishes, herein discussed, differ from centrarchid hybrids in that all the offspring reared are females. Haldane (1922) proposed the hypothesis that when one sex in the hybrid offspring is absent, rare, or sterile, that sex is the heterogametic one. If Haldane's rule holds true, the Etheostoma male is heterogametic, whereas, in their close relatives, the centrarchids, this applies to the female. It is apparently possible for such closely related groups to differ in this respect since Gordon (1933) reported that some species of Poeciliinae have XY type of inheritance in contrast to a ZW type in others.

This study shows, as was found by Linder (1955), that taxonomic characters are not exactly intermediate between the parental species in etheostomatid hybrids, as reported for centrarchid and poeciliid hybrids by Hubbs (1940), but have a relatively wide range of variation as reported by Hubbs, Hubbs and Johnson (1943) for some catostomid hybrids. The individual characters of the offspring produced by crossing Etheostoma spectabile and E. radiosum tend to range from the condition found in one to that in the other parent. The averages of the various intermediate characters tend to show all degrees of intermediacy between the averages of the parental species. The hybrid index method of describing fish hybrids was shown to have some value, in that, when an average of all intermediate hybrid index values was taken, a value approaching 50 was obtained. When individual characters were considered, however, much variation was found to occur. When compared with the parental species, these hybrids (spectabile X radiosum) gave results similar to those found by Linder (1955) in a natural hybrid presumed to have been produced by the same species.

A comparison of reciprocally-produced adult hybrids has not been made since only those produced by male *spectabile* from Dog Creek mated with female *radiosum* have been reared to adulthood. The reciprocal combination was produced using specimens of *spectabile* from Mill Creek but were not, at the time this was written, of sufficient size to be of value in a comparison. The hybrids reared to maturity in the laboratory have shown more characters to be intermediate when compared with the reciprocal cross than when compared with their own parents. These experiments indicate that it is possible to verify the identification of an assumed hybrid, collected in nature, by means of the hybrid index method; but that it is impossible (as attempted by Linder, 1955) to determine, by this method, which of the two possible reciprocal combinations produced the hybrid.

These experiments indicate that the germ plasm of female *radiosum* probably is more compatible with that of male *spectabile* from Dog Creek than is that of male *radiosum* with female *spectabile* from Dog Creek (Table 1). There was a reversal of this condition when specimens of *spectabile* from Mill Creek were used. Newman (1914) attributed the difference in the success of reciprocal crosses to a difference in egg structure. In the present experiments there seems to be very little difference in egg structure, but if this were the reason for the difference in the success in the present crosses, the results obtained when mating the two different populations of *spectabile* and *radiosum* would have been closely similar. It is possible that, by chance, the difference in success of the reciprocal crosses is an expression of sterility of some of the fish used in the matings. However, it does not seem likely that, in two successive years of experimentation with Dog Creek *spectabile* and Blue River *radiosum*, sterile fishes were always used in one mating and more prolific fishes in the reciprocal cross. Breeding experiments showed that the two populations of *spectabile* breed together in the laboratory and that reciprocal crosses between them are equally successful.

The difference in the viability of eggs produced by the breeding of *radiosum* with the two different populations of *spectabile* is possibly an expression of a genetic difference between the two populations of *spectabile*. This difference is observable when matings between distinctly different species are made, but not evident in matings between two populations of the same species. Examination of the *spectabile* populations showed no morphological differences; but since they are, geographically, widely separated, they have probably been isolated from each other for a long time. This long period of isolation has probably permitted a genetic change of sufficient magnitude to be expressed when the two forms are crossed with *radiosum*. The *spectabile* of Dog Creek have diverged in such a manner that the female germ plasm is not compatible with that of male *radiosum*. The Mill Creek fish have diverged in a manner that prohibits the development of radiosum eggs fertilized by their sperm. White (1945) indicated that it is probable that real differences exist between the species of a genus in regard to the number and type of rearrangements of the chromosomes present in natural populations. Dobzhansky (1937a) stated that rearrangements play a part in effecting differences between populations and in their evolution. Rearrangements have been reported in the sex chromosomes and autosomes of Drosophila (Dobzhansky, 1937b).

It is possible that some of the eggs produced in these Etheostoma matings were deposited, without the spawning act taking place, and underwent early parthenogenetic development, thereby lowering the percent of hatchability. In neither intraspecific nor interspecific matings were eggs obtained from females in the absence of males. However, eggs were obtained from tanks containing only female hybrids. It, therefore, seems unlikely that the low hatchability can be attributed to the deposition of eggs in the absence of a spawning act. It is probable that eggs are not deposited, in natural habitats, when males are not present. This probability is supported by the fact that a female spectabile, held in a tank with another female, retained the eggs until the body wall ruptured. If there was a tendency for eggs to be deposited in the absence of the male, the species would not be perpetuated successfully.

According to Snyder (1951) hybrid animals are more vigorous than those normally produced. Since the ecological conditions maintained in aquaria were not the same, from the standpoint of temperature, current and chemical makeup, as those found in nature, it is possible that the eggs produced by crossing different species withstood the abnormal conditions of captivity better than those produced in normal matings.

The experimental fishes subjected to increased length of day, by artificial lighting, failed to spawn any earlier than those not subjected to this treatment. Hoover and Hubbard (1937) stated that trout can be induced to spawn early by light manipulation. Modification of the sexual cycles was brought about by increased length of day in birds and mammals by Rowan (1929) and Bissonette (1935) respectively. However, Merrimas and Schedl (1941), working with the four-spined stickleback, and Harrington (1950), with the bridled shiner, indicated that both temperature and light determined time of spawning. Matthews (1939) found that light did not affect the development of the testes in Fundulus but temperature did; Craig-Bennett (1931) reported similar results for the three-spined stickleback. Petravicz (1938) found that the black-sided darter spawned soon after being placed in the laboratory in mid-winter, several months before spawning occurred in nature. Although the natural spawning time in Etheostoma is not known, it probably is not as early as mid-December, the time at which they spawned in the laboratory. This would indicate that temperature is an important factor in initiating spawning in these fishes. Some individuals, collected in August, failed to spawn after being subjected to low temperature, followed by either a gradual rise or a rapid rise in temperature. This may possibly indicate that these specimens had already finished spawning and had insufficient time to develop additional gametes. It is probable that, even though these fishes can be induced to spawn much earlier in the laboratory than in nature, they spawn only once a year.

The breeding habits of these two species (E. radiosum and E. spectabile) were found to be similar in the laboratory. The general relationship between the reciprocal matings followed the same pattern. Considerable belligerence was observed between the two species when they were first placed in the breeding tanks, but this usually subsided after a short period. This study indicates that the natural hybrid collected from Blue River probably was not produced, as indicated by Linder (1955), by sperm of a riffle spawner floating to the eggs of the form spawning below the riffle, but by the spawning together of the two species. Observations made in the laboratory indicate that neither of these species spawn in the riffle proper. The habit of diving in the gravel and sand by the females of both species indicates that these fish spawn in areas of the stream where sand and gravel are deposited.

These areas would logically be found above and below the riffles, whereas, the actual riffles usually consist of large stones and rocks and it would not be possible for the female to make a depression by diving into them head first. It is probable that a combination of incompatibility, as observed in the laboratory, and ecological factors have enabled the two species to maintain specific identity in nature. It is possible that a change in ecological factors, resulting from man's activities, has taken place and caused a limited amount of hybridization. Hubbs (1955) concluded, on the basis of extensive studies of fish hybrids, that a predominance of one species as well as changing ecological conditions brings about hybridization. He indicated that when one species is uncommon in a stream it does not readily find a spawning mate and, therefore, spawns with members of another species. E. radiosum is much more common in Blue River than is spectabile. The small number of eggs produced at each spawning and the high mortality rate in the laboratory, probably higher in nature, would explain the scarcity of natural hybrids. Study of the relationship between the two species in the laboratory also indicates that a combination of female radiosum and male spectabile probably produced the natural hybrids since the male spectabile was accepted more readily by the female radiosum than were the female spectabile by the male radiosum. It is probable that the increase in siltation brought about by farming activities has decreased the amount of gravelly habitats in the Blue River, thereby decreasing the spawning sites available to these species. With continued changes, hybridization between radiosum and spectabile will probably become more common.

Hybridization may serve an important function in nature in that occasional crossings, accompanied by backcrossing, between two species would cause an introduction of new genes into the species concerned, thereby bringing about greater variation within the species. A high degree of physiological variability probably lends survival value, whereas, a species with a narrow range of variation may face extinction in a changing environment. Hubbs (1955) in a review of natural hybridization in fishes stated, "where the environment is in a state of flux, increased premium may be inherent in the genetic variability that hybridization produces." Dobzhansky (1937b) stated that while it is impossible to appraise the evolutionary role of introgressive hybridization, it may, in some organisms, result in the emergence of superior genotypes. Moody (1953) also indicated hybrids apparently form a means by which genes of one species may be transferred to another species. However, Blair (1951) stated that he believed the amount of gene transfer between sympatric species of vertebrates is negligible. The unique presence of red pigment in the anterior interradial anal fin membranes of Blue River spectabile suggests the possibility of a gene flow between that species and radiosum.

It is also possible that the red anal fin pigment may have appeared independently from pure spectabile stock, which in all other known populations has a uniformly greenish anal fin. The first possibility is supported by researches carried out by Gordon (1946) in which he crossed two species of poeciliids. He found that, by repeated backcrosses to one parent, a stock was developed which was indistinguishable in configuration from the parent species, used in the backcrosses, but contained certain color characters of the other parent. If hybridization should become more common, with production of fertile offspring, backcrossing might cause these two species of Etheostoma to merge into one with a range in variation equal to that now possessed by the two species. Although the blurring of species lines due to hybridization has been reported for many plant species (Anderson, 1949; Stebbins, 1950), apparently only two cases have been observed in the animal kingdom. Blair (1941) reported this to occur between some populations of some Bufo species and Dobzhansky (1937b) cited one example in snails.

SUMMARY

Etheostoma radiosum and Etheostoma spectabile, with very similar breeding habits, will hybridize in the laboratory. Considerable belligerence was exhibited between some individuals of the two species when they were first placed together, but this usually subsided after a short period. It is probable that a combination of ecological factors and some degree of incompatibility has enabled both species to maintain specific identity in nature.

A putative hybrid captured in nature was found to agree with the laboratory-reared hybrids in proportional measurements, but differed in most meristic characters. The number of lateral-line scales in the laboratory-reared hybrids was lower than that of the natural hybrid, but the number of rays in the dorsal and anal fins was, in general, higher. The laboratory hybrids also differed from the parental species in these counts.

Average proportional measurements of the hybrids and their individual indexes were not exactly intermediate, but showed varying degrees of intermediacy between the averages of the two parental species. The hybrid index method of describing fish hybrids was found to be of some value in that an average of the individual

index values gave a value near exact intermediacy.

The laboratory-reared fish failed to develop an extensive lateral-line system such as is found in the parental forms in nature, indicating that the extent of development of this system is not genetically fixed.

The fishes used in this study appeared to spawn in response to temperature changes rather than variation in length of day. These experiments also indicate that these etheostomids are cyclic in that they spawn but once a year even though experimentally subjected to changes in temperature.

The embryology and early larval development of the reciprocal crosses and normal spectabile were similar with no observable differences between them. The hybrids did not breed with either parental species, although they laid eggs which developed parthenogenetically through early stages.

The eggs produced by the reciprocal crosses between the two species differed, in these experiments, in their viability. A difference in the success of the hybrid eggs was also evident when two different populations of spectabile were used, even though the two populations appeared to be morphologically alike. This poses a question of possible divergence of these two widely separated populations. The eggs produced by crossing the two species were hatched more successfully than those from normal matings.

The experiments and observations herein discussed indicate that much can be learned concerning the habits, genetics and taxonomy of fishes by laboratory experimentation. They also show that extensive work is yet needed to explain how closely related, sympatric forms remain distinct and how the environment affects the supposedly fixed taxonomic characters of these forms.

SUGGESTIONS FOR FURTHER STUDY

1. Further work is needed to determine whether the difference in success of the reciprocal crosses was caused by factors in the individual fishes or if it resulted from genetic differences between the two species. Cytological studies should be made to determine the relationship of chromosomes during early cleavage.

2. Since all the offspring produced were females it would be desirable to duplicate these experiments to determine if a lethal factor is acting on the male embryos.

3. The conditions which control the development of the lateral-line system need to be investigated thoroughly to determine the validity of some species which have been erected primarily on the basis of the extent of development of this system.

4. Further work is needed, employing careful control of temperature and length of day (by artificial light), to determine the effects of these factors on time of spawning of fishes kept in the laboratory for several successive years.

5. There is also a need for further work on the Blue River population of *spectabile* to determine whether the red on the anal fin of the males is genetically fixed and if it can be transmitted from one population to another.

6. Cytological studies of the parthenogenetic eggs are needed to determine the existence or nonexistence of the haploid condition. If the haploid condition exists, the chromosome number, at present unknown in these fishes, could be established more easily.

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APPENDIX

PLATE I

Camera lucida drawings of embryonic stages of *Etheostoma* hybrids

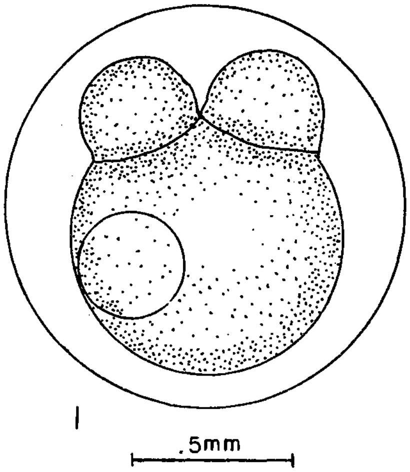
Figure 1. *E.* (*spectabile* X *radiosum*), two-cell stage.

Figure 2. *E.* (*spectabile* X *radiosum*), fourteen-hour stage.

Figure 3. *E.* (*spectabile* X *radiosum*), fifteen-hour stage.

Figure 4. *E.* (*spectabile* X *radiosum*), seventeen-hour stage.

Figure 5. *E.* (*spectabile* X *radiosum*), forty-seven-hour stage.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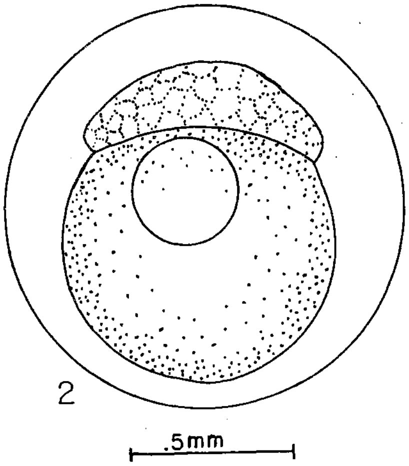
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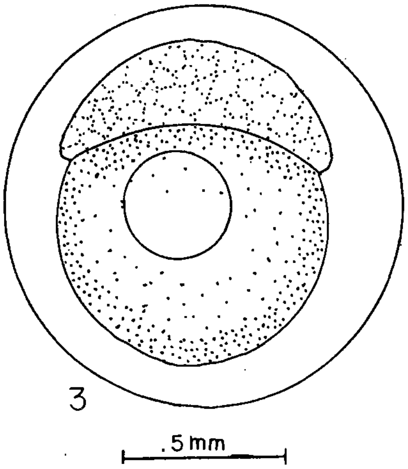
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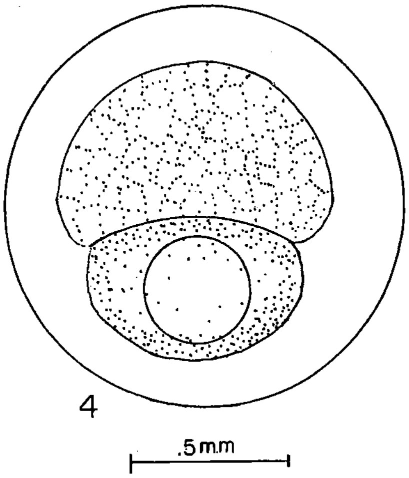
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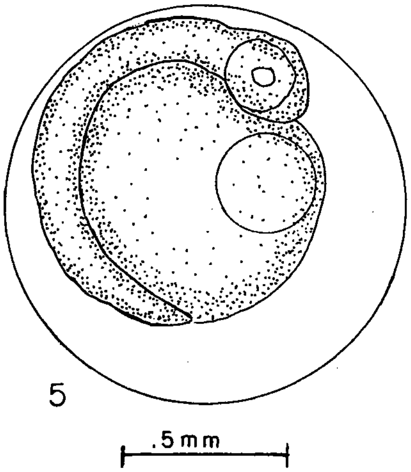
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PLATE II

Photomicrographs of embryonic stages of *Etheostoma* hybrids

Figure 6. Newly-laid egg of *E. radiosum* (fertilized by *E. spectabile*) (13.6 mm.=.5 mm.).

Figure 7. *E.* (*spectabile* X *radiosum*), five-hour stage (29.4 mm.=.5 mm.).

Figure 8. *E.* (*spectabile* X *radiosum*), fourteen-hour stage (13.6 mm.=.5 mm.).

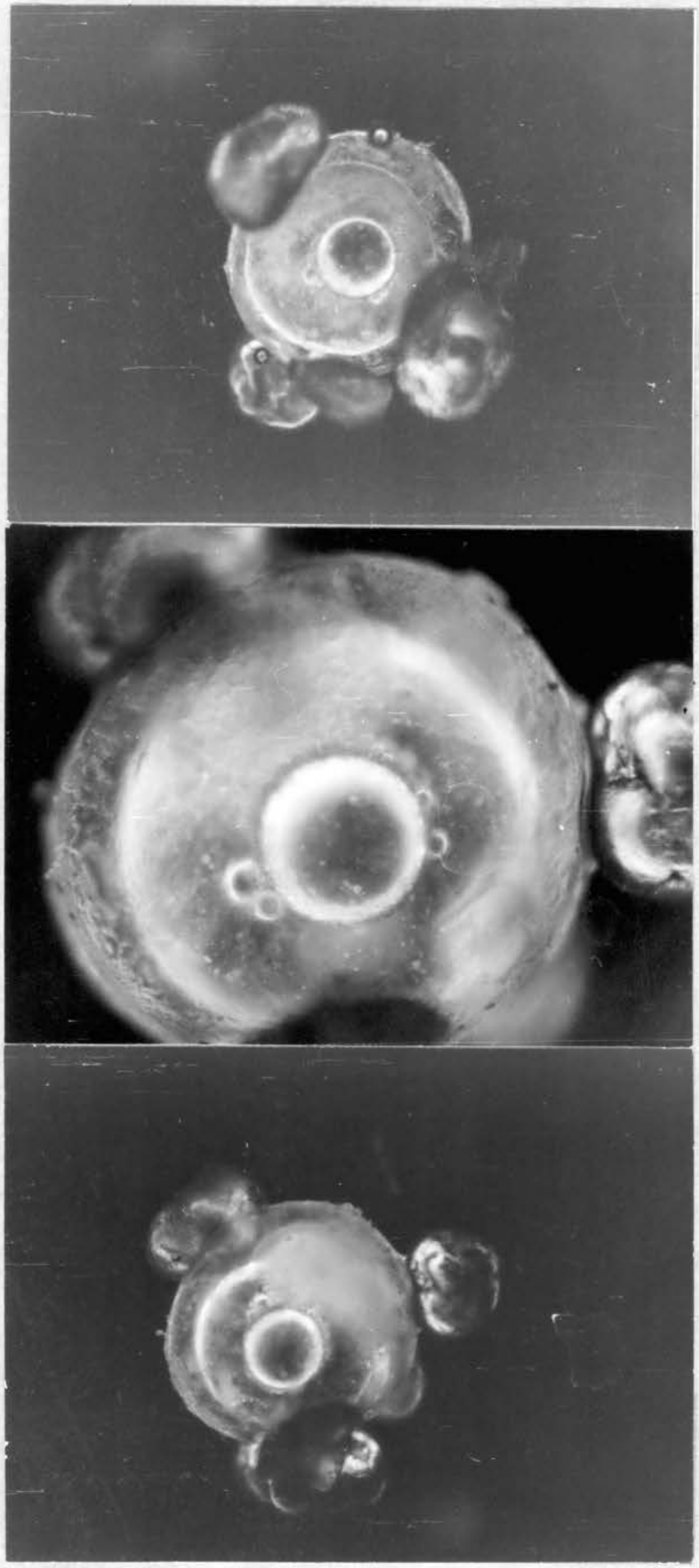


PLATE III

Photomicrographs of embryonic stages
of *Etheostoma* hybrids.

Figure 9. *E.* (*spectabile* X *radiosum*), seventeen-hour stage (13.6 mm.=.5 mm.).

Figure 10. *E.* (*spectabile* X *radiosum*), eighteen-hour stage (13.6 mm.=.5 mm.).

Figure 11. *E.* (*spectabile* X *radiosum*), twenty-hour stage (29.4 mm.=.5 mm.).

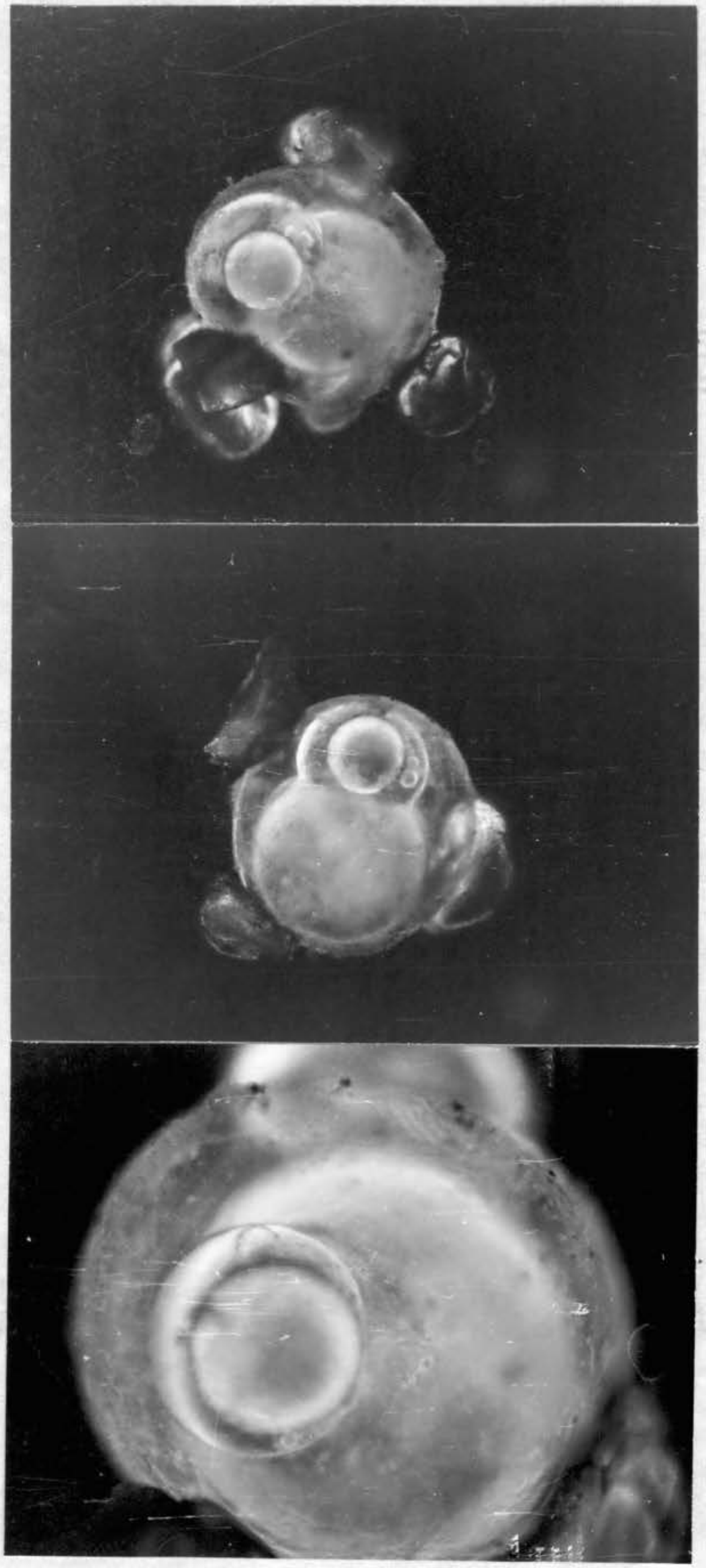


PLATE IV

Photomicrographs of embryonic stages
of *Etheostoma* hybrids.

Figure 12. *E.* (*spectabile* × *radiosum*), twenty-five-hour stage (13.6 mm.=.5 mm.).

Figure 13. *E.* (*spectabile* × *radiosum*), seventy-four-hour stage (10.6 mm.=.5 mm.).

Figure 14. *E.* (*spectabile* × *radiosum*), five-day stage (13.6 mm.=.5 mm.).

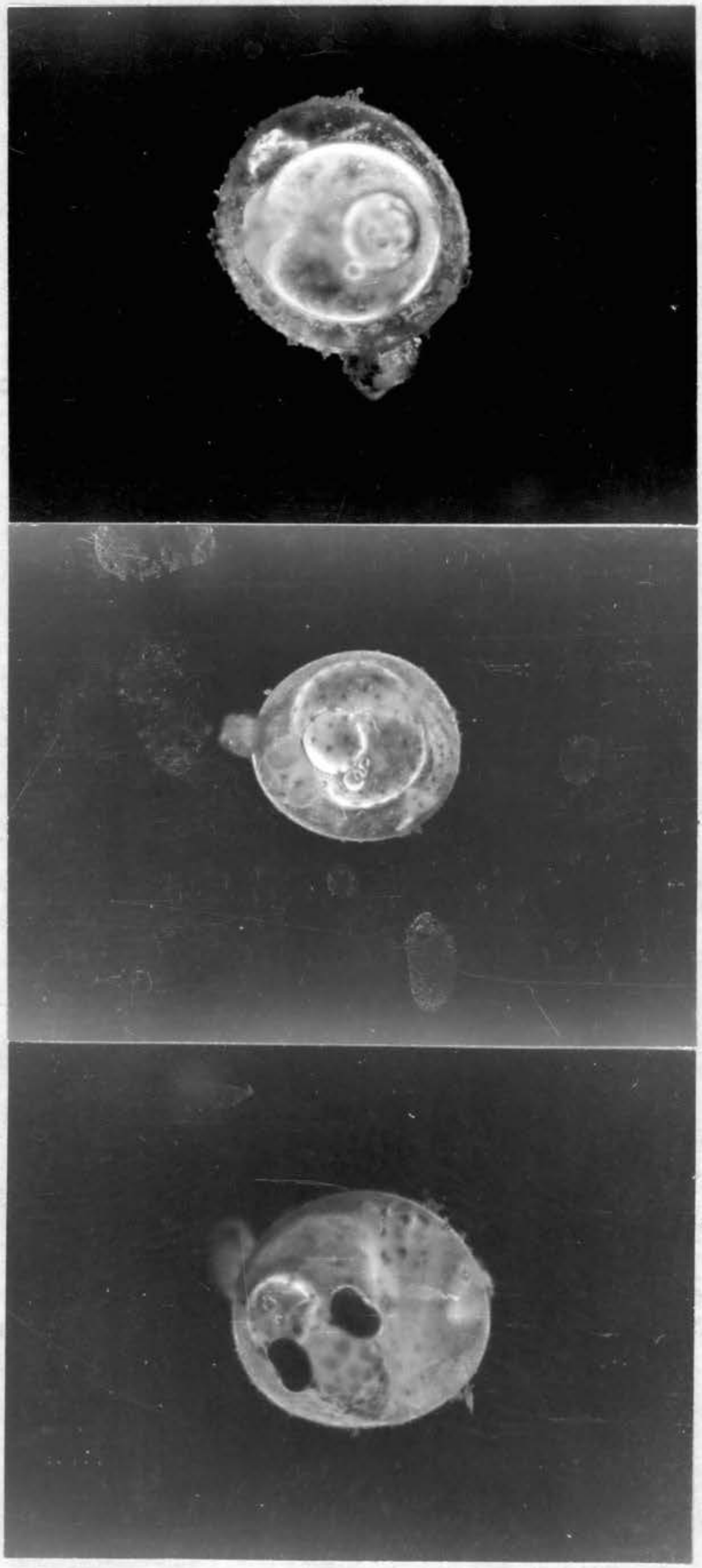
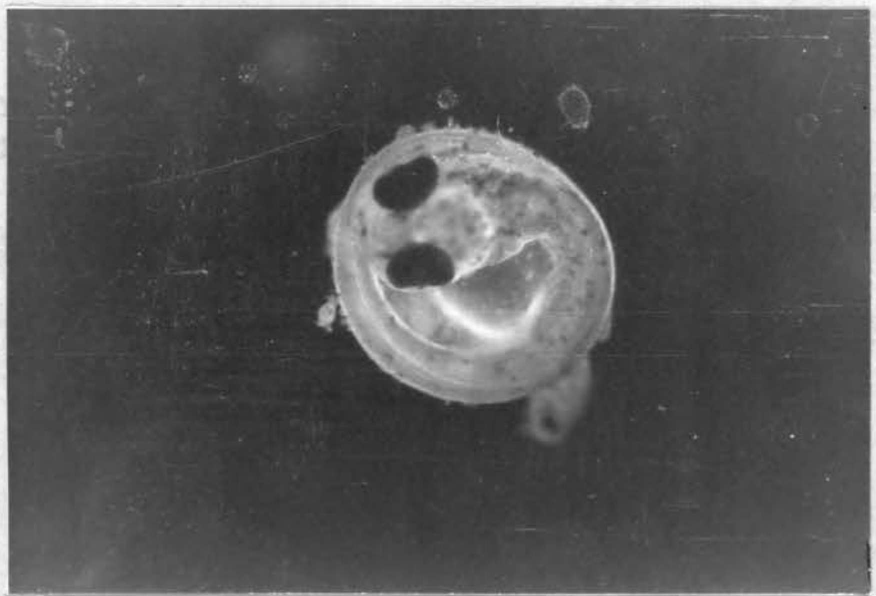


PLATE V

Photomicrographs of embryonic stages of *Etheostoma* hybrids

Figure 15. *E.* (*spectabile* × *radiosum*), seven-day stage (13.6 mm.=.5 mm.).

Figure 16. Larva of *E.* (*spectabile* × *radiosum*) immediately after hatching, 3.7 mm. total length.



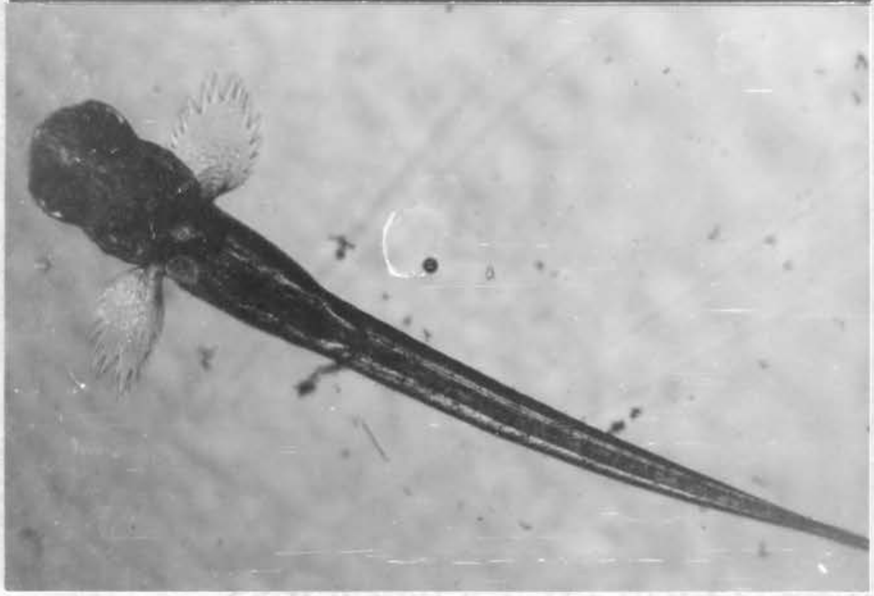


PLATE VI

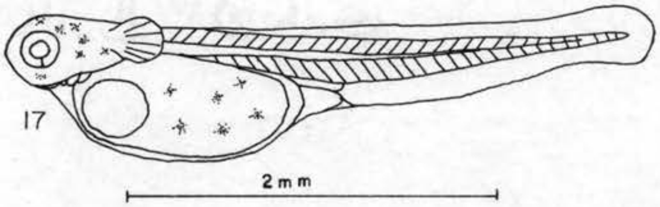
Camera lucida drawings of larval stages of *Etheostoma* hybrids

Figure 17. *E.* (*spectabile* X *radiosum*) prolarva, 3.7 mm. total length.

Figure 18. *E.* (*spectabile* X *radiosum*) prolarva, 4.5 mm. total length.

Figure 19. *E.* (*spectabile* X *radiosum*) postlarva, 6.2 mm. total length.

Figure 20. *E.* (*spectabile* X *radiosum*) postlarva, 7.4 mm. total length.



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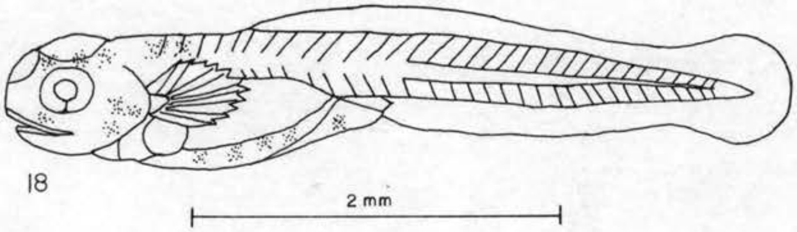

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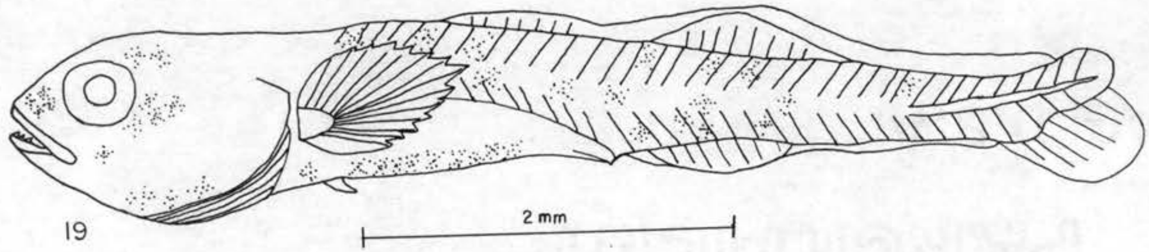

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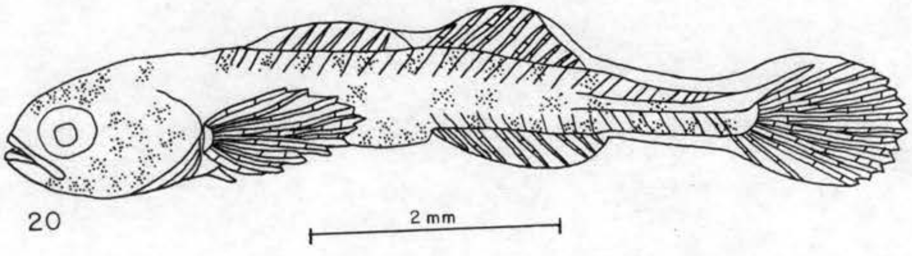

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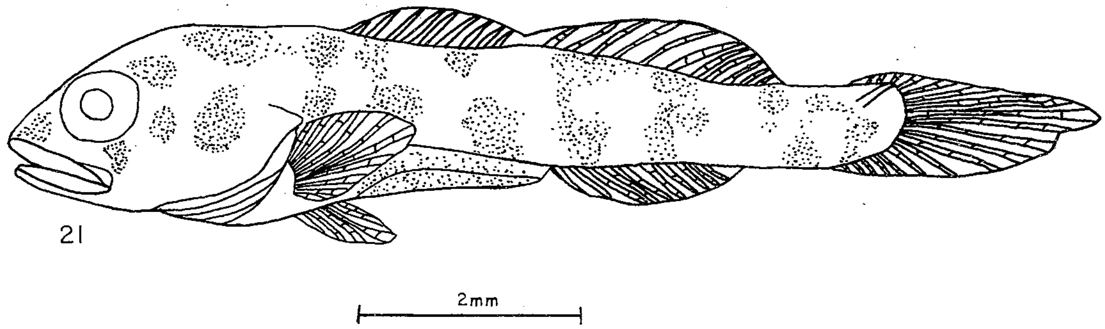
PLATE VII

Camera lucida drawings of larval stages
of *Etheostoma* hybrids.

Figure 21. *E.* (*spectabile* × *radiosum*) postlarva, 10.0 mm. total length.

Figure 22. *E.* (*spectabile* × *radiosum*) juvenile, 18.3 mm. total length.

Figure 23. *E.* (*radiosum* × *spectabile*) juvenile showing abnormal development of fins.



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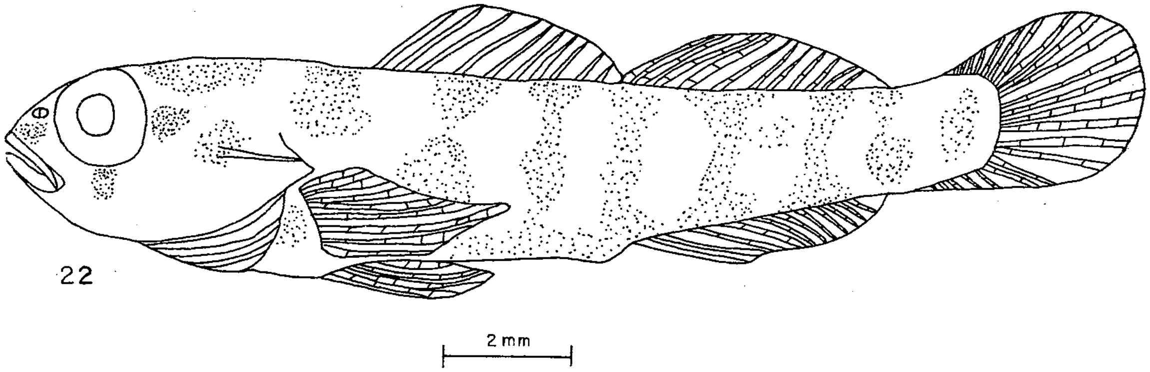

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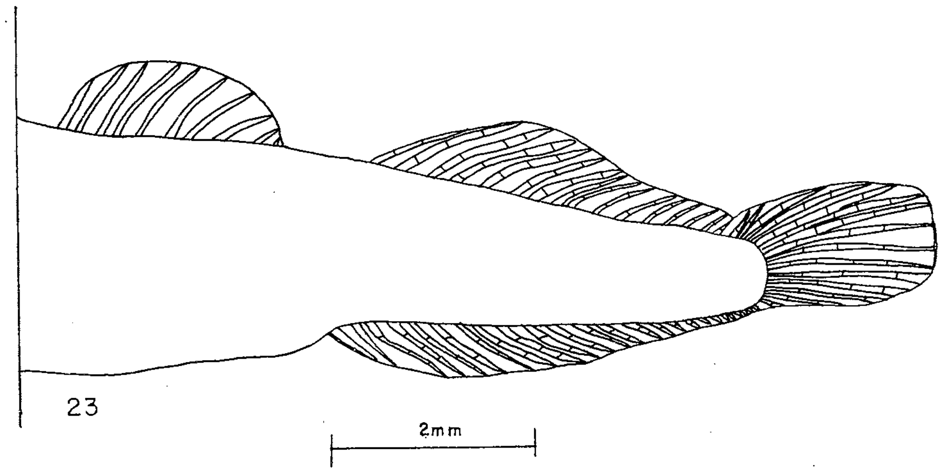

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VITA

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Doctor of Philosophy

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