STUDIES IN THE GENUS

ICTIOBUS (BUFFALOFISHES)

By

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INTRODUCTION

The study presently discussed was undertaken to investigate certain phases of the life histories and to understand more clearly relationships between two species of buffalofishes, Ictiobus bubalus (Rafinesque) and Ictiobus niger (Rafinesque).

The buffalofishes comprise the sub-family Ictiobinae of the sucker family Catostomidae. In the United States the Ictiobinae is composed of the two above-mentioned species and Ictiobus cyprinellus (Valenciennes). The bigmouth buffalofish, I. cyprinellus, is a distinctive species and is easily separated from the other two forms. The smallmouth buffalofish, I. bubalus and black buffalofish, I. niger have similar morphological characters and ecological peculiarities (Hubbs et. al., 1943 and Trautman, 1957) and frequently are confused.

It was believed that by observing the behavior and development of the young of I. bubalus, I. niger and a hybrid between the forms, that insight into the basic differences in the two species could be obtained. Controlled breeding experiments seemed the proper approach to acquire the desired information.

Throughout Oklahoma and neighboring states many large reservoirs have been constructed on major river systems.

One of the first biological considerations of these reservoirs is a greatly increased fishery resource. Fishery workers have become increasingly concerned about the magnitude and composition of fish populations in large reservoirs. A basic problem, which remains unanswered, is the ecological effects of impounded water upon species that previously occured in the streams. Many stream fishes disappear when the reservoir replaces the stream, some seem to adapt equally well to either environment and others seem to develop (in reservoirs) populations which are greatly above their stream potentials. The buffalofishes in Oklahoma reservoirs seem to belong to the latter category.

Buffalofishes from many Oklahoma waters, excluding *I. cyprinellus*, are very difficult to separate taxonomically. *Ictiobus bubalus* and *I. niger*, from Oklahoma reservoirs, have become almost impossible to separate. A study to investigate the possibilities of hybridization was considered.

The hybrid index which has been used to determine and evaluate hybrid fish populations could not be used because of the lack of distinctive characters to separate *I. bubalus* and *I. niger*. Controlled breeding experiments seemed to be the best approach to solving the problems of taxonomic confusion and hybridization. Thus known hybrids could be produced and compared with the confusing natural populations suspected of being hybrids.

The taxonomy of *I. bubalus* and *I. niger* is based on relative characters, such as mouth size, eye size and the height and thinness of the back. A taxonomic analysis of hybrid buffalofish produced from known parents could be useful in evaluating the relative characters.

REVIEW OF LITERATURE

Taxonomy

C. S. Rafinesque was the first person to describe a buffalofish. He described the brown buffalofish as *Catostomus bubalus* and the black buffalofish as *Catostomus niger* in his *Ichthologia Ohiensis*, 1820 (p. 112 of 1899 reprint). It is unfortunate that no types were preserved because some ichthologists have subsequently felt that Rafinesque's descriptions were not sufficient to validate these two species which are now accepted. It was approximately 110 years after Rafinesque described the fishes that they were assigned their present status of *Ictiobus bubalus* and *Ictiobus niger*.

Agassiz (1854, 1855) erected the genus *Bubalichthys* for the black buffalofish and corrected Rafinesque's sub-generic spelling of *Ictiobus* to *Icthyobus* for the smallmouth buffalofish. It is confusing to try to understand Agassiz's works because he used the specific taxon *bubalus* with both *Bubalichthys* and *Ichthyobus* and *niger* with *Carpiodes* and *Bubalichthys*. Agassiz also recognized the following as additional buffalofishes; *Ichthyobus rauchi*, *Ichthyobus stolleyi*, and *Bubalichthys bonasus*.

Jordan (1877) recognized *Ichthyobus bubalus*, *I.*

cyprinella, I. ischyrus, Bubalichthys altus, B. bubalus and B. niger in Northern Indiana. However, in 1896, Jordan and Evermann recognized three nominal species; Ictiobus cyprinella, I. bubalus and I. urus along with Ictiobus meridionalis (Günther) a southern form from Rio Usumacinto Guatamala. Fowler (1913) recognized the same forms from the United States, but questioned the validity of the nomenclature used by Jordan and Evermann.

Hubbs (1926) in a key to the fishes of the Great Lakes included only Ictiobus urus stating that, "It is not certain that this nominal species can be separated from I. bubalus". Green (1926) recognized both I. bubalus I. urus from Wisconsin. Hubbs and Green (1927) corrected Hubb's 1926 key, and did not include I. bubalus. Hubbs (1929) in his key included I. bubalus, and stated that his previous confusion between I. bubalus and I. urus, ". . .was due to inexperience with the group". In 1930, Hubbs resurrected niger for the black buffalofish, claiming that Agassiz's type specimen of urus was bubalus.

Hybridization

Although there has been much work done on hybrid fishes (Hubbs, 1955), and much of the work has been concentrated on hybrid suckers, (Hubbs et. al., 1943), (Hubbs and Miller, 1943), (Hubbs and Hubbs, 1947), (Hubbs and Miller, 1953), there are few references to buffalofish hybrids. Thompson (1935) suggested the possibility that I. niger is a hybrid, I. bubalus X I. cyprinella.

Hubbs et. al. (1943) refuted the idea and asserted the validity of all three forms declaring that the difficulty of identification in genus Ictiobus is due to the species being ". . .characterized by a combination of traits, none of which by itself may be invariably distinctive". However, Hubbs et. al. (1943) stated that ". . .it would be difficult to prove that hybridization does not take place between some of the more closely related species in the Ictiobinae".

Trautman (1957) recognized a hybrid, I. bubalus X I. cyprinellus, from the western end of Lake Erie. He attributed the identification to an intermediate thickness between the thin pharyngeal arch of I. cyprinellus and the thickened pharyngeal arch of I. bubalus. Although both niger and bubalus have thick pharyngeal arches, Trautman assigned the thickened-pharyngeal-arch parent to bubalus because of the hybrid's high-arched back. Trautman accounted for the hybridization in Lake Erie by the introduction of a stream-inhabiting species (bubalus) into the habitat of a lake-dwelling species (cyprinellus).

Life History

Buffalofishes normally spawn in March and April when the water temperature is about 60° F. (Canfield, 1922). Spawning is communal and the actual deposition of eggs is on materials such as aquatic plants and flooded terrestrial vegetation. Spawning is usually associated with "spring floods" or at least a rise in water level (Yeager, 1936 and Walker and Frank, 1952). The bigmouth and smallmouth buffalofishes have been cultured in ponds (Canfield, 1922, Hendricks, 1956, and Swingle, 1956) and have been propagated by stripping the eggs and hatching them in jars (Walker and Frank, 1952). Yeager (1936) recorded observing the natural spawning of buffalofish (probably I. niger) along the flooded margins of a cypress swamp in Leflore County, Mississippi. No reports were found concerning the reproductive ecology of buffalofish in reservoirs.

Although no description of young buffalofish have been found, the development of other catostomids has been observed. Crawford (1923) was apparently the first to observe changes in the position of the mouth in young suckers. Stewart (1926) observed changes in mouth position, coiling of intestine and general development of Catostomus commersoni (Lacépède). Hubbs and Creaser (1924) considered growth patterns, foods and migration of young suckers. Fish (1932) described some developmental stages of Catostomus commersoni, Hypentelium nigricans (LeSueur), Moxostoma aureolum (LeSueur) and Moxostoma anisurum (Rafinesque).

Oppenheimer (1937) gave a table of numbered stages for sequence development in Fundulus heteroclitus (Linnaeus) based on the degree of structural differentiation. Balinsky (1948) modified Oppenheimer's table to use with the Cyprinidae. Winn and Miller (1954) presented descriptions with keys to five postlarval catostomids of the lower

Colorado River Basin. They used Balinsky's modified table for both minnows and suckers.

MATERIALS AND METHODS

Collection and Care of Adults

Specimens to be used in breeding experiments were collected from the Red River in Bryan County below Denison Dam and from the Illinois River and extreme upper end of Tenkiller Reservoir in Cherokee County.

Forty buffalofish, identified as *Ictiobus niger*, were taken from the closed turbine raceway of Denison Dam on January 24, 1957. The fish were probably river fish from the Red River below the dam, however, some fishes have been known to pass from Lake Texoma through the turbines. The specimens were taken to the state fish hatchery at Holdenville, Hughes County, and released into a one-acre culture pond (designated as pond 1.).

Specimens identified as *Ictiobus bubalus* were captured in gill nets and hoop nets from the Carter's Landing and Horseshoe Bend areas of the Illinois River. The elevation of the water level in Tenkiller Reservoir determines whether this area is considered part of the Illinois River proper or the extreme upper end of the reservoir. The collections were made in quiet waters and probably should be considered as the extreme upper end of the reservoir. Seventeen specimens were collected on February 9, 1957 and carried to the Holdenville hatchery, where they were released into a separate one-acre culture pond (designated as pond 2.). Three specimens were taken from the same area on March 2, 1957 and put into pond 2. Four of the first 17 specimens had died, leaving a total, with the addition of the three specimens on March 2, of 16 specimens.

On each of the collecting trips to Tenkiller Reservoir, the fish identified as I. bubalus were selected on the relative thinness of the back, size and position of the mouth and height of the predorsal arch of the back. It is estimated that from the three collections in this area, the total catch of small-mouthed buffalofishes was 150 individuals. Of this number, 26 were selected and identified as bubalus. The remaining individuals were difficult to identify. Some of them had a small ventral "bubalus" mouth but had a low-arched, wide, elliptical "niger" back, while others had a thinner high-arched "bubalus" back with a larger more terminal "niger" mouth. A few of the fishes appeared to be niger with a thickened, low-arched, elliptical back and a large subterminal mouth.

The fish from Red River ranged in size from three to six pounds, while those from Illinois River ranged from three to eight pounds.

Natural Propagation

Ten male "niger" were taken from pond 1 and three female "bubalus" from pond 2 and were put into a separate one-acre culture pond (designated as pond 3). A reciprocal cross of the combination in pond 3 was attempted in a separate pond (designated as pond 4). Six male "bubalus" from pond 2 along with two female "niger" from pond 1 were used in the reciprocal combination in pond 4.

The sexes were easily determined, males emitting sperm when slight pressure was applied to the lower abdominal region and having pronounced tubercles over the entire body. The females generally had a well rounded, egg-filled appearance and small tubercles usually restricted to the head. No eggs could be extruded from the vent with excessive pressure.

Water levels were kept low until all four ponds were stocked. All ponds were allowed to begin to fill slowly on April 18, thus attempting to simulate a "natural rise" described by Canfield (1922). The inflow of water was adjusted so that each pond would fill in five days. One bale of native prairie hay was scattered around the margins of each pond to provide spawning media. The average surface temperature of the ponds on April 18, was 68.9° F.

Artificial Propagation

Some of the largest specimens which had been collected and deposited in the ponds were chosen for artificial spawning. One female from the Red River collection (presumed to be niger) and two males from the Illinois River (presumed to be <u>bubalus</u>) were isolated in separate holding pens on April 17, 1957. The males were readily emitting sperm and the female was well rounded, but no eggs could be obtained by pressing the abdominal region. At approximately 4:00 P.M., an injection of acetone-dried pituitary gland was injected into the coelomic cavity of the female at the base of the right pectoral fin. The female was then returned to the holding pen. The pituitary gland came from an 18-pound female flathead catfish, *Pylodictis olivaris* (Rafinesque), and was prepared by the methods described by Cozart (1954).

The following morning at 9:00 A.M. ripe watery eggs could easily be stripped from the female. As much sperm as possible from one male was stripped into a porcelain-lined basin containing a quart of water. Then, approximately one pint of eggs was stripped into the same basin and stirred with a feather. The male fish used in this spawning was tagged and preserved in formalin. The female was returned to the holding pen. The eggs were stirred for approximately two minutes then the excess water and sperm decanted. Fresh water was added to the eggs and one teaspoonful of cornstarch was added to facilitate hardening of the eggs. This technique was described by Walker and Frank (1954) and is important in keeping the highly adhesive eggs from adhering in clusters. The starch-water mixture was stirred about two minutes and then decanted. The contents of the basin were continuously stirred for one hour and frequent changes of fresh water, without starch, were made. At the end of this time the eggs were fully water hardened and did not adhere to each other when allowed to settle. About half of the eggs were then placed on a straw mat which was put into a one-eighth acre holding pond. The remaining eggs were put into a standard hatching jar with a slight current of water moving continuously over the eggs.

A second artificial spawning was performed on April 18. The same female, which still had a distended abdomen and easily obtainable eggs, was used again. The second male used (also presumed to be bubalus) had a smaller mouth than did the male used in the first spawning. The same techniques employed during the first spawning were repeated. Only one cupful of eggs was taken from the female. Both the male and female were tagged and preserved after final stripping. After the eggs had water hardened they were placed in a number 3 wash tub containing about four inches of water and carried to Stillwater.

Extreme care was exercised in keeping eggs from the two spawnings separate so that the parents of the young would be definitely known and that young with one female parent, but different male parents could be compared.

Laboratory Care of Fish

Care of Eggs

The eggs from the second artificial spawning were first put into a 20" x 30" x 15" porcelain lined tank. The water depth in the tank was about 6 inches. Then approximately 200 eggs were placed in each of three 10-gallon aquaria which contained varying depths of water. The water in both the porcelain tank and the aquaria was aerated with compressed air passing through aeration stones. One group of 25 eggs was kept at constant temperature (80° F.) in an incubator.

Care of the Young

The newly-hatched buffalofish larvae were kept in the hatching containers (porcelain tank and three aquaria) until it seemed that all viable eggs had hatched. It was estimated that 80 percent of the eggs brought to the laboratory hatched. On April 24, when the hatch was apparently complete, approximately 800 larvae, still containing yolk sacs, were transplanted into a one-quarter acre temporary pond (Hillcrest Golf Course Pond) north of Stillwater. The remaining young were transferred into clean aquaria to avoid foul water from decomposition of the egg cases. Approximately 2,500 ten-day-old young were transplanted into McElroy Pond, a one-acre farm pond on the east edge of Stillwater on May 4th. This pond had been dry the previous fall and was supposedly void of other fishes. On May 5, 370 young buffalofish were divided among and transplanted into two one-half acre farm ponds near Pawnee. The remaining young (approximately 500) were kept in two 10-gallon and one 20-gallon aquaria in the laboratory. Several specimens were preserved in either formalin or Bouin's fluid at regular intervals for developmental studies.

Feeding the Young

The young buffalofish were first fed either the fifth or the sixth days after hatching. At this time absorption of the yolk sac was complete. "Micrograin", a commercial food, prepared in fine powder form for newly hatched larvae, was fed in small amounts. Beginning on the tenth day, plankton was strained from a pond and added to each aquarium containing young buffalofish. A combination of "micrograin" and plankton was fed the fish retained in the laboratory until the 54th day after hatching when the last of this group was preserved.

Counts and Measurements

Fifty-three adult specimens from four watersheds were used to study the taxonomic characters in making counts and measurements. The number of specimens, average standard lengths and ranges from each location is listed as follows: 27 specimens from the Red River in Bryan County, average length 360 mm., range 307 to 423 mm.; 18 specimens from the Illinois River in Cherokee County, average length 401 mm., range 332 to 475 mm.; four specimens from the Fort Gibson Reservoir in Wagoner

County, average length 339 mm., range 265 to 433 mm.; four young specimens of known parentage, raised in a pond at Holdenville, Hughes County, average length 179 mm., range 173.5 to 183.5 mm.

Most counts and measurements were made according to the methods described by Hubbs and Lagler (1947). Deviations from the above methods and additional methods are listed below.

All rudimentary rays were included in fin ray counts.

The projected body depth, standard length and head width were made with wide-jawed calipers (Figure 13) to avoid influence from body curvature.

Because body depth plays an important part in the taxonomy of the forms, body depth measured over the curvature was taken by measuring the greatest distance around the body with a piece of pre-stretched nylon cord. Starting and stopping points along the cord were marked by pushing dissecting needles through the cord (Figure 14) and the length of the cord involved was measured on a linear millimeter scale (Figure 15). This distance was then divided in half and recorded as body depth taken over curvature.

A measurement from the anterior-most edge of the subopercle bone to the nearest edge of the orbital rim was taken and a measurement from the edge of the orbit to the anterior-most point of the gill cleft was also taken.

A measurement, described by Bailey (1956), from the mandibular symphysis to the end of the maxilla was taken.

All counts and measurements were taken on the left side of each specimen except lateral-line scales and paired fin ray counts, which were taken from both sides.

Illustrations

Photomicrographs and camera lucida drawings were made of larval stages (Appendix Figures 1 to 12). Regular 35 mm. photographs were also used (Figures 13 to 19). The photomicrographs were taken through a dissecting microscope, using a 10X ocular with .66X, 1X, 2X and 3X wide-field objectives, with illumination from a strobe lamp.

EXPERIMENTS AND OBSERVATIONS

Results of Propagation

The margins of each of the four ponds set up for natural propagation were observed daily by hatchery personel. No evidence of spawning activity was noted, and apparently no buffalofish reproduction occured in any of the ponds. Attempts to collect young buffalofish from each of the four ponds with a fine mesh seine on May 24, were unsuccessful. Two other attempts to collect young from each of the four ponds during the summer months were unsuccessful. The adults were seined from ponds 2 and 3, on May 24, and preserved in 20-percent formalin. Most of the adults from these two ponds still appeared to be capable of spawning. All four ponds were drained November 10, 1957. Some of the adult females from ponds 1 and 4 still appeared gravid and upon dissection were found to have developed eggs which seemed to be normal enough for spawning and fertilization. All of the adults from ponds 1 and 4 were preserved in formalin.

There were five eight-inch river carpsuckers, *Carpiodes carpio* (Rafinesque), and approximately 200 six-inch gizzard shad, *Dorosoma cepedienum* (LeSueur), recovered from pond 1 when it was drained November 10, 1957.

The eggs or small larvae of these fishes had undoubtedly passed through the one-eighth inch straining screen which separates the hatchery ponds from their water source, Holdenville City Lake. Both of the species occur in the lake.

It is not known what percentage of the eggs hatched from the first artificial spawning. About half of the eggs from this spawning were put into a hatching jar where they later developed fungus and failed to hatch. The remaining eggs were put on straw mats and placed into a small holding pond on April 18. The pond was seined with a small-mesh seine on May 24. Two young buffalofish, both 23 mm. in total length, were collected, after considerable effort, and preserved. On November 10, 1957, this pond was drained and four buffalofish that averaged 179 mm. in standard length (Figure 19) were recovered.

The second artificial spawning was highly successful with an estimated 80-percent hatch from the 5,000-6,000 eggs obtained. Young buffalofish from this spawning were used in all of the observations on development and behavior. The survival of the young put into Hillcrest Golf Course Pond, McElroy Pond and the two ponds near Pawnee was seriously hurt by flooding of the ponds. On the night of May 20, 1957, a 7.5-inch rain in the Stillwater area caused widespread flooding with tremendous overflow and water exchange in the local ponds. Two days prior to this flooding, five young buffalofish had been seined in two short hauls from Hillcrest Golf Course Pond. On May 21, 30 minutes of continuous seining in the same small pond yielded only two young buffalofish. One buffalofish was collected on June 10th. Two additional seining efforts in this pond were unsuccessful.

In addition to water exchange in McElroy Pond, it was learned, from attempts to collect young buffalofish that sizable populations of golden shiner, Notemigonus crysoleucas (Rafinesque), green sunfish, Lepomis cyanellus Rafinesque, and bluegill sunfish, Lepomis macrochirus Rafinesque, were present in the pond. Specimens of the three species, large enough to prey on the buffalofish young, were seined from the pond. A total of six separate seining trips failed to yield any buffalofish.

Only one seining effort was made in one of the two ponds near Pawnee. This was in October and was unsuccessful.

Description of Eggs

The ripe buffalofish eggs were tannish-yellow when first emitted. The adhesive mucus-like shell or chorion of the eggs caused them to adhere to each other in small clusters or to adhere to any object which they touched. At the time of stripping, the size of an individual ovum was approximately 1.5 mm. The chorion rapidly absorbed water and became fully water-hardened in about one hour. The average diameter of 10 water-hardened buffalofish eggs was 2.1 mm. The eggs ranged in width from 1.9 to 2.5 mm. Stewart (1926) reported the diameter of white sucker eggs to be 2 and 3 mm. respectively before and after water absorption. The size differences in the eggs of these species may not represent the full range of variations as both groups of measurements were taken from eggs of a single female.

The chorion of many fish eggs is transparent (Rugh, 1948) whereas the chorion of buffalofish eggs is opaque. Opaqueness made the study of cleavage stages impossible without first removing the outer capsule. The heavily pigmented eyes of the fully developed embryos were the first structures detected through the opaque chorion (Figure 9). Opaqueness of the chorion may be characteristic of all suckers as Stewart (1926) mentioned the necessity of removing the "outer shell" while studying cleavage stages of the white sucker.

Incubation

On April 18, 1957, 5,000-6,000 fertilized buffalofish eggs were carried from the Holdenville State Fish Hatchery to a laboratory in Stillwater. Upon arrival in the laboratory the temperature of the water on the eggs had changed from 69° F. to 70.7° F.

Approximately 6 hours after fertilization 25 eggs were put into a finger bowl and kept in an incubator at 80° F. ± 1°. Within 83 hours from the time of fertilization three of the eggs had hatched and the eyes of the developed embryos could be seen in most of the other eggs. By the 95th hour after fertilization four additional eggs had hatched. The activity of hatching and the increased metabolism of the newly hatched larvae had apparently depleted the dissolved oxygen to a lethal point as all the larvae and eggs in the incubator were dead.

The remaining eggs were kept in aquaria. The observed temperature of the water ranged from 70.7° F. to 69.8° F. The first eggs had hatched by the 109th hour (April 22), however, most of the eggs hatched on April 23rd between the 130th and 140th hours (5½ days) after fertilization. The water temperature on April 23rd was 71.6° F. Walker and Frank (1954) reported the incubation period for buffalofish eggs at 12 days (288 hours) with water temperatures ranging from 57° F. to 62° F. Stewart (1924) reported the hatching of white sucker eggs kept at 50.5° F. at 21 days (504 hours).

Development of Young Buffalofish

Methods of Describing Young

Hubbs (1943) pointed out the generalized usage of the term larvae for all young fishes. He divided the developmental stages of young fish into embryo, prolarva, postlarva, alevin and juvenile. The term prolarva applies to the organism between hatching and yolk absorption. Alevin applies only to those fishes, such as the catfishes and salmonids, that become juveniles after absorption of the yolk sac. Juveniles are young that have an appearance similar to that of the adults. This leaves the interpretation of the postlarval stage, according to Hubbs, to those fishes which, after absorption of the yolk, are still strikingly unlike the juvenile stage. The vagueness of the postlarval stage is clarified by Winn and Miller (1954) who redescribed postlarvae as a period after yolk absorption when, "(1) a continuous finfold is present, (2) there are pigment characters different from those of the juveniles, or (3) the fins are absent, unossified or developed to various degrees."

A more comprehensive method of describing young fish was introduced by Oppenheimer (1937), who used numbers to differentiate between various developmental stages. Balinsky (1948) modified the Oppenheimer numbers into a table of stages for the Cyprinidae. Winn and Miller (1954) condensed Balinsky's table and used the condensed form as a basis for comparison between minnows and suckers. The condensed table used by Winn and Miller is presented as Table 1.

The following descriptions of young buffalofish are listed under the headings prolarvae, postlarvae and juveniles. Descriptions of development during the prolarval period are made on a time-interval basis, with reference to the age of the fish from the time of hatching. The sequence of developmental changes during the period of

Table 1

Comparative Stages of Development for American Minnows and Suckers
(Taken from Winn and Miller, 1954)

Stage 1. Unfertilized egg.

Stages 2-27. Development of fertilized egg. Hatching occurs at about stage 27.

Stages 28-32. Prolarval stage with yolk sac.

Stage 33. Yolk is assimilated; no caudal fin rays present.

Stage 34. End of notochord straight; caudal fin with 3-4 rays; no trace of dorsal or anal fin.

Stage 35. End of notochord upturned; caudal fin with 7-9 rays; elevated fin membrane at site of future dorsal and anal fins.

Stage 36. Dorsal and anal with rudimentary, cartilaginous rays; caudal fin rounded, nearly complete, with 15-17 rays. Air bladder subdividing.

Stage 37. Rays of dorsal and anal fins partly ossified; caudal emarginate, with 19 principal rays; rudiments of pelvics appear. Air bladder subdivided into two parts.

Stage 38. Pelvic fins in the form of crescentic folds.

Stage 39. Pelvic fins in the form of paddles, with no trace of rays.

Stage 40. Pelvic fins with rudimentary, unossified rays.

Stage 41. Pelvic rays partly ossified. Continuous finfold reduced.

Stages 42-46. Finfold lost, scales develop, and lateral-line canal forms.

yolk absorption, occurs in a fixed pattern. The sequence is evident when comparing individuals of the same age raised under similar temperature conditions. However, after absorption of the yolk and feeding by mouth begins, age comparisons become unreliable because two postlarval buffalofish of exactly the same age may vary considerably in rate of development. For these reasons descriptions of the postlarval and juvenile stages are made on a size-interval basis.

Prolarvae

The prolarval buffalofish (Appendix Figure 1) were between 5.5 and 6.5 mm. long at the time of hatching. They had an average of 27 myotomes and the only pigmentation was in the eyes. The eyes were quite ventral and the anterior portion of the head was reflexed. The pericardium formed a conspicuous sac along the ventral profile between the head and yolk material. A bilobed yolk sac was present and the ventral finfold projected anteriorly only to the constriction between the two lobes. There was an inverted V-shaped notch in the ventral finfold at the point where the anus develops. The finfold was rounded on the caudal fin. The gills and otic vesicles were present but not prominent. There was no external opening into the buccal cavity.

60 hours. The most obvious change observed at the 60-hour stage was in the yolk sac. The yolk sac had been

reduced to a single lobed structure. The finfold had developed anteriorly to a point just behind the pericardium and covered the ventral margin of the yolk (Figure 2). The number of myotomes had increased to 30. A small straight-tubed intestine was present above the yolk material and the air bladder was developing above the intestine dorsal to the anterior end of the yolk. The eyes were less ventral. The external gills and otic vesicles were becoming prominent structures.

98 hours By the end of the 4th day (98 hours) the average length of the prolarvae was 7 mm. and the mouth orifice was present. The mouth was obliquely terminal, with the upper margin almost horizontal with the upper edge of the eye. The yolk was reduced to about one-third its original size. The first melanophores, other than those in the eyes, were visible on the dorsal wall of the air bladder and on the ventral wall of the otic cup.

120 hours At the 120-hour stage there was a small amount of yolk left. The lateral pigment line and the mid-dorsal pigment line were apparent (Figure 10).

144 hours By the end of the 6th day (144 hours) after hatching, the midventral pigment line and a row of internal chromatophores along the dorsal intestinal wall had developed. The latter pigmented area was called the ventro-visceral pigment line by Balinsky (1948). The number of myotomes varied from 30-32 and the average length was 7.5 mm. Since there was no yolk material left, the

prolarval period terminated sometime about the 5th and 6th days. The 144-hour stage buffalofish larva corresponds most closely to stage 33 in Table 1.

Postlarvae

8 mm. The 8 mm. postlarvae had developed a stomach. Two to four rudimentary fin rays were present in the pectoral fins. No rays were yet evident in the caudal fin (Figure 11).

10 mm. The number of myotomes apparently became fixed between the 8 and 10 mm. size as a range of 31 to 33 myotomes was counted in fish 10 mm. in length. No count above 33 myotomes was made in any of the buffalofish. By the 10 mm. stage there were 3 to 5 rudimentary rays developed in the caudal fin. The notochord was becoming upturned at the posterior end and the opercula were beginning to develop over the external gills (Figure 4). One pair of branchiostegal rays was present. A constriction on the ventral surface of the air bladder indicated the beginning of its division. The finfold was still prominent, but contained no elevations showing the formation of the dorsal or anal fin. The 10 mm. buffalofish probably approximates most closely Winn and Miller's stage 35, however, there appeared to be some significant differences in that the notochord had become upturned and no dorsal or anal fin formation was present.

13 mm. At the 13 mm. stage the caudal fin had become

forked (Figure 3) and had 10 to 12 partly ossified caudal fin rays. The dorsal fin had developed (apparently as an elevation of the dorsal finfold) and contained 6 fin rays. The anal fin was beginning to show on the ventral finfold. Fleshy pelvic fin buds were seen along either side of the ventral finfold at a point below the origin of the dorsal fin. The mouth had descended slightly ventrad from its original position (Figure 6). The suborbital lateral-line pores were evident and the opercula completely covered the gills. Winn and Miller (1954) pointed out the peculiarity that catostomids have external gills which normally become covered between stages 37 and 39. The 13 mm. postlarval buffalofish corresponded most closely to stage 39 in Table 1, however, the appearance of lateral-line pores in a postlarval stage may represent a significant difference. Winn and Miller (1954) first mention the lateral-line system in association with juveniles. Variations in the time of appearance of some larval characters for 5 species of suckers considered by Winn and Miller (1954) are compared with the buffalofish larvae used in this study (Table 2).

Juvenile

The juvenile stage is characterized by the loss of the finfold and by the formation of scales and lateral-line canals (Table 1). The juvenile period in buffalofish was also characterized by the completion of changes in position of the mouth. This metamorphosis in mouth position began during the later stages of the postlarval period. It occured in association with marked changes in the external nares (Figures 5 to 8).

17 mm. The 17 mm. buffalofish possessed characters of both the postlarval and juvenile stages. The fins were fully developed and ossified, however, a remnant of the ventral finfold was present from a point between the pelvic fins to the anal opening. No scale formation was evident.

23-27 mm. No fish were observed which showed the first appearance of scales. However, juveniles 23, 25 and 27 mm. in total length exhibited the general pattern of scale formation. It should be noted that the scale patterns were determined primarily from location of the scale pockets. The scale pockets presented a pattern visible under a magnification of 20 diameters. The scale buds within the pockets lacked ossification to take a sufficient stain of alizarin red, a technique used by Ward and Leonard (1952) for scale determination on black crappie, Pomoxis nigromaculatus (LeSueur). Although enough specimens of the critical-size buffalofish were not preserved to make conclusive comparisons, it appeared that the completion of squamation was closely associated with the completion of the ventral movement in mouth position.

Table 2

Comparisons of Variations in the Time of Appearance of Some Larval Characteristics for Five Suckers Considered by Winn and Miller (1954) and Larval Buffalofish. Sizes Are Given as Total Length in Millimeters.

Character	*Catostomus insignis*	*Catostomus latipinnis*	*Pantosteus clarki*	*Pantosteus delphinus*	*Xyrauchen texanus*	*Ictiobus sp.*
Opaque areas first appear in D. and A. fins	10.0-12.0	$\pm$ 11.5-12.5	11.5-12.5	11.0-11.5	$\pm$ 11.0	12.0-13.0
D. and A. rays completely ossified	18.5-20.5	18.5-20.5	$\pm$ 16.0	16.5-18.0	$\pm$ 17.0	17.0
Pelvic buds appear	16.5-17.5	15.5-16.5	15.0-16.0	15.0-16.0	$\pm$ 15.0	13.0
Upturning of posterior tip of vertebral column	Before 12.0	Before 12.0	Before 11.5	9.0-10.5	8.0-9.0	10.0

General Behavior of Young Buffalofish

The newly-hatched buffalofish had a definite behavior pattern. Upon emerging from the egg, the prolarva immediately worked its way to the surface by a series of lateral thrusts of the caudal fin. After reaching the surface film the prolarva relaxed for a few seconds during which time it settled, then resumed the caudal fin thrusts and again made its way to the surface. In aquaria which contained several prolarvae this continuous motion gave the impression of wriggling mosquito larvae. Throughout this entire activity the prolarvae maintained the long axis of the body in a vertical position. In two inches of water the prolarvae usually settled to the bottom of the aquaria between thrusts to the surface. In six inches of water the prolarvae rarely settled to the bottom without intermittent thrusts toward the surface. A prolarva at a depth of six inches usually reached only part way to the surface before it relaxed, settled a short distance then continued toward the surface. In no instances were prolarvae observed to remain motionless more than four or five seconds.

The activity is apparently instinctive and not a physiological necessity. Eight newly-hatched prolarvae were put into a small plastic dish which was inverted on the bottom of one of the three-gallon aquaria that contained six inches of water. Care was taken to avoid capturing any air under the dish. The prolarvae were free to swim within the dish, but could not reach the surface of the water. The prolarvae continued to swim in their vertical orientation. They rose, bumped the surface of the dish, relaxed, settled to the bottom and rose and bumped the dish again. All prolarvae both within and outside the dish continued to swim upward in the manner described. The prolarvae under the dish were still bumping the surface 48 hours later. The eight prolarvae appeared to have developed normally although they were prevented from rising to the surface of the water.

The cessation of the vertical motion was concomitant with absorption of the yolk material. Between the fourth and fifth days after hatching, the prolarvae gradually began to swim toward the surface at an oblique angle. The six-and seven-day-old prolarvae had completely absorbed the yolk, had completely abandoned the thrusting motions, and were swimming horizontally at or near the surface. When aeration created a slight current, the early postlarvae concentrated along the edge of the aquaria near the surface and orientated themselves facing the current.

A seven-day-old postlarva ingesting "micrograin" from the surface of the water was the first observed feeding. Nine-day-old postlarvae, approximately 8 mm. long, were seen to pick small bits of "micrograin" off blades of <u>Vallisneria</u> in the aquaria. No postlarvae were observed to feed from the bottom of the aquaria. On the tenth day net plankton was added to the diet of the postlarvae. The plankton was not examined critically but various Copepoda and Cladocera were recognized when the plankton was added to the aquaria. None of the plankters were seen to be ingested, however, small protozoon and algal forms were undoubtedly utilized by the postlarvae.

Juvenile buffalofish, on several occasions, were observed feeding on the larger plankters. On one occasion, when no plankton could be seen in the aquaria and none had been added during the preceeding five-day period, a large amount was strained and put into the aquaria. The juveniles, between 16 and 20 mm. long began at once to feed vigorously on the larger Copepods and Daphnia. The juveniles swam and darted vigorously about at all depths ingesting the plankton. They were very unsucker-like in appearance but resembled a group of feeding minnows.

The actual ingestion of individual plankters was accompanied by an anterior and sometimes slightly lateral quick jerk or lunge. During the feeding process the young buffalofish projected its mouthparts and appeared to "pick" the plankters from the water. The quickness of the ingestion movements made accurate observation difficult, however, the projection of the mouthparts was essentially a terminal projection rather than a ventral one common to the adult small-mouthed buffalofish. (The term small-mouthed buffalofish is used to refer collectively to _I. bubalus_ and _I. niger_.)

Taxonomy

Adults

The confused taxonomy of the small-mouthed buffalofish in Oklahoma became more fully realized when the adults in the collections from Illinois River and Red River were examined critically. When first captured, the fish could not be examined in all details and certain critical counts and measurements could not be made until the breeding experiments were completed. The characters given in taxonomic keys which seemed to more clearly separate the buffalofish into *I. bubalus* and *I. niger* were used to separate the brood stock. Field characters used were the relative height and thickness of the back and the relative size of the mouth. General appearance of the individual fish had to be used because exact measurements could not be made on live specimens.

After critically measuring the individuals of both collections, identification of the small-mouthed buffalofishes appears doubtful. Values of the variation ranges for particular characters overlap for *I. bubalus* and *I. niger* in most of the recent taxonomic keys. Projected body depth divided into standard length is an important character listed in most keys. This character is an expression of the relative height of the back in relation to the length of the fish. *Ictiobus bubalus* is generally considered as a high backed (deeper bodied) species and *I. niger* a low backed (thicker bodied and more rounded) species. In a key by Bailey (1956) the ranges used to express this character are 2.2 to 2.8 for bubalus and 2.6 to 3.2 for niger. Trautman (1957) listed the ranges for the same character at 2.2 to 3.0 for bubalus and 2.6 to 3.6 for niger. Hubbs and Lagler (1949) stated the values at "about 2.5" for bubalus and "about 3.0" for niger.

Table 3 shows the values for the projected body depths taken into standard lengths for the buffalofish from Illinois River and Red River. Collectively considering the two collections from Oklahoma all but one individual falls within the range of bubalus. However, a breakdown of the two groups shows that 50 percent of the fish from Illinois River are also in the range of niger and that 81 percent of the fish from Red River fall within the niger range. Considering only this one key character 69 percent of all the fish in the two collections could be identified as either bubalus or niger.

A second character used in many keys is the head width divided into standard length. Ictiobus niger is generally considered to be much wider at the opercular flaps in relation to body length than I. bubalus. Table 4 shows the relationships of this character to the specimens of the two collections under consideration. All but one individual in the two collections fall within Trautman's range for niger. Fifty percent of the fish from Illinois River and 48 percent of the fish from Red River

Table 3

Comparison of Buffalofish from Red River and Illinois River. Projected Body Depth Divided into Standard Length. Ranges for I. bubalus and I. niger Taken from Trautman (1957).

	Projected Body Depth into Standard Length	Buffalofish Illinois River	Buffalofish Red River
	2.2		
	2.3		
I. bubalus	2.4**	2	
	2.5	7	4
	2.6	4	9
	2.7	3	9
	2.8	1	3
	2.9	1	1
	3.0*		
	3.1		1
I. niger	3.2		
	3.3		
	3.4		
	3.5		
	3.6		

* and ** Tables 3, 4, 5, 6, 7 and 8 each have these symbols identifying particular points on the scale of values representing the character depicted in each graph. This is to identify two particular positions regarding each character with the two individual fish shown in figures 16 and 17. These two fish represent the most niger-like and bubalus-like fish in general appearance that were obtained during this study.

* Represents the niger-like fish A in figures 16 and 17. This fish was selected from 3,178 small-mouthed buffalofishes which were taken from Fort Gibson Reservoir during a rough fish removal project conducted by the Oklahoma Wildlife Conservation Department in the summer of 1957.

** Represents the bubalus-like fish B in figures 16 and 17 collected from the Illinois River at the upper end of Tenkiller Reservoir on February 10, 1957.

Table 4

Comparison of Buffalofish from Red River and Illinois River. Head Width Divided into Standard Length. Ranges for *I.* *bubalus* and *I.* *niger* Taken from Trautman (1957).

	Head Width Projected into Standard Length	Buffalofish Illinois River	Buffalofish Red River
	4.6	1	
	4.7	1	2
I. *niger*	4.8*	1	1
	4.9		2
	5.0**	4	3
	5.1	2	6
	5.2	4	5
	5.3	4	6
	5.4	1	2
	5.5		
	5.6		
	5.7		
I. *bubalus*	5.8		
	5.9		
	6.0		
	6.1		

*, ** See Table 3

also fall within the range of *bubalus*. Collectively 49 percent of the fish could be either species when only this one character is considered.

Figure I is a scattergram with the character, head width divided into standard length, plotted against the character, projected body depth divided into standard length, and the values thus obtained represent each fish from both collections. Vertical and horizontal lines drawn from the extremes of the overlapping sections of the two ranges produced nine rectangles. Each rectangle represents values of varying taxonomic significance.

Comparison of Buffalofish From Red River and Illinois River Using Head Width and Body Depth Divided into Standard Length. Ranges for I. bubalus and I. niger Taken from Trautman (1957).

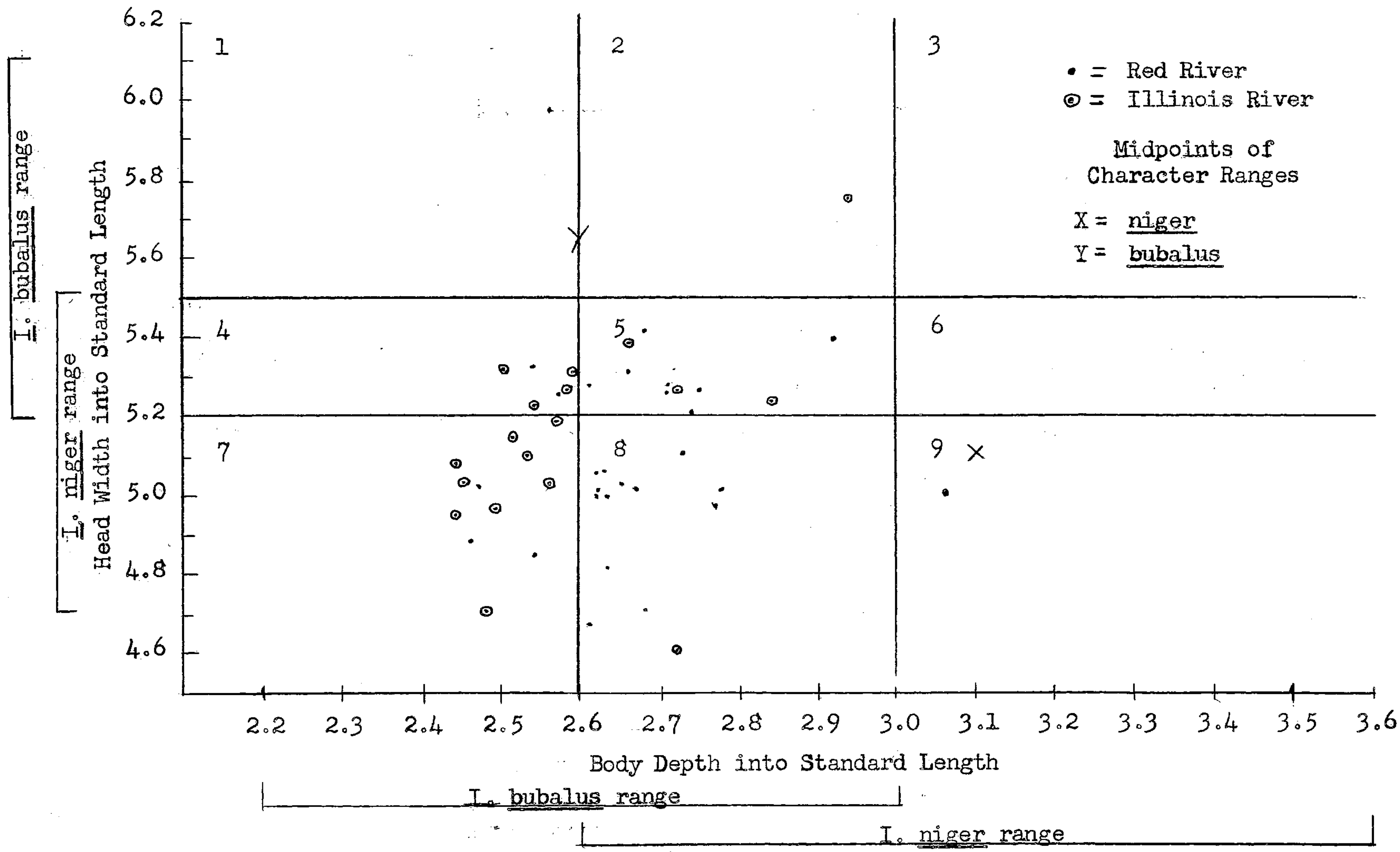

Figure I

(1) Rectangle 1 represents values which should be found only in _bubalus_. Rectangle 9 represents values which should be found only in _niger_. (2) Values in rectangle 2 could be considered _bubalus_ if we disregard the fact that the values also lie in the range of body depth divided into standard length for _niger_. Values in rectangle 8 could be _niger_ if we disregard the fact that the values also lie within the ranges of body depth divided into standard length for _bubalus_. (3) Completely disregarding one of the two characters, values in rectangles 3 and 7 could represent either _niger_ or _bubalus_, however, considering the characters as complements of each other, values in the areas could not represent either species. (4) Values in rectangle 4 might be considered _bubalus_, if we disregard their inclusion within the range of head width into standard length for _niger_. Conversely the same may be said about rectangle 6 in relation to _niger_. (5) Rectangle 5 represents the area where the values of the overlapping sections of the characters for both species meet and the fish with values in this area would be unidentifiable.

Assuming that the characters used are valid, and that the ranges listed approximate the actual differences in the two species, a certain percentage of a random sample of a normal population of _bubalus_ or _niger_ would fall within the area of rectangle 5. A proportionate part of this sample would also fall within some of the other rectangles.

By connecting the median values of the two characters for *niger*, point X is located and point Y represents a similar value for *bubalus*. Assuming that the coinciding median values of the two characters would represent the mean black or smallmouth buffalofish, a normal population of either species should have a concentration of individuals radiating around points X or Y shown in Figure 1.

Limitations of the two samples are herein recognized. The samples were not taken by random methods and they are much too small to constitute proof that *I. bubalus* and *I. niger* do not represent two valid species. The selectivity of the samples should have concentrated the individual fish more closely around points X and Y than would a random sample.

Tables 5 and 6 show relationships between the individuals in the two collections and the two additional characters listed by Trautman (1957) for which a range of values were given. Table 5 shows that all but one fish from both collections fall within the range of *niger* for eye size projected into head length. Forty-four percent of the fish from Illinois River and 74 percent of the fish from Red River are also in the range of *bubalus* for this character. Collectively, using this one character, 62 percent of both groups could be either species.

Length of orbit taken into snout length (Table 6) showed less overlap in the ranges of the two species than any of the other characters. Combining the two collections

Table 5

Comparison of Buffalofish From Red River and Illinois River. Eye Size Projected into Head Length. Ranges for *I. bubalus* and *I. niger* taken from Trautman (1957).

	Eye Size into Head Length	Buffalofish Illinois River	Buffalofish Red River
	4.4		
	4.5		
	4.6		
I. bubalus	4.7		
	4.8		
	4.9		
	5.0		1
	5.1	1	
	5.2		1
	5.3	1	
	5.4		2
	5.5		3
	5.6	1	3
	5.7	2	2
	5.8	3	4
	5.9		4
	6.0	1	1
	6.1	2	2
	6.2**	1	1
I. niger	6.3	2	1
	6.4	2	1
	6.5	1	
	6.6	1	
	6.7		1
	6.8		
	6.9*		
	7.0		
	7.1		
	7.2		
	7.3		
	7.4		

*, ** See Table 3

Table 6

Comparison of Buffalofish from Red River and Illinois River. Eye Size Divided into Snout Length. Ranges for *I. bubalus* and *I. niger* Taken from Trautman (1957).

	Eye Size into Standard Length	Buffalofish Illinois River	Buffalofish Red River
	1.5		
	1.6		1
I. bubalus	1.7		2
	1.8	1	6
	1.9	2	8
	2.0	5	1
	2.1**	6	4
	2.2*	2	2
I. niger	2.3	2	3
	2.4		
	2.5		

*** See Table 3

Table 7

Comparison of Buffalofish from Red River and Illinois River. Body Depth Taken Over Body Curvature (Figure 14) Divided into Standard Length.

Actual Body Depth into Standard Length	Buffalofish Illinois River	Buffalofish Red River
1.9	2	
2.0**	8	5
2.1	5	17
2.2*	1	3
2.3		1
2.4	1	1
2.5	1	

*** See Table 3

and using this character, 58 percent of the fish could be bubalus, 47 percent could be niger and 13 percent could be either species. Considering the collections separately, the species separation is as follows: Red River 67 percent bubalus, 37 percent niger and 4 percent either; Illinois River 44 percent bubalus, 83 percent niger and 28 percent either.

An attempt was made to find additional characters which might more distinctly separate the specimens of the two collections. The following counts and measurements were made and analyzed; number of fin rays on each fin and number of fin rays collectively on each fish, rows of scales above and below the lateral line, scales in the lateral line, rows of scales around the caudal peduncle, distance from the anterior-most edge of the sub-opercle bone to the orbital rim and the distance from the anterior-most edge of the gill cleft to the orbital rim divided into standard and head length, distance from the mandibular symphysis to the lower end of the maxilla divided into diameter of the eye, length of maxilla divided into head and standard length and the body depth measured over body curvature divided into standard length. None of these characters tended to significantly separate the buffalofish. Most of the characters actually grouped the specimens in the two collections more closely than did the data presented for the characters used by Trautman (1957). Tables 7 and 8 show two such characters that closely group the specimens.

Table 8

Comparison of Buffalofish from Red River and Illinois River. Length of Maxilla Divided into Head Length.

Length of Maxilla into Head Length	Buffalofish Illinois River	Buffalofish Red River
3.2		3
3.3*	1	2
3.4**	6	13
3.5	5	3
3.6	4	3
3.7	1	2
3.8	1	1

*,** See Table 3

Buffalofish from Known Parents

Only four young, from known parentage, large enough to compare with the parents were obtained. The young averaged 178.5 mm. in standard length at 6½ months old. The parents averaged 431 mm. in standard length at the time of spawning. The male parent had a projected body depth into standard length of 2.8, while the female parent was 2.7 for the same character. The young ranged from 2.3 to 2.5 for the same character. Comparing head width into standard length the male parent was 5.2 and the female parent 5.4. The young were 5.5, 5.6, 5.6, and 6.0 for the same character. Considering these two key characters, the adults might be considered niger and their offspring might be considered bubalus. Pictures of the parents and young are shown in figures 18 and 19.

DISCUSSION

The original purposes of the study, to observe the development and behavior of the young of _I. bubalus_, _I. niger_ and a hybrid between the two species and to gain insight into the relationships between the forms, were only partially accomplished. A supposed hybrid cross was made and the development and behavior of young buffalofish resulting from the cross were observed. Detailed measurements and other examinations of the parent fish failed to confirm that the parents were two distinctly different forms. The specific identity of the young is not known. Failure to obtain additional young from the attempted natural propagation by the adults collected from the Red River and the Illinois River prevented any comparisons of the development between the different forms.

The larval development of the young buffalofish observed was in general agreement with the larval development of other suckers (Stewart, 1926 and Winn and Miller, 1954).

The confusion of taxonomic overlap in attempts to identify the adult buffalofish used in the study is indicative of one of two conclusions. Either none of the characters or combinations of characters used were sufficient to separate the fish into two separate species, or the fish did not represent two distinct species. Assuming from the data presented that the fish did not represent two species, which of the two recognized forms, bubalus or niger, do they represent? Other considerations seem appropriate. Are there actually two valid species of small-mouthed buffalofish in Oklahoma? If there are two species, what effects have the changes from a stream environment to a reservoir environment had on individuals?

During the summer of 1957, 3,178 small-mouthed buffalofish were collected and observed from Fort Gibson, Tenkiller and Texoma Reservoirs in Oklahoma. It was estimated that had detailed examinations of the fish been made, no more than 20 percent of the total number could have been designated either as bubalus or niger. This points out the following discrepancy regarding the taxonomy of these forms.

Museums have been restricted in the number of large specimens they could keep and these suckers attain large sizes. Consequently the collections of large buffalofish have been limited to a few individuals. These few individuals may have been identified as either or both of the two species under consideration. However, all of our various concepts of a species are based on the natural populations and not selected individual specimens. It seems doubtful that the small-mouthed buffalofish in Oklahoma reservoirs can be assigned to either bubalus or niger or combinations of both.

It seems that there is only one highly variable species of small-mouthed buffalofish. The two extremes of this variable species would represent the bubalus and niger forms presently recognized. The variation of the individual fish toward one or the other of the two extremes might be enacted by ecological differences in the habitat or environment. In rivers, the fish that occupied the swifter waters could have a smaller mouth and deeper body (bubalus is considered a swift water form, Trautman, 1957) and the other fish that occupied the quieter regions of the rivers could develop a slightly larger mouth and more narrow body (niger is considered to occupy the eddies and backwaters of streams, Trautman, 1957). Therefore, in reservoirs, the small-mouthed buffalofish would not have the diversity of habitat for development of classical "smallmouth or black" forms. Additional study over the entire range of the small-mouthed Ictiobinae would be necessary to evaluate the above-mentioned theorizations.

Hybridization is another approach to an explanation of the confusion of the forms, assuming there are two species. One of the original purposes of the study, to produce a known hybrid between bubalus and niger, was not attained. Young buffalofish from known parents were obtained, however, they could not be called hybrids because the adults could not be positively identified. As previously stated, the young appear more bubalus-like and the parents more niger-like. Failure to produce a hybrid leaves the question open. As pointed out by Hubbs et. al. (1943), ". . .it would be hazardous to identify hybrids between I. niger and I. bubalus". Where two highly variable fishes are suspected of hybridizing, and the supposed hybrids can not be separated from the overlapping variability of the parent species the validity of the parent species seems questioned. This is true particularly if the supposed hybrid produced normal gametes and exibited no normal hybrid reproductive limitation.

If Ictiobus niger and I. bubalus are valid species it appears likely that hybrid introgression has all but eliminated species separation in many Oklahoma waters.

SUMMARY

1. Two collections of large adult buffalofish were put into ponds and attempted crosses by natural propagation between individuals from the collections were unsuccessful.

2. Cross breeding between individuals from the two collections was accomplished by artificial propagation. The resulting offspring could not be designated hybrids because of the inability to identify the parent fish.

3. Studies of the developmental patterns of larval buffalofish are presented.

4. Instinctive behavior patterns of larval buffalofish were observed and recorded.

5. Detailed counts and measurements were made on the adults, but definite identity was not established. Tables are presented to show the intermediacy of these adults between "classical" bubalus and niger.

6. The present status of the small-mouthed buffalofish in Oklahoma is discussed.

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APPENDIX

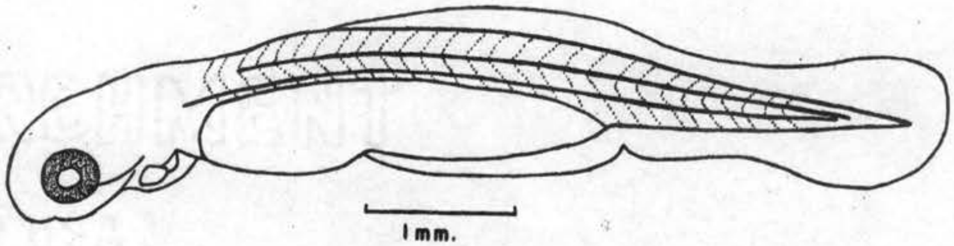


Figure 1

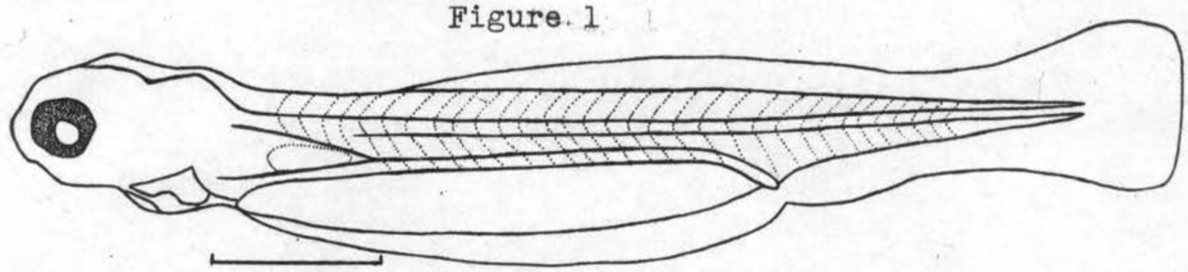
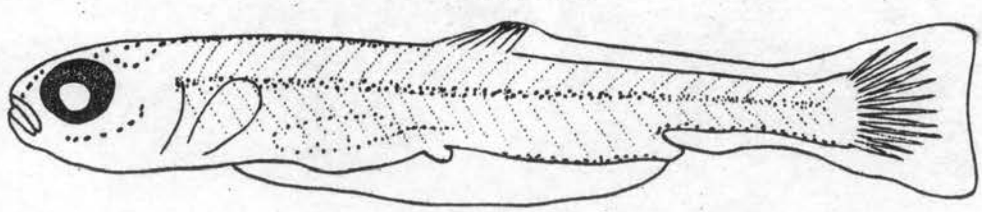


Figure 2



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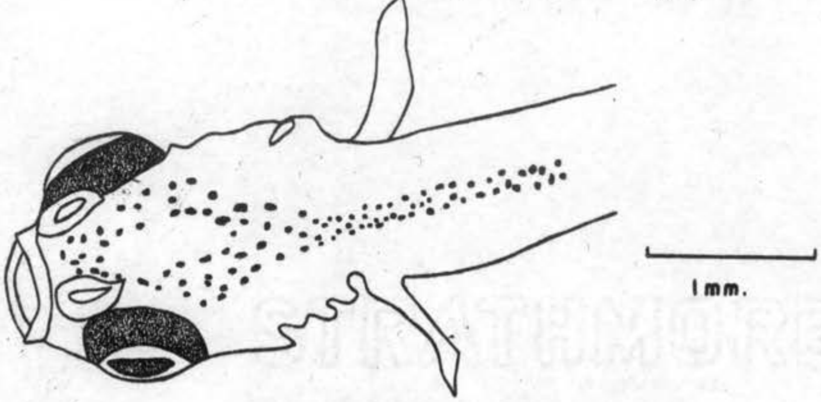

Figure 4

Figure 5. Front View of 10 mm. Postlarva Showing Terminal Mouth and Large Single External Nares

Figure 6. Front View of 13 mm. Postlarva Showing Mouth Beginning to Drop Ventrally and the External Nares Beginning to Divide

Figure 7. Front View of 17 mm. Juvenile Showing Further Ventral Movement of Mouth and Dividing of Nares

Figure 8. Front View of 27 mm. Juvenile Showing External Nares Completely Divided and Still Further Ventral Movement of Mouth

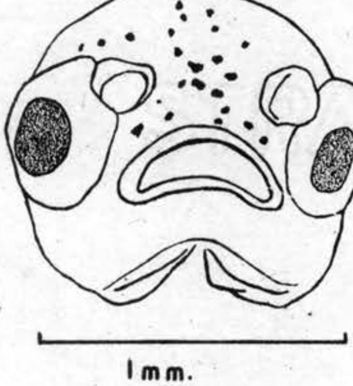

Figure 5

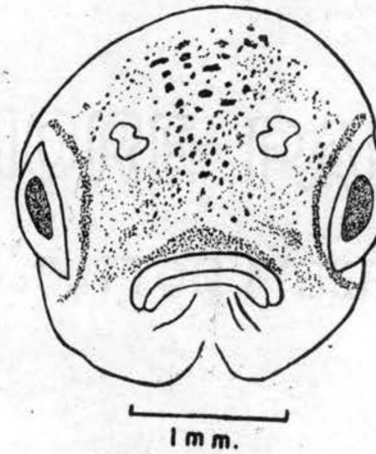

Figure 6

Figure 7

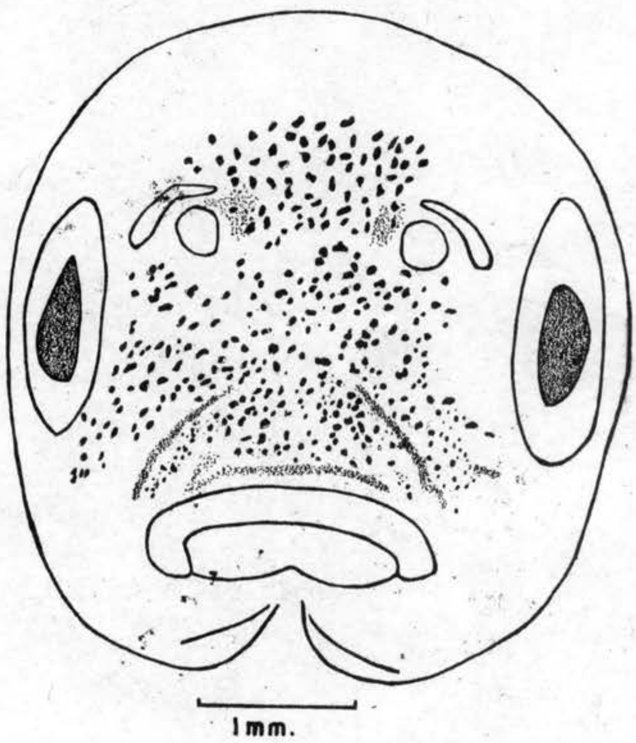

Figure 8

Figure 9. Buffalofish Egg Fully Developed and Ready to Hatch. Dark Area is Eye Seen Through Opaque Chorion

Figure 10. Buffalofish 120 Hours Old Showing Development of Lateral and Dorsal Pigment Lines

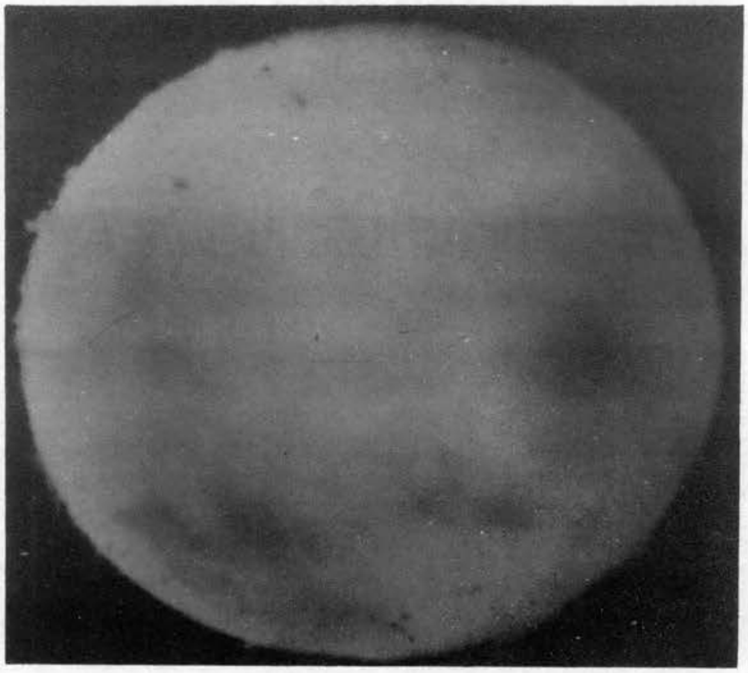

Figure 9

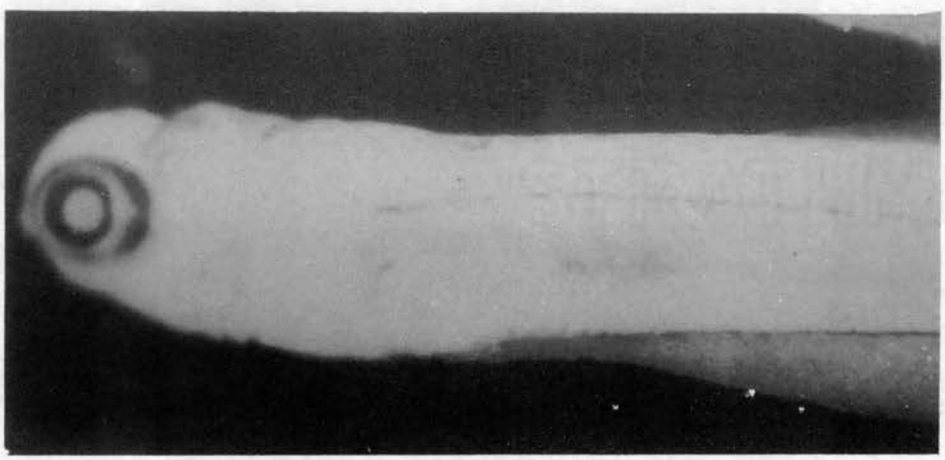

Figure 10

Figure 11. Postlarva Buffalofish 8 mm. in Total Length

Figure 12. Front View Postlarva Buffalofish 10 mm. in Total Length

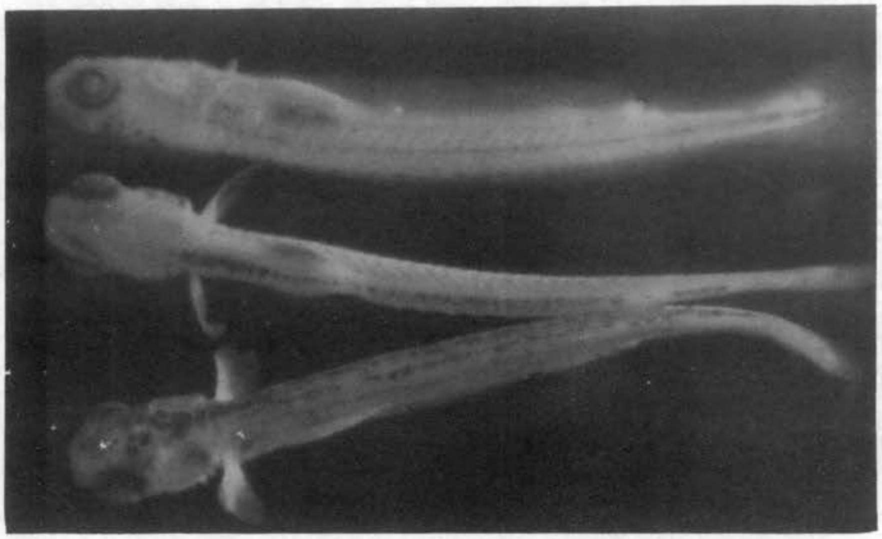

Figure 11

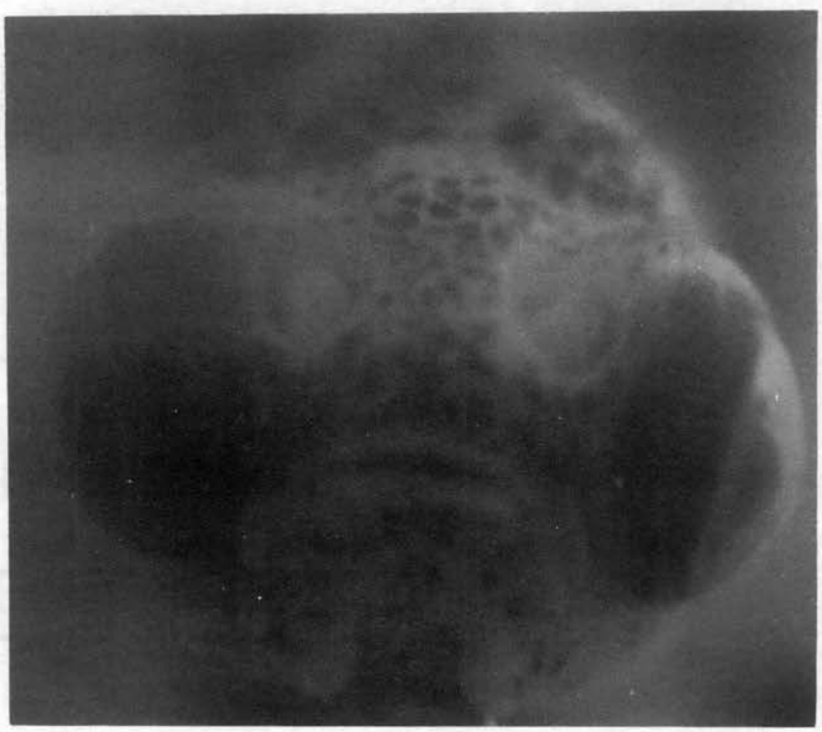

Figure 12

Figure 13. Use of Wide Jawed Calipers in Taking Measurements to Avoid Influence From Body Curvature

Figure 14. Body Depth Measurement Taken Over Body Curvature Using Nylon Cord

Figure 15. Projection of Measurement Taken in Figure 14 onto Linear Scale. This Distance is Divided in Half for Body Depth Taken Over Body Curvature

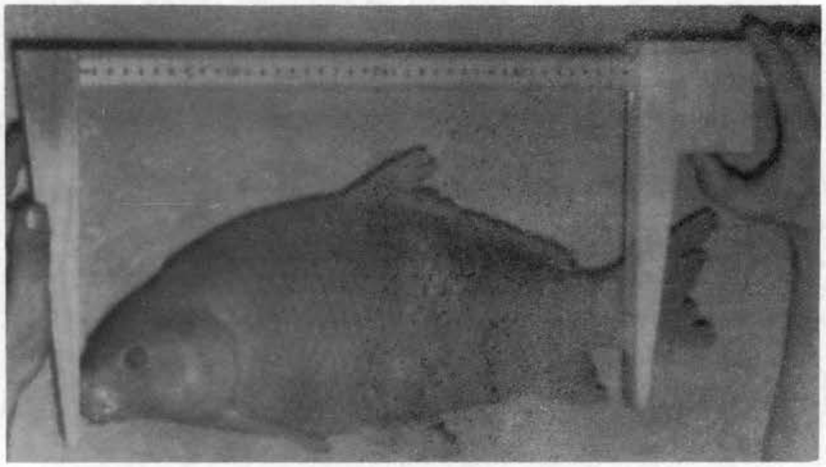

Figure 13

Figure 14

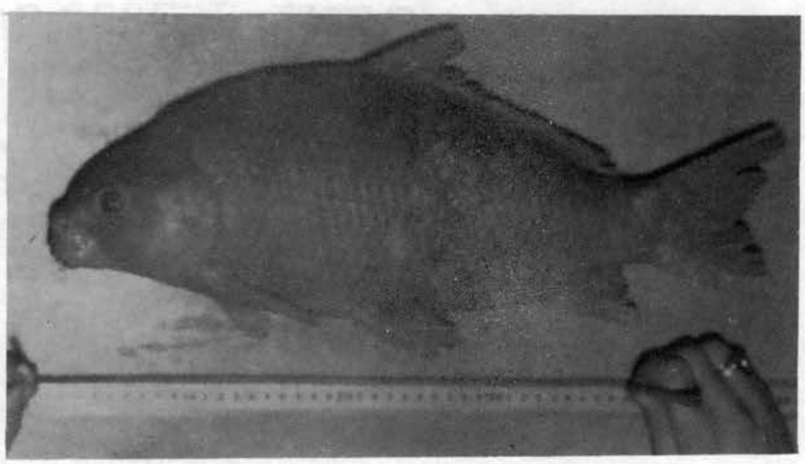

Figure 15

Figure 16. "Classical Types" of Black and Smallmouth Buffalofish. A, *Ictiobus niger*; B, *I. bubalus*

Figure 17. Dorsal View of A and B in Figure 16

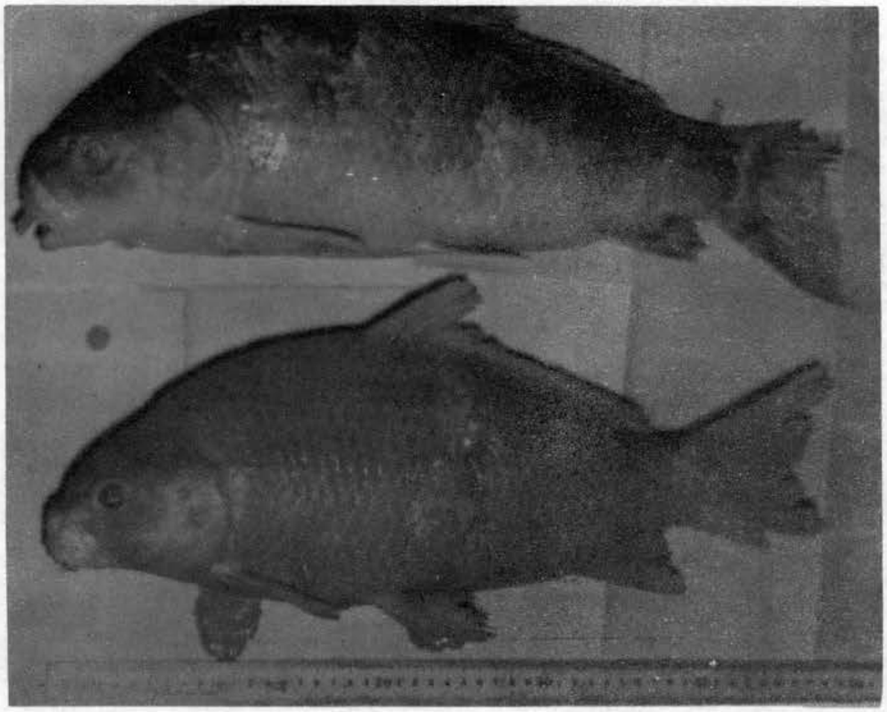

Figure 16

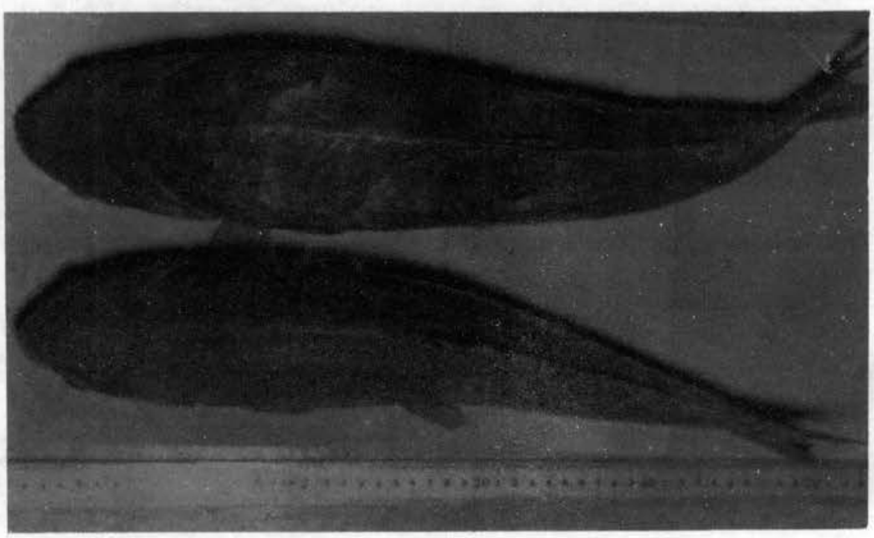

Figure 17

Figure 18. Parent Buffalofish Used in Artificial Spawning. A, Male Parent; B, Female Parent

Figure 19. Six and One-Half Months Old Young from the Parents Shown in Figure 18

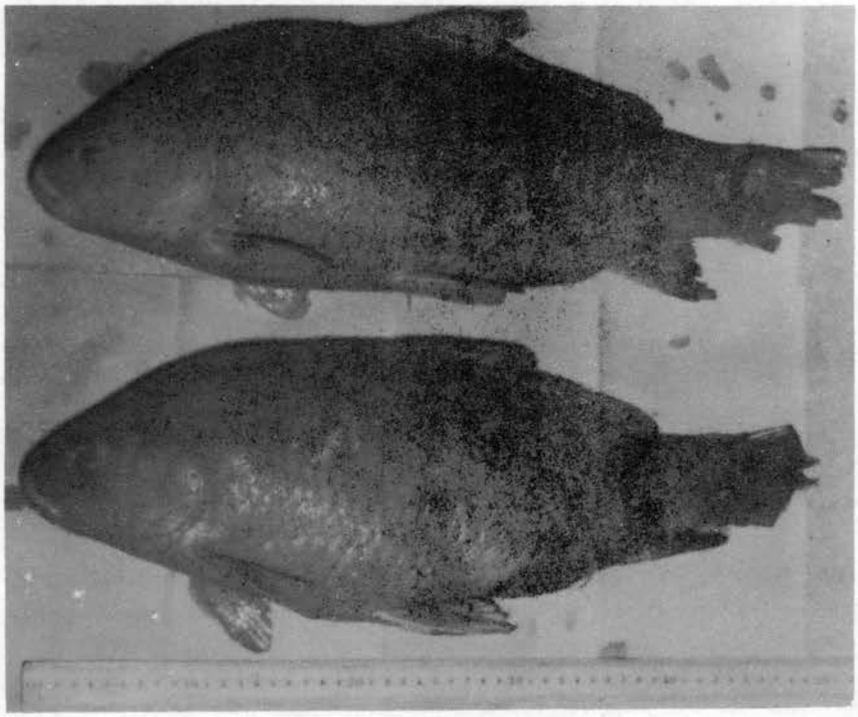

Figure 18

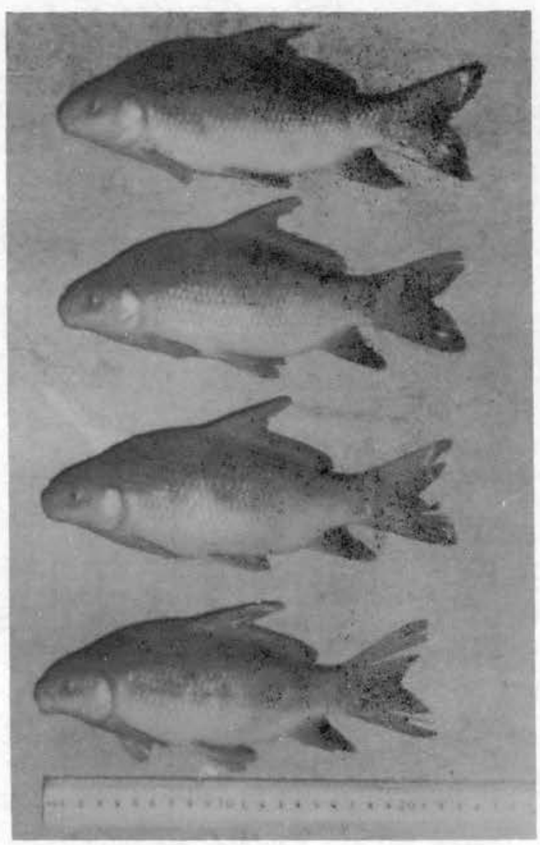

Figure 19

VITA

William Ray Heard

Candidate for the Degree of

Master of Science

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Major Field: Zoology

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Professional experience: Summer of 1954 as Nature Instructor at Philmont Scout Ranch; employed as Assistant Project Leader, Ft. Gibson Fishery Investigations with Oklahoma Wildlife Conservation Department from May 1955 until September 1956; summer of 1957 as Fishery Biologist with Oklahoma Wildlife Conservation Department; summer of 1958 as Fishery Aid with U.S. Fish and Wildlife Service, Brooks Lake, Alaska; five semesters, 1956 through 1958, as Graduate Teaching Assistant, Department of Zoology, Oklahoma State University. Member: American Fisheries Society, Wildlife Society, Oklahoma Academy of Science and Oklahoma State University chapter Sigma Xi.