THE EMBRYOLOGY, EGG STRUCTURE, MICROPYLE AND

EGG MEMBRANES OF THE PLAINS MINNOW,

HYBOGNATHUS PLACITUS (GIRARD)

By

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CHAPTER I

INTRODUCTION

This study was undertaken to describe the egg structure, micro-
pyle and egg membranes of the plains minnow, Hybognathus placitus
(Girard) and to elucidate the embryology of this little studied
species.

The plains minnow is an important bait minnow in Oklahoma. Dur-
ing years of peak abundance many thousands of individuals are col-
lected by commercial minnow dealers from the Arkansas, Cimarron, and
Red Rivers in Oklahoma.

Little is known concerning the egg structure and development of
species of Hybognathus. Most of the studies of species in the genus
are of a taxonomic nature. Raney (1939, 1941) described the breeding
behavior and propagation of Hobognathus nuchalis (Agassiz). No refer-
ence to previous studies concerning the ovary, egg structure, micro-
pyle, egg membranes or embryology was found for Hybognathus.

CHAPTER II

REVIEW OF LITERATURE

Egg Structure, Micropyle and Egg Membranes

Several workers have studied the development of the egg and seasonal changes in the ovaries of fishes. Wheeler (1924) described the development of eggs in the dab, Pleuronectes limanda (Linnaeus) and demonstrated the presence of Golgi bodies in the oöplasm. He suggested that the Golgi bodies function in yolk production. Hann (1927) described the formation of the ovary and eggs of Cottus bairdii (Girard) and found that the oögonial divisions began at the time of sex differentiation.

Marza et al. (1937) made detailed histochemical studies on the ovary and eggs of Fundulus heteroclitus (Linnaeus) and found that structural changes in the oögonia were correlated with metabolic activities leading to food storage. Matthews (1938), James (1947), Cooper (1952), Narian (1956) and Shelton (1964) discussed the structural changes in the maturing ova of several species of fishes.

Samuel (1943), James (1947), Beach (1959) and Sathyanesan (1962) described the process of resorption of mature eggs in the ovary of teleosts. Beach (1959) found atretic follicles throughout the year in the ovary of Carassius auratus (Linnaeus). Sathyanesan (1962) found that resorption occurred only in the depleted ovary of Mystus

seenghala (Sykes). Shelton (1964) found artetic follicles in the gravid ovary of Dorosoma petenense (Günther).

Several important papers concerning the micropyle and egg membranes of the teleost egg appeared before 1900. Mark (1890) described the formation of the micropyle in the gar, Lepidosteus (= Lepisosteus). He described in detail the adhesive chorion, which contained many filamentous villi, and gave a critical review of the literature concerning the egg membranes and micropyle Eigenmann (1890) observed seasonal changes in the egg membranes and formation of the micropyle of nine teleost species. Little has been reported subsequently concerning either the micropyle or the egg membranes of fishes.

Bullough (1939) mentioned the presence of a vitelline membrane in the egg of Pleuronectes limanda and indicated that late in development radial striations were present in this membrane. His description corresponds to the zona radiata described by Mark (1890) and Eigenm nn (1890).

Hayes (1949) gave the chemical composition of the egg membranes and yolk of fish eggs. He described the process of swelling of the chorion and the osmoregulatory function of the chorion in the process of "hardening" in fish eggs. Sakai (1965a) studied the changes in toughness of the chorion of Oryzias latipes (Schlegel). He found that approximately 14.5% of the apparent toughness resulted from changes in the chorion. The remainder was accounted for by the tensile strength developed by colloidal osmotic swelling of the perivitelline space. The pressure

of the perivitelline fluid corresponded to that of 9.4% gum arabic solution. Sakai (1965b) stated that the perivitelline space is formed by the osmotic pressure of the colloidal substances ejected between the chorion and the egg surface. This colloidal substance is liberated when the cortical alveoli break down.

Larimore (1950) described the follicular layer surrounding the oöcytes, a zona radiata, and a vitelline membrane in the larger ova of _Polyodon spathula_ (Walbaum). In ova 250 micra in diameter the zona radiata was not striated but radial striations were present in the mature ovarian eggs. He did not mention the presence of a micropyle.

Bottrell (1962) described the small, circular micropyle and the egg membranes of _Hybopsis aestivalis tetranemus_ (Gilbert). The micropyle compared in size to the micropyle of _Lepisosteus_. A single membrane (chorion) was present surrounding the ovum. This membrane in the ovary was radially striate and contained the micropyle.

Jollie (1964) described the formation of the vitelline membrane and chorion in the guppy, _Lebistes reticulatus_ (Peters). She found that small oöplasmic processes radiated outward from the egg surface to the theca granulosa and material was deposited between these projections to form the vitelline membrane. The filamentous terminations of the oöplasmic projections collectively constituted the chorion.

Few workers have described the semi-buoyant condition produced by the filling of the perivitelline space. As water moves into the

perivitelline cavity, the chorion swells and becomes quite turgid. The increased surface area and the turgidity of the egg produces a degree of buoyancy. Battle and Sprules (1960) described the swelling and buoyancy of the bathyplanktonic eggs of Hiodon alosoides (Rafinesque). Bottrell et al. (1964) and Moore (1944) have reported this condition in the eggs of Hybopsis aestivalis tetranemus and Notropis girardi (Hubbs and Ortenburger).

Embryology

Considerable literature exists concerning the embryology of many teleosts and most of the work has been done with important food and sport fishes.

The best means of studying the complete embryological development of fish eggs is to strip them from ripe females, artificially fertilize them with milt and carefully observe the subsequent development. Wilson (1889), Solberg (1938), Oppenheimer (1937), Jones (1939), Price (1940), Battle (1940), Budd (1940), Harrington (1948) and Weisel (1951) have successfully used this method.

Another method of obtaining eggs involves injecting the gravid females with drugs or pituitary extracts to induce spawning. Clemens and Sneed (1957), Sneed and Clemens (1959, 1960), Saksena (1962) and Saksena, Yamamoto and Riggs (1961) have used this method successfully.

A third and almost as useful method involves collecting the eggs shortly after they have been laid and fertilized. This method was used by: Kuntz and Radcliffe (1917), Fish (1932), Roosen-Runge (1938),

Budd (1940), Carr (1942), Battle (1944), Moore (1944), Ingersol
(1953), Blumenkrantz (1956), Hisoaka and Battle (1958), Piavis (1961)
and Bottrell et al. (1964).

Wilson (1889) in a classic paper, described the embryology of
the sea bass, Serranus atrarius (Linnaeus) /= Centropristes striatus
(Linnaeus)/. His work includes: gross and histological descriptions
of the fates of the primary germ layers; detailed accounts of the
formation of the periblast; and comparisons of teleost development
to that of higher vertebrates, He reviewed the literature concern-
ing teleost embryology to that date and laid the foundation for
future studies in the field.

Morgan (1895) in studying the early formation of the fish embryo
was especially concerned with concrescence (the right and left halves
of the germ ring converging to form the embryo). He was unable to
observe concrescence, and normal embryos formed from eggs in which a
part of the germ ring was removed. He concluded that if concrescence
occurred at all it played a very minor role in the formation of the
embryo.

Dean (1906), Kuntz (1913), Kuntz and Radcliffe (1917), Stewart
(1926), Bolin (1930), Fish (1932), Turner (1940), Tavolga and Rugh
(1947), Tavolga (1949) and Knight (1963) described the embryonic de-
velopment of a considerable number of fishes including fresh water,
marine, oviparous and ovoviviparous forms. They found that cleavage,
gastrulation, and neurulation were very similar in all the species
studied. Differences were noted in the length of the incubation

period and the relative times at which certain organs became visible.

Many authors believe that the chronological age of a fish, stated in hours or days, is not a satisfactory way of expressing the stage of embryonic development. Oppenheimer (1937) stated, "The chronological age of a teleostean embryo, expressed in hours or days, does not represent its actual age, which varies according to conditions of temperature, oxygen supply, etc." and that the precise state of development of older embryos cannot be expressed in somite numbers. She used a stage naming system, in which development was expressed in terms of the degree of organ and tissue differentiation. Price (1934a, b, and 1935) used a combination of chronological age and age expressed in terms of thermal units to describe the embryology of the whitefish, Coregonus clupeaformis (Mitchill). Battle (1944) found considerable variation in the thermal units required to reach the same stage in two comparable series of developing salmon eggs. This, she stated, throws some doubt on the validity of thermal units as criteria for determining precise stages in embryonic development. Jones (1939) used the mitotic index to study the relationships between differentiation, growth and cell division in Fundulus heteroclitus. He found that the miotic data agreed in most details with the observations of workers using other techniques. Hisaoka and Battle (1958) used only the most obvious morphological landmark to determine a stage. Their 25 developmental stages of the zebra fish, Brachydanio rerio (Hamilton-Buchanan) are of considerable value in comparative studies.

Changes in temperature have long been known to influence the

growth rate of organisms. Price (1940) found that whitefish embryos
hatched only at temperatures between 0 and 12 C. The percentage of
abnormal embryos which developed to hatching varied directly with
temperature between 4 and 12 C. Incubation time varied from 29.6
days at 10 C. to 141 days at 0.5 C. Piavis (1961) found a similar
narrow range of temperature tolerance in the developing embryos of
the sea lamprey, Petromyzon marinus (Linnaeus). The embryos de-
veloped only within the temperature range of 60 to 70 F (optimum
56 F).

The origin and formation of pigment in the larvae of teleosts
has been investigated by a number of workers. Rawles (1948) stated
that "In general melanin pigmentation is produced by one type of
cell, the melanophore which originates in the embryonic neural crest."
Humm and Young (1956) found the pro-pigment cells to migrate from
the nervous system to the more superficial layers of the embryo in
platyfish-swordtail hybrids. Hodges and Behre (1953) found that
melanophores were transferred from the yolk sac onto the body of the
embryo because of the expansion of the embryo and the reduction in
the size of the yolk sac.

Several members of the family Cuprinidae have received embryo-
logical study. Moore (1944) described the embryology of Notropis
girardi through postlarval development, and compared the rate of
growth with that of Hybognathus nuchalis. Harrington (1948) used
15 stages in discussing the embryology of the Bridled Shiner, No-
tropis bifrenatus (Cope). He found the newly hatched larvae to be
devoid of pigment except in the iris. Pfeiffer (1955) included

descriptions of the embryonic development of Notropis rubellus
(Agassiz) in his life history studies on the species. Reed (1958)
gave a more complete description of the development of Notropis
rubellus. He also made notes on the embryology of Campostoma
anomalum (Rafinesque).

Bottrell et al. (1964) gave a detailed account of the develop-
mental stages of Hybopsis aestivalis tetranemus and found that basic
organogenesis of the species was characteristic of teleost develop-
ment. The embryos, at hatching, were completely transparent with no
visible pigmentation. No cellular elements were observed in the
blood although circulation was established. The anterior chamber of
the swim bladder was present. The fry did not form schools, but re-
mained aloof and individualistic.

Hubbs (1943) outlined the larval stages of fishes and suggested
that they be used as a uniform standard by embryologists.

CHAPTER III

MATERIALS AND METHODS

Hybognathus placitus (Girard), the plains minnow, is a small
species native to the Arkansas, Cimarron, and Red rivers of Oklahoma
and is known from Wyoming and South Dakota to Texas and Alabama
(Moore 1957). It is most abundant in shallow, silty water adjacent
to shifting sand bottoms of the larger prairie streams that have low
gradients (Al-Rawi and Cross, 1964).

In Oklahoma, Hybognathus placitus frequents turbid, shallow
streams with sandy to muddy bottoms. The plains minnow is very com-
mon in the Cimarron river and at times becomes the most abundant
species in the river and its larger tributaries.

The plains minnow is a member of the family Cyprinidae. Moore
(1957) listed the following characters for the genus: a long doubly
coiled intestine; intensely pigmented peritoneum; mouth horizontal
and crescent shaped; pharyngeal teeth (4-4); seven or eight anal fin
rays; very slight sexual dimorphism; and no breeding colors present.
Few good characters are available to separate species of the genus.
Moore (1957) gave only three characters (the diameter of the eye, the
width of the head, and the size of caudal peduncle) to separate
Hybognathus placitus and H. nuchalis. Niazi and Moore (1962) listed
several differences in the Weberian apparatus and its associated

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muscles of the two species. Bailey and Allum (1962) noted similar
differences in the same two species. Bailey and Allum (1962) noted
similar differences in the same two species.

Egg Structure, Micropyle, and Egg Membranes

Mature adults of <u>Hybognathus placitus</u> were obtained from a bait
stand in Chandler, Oklahoma June 6, 1965 (fish obtained from Cimar-
ron), a bait stand in Stillwater, Oklahoma July 20, 1965 (fish ob-
tained from Red River, Oklahoma), and from personal collections made
in the Cimarron River near Perkins, Oklahoma on November 3, 1965 and
February 7, 1966. The minnows were fixed in either 10% formalin or
Tellyesneczky's fluid (alcohol-formalin-acetic acid) for 48 hours.
The abdominal cavities were opened to facilitate internal fixation.
After fixation, the minnows were washed in running tap water and
placed in 50% isopropanol for storage.

The ovaries were removed, dehydrated with tetrahydrafuran, and
embedded in paraffin for sectioning with a rotary microtome. There
was less hardening of the yolk with tetrahydrafuran substituted for
alcohol in the dehydration series. Sections were made at 15 and 20
micra. Longitudinal and cross sections were made of the February,
June, July, and November ovaries.

Standard staining in Mallory's triple connective tissue stain
and Harris Hematoxylin and eosin y (Guyer, 1961 and Humason, 1962)
was used. In order to make observations with a phase contrast micro-
scope some sections were not stained.

Observations, photomicrographs and measurements were made using

a Spencer triocular compound microscope equipped with a 35 mm Kodak, Pony IV camera and a filar micrometer. Observations were made of unstained sections using a Bausch and Lomb, monocular, phase-contrast microscope. Some measurements of the microphyle diameters were made by counting the number of sections in which the structure appeared and multiplying that number by the thickness of one section. Measurements of the sectioned ovarian eggs were made only on sections through the center of the ovum. The center was determined by the presence of the nucleus and by observing a series of several sections through each ovum.

B. Embryology

The eggs used in this study were collected on June 25, 1965; June 29, 1965; and August 22, 1965, from the swift swollen waters of the Cimarron River. The June 25, 1965 collection was made one mile south of Perkins, Payne County, Oklahoma. The June 29, 1965 and August 22, 1965 collections were made at the Interstate 35 bridge in Logan County, Oklahoma.

Moore (1944) used screen wire to collect the eggs of Notropis girardi. Other workers have used similar collection methods. Jones, Ingersol, Dunn et al. collected fish eggs from the Cimarron River by means of nets constructed from mosquito netting and plastic screening (Bottrell 1962). Bottrell (1962) used special nets constructed of small-mesh nylon in collecting the eggs of Hybopsis aestivalis tetranemus. The same nets were used in collecting the eggs for this study.

Over the 2 year period covered in this study, eggs were collect-
ed only when the river was swollen by recent rains. Moore (1944)
suggested that spawning of certain fishes may be correlated with the
swollen muddy condition of the stream. Bottrell (1962) implied that
the eggs of Hybopsis aestivalis tetranemus were more easily collected
when the stream was muddy and high.

Other factors which apparently influenced spawning were the time
of day and the water temperature. Eggs were collected only after
one PM when the water temperature was in excess of 78 F. Bottrell
(1962) made several unsuccessful attempts to collect eggs in the
early morning hours and at water temperatures below 85 F. He also
found that embryos collected during the early evening hours were in
late stages of development indicating that spawning probably occurred
when the sun was at its zenith or brightest point.

The eggs were washed several times in the laboratory using
aerated tap water. Separation of the eggs into five classes was
made based on the type of micropyle present in the chorion and the
diameter of the yolk material. The classes were as follows: Class
I - round micropyle; Class II - star micropyle; Class III - star
micropyle; Class IV - star micropyle; and Class V - star micropyle
(Table I). The August 22, 1965 collection was separated into two
classes, - round micropyles and star micropyles (Table II). Meas-
urements of the yolk mass and chorion diameters were made using a
Spencer binocular dissecting microscope equipped with an ocular
micrometer. The eggs were then placed in finger bowls (40 eggs per
bowl) containing freshly aerated tap water, and allowed to develop

to an identifiable age.

Observations and photomicrographs of each size class were made as follows: every 30 minutes throughout the first 24 hr of development; every 8 hr from the time of hatching until 3 days old; each day from 3 days to 2 weeks of age; and observations at 1 and 2 months. Observations were made using a tape recorder and both a Spencer triocular microscope and a Spencer binocular dissecting microscope. Photomicrographs were made using a Spencer microscope equipped with a 35 mm Kodak Pony IV camera. The objectives used for photomicrographs were the 4X, 10X, and 43X.

The observations recorded on tapes were later typewritten for a permanent record.

Small portions of commercial tropical fish food (Micrograin) were added to the containers 24 hours after hatching. During the next two weeks food was introduced at approximately 6 hour intervals. The commercial diet was supplemented with laboratory-reared protozoan cultures.

At two weeks of age the fry were transferred to 1 gal glass jars approximately one-half filled with fresh water. Aerated tap water was used to replenish water lost through evaporation. A desk lamp was used continuously for illumination. No attempt was made to control laboratory temperature which fluctuated between 70 and 83 F.

The fry were preserved from each container on October 13, 1965 and submitted to Dr. George A. Moore, Oklahoma State University for identification. The different classes were identified as follows:

Class I, only Hybopsis aestivalis tetranemus; Class II, Notropis

girardi, Notropis percobromus (Cope) and Hybognathus placitus;

and Classes III, IV, and V, only Hybognathus placitus.

CHAPTER IV

OBSERVATIONS

Egg Structure, Micropyle and Egg Membranes

Ovarian structure. The ovary of Hybognathus placitus is a bi-lobed, hollow structure located in the dorsal portion of the body cavity. Each lobe occupies a position lateral to the swim bladder and in close membranous union with it. The two lobes join posteriad to form a single oviduct which empties into a urogenital sinus behind the anus. The ovary is covered with the pigmented peritoneum char-actistic of the plains minnow. Beneath the peritoneum is a thin tunica albuginea of connective tissue.

Internally, the ovary consists of a number of ovigerous lamellae which project into a central lumen. The ovigerous lamellae contain large numbers of eggs in various stages of maturation. In the fall and early spring the lumen and the ovigerous lamellae can be rec-ognized, but as the ova fill with yolk and pass from the lamellae into the lumen the internal lumen of the ovary is obliterated.

Gravid ovary (Figure 1). A rapid increase in yolk deposition occurs during March and April. Mature ova are present in the lumen of the ovary in early May. The ovary becomes distended with mature eggs during this period and the abdomen protrudes ventrally as the

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ovary increases in size.

Three sizes of ovarian eggs could be recognized in the gravid
ovaries of specimens collected in June and July.

The smallest were primary oöcytes ranging from 35 to 248
(average, 145) micra (Figures 1 and 2). The nuclei occupy 50 to 75%
of the cell volume. There are many large nucleoli near the periphery
of the nuclei. No distinct nuclear or cytoplasmic membranes are ap-
parent. The oöplasm stains with eosin Y. No extracellular mem-
branes are formed. The oöcytes are clustered in groups of from one
to five or more and each ovum is surrounded by a follicular capsule.
These clusters are sandwiched between the larger maturing ova. No
attempt was made to differentially stain the chromosomes, but the
size and structure of the oöcytes corresponds with the early oöcytes
described by Shelton (1964) for the threadfin shad, Dorosoma
pentenense.

The secondary oöcytes range in size from 280 to 360 (average,
325) micra in diameter (Figures 1 and 3). These oöcytes are under-
going vacuolation of the cytoplasm and yolk deposition. Only a few
vacuoles are present in the smaller oocytes, but the larger ova con-
tain a continuous sphere of vacuoles midway between the outer cell
membrane and the nucleus. Eosinophilic yolk granules were present
in the immediate vicinity of the nuclei.

The nuclei are slightly acentric within the ova and less spheri-
cal than in the previous stage. As yolk deposition continues the
nuclei elongate to form rough-edged ellipsoids. The average diam-
eter of the nuclei at the onset of yolk deposition is 134 micra.

A uniform, noncellular zona radiata is present between the oöplasm and the follicular tissue. No radial striations or micropyle are apparent in the membrane. The average thickness of the zona radiata is 4 micra.

Outside the zona radiata and adhering to it, a layer of follicular cells separates from the ovarian tissue. A similar layer was observed by Shelton (1964) in Dorosoma petenense. The cell layer varies from one to several cells in thickness.

Many small darkly staining granules are present in the cytoplasm of the ovum. These appear to be concentrated in the region just outside the large vacuoles. The granules compare in size and location to the Golgi bodies observed by Narain (1956).

As deposition increases in the ovum the cytoplasm forms a peripheral layer around the yolk granules. This cytoplasmic layer gradually reduces in thickness as the yolk accumulates.

The largest ova have an average diameter of 0.88 mm. Most of the eggs in the gravid ovary are of this size (Figure 1). Many mature eggs have moved from the ovigerous lamellae into the ovarian lumen. Some, apparently mature, eggs remain in the ovarian wall.

The nucleus of the mature ovum in section appears ellipsoid to roughly rectangular, with many small pockets or folds, containing yolk granules, in the nuclear membrane. The nucleoli, still apparent and peripheral, appear to have changed very little during the maturation process.

The increase in yolk material shifts the cytoplasm outward to

the periphery of the cell. A few large vacuoles and small Golgi bodies are present in the ovum, but yolk deposition is very near completion. The cytoplasm is now only slightly basophilic.

A micropyle and radial striations are present in the zona radiata (Figures 5 and 7). The average thickness of the zona radiata of the mature egg is 10 micra. The follicular layer remains surrounding the egg in the ovarian lumen. The cytoplasm of the follicular layer and the zona radiata stain with eosin Y, while the nuclei of the cuboidal follicular cells readily stain with hematoxylin.

Ovarian egg membranes (Figures 4 and 7). The ovarian egg of Hybognathus placitus has two membranes, an outer cellular layer of cuboidal follicular cells and an inner noncellular zona radiata (Figure 4).

The outer capsular layer of cells is derived from the theca granulosa. A split develops in the granulosa cells, during vacuolation of the ooplasm, producing a distinct layer of cuboidal cells which adhere closely to the zona radiata. This capsule remains surrounding the ovum in the ovarian lumen. The capsular layer varies from one to several cells in thickness. No processes of these cells, either into the pore canals of the zona radiata, or outward into the granulosa tissue, were observed.

The zona radiata became apparent just prior to the initiation of yolk deposition. When first visible the membrane measures 2 micra in thickness. In eggs in which many large vacuoles are present the zona radiata measures 4 micra in thickness (Figure 3). The pore canals in the zona radiata did not become apparent until yolk deposition

was nearing completion. From its initial appearance until it reaches
a thickness of approximately 8 micra the membrane (zona radiata) ap-
pears as a uniform noncellular layer completely surrounding the
ovum. The zona radiata does not vary in thickness on opposite sides
of the egg. No micropyles were present until the zona radiata reach-
ed a thickness of 8 micra.

In some ova clear areas were observed just inside the zona
radiata. These regions are not continuous around the eggs. Although
the location would suggest the presence of a vitelline membrane, no
definite membrane was observed. The clear areas are believed to be
separations in the superficial layers of the ova probably resulting
from dehydration and sectioning.

Micropyle (Figures 5, 6, 7, and 8). The micropyle of Hybogna-
thus placitus is funnel shaped with a number of folds radiating out-
ward from the funnel rim (Figure 6). The funnel tapers from ap-
proximately 45 micra at the surface to 6 to 8 micra at the bottom.
The micropyles measured 85 to 105 micra in depth. The zona radiata
is continuous around the tip of the funnel and appeared much thinner
in this region. Although sections through the tip of the micropylar
funnel suggest an opening into the ooplasm no distinct canal appears
to penetrate the zona radiata. Pore canals are present in the zona
radiata in this region.

In the ovary the micropylar funnel is filled with granulosa
cells that are continuous with the capsular layer. A single large
cell is located at the tip of the funnel (Figure 5). A similar
micropylar cell is present in the ova of Lepisosteus (Mark, 1890) and

Perca (Eigenmann, 1890).

The number and length of the micropylar folds appear to be variable. In most of the stripped eggs of Hybognathus placitus six or seven folds are visible equally spaced around the micropyle and becoming deeper toward the funnel. The zona radiata appears to be thicker in the regions lateral to the micropyle, probably because of the folded nature of the membrane and not because of an increase in the thickness of the membrane in this region.

The location of the micropyles, in the zona radiata, has no apparent relationship to the points of attachment of the ova to the ovary or to the position of the ova within the ovary. Nuclei of the ova were not observed to be directly below the tip of the micropyle as described for Lepisosteus (Mark, 1890). In Hybognathus placitus eggs the nuclei are located near the center of the ova.

The manner in which the micropyle forms in the eggs of Hybognathus placitus was not determined. The structure apparently develops quite late in the maturation of the ovum. There is no indication of a micropyle or folds in the zona radiata in eggs with a zona radiata thickness of less than 8 micra. Developmental stages in the formation of the micropyle were not observed in serial sections of mature eggs.

Seasonal variation in the ovary. Primary oöcytes were present from February through November. The February ovaries contained only the primary oöcytes.

June and July ovaries contained ova in all stages of maturation. The November ova were mostly primary oöcytes, with only a few

mature ova. Many areas showed degeneration and resorption of mature ova. The November ovaries were much smaller and the ova fewer in number than in fish collected in February, June, or July.

The unfertilized egg. Eggs stripped from females have two distinct membranes. The membranes consist of on outer, noncellular capsule, without an opening, and an inner, noncellular membrane with many radial striations (zona radiata). The zona radiata contains the star shaped micropyle perviously mentioned. Inside the zona radiata a thin plasma membrane surrounds the nucleus and the yolk mass. The nucleus is located slightly to one side of the center of the yolk and is very irregular in outline. The yolk appears to be in a fine homogeneous granular state without the oil droplets characteristic of some teleost eggs.

Stripped, unfertilized eggs, when placed in tap water, are opaque, with a milky white coloration and an ovoid to spherical shape. The capsular layer and the zona radiata are closely adherent, the latter in contact with the yolk mass. Immediately after stripping, the average diameter of 16 eggs was 1.17 mm.

Eggs stripped from the female into water begin to absorb water almost immediately and assume an ovoid shape because of unequal swelling (Figure 9). As the egg continues to enlarge the outer capsule ruptures, indicating limited elasticity of the capsule. The point of rupture in the capsule has no apparent relationship to the location of the micropyle. The average diameter of the eggs (measured at the widest point and at right angles to the long axis, including the capsule and chorion), at the time of rupture is 1.53 mm.

The ovum continues to enlarge after the capsular break and slowly emerges from the ruptured structure. During emergence the ovum and the capsule together take the shape of a figure eight (Figure 9). The time, from placement of the eggs in water, to complete emergence from the capsule varied from 20 min to 2 hr.

Almost all of the eggs collected in the Cimarron River were fertile and none was obtained with the capsule still intact or adherent. Therefore, the capsule must have been removed either before or shortly after spawning or ruptured within the ovary. No evidence of retained capsules within the ovary were observed. The time factor in the emergence of the egg from the capsule would appear very important in fertilization and the bouyancy of the egg. At present we have no information as to the actual events occurring when the eggs are released in spawning. Attempts to produce spawning in the laboratory and to fertilize stripped eggs were uniformly unsuccessful although attempted many times by this author and others.

In the stripped eggs after the ova emerge from the capsule the zona radiata, now the outermost membrane, (hereafter called the chorion) quickly separates from the plasma membrane and swells to a diameter roughly twice that of the original egg. The average chorion diameter 5 hr after stripping is 2.27 mm, in contrast to 3.12 mm for the fertile eggs collected from the river. The time necessary for the chorion to reach maximum proportions varies considerably. A few of the stripped eggs fail to show swelling or capsule removal and were not measured.

There is a wide variation in the chorion diameter of both ferti-
lized and unfertilized eggs. The range for fertilized eggs is from
2.44 mm to 3.78 mm. The unfertilized eggs range from 1.95 mm to
2.74 mm.

The swelling of the chorion produces a large perivitelline space
(between the chorion and yolk mass). The ovum is freely suspended
in this fluid-filled cavity. The striations observed earlier in the
chorion disappear during its enlargement. The micropyle appears to
enlarge as swelling progresses, probably as a result of the stretch-
ing of the chorion. After the removal of the capsule and the separa-
tion of the chorion from the yolk, the stripped, unfertilized egg be-
comes translucent. The eggs are semibuoyant, remaining suspended for
several minutes near the surface after the slightest agitation of the
water. This buoyant condition undoubtedly has a survival advantage
for the fishes of this muddy river system.

From the earliest observed stages through hatching, the embryos,
yolk material, and chorions are clear and very transparent. The
chorions appear as clear glass-like bubbles surrounding the trans-
parent embryos.

Embryology

The stages presented in this study are those that best represent
the developmental landmarks for the species. Several references were
followed in selecting the stages, Hisaoka and Battle (1958), Bottrell
et al. (1964), Solberg (1938), Oppenheimer (1937), and Battle (1944).

The stages did not always exactly correspond to those of other workers because correlation of the developing parts did not conform to the stages described for other teleosts.

High blastula (Figure 10). This is the earliest stage represented in this study. The age was taken to be 3 hr after spawning. This assumes that spawning took place when the sun was it its zenith as reported by Bottrell et al. (1964). The average diameter of all the embryos examined (measured at right angles to the animal-vegetal axis) was 1.342 mm. Not all the embryos measured were in this stage of development. Most were in either the blastula or early to mid-gastrula stage. No attempt was made to count the number of cells but the embryos were comparable with the high blastula stage reported by Bottrell (1962) for Hybopsis aestivalis tetranemus. The yolk is in a fine homogeneous granular state. No oil droplets, as reported by other workers for other teleosts, were observed. The cells comprising the germ ring are large and their nuclei quite obvious.

Early gastrula (Figure 11). Gastrulation occurs by the rapid epiboly and involution of the edges of the blastoderm around the yolk mass. The germ ring is noticeably thicker and covers approximately two-fifths of the yolk mass. The embryonic shield is evident and occupies a position at right angles to the germ ring. Some thickening of the shield appears along the most dorsal and median regions. The cells in the region of the germ ring are reduced in size and are less distinct than in the previous stage.

Mid-Gastrula or equatorial ring (Figure 12). At this stage the

germ ring covers about one-half of the yolk mass. The embryonic

shield is more distinct with a slight swelling in the anterior end

of the embryo. The cells of the germ ring are smaller than those of

the previous stage.

Yolk plug stage (Figure 13). This stage occurs 5.5 hr after

the high blastula. At this point, except for the blastopore, the

embryonic tissue extends completely around the yolk material. The

embryonic shield extends four-fifths the distance around the yolk.

There is a distinct thickening of the anterior end of the shield

terminating posteriorly in a constriction opposite the blastopore.

The caudal mass is also thickened. The shield, between the constric-

tion and the caudal mass, is represented by a rather thin layer of

tissue. Differentiation is occurring in the middorsal ectoderm

anteriorly. This differentiation results in the formation of the neu-

ral tube, although the neural keel characteristic of teleost develop-

ment are not apparent. No somites are present at this stage. Granular

cells surround the yolk and appear to contain cytoplasmic yolk granules.

Blastopore closure (Figure 14). The blastopore closes approxi-

mately 7 hr after the high blastula stage. Blastopore closure oc-

curs as a continuous process of epiboly, moving the blastopore lips

together ventrally. The entire vegetal hemisphere is soon enclosed

by a thin sheet of tissue one or two cell layers in thickness. There

is no apparent change in the embryonic shield.

Formation of the optic vesicles and body segmentation (Figure 15).

Formation of the optic anlagen and the appearance of the first somites

occur simultaneously.

The first four somites develop together approximately 8 hr after the high blastula stage. The fourth somite develops a little slower than the anterior three. They begin just posterior to the developing hindbrain at a point almost opposite the vegetal pole. The lines of demarcation of the somites begin near the dorsal surface and progress ventrally with a slight posterior, circular movement. The lines separating the first four somites are at first very faint, but are quite visible at the beginning of the fifth and sixth somites.

The optic anlagen develop as rather clear hemispherical evaginations from the prosencephalon. As evagination continues they begin to bulge from the lateral surfaces of the brain.

Just previous to this stage and continuing through it the midbody region increases in height and thickness. Kupffer's vesicle forms during this period. The three primary regions of the brain are visible in profile view.

Kupffer's vesicle (Figure 16). This little understood structure develops in the ventral region just under the caudal mass simultaneously with the formation of the optic anlagen and the first somites. The vesicle reaches its maximum size at the 15-somite stage.

Otic vesicle (Figure 16). The otic placode makes its appearance as a spherical body in the surface ectoderm on the lateral surface of the hindbrain. This structure was first observed in the 15-somite embryo or approximately $11\frac{1}{2}$ hr after the high blastula. However, the placode is not distinct until 13 hr after the high blastula or in the 19-to 20-somite embryo.

Soon after the placode is visible it elongates along the antero-posterior axis of the embryo and becomes depressed dorsoventrally, forming an ovoid vesicle. Immediately after elongating the structure develops the slit described by Solberg (1938) and Bottrell (1962). No internal differentiation of the otic capsule was observed before hatching.

Sixteen-somite embryo (Figure 16). Sixteen pairs of somites are present between $11\frac{1}{2}$ and 12 hr after the high blastula. The embryo encircles four-fifths of the yolk. The height of the embryo (dorsoventral) has increased to the level of the hindbrain. Differentiation occurs rapidly in several structures at this stage. The three primary vesicles of the brain are quite distinct. The vacuolated optic vesicle is invaginating to form the optic cup. The lens placode is visible as a thin line of cells in the central region of each optic vesicle. The otic placodes are visible and elongating. The notochord extends from just posterior to the hindbrain, to a point just anterior to the caudal mass. Kupffer's vesicle is very prominent at this stage. A mass of mesodermal tissue extends from the last somite into the caudal mass and diminishes in size and thickness posteriad. Small irregular aggregations of cellular material (probably blood islands) appear in the anterodorsal regions of the yolk sac.

No muscular movement was observed at this stage of development.

Heart formation. The first indication of the heart development was observed in the 15-and 16-somite embryo ($11\frac{1}{2}$ hr after the high

blastula). When first visible the structure is a spherical mass of mesenchyme cells. The heart develops at an oblique angle to the long axis of the body. As pulsations begin ($15\frac{1}{2}$ to 16 hr after the high blastula, 29 to 30 somites) the heart is a simple straight tube. At this stage a transparent pericardium is well formed.

Thirty-two somite embryo (Figure 18). Thirty-two somites are visible 15 to 16 hr after the high blastula. The somites extend from a point just under the posterior portion of the hindbrain to a point behind the developing anus. Differentiation of the somites into metameric muscles and elongation of the embryonic body and the yolk sac are occurring. The embryos were almost as long as the diameter of the chorion. Elongation of the yolk sac results in an anterior spherical region and a posterior tapering, cone-shaped extension (Figure 17). The extension begins ventral to the twelfth somite and terminates under somite 28. This elongation when completed allows the posterior half of the body to be free of the yolk sphere (Figure 18). This freedom is apparent from the rather regular, spasmodic muscular contractions in this region of the body.

The caudal fin fold is present, beginning on the ventral surface of the yolk sac extension, encircling the tail and terminating on the dorsum above somite 12.

The anterior region of the embyro is raised slightly off the yolk mass. The otic vesicles appear to have sunk below the surface as described by Solberg (1938) and the slit observed in the otic placode earlier has disappeared. No otoliths were observed. Gill arches are visible but their number was not determined. The developing lens is

seen as a spherical body in the center of the optic cup. The optic lobes are faintly visible, developing as dorsolateral evaginations from the midbrain.

A line of endodermal cells just under the hindbrain, extending posteriorly along the junction of the yolk sac with the embryo, marks the early formation of the gut. Differentiation is occurring in the ventral region of the fin fold, just behind the termination of the yolk sac, forming the anus.

The heart lies about midway between the optics and otics at the junction of the yolk sac with the embryo. Pulsations of the tubular structure are regular and cellular material is moving back and forth in the immediate region of the heart. Circulation of blood is not apparent. No pigment is evident in the cellular material.

Kupffer's vesicle fails to increase in size as the body enlarges.

Hatching (Figure 19). The average time of hatching is 21 hr after the high blastula.

Immediately before hatching the embryos are approximately one-third longer than the diameter of the chorion. The tail is bent ventrally and curved to one side. The tail lashes violently against the chorion in all embryos and apparently ruptures the chorion to effect hatching.

Two small otoliths are present in each otic vesicle. There are approximately 38 somites present at hatching. Four gills are visible in a dorsal view. No supporting structures are present in the fin fold. No circulation of the blood or pigmentation in the embryo was observed. Small pectoral fin buds are present.

The fry exhibit swimming behavior similar to that of <u>Notropis</u> <u>girardi</u> described by Moore (1944) and <u>Hybopsis</u> <u>aestivalis</u> <u>tetranemus</u> described by Bottrell (1962). The fry swim in a spiral fashion from the bottom of the container to the surface only to slowly drift back to the bottom again. They remain motionless on the bottom for a short time and then repeat the swimming movement.

<u>Circulation</u> <u>of</u> <u>the</u> <u>blood</u>. Circulation is established soon after hatching. The first movement of blood in the anterior regions of the body occurs $2\frac{1}{2}$ hr after hatching. Pigment is not apparent in the blood until 48 hr after hatching.

<u>Pigmentation</u> <u>of</u> <u>the</u> <u>eye</u> <u>and</u> <u>body</u>. The retina of the eye first contains pigment approximately 19 hr after hatching. Pigmentation of the retina apparently nears completion 24 hr later.

Body pigmentation begins in the dorsal region of the swim bladder two days after hatching. Three days after hatching, pigment is present along the dorsal margins of the coelom. Also there are several large stellate melanophores located on the meninges of the brain and smaller ones scattered over the dorsal surface of the body.

Yellow pigment was observed in the region of the liver 4 days after hatching.

<u>Fins</u> <u>and</u> <u>fin</u> <u>rays</u>. The caudal fin develops from that part of the fin fold that encircles the tail. The dorsal portion of the fold disappears apparently because of the absorption of the tissues into the developing tail as described by Bottrell (1962). The notochord has a slight dorsal bend and enters the caudal fin at an angle.

By the third day after hatching the teardrop-shaped pectoral fins are well developed and functional.

The pelvic fins are not apparent when the fry are one month old.

Fin rays were first observed in the caudal and pectoral fins on the fourth day following hatching.

Feeding behavior. The lower jaw is apparent during the first 24 hr after hatching. Definite feeding behavior and food in the gut were observed on the second day after hatching.

As the fry begin to feed they slowly change their swimming movements from the characteristic upward spiral to definite, jerky, forward motions. Correlated with this change is the introduction of air into the swim bladder on the third day after hatching. As feeding continues the yolk sac decreases in size and disappears.

The operculum forms during the first week of development and covers the gills.

Development of neuromasts and cupulae. Since they are very transparent, the cupulae and associated neuromasts were not observed until their development was near completion. The cupulae, apparent 12 to 14 days after hatching, occupy the location of the future lateral-line canals.

CHAPTER V

DISCUSSION

Egg Structure, Micropyle and Egg Membranes

Formation and development of Hybognathus placitus ova follow

the sequence of events described for the egg of Dorosoma petenense

(Shelton, 1964). Egg production begins in the fall with rather rapid

maturation of the ova in early spring. The gravid ovary of H.

placitus contains eggs in all stages of development.

Narian (1956) described the Golgi bodies in the granulosa cells

and in the ooplasm. He concluded that the Golgi bodies moved from

the granulosa cells, through the pore canals of the zona radiata,

into the ooplasm where they functioned in yolk deposition. In H.

placitus yolk deposition is nearly complete when the pore canals be-

come apparent. Golgi bodies are present in the ooplasm long before

this. Therefore, the Golgi bodies in the egg of H. placitus could

not have migrated through the pore canals of the zona radiata.

A single egg membrane, such as that described for Notemigonus

crysoleucas (Mitchill) (Eigenmann, 1890), surrounds the developing

embryo of Hybognathus placitus. This membrane is noncellular, trans-

parent, and contains the star-shaped micropyle.

In the lumen of the ovary, the mature egg of Hybognathus placitus

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has two membranes surrounding the ovum. The outer membrane consists of the theca granulosa which forms a capsule around the ovum. The inner, zona radiata, is noncellular, radially striate, and contains the micropyle.

The outer capsular layer is removed either immediately before or after spawning. The micropylar plug is probably removed with the capsule since it is continuous with it. The zona radiata (also termed chorion), when exposed, imbibes water, swells, and separates from the yolk, in the manner described by Hayes (1949), to form the perivitelline space. Water and sperm cells are believed to enter through the micropyle (Mark, 1890 and Bottell, 1962). When expanded, the egg diameter (chorion) is approximately 3 mm. The swollen, nonadhesive, turgid membrane (chorion) provides a buoyant envelope surrounding the egg. The greatly increased surface area when acted upon by the river currents causes the egg to remain suspended near the water surface, providing protection from the abrasive sandy bottom and from being covered with silt.

The ruptured chorion does not appear to be as firm or brittle as described for the chorion of the speckled chub (Bottrell, 1962) but is quite pliable and resistant to puncture. This resistance probably results from the turgid, stretched condition of the chorion rather than a hardening process.

The micropyle of Hybognathus placitus consists of a tapering funnel in the chorion with six or seven folds radiating outward from the funnel rim, and deepening toward the rim of the funnel (Figure 8). In the ovary the funnel is filled with granulosa cells which form

the micropylar plug. A single large cell is present at the bottom
of the funnel. The size of the funnel is much larger than those de-
scribed for Lepisosteus (Mark, 1890) and Hybopsis aestivalis tetra-
nemus (Bottrell, 1962).

The mycropyles develop very late in the maturation of the ova,
as determined by the absence of micropyles in eggs with a zona
radiata thickness of less than 7 micra.

The micropyle is believed to serve as a point of sperm entrance
(Mark, 1890 and Bottrell, 1962) and as an orifice through which water
can enter the egg (Bottrell, 1962 and Jones, personal communication).
The chorion of Hybognathus placitus possibly is porous. If the ra-
diations are openings through the chorion, stretching of the membrane
during the swelling process would most probably increase the porosity
by increasing the diameter of the pore canals and the micropyle.
This is contrary to the suggestion by Mark (1890) that the stretching
results in a reduction in penetrability of the chorion and affords
the best protection for the embryo. The micropyle may be partial
compensation for such a reduction.

Direct observations of spawning of the plains minnow have not
been observed. Jones (personal communication) suggests that this
species may spawn in the main channel of the river. Unsuccessful
attempts to collect eggs and gravid adults from Skeleton Creek (1
to 5 miles above its mouth in Logan County, Oklahoma) and a success-
ful egg collection in the Cimarron River approximately 5 miles down-
stream from the mouth of Skeleton Creek, on the same day supports
Jones' hypothesis. Data on extensive collections of eggs and adults

of Hybognathus placitus by various individuals from this laboratory during the last 20 years support the belief that this species spawns only in selected regions of the river and perhaps only at the mouth of the larger tributaries. The presence of primary and secondary oocytes in the gravid ovary suggests an extended spawning period. James (1947) found a similar condition in Lepomis macrochirus (Rafinesque) ovaries. The fact that the eggs of Hybognathus placitus were collected only when temperatures were above 78 F and the river swollen indicates spawning is intermittent and dependent on temperature and stream conditions.

Embryology

The embryonic development of Hybognathus placitus follows the typical sequence of these events in teleosts.

Cleavage and early differentiation of Hybognathus placitus is faster than that of most other teleosts. In the plains minnow the blastopore closed 7 hr after the high blastula stage, and 14 hr later hatching occurred. The time for blastopore closure of some other species are: 36 days in the Atlantic Salmon Salmo salar (Linnaeus) (Battle, 1944); 22 days and 4 hr for the Whitefish Coregonus clupeaformis, (Price, 1934); 30 hr for Ictalurus punctatus (Saksena, Yamamoto, and Riggs, 1961); 26 hr for Fundulus heteroclitus (Solberg, 1938); and 10 hr for Brachydanio rerio (Hisoaka and Battle, 1958). Bottrell (1962) found that the blastopore of Hybopsis aestivalis tetranemus, an associate of Hybognathus placitus, closes 4 hr,

19 min after the high blastula, and hatching was 23 hr later.

The temperature ranges in which Brachydanio rerio (26 C)(Hisoaka and Battle, 1958), and Ictalurus punctatus (24.5 to 26.8 C), (Saksena, et al., 1961) developed are not significantly different from the 70 to 83 F range for Hybognathus placitus. Yet both species develop slower than Hybognathus placitus. The accelerated development, to hatching, of Hybognathus placitus and Hybopsis aestivalis tetranemus is probably a selective advantage in their survival since the eggs are being rapidly swept downstream during development.

The development of the somites in teleosts occur from anterior to posterior at the rate of about one every 30 min depending upon the temperature of the water (Solberg, 1938). In Hybognathus placitus the first four somites appear almost at the same time. The fourth is somewhat slower than the anterior three, but all four are visible in one 30 min interval. The fifth and sixth somites also appear in a span of one-half hour. The remaining somites develop according to Solberg's time sequence.

The swimming behavior exhibited by the newly hatched fry is identical with the descriptions given for Notropis girardi (Moore, 1944) and Hybopsis aestivalis tetranemus (Bottrell, 1962). Notropis percobromus also exhibits the spiral swimming movement from the bottom to the surface when first hatched. Moore (1944) suggested that the swimming behavior of Notropis girardi fry may be of survival value as well as aiding in locating food. This swimming adaptation may be characteristic of many of the pro- and postlarvae of minnows living in turbid, sandy bottomed prairie streams.

In many teleosts hatching is characterized by a combination of enzymatic action on the inside of the chorion, softening the membrane, and mechanical action of the tail and body as the embryo attempts to straighten (Bottrell et al., 1964). The process of hatching appears to be mechanical in Hybognathus placitus, since no hatching glands were observed. The lashing of the tail against the chorion produces a rupture in the membrane allowing the larvae to emerge. The diameter of the chorion and the length of the larvae appears to be directly correlated with the time necessary for hatching.

At hatching, the larvae of H. placitus in contrast to many other teleosts have the following: no developed circulation, although the heart is formed, pulsating, and has cells present in the lumen; and no pigmentation in the body, blood, or retina. Hybopsis aestivalis tetranemus is devoid of pigment in the body, eyes, and blood (Bottrell, 1962). Pigment develops in the retina of Hybognathus placitus during the first 24 hr following hatching, and body pigment is present 2 days after hatching. Bottrell (1962) did not report eye pigment in the speckled chub until 3 days after hatching and he saw body pigmentation 7 days later. Carr (1942) reported pigmentation of the retina in the largemouth bass on the third day after hatching.

The yolk material in the stripped unfertilized egg of Hybognathus placitus is opaque but appears as a clear, transparent mass in the high blastula stage. The chorion is a clear, transparent bubble surrounding the developing, transparent embryo.

A clearing of the yolk, sometime after fertilization, combined with the lack of pigment in the embryo and the transparency of the chorion produces a very clear, transparent condition in the entire egg from the earliest observed stage through hatching. Oppenheimer (1937) found the unfertilized egg of Fundulus heteroclitus to be opaque, but crystal clear after fertilization.

Embryos of Hybognathus placitus are free to rotate inside the fluid-filled perivitelline cavity. The embryos in the early stages do not contact the chorion. The central location of the embryo within the perivitelline cavity suggests a viscous consistency of the fluid in the cavity. Some unidentified eggs in the same collection with H. placitus had two zones within the fluid of the perivitelline cavity (Figure 20). The outer zone was more highly refractive to light. These two zones were not apparent in all of the eggs observed and disappeared in all of the eggs prior to the formation of the first myomeres.

Much of the literature concerning teleost embryology describes species that have a spherical yolk sac confined to the anterior one-half of the body. The yolk sac of H. placitus elongates during the straightening of the body to produce a cone-shaped posterior extension. Two regions, an anterior spherical sac, and the posterior tapering extension beginning at about somite 12, can be recognized at the 25- to 30-somite stage. Hybopsis aestivalis tetranemus (Bottrell, 1962) and Notropis girardi (Moore, 1944) have a similar posterior extension, but the constriction is more abrupt, and the extensions are less cone shaped than in H. placitus.

CHAPTER VI

SUMMARY

Studies of the ovaries, egg membranes, and embryonic develop-
ment of the cyprinid minnow Hybognathus placitus were conducted.
Histological studies were made of ovaries from mature females col-
lected at different times of the year. Embryonated eggs collected
in the Cimarron River were used for embryological studies.

Fertilized eggs were clear, transparent, nonadhesive and semi-
buoyant. Stripped, unfertilized eggs and mature ovarian eggs are
surrounded by an outer capsule of follicular cells and an inner
radially striate zona radiata. A single membrane (chorion) surrounds
the embryonated eggs.

A large star-shaped micropyle and micropylar plug are present.
A single micropylar cell occupies the bottom of the micropylar fun-
nel.

The embryonic development of Hybognathus placitus is similar to
that of other teleosts. Development is very rapid with embryos
hatching in 22 to 26 hr after fertilization (70-83 F). The eggs,
yolk, and embryos are devoid of pigment, clear, and transparent from
the high bastula stage through hatching.

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APPENDIX

TABLE I

YOLK AND CHORION DIAMETERS (IN MM) OF JUNE 29, 1965 COLLECTION

EGG CLASS	MAXIMUM DIAMETER	MINIMUM DIAMETER	MEAN DIAMETER	TOTAL NO. OF EGGS	SPECIES
Class I Round Micropyle					Hybopsis aestivalis tetranemus
Yolk	1.33	0.78	1.08	40	
Chorion	3.33	2.33	2.79	40	
Class II Star Micropyle					Notropis girardi, Notropis percombromis, Hybognathus placitus
Yolk	1.22	0.78	1.01	64	
Chorion	3.33	1.89	2.49	64	
Class III Star Micropyle					Hybognathus placitus
Yolk	1.67	1.00	1.31	160	
Chorion	3.78	2.44	3.11	160	
Class IV Star Micropyle					Hybognathus placitus
Yolk	1.67	1.11	1.37	40	
Chorion	3.56	2.67	3.09	40	
Class V Star Micropyle					Hybognathus placitus
Yolk	1.78	1.11	1.40	52	
Chorion	3.78	2.56	3.19	52	

TABLE II

YOLK AND CHORION DIAMETERS (IN MM) OF AUGUST 22, 1965 COLLECTION

EGG CLASS	MAXIMUM DIAMETER	MINIMUM DIAMETER	MEAN DIAMETER	TOTAL NO. OF EGGS	SPECIES
Class I Round Micropyle					Hybopsis aestivalis tetranemus
Yolk	2.22	0.78	1.01	26	
Chorion	3.44	2.44	2.98	26	
Class II Star Micropyle					Notropis girardi, Notropis percombromis
Yolk	1.44	0.89	1.12	29	
Chorion	3.78	2.33	2.98	29	

Plate I

Figure 1. Gravid ovary showing primary, secondary, and maturing oocytes. Longitudinal section of June ovary.

2. Nongravid ovary showing primary oöcytes. Longitudinal section of February ovary.

3. Secondary oöcyte showing vacuolation of cytoplasm and yolk deposition. Cross section June ovary.

4. Capsule and zona radiata of two mature ova. Cross section June ovary.

5. Micropyle (cross section) showing micropylar plug and micropylar cell. July ovary.

6. Micropyle dorsal view, fertilized egg.

7. Micropyle capsule and zona radiata. Cross section June ovary.

8. Micropyle dorsolateral view, fertilized egg.
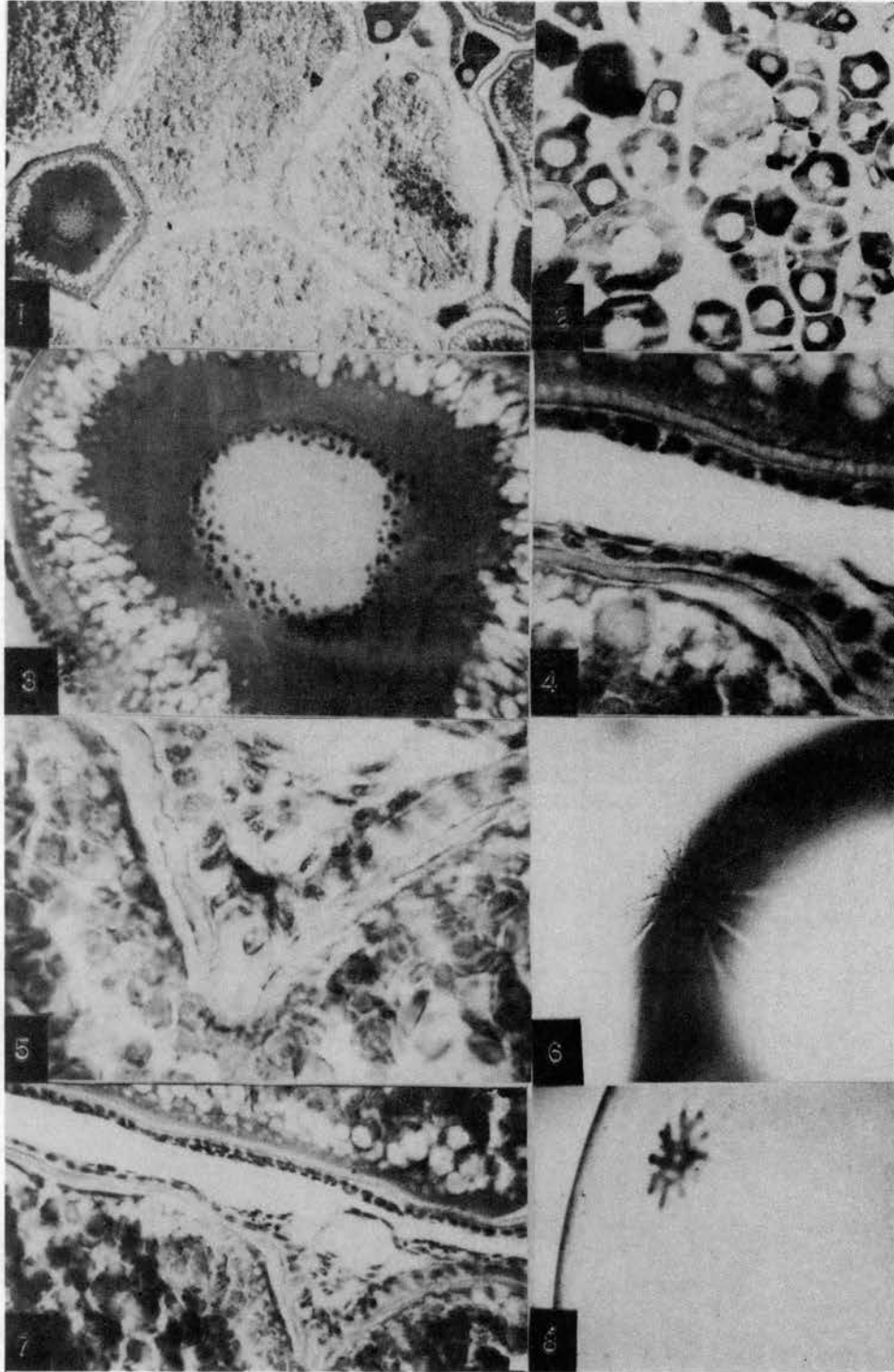
Plate 1

Plate II

Figure 9. Stripped, unfertilized egg shedding follicular capsule.
           (Photo by Ingersol)

       10. High blastula stage of *Hybognathus placitus*.

       11. Early gastrula stage of *Hybognathus placitus*. Note
            beginning of embryonic axis.

       12. Midgastrula stage of *Hybognathus placitus*. (Photo by
            Ingersol)

       13. Yolk plug stage of *Hybognathus placitus*.

       14. Embryo of *Hybognathus placitus* shortly after
            blastopore closure.

       15. First somites stage of *Hybognathus placitus*. Note
            placode and caudal mass.

       16. Sixteen somite stage of *Hybognathus placitus*. Note
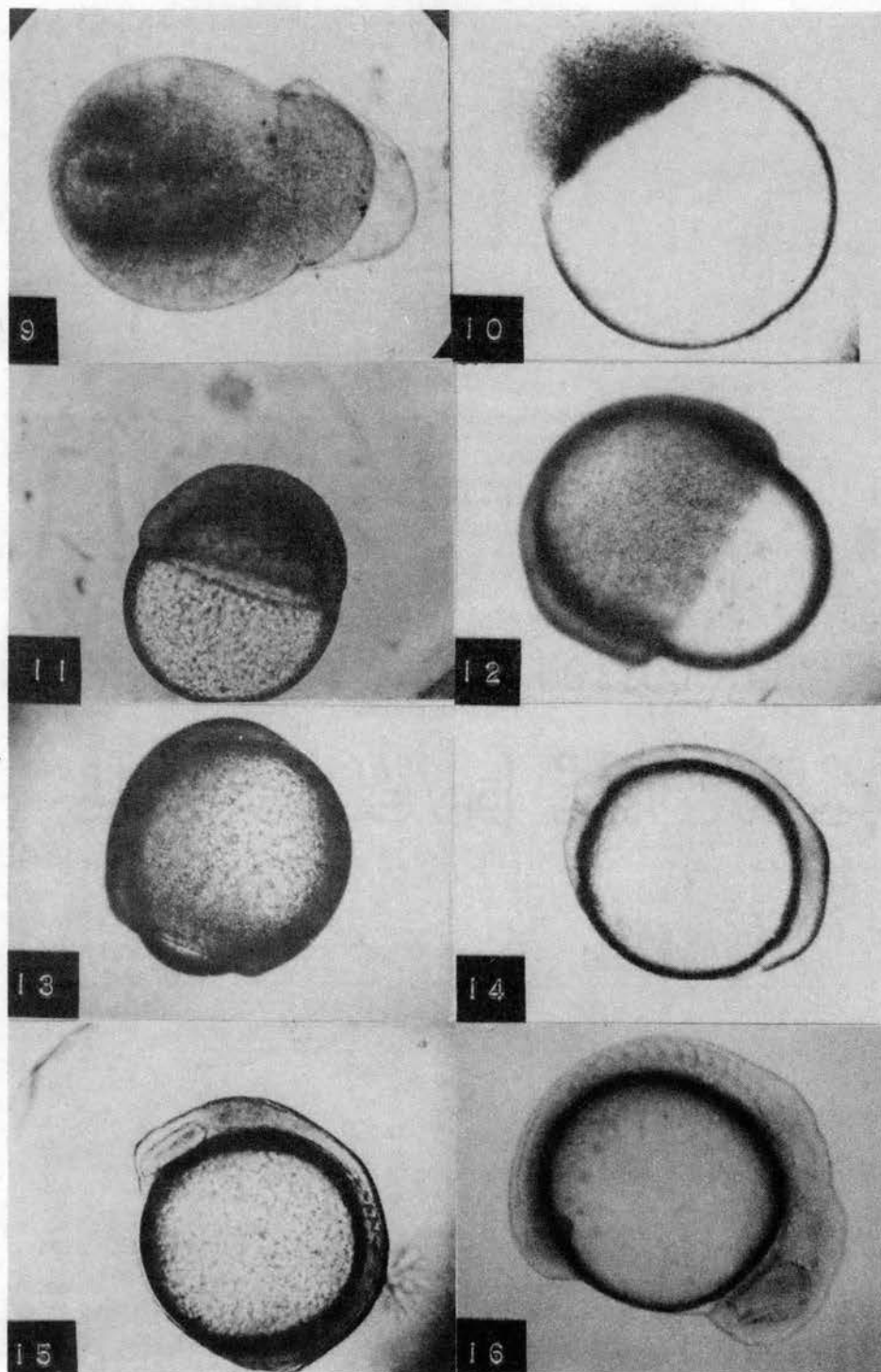   Kupffer's vesicle and otic placode.

Plate 2

Plate III

Figure 17. Twenty-three somite stage of <u>Hybognathus</u> <u>placitus</u>.
Note yolk sac elongating.

18. Thirty-two somite stage of <u>Hybognathus</u> <u>placitus</u>.
Note yolk sac extension.

19. Hatching stage of <u>Hybognathus</u> <u>placitus</u>, ventral
view.

20. Egg of unknown species showing zones within peri-
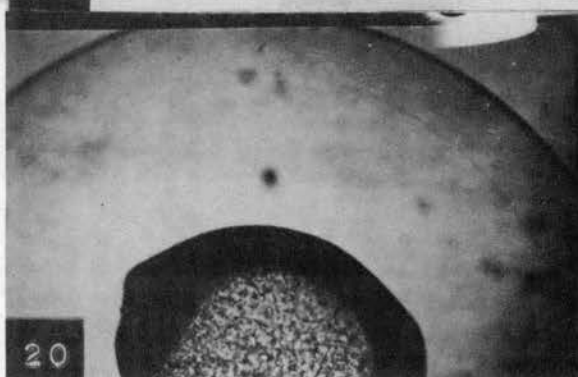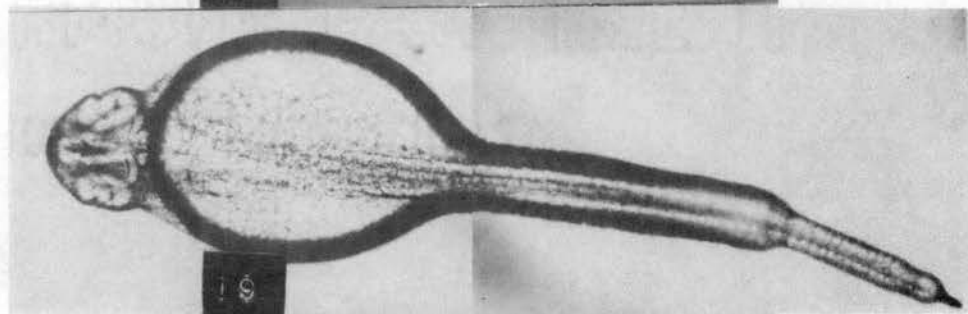vitelline space. Note fine line inside chorion
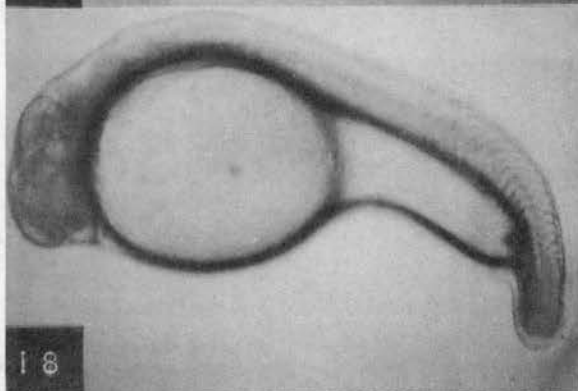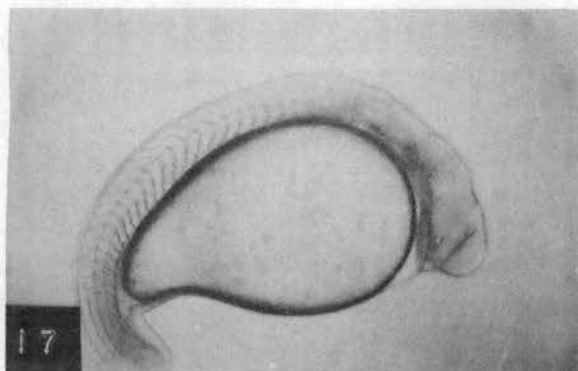encircling the embryo.

Plate 3

VITA

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Master of Science

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