NOTES ON THE EMBRYOLOGY AND EGG MEMBRANES, OF

HYBOPSIS AESTIVALIS TETRANEMUS (GILBERT)

By

CLYDE EUGENE BOTTRELL

Bachelor of Science

Oklahoma State University

Stillwater, Oklahoma

1960

NOTES ON THE EMBRYOLOGY AND EGG MEMBRANES OF

HYBOPSIS AESTIVALIS TETRANEMUS (GILBERT)

Thesis Approved:

Roy W. Jones
Thesis Adviser

W. H. Irwin

Geo. A. Moore

Racer Markear
Dean of the Graduate School

ACKNOWLEDGMENTS

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

INTRODUCTION

A. Embryology

This thesis culminates a study undertaken to elucidate
the embryology of a previously little studied minnow, *Hybopsis
aestivalis tetranemus* (Gilbert), "the speckled chub". The
embryological study consists of a gross morphological descrip-
tion of the developmental stages observed from the high
blastula to hatching. The stages observed and described are
compared and contrasted with the embryological stages of
teleosts listed by Oppenheimer (1937), Hisaoka and Battle
(1958) and Balinskey (1948). Photomicrographs are included
to illustrate the developmental stages.

Little is known concerning embryology in the genus
Hybopsis. Fish (1932) observed the breeding habits and larvae
of *Nocomis micropogon* (Cope) = *Hybopsis micropogon* (Cope). In
the same year Fish described the larvae of *Erinemus storerianus*
(Kirtland) = *Hybopsis storerianus* (Kirtland). Melvin Grubb,
in the summer of 1940, unsuccessfully attempted to determine
the spawning habits and early life history of the speckled
chub (Moore 1944). R. W. Jones, R. Ingersol, and D. Dunn
(unpublished data 1956-1959) have studied the early life
history of native minnows in Oklahoma but were unable to
include complete details concerning the development of the
speckled chub.

1

B. Micropyle and Egg Membranes

A second phase of the thesis involves a histological
study of the micropyle and egg membranes of _Hybopsis_
aestivalis _tetranemus_. Moore in 1944 observed a many rayed
"star" shaped structure in the chorion of _Notropis_ _girardi_
Hubbs and Ortenburger which he assumed was a micropyle.
Jones and his students following the suggestion of Moore col-
lected eggs from the Cimarron River over a period of several
years. These eggs were allowed to develop and the fish raised
were identified as _Notropis_ _girardi_, _Notropis_ _percobromus_ Cope,
Hybognathus _placita_ Girard and _Hybopsis_ _a_. _tetranemus_.

Jones (personal communication) suggested that these micro-
pyles may function as bouyancy regulatory structures as well
as passageway for sperm.

It was observed by Jones, Ingersol and the writer that
eggs with a large, circular, chorionic micropyle surrounded
by flaps of tissue always gave rise to larval _Hybopsis_ _a_.
tetranemus. Dissection of gravid female speckled chubs showed
the presence of similar micropyles in eggs in the ovaries.

Because this micropyle appeared to be unique it was
felt that a histological study of its origin in the ovary was
justified. No reference to previous studies concerning the
micropyle or egg membranes of this species or any _Hybopsis_
was found.

LITERATURE REVIEW

A. Embryology.

Considering the large number of teleosts, little is known concerning their detailed embryology. The more common and easily accessible groups have received the most attention. Some fishes of economic importance, food and sport fishes, have been studied in more detail.

Various approaches have been used to study the embryology of fishes. The ideal method for conducting an embryological study would be to study unfertilized eggs, their fertilization and the development of the fertilized eggs to hatching.

Stripping of gravid adult fishes is the ideal method for obtaining the eggs. Wilson (1889), Harrington (1947), Oppenheimer (1937), Solberg (1938), Jones (1937), and Hubbs and Drewry (1958) have successfully utilized this method.

A second and almost as useful method involves collecting eggs shortly after they have been laid and fertilized. Many authors including Ingersol (1953 unpublished master's thesis Oklahoma A. & M. College), Blumenkrantz (1956 unpublished master's thesis Oklahoma A. & M. College), Moore (1944), Budd (1940), and Carr (1942) have used the method in life history studies.

One paper published late in the 19th century summarized the knowledge of teleost embryology and laid the format for

3

future studies. This paper by Wilson (1889) was concerned with
embryology of the sea bass, Serranus atrarius (Linnaeus).

The sea bass is common along the Atlantic Sea Coast
where it spawns in the early summer, laying eggs 1 mm in
diameter with one large oil droplet. The eggs hatch in 75
hours at 15.5°C. Increased temperature decreased hatching time.

Wilson (1889) gave a general review and summary of fish
embryology to that date and showed, what had been well known
for a considerable time, that basic teleost embryology follows
a definite pattern of sequences. All papers reviewed since
1889 have confirmed Wilson's tenets.

Various workers in recent times have used a stage naming
classification for listing developmental stages. 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." Oppenheimer used 34 stages to classify
the development of Fundulus heteroclitus (Linnaeus), the
mummichog. She used stages characterized by obvious external
morphology.

Balinskey (1948) divided fish embryonic development into
37 stages plus larval stages. The 37 stages listed by
Balinskey include characters that are specific for fishes native
to Europe. In describing his stages Balinskey tended to list
characters that are sometimes difficult to observe without
detailed study, but wisely pointed out that the standard
length cannot be used to determine the age of a larvae. He

also incorporated the embryonic stages into a key for the
identification of embryos and larval fishes collected from
European streams. Pigmentation of the various body regions
was used as the means of identifying larval forms.

Hisaoka and Battle (1958) summarized 25 developmental
stages of the zebrafish, Brachydanio rerio (Hamilton-
Buchanan). The publication is excellent in that only the
most obvious morphological landmark is used to determine a
stage.

Blumenkrantz (1956 unpublished master's thesis Oklahoma
A. and M. College) studied the chronological development of
the zebrafish from the 16 cell stage to hatching some 72
hours later.

Various authors, Turner (1940), Tavolga and Rugh (1947),
and Tavolga (1949), have studied the embryonic development
of the live bearers. These authors all reported that the
basic teleost cleavage, gastrulation, and neurulation is
present in these forms. The live bearers (Poeciliidae) are
not truly viviparous because the young receives nourishment
from the egg yolk and not directly from the parent.

Marine fishes have been widely studied. Fry (1936),
Budd (1940), Ahlstrom and Ball (1954), have made plankton
collections and have studied the embryology of fishes that
lay pelagic eggs. The eggs were collected from the open sea
and allowed to develop, thus positive identification could
be made. Bolin (1930) stated that pelagic eggs always float
vegetal pole uppermost.

No descriptions of the embryos of the genus _Hybopsis_ were found in the literature. Fish (1932) partially described the early life history of 62 species of fishes from Lake Erie, including two species now placed in the genus _Hybopsis_. _Nocomis micropogon_ (Cope) (= _H. micropogon_) exhibits sexual dimorphism with the larger males building nests of stone and guarding the eggs. Eggs fixed in 10 per cent formalin measured 2 mm in diameter. _Erinemus storerianus_ (Kirtland) (= _H. storerianus_) spawns in June and July. The larvae were described as having a large oil droplet in the yolk sac.

B. Micropyle and Egg Membranes

The micropyle functions in the fish chorion, as it does in the insect egg, as a passageway for the sperm to enter and fertilize the ovum. However, the chorion of certain species lacks a micropyle or its presence has been overlooked. There is no obvious micropyle in the egg of the zebrafish or of the White Cloud, _Tanichthys albonubes_ Lin (personal observation). However, a complete histological study of the chorions might show such a structure.

Two outstanding papers on the micropyle were published prior to 1900. Mark (1890) described the embryology, micro- pyle, and egg membranes of the gar, _Lepisosteus_, which has an adhesive chorion with many filamentous villi. Mark figured in detail the structure and formation of the chorion and the very small micropyle, not observed by earlier workers.

Eigenmann (1890), a student of Mark, described the egg membranes and micropyle of nine osseous fishes and the complete seasonal changes in the egg membranes. The formation of the micropyle and its relation to the chorion was included for each species.

Since the two studies were completed, very little has been reported concerning the micropyle or chorion of fish eggs. Frequently persons who have studied the embryology of a fish mention a micropyle in the chorion but fail to refer the reader to a reference or give a description or illustration.

Recent histological studies concerning gametogensis in various fishes have been completed. Cooper (1952) conducted a study of the reproductive system of crappies (Pomoxis nigromaculatus and Pomoxis annularis). Cooper made no mention of a micropyle in the chorion or is there evidence of one in his photomicrographs. The chorion of the crappie is composed of two striated areas termed the vitelline membrane by Cooper.

One paper (Hayes, 1949), concerning a biochemical analysis of fish eggs, is worth mentioning. He gave the chemical composition of the egg membranes and of the yolk proper and described the osmoregulatory function of the chorion in the process of "hardening" in fish eggs. Hardening in fish eggs refers to chemical change in the chorion, involving conversion of the chorion to pseudokeratin, thus preventing the entry of other sperm.

MATERIALS AND METHODS

A. Embryology

   Hybopsis aestivalis (Girard) is a small minnow reaching
a maximum length of about 60 mm. The speckled chub is native
to the larger tributaries of the Mississippi and Rio Grande
river systems (Moore 1957). *Hybopsis aestivalis tetranemus*,
a four barbled subspecies, is a representative of the species
in the Arkansas River system of Oklahoma. The species was
usually present in main currents in the daylight hours and
was found feeding along the shores at night.

   During the springs, summers, and falls of 1960 and
1961 unsuccessful attempts were made to collect breeding
adults and to obtain embryos by stripping. Gravid females
were obtained but attempts at stripping were unsuccessful.
Gamone, a synthetic hormone, was injected as indicated by
Sneed and Clements (1960). Viable eggs were not obtained and
the treatments were discontinued. It became evident after
several attempts to utilize stripping and hormone injections
that eggs would have to be collected from the rivers where
they were laid.

   Moore (1944) noted that the eggs of *Notropis girardi*
could be collected from the flowing stream by means of a
piece of screen wire. He suggested that these fish were
stimulated to spawn when streams were swollen and muddy.

Hubbs and Ortenburger (1929) found young-of-the-year in July. May and June are the periods when we can expect heavy rains that render the plains streams muddy and high.

Jones, Ingersol, Dunn et al. in the summers of 1957, 1958, 1959, 1960, and 1961 collected fish eggs from the Cimarron River by means of nets constructed from mosquito netting and plastic screening. Embryos were hatched from the eggs and allowed to develop until large enough to identify. Several species, including _Hybopsis_ _a_. _tetranemus_, were collected and identified. Eggs of _Hybopsis_ _a_. _tetranemus_ were not found in the collections made during or immediately after flood conditions. The writer collected gravid females of the speckled chub as early as May 28, 1961, and embryonated eggs as late as August 28, 1961.

Two factors seem to play a role in the production of the eggs. The first is that of proper temperature. The eggs of the speckled chub were collected when the water temperature was in excess of $85^{\circ}F$. The temperature factor in the reproductive cycle of fishes has long been known. The second factor is that of light intensity. From the observations of Jones et al. and the writer it appears that the speckled chub spawns when the sun is at its zenith or brightest. No eggs in the early stages of cleavage were collected in the late afternoon or early morning hours. Eggs collected in the morning were always eggs of the previous day or days. Eggs collected in the afternoon were in the blastula or gastrula stages. Eggs of other species collected from the

muddy rivers of central Oklahoma (Moore 1944) tend to validate the idea that spawning occurs near noon or at the time when the sun is brightest and the water temperature the highest.

Study of dissected gravid females by Jones and Ingersol and by me established the fact that H. aestivalis eggs could be identified by the particular structure of the micropyle (Figure 1) and the size of the embryo in the early stages of cleavage. From this previous knowledge, it was easy to identify the eggs of the speckled chub at the time of collection.

The embryos utilized in the present study were collected August 17, 1961 and August 28, 1961 from the Cimarron River south of Perkins in Payne County, Oklahoma.

The eggs studied were kept in small watch glasses. Embryos while being photographed were kept in deep depression slides. Aerated tap water was added as necessary to compensate for evaporation. If an embryo was to be observed for a long period of time a thin cover glass was placed over the depression in the slide to prevent dust from collecting on the water and to retard evaporation.

No attempt was made to maintain a constant incubation temperature, but the room temperature fluctuated between 75° and 83°F.

Photographs, as deemed necessary, were taken with a Kodak 35 mm Pony IV camera mounted on a Spencer triocular microscope. Changes in development were less frequent in the early stages and called for fewer photographs, but as

details of morphogenesis became more obvious more photographs were taken.

Data concerning development were obtained in two ways. The first involved recording photographic data on specially prepared record sheets including light, speed, filter, etc. with additional notes to supplement this information. The second and most useful method involved a tape recorder. The recorder had one distinct advantage, that being freedom of the hands for manipulation of the microscope and camera. This was particularly advantageous when the embryos began to move within the chorion and keeping them in focus was difficult.

After hatching, the fry were kept in plastic one-gallon aquaria and fed powdered egg yolk. As the fry grew older, they were fed dried food, a prepared liver-cereal mixture, and white worms of the genus _Enchytrea_. This diet apparently lacked certain nutritional factors as the vertebral column became malformed in some individuals.

The juveniles were fixed in ten per cent formalin at five months of age. Positive identification was made by Dr. G. A. Moore. The specimens were deposited in the ichthyological collection at Oklahoma State University, catalog number 5561.

B. Micropyle and Egg Membranes

The ovaries used were removed from gravid females that had been fixed in 10 per cent formalin and preserved in 50 per cent isopropanol. Some females were purchased from a

minnow dealer in Ponca City, Oklahoma, July 2, 1960, and
others were seined from the Cimarron River near Okene, Oklahoma,
June 3, 1962.

After the ovaries had been dissected from the fixed and
preserved females, they were upgraded through 25, 50, 75,
and 95 per cent ethanol, placed in ether-alcohol for three
hours, infiltrated in 2, 4, 8, and 16 per cent celloidin for
24 hours each at 56°C, hardened in chloroform vapors and
cleared in Terpineol for 24 hours. Dry sections were cut
at 15 microns and placed in 95 per cent isopropanol. The
95 per cent isopropanol alcohol causes the sections to
uncurl. Some sections were stained in Mallory's triple-
connective-tissue stain and others were studied without
staining. Stained and unstained sections were mounted in
Euparal or Permount.

The micropyle study involved observation of stained
material with an ordinary compound microscope and unstained
sections with the phase microscope. Various stages and
sizes of eggs were observed from the smallest ova in the
ovary to mature eggs ready to be laid. Measurements of
the chorions and eggs were taken with the aid of an ocular
micrometer.

OBSERVATIONS

A. Embryonic Stages

Each stage used in this study is one which best fits a
descriptive developmental landmark of the species. Refer-
ences for the stages follow the criteria of Oppenheimer (1937),
Balinskey (1948), and Hisaoka and Battle (1958), although
their stages do not always correspond.

High Blastula (Figure 5). The earliest stage represented
in this study was the high blastula. The average diameter of
the yolk mass (measured at right angles to the animal-vegetal
axis) of all embryos observed was 0.95 mm. The distance called
the yolk-blastoderm, animal to vegetal poles, averaged 1.05 mm.

The yolk of all eggs observed in this stage was a
homogeneous mass except for some larger interspersed yolk
droplets. These droplets are not the oil droplets of other
authors as they lacked the characteristic transparency.

The peripheral cells of the blastoderms were large
and the nuclei quite obvious. No attempt was made to count
the number of cells but the embryos were probably in the
seventh to ninth cleavage.

Early Gastrula. The gastrula was formed by the rapid
movement of the blastoderm over the yolk mass by epiboly.
Gastrulation was marked by the thickening of the germ ring
as the cells began to differentiate and form the embryonic

13

shield. The area of the embryonic shield was recognizable but it was not a distinct structure at this stage.

Mid-gastrula or Equatorial Plate (Figure 6). At this stage the germ ring covered about one-half of the yolk material, thus, the name equatorial plate. The embryonic shield was evident as a thickening projecting at right angles to the germ ring. In Figure 6, the embryonic shield is seen in the center of the germ ring. No thickening of the shield was present. The lack of this thickening indicated that no differentiation of the central nervous system had occurred.

Late gastrula or yolk plug (Figure 7). The late gastrula showed no marked changes from the previous stage in gross examination, except that a greater amount of yolk was now covered by the blastoderm. A thickening of the embryonic shield indicated differentiation of the central nervous system. During this stage, and to the closure of the blasto- pore, 75 to 100 per cent of the yolk was covered by blastoderm.

Late in the yolk-plug stage the blastoderm had covered all of the yolk except the area of the blastopore. A protruding yolk mass appears as a plug and is the reason for the name to this stage.

Shortly after the photograph (Figure 7) was made, the embryonic shield extended from the dorsal lip of the blastopore to an area one-half of the way around the yolk mass.

Closure of the blastopore (Figure 8). The average time for closure of the blastopore was 4 hours and 19

minutes after the high blastula stage. Before the blastopore
was completely closed the cephalic thickening had become
evident. The tail bud had differentiated from the embryonic
shield in the immediate area of the blastopore, but no
somites had appeared at the time of the blastopore closure.

Formation of the optic vesicles (Figure 9). The
optic vesicles formed as a hemispherical evaginations from
the diencephalon and appeared at the two-somite stage, as
Oppenheimer (1937) and Hisaoka and Battle (1958) reported.
The optic vesicle was first evident seven hours after the
high blastula stage was observed.

Kupffer's vesicle, a little understood structure in the
tail bud region, made its appearance at this stage. Tavolga
(1947) stated that this vesicle is the urinary bladder.
However, fate and function have not been determined in
teleosts. (Bolin (1930) reported two Kupffer's vesicles
in fifty per cent of the fish embryos observed.

Otic placode (Figures 9 and 10). The first appearance
of the otic placode was marked by an ectodermal thickening in
the area of the metencephalon.

The otic placode, an ectodermal plate of cells,
became anteroposteriorly elongate and appeared to become
compressed dorsoventrally. The appearent dorsoventral
compression produced what appeared to be a slit in the
placode. The slit according to Solberg (1938) was formed as
the "placode sank to form the organ of balance". No
otoliths or their precursors were evident during this
developmental stage.

While the otic placode was forming, several other changes occurred. The optic vesicles became spherical from the previous hemispherical shape. Kupffer's vesicle was evident in the tail bud region. The tail bud which had become freed from the yolk mass now developed somites.

In agreement with Hisaoka and Battle (1958), 15 pairs of somites were present. Dorso-ventral muscular flexures of the embryo began during this period.

The heart, while not a completely formed structure, was visible in the pericardium below the head. The pericardium was quite expansive and transparent. The size of the pericardium, however, did not compare with the vascular pericardium of Fundulus heteroclitus (Tavolga and Rugh, 1947).

Heart beat and convulsive body contractions (Figures 11 and 12). When the heart began to beat, it consisted of a pulsating tube. Pulsations traversed the entire length of the heart at the rate of 15 to 20 beats per minute. No cellular material was present in the heart nor was any circulation present. The embryo in this stage had 28 pairs of somites and was threshing the tail against the chorion.

Otoliths (Figure 13). Prior to this stage no otoliths had been evident in the auditory vesicle. The otoliths first appeared as two dark spherical bodies in the auditory vesicle. Other distinct black granules much smaller than the otoliths were present. Oppenheimer (1937) stated that these granules are calcareous bodies which are incorporated into the otolith proper. Thirty three pairs of somites were present at this stage.

<u>Swim</u> <u>bladder</u> <u>and</u> <u>pectoral</u> <u>fin</u> <u>formation</u> (Figure 13).
The swim bladder first appeared as a one-chambered dorsal
evagination from the gut. Formation of the posterior chamber
occurred after hatching. The pectoral fin became visible
as a lateral projection dorsal to the air bladder. The fin
during this stage had no definite fin-like appearance or
any evidence of fin rays.

<u>Hatching</u> (Figures 14 & 15). The average hatching time
for the speckled chub was 23 hours after the high blastula
stage. Prior to hatching the continual spasmodic movement of
the embryo had damaged the chorion. The absence of two
characters at hatching marked the embryology of the
speckled chub. The first was the absence of any pigmentation.
The eye, the first structure to show pigment, was
colorless. The second character, possibly reflecting my
inability to observe, was the lack of cellular elements in
the blood.

The yolk mass was small in comparison with the original
amount. The rectum formed at right angles to the yolk mass
as an ectodermal invagination. The angle of formation makes
this structure different from that in the other species of
fry observed. The rectum in most fish species previously
described, forms at an oblique angle.

No structure that could be called the caudal fin was
evident at hatching. There was a fold of tissue extending
from the region of the anus ventrally around the posterior
end of the animal to a dorsal area just above the anus

(Figure 15). The tissue was a fin fold but there were no fin rays or other supporting structures evident.

Pigmentation of the eye. At hatching no eye pigmentation was observed, but twenty four hours after hatching embryos held over a white surface, exhibited pigment in the retina. No pigmentation was evident when the embryos were observed without the contrasting background.

The fry of the speckled chub exhibited swimming behavior, similar to that of Notropis girardi described by Moore (1944). The fry rested on the bottom of the tank and then suddenly for no obvious reason swam to the upper surface of the water. The swimming route resembled a circular staircase figure. After aimlessly following this pattern the fry dropped to the bottom and once again repeated this activity. The erratic swimming movements were continued until the yolk mass was completely absorbed at which time the fish began to move purposefully.

The gut, a structure not observed before this stage, became evident as the yolk was consumed appearing as a long tube running from the mouth to the anus. Two days after hatching the intestine was filled with food as the fry began to eat.

Presence of rays in pectoral, pelvic, and caudal fins. Three days after hatching the yolk was absorbed marking the post larval stage of Hubbs (1943). The fry lacked any pigmentation on the exterior surface of the body. The meninges of the brain were the first area to pigment other than the

retina. Scattered melanophores appeared extending from the anterior most part of the brain to the spinal column.

The pectoral fins developed from bud-like structures just dorsal to the yolk mass. At hatching the pectoral fins lacked any visible rays. Three days after hatching rays lacking branches appeared as thickenings in the pectoral fins.

The anterior portion of the caudal fin fold disappears. This seems to be due to the absorption of the tissues into the growing tail. The portion of the fold posterior to the tail remains and in it develop the caudal fin rays. The rays appear as unbranched anterior-posterior thickenings about three days after hatching.

B. Micropyle and Egg Membranes

The ovary of the speckled chub is a bilobed structure joined posteriorly. The entire mature ovary from a gravid female measured 20 mm long and 10 mm wide and filled most of the coelomic cavity.

In sections the ova were conspicuously filled with large amounts of yolky material and of various sizes, probably indicating an extended spawning period. About 90 per cent of the eggs measured 0.75 mm to 0.86 mm in diameter. Because of pressure against each other, the ova were not spherical while in the ovary. Some smaller eggs measuring less than 0.25 mm were present. Egg membranes surrounded all eggs in the ovary.

Egg membranes (chorion, zona radiata, or shell) are those structures closely associated with the ova after they have been laid. While in the ovary the chorion closely surrounds the yolk. The chorion surrounding the mature egg measured about one micron in thickness and is marked by radial striations, "pore canals" of Mark (1890) and Eigenmann (1890).

The otherwise homogeneous chorion is marked by the presence of a funnel-like structure, the micropyle (Figures 1, 2, 3 and 4). In sections the micropyle was two and one-half microns wide at the top of the funnel and nine tenths of a micron deep. The funnel tapers from the neck to the very narrow micropylar canal. This short canal terminates over the nucleus in the yolk.

The single-layered chorion forms an S shaped bend at the edge of the funnel of the micropyle, Figure 3 and 4. The funnel of the micropyle is filled with a cellular mass, the micropylar plug of modified granulos cells thought to be responsible for the formation of the micropyle. This structure appears to be composed of a large single cell in some cases and of many small cells in others.

The granulosa, surrounding the chorion, is composed of a single layer of cells which extends around the entire chorion and extends over the funnel of the micropyle, Figures 3 and 4. The granulosa plays no role in the formation of the chorion except as the donor of the micropylar plug or micropylar cells. Mark (1890) and Eigenmann (1890) speculated

that the micropyle is formed by the physical action of the micropylar cell or plug as the chorion is secreted by the ovum itself. The plug forms sort of a mold about which the chorion of the micropyle is developed.

DISCUSSION

A. Embryonic Development

The embryonic development of the speckled chub is not
markedly different from that of the previously studied
species except as follows: the rapidity with which the
animal develops, the lack of body pigmentation at hatching,
the apparent lack of formed elements of the blood at hatching
and the post embryonic behavioral pattern.

Hatching time for the speckled chub was less than 24
hours after the high blastula stage. The shortness of hatch-
ing time indicates that the developmental pattern must be an
accelerated one. This is particularly true with the speckled
chub since the blastopore closed 4 hours and 19 minutes after
the high blastula stage.

The hatching period expressed in hours must be
judiciously used and not considered as a consistant landmark.
Oppenheimer (1937) indicated that temperature plays a role
in hatching time. No control over temperature was exercised
during the incubation period but the normal range in the
rivers would not vary much from room temperature (75-83° F).
Data concerning temperature collected from the river during
the evening and night hours validate this idea.

The lack of any visible pigmentation is a second feature
that makes the embryology of the speckled chub somewhat unusual.

Most fish embryos have pigment some place on the body at hatching. Carr (1942), however, reported no pigmentation occurred in the large-mouthed bass until three days after hatching, where the retina showed the first pigmentation. The same was true with the speckled chub. Three days after hatching the retina became pigmented. The large stellate amoeboid melanophores characteristic of the adults did not appear until 10 days after hatching.

B. Micropyle and Egg Membranes

The egg membrane, the chorion, present in all mature ovarian eggs (0.25-0.87 mm) is a one-layered structure and has a striated appearance due to the radial or pore canals. Eigenmann (1890) studied the golden shiner, and found the chorion to be one layer thick.

The spawned egg of the speckled chub has a large micropyle surrounded by triangular flaps of tissue, Figure 1. The tissue flaps are not evident in the sectioned ovary. However, the folding at the edge of the micropyle and the continuous extension of the granulosa over the micropylar plug may offer an explanation as to how the flaps are formed.

When the eggs are laid, the chorion very loosely surrounds the yolk. Hayes (1949) described how the chorion in the fish egg separates from the yolk to form the perivitelline space. When the chorion expands it reaches a diameter of about two mm in Hybopsis aestivalis tetranemus. This expansion allows the folded chorion to form the micropylar

funnel. The flaps of tissue which surround the funnel mouth are probably formed from the granulosa cells that adhere to the chorion at the micropylar edge.

This conclusion is drawn from observation on eggs in the mature ovary and the eggs after ovulation. The flaps of tissue around the micropyle decay rapidly. The decay indicates that they are cellular in nature although the chorion is non-cellular. The only cellular structure in close association with the chorion is the granulosa. It appears that the granulosa adheres to the folded edge to form the flaps of tissue.

Why does the granulosa adhere in this region and not elsewhere? The only plausible explanation seems that the chorion is attached to the granulosa in this particular area. When the egg ruptures from the ovary the attachment is carried along as the flaps of tissue surrounding the micropylar funnel.

Spawning of Hybopsis a. tetranemus has not been observed. It has been suggested by Jones (personal communication) that perhaps spawning may occur in the main currents of the stream where the eggs might sink toward the bottom. However, the perivitelline fluid quickly imbibes water through the micropyle bringing in sperm cells. The swollen non-adhesive chorion soon hardens and provides a bouyant envelope around the egg. The increased surface acted upon by the river currents thus keeps the egg suspended in the muddy waters. The micropyle and chorion thus serve as bouyancy regulatory

structures which keeps the eggs from being covered with silt.

While collecting _Hybopsis_ _aestivalis_ _tetranemus_ adults from the Cimmaron River on June 3, 1961, a gravid female _Hybopsis_ _storerianus_ was taken. This fish was stripped in an attempt to determine the type of eggs and the presence or absence of a micropyle. The eggs obtained were not mature but a micropyle, similar to that of the speckled chub, was present in the chorion. Its large size in comparison to the chorion diameter and the general shape of the micropylar mouth and funnel, a circle surrounded by flaps of torn tissue, attest to a possible relationship between the two species.

SUMMARY

I. A stage sequence of the development of the speckled chub, Hybopsis aestivalis tetranemus (Gilbert), is given from the high blastula to hatching. The stages listed are compared with those of Hisaoka and Battle (1958), Balinskey (1948) and Oppenheimer (1937).

II. The embryonated eggs were collected from the Cimarron River near Perkins in Payne County, Oklahoma, August 17 and August 28, 1962 by means of a net.

III. The embryos differed from most previously described species in the following ways: rapid development, hatching in about 24 hours from the high blastula, no pigmentation at hatching, post embryonic behavior.

IV. A second phase of the study was a histological description of the egg membranes and micropyle of the speckled chub.

V. From sectioned mature ovaries, it was determined that the chorion was one layer in thickness and was radially striated. The striations are the pore canals described by Eigenmann (1890) and Mark (1890).

VI. The micropyle in the chorion was large, circular, and surrounded by flaps of tissue. The flaps of tissue were considered to be adhering granulosa.

LITERATURE CITED

Ahlstrom, E. H. and O. P. Ball. 1954. Description of eggs
     and larvae of Jack Mackerel (*Trachurus symmetricus*) and
     distribution and abundance of larvae in 1950 and 1951.
     Fishery Bull. 97 (56): 209-245.

Battle, Helen I. 1940. The embryology and larval develop-
     ment of the gold fish (*Carassius auratus* L.) from Lake
     Erie. Ohio Jour. Sci. 40 (2): 82-93.

Balinskey, B. I. 1948. On the development of specific
     characters in cyprinid fishes. Zool. Soc. of London
     Proc. 118 (1): 335-344.

Blumenkratz, Bertram L. 1956. The chronological develop-
     ment of the embro of the Zebra fish, *Brachydanio*
     rerio (Hamilton) at a constant temperature. Unpub-
     lished Master's thesis. Oklahoma State Univ.
     (Okla. A & M College), 1956.

Bolin, R. L. 1930. Embryonic development of the labrid fish,
     Oxyjulis californicus Günther. Copeia 1930 (4): 122-128.

Budd, P. L. 1940. Development of the eggs and early larvae
     of six California fishes. California Division of Fish
     and Game. Bureau of Marine Fisheries. Fish Bull.
     56: 5-53.

Carr, Marjorie H. 1942. The breeding habits, embryology, and
     larval development of the large-mouth black bass in
     Florida. Proc. New England Zool. Club 20: 43-77.

Cooper L. J. 1952. A histological study of the reproductive
     organs of crappies (*Pomoxis nigromaculatus* and *P.*
     annularis). Trans. Am. Microsc. Soc. 71 (4): 393-404.

Eigenmann, C. H. 1890. On the egg membranes and micropyle of
     some osseous fishes. Bull. Harvard Mus. Comp. Zool.
     19 (2): 129-154.

Fish, M. P. 1932. Contributions to the early life histories
     of 62 species of fishes from Lake Erie and its
     tributary waters. Bull. Bur. Fish. 47: 293-298.

Fry, D. H. Jr. 1936. A description of the eggs and larvae of
     the Pacific mackerel. Calif. Fish and Game 22 (1):
     27-29.

Hayes, F. R. 1949. The growth, general chemistry, and temperature relation in salmonid eggs. Quart. Rev. Biol. 24: 281-308.

Harrington, R. W. Jr. 1947. The early life history of the brindled shiner, Notropis bifrenatus (Cope.) Copeia 1947 (2): 97-102.

Hisaoka, K. K. and Helen I. Battle. 1958. The normal developmental stages of the Zebra fish, Brachydanio rerio (Hamilton-Buchanan). J. Morph. 102 (2): 311-328.

Hubbs, C. L. Terminology of the early stages of fishes. Copeia 43 (4): 260.

Hubbs, C. L. and A. I. Ortenburger. 1929. Further notes on the fishes of Oklahoma with descriptions of new species of Cyprinidae. Univ. Okla. Bull. (ns) 434 (Publ. Univ. Okla. Bio. Sur. 1): 17-43.

Hubbs, Clark and G. E. Drewry. 1958. Long duration of sperm function in two Euryhaline fishes and its lack of significance in hybridization. The Am. Nat. 92(867): 378-380.

Ingersol, R. H. 1949. Studies on the embryonic development of the blue gourami, Trichogaster trichopterus. Unpublished Master's Thesis, (Oklahoma A. and M. College) 1949.

Jones, R. W. 1939. Analysis of the development of fish embryos by means of the mitotic index, V. The processes of early differentiation of organs in Fundulus heteroclitus. Trans. Amer. Microsc. Soc. 58 (1): 1-23.

Mark, E. L. 1890. Studies of Lepisosteus. Bull. Harvard Mus. Comp. Zool. 19 (2): 1-129.

Moore, G. A. 1944. Notes on the early life history of Notropis girardi. Copeia 1944 (4): 209-214.

Moore, G. A. 1957. Part two. Fishes in Vertebrates of the U. S. by Blair, Blair, Brodkorb, Cagle and Moore. McGraw Hill Book Co. Inc. N. Y. p. 33-210.

Morris, R. W. 1951. Development of the Cottid fish, Clinocottus revalvus (Greely). Calif. Fish and Game 37 (3): 281-300.

Oppenheimer, Jane M. 1937. The normal stages of Fundulus heteroclitus. Anat. Rec. 68 (1): 1-15.

Sneed K. E. and H. P. Clements. 1960. Use of fish pituitaries to induce spawning in channel catfish. U. S. Fish and Wildlife Serv. Spec. Sci. Rept. Fisheries No. 329: 1-12.

Solberg, A. N. 1938. The development of a bony fish. Prog. Fish-cult. 40: 1-19.

Tavolga, W. N. and R. Rugh. 1947. Development of the platyfish, Platypoecilus maculatus. Zoologica 32 (1): 1-15.

Tavolga, W. N. 1949. Development of the platyfish (Platypoecilus), the swordtail (Xiphorus), and their hybrids. Bull. Amer. Mus. Nat. Hist. 94 (4): 161-230.

Turner, C. L. 1940a. Pseudoamnion, pseudochorion and follicular pseudoplacenta in Poeciliid fishes. J. Morphol. 67: 58-69.

Wilson, H. V. 1889. The embryology of the sea bass, Serranus atrarius. Bull. U. S. Fish Comm. 9: 209-278.

APPENDIX

31
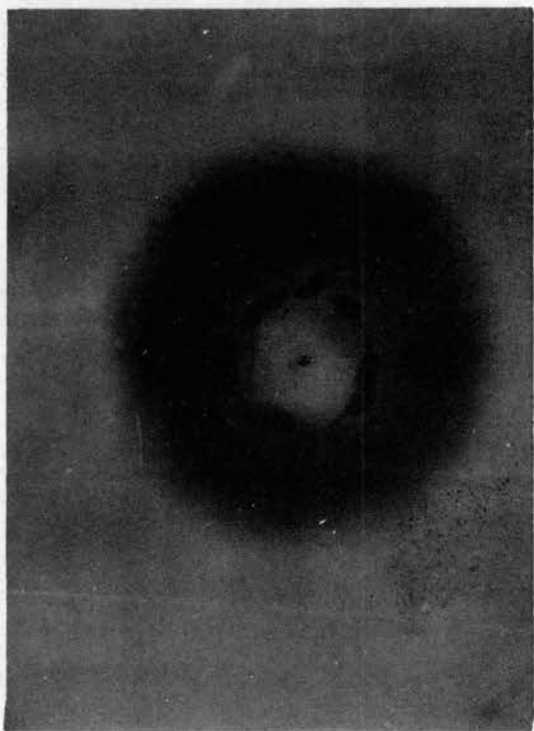
Plate I

Figure 1.

Figure 2.

Figure 3.

Figure 4.

Plate II . . . .

Figure 5. High blastula stage of *Hybopsis a. tetranemus*.
6. Mid gastrula or equatorial plate stage of *Hybopsis a. tetranemus*. Note beginning of embryonic keel.
7. Late gastrula or yolk plug stage of *Hybopsis a. tetranemus*.
8. Closure of the blastopore stage of *Hybopsis a. tetranemus*. Note micropyle in chorion.

Plate II
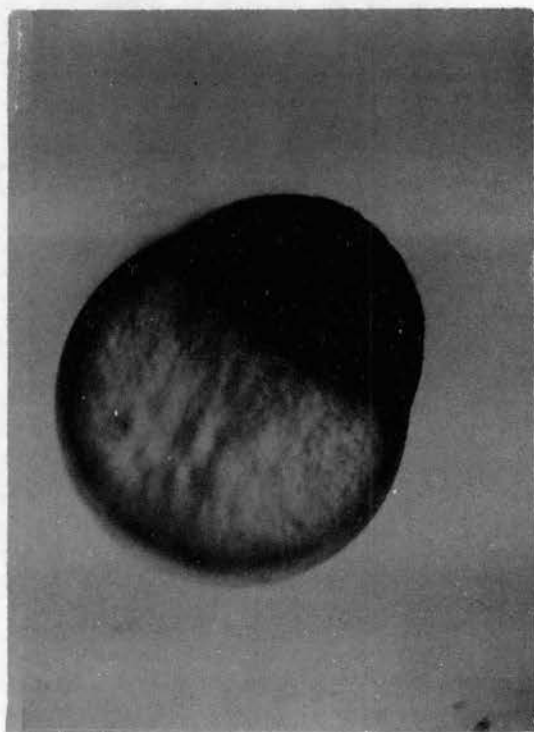
Figure 5.
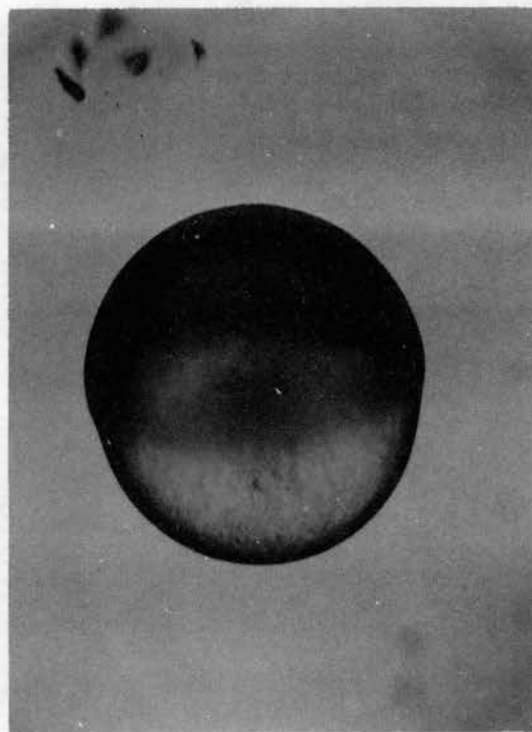
Figure 6.
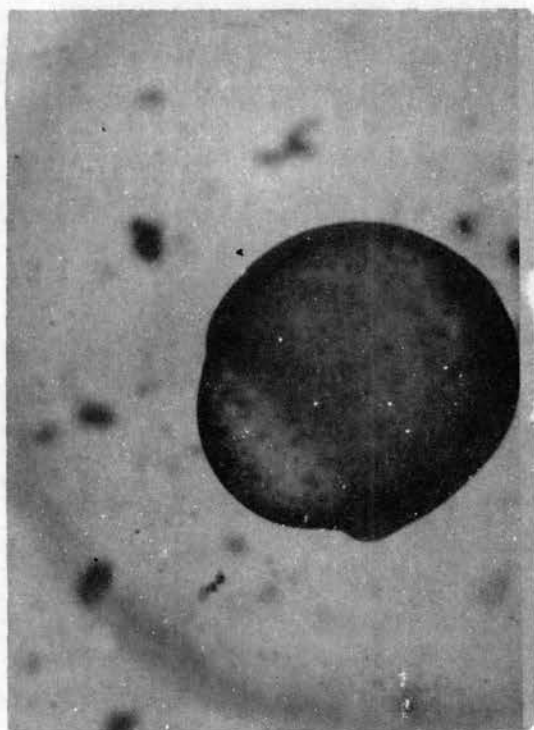
Figure 7.
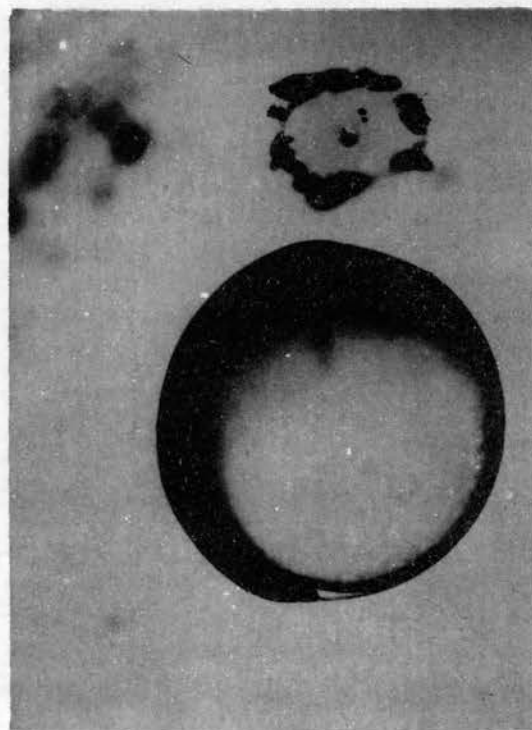
Figure 8.

Plate III
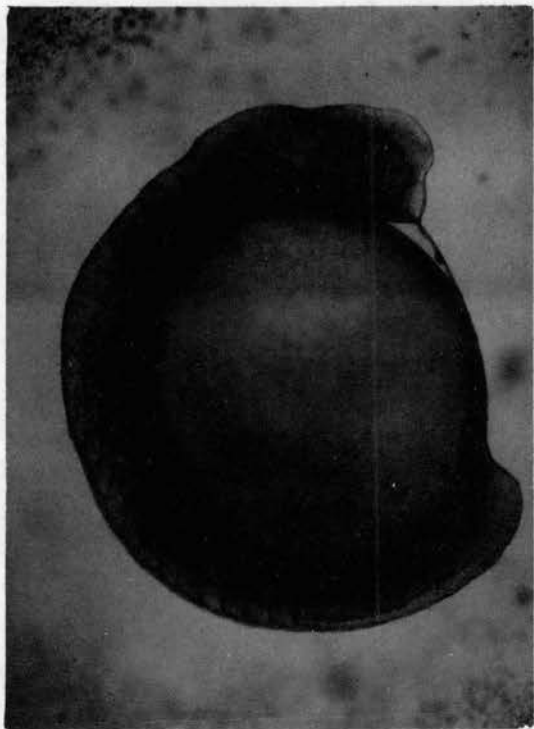
Figure 9.
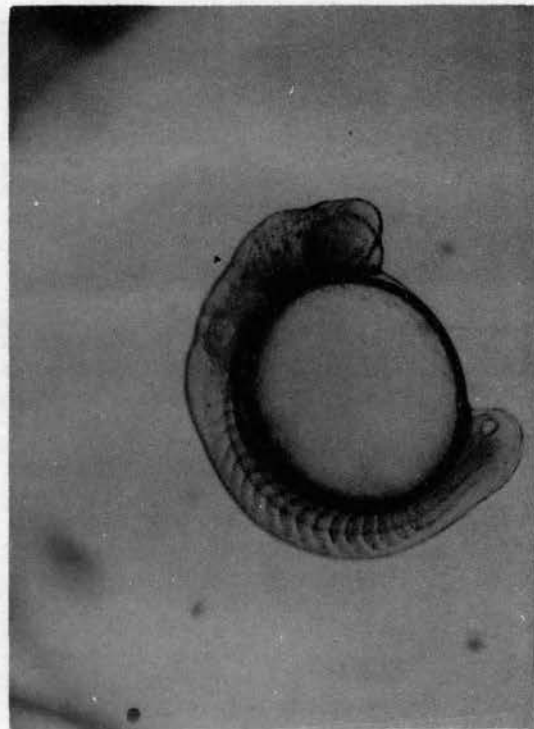
Figure 10.
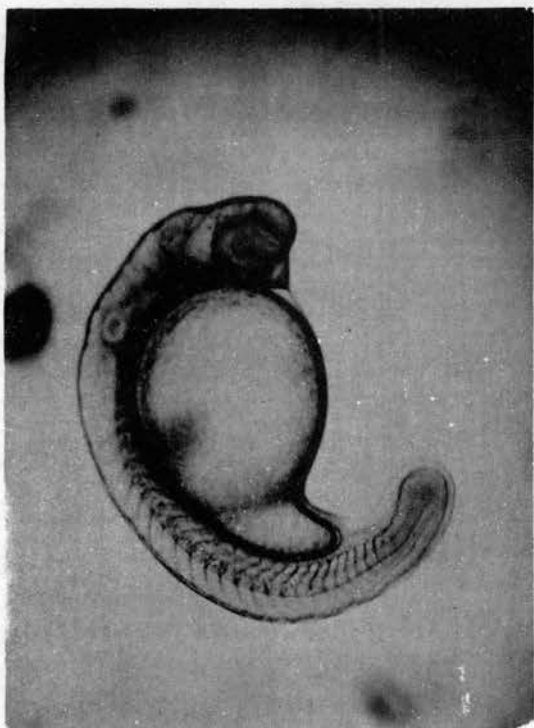
Figure 11.

Figure 12.
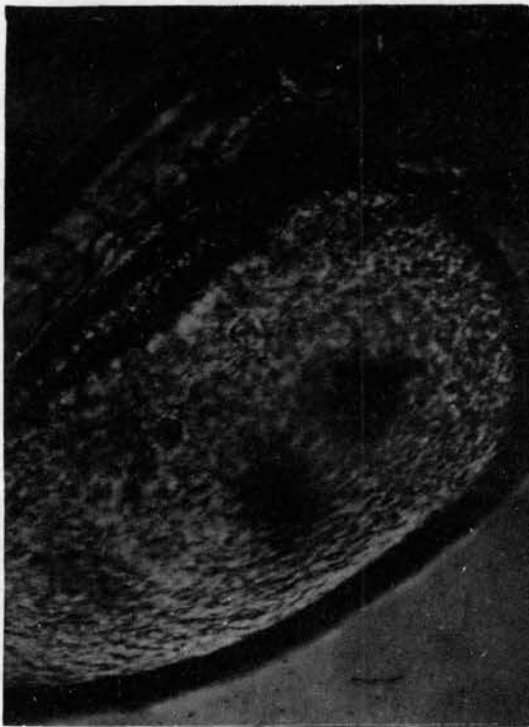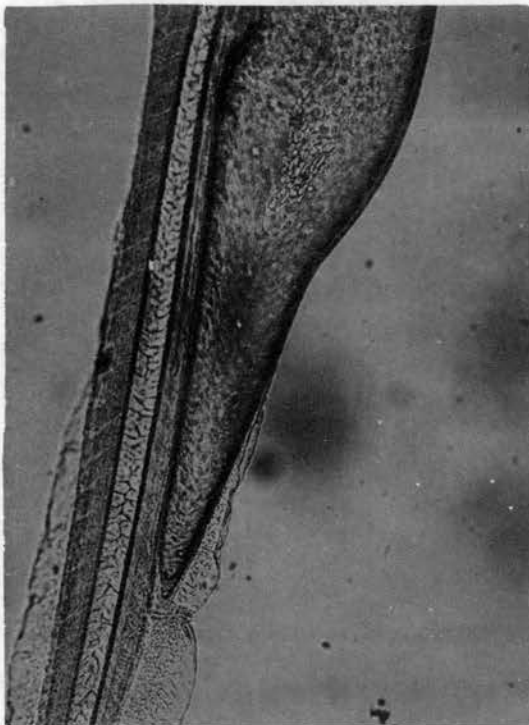
Plate IV

Figure 13.

Figure 14.

Figure 15.

VITA

Clyde Eugene Bottrell

Candidate for the Degree of

Master of Science

Thesis: NOTES ON THE EMBRYOLOGY AND EGG MEMBRANES OF
HYBOPSIS AESTIVALIS TETRANEMUS (GILBERT)

Major Field: Zoology

Biographical:

  Personal Data: Born in Ponca City, Oklahoma, September
    16, 1937, son of George H. and Leona D. Bottrell.

  Education: Attended grade school and high school in
    Ponca City, Oklahoma; graduated from Ponca City Senior
    High 1956; A. S. Northern Oklahoma Junior College,
    Tonkawa, Oklahoma, 1958; B.S. Zoology, Oklahoma State
    University, 1960; completed requirements for the
    Master of Science August, 1962.

  Professional Experience: Graduate Teaching Assistant
    Zoology Department, Oklahoma State University 1960-
    1962; Graduate Research Assistant 1960-1962.

  Organizations and Honors: Scholastic Alumni Award,
    Northern Oklahoma Jr. College, 1958; Sigma Xi,
    Phi Sigma, Oklahoma Acad. of Science.