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Title of Study: A STUDY ON THE USE OF FISH EGGS IN THE TEACHING OF EMBRYOLOGY IN HIGH SCHOOL BIOLOGY

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Scope of Study: The embryos of some teleost fishes are covered with a clear chorion. This report involves the methods by which certain of these embryos can be used in the high school biology program in the teaching of embryology. It discusses the species of fish that would be suitable for this purpose, their proper care and handling for the production of eggs, and methods of collecting fertilized eggs. Some oviparous and viviparous species are recommended for use in the classroom so that studies can be made on both methods of offspring production. Selected embryonic developmental stages that would be suitable for high school observations are discussed and some are illustrated with photomicrographs. Sources of information on the aquarium, in addition to those in the bibliography, are to be found at the end of Chapter II. Materials used in writing this report include (1) original, published research papers dealing with fish embryology, (2) unpublished Master's Theses dealing with fish embryology, (3) personal conversations with persons working in the area of fish embryology, and (4) books on the subject of the aquarium.

Findings and Conclusions: The study of embryology in the high school biology program can be made more meaningful with the use of selected teleost embryos. As a teaching tool, fish embryos can be most useful in clarifying certain developmental stages in other higher vertebrates. The embryos of many species develop rapidly; consequently, the student is able to observe several different developmental stages in one laboratory period. Many species of fishes lay eggs that can readily be used in the laboratory, and since water is the culture medium, many can be kept quite easily in the laboratory for observational purposes.

ADVISER'S APPROVAL Roy W. Jones

A STUDY ON THE USE OF FISH EGGS IN THE TEACHING OF EMBRYOLOGY IN HIGH SCHOOL BIOLOGY

By

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A STUDY ON THE USE OF FISH EGGS

IN THE TEACHING OF EMBRYOLOGY

IN HIGH SCHOOL BIOLOGY

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CHAPTER I

INTRODUCTION

The purpose of this report is to present a study on how fish eggs may be obtained and used for teaching embryology to high school biology students. Suitable species of oviparous fish will be presented for consideration and the embryonic development of two of them will be discussed at some length. Also, suitable viviparous species will be mentioned, since some teachers will want to have both available in order that reproductive methods may be compared and contrasted.

Too often the study of embryology at the high school level is brushed aside, or at best, taught with the aid of charts or diagrams only. The more energetic teacher has probably used chick or frog eggs, but their use offers several problems.

The chick egg is easy to obtain and with special equipment it is easy to incubate. But because it is fertilized internally, early cleavage stages cannot be observed. If biology classes are large, many eggs must be incubated before various embryonic stages can be observed by the student. Observation of the developing embryo necessitates the removal of the shell which leads to the death of the embryo, therefore continuous development cannot be observed. Another important factor is time. It takes 21 days for a chick egg to hatch.

Frog eggs are not as easily obtained as chick eggs. Frogs and toads lay their eggs in bodies of fresh water in the spring of the

year where they can usually be obtained in various developmental stages. It is possible to keep adult male and female frogs in the laboratory with such problems as storage, feeding, etc. When eggs are needed, the female can be artificially stimulated to ovulate if she is injected with the macerated pituitary glands of other frogs. After about 72 hours the eggs can be stripped from her body. They can be fertilized from sperm provided by killing male frogs, removing and macerating their testes, and pouring the material over the eggs. Miller and Blaydes (1962) and Moreholt, Brandwein and Joseph (1958) offer numerous suggestions on the use of chick, frog, and fish embryos in the laboratory.

A far easier method for obtaining eggs for the teaching of embryology is to keep suitable egg laying fish in the laboratory. Several species are well suited for this purpose and can be kept with little effort. Their eggs are covered with a clear transparent chorionic membrane, and since they are kept in a water medium, storage and incubation problems are diminished.

Since the developing fish embryo is not surrounded by such embryonic membranes as the allantois or amnion as in the chick embryo, the student is able to observe the complete developmental stages from fertilization to hatching.

Another point in favor of keeping fish in the laboratory, especially the tropical species to be recommended in this report, is that they have no particular laying season. When properly conditioned they can be induced to lay at any time of the year. Thus the teacher is able to schedule a discussion on embryology at a logical and convenient time.

Hatching time is also a favorable point. Some species require as little as 24 hours for development from the time of fertilization until

reaching the larval stage, while others may require from 72 hours to several weeks or months. The rapid development of some forms makes it possible for the student to observe several different developmental stages of the living specimen during one laboratory period.

Some native fresh water species also lay eggs that are suitable for use in the classroom. The adults do not reproduce readily in the laboratory however, and eggs must be collected from nearby streams or ponds. The spawning season usually stretches from early April through September, but in some species, spawning is somewhat erratic during these months. Consequently, the eggs collected from these fish would have to be used in the late spring or early fall to coincide with the school year.

Numerous biological concepts can be pointed out by the teacher. For example, the concepts of cleavage, blastulation, and gastrulation can be reinforced by actual observations of these phenomena. Also, by observing the developing circulatory system, digestive system, nervous system, etc., the student can be led to a better understanding of the events that occur in the embryonic development of other higher vertebrate animals.

Still another important biological phenomenon that can be stressed at this time is mitosis. While the embryo is developing, the student can observe the mitotic process, thus strengthening his concept of this phenomenon that is characteristic of all living things.

It has been the experience of this writer that the student shows more interest in learning biology when the specimen is alive. Something about living things increases the curiosity. For this reason, observation of hatching fish eggs should prove to be a handy tool for the teaching of embryology to the high school student.

CHAPTER II

METHODS AND MATERIALS

When keeping fish in an aquarium, certain rules should be followed to keep them alive, healthy, and productive. Axelrod (1952) offers the following suggestions:

(1) Have an aquarium with a volume of at least ten gallons.

(2) Provide a cover to control evaporation and keep out soot, dust, and other foreign matter. The cover can be of most any material but glass is preferred.

(3) Provide the aquarium with a light or place it where it will get at least two hours of sunlight daily.

(4) Before fish are placed in tap water, let it stand for at least one full day in the direct rays of the sun in order that chlorine gas can escape. Chlorine kills fish.

(5) Provide scavengers for each tank. They eat excess food and prevent it from decaying and contaminating the water.

(6) Provide plant life of some sort. Although not absolutely necessary it will offer protection to the young if they are left with the adults. An aquarium with plants is also one that is much more pleasing to the eye. However, plants should not be used with the intention that they will extract all carbon dioxide from the water. Plants utilize carbon dioxide during photosynthesis which is carried on in the presence of light of the proper wave lengths. Unless the proper light is present however, the plants will give off carbon dioxide just like any other living thing.

(7) Do not overfeed. More fish die from overfeeding than underfeeding. Adult fish should be fed once a day and only as much as they can consume in ten minutes.

(8) Do not overcrowd. Too many fish in an aquarium will cause a concentration of carbon dioxide that can prove lethal even though there is sufficient oxygen dissolved in the water. A high concentration of carbon dioxide is what causes fish

to surface and gulp in air, not a lack of oxygen. Carbon dioxide diffuses from the water to the air very slowly. This makes the surface area of the aquarium important, for the larger the aquarium the easier it is for the carbon dioxide to diffuse out of the water and into the air. A good rule to follow -- allow a minimum of 10 square inches of surface area for each inch of fish. Air pumps do not add a great deal of oxygen to the aquarium. The main function of the air that is pumped into the tank is to provide a greater surface area for the diffusion of carbon dioxide from the water. The carbon dioxide diffuses into the air bubbles as they rise to the surface.

(9) Do not allow a wide range of temperature fluctuations. Fish are cold blooded and most tropical forms are accustomed to living in water that rarely goes below 60° F.,

At least two exceptions can be taken to the above rules. First, small spawning tanks holding from one to five gallons of water can be used if they are not overloaded and are aerated properly. The second exception is the use of scavengers. Obviously they perform useful functions in the community tank, but their use in the spawning tank would be of negative value, as they will eat the eggs.

To properly maintain an aquarium, Axelrod (1952) suggests that the following basic equipment be used. This material can be bought from most any pet shop.

(1) Thermometer
(2) Thermostat
(3) Heater
(4) Air pump
(5) Filter
(6) Fish net
(7) pH test kit

Since some species will eat their own eggs, various devices can be placed in the aquarium to prevent this. One common method is to place marbles on the bottom of the tank. Since the eggs are nonadhesive, they will fall between the marbles and be relatively safe until removed.

A second method is to weave together a mat of glass rods. The glass rods can be held together with soft wire of some kind that is non-corrosive. Two glass rods of the same kind can then be placed at right angles beneath the mat on the tank bottom to act as a support.

A third method is to place a net of suitable mesh within the aquarium. Two sides of the net can then be attached to the sides of the aquarium and the fish placed within the confines of the net. The bottom of the net should be at least an inch off the bottom of the aquarium so that the eggs can fall through the mesh and be safe from the adults.

The water level above the traps should be approximately four to seven inches deep, depending on the size of the fish. If it is too shallow the fish may injure themselves and become susceptible to disease. If the water is too deep those fish that eat the eggs will have time to spear them as they fall to the bottom.

The eggs may be removed from the bottom of the aquarium by means of a pipette or siphon. This method will also pick up detritus which can be decanted off later.

For optimum viewing of the developing embryo, stereoscopic binocular microscopes with magnifications of between 10X and 30X should be used. If these are not available, it is suggested that compound microscopes be used as long as the magnification does not exceed something like 40X.

If it is desirable, small one gallon, easily cleaned, spawning tanks can be constructed. Jones, C. (1962) has utilized plastic bell jars for this purpose. These can be obtained through Doenges Supply of Guthrie, Oklahoma. When mounted on wooden frames, they require little maintenance since they are drained from the bottom.

To initiate spawning, it is suggested that plenty of live food such as Daphnia, wireworms, etc., be fed to the fish along with prepared dried foods. The presence of light after a prolonged dark spell of at least five hours also seems to cause the spawning act to start in some species. In fact, this technique is employed to get Brachydanio rerio (Hamilton) to spawn. The fish are kept in a dark area for the night and when exposed to light the next morning, a dash of live food dropped into the aquarium will usually start the spawning act. It is easily recognized and consists of a wild chase of the females by the males. Other species have their own spawning methods, but the proper stimulation by light and the use of live foods should provide the laboratory with an ample supply of eggs.

Some species of fresh water minnows lay their eggs in rivers and larger streams. One method for obtaining the eggs is to hold a finely meshed net such as plastic screen wire in the current. The eggs are very small, almost transparent and can be picked up with a pipette after they are trapped in the seine. These can be brought back to the laboratory for further observation.

Sometimes it is desirable to strip both eggs and sperm from the fish. Sperm can sometimes be obtained by holding the male with the ventral side up and stroking along the white line between the anus and the genital pore. This should cause the sperm to be forced out. If this procedure doesn't cause the sperm to appear, the fish may be killed, the testes immediately removed, placed in a small beaker containing water and macerated with scalpel or forceps.

The eggs may be stripped from the female by also holding the ventral side up and stroking with the thumb and forefinger from the anterior end

of the abdomen toward the posterior end. The extruded eggs can then be placed in the sperm charged water.

Ingersol (1953) used two methods to obtain sperm from Trichogaster trichopterus (Pallas). One method was to insert a pipette in the area where the breeding act was taking place and withdraw a sample of water and sperm. The other method was the same stripping technique described above. The stripping technique did not work successfully for him and he recommended the use of the pipette for obtaining sperm from this particular species. One important limiting factor in stripping would be the size of the fish, since some are too small to be handled in this manner.

Some teachers or students may want to preserve embryos of various ages. They can be killed and fixed in either Bouin's solution or ten percent formalin. For best results, the embryos should be left in the killing and fixing solution for 24 hours and then stored in fifty percent isopropyl alcohol or seventy percent ethyl alcohol. Stored embryos can be used by the student for further observation of some particular stage of development, and the teacher can use them for testing purposes if so desired.

Before the killing and fixing process is started however, the chorion should be removed. This can be accomplished by using sharply honed forceps to hold the embryo. A microneedle can then be used to rupture the chorion. This will allow the embryo to be squeezed out. In case the laboratory is not equipped with a microneedle, one can be made by honing an insect pin to a very fine point.

If equipment is available, photomicrographs can be made of the developing embryo by the teacher before the students begin their own

observations. The negatives can be mounted and used with a slide projector or pictures can be printed from the negatives and the time at which certain significant changes occurred after fertilization noted on each photo. Then when the students begin their own observations, they can compare the specimen that they are observing to the photos. This would allow them to calculate the approximate time for the appearance of certain organs and/or structures in their own material.

Since it has been suggested that live foods be fed to the fish on occasions, the following forms are recommended. They can easily be cultured in the laboratory and do not require a great deal of space.

The microworm Anguillula silusiae makes excellent food for all types of newly hatched fish. Cultures can be obtained from tropical fish stores and should be cultivated at room temperature. The culture medium consists of one part water to three parts yeast which has been placed in a drinking glass or small beaker to a depth of one half inch. The cultures build up rapidly, therefore a new one should be started every two weeks. New cultures are started by adding a fingerful of worms to a fresh culture medium.

A species of dwarf white worms belonging to the family Enchytraeidae can usually be obtained from damp earth near a pond or lake. They should be kept in damp earth at a temperature of about 50° F. with a very high humidity in a wooden or clay vessel. They thrive on dried milk, Horlicks malted milk, bread of any kind, or cereal boiled in milk until it is pasty. The culture should be allowed to mature for about a month before it is used.

To separate the white worms from the culture medium, a piece of window screen wire about two inches square is placed over a drinking

glass which has been filled to within an inch of the top. The middle of the screen is then pressed into the glass until it just touches the water. A tablespoon of the white worm culture is then placed on the screen and an electric light brought within three or four inches of it. The worms exhibit negative phototropism and leave the mud to enter the water. The worms can then be poured from the glass into the aquarium with a minimum of contamination.

Daphnia, a relative of the shrimps, lobsters, and crabs, is also easily kept in the laboratory. They can be obtained through pet stores or by using a plankton net to dip them from a pond or lake that contains them. To start a culture, six or eight adults should be placed in a beaker or jar with a minimum of one quart of water. They will feed on Horlick's malted milk, dried yeast, dried shredded lettuce, and hay. Each culture should be changed every month.

Other animals that will provide excellent live foods are earthworms, the brine shrimp Artemia, Drosophila larvae, mosquito larvae, and the annelid worm Tubifex.

Dry, prepared foods in several grades are on sale in pet shops and other stores. They are generally satisfactory and should be used along with the live foods. In any case, neither should be used to the exclusion of the other.

Numerous books have been written on the subject of keeping and raising tropical fish. The scope of this report does not include a detailed discussion on all the various kinds of paraphernalia and the vast numbers of different species that can be included in an aquarium. Therefore, the following books, in addition to those in the bibliography, should prove helpful in providing information on the subject.

Boulenger, E. G. 1958. The aquarium book. Robert M. McBride Co. New York. 208 pp.

Innes, Wm. T. 1949. Goldfish varieties and water gardens. Innes Publishing Co. Philadelphia. 385 pp.

McInerny, Derek, and Geoffrey Gerard. 1958. All about tropical fishes. George N. Harrap and Co. London. 480 pp.

Schneider, Earl, and Leon F. Whitney. 1957. The complete guide to tropical fishes. Thomas Nelson and Sons. New York. 549 pp.

Vondys, Horace. 1955. Tropical fish in the home aquarium. The McBride Co. New York. 157 pp.

Wells, Lawrence. 1954. Tropical aquariums, plants, and fishes. Fredrick Warne and Co. New York. 230 pp.

CHAPTER III

SOME OVIPAROUS AND VIVIPAROUS FISH SUITABLE FOR USE IN THE CLASSROOM AND LABORATORY

If both oviparous and viviparous species are kept in the laboratory, the teacher will be able to point out similarities and differences existing between the two methods of producing young. Dissections of pregnant viviparous females can be made to demonstrate that they do not have a uterus and that the developing young are borne in the ovary. During this time they receive nourishment in a way quite similar to warm blooded animals. (Axelrod, 1952). The following is quoted from Emmens (1953).

> Maternal nourishment is typically provided by a placenta, an organ in which the blood of the mother and that of the young are very closely mingled without actual mixing, and which is remarkable in that it is part of the pericardium, or membrane surrounding the heart itself. The young fishes develop in a folded position, head to tail, and are born with this fold still present.

Another interesting aspect of live bearers is that in some species, several broods may be borne by the female after only one contact with the male. It appears that the male sperm is stored in the ovary. (Axelrod, 1952).

Since most live bearers will eat their young, provisions should be made for their separation if many of them are to be saved. If the young are to be left in the same tank, a wise aquarist will include enough green plants in the aquarium to enable the fry to hide until they are big enough to fend for themselves.

Viviparous fish such as the Gambusia, the Guppy, and the Swordtail can be purchased at most any pet shop. Other species could be used but these three will provide ample material for the laboratory and classroom. They also reproduce rapidly.

The Guppy, Lebistes reticulatus (Peters), is one of the best known and most popular species of aquarium fishes. It is very hardy and will continue to breed even when subjected to all kinds of mishandling. It thrives at a temperature that varies between 65 - 80° F. and will produce from 30 - 60 fry every four to six weeks when properly conditioned. It takes about four months for the fry to mature.

The Swordtail, Xiphophorus hellerii Heckel, thrives at a temperature that ranges from 65 - 80° F. The females will produce variable numbers of young every four to eight weeks if they are kept at a temperature of 75° F. The young will mature in about six months.

The Gambusia is famous because it has an appetite for mosquito larvae. This fish is found mainly in the southern United States. It is known to have a ferrocious nature and should not be kept with other small fish. Gambusia affinis (Baird and Girard) is one of several species that can be kept in the laboratory. The Gambusia will flourish at temperatures that range between 40 - 100° F. and the optimum breeding temperature is 75° F.

As for the egg layers, there are innumerable species from which to choose for use in the laboratory. However, those to be mentioned and discussed in the next few pages should supply an abundance of eggs for the laboratory with a minimum of difficulty. Most, with the exception of the native, fresh water species, can be obtained from any pet shop.

The Blue Gourami, Trichogaster trichopterus is a native of India, and is not the only fish to be so named. The Three Spot Gourami also has the name T. trichopterus. The two are identical except that the Blue Gourami has a hazy coat of whitish blue which does not obscure the spots. (Axelrod, 1952).

The Gourami belongs to the family of fishes that are commonly called the bubble-nest builders. The nest of bubbles is built by the male and during an elaborate mating procedure, the eggs are squeezed out of the female and fertilized by the male. While the eggs are falling to the bottom, the male catches them in his mouth, places them in the bubble nest, and proceeds to stand guard. Actually the Gourami doesn't need the bubble nest, since both eggs and young float. The Gourami is very hardy, quite peaceful, and can be kept in a community tank with young and old fish. (Ingersol and Jones, 1949).

The female requires from one - three hours to spawn and will lay between 100 - 150 eggs every two weeks if properly conditioned. (Ingersol and Jones, 1949).

The eggs have an average diameter of 0.71 mm, require approximately 24 hours for incubation, and reach the free swimming stage approximately 48 hours later. (Ingersol, 1953).

Innes (1952) refers to the Gourami as the most easily bred species of the bubble nest builders. It is also recognized as a hydra eater. (Axelrod, 1952).

The Whitecloud Mountain, Tanichthys albonubes Lin, is a native of China. It is a hardy, peaceful fish which grows to a length of approximately one and a quarter inches. According to Hervy and Hems (1953), the female deposits a small number of copper colored eggs over a period of

days and it is difficult to know whether the fish has finished spawning. These authors further point out that the brood fish should be removed after spawning, for if they are left in the same tank too long, they might begin to eat their own young. According to Axelrod (1952), the incubation period ranges from 48 - 72 hours. The Whitecloud Mountain flourishes best in hard water and can withstand a temperature range between 40 - 90° F. Hervy and Hems (1953) write that it likes a comparatively low temperature of 65° F., breeds best at a range of 68 - 72° F., and is liable to succumb in a prolonged high temperature. Innes (1952) points out that these fish will eat anything but prefer small food given often.

One of the most popular of the egg layers is the Zebra fish, Brachydanio rerio. It is native of Ceylon and grows to about one and one-half inches in length. The Zebra, or Danio, as it is sometimes called, is a very peaceful and hardy fish. Creaser (1934) writes that they will produce eggs at all times of the year.

The female requires about thirty minutes to spawn and will lay up to 95 eggs with an approximate average diameter of 0.60 mm. If the female is properly conditioned she can be induced to spawn every 12 - 14 days. (Ingersol and Jones, 1949). The eggs can be placed in petri dishes, watch glasses, or finger bowls for hatching. The young will mature in three to seven months. According to Innes (1953) the best age for breeders is about one year for they are considered old at the age of two. He further states that they seldom live beyond three years.

The optimum temperature range for the adult fish is between 70 - 75° F., but they can withstand temperatures that vary from 50 - 100° F.

(Ingersol and Jones, 1949). Axelrod (1952) and Innes (1953) suggest a separate spawning tank be used for this species. When this is done, a likely looking female should be placed with two or three lively males.

Several authors have suggested ways to tell the sexes apart, but about the only sure way is to observe their behavior. The males spend a great deal of time chasing the females.

The embryonic development of B. rerio is well documented and has been observed by Roosen - Runge (1939), Hisaoka and Battle (1958), and Blumenkrantz (1956). Hervy and Hems (1953) write that it will withstand treatment that will quickly kill most tropical fish, and that it can withstand a wide temperature change. Further, they write that "perhaps it is for these reasons, as well as its beauty, its readiness to breed, and its simple requirements, that it has for long been one of the most -- if not the most -- popular of the egg laying tropicals."

There are also several species of native, fresh water minnows that will produce excellent, fast developing embryos. The Arkansas River Shiner, Notropis girardi Hubbs and Ortenburger, attains a length of four inches and is to be found in streams from Iowa to Texas. A relative to this species is the Plains Shiner, Notropis percobromus (Cope), which grows to a length of two inches and is to be found in the Arkansas and Oklahoma drainage systems.

The Speckled Chub, Hybopsis aestivalis (Girard) grows to a length of two and one half inches and is to be found from the upper Missouri drainage system to the Rio Grande River. Bottrell (1962) has described the embryonic development of this species.

The Northern Plains Minnow, Hybognathus placita Girard, is a rather large fish growing to a length of six inches. It is to be found in the upper Missouri drainage system and southward.

Jones (1962) is quoted as saying that N. girardi, N. percobromus, H. aestivalis and H. placita can be found in the Cimarron River where they lay large, free floating eggs that hatch within 24 hours.

Two other native fresh water species that lay suitable eggs are the Fathead Minnow, Pimephales promelas Rafinesque, and the Clubhead Minnow Pimephales vigilax (Baird and Girard). P. promelas grows to a length of two and one half inches and ranges from southern Canada east of the Rockies to Maine and southwest to the Gulf States.

Niazi (1962) is working out the embryonic development of P. promelas.

P. vigilax grows to a length of three inches and ranges from Minnesota and West Virginia to northern Alabama and Texas. Parker (1962) has described the embryonic development of this fish.

According to Jones (1962), the eggs laid by the last two mentioned species will be attached to floating vegetation just beneath the surface of the water. An easy way to obtain eggs from these two species is to locate a pond containing them and toss in several flat boards or logs. The female has an ovipositor that can be everted and moved from side to side. She will swim up to the floating debris, turn on her side and deposit the eggs in small clusters that can easily be scraped off. Jones (1962) further states that the eggs are quite transparent and will hatch in three to five days.

CHAPTER IV

SELECTED DEVELOPMENTAL STAGES OF THE TELEOST EMBRYO

After fertilization of the egg by the sperm (Figure 11) the mitotic process of forming a new individual begins. The following is quoted from Shumway and Adamson (1954).

> The process of fertilization supplies some stimulus whereby the development of a new individual is set in motion. This is manifested by the occurrence of a series of mitotic cell divisions. In this way a large number of cells is formed and the process of cleavage culminates in the formation of a blastula where the embryo is in the form of a hollow sphere or disc. Following the formation of the blastula, further changes occur in the embryo resulting in the segregation of the three primary germ layers. This process is called gastrulation and as a result of it an outer layer of ectoderm, an inner layer of endoderm, and an intermediate layer of mesoderm are formed.

The three cell layers or germ layers give rise to various organs and structures. Patten (1958) writes that even though a layer of cells looks all alike to us, they are gradually being established into localized groups with different developmental potentialities. He goes on to say that the endoderm gives rise to the primitive gut which in turn gives rise to parts of the liver, pancreas, lungs, digestive tube, etc. The mesoderm gives rise to the skeleton, muscles, circulatory system, parts of the excretory system, parts of the reproductive system, mesenteries, peritoneum, connective tissue layers of skin, etc. From the ectoderm, such things as the brain, spinal cord, hair, nails, claws, sense receptors, enamel of teeth, parts of sweat glands and sebaceous glands, etc. are derived.

The remainder of this chapter will deal primarily with the embryonic development of B. rerio and T. trichopterus. Their developmental stages are somewhat similar to the species of oviparous fish that were discussed in the previous chapter, but they differ in the amount of time required for incubation. For example, T. trichopterus requires about a third as much time for incubation as B. rerio. Incubation time, as used in this report, means the amount of time required for the embryo to develop and emerge as a larva from the chorion.

Blumenkrantz (1956) observed that it required from 60 - 77 hours for the incubation of B. rerio at a temperature around 26° C., while Ingersol (1953) noted that approximately 25 hours were required for the incubation of T. trichopterus at a temperature around 23° C. The latter species is comparable to some of the native fresh water species of this area in the amount of time required for incubation.

Since the development of T. trichopterus is one of the most rapid embryonic developments known among vertebrates, Ingersol (1953) offers the explanation that this very rapid cell division appears to be the best answer for the formation of a new individual in such a short time.

Only the more obvious embryonic developmental stages and/or structures will be discussed in this report since most high school students will not be acquainted with the more technical aspects of embryology. Even without knowing the complete nomenclature and function of each tiny bit of tissue it is felt that students at this level will gain a greater insight into the mystery of life by observing the developing embryo. Further, it is hoped that an experience of this nature will provide motivation for future studies in this area.

In their observations of B. rerio, Roosen - Runge (1939), Blumenkrantz (1956), and Hisaoka and Battle (1958) observed the first cleavage to occur approximately 34 minutes after fertilization. Thereafter, each successive cleavage to the tenth occurred at 17 to 20 minute intervals. A situation somewhat similar to this was noted by Ingersol (1953) in his observations of T. trichopterus in which he reported that the first cleavage after fertilization occurred in approximately 30 minutes. Subsequent cleavages thereafter occurred at approximately 14 minute intervals. It would appear then, that the shorter mitotic cycle of approximately 14 minutes in T. trichopterus as compared to B. rerio and others, accounts for its short incubation period. Jones (1939) on the other hand, while working with Fundulus heteroclitus (Linne) reported that "increases in the rates of cell divisions were found to precede or occur simultaneously with the growth (increase in protoplasmic volume) of an organ."

Figures 1 and 2 are photomicrographs of the four and eight cell stage of B. rerio and show the results of the second and third cleavages respectively. In both figure 1 and figure 2 the blastodisc cells are all lying above the large rounded yolk mass.

Another easily identified stage in the developing embryo is the blastula which has developed from the blastoderm. Figure 3 is a photomicrograph of B. rerio which shows the cap like blastula sitting atop the yolk. Here the chorion can be observed as an outer darkened circle surrounding the entire blastula and yolk mass. At this stage the chorion has not picked up detritus and is quite clear and transparent.

Figure 4 is a photomicrograph of a developing minnow taken from the Cimarron River. The germ ring, arising from the blastodisc or

blastula, can be seen as a descending layer of cells that have reached the equatorial region of the yolk mass. The germ ring will continue to move down, over, and around the yolk.

According to Blumenkrantz (1956) the germ ring stage can be observed during the fourth hour of development in B. rerio while Ingersol (1953) has reported this stage to occur in T. trichopterus at the middle of the sixth hour after fertilization.

Figure 5 is a photomicrograph of another embryonic minnow taken from the Cimarron River. Its approximate age is two to four hours. Note that even in this short space of time the embryo has developed a well defined anterior end which can be seen as a swelling on the left side and a well developed posterior end which is to be seen as a swelling on the right side. Note also the large yolk mass that is present.

Figure 6 is also a photomicrograph of an embryonic minnow taken from the Cimarron River and its approximate age is four to seven hours. The yolk plug stage is quite evident in this lateral view as a slight swelling in the lower middle part of the photomicrograph. At this stage, the germ ring has almost encircled the yolk. The constriction created by the germ ring has forced the yolk into an ellipsoidal shape with the small end forming the plug. This stage was observed to occur in B. rerio at the middle of the seventh hour and was observed to occur in T. trichopterus during the last quarter of the eighth hour.

Figure 7 is a photomicrograph of B. rerio shown developing within the chorion. The embryo at this stage is approximately 25 hours old. One cannot help but notice the detritus on the chorion. As the embryo grows older, dust particles, fungi, and other materials will collect on the chorion and obscure or partially obscure the view of the developing

embryo. Consequently, it is best that the chorion be removed sometime around the 20th hour after fertilization for optimum viewing. Removal before this time is possible but it seems to lessen the chances that the embryo will survive.

Figure 8 is a photomicrograph of another minnow that has been taken from the Cimarron River. Here one can see well developed somites and the beginning eye development. The age of this embryo is unknown.

Figure 9 is a photomicrograph of B. rerio that has been removed from the chorion. Its approximate age at this time is 26 1/2 hours which would approximate the age of the embryo in figure 7. When these two are compared, one cannot help but notice how many more structures can be observed after the chorion has been removed.

Figure 10 is a photomicrograph of a minnow from the Cimarron River taken a short time after hatching. This is the larval stage in which the fish does not swim actively but lies on the bottom of the tank. If it is disturbed, the fish will swim for a short distance and then settle back to the bottom. After the larval stage is past the fish become free swimming.

There are many other structures and/or physiological functions for which there are no photomicrographs available, but never the less they can be quite readily observed. Blumenkrantz (1956) working with B. rerio and Ingersol (1953) working with T. trichopterus recorded the following events.

a. Somite formation -- First observed in B. rerio during the 12th hour of development while it was first observed in T. trichopterus during the middle of the 11th hour.

b. Eye formation -- The eyes had begun to form the characteristic cup shape in B. rerio during the 12th hour while this development was first observed in T. trichopterus during the middle of the 14th hour.

c. Motion of the embryo -- Movement was first observed in the developing embryo of B. rerio during the 21st hour of development indicating that the nervous and muscular systems had started to function. There was no report on the earliest movement of T. trichopterus.

d. Appearance of the heart beat -- The heart beat was first observed in B. rerio during the 27th - 28th hours. It appeared in T. trichopterus during the 17th hour of development. Both authors reported that the heart beat was slow and erratic at first, but soon became regular.

e. Blood circulation -- Blood circulation was observed to begin some 30 minutes after the heart beat had started in both B. rerio and T. trichopterus.

f. Early pigmentation -- Pigment cells were first observed in B. rerio during the 27th - 28th hours of development on the ectoderm of the sides of the embryonic axis. This same phenomenon occurred in T. trichopterus during the 15th hour.

g. Appearance of fin buds -- These were first observed in B. rerio during the 30th hour of development but were observed during the 23rd hour in T. trichopterus.

h. Hatching -- Hatching occurred during the 60th - 77th hours in B. rerio and during the 24th - 26th hour in T. trichopterus. The lashing movement of the embryo's tail causes the chorion

to rupture. The time required for hatching depends upon the activity of the embryo and the condition of the chorion.

The teacher should bear in mind that the observations just presented were performed in laboratories where the temperature could be kept fairly constant. More than likely this will not be possible in the average high school. However, this should not be of too great a significance since high school students are to act only as observers of these phenomena and an exact time for the appearance of some certain structure or organ more than likely will not be needed. Consequently, it is suggested that the time tables just presented be used as rough guides only.

Some authors express doubt that laboratory conditions can be standardized to the point where the same observations can be made time after time. Oppenheimer (1937) is of the opinion that the chronological age of a developing teleost embryo cannot be expressed in hours or days because of varying conditions of temperature, oxygen supply, etc. Blumenkrantz (1956) did not agree however, and expressed the opinion that the environment could be standardized within the laboratory to such a point that a chronological description could be presented. He further went on to say that because the eggs of B. rerio are fertilized externally, the various facets controlling the developmental rate of the embryo from the time of fertilization to the termination of the experiment can be controlled and recorded.

Regardless of ones feelings on the matter of environmental standardization, this author still feels that the use of certain fish eggs in the classroom can contribute significantly to the high school biology program.

CHAPTER V

SUMMARY

Fish embryos can be useful for teaching embryology to high school biology students. The embryos of some species are covered with a clear chorion and develop so rapidly that several different developmental stages may be observed by the student during a single laboratory period. Many suitable species are of such a size that several may be kept in the laboratory for the specific purpose of supplying eggs for observational purposes. Also, many fish embryos are of such a size that large numbers may be kept and incubated in the laboratory in a minimum amount of space.

Many basic and important biological concepts pertaining to embryology can be stressed by the teacher. After observing such embryonic stages as the early cleavage stages, germ ring stage, yolk plug stage, early eye formation, early somite formation, heart formation, blood circulation, etc., the phenomena of cleavage, gastrulation and blastulation should become more clear cut in the student's mind.

Some tropical species, as well as some species of native fresh water fish, lay eggs that are suitable for laboratory use. Many tropical species are easily kept in the laboratory and will usually lay eggs the year round when properly conditioned by such factors as light, temperature, and food. On the other hand, the native species are not kept in the laboratory because they do not readily reproduce in such an environment. Rather, the eggs are taken from fresh water ponds and streams containing them.

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APPENDIX

PLATE I

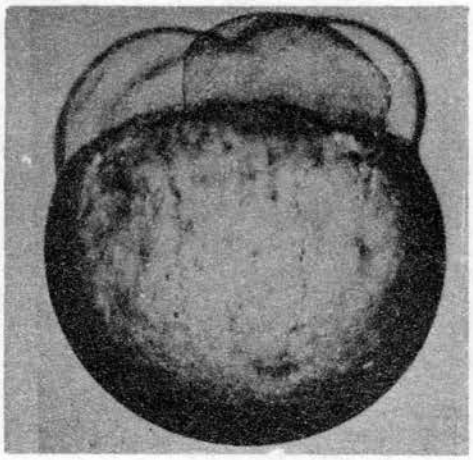


Figure 1. (Photomicrograph by Dr. Roy W. Jones) The four cell stage of B. rerio as a result of the second cleavage.

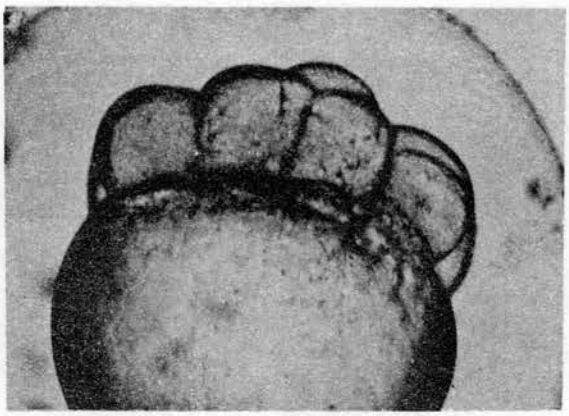


Figure 2. (Photomicrograph by Dr. Roy W. Jones) The eight cell stage of B. rerio as a result of the third cleavage.

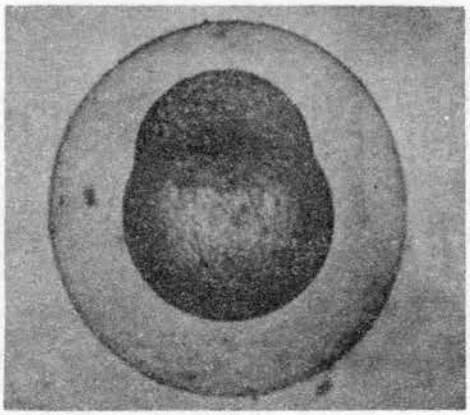


Figure 3. (Photomicrograph by Dr. Roy W. Jones) Blastula formation in B. rerio. Note the clear chorion.

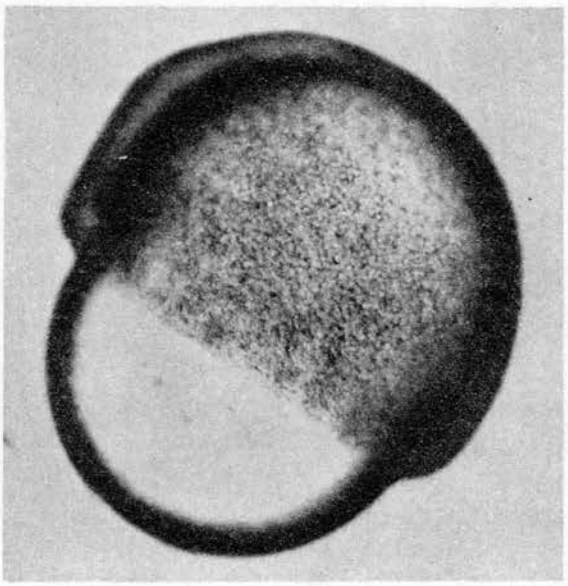


Figure 4. (Photomicrograph by R. H. Ingersol) Germ ring formation in a minnow taken from the Cimarron River.

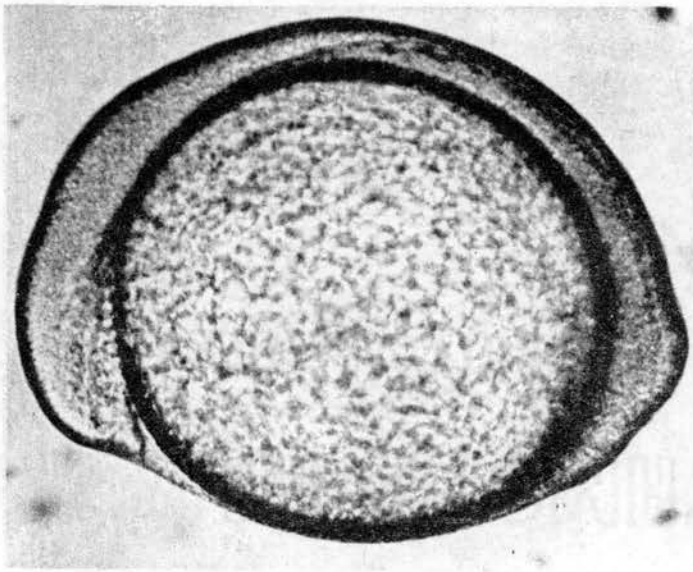


Figure 5. Left. (Photomicrograph by R. H. Ingersol) Embryonic minnow taken from the Cimarron River. Age - approximately 2 to 4 hours.

Figure 6. Below. (Photomicrograph by R. H. Ingersol) Embryonic minnow taken from the Cimarron River. Note formation of yolk plug. Age - approximately 4 to 7 hours.

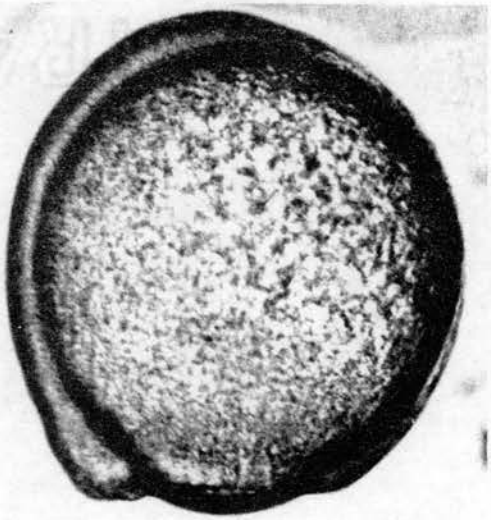


Figure 7. Below. (Photomicrograph by the author) Embryo of B. rerio shown developing within the chorion. Note that the tail is free of the yolk sac at this time. Age - approximately 25 hours.

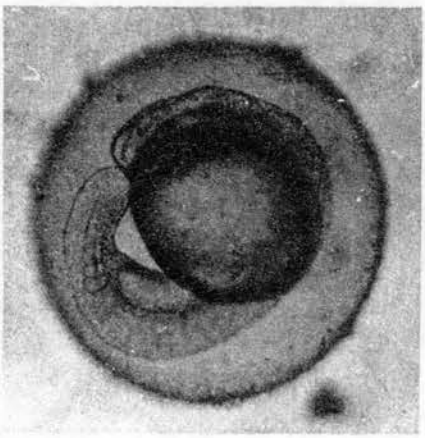
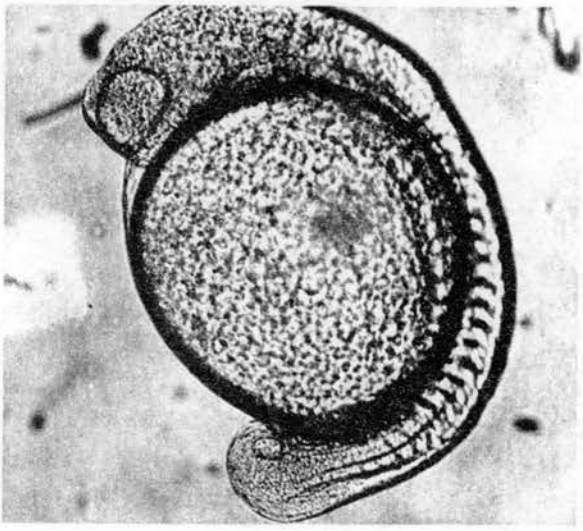


Figure 8. Right. (Photomicrograph by R. H. Ingersol) Embryonic minnow taken from the Cimarron River. Note the somite formation and the beginning eye development.



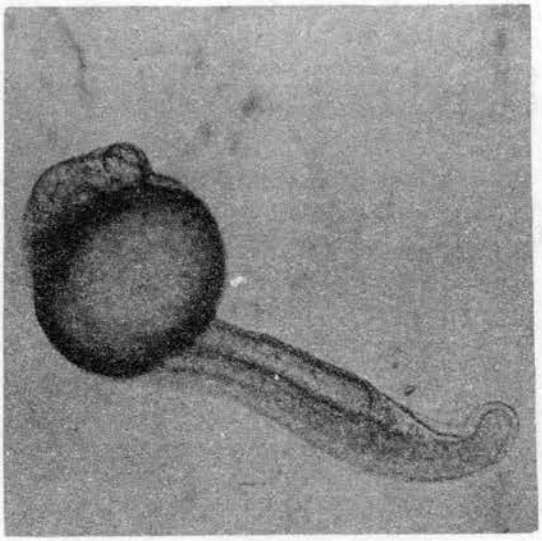


Figure 9. (Photomicrograph by the author) B. rerio embryo with the chorion removed. Age - approximately 26 1/2 hours.

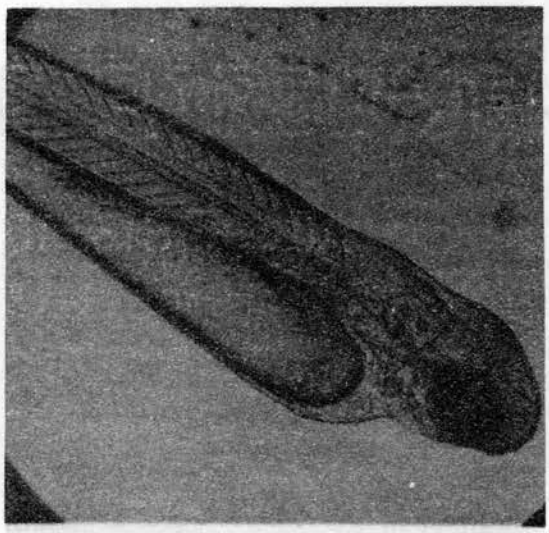


Figure 10. (Photomicrograph by Dr. Roy W. Jones) Minnow from Cimarron River. Age - approximately 24 hours.

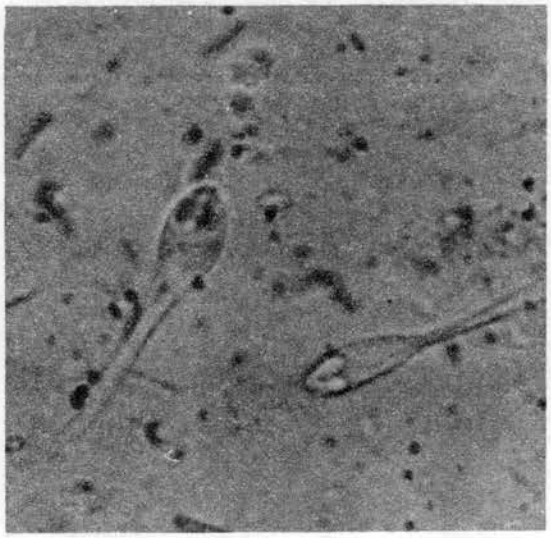


Figure 11. (Photomicrograph by R. H. Ingersol) Sperm cells of the Orange Spotted sunfish, Lepomis humilis (Girard).

VITA

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