

## A DEVICE FOR OBSERVING LIVING FISH EMBRYOS AT CONTROLLED TEMPERATURES

JOHN W. PRICE

Department of Zoology and Entomology  
The Ohio State University

In many species of fishes, living embryos together with their yolk sacs lie partially suspended within the perivitelline space. They rotate freely when the eggs are disturbed, but otherwise they assume a position of equilibrium in relation to the center of gravity. During cleavage stages, the early blastomeres and later the blastoderm at the animal pole are uppermost. During gastrulation, the center of gravity may shift as oil globules disappear or coalesce and the embryo grows forward from one side of the germ ring. As a result, before the hatching stage is reached, the embryo at different times may appear to be attached to the yolk at its side or below or above it.

Such changes in relative position of the fish embryo in relation to the yolk sac make desirable its observation through a microscope held in a horizontal position. At such an angle, the embryo may be viewed in profile against an illuminated background, rather than by reflected light against the yolk as a background. From the vertical position, the observation of cell boundaries of the superficial blastomeres and the topography of the relatively translucent embryo is not satisfactory.

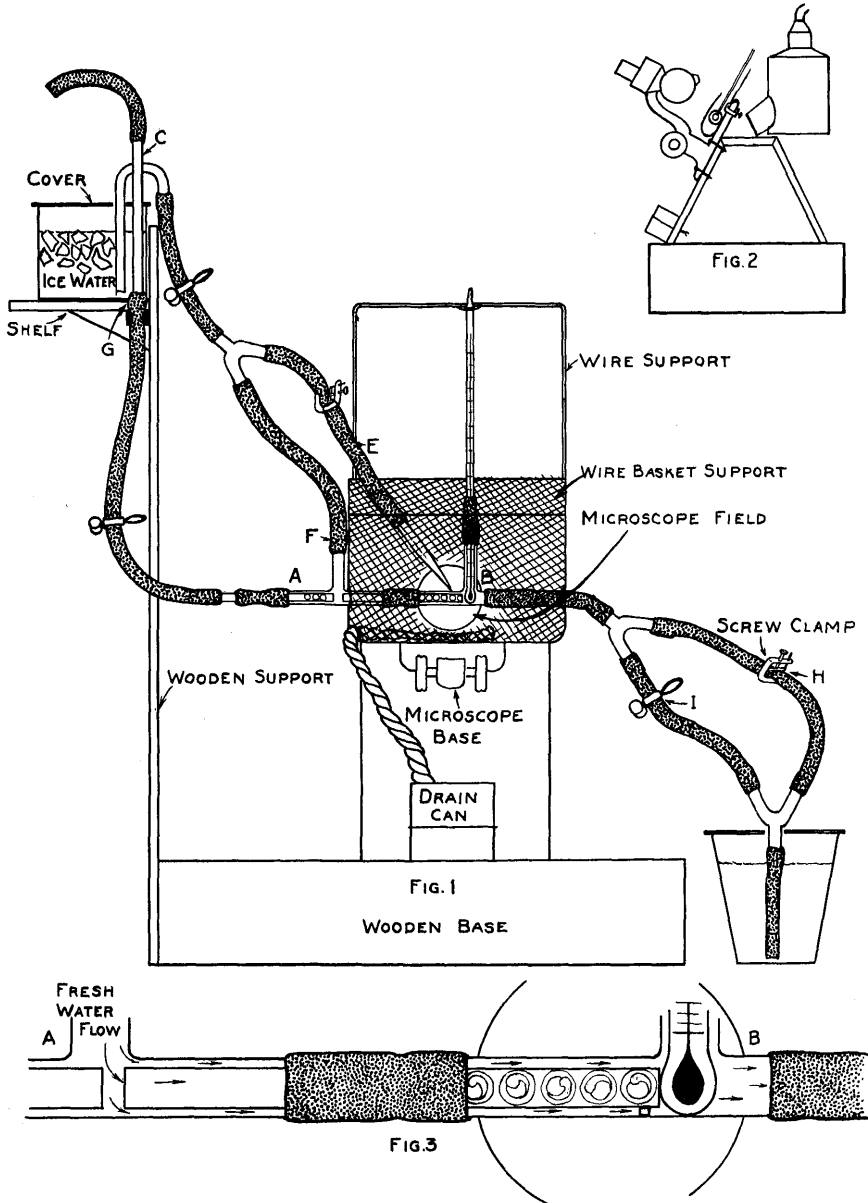
The device described below permits the observation of such embryos in profile from the side view, and at the same time under controlled temperature conditions.

The essential features of this apparatus, shown in the accompanying figures, include a series of glass tubes which convey non-adhesive fish eggs into the field of vision of a binocular dissecting microscope. The eggs are viewed from the side. A lighted background throws the blastodisc or embryo into bold relief. Prechilled water, flowing over these eggs at a controlled rate, makes possible the retention of temperatures below that of the surrounding air and provides an oxygen supply to the eggs while they are being examined.

The design of the apparatus is evident for the most part from the drawings of the front view (Fig. 1) the side view (Fig. 2) and the detail of the tubes that bring the eggs into the field of vision (Fig. 3). The box-like wooden base has four sides. From its front and rear sides rise standards at the angle desired to give a slight tilt to the microscope bolted to the front at the top (See Fig. 2). The U-base of the microscope projecting above the wooden base gives an anchorage to the wire basket support of the tubing and thermometer. A focusing lamp in its housing is mounted on a vertical rod behind the wire basket, and the flat top of the wooden frame permits the use of a liquid heat filter in front of the lamp housing.

The rubber tubing, the straight glass tubing and the Y- and T-tubes used are of standard laboratory design. Metal screw clamps at *E* and *H* are used for maintaining a controlled steady flow of water, while pinch clamps are used elsewhere to obtain an on and off effect. The two T-tubes between positions *A* and *B* in Figs. 1 and 3 are of such a size as to permit the insertion of the base of a thermometer at *B* and of two straight glass tubes at *A* which carry the eggs as desired into the field of vision at *B*. These inside tubes are just large enough to carry eggs freely in single file without their piling up. The thermometer bulb acts as a barrier to their further passage at *B*. The rubber tube which seals the union between the T-tube and the stem of the thermometer permits the thermometer to be drawn

slightly out of the T-tube sufficient to clear the end of the horizontal tube and permit the passage of eggs out of the system into the catch basin to the lower right, when the clamp at *I* is opened.



Figs. 1-3. Apparatus for observing living fish embryos at controlled temperatures. Figure 1 shows the front view; fig. 2, the side view; fig. 3, detail of tubes that convey eggs into the field of vision.

The temperature inside the egg chamber, *A-B*, is read directly from the thermometer at *B*. The wires of a thermocouple may be wrapped around the bulb of the thermometer and their free ends connected to a galvanometer. The writer made such thermocouple readings with a Leeds and Northrup potentiometer, but when these readings were found repeatedly to be in close agreement with those of the thermometer used, the thermocouple was dismantled.

Effective temperatures below that of the surrounding air are obtained by siphoning prechilled or ice water from the supply vessel at the upper left along the route *F, A-B, H*, to the catch basin at the lower right. As water enters the horizontal double tubing from *F*, it flows through both the inner tubing over the eggs and outside this tubing. At the point of water entrance, *A* in Fig. 3, it is essential to have an open joint in the inner tubing, permitting the entrance of water as shown. The rate of flow of water is controlled by regulating the screw clamp at *H*. Temperatures on the eggs at *B* are elevated by a decrease and are depressed by an increase in rate of water flow. A further depression of the temperature may be brought about by packing the wire basket with cracked ice. Waste water from the melted ice follows the wicking into the drain can. The moisture that collects on the outside of the egg-conveying tube when temperatures below the dew point are reached is flushed off by a steady flow of water onto that surface from the pipette *E* regulated by the screw clamp above it.

Eggs to be observed with this device are introduced into the system by removing the glass tube *C* from its lower connection at *G*, and using it as a siphon for drawing eggs from the incubation jar. This tube also should carry eggs in single file. This tube, once filled with eggs, is connected with the rubber tube and held vertically in a clamp at *G*. During this operation, the flow of ice water from the supply jar is momentarily checked by the pinch clamp above the main line at *F*. The pinch clamp below *G* is then opened and the eggs pass into the horizontal tubing. The two lengths of inside tubing at *A* are then held end to end by hand pressure, and the eggs are drawn into the observation area of the tube *A-B*, against the bulb of the thermometer at *B*.

Preliminary to the examination of eggs, certain adjustments must be made. These include the focusing of the lamp, the placement of its water filter, the filling of the wire basket with ice, and the regulation of the temperature at *B* as desired by starting the siphon flow of water and its regulation at the screw clamp *H*. In addition, the screw clamp above *E* is adjusted to produce a water film over the outside of the observation tube. Once these preliminary adjustments are made, a dozen or more eggs may be introduced. Since the tube *A-B* is free to be moved in the wire-basket frame, it can be shifted from left to right to permit the examination of a number of eggs simultaneously. Once examined, eggs may be released by elevating the thermometer bulb as described above, and additional eggs be brought into the field of vision.

This device, while undoubtedly still subject to considerable refinement, has proved especially helpful in bringing such eggs into a profile view for protracted periods of observation. It was developed as a means of enabling the writer to observe the chronological sequence in fish embryonic bodily movements that are characteristic of certain egg stages, and to study bloodflow and heart rhythm at given desired temperatures. For prolonged observation of fish embryos incubated at temperatures below that of the laboratory air, a continuous flow of fresh aerated water controlled as to temperature is desirable. The above device has been found to provide these conditions reasonably satisfactorily. It lends itself for use with non-adhesive fish eggs of different diameters, since the egg-conveying horizontal tube may be replaced by one of any desired diameter, within limits usually encountered in such material.