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EMBRYOLOGICAL STAGES IN THE SEA LAMPREY AND EFFECTS OF TEMPERATURE ON DEVELOPMENT

By George W. Piavis

FISHERY BULLETIN 182
From Fishery Bulletin of the Fish and Wildlife Service
VOLUME 61

Published by the U.S. Fish and Wildlife Service • Washington • 1961
Printed at the U.S. Government Printing Office, Washington, D.C.

For sale by the Superintendent of Documents, U.S. Government Printing Office
Washington 25, D.C. - Price 30 cents

Library of Congress catalog card for the series, Fishery Bulletin of the Fish and Wildlife Series:

U.S. *Fish and Wildlife Service.*
Fishery bulletin. v. 1–
Washington, U.S. Govt. Print. Off., 1881–19

v. in illus., maps (part fold.) 23–28 cm.

Some vols. issued in the congressional series as Senate or House documents.
Bulletins composing v. 47– also numbered 1–
Title varies: v. 1–49, Bulletin.
Vols. 1–49 issued by Bureau of Fisheries (called Fish Commission, v. 1–23)

1. Fisheries—U. S. 2. Fish-culture—U. S. I. Title.

SH11.A25 639.206173 9–35239 rev 2*

Library of Congress [r55e½]

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ABSTRACT

Early embryology of the sea lamprey has been subdivided into 19 stages. The stages are based largely on external morphology, behavior, and organ function. The holoblastic cleavage of sea lamprey eggs exhibited two types of third and fourth cleavages, equatorial and meridional. The open blastopore had an apparent migration over the surface of the embryo until it became the anus. Gastrulation resembles teleostean gastrulation in some characteristics and amphibian in others. Lamprey neurulation resembles teleostean neurulation more closely than it does amphibian.

Sea lamprey eggs were reared experimentally at 10 constant temperatures (at 5° intervals from 45° to 80° F., inclusive, and at 52.5° and 77.5° F.). No viable, burrowing larvae were produced at any temperature below 60° F. or above 70° F. Optimum temperature was 65° F. which yielded 78 percent survival to the burrowing stage; survival to the same stage was much lower at 60° F. (12 percent) and 70° F. (5 percent). The stage attained before all embryos were dead decreased as the temperatures were shifted in either direction from the 60°–70° F. interval. In general, developmental rate became faster, lengths of stages became shorter, and overlap between stages was lessened as temperature increased.

The evidence that sea lamprey eggs can develop successfully only within a limited temperature range suggests that unfavorable temperatures may account for the failure of certain apparently suitable streams to produce larval lampreys.

EMBRYOLOGICAL STAGES IN THE SEA LAMPREY AND EFFECTS OF TEMPERATURE ON DEVELOPMENT

By GEORGE W. PIAVIS, *Fishery Research Biologist*

The Great Lakes fisheries, the Nation's richest source of fresh-water fishes for both commercial and recreational fishing, have suffered depletion of catch and the threat of disaster. The danger has its origin in an increase in abundance of the sea lamprey, *Petromyzon marinus*, which is parisitic on and highly destructive of fish. Major goals of the Great Lakes research program of the U.S. Fish and Wildlife Service have been to develop techniques for controlling this menace, to restore the Great Lakes fishery stocks to an economically profitable level of abundance, and to sustain them at that level.

Others have outlined in detail the history of the sea lamprey within the St. Lawrence drainage and their invasion of the upper Great Lakes: (Gage, 1928; Creaser, 1932; Hubbs and Pope, 1937; Applegate and Moffett, 1955). The sequence of this invasion was summarized by Applegate (1950) as follows: 1921, Lake Erie; 1934, Lake St. Clair; 1936, Lake Huron; 1937, Lake Michigan; 1946, Lake Superior.

In planning an attack against the ever-increasing numbers of sea lampreys, practically every phase of their life history has been investigated except that of early embryology. A search of the literature reveals little on the embryology of *P. marinus*, *Lampetra fluviatilis*, *L. planeri*, and *Ichthyomyzon unicuspis*, the significant predators. Still less has been written concerning the other lampreys. Clear-cut stage designations are lacking for all lampreys. The usual embryological designations for the common early stages of development are mentioned through the gastrula stage, but even these lack clear definition.

In order to portray accurately the embryology of *P. marinus*, a study was undertaken which had as its objective the determination and definition of the various stages of development.

In these studies, staging of lamprey development has been considered essential to a better understanding of the results of the series of experiments on effects of temperature. It was immediately obvious that differences in developmental time periods would result from variations in temperatures. In order to place the entire series of temperature experiments on a common basis, an accurate series of stages was of the utmost importance.

A second objective of this study was to determine the range of temperature for development of *P. marinus* eggs as well as their optimum developmental temperature.

This work began with a preliminary investigation conducted on a part-time basis during the summer of 1954. Intensive investigations were carried on throughout the spring and summer of 1955 when temperature experiments were undertaken. Confirmatory temperature experiments at 65° F. (18.4° C.) were conducted in the summer of 1956. These latter experiments also provided materials for normal staging.

This research was conducted as part of my graduate training at Duke University while employed at Hammond Bay as a fishery research biologist by the Great Lakes Biological Laboratory of the U.S. Fish and Wildlife Service.

Embryological studies and experimental work on living materials were conducted at Hammond Bay where the facilities of the sea lamprey research laboratory were placed at my disposal by Dr. James W. Moffett, Director of the Bureau's Great Lakes Biological Laboratories, and Dr. Vernon C. Applegate, Chief of the Hammond Bay Laboratory. Sectioning and statistical work were conducted at Duke University. The University also provided me with a refrigeration unit for use at Hammond Bay and the other facilities and materials necessary to the investigation.

Dr. Edward C. Horn, Duke University, knows of my appreciation for his many criticisms and patient guidance. I wish also to thank the staff

NOTE.—Dr. Piavis is presently Assistant Professor of Anatomy, Baltimore College of Dental Surgery, Dental School, University of Maryland, Baltimore 1, Md.

Fishery Bulletin 182. Approved for publication May 4, 1960.

of the Hammond Bay Laboratory, and especially John Howell, for courtesies shown me and the time spent in my behalf.

STAGES IN NORMAL DEVELOPMENT

MATERIALS AND METHODS

Eggs of the sea lamprey were taken from mature, nest-building or spawning lampreys found in either the Trout River or the Ocqueoc River, both tributary to Lake Huron, Presque Isle County, Michigan.

Spawning lampreys were seized in a forked grip which placed the lamprey between the forefinger and the middle finger while the thumb anchored the animal. Capture by hand, in this way, minimized the loss of specimens by injury. The lampreys were kept in stream water throughout the transfer from the stream to the central collecting point, and to the laboratory where they were tempered and stripped in preparation for fertilization. To condition the eggs and sperm adequately to the test temperatures the lampreys were tempered in running lake water 2 to 3 hours prior to the initiation of the experiments.

Eggs were removed rapidly from female lampreys, held by two people. One held the anterior end by placing a thumb within the oral disc, thus taking advantage of the cusps to prevent slippage. The other held the tail by means of a pair of pliers. While the lamprey was thus outstretched, a transverse slit was made with scissors in the mid-ventral body wall at a point behind the heart and the liver, i.e., at the anterior level of the ovaries. If the females were ripe, eggs began to extrude from this initial opening, whereupon the lamprey was slit to the vent by a rapid stroke with half-open scissors. Since the eggs are completely free in the coelom of a ripe female, they were allowed merely to flow into a 10-liter battery jar which contained a few liters of lake water from the trough where the animals were tempered. Any eggs entrapped within the coelom or folds of the ovary were removed rapidly by dipping the lamprey into the water with the slit open. This method of removing the eggs was quicker and far superior to the milking procedure used by McClure (1893) and others (including the author) because the time required to clear the female of eggs was limited only by the dexterity of the operators. Furthermore, the eggs are not distorted or damaged. Blood was not introduced into the fertilization jar since the slit produced no blood providing neither the liver nor the heart was pierced.

The males were grasped in the manner described for the females. The individual who was holding the lamprey by the oral disc forced the milt from within the coelom through the genital papilla in a stream directed over the eggs in the battery jar. Two males were used for each female; four males and two females constituted the usual batch.

Immediately after the addition of sperm, the jar was provided with an air bubbler and placed in a trough previously brought to the desired temperature. The entire operation from stripping to placing in the jar was completed within 60 seconds. Twenty to 30 minutes after fertilization the eggs were washed. Washing was repeated at least two more times during the first hour to insure complete removal of excess sperm. After the first hour the eggs were apportioned among enamel pans (8″ x 12″ x 2″) or, on occasion, 4-inch glass bowls. These containers were covered with glass plates to minimize the accumulation of water-borne debris, and submerged. Circulation of water beneath the glass covers and over the developing embryos was insured by allowing a small area of the pans and bowls to remain uncovered. Care was taken not to crowd the eggs in the containers. Preliminary work had shown that eggs arranged in more than a single layer were highly susceptible to attack by fungus.

Development of the embryos took place in the pans or bowls which were held at the desired temperature in either constant-temperature troughs or in 20-gallon aquariums. Each insulated trough measured 12 feet by 2 feet by 9 inches and contained an inner-water chamber surrounded by a 3-inch outer-water chamber. Spaces beneath the metal divider provided free access between the inner and outer chambers. Water within the inner chamber was provided with air from controllable bubblers.

The troughs were equipped with thermostatically controlled heating units and refrigeration units which provided temperature control within $\pm 0.5°$ F. The refrigeration tubing, the heating elements, and the thermostat bulbs lay within the 3-inch outer chamber.

Water was circulated over the thermal units by a continuously revolving 6-vane water wheel driven by a Ratiomotor. Since water flowed freely between the outer and inner chambers, the

action of the thermal units was transmitted with little time lag to the inner chamber which held the container of eggs.

Heat for the high temperatures was provided by Bronwil circulators and heaters used in aquaria filled with lake water and provided with a controllable air bubbler. Bowls and pans were used to contain the eggs within the aquaria as in the troughs. Air bubblers and water circulation by the Bronwil circulators provided air for the aquaria. The circulators equipped with a contact-thermostat and thermometer can be utilized for any temperature setting from room temperature to boiling. In order to attain the maximum range from this piece of apparatus the aquaria were located within a cool room where it was expected that the ambient temperature would not rise above the desired temperature. The temperature variation for the circulator was advertised as $\pm 0.18°$ F. In actual practice, however, temperature variation could not be noticed. The apparatus just described was utilized for experiments at temperatures of 65° to 80° F.; the troughs were used for temperatures from 45° to 70° F.

Prior to each experiment, troughs and aquaria were washed, air-dried, and refilled with lake water; the thermostat was then set at the desired temperature. Observations of the temperature at 5-minute intervals for a period of 4 to 8 hours and occasional readings in the remainder of a 24-hour interval, assured stabilization at the correct temperature. After the desired temperature was established, Taylor thermographs and hourly readings of total-immersion thermometers (placed on submerged rubber stoppers grooved to receive them) gave a further check. The thermometers were set in such a manner as to be readily visible without handling. Thermographs were not used with the circulator but the temperature was watched for 4 to 12 hours prior to the initiation of the experiment. Because of the small variation in temperature delivered by the circulator, a thermograph record was considered unnecessary except when the ambient temperature was expected to rise above that desired. Furthermore, a submerged thermometer was compared periodically with the contact-thermostat and thermometer.

All sampling was random throughout the experiments, and in general, the procedure varied only slightly from that outlined below. Fertilization was considered zero time; the first sample was taken 20 to 30 minutes after fertilization. Thereafter, samples were taken at the following hours: 1, 2, 3, 4, ___ 12, 14, 16, 18, 20, 24, 28, 32, 40, 48, ___ 72. After the 72d hour samples were taken at 12-hour intervals until the end of the experiment. In some of the longer experiments, samples were taken each 24 hours after about the 18th day. In addition to the samples taken during the run, all remaining eggs and larvae were kept as a final sample.

Samples of specimens were placed in Syracuse dishes and the gross morphological characteristics of the embryos were observed under a binocular dissecting microscope. The microscope was equipped with a calibrated ocular micrometer with which all measurements were made. Immediately after these observations all samples were placed in Smith's fixative[1] for 12 to 24 hours, washed in several changes of water during a 24-hour period, and preserved in a 4-percent solution of formalin. This method of fixation and preservation was most satisfactory as judged by the pliability of the heavily yolk-laden eggs after 3 years' preservation.

Specimens to be sectioned for microscopic examination were washed overnight in running tap water, stained in alum-cochineal 16 to 24 hours, then dehydrated and embedded in paraffin. Sections cut at 5 to 10 micra were mounted and counterstained in a 0.5-percent solution of fast green in 95-percent ethyl alcohol and covered permanently.

DESCRIPTION OF STAGES

The following description of stages was based on materials which were taken from all of the batches of the different temperature series; but greatest emphasis was placed on observations of the 65° F. batches. Most characteristics of embryos of the other batches were identical with those reared at 65° F. Those differences which were observed are pointed out in the discussion of the particular stage. The time intervals listed for each stage include the time between the first and last appearance of the stage in the samples reared at 65° F.

The end-points selected for each stage were established after numerous observations of both

[1] Solution *A*: Potassium bichromate 0.5 gm., water 87.5 cc. Solution *B*: Formalin 10 cc., glacial acetic acid 2.5 cc. Mix solutions *A* and *B* immediately before using.

living and preserved materials. It was considered essential to be able to recognize all preserved stages at a later time as well as living stages at the moment of collection.

In addition to the gross morphological end-points, histological examination was used on occasion to define the stage more critically. The criteria selected were such as to include also the physiological differences. Furthermore, since the status of an animal is evidenced in part through its activity and movements, natural movements or activity were incorporated as far as possible into the staging criteria.

The method of fixation and preservation proved to be suitable for recognition of practically all end-points. The normally transparent prolarvae[2] became opaque in Smith's fixative. When selected specimens were fixed and preserved in 4-percent formalin, however, retention of all pigmentation and transparency aided staging. The formalin, of course, hardened the yolk mass and the notochord enough to prevent the use of these specimens for histological preparations.

Stage 0: Ovulated but unfertilized egg

Animal-pole depression: Present but just visible.

Cellular areas: Nuclear; animal-hemisphere cytoplasm; vegetal-hemisphere cytoplasm.

Size: 1.0 ± 0.2 millimeters.

Ovulated eggs within the coelom are assigned this stage. These creamy-white eggs are surrounded by a relatively thin jelly coat which expands when the eggs are shed into water. The stickiness of this coat causes sand grains stirred up by spawners to adhere to the surface of the egg.

The surface of the unfertilized egg has a small depression in the egg membrane over the nucleus and extends into the nucleus proper. A demarcation separates the nucleus and the surrounding cytoplasm. One-third the distance down the animal-vegetal axis the cytoplasm contains another demarcation between the cytoplasm surrounding the nucleus and the remaining cytoplasm.

The egg of the sea lamprey is telolecithal in that the egg consists of a relatively large amount of yolk and the nucleus is located at the center of the animal hemisphere.

[2] Hubbs (1943) defined a prolarva as a "larva still bearing yolk." In the present work prolarva includes stages 14–17.

This stage is initiated when the eggs are ovulated into the coelom and ends with fertilization.

Stage 1: Zygote (fig. 1) hours 0-2

Animal-pole depression: Increases in diameter and depth; disappears within about 1 hour.

Cellular areas: Identical to stage 0.

Size: 1.0 ± 0.2 millimeters.

Fertilization membrane: Appears within 20–30 minutes after fertilization; is retained through stage 13.

FIGURE 1.—Lateral view of stage 1, zygote, showing cellular areas.

This stage extends from the time of fertilization to the time when the fertilized egg begins to undergo first cleavage. A meridional section of a fertilized egg (fig. 2) shows the yolk platelets

FIGURE 2.—Meridional section of stage 1 prior to formation of fertilization membrane.

within the cytoplasm surrounding the nucleus and the remaining cytoplasm. The yolk of the animal and the vegetal areas differs in that the yolk platelets within the vitelline area are much larger than those in the area around the nucleus.

The depression at the animal pole seen in stage 0 persists through this stage. The first indication of significant morphological change is a noticeable deepening of this depression which will remain until about the first hour after fertilization.

Three distinct external areas indicate the presence of the three internal areas: nucleus; animal-pole cytoplasm; and vegetal-hemisphere cytoplasm. Below the nucleus the cytoplasmic area is demarcated by a band extending approximately one-third the distance down the animal-vegetal axis. The remainder of the egg consists of cytoplasm heavily laden with yolk. Each of the areas of the egg is visible both externally and internally as early as the unfertilized egg and up to the initiation of first cleavage.

Shortly after the animal-pole depression deepens, the fertilization membrane appears.

Stage 2: Two cells (fig. 3) hours 2-8

Cellular areas: Visible in the daughter cells.

Size: 1.0 ± 0.2 millimeters.

Cleavage: First furrow appears. Prominent peaks in daughter cells. Holoblastic. Completed within 5–6 hours.

The external topography of the 2-cell stage is comparable to that of the zygote in that the nuclear, and the animal- and vegetal-hemisphere cytoplasmic areas are readily visible in both daughter cells immediately after reconstitution of the nucleus (fig. 3).

This stage begins when the animal pole of the zygote begins to furrow and dimple in preparation for first cleavage, and ends at the beginning of the second cleavage furrow and dimple at the animal pole. Cleavage is total, usually slightly unequal (fig. 4) but occasionally (less than 1 percent) grossly unequal. As mentioned above, cleavage begins as a small furrow; a slight uprising of the cell membrane lateral to the furrow produces the dimpled effect. As cytokinesis progresses the cell membrane expands to a greater and greater extent while the fertilization membrane remains unchanged. The cell membrane continues to expand until the cell reaches the stage seen in figure 3, where the expanded cell membrane can be seen as twin peaks lateral to and above the furrow. As the cleavage furrow progresses meridionally over

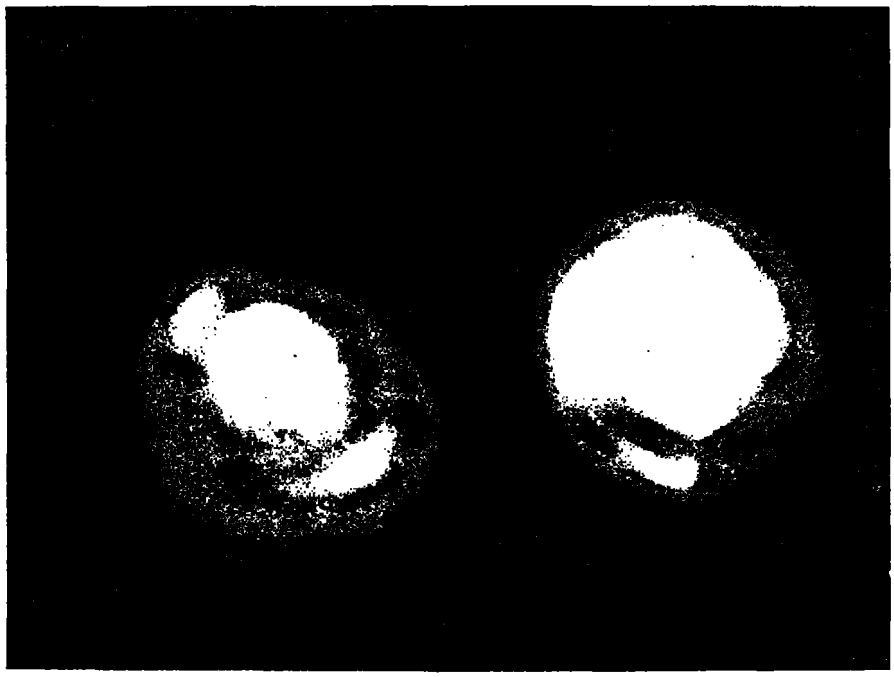

FIGURE 3.—External views of stage 2, two cells, showing the expanded cell membrane (right side) and daughter cells showing the cellular areas.

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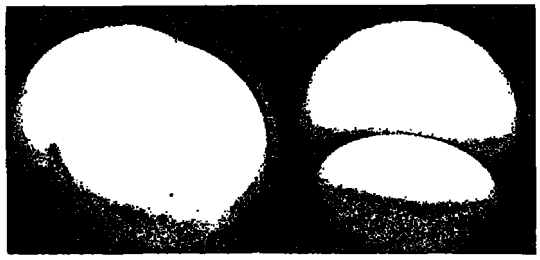

FIGURE 4.—External views of cleavage extremes of stage 2.

the cell, the cell membrane expands in advance of the progressing furrow.

Stage 3: Four cells (fig. 5) hours 8-11

Cellular areas: Visible in four daughter cells.

Size: 1.0 ± 0.2 millimeters.

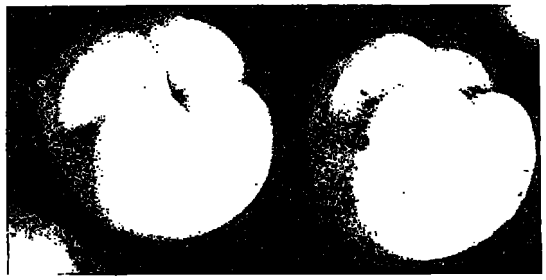

FIGURE 5.—Various external views of stage 3, four cells.

Cleavage: Second furrow appears: peaks are less prominent than in first division. Holoblastic. Completed within 3 hours.

Despite cellular division the three distinct topographical areas are still discernible in each of the four cells.

Stage 3 begins with the advent of the second cleavage furrow and dimple which starts in much the same manner as the first; the furrow and dimple are observed at the animal pole to either side of the first cleavage furrow and progress meridionally at right angles to the first cleavage furrow. As in stage 2, the expanding cell membranes are observed during cytokinesis.

The end-point of stage 3 is the appearance of third cleavage furrow and dimple.

Stage 4: Eight cells (fig. 6) hours 10-15

Cellular areas: Visible in meridional type cleavage.

Size: About 1.0 millimeter for equatorial division. Meridional division increases equatorial diameter and shortens meridional diameter.

Cleavage: Two types; meridional or equatorial. Holoblastic. Completed within 2 hours.

Stage 4 begins with the appearance of the third cleavage furrow, which may be either a double

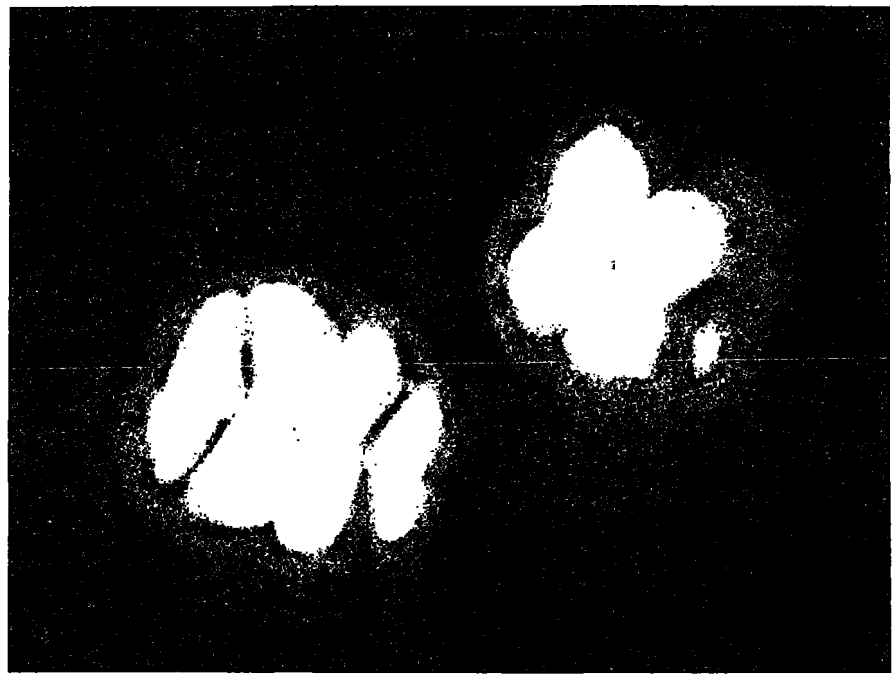

FIGURE 6.—Polar views of meridional and equatorial cleavages forming stage 4, eight cells.

meridional furrow or a single equatorial division. The meridional type was described by McClure (1893) for *P. marinus* eggs held at 6°–8° C. (42.8°–46.4° F.) or at room temperature, whereas both types of cleavage were found in this study. The equatorial cleavage was not mentioned by McClure, although it predominated in the present study in all experimental batches, regardless of temperature.

Embryos formed after meridional cleavage can be distinguished by the flatness at the animal pole. The nuclei are aligned four on each side of the first cleavage plane (fig. 6). In some embryos the segmentation cavity can be seen because the embryo splits along the first cleavage plane.

The flatness of stage 4 embryos formed by meridional divisions produces a large perivitelline space which facilitates removal of the fertilization membrane. The same operating space is not encountered again until stage 9.

The embryos formed by equatorial divisions have 4 micromeres resting upon 4 macromeres. In them, the space available for removal of the fertilization membrane is relatively small.

The end-point of this stage is the appearance of the fourth cleavage furrow.

Stage 5: Sixteen cells (fig. 7) hours 13-15

Cellular areas: No longer recognizable from external view.

Size: 1.0 millimeter.

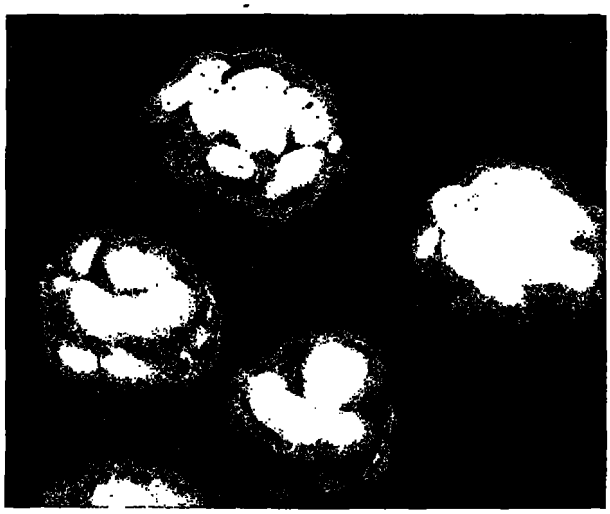

FIGURE 7.—Several views of stage 5, sixteen cells, illustrating differences in size of animal and vegetal cells.

Cleavage: Equatorial or meridional, depending upon type in stage 4. Pattern irregular. Completed within less than 2 hours.

Stage 5 begins with the appearance of the fourth cleavage, the plane of which varies according to the type of cleavage which formed stage 4; a third-stage equatorial division, near the animal pole, is followed by a fourth meridional division and vice versa. Cleavage irregularity becomes apparent during this stage; embryos composed of 9 to 16 cells are found and included in this stage.

The end-point of stage 5 is reached when the embryo is composed of 17 or more cells.

Stage 6: Thirty-two cells (fig. 8) hours 16-19

Size: 1.0 millimeter.

Cleavage: Random and indeterminate. Completed within 1 hour.

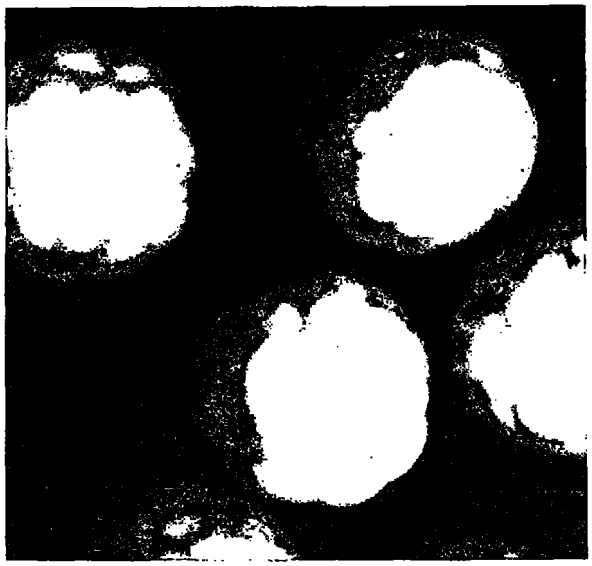

FIGURE 8.—Several polar views of stage 6, thirty-two cells.

Embryos were assigned to this stage when 17 to 32 cells were distinguishable. Cytokinesis at this point, however, became indeterminable so that the fifth cleavage appeared to take place at random. Cell counts were made on all embryos that could not be identified on the basis of relative cell sizes by comparison with both stages 5 and 7. When animal cells are compared with animal cells, and vegetal cells with vegetal cells, the cells of stage 6 embryos will be about one-half the size of stage 5 cells and approximately twice the size of stage 7 cells. The relative sizes of the animal-

and vegetal-cells can be ascertained in figure 9, a meridional section of stage 6.

The extent of the blastocoel can also be seen in figure 9. The roof of the blastocoel is composed of a single layer of relatively large animal cells whereas large vegetal cells extending from the vegetative pole to the blastocoel constitute the floor. The end-point of stage 6 is reached when the embryo has 32 cells.

FIGURE 9.—Meridional section of stage 6.

Stage 7: Sixty-four cells (fig. 10) hours 19-24

Size: 1.0 millimeter.

Cleavage: Indeterminate.

Stage 7 is considered to have begun when the embryo has more than 32 cells. The cleavage continues indeterminable in this and later stages. Recognition of the stage becomes a matter of cell counts and comparison of cell sizes with those of the preceding and succeeding stages. A cursory count of the animal cells is made to get an approximate estimate of the stage to which the embryo should be assigned; the final assignment is based on a combination of cell size and cell count. As cell size diminishes the contour of the embryo becomes smoother. This change can be seen in a comparison of figures 8 and 10. The animal cells are still smaller than the vegetal cells, which have

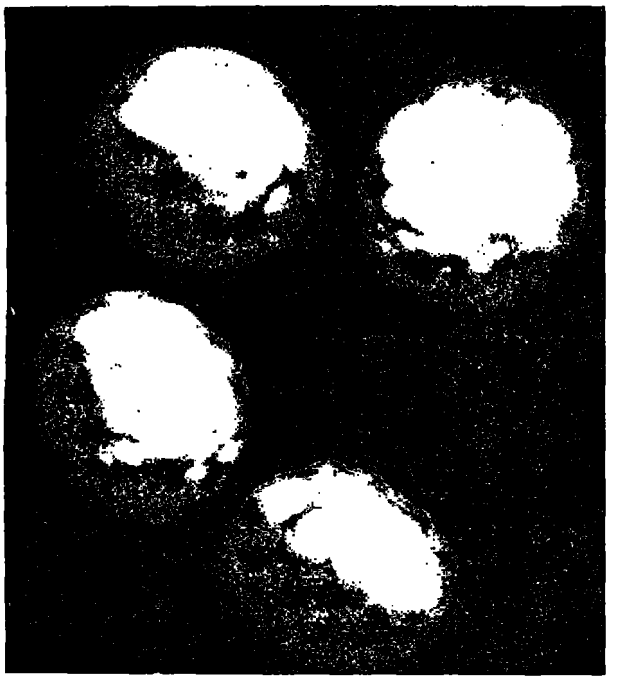

FIGURE 10.—Polar and lateral views of beginning stage 7, sixty-four cells.

since divided and are now only about twice the size of the animal cells.

The end-point of stage 7 is reached when the embryo has more than 64 cells. Division of all animal cells along with division of the vegetal cells can be taken as an approximate end-point.

Stage 8: Full blastula (fig. 11) hours 24-64

Size: 1.0 millimeter, increasing to 1.2–1.4 millimeters.

Cleavage: Furrows seen on individual cells.

Animal hemisphere: Becomes translucent.

Blastocoel: Visible through animal cells.

When the animal pole cells of stage 7 undergo further cell division as evidenced by further reduction in cell size and the appearance of cleavage furrows, the embryo is considered to have entered stage 8. The most striking external feature of this early phase of the stage is its contours (compare to stage 7) which become progressively smoother as the number of cell divisions increases and cell size decreases.

In the final phase of this stage the volume of the embryo increases by some 3 to 5 times. Measurements of living specimens made within a calibrated ocular micrometer at the initiation of and

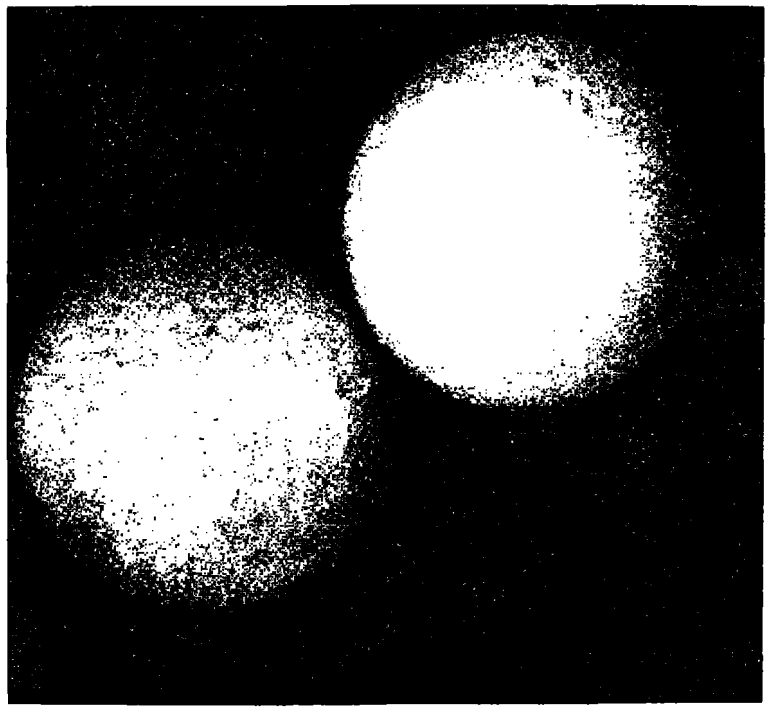

FIGURE 11.—Polar views of stage 8, full blastula, arranged to illustrate changes in cell size.

just prior to this stage were consistently about 1.0 millimeter across as viewed and measured in optical section perpendicular to the animal-vegetal axis as well as along that axis. Subsequent measurements prior to the appearance of the dorsal lip of the blastopore, the end-point of stage 8, ranged from 1.2 to 1.4 millimeters. The increase in volume is accompanied by translucency of the animal-pole cells so that the outline of the blastocoel becomes visible through approximately one-third the surface of the embryo. The size of the animal cells does not change during the expansion process; the increase of the blastocoel indicates that the expansion is due to blastocoel enlargement. The blastocoel is at its greatest volume at the end of stage 8. The animal cells are still about one-half the size of the vegetal cells. This size relation is maintained as long as the epibolizing animal cells can be compared externally with the underlying vegetal cells.

The end-point of stage 8 is the appearance of the blastopore.

Stage 9: Gastrula (fig. 12) hours 64-104

Size: 1.2 to 1.4 millimeters decreasing to 1.0 millimeter.

Animal hemisphere: Translucent to transparent to opaque. Opacity extends progressively forward from blastopore. Begins to flatten, forming neural plate.

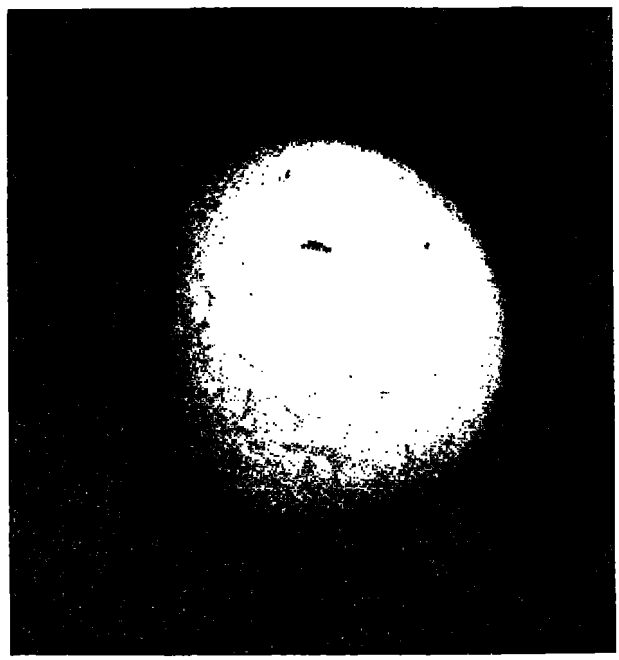

FIGURE 12.—Posterior view of stage 9, gastrula, illustrating the blastopore.

Blastocoel: Progressively obscured by opacity of animal hemisphere. Decreases in volume.

Blastopore: Forms as wide arched slit. Hooded as neural plate begins to form. Apparently migrates.

The translucency of the animal hemisphere of stage 8 changes to transparency after the appearance of the blastopore. Through the transparent animal hemisphere the underlying chorda-mesoderm is visible as it undergoes its morphogenetic movements. During this period the volume of the blastocoel decreases when the chorda-mesoderm reaches a position two-thirds the distance across the animal hemisphere. The most advanced portion of the chorda-mesoderm is in the mid-sagittal plane; the material in the parasagittal planes lags behind these more advanced cells. As this material progresses beneath the animal hemisphere, the dorsal lip of the blastopore progressively increases in thickness and begins an apparent migration from its original position at or near the overlap of animal and vegetal cells one-third the distance from the center of the animal hemisphere, toward the center of the animal hemisphere. As the chorda-mesoderm moves to its anteromost position in the embryo, the transparency of the animal hemisphere decreases, and the decrease in the volume of the embryo continues.

The typically circular blastopore of later stages is formed by progression of the animal cells in their epibolic movements to produce a changing pattern to the dorsal lip and, necessarily, the blastopore. At the time of the blastopore's inception the advancing margins of epibolizing cells beneath the blastopore approach each other laterally as a wide-open *V* with the apex at the center of the dorsal lip of the blastopore. As gastrulation progresses, these V-arranged margins close toward each other until only the vegetal cells below the blastopore remain uncovered. These vegetal cells are covered by epibolizing cells which move upward along the mid-sagittal plane (fig. 12).

As the chorda-mesoderm advances farther into the anterior portion of the embryo, the blastopore begins an apparent migration along the mid-sagittal plane from its original position to one located at the posterior limit of the mid-sagittal plane in stage 10. Histological comparisons between stages 8 and 9 indicate that this apparent movement resulted from reduction in the size of the blastocoel. At this time the dorsal region of the entire embryo begins to flatten and to thicken from the dorsal lip of the blastopore to the anterior region.

Stage 9 is marked by the appearance of the dorsal lip of the blastopore, a flat crescentic-shaped furrow within the overlapping line of the animal cells epibolizing over the vegetal cells. Recognition of the stage depends upon locating the blastopore half-way beneath the embryo; the observer must rotate the embryo to find it.

The end-point of stage 9 is reached when the flattening process reaches the anterior end of the embryo.

Stage 10: Neural plate and groove (fig. 13) days 4-5

Size: 1.1 to 1.3 millimeters.

Blastopore: Triangular to ovoid. Reaches its dorsalmost point.

Neural tissue: Neural plate forms and thickens. Groove and folds form.

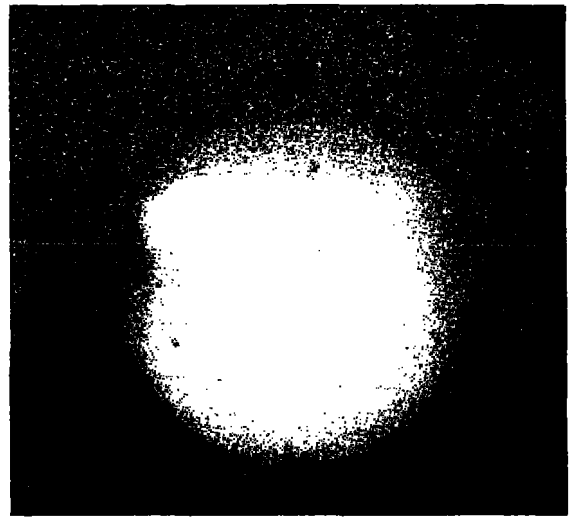

FIGURE 13.—Lateral view of stage 10, neural plate.

Stage 10 begins when the flattening of the dorsal ectoderm mentioned in stage 9 has reached the anterior extremity of the embryo (fig. 14). At the same time, the nearly completed blastopore is near or at the dorsalmost point of the posterior area of the mid-sagittal plane of the embryo (fig. 15). Further thickening of the depth of the flattened area follows almost immediately after the flattening of the dorsal ectoderm extends from the blastopore to the anterior region. These animal cells are no longer transparent. The ovoid to triangular

FIGURE 14.—Dorsal view of stage 10 illustrating neural groove.

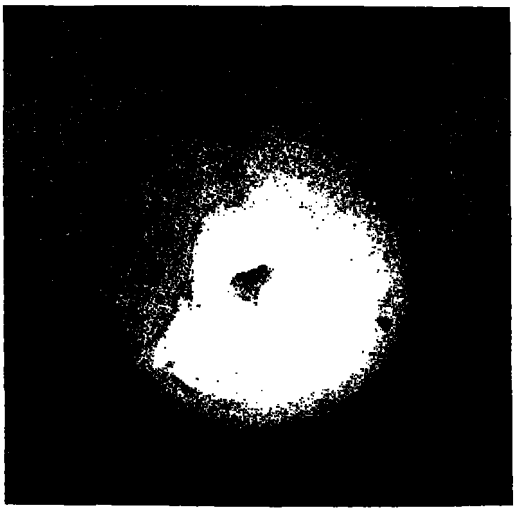

FIGURE 15.—Posterior view of stage 10 illustrating blastopore and neural plate with neural groove.

blastopore is now at the uppermost point of its apparent migration along the mid-sagittal plane and the flattened ectodermal cells (neural plate) are at the height of their thickening. Practically all the vegetal cells are covered by the epibolizing animal cells. Immediately after the neural plate thickens, the central portion of the plate begins to form a trough, producing a neural groove and fold stage (fig. 16). As the groove in the neural plate deepens the "folds" begin to approximate each other and unite. The first actual union occurs in the mid-dorsal region of the embryo. This union is the end-point for stage 10.

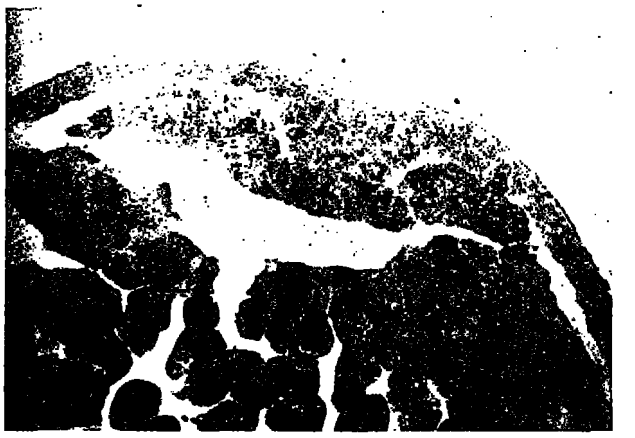

FIGURE 16.—Cross-section of stage 10

Stage 11: Neural rod (fig. 17) days 5-6

Size: 1.1 to 1.3 millimeters.

Blastopore: Circular. Apparent migration toward ventral surface of embryo.

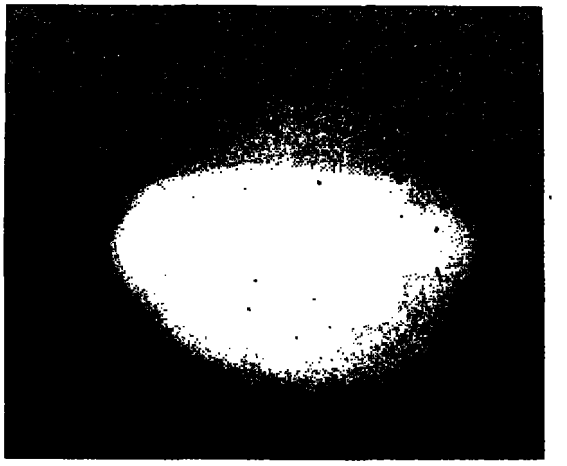

FIGURE 17.—Oblique lateral view of stage 11, neural tube.

Neural tissue: Union of folds middorsally on embryo. Neural tube lacks neurocoel, and thus is a neural rod. Becomes prominent across dorsum of embryo; circumscribes approximately two-thirds of embryo.

In the late phase of this stage the dorsoposteriorly located, circular blastopore begins an apparent migration toward the ventral surface accompanied by a similar ventral movement of the prospective head. Toward the end of this stage the circular blastopore is at the ventralmost point of the posterior as contrasted with its

earlier position at the dorsalmost point of the posterior. These movements continue until the neural rod occupies approximately two-thirds of the circumference of the embryo.

The union of the folds marks the beginning of stage 11. The neural plate begins to round up and appears in external view to have become a neural tube with anterior and posterior neuropores. This appearance is a result of the apparently simultaneous, progressive union of the folds in anterior and posterior directions from the site of first union, the middorsum.

The external morphology of this stage is as deceptive as that of the preceding stage since histological examinations reveal that the neural tube does not possess a neurocoel; in reality the neural tube is a neural rod as described by Shipley (1885). In the anteromost region the neural rod seems to elevate from the surrounding and underlying tissue.

The end-point of this stage is reached when the anterior region is raised above the globular yolk mass.

Stage 12: Head (fig. 18) days 6-8

Size: 1.1 to 1.4 millimeters.

Blastopore: Below posteriormost point of neural rod, at ventral surface of embryo. Circular.

Neural tissue: Very prominent from head to blastopore. Neurocoel over presumptive pharynx.

Head: Elevated from yolk mass. Free length approximately 1.0 millimeter.

Yolk: Globular as in preceding stages.

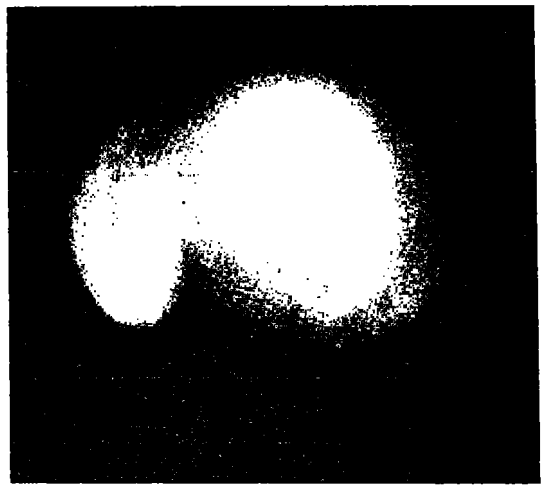

FIGURE 18.—Lateral view of stage 12, head.

Stomadaeum: Invagination begins.

Measurements of the embryo from the tip of the head to the posteriormost point of the yolk mass along the neural rod gave a range from 1.0 to 1.1 millimeters when the head was just beginning to form and 1.3 to 1.4 millimeters when the head was fully formed and elevated from the yolk mass. Although sections show that some somites have formed, they are not discernible externally.

The blastopore is below the posteriormost point of the neural rod and faces directly ventral during the initial period of this stage. When the head is fully formed, the blastopore faces anteriorly along the ventral surface toward the head.

Histological examination proves that the neural rod forms its neurocoel over the presumptive pharynx only. By this time the neural rod extends along approximately 75 percent of the periphery of the embryo (fig. 19). The rod is prominent and extends from the head to the blastopore.

FIGURE 19.—Parasagittal section of stage 12.

Elongation takes place faster in the head region than in the tail region. The anterior part becomes well elevated and protrudes between the lateral swellings. The length of the head and the presumptive branchial region is about 1.0 millimeter at the end of the head stage. The appearance of a head on the exterior of the embryo marks the beginning of stage 12. This change is caused by the sudden increase in length of the neural rod during stage 11 and the sudden expansion of the presumptive pharyngeal cavity both dorsally and laterally to produce an upswelling of the prospective head from its original position.

The yolk mass retains the globular shape it had in the preceding stage. The stomodaeal invagination begins in the ventral portion between the lateral swellings.

The end-point of this stage is reached when the head region begins muscular activity.

Stage 13: Prehatching (fig. 20) days 8-12

Size: 1.4 to 2.5 millimeters.

Blastopore: Circular. Much reduced. Located at anteriormost point of apparent migration.

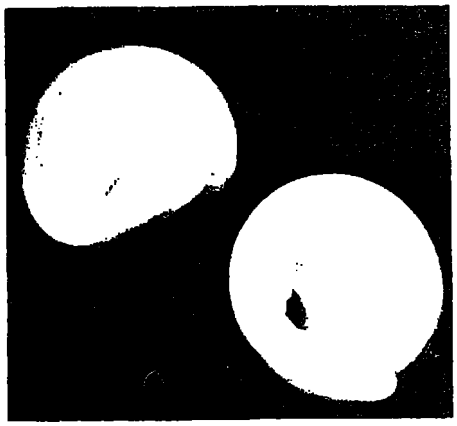

FIGURE 20.—Lateral view of stage 13, prehatching.

Neural tissue: Neural rod now a true neural tube. Prominent above somites from blastopore to head.

Head: 2.0 millimeters.

Yolk: Obovate, blunt end posterior.

Stomodaeum: Deepens and widens.

Somites: 5 to 20; not easily distinguished externally.

Locomotion: First muscular activity. Movement of free embryo, head.

The length of the head region has increased from 1.0 millimeter at the beginning to 2.0 millimeters at the end of this stage. Measurements were taken from the tip of the head to the anteriormost part of the yolk mass.

The blastopore, which is situated anteroventrally lies at its anteriormost position directly opposite the stomodaeal invagination.

Stage 13 begins with the advent of muscular activity. These movements initially are mere lateral flexions of the head and "neck" portion of the embryo; up-and-down activity is not apparent. As the embryo enlarges, muscular contraction includes dorsoventral flexion and becomes undulating rather than wagging. With increase in in the embryo's size the perivitelline space becomes fully occupied, since the embryo, arranged in circular fashion within the membrane, begins to spiral upon itself. During this growth the above-mentioned movements become more forceful and more frequent.

Embryos that show movement but still have intact fertilization membranes are in stage 13. The endpoint of stage 13 comes when the head protrudes through the fertilization membrane to initiate hatching.

Stage 14: Hatching (fig. 21) days 10-13

Size: 3.0 to 5.0 millimeters.

Blastopore: Minute opening at apex of 90° ventral flexion of posterior. Dubious structure for staging.

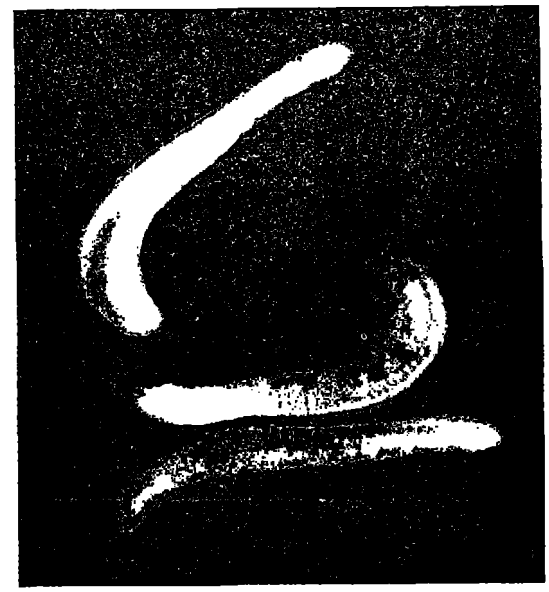

FIGURE 21.—Lateral view of several prolarvae of stage 14, hatching, showing posterior curvature, somites, yolk mass, and condition of mouth and nostril.

Neural tissue: Neural tube still very prominent above somites.

Yolk (gut): Becomes slender and assumes a spatulate shape. Posterior region has 90° ventral flexion. Anteriormost portion greenish.

Stomodaeum: Deep pit located ventrally.

Somites: 18–20 to 30–35. Extend from neural tube to gut.

Locomotion: Hatching movements. Undulation of anterior body region only.

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Nostril: Begins as single invagination in midventral line, anterior to the stomodaeum.

Transparency: First appearance of ectodermal transparency over pericardium. Extends posteriorly and anteriorly in later phases of this stage.

Circulatory system: Pericardium visible through transparent ectoderm. Straight tubular heart. Begins beating 40 times per minute.

Liver: First indication posterior to pericardium in anterior part of gut. Appears in late period of stage.

Stage 14 starts when the embryo breaks through the fertilization membrane. The constant movement observed in stage 13 finally becomes sufficiently strong to extrude the head through the membrane. Further activity enlarges the tear in the membrane and eventually leads to hatching.

The pericardial cavity appears as a ventral swelling approximately 1.0 millimeter posterior to the tip of the head. It is also present in late stage 13 but is not easily recognized in most embryos. Shortly after hatching the ectoderm overlying the pericardial region and the tissue beneath gradually become transparent. A short time later, the body anterior to the pericardial cavity becomes transparent.

Through these transparent tissues can be seen the straight tubular heart which begins to pulsate during the eleventh day at 40 beats per minute (at 65° F.). There is no sign of blood in the heart or anywhere in the prolarva.

The end-point of stage 14 is reached when melanophores appear on the embryo.

Stage 15: Pigmentation (fig. 22) days 13-16

Size: 5 to 6 millimeters.

Neural tissue: Brain and tube visible through ectoderm.

Gut: Spatulate, changing to cylindrical. Ventral flexion of about 10° remains. Anterior face greenish.

Stomodaeum (mouth): Transverse slit bounded by thickened lips. Opens into oral cavity.

Somites: 35 to 50.

Locomotion: Undulation of entire body slightly restricted by yolk-filled gut in early stage. Full swimming movements in late period.

Nostril: Single, median at anteriormost point on ventral surface.

Transparency: Extends anteriorly to branchial region and posteriorly to about two-thirds the length of the prolarva.

Circulatory system: Heart becomes S-shaped. Heart walls thicken. Grayish channel forms in midventral gut and turns red as hemoglobin appears. Bilateral channels appear in 15th day. Heart beat, 100 per minute.

Liver: Becomes larger and vascularized.

Pigmentation: First appears as bilateral melanophores dorsal to the midbrain.

The stomodaeal pit has become a slit opening into the anterior (oral) chamber of the pharynx which is separated from the posterior (pharyngeal) chamber by the velum. The transverse slit is bounded by a thickened ectodermal lip anteriorly and posteriorly. The pharynx has its full complement of 7 visceral pouches.

Deftness of swimming increases as the shape of the yolk mass changes from spatulate to cylindrical. At the same time the tail straightens from its ventral flexion. The prolarvae progress from the awkward movements of stage 14 to an undulating movement, the smoothness of which depends on the amount of the ventral flexion of the tail.

The heart has enlarged and assumes an S-shape

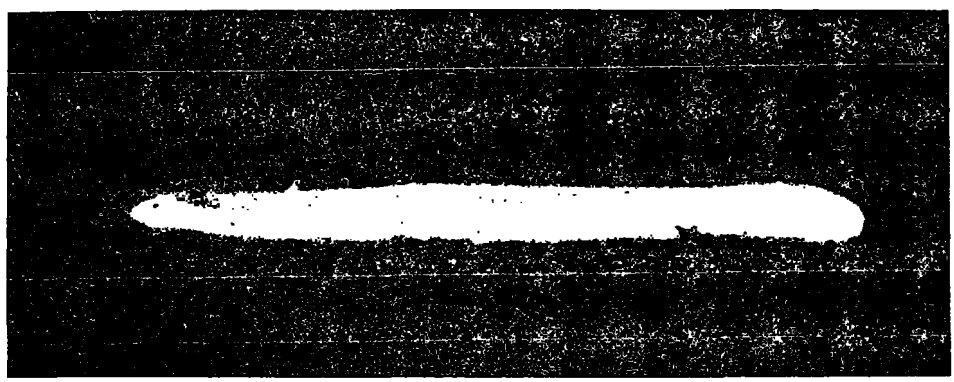

FIGURE 22.—Lateral view of stage 15, pigmentation, showing somites, pericardial area, and condition of yolk.

as the auricle moves dorsal to the ventricle. Both auricular and ventricular walls begin to thicken but remain transparent. The rate of heartbeat increases from the 40 per minute of stage 14 to 100 per minute at the end of stage 15.

Between the 13th and the 14th days a grayish channel develops along the midventral line of the cylindrical yolk mass. The channel extends from the anterior portion to the midpoint of the yolk-filled gut and within a short time turns red as hemoglobin is produced. During the 15th day bilateral channels appear along the midlateral line of the yolk mass and then converge to form a single vessel to the heart.

Stage 15 is initiated by the appearance of a pair of dorsal melanophores bilateral to the midbrain. Second and third pairs appear in sequence immediately posterior to the original ones. Shortly after the appearance of the third pair, a melanophore can be seen above the anterior branchial region. Pigmentation spreads anteriorly and posteriorly from the dorsal pairs along the neural tube (fig. 23); it spreads posteriorly and ventrally from the lateral pair along the line between the yolk and the somites, as far as the anterior limit of opaque or undifferentiated tissue. As transparency progresses, the posterior distribution of melanophores is extended correspondingly.

The end-point of stage 15 is reached with the appearance of the gill slits.

Stage 16: Gill-cleft (fig. 24) days 15-17

Size: 6 to 7.5 millimeters.

Neural tissue: Divisions of central nervous system recognized through ectoderm.

Gut: Cylindrical yolk mass. Increases in length and becomes more slender. No ventral flexion. Anterior portion greenish. Postanal gut present. Anus forms at persistent blastopore.

Mouth: Semicircular slit. Enlarges to hooded mouth. Oral cirri make first appearance.

Somites: No longer useful as staging criterion.

Locomotion: Larval swimming pattern. Very adept at end of stage.

Nostril: Migrates from anteroventral to anterodorsal extremity.

Transparency: Practically entire prolarva becomes transparent except gut.

Circulatory system: Ventricular wall has thickened. Heart beat, 150 per minute. Flow of blood readily visible.

Liver: Size increases. Distinctly separated from gut.

Pigmentation: Melanophores extend along dorsum and sides of embryo from anterior to posterior. Bilateral eyespots appear at end of stage.

Gill clefts: First appearance. They become functional.

Respiratory rate: 120 per minute.

Fins: Caudal and anal fins appear.

The mouth continues to migrate anteriorly and

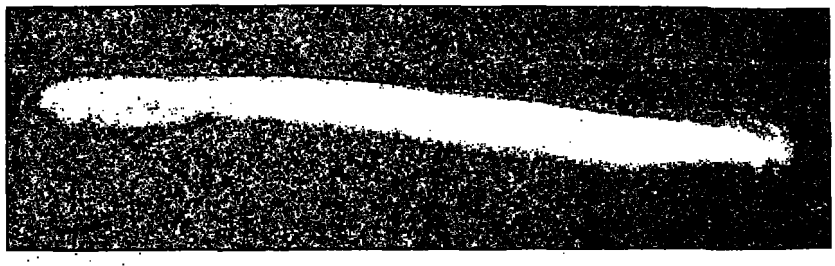

FIGURE 23.—Dorsal view of stage 15 showing condition of pigmentation over the head region.

FIGURE 24.—Lateral view of stage 16, gill cleft, showing gill clefts, somites, pericardial area, and pigmentation over yolk mass.

the nostril dorsally. The anterior lip of the slit-like mouth of stage 15 moves anteriorly at the midline only, producing a semicircular mouth. Migration of the anterior lip and nostril continues until the mouth lies at the anteroventral extremity and the nostril is at the anterodorsal extremity. In later phases of the stage the mouth enlarges and is hooded by the anterior lip. Oral cirri begin to form posterior to the anterior and posterior lips.

Movement during this stage is by a rapid undulation of the trunk and tail. All ventral flexion of the tail region has disappeared during the transition from stage 15 to 16.

The anterior boundary of the auricle lies over the center of the ventricle. The flow of blood through the heart and other parts of the prolarva is seen readily.

In the latter period of this stage the bilateral pigmented retina (eyespot) appears anterior and dorsal to the velum. Melanophores which extend down the length of the neural tube and along the yolk-filled gut begin to migrate ventrally from both levels. Melanophores migrate along the myosepta from the neural-tube level and ventrally from the yolk-somite line. The anterior lip and head region also are well pigmented.

The appearance of gill clefts marks the initiation of stage 16. They appear and start functioning in order from the first to the seventh. Functioning of the branchial apparatus can be determined by two means: beating of the velum (between the oral cavity and the first visceral cleft) as it forces water through the pharynx; or, contractions of the gill clefts. The respiratory rate (velum beat) is 120 per minute.

The end-point of stage 16 is reached when the prolarvae acquire bilateral eyespots and are about to burrow.

Stage 17: Burrowing (fig. 25) days 17-33

Size: 7.5 to 9.0 millimeters.

Gut: Cylindrical and yolk-filled. Postanal gut absorbed. Esophagus visible on left side. Cloaca transparent. Peristaltic movements in hind gut. Lumen of gut opens at end of stage.

Mouth: Oval with dorsal and ventral lips. Opens anteriorly. Oral cirri.

Locomotion: Swimming movements same as in stage 16. Prolarvae burrow.

Nostril: On dorsal aspect, its ultimate position.

Transparency: Complete except for yolk-filled gut.

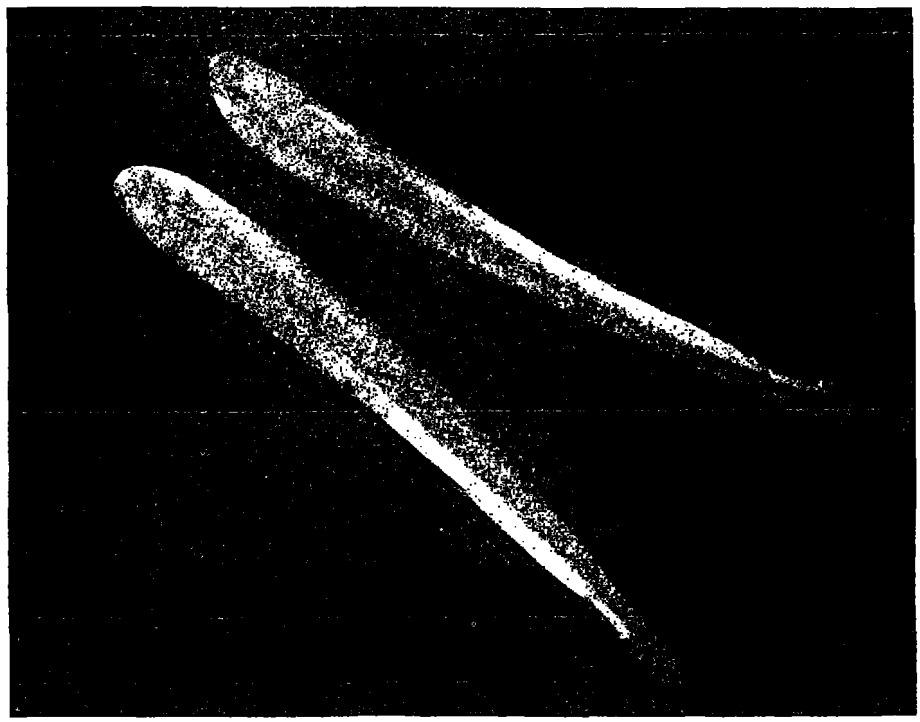

FIGURE 25.—Lateral views of stage 17, burrowing, showing the condition of the mouth and the lips.

Circulatory system: Heart walls thicken. Heart beat more than 200 per minute. Flow of blood visually traceable throughout most of prolarva.

Liver: Further increases in size. First indication of gall bladder on right side.

Pigmentation: Melanophores extensively distributed. Aggregation over pronephric region and caudal end of notochord and neural tube.

Respiratory rate: 200 per minute.

Pronephros: First detectable externally.

The posterior region of the gut becomes transparent as the cloaca is formed with the opening of the pronephric ducts into the gut (histological observations). A yolk pellet forms in the gut anterior to the cloaca as the result of peristaltic movements of the gut. These movements move the pellet toward the anus and then back to the remaining yolk mass.

The lips take on the characteristic larval form. The mouth opens directly anteriorly, so that the anterior lip has now become the dorsal lip and the posterior lip the ventral.

Stage 17 begins when the prolarvae burrow into the bottom mud. Burrowing is the result of the action of both the tail and the head regions. As the head moves from side to side to create space within the mud, the lashing of the tail drives the prolarva into the mud. Prolarvae of this stage placed in aquaria or beakers first swim near the surface of the water and then suddenly plunge downward with rapid swimming movements until they reach the bottom, when they immediately begin to burrow. Prolarvae of stage 16 merely drift down to and lie on the bottom. Swimming movements do not differ from those of stage 16.

The liver is extended farther and the presence of the gall bladder in the later period is marked by its bile-green color.

The eyespot or retina is very prominent anterior and dorsal to the velum. Melanophores have spread completely around the gut region to the ventral surface. They have migrated down the lateral surface of the velum and the gill bars, and have completely outlined the branchial basket. The dorsal lip is covered completely with melanophores.

A pronephros dorsal and posterior to either side of the pericardial cavity is visible externally because of the presence of much blood. This stage is the first in which the pronephros is visible externally.

The end-point of stage 17 is reached when the lumen of the yolk-filled gut is opened.

Stage 18: Larva (fig. 26) days 33-40

Size: 9 millimeters and longer.

Gut: Lumen completely opened. Yolk extruded from gut. Gut tissue becomes transparent.

Respiratory rate: 200 per minute.

FIGURE 26.—Various views of several stage 18 larvae on a black background. Shown are: condition of the mouth and lips, eyespot, pigment outlining branchial region, liver immediately posterior to the heart, somites, fins, and the gut from the liver to the cloaca. The granular appearance along the gut is caused by pigmentation.

The transition from the prolarval to the larval condition (all systems differentiated save genital) is marked by the differentiation of the formerly yolk-filled gut into its definitive form. This change is seen outwardly as the gut becomes transparent at the "stomach" region behind the liver. Transparency progresses posteriorly until the entire gut has differentiated and the digestive system is open from the mouth to the anus. After the pellet described in stage 17 is passed, yolk remaining within the lumen of the gut is extruded constantly from the anus.

Stage 18 larvae are between 9 and 10 millimeters long at the time the gut becomes fully differentiated. They belong to the first larval stage which would be equivalent to age-group 0, 11 to 21 millimeters long, of Applegate (1950).

REVIEW OF STAGES AND COMPARISON WITH EARLIER STUDIES

Staging of lampreys in most early literature lacked precision. Investigators, for example, Shipley 1885, Scott 1887, McClure 1893, Hatta 1914, 1915, who studied early embryology, gave little or no attention to the need for staging. Developmental sequence was described in conventional embryological terms.

The first serious attempt to show stages of lamprey development was by Damas (1944) in his excellent histological study of *L. fluviatilis*, concerned primarily with tracing development of the head. Each section (comparable to a stage) comprised a short account of external features and a thorough histological description. His staging series began with embryos possessing 3 somites (section 1). The next section included embryos with 10 somites. (Every somite listed by Damas was not visible in external view, but each was seen histologically.) On the basis of somite numbers Damas was able to describe several stages slightly different from each other which could be described also by staging based on external morphological features. Some of his sections, based usually on intervals of 10 somites, have been translated into stages of the present study for comparison in table 1.

Stages described in the present work have been based primarily on changes of external features. Histological observations have been used sparingly and merely for clarification. Damas, who began his study after development had started, omitted the first 9 stages of the present work. Because his criteria were histological, his stages are overlapped by stages in the present study (table 1).

TABLE 1.—*Equating of "sections" of Damas (1944) study with "stages" of the present research*

Damas (section)	I	II	III	IV	V
Plavis (stage)	10	11	12	12	13
Damas (section)	VI	VII	VIII	IX	X
Plavis (stage)	13	13	14	14	14
Damas (section)	XI	XII	XIII	XIV	XV
Plavis (stage)	15	15	15	16	18

Staging, to be of practical value, should be simply, easily, and promptly recognized by investigators in both living and preserved materials. By far the easiest method of designating stages is one based on morphological characteristics since the resulting demarcations are relatively sharp and distinct and the diagnostic features are visible without elaborate histological preparation. They are natural divisions in a developmental sequence. Particular effort was made in this study to avoid dependence on measurements and counts. Ideally, observation alone should suffice for recognition of stages.

Applegate (1950) divided the larval forms of *P. marinus* on the basis of length and weight into age groups, beginning with age-group 0. He stated that the larvae ". . . upon leaving the nest have completed their early developmental stages and are perfectly formed but diminutive ammocoetes." These ammocoetes are in reality stage 18 larvae, for specimens from Applegate's collections match specimens from my collections in their external as well as histologic characteristics.

Stages, as designated here, can be determined by the naked eye or with a hand lens. Separation of stages 12 and 13 might conceivably cause some difficulty since transition between them is based on muscular activity. It is easy, however, to recognize preserved specimens, for the head region of embryos of stage 13 is displaced to either side of midline whereas heads of stage 12 embryos are medial.

Cleavage in the sea lamprey has several interesting characteristics, the first of which is the appearance of relatively high prominences lateral to and above the first cleavage furrow. Similar prominences occur during second cleavage (stage 3).

McClure (1893) noted the unusual cleavage pattern of *P. marinus*, especially in the third and fourth cleavages. He described the third cleavage as meridional and considered this to be the sole type of third cleavage. Actually, both meridional and equatorial types of third cleavage occur, but their relative abundance fluctuated widely irrespective of temperature.

Teleostei, Gymnophiona, Gallus, and other forms exhibit a meridional third cleavage, which, however, is accomplished in meroblastic division. In the sea lamprey the meridional third cleavage is holoblastic.

The cleavage of stage 4 embryos determines the type of cleavage for stage 5, since an equatorial stage 4 is followed by meridional stage 5 and vice versa.

Cleavage of stage 6 embryos is indeterminable and is accompanied by a lag in the cleavage of the vegetal cells. After stage 6, demarcation between the animal and vegetal cells is sharp until

the vegetal cells are covered by the epibolizing animal cells.

Blastopore formation, as was observed by Shipley (1885), involves behavior of animal cells and vegetal cells similar to that in amphibians and teleosts. When invagination of animal cells forms the blastopore, the margin of animal cells is arranged latitudinally around the embryo from the blastopore. As the animal cells continue to epibolize, the vegetal cells are covered by animal cells from the anterior and dorsolateral portions of the animal hemisphere and subsequently from the posterolateral and ventral positions in the fashion described for stage 9. Thus, migration of animal cells over vegetal cells in the lamprey matches closely the epiboly of animal cells in amphibians. The dorsal lip does not curve as much, however, in the sea lamprey as in amphibians. If the blastopore is considered to be defined by the line of epibolizing animal cells, as it is in teleosts, the blastopore of the sea lamprey is at first oval. This shape results from alignment of animal cells lateral to the midsagittal plane. A circular blastopore is formed when animal cells at the ventralmost point of the oval-shaped blastopore cover the oval yolk plug in a ventral-to-dorsal direction along the mid-sagittal plane.

The circular blastopore of the sea lamprey apparently migrates toward the center of the animal hemisphere whereas the teleost blastopore migrates toward the vegetative pole as the embryo lengthens. The amphibian blastopore eventually reaches and passes beyond the vegetative pole at a stage equivalent to stage 13 of the lamprey. Because teleost embryos do not extend ventrally over the yolk mass the blastopore does not reach the vegetative pole.

The apparent migration of the lamprey blastopore might possibly be attributed to the decrease in the volume of the blastocoel during stages 9 and 10. An increase in embryo volume which takes place in stage 8 is apparently due primarily to the increased volume of the blastocoel. During stage 9, after involution is completed, the volume of the embryo decreases as the result of a decrease in the volume of the archenteron. The archenteron, in reality, is the original blastocoel since the blastocoel is not obliterated during gastrulation (histological observations) as it is in amphibians. In this feature of gastrulation the lamprey closely resembles teleosts. Since decrease in the size of the archenteron shortens the embryo and since growth of the neural tube in stages 12 and 13 moves the blastopore along the mid-sagittal plane, the blastopore appears to migrate.

The present study also corroborates Shipley's (1885) observation that the open blastopore persists, eventually becoming the anus. Histological sections permit tracing the archenteron to an open blastopore through stage 15. During stage 16, the diameter of the blastopore widens and the lips thicken to form the anus. The archenteron can be traced to the anus in stage 16 as it was traced to the blastopore in stage 15.

Shipley recognized that the early neural tube did not possess a neurocoel and called it a neural rod, which term has been retained here. Selys-Longchamp (1910) described neural-tube formation in lampreys as intermediate between the keel method of teleosts and neural-fold method of other vertebrates.

Among the morphological features prominent during development were the gut, liver, gall bladder, hemoglobin and vascularization, and pigment. The gut opens from the oral cavity to the anus at stage 18. The stomodaeum opens in stage 16, the esophagus in stage 17, the cloaca in stage 17, and finally the portion between esophagus and cloaca at stage 18.

The formation of the liver in late stage 14 is indicated by the greenish cast of the anteriormost portion of the gut. Vascularization of the liver occurs during stage 15. The size of the liver continues to increase through stages 15 and 16. A gall bladder forms in stage 17; it is recognizable externally by its accumulation of blue-green bile.

Additional changes in stage 15 embryos include the appearance of hemoglobin within the blood channels which had formed in the yolk-filled gut. Blood cells appear first in the midventral channel and soon are in all three major channels. Blood formation and the bulk of vascularization take place in stages 15 and 16. Vascularization is extended in stage 17, when practically all major vessels can be traced by following red corpuscles within the transparent tissues.

Pigmentation of the embryo begins as two melanophores bilateral to the midbrain. Successive pairs of melanophores appear posterior to the initial one; next is the appearance of a melanophore above the branchial region. The number of melanophores increases throughout stage 15 when

they loosely cover the dorsum and form a line posteriorly from the branchial region between yolk and somites. In stage 16 melanophores extend along the dorsum and sides of the embryo. Bilateral eyespots appear at the end of stage 16. The melanophores become more extensively distributed in stage 17. Aggregations appear over the pronephric region and the caudal end of the notochord and neural tube. The anterior lip, and the head region and the gut become profusely covered with pigmentation. The pattern of pigmentation changes little in stage 18 except that the pigmented area is extended.

Activity in the embryo included locomotory movements, heart beat, and velum beat. First evidence of activity is the movement of the head region, produced by muscles of somites of stage 13 embryos. Greater muscular movements take place during later stages, including the movements that lead to: hatching; poor swimming movements of stage 14; somewhat better swimming in stage 15; the greatly increased deftness of swimming in stage 16; and movements which accomplish burrowing at stage 17.

Changes in the heart region in stage 14 embryos can be seen through the ectoderm over the pericardial cavity as it becomes transparent. The heart begins to beat at a rate of 40 times per minute, increasing to 100 per minute in stage 15, 150 per minute in stage 16, and more than 200 per minute in stage 17.

The respiratory system becomes functional at stage 16 when the initial respiratory rate (velum beat) is 120 per minute. This rate increases to 200 per minute in stage 17 and is maintained at this rate into stage 18.

In general, lamprey development resembles amphibian development in cleavage (stages 1–8). Lamprey gastrulation resembles amphibian gastrulation in some respects and teleostean in others. Epiboly is more like that of amphibians than of teleosteans. Invagination and involution of chorda-mesoderm seem similar to that in amphibians. Formation of the lamprey archenteron produces a situation comparable to that of a teleost. In both, the developing embryo is located on a large yolk mass but separated from the yolk by an archenteron which was the original blastocoel. A major difference between the two is that lamprey yolk is divided whereas teleostean yolk remains undivided. Another similarity to teleostean development is the formation of a solid neural rod which develops a lumen only after neurulation has been completed.

DEVELOPMENT AT DIFFERENT CONSTANT TEMPERATURES

The primary objective of the experimental rearing of sea lampreys at a series of constant temperatures was to determine the temperature levels at which the eggs were capable of developing into normal, viable larvae. As part of the work, detailed records were kept on: the relation between temperature and progression of development; mortality rate during development; occurrence and nature of developmental abnormalities. Information of this type may help explain the failure, noted both in the United States and in Canada, of the sea lamprey to utilize certain apparently suitable spawning streams as extensively as other apparently similar waters.

MATERIALS AND METHODS

The experiments were conducted at temperatures and with the aid of equipment indicated in table 2. Throughout this work, control lots at 65° F. were maintained as an index to development. Thus, any one control lot could serve effectively as an indicator to several other experiments. Eggs from 2 to 4 females were mixed with sperm from 4 to 8 males. These eggs were then apportioned into containers in the numbers (by actual count) indicated in table 3.

TABLE 2.—*Equipment used in experiments on development at constant temperatures*

Temperature (° F.)	Temperature-control equipment	Temperature (° F.)	Temperature-control equipment
45	Refrigeration.	65	Heat, refrigeration (Bronwil circulator).
50	Refrigeration.	70	Heat.
52.5	Refrigeration.	75	Circulator.
55	Refrigeration.	77.5	Circulator.
60	Heat and refrigeration.	80	Circulator.

The sampling schedule differed somewhat among the experiments (as may be seen from later tables that give details for individual samples) but the differences of schedule and the unavoidable occasional interruptions of timing were not sufficient to impair comparisons between series or to hamper the description of the progress of development. In the main, the earlier samples were taken at 1- or 2-hour intervals; the time between samples

TABLE 3.—*Specimens and number of eggs used in experiments on development at constant temperatures*

[One group of lampreys provided the eggs for experiments conducted at 45°, 55°, 70°, 75°, and 80° and another group for those at 52.5°, 60°, and 77.5°. Eggs from the lots that supplied materials for experiments at 50° and 65° were reared at the one temperature only]

Water temperature (° F.)	Number of females	Number of males	Number of eggs
45	4	8	10,000
50	2	4	10,000
52.5	2	4	15,000
55	4	8	25,600
60	2	4	9,000
65	2	4	33,000
70	4	8	18,000
75	4	8	13,500
77.5	2	4	9,000
80	4	8	5,000

was increased to 4 hours at about 32 hours; later samples (that is, after 2–3 days) were obtained about 12 hours apart; and, finally in the longer experiments sampling was daily. Sampling after stage 14 appeared was biased because the prolarvae had to be pursued as they became older; thus the samples were non-representative in comparison with the random samples for all earlier stages. In the main the bias led to over-representation of live specimens.

Several criteria were used to separate live and dead embryos. The most obvious indication of death was disintegration of the embryo—a separation of cells and subsequent filling of the intramembrane volume with loosely arranged cells, within an intact membrane. Furthermore, the fertilization membrane became translucent with a cloudy cast in contrast to the transparency of a living membrane. This difference was most apparent in later development, stages 7–14; the earlier stages (1–6) became vacuolated as they underwent changes after death. Eventually, the membranes of eggs in these earlier stages also became cloudy. Another indicator, particularly in early development, was a change of color from the creamy white of normal eggs to a brownish tan, accompanied by a fuzzy appearance of the surface. Dying embryos of stages 9–11 possessed appreciably widened blastopores, which in some extended across the entire diameter of the embryo.

The basic data on the several temperature experiments are given in the records of number of dead embryos and number of living embryos in various stages of development. In conjunction with these records, information was recorded relative to the elapsed time and temperature and remarks were noted concerning the general composition of each sample.

The percentage of dead embryos was computed for each sample. Until stage 14 appeared this percentage provided an estimate of mortality up to the time of sampling since all embryos regardless of time of death were included in the computations. This procedure was possible since all eggs used in any one experiment were recoverable throughout the experiment. Although disintegration of dead embryos did occur, the embryonic membrane remained intact to the end of the experiment. The information on dead embryos per sample does not include data on time of death, since stages could not be determined for embryos that were decomposing, but it does provide good information on the progression of mortality with development.

Because, as has been stated, the quantitative sampling of lots of eggs become biased after the appearance of stage 14 (hatching) and later stages, none of the tables that carry details on individual samples goes beyond the last sample that contained stage 13 embryos (the bias starts, of course, with the first sample containing stage 14 specimens, but full records for stage 13 appear to be desirable). In the experiments in which stage 14 was not reached, the tabulation ends with the last sample that contained live embryos. Similarly, the tables showing mortality by 1-day intervals end with the records for the first day on which stage 14 appeared, or, if that stage was not reached, with the last day on which samples contained live embryos. Terminal samples are included for those experiments in which some embryos survived the full term of the experiment or in which there was cause to suspect that a few live embryos might still be present. These terminal samples which contained all eggs remaining at the end of the experiment are considered, for practical purposes to be unbiased. They were affected to some degree by the earlier removal (after the start of stage 14) of samples in which living embryos were taken out of proportion to their true abundance, but the numbers were so much greater in the terminal sample than in these earlier biased samples as to minimize the distorting effect.

Records from biased samples were of course useful for showing the time of first appearance and duration of the later stages.

ACCOUNT OF INDIVIDUAL EXPERIMENTS

Development at 45° F.

The sea lamprey eggs could not make even a good start toward development in a constant temperature of 45° F. The first two cleavages (stages 1 and 2) proceeded very slowly and all eggs were dead before the third cleavage was completed (tables 4 and 5; fig. 27).

TABLE 4.—*Living and dead lamprey embryos per sample and their stage of development at 45° F. (7.2° C.)*

Sample	Number of hours	Number dead	Stage of living embryos			Sample	Number of hours	Number dead	Stage of living embryos		
			1	2	3				1	2	3
1	1	67	213	----	----	14	27	91	----	23	----
2	3	15	105	----	----	15	31	105	----	57	----
3	5	10	94	----	----	16	35	120	----	23	----
4	7	13	125	----	----	17	39	123	----	32	----
5	9	16	169	----	----	18	43	85	----	16	----
6	11	17	120	----	----	19	47	177	----	22	2
7	13	11	113	----	----	20	51	207	----	8	4
8	15	73	92	----	----	21	58	175	----	----	10
9	17	38	233	----	----	22	71	252	----	----	14
10	19	82	84	33	----	23	82	322	----	----	3
11	21	45	76	109	----	24	95	363	----	----	2
12	23	45	----	101	----	25	103	341	----	----	2
13	25	62	----	63	----						

TABLE 5.—*Mortalities of sea lamprey eggs reared at a constant temperature of 45° F. (7.2° C.)*

Time		Stage span	Number of samples	Number of embryos			Percentage dead
Hours	Days			Alive	Dead	Total	
0–24	1	0–2	12	1,667	432	2,099	21
25–48	2	2–3	7	238	763	1,001	76
49–72	3	2–3	3	36	634	670	95
73–96	4	3	2	5	685	690	99
97–120	5	3	2	2	579	581	[1] 100

[1] More than 99.5; actually all embryos (238 specimens) were dead in the sample taken at 116 hours.

Stage 1 lasted about 20 hours; stage 2 commenced at the 19th hour and lasted beyond the 47th hour, when a highly defective stage 3 appeared (tables 4 and 6; fig. 28). Many 3-cell embryos were found along with a few abortive 4-cell embryos, in which the cleavage furrow seemed to undergo regression.

Throughout the samples, beginning with the sixth at 11 hours, the eggs began to vacuolate and to fragment yolk into the perivitelline space, in short, to die and disintegrate. Beginning with the 19th sample, at 47 hours nearly 90 percent

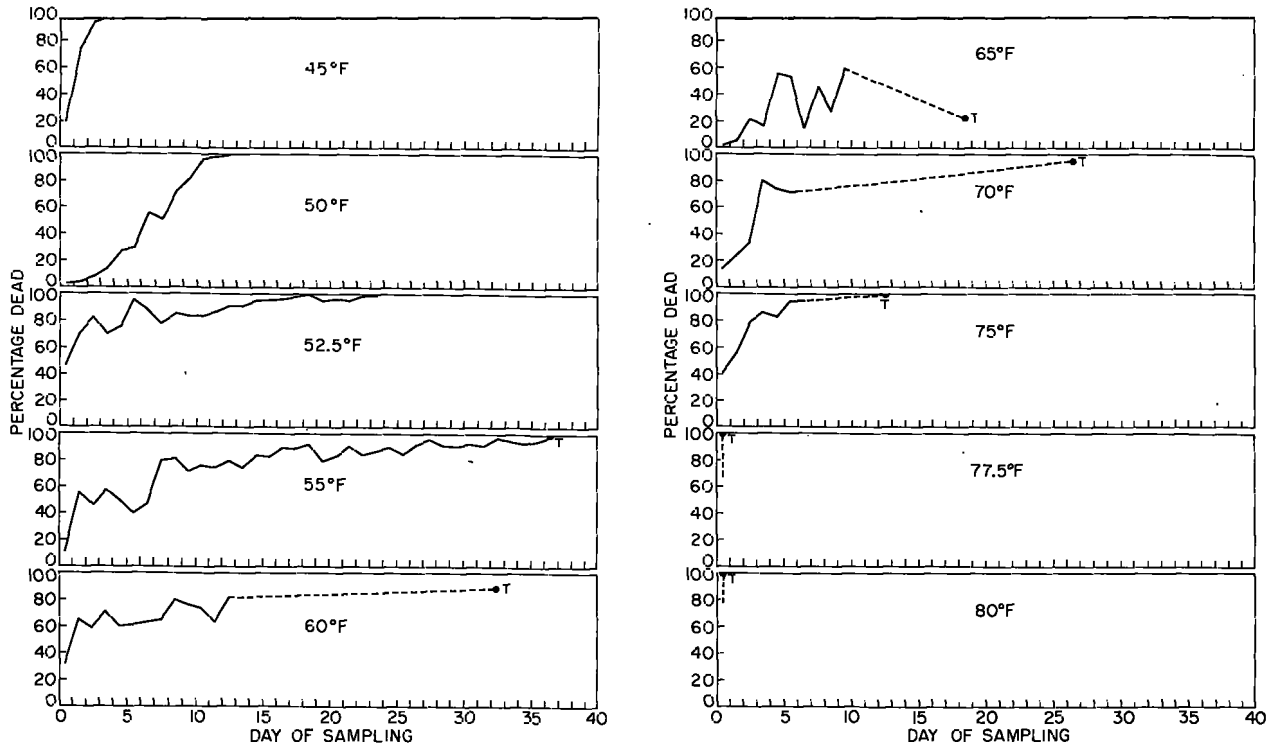

FIGURE 27.—Percentage of dead embryos in daily samples from sea lamprey eggs reared at 10 different constant temperature levels. The day-by-day records end when the percentage of dead in the samples reached and remained at 100, or with the onset of hatching. A broken line connects the last recorded daily sample with the terminal sample (T). At 77.5° and 80° live embryos occurred in the earlier samples of the first day but none in the later ones.

TABLE 6.—*Hours at which the first and last specimens of the various stages appeared in samples during each experiment and the duration of stages (in parentheses)*

Stages	Constant temperature (F.) at which embryos were reared									
	45°	50°	52.5°	55°	60°	65°	70°	75°	77.5°	80°
1	1–21 (21)	1–16 (16)	1–10 (10)	1–13 (13)	1–6 (6)	1–2 (2)	1–3 (3)	1–3 (3)	1–4 (4)	1–7 (7)
2	19–51 (33)	6–25 (20)	3–22 (20)	3–23 (21)	3–10 (8)	2–8 (7)	5–7 (3)	5 (2)	4–6 (3)	5–9 (5)
3	47–103 (57)	19–34 (16)	13–28 (16)	15–31 (17)	13–16 (4)	8–11 (4)	7–11 (5)	7 (2)	6–10 (5)	9–11 (3)
4	----------	28–42 (15)	20–36 (17)	23–35 (13)	16–18 (3)	10–15 (6)	11–13 (3)	7–11 (5)	10 (4)	[3]
5	----------	34–48 (15)	32–44 (13)	31–43 (13)	20–22 (3)	13–15 (3)	13–15 (3)	9–13 (5)	[2] 13	[3]
6	----------	40–58 (19)	40–48 (9)	39–47 (9)	22–28 (7)	16–19 (4)	15–17 (3)	13–15 (3)	[2] 13	[2] 15
7	----------	44–78 (35)	48–64 (17)	47–103 (57)	28–34 (5)	19–24 (6)	17–21 (5)	15–17 (3)	----------	----------
8	----------	62–126 (65)	68–136 (69)	51–151 (101)	36–89 (54)	28–64 (37)	19–51 (33)	19–43 (25)	----------	----------
9	----------	114–270 (167)	160–338 (179)	127–271 (145)	100–148 (49)	64–104 (41)	47–82 (36)	43–82 (40)	----------	----------
10	----------	----------	338–362 (25)	211–295 (185)	136–172 (37)	104–128 (25)	82–103 (22)	71–103 (33)	----------	----------
11	----------	----------	362–470 (109)	295–405 (111)	172–220 (49)	128–152 (25)	103–127 (25)	95–116 (22)	----------	----------
12	----------	----------	470–570 (101)	369–525 (157)	208–283 (76)	152–200 (49)	116–151 (36)	103–139 (37)	----------	----------
13	----------	----------	----------	[1] 490–874	283–409 (127)	200–296 (97)	151–235 (85)	151–175 (25)	----------	----------
14	----------	----------	----------	[4] (--------)	317–409 (93)	[4] 248–------ [4] (--------)	175–247 (73)	175–211 (37)	----------	----------
15	----------	----------	----------	----------	352–469 (118)	[4] 308–------ [4] (--------)	225–307 (83)	211–225 (15)	----------	----------
16	----------	----------	----------	----------	446–569 (124)	[4] 363–------ [4] (--------)	295–343 (49)	[1] 225–307 [4] (--------)	----------	----------
17	----------	----------	----------	----------	[1] 594–782 [4] (--------)	[1] 405–437 [4] (--------)	[1] 357–633 [4] (--------)	----------	----------	----------

[1] Termination of experiment.
[2] 100-percent mortality subsequent to this hour.
[3] No embryo of this stage found in any sample.
[4] These blanks cannot be filled properly for these stages were sampled either in or immediately preceding the terminal sample.

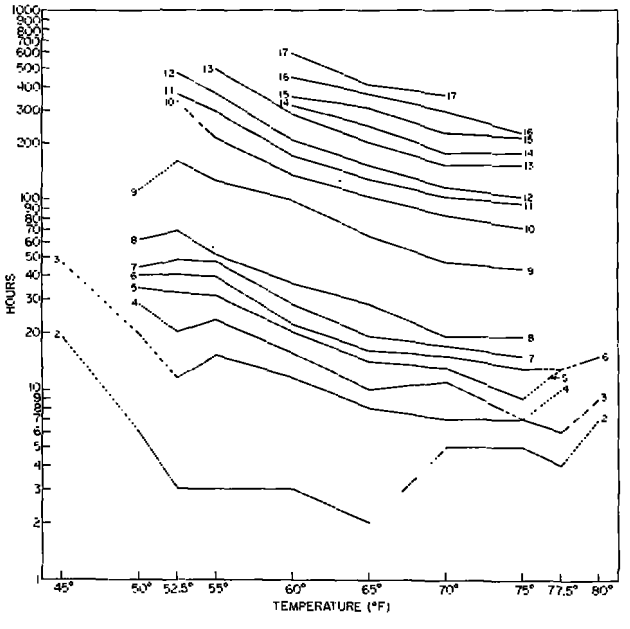

FIGURE 28.—Number of hours required by sea lamprey embryos to reach different developmental stages when reared at various constant temperatures.

of the eggs were dead. All eggs obviously were dead in samples taken after the 5th day (table 5). Eggs marked by lesions as described by Damas (1948) did not undergo first cleavage.

The highest stage completed in this experiment was stage 2, since stage 3 was not consummated.

Development at 50° F.

The rate of development was decidedly faster at 50° F. than at 45° F. (table 6; fig. 28) and development proceeded farther. Stage 9 was reached but few embryos survived to the end of the stage (tables 7 and 8; fig. 27). A single sample contained eggs that seemed to be intermediate between stages 9 and 10. No embryos were alive after the 270 hours (sample 65, table 7; table 6, also fig. 27).

The duration of each of the first 6 stages fell within the limited range of 15–20 hours (table 6). Stage 7, however, lasted 35 hours, and stage 8 approximately 65 hours, the longest save for the last stage reached (stage 9). The greatest period overlap of stages occurred between stages 7 and 8

TABLE 7.—*Living and dead embryos per sample and their stage of development at 50° F. (10° C.)*

Sample	Hours	Number dead	Stage of living embryos						Sample	Hours	Number dead	Stage of living embryos					
			1	2	3	4	5	6				4	5	6	7	8	9
1	½		93						34	40	3	7	60	5			
2	1		140						35	42	3	5	19	89			
3	2		215						36	44	17		69	232	8		
4	3		184						37	46	14		49	187	5		
5	4		133						38	48	10		8	83	30		
6	6	1	200	20					39	50	13			108	107		
7	7	1	157	43					40	54	25			41	163		
8	8		132	34					41	58	11			17	132		
9	10	1	108	30					42	62	15				87	183	
10	11		129	18					43	66	14				13	125	
11	12		132	27					44	70	13				11	271	
12	13		112	68					45	74	35				5	335	
13	14		31	84					46	78	71				2	236	
14	15		42	139					47	90	32					238	
15	16	1	14	111					48	102	87					119	
16	17	6		139					49	114	42					209	7
17	18	6		151					50	126	32					77	12
18	19	8		283	2				51	137	93						217
19	20	11		304	62				52	146	43						57
20	21	12		271	127				53	150	123						125
21	22	8		141	213				54	163	119						41
22	23	10		72	210				55	171	118						210
23	24	6		47	262				56	174	207						135
24	25	7		13	95				57	182	169						205
25	26	5			117				58	187	232						134
26	27	4			72				59	198	337						106
27	28				48	12			60	211	315						123
28	29				50	48			61	222	283						82
29	30	18			137	190			62	234	254						25
30	32	23			90	344			63	246	375						10
31	34	19			26	453	23		64	260	435						2
32	36					43	60		65	270	634						6
33	38	3				24	80										

TABLE 8.—*Mortalities of sea lamprey eggs reared at a constant temperature of 50° F. (10° C.)*

Time		Stage span	Number of samples	Number of embryos			Percentage dead
Hours	Days			Alive	Dead	Total	
0–24	1	0–3	23	4,680	71	4,751	2
25–48	2	3–7	15	2,781	126	2,907	4
49–72	3	6–8	6	1,258	91	1,349	7
73–96	4	7–8	3	816	138	954	14
97–120	5	8–9	2	335	129	464	28
121–144	6	8–9	2	306	125	431	29
145–168	7	9	3	223	285	508	56
169–192	8	9	4	684	726	1,410	52
193–216	9	9	2	229	652	881	74
217–240	10	9	2	107	537	644	83
241–264	11	9	2	12	810	822	99
265–288	12	9	2	6	1,222	1,228	99.5

(20 hours) and the next longest between stages 8 and 9 (12 hours). Mortality was light the first 3 days (2 to 7 percent dead; table 8) and was moderate (14 to 29 percent dead) the next 3 days. The death rate increased considerably shortly after the embryos entered stage 9 (gastrula). Samples on the seventh day contained 56 percent of dead eggs. The percentage had reached 99 and 99.5 percent on the 11th and 12th days, respectively, and all eggs were dead on the 13th day.

Development at 52.5° F.

Again, an increase of temperature (this time only 2.5° above that of the experiment described in the preceding section) permitted development at a faster rate and through a greater number of stages (table 6; fig. 28). Heavy mortality started early, however, the death rate increased as development progressed (tables 9 and 10; fig. 27), and no embryos survived beyond stage 12 (head stage).

The early stages (1 to 6) of this experiment proceeded slightly more rapidly than the corresponding stages of the 50° F. experiment. The periods of overlap of the earlier stages were nearly equal to those of the 50° F. test, although the length of time for each stage was less at 50° F. than at 52.5° (tables 6, 7, and 9). Stage 9 was prolonged at 52.5° F. over a period of approximately 9 days. During this time the number of deaths increased. The relatively few embryos that developed beyond stage 9 progressed through a short stage 10 and a much longer stage 11 (about 4 days). Stage 12 was reached by approximately 40 embryos, all of which died. The most frequent symptom of death was disintegration of the anterior region of the embryos.

Development at 55° F.

Development at 55° F. proceeded as far as stage 13 (prehatching). Indeed, the terminal sample included 11 live embryos in this stage (along with some 16,000 dead embryos) but all of them were so defective as to make early death almost certain; they were accordingly classed as

TABLE 9.—*Living and dead embryos per sample and their stage of development at 52.5° F. (11.4° C.)*

Sample	Hours	Number dead	Stage of living embryos								Sample	Hours	Number dead	Stage of living embryos				
			1	2	3	4	5	6	7	8				8	9	10	11	12
1	1	2	76								28	136	142	6				
2	3	11	29	2							29	148	37					
3	5	15	23	4							30	160	33		10			
4	6	7	76	3							31	172	58		20			
5	8	8	36	2							32	814	57		12			
6	10	21	31	1							33	196	76		13			
7	13	31		15	1						34	281	44		12			
8	14	47		23	2						35	230	70		10			
9	16	38		9	1						36	242	65		12			
10	18	74		12	3						37	254	86		17			
11	20	27		2	10	1					38	269	40		6			
12	22	37		2	12	3					39	293	75		9			
13	24	48			7	3					40	302	78		7			
14	26	33			2	10					41	327	53		5			
15	28	39			2	16					42	338	64		4	1		
16	32	29				13	12				43	349	96			4		
17	36	44				2	11				44	362	85			2	1	
18	40	49					2	10			45	375	96				5	
19	44	66					2	15			46	386	70				2	
20	48	29						4	21		47	419	117				2	
21	54	51							18		48	447	44					
22	64	65							5	2	49	470	83				1	3
23	76	21								9	50	494	98					3
24	89	46								20	51	518	68					3
25	100	32								13	52	543	79					1
26	112	72								21	53	570	92					1
27	124	50								5								

TABLE 10.—*Mortalities of sea lamprey eggs reared at a constant temperature of 52.5° F. (11.4° C.)*

Time		Stage span	Number of samples	Number of embryos			Percentage dead
Hours	Days			Alive	Dead	Total	
0–24	1	0–4	13	389	366	755	48
25–48	2	3–7	7	122	289	411	70
49–72	3	7–8	2	25	116	141	82
73–96	4	8	2	29	67	96	70
97–120	5	8	2	34	104	138	75
121–144	6	8	2	11	192	203	95
145–168	7	9	2	10	70	80	88
169–192	8	9	2	32	115	147	78
193–216	9	9	1	13	76	89	85
217–240	10	9	2	22	114	136	84
241–264	11	9	2	29	151	180	84
265–288	12	9	1	6	40	46	87
289–312	13	9	2	16	153	169	91
313–336	14	9	1	5	53	58	91
337–360	15	9–10	2	9	160	169	95
361–384	16	10–11	2	8	181	189	96
385–408	17	11	1	2	70	72	97
409–432	18	11	1	2	117	119	98
433–456	19		1		44	44	100
457–480	20	11	1	4	83	87	95
481–504	21	12	1	3	98	101	97
505–528	22	12	1	3	68	71	96
529–552	23	12	1	1	79	80	99
553–576	24	12	1	1	92	93	99

"dead" in the records for that sample (tables 11 and 12; fig. 27). The incidence of dead embryos in the samples increased rather consistently as development proceeded and was high in the later stages.

Overlapping was prominent between later stages since individual stages lasted from 4 to 16 days (table 6; fig. 28). Stage 13, the highest stage reached, had lasted 384 hours (16 days) when the experiment was finally terminated at 874 hours (ca. 37 days). Stages 12 and 13 overlapped 36 hours.

A most interesting feature of this experiment was the condition of embryos that finally reached and remained in stage 13. Although they were in stage 13, they were developing within the fertilization membrane (chorion). The spirally curled embryos developed transparent pericardia, pigment spots, and hemoglobin which had a muddy red to brown appearance in contrast to the normal bright pink to red. Some of these embryos finally were released when their membranes disintegrated. After release, however, the embryos did not straighten out or develop further but remained in this condition until death.

During this test several abnormalities were noted in the embryos: enlarged pericardia; straight tubular hearts; shorter but heavier bodies; enlarged gut region; a separation of yolk cells from the gut walls; and failure to straighten from the spirally curled position after removal of the chorion.

Development at 60° F.

The rate of development was much more rapid and the overlap of stages was less at 60° F. than at lower temperatures (tables 6, 13, and 14; fig. 28). The temperature of 60° F. was the lowest at which viable, burrowing prolarvae (stage 17) were produced. Mortality was generally less than on corresponding days in experiments at lower temperatures (fig. 27) and abnormalities were relatively few.

TABLE 11.—*Living and dead embryos per sample and their stage of development at 55° F. (12.8° C.)*

Sample	Hours	Number dead	Stage of living embryos								
			1	2	3	4	5	6	7	8	9
1	1		143								
2	3		80	8							
3	5		91	12							
4	7		27	53							
5	9		64	67							
6	11		32	114							
7	13		9	92							
8	15	22		91	19						
9	17	48		31	91						
10	19	14		56	65						
11	21	15		26	40						
12	23	47		5	34	48					
13	25	61			26	40					
14	27	83			16	35					
15	31	81			4	52	23				
16	35	81				9	75				
17	39	81					25	57			
18	43	62					11	16			
19	47	87						25	52		
20	51	70							36	39	
21	58	80							50	77	
22	71	118							50	75	
23	82	157							38	83	
24	95	151							40	81	
25	103	111							28	98	
26	116	133								135	
27	127	51								34	49
28	139	89								82	59
29	151	20								152	68
30	163	226									61

Sample	Hours	Number dead	Stage of living embryos				
			9	10	11	12	13
31	175	91	26				
32	187	141	34				
33	199	146	41				
34	211	133	27	3			
35	225	80	21	14			
36	235	49	5	16			
37	247	54	6	10			
38	260	61	3	19			
39	271	30	2	13			
40	283	47		12			
41	295	41		8	10		
42	307	83			14		
43	319	47			18		
44	331	37			11		
45	343	64			15		
46	357	71			12		
47	369	97			17	2	
48	381	86			12	9	
49	393	63			3	5	
50	405	56			2	4	
51	417	62				6	
52	432	48				9	
53	456	108				11	
54	465	73				19	
55	490	97				12	6
56	501	89				7	12
57	512	99				2	6
58	525	148				3	15
59	538	71					18
60	549	92					12

TABLE 12.—*Mortalities of sea lamprey eggs reared at a constant temperature of 55° F. (12.8° C.)*

[No sample on 24th day]

Time		Stage span	Number of samples	Number of embryos			Percentage dead
Hours	Days			Alive	Dead	Total	
0–24	1	0–4	12	1,298	146	1,444	10
25–48	2	4–7	7	466	536	1,002	54
49–72	3	7–8	3	327	268	595	45
73–96	4	7–8	2	242	308	550	56
97–120	5	7–8	2	261	244	505	48
121–144	6	8–9	2	224	140	364	38
145–168	7	8–9	2	291	246	537	46
169–192	8	9	2	60	232	292	79
193–216	9	9–10	2	71	279	350	80
217–240	10	9–10	2	56	129	185	70
241–264	11	9–10	2	38	115	153	75
265–288	12	9–10	2	27	77	104	74
289–312	13	10–11	2	32	124	156	79
313–336	14	11	2	29	84	113	74
337–360	15	11	2	27	135	162	83
361–384	16	11–12	2	40	183	223	82
385–408	17	11–12	2	14	119	133	89
409–432	18	12	2	15	110	125	88
433–456	19	12	1	11	108	119	91
457–480	20	12	1	19	73	92	79
481–504	21	12–13	2	37	186	223	83
505–528	22	12–13	2	26	247	273	90
529–552	23	13	2	30	163	193	84
577–600	25	13	1	15	113	128	88
601–624	26	13	1	18	93	111	84
625–648	27	13	1	12	125	137	91
649–672	28	13	1	5	104	109	95
673–696	29	13	1	12	126	138	91
697–720	30	13	1	9	86	95	91
721–744	31	13	1	10	108	118	92
745–768	32	13	1	6	61	67	91
769–792	33	13	1	4	109	113	96
793–816	34	13	1	6	103	109	94
817–840	35	13	1	8	108	116	93
841–864	36	13	1	8	128	136	94
865–888	37	13	1	3	105	108	97
875 [1]	37		1		16,250	16,250	100

[1] Terminal sample includes 11 live embryos mentioned in text as being incapable of survival.

Overlapping of stages was most limited with the exception of stages 1 and 2 (overlap of 3 samples) and the last 2 (stages 13 and 14) (overlap of 7 samples). Stage 13 was longest (127 hours or about 5 days). Stage 8 lasted more than 54 hours and was also represented in 8 samples (tables 6 and 13). Stage 14 was attained within 317 hours (about 13 days for first appearance).

Percentages of dead embryos in samples rose from a low of 32 percent during early cleavage stages (1–6) to 88 percent in the terminal sample. The rate of increase was highest in the early stages actually during the first 2 days. Percentage hatch (embryos that survived through stage 14) though not accurately measurable from the biased samples, obviously was good.

The relatively few abnormalities during this experiment (approximately 20 percent) took several forms, some of which were similar to those described earlier. Among the more common were enlargement of the pericardial area, and the straight tubular heart which, nevertheless, maintained a regular beat. Other specimens exhibited abnormal curvatures, balloon mouths, or cleft-lip. Abnormal curvatures of the trunk region produced embryos with "C," "J," "O," and "L" shapes. Balloon mouths were caused

TABLE 13.—*Living and dead embryos per sample and their stage of development at 60° F. (15.5° C.)*

Sample	Hours	Number dead	Stage of living embryos								Sample	Hours	Number dead	Stage of living embryos					
			1	2	3	4	5	6	7	8				9	10	11	12	13	14
1	1		143								25	100	20	15					
2	3		31	13							26	112	22	13					
3	4		14	20							27	124	26	14					
4	6	12	8	56							28	136	35	19	6				
5	8	11		38							29	148	23	5	7				
6	10	13		26							30	160	36		23				
7	13	17			26						31	172	29		11	7			
8	14	22			25						32	184	31			14			
9	16	29			9	14					33	196	55			5			
10	18	37				29					34	208	23			9	6		
11	20	24					17				35	220	37			2	12		
12	22	22					23	3			36	232	68				18		
13	24	53						23			37	244	40				20		
14	26	36						11			38	259	49				11		
15	28	31						4	13		39	283	45				9	16	
16	32	34							19		40	292	46					10	
17	36	30								13	41	317	51					20	4
18	40	28								10	42	328	36					4	5
19	44	28								23	43	339	35					8	12
20	48	31								26	44	352	5					1	6
21	54	17								15	45	365						5	3
22	64	26								15	46	376	3						
23	76	16								13	47	409	32					4	11
24	89	31								5									

TABLE 14.—*Mortalities of sea lamprey eggs reared at a constant temperature of 60° F. (15.5° C.)*

[No sample on 17th day]

Time		Stage span	Number of samples	Number of embryos			Percentage dead
Hours	Days			Alive	Dead	Total	
0–24	1	0–6	13	518	240	758	32
25–48	2	6–8	7	118	218	336	65
49–72	3	8	2	30	43	73	59
73–96	4	8	2	18	47	65	72
97–120	5	9	2	28	42	70	60
121–144	6	9–10	2	39	61	100	61
145–168	7	9–10	2	35	59	94	63
169–192	8	10–11	2	32	60	92	65
193–216	9	11–12	2	20	78	98	80
217–240	10	11–12	2	32	105	137	77
241–264	11	12	2	31	89	120	74
265–288	12	12–13	1	25	45	70	64
289–312	13	13	1	10	46	56	82
313–336	14	13–14	2			120	
337–360	15	13–15	2			75	
361–384	16	13–15	2			31	
409–432	18	13–15	1			70	
433–456	19	15–16	1			22	
457–480	20	15–16	1			20	
481–504	21	16	1			16	
505–528	22	16	1			17	
529–552	23	16	1			42	
553–576	24	16	1			19	
577–600	25	17	1			18	
601–624	26	17	1			24	
625–648	27	17	1			35	
649–672	28	17	1			17	
673–696	29	17	1			12	
697–720	30	17	1			14	
721–744	31	17	1			8	
745–768	32	17	1			15	
769–792	33	17	1			7	
783 [1]	33	17	1	795	5,606	6,401	88

[1] Terminal sample.

from a failure of the stomadaeum to open. An enormous enlargement or ballooning of the pharyngeal region resulted, especially in the anterior portion, which tended to compress the branchial structures and cause them to become malformed and from all indication, non-functional. A midline cleft in the dorsal lip was designated a "cleft-lip" abnormality.

Development at 65° F.

The rearing of sea lamprey eggs under constant temperature was more successful at 65° F. than at any lower or higher level (tables 15 and 16; fig. 27). Development was rapid (tables 6 and 15; fig. 28), overlapping of stages was practically nil, mortality was low, and abnormalities were few. The details offered in this section are based on an experiment conducted under refrigeration. Essentially the same results were obtained in experiments conducted with heat and the circulator.

Stages did not overlap at 65° F. with the exception of the overlap of stages 4 and 5 and of stages 13 and 14.

The percentage of dead embryos was low in most samples but the day-to-day variation of that percentage (fig. 27) was highly erratic and for several days the percentage exceeded that of the terminal sample taken on the 19th day. No verifiable explanation can be offered for this behavior of the percentages, but it is possible that dead eggs were not distributed evenly through the experimental lot and that certain samples happened to be taken from points at which dead embryos were concentrated. The 78-percent survival to the end of the experiment (only 22 percent dead in the terminal sample) was by far the highest

TABLE 15.—*Living and dead embryos per sample and their stage of development at 65° F. (18.4° C.)*

Sample	Hours	Number dead	Stage of living embryos								Sample	Hours	Number dead	Stage of living embryos						
			1	2	3	4	5	6	7	8				8	9	10	11	12	13	14
1	½		195								26	32	10	208						
2	1		330								27	45	9	218						
3	2		112	1							28	53	22	227						
4	3			279							29	56	81	247						
5	4			224							30	64	91	7	189					
6	5			433							31	69	82		304					
7	6	2		272							32	80	65		337					
8	7	1		287							33	93	65		365					
9	8	2		11	500						34	104	231		96	78				
10	9	3			169						35	116	181			161				
11	10	5			376	1					36	128	113			99	194			
12	11	3			51	307					37	142	284				61			
13	12	3				315					38	152	80				74	258		
14	13	12				303	62				39	164	6					139		
15	14	11				36	381				40	176	118					231		
16	15	9				4	274				41	188	179					135		
17	16	14						346			42	200	51					64	171	
18	17	14						329			43	212	107						170	
19	18	4						97			44	224	100						60	
20	19	14						19	324		45	236	97						84	
21	24	11							245		46	248	38						39	43
22	28	24								476	47	271	3						20	135
23	29	19								361	48	285	5						10	129
24	30	23								329	49	296	6						19	205
25	31	7								334										

TABLE 16.—*Mortalities of sea lamprey eggs reared at a constant temperature of 65° F. (18.4° C.)*

[No samples on 15th and 18th days]

Time		Stage span	Number of samples	Number of embryos			Percentage dead
Hours	Days			Alive	Dead	Total	
0–24	1	0–7	21	6,283	108	6,391	2
25–48	2	8	6	1,926	92	2,018	5
49–72	3	8–9	4	974	276	1,250	22
73–96	4	9	2	702	130	832	16
97–120	5	9–10	2	335	412	747	55
121–144	6	10–11	2	354	397	751	53
145–168	7	11–12	2	471	86	557	15
169–192	8	12	2	366	297	663	45
193–216	9	12–13	2	405	158	563	28
217–240	10	13	2	144	197	341	58
241–264	11	13–14	1			120	
265–288	12	13–14	2			302	
289–312	13	13–15	2			384	
313–336	14	14–15	2			271	
361–384	16	15–16	1			31	
385–408	17	15–17	2			117	
433–456	19	17	1			114	
438 [1]	19	14–17		11,918	3,355	15,273	22

[1] Terminal sample.

among the experiments and represents unusually good results for the artificial rearing of fish eggs of any kind.

Abnormalities were extremely few, approximately 2 to 5 percent, mostly in the last samples. A few specimens had enlarged pericardia with a straight, tubular heart, and several had yolk separation and the associated hydrocoelus gut. One "twin" embryo was seen. These twins, in early stage 12, possessed a common but somewhat enlarged blastopore (fig. 29).

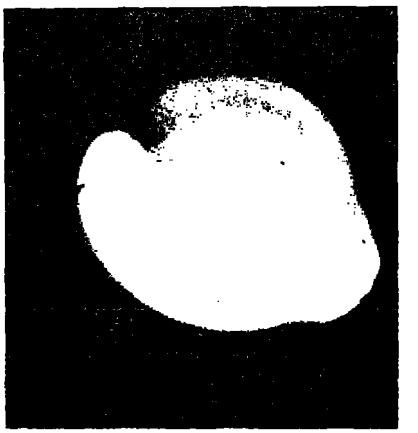

FIGURE 29.—Posterolateral view of twin embryos, showing the common, but enlarged, blastopore.

Development at 70° F.

The rate of development at 70° F. was only slightly accelerated over that at 65° F., in fact embryos reared at 70° F. reached stages 2 and 4 later than did those reared at 65° F. (table 6; fig. 28), and the limited overlap of stages characterized embryos at both temperatures. More pronounced and more significant were the increase of mortality (tables 17 and 18; fig. 27) and the greater number of abnormalities at 70° F. as compared with the "optimum" of 65° F.

At 70° F. as in the 65° test, the overlapping of stages was limited to a slight overlap between stages 11 and 12 and a somewhat greater one between stages 13 and 14 (tables 6 and 17). Elsewhere, the progression from one stage into the next was precise. Stage 8 was prolonged over 11 samples covering 32 hours, as against 2 to 3 for all other early stages.

TABLE 17.—*Living and dead embryos per sample and their stage of development at 70° F. (21.1° C.)*

Sample	Hours	Number dead	Stage of living embryos								Sample	Hours	Number dead	Stage of living embryos						
			1	2	3	4	5	6	7	8				8	9	10	11	12	13	14
1	1		277								19	47	45	99	24					
2	3		195								20	51	38	48	61					
3	5			130							21	58	65		122					
4	7	50		84	74						22	71	92		160					
5	9	8			121						23	82	89		62	31				
6	11	13			5	124					24	95	325			13				
7	13	43				56	93				25	103	221			58	61			
8	15	31					58	69			26	116	258				41	6		
9	17	39						45	96		27	127	44				12	53		
10	19	42							76	71	28	139	206					30		
11	21	41							39	81	29	151	139					5	35	
12	23	26								151	30	163	195						87	
13	25	40								113	31	175	64						33	13
14	27	34								122	32	187	48						15	20
15	31	38								138	33	199	25						18	26
16	35	28								123	34	211	46						8	44
17	39	38								135	35	225	24						1	32
18	43	16								71	36	235	24						1	41

TABLE 18.—*Mortalities of sea lamprey eggs reared at a constant temperature of 70° F. (21.1° C.)*

[No sample on 24th day]

Time		Stage span	Number of samples	Number of embryos			Percentage dead
Hours	Days			Alive	Dead	Total	
0–24	1	0–8	12	1,845	293	2,138	14
25–48	2	8–9	7	825	239	1,064	22
49–72	3	8–9	3	391	195	586	33
73–96	4	9–10	2	106	414	520	80
97–120	5	10–12	2	166	479	645	74
121–144	6	11–12	2	95	250	345	72
145–168	7	12–13	2	127	334	461	72
169–192	8	13–14	2			193	
193–216	9	13–14	2			167	
217–240	10	13–15	2			160	
241–264	11	14–15	2			149	
265–288	12	15	2			124	
289–312	13	15–16	2			118	
313–336	14	16	2			32	
337–360	15	16–17	2			69	
361–384	16	17	2			91	
385–408	17	17	2			98	
409–432	18	17	2			21	
433–456	19	17	1			68	
457–480	20	17	1			109	
481–504	21	17	2			65	
505–528	22	17	2			59	
529–552	23	17	1			28	
577–600	25	17	1			29	
601–624	26	17	1			5	
625–648	27	17	1			50	
634 [1]	27	17	1	528	10,291	10,819	95

[1] Terminal sample.

The percentage of dead embryos in the samples rose rapidly to 80 percent on the fourth day and continued above 70 percent through the seventh day after which hatching made the further samples undependable as measured at mortality (table 18; fig. 27). The percentage of embryos dead in the 70°-terminal sample (95 percent) indicates final production a little less than half that at 60° F. (88 percent dead in 60° terminal sample).

Abnormalities in this experiment were relatively few (15 to 20 percent of the prolarvae), but more numerous than at 65° F. The abnormalities were similar to those listed for the 60° F. test, especially with respect to body shape.

Development at 75° F.

No viable, burrowing larvae were produced at a constant temperature of 75° F. Heavy mortality appeared early and persisted throughout development (tables 19 and 20; fig. 27) with the result that few embryos survived even to hatching. With the exception of stages 2 and 3 the various

TABLE 19.—*Living and dead embryos per sample and their stage of development at 75° F. (23.9° C.)*

Sample	Hours	Number dead	Stage of living embryos								Sample	Hours	Number dead	Stage of living embryos						
			1	2	3	4	5	6	7	8				8	9	10	11	12	13	14
1	1		129								17	39	54	69						
2	3	25	200								18	43	60	17	36					
3	5	25		113							19	47	207							
4	7	102			57	1					20	51	153		54					
5	9	186				40	2				21	58	268		81					
6	11	71				12	148				22	71	260		45	4				
7	13	81					113	40			23	82	322		8	23				
8	15	88						133	81		24	95	188			21	27			
9	17	85							177		25	103	287			4	26	3		
10	19	172								175	26	116	124				14	37		
11	21	191								102	27	127	202					13		
12	23	103								62	28	139	210					7		
13	25	53								89	29	151	265						14	
14	27	103								80	30	163	176						8	
15	31	163								89	31	175	106						1	11
16	35	105								202										

TABLE 20.—*Mortalities of sea lamprey eggs reared at a constant temperature of 75° F. (23.9° C.)*

Time		Stage span	Number of samples	Number of embryos			Percentage dead
Hours	Days			Alive	Dead	Total	
0–24	1	0–8	12	1,585	1,129	2,714	42
25–48	2	8–9	7	582	745	1,327	56
49–72	3	9–10	3	184	681	865	79
73–96	4	9–11	2	79	510	589	87
97–120	5	10–12	2	84	411	495	83
121–144	6	12	2	20	412	432	95
145–168	7	13	2	22	441	463	95
169–192	8	13–14	2	------	------	325	------
193–216	9	14–15	2	------	------	355	------
217–240	10	15–16	2	------	------	265	------
241–264	11	16	2	------	------	94	------
265–288	12	16	1	------	------	27	------
307 [1]	13	------	1	------	5,600	5,600	100

[1] Terminal sample.

stages were reached more rapidly than at lower temperatures and the overlap of stages was limited (tables 6 and 19; fig. 28).

Transition from one stage into the next was rather precise, as in the two preceding experiments. No stage overlapped another in more than 2 samples (tables 6 and 19). Stage 8 was projected over 9 samples covering 24 hours as contrasted with 2 or 3 for each of the earlier stages.

Substantial mortality appeared early at this temperature, especially between stages 0 and 8. The percentage of dead embryos had reached 95 percent on the seventh day, and the terminal sample did not include any live embryos. Production at this temperature was nil, despite the survival of some individuals as far as stage 16.

It is conceivable that stage 17 possibly could have been reached since the experiment was terminated when the supply of stage 16 prolarvae was exhausted. Were it possible to have allowed development to proceed without sampling, the probability of stage 17 being reached would have been improved.

Abnormalities occurred in 35 to 40 percent of the prolarvae. These abnormalities were similar to those already listed except that deformed specimens were especially numerous.

Development at 77.5° F.

A constant temperature of 77.5° F. was decidedly above the maximum at which sea lamprey eggs could develop successfully. Mortality was so great that all embryos were dead after 13 hours and none developed beyond stage 6, 32 cells (tables 6, 21, and 22; fig. 27).

Stage 1 was taken in the 1- to 4-hour samples, stage 2 in the 4- and 6-hour samples, and stage 3 in the 6- to 10-hour samples. Only 4 embryos developed beyond stage 3, and only 3 (stages 5 and 6) were still alive in the sample taken at 13 hours. All were dead in samples from 14 hours to the termination of the experiment at 24 hours. Some stages were reached earlier at 77.5° F. than at 75° F. (stages 2 and 3), but others were attained later at the higher temperatures (stages 4 and 5). See table 6 and fig. 28.

TABLE 21.—*Living and dead embryos per sample and their stage of development at 77.5° F. (25.3° C.)*

Sample	Hours	Number dead	Stage of living embryos					
			1	2	3	4	5	6
1	1	------	163	------	------	------	------	------
2	3	1	67	------	------	------	------	------
3	4	10	29	36	------	------	------	------
4	6	58	------	32	4	------	------	------
5	8	20	------	------	31	------	------	------
6	10	64	------	------	8	1	------	------
7	13	70	------	------	------	------	1	2

TABLE 22.—*Mortalities of sea lamprey eggs reared at a constant temperature of 77.5° F. (25.3° C.)*

Time		Stage span	Number of samples	Number of embryos			Percentage dead [1]
Hours	Days			Alive	Dead	Total	
0–24	1	0–6	13	364	708	1,072	66

[1] All embryos (485) dead in the 6 samples taken after 13 hours.

Development at 80° F.

Development was brief and erratic at a constant temperature of 80° F. A few embryos reached the 32-cell stage (stage 6) but most were dead long before that stage was reached (tables 6, 23, and 24; figs. 27 and 28).

No signs of cleavage had appeared at the end of the first 3 hours but at the fifth hour practically all of the eggs had begun to dimple, the initiation of first cleavage and stage 2. By the ninth hour all eggs had completed first cleavage and many had started the second cleavage (start of stage 3). At the 11th hour the pattern of cleavage had become rather erratic, since the second cleavage furrow often began while the first was less than half-completed. At times the first cleavage furrow seemed to regress.

Mortality which was slight in the first 2 samples had risen to about 35 percent in the seventh hour. All embryos were dead in the sample at 13 hours, but the next sample at 15 hours contained eggs

that had reached stage 6 (32 cells) but had begun to vacuolate. All subsequent samples had only dead embryos.

TABLE 23.—*Living and dead embryos per sample and their stage of development at 80° F. (26.6° C.)*

Sample	Hours	Number dead	Stage of living embryos					
			1	2	3	4	5	6
1	3	6	131	------	------	------	------	------
2	5	7	107	16	------	------	------	------
3	7	85	35	131	------	------	------	------
4	9	124	------	11	55	------	------	------
5	11	149	------	------	31	------	------	------
6	13	165	------	------	------	------	------	------
7	15	303	------	------	------	------	------	2

TABLE 24.—*Mortalities of sea lamprey eggs reared at a constant temperature of 80° F. (26.6° C.)*

Time		Stage span	Number of samples	Number of embryos			Percentage dead [1]
Hours	Days			Alive	Dead	Total	
0–24	1	0–6	11	519	1,753	2,272	77

[1] Only 2 live embryos were taken after 11 hours and all (914) were dead after 15 hours.

SIGNIFICANCE OF OBSERVATIONS

Effect of temperature on development

The most significant result of the experimental rearing of sea lamprey eggs at 10 different constant temperatures (ranging from a minimum of 45° F. to a maximum of 80° F.) was the clear demonstration that successful development through to the production of viable burrowing larvae was possible only within a relatively narrow range. No live larvae were produced at any temperature below 60° F. or above 70° F. Further evidence of the extreme sensitivity of sea lamprey eggs to temperature comes from the much lower survival at 60° F. (12 percent), and 70° F. (5 percent), than occurs at the "optimum" temperature of 65° F. (78 percent). It is to be regretted that experiments were not made at 62.5° F. and 67.5° F. to define more clearly the trends within the 60°–70° range, but no further time was available when the importance of tests at these two intermediate temperatures became obvious.

Mortality was so heavy at the highest and lowest temperatures that all eggs had died before development had proceeded beyond very early stages. In general, the highest stage reached increased as the temperature approached the "successful" levels of 60°–70° F. This relationship is brought out by the following listing:

Temperature (F.)	*Highest stage reached*
45°	3 (4 cells)
50°	9 (gastrula)
52.5°	12 (head)
55°	13 (prehatching)
60°	17 (burrowing)
65°	17 (burrowing)
70°	17 (burrowing)
75°	16 (gill clefts)
77.5°	6 (32 cells)
80°	6 (32 cells)

Developmental abnormalities were least plentiful at 65° F. and increased as the temperature deviated from that value in either direction. In some tests the incidence was high, and the abnormalities (described briefly in the accounts of the experiments) involved monstrous distortions of the embryos.

In general, developmental rate (notably, the time required to reach the various stages) became faster, lengths of stages became shorter, and overlap between stages was lessened as temperature increased. Some of the exceptions to this statement no doubt represent the random variability of the data. Others, as for example, the seemingly depressing effect of the highest temperatures on the rate of development in the early stages may reflect a real cause-and-effect relationship.

The clear demonstration in the present studies that sea lamprey eggs are capable of full and normal development only within a relatively narrow temperature range brings out the great importance of controlling temperature at the correct level in developmental studies and experimental research. Consideration of the proper temperature had little place, nevertheless, in past studies of sea lamprey eggs. Authors failed to state the temperatures at which the eggs were reared or reared them at levels at which full, normal development could not be expected. Shipley (1885) did not state the temperature at which his sea lamprey embryos developed and McClure (1893) reared his embryos at 6°–7° C. (42.8°–44.6° F.). Damas (1948) mentioned temperatures of 12° and 18° C. (53.6° and 64.4° F.) on the development of *Lampetra* (it is not to be assumed, of course, that the effects of temperature on development are the same for *Petromyzon* and *Lampetra*, but certain parallels must be considered highly probable).

Shipley's account of the persistence of the blastopore and his statement that invagination took place at 130 hours suggest that embryos of the lamprey developed at a temperature of about 55° F. It appears then that neither he nor McClure conducted their experiments within the range at which normal development could be expected. If the relation of temperature to development in *Lampetra* is similar to that in *Petromyzon*, Damas' experiments at 18° C. (64.4° F.) should have been at nearly the optimum temperature, but those conducted 12° C. (53.6° F.) were well below the optimum. Thus, certain of the abnormalities (most of them duplicated in the present study, particularly at the higher and lower temperatures) that he interpreted as the effect of light intensity may actually have been caused by temperature.

Although the findings of the present experiments offer the strongest evidence that unsuitable temperatures may account for the failure of certain apparently suitable streams to produce larval sea lampreys, a too close application of the results to problems in nature is not advisable. The sea lamprey eggs were reared at constant temperatures in this study, whereas the temperatures in natural streams are subject to diurnal fluctuations, to substantial short-term increases and decreases along with changes of weather, and finally to a longer term, seasonal, upward trend as development proceeds. These fluctuations may have a profound effect on the tolerance of the developing egg. Temperature surely is an important, sometimes a critical, factor in the production of viable larvae in nature, but a good understanding of its operation would require controlled investigations in which eggs develop under fluctuating temperatures which are made to vary much as they do in natural streams.

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