Staging Anchovy Eggs

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ABSTRACT

The developmental period of northern anchovy eggs is divided into eleven stages. These stages are defined by structural criteria chosen from the sequence of morphological changes which occur during embryogenesis. Samples of eggs, preserved in 3% formaldehyde solution, are examined under a dissecting microscope using soft transmitted light. In Stage I eggs, cell division has not yet begun and the cytoplasm appears as a cap at one pole of the elliptical yolk mass. Cell division occurs during Stage II to produce a blastodisc. At Stage III the size of the individual blastomeres is greatly reduced and the blastodisc has the appearance of tissue. Stages IV, V, and VI are defined by the fraction of the yolk mass covered by the blastoderm. The proportion of tail length to head length defines Stages VII and VIII, and the proportion of tail length to yolk mass length defines Stages IX, X, and XI. The procedure for staging eggs with disintegrated embryos is described.

INTRODUCTION

The eleven egg stages used since 1980 in egg production biomass estimates are modified from stages established by Ahlstrom (1943) for the Pacific sardine, *Sardinops sagax*. Northern anchovy eggs differ markedly from those of the Pacific sardine in size, shape, and in the absence of an oil globule. The strongly elliptical eggs of *Engraulis mordax* measure 1.23-1.55 mm at the major axis and 0.65-0.82 mm at the minor axis (Bolin 1936), whereas developing sardine eggs range from 1.35 to 2.05 mm with a mean of 1.70 mm (Ahlstrom 1943). The sardine embryo, however, is only slightly larger than the embryo of the anchovy since the wide perivitelline space of the sardine accounts for almost half the total egg diameter. Also, the rates of development of major embryonic features and organ systems are comparable in the two species.

METHODS

Eggs are sorted from the plankton samples as described by Stauffer and Picquelle (1985) and preserved in 3% buffered Formalin in 2-dr vials. For staging, each sample is placed in a watch glass with water and examined under a dissecting microscope, using the microscope mirror to produce a soft transmitted light. A direct light source does not allow one to distinguish fine structures of the embryos.

Most samples contain several groups of eggs representing widely separated stages from several days' spawnings. An initial cursory examination allows one to sort most of the eggs into these several subgroups, which can then be assigned to stages. The stages are based on structural criteria chosen from the sequence of morphological changes which occur during embryogenesis. These are described below and illustrated in Figures 1 and 2.

EGG STAGES

Stage I

Cell division has not yet begun. In intact eggs the cytoplasm of the single cell appears as a clear hemisphere at one pole, easily differentiated from the yolk mass which is divided into granules. The cytoplasm may be displaced to other locations around the periphery of the yolk mass, but there is usually some accumulation at one pole, which allows the stage to be identified.

Stage II

This begins with the division of the single cell into two cells or blastomeres. The division is first noticeable when a furrow develops in the middle of the cytoplasmic cap. Small bubble-like structures (probably artifacts) are often visible along the furrow and help identify it. The next cleavage plane is at right angles to the first, and subsequent synchronous divisions in both meridional and latitudinal planes produce a hemispherical mound of cells, termed the blastodisc. After the 5th or 6th division, the blastodisc has a berry-like appearance, the so-called "mulberry stage," and with subsequent divisions the blastomeres become increasingly smaller and more difficult to distinguish individually. During a certain phase of the early divisions the blastomeres are about the same size as the yolk granules. If the blastodisc and yolk mass become disrupted during collection or preservation, the blastomeres may become distributed among the yolk granules. They may be distinguished from one another since they have different refractive indices and the blastomeres appear darker when viewed with transmitted light.

In: R. Lasker (editor), An Egg Production Method for Estimating Spawning Biomass of Pelagic Fish: Application to the Northern Anchovy, *Engraulis mordax*. NOAA Tech. Rep. NMFS 36. U.S. Dep. Commer., Wash., D.C., 99 p.

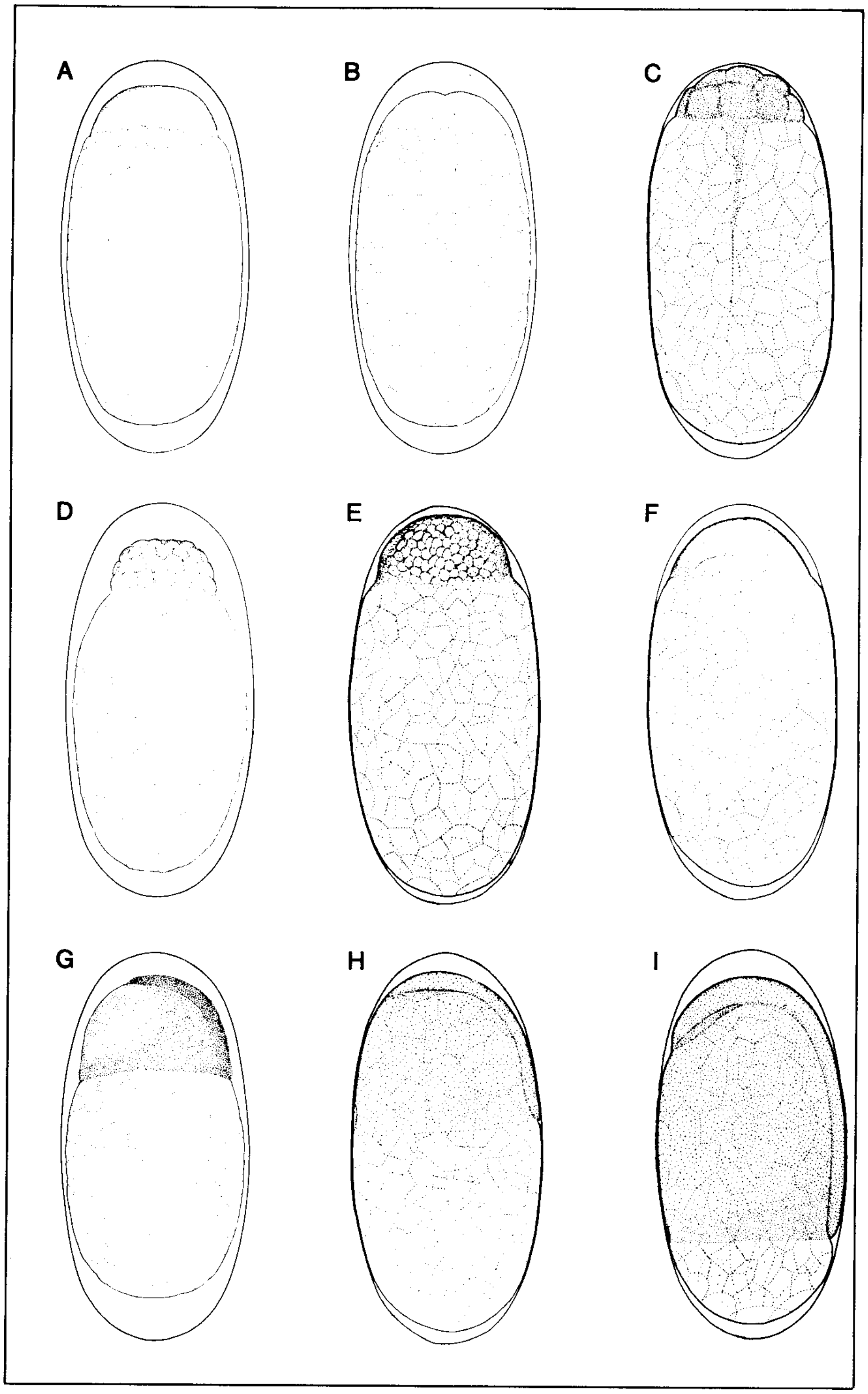

Figure 1.—Stages of northern anchovy eggs. A. Stage I; B. Stage II (2 cells); C. Stage II (16 cells); D. Stage II ("Mulberry"); E. Stage II (late); F. Stage III (mid); G. Stage III (late); H. Stage IV (mid); I. Stage V (mid). Original illustrations of A, B, D, G, by G. Mattson; C, E, F, H, I from Bolin (1936).

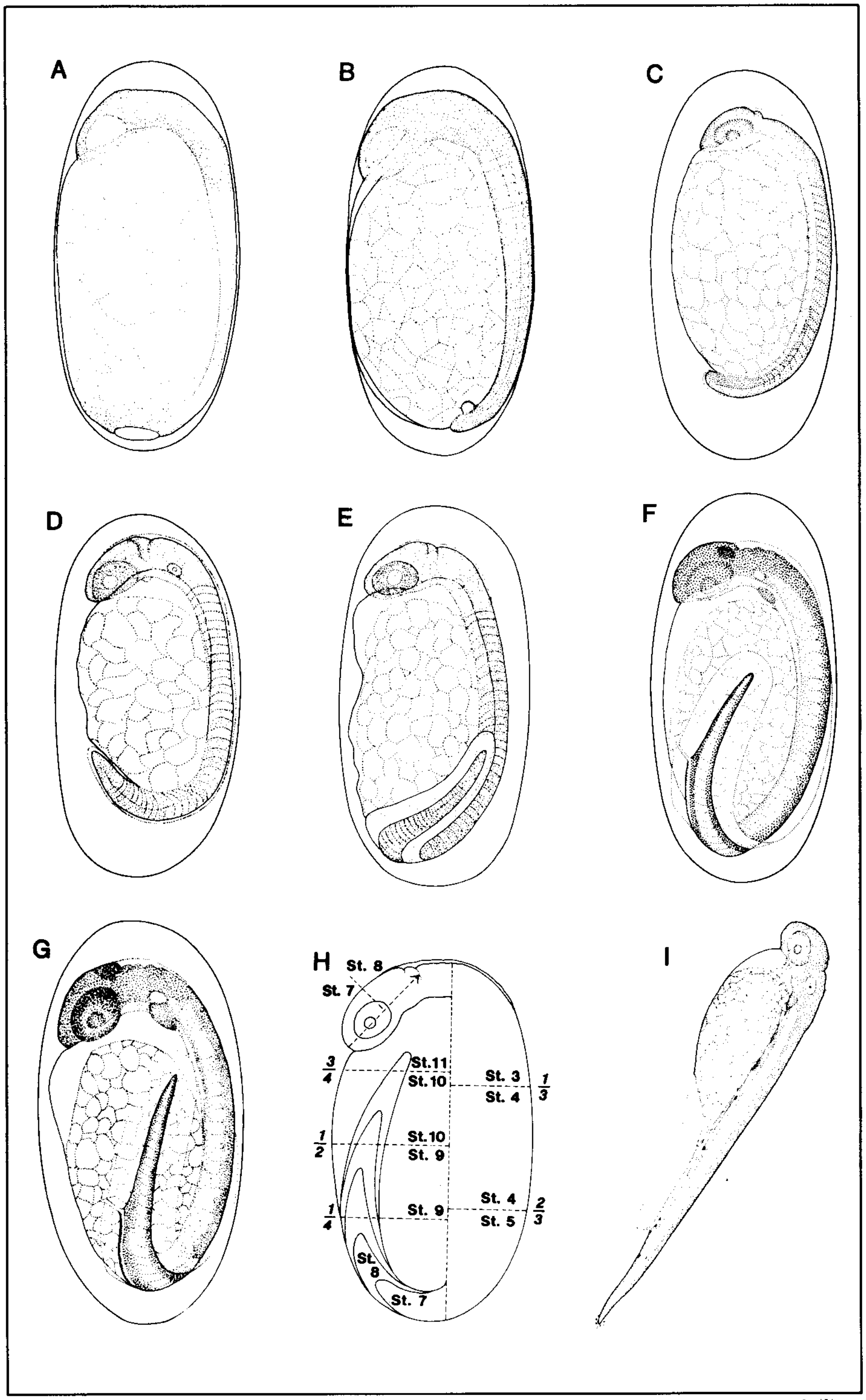

Figure 2.—Stages of northern anchovy eggs. A. Stage V (late); B. Stage VI; C. Stage VII; D. Stage VIII; E. Stage IX; F. Stage X; G. Stage XI; H. Diagrammatic egg showing relationship of epiboly and tail length to stage; right side indicates fraction of the yolk mass covered by the blastoderm in Stages III, IV, and V; left side shows tail length/head length proportions which define Stages VII and VIII and tail length/yolk mass length proportions which define Stages IX, X, and XI; I. Newly hatched anchovy. Original illustrations of A, C, and F by G. Mattson; B from Bolin (1936); original illustrations of D and E by H. Orr; original illustration of G by G. Moser; H prepared by B. Sumida; I from Kramer and Ahlstrom (1968).

Stage III

Ahlstrom (1943) defined this stage in sardine eggs as beginning with the appearance of the segmentation cavity. The segmentation cavity of teleost eggs is the space formed between the blastodisc and the yolk mass during late cleavage. In most anchovy eggs in our collections the blastodisc is shrunken and somewhat cup-shaped and consequently the segmentation cavity, which is a delicate structure, is obliterated. We have found it preferable to define the beginning of Stage III on the basis of the appearance of the blastoderm, i.e., when it has the appearance of tissue rather than of a collection of individual cells. This stage marks the beginning of gastrulation. The margin of the blastodisc becomes slightly thickened and is termed the germ ring. At one region of the germ ring the thickening extends inward to form the embryonic shield, which defines the future axis of the embryo. Gastrulation proceeds by further proliferation and downward movement of cells in the region of the germ ring by a process known as epiboly. Simultaneously, proliferation and inward migration (emboly) of cells from the margin of the embryonic shield produce the organ-forming cell layers of the primordial embryo. At the end of Stage III the germ ring is one-third down the yolk mass and the bilateral nature of the primordial embryo is apparent.

Stage IV

At the beginning of this stage the germ ring has enclosed one-third of the yolk mass and the embryo is beginning to form along the median region of the embryonic shield. At the end of this stage, defined by the germ ring enveloping two-thirds of the yolk, the head region of the embryo is becoming apparent.

Stage V

This stage begins with the germ ring two-thirds down the yolk and ends with the closure of the blastopore and the complete enclosure of the yolk by the cellular sheath of the embryo. The stage is characterized by rapid differentiation resulting in the formation of several somites in the midregion of the embryonic axis, development of the notochord which can be seen from a dorsal viewpoint, and differentiation of the optic vesicles from the brain.

Stage VI

This stage begins with the closure of the blastopore and ends when the tail starts to separate from the yolk mass. The embryonic sheath of cells is extremely thin and, in some samples, it may be difficult to determine the point of blastopore closure. In these cases the event can be estimated from the position of the caudal terminus of the embryonic axis, since it grows toward the pole with the edge of the cellular sheath. Initially the caudal region lies flat against the polar region of the yolk, then gradually thickens and becomes more rounded at the tip until it is clearly separate from the yolk. During this stage the somites are apparent along the entire body axis (except at the caudal portion), the lens primordium appears in the eye, and the regions of the brain begin to differentiate.

Stage VII

At the beginning of this stage the tip of the tail free from the yolk is broadly rounded, then begins to narrow as it elongates. The notochord extends almost to the tip and the finfold is just becoming visible. At the end of this stage the length of the free tail is one-half the length of the head. For this purpose, head length is considered the distance from the tip of the snout to the back of the cerebellum (see Fig. 2H). Relative tail length is the criterion for each remaining stage.

Stage VIII

This stage begins when the free tail length is greater than one-half the head length and ends when tail length equals head length. The tail becomes pointed during this stage and begins to bend away from the axis of the body, to the right or left side. The curvature of the tail generally increases with development, but is subject to individual variability (Fig. 2). Judgement is required in compensating for curvature in estimating relative tail length; however, accuracy and precision increase rapidly with practice.

Stage IX

This stage begins with the tail extending one-quarter the length of the yolk sac and ends when it reaches one-half the yolk sac length. The gut is now apparent along the ventral surface of the tail, and its terminal section passes through the finfold which is now considerably wider than in the previous stage. The pectoral fin buds appear as lateral thickenings as do the otic vesicles.

Stage X

This stage starts when the tail is one-half the length of the yolk sac and ends when it reaches three-quarters of the yolk sac length.

Stage XI

This is the final stage before hatching and is defined by a tail length greater than three-quarters of the length of the yolk sac.

Disintegrated (Dis) Eggs

Eggs with embryos in various states of disintegration are found in many samples, and some samples contain a large proportion of disintegrated eggs. Despite some preliminary field and laboratory experiments, we are not able to determine whether disintegration is caused by net damage, fixation, mortality prior to capture, or some combination of these. The sampling design requires that all eggs be assigned a stage regardless of condition. Empty egg shells and eggs which have no identifiable morphological features are assigned the "Dis" (disintegrated) category. These are later assigned a stage, by pro rating, during the aging procedure (see Lo 1985). With detailed examination, eggs containing damaged or shrunken embryos can be staged. Usually they are part of a mode which is present in the sample and thus can be staged by comparison with the groups of intact eggs. In the early-stage disintegrated eggs, the blastomeres and yolk granules are intermingled but can be distinguished since the former appear darker in transmitted light. In eggs beyond Stage III, the embryos are rarely disassociated but may be distorted or shrunken and oriented abnormally because of the disintegration of the yolk mass. Despite their condition, these embryos have the morphological features described for intact embryos and can be staged, although this requires more attention to morphological details; particularly useful are careful observations on the degree of differentiation of myomores, median finfold, and head structures such as eyes, brain lobes, and otic vesicles.

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