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HISTOLOGICAL CRITERIA FOR DIAGNOSING THE STARVING CONDITION IN EARLY POST YOLK SAC LARVAE OF THE NORTHERN ANCHOVY, *ENGRAULIS MORDAX* Girard

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Abstract: The identification of fish larvae that are in starving condition in plankton samples would be a valuable aid in estimating early mortality rates of populations in the sea. Histological criteria are given for determining the degree of starvation-induced emaciation, with an indication of its relevance to mortality, for larvae of the northern anchovy, *Engraulis mordax* Girard. Larvae were reared with and without food to age 9 days, *i.e.*, beyond the point of yolk absorption.

Eleven histological characteristics were each graded on scales of poor, intermediate or good, depending variously on texture, shape and fullness of nuclei, cytoplasm, extracellular substance, and cellular products and storage material. Grades varied with food deprivation, so that increasing emaciation may be estimated from histological variables. Distribution of the grades also paralleled trends in survival and length, indicating that degree of emaciation reflects the probability of survival.

The histological variables that best classified larvae as severely emaciated, moderately emaciated, or robust in a stepwise discriminant analysis were pancreas condition, trunk muscle fiber separation, intermuscular tissue, and liver cytoplasm. The discriminant analysis suggests that an average of grades for three or four characteristics is sufficient for determining the state of emaciation of a larva.

INTRODUCTION

In attempting to understand the causes of fluctuations in year-class size of marine teleosts, Hjort (1914, 1926) proposed the concept of a critical period, according to which the strength of a year class is largely determined by survival of the larvae immediately after yolk is exhausted and so probably depends on the availability of planktonic food at that time. May (1974) concluded from a review of both laboratory and field studies since the time of Hjort that while the relation between year-class strength and early mortality remains unclear, starvation beyond the end of the yolk sac stage could be an important cause, both directly and indirectly, of larval mortality. Evidence of starving larvae in the sea is, however, slight and this is due in part at least to difficulties in diagnosing the starving condition. Condition factor (wt/l^3) (Blaxter, 1971) and chemical indices (Ehrlich, 1974) both show changes with starvation in herring and plaice but are complicated by problems of measurement and changes with growth. Morphometric methods showed some possibilities for the anchovy (Nakaii *et al.*, 1969) and the plaice (Shelbourne, 1957), and recently have been used to characterize the progressive collapse of the head and pectoral region of starving herring and plaice larvae (Ehrlich, Blaxter & Pemberton, 1976). Some histological changes were also described in the latter study, and a histological approach was used

by Umeda & Ochiai (1975) to classify laboratory-reared larvae of the yellowtail, *Seriola quinqueradiata*, on the basis of condition of the cells of the digestive tract, liver and pancreas into feeding, semi-starved, and starved types.

In the present study, criteria for judging starvation in first-feeding larvae of the northern anchovy, *Engraulis mordax* Girard, are based on histological changes rather than on morphometrics. There can be little doubt that morphological characteristics change with starvation and that they are relatively simple to measure, but there is uncertainty in regard to the validity of laboratory derived criteria for populations in nature. Body proportions and condition factor have been shown to differ for laboratory reared and wild herring down to lengths of 16 mm (Blaxter, 1971; Balbontin, De Silva & Ehrlich, 1973). It is, however, difficult to establish criteria except by laboratory rearing, where changes can be related to food deprivation and survival. The histological approach appeared to hold good possibilities for developing criteria based on deterioration of cell and tissue structure and integrity that would be largely free of dimensional considerations, and fundamentally the same for laboratory-reared and ocean-caught animals.

In addition to the kinds of degeneration described by Umeda & Ochiai (1975) for the digestive tract and its associated glands, the decrease in protein and relative increase in water content that might be expected to accompany starvation were considered likely sources of histological indicators. Love (1974) states that cell size in several animals decreases and extracellular space increases as the relative water content rises during starvation. This has been demonstrated histologically for the trunk musculature of mature cod, *Gadus morhua* (Love, Robertson & Strachan, 1968), and the rough dab, *Hippoglossoides platessoides* (Templeman & Andrews, 1956). Love (1974) found that in such cases muscle protein is not mobilized until relatively late in starvation, after lipid reserves have been depleted, but as Ehrlich (1974) has shown for both herring and plaice, early larvae have negligible lipid reserves, and protein mobilization starts immediately on starvation.

Materials and Methods

Eggs were collected early in the morning following night-time spawning in the aquarium of the Southwest Fisheries Center (SWFC) of a captive school of the northern anchovy, *Engraulis mordax*. Spawning was induced by gonadotropin injections (Leong, 1971). The eggs were incubated for 24 h at 16 °C, when 100 eggs showing normal development were transferred by pipette to each of 16 glass beakers containing 3200 ml of sea water. The beakers were held in a water bath at 17 °C ($\pm$ 0.5 °C) under a 12-h light, 12-h dark cycle. A dim light remained on during the dark phase.

Hatching was largely completed 72 h after eggs had been collected, *i.e.*, 48 h after they had been put in the glass beakers. The time at which the larvae were $\approx$ 24 h old, was designated as the start of day 1 in the life of the larvae.

One half of the larvae were fed, the other half were starved. On day 1 all fed larvae received *Gymnodinium splendens* at a density of ≈ 150/ml as initial diet food (Lasker *et al.*, 1970). At the start of day 3 the rotifer *Brachionus plicatilis* was added as a second food (Theilacker & McMaster, 1971) at a density of ≈ 100/ml; the densities of these organisms were estimated every other day and supplements were added to maintain the initial levels.

Dead eggs and larvae were removed by pipette and counted on day 1. On all subsequent days, dead larvae that were readily detected were removed, but not counted.

Samples of larvae on each successive day from age 2 days were obtained by sacrificing the entire remaining population from one container in the fed series and one in the starved series. After the readily visible dead larvae were removed, the living ones were captured one or a few at a time by pipette or small beaker and immediately dropped into Bouin's fluid or in some cases 10 % buffered neutral formalin. Each larva preserved was alive at the time of capture.

Two to 4 days after fixation, the fixing fluid was replaced by two changes of 70 % ethyl alcohol at 24 h intervals. Larvae were then measured and stored individually in small vials of 70 % ethyl alcohol. Less than 2 weeks after fixation all larvae were dehydrated and embedded in 56° to 57 °C Paraplast *plus*. Dehydration was carried out automatically in a Fisher Tissuematon with an ethyl alcohol–*n*-butyl alcohol series, the final solution being pure *n*-butyl alcohol (three changes).

Larvae were serially sectioned at 6 μm approximately in the sagittal plane, mounted, and stained in Harris's hematoxylin–Eosin. Coverslips were mounted with a synthetic resin medium. The plane of sectioning was only approximate because no attempt was made to straighten larvae before or during the embedding step. Although most larvae were straight or nearly so, some had strong lateral bends; for these the sectioning plane tended to be frontal. Larvae were not forcibly straightened for measurement, although they were placed on a wet slide where most assumed a reasonably straight posture. For those with noticeable bends, standard length was measured in successive increments. About 10 % of all larvae were too irregular to be measured.

The microscopic examination of larvae was carried out in two stages. First, the largest and the smallest individual from the fed and starved groups terminated on each of the days, 3 through 6, were separated from the collection and labelled with random numbers to mask their identity. Examination of this group of 16 specimens, presumed to represent the extremes of variability *vis à vis* the availability and non-availability of food, indicated the features that should be examined in all and also suggested a format for the examination. Following this preliminary study, the remaining 204 fixed specimens were randomly labelled for examination.

Results

Survival

Lasker *et al.* (1970) showed that populations of anchovy larvae reared at 22 °C declined only moderately over 12 days in the presence of food, but suffered almost

total mortality after 6 days if starved. They further established a "point of no return," *i.e.*, the latest time that food must be made available if the larvae were to have a good chance of surviving, at age 3 days, which was 2 days before increased mortality appeared and 3 days before it was virtually total in the absence of food. Ages 4 and 5 days were, therefore, considered to constitute a period of irreversible starvation for larvae that had not yet received food. In the present work, survival is given by the percentage of the initial population recovered on the day it was terminated and harvested:

Day	2	3	4	5	6	7	8	9
Fed	57	51	72	55	75	71	27	42
Starved	56	56	48	38	40	11	0	0

The initial populations were determined from the numbers of eggs originally introduced (100) minus the numbers that were found dead and unhatched, along with some dead larvae, on day 1 ($\leqslant$ 22 for most containers). In the starved series, mortality was very high on day 7 and total on day 8. This was preceded by slightly increased mortality on days 5 and 6. These events occur a day later than shown by Lasker *et al.* (1970), undoubtedly because this experiment was carried out at a temperature of 17 °C *i.e.*, about 5 °C lower, and so 7 days is considered here to be the age at which there is high mortality from starvation, while 5 to 6 days is the period during which many larvae reach a state of irreversible starvation. In the fed series, moderately good proportions of larvae survived in all containers, but it does appear that there was some increase in mortality at or just after day 7, the time when populations without food rapidly died. This pattern resembles those arising when food densities are inadequate (O'Connell & Raymond, 1970), or when food introduction is delayed (Lasker *et al.*, 1970).

LENGTH

The mean and range of standard length is shown in Fig. 1 for each of the age-food treatment groups. These data are based on about half of the larvae removed from containers at termination and fixed in Bouin's fluid. The others were fixed in formalin. Comparison of standard length measurements, taken after fixation, showed that the Bouin-fixed material adequately represented the length range for each of the container populations. One point of interest is that the Bouin-fixed material was consistently about 6 % less than the formalin-fixed material.

The mean length of fed larvae increased more or less steadily, with the greatest increase between 5 and 7 days. The means and ranges agree closely with those given by Kramer & Zweifel (1970), except at day 9 where it was 20 % lower, indicating that the growth of fed larvae was suboptimal during the last day or two. For the starved larvae, mean length tended to decrease, especially between age 5 and 6 days, to a point much lower than that for fed larvae at day 6. The decreasing mean length with age for the

starved groups may be assumed to represent shrinkage, which has been shown to occur in starved herring larvae after yolk absorption (Blaxter & Hempel, 1963).

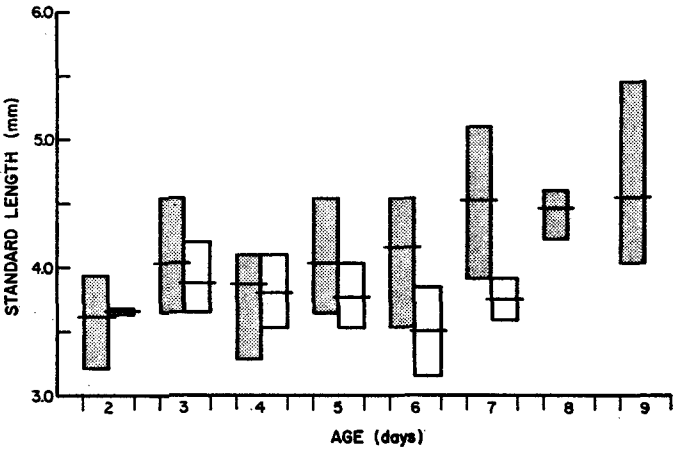

Fig. 1. The mean and range of standard length for larvae preserved from each of the age-food groups: dark bars, fed; light bars, starved.

Although increase in length is adversely affected by starvation, it must be noted that a large proportion of larvae in the 5 to 7 day old starvation groups would not be distinguishable from many of the larvae in fed groups of various ages on the basis of length alone. The ranges are broadly overlapping among all groups.

In addition to standard length other measurements were made on the formalin-fixed larvae to determine whether body proportions changed in relation to food deprivation. Dorsal finfold depth, body depth (including the digestive tract), and ventral finfold depth were measured at the level of the pyloric valve. Midgut length was measured as the distance from the pyloric valve to the ileo-caecal constriction, and hindgut length as the distance from the latter to the tip of the anus. The averages of these measurements were constant proportions of standard length over all age-food treatment groups. None of these proportions could be used to diagnose the starving condition over the first 9 days of life. The morphometric features, pectoral angle and the eye height: head height ratio, shown to change with starvation for herring and plaice larvae by Ehrlich, Blaxter & Pemberton (1976), were not measured on the above anchovy larvae. In view of their small size, it is uncertain whether these anchovies would have shown changes with starvation in such measurements. The largest fed animals were no more than 5.5 mm S.L. at age 9 days, and the largest starved ones no more than 4.5 mm S.L. The morphological changes were not distinguishable for herring < 2 weeks old and 10 mm S.L. These measurements might give useful indications of starvation for more advanced anchovy larvae.

HISTOLOGICAL VARIATION

Ultimately, 11 histological variables were used to analyse the differences relative to starvation. These were characteristics of the notochord, cartilage, trunk musculature, digestive tract, liver, and pancreas, all of which show growth and differentiation but no striking changes in morphological organization between the ages of 2 and 9 days. General descriptions are given below, followed by an outline of the criteria used in grading the observed variability.

Notochord. The notochord, composed of vesicular connective tissue in which fluid-filled vacuoles predominate, is by far the largest tubular structure of the body. The spinal cord lies along its dorsal midline and the dorsal aorta along its ventral midline. It is encircled by a layer of transverse bands which eventually form the outer sheath of dense fibrous connective tissue. Generally the notochord is turgid and fully distended, but it can show degrees of collapse.

Cartilage. The jaws and branchial elements, along with certain pectoral girdle and cranial elements, have the characteristics of procartilage, the elements being composed of chondrocytes in close proximity, separated by no more than thin shells of capsular or territorial matrix. The appearance of inter-territorial matrix and the consequent separation of chondrocytes starts at age 8 or 9 days. During the procartilage stage the basophilic chondrocytes ordinarily have round, light granular or mottled nuclei and lightly stained cytoplasm which largely or entirely fills the capsular spaces; however, nuclei can be small and dense and cytoplasm partially or completely lacking.

Trunk musculature. The trunk musculature is closely applied to the lateral surfaces of the notochord between the spinal cord and the dorsal aorta. It is segmented, the myomeres having a weak chevron form and overlapping slightly; the layer is, nevertheless, relatively thin during this early period. The major components of the trunk musculature are a layer of internal oblique fibers (antero-ventral to postero-dorsal) and a layer of external oblique fibers (opposite alignment). The fibers, which contain a number of axial nuclei surrounded by myofibrils that run the full length of the fiber, are broadly attached to the myosepta, but they are precisely aligned in successive myomeres to form fiber trajectories that apparently extend over two to three myomeres. Striations are evident in the fibers. Within each myomere the internal and external oblique layers are separated by a moderately thick layer of basophilic substance with a light granular texture and scattered nuclei. The granular substance appears to be cellular cytoplasm, and this intercellular layer is probably composed mainly of myoblasts. The muscle fibers themselves may vary both in width and lateral separation and the intermuscular tissue may vary from thick and relatively dense to virtually complete absence.

By the end of the first week an outer or superficial layer of slender, horizontal muscle fibers is visible over the entire surface of each myomere. The lateralis branch of

the vagus nerve, which subtends the lateral line of neuromasts, lies between the superficial muscle layer and the epidermal integument. The superficial muscle layer and neuromasts were not involved in evaluating the condition of larvae.

The digestive tract. The digestive tract is composed of a long foregut, a straight midgut of lesser length but greater diameter, and a short hindgut. The foregut is made up of cuboidal mucosa cells and a coat of transverse muscle fibers. The mucosa shows weak longitudinal ridges over its entire length. It is stratified and folded at the pharyngeal port, where the only goblet cells in the digestive tract are found. Although there was some variation in size and clarity of mucosa cells and in the mass of the pharyngeal port tissue, the range of variation was small and no attempt was made to evaluate the foregut. It should be noted that the anchovy larva does not have a stomach, in either the morphological or glandular sense, until much later in its development.

Transition to the midgut at the pyloric port involves an increase in cell height and in longitudinal folds that reduce the lumen to valvular proportions. The midgut mucosa is composed of one type of columnar cell with microvilli on the mesial border facing the lumen. Cytoplasm tends to be homogeneous and lightly granular, but sometimes has a coarse, dark appearance. Cells are closely joined laterally and nuclei are in the basal half so that, in tangential section, the apical halves generally form a well defined unnucleated mosaic. At age 2 days the lumen surface of the midgut mucosa is essentially flat, although there may be some degree of longitudinal folding. The mucosa cells are somewhat shortened when the lumen is well expanded. The strong transverse folds characteristic of the species begin to take form at days 8 and 9.

The chief indicators used in evaluating the midgut were the texture and color of the cytoplasm, the prominence of the tangential mosaic pattern, or conversely the tendency for mucosa cells to be separated rather than well joined; the band of microvilli did not seem to vary. The midgut showed vacuolization and hypertrophic cells in a small number of specimens. These conditions were recorded and will be discussed later.

Transition to the hindgut is marked by a low ileocaecal ridge, visible from the exterior in many larvae as a shallow furrow. The cells of the hindgut mucosa did not differ in appearance from those of the midgut except for the presence of supranuclear eosinophilic inclusion bodies in many feeding larvae. Evaluation of the hindgut was based on the absence or presence and extent of such inclusions which could be considerably larger than the cell nuclei and very abundant.

Iwai (1968, 1969) and Iwai & Tanaka (1968) described inclusion bodies in the hindgut of larvae of some freshwater teleosts and suggested that these were indicative of intracellular digestion of food particles engulfed by pinocytosis in the hindgut. Though it may occur, pinocytosis was not identified in the hindgut of the anchovy larva. The biochemical composition of the inclusions is uncertain. A few dozen formalin-fixed specimens were post-fixed in osmium tetroxide, which stains several kinds of lipid droplets black (Chang, Lalich & Alden, 1974). Hindgut inclusions in

these larvae were strikingly black, but no structures in the midgut mucosa gave this reaction. This tentative result contrasts with the finding of Iwai that hindgut inclusions in the larvae studied by him were probably proteinaceous (electron lucent) and that lipid droplets occurred in the midgut mucosa.

The liver. The two large glands of the digestive tract, liver and pancreas, are elongate, compact organs beneath and partially surrounding the posterior half or more of the foregut. Both give ducts into the pyloric region of the midgut along with the cystic duct extending from the gall bladder, which is a cylindrical, membraneous structure lying between, and somewhat rostral, of the anterior ends of the liver and pancreas. The gall bladder, always visible and at least moderately well distended, was measured but not otherwise graded.

The liver is composed of close-packed tubules of pyramidal hepatocytes. The tubule lumina, sometimes clearly visible, are short projections from the hepatic vein which traverses the organ longitudinally. The dilation of the hepatic vein and tubules was often difficult to judge, so this feature was not graded. Both the nuclei and the cytoplasm of the hepatocytes showed considerable variability. Nuclei most often contained a distinct nucleolus with relatively few surrounding dark granules, but in some specimens the dark granules were widespread, sometimes submerging the nucleolus or even occupying the entire nucleus. It is possible that the relative amount of dark material indicates the extent to which chromatin is 'condensed' and not actively synthesizing RNA and ultimately protein (Stein, Stein & Kleinsmith, 1975). At worst, nuclei became pycnotic, *i.e.*, black and irregular. Cytoplasm was hyaline in some specimens, uniformly or irregularly granular in others, and dispersed in yet others. The dispersed state accompanied clear intracellular spaces of considerable size. Intracellular spaces sometimes occurred when the cytoplasm was more homogeneous, but then the spaces were small and generally infrequent. Presumably the intracellular spaces are sites of glycogen or lipid storage in the liver.

The pancreas. The exocrine pancreas, lying along the right side of the foregut and occasionally extending rostral of the gall bladder, is composed of pyramidal acinar cells surrounding the terminal branches of a pancreatic duct system. In the early anchovy larva the branches are not discernible and are probably in an early stage of budding from the axial main duct, part of which is visible as a relatively large, more or less spherical sinus in the posterior half of the organ. The sinus was usually filled with homogeneous zymogen, probably available for immediate transport to the midgut. There was wide variation in the state of fullness of the sinus and this feature as well as the pancreas proper was graded.

In the pancreas proper the basal part of the pancreatic acinar cells contain, along with the cell nucleus, strongly basophilic ergastoplasm or chromaffin. The apices of the cells contain membrane bound droplets of zymogen (acidophilic). Zymogen is synthesized in the ergastoplasm, concentrated in the Golgi apparatus above the

ergastoplasm, and accumulated in the apical region, from which it is secreted into the acinar ducts by fusion of the droplet membranes with the cell membrane (Bloom & Fawcett, 1968). For a number of vertebrates it has been shown that the production of zymogen tends to vary with the intake of food (Bloom & Fawcett, *loc. cit.;* Patt & Patt, 1969). In the resting and/or fasting state accumulated zymogen is prominent and chromaffin minimal. Following a large meal, zymogen is reduced and the chromaffin becomes more prominent as the rate of synthesis increases. Although cyclic patterns were not found, the anchovy larva showed considerable variation in the relative prominence of zymogen and chromaffin. The pancreas was graded with respect to this variability as well as on indications of cell diminution and separation.

The endocrine pancreas is a single large Islet of Langerhans located immediately behind the sinus of the pancreatic duct. Although the condition of the Islet tended to vary with the condition of the organ, it was not graded as a separate feature.

Grading of variability

Differences in each of the 11 characteristics studied were graded as 1) poor, 2) intermediate, and 3) good. For a few of the features intermediate grade were used. The grade for a characteristic was based on its appearance in all sections of the larva in which it occurred. Larvae were never identified as to age and food treatment before the grades were assigned; indeed, the majority of larvae were not identified until all examinations were completed. The grading scales were as follows:

Notochord shrinkage:

1. *Severe*; notochord extensively shrunken away from surrounding trunk musculature (Fig. 2).
2. *Medium*; some areas of separation, but most of notochord surface in contact with musculature (Figs 3, 27).
3. *Negligible*; virtually no separation of notochord and muscle (Fig. 4).

Cartilage:

1. *Poor*; nuclei condensed, irregular, cytoplasm largely or entirely absent: this condition was characterized by considerable empty space since the framework of capsular shells did not collapse along with the chondrocytes (Fig. 5).
2. *Intermediate*; nuclei with variable degrees of condensation, presence of cytoplasm variable and often contracted to occupy only a portion of the capsular space (Fig. 6).
3. *Good*; nuclei relatively round and granular, or mottled; capsular spaces largely filled with cytoplasm (Fig. 7).

Trunk musculature:

Fiber separation

1. *Severe*; parallel fibers separated by wide spaces, sometimes in disarray; fibrils within the fibers indistinct (Figs 8, 9).
2. *Intermediate*; parallel fibers separated by narrow spaces over much of larva; fibrils within fibers distinct (Figs 10, 27).

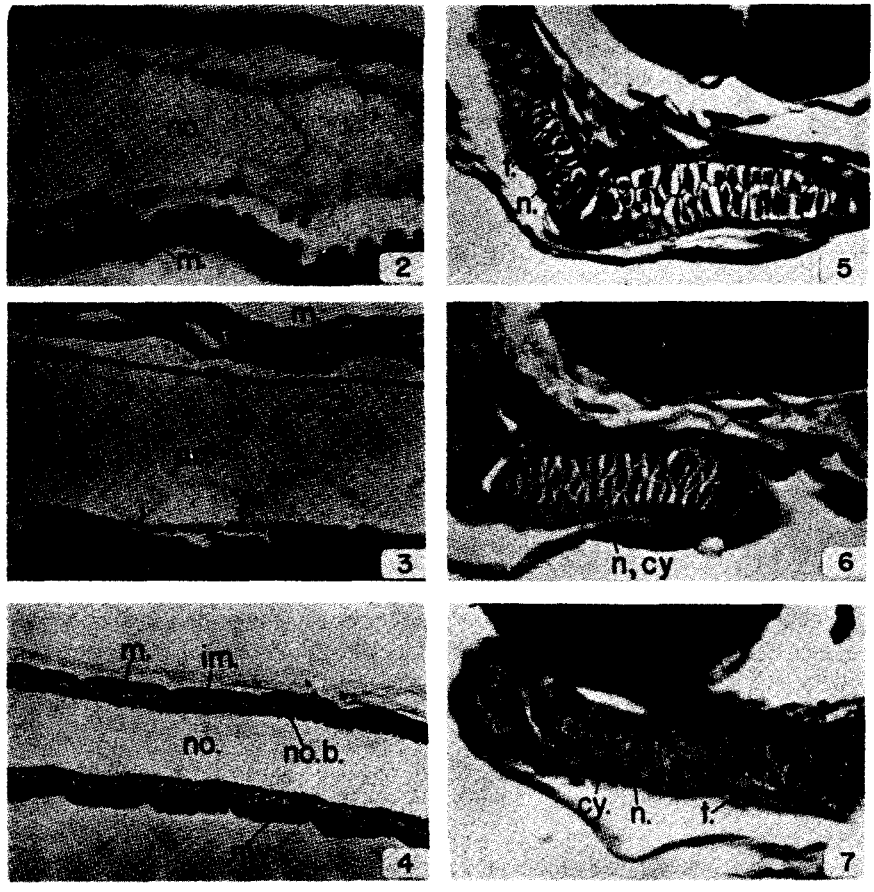

Fig. 2. Severe notochord shrinkage: 6S-1: ×393. The designations are the same in all figures: the first number refers to age, the last to grading, and the letter indicates whether starved or fed, *i.e.* 6S-1 denotes six day larva, starved, condition grade 1.

Fig. 3. Medium notochord shrinkage: 5F-2: muscle fiber shrinkage and intermuscular tissue also medium: ×393.

Fig. 4. Negligible notochord shrinkage: 6F-3: also negligible muscle fiber separation and abundant intermuscular tissue: note prominent transverse bands that become the perichordal sheath: ×155.

Fig. 5. Cartilage in poor condition: 6S-1: nuclei pycnotic and cytoplasm lacking: ×393.

Fig. 6. Cartilage in intermediate condition: 2F-1: nuclei tending to pycnotic, cytoplasm reduced: ×393.

Fig. 7. Cartilage in good condition: 6F-3: ×393.

cy., cytoplasm; im., intermuscular tissue; m., muscle; mys., myoseptum; n., nucleus; no., notochord; no. b., bands of notochord sheath; t., territorial matrix of cartilage.

3. *Negligible*; parallel fibers largely in contact laterally; fibrils within fibers distinct; transverse striations usually detectable in any of the three conditions (Fig. 11).

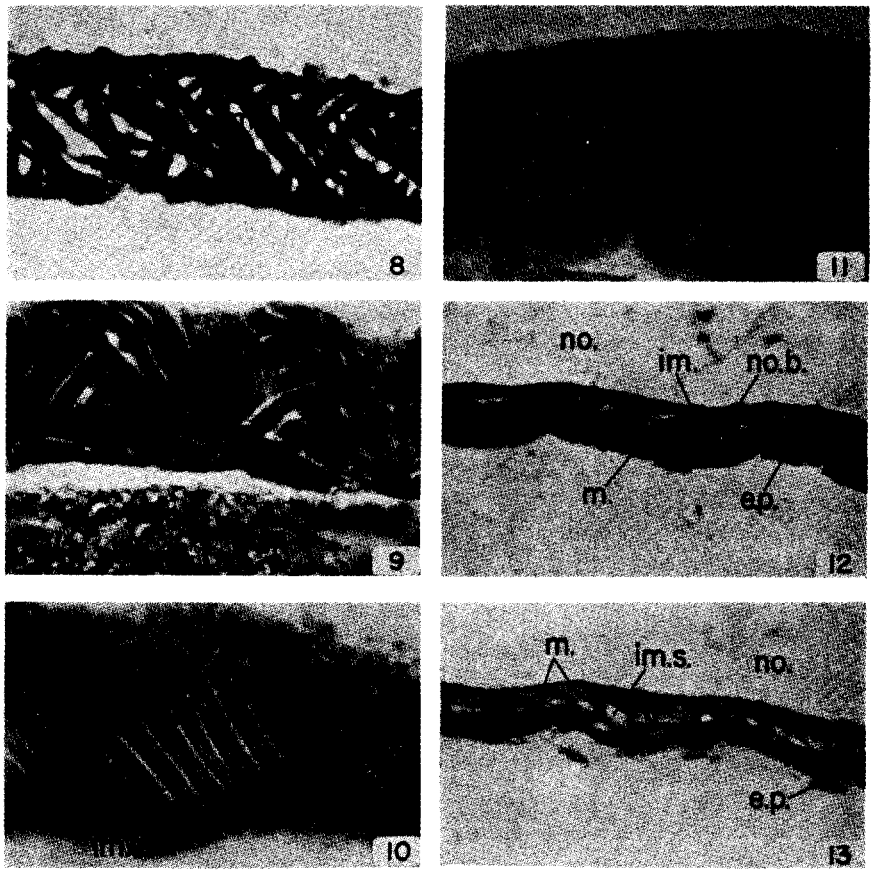

Fig. 8. Severe muscle fiber separation and lack of intermuscular tissue: 6S-1: note loss of integrity of myofibrils within fibers: tan: ×393.
Fig. 9. Severe muscle fiber separation and lack of intermuscular tissue: tan for muscles: 6F-1: ×393.
Fig. 10. Intermediate muscle fiber separation: intermuscular tissue lacking: 5S-1: tan: ×393.
Fig. 11. Negligible muscle fiber separation: 5F-3: note distinct myofibrils in this and previous Fig.: transverse striations present but faint and did not register well: tan: ×393.
Fig. 12. Thick intermuscular tissue between the two layers of muscle fibers: 6F-3: also both negligible muscle fiber separation and notochord shrinkage: ×393.
Fig. 13. Medium intermuscular tissue, but muscle fiber separation and notochord shrinkage are negligible: 3F-2: ×393.

e.p., epithelial plates; im., intermuscular tissue; im. s., intermuscular space; l., liver; l.l., lateral line neuromast; m., muscle; no., notochord; no. b., bands of notochord sheath; p., pancreas.

Intermuscular tissue

1. *None*; the two layers of muscle separated by some open space; scattered nuclei sometimes visible in spaces (Figs 8, 9).
2. *Medium*; basophilic tissue present between muscle fiber layers but sparse and largely lacking in granular substance (cytoplasm) (Figs 13, 27).

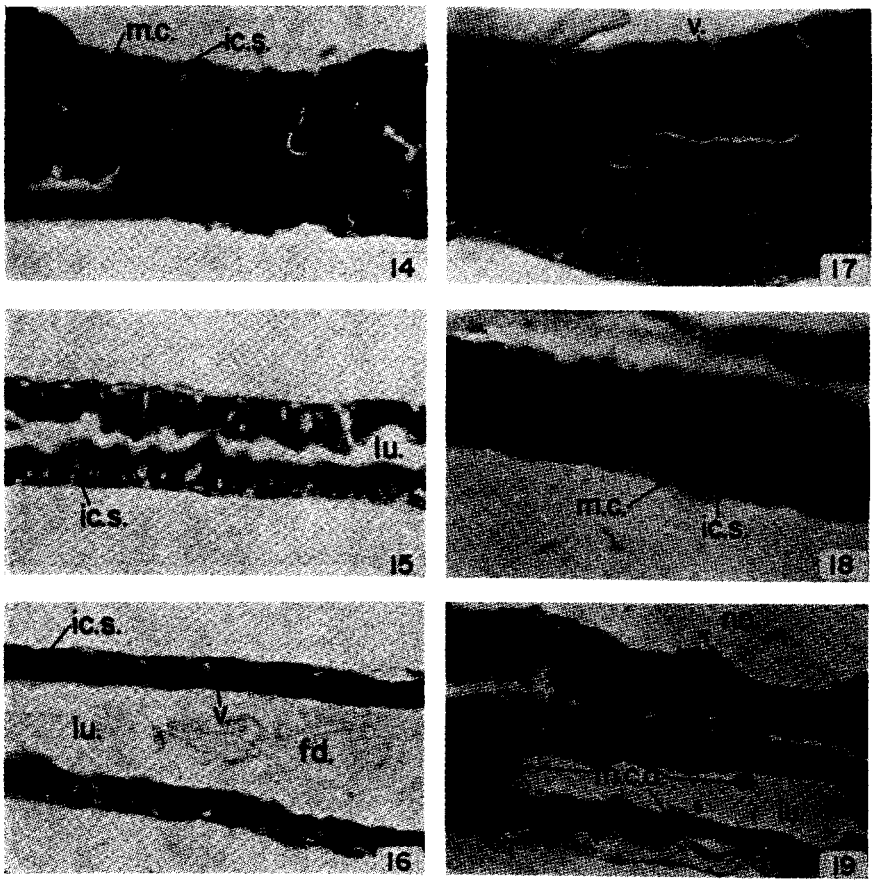

Fig. 14. Poor midgut integrity: cells are dark and separating: 6F-1: tan: ×393.
Fig. 15. Poor midgut integrity. Spaces are cell separations, not vacuoles: 9F-2: ×393.
Fig. 16. Intermediate midgut integrity: separations are of a lesser degree than above: 3F-2: food in lumen has appreciable chromatophore content: ×393.
Fig. 17. Good midgut integrity: tall, well joined mucosa cells produce a mosaic pattern at the supranuclear level: 5F-3: tan: ×393.
Fig. 18. Intermediate midgut integrity: mucosa cells in early stages of separation: 5S-2: tan: ×393.
Fig. 19. Intermediate midgut integrity: mucosa cells somewhat reduced in size: atrophy shown by sloughing cells: 5S-2: ×393.

fd., food; ic.s., intercellular space; lu., lumen; mos., supranuclear mosaic pattern; m.c., mucosa cell; m.c.a., mucosa cells in atrophy; no., notochord; v., microvilli.

3. *Abundant*; basophilic tissue relatively thick and uniformly present between muscle fiber layers; nuclei common (Fig. 12).

Digestive tract:

Midgut integrity

1. *Poor*; cells tend to be small, dark, and separated (Figs 14, 15).
2. *Intermediate*; tangential mosaic pattern at supranuclear level of mucosa cells

indistinct or absent; separations appearing especially between basal (outer) portions of cells; cytoplasm often dark and coarse (Figs 16, 18, 19).

3. *Good*; supranuclear mosaic pattern of mucosa cells prominent; no cell separations; cytoplasm usually homogeneous and light in color (Fig. 17).

Hindgut inclusion bodies

1. *None*; no inclusion bodies in cytoplasm of mucosa cells (Fig. 20).
2. *Stratified*; most cells with distinct supranuclear zone of relatively pale, coarse material not in the form of discrete spheroid bodies (Fig. 21).
3. *Moderate inclusions*; low to moderate proportion of cells contain one to a few spheroid eosinophilic bodies of small to medium size, *i.e.*, generally smaller than the nucleus (Fig. 22).
4. *Massive inclusions*; large proportion of cells contain one to a few spheroid eosinophilic bodies, often larger than the cell nuclei (Figs 23, 24, 25).

Liver:

Nuclei

1. *Poor*; usually totally black (Fig. 26).
2. *Intermediate*; nuclei with abundant dark granules; nucleoli enlarged or indistinct (Fig. 27).
3. *Good*; nuclei with lightly granular chromatin; nucleoli usually small and distinct (Figs 28, 29, 30, 31, 36).

Cytoplasm

1. *Hyaline*; cytoplasm lacks texture and/or appears dark; cells usually small, often separated (Fig. 26).
2. *Homogeneous*; cytoplasm granular, often with slight variability in coloring (Figs 28, 29).
3. *Variable*; cytoplasm contains numerous eosinophilic granules of various sizes (Figs 30, 36).
4. *Structured*; cytoplasm varies in texture from scattered granules to distinctive eosin patches (Fig. 31).

Intracellular space

1. *None*; cytoplasm of hepatocytes completely devoid of open gaps; (does not include the highly variable, sometimes large sinusoidal spaces) (Figs 26, 28).
2. *Slight*; occasional small gaps in cytoplasm of hepatocytes, usually scattered (Fig. 29).
3. *Medium*; small gaps in cytoplasm of hepatocytes common throughout the organ (Fig. 30).
4. *Extensive*; gaps in cytoplasm large and present in most hepatocytes, suggestive of vacuolization; boundaries of hepatocytes usually prominent (Figs 31, 36).

Pancreas:

Pancreas proper

1. *Poor*; cells usually small, black and separating; organ appears thin, with little or no eosin coloration (Figs 26, 32).

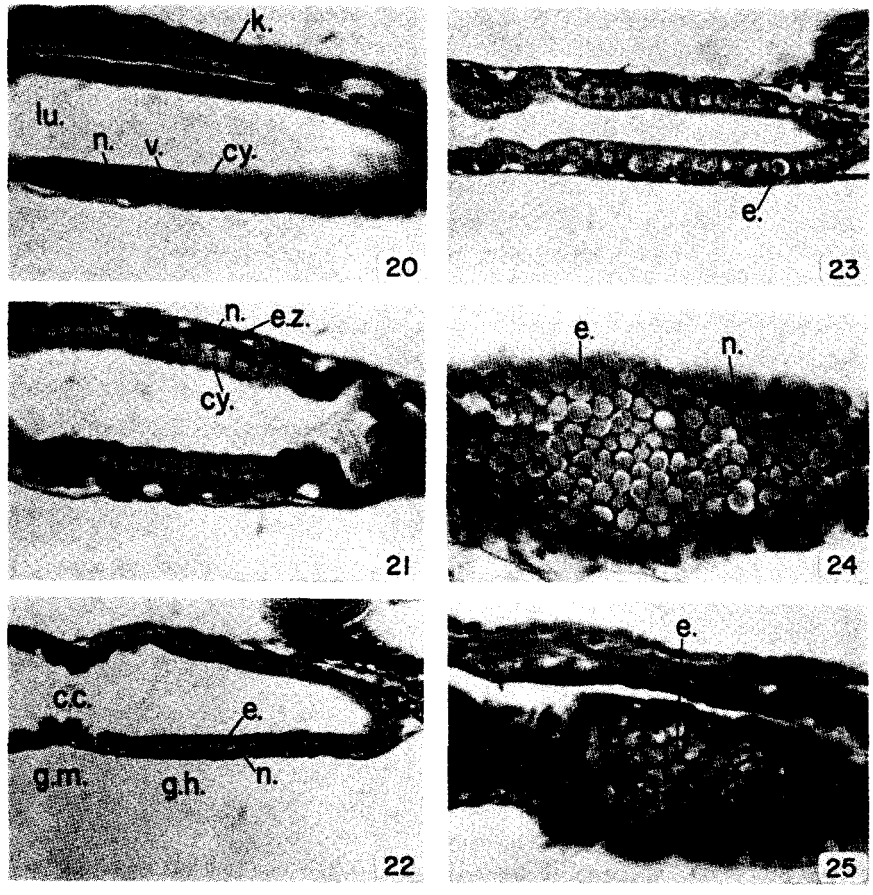

Fig. 20. Hindgut mucosa cells with no eosinophilic inclusions: structure above the hindgut is left kidney duct: 5S-2: ×393.

Fig. 21. Hindgut mucosa showing the stratified condition: eosinophilic material above nuclei light in color and granular and not in form of large spheroid bodies: 3F-2: ×393.

Fig. 22. Hindgut with inclusion bodies of moderate size: these are deeper in color and far more variable in size than previous: constriction in the digestive tube is ileocaecal furrow separating hindgut and midgut: 7F-2: ×250.

Fig. 23. Hindgut with large eosinophilic inclusion bodies: noticeable variation in size: 7F-3: ×250.

Fig. 24. Hindgut with large spheroid eosinophilic inclusions: size and texture quite uniform: 5F-3: tan: ×393.

Fig. 25. Hindgut with large spheroid inclusions, but inclusion bodies have coarse, granular texture suggestive of breakdown (intracellular digestion?): 5F-3: tan: ×393.

c.c., ileocaecal constriction; cy., cytoplasm; e., eosinophilic inclusion bodies; e.z., supranuclear zone of pale eosin granules; g.h., hindgut; g.m., midgut; k., kidney duct; lu., lumen; n., nucleus; v., microvilli.

2. *Fair*; at best basophilic chromaffin appears definitely more abundant than eosinophilic zymogen droplets; at worst zymogen negligible with some peripheral cells showing blackness (Figs 27, 33, 34).

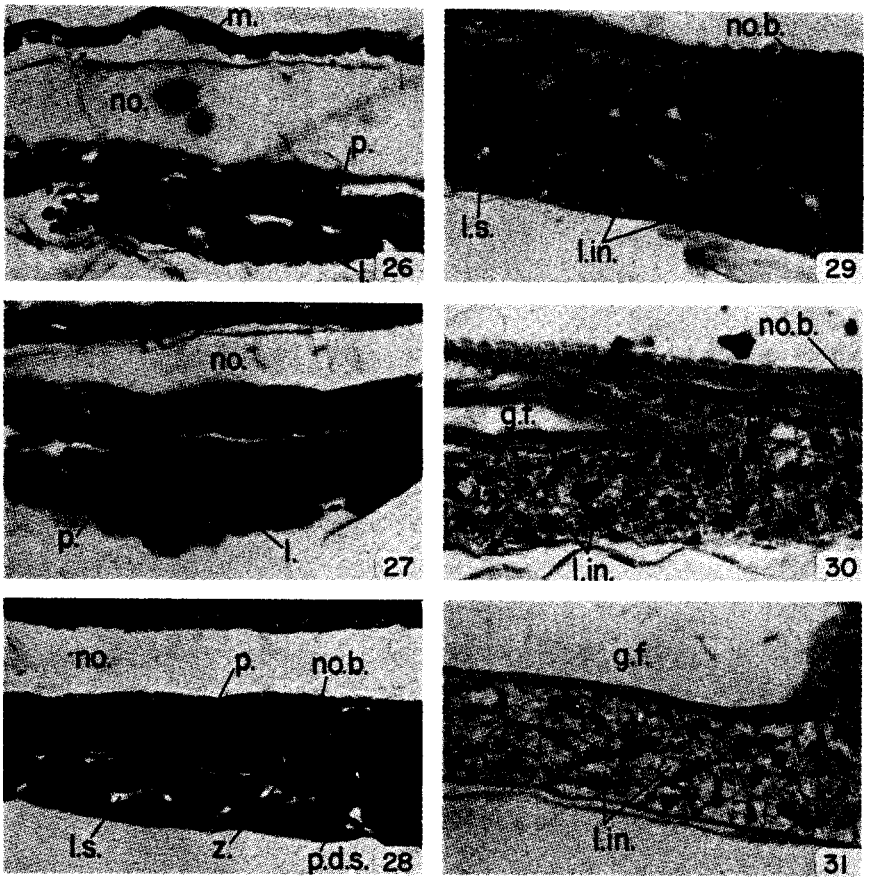

Fig. 26. Liver nuclei poor, cytoplasm hyaline, and intracellular space absent: cells appear to be disintegrating: organ diminished in size and dark: pancreas is also poor, dark, and diminished: 6S-1: ×250.

Fig. 27. Only portion of liver visible showing nuclei in intermediate condition: nuclei contain considerable dark chromatin: pancreas in fair condition, organ is dark but some zymogen throughout: muscle fibers, intermuscular tissue, and notochord shrinkage all intermediate: 6F-2: ×393.

Fig. 28. Liver with good nuclei, homogeneous cytoplasm and no intracellular space: liver sinuses moderate in size: pancreas good though this is not the definitive section for this organ: pancreatic duct sinus filled with fine-textured zymogen: 6F-2: ×250.

Fig. 29. Liver with good nuclei, cytoplasm showing slightly variable texture and also slight intracellular spaces: 6F-3: ×393.

Fig. 30. Liver with good nuclei, variable cytoplasm and medium intracellular spaces: 6F-3: ×393.

Fig. 31. Liver with good nuclei, structured cytoplasm, and extensive intracellular spaces: 7F-3: ×393.

g.f., foregut; l., liver; l. in., liver intracellular spaces; l.s., liver sinus; m., muscle; no., notochord; no. b., bands of notochord sheath; p., pancreas; p.d.s., pancreatic duct sinus; z., zymogen.

3. *Good*; chromaffin and zymogen appear to be in about equal proportions throughout (Figs 35, 36).
4. *Resting*; zymogen droplets clearly dominant and chromaffin minimal through-

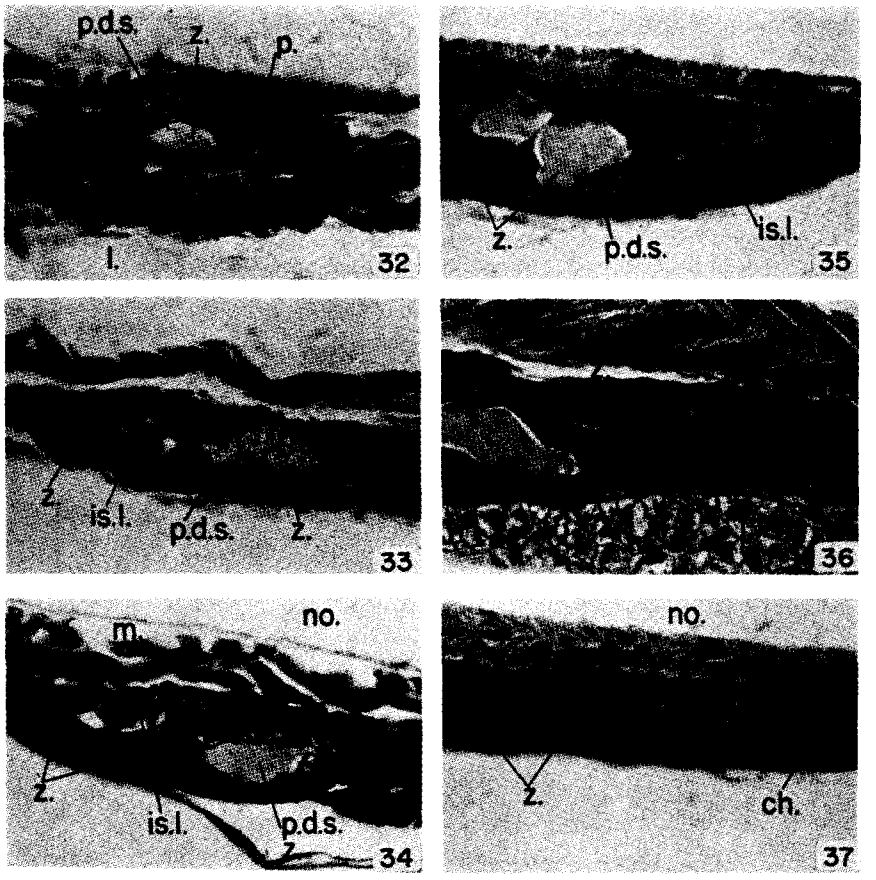

Fig. 32. Pancreas in poor condition: nuclei pycnotic, cell structure disintegrating: pancreatic duct sinus with a few loose granules of zymogen: little if any zymogen elsewhere in the organ: liver also in poor condition: 6S-1: ×393.

Fig. 33. Pancreas in fair condition: zymogen present but not abundant: nuclei tending towards pycnotic and traces of cell separation: islet of Langerhans composed of contracted cells with pycnotic nuclei: pancreatic duct sinus filled only loosely with zymogen: 5S-1: ×393.

Fig. 34. Pancreas in fair condition: this posterior portion of organ shows cells with zymogen, as in other areas, but also a tendency towards pycnotic nuclei and cell separation in some areas: organ tends to be dark: islet of Langerhans tends to be contracted and pycnotic: duct sinus filled with homogeneous zymogen: typical of many of the day 2 larvae: 2F-1: ×393.

Fig. 35. Posterior portion of pancreas in good condition showing extensive zymogen as well as chromaffin in cells and well expanded duct sinus filled with fine-textured zymogen: note also well expanded islet of Langerhans. 6F-3: ×393.

Fig. 36. Pancreas in good condition showing considerable zymogen but much dispersed chromaffin: duct sinus filled with fine-textured zymogen: liver has good nuclei, variable cytoplasm, and extensive intracellular spaces: 5F-3: ×393.

Fig. 37. Middle region of pancreas in good condition showing peripheral and some dispersed light chromaffin, and extensive complement of zymogen globules in acinar cells: 6F-3: ×393.

ch., chromaffin (basophilia); is. l., Islet of Langerhans; l., liver; m., muscle; no., notochord; p., pancreas; p.d.s., pancreatic duct sinus; z., zymogen.

out (only 12 % of all larvae were given this grade and these were largely day 4 and never beyond day 5).

Pancreatic duct sinus

1. *Empty*; sinus contains no zymogen or only a few scattered granules; sinus usually small and sometimes not visible (Fig. 32).
2. *Partially filled*; zymogen fills most of the sinus but is either very coarse in texture or in separated granules (Fig. 33).
3. *Filled*; zymogen in sinus fine textured or hyaline; sinus always full and relatively large (Figs 28, 34, 35, 36).

The larvae were also graded for the relative quantity and appearance of food in the digestive tract, and for the presence of yolk. Even the largest yolk residues were quite small relative to the hatching larva. Although such information helps to judge the feeding status of a larva, it does not necessarily indicate a state of good health. There were even a few instances where yolk or food was present but in an apparent state of degeneration. Grading was as follows:

Digestive tract contents:

Quantity

1. *None.*
2. *Small to moderate amount*; scattered traces of material or one mass of material occupying a small part of the lumen.
3. *Large amount*; lumen relatively large and at least half filled with material.

Appearance

1. *Debris*; scattered flocculent material, sometimes identifiable as cellular debris associated with atrophy of midgut cells.
2. *Granular*; material granular, loose, pale; often with high chromatophore content.
3. *Mixed*; contents a mixture of granular, fibrous, and membranous elements; chromatophore content low; organism configurations sometimes recognizable.
4. *Membranous*; long membranous elements dominant; usually orientated longitudinally and sometimes densely packed.

Yolk:

1. *Moderate remnant*; small but compact mass, usually teardrop shaped; often surrounded by periblast nuclei.
2. *Trace amount*; one or two small clusters of yolk globules; usually only between bases of pectoral fins; few if any periblast nuclei.
3. *None.*

In addition to the histological characteristics, the lengths of the gall bladder, liver, pancreas, and pancreatic duct sinus were measured. These lengths were, in part, directly related to standard length. Their ratio to standard length did not vary relative to starvation and they were not used in estimating the condition of larvae.

Missing grades. Occasionally it was not possible to grade a given histological characteristic. In some instances the reason was technical, *e.g.*, missing or damaged sections, distorted specimens, *etc.*, but for the most part it was because that particular organ, or the entire larva, was badly disarrayed – had literally come apart in processing – probably because of its frail condition. Since analysis based on the numerical grade data would be complicated by missing values, and since frail condition appeared to be the main reason for missing values, all missing entries were assigned the minimum grade of unity. The effect of this on grade means is small: no one of the 11 histological characteristics included more than 4 % of such assignments. The only extreme cases were three larvae where grades of 1 were assigned for seven or eight of the 11 histological characteristics.

VARIATION BETWEEN AGE-FOOD GROUPS

Fig. 38 shows the mean grade for each of the 11 histological characteristics by age-food group, and in the upper right hand corner for all 11 characteristics averaged: the latter does not include the data for yolk and food in the digestive tract. The number of larvae represented by each age-food group (for all graphs) is as follows:

Age in days	2	3	4	5	6	7	8	9
Fed	15	21	26	18	18	30	8	19
Starved	8	15	15	14	13	1	0	0

In general, average grades for the fed larvae rise over the first few days and remain about the same thereafter, while those for the starved larvae are higher at age 2 days but decline steadily to a low level by age 6 or 7 days. There are some noticeable deviations from this general pattern. Hindgut inclusions, for example, cannot show a decline for the starved group because it starts and remains at the minimum grade, signifying absence of inclusions. While this demonstrates a lack of feeding in contrast to the higher grades for the fed group, it does not necessarily indicate poor health or condition. Many larvae without inclusions during the first few days appeared to be in good condition on the basis of other features. Conversely, the fed group contained individuals at different ages that appeared to be in good condition despite the absence of inclusions, or in poor condition despite the presence of inclusions. In the latter instances the inclusions themselves were oddly colored and misshapen, apparently in a degenerating state.

Another notable feature in the figure for hindgut inclusions is the rise in grade for fed larvae between days 2 and 5. This would seem to indicate increasing hindgut activity following the initiation of feeding, but it may also reflect a change from *Gymnodinium* to *Brachionus* as the main food. While the higher grades, prevalent in later days, undoubtedly represent digestion of *Brachionus* in the hindgut, the rather different stratified condition represented by Grade 2 and prevalent during the first few days, may be a histological expression of *Gymnodinium* digestion. The contents of the

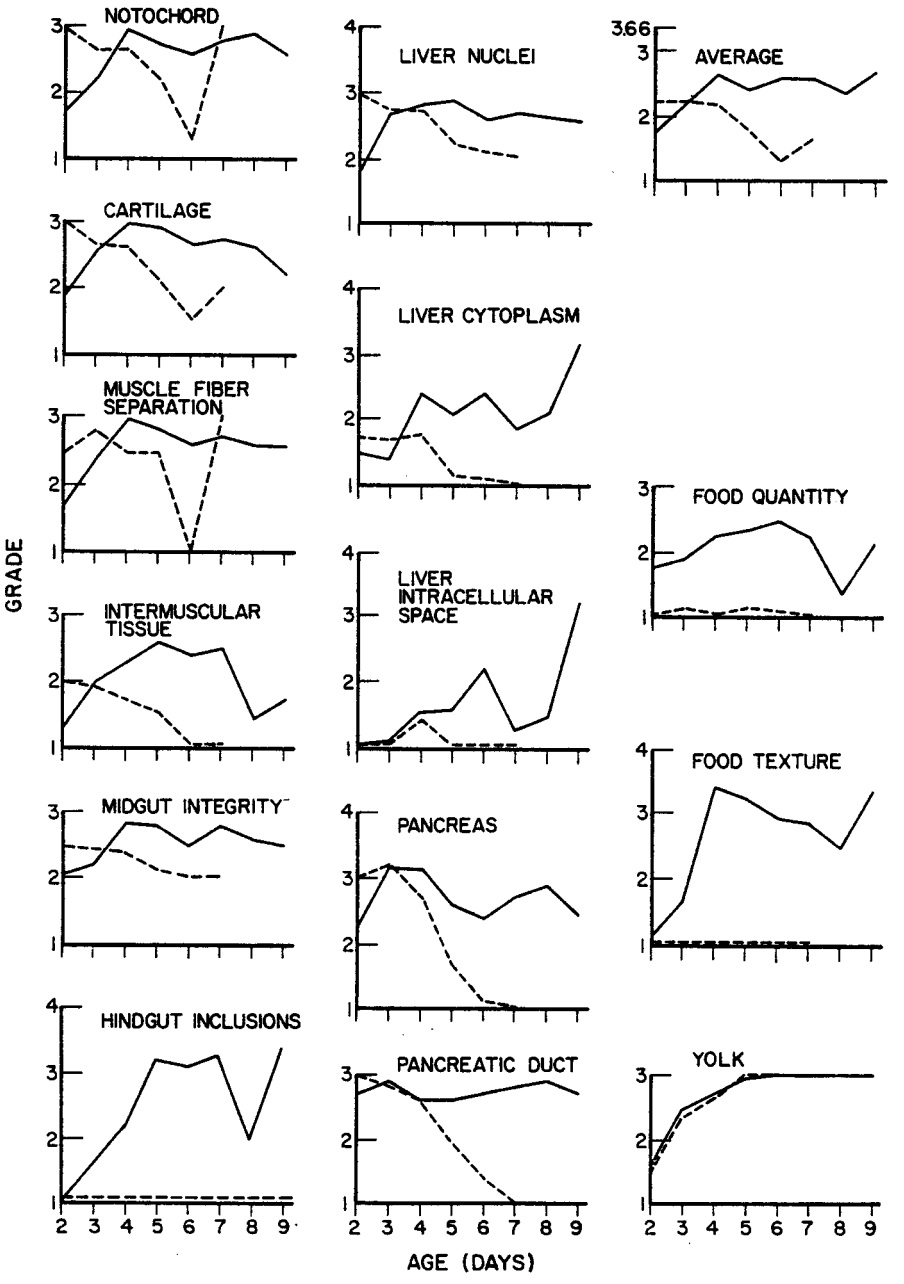

Fig. 38. Relation between average grade and age for fed (solid lines) and starved (dashed lines) larvae for each of the 11 histological features, and for food texture, quantity, and yolk: upper right, average for all histological features: highest grade, healthiest condition: for yolk highest grade, complete absence: see p. 302 for number of larvae.

digestive tracts of larvae showing the stratified state contained an abundance of light greenish granules, probably chromatophores of *Gymnodinium*. Some older larvae were also graded 2 for the hindgut, but re-examination showed that in these instances the condition was one of large inclusions composed of loose granules, which may represent a breakdown phase in the hindgut digestion of *Brachionus*. Despite these uncertainties with regard to the interpretation of hindgut grades, the pattern of averages shows that this characteristic is at least relevant as an indicator of recent feeding status of the larva.

Liver intracellular space is the only other histological characteristic giving minimum average grades for both fed and starved larvae at age 2 days and, with one small exception, for starved larvae throughout. Another deviation in this pattern is the sharp rise to a high grade at age 9 days for fed larvae following a more or less gradual rise through the earlier ages. This probably indicates low but increasing stores of energy reserves (glycogen?) in hepatocytes of fed larvae culminating in abundant large vacuoles with appreciably greater stores, as compared with the absence of stored reserves in hepatocytes of starved larvae. The characteristic indicates differences contingent on the availability of food and is pertinent to health or condition; even so it must be noted that the pattern of grades, like that for hindgut inclusions, differs from those for other characteristics in not showing an appreciable decline in the starved larvae.

In certain respects, the pattern for liver cytoplasm resembles that for liver intracellular space including a high average grade for 9-day-old larvae; this is because the two characteristics are to some extent interdependent. The nature of the variation in liver cytoplasm and the criteria for grading it were, however, such that this characteristic does show a definite element of decline for starved larvae and of increase for fed larvae.

Muscle fiber separation and notochord shrinkage each contain a peculiarity in their high grade for 7-day-old starved larvae. These values indicate no separation or shrinkage, phenomena that were increasingly apparent in starved larvae over the preceding few days. Day 7 is, however, represented by only one specimen at a time when most larvae without food had already died, and it appears that this one may have reached a degree of emaciation not anticipated in the grading criteria for muscle and notochord. (My notes on this specimen contained the observation that although muscle fibers did not show separations, they were thin and the organelle structure seemed degenerate. Thus, it seems possible that the fibers had gone through a separation stage but had finally shrunken to secondarily eliminate spaces, including those around an already shrunken notochord.) It may be seen that all other characteristics of this specimen were graded low, and that the high values for muscle and notochord do not appreciably raise the average for all 11 characteristics at the day 7 starved point.

The above mentioned deviations notwithstanding, it is evident that the patterns of grades for the histological characteristics tend to parallel the trends in the survival and length, and that the grading scales are, therefore, good indices of the effects of

starvation on the larvae. This is especially true for the pattern of averages based on all 11 characteristics, indicating that such averages will provide the best index of condition. The pattern for pooled grades shows that values approximating unity are primarily associated with the greatest mortality in the starved series and reflect severe emaciation, while values approaching three are associated with good survival in the fed series and reflect, therefore, the robust condition. Values approximating to two are primarily associated with the starved series, especially with the few days just preceding total mortality from starvation, and may be considered to represent moderate emaciation. There is an anomaly at age 2 days in that fed larvae had relatively low grades at a time when yolk remnants were still present and adverse effects from food deprivation would not be expected. This will be discussed below.

VARIATION WITHIN AGE-FOOD GROUPS

When grades for the 11 histological variables are averaged for a single larva the lowest possible score is 1.00 and the highest score 3.36. When this range is divided into three equal classes, the class intervals are 1.00 to 1.78, 1.79 to 2.56, and 2.57 to 3.36. In view of the preceding section, these classes may be considered to represent severe emaciation, moderate emaciation, and the robust condition, respectively. The percentage distributions of larvae by average grades among these three classes (Fig. 39) show that the composition of the age-food groups vary in a way that is consistent with the survival and length patterns for fed and starved larvae. In the fed series larvae are equally divided between the moderate emaciation and robust classes at age 4 days, but beyond this age the majority are in the robust class. In the starved series, the majority are initially in the moderate emaciation class but the proportion in this class decreases, while the proportion in the severe emaciation class increases to 100 % at ages 6 and 7 days, as mortality also increases and eventually becomes total.

The presence of some larvae in the two emaciated classes in almost every age group is not inconsistent with the survival and length data. There was some mortality in all groups, and average length was lower than expected in the day 8 and especially the day 9 groups, both of which had relatively high proportions of moderately and severely emaciated larvae. The interesting point with regard to length at ages 8 and 9 is that poor condition probably indicates suboptimal growth for an identifiable portion of the sample. Why severely and moderately emaciated larvae occur in the fed groups is not known, but it would appear that unavailability of food is not always the underlying cause of declining condition.

On the other hand, the high proportion of severely emaciated larvae in the 2-day-old group of the fed series is not easily reconciled with the circumstances of the experiment. Starvation should not have been a danger. Many of the larvae still had vestiges of yolk and *Gymnodinium*, known to be an adequate food for early feeding of the anchovy (Lasker *et al.*, 1970) was available; however, the hindguts showed no evidence of intracellular digestion and the midguts were either empty or contained only small amounts of material regarded as debris. The debris may have been the residue of

ingested *Gymnodinium*, but in some instances at least, it was the residue of disintegrating, atrophied midgut cells. Atrophied cells, though more prevalent in the 3-day-old fed group, were identifiable in some of these day 2 specimens. They are mucosa cells which become spherical and pass into the lumen before disintegrating. The process is probably an aspect of tissue retrenchment. The poor condition shown by most of the larvae of this group could also have been a behavioral phenomenon. If success were low in early attempts to capture food, perhaps because of a brief learning period and/or because food density was low, the larva might have incurred an energy deficit accompanied by symptoms of emaciation. Two-day old larvae with no food available, on the other hand, might have remained in somewhat better condition because they were not stimulated to expend energy in attempting to capture food.

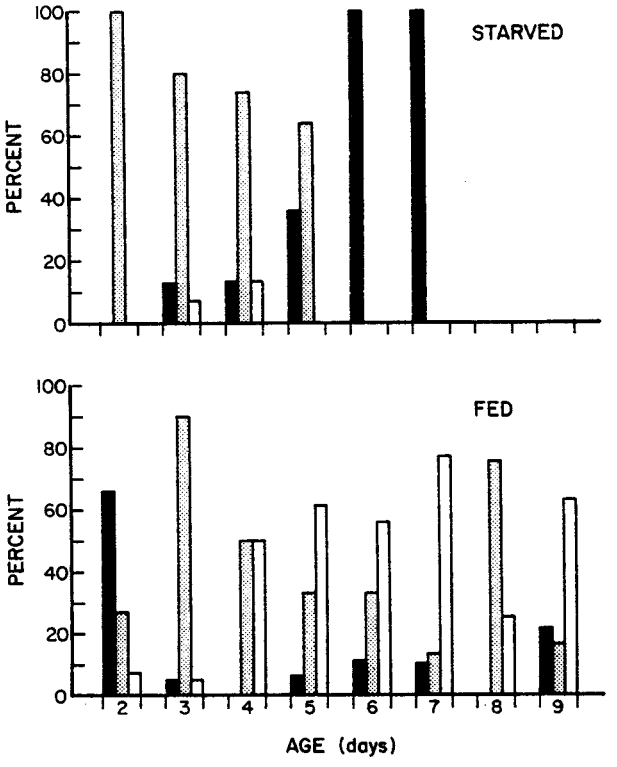

Fig. 39. Percentage of larvae in each of three classes, severe emaciation (dark), moderate emaciation (gray), and robust (white) for each age group in fed and starved series: the three classes represent equal intervals of the range of average grade for a single larva, where the average is based on grades for all 11 histological features pooled: no. of larvae, see p. 302.

Whatever the reason for the relatively poor condition of most of the 2-day old larvae, the pancreas differed in an interesting way from that of larvae in the severely emaciated class after 5 or 6 days of starvation. The latter had low grades for both the

pancreas proper and the pancreatic duct, indicating that they were not synthesizing digestive enzymes and that the duct sinus contained little, if any, reserve for immediate digestive activity. By contrast, despite the low and intermediate grades for the pancreas proper, almost all of the 2-day old fed larvae had high grades for the pancreatic duct, which was well expanded and filled with homogeneous zymogen. It is possible that many of these larvae, though considerably emaciated, were still capable of immediate digestive activity.

Apart from the special problem with 2-day old larvae, the general compatibility of the distribution of average grades within the age-food groups with survival and length demonstrates that the condition of individual anchovy larvae may be evaluated from histological parameters independent of age or size for the range studied, regardless of the factors producing the condition. On the same grounds, it appears that the three class intervals used to describe the distribution of average grade may be considered to approximate severe emaciation that is irreversible, moderate emaciation that is reversible, and robust health.

ORDER OF IMPORTANCE OF HISTOLOGICAL CHARACTERISTICS

To determine how many of the 11 histological variables would be required to establish the condition of the larva, and also the relative importance of the variables the data were submitted to a stepwise discriminant analysis (Dixon, 1965). The analysis assembles variables in the order by which they reduce the ratio of within to total generalized variances among predetermined groups into which the items are being classified (Rao, 1952). The items are larvae, each with an average grade for all variables that identifies it as belonging to one of three pre-determined groups, severely emaciated, moderately emaciated, or robust, which were defined as specific intervals of

TABLE I

Order of selection of histological variables by discriminant analysis for four age arrays: lower half is repeat of analysis with variable HG excluded. C, cartilage; HG, hindgut inclusions; IM, intermuscular tissue; Lc, liver cytoplasm; Ln, liver nuclei; Ls, liver intracellular space; M, muscle fiber separation; MG, midgut integrity; NO, notochord shrinkage; P, pancreas condition; PD, pancreatic duct sinus.

Age in days	No. of larvae	Selection steps										
		1	2	3	4	5	6	7	8	9	10	11
All variables												
5–9	121	P	M	IM	HG	NO	Lc	C	PD	Ln	MG	Ls
4–9	162	P	M	HG	IM	C	Ls	PD	NO	MG	Lc	Ln
3–9	198	M	HG	P	IM	C	Lc	MG	PD	NO	Ls	Ln
2–9	220	M	HG	P	IM	C	Ls	MG	NO	PD	Lc	Ln
Variable HG excluded												
5–9	121	P	M	IM	Lc	NO	PD	MG	C	Ln	Ls	—
4–9	162	P	M	IM	Lc	NO	PD	MG	C	Ln	Ls	—
3–9	198	P	NO	IM	Lc	M	PD	MG	C	Ln	Ls	—
2–9	220	P	NO	IM	Lc	Ln	M	PD	C	MG	Ls	—

average grade. For the present purpose, the percentage of larvae that are correctly placed in the predetermined groups as each variable is added is taken as an indication of the improvement in discrimination. The first variable selected, of course, is the one that effects the greatest reduction in variance when the variables are compared individually.

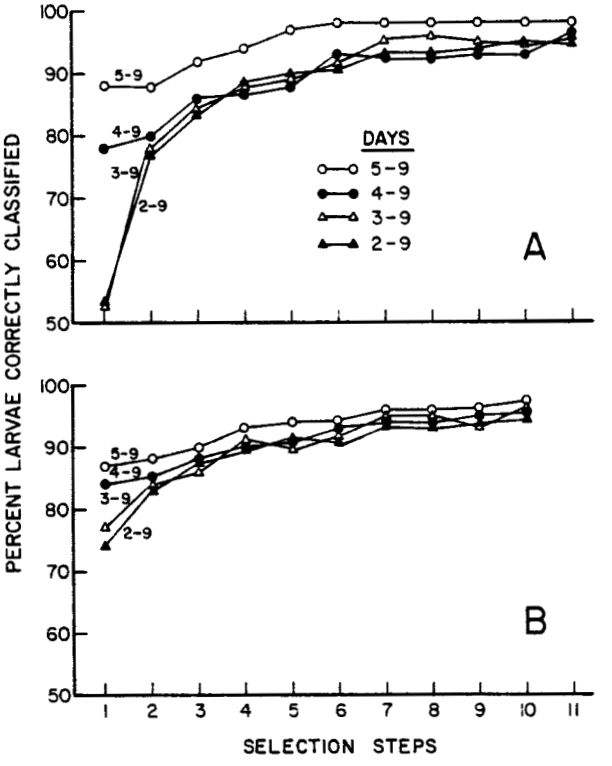

Fig. 40. Percentage of larvae correctly identified (as severely emaciated, moderately emaciated, or robust) in relation to the successive addition of histological variables in stepwise discriminant analysis: 'correct' class for a larva based on its average grade for all the histological variables: see text and Table I for further details: A, all 11 histological variables included in analyses: B, the variable, hindgut inclusion bodies (HG), not included in analysis.

The discriminant analysis was run on eight different arrays of larvae, namely, days 5 to 9 inclusive (*i.e.*, encompassing all larvae from both the fed and starved series for these days and without regard to age or food treatment) days 4 to 9, days 3 to 9, and days 2 to 9, and the same sets repeated but with one of the variables, hindgut inclusions, not included. The last of these arrays includes all the larvae studied, while the first includes larvae for the age span when histological differences between the fed and starved groups were distinct. The order in which variables were selected for each array is shown in Table I, and the percentage of larvae correctly classified (into the three classes) as each variable was added in Fig. 40.

In the arrays where all 11 variables were used, the same four features rank at the top but their order changes as younger larvae are included. Pancreas condition (P) falls to third place while muscle fiber separation (M) and especially hindgut inclusions (HG) advance; however, the percentage correctly classified also declines with these changes. Thus, where the youngest larvae are included along with all others, muscle fiber separation (M) yields the best discrimination for classification on the basis of a single feature, but this discrimination is not nearly so good as that shown by pancreas condition (P) where only the 5 to 9-day old larvae are involved. On the other hand, discrimination is quite high for the arrays including the 2 and 3-day old larvae if it is based on four or five histological features. Approximately 90 % of all larvae are then correctly classified as severely emaciated, moderately emaciated, or robust. For the array of 5 to 9-day old larvae correct classisfication is equally good on the basis of the pancreas condition (P) alone.

The arrays were rerun with all information on hindgut removed because it ranked high in the order of selection given above and yet had previously been described as an indicator of feeding or non-feeding but not necessarily a reliable indicator of the state of emaciation of the larva; in the youngest larvae making their first attempts at feeding, for example, even the most robust individuals are not likely to have large inclusion bodies in the hindgut. At best, they might show the stratified condition, which recieved a low grade. Elimination of the hindgut feature from the average score for all variables slightly changed the intervals defining the severely emaciated, moderately emaciated, and robust classes. The class intervals now become 1.00 to 1.76, 1.77 to 2.53, and 2.54 to 3.30. There were also changes in the distribution of larvae among the three classes, amounting to 2 % for the 5 to 9, 5 % for the 4 to 9, and 9 % for the 3 to 9 and 2 to 9-day old arrays. These were almost entirely due to transfer of larvae from the moderate emaciation to the robust class. They do not alter the essential compatibility of the distribution of histological grades with the survival pattern.

With hindgut eliminated, pancreas condition (P) occupies the position of first importance for all the arrays. Muscle fiber separation (M) is replaced in second position by notochord shrinkage (NO) when the arrays include 3 and 2-day old larvae, but intermuscular tissue (IM) and liver cytoplasm (Lc) occupy the third and fourth positions respectively for all arrays. Fig. 40 shows, moreover, that with hindgut removed, the first variable, *i.e.*, pancreas condition (P), produces a relatively high percentage of correct classifications for the arrays containing the youngest larvae. Nevertheless, correct classification does not exceed 90 % until the evaluation is based on four variables. Little is gained by the addition of more variables.

From the foregoing considerations it may be stated that the condition of the anchovy larva can be evaluated with confidence on a histological grading scale that does not include the status of the hindgut inclusions, but does include pancreas condition and at least three other features, preferably muscle fiber separation, intermuscular tissue, and liver cytoplasm. Substitutions could be made for any of these last three, if necessary, from among the variables that were placed lower in the order of selection

without seriously compromising an evaluation. All of them had significant F ratios (likelihood ratio tests) when tested for inclusion, so that each variable still showed some capacity for discriminating larvae of the three classes, given the variables already entered.

Discussion

These results on the anchovy are similar in many ways to those obtained by Umeda & Ochiai (1975) on the yellowtail. In both cases food deprivation results in signs of degeneration of the cells in the intestine, the pancreas, and liver, and the degeneration tends to become more severe with starvation. The availability of food, on the other hand, tended to promote production and storage activities of cells and tissues. For the anchovy, additional signs of decline were evident in cell shrinkage, separation, and the loss of intercellular substance, particularly in the trunk musculature and cartilage elements. The presence or absence of eosinophilic inclusion bodies in the hindgut is, in both species, strongly associated with the availability and non-availability of food respectively. In both studies, three classes of larvae were defined. For the yellowtail these were starved, semi-starved, and feeding, and among larvae provided with food the semi-starved group was distinguished from the feeding group only by its lack of eosinophilic inclusions in the hindgut cells and lack of food in the lumen of the digestive tract. Umeda & Ochiai concluded that larvae in this condition would have the ability to absorb and digest food if it were to become available. They recognized, on the other hand, that even with food available some of the larvae weakened and, for unknown reasons, died as was also the case with the anchovy. For the anchovy the three classes, severe emaciation, conditional emaciation, and robust were based on several histological characteristics. Hindgut inclusions ranked high in discriminating among the classes but this was considered to be more an indication of feeding status in the particular circumstances of the experiment than of condition or health. Discrimination of the three classes was actually improved if hindgut was excluded from consideration. In samples of sea-caught larvae the characteristic should no doubt be taken into consideration but interpreted with caution. The absence of inclusions, for example, might be a regular feature of the daily feeding cycle, but in some instances their absence might be a sign of low food availability with the threat of emaciation and reduced probability of survival. Not enough is yet known about the dynamics of intracellular digestion in the hindgut.

Another circumstance suggesting a need for special consideration is the relatively poor condition of 2-day old fed larvae and the fact that the inclusion of these larvae diminished the adequacy of the discrimination analysis. This indicates that grades for some of the characteristics do not reflect the average grade based on all characteristics as well in the 2-day old group as in the older age groups, where grades, moreover, were better related to the survival pattern. One reason for the lack of consistency in grades might have been the relatively high grades for the pancreas and

especially for the pancreatic duct sinus in the otherwise emaciated 2-day-old larvae. This suggests that at this age they retain a ready reserve of zymogen which gives them a capacity for immediate digestive activity and rapid recovery if food were encountered. If so, signs of emaciation have less import as an indication of imminent mortality from starvation at 2 days of age than they do at 4 to 6 days of age. In view of the uncertainties, larvae from sea samples suspected of being 1 or 2 days old, perhaps on the basis of yolk remnants, should probably be treated as a special group or disregarded in making estimates of condition.

Although there are some points of ambiguity, it has been demonstrated that at least for anchovy larvae at and just beyond yolk absorption, signs of emaciation are a cumulative function of starvation, and that they may be expressed as a numerical index of condition or health. For the experimental material the numerical index varied with survival, from which it is inferred that degree of emaciation indicates the probability of survival. Where minimum and maximum possible grades are assumed to represent zero and 100 % survival potential respectively, experimental larvae categorized as moderately emaciated would have survival potentials ranging between 33 and 66 %. Such ratings, whether based on the foregoing variables or on some modified or different numerical coding, may undoubtedly be determined for larvae taken from the ocean. While such values might not be direct estimators of survival of larvae, they should at least be useful measures of the starvation parameter for multifactor estimates of survival.

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