EFFECT OF STARVATION ON THE HISTOLOGICAL AND MORPHOLOGICAL CHARACTERISTICS OF JACK MACKEREL, *TRACHURUS SYMMETRICUS*, LARVAE

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ABSTRACT

Histological and morphological criteria were developed to assess the nutritional condition of laboratory-reared jack mackerel, *Trachurus symmetricus*, larvae. A comparison of the histological features of fed and starved larvae revealed that the digestive tract and its associated glands were the first tissues to be affected by starvation. The extent of cellular deterioration increased with time of starvation. To classify larval condition, histological characteristics of the pancreas and gut were given numerical grades. The histological technique correctly classified 83% of the feeding and starving larvae.

The morphometric analysis relied upon a stepwise discriminant analysis that used a combination of five measurements (standard length, head length, eye diameter, body depth at the pectoral, and body depth at the anus) to estimate individual larval condition. The morphometric method was as sensitive as the histological examination in determining whether or not a larva was fed or starved. Ultimately, these histological and morphological criteria may be useful for estimating larval survival in the field by assessing the condition of sea-caught larvae.

Fishery scientists generally agree that observed fluctuations in recruitment of young fish to a fish stock may be the consequence of mortality during the larval stage. Because starvation is probably one of the principal causes of mortality (Hunter 1976a), a need exists to develop criteria for detecting the incidence of starvation in sea-caught specimens. Several scientists have suggested that the differences in body form between feeding and starving larvae could be used to identify the nutritional status of larvae caught in sea surveys. For example, Shelbourne (1957) based his assessment of the condition of ocean-caught plaice, *Pleuronectes platessa*, larvae on their external appearance. Certain morphometric measurements also can be indicative of starvation. A decrease in thickness of the larval fish body has been correlated with starvation for several marine and freshwater fish larvae [herring, *Clupea harengus*, and plaice (Ehrlich et al. 1976); northern anchovy, *Engraulis mordax* (Arthur 1976); anchovy, *E. japonica* (Honjo et al. 1959; Nakai et al. 1969); pike, *Esox lucius*, and carp, *Cyprinus carpio* (Kostomarova 1962)]. Other morphological features (Ehrlich et al. 1976) considered to be indicative of starvation in herring and plaice were a decrease in the angle of the pectoral girdle, a change in the ratio of the head to eye height (herring only), and a decrease in the relative condition factor. Coincident with morphometric differences caused by starvation, Ehrlich et al. (1976) described histological changes in the gut and liver. The histological approach was used to classify yellowtail, *Seriola quinqueradiata*, larvae into "feeding," "semi-feeding," and "starving" groups by Umeda and Ochiai (1975). This technique was also effective for diagnosing starvation in northern anchovy larvae (O'Connell 1976). In both species, degeneration of cells of digestive organs was the best indicator for identification of starvation. Several other studies also have correlated starvation in fish larvae with degeneration of the digestive organs, mainly the gut. Kostomarova (1962) described a retardation in development of the gut in larvae of starved carp and pike and a reduction in the depth of the epithelial cells lining the gut. Reduced gut cell height was also reported for the larvae of starved yellowtail (Umeda and Ochiai 1975), herring, and plaice (Ehrlich et al. 1976).

Morphological criteria are preferable to histological ones because they take much less time to determine and require no special preservation techniques. However, histological criteria may be more accurate for classifying individual larva.

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The purpose of this study was to develop morphological and histological criteria for assessing the nutritional condition of jack mackerel larvae and to evaluate these criteria by comparing their success in identifying fed and starved larvae reared in the laboratory. Ultimately, criteria based on these results may be useful for estimating larval survival in the field by assessing the condition of sea-caught larvae.

MATERIALS AND METHODS

Jack mackerel eggs were collected by towing a 1-m (mouth diameter, 0.505-mm mesh) plankton net just below the sea surface at various locations between 20 and 200 mi (32 and 320 km) off the coast of southern California in June and July 1975 and in May 1976. The eggs were separated from most of the plankton at sea and then sorted by developmental stage at the Southwest Fisheries Center, La Jolla, Calif. Temperature was maintained at 15°C during sorting and in the larval rearing containers. The light cycle was 12 h light and 12 h dark. Five hundred normally developing eggs from a single day's spawning were transferred into 100 l black Kydex[2] circular rearing tanks containing filtered seawater (5μm, Cuno filtered). There were three experiments and two treatments in each experiment; larvae in one tank were offered food while those in the other were not. The fed larvae were given a diet of a naked dinoflagellate, *Gymnodinium splendens* (50/ml), a rotifer, *Brachionus plicatilis* (30-40/ml), and a copepod, *Tisbe* sp. (1 or 2/ml). This feeding method has been described (Lasker et al. 1970; Theilacker and McMaster 1971; Hunter 1976b).

Histological criteria were developed in the first two experiments. The sampling procedure and the number of larvae sampled differed depending on the requirements of the analysis. Collectively, a total of 152 larvae were examined. In the third experiment, usually 15 larvae were sampled daily for 5 days from the "fed" tank (n = 69) and 3 days in the "starved" tank (n = 48). All larvae were examined both histologically and morphologically. No dead larvae were sampled because the postmortem change which takes place in tissues of fish larvae, due to digestion by their own enzymes (autolysis), resembled antemortem destruction caused by starvation. Standard length of each larva was measured on a slide; then seawater was removed and replaced by Bouin's fixative. Preserving individual larvae in this manner assured that each would be straight and flat, facilitating subsequent morphometric measurements. Five measurements were taken after preservation to monitor daily changes in larval body form and determine effects of starvation: standard length (SL, tip of upper jaw to tip of notochord), head length (HL, tip of upper jaw to cleithrum), eye diameter (ed), body depth at the pectoral (bd-1), and body depth at the anus (bd-2). Standard length shrinkage in Bouin's fixative was 11.5%. Next, measured larvae were prepared for histological examination using standard techniques. Larvae were transferred from Bouin's to 70% ethyl alcohol after 24 h, dehydrated with an ethyl n-butyl alcohol series in a Fisher Tissuemation, and embedded in Paraplast-plus. The Paraplast paraffin blocks were frozen with fluorocarbon spray (Cryokwick) just before the larvae were serially sectioned at 5 μm in a sagittal plane. The mounted sections were stained with Harris' hematoxylin and eosin and mounted in synthetic resin.

Histological Grading System

Recently O'Connell (1976) developed a numerical, histological grading system to characterize the nutritional condition of individual northern anchovy larvae. He examined tissues of the larvae microscopically to determine the features of tissue microstructure that were affected by starvation. A grade was assigned to each feature based on the degree of similarity or dissimilarity of the histological microstructure between the starved larvae and fed larvae. I followed this method and modified it as necessary for the tissues of jack mackerel.

Each of the histological characteristics used to assess starvation in jack mackerel larvae was evaluated and assigned a grade. A grade of "3" was given to a characteristic which resembled that in "normal or healthy" larvae, a grade of "2" was given to an intermediate condition, a grade of "1" to the starved condition. Since these criteria were established by comparing actively feeding, seemingly healthy larvae with moribund larvae, it was assumed that an average of the 12 graded features examined for each larva classified the larva into the correct nutritional group: "healthy group," average grade = 2.34 to 3.00; "intermediate group" = 1.67 to 2.33; and "starved" = 1.00 to

[2]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

1.66 (the break points establish three equal groups).

Data Analysis

In the main, conclusions are based on the results of a stepwise discriminant analysis (SWDA). A discriminant analysis allows one to distinguish between two or more groups, given a set of variables that describe the characteristics in which the individuals in each group are expected to differ. In the stepwise discriminant analysis, all the variables are introduced into a SWDA computer program and the best set of variables, based on the generalized Mahalanobis distance (Rao 1952), is selected. The first variable chosen will usually be the one which gives the best score when classifying the individuals into their predetermined groups. The score is equal to the number correctly classified. The selection of each succeeding variable improves the score until a subset is chosen which is as good as the full set of variables for discriminating the groups. All variables not included in the final subset are considered superfluous, or not necessary for classification.

RESULTS

Histological

The histological condition of yolk-sac and actively feeding larvae ("normal") was compared with that of 3-day starved larvae and many differences were noted in the cells, tissues, and organs. The degree of apparent histological deterioration of 1- and 2-day starved larvae was intermediate between the "normal" and moribund status. This condition was termed "semi-starved" by Umeda and Ochiai (1975) and "intermediate" by O'Connell (1976).

The following section describes the normal histology of actively feeding jack mackerel larvae and that of starving larvae. Twelve histological criteria, which appeared to be indicators of starvation, were identified; they are numbered in the test below and are referenced in the photomicrographs (Figures 1-12).

Brain (Figures 3, 4)

The primitive brain cells exhibited a high incidence of mitotic activity (1) in normal larvae and there was relatively little intercellular space (2) between the round cells. In starved larvae, mitotic activity was arrested and many of the cells were shrunken, which caused large clear areas to appear between densely stained, atrophied cells.

Liver (Figures 5-8)

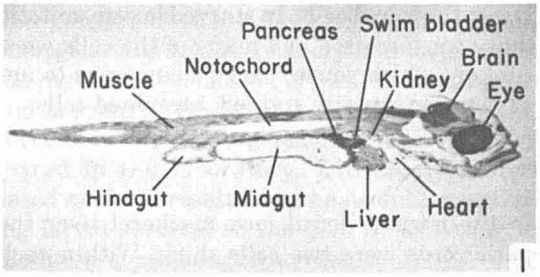
In the normal larval jack mackerel liver the hepatic cords were two cells thick. Within each hepatocyte, the nucleus (3) was regular in shape and distinct. The cytoplasm (4) was well dispersed with intracellular spaces, probably an area where glycogen and lipid are stored. Sinusoid areas, where metabolic exchanges take place between the hepatic cords, contained blood cells. After starvation for a few days, the liver atrophied, the cytoplasm condensed, stained darkly, and intracellular spaces had disappeared. There were focal degenerative or necrotic areas and accumulations of eosinophilic granules and masses. Nuclei were often irregular in shape and granules appeared around their periphery; these darkened areas are presumed to be condensed, inactive chromatin (Stein et al. 1975). The gallbladder (5) in starved larvae was always enlarged; normally it discharges its contents under the stimulus of food (Love 1970).

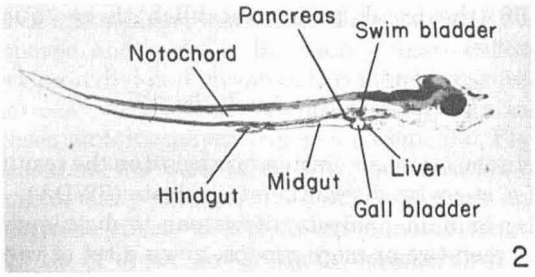
Pancreas (Figures 5-8)

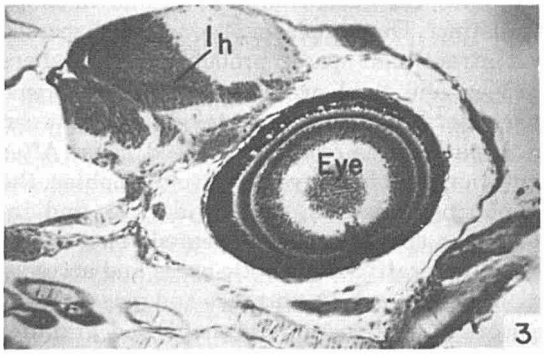
Cells of the exocrine pancreas were arranged in series, in a circular fashion, as a secretory unit called an acinus. The nucleus (6), clear and distinct, was located in the basal portion of the pyramidal cells. The acinar arrangement of the pancreatic cells (7) was found to be very sensitive to deprivation of food. A breakdown in the symmetry in the acinus was slight but usually detectable after 1 day of starvation. Tissue degeneration after 3 days of starvation was extreme. The nucleus was irregular and uniformly stained and there was no detectable acinar arrangement. The presence of zymogen, digestive proenzyme secreted by the acinus and stored as granules at its central apex, was usually associated with starvation.

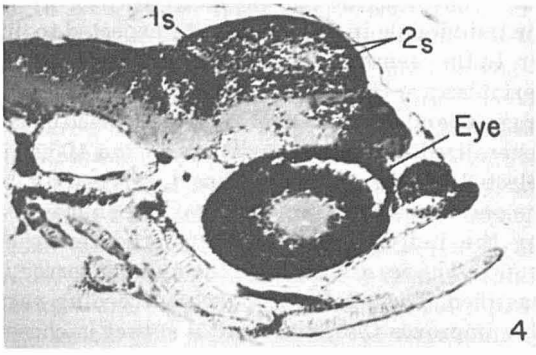
Digestive Tract (Figures 7-12)

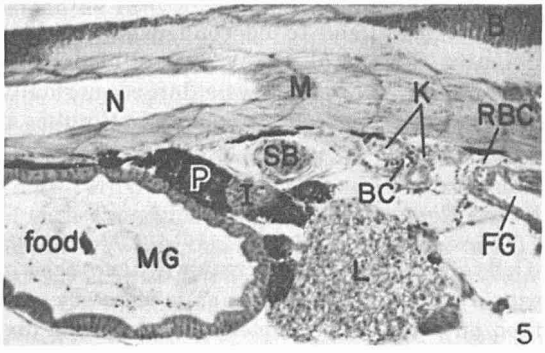
The columnar epithelial cells of the midgut were closely united (8) in a single layer. Microvilli were visible along the border of the lumen giving a brush effect. In starved larvae, the midgut cells











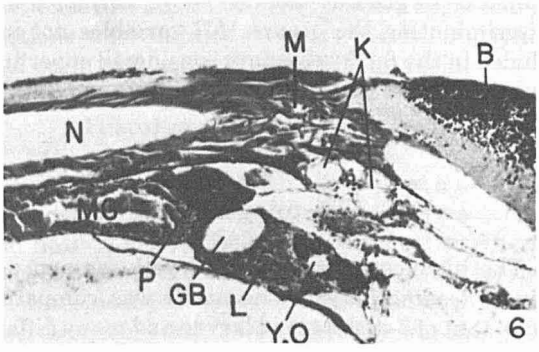


FIGURE 1.—*Trachurus symmetricus* larva, day 8, fed for 3 days. All 12 histological criteria graded as "healthy." 32 ×.

FIGURE 2.—*Trachurus symmetricus* larva, day 8, starved for 3 days. All 12 histological criteria graded as "starved." 32 ×.

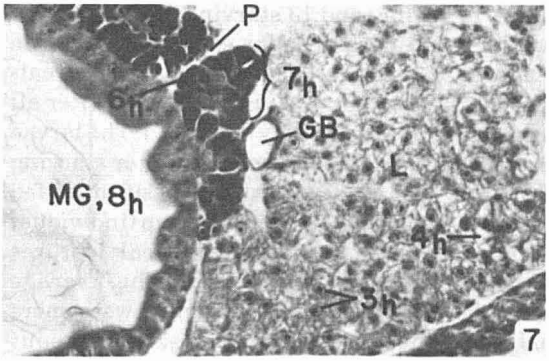
FIGURE 3.—Head of fed larva, graded "healthy." Mitotic activity (1 h) is indicated. Note close proximity of primitive brain cells to each other. 200 ×. h = histological grade = healthy.

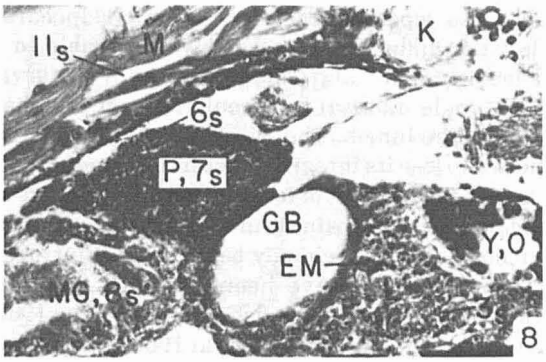
FIGURE 4.—Head of starved larva, graded "starved." Atrophied and darkly stained primitive brain cells (1 s); large intercellular spaces (2 s). 200 ×. s = histological grade = starved.

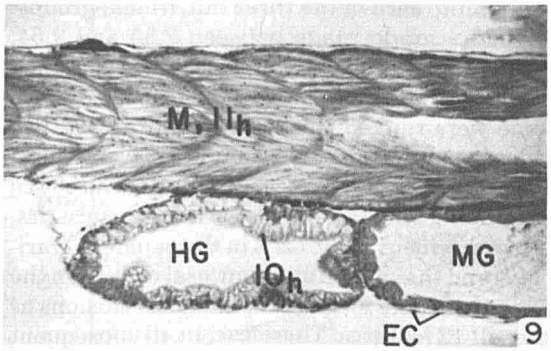
FIGURE 5.—Day 8 fed larva. Histological features graded "healthy." 200 ×. See enlargement, Figure 7. B = brain, BC = blood cells (white or immature red), FG = foregut, I = Islet of Langerhans (endocrine pancreas), K = kidney, L = liver, MG = midgut, M = muscle, N = notochord, P = exocrine pancreas, RBC = red blood cells, SB = swim bladder.

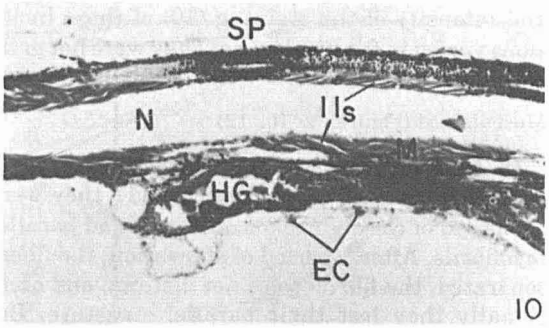
FIGURE 6.—Day 8, starved larva. Histological features graded "starved." 200 ×. See enlarge, Figure 8. GB = gallbladder, O = oil, Y = yolk, see Figure legend 5 for rest of symbols.

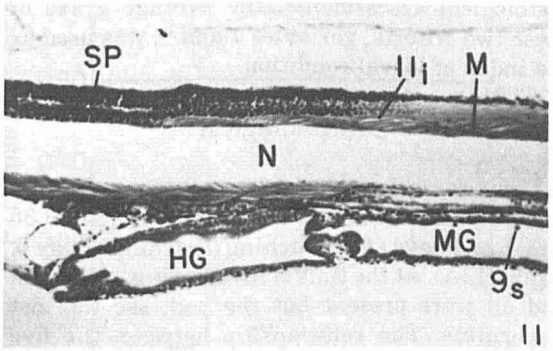
FIGURE 7.—Day 8 fed *Trachurus symmetricus* larva. Enlargement of Figure 5. Midgut cells in close union (8 h); prominent nuclei (3 h) and large intracellular spaces (4 h) in the liver; pancreatic nuclei distinct (6 h) and cells arranged in a circular unit (acinus) (7 h); gallbladder (GB) "normal." 480 ×. h = histological grade = "healthy," L = liver, MG = midgut, P = exocrine pancreas.











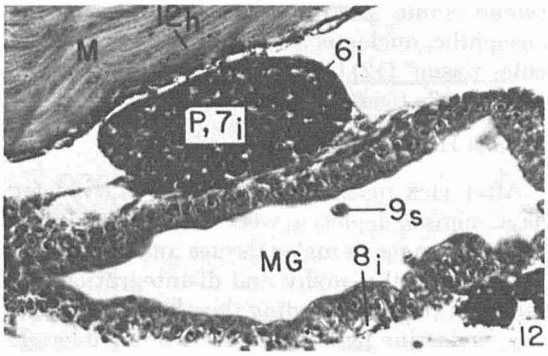


FIGURE 8.—Day 8, starved *Trachurus symmetricus* larva. Enlargement of Figure 6. Loss of integrity of midgut cells (8 s); atrophied liver with dark staining and irregular nuclei (3 s); no acinar cellular arrangement in pancreas (7 s); separated muscle fibers (11 s); swollen kidney (K); distended gallbladder (GB); note presence of yolk and oil (Y, O), and eosinophilic mass (EM) in liver. 480 ×. s = histological grade = "starved," M = muscle, MG = midgut, P = exocrine pancreas.

FIGURE 9.—Day 8 fed larva. Large eosinophilic inclusions in hindgut (10 h); muscle fibers closely packed (11 h); thin, epithelial integumental cells (EC) are prominent below gut and above trunk musculature. 200 ×. h = histological grade ="healthy," HG = hindgut, M = muscle, MG = midgut.

FIGURE 10.—Day 8, starved larva.Loss of cellular structure in hindgut; enlarged epithelial integument cells (EC); separated muscle fibers (11 s). 200 ×. s = histological grade = "starved," HG = hindgut, M = muscle, N = notochord, SP = spinal cord.

FIGURE 11.—Day 7 larva, starved 2 days and histologically graded "intermediate." No inclusions in hindgut; cellular separation in midgut (MG) and hindgut (HG); midgut cells sloughing (9 s); muscle fibers beginning to separate (11 i). 200 ×. i = histological grade = intermediate, s = histological grade = "starved," M = muscle, N = notochord, SP = spinal cord.

FIGURE 12.—Day 7 larva, starved 2 days, graded "intermediate." Abundant intermuscular tissue (12 h); no muscle (M) fiber separation; pancreatic nuclei (6 i) not distinct and cellular acinar arrangement lacking (7 i); midgut cells separating (8 i) and sloughing (9 s). 480 ×. i = histological grade = intermediate, s = histological grade = "starved," MG = midgut, P = exocrine pancreas.

began to separate from each other. It appeared that the midgut was extremely vulnerable to a deficiency of food and usually after 1 day of starvation, single mucosal cells could be seen sloughed (9) into the lumen. The margin of the lumen continued to lose its integrity as starvation advanced. Cells of the hindgut of feeding larvae exhibited an accumulation of eosin staining inclusions. The inclusion bodies, which may be the sites of intracellular digestion, have been observed in other marine and freshwater fish (Kostomarova 1962; Iwai and Tanaka 1968a, b; Iwai 1968; Umeda and Ochiai 1975; O'Connell 1976). The amount, size, and intensity of the staining (10) of these inclusions varied in feeding larvae. They were not present in starving larvae.

Musculature (Figures 9, 10, 12)

In feeding jack mackerel larvae, individual muscle fibers were close together (11); they were composed of closely packed, striated, and parallel myofibrils. After a period of starvation, the fibers separated, the fibrils were not distinct, and occasionally they lost their parallel structure. Between some fibers there was a granular, basophilic, nucleated substance called "intermuscular tissue" (12) by O'Connell (1976). In starved larvae, this tissue was usually absent.

General Histological Characteristics

After jack mackerel larvae had starved for 3 days, signs of depletion were widespread. In addition to changes in major tissues and organs there was a general atrophy and disintegration of all cells and tissues including those of cartilage, kidney, endocrine pancreas, and swim bladder. The number of pyknotic nuclei (i.e., darkening and shrinking nuclei, which give the first indication that a cell is dying) increased in all tissues (see eye and brain, Figure 4). Epithelial cells of the integument were hypertrophic, twice as large as normal in 3-day starved larvae (Figure 10), and kidney tubules were swollen (Figure 6). There was always a larger yolk reserve retained by starving larvae (Figure 6). A decrease in yolk absorption in starving larvae was also reported by Kostomarova (1962) for pike and carp and by Umeda and Ochiai (1975) for yellowtail.

Histological Grading

To determine whether the classification of a jack mackerel larva required the grading of all histological features or a lesser number, a group of 27 larvae, 14 feeding and 13 starving, was examined and the resulting grades for each criterion were submitted to a SWDA. The experimental treatment (fed or starved) was unknown until after all larvae were microscopically examined. The larvae were 7 days old and had been feeding or starving for 2 days. The grading system classified all fed larvae (n = 14) into the healthy group (individual average grade of the 12 histological features ranged between 2.42 and 2.92). The average grades for the 2-day starved larvae were more variable. The larvae were classified, about equally, into each of the three nutritional groups: four had a grade range between 2.35 and 2.54, ranking in the healthy group; four were classified as intermediate, grade range 2.08 to 2.31; and five larvae were ranked as starved with the average grades ranging between 1.15 and 1.54.

Results of the SWDA on the above data disclosed that grading only two histological characteristics, the arrangement of the cells in the pancreas (variable 7) and the sloughing of mucosal cells from the midgut (variable 9), gave the same conclusions as using all 12 features. Therefore, in all subsequent histological assessments, the average grade of these two criteria, variables 7 and 9, was used as the index of larval condition.

Morphological

The jack mackerel larvae were 2.45 mm SL (preserved) at hatching and initiated feeding at 3.35 mm, 5 or 6 days after hatching (hatching = day 0, Figure 13A). At the time of first feeding, some yolk and oil were present but the yolk sac was not discernible. The relationship between the five morphological characteristics, measured to determine the effects of starvation, and days of starvation is illustrated in Figure 13B-F. Since no data have been published on the daily growth rate of field-caught jack mackerel larvae, I used length as an estimate of age. When the morphometric measurements were plotted against length, no single measurement was a reliable index of starvation, as illustrated by pectoral body depth plotted for fed and starved larvae (Figure 14). However, some limits can be set from this graph: 1) all larvae <3.30 mm SL that do not have a yolk sac probably are starving (feeding is initiated at 3.35 mm); and 2) larvae with a body depth >0.47 mm are feeding. This leaves the size class between 3.30 and 3.55 mm where the cases cannot be separated. Most individuals in this class (29 fed and 24

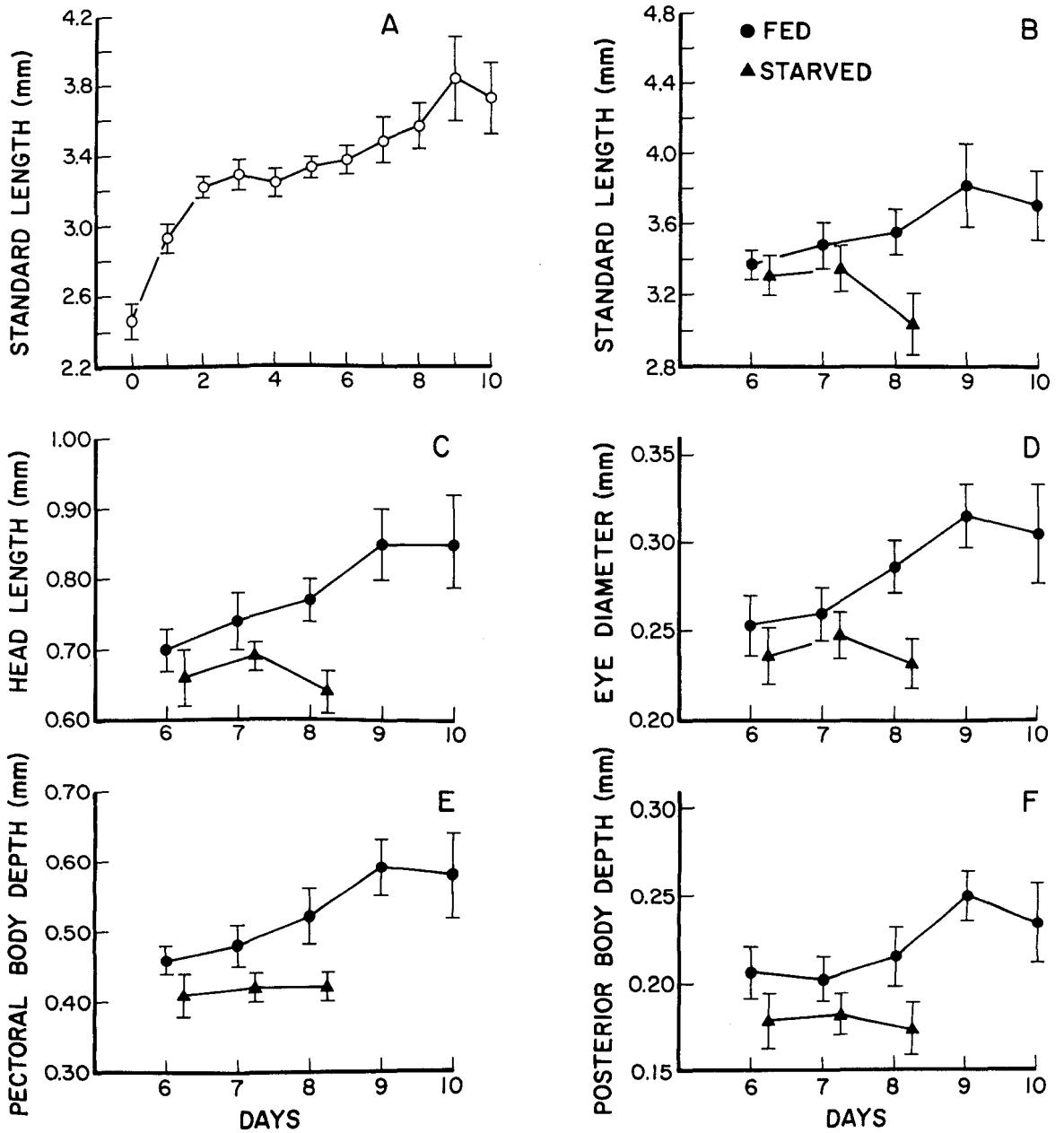


FIGURE 13.—A. Growth of *Trachurus symmetricus* larvae, with means and standard deviations. Sample size on any day was usually 15. B-F. Relationship between five morphological characteristics of *T. symmetricus* larvae and days of starvation, with means and standard deviations. Sample size was usually 15.

starved) have been feeding or starving for 1 or 2 days.

To determine whether a set of several morphometric variables could predict the condition of larvae in the 3.30 to 3.55 mm size class, a SWDA was run. Eleven morphometric variables, in which the two predetermined groups (fed and starved) were expected to differ, were entered into the SWDA: 1) HL, 2) ed, 3) bd-1, 4) bd-2, 5) HL/SL, 6) ed/SL, 7) bd-1/SL, 8) bd-2/SL, 9) ed/HL, 10) bd-1/

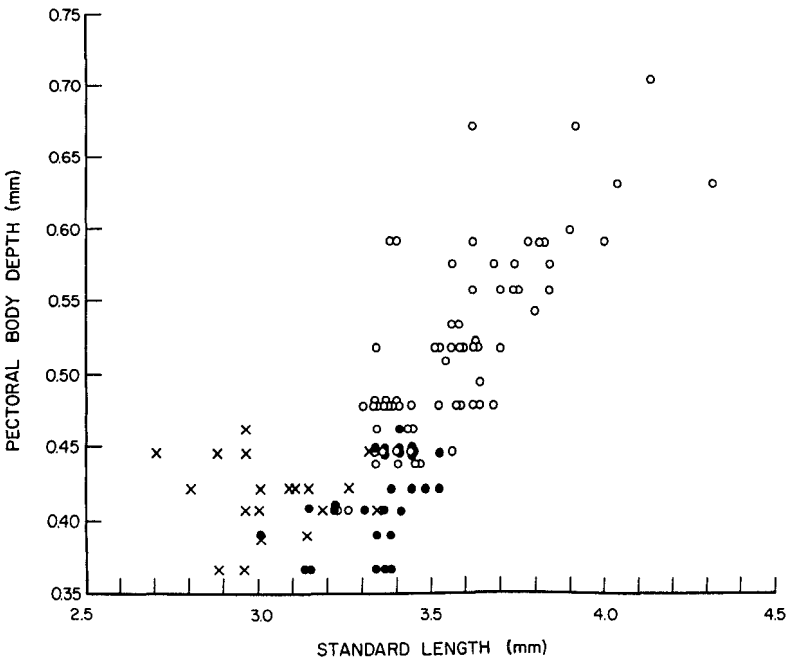


FIGURE 14.—Relationship between pectoral body depth and standard length of *Trachurus symmetricus* larvae which were fed (open circles), starved 1 and 2 days (dots), and starved 3 days (x's).

HL, and 11) bd-2/HL. Standard length was used in ratios and not as a unit to allow discrimination between fed and starved larvae of the same length. To discriminate between the two groups, the analysis selected a set of five variables, listed in the order of selection: 1) bd-2/SL, 2) bd-2/HL, 3) bd-1/HL, 4) ed/SL, and 5) bd-1. With these five variables, 83% of the fed and 86% of the starved larvae in the select size class were correctly classified. When all the larvae, 69 fed and 48 starved (Figure 14), were included in the analysis and the discriminant functions derived from the above five variables were used, 87% of the fed and 94% of the starved were correctly classified.

Comparison of Histological and Morphological Techniques

All larvae classified using morphometric characters were also analysed histologically (Table 1). The histological grading system classified 83% of the larvae which were fed into the healthy group and 83% of the larvae deprived of food for 3 days into the starved group. The histological grades of 1- and 2-day starved larvae were not as well defined; 19% of the cases were graded as healthy, 44% were graded intermediate, and 37% were graded starved. This outcome was similar to results of the initial histological study of 2-day starved larvae reported under Histological Results.

In the morphometric SWDA (Morphological Results), all the starved larvae (1-, 2-, and 3-day starved) were entered into the analysis as one group (n = 48). However, from the above histological grades of the starved larvae, the class should be divided into two groups, early starvation (including 1- and 2-day starved larvae which received all three histological grades) and prolonged starvation (including 3-day starved larvae which were mostly graded as starved). Preliminary laboratory experiments supported this decision. Food was offered to 1-, 2-, and 3-day starved larvae and some 1- and 2-day starved larvae did initiate feeding. No 3-day starved larvae ate food. The

TABLE 1.—Histological classification of *Trachurus symmetricus* larvae.

Item	n	Average histological grade		
		Healthy 2.34-3.00	Intermediate 1.67-2.33	Starved 1.00-1.66
Days fed:				
1	14	12	1	1
2	15	13	2	—
3	15	11	4	—
4	6	6	—	—
5	14	11	3	—
Days starved:				
1	13	3	5	5
2	14	2	7	5
3	18	2	1	15

feeding capability reported for other pelagic fish larvae, after an initial period of starvation, was similar to jack mackerel (Blaxter 1965; Lasker et al. 1970; May 1971). Because of these variables, the morphometric analysis was refined further to differentiate three groups.

Two methods of analyzing the morphometric data for three predetermined groups (fed, early starvation, and prolonged starvation) were examined using 1) all 11 variables previously described, and 2) the set of 5 variables chosen to discriminate between fed and starved larvae morphometrically. In the first test, the SWDA selects the best set of variables to discriminate between the three groups and in the second test, the analysis separates groups with a given set of variables.

The variables selected by the first test (in order of selection) were: 1) bd-2, 2) bd-2/SL, and 3) HL. The set of three variables correctly classified 84% of the fed larvae, 93% of the early starved larvae, and 79% of the prolonged-starved larvae (Test 1, Table 2). The inclusion of two more variables, bd-2/HL and bd-1/HL, did not improve the classification scores. The second test, using the previously chosen set of five variables, correctly classified 84% of the fed larvae, 90% of the early starved, and 74% of the prolonged-starved larvae (Test 2, Table 2). The first test had a better score for both the early and prolonged starvation groups, but either set of variables is a reliable predictor of larval condition.

TABLE 2.—Morphological classification of *Trachurus symmetricus* larvae. The number of larvae in the predicted groups (experimental conditions) is compared with the actual group membership determined by a stepwise discriminant morphometric analysis. Test 1 uses a set of three variables: posterior body depth, posterior body depth divided by standard length, and head length, and Test 2 uses a set of five variables: posterior body depth divided by standard length and head length, pectoral body depth divided by head length, eye diameter divided by standard length, and pectoral body depth.

Experimental conditions	*n*	Fed	Starved	
			1 and 2 days	3 days
		Group membership test 1		
Fed	69	58	10	1
Starved:				
1 and 2 days	29	1	27	1
3 days	19	0	4	15
		Group membership test 2		
Fed	69	58	10	1
Starved:				
1 and 2 days	29	2	26	1
3 days	19	1	4	14

Either the morphological or the histological technique can be used to identify fed larvae and 3-day starved larvae (Table 3). Fifty-three of the 64 (83%) larvae which were offered food were classified as fed (column 2) by the morphometric SWDA and as healthy (column 3a) by the histological method; but, only 44 of these larvae had the score in common (Table 3). Within the 3-day starved group, 14 of the 18 (78%) larvae were correctly classified (column 2) with the morphometric analysis and 15 (83%) were labelled correctly with the histological technique; 13 larvae had the starved score in common. It is difficult to compare the morphological and histological scoring of the early starvation (1- and 2-day starved) group. The morphometric analysis was extremely sensitive for selecting this group, 96% were correctly classified; however, the histological analysis may be more accurate for indicating which larvae in this group can still feed. Five of the 27 (19%) early starved larvae were histologically graded as healthy and may be capable of feeding. It is also possible that intermediate grade larvae (44%) would eat if exposed to food. Eleven of the 12 fish in this intermediate group had a score in common with the morphometric analysis. Intermediate larvae exhibit some tissue degeneration which may be reversible. Additional laboratory experiments are required to determine survi-

TABLE 3.—*Trachurus symmetricus* larvae were fed or starved for 1, 2, or 3 days (column 1). Each larva was classified with the morphometric method (column 2) and the histologic method (column 3). The percent of the larvae correctly classified with each method and the number of larvae correctly classified by both methods is also indicated.

Experimental conditions[1] (1)	Morphometric analysis (2)		Histological analysis (3)		
			Healthy	Intermediate	Starved
		n	53/83%	10	1
Fed *n* = 64	Fed	53/83%	44	8	1
	Starved 1 and 2 days	10	8	2	0
	Starved 3 days	1	1	0	0
		n	5	12/44%	10
Starved 1 and 2 days *n* = 27	Fed	1	0	1	0
	Starved 1 and 2 days	26/96%	5	11	10
	Starved 3 days	0	0	0	0
		n	2	1	15/83%
Starved 3 days *n* = 18	Fed	0	0	0	0
	Starved 1 and 2 days	4	1	1	2
	Starved 3 days	14/78%	1	0	13

[1]The total number of larvae within each experimental group agrees with Table 1 but differs from Table 2 because several larvae were lost during the microtechnique procedure.

val when feeding is only delayed for 1 or 2 days and to ascertain whether tissue degeneration noted during the period of early starvation is reversible.

DISCUSSION AND CONCLUSION

The effects of starvation could be seen throughout the body of larval jack mackerel. There was atrophy of all tissues, with the digestive tract and its associated glands the first tissues affected by starvation. The extent of cellular deterioration increased with time of starvation. Many histological changes, which were associated with starvation in jack mackerel, were similar to changes described in other starving fish larvae: 1) atrophy and cellular and nuclear degeneration of the liver (yellowtail, Umeda and Ochiai 1975; northern anchovy, O'Connell 1976; plaice, Karl Ehrlich pers. commun.), 2) separation of muscle fibers (northern anchovy, O'Connell 1976; herring and plaice, Ehrlich pers. commun.), 3) cellular and nuclear degeneration in the pancreas (yellowtail, Umeda and Ochiai 1975; northern anchovy, O'Connell 1976), and 4) decrease in the size of the epithelial cells in the digestive tract (pike and carp, Kostomarova 1962; yellowtail, Umeda and Ochiai 1975; herring and plaice, Ehrlich et al. 1976; northern anchovy, O'Connell 1976). Some histological changes present in starved jack mackerel larvae, enlargement of the gallbladder and deterioration of the primitive brain cells, have not been reported for other starved fish larvae.

The onset of starvation in jack mackerel larvae was manifested as 1) a change in acinar arrangement of the pancreatic cells and 2) a sloughing of mucosal cells from the midgut into the lumen. These two criteria were shown to be critical variables and were histologically graded to assess the nutritional condition of jack mackerel larvae. O'Connell (1976) reported that the condition of the pancreas was of primary importance for classifying starving northern anchovy larvae. His criteria for good pancreatic condition depended on the abundance of zymogen as well as continuity of cellular structure. The presence of zymogen was not used as a criterion to classify jack mackerel larvae; absence of zymogen was usually associated with feeding, however, the correlation was not consistent. O'Connell also noted mucosal cells in the midgut of larval anchovy but he did not grade this feature. Love (1970) suggested that sloughed midgut cells may be used as an energy source.

During starvation, growth was retarded, larvae shrank, and the soft tissues collapsed causing larvae to look abnormal; the body became bent and thin and the head disproportionately large. An array of larval fish morphometric characteristics have been associated with nutritional condition. A decrease in depth of the larval body has been considered to be an important indicator of starvation although Blaxter (1971) did not find it to be a sensitive character for elongate clupeoid larvae. However, body depth divided by standard length appears to reflect the condition of postlarval anchovy (Nakai et al. 1969) and older, larval northern anchovy (Arthur 1976), but the ratio did not identify feeding and starving post yolk-sac northern anchovy (O'Connell 1976). Nakai et al. (1969) also related the diameter of the posterior digestive tract to condition in postlarval anchovy. The height of the body in the posterior part of the trunk was measured by Honjo et al. (1959) and was found to differ between postlarval anchovy collected from two fishing grounds. Honjo et al. (1959) suggested that the anatomical difference was indicative of a higher availability of food organisms in one area than in the other. Another predictive parameter for the condition of post yolk-sac larvae was the relative condition factor reported by Ehrlich et al. (1976) for herring and plaice. Its estimation involves weighing each larva. All of the above mentioned morphological criteria reflect larval nutritional condition and may be considered reference parameters, but no one criterion accurately identifies larval condition on an individual basis. The lack of sensitivity of a single morphometric indicator may be due to natural variability alone or more probably to the rapid morphological changes which occur in larval fish. For example, Zweifel[3] using the data of Hunter (1976b) has shown that for feeding northern anchovy larvae the condition factor weight/length^3 was neither constant nor monotonic even when food densities were carefully controlled. For variation in food levels (including no food) it would be expected that there should be even more complex relationships.

Even though the histological and morphological techniques have proven to be effective for predicting condition of laboratory-reared animals, both techniques must be field tested with wild-caught

[3]Zweifel, J. 1977. A non-linear model for allometry in larval fish. Unpubl. manuscr., 25 p. Southwest Fisheries Center La Jolla Laboratory, National Marine Fisheries Service, NOAA, P.O. Box 271, La Jolla, CA 92038.

larvae. Evidence of morphological differences between laboratory-reared and sea-caught larvae indicates that it may be difficult to transfer laboratory results to the sea. Blaxter (1968, 1971), for example, found laboratory-reared herring larvae exhibited a greater size variation and a higher condition factor than sea-caught larvae. Balbontin et al. (1973) reported differences in body depth, head depth, and head length between laboratory and wild herring juveniles of the same length. The laboratory-reared animals had a deeper body and a deeper or longer head.

The usefulness of the morphometric discriminant technique will depend upon the consistency of changes in morphological pattern in individuals when feeding and starving. However, even if the changes are not constant, useful indicators of larval survival will not require the exact allocation of all individuals into feeding and starving groups but rather only reasonable estimates of the relative proportions will be needed. My results indicate that a multivariate statistical approach, combining several morphometric measurements in a SWDA, may provide this information. Species that do not yield to a morphological analysis should yield to the histological approach.

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