Midgut Cell Height Defines Nutritional Status of Laboratory Raised Larval Northern Anchovy, *Engraulis mordax*

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ABSTRACT: The height of the midgut mucosal cells was developed as a diagnostic index to assess the past feeding history of larval northern anchovy and to estimate starvation mortality of larval fishes in the sea. The index was compared to traditional histological indices and tested for field use. The height of the mucosal cells was a sensitive index, yielding reliable estimates of larval condition. Because it was sensitive, easy to measure and dependable in formalin-fixed tissue, this diagnostic index should be useful and practical for field work. Additionally, unlike other diagnostic criteria, it resisted the effects of autolysis (withstood prolonged time in the collecting net). Also included is a discussion of the growth characteristics of northern anchovy larvae that experienced a delay in feeding.

Larval mortality may determine recruitment of young fish to a fish stock. A major cause of larval mortality is starvation (Hunter 1976a; Lasker 1981). Several techniques have been recently developed to estimate the proportion of natural mortality caused by starvation. A histopathological technique that uses cellular criteria to identify larval nutritional condition was calibrated in the laboratory (O'Connell 1976; Theilacker 1978; Martin and Malloy 1980), applied to field studies to yield assessments of larval nutritional condition (O'Connell 1980; Theilacker 1986; Margulies 1986; Setzler-Hamilton et al. 1987), and successfully used to estimate rates of starvation-induced mortality (Theilacker 1986). Another technique that employs a morphometric approach generated good predictions of nutritional condition for several larval fish species (Theilacker 1978, 1986; Martin and Malloy 1980; Martin and Wright 1987; Setzler-Hamilton et al. 1987) as did the use of an RNA/DNA index (Buckley 1979; Wright and Martin 1985; Clemmesen 1987; Buckley and Lough 1987; Setzler-Hamilton et al. 1987).

Each of these diagnostic techniques suffers from various constraints. To develop histological criteria, an accurate representation of the structure of live tissue is needed for identifying larval fish condition. The usefulness of these criteria depends on the tissue quality of the field-collected specimens. For many fishes (northern anchovy and striped bass, *Morone saxatilis* (O'Connell 1980; Setzler-Hamilton et al. 1987)), autolytic tissue decomposition occurs within 2–3 minutes after sampling. Because routine ichthyoplankton collections usually take 21 minutes (Smith and Richardson 1977), special plankton collections are required for histopathology. Additionally the routine solution (3–5% formalin) used to preserve plankton does not adequately preserve cellular structure of larval fishes and special solutions (Bouin's fixative) must be used (O'Connell 1976; Theilacker 1978). The morphometric analysis requires extensive calibration studies to estimate the effect of the net and preservatives on shrinkage of body parts because the morphometric indices are very sensitive to shrinkage (Theilacker 1980; 1986). The RNA/DNA index must be calibrated for temperature effects and animal age (Ota and Landry 1984; Buckley 1984; Buckley and Lough 1987); however, there are other considerations that may limit its application. Furthermore, RNA/DNA is generally regarded as an index of potential growth (protein synthesis rates) not starvation mortality.

In March of 1985, we anticipated applying the histopathological technique developed by O'Connell (1976) to estimate starvation rates of northern anchovy collected at two sites off the coast of California (see Owen et al., 1989). However, when aboard ship, we found that it was impossible to preserve the larvae within the required 2–3 min time period (needed to maintain tissue quality) and take a sample that was representative of the zone inhabited by larval anchovy. We were unable to use the established histological criteria because it took us 5 or more minutes to sample larval anchovy from 50 m to the surface and process our collection. Thus, to estimate anchovy starvation rates, we needed to develop

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new criteria that were stable for a longer period.

Here we describe and evaluate a diagnostic index, the height of midgut mucosal cells of larval northern anchovy. The index is sensitive to feeding history, resistant to autolysis (withstands prolonged time in a collecting net), and simple to measure. It is reliable for fish fixed in formalin, the commonly used fixative for field collections.

METHODS

Experimental Design

We raised larval northern anchovy under different feeding conditions to produce specimens that exhibited various health states. These larvae were used to describe growth, determine the dominant midgut height measurement within each feeding treatment, evaluate the midgut index, and estimate response times. In a second series of experiments, groups of fed and starved fish were treated with nets in a manner designed to simulate plankton collection methods in the sea. These studies were used to evaluate how useful the new criterion would be in a field situation.

Rearing Treatments

Five groups of anchovy were raised at 15.5°C for three weeks in 100 L containers. The eggs, stocked at 10/L, were collected from a hormone-injected broodstock maintained at the Southwest Fisheries Center aquarium (Leong 1971). We fed the control group ad libitum on *Gymnodinium* and *Brachionus* (Lasker et al. 1970; Theilacker and McMaster 1971) at yolk absorption (four days after hatching at 15.5°C; hatching = day 0), and delayed feeding 1–4 days in the other groups. Fish were sampled daily from the fed, starved, and delay-fed treatments and preserved in Bouin's fixative (Table 1).

Field Simulation Experiments

One group of northern anchovy was fed for 3 days and one was starved for 3 days. We treated samples from each group of 7 d old fish in a net by flushing the submerged net with 15.5°C seawater for 0, 2, 5, 10, 15, 20, and 25 minutes (see Theilacker 1980 for details of this method). After the net treatment, fish were preserved in either 5% buffered formalin (standard shipboard fixative) or Bouin's solution (required fixative for histopathology).

Histological Preparation

We measured the standard length (SL) of preserved larvae to the nearest 0.01 mm and subsequently prepared them for histopathology using standard microtechniques (O'Connell 1976). Tissue was dehydrated, embedded in paraffin, serially sectioned at 6 μm in the sagittal plane, mounted, and stained with hematoxylin and eosin.

Measurement of Midgut Cell Height

The midgut is the major part of the intestine of

TABLE 1.—Standard length (SL) and

	Fed					
	Growth			Midgut		
Age (days)	*n*	SL[1] $\bar{x}$ mm	SD	*n*	SL[1] $\bar{x}$ mm	mght[2] $\bar{x}$ μm
4	FED					
5	32	3.95	0.23	10	3.93	19.63
6	34	3.92	0.21	17	3.87	20.13
7	26	4.33	0.24	13	4.29	19.52
8	33	4.59	0.46	19	4.67	19.13
9	33	5.29	0.40	10	5.26	22.13
10	38	5.54	0.33	10	5.85	21.88
11	26	6.22	0.47	10	6.09	21.13
12	55	6.36	0.48	10	6.52	20.75
13	38	6.85	0.43			
14	36	7.20	0.54			
17	66	7.22	0.74			
19	35	7.43	0.80			
Total *n*	452			99		

	Starved 3 d/fed					
	Growth			Midgut		
Age (days)	*n*	SL[1] $\bar{x}$ mm	mght[2] $\bar{x}$ SD	*n*	SL[1] $\bar{x}$ mm	mght[2] $\bar{x}$ μm
4	STARVED					
5	STARVED					
6	STARVED					
7	28	3.37	0.22	13	3.29	10.19
8	25	3.35	0.15	13	3.35	9.90
9	29	3.79	0.24	10	3.83	11.38
10	26	3.90	0.25	17	3.93	12.28
11	36	3.92	0.19	11	3.96	14.21
12	34	4.00	0.35	10	4.00	14.88
13	31	4.25	0.38			
14	26	4.63	0.41			
17	31	5.27	0.45			
19	30	5.51	0.68			
24	34	6.80	0.90			
Total *n*	330			74		

[1] SL taken on Bouin's fixed larvae
[2] Midgut cell height (see text)

larval northern anchovy; it extends from behind a constriction at the pylorus to another constriction at the beginning of the hindgut (Fig. 1). We measured the cell heights under a light microscope using the histological preparations. Epithelial cells of the midgut tend to decrease in height from the anterior part to the posterior end. We chose the midpart of the midgut (from 2/5 to 3/5 of the entire length) as the measurement site. The area is easily located in histological sections that are cut parallel to the median plane. The dorsal and ventral rows of midgut cells appeared in these sections. We measured the cells of the ventral row that were larger than the dorsal row, probably because of greater absorptive activity. We selected a section having four to six neighboring cells in which the nuclei, brush border, and cell base were clearly defined; brush border may be indistinct in larvae of poor condition. Measurements were taken from the luminal surface of the brush border to the cell base delimited by a basement membrane, using a micrometer attached to an eye lens. Usually, in sagittal sections, the cell heights are not so varied within the middle part of the midgut. If, however, there were slight differences between cells due to a slight angle of the section, we took an average of several measurements.

midgut cell height (mght) statistics for fed northern anchovy by diet and age.

	Starved 1 d/fed						Starved 2 d/fed					
	Growth			Midgut			Growth			Midgut		
Age (days)	*n*	SL[1] $\bar{x}$ mm	SD	*n*	SL[1] $\bar{x}$ mm	mght[2] $\bar{x}$ µm	*n*	SL[1] $\bar{x}$ mm	SD	*n*	SL[1] $\bar{x}$ mm	mght[2] $\bar{x}$ µm
4	STARVED						STARVED					
5	33	3.55	0.20	12	3.95	14.27	STARVED					
6	47	3.59	0.37	15	3.60	15.83	27	3.58	0.22	18	3.57	13.47
7	61	3.46	0.23	11	3.45	14.89	33	3.39	0.18	10	3.35	11.88
8	23	3.81	0.31	10	3.77	16.00	31	3.58	0.21	17	3.60	12.21
9	49	4.34	0.46	11	4.79	19.32	18	4.07	0.21	10	4.02	14.63
10	46	4.90	0.39	10	4.80	18.63	38	4.25	0.32	11	4.19	14.77
11	55	5.09	0.56				41	4.58	0.31	10	4.60	15.63
12	71	5.56	0.47				28	4.95	0.39			
13	47	5.86	0.45				27	4.99	0.41			
14	61	6.32	0.62				46	5.63	0.45			
17	121	6.84	0.86				33	6.25	0.73			
19	26	7.19	0.95				39	6.65	0.93			
Total *n*	640			69			361			76		

	Starved 4 d/fed					
	Growth			Midgut		
Age (days)	*n*	SL[1] $\bar{x}$ mm	SD	*n*	SL[1] $\bar{x}$ mm	mght[2] $\bar{x}$ µm
4	STARVED					
5	STARVED					
6	STARVED					
7	STARVED					
8	19	3.40	0.19	10	3.44	10.51
9	14	3.58	0.22	6	3.59	10.00
10	6	3.66	0.12	6	3.66	8.13
11						
12						
13						
14						
17						
19						
24						
Total *n*	39			22		

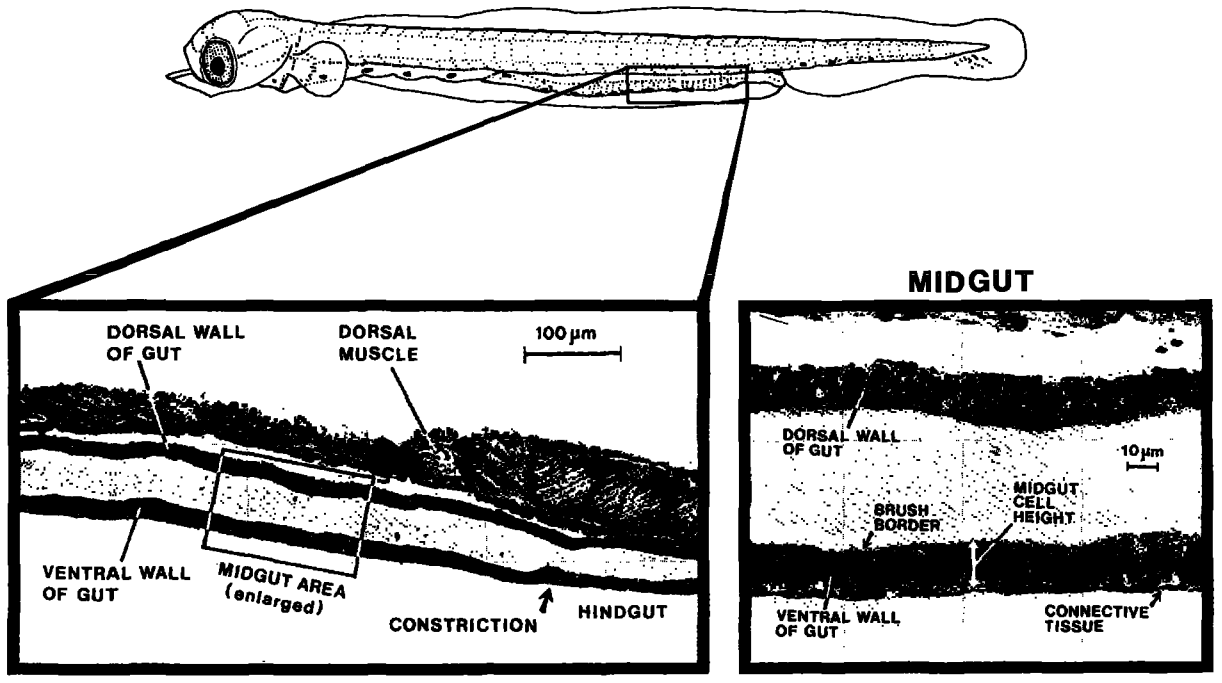

FIGURE 1.—*Engraulis mordax* larva showing measurement site for midgut cell height.

Evaluation of Midgut Criteria

To evaluate the capacity of the midgut cell height measurement to classify larval anchovy condition, we graded 5–10 larvae taken daily from 3 treatments (fed, feeding delayed 1 day, and starved) using both O'Connell's (1976) histological criteria and the midgut cell height. We assumed that the histological criteria (the sum of the cellular grades for the brain, cartilage, notochord, musculature, liver, and midgut) would yield the best estimate of larval condition, and we compared the power of each index to predict past feeding history.

RESULTS

Diet-Induced Differences in Growth

Growth of the fed control group was 0.41 mm/d, similar to published reports for northern anchovy raised in the laboratory (Kramer and Zweifel 1970; Hunter 1976b; Theilacker 1987). Larvae experiencing a feeding delay of 1, 2, and 3 days were smaller at 10.8 days than larvae not experiencing a delay, and as the starvation interval increased, their final size decreased (Table 2). For the test of significance of size at age, P was <0.01 for all delay combinations except the starved 3 d/fed (S3/F) vs. the 4 d delay in feeding

TABLE 2.—Diet-induced differences in growth of northern anchovy at a common age and at a common feeding period (n's for common feeding period slightly less than n's for common age).

		Common age			Common feeding period		
Diet	n	Age d	Feeding period d	SL $\bar{x}$ mm	Age d	Feeding period d	SL $\bar{x}$ mm
Fed	452	10.8	6.8	5.58	10	6	5.51
Starved							
1 d/fed	640	10.8	5.8	4.87	11	6	4.96
2 d/fed	394	10.8	4.8	4.48	12	6	4.75
3 d/fed	372	10.8	3.8	4.00	13	6	4.32
4 d/fed	114	10.8	2.8	3.98	—	—	—

(S4/F) where $P = 0.8$, indicating that delaying feeding affected the final fish size at a given age (ANOVA; Table 2).

Progressively increasing the periods of starvation before feeding also caused a decrement in the growth rate after feeding resumed (Fig. 2; Table 2). Larvae were unable to compensate for a delay in feeding at the onset, and fish length at an age where all fish had been feeding for 6 days differed for each treatment [$P = < 0.01$ for all diet combinations except starved 1 d/fed (S1/F) vs. 2 d delay in feeding (S2/F) where $P = 0.08$ (ANOVA; Table 2)]. Growth rates for the 6 d period ranged from 0.41 mm/d for the fed group, 0.39 mm/d for 1 d delay in feeding (S1/F), 0.34 mm/d for 2 d delay (S2/F), 0.31 mm/d for 3 d delay (S3/F). Length was transformed to natural logs for the ANOVAs. The test for the equality of slopes showed that all slopes differed ($P = <0.0001$ for all combinations). Thus, delaying feeding affected the final fish size and caused a decrement in the growth rate after feeding resumed.

Evaluation of Midgut Cell Height Criterion

For a subset of northern anchovy larvae taken from the treatments where larvae were fed, starved, and feeding was delayed 1 day (S1/F), we compared the number of larvae correctly classified to feeding treatment by the midgut cell height and by the traditional histological index (O'Connell 1976). To define the midgut cell height interval for each feeding treatment, we selected the midpoint between successive cell height means (Table 3) as the interval breakpoint for predicting feeding history from cell height. The midgut cell height was correlated with the histological score (Fig. 3, $n = 38$, $P < 0.001$, t-ratio = 25.85). Both the cell height and the histological score correctly classified all of the subset larvae that were fed to the correct group and 78 and 79% of the starved larvae to the correct group. On the other hand, for the delayed feeding group (S1/F), the midgut cell height was a better predictor of feeding history than the histological score. The midgut measurement classified 78% correctly whereas the histological score classified only 50% correctly.

After the apparent success of this evaluation, we used the midgut cell height data set (Table 1; Fig. 4) to establish the criteria needed to define and calibrate the midgut cell height measurement for predicting feeding history and for calculating starvation rates of anchovy larvae in the sea.

Diet and Midgut Cell Height Categories for Larvae <4.0 mm

We defined the diet and cell height categories for first-feeding larvae (<4.00 mm SL) which are vulnerable to starvation. The height of the

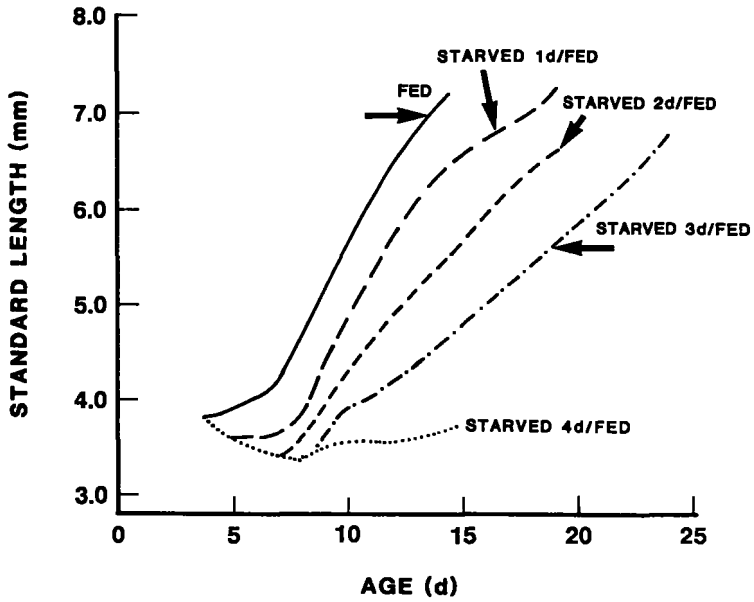

FIGURE 2.—Growth of *Engraulis mordax* larvae under different feeding conditions.

TABLE 3.—Midgut cell height (mght) statistics for six diets by northern anchovy size.

Size Interval (mm)	Fed n $\bar{x}$ mght (μm) SD	Starved 1 d/fed n $\bar{x}$ mght (μm) SD	Starved 2 d/fed n $\bar{x}$ mght (μm) SD	Starved 3 d/fed n $\bar{x}$ mght (μm) SD	Starved 1 & 2 d n $\bar{x}$ mght (μm) SD	Starved 3 & 4 d n $\bar{x}$ mght (μm) SD	All
<4.00	20 20.50 2.27	31 15.56 3.18	33 12.58 2.12	41 11.77 2.44	28 13.48 2.81	24 10.38 2.00	176
4.00–4.99	40 19.47 3.67	18 17.36 3.58	27 15.28 2.51	20 13.68 2.64			105
5.00–5.99	21 20.95 2.71	8 20.63 3.78					29

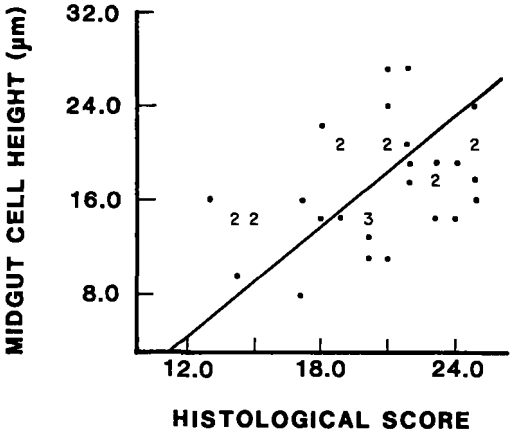

FIGURE 3.—*Engraulis mordax* larvae, regression of midgut cell height measurement on histological score.

midgut cells decreased from the fed to the starved state. The average height of the midgut cells in fed fish <4.00 mm was 20.5 μm. The height averaged 15.6 μm when feeding was delayed one day, 12.6 μm for a 2 d delay and 11.8 μm for a 3 d delay in feeding. The mean midgut height for larvae starved 1 and 2 days was 13.5 μm and for the 3 and 4 d starved group was 10.4 μm (Table 3; Fig. 5).

The height of midgut cells of the fed group (fed) and those where feeding was delayed 1 day (S1/F) was different from all other groups (ANOVA P's < 0.01; Table 4). Fish starved 3 and 4 days (S3&4) also differed from all others (P's < 0.05 level). On the other hand, midgut cell heights of larvae starved 1 and 2 days (S1&2) were not different from those of larvae in which feeding was delayed 2 or 3 days (S2/F and S3/F) (P's = 0.16 and 0.17; Table 4). The t-test matrix indicated four distinct diet and cell height

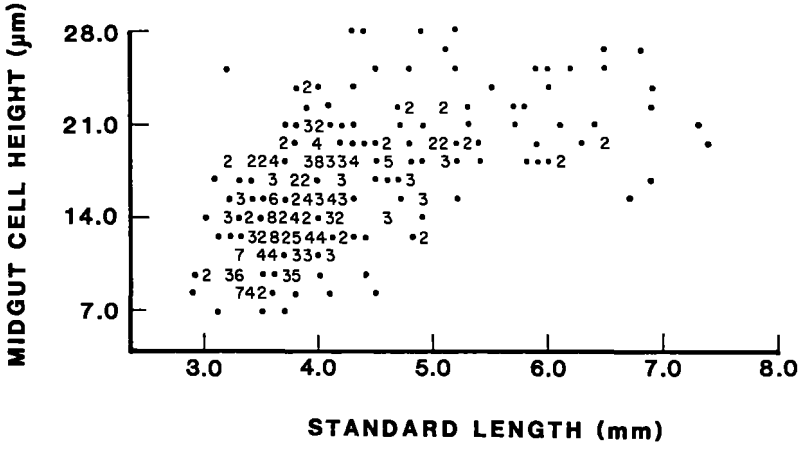

FIGURE 4.—Midgut cell height measurements for *Engraulis mordax* larvae that were raised in the laboratory. Fish were either fed, starved, or feeding was delayed.

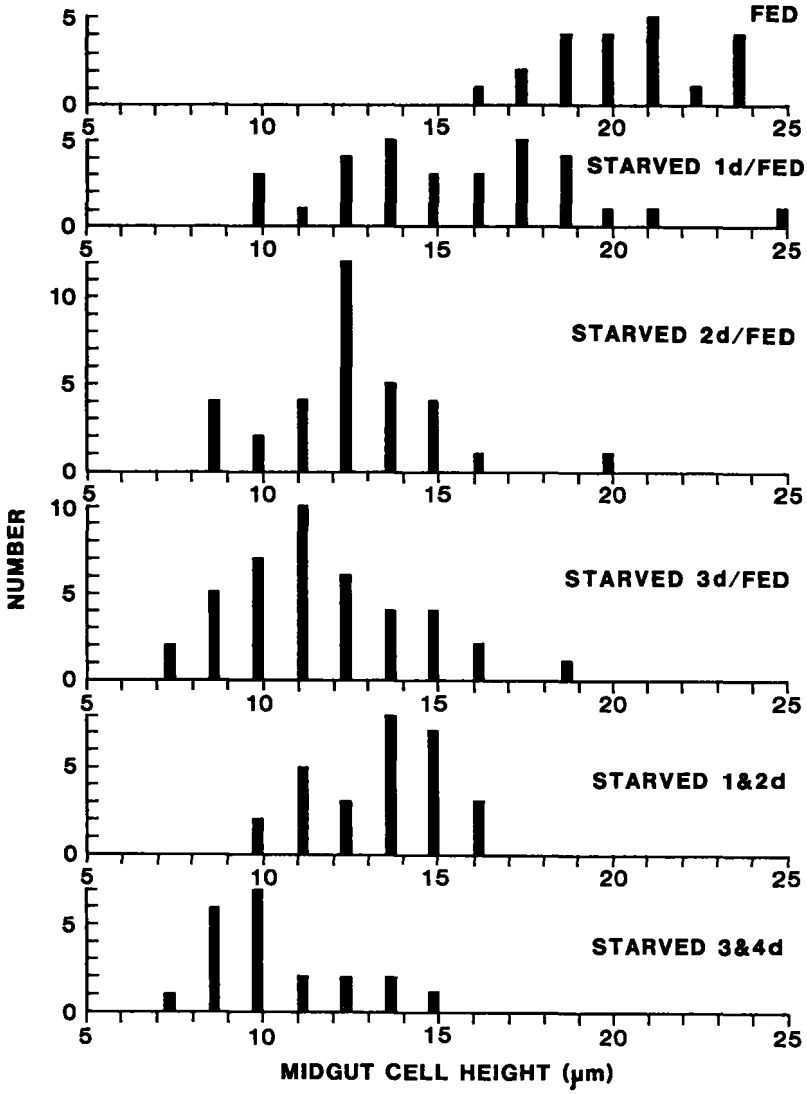

FIGURE 5.—Midgut cell height measurements for *Engraulis mordax* larvae <4.00 mm raised using various feeding conditions.

TABLE 4.—Paired comparison of midgut cell heights for six diets for northern anchovy <4.00 mm SL: *t*-test matrix and probabilities ($P = < 0.01$, **; $P = < 0.05$,*; $P = > 0.05$, value).

		Starved				
	Fed	1 d/fed	2 d/fed	3 d/fed	1 & 2 days	3 & 4 days
Fed	0.0000					
Starved						
1 d/fed	− 6.8438**	0.0000				
2 d/fed	−11.1211**	−4.7522**	0.0000			
3 d/fed	−12.7320**	−6.3433**	−1.3731/.17	0.0000		
1 & 2 days	− 9.5331**	−3.1765**	1.4029/.16	2.7802**	0.0000	
3 & 4 days	−13.1632**	−7.4916**	−3.2143**	−2.1187*	−4.3834**	0.0000

categories for first-feeding larvae: 1) a fed category, 2) a category for 1 d delay in feeding (S1/F), 3) an intermediate category (S2/F + S3/F + S1&2), and 4) a starved category (S3&4).

Calibrations for Field Studies

To estimate daily starvation rates in the sea using cell height, an approximation of the dura-

tion or response time for each size, diet, and cell height category is needed. The duration is the number of days that larvae belonging to the size, diet, and cell height category remain within that category. Daily rates in the sea (estimates of numbers of larvae per day) are determined by dividing the number of field-collected larvae classified to each category by the category duration. See the following section on "Applying the Calibration Criteria" for a more detailed explanation of this procedure.

Duration for Fed Category, <4.00 mm SL

Fed larvae began to eat at 3.8 mm (preserved length), yet there was little or no growth for 2 days (Fig. 2). Even though, on the average, the fed anchovy grew at 0.41 mm/d, when the delay in growth was considered, the resulting duration for the <4.00 mm fed group was 2.5 days. Thus it took 2.5 days for the fed fish to move out of this size, diet, and cell height category. This initial lag in growth was reported earlier for northern anchovy (Theilacker 1987).

Durations for Intermediate Categories, <4.00 mm SL

The durations were 5 days for diet groups where feeding was delayed 1 and 2 days (S1/F and S2/F). The group that was starved 3 days before feeding (S3/F) remained within the midgut cell height interval for 8 days (Table 1). The duration for larvae that were starved for 1 and 2 days (S1&2) was 2 days.

Duration for Starved Category, <4.00 mm SL

The duration for the group starved 3 and 4 days (S3&4) was 2 days. Northern anchovy larvae died after starving 3–4 days. No larvae belonging to the starved category were larger than 4.0 mm.

Field Feasibility Study

Because larval fish tissues decompose rapidly due to autolysis (Theilacker 1978; O'Connell 1980), we tested the effects that the prolonged processing periods encountered at sea have on the integrity of the midgut cells. In this study, after 5 minutes in the net, the condition of both formalin- and Bouin's-fixed larval tissues could not be graded using traditional histological criteria due to indistinct nucleoli, diffused nuclei, and intercellular spaces. However, measurement of midgut cell height was still possible after 25 minutes.

The height of the midgut cells, whether measured in formalin- or Bouin's-fixed individuals, showed little change in height over the 25 min processing period (Fig. 6a–d; slope b is not significant from 0; $P = 0.494$ and 0.596 for the formalin group and 0.077 and 0.039 for the Bouin's group, Table 5). Although the height of midgut cells is stable within each diet and 1 mm interval size category (Table 3), we weighted the midgut cell height by fish size for this analysis because it included both fed and starved larvae ranging between 2.8 and 5.6 mm. When the midgut height of fish belonging to all diets was plotted over the 2.8–5.6 mm size range, midgut cell height increased linearly with size (Fig. 4). And, because the fish shrink in the collecting net and the amount of shrinkage is related to the time elapsed in the net, we adjusted fish lengths in this analysis to equal "capture" size using the model developed by Theilacker (1980).

Applying the Calibration Criteria

In practice, when applying this analysis to the field to estimate starvation-induced mortality rates (Owen et al. 1989), it was deemed necessary to use only three diet categories (fed, intermediate, and starved) instead of the four distinct categories determined by ANOVA (Table 4), and discussed earlier. Because the durations were 5 days for both diets where feeding was delayed 1 and 2 days (S1/F and S2/F), there was no need for the S1/F larvae to be a separate, fourth category. Thus we included S1/F in the intermediate category and selected 5 days as an average duration for the <4.00 mm larvae in this category.

The cell-height intervals for the three categories were the midpoints between the means of the group cell heights determined for the laboratory-reared larvae. We regarded all larvae with a midgut height measurement >17.5 μm as belonging to the fed category; the break between the intermediate and starving categories was 11.25 μm. This classification scheme correctly identified 95% of the fed larvae, 77% of the starved larvae, and 74% of the intermediate larvae <4.00 mm SL.

To calculate the starvation rates of northern anchovy larvae in the sea, we measured the length of each field-collected larva (corrected for

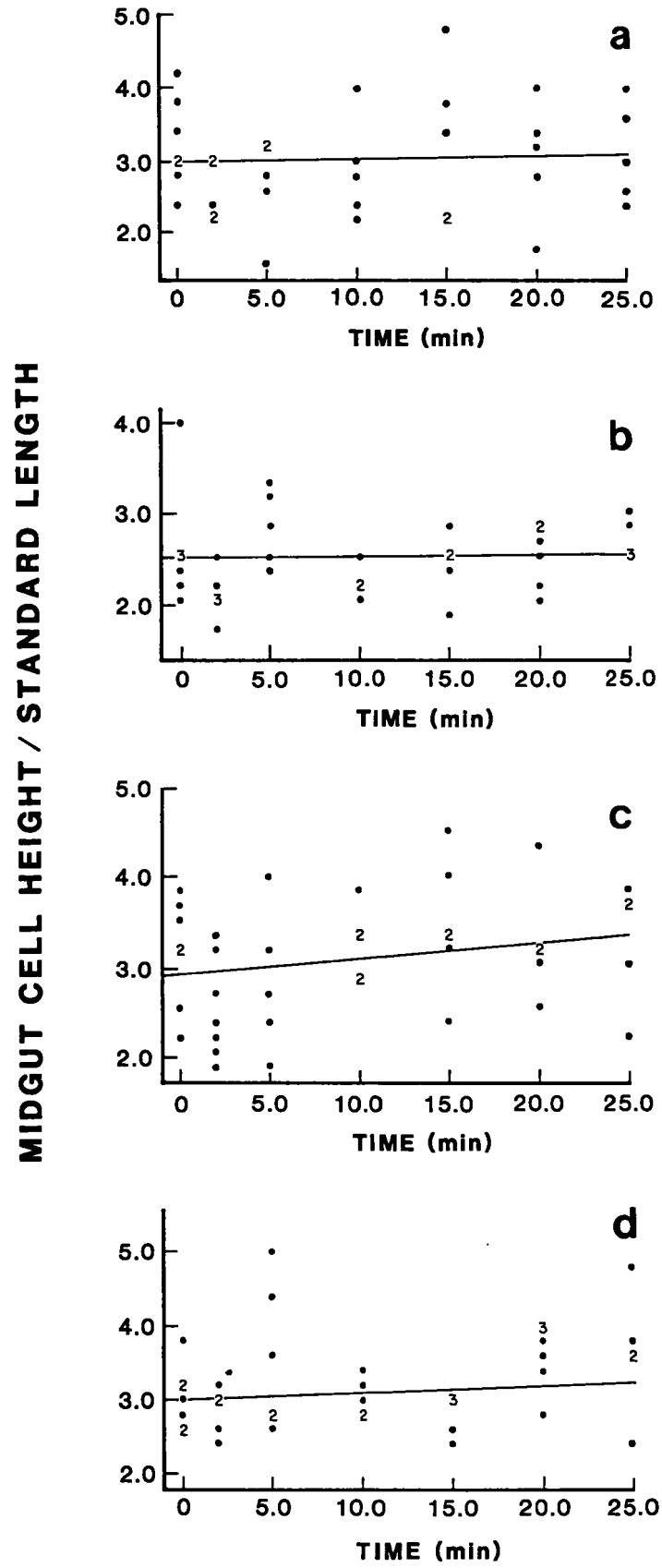

FIGURE 6.—a) Change in midgut cell height measurement over time for formalin-fixed *Engraulis mordax* larvae that were fed. b) Change in midgut cell height measurement over time for formalin-fixed *Engraulis mordax* larvae that were starved. c) Change in midgut cell height measurement over time for Bouin's-fixed *Engraulis mordax* larvae that were fed. d) Change in midgut cell height measurement over time for Bouin's-fixed *Engraulis mordax* larvae that were starved.

shrinkage due to time-in-net and preservation (Theilacker 1980)) and the height of the midgut cells. The adjusted SL and absolute midgut cell height classifies each larva into a size, diet, and midgut cell height category. The numbers of larvae belonging to each category are divided by the category durations to yield the number of larvae per day per category. To estimate the percentage of larvae dying per day due to starvation, the starved category is divided by the total number. An example of these manipulations in Table 5 (taken from Owen 1989; this issue) shows that mortality due to starvation was estimated to be 24%/d for first-feeding anchovy larvae collected off southern California.

(taken on sagittal sections that were prepared for histological examination) defines past feeding history. Kostomarova (1962) also measured the height of intestinal cells of larval fishes to evaluate their state of health. Although she found a relation between fish condition and size of midgut cells, the relation was not quantified. In our study, we establish criteria for discriminating the condition of laboratory-raised larvae using the midgut cell height and for estimating rates of starvation in the field.

To validate this technique, it was necessary to measure the effects on the midgut cells of the prolonged processing periods, commonly encountered at sea. The elapsed time for standard

TABLE 5.—Histological condition of larval northern anchovy off southern California. (From Owen et al. 1989: table 7.)

		INSHORE SITE I				
		Categories				
SL mm		Fed "healthy"	Intermediate	Starved	Total	%/d
<4.00	Number[1]	28	17	9		
	Duration[2]	2.5	5	2		
	No./d[3]	11.2	3.4	4.5	19.1	24

[1]Number of field-collected larvae belonging to each size, diet, and midgut-cell-height category.
[2]Response times for each size, diet, and midgut-cell-height category (see text).
[3]Number/duration = No./d.

To complete these manipulations for the next size class, 4–<5 mm, fed and intermediate larvae were discriminated using the same cell height measurement, 17.5 μm, and starved larvae did not grow into this size class (Table 3). The duration for the fed category was 2.5 days and for the intermediate category averaged 3 days. The 2.5 d duration for the fed category was obtained by dividing the size-class interval by the growth rate, 0.41 mm/d. Larvae from the delayed-feeding treatments that belonged to the intermediate category outgrew this size class within 2–4 days (Table 1). Some larvae belonging to the group where feeding was delayed 1 day (S1/F) had recovered and entered the fed category by the time they grew to 4.00 mm.

Cell heights cannot be measured for northern anchovy larger than 6 mm SL as the midgut folds, increasing the absorptive area and making cell heights difficult to measure.

DISCUSSION

The height of northern anchovy midgut cells

ichthyoplankton net hauls to 225 m is 21 minutes, and additional time is needed for washing down the net and for sample preservation (Smith and Richardson 1977). To quantify the effect of collecting time and processing time on autolysis, we simulated the net collection and used a matrix of 8 time periods, ranging from 0 to 25 minutes, followed by preservation in either of two fixatives, Bouin's or formalin. Initially, alcohol also was tested because we anticipated using the same fish to study both growth and condition. (80% alcohol is routinely used to preserve otoliths.) Unfortunately, we found alcohol useless for histopathology.

The height of the midgut cells did not change over the 25 m net treatment, but the standard length decreased. Thus, for field collections, it is not necessary to adjust midgut height for time-in-net, but it is important to correct standard length of field-collected larvae for shrinkage in order to allocate the larvae into the correct length categories. The model (Theilacker 1980) used to adjust length was generated for formalin-preserved anchovy. Perhaps the slight

increase noted in the midgut index (midgut cell height/SL) of the Bouin's-fixed specimens (Fig. 6c, d) indicates that the model is overcorrecting for Bouin's. That is, the additional shrinkage caused by fixation (shrinkage in addition to that due to abrasion by the collecting net) is less when Bouin's is used for the final fixative than when formalin is used. This logic, however, is hard to reconcile because Bouin's, made with 20% formalin, is stronger than the 5% formalin solution we used.

Starved fish may be shrinking more during the net treatment than their fed counterparts, further complicating the interpretation of the shrinkage adjustment. But we cannot be certain that this occurred because we did not follow individual larvae during the net treatment. Yet there was a significant decrease in length of starved larvae when the adjusted lengths (corrected to "live length") were regressed and plotted over time (Fig. 7b; Table 6). The decrease in length was not evident for the fed larvae (Fig. 7a). This apparent difference in shrinkage rates between fed and starved larvae needs to be tested.

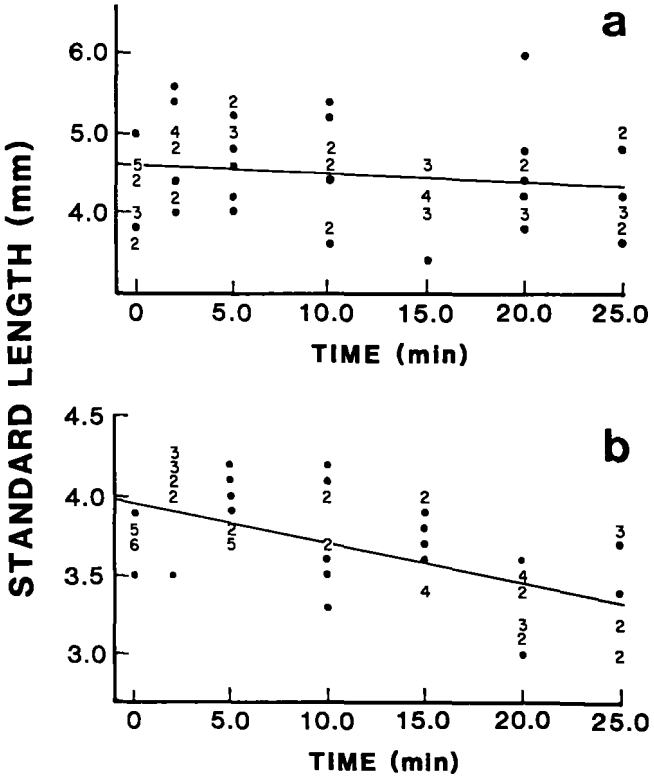

FIGURE 7.—a) Change in standard length over time for fed *Engraulis mordax* larvae that were fixed in formalin and Bouin's. b) Change in standard length over time for starved *Engraulis mordax* larvae that were fixed in formalin and Bouin's.

TABLE 6.—Coefficients for regressions of midgut indexes (midgut cell height/adjusted standard length (mght/SL)) and of adjusted standard length (SL) with elapsed time-in-net (t).

Diet	Measurement	Fixative	Mght/SL = a + bt a	b	P	Reference figure
Fed	Mght/SL	5% formalin	2.8941	0.0092	0.494	6a
Starved	Mght/SL	5% formalin	2.4869	0.0041	0.596	6b
Fed	Mght/SL	Bouin's	2.8961	0.0212	0.077	6c
Starved	Mght/SL	Bouin's	2.9839	0.0231	0.039	6d
Fed	SL	Formalin & Bouin's	3.9605	−0.0251	0.000	7a
Starved	SL	Formalin & Bouin's	4.5813	−0.0124	0.079	7b

Incidental information from delayed feeding experiments (Table 1) revealed that, depending on diet, age of 5 mm northern anchovy ranged between 9 and 19 days. This 10 d range in age at size (due to feeding history) has important implications for size-at-age mortality estimates.

In conclusion, our study indicates that the absolute midgut cell height of northern anchovy larvae is a practical criterion to use for estimating rates of starvation. The cell height measurement yields reliable estimates of feeding history, is resistant to autolysis, and is reliable in formalin-fixed specimens. Thus, the special ichthyoplankton tows and preservatives required for a histopathological index are not needed when the midgut index is used. The midgut index is as sensitive to nutritional conditions as the histological index and does not require the rigorous calibration needed for the morphometric technique (Theilacker 1986; Setzler-Hamilton et al. 1987). Also the midgut character is much easier and faster to measure than scoring the histological features of northern anchovy tissues, and it does not require a solid background in histology for the person scoring. Because of these features, the midgut index is practical for routine estimates of starvation rates of larvae in the sea. In addition, unlike previous methods, the duration of tow is not a constraint because the character does not degrade with time and a sample representative of the water column can be taken.

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