

CHANGES IN BODY MEASUREMENTS OF LARVAL
NORTHERN ANCHOVY, *ENGRAULIS MORDAX*, AND OTHER FISHES
DUE TO HANDLING AND PRESERVATION

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ABSTRACT

The relation between northern anchovy length and body parts was compared for live and laboratory-preserved larvae as well as larvae treated in a net to simulate field collection conditions. Larvae were damaged by net abrasion, and those netted before preservation shrank more than those that were laboratory preserved (that is, larvae pipetted directly into preservative). Shrinkage of net-treated individuals decreased with age and increased with handling time, but shrinkage of laboratory-preserved larvae was constant for the size class studied. The results show that morphological differences reported for laboratory-reared and sea-caught larvae of the same length may result from the method of handling larvae prior to preservation.

To describe life stages of larval fish, field and laboratory studies rely on length measurements of preserved sea-collected and preserved laboratory-collected larvae. Sea-collected larvae incur mechanical damage, abrasion from the collecting net and from other plankters, while the net is being towed and washed down (Ahlstrom 1976). When damaged, delicate larvae shrink. This initial shrinkage usually occurs before death, and this shrinkage is compounded by preservative shrinkage (Blaxter 1971). Conversely, laboratory handling of larvae prior to preservation is less damaging than net abrasion. In the laboratory, individual larvae are usually transferred by pipette or beaker to preservative, and they die and shrink in the preservative.

Laboratory-reared fish larvae differ morphologically from sea-caught larvae. Body depth of wild herring, *Clupea harengus*, larvae was smaller than that of starved laboratory-reared larvae of the same preserved length (Blaxter 1971). Ryland (1966) observed that sea-sampled larval plaice, *Pleuronectes platessa*, were smaller than laboratory larvae at a comparable stage and suggested that a factor for shrinkage was needed to equate field with laboratory measurements. I have noticed a similar discrepancy in preserved length of sea-collected yolk-sac larvae and laboratory-hatched and preserved yolk-sac larvae of the jack mackerel, *Trachurus symmetricus* (Theilacker

unpubl. data). These morphological differences may be the result of the method of handling (laboratory capture or net capture) prior to preservation. Since it is necessary to compare animals at the same developmental stage to relate laboratory larval fish studies to the field, there is a need to intercalibrate field (preserved) and laboratory (live and preserved) larval fish measurements.

METHODS

Adult northern anchovy, *Engraulis mordax*, maintained in the Southwest Fisheries Center's aquarium, were spawned by hormone injection (Leong 1971). I reared the anchovy larvae at 15.5° C on cultured food organisms (*Gymnodinium splendens*; rotifers, *Brachionus plicatilis*; and copepods, *Tisbe furcata*) in 100 l tanks using methods described by Lasker et al. (1970), Theilacker and McMaster (1971), and Hunter (1976).

I considered several factors that could affect shrinkage of larval fish: 1) size, 2) type of fixative, 3) treatment of larvae before fixation (net or laboratory capture), and 4) duration of net retention. Larval fish measurements fit into four treatment categories (Figure 1): 1) live, 2) laboratory pipetted and preserved, 3) net treated, and 4) preserved after net treatment (equivalent to "field-collected" larvae). Five body measurements (in millimeters) were taken: standard length (SL), tip of upper jaw to perpendicular at end of notochord; head length, tip of upper jaw to cleithrum; body depth at the pectoral (not measured

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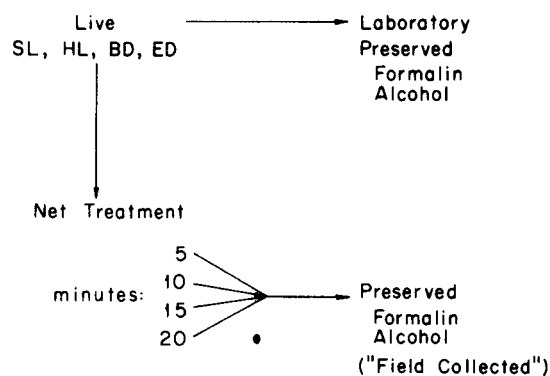


FIGURE 1.—Experimental design. Four measurements of larval northern anchovy were taken to estimate shrinkage during handling treatments: standard length, SL; head length, HL; body depth at the anus, BD; and eye diameter, ED.

for northern anchovy); body depth at the anus; and eye diameter. I kept track of individual larvae during all treatments and determined body part shrinkage on an individual basis. The same larva could be measured as many as six times; e.g., a "field-collected" larva was measured live, after four time intervals in the net, and again after preservation. However, not all net-treated larvae were measured for four time intervals. I used several preservatives: Bouin's fixative, usually used for histological studies; 5% buffered Formalin² (2.2% formaldehyde), the standard ichthyoplankton-survey preservative (Ahlstrom 1976; Smith and Richardson 1977); and 80% ethyl alcohol, preservative for otoliths (Methot and Kramer 1979). In treatments (2) and (4), larvae were kept in preservative for 4-5 wk before remeasuring.

As an example of laboratory handling procedures, I have included results from ongoing studies on morphology of jack mackerel and Pacific barracuda, *Sphyraena argentea*, larvae. Eggs of jack mackerel and Pacific barracuda were collected 30-50 km off the coast of southern California in June and July 1977, and rearing procedures were the same as for northern anchovy. Laboratory handling in this study consisted of pipetting live larvae 1) onto a slide for measurement and 2) into preservative. Time spent handling was an important factor affecting shrinkage. Larvae shrink during the measuring process. In this study, all live and laboratory shrinkage mea-

surements include about a 30 s handling time. Some scientists measure laboratory-reared larvae only after preservation; these larvae are probably handled <30 s.

In his paper on the quality of field-collected fish larvae, Ahlstrom (1976) noted several conditions that damaged specimens: fast net speeds, high temperatures, and increased time in the net. During standard ichthyoplankton surveys, larvae in the nets could be damaged by abrasion for up to 20 min before preservation, the net is towed for 20 min, ascending 15 min, and then the collected sample is washed down into the cod end and preserved (Ahlstrom 1976; Smith and Richardson 1977). Considering these variables, I designed a net treatment to simulate shipboard procedures. For the treatment, seawater was circulated over a single larva in a submerged net container. (Small larvae, 4-7 mm, were treated in groups of 10.) To obtain conservative results, the water temperature was cool, 13° C, and the net-treatment time varied: 5, 10, 15, and 20 min. The net-treatment time included the pipetting and measuring as well as the time in the net. After net treatment, larvae were preserved; I equate these net-treated and preserved larvae with field-collected larvae (Figure 1).

RESULTS

Live Body Parts

Head length, body depth, and eye diameter were examined as functions of standard length for live northern anchovy larvae (Figure 2, Table 1). On a double logarithmic scale (Figure 2) both head length and body depth relationships show curvature, but the eye diameter relation appears to be nearly linear. According to Zweifel,³ the simple allometric body part relationships used for juvenile and adult fish are not adequate for describing body part relationships of larval fish, except for very limited ranges of size or age. Therefore, I assumed that the larval body proportions (y) change continuously during growth, varying according to a nonlinear allometric growth model,

$$\ln y = a - b(c - \ln x)^d,$$

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

³Zweifel, J. T. Equations of growth and allometry in larval and adult fish. Unpubl. manusc. Southwest Fisheries Center La Jolla Laboratory, National Marine Fisheries Service, NOAA, P.O. Box 271, La Jolla, CA 92038.

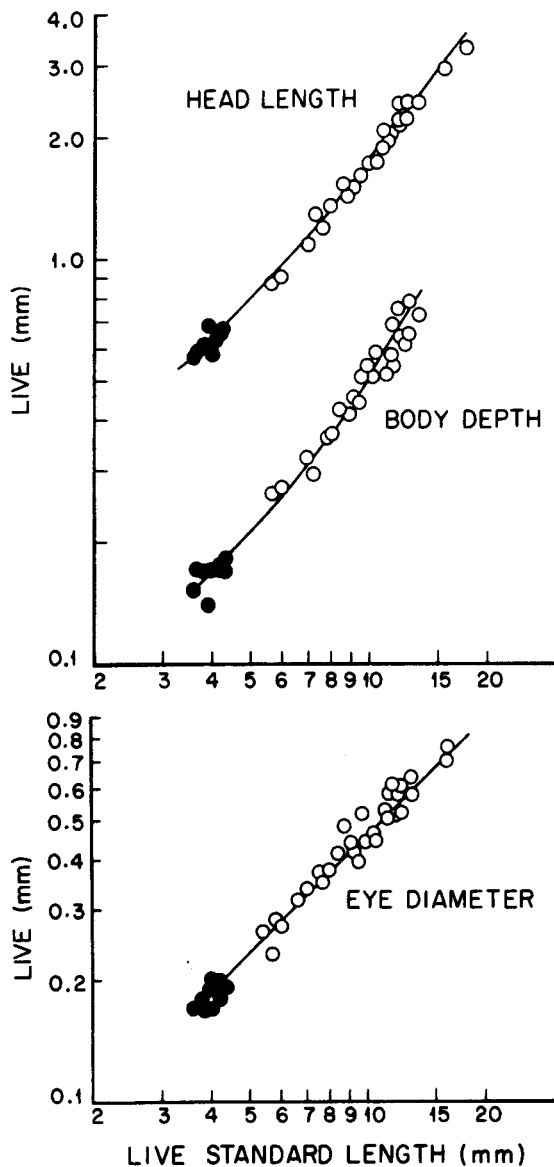


FIGURE 2.—Head length, body depth, and eye diameter as functions of standard length in live northern anchovy larvae. Non-linear models for live head length and body depth with standard lengths and linear model for eye diameter with standard length are described in the text; estimated parameters are in Table 1. Dots are means of 10 larvae. Circles represent individual fish.

obtained by eliminating time (*t*) from two Gompertz equations

$$y = y_0 \exp(A_0 [1 - \exp(-\alpha t)] / \alpha) \text{ and}$$

$$x = x_0 \exp(B_0 [1 - \exp(-\beta t)] / \beta)$$

TABLE 1.—Estimated parameters for nonlinear and linear models relating live body part measurements (*y*) with standard length (*x*) of northern anchovy larvae.

<i>y</i>	<i>n</i>	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>
Head length ¹	86	4.120	2.456	4.189	0.607
Body depth ¹	38	2.922	3.699	3.241	0.389
Eye diameter ²	44	-3.021	0.976		

¹ $\ln y = a - b(c - \ln x)^d$.
² $\ln y = a + b \ln x$.

(Zweifel footnote 3). *a* corresponds to the natural logarithm of the asymptotic size of *y*; *c* represents the natural logarithm of the asymptotic size of *x* (standard length); and *d* is the ratio of the decay parameters α and β in the individual Gompertz growth curves. The parameter *b* has no simple biological interpretation except when the decay parameters are equal (*d* = 1); in this case the equation is reduced to a simple allometric growth model. The model was fit to the observed head length and body depth measurements using Marquardt's algorithm for fitting nonlinear models (Conway et al. 1970). The equations (Table 1) gave a good fit to the data (Figure 2). The relation between eye diameter and all treatments is discussed in the section on Eye Diameter.

Laboratory Shrinkage

For northern anchovy larvae preserved in Formalin, the ratio of preserved to preceding live size for standard length (Figure 3), head length, and body depth did not increase with length; i.e., shrinkage did not decrease with age. The ratio averaged 0.92 for standard length after shrinkage in Formalin, and this relation also held for shrinkage in standard length of northern anchovy, jack mackerel, and Pacific barracuda larvae preserved in Bouin's fixative (Table 2). Shrinkage of other body parts differed among species, but the measurements were not made on all three species in

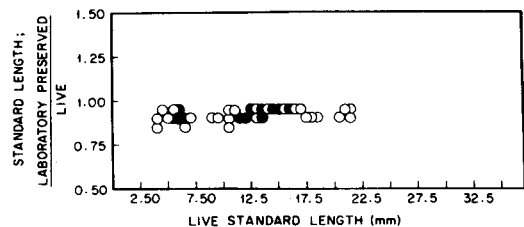


FIGURE 3.—Ratio of subsequent laboratory-preserved standard length to live standard length in northern anchovy preserved in Formalin. Dots are means of two or three larvae. Circles represent individual fish.

TABLE 2.—Shrinkage of laboratory-preserved northern anchovy, jack mackerel, and Pacific barracuda. Ratio is laboratory-preserved size divided by previous live size (1.00 = no shrinkage). Standard length; head length; eye diameter; body depth at the pectoral, BD-1; and body depth at the anus, BD-2. Measurements in millimeters.

Species	Fixative	Standard length				Head length				Eye diameter				BD-1			BD-2		
		No.	Range	Ratio	SD	No.	Range	Ratio	SD	Range	Ratio	SD	Range	Ratio	SD	Range	Ratio	SD	
Northern anchovy	Formalin	61	3.9-21.6	0.92	0.03	23	0.58-2.41	0.91	0.07	0.17-0.61	1.05	0.08	—	—	—	0.14-0.82	0.90	0.10	
	Alcohol	26	3.7-19.0	1.00	.04	—	—	—	—	—	—	—	—	—	—	—	—	—	
	Bouins ¹	224	3.8-15.7	.92	.00	—	—	—	—	—	—	—	—	—	—	—	—	—	
Jack mackerel	Bouins	45	3.25-3.90	.92	.03	43	0.74-0.97	.82	.04	0.26-0.33	.90	.06	0.48-0.67	0.75	0.05	0.20-0.26	.75	.05	
Pacific barracuda	Bouins	54	3.75-5.23	.92	.02	56	1.00-1.63	.79	.05	0.30-0.48	.82	.04	0.58-0.96	.75	.04	0.24-0.41	.77	.05	

¹P. Paloma, Fishery Biologist, National Marine Fisheries Service, La Jolla, Calif. Unpubl. data.

the same preservative. Jack mackerel and Pacific barracuda, deeper bodied than northern anchovy at the same length, were preserved in Bouin's fixative, and northern anchovy were preserved in Formalin. Head length, eye diameter, and body depth shrank more in jack mackerel and Pacific barracuda larvae than in northern anchovy larvae (Table 2). Eye diameter of northern anchovy increased in size after Formalin preservation; the increase was significant ($P = 0.058$; paired t -test) but small (0.0145 ± 0.0031 mm).

Alcohol preservation did not cause a change in northern anchovy standard length (Figure 4, Table 2); smaller body parts were not measured because alcohol distorted the larvae and they were extremely difficult to remeasure after preservation.

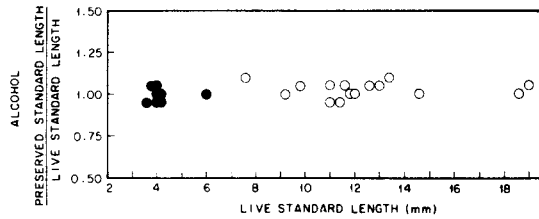


FIGURE 4.—Ratio of subsequent laboratory-preserved standard length to live standard length in northern anchovy preserved in alcohol. Dots are means of 10 larvae. Circles represent individual fish.

Net-Treatment Shrinkage

Shrinkage of net-treated larval northern anchovy varied with handling time and fish size (Ta-

TABLE 3.—Shrinkage in standard length (millimeter) of northern anchovy larvae after net handling for four time intervals (X_2). Ratio (R) = mean net standard length (X_1) divided by mean live standard length (L). Ratio of 1.00 = no shrinkage.

Live size	n	Mean size		Ratio		n	Mean size		Ratio	
		Live	5 min net	Observed	Estimated ¹		Live	10 min net	Observed	Estimated ¹
4.00- 5.99	3	4.27	3.46	0.81	0.84	8	4.40	3.55	0.81	0.81
6.00- 7.99	3	7.57	6.87	0.91	0.90	9	7.17	5.99	0.84	0.85
8.00- 9.99	5	9.02	8.16	0.90	0.92	11	8.87	7.71	0.87	0.87
10.00-11.99	9	10.98	10.21	0.93	0.94	21	11.11	9.85	0.89	0.89
12.00-13.99	6	12.63	12.12	0.96	0.95	29	12.90	11.58	0.90	0.90
14.00-15.99	6	14.60	13.83	0.95	0.96	18	14.85	13.45	0.91	0.92
16.00-17.99	2	16.70	15.80	0.95	0.97	11	16.65	15.22	0.91	0.93
18.00-19.99	4	19.20	18.73	0.98	0.98	11	18.93	17.71	0.94	0.94
20.00-21.99	3	21.10	20.67	0.98	0.99	7	20.80	19.71	0.95	0.95
22.00-23.99	—	—	—	—	—	2	22.75	21.50	0.95	0.96
24.00-25.99	1	24.70	24.50	0.99	0.99	2	24.85	24.10	0.97	0.97
26.00-27.99	1	26.70	26.00	0.97	0.99	1	26.70	25.50	0.96	0.97

Live size	n	Mean size		Ratio		n	Mean size		Ratio	
		Live	15 min net	Observed	Estimated ¹		Live	20 min net	Observed	Estimated ¹
4.00- 5.99	2	4.22	3.41	0.81	0.80	—	—	—	—	—
6.00- 7.99	2	7.35	6.20	0.84	0.83	1	7.50	6.50	0.87	0.82
8.00- 9.99	4	8.96	7.55	0.84	0.84	—	—	—	—	—
10.00-11.99	5	11.44	9.84	0.86	0.86	3	11.73	10.30	0.88	0.85
12.00-13.99	4	12.85	11.29	0.88	0.88	1	13.70	11.50	0.84	0.86
14.00-15.99	7	14.57	12.73	0.87	0.89	6	14.60	12.55	0.86	0.87
16.00-17.99	4	16.57	14.53	0.88	0.90	2	16.70	14.30	0.86	0.88
18.00-19.99	5	19.22	17.72	0.92	0.92	5	19.22	17.22	0.90	0.90
20.00-21.99	3	21.10	19.67	0.93	0.93	3	21.10	19.37	0.92	0.91
22.00-23.99	1	23.50	21.40	0.91	0.94	1	23.50	21.40	0.91	0.92
24.00-25.99	1	24.70	23.40	0.95	0.94	1	25.00	23.80	0.95	0.93
26.00-27.99	1	26.70	24.60	0.92	0.95	—	—	—	—	—

¹Estimated ratio = $\ln X_1 - \ln L$; Equation (4), see text and Table 4.

ble 3). In larvae 6 mm SL or less, maximum shrinkage (19%) occurred after 5-10 min treatment in the net; larvae were usually dead at the end of the treatment. Older larvae shrank throughout the 20-min period and were often alive at the end. For example, 18-22 mm larvae were 2% smaller after 5 min and 8-10% smaller after 20 min in the net. Further net treatment of larger larvae caused an additional 1-2% shrinkage. Figure 5

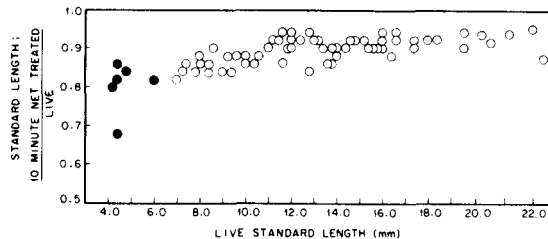


FIGURE 5.—Ten-minute net-treated shrinkage of standard length as a function of size for northern anchovy larvae. Dots are means of 10 larvae. Circles represent individual fish.

shows that measurable shrinkage decreases for older, larger larvae, and that the ratio (R) of the size of net-treated (X_1) to live (L) size larvae rises rapidly from about 0.7-0.8 at 4 mm SL to 0.9 by 11-12 mm SL. Although shrinkage appears nearly constant for larvae from 12 to 22 mm (Figure 5), I measured few older, larger larvae. Conceptually, shrinkage is probably related to the degree of ossification; ossification of northern anchovy vertebrae begins at 14 mm SL and is complete at transformation, about 35 mm (E. H. Ahlstrom⁴). At transformation, shrinkage should be negligible or zero, and the ratio should approach an asymptote of one. To characterize this relationship, I used the equation

$$R = \exp[(-f_1) \exp(-f_2 X_1)]. \quad (1)$$

Equation (1) may be transformed so that the double logarithm of R is a linear function of size, X_1 , i.e.

$$\ln[-\ln(R)] = \ln f_1 - f_2 X_1. \quad (2)$$

For standard length measurements, the parameters of Equation (2) were estimated for each of the four net-treatment periods as shown below:

Net-treatment time = t	$\ln f_1$	f_2
5	-1.3436	0.1209
10	-1.2708	0.0752
15	-1.2479	0.0509
20	-1.3759	0.0430

The logarithm of f_2 is linear with net-treatment time, i.e.

$$f_2 = g_1 t^{k_2}, \quad (3)$$

while f_1 shows no trend. Combining these two relationships, i.e., R with size (Equation (1)) and f_2 with time (Equation (3)), and inverting the equation to solve for live size in terms of treated larvae, the resultant relationship is

$$\ln L = \ln X_1 + P_1 \exp(-P_2 X_1 X_2^{P_3}) \quad (4)$$

where L is live size, $P_1 = f_1$, $P_2 = f_1 g_1$, $P_3 = g_2$, X_1 is treated size, and X_2 is time (t) in minutes. Equation (4) was then fit directly using a nonlinear fitting procedure (Conway et al. 1970) to obtain the final parameter estimates. The same procedure for estimating parameters and fitting equations was followed for shrinkage of head length and body depth. All equations gave a good fit to the observed data (Figure 6, Table 3); estimates of the param-

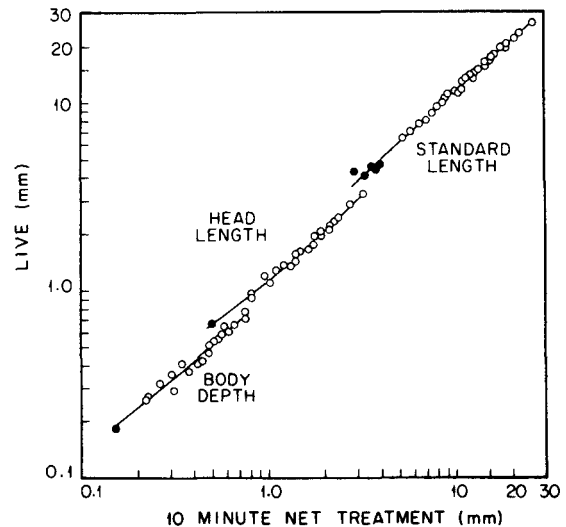


FIGURE 6.—Fit of models (Equation (4)) describing net-treatment shrinkage of larval northern anchovy body parts. Estimates of parameters for models are given in Table 4. Models predict live size from net-treated size.

⁴E. H. Ahlstrom, Senior Scientist, Southwest Fisheries Center La Jolla Laboratory, National Marine Fisheries Service, NOAA, P.O. Box 271, La Jolla, CA 92038, pers. commun. December 1978.

TABLE 4.—Estimated parameters for models that predict live northern anchovy body part size (L) from net-treated size (X_1).

Model ¹	P_1	P_2	P_3
Standard length	0.289	0.434	-0.680
Head length	0.177	3.858	-0.605
Body depth	0.413	29.746	-0.620

¹Equation (4), see text.

ters for the standard length, head length, and body depth models are given in Table 4. These equations can be used to estimate live size of each body part from measurements of northern anchovy larvae after net capture.

Preservation Shrinkage (After Net Treatment)

After larvae shrank during net treatment, additional shrinkage caused by Formalin was nearly a constant proportion of length (Figure 7). The ratio of preserved length to net-treated length (Figure 7) may decrease slightly (i.e., shrinkage may increase) with increasing fish size, but this slight decrease has no practical significance for length calibration of larvae taken in routine plankton samples. Because ossification begins at 14 mm SL, I expect large fish would shrink less, not more, than small fish. The overall mean ratio (preserved size/size after each timed-net treatment) of 104 standard length measurements was 0.9668 ± 0.0020 ; percent shrinkage of the other body parts was the same as standard length shrinkage. I recommend using 3% shrinkage for all body parts in Formalin after net treatment. Preservation in alcohol after net treatment did not cause further shrinkage (only standard length measured).

To adjust the shrinkage models (Table 4) to predict live size from net-treated and Formalin-preserved size (equivalent to field-collected larvae), the preserved size is multiplied by 1.03. No

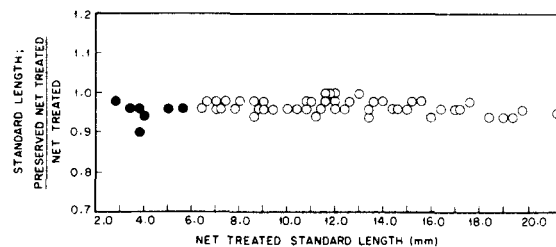


FIGURE 7.—Size specific shrinkage of northern anchovy larvae preserved in Formalin after 10-min net treatment. Dots are means of 10 larvae. Circles represent individual fish.

adjustments are needed for alcohol-preserved samples.

The difference in shrinkage between laboratory-preserved larvae and net-treated and preserved larvae of the same initial live size decreased with age. For example, 3 mm larvae that were net treated and preserved in Formalin shrank 15% more in standard length than 3 mm larvae that were laboratory preserved in Formalin, but shrinkage of 20 mm larvae was the same for both treatments (Table 5). Shrinkage of

TABLE 5.—Comparison of standard length for live (L), laboratory-preserved and net-treated northern anchovy larvae (X_1). Numbers in parentheses are preserved length divided by live length (ratio of 1.00 = no shrinkage).

Live size (mm)	Estimated preserved size (mm)			
	Laboratory ¹		Net-treated ²	
	Alcohol	Formalin	Alcohol	Formalin
3	3 (1.00)	2.76 (0.92)	2.38 (0.79)	2.31 (0.77)
5	5 (1.00)	4.59 (0.92)	4.10 (0.82)	3.98 (0.80)
10	10 (1.00)	9.18 (0.92)	8.77 (0.88)	8.51 (0.85)
15	15 (1.00)	13.78 (0.92)	13.81 (0.92)	13.40 (0.89)
20	20 (1.00)	18.37 (0.92)	18.99 (0.95)	18.42 (0.92)
25			24.21 (0.97)	23.48 (0.94)
30			29.40 (0.98)	28.52 (0.95)
35			34.56 (0.99)	33.52 (0.96)
40			39.68 (0.99)	38.49 (0.96)
45			44.77 (0.99)	43.43 (0.97)
50			49.84 (1.00)	48.34 (0.97)

¹Includes 30 s handling time; no shrinkage in alcohol and 8% shrinkage in Formalin (see text).

²Estimated preserved size calculated from Equation (4) for 10 min net treatment; size adjusted for 3% additional shrinkage in Formalin (see text and Table 4).

laboratory-preserved larvae in Formalin probably decreases to something <8% (Table 5) as the skeleton develops and ossification occurs. Formalin preservation of 90 mm and larger salmon, *Oncorhynchus* spp., smolts caused 3-4% shrinkage in length (Parker 1963). Laboratory shrinkage of northern anchovy after transformation may be similar; thus, shrinkage of net-collected and preserved northern anchovy >35 mm (Table 5) should be similar to shrinkage of laboratory-preserved fish >35 mm.

Eye Diameter

Netting live larvae for 10 min caused the eye diameter to shrink an average of 0.0443 ± 0.0069 mm, and Formalin preservation after net treatment caused an increase in eye diameter that averaged 0.0177 ± 0.0046 mm. The increase in eye diameter after preservation was similar to the increase after preservation noted for eye diameter of laboratory-preserved larvae. The t -tests for paired data ($n=23$) showed that in all cases the differ-

ences between treatments (live, net treated, and preserved) were significant ($P < 0.01$). Even though these differences were significant, the small changes in eye diameter size caused by net treating and preserving probably are not important for calibration of size of field-collected larvae. Thus eye diameter should be a useful parameter for estimating average live standard length of field-collected larvae (Table 1).

DISCUSSION AND CONCLUSION

The causes of antemortem shrinkage of fish larvae are not completely understood. Before death, appearance of the body changes from translucent to opaque. This phenomenon is an indicator of ensuing death of larvae in rearing experiments. Autolysis, digestion of tissues by their own enzymes, is occurring during this antemortem period (Theilacker 1978), and the enzymatic action on proteins may cause denaturation, thus the color change and shrinkage. Shrinkage also may be caused by an osmoregulatory problem. An inability to osmoregulate may develop from loss of mucus by abrasion after contact with a surface. The internal osmolar concentration of another clupeoid larva, Pacific sardine, *Sardinops sagax*, is 0.24 M and that of seawater 0.56 M (Lasker and Theilacker 1962). If a larva were unable to osmoregulate, this difference in osmolarity would cause it to lose fluid and shrink.

The amount of shrinkage that occurred before larvae were killed in a preservative was dependent on larval fish size and the extent of "handling" (measuring and netting). The elapsed time of surface contact was the main determinant of final length. This was especially noticeable while measuring small, 3-7 mm larvae. As larvae increased in size and ossification progressed, net-treatment shrinkage decreased.

Preserving larvae after handling caused additional shrinkage that was a constant proportion of size. Laboratory-preserved shrinkage in Formalin included a 30 s handling time; shrinkage in Formalin was constant at 8% and independent of size. Preserving larvae that had been retained in a net caused an additional 3% shrinkage; the additional shrinkage was nearly a constant proportion of size.

Farris' (1963) results on shrinkage of laboratory-preserved, 3-6 mm yolk-sac Pacific sardine larvae agree with my results. He found Formalin shrinkage of standard length ranged between 7

and 11%, similar to the 8% shrinkage for laboratory-preserved northern anchovy in my study. Rosenthal et al. (1978) reported a 16% shrinkage of newly hatched, 2 mm larvae of the sea bream, *Chrysophrys major*. The larvae were anesthetized with MS-222 and measured with a projector prior to preservation in Formalin. It appears that handling of the sea bream was minimal; however, MS-222 has been reported to interfere with osmoregulation (Parker 1963), and an inability to osmoregulate would cause a greater shrinkage. Blaxter (1971) reported on a net-shrinkage experiment that was similar to my study. After his net treatment, mean live size of 22 herring larvae (10.77 mm) decreased by 17%; Formalin fixation caused an additional 3-5% shrinkage for a total of 20-22%. He noted the larvae were dead after netting, but the elapsed time is unknown. In this study, the netted 11 mm northern anchovy were usually dead after 20 min, and the total shrinkage of the 20-min treated 11 mm northern anchovy was about 18%, similar to Blaxter's experiment.

If the larvae to be measured are badly damaged or partially digested, the models generated in this study, which describe live body proportions and shrinkage, could be used to estimate average fish length from size of head or eye. Packard and Wainwright (1974) found that eye diameter of young herring (up to 100 mm) was a useful reference parameter for estimating both size and weight. Because eye diameter of northern anchovy changed little during netting and preservation, eye diameter may be a useful parameter for estimating average live size of field-collected larvae. However, use of eye diameter to estimate live standard length assumes that the relation between eye diameter and standard length is the same for laboratory-reared and field-collected larvae. Balbontin et al. (1973) and Blaxter (1976) have shown that morphological differences exist between reared and wild fish of the same length, thus the assumption, that the body forms of reared and wild northern anchovy larvae are similar, may be invalid. However, as I have shown in this study, the method of handling larvae prior to preservation causes shrinkage differences that could be interpreted as morphological differences.

The most important use of the shrinkage models is to predict live size, and thus age, of sea-collected northern anchovy larvae so that results from laboratory larval fish studies can be related to the sea. Use of the standard length shrinkage model (Table 4) should give the best estimate of live size

for field collected larvae. The standard length model can probably be applied to shrinkage of all clupeidlike larvae if the patterns of calcification are similar.

FORTTRAN computer programs for the nonlinear models are available at the Southwest Fisheries Center La Jolla Laboratory.

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