CULTURE AND GROWTH OF NORTHERN ANCHOVY, ENGRAULIS MORDAX, LARVAE

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ABSTRACT

Culture techniques used to rear larval anchovy through metamorphosis using laboratory cultured foods are described. Anchovy larvae fed dinoflagellates Gymnodinium splendens, rotifers Brachionus plicatilis, harpacticoid copepods Tisbe furcata, and brine shrimp nauplii Artemia salina, completed metamorphosis (35 mm) in 74 days at 16°C with a minimum survival of 12.5%. Growth in length and weight were recorded over this interval and an excellent fit to the Laird-Gompertz growth equation was obtained. Growth was comparable to that on a wild plankton diet. In a starvation experiment, most of the fish that completed metamorphosis withstood a starvation period of 12-15 days, whereas those that had not completed metamorphosis did not.

Knowledge of the growth rate of northern anchovy, Engraulis mordax Girard, is essential for estimating year class success or larval survival. Another important element in estimating survival is the time fish or larvae can withstand starvation. In this report I describe the growth rate of larval anchovy to metamorphosis and present data on the ability of newly metamorphosed juveniles to withstand starvation. Special attention is also given to culture techniques because this is the first time northern anchovy have been reared through metamorphosis entirely on cultured foods.

Kramer and Zweifel (1970) recorded the growth of anchovy larvae at 17° and 22°C for periods of up to 34 days. In their experiments larvae attained an average length of 17 mm but did not reach metamorphosis, which is complete at about 35 mm standard length. Their larvae were fed wild plankton supplemented by Artemia salina nauplii. In the ensuing years, rearing techniques using cultured foods have gradually been developed: Gymnodinium splendens for 3- to 5-dayold larvae (Lasker et al. 1970), and Brachionus plicatilis for 5- to 20-day-old larvae (Theilacker and McMaster 1971). This paper describes the use of the harpacticoid copepod Tisbe furcata which are the proper size food for larvae older than 20 days (10 mm). All previous attempts to rear anchovy larvae beyond 35 days on cultured foods have failed. In all attempts Artemia nauplii were used after 20 days.

METHODS

Five rearing experiments were done, four at 16°C and one at 17° to 18°C (Table 1). Eggs for all experiments were obtained from a captive population of anchovy which were maintained in breeding condition continuously at the Southwest Fisheries Center La Jolla Laboratory (Leong 1971).

Rearing tanks were cylindrical, black fiberglass, 122 cm diameter, 36 cm deep, covered with a transparent acrylic plastic top, and immersed in a water bath regulated by a refrigeration unit. Temperature was maintained near 16°C in all but one experiment, and the salinity was 35%. Fluorescent lamps suspended directly over each tank provided about 2,000 lx at the water surface. The volume of water in the tanks gradually increased from an initial volume of 200 liters of filtered seawater to 400 liters by about 20 days because of additions of seawater containing algae and food organisms. Thereafter, the volume was maintained at about 400 liters by siphoning water from the bottom from time to time which also cleaned the tank.

Records were kept of the quantity of food or algae added to tanks and on alternate days 16, 0.20-ml aliquots were taken to measure the density of *Brachionus plicatilis*, *Gymnodinium splendens*, and *Artemia salina* nauplii in the tanks. Concentrations of *Tisbe furcata* in the tanks were not recorded because they were concentrated on or near the walls and bottom of the tank, but records were kept of the numbers added to the tank. Details regarding the feeding of

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TABLE 1.—Characteristics of five larval anchovy rearing experiments.

Experiment Number of eggs		3,000 15.7 ± 0.21	_		2 6,000 17.7 ± 0.52			3 3,000 15.8 ± 0.53			6,000 15.7 ± 0.71			5 3,000 16.2 ± 0.33	
(±2.5E) Percent survival¹ (age in days)		7.0 (34)			0.1 (46)			6.0 (42)			0.1 (38)			12.5 (74)	
Type additions ²	Larval age days	Numbers ii added/day	Mean density in tank no./ml	Larval age days	Numbers added/day	Mean density in tank no./ml	Larval age days	Numbers added/day	Mean density in tank no./ml	Larval age days	Numbers added/day	Mean density in tank no./ml	Larval age days	Numbers added/day	Mean density in tank no./ml
Millions of	5-20	0.8	30	4-20	1.7		5-20	2.9	27	4-20	2.0	27	5-20	1.9	118
Brachionus	21-34	0.5	19	21-46	0.3	80	21-42	2.3	93	21-38	0.1	61	21-74	4.0	15
Millions of	5-20	11.7	ì	4-20	28.6	ļ	5-20	36.4	I	4-20	26.0	ł	5-20	36.4	1
Nannochloris	21-34	14.3	į	1	I	ı	21-42	7.8	1	21-38	808	1	21-74	11.7	I
Millions of	24-34	0.3	-	19-46	0.7	5	ł	!	1	ı	ł	ļ	48-74	1.6	8
Artemia nauplii															
Thousands of	i	I	I	I		ı	l	1	I	12-20	180.0	1	6-20	260.0	1
Tisbe	ļ	1	1	1	I	١	ļ	ł	1	21-38	200.0	ì	21-48	300.0	I
Thousands of	1	ı	I	ı	ļ	ļ	ı	I	ı	I	I	1	66-74	12.0	1
Artemia adults															
¹ Mortatity includes 15 larvae remov ² Forty liters of Gymnodinium, 1,70	15 larvae rer nodinium, 1,		ernate days , were adde	for length d at hatchir	ved on atternate days for length measurements. 0 cells/ml, were added at hatching in all groups.	ıts.									

anchovy larvae on *Tisbe* will be given in a separate section.

To obtain growth rates, 15 or more larvae were removed every other day from each tank, then measured, rinsed in distilled water, dried, and weighed in groups of 15.

CULTURE

Not until the fifth experiment was the procedure developed sufficiently to rear anchovy through metamorphosis. The first four experiments ended when it became obvious that it would be impossible to rear them to metamorphosis because of slow growth and high mortality. Data are included from the first four experiments to provide the background information for the final successful rearing procedure.

In all experiments, a single inoculation of 40 liters of Gymnodinium splendens (1,500-2,000 cells/ml) was given at age 0 days. This was sufficient to provide a final density in the tank in excess of 100 cells/ml for about 12 days. Gymnodinium was cultured using techniques described by Thomas et al. (1973). If fed only Gymnodinium, survival of anchovy larvae remains high for at least 12 days (about 45% at 12 days) but growth is depressed (Hunter in prep.).

In all experiments *Brachionus* was added on the 4th or 5th day in numbers calculated to yield a density of 30 to 50/ml in the tank (Table 1). Subsequent additions were made daily or on alternate days until day 20 in all experiments.

Nannochloris sp. was used to culture the rotifer Brachionus (Theilacker and McMaster 1971), and as a consequence Nannochloris was added to larval rearing tanks in all experiments to maintain a food supply for the rotifers. Four liters (about 13,000 cells/ml) were added on days 4 and 5, and further additions were made on the basis of water color. If water in the rearing tank was faintly green none was added as I wished to avoid creating a bloom in the tank because it is difficult to see the larvae in a dense bloom. To avoid a bloom usually required a reduction in the quantity added after 20 days. Nannochloris sp. is too small (about 7 μ m) to be directly fed upon by larval anchovy although larvae might ingest cells accidently.

In experiments 1 and 2 Artemia was added at about 20 days and the level of Brachionus was allowed to slowly decline thereafter. In experiment 3, Brachionus was maintained at a high

level to the end and no Artemia was used. Although high mortalities on the order of 30 to 300 larvae/day occurred in all three experiments between ages 20 to 30 days, the larvae in experiment 3, those fed only Brachionus, grew faster (Figure 1) and had a higher survival than in the two groups fed Artemia. From these three experiments I concluded that Artemia was an inadequate food for 20-day-old anchovy larvae and that growth and survival could be increased by continuing to add large quantities of Brachionus after 20 days. Clearly, an adequate food larger than Brachionus was needed for 20-day-old larvae.

The food selected was the harpacticoid copepod Tisbe furcata. Tisbe is a common contaminant in the seawater system of the Southwest Fisheries Center and can be easily reared on dried foods (Johnson and Olson 1948) or algae (DeVauchelle and Girin 1974). Copepods collected from cultures ranged from $50-\mu m$ nauplii to $1,000-\mu m$ adult females but the typical size was about 650 μ m and comparable in size to Artemia nauplii. The first attempt to rear anchovy using Tisbe (experiment 4) began as the other experiments except that I began adding Tisbe at age 12 days at the average rate of 180,000/day. At age 20 days the rate was increased to 240,000/day and the Brachionus was allowed to decline. The larvae fed on Tisbe but growth was slow and survival low. The low survival was attributed to an insufficient number of Tisbe in the tank, failure to maintain Brachionus at a high level after 20 days, as I had

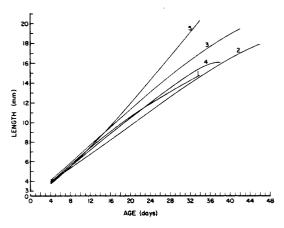


FIGURE 1.—Laird-Gompertz growth curves for lengths of anchovy larvae in five rearing experiments. Growth equation given in text; parameters for equation in Table 2. (Foods used in experiments 1-5 in Table 1.)

in experiment 3, and too high an egg stocking density (6,000 eggs).

The first four experiments established the guidelines needed for experiment 5, the final and successful rearing experiment. Over the first 20 days Brachionus, Nannochloris, and Gymnodinium additions were managed in the same way as in experiment 3. After 20 days, additions of Brachionus were increased above that used in experiment 3 and maintained at a high level until the end of the experiment on day 74. Tisbe additions were begun at age 6 days at an average rate of 260,000/day and increased to 306,000/day after 20 days. These additions were begun before most larvae were capable of feeding upon them in order to bring the copepod density in the tank to a high level at the time feeding on Tisbe became common (about age 12 days, anchovy length, 7-8 mm). This procedure is practical because survival of *Tisbe* in the tank is high and consequently, uneaten animals accumulate. Tisbe additions ended at age 48 days (26 mm) because the quantities needed exceeded the capacity of my cultures. Although younger larvae did not survive on a diet of Artemia nauplii it seemed possible that larvae 26 mm long might survive because they have a differentiated digestive tract, not simply a straight tube as do younger larvae, and they have a larger gut capacity (C. O'Connell, Southwest Fisheries Center La Jolla Laboratory, pers. commun.) Rosenthal (1969) showed that Artemia nauplii in the guts of herring larvae were only partially digested whereas digestion of copepods was nearly complete. From this he concluded that poor survival of herring fed Artemia could be attributed to digestive inefficiency. Past experience in maintaining adult anchovy at the Southwest Fisheries Center showed that they survived on Artemia; thus, it seemed reasonable that this might first occur when the digestive tract became differentiated. For these reasons I decided to change from a diet of Tisbe and Brachionus to one of Artemia nauplii and Brachionus at age 48 days. The change from copepods to Artemia nauplii did not cause a noticeable mortality nor a change in growth rate. Adult Artemia were added at age 69 days as some of the fish had metamorphosed and readily ingested adult Artemia.

In this description of culture I have stressed additions rather than density of food in the tank because I felt they provided a more reliable outline of culture procedures. Density in the tank was measured before food was added and served as a guide for the quantity of food to be added. Where losses from ingestion or other sources of mortality were high, the density measurements tended to be lower than the level we attempted to maintain. In experiment 5 we attempted to maintain the density of *Brachionus* between 50 and 100/ml and that of *Artemia* nauplii at 2 to 3/ml.

In all experiments, 15 or more larvae were removed on alternate days and consequently, survival estimates include the effect of this sampling. In experiments 1 to 4 no daily counts of dead larvae were made until heavy mortalities occurred after age 20 days. In experiment 5, daily records of dead larvae were begun at age 54 and continued to the end of the experiment (age 74 days). At age 54 days 20% of the larvae were alive and at age 74 days, 374 larvae or 12.5% were alive. If the tank had not been sampled survival would probably have been greater because between 54 and 74 days the number of larvae sampled, 151, exceeded the number that died in the tank, 70. A total of 387 larvae were removed during the experiment. A method exists for estimating mortality in rearing work independent of the effect of sampling (Laurence 1974) but the programming effort required seems unwarranted for the objective of this paper. Collision with the walls of the container was a frequent cause of mortality over the last 3 weeks.

A survival of 12.5% at 74 days contrasts sharply with the other four experiments where nearly all larvae died by 30 to 40 days. Prior to the study described here, marked mortalities were common after 20 days and in all of the attempts Artemia was used as food. The pattern had become so typical at this laboratory that we have called it the "Artemia syndrome" for some years. The results of the current study suggest that the cause of the Artemia syndrome may simply be an inability of young clupeoid larvae with straight tube digestive tracts to digest Artemia nauplii but that Artemia may be used once the gut becomes differentiated.

It is important to call attention to the fact that 6% of the anchovy larvae in experiment 3 were able to survive for 42 days on a diet of only Brachionus. Plaice, Pleuronectes platessa, larvae have been reared through metamorphosis on only Brachionus although growth was slower than that on Artemia nauplii (Howell 1973). Howell found that plaice larvae, immediately prior to metamorphosis (12.7 mm), consumed 1,400 roti-

fers per day. In experiment 3, at age 42 days, the mean length of the anchovy larvae was 21.6 mm and dry weight was 5.5 mg. Assuming a digestive efficiency of 100%, larvae of this weight would have to ingest about 3,800 rotifers per day to meet metabolic requirements (calculation based on caloric value of *Brachionus* and anchovy respiration data given by Hunter 1972). These results illustrate the value of maintaining a high density of rotifers in culture containers long after a larger food has been added. They also suggest that some fish larvae have the ability to ingest large quantities of small prey and this could be of considerable benefit under natural conditions.

TISBE FURCATA AS A FOOD FOR LARVAL FISH

The evidence for the use of *Tisbe* as a food for rearing larval anchovy to metamorphosis is a single rearing experiment. It would be preferable to have additional experiments but none are planned at present because current work is concerned with only young stages and other species. Two groups of Pacific mackerel, *Scomber japonicus*, have been reared to metamorphosis using *Brachionus* and *Tisbe* as foods and this supports the contention that *Tisbe* is a satisfactory food for pelagic marine fish larvae. The work on *Scomber* will be reported at a later date.

That larval anchovy ate Tisbe is supported by records of stomach contents of larvae examined during the course of the rearing work. Seventy-four percent of the stomachs examined in experiment 5 contained only Tisbe or Tisbe and Brachionus and 26% contained only Brachionus (N=69, larval length =8.6-18.8 mm). The number of Tisbe in stomachs of larvae increased from 2.8 per larva (5.6-8.5 mm) to 18 per larva (17.6-20.5 mm). (Data from experiments 4 and 5 combined—Table 2.) The average length of the

TABLE 2.—Number and mean length of *Tisbe furcata* in the stomachs of anchovy larvae in experiments 4 and 5.

Larval a	nchovy		Tisbe in stor	nachs
Length class (mm)	Number	Total	Number per larva¹	Mean length μm ± 2 SE
5.6- 8.5	12	34	2.8	506 ± 57
8.6-11.5	25	90	3.6	681 ± 28
11.6-14.5	16	102	6.4	714 ± 28
14.6-17.5	9	98	10.9	758 ± 32
17.6-20.5	3	54	18.0	734 ± 43

^{*}Includes only larvae that had either Tisbe and Brachionus or only Tisbe in stomachs.

copepods ingested by larvae also increased with larval length as expected (Arthur 1956).

Tisbe occurred throughout the rearing tank but the greatest concentrations occurred on or near the walls and on the bottom. Free swimming copepods were plentiful near the walls of the tank because Tisbe frequently leave the wall for short periods. Anchovy larvae captured Tisbe that were on the walls as well as free-swimming individuals. A pelagic copepod would be preferable to one that prefers surfaces such as Tisbe furcata but I have not been able to culture pelagic species in sufficient quantities for rearing work.2

GROWTH

The length data from each of the five experiments were fitted to the Laird-Gompertz growth equation (Laird et al. 1965) using Marquardt's Algorithm for fitting nonlinear models (Conway et al. 1970). The equation for length was:

$$L = L_0 e^{K_L (1 - e^{-\alpha t})}$$

where L =standard length in millimeters

 L_0 = initial length at time 0 $K_L = A_0/\alpha$

 A_0 = rate growth at time 0

 α = rate of decay of growth.

A fit of the weight data from experiment 5 was also made to the Laird-Gompertz equation:

$$W = W_0 e^{K_W (1 - e^{-\beta t})}$$

where W = dry weight in milligrams

 W_0 = initial weight at time 0

 $K_W = B_0/\beta$ B_0 = rate growth at time 0 β = rate of decay of growth.

The length-weight relationship for larvae in experiment 5 was derived from the above two equations by James Zweifel (Southwest Fisheries Center) and had the form

$$\ln W = \ln W_0 + K_W \left[1 - \left(\frac{K_L - \ln (L/L_0)}{K_L} \right)^{\beta/\alpha} \right]$$

The Laird-Gompertz equation gave an excellent fit to the growth in length and in weight and to the length-weight relationship (Figures 2-4, Table 3). The curvilinear nature of the lengthweight data evident in the log-log plot (Figure 4) clearly indicates that a linear fit to log of length and weight would lead to inaccurate estimates.

The growth of anchovy larvae in experiment 5 was about the same as that recorded by Kramer and Zweifel (1970) for anchovy fed wild plankton at 17°C. At age 34 days, the last day of their experiment, the mean length of larvae was 17.4 ± 1.8 mm and that in experiment 5 at age 34 days was 19.7 ± 1.0 mm. Thus, over at least the first 34 days, growth on the cultured food diets was about the same as that on wild plankton.

SURVIVAL AT METAMORPHOSIS

The object of this experiment was to determine how long newly metamorphosed anchovy larvae can survive without food. Most adult fishes and presumably the anchovy can withstand prolonged periods of starvation of weeks or months. On the

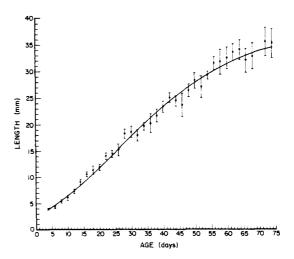


FIGURE 2. - Laird-Gompertz growth curve for length of anchovy larvae in experiment 5 and mean length ± 2 SE. Parameters for equation given in Table 2.

²At present our copepod culture system is composed of 10, 90-liter, glass, rectangular tanks maintained at 17° to 19°C. The Tisbe are given green algae, either Tetraselmis or Nannochloris, which is grown using commercial plant fertilizer (fish emulsion). An inoculation of 50,000 to 100,000 copepodid-adult stages yields on the average 500,000 copepods in these stages in 2 weeks. A tank is drained, harvested, and reestablished 5 days a week producing about 2.5×10^6 copepodid-adult stages per week, which is sufficient to rear one group of anchovy in the manner described. Occasional harvests of over a million in 2 weeks have been obtained suggesting that major improvements in the technique are possible. Contamination by Brachionus has been a problem because it increases the amount of algae that must be added to the culture. A more detailed description of this culture system would be premature but a description of a similar method of mass culture exists (De-Vauchelle and Girin 1974).

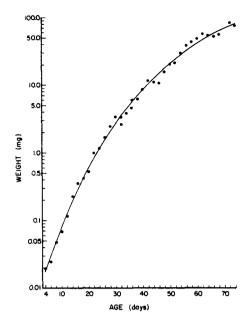


FIGURE 3.—Laird-Gompertz growth curve for dry weight of anchovy larvae in experiment 5. Points are average weight of larvae weighed in groups of 15-26 larvae each. Equation for curve given in text; parameters for equation in Table 2.

other hand, larval anchovy, after they absorb their yolk, survive only 1 to 2 days without food (Lasker et al. 1970). The point at which this extreme vulnerability to starvation ends is essential information for any model of anchovy ecology and survival.

In this experiment fish reared to metamorphosis in experiment 5 were used. At age 74 days a group of 53 fish (group 1) and one of 73 (group 2) were placed into tanks containing only filtered seawater and a sample of 29 fish was taken for length and weight measurements. The tanks

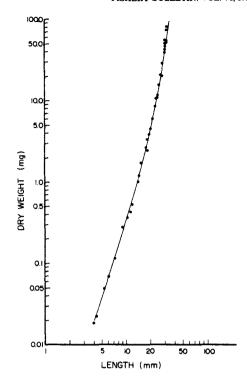


FIGURE 4.—Length-weight relationship of anchovy larvae reared in experiment 5. Equation for curve given in text; parameters for equation in Table 2.

were the same as those described for the rearing experiments and temperature was maintained at 16°C. Artemia nauplii were offered to group 1 after 12 days of starvation and to group 2 after 15 days; the experiment ended after 20 days. Daily records were kept of water temperature and lengths of dead fish; after 20 days all surviving fish were measured. Total lipid content was also monitored through the course of the experiment.

TABLE 3.—Parameters and 95% support plane¹ for Laird-Gompertz growth equation for length, experiments 1-5, and weight for experiment 5. Symbols and equations are given in text.

	Lo				Ao		α			
		Suppo	rt plane		Suppo	rt plane		Suppo	rt plane	Number of
Experiment	Parameter	Lower	Upper	Parameter	Lower	Upper	Parameter	Lower	Upper	observations
Length										
1	2.4378	2.0856	2.7901	0.1349	0.1040	0.1658	0.06939	0.05366	0.08512	289
2	3.0600	2.5512	3.5688	0.0835	0.0614	0.1056	0.03936	0.02700	0.05171	358
3	2.8361	2.3894	3.2829	0.1088	0.0835	0.1342	0.04951	0.03696	0.06206	345
4	2.6711	2.1648	3.1774	0.1098	0.0792	0.1404	0.05185	0.03591	0.06779	345
5	2.4928	2.2219	2.7636	0.1167	0.1042	0.1292	0.04264	0.03865	0.04663	553
		W _o			₽0			β		
Weight	0.005758	0.003046	0.008470	0.2997	0.2552	0.3442	0.02725	0.02243	0.03206	35

¹An approximation of the 95% confidence limits (Conway et al. 1970).

Fat was removed by Soxhlet extraction with chloroform-methanol (Krvarić and Mužinić 1950) from batches of 5 to 9 fish each. One such sample was taken at the beginning of the experiment, one from each group just before food was added, and one from each group when the experiment ended after 5 to 8 days of feeding.

A marked initial mortality occurred on the day following the transfer of the two groups (Figure 5) which was probably caused by handling. For this reason the first day's mortality is excluded from the analysis presented below, but the survival is given for all days in the figure.

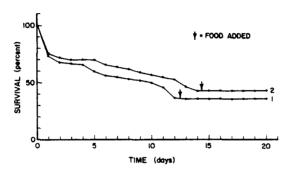


FIGURE 5.—Percent survival of metamorphosed larvae reared in experiment 5 during starvation periods of 12 and 15 days. Arrow indicates end of starvation period.

After 12 days of starvation, 50% of the fish were alive in group 1 (excluding the first day mortality) and 58% were alive in group 2 after 15 days of starvation. One fish in group 1 died the day after the first feeding. This was the only fish to die after feeding began. Thus for fish averaging 35 mm in length, about 50% mortality is reached after about 15 days of starvation and nearly all surviving fish are able to recover from a starvation period of that duration. Mortality during starvation appeared to be dependent on size or state of maturity, however. Metamorphosis is completed in the northern anchovy when they reach 35 mm standard length (E. H. Ahlstrom, Southwest Fisheries Center La Jolla Laboratory, pers. commun.). Eighty-three percent of the fish that died were less than 35 mm whereas only 17% of those longer than 35 mm died (Table 4). About 45% of the fish were less than 35 mm long at the beginning of the experiment. These results are similar to those obtained for herring larvae, Clupea harengus. The number of days to irreversible starvation for herring larvae increased from

TABLE 4.—Lengths of fish in starvation groups and lengths of fish that died during starvation.

	Nu	mber of fis	h		
Group	Length <35 mm	Length ≥35 mm	Total ¹	Percent <35 mm	Mean length mm ± 2 SE
Sample before					
starvation	13	16	29	45	35.4 ± 1.8
All fish2:					
1	29	25	54	54	34.4 ± 1.5
2	16	24	40	40	36.0 ± 1.7
1 + 2	45	49	94	48	35.1 ± 1.1
Dead fish:					
1	21	4	25	84	30.9 ± 1.4
2	14	3	17	82	31.8 ± 1.6
1 + 2	35	7	42	83	31.2 ± 1.1

¹Fish that died on first day of starvation in groups 1 and 2 not included. ²Surviving fish measured at end of experiment after 5- to 8-day feeding period.

6 days at the end of the yolk-sac stage to 15 days at age 88 days (Blaxter and Ehrlich 1974).

Lipid content of fish declined during the starvation period from about 30% of dry weight to about 12% (Table 5). Recovery for the surviving fish was rapid, as they returned to the 30% level after 5 to 8 days of feeding. Water content was inversely related to fat as expected (Iles and Wood 1965). Fat content of muscle of adult anchovy is about 30 to 40% of dry weight during late summer and fall when gonadal fat is low (Lasker, Southwest Fisheries Center La Jolla Laboratory, unpubl. data). Thus, fat levels of these newly metamorphosed larvae appeared to be about the same as that of adult fish.

TABLE 5.—Total lipid and water content of anchovy at metamorphosis before, during, and after starvation.

Treatment	Elapsed time (days)	Water (%)	Total lipid dry wt (%)	Dry wt (mg)	Mean length (mm)	N
Before starvation End starvation:	0	78.1	30.6	74.6	35.0	7
Group 1	12	83.2	10.5	33.3	32.8	7
Group 2	15	82.9	13.4	54.2	36.9	5
End feeding:						
Group 1	20	79.5	32.3	90.3	39.6	9
Group 2	20	79.2	32.3	83.7	39.8	6

Extreme vulnerability to starvation appears to be characteristic of only the larval phase of the northern anchovy and it is over by the time the fish completes metamorphosis. There is a danger in interpreting these data beyond these general conclusions because reared fish may have more fat than wild ones and this could alter the results (Balbontin et al. 1973).

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