EFFECTS OF DELAYED INITIAL FEEDING ON LARVAE OF THE GRUNION, *Leuresthes tenuis* (AYRES)

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ABSTRACT

The initial feeding of newly hatched larvae of the grunion, *Leuresthes tenuis* (Ayres), was delayed for various periods of time under laboratory conditions at 18° C. Unfed larvae did not develop morphologically beyond the stage reached at the time of yolk absorption, about 4 days after hatching, although some survived as long as 3 weeks. Regardless of how long initial feeding was delayed, 80% or more of previously unfed larvae began feeding when food was made available to them, and at least 40% of the larvae alive when food was offered were able to survive to the end of a 20-day experiment. Some larvae feeding for the first time after 1 to 2 weeks without food, died after gorging themselves with *Artemia* nauplii. When food was offered to starved larvae, growth began and generally proceeded at about the same rate as in larvae fed from day 1, although there was some indication that a few days' delay in initial feeding increased the conversion efficiency of grunion larvae feeding on *Artemia* nauplii. Catabolism of fat provided most of the energy for metabolic processes during starvation. The condition factors and carbon/nitrogen ratios of unfed larvae were below those of fed larvae; condition factor seemed to be the better index of nutritional state. Grunion larvae probably do not experience high mortality at sea due to starvation, nor do they exhibit a classical "critical period" at the time of yolk absorption.

Most marine fishes pass through a free-swimming larvae stage, and it is well documented that survival through this stage is very low, generally being much less than 0.1% (e.g., Sette, 1943; Ahlstrom, 1954; Pearcy, 1962; Iizuka, 1966). The rate of survival through the larval stage is probably the most important factor determining the strength of year classes (Beverton, 1962; Gulland, 1965). Hjort (1914, 1926) advanced the hypothesis that larval survival was drastically affected by the abundance of food at the time the yolk was completely absorbed, and that poor year classes resulted when insufficient food was available to larvae at this "critical period." As Marr (1956) pointed out, Hjort's "critical period" concept has had a profound effect upon the thinking of fishery biologists.

Increased larval mortality at the time of yolk absorption has, however, proved difficult to demonstrate in nature. Marr (1956) concluded that the published evidence did not establish the existence of such increased mortality at sea; even in the light of more recent field data (Farris, 1961; Pearcy, 1962; Stevenson, 1962; Iizuka, 1966; Karlovac, 1967), it is difficult to decide from survival curves whether increased mortality at yolk absorption does in fact occur in nature. It has proved equally difficult to demonstrate that starvation is a major cause of larval mortality in the sea. Wild larvae found with empty guts (Lebour, 1920; Bowers and Williamson, 1951; Arthur, 1956; Bhattacharyya, 1957; Berner, 1959) may indicate imminent death by starvation or may reflect artifacts such as defecation or selective capture by plankton nets (Blaxter, 1965, 1969). Reports of apparently emaciated larvae, sometimes caught in regions where food is scarce (Soleim, 1942; Arthur, 1956; Shelbourne, 1957; Nakai, 1962; Hempel and Blaxter, 1963; Nakai et al., 1969), are suggestive but inconclusive (Marr, 1956; Blaxter, 1965, 1969). Field data thus indicate the possibility of high larval mortality due to starvation after the yolk has been absorbed but have not demonstrated its existence conclusively

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or determined its significance for year-class strength.

The response of larvae to food deprivation in the laboratory may provide badly needed evidence of how susceptible they are to starvation at sea. There have, however, been few attempts to determine experimentally the effects of delayed initial feeding on the larvae of marine fishes. Fabre-Domergue and Biétrix, the two pioneers of marine fish culture who coined the term "critical period," which Hjort later adopted, believed that feeding before the yolk supply was exhausted was essential to assure larval survival in laboratory rearing attempts. They stated that among larvae which received food prior to yolk absorption, ". . .*la phase que nous avions nommée période critique post-larvaire n'existe pas*" (Fabre-Domergue and Biétrix, 1898: 468). These authors went on to say that larvae which did not receive food early would subsequently become weak and unable to capture food and would exhibit considerable, if not total, mortality (Fabre-Domergue and Biétrix, 1898). The importance of early feeding for marine fish larvae was not further investigated in the laboratory until Blaxter and Hempel (1963) studied the effects of delayed initial feeding on the behavior of larval herring, *Clupea harengus* L. By feeding larvae after successively longer times without food, Blaxter and Hempel determined the time beyond which the larvae failed to exhibit feeding movements when supplied with food, a time they called the "point of no return." This point came 5 to 9 days after complete yolk absorption, at temperatures of 12° to 8° C, much later than the statements of Fabre-Domergue and Biétrix would have led one to expect. Recently, Lasker et al. (1970) observed the mortality of larvae of the northern anchovy, *Engraulis mordax* Girard, which had been fed at progressively later times after hatching. At temperatures of 15° to 22° C, larvae for which initial feeding had been delayed until 2.5 days after complete yolk absorption, showed the same pattern of mortality as groups of starved controls, while larvae receiving food 1.5 days after yolk absorption exhibited good survival—a phenomenon which these authors termed "irreversible starvation."

The purpose of the present study was to investigate in detail the changes which take place in starving larvae and in larvae whose initial feeding is delayed for various lengths of time, and thus to bring more evidence to bear upon the perennial questions of how susceptible larval fishes are to food deprivation and whether they do pass through a "critical period" at the time of yolk absorption. This study also sought to broaden the range of our knowledge of larval fish ecology by utilizing a species belonging to a group other than the Clupeiformes or Pleuronectiformes, or which nearly all of our information has hitherto been based. The fish chosen for study was the grunion, *Leuresthes tenuis* (Ayres), a member of the family Atherinidae. Atherinids produce rather large demersal eggs (Breder and Rosen, 1966), and the well-developed larvae which hatch provide an interesting contrast with flatfish and clupeoid larvae. Specifically, these experiments were designed to determine the effects of delayed initial feeding on mortality, on growth, and on the ability of grunion larvae to begin feeding and to utilize ingested food, and to ascertain what changes in the morphology and chemical composition of the larval body occur during starvation.

MATERIALS AND METHODS

SOURCE OF EGGS; HATCHING

The grunion is best known for its unusual habit of spawning on the sandy beaches of southern California and northern Baja California (Thompson and Thompson, 1919; Walker, 1952; Breder and Rosen, 1966). The eggs are deposited in the sand at night at certain times in the tidal cycle and are washed free some days later by a succeeding high tide, at which time the larvae hatch if the developmental period has been sufficiently long. The spawning season extends from late February or early March to late August or early September, with spawning intensity reaching a peak in April and May (Walker, 1952). During this time it is relatively easy to collect grunion eggs, which therefore provide

convenient material for the study of embryonic and larval development.

Eggs for the present study were collected during a spawning run on the night of March 24, 1970, at the beach in front of Scripps Institution of Oceanography, La Jolla, Calif. The eggs from a running ripe female were expressed into a small plastic container and artificially fertilized by adding milt from one male and a small amount of seawater; after approximately 1 min the water was decanted and sperm removed with several washes of fresh seawater. At the National Marine Fisheries Service Fishery-Oceanography Center in La Jolla, developing eggs were dispersed in a layer of washed, slightly moist beach sand approximately 1 cm deep at the bottom of rectangular plastic containers (16 × 12 × 11 cm), and a paper towel moistened with seawater was placed on the surface of the sand. The tops of the containers were covered with aluminum foil. This incubation procedure, essentially the same as one described by Morris (1956), kept the eggs moist and produced good hatching when excess water (which quickly brought on anoxic conditions) was avoided. The containers were placed in a water bath held at 20° ± 1° C by manually mixing water from the warm and cold seawater systems of the Fishery-Oceanography Center (see Lasker and Vlymen, 1969). The day before hatching, the temperature of the water bath was lowered to 18° C over a period of 3 hr.

On April 3, 1970, after 10 days of incubation (a common incubation time in nature, according to Walker, 1952), hatching was induced by adding filtered seawater at 18° C to the incubation containers and agitating the water and sand by drawing water rapidly in and out of a pipette. Hatched larvae were immediately transferred via pipette (4-mm bore) to 18° C water in rearing containers. In this paper the day of hatching will be referred to as day 0, the day after as day 1, and so forth.

DESIGN OF EXPERIMENT

A total of 20 rearing containers was set up, each with approximately 50 newly hatched grunion larvae. Seven containers (#1-7) were used to determine the effect of delayed initial feeding on mortality and growth; in six containers in this group, feeding was begun at progressively later times at 3-day intervals beginning on day 1—i.e., on days 1, 4, 7, 10, 13, and 16—while the larvae in one container (#7) were not fed and served as a control. On the 20th day after hatching, all surviving larvae which had received food in this series were collected, and the length, weight, and chemical composition of the larvae were determined. Seven of the other containers (#8-14) were fed daily beginning on day 1, and six (#15-20) were given no food; these containers, referred to as "supply containers" in what follows, supplied fed and unfed larvae for measurements of length, weight, and chemical composition and also for experiments on feeding and growth. On the same days when feeding was begun in a new container in the delayed feeding series (containers #1-7), larvae from both the "fed" and the "unfed" supply containers were used to determine the incidence of feeding and to begin quantitative feeding experiments.

PHYSICAL CONDITIONS

Water of approximately 33 ‰ salinity was taken from the seawater system of the Fishery-Oceanography Center. The larval fish containers were originally filled with HA Millipore-filtered seawater, and at weekly intervals filtered water was added to the fed containers in order to replace water removed with uneaten food and fecal matter (see below), the volume added usually being between 1 and 2 liters. The temperature in the water bath was kept at 18° ± 1° C, and temperatures in the larval containers were within 0.5° C of the bath temperature. Eighteen degrees is the midpoint of the 14°-22° C range of water temperatures which occurs off La Jolla during the spawning season of the grunion (Reid et al., 1958). Banks of two 40-watt "daylight" fluorescent lamps positioned 76 cm above the surface of the water illuminated the containers for 12 hr each day. The lights were timed to go on after sunrise, so that diffuse light entering through windows increased

slowly in intensity as the sun rose and no abrupt dark-light transition was imposed upon the larvae.

CONTAINERS

The containers used for rearing larvae in this study were the same as those described by Lasker et al., 1970), being circular (35 cm diameter, 14 cm deep) and made of the plastic alloy Kydex.[2] These containers have nonglossy black surfaces and hold 10 liters of seawater. For feeding studies with individual larvae, smaller Kydex containers were used (10.5 cm diameter, 4.2 cm deep) which held 300 ml of water. Both large and small containers were covered with clear plexiglass tops to reduce evaporation and keep out dust particles.

FEEDING

Nauplii of the brine shrimp, *Artemia salina*, were used exclusively as a food source. *Artemia* nauplii have proven to be poor food for larval clupeids but excellent for several other types of larvae (May, in press), and they appear to satisfy the nutritional requirements of larval grunion. Nauplii were obtained by hatching San Francisco brine shrimp eggs in trays modeled after those described by Riley (1966). Two trays were used, which allowed harvesting of trays on alternate days with a time lapse of 48 hr between inoculation of eggs and harvesting of nauplii. The water in the trays was kept at about 20° C. Nauplii were rinsed in filtered seawater and added to rearing containers shortly after the lights went on each morning, and more were added during the day if the concentration dropped low enough to prevent *ad libitum* feeding. If any uneaten nauplii remained from the previous day, as many as possible were removed by pipette before the morning addition of new nauplii. Fecal matter was siphoned daily from the bottoms of the "fed" containers.

[2] Kydex is manufactured by Rohm and Haas, Philadelphia, Penn. Use of trade name does not imply endorsement of the product.

QUANTITATIVE FEEDING STUDIES

At 3-day intervals beginning on day 1, 6-day quantitative feeding experiments were begun to measure the food consumption and growth of previously fed and unfed larvae. Larvae were transferred individually, from both the "fed" and the "unfed" supply containers, to small (300 ml) containers late in the afternoon on the day before the beginning of the feeding experiment. Three larvae from a "fed" container and three from an "unfed" container were used in each feeding experiment; individual larvae were kept in separate containers during the feeding study.

On the morning following transfer to the feeding containers, and for six mornings thereafter, a known number of *Artemia* nauplii was counted out with a pipette under a dissecting microscope and added to each container. Shortly before the lights went off at the end of the day, the grunion larvae were transferred by pipette to new containers, and the uneaten *Artemia* in the old containers were concentrated on a nylon mesh, preserved in Formalin and later counted. The difference between the number of nauplii added in the morning and the number left at the end of the day gave the number eaten by a larva. At the start of the series of feeding experiments, on day 1, 100 nauplii were added to each experimental container; when larvae consumed 70% or more of the nauplii offered, the number offered the following day was increased by 50 nauplii. On the morning following the final (6th) day of feeding, the experimental larvae were collected and analyzed as described below. The weight of a larva at the start of the feeding experiment, estimated from the mean weight of 10 larvae sampled at that time, was subtracted from the weight of the experimental larva at the end of the feeding period to yield its gain in dry weight.

In order to determine the weight of the ingested material, the weight of a single *Artemia* nauplius was estimated by making several weighings on an electrobalance[3] of groups of 5 to 20 nauplii, collected at the same interval after inoculation of eggs as the nauplii used in the feeding study. The nauplii were rinsed with distilled water and dried to constant weight at

60° C before weighing. The mean weight per nauplius was 1.64 μg, almost identical to Paffenhöfer's (1967) value of 1.65 μg per newly hatched *Artemia* nauplius. Where the larvae had yolk sacs at the beginning of the feeding period, the mean weight of the yolk masses dissected off the 10 larvae sampled at the start of the experiment (the dissection technique is described below) was added to the weight of the nauplii consumed to yield the total dry weight of the consumed material.

The growth and food consumption of individual larvae during the feeding period were thus known and allowed calculation of the efficiency of food conversion:

$$\text{percent conversion efficiency} = \frac{\text{dry weight gained}}{\text{dry weight consumed}} \times 100.$$

INCIDENCE OF FEEDING

The percentage of larvae which fed after progressively longer times without food, termed here the incidence of feeding, was determined in separate experiments in 10-liter containers. Approximately 25 larvae were transferred from one of the "unfed" supply containers to a 10-liter container with filtered seawater late in the afternoon preceding the experiment, and on the following morning large numbers of *Artemia* nauplii were introduced into the container. One hour later, the anesthetic MS-222 (tricaine methanesulfonate) was added to the container to a concentration of 132 mg/liter, and the anesthetized larvae were examined under a dissecting microscope for the presence of an orange-colored gut indicative of feeding on *Artemia* nauplii. Simultaneously, the incidence of feeding among larvae from one of the "fed" supply containers was determined in the same way, to serve as a control with which hitherto unfed larvae could be compared. Experiments of this sort were conducted on the same days on which food intake and conversion experiments were started, beginning with day 4. Owing to mortality from starvation, the numbers of larvae available in the "unfed" containers dwindled so that only 13 larvae were available for the feeding incidence experiment on day 13 and 4 on day 16. By day 16, body pigmentation had developed in larvae from the "fed" containers to such an extent that feeding incidence could not be assessed by examining the coloration of the gut, and no value was obtained for previously fed larvae on this day.

Anesthesis stimulated peristalsis in grunion larvae, as Blaxter (1965) observed in larval herring, but the procedure in the present experiment was rapid enough that at most only the contents of the rectum were being extruded during examination and all larvae which had in fact fed were recorded as such. It should be pointed out that, unlike the straight, tubelike gut of clupeid larvae, the gut of larval grunion is already differentiated at hatching into three more or less distinct portions, the last of which, the rectum, is separated from the rest of the gut by an "ileorectal valve" (Al-Hussaini, 1947) which inhibits rapid defecation of material not in the rectum.

MORTALITY

Dead larvae were removed from the containers each morning by pipette. A larva was considered dead when its brain had become opaque and it did not respond to water current or to tactile stimulation. Dead larvae were routinely examined with a dissecting microscope.

SAMPLING PROCEDURE AND ANALYSIS OF LARVAE

Larvae were collected by pipette, and their lengths measured with an ocular micrometer from snout to tip of notochord, or, after upward flexion of the tip of the notochord had taken place, to the posterior edge of the hypural elements (standard length). Only free-swimming larvae were sampled, although in "unfed" containers these became increasingly rare toward the end of the experiment. Sampled larvae were rinsed quickly in distilled water and placed on glass microscope slides. Since larvae which were sampled on days 1 and 4 still possessed yolk sacs, they were preserved in 3%

[3] Cahn Instrument Company, Paramount, Calif.

Formalin (in 50% seawater). Within 1 week of sampling they were rinsed in distilled water and their yolk dissected off, separated larvae and yolk masses being placed on microscope slides. The samples on slides were dried to constant weight at 60° C and weighed to the nearest microgram on an electrobalance. In agreement with the results of Blaxter and Hempel (1966), no effect of Formalin on the dry weight of larvae was found, nor was there a significant effect of Formalin on the dry weight of yolk masses (this was tested in a previous experiment by comparing dry weights of yolk dissected from preserved larvae with yolk dissected from frozen larvae or collected in preweighed capillary tubes).

Larvae sampled from the supply containers at 3-day intervals, and those collected from the delayed-feeding series on day 20, were analyzed for their ash, carbon, hydrogen, and nitrogen content. Percent ash was determined by weighing separately three randomly chosen larvae from each sample before and after combustion at 500° to 520° C. In the case of larvae fed from day 16 and sampled on day 20, only one larva was available for the ash determination. During combustion, larvae were held on tarred pieces of precombusted aluminum foil, and weighings were made on an electrobalance. The remaining larvae in each sample were ground into fine particles with an agate mortar and pestle, and two aliquots of this material from each sample were analyzed for carbon, hydrogen, and nitrogen content with a Model 185 carbon-hydrogen-nitrogen analyzer.[4] The number of replicates was limited by the amount of material available, but variation between replicate determinations was small, and means calculated from the replicates were accepted as the ash, C, H, and N values for the sample. This approach to the chemical analysis of larvae was chosen because it allowed determination of C/N ratios, estimation of protein and fat content, and calculation of caloric content (see Results section).

Larvae which had been used in the feeding incidence experiments described above were preserved in 3% Formalin and later cleared in KOH and stained with Alizarin Red-S, the standard stain for bone, to allow comparison of ossification in fed and unfed larvae.

RESULTS

BEHAVIOR

Newly hatched grunion larvae have functional eyes and jaws and are extremely active (Thompson and Thompson, 1919; David, 1939). Grunion larvae which received food in the present experiment remained very active as they grew, and some schooling behavior was noted as early as day 6. Of more immediate concern was the behavior of starved larvae. On day 7 it was noted that unfed larvae were much easier to catch with a pipette than fed larvae. As the period of starvation lengthened, larval activity declined and the number of larvae remaining quiescent on the bottom increased. Near the end of the experiment, no starved larvae were swimming freely above the bottom, and their activity consisted in occasional erratic movements, followed by long quiescent periods.

SURVIVAL

Figure 1 shows the survival to day 20 of larvae which were fed at various times after

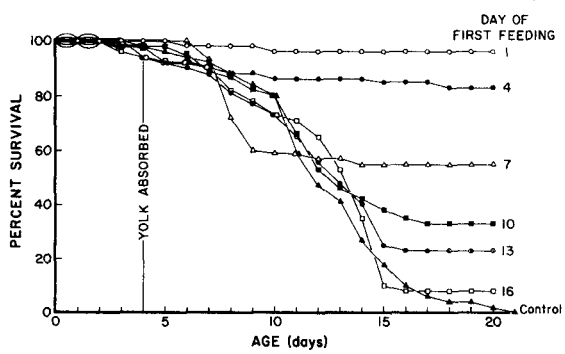

FIGURE 1.—Survival curves for larvae with different times of initial feeding, at 18° C. The number at the end of each curve indicates the day of initial feeding. The control group was given no food during the experiment.

[4] Hewlett Packard Corporation, Palo Alto, Calif.

hatching and a starved control. Yolk was completely used up in unfed larvae by day 4, and on this day only a minute amount was left in fed larvae. The survival curve for unfed larvae passed the 50% line between days 11 and 12, roughly the same as the starvation time given by Hubbs (1965) for larval grunion at 18° C. The starved control larvae were all dead by day 21. There is a direct relationship between percent survival of original larvae to day 20 and the day of first feeding (Table 1). The number

TABLE 1.—Survival to day 20 of larvae with different times of initial feeding.

Day of first feeding	Original number of larvae	Number of larvae alive when food first offered	Number of larvae alive on day 20	Percent survival to day 20	
				Original larvae	Larvae alive when food first offered
1	51	51	49	96.1	96.1
4	52	51	43	82.7	84.3
7	53	49	29	54.7	59.2
10	55	44	18	32.7	40.9
13	48	23	11	22.9	47.8
16	51	4	4	7.8	100.0

of larvae which survived to day 20, expressed as a percentage of those which were alive when food was first offered, is also listed in Table 1. This figure never dropped below 40%, and all of the previously unfed larvae alive in container #6 on day 16 (i.e., four larvae) began feeding when food was supplied and survived to day 20, and their general appearance and behavior indicated that they would easily have survived longer, had the experiment been prolonged.

In the group of larvae which was fed for the first time on day 7, 11 larvae were found dead on the morning following the day of first feeding; 9 dead larvae had food in their guts and 5 of these had guts which were bright orange and so stuffed with *Artemia* nauplii that the abdomen was noticeably distended. In the group fed initially on day 10, all eight larvae found dead the next morning had food in their guts, and four of these had bright orange, packed guts. On day 14, only one out of four dead larvae, found in the container which had been fed for the first time on the previous day, had food in its gut and showed the orange and bulging abdomen noted in dead larvae from the previous two groups.

GROWTH

The increase in length and dry weight of larvae sampled from the "fed" supply containers is presented in Table 2. The rate of growth was higher from day 16 on; variability likewise increased after this time, an example of the "growth depensation" which is commonly found in growing fish (Ricker, 1958). On day 7, the hypural elements were beginning to form along the posterior ventral margin of the notochord, and on day 10 the tip of the notochord was beginning its upward flexion. The greatest increase in length occurred between days 1 and 4, and owing to the upturning notochord the mean length actually decreased between days 13 and 16 (Table 2). On day 4, only the cleithrum and a very few cranial and branchial elements were ossified, but by day 10 about half of the vertebrae (the anterior ones) and some of the caudal rays were beginning to take up alizarin, and by day 16 all vertebrae and hypural elements were ossified.

TABLE 2.—Length and weight of fed and unfed larvae. $\bar{x}$ = mean, *SD* = standard deviation, n = number of larvae measured.

Age (days)	Length (mm)						Dry weight (mg)					
	Fed			Unfed			Fed			Unfed		
	$\bar{x}$	*SD*	n	$\bar{x}$	*SD*	n	$\bar{x}$	*SD*	n	$\bar{x}$	*SD*	n
1	--	--	--	8.96	0.17	9	--	--	--	0.362	0.021	9
4	9.64	0.34	10	9.31	0.20	10	0.428	0.038	10	0.386	0.030	10
7	10.70	0.59	10	9.27	0.12	10	0.771	0.130	10	0.409	0.015	9
10	11.66	0.28	10	9.11	0.23	10	1.027	0.100	10	0.355	0.032	10
13	12.28	0.34	10	8.98	0.19	10	1.340	0.183	10	0.311	0.013	10
16	12.22	0.60	10	8.78	0.19	10	1.517	0.361	10	0.266	0.018	10
19	13.53	0.58	10	--	--	--	2.433	0.458	10	--	--	--
25	15.12	0.45	10	--	--	--	3.804	0.464	10	--	--	--

Starved larvae exhibited a slow decline in dry weight after yolk absorption, with little variability between larvae (Table 2). Although rudiments of the hypural elements were just discernible in starved larvae on day 7, their notochords never showed evidence of upward flexion, even as late as day 16. On day 16, ossification in starved larvae was comparable to that in fed larvae from day 4 or day 7, with only the cleithrum and a few elements of the cranium and visceral skeleton taking up alizarin.

The lengths and weights of 20-day-old larvae from the delayed feeding series (containers #1-6) are listed in Table 3, and in Figure 2 the weights are plotted and connected by dotted lines to the weights of starved larvae on the days when food was first offered. As expected, the later the initial feeding, the lower the mean weight on day 20, although the range of weights of larvae fed from day 4 falls within that of larvae fed from day 1, and larvae fed from days 13 and 16 likewise overlap (Figure 2). The rate of gain in weight decreases with delay of initial feeding, as indicated by the decreasing slopes of the dotted lines in Figure 2, but these rates are similar to those of larvae feeding for comparable lengths of time from day 1 (e.g., the weight gain of fed larvae between days 1 and 10 is about the same as that of larvae fed initially on day 10 and sampled on day 20). Larvae fed from days 1, 4, and 7 had completed notochordal flexion by day 20, and those fed from day 10 were at an intermediate stage of flexion at this time; in those fed from days 13 and 16, the process of flexion had not yet begun by day 20. Larvae from both the fed series and the delayed-feeding series indicated that notochordal flexion did not begin until a length of about 11 mm and a dry weight of about 1 mg had been reached.

TABLE 3.—Length and weight of 20-day-old larvae with different times of initial feeding. $\bar{x}$ = mean, SD = standard deviation, n = number of larvae measured.

Day of initial feeding	Length (mm)			Dry weight (mg)		
	$\bar{x}$	SD	n	$\bar{x}$	SD	n
1	14.24	0.61	20	2.702	0.420	20
4	14.03	0.36	20	2.513	0.193	20
7	12.38	0.31	20	1.638	0.148	20
10	11.10	0.38	18	0.995	0.086	18
13	9.87	0.51	11	0.561	0.137	11
16	9.82	0.35	4	0.436	0.036	4

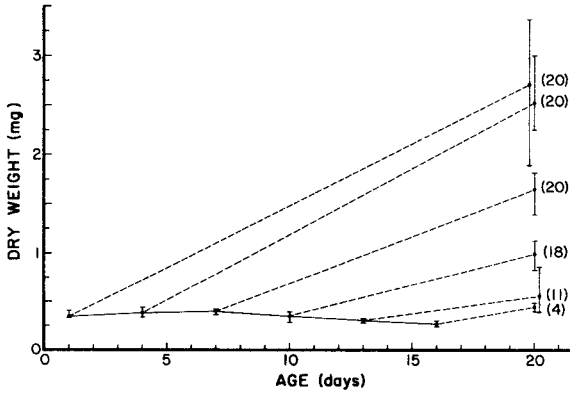

FIGURE 2.—Dry weights of 20-day-old larvae with different times of initial feeding. Means and ranges are plotted, and the number of 20-day-old larvae measured in each group is given in parentheses. Dashed lines connect mean weights at day 20 with the mean weights of unfed larvae on the days when feeding was initiated.

Condition factors have been used in the past to assess the nutritional state of fish larvae (Hempel and Blaxter, 1963; Blaxter, 1965) and were calculated for each sample in the present experiment as

$$\frac{\text{(mean dry weight, mg)}}{\text{(mean standard length, mm)}} \times 10^3$$

Figure 3A shows that after day 4, the condition factors for fed larvae increased until the end of the experiment on day 25, while those for starved larvae, after showing a slight rise on day 7, decreased until the final sampling on day 16. As shown in Figure 4A, the condition factors of 20-day-old larvae decreased in groups for which initial feeding had been delayed 7 days or more, with larvae fed from day 16 showing a condition factor between those of starved larvae 10 and 13 days old.

FEEDING INCIDENCE

On day 4, larvae which had been offered food since day 1 showed a higher feeding incidence (88%) than those which were given food for

the first time on this day (35%)—Table 4. However, on days 7, 10, and 13 the feeding incidence was similar (between 80 and 96%) in larvae which had fed and those which had not fed prior to the test. On day 16, the darkly pigmented abdomen of previously fed larvae made it impossible to determine their feeding incidence, but all of those larvae tested which had received no food prior to day 16 did consume food on this day. The failure of previously fed larvae to show a feeding incidence of 100% in these experiments probably reflects the stress associated with transfer between containers.

TABLE 4.—Feeding incidence of previously fed and unfed larvae. Larvae were exposed to *Artemia* nauplii for 1 hr, after which they were examined for evidence of feeding.

Age (days)	Larvae previously fed			Larvae previously unfed		
	Number of larvae feeding	Number of larvae not feeding	Percent feeding	Number of larvae feeding	Number of larvae not feeding	Percent feeding
4	22	3	88.0	9	17	34.6
7	21	2	91.3	23	1	95.8
10	22	2	91.7	24	1	96.0
13	20	4	83.3	8	2	80.0
16	--	--	--	4	0	100.0

FOOD INTAKE AND CONVERSION

On day 1, the mean dry weight of the larval yolk supply was 0.027 mg (range, 0.015-0.039 mg), and on day 4, fed larvae retained 0.003 mg of yolk (range, 0-0.011 mg) while starved larvae had no yolk left.

In the quantitative feeding experiments, conducted in small, 300-ml containers, some larvae did not survive the 6-day experimental period, and some exhibited erratic swimming behavior. Only data from the surviving larvae which displayed normal behavior have been retained. Larvae did not begin feeding until day 2, although food was available to them on day 1. The number of nauplii consumed daily per larva increased as larvae grew, from less than 50 in first-feeding larvae to almost 300 in larvae 2 weeks old and older. Table 5 gives the total food consumption, growth, and conversion efficiencies of all healthy larvae which survived the feeding experiments in small containers. Larvae which displayed growth comparable to that of larvae in 10-liter containers may be expected to give the most reliable conversion efficiency values and are identified by asterisks in Table 5. One larva, fed from day 1, showed the extremely high efficiency of 73%. Table 5 suggests a trend toward decreasing conversion efficiency as larvae get older. In the experiment begun on day 7, the previously unfed larva for which data are available showed a much higher efficiency than the previously fed larva.

BODY COMPOSITION

Results of the analyses of carbon, hydrogen, nitrogen, and ash in sampled larvae are given

TABLE 5.—Food consumption, growth, and conversion efficiencies of individual larvae in small containers during 6-day feeding experiments. Asterisks identify larvae which exhibited growth comparable to that of larvae in large containers and hence probably provide the most reliable conversion efficiency figures.

Age at start of feeding experiment (days)	Previous treatment	Dry weight (mg)				Percent conversion efficiency [= (gain/total food consumed) × 100]
		Total food consumed	Larva, initial	Larva, final	Larva, gain	
1	--	[1]0.433	0.362	0.678	0.316	73.0*
1	--	[1]0.771	0.362	0.700	0.338	43.8*
4	fed	[2]1.321	0.428	1.189	0.761	57.6*
4	unfed	1.074	0.386	0.880	0.494	46.0
7	fed	1.053	0.771	0.962	0.191	18.1
7	unfed	1.026	0.409	0.826	0.417	40.6
10	fed	2.250	1.027	1.940	0.913	40.6*
10	fed	1.640	1.027	1.528	0.501	30.5*
10	unfed	0.978	0.355	0.668	0.313	32.0
13	fed	2.000	1.340	1.657	0.317	15.9
16	fed	2.365	1.517	2.002	0.485	20.5
		2.155	1.517	2.418	0.901	41.8

[1] Includes 0.027 mg of yolk.
[2] Includes 0.003 mg of yolk.

TABLE 6.—Carbon, hydrogen, nitrogen, and ash in larval samples, as percentages of total dry weight.

Age (days)	Treatment	C (%)	H (%)	N (%)	Ash (%)
1	--	45.7	7.0	10.2	5.1
4	fed	45.5	7.0	10.5	5.4
7	fed	46.2	7.1	10.5	7.9
10	fed	47.1	7.1	10.8	8.6
13	fed	46.2	7.2	11.3	8.6
16	fed	45.2	7.0	10.9	8.7
19	fed	45.0	7.0	10.7	9.2
25	fed	43.1	6.6	10.9	10.0
4	unfed	46.5	6.9	10.4	6.6
7	unfed	44.5	6.9	10.6	7.8
10	unfed	44.0	6.8	11.1	9.4
13	unfed	44.0	6.8	11.2	9.2
16	unfed	43.2	6.4	11.3	7.3
20	fed from day 1	45.0	6.7	11.3	9.9
20	fed from day 4	43.1	6.7	11.1	9.8
20	fed from day 7	44.8	6.8	11.4	9.4
20	fed from day 10	44.8	6.7	10.8	8.2
20	fed from day 13	44.6	6.7	10.9	8.8
20	fed from day 16	43.3	6.5	10.6	11.4

in Table 6. In this paper the term *level* will be used in the sense of Giese (1969) to denote the percentage of the total dry weight which a particular body component constitutes. In fed larvae, the level of ash increased from 5.1% on day 1 to 10.0% on day 25. The ash level was higher in unfed than in fed larvae, except on days 7 and 16. The nitrogen level increased with age in both fed and starved larvae, but the increase was more steady in the latter. Fed larvae fluctuated between 10.7 and 11.3% nitrogen from day 10 to day 25. Accompanying the increase in nitrogen was a decrease in the level of carbon. Among 20-day-old larvae, the level of ash was higher, nitrogen lower, and carbon the same or slightly lower in larvae whose initial feeding had been delayed for 16 days than in larvae fed earlier. Nitrogen in 20-day-old larvae decreased with time of first feeding, from day 7 to day 16. The ratio of carbon to nitrogen has been plotted in Figures 3 and 4 along with condition factors. The C/N ratio is lower in starved than in fed larvae after day 4 but shows a decreasing trend with time even in fed larvae whose condition factor is increasing (Figure 3). On day 20, larvae whose initial feeding had been delayed 10 or more days had higher C/N values than larvae fed earlier; here too, decreasing condition factors accompanied increasing C/N values. Since preservation in Formalin has been shown to affect the C, H, N, and ash levels of copepods

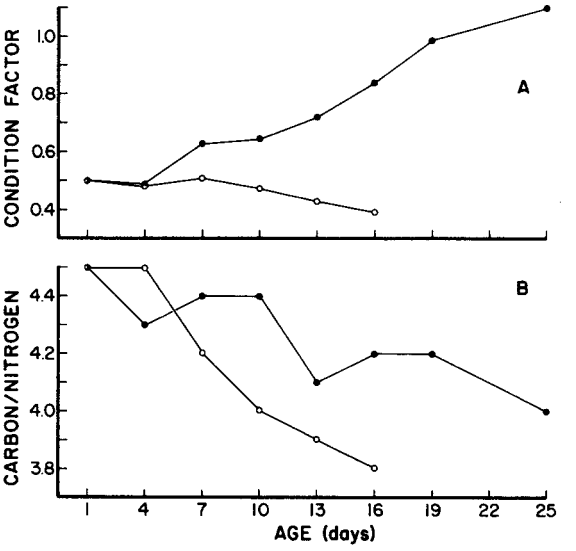

FIGURE 3.—Condition factors and carbon/nitrogen ratios of fed and unfed larvae. Condition factors were calculated as [(mean dry weight, mg)/(mean standard length, mm)] $\times 10^3$. Closed circles = fed larvae, open circles = unfed larvae.

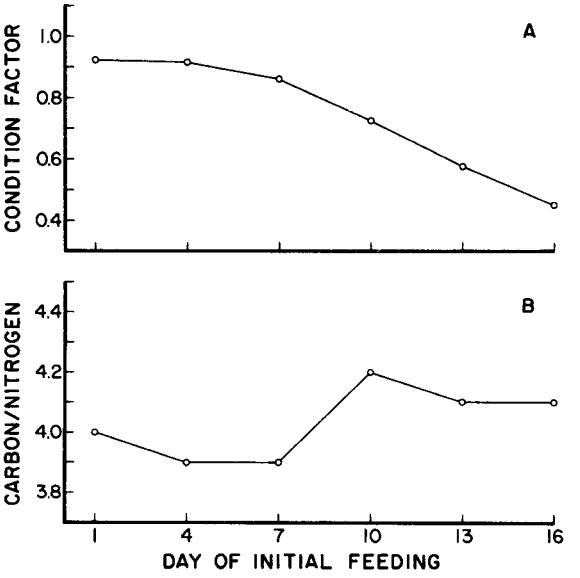

FIGURE 4.—Condition factors and carbon/nitrogen ratios of 20-day-old larvae with different times of initial feeding. Condition factors calculated as in Figure 3.

(Omori, 1970), the values given here for larvae 1 and 4 days old, which had been preserved in Formalin to allow removal of yolk by dissection, may be somewhat in error.

The level of protein in larval samples was estimated by multiplying the nitrogen level by 6.25 (White, Handler, and Smith, 1968), and fat was calculated by difference: 100 - (percent ash + percent protein) = percent fat. Nonprotein nitrogen and carbohydrate were assumed to be present in negligible amounts in this material (Lasker, 1962). Caloric content was calculated by multiplying weights of fat and protein in average larvae by 9.5 cal/mg and 5.7 cal/mg, respectively (Brody, 1945; Kleiber, 1961). Table 7 lists the resulting values. The most notable trends are an increase in protein level and decrease in fat level, in both fed and unfed larvae (Table 7). Unfed larvae had lower fat and about the same or somewhat higher protein levels than fed larvae. Among 20-day-old larvae, those for which initial feeding had been delayed tended to have higher fat and lower protein levels than those fed early (Table 7).

TABLE 7.—Protein, fat, and caloric content of larval samples. Protein and fat are given as percentages of total dry weight. Protein was calculated from the nitrogen content of samples, fat by difference, and caloric content by multiplying weights of protein and fat by standard conversion factors.

Age (days)	Treatment	Protein (%)	Fat (%)	Caloric content	
				cal/mg of dry weight	cal/mg of ash-free dry weight
1	--	63.8	31.1	6.60	6.97
4	fed	65.6	29.0	6.50	6.86
7	fed	65.6	26.5	6.25	6.79
10	fed	67.5	23.9	6.12	6.70
13	fed	70.6	20.8	6.00	6.54
16	fed	68.1	23.2	6.11	6.68
19	fed	66.9	23.9	6.09	6.70
25	fed	68.1	21.9	5.97	6.64
4	unfed	65.0	28.4	6.43	6.89
7	unfed	66.3	25.9	6.26	6.79
10	unfed	69.4	21.2	5.94	6.55
13	unfed	70.0	20.8	5.98	6.60
16	unfed	70.6	22.1	6.13	6.60
20	fed from day 1	70.6	19.5	5.89	6.54
20	fed from day 4	69.4	20.8	5.94	6.59
20	fed from day 7	71.3	19.3	5.90	6.50
20	fed from day 10	67.5	24.3	6.16	6.71
20	fed from day 13	68.1	23.1	6.07	6.65
20	fed from day 16	66.3	22.3	5.89	6.64

Reflecting changes in proximate composition, the caloric content of larval tissue showed an early decrease and from day 10 on showed no increasing or decreasing trend, in both fed and unfed larvae (Table 7). From day 10 on, starved larvae tended to have a lower caloric content than fed larvae. The caloric contents of 20-day-old larvae showed no consistent trend with time of initial feeding (Table 7).

DISCUSSION

The developmental process requires a nutritional input to supply energy and raw materials. In larval grunion which receive no food, development does not progress beyond the stage reached when the yolk is absorbed, although the larvae survive well beyond this point. The process of ossification is halted and the upward flexion of the notochord does not take place in unfed larvae, while tissue resorption, supplying energy for metabolic processes during starvation, results in a slow decrease in larval mass. Fat seems to be utilized most during starvation. The amount of fat in an average starving larva decreases by 0.071 mg, or 0.689 cal, during 16 days of starvation, while protein decreases by only 0.043 mg, or 0.245 cal (Tables 2 and 7). The fat and protein levels of feeding larvae are not greatly different from those of starving larvae (Table 7), but in the former case the observed increase in protein level with time must be a consequence of rapid protein synthesis in the growing organism, whereas in the latter it reflects the utilization of the body's fat reserves.

When food is offered to unfed larvae, growth begins and proceeds at about the same rate as in larvae fed from day 1 (Figure 2). Weight and body composition at day 20 in larvae whose initial feeding was delayed for various periods is close to that of larvae fed for similar lengths of time from day 1 (Tables 2, 3, and 7), though fat is much more depleted in larvae fed for 4 and 7 days starting on days 16 and 13, respectively, than in larvae fed for 4 and 7 days starting on day 1. Larvae fed for a period of 16 days, from day 4 to day 20, gained more weight and had higher protein levels than larvae fed from days 1 to 16 (Tables 2, 3, and 7), suggesting that a few days' delay in initial feeding caused

an increase in conversion efficiency. A similar effect has been found in adult fish (Ivlev, 1939; Pandian, 1967). There is some indication in the results of the quantitative feeding experiments that larvae convert food more efficiently after 7 days without food than after 7 days of feeding (Table 5), but the data are too meager to justify any conclusion on this point.

Omori (1970) showed that copepods from areas poor in food tended to have lower C/N ratios than copepods from rich areas. In larval fishes, condition factors have been used in attempts to assess nutritional state (Hempel and Blaxter, 1963; Blaxter, 1965). Both measures were compared in the present study (Figures 3 and 4). Although starved larvae had lower C/N ratios than fed larvae after day 4, due presumably to catabolism of fat, the C/N ratios of growing, fed larvae decreased with age as a consequence of the rapid elaboration of protein, while their condition factors increased. Reflecting this same tendency, larvae 20 days old had higher condition factors but lower C/N ratios, the longer they had been feeding. Condition factor seems to be somewhat more consistent and reliable as an index of the nutritional state of larval grunion than C/N ratio.

In sum, larval grunion appear to be extremely resistant to food deprivation. Under laboratory conditions it takes 3 weeks for all larvae to die of starvation at 18° C (Figure 1). No matter how long initial feeding is delayed, over 40% of the larvae alive when food is offered will survive, and all larvae which survive 16 days without food can commence feeding at this time and survive (Table 1). Since grunion larvae hatch from eggs deposited in the beaches of southern California and northern Baja California and must inhabit inshore waters almost exclusively, and since the abundance of microplankton is extremely high in inshore as compared with offshore waters in this region (Beers and Stewart, 1967), it seems unlikely that these larvae ever experience high rates of mortality due to starvation. Major sources of mortality among grunion larvae must be sought, rather, in predation and physical damage from waves. Tidal variations may result in different incubation periods in grunion eggs from different spawnings (Walker, 1952), but the effect of this on larval viability has yet to be determined.

These findings differ from results for clupeoid larvae. In the northern anchovy a delay in initial feeding of 2.5 days after yolk absorption resulted in nearly complete mortality, even though many larvae were alive when food was administered (Lasker et al., 1970). This "point of irreversible starvation" appears not to exist for larval grunion, as starvation can in fact be reversed at any point along the survival curve of starved larvae (Figure 1).

Larvae of the herring (*Clupea harengus*) show a decrease in the percentage of larvae which commence feeding as the period of food deprivation is lengthened, and the point at which the percentage feeding is half that at the start of the experiment has been termed the "point of no return" (Blaxter and Hempel, 1963; Blaxter, 1965). Again, the grunion larvae show a different pattern, with at least 80% of the larvae commencing feeding when food is offered after periods of starvation ranging from 7 to 16 days (Table 4). Some larvae which did commence feeding after 7, 10, and 13 days without food were nevertheless unable to survive and died after gorging themselves with *Artemia* nauplii. The interesting fact that all of the larvae alive after 16 days of starvation commenced feeding and survived, while the percentage feeding was lower in larvae starved for shorter periods of time, may be explained as a result of mortality among the weakest larvae, so that by day 16 only the most hardy individuals were still alive.

Thus, certain types of larvae would be more likely than others to show a "critical period" pattern of mortality at sea under conditions of low food availability. If northern anchovy larvae were not to encounter food within 2.5 days after yolk absorption, there would ensue a catastrophic mortality concentrated in time (Lasker et al., 1970). In contrast, grunion larvae, which hatch in a more well-developed and robust state, would exhibit mortality extending over a number of days if deprived of food and hence would not show a "critical period" in the classical sense of Hjort. Obviously a sudden increase

in mortality at sea could come about after yolk absorption, or at any other time, owing to factors other than the availability of food. Hjort was, of course, not referring to larvae of the atherinid type when he enunciated his "critical period" hypothesis, but with the large volume of published material now available concerning the larvae of a few commercially important species, it would be easy to lose sight of the great diversity of larval forms and to apply ideas which may have validity in some groups to groups in which they have no place.

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