THE PHYSIOLOGY OF PACIFIC SARDINE EMBRYOS AND LARVAE

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INTRODUCTION

The purpose of this paper is to review the present state of knowledge on the physiology of sardine eggs and larvae, to show the relationship between the developing larva and its yolk supply, and finally to offer some deductions on what the available food supply for larvae must be after the yolk supply is exhausted.

GENERAL BIOLOGY OF THE SARDINE EGG AND LARVA

The sardine egg after fertilization is planktonic, has a chorion about 1.7 mm in diameter, a perivitelline space containing sea water and a spherical yolk sac about 1 mm in diameter. The chorion is freely permeable to water, salts and respiratory gases (Lasker and Theilacker, 1962). The yolk mass and developing embryo is less dense than the chorion and floats within the subchorionic space abutting the inside of the chorion at the top. During stage XI (see Ahlstrom, 1943 for stage descriptions) with development of the embryonic tissue and the concomitant utilization of the light yolk the entire egg sinks. This sinking period (depending on the temperature and density of the water) can be as long as 2-3 hours and at a rate of about two meters per hour whereupon the animal hatches out, probably by utilizing a hatching enzyme. Unencumbered by the heavier chorion the larva floats upward. At this stage the sardine larva lacks a mouth, an open gut, gills, pigment in the eyes, and its organ systems are virtually undeveloped. An electron micrograph (Threadgold and Lasker, unpublished) of the skin of the newly hatched larva is shown in Figure 1. The entire skin is composed of two cell layers, is 1.7μ thick in the fin-fold region, and 3μ in the main body region. In cross section each cell of the outer layer has an internal densely staining rod-shaped structure which may be the precursor of the true scale. There are structures in the outer cell layer which suggest a secretory function for the epithelium but an analysis of the role of subcellular structures in osmoregulation is yet to be done. As the larva develops (at 15° C for example) the mouth begins to form at 162 hours post-spawning and the gut opens four hours later. At this point the bulk of the yolk has been consumed and only a trace remains. Fully pigmented eyes and a movable jaw are complete at approximately 170 hours and the last trace of yolk is utilized seven hours later. At first opening the gut is 40μ in diameter and can be distended to 100μ the following day with food. In dense suspensions of unicellular algae (e.g. *Platymonas subcordiformis*) larvae with their intestines fully opened rapidly fill their gut by swallowing.

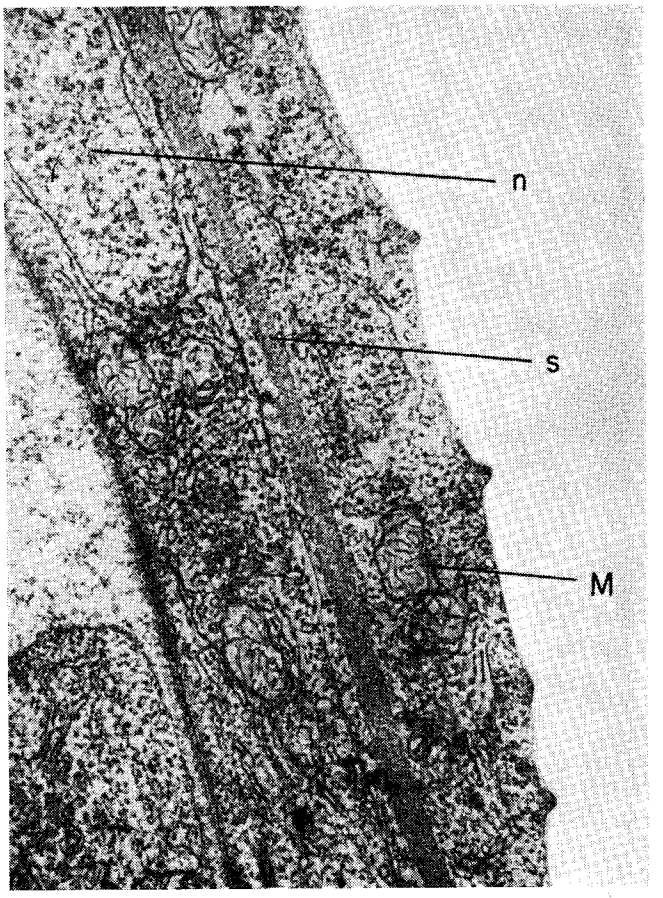

FIGURE 1. Electron micrograph photograph of a cross section of larval Pacific sardine skin. M, mitochondria; n, nucleus; s, scale anlage (?) photo by Dr. L. T. Threadgold, Queen's University, Belfast, Ireland). Larval skin is approximately 3 μ across.

ENERGY REQUIREMENTS

During development, as organized tissue is added, there is a gradual but definite increase in oxygen uptake (Fig. 2) and at the time the yolk is completely consumed the animal has its highest basal rate. Swimming movements are frequent and cause an increase in respiration. A balance sheet has been

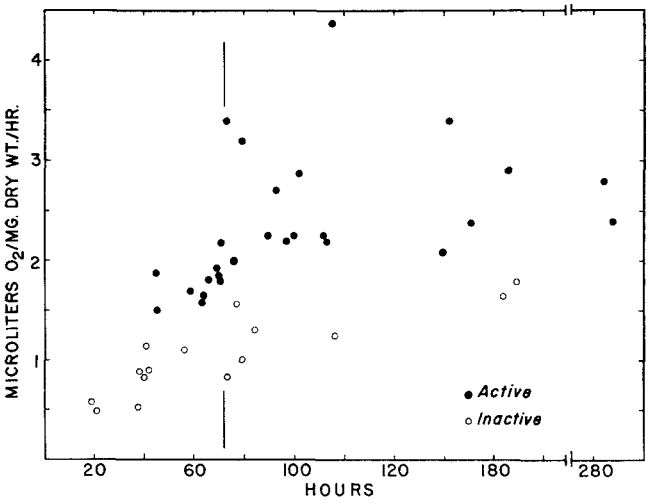

FIGURE 2. Q_{O_2}'s of active and inactive Pacific sardine eggs and larvae (14° C). The vertical line at 72 hours indicates time at hatching. Each dot represents an individual egg or larva. (Lasker and Theilacker, 1962).

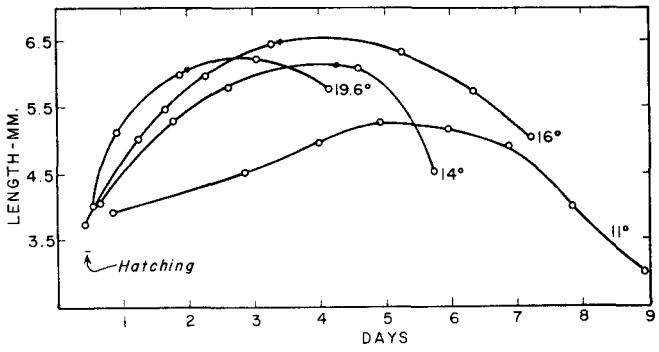

FIGURE 3. Increase in length of sardine larvae at four different temperatures. (Lasker, 1964).

constructed (Table 1) which shows that at 14°C the sardine larva metabolizes at a steady high rate and reaches a point where there is not enough yolk remaining to provide all the energy needed. This time is approximately 10 hours prior to complete eye pigmentation and development of the functional jaw. Conversion efficiency of the yolk to somatic tissue over the entire yolk sac period is high (78 per cent at 14°C), and may be greater at slightly higher temperatures (16° C) if the attainment of maximum length is taken as the criterion (Fig. 3). Calculations of efficiency with time over the developmental period show that conversion efficiency declines as the embryo grows older (Table 2) and a greater proportion of the energy available is used up in catabolic processes.

Since the yolk supply is presumably ideally suited to the metabolic and growth needs of the embryo and larva, it should be possible to deduce from information of the energy value of yolk and the metabolic requirements of sardine larvae at yolk sac absorption, the food requirements of the larva when it begins to feed. Knowledge of the Q_{10} of yolk absorption (i.e., the relative rates over a span of 10°C) permits these calculations to be made for a range of environmental temperatures.

The caloric value of a sardine egg yolk of 0.56 mm^3 averages 0.3 calorie and at 14°C this is consumed in 180 hours (Lasker, 1962). Table 1 also shows the calorie requirement for catabolism by a sardine larva with time. The remainder of the yolk is utilized for growth. During the last day of yolk absorption the animal continues its high rate of metabolism but there is less yolk energy available than the catabolic demand. To maintain the same catabolic rate with no growth, the larva must eat and digest the caloric equivalent of 4.4×10^{-4} cal/hr. If the same caloric uptake is needed that was supplied by the yolk, the larva must ingest the equivalent of 17×10^{-4} cal/hr.

Food organisms taken by 4–6 mm sardine larvae range in cross sectional diameter from 25–125μ and are chiefly copepod nauplii (Arthur, 1956). The mode falls between 75 and 80μ. The volume of a nauplius 80μ wide is approximately 4.0×10^{-4} mm^3 and the dry weight of an animal of this size is 1.2×10^{-7}g if the animal is 70 percent water. The caloric content of the copepods *Calanus helgolandicus* and *Tigriopus cal-*

TABLE 1

AVERAGE YOLK UTILIZATION BY SARDINE EMBRYOS AND LARVAE AT 14° C EXPRESSED AS DIMINISHING VOLUME AND CALORIC UPTAKE

(Lasker, 1962)

Elapsed hours from spawning	Yolk volume (mm^3)	Calories remaining	Catabolic calories
0	0.56	0.300	0
42	0.48	0.260	0.0063
71	0.29	0.160	0.0096
80	0.25	0.130	0.0040
100	0.16	0.085	0.0088
120	0.09	0.048	0.0088
140	0.04	0.021	0.0088
160	0.01	0.005	0.0088
180	0	0	0.0088
		Total	0.064

$$\text{Per cent efficiency} = 100 \times \frac{0.300 - 0.064}{0.30} = 78.7\%$$

TABLE 2

CHANGING INCORPORATION EFFICIENCY WITH YOLK UTILIZATION IN SARDINE EMBRYOS (14° C)

1 Elapsed hours from spawning	2 Time intervals	3 Total calories consumed per time interval	4 Catabolic calories per time interval	5 Incorporated calories 3–4	6 Incorporation efficiency
42	42	.040	.0063	.0337	84
71	29	.100	.0096	.0904	90
80	9	.030	.0040	.0260	87
100	20	.045	.0088	.0362	80
120	20	.037	.0088	.0282	76
140	20	.027	.0088	.0182	67
160	20	.016	.0088	.0072	45

$$\text{Per cent incorporation efficiency} = 100 \times \frac{\text{Incorporated calories}}{\text{Total calories consumed}}$$

ifornicus is shown in Table 3 (mean caloric value = 5100 cal/g dry weight).

TABLE 3

CALORIC CONTENT OF TWO SPECIES OF COPEPOD, *TIGRIOPUS CALIFORNICUS* AND *CALANUS HELGOLANDICUS*

(Measurements made by Drs. B. L. Slobodkin and S. Richman, University of Michigan)

Dry wt. (mg)	cal/g dry wt.
T. californicus, 0.9–1.1 mm	
13.66	5250
14.63	5268
15.00	5374
C. helgolandicus, stage V and adults + 6.5% other planktonic material	
25.26	5125
20.10	5103
24.95	4917
24.05	4910

Average of 7 determinations: 5135 cal/g dry wt.

Therefore at 14°C, to maintain catabolism, 0.7 copepod nauplius/hr must be ingested and digested by the newly feeding larva. To continue energy uptake equivalent to average yolk absorption, 2.8 copepod nauplii/hr are required. Per cent digestion is very high in fishes, usually over 80 per cent (Winberg, 1961). With a correction for per cent digestion these values can be adjusted to 0.9 nauplius/hr for catabolism only and 3.5 for total metabolism (which includes growth).

The effect of temperature on yolk absorption was determined and is plotted in Figure 4; a regression line is fitted to the data. The Q_{10} for this process is 4 for the environmental temperature interval from 15 to 21°C. Therefore, for each 5°C rise in temperature the rate of yolk utilization is doubled and will be reflected in the food requirement by doubling the number of food organisms required when the larva starts feeding.

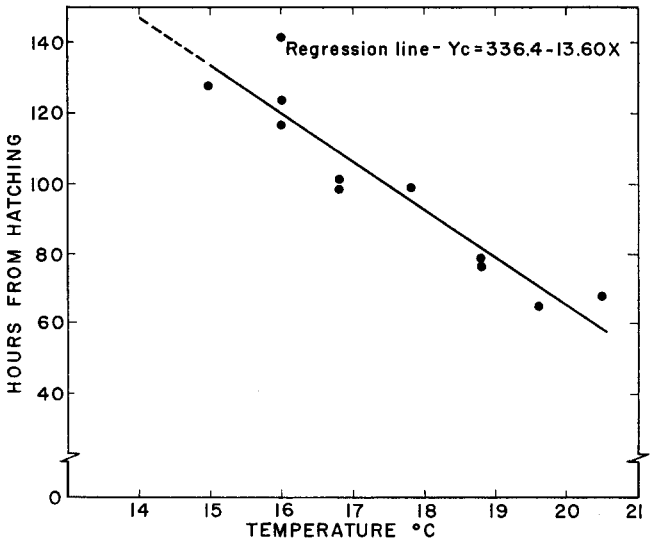

FIGURE 4. The effect of temperature on yolk absorption ($Q_{10}=4$).

VARIATIONS IN THE YOLK SUPPLY

Basing metabolic measurements on an average larva avoids the obvious fact that all eggs are not alike and all do not have the same potential. For example, there are small but significant differences in the amount of yolk available to the newly fertilized sardine egg, and in the chemistry and caloric value of the yolk. Yolk volumes may vary from 0.5 mm^3 to 0.6 mm^3 and the per cent dry weight of the yolk may span in one standard deviation 7.4 to 10.2 per cent. Although the nitrogen content (and therefore the protein) of the yolk is very constant, variations in the fat can be great. Phospholipids varied from 10.3–17.5 per cent in six individual determinations, total fat from 11.0-14.4 per cent in 11 determinations and caloric value for yolk from 5007 to 5598 cal/g in 9 determinations. An egg having a yolk volume of 0.5 mm^3, could provide as little as 0.185 cal to the growing embryo which may be compared with the 0.3 cal available in an egg of average volume, dry weight and caloric value.

Figure 6 illustrates the variation in growth of sardine larvae kept at 17°C in the laboratory. All larvae hatch out at approximately the same length, but as time progresses and yolk reserves are depleted, the divergence in length becomes great. The consequences of variations in the yolk supply of individual embryos is not known as yet, but it seems safe to conclude that this may contribute to the generally observed wide differences in growth of sardine larvae at all temperatures.

Growth curves of yolk sac larvae at different temperatures (Fig. 3) show that after reaching a maximum length the sardine larva begins to shrink. Shrinking is a result of starvation since weight loss is a concomitant feature of this phenomenon and it can be shown that there is a sharp decrease in the quantity of structural proteins associated with this shrinking. The change in amount of cold trichloroacetic acid precipitable proteins with time and development is plotted in Figure 7. The curve shows the build-up of the somatic proteins and the subsequent decrease in these proteins with starvation; a horizontal bar indicates the time span over which the articulated jaw may complete its development. It is prior to this period that a metabolic deficit begins, and this once again illustrates the delicate balance between metabolic needs and the onset of feeding.

As far as growth of the post yolk sac larva is concerned no information is as yet available and until we are able to rear them in the laboratory, energy and food requirements will not be precisely known.

SWIMMING

The most important drain on the energy resource of the sardine larva is its swimming activity. Lasker and Theilacker (1962) have shown that swimming can increase the oxygen consumption of a sardine larva as much as 3.5 times its basal uptake but the average increase in Q_{O2} (μl O_2/larva/hr) is 2 × the basal rate (Table 4). Dr. Schumann discusses in this symposium

TABLE 4

AVERAGE INCREASE IN OXYGEN CONSUMPTION BETWEEN INACTIVE AND ACTIVE EGGS AND LARVAE (14° C)

Q_{O_2} = μl O_2/egg or larvae/hour (Lasker and Theilacker, 1962)

	Number	Q_{O_2} inactive	Number	Q_{O_2} active	Increase O_2 consumption with activity
Eggs	8	0.0321	10	0.0714	2.2×
Larvae	7	0.0533	17	0.1072	2.0×

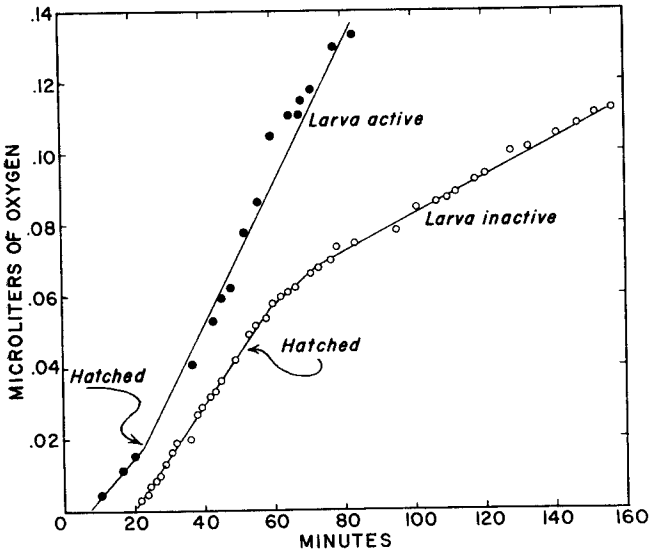

FIGURE 5. Effect of activity on oxygen consumption of sardine larvae. (Lasker and Theilacker, 1962).

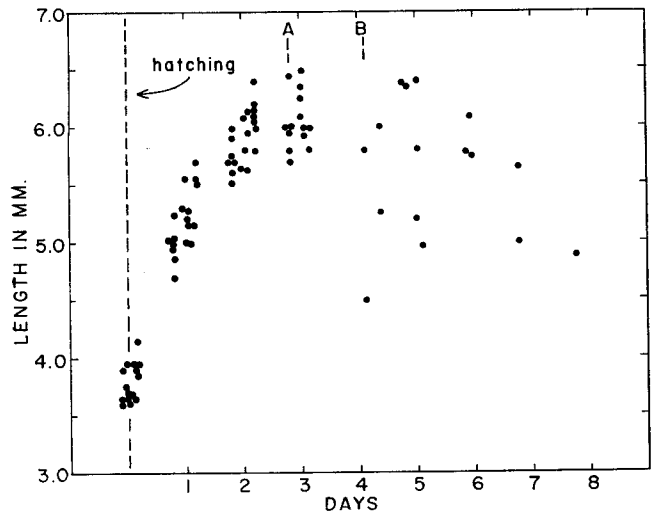

FIGURE 6. Variation in length of developing sardine larvae at 17° C. "A" indicates the time when development of a moveable jaw and eye pigmentation is completed; "B" is the time when the yolk is completely consumed.

the behavior of sardine larvae after yolk absorption and during the onset of their feeding. He has found that swimming and feeding alternate with periods of rest, so that on the average a larva spends about 40% of its time swimming and 60% resting. As the larva grows, swimming time gradually increases and is continuous when the animal reaches about 9 mm in length. Figure 5 illustrates the effect of swimming activity on the energy requirement of the larva. The fact that the newly-feeding animal has alternate periods of rest and swimming allows the larva to conserve its energy at a critical time in its life cycle. Were swimming to be continuous at this time and no food found, the two-fold increase in its food requirement would further aggravate the energy deficit it is already experiencing.

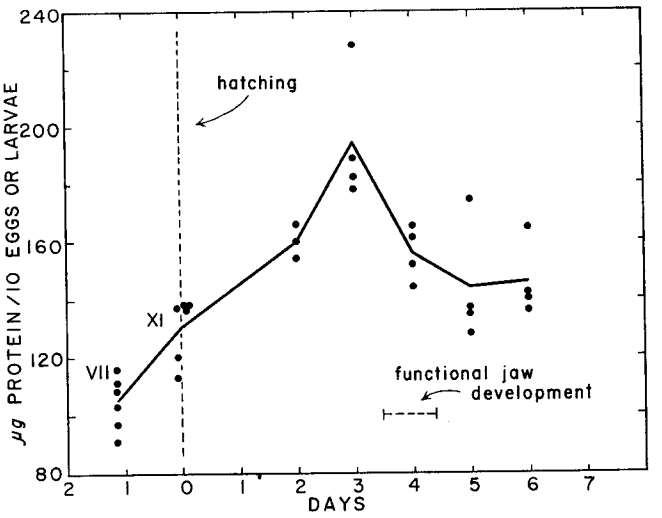

FIGURE 7. The quantitative change of somatic protein with time of development (16° C). Protein was precipitated with cold trichloroacetic acid.

OSMOREGULATION

The sardine larva is hypotonic to sea water and thus must osmoregulate. Figure 8 presents the osmolar concentrations of sardine embryos and larvae compared with adult sera and plasma (sea water M = 0.56). The surface membranes are permeable to the

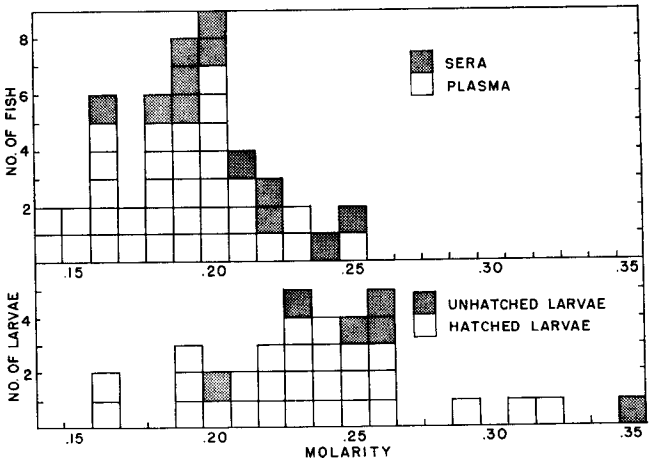

FIGURE 8. Osmolarities of sardine larval yolk and adult sardine sera and plasma.

environment since a loss of salts can be demonstrated after placing the animal in fresh water. Electron micrographs show a typical naked epithelium exposed to sea water. To maintain the hypotonic internal concentration a steady flow of salts must be excreted and water conserved (presumably by the epithelium). No significantly different oxygen uptake was found in larvae at different salinities and our conclusion was that the energy requirement for this physiological function was negligible (Lasker and Theilacker, 1962).

TEMPERATURE

Incubation time, development of a functional jaw and fully pigmented eyes and yolk utilization (Fig. 4) all have a Q_{10} of 4 over the temperature range of 14 to 21°C (Lasker, 1964). At temperatures below 13° C sardine eggs hatch and the larvae develop to some extent, but there is no formation of a jaw and the eyes fail to pigment, therefore 13°C must be considered the lower limit for survival of sardine larvae.

The relative rates of development given in Table 5 indicate that at the lower temperature range, 15°–13° C, sardine larvae spend respectively 2 and 3 times as long in the larval stage as they do at 21°C, but differences in developmental rate at higher environmental temperatures are slight because the time-temperature curve approaches an asymptote. For example development is only 1.2 times longer at 19°C than at 21°C.

TABLE 5

COMPARATIVE RATES OF EGG INCUBATION AND LARVAL JAW DEVELOPMENT—EYE PIGMENTATION AT DIFFERENT TEMPERATURES. RATE AT 21° = 1

(Lasker, 1964)

Incubation Time		Eye Pigmentation and Jaw Development
Temp.		
11	4.12	----
12	3.38	----
13	2.74	2.77
14	2.31	2.33
15	2.00	2.02
16	1.77	1.76
17	1.58	1.54
18	1.42	1.37
19	1.27	1.22
20	1.16	1.11
21	1.00	1.00

The Northern Anchovy (*Engraulis mordax*) was also studied with respect to temperature and presents an interesting comparison to the sardine. The anchovy's larval development is normal at 11 and 12° C and at these lower temperatures the anchovy hatches about a day earlier than the sardine. However, at higher temperatures the difference in rates between the two species diminishes although the anchovy always hatches and develops earlier. This is illustrated in Figure 9 and shows incubation time curves with temperature obtained for both species.

It is of interest to note that the lower temperature thresholds for both sardine (13°C) and anchovy

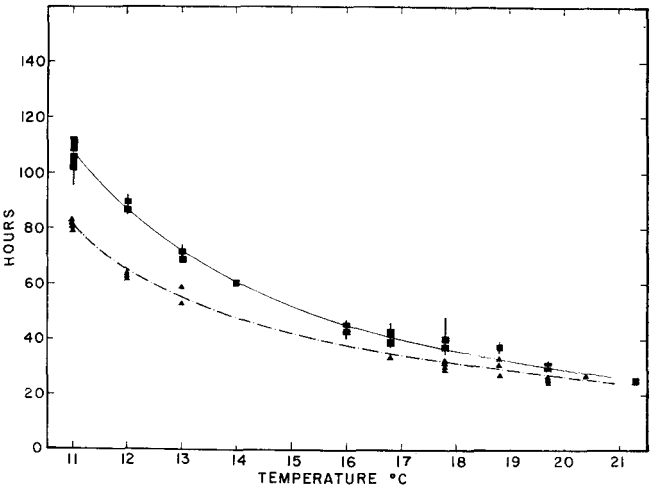

FIGURE 9. Observed incubation times for Pacific sardine and northern anchovy eggs (from stage IV). For the sardine the vertical lines indicate the range at each temperature and the bars the standard deviations around the mean; Δ = anchovy individual.

(11°C) were determined and the effect of temperature on incubation time were deduced from field collections by Ahlstrom (1943, 1956). These have largely been borne out by laboratory results. A comparison of incubation times with temperature obtained by the two methods is given in Table 6.

TABLE 6

INCUBATION TIMES OF PACFIC SARDINE EGGS AT DIFFERENT TEMPERATURES. LABORATORY STUDIES COMPARED WITH FIELD STUDIES

(Lasker, 1964)

Temp. °C	Incubation time in hours* (this study)	Incubation time in hours (Ahlstrom, 1954)	Percent difference†
11	140	114	18.5
12	115	100	13.0
13	93.0	88	5.3
14	78.5	77	1.9
15	68.1	68	0.1
16	60.2	60	0.3
17	53.7	53	1.3
18	48.4	46	5.0
19	43.2	41	5.1
20	39.3	36	8.4
21	34.0	31	8.8

* Corrected for time from spawning to stage IV based on field observations by Miller (1952).

† Percent difference is calculated by dividing the difference in hours at a temperature with the time found experimentally (× 100).

DISCUSSION

In 1926, Hjort, in discussing the concept of the "critical period" suggested "that those individuals which at the very moment of their being hatched did not succeed in finding the very special food they wanted would die from hunger. That in other words the origin of a rich year-class would require the contemporary hatching of the eggs and the development of the special sort of plants or nauplii which the newly hatched larvae needed for its nourishment." As this presentation has shown, the sardine larva

seems to fit this pattern in most of its details. Toward the end of yolk absorption and before feeding, the larva is on an energy deficit. This becomes particularly acute when the yolk is completely gone. The timing of development and the metabolic deficit are in delicate balance, and depending on the temperature, a day, or perhaps a few hours in the life of the larva will decide whether it will live or die. Because tissue resorption inevitably ensues, a sardine larva must encounter a particle of food soon after its mouth is formed if it is to have enough energy for succeeding food excursions. It also seems that this encounter may be a chance affair because the larva has not yet fully developed its vision (Schwassmann, this symposium) for accurate hunting.

A true "critical period" for a Pacific sardine larva could be the result of the lack of copepod nauplii or other suitable food organisms of the proper size and in sufficient density to ensure contact the first or second time the sardine larva hunts for food. Dr. Schumann discusses the implication of larval behavioral aspects in relation to feeding elsewhere in this symposium.

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