GROWTH AND SURVIVAL OF CHILEAN SARDINE, *SARDINOPS SAGAX*, LARVAE REARED AT DIFFERENT DENSITIES OF FOOD. *

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Fernando Balbontín [1] and Alejandro Cannobbio [2]. : Growth and survival of Chilean sardine, *Sardinops sagax*, larvae reared at different densities of food.

Growth and survival of larval sardine, *Sardinops sagax*, were determined in relation to discrete prey densities and delayed feeding under laboratory conditions. In replicate experiments, larval sardine were fed at prey densities of 1, 3, 6, 12, and 24 rotifers/ml for 19 days. Growth in length during the first two weeks after yolk absorption was not significantly different among sardine fed 3 rotifers/ml or above this density. Larvae reared at a prey density of 1 rotifer/ml died prior to 2 weeks of age. Survival at the end of the experiment exceeded the 50% level in larvae fed more than 1 rotifer/ml. The point of irreversible starvation was reached in groups of larvae when the first feeding was delayed more than 3 days after yolk absorption. The high vulnerability of early larvae to the lack of food at first feeding in the laboratory is compared to the high feeding incidence of larvae caught off Valparaíso. It is estimated that due to suitable coastal environmental conditions in the study area, first feeding may not be necessarily critical for sardine larvae.

Key words: fish larvae, delayed feeding, starvation, Valparaíso, Chile.

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* *Presented at the Early Life History Symposium, Norway, October 1988.*

RESUMEN

Crecimiento y supervivencia de las larvas de la sardina chilena *Sardinops sagax*, criadas bajo diferentes densidades de alimento.

Se determinó el crecimiento y supervivencia de larvas de sardina *Sardinops sagax*, bajo condiciones de laboratorio en relación a diferentes densidades de alimento. En experimentos con réplicas, las larvas de sardina se alimentaron con densidades de alimento de 1, 3, 6, 12 y 24 rotíferos/ml durante 19 días. El crecimiento en longitud durante las primeras dos semanas después de la absorción del vitelo, no fue significativamente diferente en sardinas alimentadas con 3 rotíferos/ml o sobre esta densidad.

Las larvas criadas a una densidad de 1 rotífero/ml, murieron antes de las 2 semanas de edad. La supervivencia al final del experimento sobrepasó el nivel del 50% en larvas alimentadas con más de 1 rotífero/ml. Se alcanzó el punto de inanición irreversible en los grupos de larvas en que la primera alimentación se postergó más de 3 días después de la absorción del vitelo .

Se compara la alta vulnerabilidad de las larvas en el momento que deben alimentarse por primera vez en el laboratorio con los altos valores de incidencia alimentaria de las larvas capturadas frente a Valparaíso. Se estima que existirían condiciones favorables en el ambiente costero del área de Valparaíso, por lo que la primera alimentación no sería necesariamente crítica para las larvas de sardina.

INTRODUCTION

The dynamics of growth and mortality rates during the early life stages of fish are critical aspects of recruitment variability (Houde 1987). Since starvation is considered as one of the major sources of larval mortality (Hunter 1981), experimental studies on prey availability and feeding of larvae may contribute to a better understanding of the mechanisms involved in larval survival.

The effect of delayed feeding on larval survival has been tested in various species of fish (see Theilacker & Dorsey 1980; Yin & Blaxter 1987; Strüssmann & Takashima 1992). Species in which embryos have large amounts of yolk, should be able to survive in the absence of food better than species with little yolk reserve (Hart & Werner 1987). Chilean sardine, *Sardinops sagax* (Jenyns, 1842), is a member of the latter group (Balbontín & Garretón 1977). At some point in time, larvae deprived of food will no longer survive, reaching the point of irreversible starvation (Lasker *et al.* 1970), which is similar to the point of no return (Blaxter & Hempel 1963) except that the last term refers to larvae before dying.

Prey should reach a threshold number to assure larval survival (Lasker 1987). Laboratory estimates of food density required for larval growth and survival have been much higher than the observed density of prey in the sea. To explain this discrepancy, it has been suggested that larvae in the sea are able to remain in plankton patches (Hunter & Thomas 1974). Recent reanalysis of data on zooplankton distribution supports this explanation, and suggests that larvae remain in areas where food resources are available (Frank 1988).

No data have been published on the growth and survival of larval sardine from Chile in relation to prey density. This study was designed to determine the effect of delayed feeding on larval survival, and to determine the effect of prey density on growth and survival of larvae.

METHODS

Sardine eggs were obtained from plankton samples in the bay of Valparaíso (Lat. 32° 57' S) and incubated in the laboratory until hatching. The onset of feeding was determined by periodic microscopical observation of larvae. Seventy first feeding larvae were transfered to 15-l black, circular tanks. Sea water was filtered through cartridges of 5 and 2 µm pore sizes respectively, and kept unaereated during the experiments. Light was provided by two sets of two fluorescent lamps placed 70 cm above the tanks. Larvae were exposed to a daily cycle of 16 h light : 8 h dark. Two experiments were undertaken:

The first experiment was designed to study the effect of delayed feeding on larval survival and to determine whether a point of irreversible starvation exists in sardine. Larvae in replicate sets from eight tanks, were fed rotifers, *Brachionus plicatilis*, at progressively later time intervals (days). In addition, four tanks were kept as controls with unfed larvae. Food was added to give a final concentration of 40 rotifers/ml. Water temperature was maintained at 18.2 ± 0.5°C. Mortality was determined each day.

The second experiment examined the effect of food density on growth and mortality of first feeding larvae. Food densities in the containers were maintained at 1, 3, 6, 12, and 24 rotifers/ml. Each feeding condition had two replicates. The contents of one of the tanks with 24 rotifers/ml was accidentally lost on the seventh day. Nominal food density in the experimental containers was controlled daily by counting the rotifers from two samples after a gentle stirring of the water. Food concentration was adjusted by adding or subtracting known quantities of rotifers. In addition, algal cultures of *Tetraselmis suecica* and *Monochrysis* sp were periodically added to the tanks to maintain a green tint in the water. One tank was treated with algae alone and an additional tank was maintained without food or algae. Water temperature was 19.0 $\pm$ 0.5º C. Larval growth, expressed as change in length, was determined every other day in a random sample of 5 specimens from each container, except on the last day of the experiment (day 19) when all the surviving larvae were measured. Sampled larvae were anaesthetized with tricaine methane sulphonate to measure the standard length (SL) under the microscope, and were later discarded. Mortality was determined each day.

The instantaneous rate of growth was estimated by the relation $G = ((\ln l_2 - \ln l_1)/t) \cdot 100$, where l_1 and l_2 are the SL of larvae at the beginning and end of the time interval, t. Similarly, the instantaneous rate of mortality was calculated by the relation $Z = ((\ln S_2 - \ln S_1)/t) \cdot 100$, where S_1 and S_2 are the percentages of expected survival at the beggining and at the end of the time interval, t.

The effect of sampling of larvae for growth determinations, was accounted for by using the following relations to calculate the number of potential survivors:

$$n_s = n_1 + (n_2 - m_1) - (n_2 + m_2)$$

and

$$m_1 = \sum_{i=1}^{k} \frac{m_2 \cdot n_2}{n_3}$$

where n_s = expected number of survivors
n_1 = number of larvae at the beggining of the time interval
n_2 = number of larvae removed in each sampling
n_3 = number of survivors in the container
m_1 = number of larvae that would have died if they had not been removed from the container, calculated iteratively
m_2 = observed mortality
k = number of samplings

The differences in the instantaneous rates of growth and mortality calculated for larvae from each experimental tank were subjected to a 1-way analysis of variance (ANOVA). Length data for feeding treatments were fit to an exponencial growth model, $SL = \exp(a+b \cdot t)$, where SL is the standard length of larvae and t is age in days.

RESULTS

DELAYED FEEDING

The daily count of dead larvae revealed that initial mortality was similar in all the experimental tanks up to the fourth day, regardless of the presence or absence of food (Fig. 1). Unfed controls showed a drastic increase in mortality on the fifth day, attaining 96 to 99% mortality the following day. In larvae deprived of food for 3 or 4 days, the percentage of mortality increased sharply on the fifth day, but only in the second case exceeded the 50% level, that is to say, the population reached the point of irreversible starvation. In these containers, the shape of the mortality curve of larvae was similar to unfed controls.

GROWTH

During the course of the experiment, prey density in the tanks showed daily fluctuations. However, the calculated mean values were very close to the nominal values fixed at the beginning for each tank (Fig. 2).

In order to determine the influence of prey density on the growth of larvae, the G value of larvae for the growth stanza where the effect of exogenous food was most pronounced was tested. Therefore, lengths of larvae prior to day 1 first feeding were not included in the calculations because of the effects of yolk reserves on growth. Similarly, data for larvae beyond the first 2 wk after hatching were not included because the negative values reached by G, indicated that growth under the present experimental conditions had attained a plateau (Table 1).

There were no differences in G between the two replicates of each feeding condition ($p<0.05$, 1-way ANOVA); therefore, these data were pooled. The differences across prey densities of 3, 6, 12, and 24 rotifers/ml were not statistically significant ($F=0.21$; $p<0.05$, 1-way ANOVA of G). Larvae fed 1 rotifer/ml were not included in the test since they died before the end of the experiment.

The parameters of the equations for the exponential growth model are summarized in Table 2.

Larvae supplied with algal cultures alone showed a maximum length similar to those which had been starved. The main difference between the two groups was that the growth increment exhibited by unfed larvae depending on yolk reserves alone declined more rapidly than in the case of larvae maintained with algae (Table 1). Larvae fed 1 rotifer/ml exhibited a larger G value for the last survivors. This value may have been biased towards the last healthy larvae remaining in the tank.

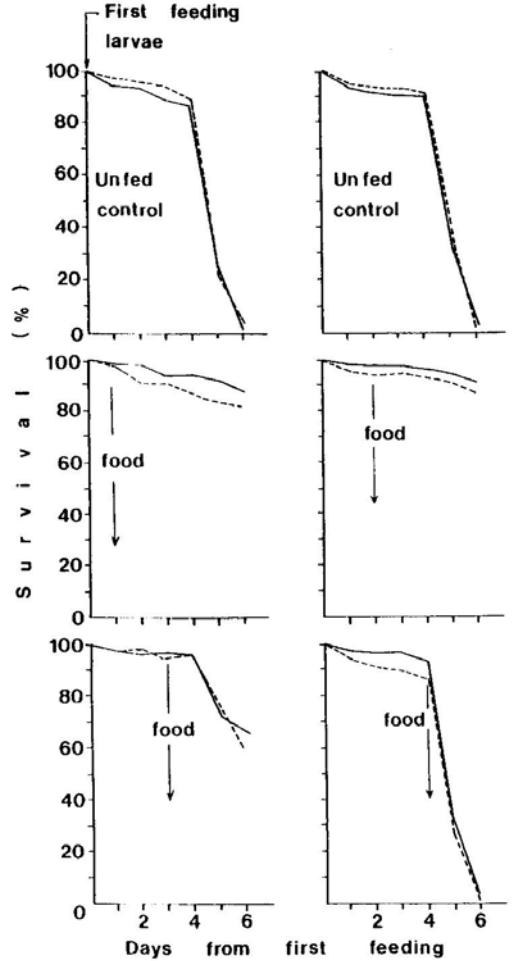

Figure 1. Effect of delayed feeding on survival of *Sardinops sagax*, first feeding larvae. The solid line is replicate 1 and the broken line is replicate 2. Unfed controls had four replicate tanks kept under similar conditions.

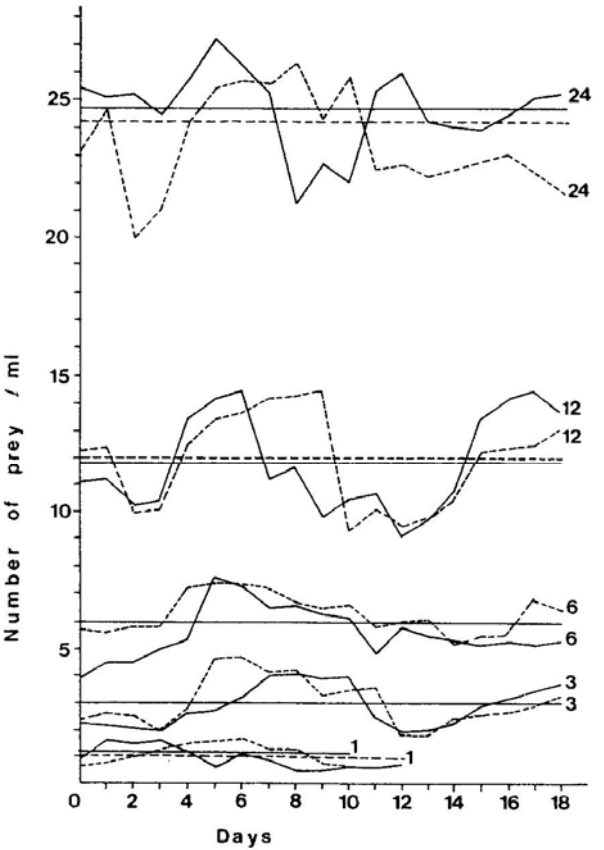

Figure 2. Daily fluctuations in prey density, expresed as number of rotifers/ml in each experimental tank with larval sardine, *Sardinops sagax*. The solid line is replicate 1 and the broken line is replicate 2. Mean values are indicated by a horizontal line corresponding to each replicate.

SURVIVAL

The experiment was terminated when larvae were 19 days old, although not all larvae survived to this point. The addition of algae to the container without rotifers prolonged larval survival two days compared to the group in starvation (Table 1). Larvae fed 1 rotifer/ml died before reaching two weeks of age. Final expected survival did not reach the 50% level only in larvae reared at a prey density of 1 rotifer/ml (Fig. 3). As in the case of G, there were no differences in Z between the two replicates of each feeding condition ($p<0.05$, 1-way ANOVA). Therefore, these data were pooled. The differences across prey densities of 3, 6, 12, and 24 rotifers/ml were not statistically significant ($F= 0.53$; $p<0.05$, 1-way ANOVA of Z).

DISCUSSION AND CONCLUSIONS

At the onset of exogenous feeding, sardine larvae still have some yolk reserves (Garretón & Balbontín 1982). Nevertheless, they are vulnerable to starvation, comparable to some extent to the findings on *Engraulis mordax* (Lasker *et al.* 1970). Larval sardine experienced more than 50% mortality when deprived of food for 4 days, but it is reasonable to suppose that the point of irreversible starvation could be reached between day 3 and day 4 from first feeding (5.5-6.5 days from hatching). Histological analysis of larvae starved for 3 days in the laboratory at 16º C indicated that they were in a semi-healthy condition; after 4 days of starvation sardine were classified as moribund (Uriarte & Balbontín 1987). These results are in agreement with the data in the present study.

As sardine larvae grow, the caloric value of rotifers apparently does not meet their energy needs, reflected in a negative G at 10.3-11.6 mm SL. Hunter (1981) described the asymptotic growth of larval fish over the first weeks of feeding solely on rotifers, concluding that the lower size limit of prey eaten by larvae seems to be set by metabolic relations. In accordance to this, analysis of gut contents of *Sardinops sagax* larvae from the coasts of Valparaíso and California indicate that as larvae grow they eat larger prey (Herrera & Balbontín 1983; Arthur 1976).

Growth values obtained in the present study are similar to the estimates for sardine from other geographic areas at approximately the same temperature. At age 10 days, Chilean sardine reached a size comparable to that of sardine from Chimbote (Perú) and California (Butler & Rojas de Mendiola 1985; Kimura & Sakagawa 1972, according to the figure redrawn in Theilacker & Dorsey 1980), but beyond this age, our estimates are 10 to 20% lower.

No significant differences in growth rate were observed in larval sardines reared at prey densities over 1 rotifer/ml. A similar result was found by Werner & Blaxter (1980) in herring larvae, using nauplii of *Artemia salina* as food, indicating that this relation is an asymptotic one at high prey densities. The same kind of relation was not clear with respect to larval survival, where higher values of this parameter were obtained at more elevated prey densities.

Table 1. Number of larvae (N), mean standard length (SL), standard error of the mean (S.E.), and mean instantaneous rate of growth (G) of *Sardinops sagax*, first feeding larvae reared under different prey densities. Exogenous feeding was initiated 2.5 days after hatching. The group of larvae fed algae is included. Age expressed in days.

Nominal prey density (rotifers/ml)

	0				1				3				6				12				24			
Age	N	SL	S.E.	G	N	SL	S.E.	G	N	SL	S.E.	G	N	SL	S.E.	G	N	SL	S.E.	G	N	SL	S.E.	G
												Replicate 1												
3	5	5.93	0.32	4.5	6	6.14	0.24	6.3	5	6.11	0.16	6.0	4	6.06	0.36	5.6	5	5.97	0.35	4.9	5	6.09	0.27	5.8
5	5	5.83	0.36	-0.8	5	5.83	0.27	-2.5	5	6.78	0.80	5.2	5	6.44	0.51	3.0	5	6.93	0.90	7.5	5	7.60	0.54	11.1
7	5	5.54	0.51	-3.4	5	6.66	1.03	6.6	5	7.21	0.39	3.1	5	7.84	0.97	9.8	5	8.53	0.83	10.4	5	7.96	0.45	2.3
9					5	7.74	1.60	7.6	5	8.35	0.89	7.4	5	8.35	1.18	3.2	5	9.20	0.39	3.8	5	9.33	1.38	8.0
11					5	9.30	1.52	9.2	5	9.38	2.07	5.8	5	10.01	1.87	9.0	5	10.01	2.08	4.2	5	10.84	0.73	7.5
13					3a	11.82	1.78	12.0	5	10.74	1.44	6.8	5	10.28	1.38	1.4	5	10.83	1.78	4.0	5	11.60	0.85	3.4
15									5	10.48	0.99	-1.2	5	11.13	1.06	4.0	5	11.06	0.67	1.0	5	10.93	0.99	-5.5
17									2a	10.45	2.37	-0.2	5	10.92	1.20	-1.0	5	12.35	0.88	5.5	5	11.37	1.38	4.5
19													5	11.52	1.38	2.7	3	11.56	0.58	-3.3	11	11.02	1.21	-1.5
												Replicate 2												
	[Algae]																							
2.5	5	5.79	0.16	4.5	4	6.08b	0.22	5.8	5	5.91	0.22	6.3	5	5.95	0.38	6.8	6	5.89	0.49	6.2	5	6.07	0.38	8.1
5	5	5.97	0.31	1.5	5	5.99	0.19	-0.8	5	6.60	0.91	4.4	5	6.82	0.88	5.4	5	7.15	0.76	7.7	6	6.29	0.63	1.4
7	5	6.01	0.41	0.3	5	6.74	0.68	5.9	5	8.38	0.51	11.9	5	7.13	0.73	2.3	5	7.86	0.49	4.7	5	7.23	0.96	7.0
9	5	5.89	0.54	-1.0	5	8.37	0.43	10.9	5	9.65	0.87	7.1	5	8.94	1.36	11.3	5	9.26	0.77	8.2	d			
11					4a	8.37	0.92	0	5	9.08	1.39	3.0	5	10.36	1.59	7.4	c							
13									5	10.82	1.32	8.7	5	11.62	1.03	5.8	5	11.19	0.82	4.7				
15									5	10.83	0.57	0.1	5	11.31	1.44	-1.4	5	11.25	0.66	0.3				
17									5	10.34	1.12	-2.3	5	12.08	0.82	3.3	5	11.29	0.84	0.2				
19									12	10.96	1.21	2.9	3	12.35	0.41	1.1	25	11.60	1.34	1.3				

a= Last survivors; b= larvae measured on day 3; c= not sampled; d= contents lost.

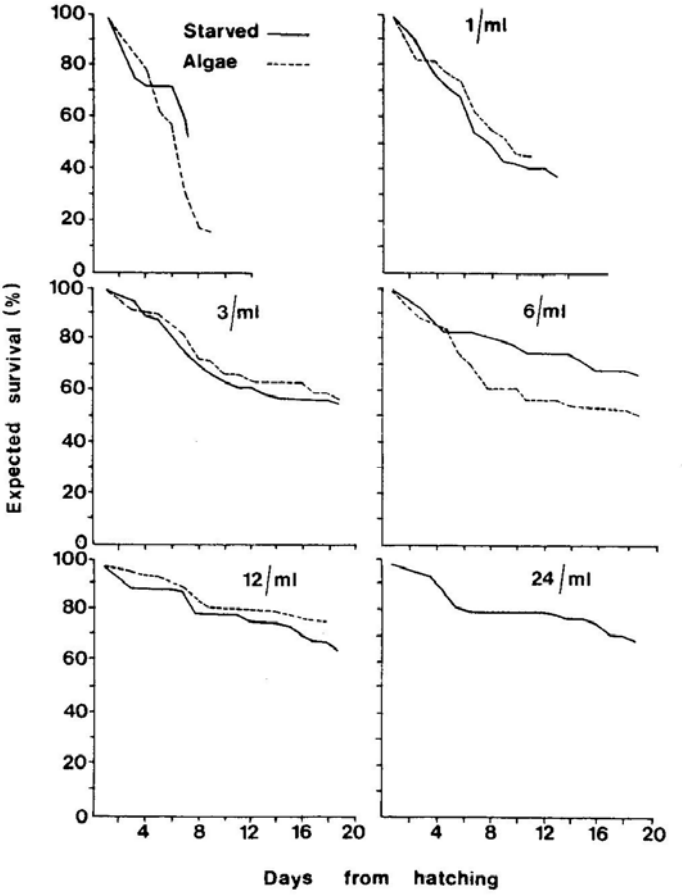

Figure 3. Expected survival of *Sardinops sagax*, larvae reared under different prey densities (number of rotifers/ml. The solid line represents replicate 1 and the group of starved larvae. The broken line indicates replicate 2 and the group of larvae plus algae.

Table 2. Parameters of the growth equation for *Sardinops sagax*, larvae during the first 15 d. SL= a+b·t, where SL is standard length of larvae and t is time in d. Prey density expressed as number of rotifers/ml.

Prey density	Replicate	Parameters a	b	S. E. of estimate	r
1	1	1.58824	0.05542	0.14089	0.85
1	2	1.62898	0.04614	0.08187	0.90
3	1	1.64799	0.05061	0.11085	0.91
3	2	1.65567	0.05370	0.10888	0.93
6	1	1.63787	0.05358	0.11324	0.92
6	2	1.62453	0.05851	0.11508	0.93
12	1	1.66282	0.05467	0.12060	0.91
12	2	1.64885	0.05646	0.08074	0.96
24	1	1.68613	0.05510	0.11474	0.92

The threshold limits of food density for adequate growth and survival of sardine larvae in the laboratory are much higher than average values of planktonic prey in coastal areas (Houde 1978). High values of phytoplankton production have been estimated in spawning areas of sardine and other fishes (Avaria & Muñoz 1991), giving indirect evidence of an adequate food availability for the larvae. Besides, gut contents analysis of sea-caught sardine larvae have shown a feeding incidence of 86% in early larvae (Herrera & Balbontín 1983). Five miles offshore Valparaíso, the feeding incidence of sardine larvae of various sizes was 80% (Valenzuela 1991)[1].

In the laboratory, first-feeding larval sardine must find food resources within a few days or they reach the point of irreversible starvation. A critical period at the time when larvae start exogenous feeding has been considered to be a determinant of the resultant year-class strength (Hjort 1914). The expansion of the spawning area and the large size of the adult biomass in the last decade (Bernal *et al.* 1982; SERNAP 1992), becoming one of the most important fisheries of the world, indicates that *Sardinops sagax* larva has succesfully adapted to planktonic conditions in the Chilean coastal ecosystem. In this sense, we may speculate, considering the high feeding incidence in early larvae in the sea, that first feeding may not be necessarily a critical period for sardine.

Analysis of size-dependent processes in fish larvae, has indicated that the higher mortality experienced by first feeding larvae in comparison to larger larvae, is due solely to their small size, which make them more vulnerable to starvation (Miller *et al.* 1988).

(1) XI Jornadas de Ciencias del Mar, Viña del Mar, Chile, p.73 (abstract).

ACKNOWLEDGEMENTS

The financial support provided by the University of Valparaíso and Andes Foundation is gratefully acknowledged. E. M. Thompson kindly reviewed an earlier version of the manuscript. J. I. Sepúlveda hellpfully contributed to improve the statistical analysis of data.

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Trabajo recibido en agosto de 1992 y aceptado en septiembre de 1992