

SURVIVAL AND GROWTH OF NORTHERN ANCHOVY LARVAE ON LOW ZOOPLANKTON DENSITIES AS AFFECTED BY THE PRESENCE OF A *CHLORELLA* BLOOM

NANCY M. MOFFATT

Southwest Fisheries Center,
National Marine Fisheries Service,
La Jolla, California 92038, USA

First feeding northern anchovy, *Engraulis mordax*, were reared on low densities (100, 150 and 300 items/l) of wild zooplankton in a medium containing 10 000 to 19 000 cells/ml of the green alga *Chlorella*. After 16 days, mean survival was five times greater than, and growth equivalent to, that usually sustained in the laboratory at much higher food densities.

Feeding experiments, coupled with larval and dietary fluorescence measurements, showed that larvae not only ingest the small *Chlorella* cells, but are capable of substantial intestinal degradation of the algal cell wall and intracellular chlorophyll. Thus anchovy larvae potentially derive direct nutritional benefit from the algal cells. It was also shown that these larvae can derive indirect nutritional benefit from the algal cells present in the guts of well-fed zooplankton ingested by the larvae.

Therefore, it appears that northern anchovy larvae are capable of significant survival and growth at low zooplankton densities in the presence of dense algal blooms, because the larval diet is supplemented both directly and indirectly by algal nutrients.

INTRODUCTION

Most laboratory studies indicate that significant survival in larval fishes requires much higher food densities than those normally found in the sea (O'Connell and Raymond, 1970; Lasker et al., 1970; Theilacker and McMaster, 1971; Hunter, 1972, 1976, 1980; Hunter and Thomas, 1974). Several recent reports, however, suggest that a number of larval fishes are capable of significant survival and growth at low wild zooplankton densities (25, 50, 100, 150, 300 items/l) when reared in the presence of dense algal blooms (Detwyler and Houde, 1970; Houde and Palko, 1970; Saksena and Houde, 1972; Houde, 1972, 1973, 1974, 1975, 1977, 1978; Stepien, 1976; Houde and Schekter, 1978).

Houde and his colleagues believe that green water functions to reduce the detrimental effects of metabolic by-products which accumulate in a static rearing system. They further contend that *Chlorella* cells (<10 μ m) are not purposefully ingested and, when taken accidentally, the larvae are not capable of breaking them down to derive nutritional benefit (Houde, 1973, 1975, 1977 unpublished research proposal). Green water may well function as these gentlemen suggest, however, it is unrealistic to assume

that algal cells present in the gut do not provide at least minimal nutrient value for the larvae. Neither does their hypothesis consider the possibility that the green water actually provides a rich nutrient source for the zooplankton, thus enhancing the larval diet indirectly.

To investigate these possibilities, feeding and rearing experiments were performed on *Engraulis mordax* to determine the effects of low wild zooplankton densities with and without an algal bloom. Subsequently, fluorescence measurements of pure algal cultures and lab-cultured foods were compared with those of larvae fed various combinations of these cultures, to determine whether larvae potentially derive any direct or indirect nutritional benefit from the alga.

MATERIALS AND METHODS

In order to determine the feeding response of first feeding larvae to the presence or absence of the alga *Chlorella*, the dinoflagellate *Gymnodinium splendens*, and the rotifer *Brachionus plicatilis*, feeding experiments similar to those described by Lasker (1975) and Scura and Jerde (1977) were performed in blackened 8 liter battery jars submerged in a 17°C water bath. The light source (four, 40W daylight

fluorescent bulbs) was suspended 60 cm above the surface of the feeding medium. Battery jars contained *Chlorella* blooms of densities varying from 0 to 19 000 cells/ml. To some of these jars *Gymnodinium* and *Brachionus* were added to achieve densities of 300/ml and 100/ml, respectively. To other jars no additional food was introduced. *Brachionus* were sieved so that only individuals < 100 μm were offered as food for the lab-spawned, first feeding larvae. Algal and food density counts were made using a 16-channel model Ta Coulter counter. The larvae were allowed to feed for 3 h, after which the larvae were vacuum siphoned onto a nitex filter disc for microscopic inspection for feeding condition and gut fullness.

Rearing experiments were performed to evaluate the growth and survival of first feeding larvae at low zooplankton densities in the presence and absence of dense algal blooms. Rearing procedures were similar to those reported by Detwyler and Houde (1970); Houde and Palko (1970); Saksena and Houde (1972); Houde (1972, 1973, 1974, 1975) and Stepien (1976). Larvae were reared from first feeding (3 to 4 days after hatching) to 16 days in a static system consisting of 75 liter glass aquaria submerged in a 17°C water bath. Aquaria were filled with either sea water filtered to 1 μm or a dense *Chlorella* bloom (10 000 to 19 000 cells/ml) to within 2.5 cm of the rim. Low aeration was used to keep *Chlorella* cells in suspension. One-fifth of the rearing medium was exchanged bidaily, and fresh algal cells were added to maintain the culture. A light source similar to that described in the feeding experiments illuminated the aquaria on a 13 light, 11 dark light cycle. The initial stocking density was 2/l or 130/aquarium.

Wild zooplankton was collected three times weekly in 5 min surface to 1 m tows using a 3/4 m, 35 μm net. Zooplankters were segregated by size using nitex sieves and maintained at 10°C in glass aquaria containing high concentrations of *Chlorella* (5000 to 10 000 cells/ml). Approximately 95% of the zooplankters introduced to rearing tanks were various life stages of copepods.

On the fourth day after the larvae hatched, zooplankton between 35 and 64 μm (predominately copepod nauplii) were added to the tanks in densities of 0/l, 100/l, 150/l or 300/l. Food levels were monitored daily (1200/ml/tank), and the appropriate zooplankton additions were made. Progressively larger food items (copepodites and small adults to 350 μm) were added during the 16-day experimental period. Larvae were sampled only at the termination of the experiment by siphoning the contents of each tank through a 150 μm sieve. Counts and total lengths of the survivors were determined and compared.

Larvae used for fluorescence measurements were allowed to feed for 3 h in the same manner described for the feeding experiments. Once vacuum siphoned onto a nitex filter, larvae were microscopically inspected, and 10–20 of those containing food items were placed on a ground glass filter. Known quantities of *Gymnodinium*, *Chlorella*, and both starved and *Chlorella*-fed *Brachionus* were also placed on separate ground glass filters.

Following procedures described by Holm-Hansen et al. (1965), each filter was ground in a tissue grinding tube with 5 ml of 90% acetone for 1 min, until the filter disintegrated. The resulting suspension was poured into a vial and the volume increased to 10 ml with acetone. Vials were stored in a refrigerator overnight to allow complete pigment extraction in the dark. The suspension was then centrifuged at 2 500 \times g for 10 min and the supernatant decanted into a fluorometer tube and stored in the dark until fluorescence was measured.

To afford more consistent sensitivity the third slit of a Turner model 111 fluorometer was used exclusively. After the initial reading, three drops of 0.5 N HCl were added to the sample; within 1 min after thorough shaking, the second reading was taken. Acid factors for each sample were calculated by dividing the initial reading (the fluorescence of the chlorophyll *a* present before acidification) by the fluorescence of the chlorophyll *a* after acidification. Comparisons were made between the acid factors of lab cultures and those calculated for larvae and/or *Brachionus* containing ingested food items.

RESULTS

Results of feeding experiments indicate that of the larvae placed in a *Chlorella* bloom of at least 5000 cells/ml with no other food items present, about 20% had guts half- to completely-full of algal cells in 3 h. The degree of gut coloration was qualitatively proportional to the algal density in the feeding medium. Of those larvae offered either *Gymnodinium* or *Brachionus* in addition to the algal medium, over 50% contained accumulations of algal cells surrounding other ingested items. Larvae were not found to accumulate algal cells in the absence of light.

Forty to 60% of the larvae reared at 100 items/l in green water survived with a 67.5% increase in the mean total length (Table 1A and B). At 150 items/l survival varied from 31 to 84% with a mean length increase of 98%. Survival at 300 items/l in green water averaged 61.5% with a 72% length increase. When *Chlorella* cells were absent, survival rates were 6% or less at all

Table 1. Survival and growth percentages of *E. mordax* larvae reared for 16 days on wild zooplankton with and without a *Chlorella* bloom.

A. Larval Survival (%)							
<i>Chlorella</i> present				<i>Chlorella</i> absent			
Zooplankton densities				Zooplankton densities			
0/l	100/l	150/l	300/l	100/l	150/l	300/l	
0	45	31 ^a	84 ^a	0	1	0.8	
0	44	63 ^a	39 ^a	6	4		
0	39 ^b	39					
0	60 ^b	44					
		64 ^b					
		84 ^b					
$\bar{x} = 0$	47	54	61.5	3	2.5	0.8	

B. Larval Growth (% total length increase)							
<i>Chlorella</i> present				<i>Chlorella</i> absent			
Zooplankton densities				Zooplankton densities			
0/l	100/l	150/l	300/l	100/l	150/l	300/l	
0	35	58 ^a	75 ^a	—	12	78	
0	70	65 ^a	68 ^a	12	40		
0	80	118					
0	85	82					
		120 ^b					
		142 ^b					
$\bar{x} = 0$	67.5	97.5	71.5	12	26	78	

^a *Noctiluca* bloomed during the last 4 days of the experiment.

^b Barnacle cyprid larvae contamination during last week.

three food densities with 28% mean total length increase.

The guts of surviving larvae commonly contained *Chlorella* cells in the "soup" surrounding ingested

green copepodites and whole adult copepods. Zooplankters sampled daily from each aquarium ranged from clear in non-green water to very green in tanks containing *Chlorella*. During the last 4 days of the first experiment, *Noctiluca* "bloomed" in only the green tanks. These dinoflagellates were very green in color compared to the few sampled in non-green tanks. *Noctiluca* cells were observed in the guts of surviving larvae. During the last week of the third experiment, barnacle cyprid larvae (<40 μ m) were a large contaminant of only the green tanks, although zooplankton additions were identical between tanks. These cyprids were not detected in the guts of surviving anchovies.

The Turner fluorometer had a fluorescence range from 1.0 for pure phaeophytin *a* (the breakdown product of chlorophyll *a*) to 2.0 for pure chlorophyll *a*. Table 2 gives the number of samples, the acid factor ranges and mean acid factors for the measurements made on pure cultures of *Chlorella*, *Brachionus* (starved and fed), and *Gymnodinium*, as well as the larvae fed these cultures.

Acid factor comparisons (Fig. 1) indicate that chlorophyll levels in *Gymnodinium*-fed larvae tended to be 25% lower than those measured for pure cultures of *Gymnodinium*. Comparison between pure *Chlorella* and *Chlorella*-fed larvae shows a 27% difference, presumably the result of the digestive activity of the larvae. Chlorophyll *a* levels decreased by 35% when *Chlorella* was ingested by *Brachionus*; larvae containing *Chlorella*-fed *Brachionus* show this same chlorophyll level. Similarly both starved *Brachionus* and larvae containing starved *Brachionus* show the same low level of chlorophyll. Starved larvae show the lowest chlorophyll level.

Table 2. Sample size, mean acid factors and ranges are given for fluorescence measurements made on each lab culture and the larvae used in each feeding treatment. Acid factors are an index of the phaeophytin *a* levels present (1.0 = pure phaeophytin and 2.0 = pure chlorophyll *a*). Phaeophytin is the metabolic breakdown product of chlorophyll.

Culture	Sample size	Acid ratio range	Mean acid ratio
<i>Chlorella</i>	8	1.56-1.78	1.66
<i>Gymnodinium</i>	6	1.83-2.00	1.93
<i>Brachionus</i>			
Starved	7	1.23-1.43	1.34
<i>Chlorella</i> -fed	6	1.37-1.58	1.43
Larval Feeding Treatments			
Starved	16*	1.00-1.30	1.12
Fed <i>Chlorella</i>	10*	1.15-1.64	1.48
Fed <i>Gymnodinium</i>	8*	1.53-1.89	1.70
Fed <i>Brachionus</i> (starved)	7*	1.10-1.57	1.35
Fed <i>Brachionus</i> (fed)	6*	1.33-1.62	1.45

*Each sample contained 10-20 larvae.

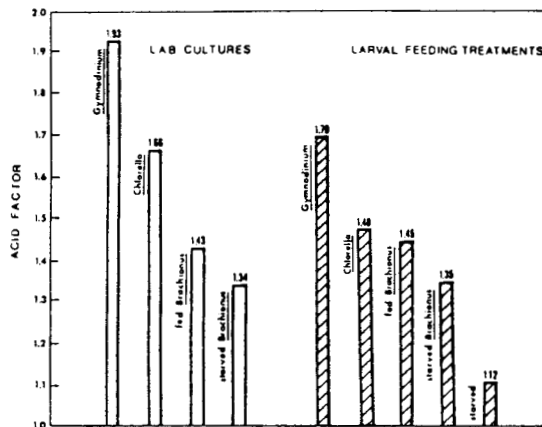


Figure 1. Bars represent the acid factors (relative chlorophyll *a* levels, where pure chlorophyll *a* = 2.0 and pure phaeophytin *a* = 1.0) for each lab culture or larval feeding treatment. Comparisons show that about 25% of the chlorophyll *a* in *Gymnodinium* and *Chlorella* is reduced to phaeophytin *a* by the larvae in 3 h. About 35% is reduced by *Brachionus*. Larvae fed *Brachionus*, either well-fed or starved, show no difference from these *Brachionus* cultures alone.

DISCUSSION

Laboratory and field evidence indicates that feeding in northern anchovy is highly size selective, limited primarily by mouth size and prey visibility (Hunter, 1972, 1980; Arthur, 1976). Lasker (1975) found that items smaller than 30 μm in effective diameter were taken rarely. In the present study, *E. mordax* first feeding larvae were found to accumulate *Chlorella* cells in the gut. This is somewhat surprising, since the effective diameter of *Chlorella* is $<10 \mu\text{m}$ and first feeding larvae can not filter feed (Hunter, 1972). Since larvae were not found to accumulate algal cells in the dark, simple drinking of algal cells is unlikely. Therefore, it is feasible, particularly in light of the correlation between cell density in the medium and the degree of gut coloration, that while individual cells are not perceived, their cumulative presence and motion in the water stimulate some of the larvae to strike at the "green" mass. Thus algal cells are ingested in proportion to their relative abundance in the medium.

When offered either *Brachionus* or *Gymnodinium* in green water, larvae striking at the larger prey items ingest *Chlorella* cells whether prey capture is successful or not. This accounts for algal cell accumulations observed in rearing experiment survivors as well.

Zooplankton densities found previously to be required for 50% survival varies greatly between the northern anchovy, 4000 items/l (O'Connell and

Raymond, 1970), and the species investigated by Houde (1975, 1977, 1978); 1751/l for bay anchovy, 199/l for sea bream, and 854/l for lined sole. Densities required for 10% survival were 900/l for northern anchovy (O'Connell and Raymond, 1970); and 75/l, 28/l and 115/l for bay anchovy, sea bream, and lined sole, respectively (Houde, 1975, 1977, 1978). Houde attributes the "significant" survival at lower food densities to lower stock densities, daily food monitoring and additions and the reduced metabolic by-product accumulations afforded by the algal bloom. By employing the same techniques described by Houde and his colleagues, northern anchovy survival averaged 47% at 100 food items/l, 54% at 150/l, and 61.5% at 300/l in green water. Survival in tanks where green water was absent was 6% or less.

Hunter (1977) calculated that about 230, 50 μm food items must be ingested and metabolized daily by a single first feeding anchovy in order to survive. Lasker (1975) showed that in order to fill or partially fill a larval gut in an 8 h feeding period, 20 to 40 items/ml are required in the feeding medium. The food levels available per larva in the present study (50, 75, 150/larva) were far below these "required" ones. Perhaps the differences can be partially explained by the presence of the dense *Chlorella* bloom, if the larvae are capable of breaking through the tough cell wall to digest and assimilate any of the algal nutrients.

Holm-Hansen et al. (1965) show that the higher phaeophytin levels detected in phytoplankton field samples, as compared to pure lab cultures, are the direct result of grazing and the chlorophyll reduction processes of zooplankton. The acid factor of zooplankton fecal pellets is 1.05, indicating the total reduction of chlorophyll *a*. The evidence provided herein suggests that this is not only the case in grazing by *Brachionus*, but also in grazing by anchovy larvae. About 25% of the chlorophyll *a* in *Chlorella* and *Gymnodinium* is available to the larvae in only 3 h of their 5 h gut residence time. More would probably be detected at the end of 5 h and by the fluorescence of fecal pellets. The rather wide ranges observed in the acid factors may be correlated with the variability in larval gut fullness and differences in the onset of feeding between individual larvae. In the same length of time (3 h) 35% of the chlorophyll *a* in *Chlorella* is available to *Brachionus*. These results lend strong support to the direct availability of algal nutrients to anchovy larvae.

While *Chlorella* would never be encountered in nature by *Engraulis mordax*, since it is a fresh water alga, nor would the density of an alga be likely to reach such high levels, the evidence presented here emphasizes the fact that small particles can not be ignored as a potential source of nutrients either in the laboratory or under field conditions.

In these experiments, larvae fed *Brachionus* which had been fed *Chlorella*, show the same phaeophytin level as the culture of well-fed *Brachionus*. This suggests that the larvae need not process the *Chlorella* further upon ingesting and digesting *Chlorella*-fed *Brachionus*, and that they do actually benefit from the green water indirectly via the zooplankters. Theilacker and McMaster (1971) found that well-fed *Brachionus* provide a better food source for anchovy larvae than poorly-fed *Brachionus*.

I suggest that the presence of the dense algal bloom in the laboratory functions in the same manner described by Lasker (1975) and Smith and Lasker (1978) for the chlorophyll maximum layer under field conditions. Available primary producers are concentrated in a stratified layer, and thus, provide a rich feeding ground for grazing zooplankters, which in turn provide a concentrated food source for larger heterotrophs. The blooms of *Noctiluca*, high barnacle larvae survival, and the green zooplankters in the green rearing tanks simulated this effect in the laboratory.

While these conditions augmented survival in these experiments, growth rate was not significantly different from that sustained when anchovy were reared on a combination of *Gymnodinium* and *Brachionus* at much higher densities. Arthur (1977, 1980) suggests that larval feeding behaviour may be more active in the lab, enhancing heart and musculature development, while at the same time depressing growth by the greater metabolic demand of the activity. Hunter (1977) contends that prey items too small to quantitatively sustain growth exclusively, may indeed afford dietary supplement provided larger food items are also available. In addition, it may be that qualitatively, the nutrients available from the algal cells are not appropriate for growth, but serve to reduce the energy deficit between food items of the appropriate size and nutrient value and/or provide a more abundant supply of trace nutrients.

It is quite probable the presence of an algal bloom does stabilize water quality in a static rearing system, thus promoting the survival of fish larvae. Contrary to the reports of other investigators, this study indicates that the algal cells provide both direct and indirect supplement for northern anchovy larvae reared at exceptionally low food densities. Whether these contradictory results are due to subtle differences in rearing technique, interspecific differences or interpretation must be examined further. However, it is clear from this investigation that small organic particles, especially in large concentrations, can no longer be ignored as potential nutrient sources in future studies of larval fish ecology.

ACKNOWLEDGEMENTS

This research was supported by a National Research Council Postdoctoral Associateship in residence at the National Marine Fisheries Service, Southwest Fisheries Center, La Jolla, California.

I express my sincere thanks to Reuben Lasker and John Hunter for their guidance, encouragement, and friendship during the progress of this research. I also thank Robert Owen, Paul Smith, Gail Theilacker, John Chandler, Eric Lynn, Roger Leong, William Kane, and others for their valuable assistance during the research and preparation of this manuscript.

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