
Responsiveness of Starved Northern Anchovy *Engraulis mordax* Larvae to Predatory Attacks by Adult Anchovy

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Most studies of larval mortality have been on food, feeding, and starvation, with less work done on predation and even fewer studies on the interaction between starvation and predation (Hunter 1984). Vulnerability to predation is a product of the probability of encounter between prey and predator, and prey susceptibility (Bailey and Houde 1989). Starvation could affect one or both of these components: starving larvae might be less able to avoid attacks by predators than fed ones (Rothschild et al. 1982). On the other hand, a decrease in conspicuousness of starved larvae might reduce their probability of encounter with predators (Gamble and Fuiman 1987). In addition, slow growth caused by an inadequate food supply could result in larvae remaining in the length-classes most vulnerable to predation and thereby increase larval losses (Shepherd and Cushing 1980).

In northern anchovy *Engraulis mordax* larvae, vulnerability to predation by fishes is size-specific, thus slower growth could result in greater losses (Webb 1981, Folk-

vord and Hunter 1986). The objective of this study was to evaluate the effect of starvation on avoidance performance of northern anchovy. We estimated the responsiveness and escape ability of 9 mm SL (standard length) anchovy to predatory attacks by adult anchovy as a function of days of starvation. We also developed histological starvation criteria which link the nutritional condition of larvae to their responsiveness to attacks by fish predators. This could allow an assessment of the interaction between predation by fishes and starvation in the sea, because the nutritional condition of sea-caught larvae could be assessed using histological criteria (O'Connell 1976 and 1980, Theilacker 1978 and 1986).

Materials and methods

Rearing and experimental design

About 4000 northern anchovy eggs produced by a captive brood stock (Leong 1971) were placed in a 100-liter black fiberglass tank main-

tained at 16.0–17.4°C (mean 16.2°C). Except for minor differences, larvae were reared following the procedure outlined by Hunter (1976). Larvae were reared until they reached 9 mm live standard length (about 20 days posthatch) at which time they were used in our experiments. In the experiments, 9 mm larvae were used because smaller anchovy larvae respond less frequently (<36%) to predators (Folkvord and Hunter 1986).

When larvae reached 9 mm, they were separated into two groups by transferring about half of them to another 100-liter tank containing only filtered seawater. The transferred larvae were never fed. Avoidance performance of the starved larvae was compared with that of larvae fed daily in the original tank. Initially a series of tests was conducted to estimate if the transfer of larvae affected avoidance performance. In these tests both groups were fed and one was transferred. Mortality was higher in the transferred group over the first day, but no significant difference existed between the groups thereafter in mortality or in their response to predators using the behavioral methods outlined below.

Larvae from three different rearing groups were used. In two groups, none of the starved larvae survived more than 6 days, and in one group a few survived 7 days. Considering all groups together, about 50% of the starved larvae survived 4–5 days and only 1% survived 7 days of starvation. Thus, most of the data are for larvae starved for 1–5 days. Four groups of five adult anchovy (90–107 mm SL) were used as predators. Predators were placed in the test container (a fiberglass tank 0.75 × 2.15 × 0.83 m with a glass window on one side) about 3 weeks prior to a series of experiments. Ambient seawater

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(15.1–17.8°C) flowed continuously through the tank, except during the experiments when water flow was stopped. Two household tungsten lamps above the tank provided about 2000–3000 lux at the water surface.

The experimental procedure was similar to Folkvord and Hunter (1986). Experiments were run in the morning and early in the afternoon starting about 24 hours after onset of starvation. Adult brine shrimp (*Artemia* sp.) were used to identify variation in predator feeding behavior between and during experiments. The addition of three brine shrimp or three starved anchovy larvae or three fed anchovy larvae constituted a single trial. Each experiment started with 3 *Artemia* trials followed by 15 trials repeating the sequence; fed larvae, starved larvae, and *Artemia*. The initial three brine shrimp trials insured that the feeding performance of the predators was stable, and subsequent brine shrimp trials were to check for effects of satiation (Folkvord and Hunter 1986). The brine shrimp trials were also used to test for differences in feeding performance among predator groups. No significant differences existed among the 4 predator groups in the proportion of brine shrimp eaten in 5 minutes (99% of the brine shrimp were eaten), or in the proportion of fed or starved larvae responding to attacks (logistic regressions, $P > 0.40$).

A trial began when the prey were gently poured from a beaker into the test container and ended when all three prey were consumed or when 5 minutes had elapsed. During a trial, each time a prey was attacked by a predator, we recorded whether or not the prey responded to the attack, whether or not it escaped, and the time required to capture the prey. A predator attack was defined as a change in swimming speed or direction towards a prey followed by an opening of the mouth. A response to a predator was defined as a change in larval swimming speed or direction that occurred during an attack. An escape was defined as a predator attack in which the larva responded and successfully avoided the predator. A total of 345 fed and 345 starved larvae were used in the experiments, but the results are based on the number of predatory attacks and not on the total number of larvae tested. Some larvae were attacked more than once and others were not attacked at all. If a larva was attacked more than once in an experiment, each interaction was considered a separate event. When a larva was taken immediately after release or before it had moved, the interaction was not assessed. The responsiveness to attacks was assessed in 295 out of 325 attacks by predators on fed larvae and in 266 out of 323 attacks on starved larvae.

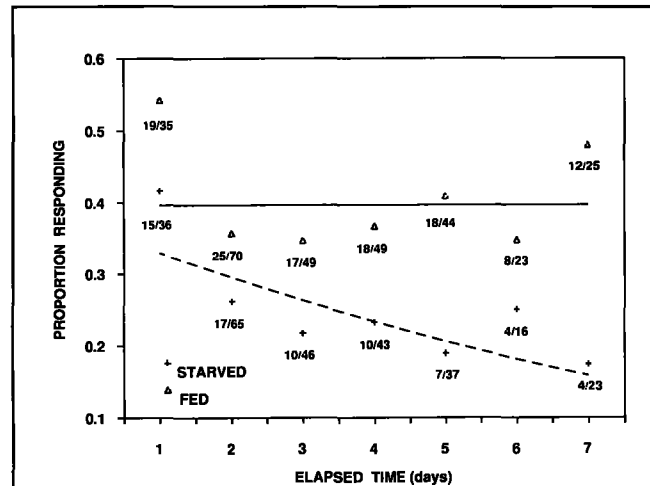


Figure 1

Proportion of fed and starved 9mm SL northern anchovy larvae that responded to attacks of adult northern anchovy (Y), as a function of the duration of the starvation period (X = elapsed time in days). Horizontal line represents average proportion of fed larvae responding (0.4 ± 0.03); logistic regression, effect of day, $P > 0.8$. Dashed line for starved larvae is given by the equation: $Y = e^{(-0.55-0.16 \cdot X)} / (1 + e^{(-0.55-0.16 \cdot X)})$; logistic regression, effect of day, $P 0.044$. Numbers next to points are number of responses and interactions.

Assessment of nutritional condition

Histological criteria were developed to link starvation-induced changes in avoidance behavior of larvae to their nutritional condition. Samples of starved and fed larvae from each of the three groups were taken for histological analysis. Twenty starved and 10 fed larvae were collected every morning starting about 24 hours after onset of starvation. Larvae were fixed in Bouin's solution, embedded in Paraplast-plus, serially sectioned at $5 \mu\text{m}$, and stained with Harris hematoxylin eosin-phloxin B.

O'Connell (1976) found that pancreas condition, notochord shrinkage and muscle fiber separation were the histological characteristics that best distinguished starving anchovy larvae from healthy ones during the first feeding stage (4 mm SL, about 4 days posthatch). These characteristics did not prove to be useful for the diagnosis of starvation in the 9mm (20 day posthatch) larvae. For example, fibers of the trunk musculature were perfectly arrayed in starved 9 mm larvae, and no interfiber spaces existed in any of the larvae analyzed even after 6 days of starvation. We also found no correlation between the condition of the notochord and starvation. Also presence, size, and abundance of hindgut vacuoles were interpreted as indicators of recent feeding and not of larval condition.

The histological condition of the liver proved to be the best character for diagnosis of starvation in 9 mm larvae. Liver condition has been used for the diagnosis of starvation in fishes in combination with other characters by O'Connell (1976), Theilacker (1978), and Watanabe (1985), and liver alone was used by Storch and Juario (1983) and Storch et al. (1984). In the results section, we relate histological condition of the liver to days of starvation and responsiveness to predatory attacks.

Results

Response and escape probabilities

The proportion of larvae responding to attacks of adult anchovy progressively decreased with starvation. Forty percent of fed larvae responded to the attacks of predators, about the same proportion determined for 8.5 mm anchovy by Folkvord and Hunter (1986, test of binomial proportions, P 0.64). After 4 days of starvation 23% responded, and by 7 days of starvation 17% responded (Fig. 1). The higher value for both fed and starved larvae on day 1 could partly be due to the predator inexperience in preying on anchovy larvae, since during the acclimation period the predators had been fed only live adult *Artemia*.

Considerable variability existed in the percentage of larvae that responded to predators in the fed groups from day to day (range of daily averages 35–54%) and among predator and prey groups (range of group averages 36–45%). Since a series of trials using fed larvae always accompanied those with starved larvae, it was possible to adjust for this interexperiment variability by dividing the fraction of starved larvae that responded by the fraction of fed larvae that responded during the same experiment. After one day of starvation (that is, 24–30 hours after transfer) this ratio was 0.77, indicating that the response probability for larvae deprived of food for 1 day was 23% lower than that of fed larvae. The regression for these calculated ratios indicates that larvae starved for 4 days were 40% less likely to respond than fed larvae, and those starved for 7 days were 58% less likely to respond (Fig. 2).

Very few larvae escaped predators regardless of their nutritional state. Thus, it was not possible to evaluate the proportions escaping as a function of the duration of starvation. Considering all data combined, starved larvae were less successful in escaping predators (0.8% vs. 3.4% fed larvae; test of binomial proportions, P 0.027). Escape probabilities were lower in fed larvae in this study (3.4%) than in Folkvord and

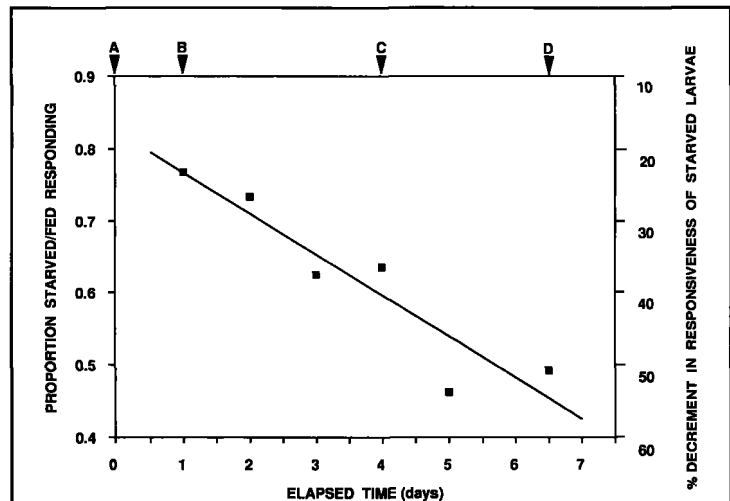


Figure 2

Responsiveness to anchovy predators of starved northern anchovy larvae (relative to fed larvae tested in the same experiment) shown in relation to duration of the starvation period and the condition of larval hepatic tissue. Data from days 6 and 7 were combined to obtain sufficiently high numbers of starved larvae. Equation for the line is $Y = 0.824 - 0.057X$ (R^2 0.87, SE est. 0.05). Histological characteristics of the liver of starved larvae are indicated with letters and arrows. (A) Glycogen vacuoles in hepatocytes, (B) no glycogen, structures unaltered, (C) hepatocytes membranes damaged, dark nuclei, and (D) cell atrophy, nuclei pycnotic and irregular.

Hunter (1986, 12%) for 8.5 mm larvae (test of binomial proportions, P 0.01). Some obvious differences existed between the studies. In the present study, body size of the anchovy predators was about 10% longer, temperature in the experimental tank about 5°C lower, and the test tank acclimation period of predator groups was longer. Any of these factors could affect the probability of escape.

Relation between prey responsiveness and histological condition

Larvae deprived of food for 1 day had no vacuoles in their liver, but cellular structures showed no alterations (Fig. 3). The vacuoles seen in fed larvae presumably contain glycogen (O'Connell and Paloma 1981). Disappearance of vacuoles after 24 hours of food deprivation has also been observed by Watanabe (1985) in freshwater gobiid larvae reared at 15–20°C. At this stage of starvation, anchovy larvae were 23% less responsive to predators than were fed larvae. After 4 days of starvation, the hepatic tissue began to deteriorate; cell outlines were hardly distinguishable, and the nuclei stained darkly. Larvae with these characteristics were about 40% less responsive than were fed larvae.

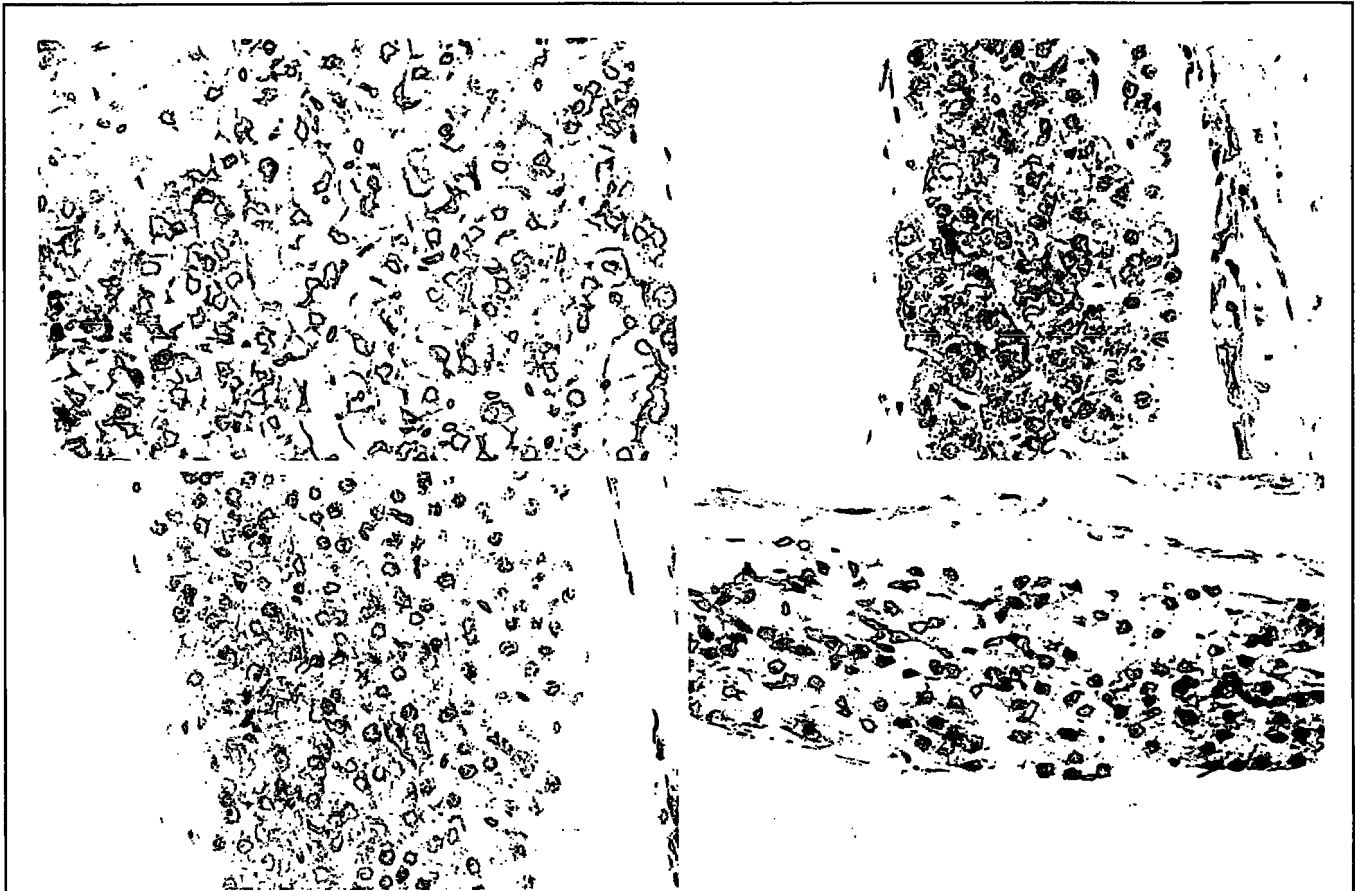


Figure 3

Condition of the hepatic tissue of larval northern anchovy after 0, 1, 4, and 6 days of starvation (white bar = 0.01 mm). (Upper left) fed larvae. White vacuolated areas may be glycogen deposits. (Upper right) 1 day of starvation. No vacuolated areas and no cellular deterioration. (Lower left) 4 days of starvation. Damaged membranes and dark nuclei. (Lower right) 6 days starvation. Hepatocytes atrophied, nuclei shrunken and pycnotic.

Hepatocytes of larvae starved for 6–7 days had atrophied, making the nuclei appear to be more densely distributed. Nuclei were pycnotic and irregular in shape. Such larvae were about 55% less responsive to predators than fed larvae.

Discussion

Starvation may affect responsiveness of larger anchovy larvae in a fashion similar to the one here described for 9 mm larvae. Preliminary measurements by Folkvord (1985) indicated that newly metamorphosed juvenile anchovy (35 mm SL) were less likely to respond if deprived of food. When deprived of food for 5 days, only 50% of the fish responded to predatory attack whereas 100% of fed fish did so. Håkanson (1989) analyzed the lipid contents of fed and starved anchovy

larvae of similar age and reared at similar temperatures as in this study. He found a decrease in the content of triacylglycerol, an energy storage component, after 3 days of starvation. On the other hand, we found that the glycogen reserve in the liver was nearly depleted after one day of starvation. Lipid reserves greatly increase after metamorphosis (J.R. Hunter, unpubl. data) and consequently such a decrement in avoidance behavior with a few days of food deprivation may be limited to the larval and early juvenile period. Yin and Blaxter (1987) also determined a decrease in the response rates of unfed yolk sac herring, cod and flounder larvae to tactile stimuli, especially after the point of no return (PNR). These authors determined a similar effect in the response rate of older Clyde herring larvae (36 days, 14 mm) to contact with a pipette after the PNR (6 days of starvation at 9–10 °C). A starvation-induced decrease in vulnerability to

predation because of a decrease in encounter rates between predators and prey (Gamble and Fuiman 1987) is not supported by our data. A decrease in encounter rates does not seem to have occurred, since attack rates on fed and starved larvae were the same. A change in larval activity may, however, have a greater effect on prey visibility among smaller larvae than larger ones.

Our scale of nutritional condition based on liver characteristics could be used to assess the extent of the interaction between starvation and predation in the sea. The level of cell deterioration found in larvae starved 4 days or more at 16°C most likely represents the PNR, and the starvation-induced vulnerability to predation could amplify the losses caused by starvation only up to this point. A sharp decline in cholesterol and polar lipid content was found in larval anchovy after 5 days of starvation at 15.5°C (Håkanson 1989). These lipids are cell membrane constituents. The high vulnerability of larvae starved for periods longer than 4–5 days seems of less ecological consequence, since nearly all such severely emaciated larvae would probably ultimately die of starvation. However, the increase in vulnerability over the entire starvation period is of interest, because it may affect estimates of starvation rates in the sea and possibly estimates of natural larval mortality. Clearly, rates of starvation based on daily incidence of starvation classes (Theilacker 1986) may underestimate the actual losses if starvation results in an increase in the vulnerability to predators. In addition, our results may support the hypothesis of Isaacs (1964) that daytime plankton samplers may be selective for weaker and less-responsive larvae. Such a hypothesis requires vision to be the primary sensory modality for the larval avoidance response. If this were the case, the reduced responsiveness caused by starvation could result in daytime plankton catches containing proportionately more starving larvae than those taken at night.

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