EUPHAUSIID PREDATION ON LARVAL ANCHOVY AT TWO CONTRASTING SITES OFF CALIFORNIA DETERMINED WITH AN ELISPOT IMMUNOASSAY

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INTRODUCTION

Understanding the processes affecting recruitment is a fundamental objective in fishery research. Starvation and predation are believed to be the major factors influencing survival of young fish (Hunter, 1984), and mortality due to these two factors must be quantified to understand how they control recruitment of young fish to a fish stock.

Evidence supporting the occurrence of starving larval fish in the ocean was mainly circumstantial (May, 1974; Jones and Hall, 1974; Lasker, 1975) until recently, when the presence of starving ocean-caught larval northern anchovy, *Engraulis mordax*, was documented using histological criteria (O'Connell, 1980). Subsequently, using similar criteria, Theilacker (1986) quantified starvation-induced mortality for ocean-caught larval jack mackerel, *Trachurus symmetricus*, and showed that starvation was a significant source of mortality for first-feeding fish.

Limited information is available on predation of larval fishes. Field evidence of predation by crustaceans has been mainly circumstantial (Westernhagen and Rosenthal, 1976; Alvariño, 1980; Frank and Leggett, 1982; Bailey and Yen, 1983; Brewer *et al.*, 1984), yet many crustaceans eat larval fish under laboratory conditions (reviewed by Hunter, 1984). Little field information exists for carnivorous Crustacea because they macerate their prey and prey remains cannot be visually identified. However, predation on larval fishes by pelagic cnidarians and ctenophores, gelatinous predators, was documented in the field and, in some cases, quantified (Moller, 1984; Purcell, 1981; 1984). Furthermore several field studies have demonstrated that planktivorous fishes also consume fish eggs and larvae (reviewed by Hunter, 1984).

Off California, the euphausiid crustacean *Euphausia pacifica* co-occurs temporally and spatially with northern anchovy larvae. In February and March, during northern anchovy spawning season, field collections containing 5000 adult euphausiids per m^3 are common (Brinton and Wyllie, 1976). In the laboratory, adult *E. pacifica* can consume up to 25 larvae per day (Theilacker and Lasker, 1974). If this feeding rate occurs in the ocean, euphausiids could be an important predator of larval anchovy.

These results are of a preliminary field study conducted to test the feasibility of using a newly developed immunoassay to assess predation on larval anchovy by euphausiids. *E. pacifica was sampled* from two contrasting sites off California. The inshore site was within an area of intense anchovy spawning, and the offshore site was outside of the main spawning area. An ELISPOT immunoassay was used to detect the presence of northern anchovy yolk protein in the euphausiids' stomachs (Theilacker *et al.*, 1986). Evidence obtained shows that euphausiids eat anchovy eggs and/or larvae; quantification of the euphausiid consumption rate in the field; and suggests that the predation by euphausiids may be significant.

This preliminary study proved the feasibility of using the ELISPOT technique for field samples. A more comprehensive spatial and temporal estimation of anchovy egg and larval mortality due to predation by euphausiids will be made next year. The proposed research is discussed.

METHODS

Field Collection

Inshore collections were made in March 1985 at two sites, 8 miles apart, 50 miles off the coast of southern California where anchovy spawning was intense. The offshore collection was made in February 1986, 100 miles off the southern California coast where anchovy spawning was minimal. These collections were made aboard the NOAA Ship David Starr Jordan. Between 2200 and 0200, 5 minute oblique tows were taken to 50 m, the depth where euphausiids and larval northern anchovy co-occur for about 10 h at night (Ahlstrom, 1959; Brinton, 1976). A Bongo frame was used, fitted with a 505 µm mesh net and plastic cod ends. After carefully diluting the live euphausiids into several buckets containing 14°C surface seawater, swimming animals were selected, blotted and placed individually into

1 ml Eppendorf tubes set in crushed ice. The sorting process, from seawater to cooled tube, took about 1 min per animal. Groups of animals were frozen every 10 min in liquid nitrogen and stored at -80°C.

In addition to the euphausiid collections, quantitative, oblique plankton tows were taken with a 505 µm mesh Bongo to a depth of 200 m at the inshore and offshore sites. Abundances of anchovy eggs, yolk-sac larvae, and adult (12-22 mm) and juvenile (6 - 11 mm) euphausiids were estimated from these samples.

Laboratory immunoassay

To test for the presence of anchovy yolk protein (which cannot be visually detected or identified) in the stomachs of field-collected *E. pacifica*, an immunoassay (Enzyme-Linked Immunospot; ELISPOT) was used that detected nannogram quantities of anchovy egg-yolk protein in stomach of laboratory-fed euphausiids. The details of the ELISPOT assay are given by Theilacker *et al.* (1986). The individual, field-collected animals were prepared for the assay by dissecting the stomach, hepatopancreas, and hindgut, teasing the tissues in buffer, and dotting the extract, about 10 µl, onto nitrocellulose paper (0.45 µm pore size). For the ELISPOT procedure, after the antigen (unknown gut contents; potentially, anchovy yolk protein) was immobilized on nitrocellulose, the antigen was then reacted with the primary antibody to anchovy yolk protein which was produced in rabbits. The resulting antigen-antibody complex was treated with a secondary antibody which had been tagged with the enzyme alkaline phosphatase. This secondary antibody was produced in goats by immunizing them with rabbit sera. The complex on the nitrocellulose was visualized with a dye, and positive phosphatase activity produced a blue-black reaction.

Control euphausiids, fed algae in the laboratory for 24-48 h at 14°C to clear their guts of non-algal prey, were dissected and run with each test. A test consisted of one control animal with 6-9 unknowns; as many as 6 tests were run simultaneously with this assay. Dotted gut contents from field animals that developed color visually darker than the gut contents of the control animals were counted as positive. In this application, the immunoassay was used to detect presence or absence of yolk in the predator's stomach. That is, even though the protein quantity is measurable with the assay, the number

of larvae eaten cannot be quantified because the absolute amount of yolk contained in an individual egg and yold-sac larva descreases with age.

RESULTS

A total of 114 adult *E. pacifica* were taken at the two inshore collections. The fraction positive at the first site was 43/55 (78%) and at the second site was 47/59 (80%). Offshore, the fraction positive was 5/65 (7%).

At the two inshore sites euphausiid abundance, estimated as the number of individuals under 10 m^2 sea surface, was 192 and 542 adults and 526 and 927 juveniles; offshore, the number of individuals was 3376 adults and 399 juveniles (Table 1).

Estimates of anchovy egg and larval abundance inshore (estimated, as above, for numbers/10 m^2 sea surface and summed over the duration, 6 days, that eggs and larvae are eaten by predatory euphausiids, see Theilacker and Lasker, 1974) were 18207 eggs and 3200 yolk-sac larvae; estimates for the offshore site were 1942 eggs and 240 yolk-sac larvae (Table 1).

To estimate daily consumption by the euphausiid population, additional field and laboratory information was needed. Brinton's (1976) field data on vertical distribution of *E. pacifica* showed that for 10 hours at night 87% of the animals occur in the upper 50 meters of the sea surface, which is the depth distribution for northern anchovy eggs and larvae (Ahlstrom, 1959). Earlier laboratory studies revealed that juvenile *E. pacifica* feed at one third the rate of adults (Theilacker and Lasker, 1974). Thus to calculate total predation by euphausiids the number of juveniles was adjusted by 0.3 (Table 1; footnote 2). The final information needed, euphausiid digestion times at *in situ* temperatures, was taken from the results of a digestion experiment where 12 adult euphausiids that had eaten 1 or 2 anchovy larvae were subsequently fed copepods and algae for 1 to 6 h. More experiments are needed, but preliminary results showed that yolk protein was undetectable in the euphausiid guts after 4 h digestion. For this study, a digestion time of 4 h was assumed to calculate euphausiid feeding rates.

A Poisson distribution was used to calculate the mean number of anchovy eggs and larvae in a euphausiid's stomach because the mean of a Poisson distribution can be easily estimated directly from the frequency of "0" counts, or negative assays ($\mu = -ln\ f(0)$). As mentioned earlier, the immunoassay technique determines presence or absence of anchovy yolk protein and does not provide information on how many prey were consumed. Thus the positive assays combine counts of one or more eggs or larvae, and the negative assays identify the euphausiids that had not eaten anchovies in the last 4 h, the assumed digestion time. Using the Poisson distribution, the mean number of eggs or larvae eaten per 4 h is equal to $-ln$ of the frequency of negative assays. Because the euphausiids and anchovy co-occur for 10 h per day, the number of anchovy eggs and larvae eaten per day is 2.5 μ (Table 1; footnote 2). This relation was used to estimate the impact of euphausiid predation on the anchovy population at the two contrasting sites given the above laboratory and field information (Table 1).

Table 1.

	ESTIMATED ABUNDANCE/10m²		PREDATION BY EUPHAUSIIDS		
	ANCHOVY (eggs/larvae)	EUPHAUSIIDS (juv./adults)	ELISPOT (fraction +)	EST. FEEDING RATE/10h (indiv./pop.)	% ANCHOVY POPULATION EATEN
Inshore$_1$	18,207/3200	526/192	43/55	3.8[1]/1,156[2]	5
Inshore$_2$		927/542	47/59	4.0/2,854	13
Offshore$_1$	1,942/240	399/3,376	5/65	0.2/608	28

1 Poisson distribution $f(0) = e^{-\mu}$; $\mu = -\ln f(0)$ = mean number eaten/4h

2 Number eggs or larvae eaten/10h = (number adult euphausiids + 0.3 number juveniles) x 2.5μ x 0.87

The egg and larval (yolk-sac) mortality rates due to euphausiid predation at the inshore sites were 0.05 and 0.13/d, with an average of 0.09 (percent of anchovy eaten; Table 1). At the offshore station, daily anchovy mortality due to euphausiid predation was 0.28. The total instantaneous mortality rate (Z) calculated from the above egg (N_1) and larval (N_2) data for the inshore site was 0.6/d and for the offshore site was 0.7/d ($Z = ln\ N_1 - ln\ N_2)/(t2-t1)$). Thus the results from this preliminary study show that the difference in mortality between the inshore and offshore sites can be accounted for by differences in rates of euphausiid predation.

DISCUSSION

This preliminary study shows that invertebrate predation rates can be estimated by using the ELISPOT immunoassay together with additional laboratory and field data. Refining recruitment estimates may be possible by combining information on predation mortality together with estimates of total larval fish mortality and starvation-induced mortality.

Efforts to make a comprehensive spatial and temporal evaluation of anchovy mortality due to predation by euphausiids are being planned. Three two-week cruises, beginning in November, 1986, will be conducted to sample the northern anchovy habitat off California before, during and after peak spawning. A net with a larger mesh, 2 mm, will be used to collect the animals. This net allows eggs and larvae to pass through, precluding biases caused by euphausiids feeding in the cod-end (Nicol, 1984). Short, 5 minute tows were used in the preliminary study reported here to decrease the probability of cod-end feeding. In the laboratory, additional euphausiid digestion experiments will be conducted with adult and juvenile animals.

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REFERENCES

Ahlstrom, E.H. 1959. Vertical distribution of pelagic fish eggs and larvae off California and Baja California, U.S. Wildl. Serv. Fish. Bull. 60: 107-146.

Alvariño, A. 1980. The relation between the distribution of zoopalnkton predators and anchovy larvae. Calif. Coop. Oceanic Fish. invest. Rep. 21: 150-160.

Bailey, K.M. and J. Yen. 1983. Predation by a carnivorous marine copepod, *Euchaeta elongata* Esterly, on eggs and larvae of the Pacific hake, *Merluccius productus*. J. Plankton Res. 5: 71-82.

Brewer, G.D., F.S. Kleppel and M. Dempsey. 1984. Apparent predation on ichthyoplankton by zooplankton and fishes in nearshore waters of southern California. Mar. Biol. 80: 17-28.

Brinton, E. 1976. Population biology of *Euphausia pacifica* off southern California. Fish. Bull. U.S. 74: 733-762.

Brinton, E. and J.G. Wyllie. 1976. Distributional atlas of euphausiid growth stages off southern California, 1953 through 1956. CalCOFI (Calif. coop. ocean. Fish. Invest.) Atlas No. 24.

Frank, K.T. and W.C. Leggett. 1982. Coastal water mass replacement: its effect on zooplankton dynamics and the predator-prey complex associated with larval capelin (*Mallotus villosus*). Can. J. Fish. Aquat. Sci. 39: 991-1003.

Hunter, J.R. 1984. Inferences regarding predation on the early life stages of cod and other fishes. *Flodevigen rapportser.* 1, The propagation of cod, *Gadus morhua* L.: 533-562.

Jones, R. and W.B. Hall. 1974. Some observations on the population dynamics of the larval stage in common gadoids. IN: Early life history of fish, J.H.S. Blaxter (ed.). Springer-Verlag. Berl. pp. 87-102.

Lasker, R. 1975. Field criteria for survival of anchovy larvae: the relation between inshore chlorophyll maximum layers and successful first feeding. Fish. Bull. U.S. 73: 453-462.

May, R.C. 1974. Larval mortality in marine fishes and the critical period concept. IN: The early life history of fish. J.H.S. Blaxter (ed.). Springer-Verlag, NY. pp 3-19.

Moller, H. 1984. Reduction of a larval herring population by jellyfish predator. Science 224: 621-622.

Nicol, S. 1984. Cod end feeding by the euphausiid *Meganyctiptianes norvegica*. Mar. Biol. 80: 29-33.

O'Connell, C.P. 1980. Percent of starving northern anchovy larvae (*Engraulis mordax*) in the sea as estimated by histological methods. Fish. Bull. U.S. 78: 475-489.

Purcell, J.E. 1981. Feeding ecology of *Rhizophysa eysenhardti*, a siphonophore predator of fish larvae. Limnol. Oceanogr. 26: 424-432

Purcell, J.E. 1984. Predation on larval fish by the Portuguese man of war, *Physalia physalis*. Mar. Ecol. Prog. Ser. 19: 189-191.

Theilacker, G.H. 1986. Starvation-induced mortality of young sea-caught jack mackerel, *Tranchurus symmetricus*, determined with histological and morphological methods. Fish. Bull. U.S. 84: 1-17.

Theilacker, G.H., A.S. Kimball and J.S. Trimmer. 1986. Use of an ELISPOT immunoassay to detect euphausiid predation on larval anchovy. Mar. Ecol. Prog. Ser. 30: 127-131.

Theilacker, G.H. and R. Lasker. 1974. Laboratory studies of predation by euphausiid shrimps on fish larvae. IN: The early life history of fish. J.H.S. Blaxter (ed.). Springer-Verlag, Berlin. pp. 287-299.

Westernhagen, H. von and H. Rosenthal. 1976. Predator-prey relationship between pacific herring, *Clupea harengus* pallasi, larvae and a predatory hyperiid amphipod, *Hyperoche medusarum*. Fish. Bull. 74: 669-674.