UC San Diego
UC San Diego Previously Published Works

Title
Immunochemical Detection of Predation on Ciliate Protists by Larvae of the Northern Anchovy (Engraulis mordax).

Permalink
https://escholarship.org/uc/item/6k9113ns

Journal
The Biological bulletin, 181(3)

ISSN
0006-3185

Authors
Ohman, MD
Theilacker, GH
Kaupp, SE

Publication Date
1991-12-01

DOI
10.2307/1542370

Peer reviewed

eScholarship.org Powered by the California Digital Library
University of California

Reference: *Biol. Bull.* **181**: 500–504. (December, 1991)

Immunochemical Detection of Predation on Ciliate Protists by Larvae of the Northern Anchovy (*Engraulis mordax*)

MARK D. OHMAN[1], GAIL H. THEILACKER[2], AND SANDOR E. KAUPP[1]

[1]*Scripps Institution of Oceanography, U.C. San Diego, La Jolla, California 92093-0227 and*
[2]*Alaska Fisheries Science Center FAKC1, National Marine Fisheries Service, 7600 Sand Point Way, Seattle, Washington 98115*

New approaches are needed for investigating planktonic prey-predator interactions *in situ,* free from enclosures and long term incubations. Lengthy incubations can lead to several artifacts, including selective mortality of planktonic organisms (1), lysis of some taxa upon fixation (2), alteration of prey-predator encounter rates through perturbations of the turbulent flow field (3), and modification of natural search and avoidance behaviors of predators and prey (4). Microscopic analysis of the gut contents of predators is a feasible alternative only if prey leave digestion-resistant hard parts. Here we describe a different approach involving immunochemical methods. We report the development and first application of an immunoassay that permits detection of predation on soft-bodied, nonloricate ciliates (*Strombidium* sp.). Polyclonal antibodies raised against *Strombidium* sp. recognize both intact ciliates and partially assimilated ciliate antigens occurring in a predator's gut. We demonstrate, by both immunochemical and conventional methods, unequivocal predation by first-feeding larvae of the northern anchovy (*Engraulis mordax*) on nonloricate ciliates. The intensity of the immunochemical reaction, quantified by enzyme-linked dot blots and reflection densitometry, is proportional to prey density and to the predator's ingestion rate.

Polyclonal antisera were produced in New Zealand white rabbits (5) against *Strombidium* sp. cultured on a diet of the bacterium *Vibrio natriegens* (6). Immunoglobulin G (anti-*Strombidium* IgG) was isolated from the antiserum by precipitation in 45% (w/v) ammonium sulfate, desalted by gel filtration, then fractionated by diethylaminoethyl ion exchange chromatography. Detailed protocols will be presented elsewhere. No significant cross-reaction was found between anti-*Strombidium* IgG and *V. natriegens* or 25 other species of planktonic algae, ciliates, and metazoans (cross-reactions were analyzed by dot blots, using at least 200 ng protein per spot). However, a significant cross-reaction was found with larval anchovy, *Engraulis mordax,* the predator of interest in this study. The reaction occurred with homogenates of isolated guts dissected from the larvae as well as with whole larvae. Pre-immune sera drawn from five rabbits and tested individually by dot blots also showed a strong cross-reaction with larval anchovy. We do not know the reason for this unexpected cross-reaction, but we successfully eliminated it by immunoadsorption against homogenates of larval anchovy bound to an affinity column.

We briefly present the method here; it is derived from the recommendations of the supplier of Sepharose 6MB (Sigma Chemical Co). A group of 100 unfed first-feeding larvae was homogenized in coupling buffer (0.1 *M* $NaHCO_3$, 0.5 *M* NaCl, pH 8.0), then sonicated in an ice water bath. This extract was mixed with cyanogen bromide-activated Sepharose 6MB overnight at 4°C. Unbound material was washed away with coupling buffer, then the remaining active groups reacted with 1 *M* ethanolamine (pH 8.0) for 2 h. Three washing cycles were then done, first with 0.1 *M* acetate buffer containing 0.5 *M* NaCl (pH 4.0), then with 0.1 *M* borate buffer containing 0.5 *M* NaCl (pH 8.0). The Sepharose beads were then packed into a capillary pipet to form a micro affinity column. Anti-*Strombidium* IgG was adsorbed against the affinity column-coupled larval extract after the procedure in reference (7) (section 10.3.1, substituting 0.1 *M* glycine HCl, pH 2.5, as elution buffer). The residence time of the

Received 7 June 1991; accepted 20 September 1991.

IgG solution in the column was 24 min. Dot blot immunoassays revealed remarkably effective elimination of the cross-reaction with anchovy larvae by this procedure. The optical density of the dot blot reaction with dissected anchovy guts decreased from a mean of 0.154 (maximum 0.225) prior to adsorption to a mean of 0.049 after adsorption.

Following immunoadsorption, the optical density of immunochemical dot blots varied with the quantity of *Strombidium* antigen (Fig. 1). Log-linear calibrations of this kind are not uncommon (*e.g.*, ref. 8) and may reflect quenching at high antigen concentrations. The antibody used for this calibration curve and for all other assays was affinity-purified anti-*Strombidium* IgG at a concentration of 0.4 μg IgG ml^{-1}.

In predation experiments, the quantity of ciliate remains detected in the gut contents of first-feeding anchovy larvae varied as a function of prey density (Fig. 2A). Even at the lowest prey concentration, 0.8 ciliates ml^{-1}, ingestion of ciliates occurred. The mass of *Strombidium* protein detected per larval gut was estimated from the calibration relation in Figure 1, and is illustrated in Figure 2B. A significant ($r^2 = 0.89$, $P < 0.05$) linear relation was found between the density of ciliate prey offered and the amount of ciliate protein in larval guts. (The values in Fig. 2A have been corrected for the blank.) Visual examination of larval gut contents at the end of the incubations confirmed that guts were at times packed full of amorphous, nearly transparent, and otherwise unidentifiable ciliate remains.

The time course of uptake of *Strombidium* sp. was evaluated in a separate experiment. The first sample of fed larvae was taken after 80 min, based on earlier results (9) suggesting that naive larvae take 1-2 h to learn to capture a new prey item. Indeed, the amount of *Strombidium* antigen in larval fish guts did not reach a maximum value until 2 h after the experiment began (Fig. 3). No significant variation ($P > 0.05$, ANOVA) in ciliate protein $larva^{-1}$ was found from 80 min onward, but the high variability among individuals could have masked a trend. The mean quantity of immunoreactive gut contents appeared to decrease after 2 h, perhaps due to the egestion of fecal matter, and then to attain the previous maximum after 6 h of feeding.

Prey disappearance experiments were carried out to describe the dependence of ingestion rate of first-feeding anchovy larvae on the density of the ciliate *Strombidium* sp. (Fig. 4). Again, even at the lowest prey concentration tested (4 ciliates ml^{-1}), ingestion of ciliates was detectable. The ingestion rates can be converted to daily carbon-specific rates, assuming 9 μg C $larva^{-1}$ and 12 h feeding per day. Accordingly, the carbon-specific ingestion rates reached 85–95% per day (right hand ordinate in Fig. 4).

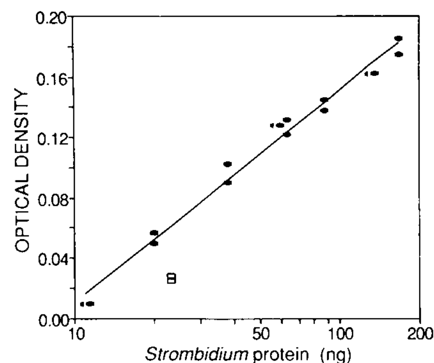

Figure 1. Calibration relation between the optical density of immunochemical dot blots and the quantity of *Strombidium* protein (OD = −0.131 + 0.141 [$\log_{10}$ protein], $r^2 = 0.987$, $P < 0.001$). The outliers indicated by open squares were excluded from the regression. Overlapping data points are offset. Dot blot assays were done on 0.45 μm nitrocellulose (Schleicher and Schuell) by a modification of the methods in refs. (17) and (27). Following incubation with the primary antibody (anti-*Strombidium* Ig), the blots were reacted with secondary antibody (goat anti-rabbit IgG conjugated to alkaline phosphatase), then with chromogenic substrate for alkaline phosphatase (8). Optical density was read at 530 nm with a calibrated reflection densitometer interfaced to a microcomputer.

There was a significant positive relation between immunochemical estimates of gut contents (G; ciliate protein $larva^{-1}$) and ingestion rates (I; ciliates $larva^{-1}$ h^{-1}) from prey disappearance experiments: $G = 9.53 + 0.22$ (I), $r^2 = 0.834$, $P < 0.05$. For this comparison, the ingestion rate was estimated from the curve illustrated in Figure 4, using the average prey density in each of the immunochemical treatments. Note that G is a static measure of gut contents, while I is a rate. This relation (and that in Fig. 2B) should not be extrapolated to $x = 0$, which would imply a positive y-intercept. There appears to be a threshold prey density below 0.8 ciliates ml^{-1} that elicits a rapid, nonlinear increase in capture rates of ciliates by anchovy larvae.

We have demonstrated the ability to detect soft-bodied nonloricate ciliates in the gut contents of predators using immunochemical methods. Unlike tintinnid ciliates, which leave loricae that can be identified by microscopy in predator guts (10), ingested nonloricate ciliates cannot be recognized by conventional means. Because nonloricate ciliates are more abundant and constitute greater biomass than tintinnids in most regions of the ocean (11), this technique permits a potentially significant food web linkage (12, 13) to be investigated *in situ.* Although we have initially used controlled laboratory experiments to establish the relationship between immunochemical measures and ingestion rates, the immunoassay presented here is suitable for analysis of field-collected predators.

Overviews of marine ecological applications of immunochemistry can be found in refs. (14) and (15). Feller *et al.* (16) identified unanticipated prey-predator links in

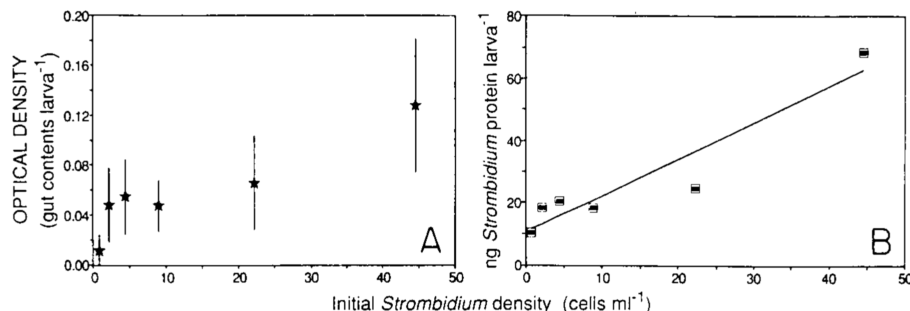

Figure 2. (A) Relation between the gut contents of first feeding anchovy larvae as determined from immunochemical dot blots ($\bar{x} \pm 95\%$) and the concentration of ciliate prey. Optical density readings have been corrected for the reaction blank from starved larvae. (B) As in part A, with gut contents expressed as ingested ciliate protein content larva^{-1} from the relation in Figure 1.

Anchovy larvae were raised from eggs collected in surface waters off La Jolla, California, and maintained in glass-fiber (GF/F) filtered seawater at 15°C under fluorescent lamps (12 μEin m^{-2} s^{-1}) on a 12:12 light: dark cycle. Containers for predation experiments were black polypropylene beakers containing 800 ml of filtered seawater, covered with Mylar lids. Only first-feeding larvae (3–4 days post-hatch, 4.0 mm standard length) were used. Ciliates (*Strombidium* sp. clone AH) were grown on a monoxenic diet of the bacterium *Vibrio natriegens* (6). The dimensions of stationary phase *Strombidium* sp. are 25 × 30 μm (width × height; preserved in 2% acid Lugols and thus reflecting cell shrinkage), cell biovolume 12,500 μm^3 (live volume), 1.4 ng protein ciliate^{-1}, and 1.6 ng C ciliate^{-1} (6). All *Strombidium* used in predation experiments were in stationary growth phase. Larvae were incubated for 6–8 h with *Strombidium* sp. Seven to 13 individual larvae were analyzed per treatment.

After predation experiments, larvae were quickly frozen in liquid N_2, then transferred to a −80°C freezer. Control first-feeding larvae (maintained continuously in filtered seawater) were treated in the same manner. For all immunoassays, guts of thawed larval anchovy were individually dissected onto a glass microscope slide. The gut contents were teased into 2–4 μl of PBS buffer, then blotted onto pre-washed nitrocellulose. A standard of known concentration of *Strombidium* sp. protein was blotted to ensure consistent reaction intensity.

a marine benthic assemblage using the precipitin reaction. Theilacker *et al.* (17) estimated the frequency of occurrence of euphausiid predation on larval anchovy using dot blots. Hentschel and Feller (18) established a quantitative relation between the intensity of the precipitin reaction (determined by rocket immunoelectrophoresis)

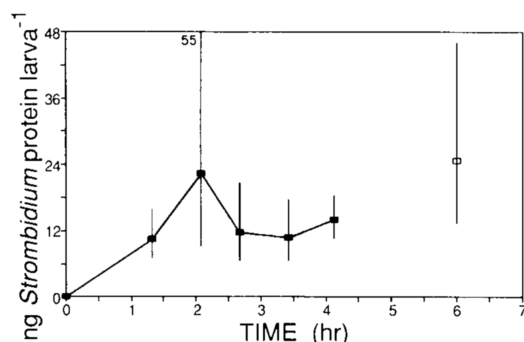

Figure 3. Time course of uptake of planktonic ciliates, as measured by immunochemical dot blots ($\bar{x} \pm 95\%$). The values at 6 h were obtained in a separate experiment. The initial prey concentration was 22 *Strombidium* ml^{-1} for all treatments. Five to eleven individual larvae were analyzed per treatment. Optical density readings were corrected for the reaction blank from starved larvae and converted to protein from the relation in Figure 1.

and the mass of prey in the proventricular contents of a penaeid shrimp.

Our dot blot method, quantified by reflection densitometry, appears to be 10^4 times more sensitive than the immunoelectrophoretic methods employing the precipitin reaction (18, 19). Still greater sensitivity could be obtained by use of chemiluminescence to visualize the reaction, or perhaps with ELISA (enzyme-linked immunosorbent assays), provided the antigens of interest bind effectively to polystrene surfaces. The dot blot procedure has the additional advantages that it is relatively fast (about 4 h), is sparing of antibody, and provides a permanent record of the reaction.

Reflection densitometry makes it possible to quantify the amount of dye product formed from a known quantity of ciliate antigen, as exemplified by the calibration curve above (Fig. 1). However, quantification of the gut contents of predators requires the following additional assumptions: (1) a constant number of secondary antibody molecules bind to the primary antibody; (2) the antigenic fraction of ciliate protein bears a constant (or known) relationship to total ciliate protein; and (3) the gut residence time of antigens is known.

Assumption (1) appears to be reasonable over the range of antigen concentrations used here (Fig. 1). Assumption

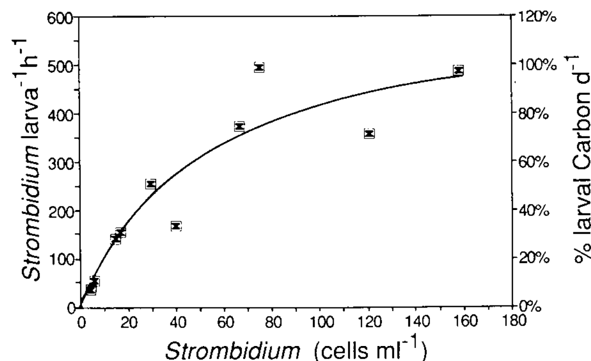

Figure 4. Ingestion rate (I) of first-feeding anchovy larvae as a function of concentration of the ciliate *Strombidium* sp. (C), determined by prey disappearance experiments. The right hand ordinate indicates daily carbon-specific ingestion rates, assuming feeding for 12 h each day. The ingestion rate is described by:

$$I = \frac{639.5 * C}{54.6 + C}$$

This curve was fitted by nonlinear least squares using the Simplex algorithm. Monte Carlo simulations (n = 1000) based on standard deviations of replicate cell counts indicated that the coefficient of variation about individual ingestion rates was approximately 15%.

Seven to twenty-five first-feeding larvae were incubated for 6–8 h with ciliates in illuminated 800-ml containers. Aliquots were preserved in 2% acid Lugols solution, then four subsamples settled overnight by the Utermöhl procedure and ciliates enumerated by inverted microscope. Control containers lacking larvae were run at each experimental density (average ciliate recovery 102%). Predation rates were calculated from the equations in (28).

(2) implies that if differential digestion of antigenic and non-antigenic protein occurs, the time course of this relationship must be determined. The antigenic properties of the prey ingested by a penaeid shrimp did not change over time during digestion (18), although this was not the case with a carid shrimp ingesting different prey (20). The immunochemical detectability of ingested prey varied with location in the intestinal tract of *Octopus vulgaris* (19). However, because first-feeding larval anchovy have a relatively simple tubular gut, we do not expect digestion to be compartmentalized. As an alternate solution to the issue of differential digestion, we have produced antibodies against partially assimilated ciliate protein, rather than against intact ciliates; these results will be reported elsewhere. Assumption (3), concerning gut residence time, requires considerable attention. Predation rate estimates are sensitive to both the speed of gut passage and to the theoretical model of digestion. The model of digestion is important because either ingestion or digestion may be discontinuous processes (Fig. 3).

Conventional prey disappearance experiments corroborated the ingestion of *Strombidium* sp. The maximum ingestion rate observed in these experiments would require 7–8 successful strikes min^{-1}. This compares with Hunter's (21) determination of 5–7 strikes min^{-1} for 4.5–4.9 mm anchovy larvae feeding on *Gymnodinium sanguineum* (=*splendens*, 40–50 μm), and implies that 25–35 μm ciliates are captured at rates comparable to those when larvae are fed an unarmored dinoflagellate that is thought to be an optimal prey item. The high rate of successful attacks on *Strombidium* sp. is somewhat surprising, given the relative transparency of these cells when in stationary growth phase and their size at the lower end of the range (22–100 μm) of prey previously documented from field-caught larvae (22). The distinctive swimming motions of these ciliates may enhance their detectability to visual planktivores.

Ingestion of ciliates by larval anchovy was detectable at prey concentrations as low as 0.8 ciliates ml^{-1}. An estimate of the ciliate density required to meet the daily metabolic requirements of first-feeding anchovy larvae can be obtained from Figure 4 combined with reported respiration rates (9, 23). *Strombidium* densities of 5–8 cells ml^{-1} are sufficient to meet basal metabolic requirements. Ambient densities of ciliates in the habitat of the northern anchovy range to at least 45 ciliates ml^{-1} (11). This is likely an underestimate due to difficulties in preserving some ciliates (2), combined with undersampling of microscale layers of organisms in the sea (24).

Among other evidence for the importance of ciliates in the diet of fish larvae, larval sea bream (*Lithognathus mormyrus*) appeared to grow faster, and with lower mortality, when ciliates were available as prey (25). Stoecker and Govoni (10) observed disproportionate consumption of the tintinnid *Favella* sp. by larval gulf menhaden (*Brevoortia patronus*), and noted that many ciliates and dinoflagellates lacking hard parts may have been overlooked in larval fish feeding studies.

Despite their small size and relative transparency, nonloricate ciliates are captured readily by first-feeding larval anchovy, at low prey concentrations and with high ingestion rates. Soft-bodied ciliates lacking hard parts may be significant links between microbial food webs and larger planktonic predators. Small scale "Lasker" patches of nonloricate ciliates, in addition to dinoflagellates (26), require attention as enriched sites of prey for teleost larvae and other planktonic predators in nature.

Acknowledgments

We thank J. R. Wilkinson for assistance with culturing ciliates, E. L. Venrick for the Monte Carlo simulations, and E. D. Gallagher for comments on the manuscript. Supported by NOAA Sea Grant NA89AA-D-SG138 and by NSF OCE-8812123.

Literature Cited

1. **Venrick, E. L., J. R. Beers, and J. F. Heinbokel. 1977.** Possible consequences of containing microplankton for physiological rate measurements. *J. Exp. Mar. Biol. Ecol.* **26:** 55–76.

2. **Snyder, R. A. and M. D. Ohman. 1991.** Description of a new species of Strombidinopsidae (Ciliophora: Choreotrichida) from coastal waters of southern California, U.S.A. *Trans. Am. Microsc. Soc.* **110:** 237–243.
3. **Yamazaki, H., T. R. Osborn, and K. D. Squires. 1991.** Direct numerical simulation of planktonic contact in turbulent flow. *J. Plank. Res.* **13:** 629–643.
4. **Ohman, M. D. 1988.** Behavioral responses of zooplankton to predation. *Bull. Mar. Sci.* **43:** 530–550.
5. **Vaitukaitis, J. L. 1981.** Production of antisera with small does of immunogen: multiple intradermal injections. *Meth. Enzymol.* **73:** 46–52.
6. **Ohman, M. D., and R. A. Snyder. 1991.** Growth kinetics of the omnivorous oligotrich ciliate *Strombidium* sp. *Limnol. Oceanogr.* **36:** 922–935.
7. **Johnstone, A., and R. Thorpe. 1987.** *Immunochemistry in Practice,* 2nd ed. Blackwell Scientific, Oxford.
8. **Blake, M. S., K. H. Johnston, G. J. Russell-Jones, and E. C. Gotschlich. 1984.** A rapid, sensitive method for detection of alkaline phosphatase-conjugated anti-antibody on Western blots. *Analyt. Biochem.* **136:** 175–179.
9. **Theilacker, G. H. 1987.** Feeding ecology and growth energetics of larval northern anchovy, *Engraulis mordax. Fish. Bull.* **85:** 213–228.
10. **Stoecker, D. K., and J. J. Govoni. 1984.** Food selection by young larval gulf menhaden (*Brevoortia patronus*). *Mar. Biol.* **80:** 299–306.
11. **Beers, J. R., F. M. H. Reid, and G. L. Stewart. 1980.** Microplankton population structure in southern California nearshore waters in late spring. *Mar. Biol.* **60:** 209–226.
12. **Stoecker, D. K., and J. M. Capuzzo. 1990.** Predation on protozoa: its importance to zooplankton. *J. Plank. Res.* **12:** 891–908.
13. **Gifford, D. J. 1991.** The protozoan-metazoan trophic link in pelagic ecosystems. *J. Protozool.* **38:** 81–86.
14. **Yentsch, C. M., F. C. MaGue, and P. K. Horan (eds.). 1988.** *Immunochemical Approaches to Coastal, Estuarine and Oceanographic Questions.* Lecture notes on coastal and estuarine studies. v. 25, Springer-Verlag, New York.
15. **Ward, B. B. 1990.** Immunology in biological oceanography and marine biology. *Oceanography* **3:** 30–35.
16. **Feller, R. J., G. L. Taghon, E. D. Gallagher, G. E. Kenny, and P. A. Jumars. 1979.** Immunological methods for food web analysis in a soft-bottom benthic community. *Mar. Biol.* **54:** 61–74.
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. Progr. Ser.* **30:** 127–131.
18. **Hentschel, B. T., and R. J. Feller. 1990.** Quantitative immunoassay of the proventricular contents of white shrimp *Penaeus setiferus* Linnaeus: a laboratory study. *J. Exp. Mar. Biol. Ecol.* **139:** 85–99.
19. **Grisley, M. S., and P. R. Boyle. 1988.** Recognition of food in *Octopus* digestive tract. *J. Exp. Mar. Biol. Ecol.* **118:** 7–32.
20. **Zagursky, G., and R. J. Feller. 1988.** Application of immunoblotting for dietary analysis. Pp. 117–129 in *Immunochemical Approaches to Coastal, Estuarine and Oceanographic Questions.* Lecture notes on coastal and estuarine studies. v. 25, C. M. Yentsch, F. C. MaGue and P. K. Horan. eds. Springer-Verlag, New York.
21. **Hunter, J. R. 1977.** Behavior and survival of northern anchovy *Engraulis mordax* larvae. *Calif. Coop. Oceanic Fish. Inv. Rep.* **19:** 138–146.
22. **Arthur, D. K. 1976.** Food and feeding of larvae of three fishes occurring in the California Current, *Sardinops sagax, Engraulis mordax,* and *Trachurus symmetricus. Fish. Bull.* **74:** 517–530.
23. **Hunter, J. R. 1972.** Swimming and feeding behavior of larval anchovy *Engraulis mordax. Fish. Bull.* **70:** 821–838.
24. **Owen, R. W. 1989.** Microscale and finescale variations of small plankton in coastal and pelagic environments. *J. Mar. Res.* **47:** 197–240.
25. **Kentouri, M., and P. Divanach. 1983.** Contribution à la connaissance du comportement et de la biologie des larves de marbré *Lithognathus mormyrus* (sparides) en élevage. *Ann. Zootech.* **32:** 135–152.
26. **Lasker, R. 1975.** Field criteria for survival of anchovy larvae: the relation between inshore chlorophyll maximum layers and successful first feeding. *Fish. Bull.* **73:** 453–462.
27. **Monroe, D. 1985.** The solid-phase enzyme-linked immunospot assay. Current and potential applications. *BioTechniques* **3:** 222–229.
28. **Frost, B. W. 1972.** Effects of size and concentration of food particles on the feeding behavior of the marine planktonic copepod *Calanus pacificus. Limnol. Oceanogr.* **17:** 805–815.