a greater proportion of obviously degenerate larvae reported in the day catch (*5*).

It is surprising that the factor K in Eqs. 1 and 2 has a value near unity. Although the selected length interval affects this factor, the intervals of time that both categories of larvae spend in the environment at each length interval (and hence their relative availability) are similar (*4*), and the day-caught larvae must be alive and growing. This is also supported by the evidence that the day-caught larvae begin to escape the net at the same length ($>$ 15.75 mm) as do the night-caught larvae, probably by swimming out of the mouth of the net after entering (*2, 3*).

An hypothesis to explain these results is that the relationship of these larvae to their food and predators is a highly visual one. At night the plankton net acts unlike a predator, and adequately samples the population up to a larval length of 15.75 mm. During the day, however, it acts much like a predator. Most larvae above 5.75 mm dodge the net, and the larvae that are caught are a measure of their availability to predation. This would be expected to include disproportionate numbers of the less active and visually alert, starving, maimed, moribund, and dead larvae. This category should closely represent the fraction of the population that is being removed by natural mortality.

The day- and night-caught larvae of the only other species subjected to this analysis (that is, larvae of the Northern Anchovy, *Engraulis mordax*) display a similar relationship. This species, while more numerous, is adequately sampled only over a very small range of lengths (approximately 5.75 mm to 9.75 mm) (*2, 3*), and hence the relationship is less extensive than in the case of the sardine.

It has long been observed that net collections of zooplankton are sparse in daytime. Although much of this sparseness undoubtedly is due to vertical migration of the organisms out of the range of the nets, much also is due to direct avoidance of the net. Hence the day collections of many active nonmigrant species also may be related to the natural mortality of the species. If so, the standard plankton net, in being an "imperfect" collector of representative samples of the population during daytime, may in fact, be a quite sophisticated collector.

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29 MAY 1964

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1. E. H. Ahlstrom, "A record of Pilchard eggs and larvae collected during surveys made in 1939 to 1941," *U.S. Fish Wildlife Serv., Spec. Sci. Rept. No. 54* (1948), and subsequent reports of this series.
2. The interval from $L = 5.75$ to $L = 15.75$, inclusive, is the significant interval in this study. There are reasons to believe that this population is adequately sampled to a length of 15.75 mm, after which escape out of the net becomes increasingly important (*3*). The dominant statistical basis for this belief is that when total larval numbers at each length are converted into total biomass, the total biomass increases sharply and almost linearly with length from 5.75 mm to 15.75 mm, and then decreases sharply for all greater lengths. This sharp maximum and subsequent decrease of population biomass cannot be a reasonable characteristic of the population, but it can be shown to be consistent with a model of escape from the net beginning at 17.25 mm. This holds for both day- and night-caught larvae. At the other end of the range, larvae smaller than 5.75 mm still retain portions of the yolk sac, have incomplete eye development, and are approximately equally available to the net both at day and night (Fig. 1). Hence, this study is confined to the length interval of similar behavior, 5.75 to 15.75 mm, inclusive. For the purposes of determining slope (in Fig. 2) these two limiting points of this interval (5.75 and 15.75 mm) are subject to error from the effect of adjacent points, and hence are excluded from the significant interval in Fig. 2. In the case of the anchovy the small larvae ($>$ 5.75 mm) escape through the net, and from an equivalent biomass analysis as above, they appear to escape out of the mouth of the net at sizes $>$ 9.75 mm. Hence, the length interval of adequate sampling for this species is very small.
3. J. D. Isaacs, "Larval sardine and anchovy interrelationships," *Calif. Coop. Oceanic Fisheries Investigation Rept. Ser.*, in press.
4. The slight slopes to curve *D* (Figs. 1 and 3) can be interpreted as the effect of changing linear growth rates. An accelerating growth rate causes the curve to slope downward with increasing length, and a decelerating growth rate results in an upward slope. Years qualitatively ranked by this criterion display accelerating growth in years of high survival and decelerating growth in years of poor survival.
5. D. Arthur, unpublished thesis.
6. Contribution from Scripps Institution of Oceanography, New Series. This paper represents one of the results of research conducted under the Marine Life Research Program, the Scripps Institution's component of the California Cooperative Oceanic Fisheries Investigations, a project sponsored by the Marine Research Committee of the State of California. It is part of an extensive study of sardine and anchovy larvae and will be reported in detail elsewhere (*3*).

24 February 1964

2-Aminoethylphosphonic Acid in Insoluble Protein of the Sea Anemone Metridium dianthus

Abstract. *2-Aminoethylphosphonic acid has been isolated from a hydrolysate of insoluble proteinaceous material of* Metridium dianthus *in 1.1 percent yield. It appears to be incorporated in the protein structure; when trichloroacetic acid is added to partial hydrolysates, precipitates form from which this compound is released on complete hydrolysis. The phosphonic acid was also detected in hydrolysates of the 70-percent ethanol- and chloroform-soluble fractions of the sea anemone. The total amount isolated accounted for 0.99 percent of the dry weight of the animal.*

2-Aminoethylphosphonic acid ($NH_2CH_2CH_2PO(OH)_2$) was recently reported as the first compound found in biological material to have a carbon-phosphorus bond (*1–3*). That it was isolated from an ether-ethanol extract of ciliate Protozoa (*1*) and from an aqueous ethanol extract of the sea anemone *Anthopleura elegantissima* (*3*) suggested a possible lipid structure, and the isolation of a glycerol ester (*3*) supports this idea.

The sea anemone *Metridium dianthus* has now been found to contain carbon-phosphorus bonded compounds in an insoluble proteinaceous fraction, as well as in an aqueous ethanol and a chloroform extract of anemone homogenate. The phosphorus has so far been isolated only in the form of 2-aminoethylphosphonic acid from the three fractions. This compound amounted to a surprising 1.1 percent of the insoluble protein fraction, and 0.99 percent of total dry material. In order to detect these compounds, advantage has been taken of the stability of the carbon-phosphorus bond to hydrolysis; a difference between phosphate formed on combustion and on hydrolysis should be an indication of phosphorus bonded to carbon.

Animals (467 g) collected from Vineyard Sound, Mass., were washed, chopped, and then homogenized in a Waring Blendor with three separate portions of 70-percent aqueous ethanol. The extract on evaporation left 16.6 g of hygroscopic solid. The dried insoluble residue (45.2 g) was further defatted (4.47 g) in a Soxhlet apparatus with chloroform.

Total phosphorus was determined colorimetrically (*4*) after combustion of 30- to 50-mg samples by the Schöniger oxygen-flask method. Hydrolyzable phosphorus was found by refluxing 50-mg samples in 30 ml of 6*N* hydrochloric acid for 24 hours, removing any colored matter with Norite, and then applying the colorimetric procedure (Table 1). The validity of the methods was tested with known compounds. For triphenyl phosphate,

Table 1. Phosphorus analyses of anemone fractions.

Fraction	Phosphorus (%)	
	Total	Hydrolyzable
70% ethanol soluble	0.68	0.47
Chloroform soluble	2.28	1.53
Insoluble residue	0.95	0.66

total and hydrolyzable phosphorus were 9.39 and 9.45 percent, respectively (calculated for $C_{18}H_{15}O_4P$: P, 9.49 percent for both). For 2-aminoethylphosphonic acid, total and hydrolyzable phosphorus were 25.1 and 0.13 percent, respectively (calculated for $C_2H_8NO_3P$: P, 24.8 percent; no hydrolyzable phosphorus).

A portion (10 g) of the insoluble proteinaceous residue was hydrolyzed by refluxing for 24 hours in 4*N* hydrochloric acid. After filtration and evaporation, the hydrolysate was placed on a Dowex-50 (H^+) column. Compounds eluted with water had no carbon-phosphorus bonds detectable by analysis (total phosphorus, 14.3 percent; phosphate P, 14.0 percent). Elution with 1:9 concentrated ammonia-water gave 8.1 g of amino acids (total P, 0.66 percent). A 3.0-g portion was fractionated on a 2.8-cm by 28-cm column of Dowex 1 (×8, 200 to 400 mesh, acetate form) with 0.5*N* acetic acid (*3*). There was obtained from the 50- to 110-ml fraction, 2.4 g of solid (0.63 percent P), and from the 110- to 260-ml fraction, 0.3 g (0.085 percent P). Elution of the column with 3*N* acetic acid gave an additional 0.5 g (0.094 percent P). The first of these fractions was chromatographed again (in two portions) on the Dowex-1 column with water. The bulk of the solids (2.25 g) was eluted in the 50- to 130-ml fraction, but contained little phosphorus (0.088 percent). However, the 170- to 570-ml eluate contained a solid rich in phosphorous. Some amino acid impurities were removed from this solid by a second passage with water through the same column, and color was then removed with a pinch of Norite. The white solid (40 mg) obtained on evaporation gave a single spot (R_F, 0.24) on paper chromatography in a fresh mixture of butanol, acetic acid, and water (4 : 1 : 2) (*1*). Material accumulated in this fashion was twice recrystallized by adding ethanol to a concentrated water solution. The product melted at 282° to 283°C (with decomposition), contained 25.1 percent phosphorus, and had R_F of 0.54 in a pyridine and water mixture (65 : 55). Synthetic (*5*) 2-aminoethylphosphonic acid (calculated for $C_2H_8NO_3$ P: P, 24.8 percent) melted at 282° to 283°C (with decomposition) and gave R_F of 0.23 and 0.54 in the butanol and pyridine systems, respectively. The natural and synthetic compounds had identical proton magnetic resonance spectra (D_2O solution) in accord with the assigned structure. The amount of isolated phosphonic acid, scaled to the entire insoluble protein fraction, was 0.440 g. Additional small amounts were indicated by paper chromatography in various fractions from the ion-exchange chromatographic separation.

Application of the foregoing hydrolysis and separation procedures to 5.4 g of the 70-percent ethanol-soluble fraction gave 35 mg of 2-aminoethylphosphonic acid (107 mg for the entire fraction), and to 2.5 g of the chloroform-soluble fraction gave 37 mg (66 mg for the entire fraction). The total yield of 2-aminoethylphosphonic acid isolated from 467 g of *Metridium dianthus* was 0.613 g.

That 2-aminoethylphosphonic acid may be incorporated in the protein structure is suggested by its inclusion in material precipitated from partial hydrolysates by trichloroacetic acid. The free amino acid is not precipitated under similar conditions. Exposure of 2.0 g of insoluble proteinaceous material to 25 ml of 1*N* sodium hydroxide for 30 minutes at room temperature resulted in almost complete solution. The mixture was centrifuged; the supernatant liquid was adjusted to *p*H 7, and again centrifuged to remove a slight precipitate. The addition of 30-percent trichloroacetic acid caused immediate precipitation of 0.39 g (total P, 0.69 percent; hydrolyzable P, 0.53 percent). This precipitate was hydrolyzed, and the ion-exchange procedures applied. The presence of 2-aminoethylphosphonic acid in the proper fraction was demonstrated by two-dimensional paper chromatography in the butanol and pyridine systems.

Partial basic hydrolysates were also passed through a column of Dowex-50 (H^+). Material eluted with water, as well as that retained on the column and eluted with a mixture of ammonia and water (1 : 9), contained the carbon-phosphorus bond. These and enzymatic partial hydrolysates should be useful in studies to confirm and define a role for 2-aminoethylphosphonic acid in protein structures (*6*).

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6. I thank Max Blumer, in whose laboratory this work was performed, for his interest and assistance. I was on sabbatical leave from Duke University, 1963—1964. Supported by research contract Nonr-2196(00) with the U.S. Office of Naval Research and by the Ford Foundation through a postdoctoral fellowship at Woods Hole Oceanographic Institution. Contribution No. 1461 of Woods Hole Oceanographic Institution.

17 Febuary 1964

Chlorophyll Content and Growth of Soybean Plants: Possible Interaction of Iron Availability and Day Length

Abstract. *Data indicate a relationship between the availability of iron and the response of "Hawkeye" soybeans to day length. The more readily available the iron, the less influence an increase in day length on dry weight. When iron was present but not chelated the plants remained green in a 16-hour day but became chlorotic in a 9-hour day.*

In some greenhouse studies on the responses of soybean plants to different iron chelates (*1*), it was noted that as the summer season approached, more and more days in iron-deficient solutions were required to induce the degree of chlorosis desired in the experimental plants. This suggested that day length might be a factor in iron availability or iron utilization by soybeans or, in turn, that the availability of iron might influence the response of the plants to day length.

Soybean plants *Soya max* var. Hawkeye were grown from seed in a complete nutrient solution, at *p*H 6, con-