THE SIGNIFICANCE OF ORGANIC COMPOUNDS IN SEA WATER

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INTRODUCTION

Since the time of Pütter (1909) much effort has been expended on the problem of the direct utilization of dissolved organic compounds by marine organisms. Most of the consideration has been on the basis of total carbon in conjunction with such criteria as over-all growth and respiratory equivalents. As recently as last year Jørgenson (1952) stated: "It is not finally settled whether filter feeders obtain their food from the particulate fraction only, or whether they are also able to utilize the so-called dissolved organic matter in the sea." The use of the term "dissolved" is to some extent ambiguous and in the sense used here should be considered to include colloidal organic materials. Fox *et al.* (1952) and Goldberg *et al.* (1952) have developed methods for collecting these materials (not necessarily those which are dissolved in the strict sense) by a special filtration technique. They introduced the term *leptopel* to include the finely divided particulate materials and organic dispersions. Their quantitative results were given in terms of total carbon.

A somewhat different approach has been taken by Wilson (1951, 1952) who adapted a type of bio-assay to the demonstration of the biological differences between waters of different origins. Cooper (1951) conducted a brief chemical study of the areas involved and found that the water which was most favorable to the development of the invertebrate larvae used as test organisms by Wilson came from the areas showing a comparatively high rate of phosphate regeneration. It was not claimed that the phosphate was the critical factor, but simply that the phosphate regeneration indicated a level of biological activity at which growth promoting substances might have been formed.

Fox (1950) gave a general review of the metabolism of detritus as food for detritus feeders and discussed briefly the lipochromes to be found in the sediments. Kalle (1949) reported certain yellow substances which he isolated from the sea water and which fluoresce under ultraviolet light.

In 1948 my co-workers and I (Collier *et al.*, 1950) began an investigation of this problem along an entirely different line. This approach was based on the study of the existence of specific organic compounds in natural sea waters and their effects on marine organisms. We started out by testing for tyrosine-tryptophane and carbohydrates. It soon developed that there were susbtances present which influenced the rate of filtration of the oysters and which responded to the carbohydrate test used (N-ethyl-carbazol). This was encouraging, for it meant that we had found a biologically active compound (or group of compounds) to which an organism would respond quantitatively.

These findings bring into sharp focus for the marine biologist Lucas' (1949) treatment of external metabolites, or ectocrines, as he called them.

PROGRESS IN WORK ON SPECIFIC COMPOUNDS FOUND IN SEA WATER

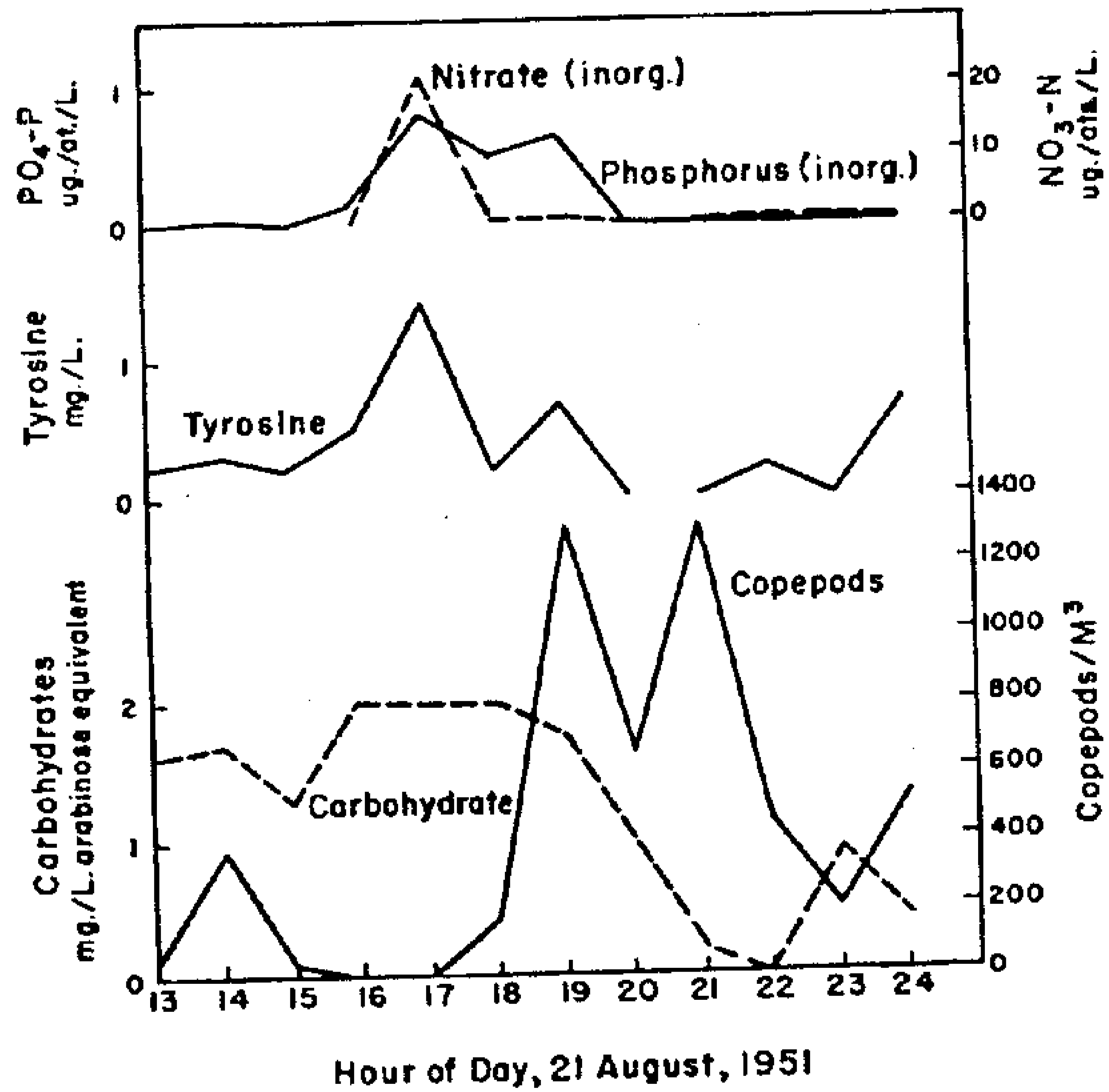
In general, we would expect all sorts of compounds to be present as degradation products of dead and dying animals and plants. Also, we would expect some quantity of excretory products, as well as diffusable metabolites which passively escape the more permeable boundaries of plant and animal masses. Some could result from extrinsic bacterial activity and many others from an intrinsic enzymatic system. The general scheme for this complex set of factors is graphically figured by Fox (*loc. cit.*).

Neritic waters can be viewed much as the mammalian physiologist would view blood: as a transport system charged with living cells and agglomerates of proteinaceous and carbohydrate complexes, as well as dissolved gases. There is good reason for the marine biologist to approach the problems from the point of view of blood chemistry. The following is a brief outline of some of the progress on specific compounds or classes of compounds being studied at the Galveston laboratory.

The N-ethyl-carbazol substances ("carbohydrates"). There are substances in sea water which respond as carbohydrates to the N-ethyl-carbazol test. These may or may not be true carbohydrates, and until we are certain of their identification the provisional term "N-ethyl-carbazol substances" has been adopted. Accordingly, the values given as "carbohydrates" are always in terms of arabinose equivalents because we use arabinose standards for reference. We have isolated and concentrated two substances which respond to the test and our chemists are now at work on the identification of these compounds.

We make routine determinations of these substances on all of our oceanographic stations. They are not homogeneously distributed at all, and we feel that they are associated with phytoplankton production. The reasons for this are: (1) in the early work (Collier *et al.*, in press) we found that in five-gallon bottles the production of the substances was associated with light as well as with aeration; (2) there was a clear diurnal variation of concentration in the natural waters, with

FIGURE 1

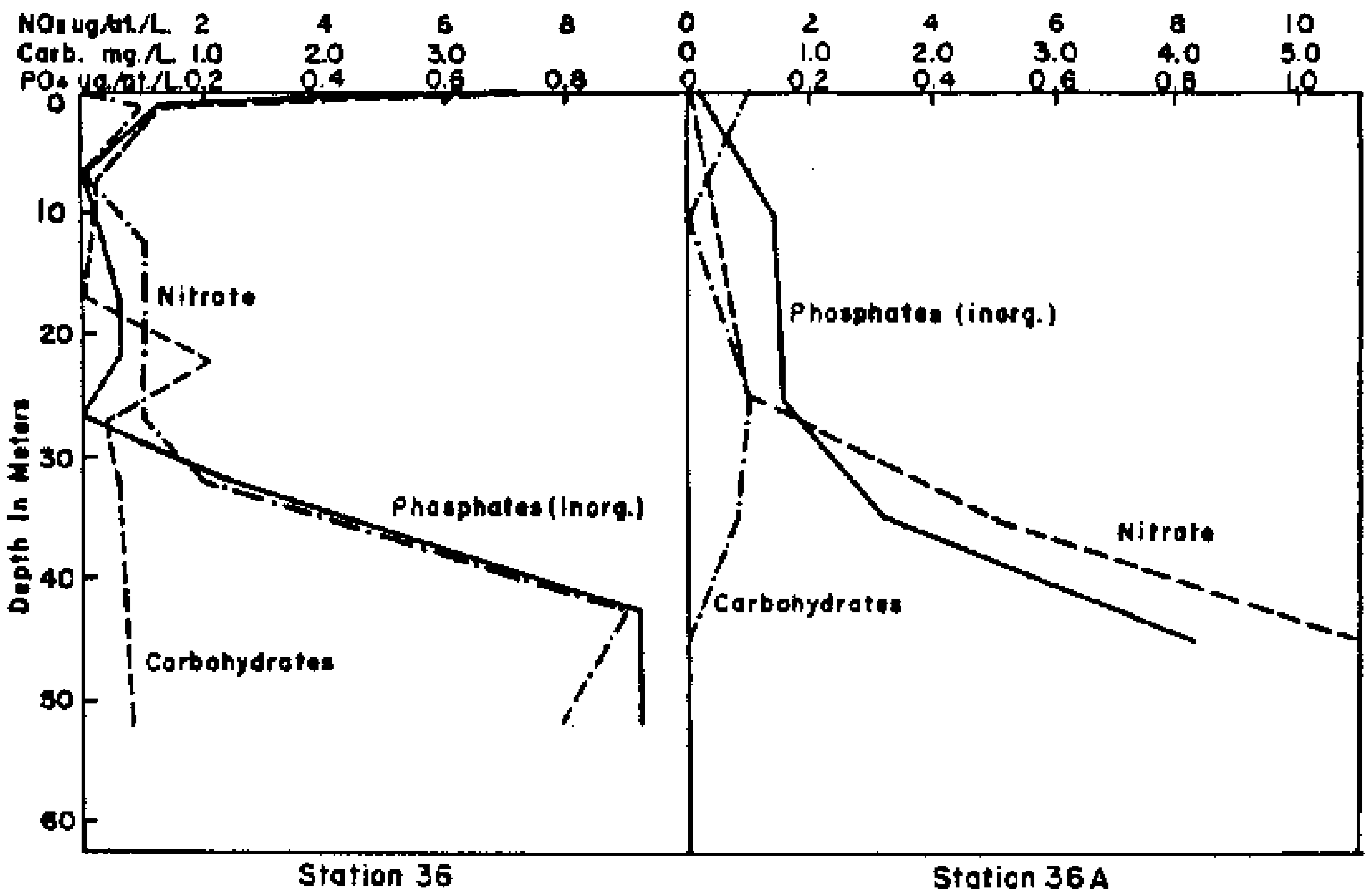


the minimum coming about 1 o'clock in the morning and the maximum in mid-afternoon.

On one of our routine cruises in the Gulf of Mexico a bloom of microorganisms was encountered and some detailed studies were made. Figure 1 shows the vertical distribution of the carbohydrates and other factors as they occurred in the bloom and just at its edge. The bloom was mainly composed of an unidentified ciliate.

The research vessel *Alaska* is equipped with an automatic plankton sampler which was designed to sample copepod populations continuously in hourly components. Figure 2 illustrates the hourly variations or carbohydrates and other chemical parameters sampled simultaneously with the hourly fractions of the plankton sampler. There are a number of important implications here that do not fall within the

FIGURE 2



scope of this discussion, but the point to be demonstrated is the type of distribution to be expected and how it fits in with the idea of patchiness in plankton distributions. The area in which the chemical factor showed pronounced maxima is biologically distinct from that in which they are at their minima.

Tyrosine and tryptophane. These amino acids are being determined routinely on all oceanographic stations occupied by the *Alaska* and their vertical and horizontal distributions studied in relation to the plankton community. We cannot, of course, say that the values we get for these represent amino acids in solution, because they may indicate the presence of intact proteins from which we get the acids on hydrolysis. The important thing is that we find measurable quantities which may represent up to 50 mg. of protein per liter if tyrosine is taken as 4 per cent of a protein molecule.

Rhamnoside. This sugar complex has been isolated (Wangersky, 1952) and concentrated in crystalline form in a number of instances from tidal marshes, open ocean, and more recently from the Florida red tide of November, 1952. In the latter case it was extracted at the rate of 50 mg. of crystalline material per liter of water. We have no routine test for this material, and, as yet, are not sure of its chemical structure.

Ascorbic acid. Minute quantities of this compound were isolated and

identified spectrometrically (Wangersky, *loc. cit.*). It is only to be expected that this and other vitamins would be in sea water which is supporting a great amount of activity, both plant and animal.

Unidentified compounds. In the course of this work a number of distinct fractions have been isolated chromatographically and await chemical identification and testing for biological activity. Among these are some relatively abundant substances which appear to be carbohydrate derivatives of low molecular weight (two or three carbon atoms). These are particularly interesting because some of their characteristics fit in the pattern of the N-ethyl-carbazol substances mentioned above.

In the bottom sediments from the Gulf of Mexico taken at 1,700 fathoms we have found yellow lipoid materials which give a bluish fluorescence under ultraviolet light. These may or may not be related to the substances mentioned by Kalle (*loc. cit.*).

THE SIGNIFICANCE OF THE COMPOUNDS IN THE MARINE COMMUNITY

1. As energy source.

As already mentioned, much of the interest in this subject has been in the nature of controversy regarding Pütter's theory. It is entirely possible that at least some organic materials are adsorbed on inert particles and then ingested to be stripped, as it were, for easy assimilation by the organisms. We are particularly interested in investigating the possibility of using this mechanism in the rearing of very young fish.

2. As growth promoters and inhibitors.

This may well be the field in which dissolved organic compounds have their greatest significance. The importance of the micro-biota in the marine economy needs no emphasis. Neither is it necessary to emphasize the fact that a great proportion of this segment of marine life derives its nourishment from the utilization of dissolved substances. If growth-promoting substances are found to be necessary in experimental nutrient solutions used in the culture of various planktonic organisms, it is likely that they will also be required in a normal environment. For instance, it has been found that several planktonic organisms will not flourish without the addition of soil extract to the artificial media used in their culture (Sweeney, 1951). Hutner and Provasoli (1951) reflect that "A mud or soil with an abundant and varied microflora should contain very nearly the gamut of microbial metabolites; . . ." Lake drainage, tidal marsh and estuarial effluents can all be considered as soil extracts which have been manufactured on a grand scale.

Once enriched by an organic bearing land effluent, some sea areas establish their own milieu which, in turn, evidently can produce growth promoting substances of their own. Large over-blooms of dinoflagellates must have their origin in something of this sort.

We plan to try some of the substances that have been isolated from such blooms and purified in our laboratories as growth promoters on young fish, as well as on various microorganisms, particularly the dinoflagellates associated with the Florida red tide.

Another candidate for significance in this area would be the caretenoid residues from the phytoplankton. These can be found in both sediments and water columns in extractable quantities and their influence on the marine fauna, micro and macro, should most certainly be given detailed attention.

Metabolites can limit populations as well as assist their proliferation. Pratt (1943) found that a water soluble extract from old cells of *Chlorella Vulgaris* retarded photosynthesis in young cells. He improved the growth of his cultures by providing means of removing these metabolites. The importance of the study of such compounds in connection with phytoplankton blooms is obvious.

3. As regulators of feeding activity.

Collier *et al.* (*loc. cit.* in press) have demonstrated the relationships existing between unknown "carbohydrate" compounds (N-ethylcarbazol substances) and the filtration rate of oysters. There appeared to be a threshold beneath which the oysters would not pump water, but above which there was a quantitative association. More carbohydrates seemed to be required when the water temperatures were higher. The oysters exhibited testing periods during which the valves opened only to a certain point, and beyond which they would not open unless the carbohydrates were at threshold level or over. This testing was at random, and at times periods of high carbohydrate would be allowed to pass undetected because the oyster failed to make a test opening.

It is possible that this mechanism serves to keep the oyster from expending stored energy on pumping sterile water.

4. Effects on the movements of animals.

An extension of the above effects of organic compounds on oysters could be made into the field of fish behavior. If oysters have the neurosensory systems capable of detecting and acting on such stimuli, there is no reason to think that a similar mechanism could not exist in fish.

Stevens (1949) cites supporting evidence that fish such as mackerel and herring avoid water heavily populated with phytoplankton such as the diatom *Rhizosolenia* or the flagellate *Phaeocystis*. On the other hand he relates that mackerel are caught most in so-called yellow water which is rich in copepods.

A study of the organic compounds exuded into the water by these different populations and the effects of these compounds on fish would certainly be rewarding.

5. As toxins.

We have here only to mention the great fish kills which are laid at the door of large blooms of dinoflagellates of various species. Riegel *et al.* (1949) isolated definite toxins from blooms of *Gonyaulax catenella* which had a toxicity of 1.65 mouse units per microgram. A mouse unit is that quantity of poison which will kill a 20-gram mouse in 15 minutes when injected intraperitoneally.

The author experimentally killed *Fundulus* sp. and *Cyprinodon* sp. with small quantities of water from the November red tide in Florida. This water was quick frozen upon collection and was held in the frozen state for a month before the experiment, and fish were killed in less than 20 minutes by a 4 per cent mixture of this water. The same water held at room temperature from the time of original collection was not toxic. It is hard to avoid the conclusion that this water, in its original state, held in solution some organic compound which acts as a poison to fish and which was stabilized by quick freezing.

6. As aid in evaluating biological activity of discrete water masses.

It appears that some of these compounds might be produced in the early phases of the photosynthetic process, and some in the anabolic phases of a phytoplankton community. In any case, their concentration and distribution would be dependent upon the size of the colony of organisms producing them, the activity level, and the potential productivity of the water supporting the growth. It should be possible, then, to obtain valuable information on the productivity of ocean waters by an analysis of these factors. Such studies could be at the level of short-term variations of activity within patches of plankton by analysis for the more volatile components, while a long-range approach over a greater area could be had through a study of the more stable residues.

SUMMARY

That sea water contains a significant quantity of dissolved organic compounds is a long recognized fact. More recently, biological differences between waters have been proven and their effects on the survival of invertebrate larvae demonstrated. It has been shown that substances which may originate in the photosynthetic process have a direct and quantitative influence on the feeding rate of oysters.

In the laboratories of the Gulf Fishery Investigations of the U. S. Fish and Wildlife Service carbohydrate and proteinaceous substances are being isolated and purified for experimental testing on living organisms, including young fish and dinoflagellates.

The principal fields of possible significance of these compounds are: as an energy source; as regulators or stimulators of feeding activities; effects on movements of marine animals; toxins; and in the evaluation of the biological activity within discrete bodies of water.

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DISCUSSION

DR. MCHUGH: One of the chief criticisms of the conventional method of measuring productivity of the sea has always been the fact that what you were measuring is really a standing crop rather than the total amount of potential productivity of the area.

I think this work of Mr. Collier's has great promise of perhaps bypassing that difficulty; also, as he pointed out toward the end of his paper, these substances

are supposedly uniformly distributed throughout the water mass, rather than occurring in chunks as plankton does. We will look forward to the development of this work with interest, I am sure.

I am sure some of us must have some questions to ask Mr. Collier in regard to his methods and to the possibility of utilizing his work for these various purposes he has described.

CHAIRMAN HUBBS: I would like to ask Mr. Collier a point which he did not bring out particularly, whether he is inclined to believe that these sugars or other dissolved materials of that sort are actually utilized by the fish by being absorbed through the gills and utilized in the nutrition of the fish?

MR. COLLIER: I am afraid, Dr. Hubbs, in this case, I might have to resort to the work on oysters; I hope I might be forgiven for that. We did measure the removal of the carbohydrate substances from the sea water by the oyster in our work, and we found it was removed as the water passed through the oyster. We are not prepared to say that this was actually utilized by the oyster physiologically because it is entirely possible that the material might have been absorbed on mucous train and voided with the feces, rather than being ingested. There is, of course, in the literature now a rather increasing amount of evidence that these compounds, after all, might be absorbed by organisms from a solution of water.

CHAIRMAN HUBBS: Even if the concentrations were fine in the sea water? I think that is one criticism we have always put to the old belief that these materials were significant.

MR. COLLIER: Do I gather that you mean the amounts present in sea water would not be sufficient to support an organism?

CHAIRMAN HUBBS: Either that or that they would not utilize it at that concentration.

MR. COLLIER: Well, of course, that would depend on the size of the organism, its body surface and so on, the demand of the organism for such substances, and whether you would take the point of view that the organism would survive on these substances.

It is entirely possible that, even at low concentrations, they might provide for supplements to the field.

CHAIRMAN HUBBS: Have you in mind any investigations with radioactive material to determine if the material or organic matter actually finds a place in the oyster or fish?

MR. COLLIER: No, sir, not in the immediate future. Of course, we realize the value of that particular technique and that is being used in certain cases by another laboratory of the Service. We have considered that; but, in our laboratory, we do not have the facilities and it is not in sight.

DR. HUGH BENNETT: I just wanted to ask if these organic substances are in complete solution or possibly some of them in some such state as colloidal suspension.

MR. COLLIER: In our work, we did run water through bacteriological filters; but, of course, that does not mean that we might not have had some very fine colloidal suspensions. It all depends on whether you want to establish the boundary.

DR. MCHUGH: I would like to ask just one more question. That is, you have shown that these substances have some effect on the feeding activities of the oysters. I wonder if you have considered any work on the possibility of their having an effect on the spawning activities of oysters and other mollusks.

MR. COLLIER: We have not studied them in relation to spawning activities. Of course, we all know of Nelson's work with the hormone, dianaline. That is something produced by the oyster itself. We have not gone into this, after all.

CHAIRMAN HUBBS: In partial answer to Dr. Bennett's question, I might say that we have research going on at the Scripps Institution of Oceanography, which indicates that the particular organic material in the sea, the remains of the organisms, some of which is in virtually colloidal state, is probably of tremendous significance in the nutrition of the filter-feeding organisms, such as the mussel and oyster, and a great many others. Some of our men there in biochemistry feel that this sort of material is probably more significant in many places, and at many times, than the actual living plankton itself.

DR. MCHUGH: If there is no more discussion of this paper, I will turn the floor back again to Dr. Hubbs.

CHAIRMAN HUBBS: We now have a bit of shift in our line of papers here, from those dealing with the fishery products and the organic materials which are basic thereto, to a series of papers on marine mammals, a subject which I know will be of very great interest to all of us.

The first of these papers is by Richard E. Griffith, of the Fish and Wildlife Service, dealing with the future of the sea otter.