Marine Biology 58, 205–209 (1980)

MARINE BIOLOGY

Siliceous Nanoplankton I. Newly Discovered Cysts from the Gulf of Alaska*

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Abstract

Siliceous nanoplankton in the size range 2.5 to 5.5 μm and of a type hitherto undescribed are reported from Eastern Subarctic water samples. Nine distinct cell types were recognizable, each possessing an unusual tetrahedral symmetry, resulting from the arrangement of 8 siliceous plates. Since the cells were abundant (maximum concentration of about 7×10^5 cells 1^{-1}) and were distributed over a wide oceanic area (136° to 149°W), they could well play an important role in the food web in Subarctic seas. Similar cells were found simultaneously in Antarctic waters (see following paper: Silver *et al.*, 1980), where they were as abundant and widespread as in the Subarctic. Evidence that the siliceous forms are likely a cyst stage and that they may be part of the life cycle of species of siliceous oceanic choanoflagellates is presented.

Introduction

In May 1978, during the course of a nanoplankton (cell diameter 2 to 20 μm) survey of Subarctic waters in the Gulf of Alaska, we first observed a number of cell forms of a type hitherto unreported. The cells bore little similarity to well-known planktonic taxa. It seemed unlikely that they could be diatoms, coccolithophorids, or dinoflagellates, and on the basis of electron micrographs alone they could not be assigned to any other major biological group. The present study describes these unusual forms and discusses their possible taxonomic affinities and ecological importance.

*Contribution No. 1149 from the Department of Oceanography, University of Washington

Materials and Methods

Samples were collected on two cruises in the Gulf of Alaska at 13 stations (Table 1). A 20 ml subsample of each water sample was filtered onto a 0.8 μm Nuclepore filter and prepared for scanning electron microscopy (SEM) using the method of Paerl and Shimp (1973). A 200 ml subsample was preserved in 0.2% buffered formalin (using 40% formaldehyde saturated with sodium acetate). The filtered samples were examined in a JEOL U3 SEM after critical point drying from Freon and coating with carbon and gold-palladium; some of the formalin samples were examined using a Zeiss inverted microscope (Utermöhl, 1931). Part of one of the Nuclepore filters containing numerous cells was boiled in concentrated nitric acid for 7 min, cooled in the acid for 20 min, rinsed in distilled water 5 times, then dried, coated with gold-palladium and examined in the SEM.

Table 1. Locations of water samples from the Gulf of Alaska in which siliceous cysts were found

Collection date and station	Location	Time (hrs)	Depth (m)
4 May, 1978			
GOA St 1	56° 15′N; 135° 48′W	08.02	8
GOA St 2	56° 23′N; 136° 32′W	10.10	8
GOA St 3	56° 34′N; 137° 27′W	12.50	8
GOA St 4	56° 36′N; 138° 37′W	16.00	8
GOA St 5	56° 47′N; 139° 45′W	19.00	8
GOA St 6	56° 59′N; 141° 27′W	23.25	8
5 May, 1978			
GOA St 7	57° 17′N; 143° 45′W	04.40	8
GOA St 8	57° 21′N; 144° 32′W	06.40	8
GOA St 9	57° 26′N; 145° 38′W	09.23	8
GOA St 10	57° 31′N; 146° 49′W	12.20	8
GOA St 11	57° 34′N; 147° 44′W	16.08	8
GOA St 12	57° 39′N; 149° 21′W	21.25	8
13 June, 1978			
St "P"	50° N; 145° W	01.30	19
St "P"	50° N; 145° W	01.30	51

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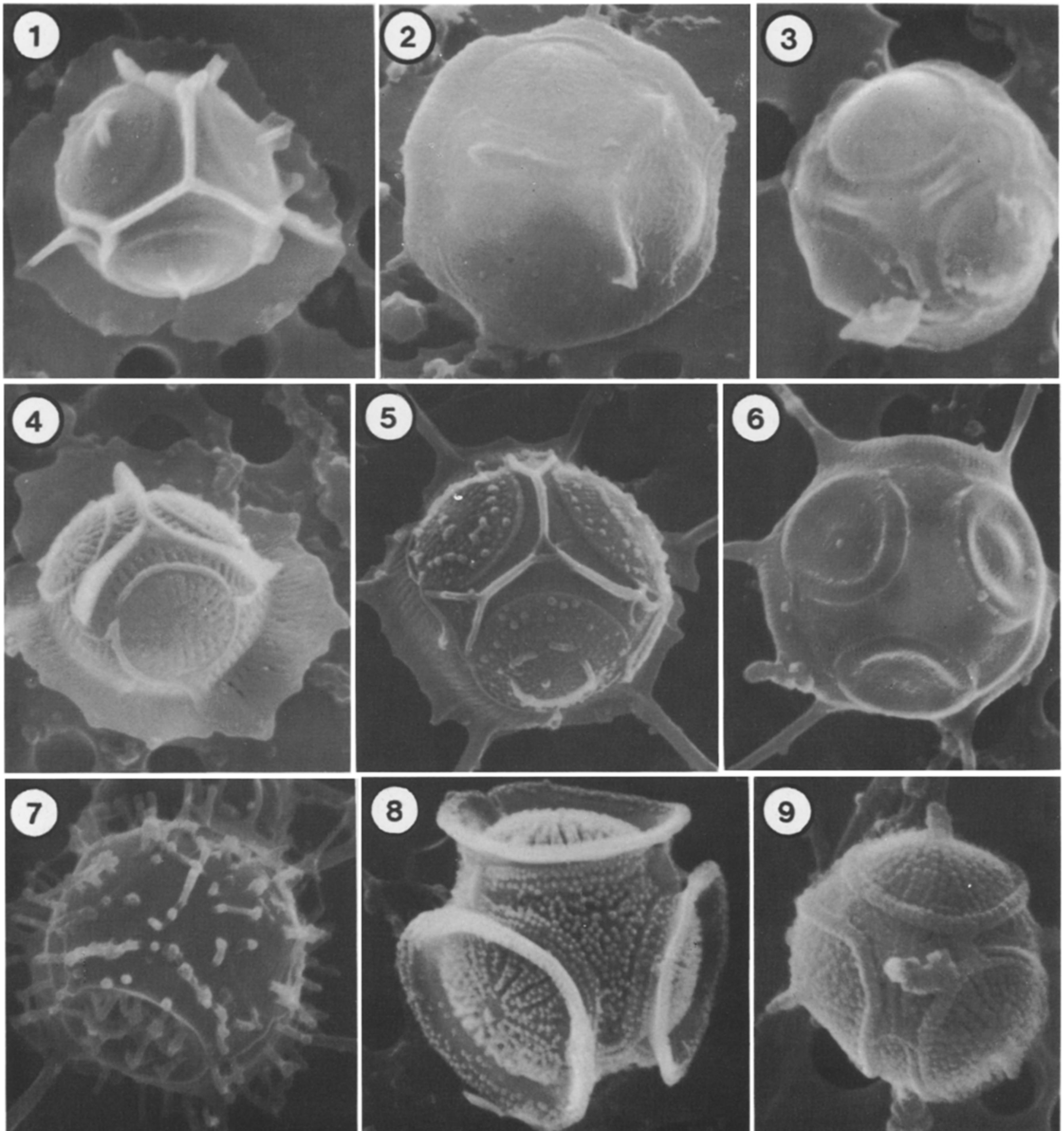

Fig. 1. The 9 types of siliceous cyst found in nanoplankton samples from Gulf of Alaska. Cell diameters range from 2.5 to 5.5 μm. Shield plates are clearly visible in all examples; triradiate plates are clearest in *1–5* and *8*; girdle plates in *2* and *4–6*. (Scale: Nuclepore filter hole diameter = 0.8 μm)

Results and Discussion

The new cells were observed in near-surface waters of the Gulf of Alaska in May and at 19 and 51 m in June (Table 1). (We could not determine whether the new cells were present in surface waters in June or not, because no surface sample was collected then. Similarly no samples were collected at depth in May.) Some of the forms were more abundant than others, and the relative abundance varied with the station. Cell concentrations

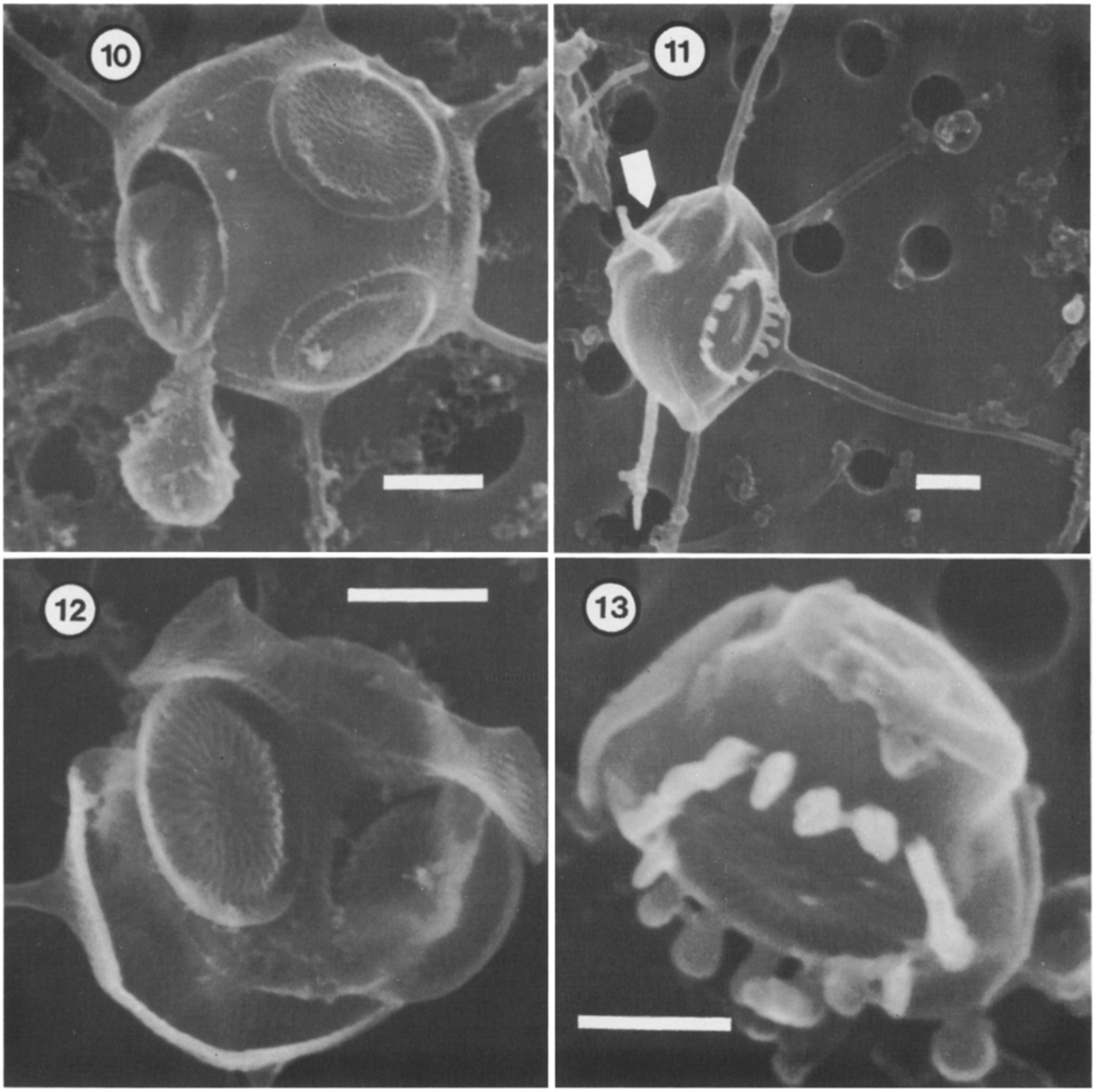

Fig. 2. One of the 9 types of siliceous cysts. *10:* Dorsal surface showing 3 shield plates, 1 triradiate plate, 3 girdle plates with attached spines; note material emerging from under one shield plate. *11:* Ventral surface with ring of ventral processes, girdle plates with attached spines; one shield plate seen in lateral view (arrow). *12:* Cyst with partially dissociated plates; internal surfaces of the triradiate plate and 2 of the shield plates; 1 girdle plate with 2 attached spines (lower left). *13:* Immature cyst, ventral view; note lack of spines and different proportion of component parts compared with *10* and *11*. (Scale bars = 1 μm)

ranged from approximately 4×10^3 cells l^{-1} to a maximum of 7×10^5 cells l^{-1}. In June, one of the forms was almost as abundant as each of the predominant phytoplankton species which were *Nitzschia cylindrus* and *Emiliania huxleyi*. The cells occurred in fecal pellets and in clumps of phytoplankton debris as well as singly at 19 m in June.

A total of nine distinct cell types was observed (Fig. 1). An unusual tetrahedral symmetry was common to all, yet they displayed a considerable diversity of shape and appearance. There were no intergradations between the 9 forms. The cells ranged from 2.5 to 5.5 μm in diameter; their component plates were as small as 1.5 μm in diameter. These plates were separable and became detached from one another on acid treatment. All of the cell wall ornamentation as well (spines, processes, knobs etc.) remained intact through treatment with nitric acid indicating that the cell walls were composed of silica.

The geometry of 7 of the cell types (Fig. 1: *1-7*) was that of a tetrahedron with 3 equal parts, which we have arbitrarily termed the dorsal surface, and 1 unequal part (the ventral surface). The dorsal surface was strongly convex; the ventral surface varied from flat to strongly convex in the various forms. The dorsal wall was composed of 3 round plates termed shield plates (Fig. 1: *1-7*) and 1 triradiate plate (most clearly visible in Fig. 1: *1-5* and Fig. 2: *12*) fitting between the shield plates. The ventral surface was a single, round plate of wider diameter than the shield plates (Fig. 2: *11*). A girdle zone between the dorsal and ventral surfaces was composed of a ring of 3 plates (Figs. 1: *2, 4-6*; Fig. 2: *10-12*). Two of the cell types (Fig. 1: *8, 9*) were spherical in shape, but still possessed a tetrahedral symmetry created by 4 round plates of equal diameter and 4 triradiate plates.

The plates of the different forms were variously ornamented with striae (Fig. 1: *2-6*), papillae (Fig. 1: *5, 8, 9*), rounded or forked processes (Fig.1: *7*), knobs (Fig. 1: *9*) or ridges (Fig. 1: *1, 4, 5*). In the 7 cell types with unequal symmetry (Fig. 1: *1-7*), ornamentation of the round ventral plate was often different from that of the dorsal shield plates (e.g. cf. Fig. 2: *10* and *11*). Each of the 3 girdle plates in these 7 forms usually gave rise to a wing or to 2 spines. Two of the cell types had spines (Fig. 1: *6, 7*), 2 had wings (Fig. 1: *1, 4*), 1 had both spines and wings (Fig. 1: *5*), and 2 had neither (Fig. 1: *2, 3*). The spines were straight, forked, or rarely with multiple dichotomies.

We have observed 3 cells with material emerging from under one of the shield plates (Fig. 2: *10*). This material was about 1 μm in diameter with some trailing substance, but no evidence of a flagellum. In a number of instances cells were encountered with one shield plate askew or missing.

We also observed 4 interesting small forms, each of which was clearly related to a single, mature cell type, but was smaller, rounder, had fewer spines and different plate proportions. Fig. 2: *13* is an example of one of these forms, which we think may be an immature stage. Five specimens of this particular immature form were observed, an observation which discredits the idea that they were mature cells with the spines broken off. These immature forms may enlarge by growth of portions of the ventral and girdle plates that have not yet become silicified, thereby changing the plate proportions between immature and mature cells.

Although the unknown cell forms were an important part of the nanoplankton and occurred at all 13 stations sampled across the Gulf of Alaska, no cell-division stages were ever observed. This fact coupled with the consistent opening of the cell and subsequent release of cell contents suggests that these cells are cysts. Their morphology is unlike any cysts so far described from marine plankton. Organisms similar to Figs. 1: *3*, 1: *9* have been observed in high concentrations (2×10^5 cells l^{-1}, our calculations) in the Subarctic Current (44 °N to 50 °N; 162 °E to 180 °E) in July, 1974, when they comprised up to 98% of the nanoplankton community (Nishida, 1979). Similar cells have also been discovered in Antarctic seas, where they occurred in large numbers in association with equally dense concentrations of choanoflagellates, leading Silver *et al.* (1980) to propose that such cells may be cysts of choanoflagellates. X-ray analysis by Silver *et al.* (1980) of the walls of cells very obviously similar to ours (Silver *et al.*, 1980, their Fig. 1) confirmed the results of our chemical test (nitric acid) and demonstrated definitively that the cell walls are composed of silica.

The deposition of silica walls is uncommon in algae. Of the groups of organisms represented in our samples, only diatoms, silico-flagellates and choanoflagellates exhibit a "silicon chemistry". Diatom resting-spores match the parent cell in basic symmetry (radial or bilateral) and usually in diameter (Hendey, 1964; Hargraves, 1976). The new cells we observed are considerably smaller than most diatom cells and exhibit a different symmetry from diatom resting-spores. Only one species of silico-flagellate (*Dictyocha fibula*) was found in our samples; it probably could not produce 9 types of cyst. We have identified 9 species of choanoflagellates (all in the genera *Pleurasiga, Parvicorbicula, Calliacantha*, and *Bicosta*) from our samples and 9 types of "cyst". Except for the diatoms there is no other major group of organisms (siliceous or not) in our samples for which we have as many as 9 species. The size of the choanoflagellate cells (not including the loricae) range from 2.5 to 3.2 μm, a range comparable to that of the unknown cells. The tetrahedral symmetry of the unknown cells can be derived from the conical symmetry of choanoflagellates, if the tetrahedron is viewed as a truncated cone. The above evidence, along with that in the following paper (Silver *et al.*, 1980), although admittedly circumstantial, points to the possibility of the unknown cells being choanoflagellate cysts. However, none of the many records of acanthoecacean choanoflagellates from northern waters (e.g. Throndsen, 1970a, b; Manton *et al.*, 1976; Manton and Oates, 1979), includes a record of any cells similar to the unknowns described here.

The relative abundance of choanoflagellates and cysts in the North Pacific Ocean differed from those found by Silver *et al.* (1980) in Antarctic water samples. In our samples, maximum choanoflagellate concentrations were observed at GOA Station 4 (approximately 1×10^5 cells l^{-1} for *Calliacantha simplex* and *Bicosta spinifera*), while *Pleurasiga* spp. and *Parvicorbicula socialis* averaged around 1×10^4 cells l^{-1} at 7 stations. Cyst concentrations were greater than those of choanoflagellates at all but GOA Stations 1, 4, 5, 8, with a maximum for one form (Fig. 1: *7*) of 7×10^5 cells l^{-1} at GOA Station 12. Other nanoplankton species were also more abundant than the choanoflagellates, for instance, a number of species of diatoms, cryptomonads, and Prymnesiophyceae reached maximum concentrations of 5×10^5

cells l^{-1}. If the new cells are cysts of choanoflagellates, they would not necessarily be found in equal concentrations with choanoflagellates; in fact, cyst and loricate forms might be expected to concentrate at different depths except at the precise time of cyst formation. Therefore, the differences between Antarctic and North Pacific samples as to cell concentration should not affect the hypothesis that the cysts are stages in the life cycle of choanoflagellates.

None of the mature or immature forms of the cysts was ever observed physically attached to any choanoflagellate lorica. If the cells are choanoflagellate cysts, it is possible then, that they may form and develop to maturity independent of the loricae. It follows that positive identification of the cysts, both as to class and to species, will be quite difficult, and linking evidence will probably remain circumstantial until observations of cysts from monospecific cultures have been made. For this reason a detailed description of the structure of each of the 9 cell types is now in preparation.

Using the light microscope with phase-contrast optics, we examined a formalin-preserved sample (from GOA Station 6) which had a high density of the unknown cells when viewed in SEM. In a volume equivalent to that collected on the SEM filter, we thus far have been unable to recognize any of the unknown cells; although this is puzzling, it may explain the absence of any previous record based on formalin-preserved samples.

The implications of the present study for paleo-oceanography are interesting, regardless of the taxonomic affinities of the new cells. Most nanoplankton species do not have mineralized walls and therefore are not preserved in the sediments. If the new cysts are found in sediments (which they most probably will be), they can provide an indicator for paleo-distribution of nanoplankton with implications in current and climate changes, especially if recent distributions are limited in space and time.

The importance of nanoplankton as a group has been demonstrated in coastal waters (e.g. Anderson, 1965; McCarthy *et al.*, 1974; Takahashi *et al.*, 1978) and in oceanic areas (Saijo, 1964; Mullin, 1965; Holligan, 1979), but studies of non-calcareous nanoplankton species from the Pacific Ocean have been limited to coastal waters (Manton, 1977; Moestrup, 1979). The discovery of new entities in the nanoplankton of the oceanic Pacific underlines our limited understanding of this community. The small size, considerable concentrations, and widespread distribution of the cells suggests their importance in oceanic food webs and emphasizes the need for more thorough research into temporal and geographic distribution of nanoplankton species.

Acknowledgements. This research was supported by NSF Grant (OCE) 77-25957. We thank G. C. Anderson, K. Banse, T. S. English, and R. Horner for helpful discussions and critical reviews of the manuscript and F. Reid for calling our attention to the work of S. Nishida. L. Kniffen prepared Fig. 2 and printed both figures. S. Chamberlain collected the Station "P" samples. We thank J. Larrance and A. Chester for their cooperation and for furnishing laboratory space on the NOAA ship "Miller Freeman".

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Date of final manuscript acceptance: May 30, 1980.
Communicated by N. D. Holland, La Jolla