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Cytometric diversity in marine ultraphytoplankton

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Abstract

The concept and methods of ecological diversity in communities were applied to phytoplankton categorized by flow cytometric measurements related to size and chlorophyll content. Each cytometric signature was condensed to single numerical values indicative of diversity and evenness. Measurements pooled from studies disparate in temporal and spatial scales indicated greater chlorophyll biomass and primary production with greater cytometric diversity and evenness. Future development of these ideas may help link biological oceanographic processes with patterns established through ecological processes at the community level.

The concept of biotic diversity is a central theme in community ecology, both in general (Pielou 1975; Magurran 1988) and in particular for marine communities (Frontier 1985; McGowan and Walker 1993). Diversity embodies two notions: richness, which indicates the number of different kinds of organisms; and evenness, which indicates the relative "importance" (e.g. abundance) of the different kinds of organisms. Thus, a community with say 100 species is more diverse (rich) than a community with 10 species. Further, a community with 5 species each of which has 10 individuals is more diverse (even) than a community with the same 5 species, one of which has 46 individuals and the other 4 species having only 1 individual each.

Diversity is an expression of the organization or structure of a community; it is a collective property revealed at a high level of organization (the community) but not at lower hierarchical levels (populations and individuals). Usually, the purpose of measuring diversity is to judge its relationship to other community properties (e.g. productivity) or to the prevailing environmental conditions (Pielou 1975). Generally, but with important exceptions, high diversities are related to high environmental stability, high environmental predictability, and high productivity. Recent discussions (Kareiva 1996; Moffat 1996) on new experiments in grassland ecosystems (Tilman 1996; Tilman et al. 1996) have brought into sharp focus the earlier ideas of Charles Darwin, Charles Elton, and Robert May on the relation between diversity and productivity. These new discussions have not included phytoplankton.

In phytoplankton ecology, the singular efforts of Margalef (1960, 1967, 1968) have led to a widespread study of diversity indices (Hulburt et al. 1960; Patten 1962; Travers 1971). In most cases, these indices refer to phytoplankton identified by species and whose importance is rated by abundance (cells ml^{-1}). In other words, the classifications are based on conventional binomial taxonomic nomenclature. However, as noted by Margalef (1968), "the notion of diversity can be applied to anything that can be distributed into categories." Furthermore, importance can be rated by measures other than a count of individuals. It is therefore feasible to speak of diversity when the organisms are categorized by photosynthetic pigments (Margalef 1967, 1968), cell size (Parsons 1969; Ruiz 1994), biomass (Lurié and Wagensberg 1983), or when the "importance" of the organisms is expressed as ash-free weight (Wilhm 1968) or as biovolume (Parsons 1969). In fact, if the emphasis of the research goal so requires, importance could presumably be expressed as energy flow, productivity, calorific values, respiratory gas exchange rate, and other measures of resource utilization (Tokeshi 1993).

In this paper, I explore the application of ecological diversity indices for phytoplankton that are classified in a novel way: namely by their bio-optical properties measured on an individual cell basis by flow cytometry. The purpose of this study in cytometric diversity is to discern whether this collective property of the phytoplankton community, which can be the same for greatly different collections of individuals, bears any relationship to the biomass and productivity of phytoplankton.

Concept of cytometric diversity

To calculate diversity, it is first necessary to identify each phytoplankter by category and also to rate the importance of each category. Here, I present the notion that this identification and rating can be made within a classification scheme of cytometric properties instead of the conventional taxonomy of algal systematics. A cytometric classification can be based on as few or as many criteria as are deemed appropriate by the objective of enquiry. The minimum number is of course one, and this limiting case is parallel to the classification of particles by volume as measured by a Coulter counter (Parsons 1969). The maximum number is the total number of different measurements simultaneously made on each particle; this may be eight (Hofstraat et al. 1994) or even more, dependent on instrument construction. The maximum number of distinct categories in cytometric

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classification depends on the resolution at which each variable is measured. In a typical flow cytometer, each variable is resolved to 256 or 1,024 discrete values. Thus, at the lower resolution, if only one variable is used for classification, there would be a maximum of 256^1 categories; if eight variables were considered simultaneously, the maximum would be 256^8 categories. For calculating diversity, a nomenclature for the categories is not required, although it is easy to see that each category can be named by its coordinate values in multivariate space. If the measured properties (light scatter, fluorescence) are calibrated, it would be possible to name each category in absolute units of, say, cell size and pigment content.

In the present preliminary work, each cell is identified by only two criteria: how large it is (as indicated by forward light scatter) and how much Chl *a* it has (as indicated by red fluorescence). The importance of each category is rated simply by the numerical count of cells that meet the joint criteria for that category. This means that a small, Chl-poor cell is as important to the diversity of the community as a large, Chl-rich cell. This is of course the same way that cells are rated when conventional species diversity indices are calculated.

Many indices can be used to characterize diversity depending on whether richness or dominance is to be emphasized (Magurran 1988). An elegant notation developed by Hill (1973) unifies the commonly used, but apparently disparate, indices such as those of Shannon–Wiener and Simpson:

$$N_a = \left(\sum_i p_i^a \right)^{1/(1-a)}$$

where N_a is Hill's diversity measure of order *a*, *a* is any real number, and p_i is the proportional abundance of the *i*th category of organism. Various properties of Hill's family of functions have been investigated by others (Peet 1974; Kempton 1979). The following members of this family have particular significance (Hill 1973): $N_{-\infty} = (p_i)^{-1}$ for the rarest category; $N_0 = \Sigma_i p_i^0$ = total number of categories present; $N_1 = \exp[-\Sigma p_i \cdot \ln(p_i)] = \exp(H')$ = exponential Shannon–Wiener index; $N_2 = (\Sigma p_i^2)^{-1}$ = reciprocal of Simpson's index; and $N_\infty = (p_i)^{-1}$ for the commonest category. It is evident that as *a* varies from $-\infty$ to ∞, N_a depends increasingly more on the common kinds of cells and increasingly less on the rare ones. The degree of evenness can therefore be surmised by the relationship between different members of this family. A commonly used measure of evenness is J' (Pielou 1975).

$$J' = \frac{\ln(N_1)}{\ln(N_0)}.$$

Methods

Many of the sampling locations and procedures have already been described (Li 1994*a,b*, 1995; Li et al. 1995). Briefly, seawater was collected by Niskin bottles from Bedford Basin (1993–1996), inshore-to-offshore transects from Morocco (October 1992) and Delaware (April 1995), the Labrador Sea–Grand Banks–Scotian Shelf regions (July 1995), and a North Atlantic transect from the Canary Islands to Nova Scotia (September–October 1992). Additionally, new sampling was conducted during March 1996 in the southeastern inlets of Vancouver Island (BC), mostly in Saanich Inlet (123.50°W long.) but also including Swanson Channel. Standard hydrographic information (Seabird CTD) and bulk phytoplankton Chl *a* measurements (assay of acetone extracts using a Turner 10-AU fluorometer) accompany each sample. For flow cytometry, samples were fixed with glutaraldehyde or paraformaldehyde and stored at cryogenic temperature (Li et al. 1995) when the preferred shipboard analysis of live samples was not possible.

Flow cytometric analysis was performed as previously described (Li 1995). Measurements of single-cell forward light scatter and red fluorescence were collected using logarithmic amplification and recorded in relative units in a four-decade range spanned by 256 channels. To ensure that only phytoplankton were enumerated, particles with red fluorescence weaker than that emitted by *Prochlorococcus* were rejected (Olson et al. 1993). Offline listmode analysis was performed using WinMDI (Joseph Trotter) on a PC. For calculation of diversity (N_1, N_2) and evenness (J') indices, data resolution of each variable was reduced to 16 channels by successively binning the counts of four adjacent channels. The maximum number of possible categories in the light scatter-fluorescence domain was therefore 16^2 (=256). The degree of correlation between N_1 and N_2 was very high (r = 0.98, n = 353) for the ultraplankton samples so that only values of N_1 (i.e. $\exp^{H'}$) are reported.

Because neither light scatter nor fluorescence were calibrated, it is not possible to specify precisely the true nature of any of the measured cells. However, in comparisons against synthetic fluorescent beads, the cells measured in this study range between 0.5 and 20 μm in equivalent spherical diameter. This size range cannot be considered accurate but merely serves to indicate that only picoplankton and nanoplankton were measured but not microplankton. For convenience, the term ultraphytoplankton is used to indicate the cells detected by flow cytometry: it includes the two picoplankters *Prochlorococcus* and *Synechococcus,* as well as small eukaryotic phytoplankton ranging from <1 μm to an undetermined larger size.

Primary production (mg C m^{-3} h^{-1}) was measured as ^{13}C or ^{14}C incorporation into total phytoplankton collected on glass-fiber filters (Whatman GF/F) after incubations under simulated in situ conditions or in temperature-controlled artifical light gradients. These data are courtesy of Glen Harrison and Brian Irwin (unpubl.).

Results

Surveys of diversity and evenness—Measures of cytometric diversity and evenness were made in six separate studies. Results for a transect along a short coastal inlet (Saanich Inlet, Canada) and for a basinwide transect of the North Atlantic from the Canary Islands to Nova Scotia are not presented in detail but are included in the correlation analysis of the following section. The other four studies were: (1) a

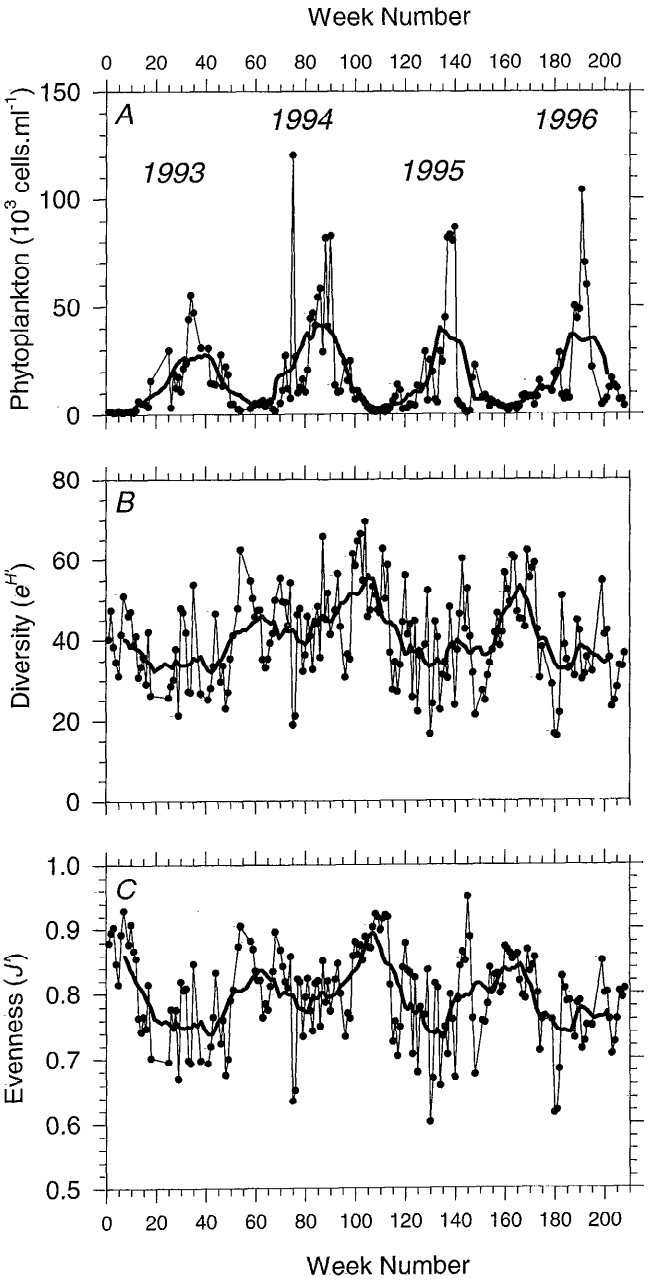

Fig. 1. Ultraphytoplankton in Bedford Basin (5 m) from January 1993 to December 1996. (A) Cell abundance. (B) Diversity $N_1 = e^{H'}$. (C) Evenness J'. Bold trend lines are 13-week running averages.

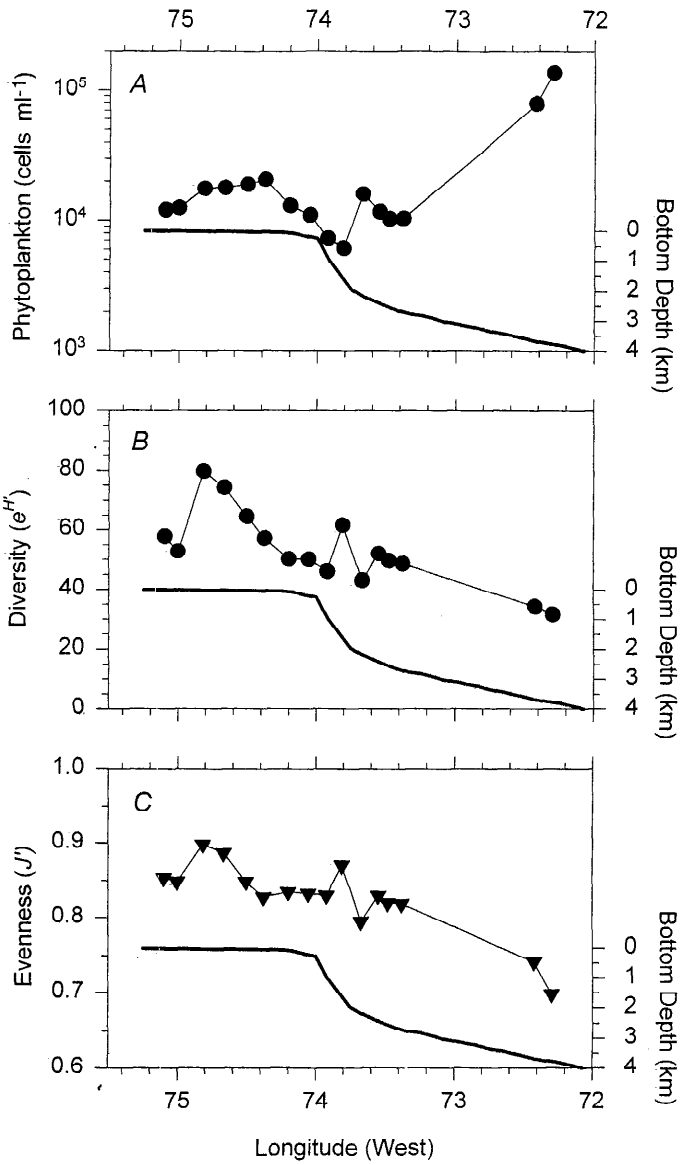

Fig. 2. Ultraphytoplankton in the western North Atlantic offshore from Delaware. (A) Cell abundance. (B) Diversity $N_1 = e^{H'}$. (C) Evenness J'. Bold line indicates ocean depth (right ordinate).

time series of observations in a harbor (Bedford Basin, Canada); (2) an inshore–offshore transect from Delaware to the Sargasso Sea; (3) an inshore–offshore transect from Morocco to the oceanic eastern North Atlantic; and (4) a wide-area survey of the Labrador Sea, Grand Banks, and Scotian Shelf.

(1) Over 4 yr in Bedford Basin, diversity fluctuated greatly from week to week (Fig. 1B). However, running averages indicated that within each year, diversity (Fig. 1B) and evenness (Fig. 1C) were low in the summer when the numbers of ultraphytoplankton were high (Fig. 1A).

(2) Traversing west to east out from Delaware to the Sargasso Sea, diversity (Fig. 2B) and evenness (Fig. 2C) reached maxima over the middle of the continental shelf, then decreased over the continental rise where the phytoplankton were dominated by large numbers of the picoplankter *Prochlorococcus* (Fig. 2A).

(3) On another transect, traversing east to west from Morocco out to the oceanic eastern North Atlantic, a different observation scale was chosen for diversity analysis. Here, the example of Frontier (1985) was followed whereby cytometric counts from many depths at each location were pooled together to yield values for diversity and evenness characterizing the water column as a whole. Results indicate diversity (Fig. 3B) and evenness (Fig. 3C) were greatest at the edge of the continental shelf, decreased rapidly over the

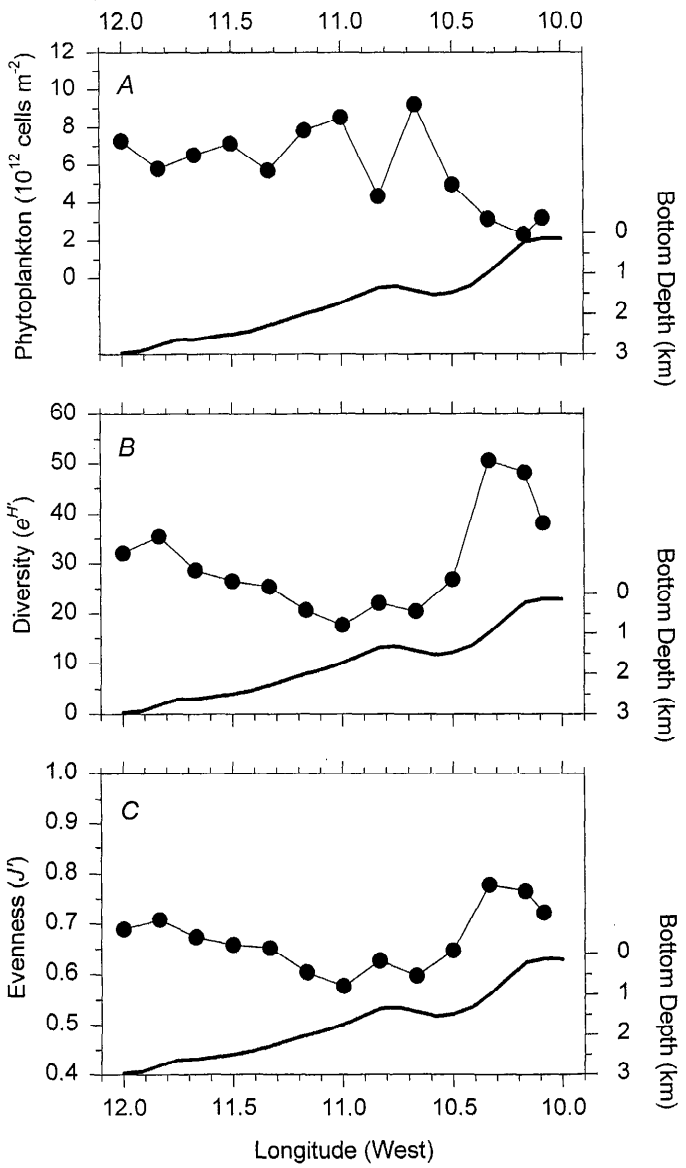

Fig. 3. Ultraphytoplankton in the eastern North Atlantic offshore from Morocco. At each location, phytoplankton counts from 1, 10, 20, 40, 60, 80, 100, and 120 m were pooled together for analysis. (A) Cell abundance. (B) Diversity $N_1 = e^{H'}$. (C) Evenness J'. Bold line indicates ocean depth (right ordinate).

slope, and then increased slowly further offshore over the rise. Once again, low diversity and evenness occurred when *Prochlorococcus* and *Synechococcus* (to a lesser extent) were dominant. The gradual offshore increase in diversity was due to increasing relative importance of picoeukaryotic algae.

(4) In a wide-area survey of 40 stations on the continental shelf of eastern Canada, the Labrador Sea, and the northern Sargasso Sea (Fig. 4A), diversity (Fig. 4C) and evenness (Fig. 4D) were highest in the central Labrador Sea. Areas of high cell concentrations >40,000 cells ml $^{-1}$ (Fig. 4B) were in the Sargasso Sea dominated by *Prochlorococcus*; and on the Nova Scotian Shelf dominated by *Synechococcus*: these were regions of low diversity and low evenness. An area of unforeseen low diversity was the Grand Banks east of St. John's, Newfoundland, dominated by neither *Prochlorococcus* nor *Synechococcus*. Here, the ultraphytoplankton community was dominated by an unidentified phytoplankter at concentrations of ~10,000 cells ml $^{-1}$.

Correlations—Total phytoplankton chlorophyll biomass (logarithmic units) was significantly correlated ($P < 0.01$) with diversity (Fig. 5A) and evenness (Fig. 5B) of the ultraphytoplankton community. As well, but based on fewer measurements, total primary production (logarithmic units) was likewise correlated ($P < 0.01$) with diversity (Fig. 5C) and evenness (Fig. 5D) of the ultraphytoplankton community.

Discussion

The concept and methods of ecological diversity have been presented here for organizing the ataxonomic aggregations of phytoplankton as revealed by flow cytometry. This approach differs from other multivariate cytometric methods such as neural network classification, cluster analysis, and curve fitting in that a single number, the diversity index, summarizes the structure (richness and evenness) of the phytoplankton sample. The heuristic benefit of condensing each cytometric signature to a single numerical value is the ease with which questions about ecological communities can be addressed. Information about patterns of abundance that is lost in the condensation can be re-examined in rank-abundance plots, but this fruitful area of research (Frontier 1985; Tokeshi 1993) is not exploited in the present preliminary work.

An important distinction must be made between the diversity of an entire community and that of a sample taken from the community. It is of course only possible to estimate, but not fully census, the entire phytoplankton community. Thus any impression about diversity is circumscribed by the limitations of sampling and analysis. In conventional taxonomic studies of species diversity, attention is usually focused on cells large enough to settle in counting chambers (Hulburt et al. 1960; Margalef 1994) or restricted to only the net phytoplankton (Patten 1962; Uysal and Sur 1995). On the other hand, flow cytometry emphasizes the ultraphytoplankton, not inherently because they are small, but because they are most numerous in the plankton. That there are fewer large cells than small cells means a greater volume of sample is required to encounter a (statistically) reasonable number of large cells. Although flow cytometers can be made (Dubelaar et al. 1989) or modified (Olson et al. 1993) to achieve high sample throughout, most only operate at ~1 μl s^{-1} or less. In the present study, a sample volume of no more than 1 ml was analyzed in each case. There can be no expectation that phytoplankton groups of fewer than ~10^2–10^3 cells ml^{-1} would have valid statistical representation. For this reason, conclusions about cytometric diversity are biased toward the low end of the phytoplankton size spectrum, in contrast to the bias of species diversity toward the high end.

There is a second consideration related to instrument configuration that is relevant: namely, the occasional inadequacy

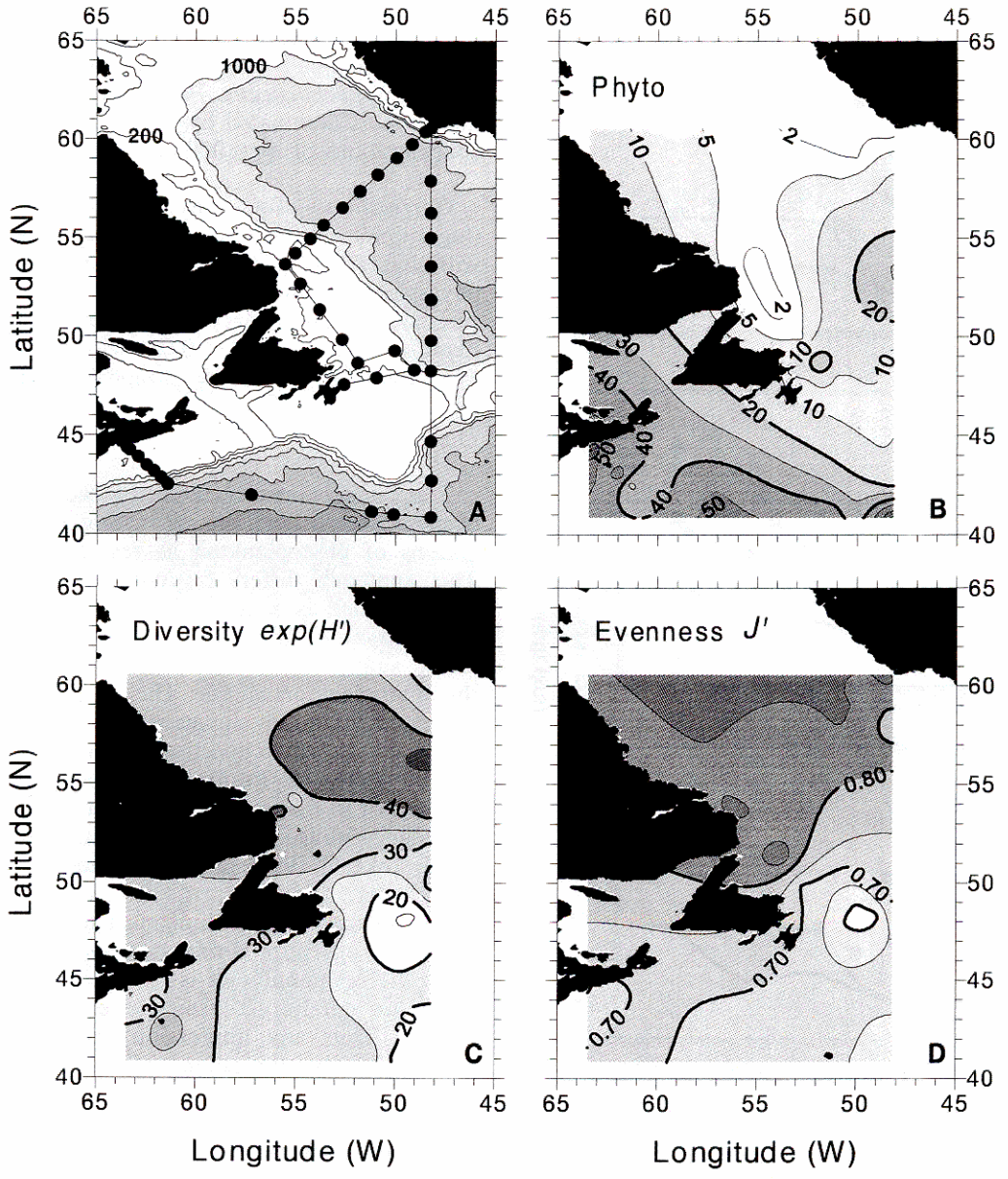

Fig. 4. Ultraphytoplankton in the western North Atlantic sampled on a mission from St. John's, Newfoundland to Halifax, Nova Scotia. (A) Map indicating 40 stations and bathymetric contours. (B) Cell abundance (10^3 cells ml^{-1}). C. Diversity $N_1 = e^{H'}$. (D) Evenness J'.

of a four-decade logarithmic dynamic range to span the light scatter and fluorescence of particular cell assemblages. When cells are too small, too big, too dim, or too bright to be properly accounted, estimated diversities will be inaccurate. It is possible to circumvent this problem by making two (or more) separate measurements of the assemblage: first with the detectors set at high sensitivities for the small, dim cells; then at low sensitivities for the large, bright cells. In this preliminary work, the cytometer detectors were set at only one configuration that was chosen to encompass as many cells as possible and proved adequate for most samples.

Cytometric diversity, as described here, refers to categories of phytoplankton that do not necessarily correspond to taxonomic groupings. Although some cytometric categories are easily matched to particular taxa, most notably *Prochlorococcus* and *Synechococcus* (Olson et al. 1993), many categories would simply be termed eukaryotic ultraphytoplankton with no further indication of taxonomic identity. The intensities at which a phytoplankter scatters light and emits pigment fluorescence depend on various factors including its taxonomic affiliation, its age (on which size depends), its physiological adaptive state (on which fluorescence depends), and others. Cytometric diversity differs most from species diversity in that the former indicates richness in physiological as well as genetic variations. In the extreme, cytometric diversity could be the same whether all variability was intraspecific (physiological) or interspecific (genetic). This view of diversity emphasizes the fact of oc-

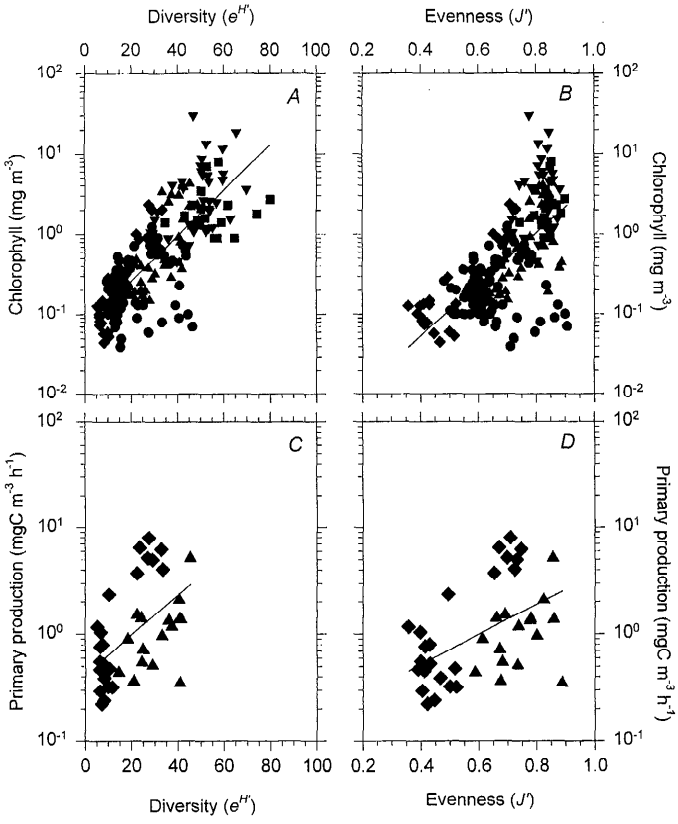

Fig. 5. Correlations between diversity ($e^{H'}$) and evenness J' versus the logarithms of phytoplankton chlorophyll biomass (CHL) and primary production (PP). (A) CHL versus $e^{H'}$ ($r = 0.80$, $n = 217$, $P < 0.01$). (B) CHL versus J' ($r = 0.69$, $n = 217$, $P < 0.01$). (C) PP versus $e^{H'}$ ($r = 0.53$, $n = 39$, $P < 0.01$). (D) PP versus J' ($r = 0.50$, $n = 39$, $P < 0.01$). Data from the time series in Bedford Basin (Fig. 1) were not included here; but inclusion would not have altered the statistical significance of CHL versus $e^{H'}$ ($r = 0.65$, $n = 343$, $P < 0.01$) or of CHL versus J' ($r = 0.63$, $n = 343$, $P < 0.01$). Different symbols refer to different study missions stated in the results.

cupation of photoautotrophic niches in the size-chlorophyll domain rather than the identity of the occupier. Yentsch and Cucci (1991) made the following intriguing observation regarding occupation in the joint size-chlorophyll domain: phytoplankton almost invariably, without reference to species composition, manifest only a band from the lower left (small, dim cells) to the upper right (large, bright cells) of flow cytograms. Ultimately, this distribution may due to biophysical constraints: small cells cannot physically contain a lot of pigment, and large cells cannot survive with too little pigment. However, the proximate factors that underly the realized apportionment of cells might be related to ecological interactions such as resource utilization, competition, and grazing. The description of cytometric signatures in terms of ecological diversity points to the yet unexplored possibility of studying ultraphytoplankton community structure using species abundance models (but *see* Venrick 1982) in addition to statistical models (Tokeshi 1993). Moreover, future cytometric descriptions could be extended beyond properties measured here; and the importance of cells could be rated by other criteria, for example the cellular rate of photosynthesis (Li 1994*b*).

Studies of cytometric diversity at various time and space scales (Figs. 1–4) indicate the responsiveness of indices of diversity and evenness to variations at those scales. In each one of those studies, it is possible to examine in detail the changes occurring week after week or station after station. However, a broader perspective was sought by seeking correlations within the pooled results in spite of different scales. The nature of relationships among the measures of diversity and evenness are a consequence of definition (Hill 1973), but the degree of data scatter indicates ecological variability. Thus by definition, N_1 and N_2 are directly related with N_1 always greater than N_2. However, samples with the same value of N_1 will have different values of N_2 depending on evenness. Given the data at hand and the instrument limitations described above, cytometric diversity of ultraphytoplankton appears to be characterized equally well by N_1 or N_2. In other words, samples of ultraphytoplankton having the same diversity do not differ very greatly in their evenness. It also appears that increased cytometric diversity is largely a reflection of increased evenness rather than richness (given by N_0). In other words, the ultraphytoplankton community becomes diverse by lessening the numerical dominance of some categories rather than by greatly increasing the number of other categories. Because flow cytometry gives a proper accounting of the large numbers of picoplankters, it can be said that diversity is low in oligotrophic waters, not because of low richness, but because of the extremely uneven apportionment of cells: *Prochlorococcus* and *Synechococcus* are highly dominant in numbers. However, there is a crucial need to re-assess this conclusion when cytometric diversity can be measured routinely on much larger volumes of samples (to detect rare cells) and over dynamic ranges that span the entire phytoplankton spectrum. Using microscopy, which allows a very refined categorization and which does not impose stringent limitations on sample volume, Venrick (1990) described highly diverse oligotrophic phytoplankton communities in which a large number of rare and very rare species persist over long periods.

The long-standing discussion of whether ecosystem functioning depends on ecological diversity has recently gained momentum in view of new experiments in grassland ecosystems (Tilman 1996; Tilman et al. 1996). Through direct experimentation, these authors documented a significant correlation between peak standing crop (their estimate of plant productivity) and the number of species seeded into plots. The conclusion that greater diversity led to greater productivity was supported by observations in native, undisturbed grassland that showed a similar relationship between total vascular plant cover and species richness. In this respect, the present observations of native, undisturbed phytoplankton communities also resulted in significant correlations between standing crop (i.e. chlorophyll biomass) and diversity, and also between primary productivity (i.e. rate of ^{14}C incorporation) and diversity. The large degree of scatter in these results (Fig. 5) is likely due in part to the many different scales at which the plankton were sampled. McGowan and Walker (1993) remarked that diversity patterns are most clearly delineated at large climatic scales where habitat fea-

tures are persistent and in situ processes predominate. On the other hand, at smaller scales such as mesoscale eddies, rings, and small ocean basins, diversity is much more dependent on the physical movement of water. In the grassland experiments, it was postulated that the diversity–productivity (or diversity–stability) relationships could be explained by a mechanism of compensatory competitive interaction. In essence, because species compete and because they differ in their susceptibility to disturbance, some species compensate for susceptible ones in the face of disturbances; in other words, species richness stabilizes community biomass (Tilman 1996). It remains to be considered whether phytoplankton communities are governed by similar principles. In the copepod (McGowan and Walker 1985) and phytoplankton (Venrick 1990) communities of the central North Pacific, the observational evidence does not indicate that comparable mechanisms are operative (McGowan and Walker 1993). Experimental work is lacking.

In summary, this work has explored the concept and methods of ecological diversity as applied to phytoplankton categorized by cytometric measurements related to size and chlorophyll content. Given the statistical result that higher biomass and higher production are related to higher diversity and evenness, there is the tantalizing prospect of integrating large-scale remotely sensed measures of bulk phytoplankton properties (biomass and production) with fine-scale measures of individual cells (diversity and evenness) using the models and principles of general community ecology.

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