THE IMPACT OF PROTO- AND METAZOOPLANKTON ON THE FATE OF ORGANIC CARBON IN CONTINENTAL OCEAN MARGINS

FINAL PROGRESS REPORT

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BY

SKIDAWAY INSTITUTE OF OCEANOGRAPHY
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COVERING THE PERIOD MAY 1992 - JULY 1995

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Final Report

Introduction

Three fates potentially consume primary production occurring on ocean margins: portions can be oxidized within the water column, portions can sediment to shelf/slope depots, and portions can be exported to the interior ocean. Zooplankton mediate all three of these processes and thus can alter the pathway and residence time of particulate organic carbon, depending on the size structure and composition of the zooplankton (and phytoplankton). (1) If the zooplankton community is dominated by protozoans then grazing rates and gross growth efficiencies will be high (Goldman *et al.*, 1985; Verity, 1985) but fecal matter will remain in suspension (Stoecker, 1984). Thus protozooplankton grazing would be a moderately large sink for primary production, as documented elsewhere (Watras *et al.*, 1985; Verity, 1986), but considerable suspended POC would follow the path of water into the Atlantic basin. Note that significant predation of protozoans by metazoans would reduce the importance of the latter transport of POC. (2) If thaliacea (doliolids, salps) are abundant, they most likely control phytoplankton because of the following characteristic population dynamics variables: (a) high growth rates of individuals, and high asexual reproduction rates (Heron and Benham, 1984, 1985; Paffenhöfer and Lee, 1987); and (b) high weight-specific feeding rates, estimated to amount to 430% $\cdot$ d^{-1} (Tsuda and Nemoto, 1992). These weight-specific ingestion rates are far above experimental rates which range from 48 to 199% (Mullin, 1983; Andersen, 1985; Deibel, 1985a). Lower values are attributed to inadequate methodology (Reinke, 1987) because the almost continuously swimming salps frequently encountered walls in small containers, leading to suppressed feeding behavior. High salp feeding rates cause high pellet production rates. Pellets from freshly-collected salps are compact and dense, resulting in high sinking rates (Bruland and Silver, 1981). Observations on the production of fluffy, slow-sinking fecal strings by salps and doliolids (Pomeroy and Deibel, 1980) were attributed to laboratory conditions which differ from those *in situ* (Bruland and Silver, 1981). The large contribution of salps to vertical flux of fecal material has been repeatedly observed (e.g. Iseki, 1981; Matsueda *et al.*, 1986). Therefore, in the presence of thaliacea a high percentage of the daily primary productivity would be grazed, and a large part deposited as fecal pellets onto shelf sediments. (3) If copepods are the most abundant zooplankton, then their relatively low weight-specific ingestion (e.g. Paffenhöfer, 1984) and growth rates (generation times of middle and outer shelf calanoids range from about 12 to 20 days, Paffenhöfer, unpubl. data), would result in a rather small impact on primary production. In this case, a larger fraction of phytoplankton carbon would be available for export into the open ocean.

To achieve our long-term goal of quantifying the role of proto- and metazooplankton in removing newly formed POC (primary production), we must accomplish two major component objectives: (a) determine plankton carbon biomass at relevant temporal and spatial scales; and (b) measure zooplankton carbon consumption rates and (for metazoan zooplankton) fecal pellet production. These measurements will specify the importance of different zooplankton groups as

consumers and transformers of phytoplankton carbon. During Phase I, we concentrated on methodological and technological developments prerequisite to an organized field program.

Specifically, we proposed to develop and test an optical zooplankton counter, and to fully enhance our color image analysis system. In addition, we proposed to evaluate a solid-phase enzyme-linked immunospot assay to quantify predation by metazoan zooplankton on protozoans; and to improve methodology to determine ingestion and growth rates of salps, and accompanying pellet production rates, under conditions which very closely resemble their environment. The image analyzer data provide insights on basic ecosystem parameters relevant to carbon flux from the continental ocean to the deep ocean. Together these approaches provide a powerful set of tools to probe food web relationships in greater detail, to increase the accuracy and speed of carbon biomass and rate measurements, and to enhance data collection and analysis.

Protozoan Studies

Progress during Phase I can be described from two perspectives: that toward projected goals, and progress in unexpected directions which enhances our potential contributions to Phase II field studies. The first two items below represent our original planned efforts; the remaining three subjects are related but unanticipated successes.

Biomass of Phytoplankton and Protozoan Zooplankton. Samples for measurement of plankton carbon biomass are initially fixed with gluteraldehyde (0.3% final concentration). They may be stored at 4°C for up to 24h or (preferably) filtered immediately. Separate slides are typically prepared for picoplankton (10-20 ml) and nano/microplankton (20 - 100 ml). Samples to be analyzed for picoplankton are stained with DAPI (5.0 µg/ml final concentration) for 4 minutes and filtered onto black 0.2 µm Nuclepore filters. Samples for nano/microplankton analyses are stained with DAPI as above, then momentarily stained with proflavine (5.0 µg/ml final concentration), and finally collected on black 0.8 µm Nuclepore filters. Filters are covered with a small drop of low-fluorescence immersion oil (Resolve) and a coverslip. They are either analyzed immediately or stored frozen. Proflavine and DAPI are ideal stains for image analysis studies due to their bright fluorescence.

Under the microscope, a new field is manually located, either randomly or on a transect, and focused on the video monitor visually. It is then digitized using frame-rate image averaging to reduce noise. Digitization with 16 frames averaged takes less than 2 seconds so fluorescence fading is avoided. Once the image is judged to be adequate either the whole image is analyzed or individual cell sub-images are saved for later analysis. The volume of sample filtered per slide is adjusted according to the populations we encounter to optimize the cell density on the slide for image analysis. It is generally preferable to have more cells per field for image analysis than visual enumeration. Since each field image is entirely scanned by the computer regardless of the number of cells it contains, it is more efficient for each image to contain as many cells as possible without excessive cell overlap. Our microscope gives us considerable flexibility in optimizing the analysis to different plankton communities. Two approaches are used depending upon the density of the particular cell population being analyzed.

For cells which are numerous per field and relatively uniform in brightness in a given sample, randomly chosen whole fields are analyzed. In this case all the cells in a field are segmented and measured automatically. This approach requires that a single threshold be used for all cells in the image, but is faster than automatically finding individual cell thresholds. Acquisition and analysis of an image with 40 cells takes about 4 s. For rare cells, transects of the slide will be scanned and individual cells will be isolated and identified by the operator interactively. Sub-images containing the individual cells are temporarily stored and analysis done overnight. The analysis procedure for these sub-images involves automatic threshold determination using the second derivative method (Sieracki et al., 1989), segmentation and cell measurement. A combination of these two approaches is used to optimize sample analysis for the particular populations we encounter. Cell measurement data and example images are archived on disk for later analysis. Individual cell biovolume measurements are converted to carbon biomass for intercomparison of trophic and size classes of the plankton community (Verity and Sieracki, 1993).

The imaging system, which was state-of-the-art in 1992, was composed of an Olympus BH-2 epifluorescence microscope with 100w mercury burner and superior dry/oil objectives from 10x-100x; a Dage-MTI B&W charge coupled device (CCD = a high-resolution, solid-state video frame rate camera); a Hitachi DK-7000 3-chip color CCD; a Sony RGB monitor; a 386-25 desktop computer housing a Matrox framegrabber and video board; and proprietary image and data analysis software (Analytical Imaging Concepts, Inc.). We worked with AIC to modify the source code on their generic software package (IM-3000) to optimize image digitization in true-color, background subtraction, image averaging, and contrast enhancement. We also successfully ported over to our system algorithms which automate threshold selection and which calculate cell volume from cell dimensions (Sieracki and Viles, 1989; Sieracki *et al.*, 1989).

During our OMP Phase I research, we improved our imaging system in several regards, most notably by interfacing a Ludl motorized stage and associated modular automation controller with the epifluorescence microscope. This permits the operator to scan transects of variable length across the plankton slides while the computer records the fraction of the surface area of the slide which has been examined. For abundant populations, the computer can randomly select individual locations on the slide. AIC is no longer in operation, but we have maintained a working relationship with its major software engineer and we continue to broaden our software to keep pace with changes in computer architecture. We modified the driver software so that the entire process (moving to a given location, focussing, opening an electronic shutter, grabbing an image, closing the shutter, and moving to a new location) is automated and computer-controlled. Our system now has a Pentium CPU; a second digitizing card, mounted piggyback on the original Matrox board, to perform image averaging and background subtraction in color and in real time; an electronic shutter interfaced to our software to minimize sample burnout by the high-intensity light source; a new Sony RGB monitor; and a state-of-the-art electronically cooled, integrating color CCD (Optronics). It is the most sophisticated and sensitive true-color image analysis system in use for plankton research that we are aware of. We also completed the process of empirically deriving regression models to convert cell volume to cell carbon and

nitrogen contents (Verity *et al.*, 1992; Verity and Sieracki, 1993)

The approach and technology described above would be used in the Phase II field program to determine the abundance, size distribution, composition, and biomass of plankton in samples from vertical profiles during shipboard transects and process studies, dilution experiments to measure herbivory by microzooplankton, and grazing experiments with copepods.

Predation on Microzooplankton. Among microzooplankton, ciliates are generally considered to be the most significant component which links pico- and nanoplankton to the larger metazoans which cannot efficiently feed on these tiny cells directly. Ciliates are often major herbivores, along with other nano- and microzooplankton (see Cruise Data below).

Molecular techniques can be particularly useful in predator-prey studies because of their extreme specificity and sensitivity (Powers *et al.*, 1990). Immunochemical detection of prey antigens in gut contents has already been successfully applied to euphausiid predation on anchovy larvae (Theilacker, 1988), and copepod predation on ciliates (Ohman, 1992). Given the significance of thaliaceans and ciliates in the southeastern U.S. ocean margin (and other regions), we proposed to investigate predation by thaliaceans on ciliates using a solid-phase enzyme-linked immunospot assay. We have preliminary data strongly suggestive of a significant predator-prey interaction (Paffenhöfer *et al.*, 1994). Because of the difficulty in culturing thaliaceans, and container-induced reduction in thaliacean feeding (e.g. Deibel, 1982), this trophic link is challenging to quantify in standard incubations. Moreover, the large size of thaliaceans (their gut contents can approximate the volume of an entire copepod) and ease of teasing away the gut are features particularly amenable to immunochemical approaches (Feller and Ferguson, 1988). There are, however, numerous problems which must be overcome, and we predicted that this was a high risk component of our overall research.

The immunological procedures we used were similar to those described elsewhere (Ohman, 1992) and will not be presented in detail here. The general steps include (1) preparation of antigen, which includes isolation and growth of ciliate clones in culture. The desired immunogen, which should be separated from other non-desired potential antigens, e.g. prey items, is then concentrated into pellets. (2) The antigen is injected (by a commercial immunization firm) in New Zealand white rabbits, which subsequently produce polyclonal antisera. Booster injections stimulate production of antibodies, which are harvested after a period of 60-90 days. The immunoglobulin G (IgG) fraction is then isolated from the crude serum and purified via ion exchange chromatography. (3) The primary antibody is reacted with a secondary antibody (goat anti-rabbit IgG) conjugated to alkaline phosphatase, and the antigen-antibody reaction is visualized colorimetrically on nitrocellulose using dot blots. Color development is proportional to antigen protein present. (4) The specificity of the antibody is evaluated by testing for cross-reactions with antigens from the original clone and related taxa (desired responses), and with other antigens which might co-occur with ciliates, e.g. bacteria, eucaryotic and procaryotic phytoplankton, and other micro-heterotrophs (undesirable). A calibration curve is prepared by serial dilution of the original immunogen, and controls for false positives are run using pre-immune serum. (5) The

utility of the antibody is tested in the lab by feeding ciliates of the original immunogenic clone to starved predators (thaliaceans). Ingestion of ciliates is calculated from their disappearance over several hours, after which thaliaceans are frozen in liquid nitrogen. Their gut contents are subsequently teased apart on nitrocellulose and incubated with antibodies as above. Gut residence time of the antigens is determined, as well as stability in immunoreactivity of antigens. (6) Finally, individual thaliaceans from natural communities are sorted and frozen at sea, and their guts are tested for presence of ciliate proteins upon return to the lab, as in (5). The type of field samples and procedures required to estimate predation rate by thaliaceans on ciliates according to this approach are given in the Renewal Proposal.

This component of our research has progressed in the form of punctuated equilibria, i.e. progress and stasis. Due to our expertise in culturing protozooplankton (Verity 1985, 1986, 1991), clones of several ciliate species were established efficiently. However, the mass requirement for successful antibody stimulation (e.g. 100-1000ug protein/ml concentrate) required huge volumes of cultured antigen (ciliates) when they were grown at standard concentrations of 5-50/ml. To obviate this logistical issue, we chose to focus on bacterivorous species and were successful in culturing three taxa at densities of 500-1000/ml. These were concentrated by centrifugation, and polyclonal antisera directed against whole cell homogenates and cell surface antigens were produced according to the procedures outlined above.

Our ciliate antibodies showed strong positive responses with antigens from the original clones plus those of other oligotrich ciliate taxa, as desired. The most significant problem has been unexpectedly large cross-reactions between the antibodies and bacterial antigens. That is, the colorimetric antigen-antibody test cannot, at present, distinguish between bacteria and ciliate proteins. This likely reflects the difficulty of completely separating ciliates from their bacterial prey at the time of culture harvest. We are trying two approaches to negate this problem: gradient centrifugation to better separate the two potential antigens, and new culture techniques to grow ciliates on larger phytoplankton prey (which can be more easily separated from ciliates at time of harvest) rather than bacteria. We believe that we will have resolved this problem by the end of the grant period; if not, we will utilize other methods during the Hatteras field studies to estimate the transfer of protozooplankton carbon to higher trophic levels (see below).

Concurrent with the immunochemical studies, we have also pursued indirect estimates of predation. These take the form of more traditional experiments in which natural plankton communities are incubated with and without added metazooplankton (see Metazoan Studies section for experimental details). But instead of using chlorophyll or particle volume as a proxy for grazing, samples are analyzed using the imaging system previously described. This approach allows us to specify which components (by taxa, size class, and trophic status) of the entire autotrophic and heterotrophic community are receiving unusually strong or weak grazing/predation pressure. We have conducted these experiments in the lab at SkIO and aboard the R/V Columbus Iselin off Cape Hatteras: an example of the latter is shown in Table 2 and is discussed in the Metazoan Studies section of this report. This experimental approach is time-consuming but provides more insight into plankton community interactions and carbon transfer than any other approach available.

Cruise Data. Three cruises to the Hatteras ocean margin, which focussed on physical and biological parameters, were conducted during Phase I: May 1993, April and June 1994. Their major purpose was to provide background data on hydrography and distributions of major nutrients, gases, dissolved inorganic carbon, dissolved and particulate organic carbon, and important biological components and processes. We participated directly in all these efforts, which yielded valuable insights into temporal and spatial patterns in plankton carbon distributions, composition, size structure, and coupling to physical circulation processes. But the time dedicated to sea and subsequent analyses was substantial and did detract from the intensity of effort which was directed at our planned Phase I objectives.

During May 10-16, 1993, the R/V Gyre conducted three transects across the shelf just north of Cape Hatteras, which were run sequentially, with ca. 50 km between transects. Each transect required ca. 24h to complete. Twelve stations were spaced on each transect at intervals of 6 km, with the innermost station coinciding approximately with the 20m isobath and the outer stations exceeding the 500m isobath. Samples for vertical profiles were collected using CTD rosettes, with typically 5m between samples at stations <30m deep, 10m between samples at stations 30-60m deep, and 15m between samples at stations >60m deep. The following measurements were included in virtually all of the above samples: salinity, temperature, and pressure (depth) via CTD; phytoplankton chl a via fluorometry; O_2 concentration via Winkler titration; and plankton composition, abundance, size distribution, and biomass via microscopy. At selected stations, primary production was measured via C14 incubations, and metazooplankton abundance and biomass via stratified net sampling.

From these data, detailed carbon budgets were estimated in the cross-shelf and alongshore directions. These indicated that inner shelf waters contained ca. 50% more living POC than outer shelf waters. The relative importance of large phytoplankton and grazers decreased with distance offshore, and they were replaced by photosynthetic nanoplankton and nano/microzooplankton. Even greater changes in living POC occurred in the alongshore direction than cross-shelf, primarily due to the dramatic reduction in large phytoplankton in southern waters. The ratio of heterotrophic:autotrophic POC increased from 38% in northern waters to 137% in southern waters, suggesting that phytoplankton carbon was being converted into consumer carbon as shelf waters advected south. Estimated carbon ingestion by zooplankton was ca. 2-4 $gC/m^2/day$, and the patterns of relative importance of large and small zooplankton were consistent with changes in the size structure of the phytoplankton community.

To the extent that declines in diatoms were due to grazing by larger crustacean zooplankton, whose fecal material typically sediments rapidly to depth, then considerable diatom carbon may have remained on shelf/slope sediments. However, the dominant primary producers on all three transects were apparently small photosynthetic nanoplankton, which supported an active protozooplankton community, whose fecal products generally remain in suspension. Moreover, fecal pellets formed by copepods feeding on nanoplankton sink significantly more slowly than those of copepods feeding on diatoms. Since most of the metazooplankton biomass on the Hatteras shelf in May was in the form of juvenile and adults small copepods (e.g. *Oithona, Temora*), which can feed upon nanoplankton and whose fecal pellets also remain in suspension in shelf

environments, much of the non-diatomaceous POC may have been exported with shelf waters as they were entrained offshore at Cape Hatteras.

The details of these data are given in Verity *et al.* (in press) which is attached to this report. An additional manuscript(s) is anticipated from the results of the 1994 cruises, analyses from which are underway.

Community Respiration. A major focus of the shipboard and moored sensor approach to meeting OMP objectives in Phase II is to use changes in dissolved oxygen as a proxy for patterns in uptake of carbon dioxide into organic carbon (photosynthesis) and its subsequent regeneration (respiration). The moored box volume array of O_2 sensors is expected to provide detailed meter scale (z-dimension) and kilometer scale (x- and y-scale) resolution of diel patterns in O_2 production and consumption. We propose to help provide a mechanistic interpretation of those patterns by measuring changes in oxygen in the control bottles which are part of zooplankton grazing experiments (described in the Metazoan Studies section). These control bottles, which contain unmodified natural plankton assemblages, will be incubated in flowing seawater under dim natural irradiances. Changes in oxygen during the light period of the incubation will not simulate those occurring in situ, but changes during the dark period should reflect processes occurring in situ. It is this latter net community respiration which interests us.

Two test experiments were conducted on the June 1994 OMP scoping cruise. Basically, extra control bottles were prepared at the initiation of each experiment and these were treated identically to the others: bottles with and without added zooplankton were incubated on a ferris wheel rotating in a bath of flowing seawater. To measure respiration, control bottles were sacrificed at the beginning of darkness and again at sunrise. The O_2 concentrations at those times were determined by Brookhaven National Lab personnel using Winkler titration (WOCE Protocol precision +/- 0.5 µmole/kg). In both cases (Table 1), significant measurable decreases in O2 occurred during the 9hr dark period, with rates of 0.8 µmole/kg/hr. Both communities had similar carbon biomasses of bacteria, nano- and microzooplankton, which are considered to be the primary contributors to community respiration. If sufficient experiments are conducted in Phase II, it may prove possible to construct multiple regression models to predict respiration from microbial carbon, chl *a*, and temperature. Given these rates and analytical sensitivities, future incubations could be considerably shorter.

Detrital Carbon. One of the biggest uncertainties in phytoplankton ecology remains that associated with conversion from unequivocal estimates of algal biomass, i.e. chlorophyll, to units of greater geochemical significance, i.e. carbon. This conversion factor (C:Chl*a*) varies with algal physiological state and recent history, and is therefore not constant. Nor can phytoplankton carbon be directly measured due to the confounding effects of non-living POC, e.g. detritus. It is not a trivial issue, because often detrital carbon exceeds that of living POC, as will be shown below. Unfortunately, no satisfactory techniques have been developed which permit direct measurement of the carbon content of detritus. Banse (1977) states "it would be desirable to obtain a better supported conversion factor from .. organic volume to

carbon content .... new field studies should focus on (microscopically derived detrital carbon)" (pp. 209-210). This objective has yet to be met.

As a by-product of studies outside of OMP, we have adapted 3D visualization techniques to the study of plankton. Because the concepts behind this approach are new, a brief overview follows here. In medicine, 3D visualization is publicly recognizable as cat-scans or tomography, typically done on objects the size of the human body. With plankton, however, the object to be viewed is the size of a red blood cell within the human body, so there is a tremendous scaling problem. Now, consider that a camera on a microscope looking down on a plankton cell does not use focussing lenses. By analogy, it would be as though your eyes only focussed on objects which are a constant distance away from you: as if everything else closer or further away was blurry or out-of-focus. So the microscope stage which holds the specimen (= plankton cell, in this case) can be moved closer or further from the camera in order to focus on it. Now, consider further that the plane of focus is very thin, and the other sections of the cell (above and below the plane in focus) are blurry.

The trick in 3D imaging is to remove that out-of-focus light. There are two ways. One is to allow only the in-focus light to reach the camera, by forcing it to pass through a very tiny pinhole which can be set to stop passage of the out-of-focus light. The trouble is that this approach reduces the light coming from the object by 99%, so that a serious excitation light source is required. This is typically a scanning laser, which has three negative attributes: (1) it is very high energy and quenches autofluorescence; (2) the illuminating beam and return light aperture must be precisely aligned, and the scan must be uniform; and (3) it is therefore quite expensive. The other approach, which we use in our lab, depends upon the theoretical distribution of light energy around a point source (= point spread function), which is understood quite well and described mathematically (Gibson and Lanni, 1990; Hiraoka *et al.*, 1990). Thus we can computationally remove the out-of-focus haze, obviating the need for lasers and allowing samples to be imaged on standard microscopes.

Our approach is to selectively label detritus in a plankton sample using a fluorescent stain, and then concentrate the detritus on a black filter as described previously for determination of plankton carbon. Presently we use DAPI and propidium iodide in conjunction with modified UV excitation filters from Olympus, so that detritus stains orange, distinct from the stain-induced fluorescence of cytoplasm and nuclear material, and the autofluorescence from pigments; thus, detritus and plankton cells are visually distinct (Williams *et al.*, in press). This has the advantage of allowing measurement of both living and non-living POC on the same samples. Thus sample preparation at sea does not require any additional time nor personnel. We have also been successful using colorimetric stains (e.g. alcian blue) in settled samples. We have calibrated both stains against direct POC measurements using artificial detritus derived from aging phytoplankton cultures.

In the lab, the sample is placed on an Olympus microscope equipped with a z-axis motorized stage slaved to a desktop computer (Verity *et al.*, subm.). The computer drives the microscope so that it automatically focuses on the top of a blob of detritus, electronically opens a shutter, the camera grabs an image (= an "optical slice") in 1/30 sec, the shutter closes, the motorized microscope stage moves down an exact prescribed amount so that a new "slice" is in focus, and the procedure is repeated until

the bottom of the detritus is reached. The distance between slices is precisely controlled and is very small, typically 0.5 microns, which is less than the diameter of a bacteria cell. Then we apply the algorithm which removes the blur cause by the out-of-focus light. The resulting sharply focussed optical slices are fed into a sophisticated graphics computer (Silicon Graphics Indigo 2), aboard which a high-end volume rendering software package (VoxelView) interpolates between the image planes and draws the "blob" of detritus in 3D. One of the significant features of VoxelView is that it can calculate the number of voxels (a voxel is a 3D picture element, or pixel) in a 3D volume, or any subvolume thereof. A typical 512x480 pixel image containing 60 slices is thus composed of a total of 14.7 million voxels. VoxelView calculates the number of those voxels which correspond to detritus. Since the exact size of each voxel in x-, y-, and z-dimensions is known (e.g. 0.26 x 0.21 x 0.50um at 500x magnification), the volume of detritus in μm^3 can be calculated. This has been confirmed using commercial fluorescent microspheres of known size.

The volume of detritus is of little use unless it can be converted to carbon units. There are two methods. One is to measure detrital carbon directly by allowing algal cultures to decay over time (weeks to one year) and directly measuring the total POC by CHN analyses. Such an experiment is presently being conducted, but this "detritus" bears an unknown chemical fidelity to that found in nature. The other approach is to measure total POC by CHN, measure autotrophic and heterotrophic plankton POC by image analysis (as described in the section on Biomass), and then calculate the difference as detrital POC. Detrital carbon is then regressed upon detrital volume. An example of this approach applied to samples from the May 1993 Gyre cruise is illustrated in Figure 1 (Total POC data were kindly provided by OMP PIs at Brookhaven). The linear regression model suggests that detrital carbon could be estimated from detrital volume as 0.23 pg C per cubic micrometer of detrital volume. This factor may well vary over large spatial and long temporal scales, but it has yet to be quantitatively evaluated in any area due to prior limitations in measuring detrital volume. It can be calculated for any sample in which total POC, plankton carbon, and detrital volume are all measured. Comparing detrital C : total C to total C : chl *a* (again kindly provided by BNL scientists) suggests that detritus is the most important carbon depot in high C:Chl*a* waters (Figure 2). With sufficient data, it may be possible to derive predictive regression models to estimate detrital carbon from C:Chl *a* or Chl *a* directly (Figure 3).

Metazoan Studies

Optical Zooplankton Counter (Critter Cam®). To obtain estimates of metazooplankton abundance we proposed two approaches: (1) sample a moving water mass repeatedly with our multiple net system (Lagrangian) during a cruise; since this can only be conducted during part of the year we wanted to add (2) a Eulerian approach to observe between cruises i.e, employ optical zooplankton counters which would be deployed at different sites at 3 different depths on current meter arrays.

These zooplankton counters whose design was completed by mid 1991 could not be manufactured for use at sea until late spring of 1994 when we received our first seagoing Critter Cam® (Strickler, 1985). This instrument will be tested and operated

at sea during the remaining months of Phase I.

In the meantime, however, we assembled and operated a Critter Cam® in the laboratory to study zooplankton recognition and behavior. With a resolution between 5 and 30 microns, depending on magnification, copepod species can be easily identified using morphology and swimming behavior of copepodid stages and adults. Even nauplii of related species can be distinguished (*Temora stylifera* vs. *T. turbinata*). In addition, we utilized this instrument to determine feeding rates of individual copepods on larger phytoplankton cells (see enclosed manuscript). Continuous observations for 30 to 60 minutes of single adult females of *Paracalanus aculeatus* allowed quantification of particle capture, ingestion and rejection in relation to food concentration, and also fecal pellet production, and resulted in an assessment of interindividual variability of feeding and associated processes.

Rate Measurements on Thaliacea (Tunicata). Among thaliacea, doliolids occur during most of the year in ocean margins off the southeastern U.S.A., frequently being among the dominant zooplankton taxa (Spring: Deibel 1985b, Summer: Paffenhöfer and Lee, 1987, Winter: Paffenhöfer *et al.*, 1995). Our preliminary data from Cape Hatteras show their importance there as well (Fig. 10). Since rate measurements (feeding, growth and reproduction) on these animals have been difficult to accomplish, partly because of their sensitivity to walls and surfaces, we focussed on improving culture conditions and succeeded in rearing *Dolioletta gegenbauri* through two complete generations. Generation time at 20°C was on average 29 days at low concentrations (ca. 30 µg C · l^{-1}) of phytoplankton. Feeding rates (volume swept clear · $zooid^{-1}$ · day^{-1}) covered three orders of magnitude from early oozooids to near fully-grown gonozooids (Fig. 4), and increased linearly in a log-log plot. Phorozooids reproduced asexually (budding gonozooids) for up to 20 days at rates between 9.2 to 13.7 gonozooids released · $phorozooid^{-1}$ · day^{-1} (n = 5), with a maximum of 252 gonozooids per phorozooid during its lifetime. Gonozooids produce sexually oozooids which start producing trophozooids by the time they reach a length of 3 mm. At a length of 4 mm, an oozooid has a chain of 3 mm length to which 4 trophozooids are fastened. By that time the oozooid stops feeding and becomes a nurse, which is solely needed for locomotion and is nourished by the trophozooids. By the time the nurse is ca. 12 mm long, its chain is ca. 35 mm long with 50 - 60 trophozooids, and the first phorozooids are released which are ca. 1 mm long. All experiments were conducted in volumes ranging from 2 to 6 liters at 20 °C.

Over a range of naturally occurring concentrations of particulate matter (0.16 to 1.10 mm^3 · l^{-1}) ingestion rates increased evenly with increasing weight of doliolids. This is in contrast to planktonic copepods the oldest stages of which do not ingest amounts as high as earlier copepodid stages per unit weight. Partly because of the largely unselective, non-time consuming food uptake of doliolids we expect for higher feeding impact of doliolids (per unit weight) than copepods at high environmental food concentrations which are repeatedly observed in ocean margins. Their abundant presence as observed earlier (Paffenhöfer et al. 1995) should have a profound effect on the fate of particulate organic carbon.

Results from Pilot Cruises. Since oceanographic data about the OMP study region is scarce, it was decided to conduct pilot cruises during different times of the year to improve the design of the major field study. Late March - early April was the time prior to seasonal stratification, early May when stratification had just started, and late June when stratification was pronounced. Sampling was conducted with a Multiple Net System (MNS) which could be operated with up to 6 nets consecutively. This allowed us to sample various depth strata consecutively. During 1993 we operated with 73 µm mesh, and in 1994 with 105 µm mesh. The MNS was equipped with a flowmeter, a depth and a temperature sensor the data of which were continuously observed on deck, and recorded on a PC.

May 1993. We operated, as planned, between Cape Hatteras and Chesapeake Bay, conducting 3 transects from nearshore to ~1000 m isobath at 35°30′, 36°00′ and 36°30′ N. Metazooplankton was dominated numerically and in weight by small copepods of the genera *Paracalanus, Oithona* and *Temora*, and occasionally *Centropages*. Utilizing feeding rate data from the literature and unpublished data, we estimated the percentage of the phytoplankton standing stock which was daily ingested by the metazooplankton (Figures 5 and 6). The general findings were that these percentages decreased from nearshore to offshore, and from south to north, being mainly a function of zooplankton abundance. In the nearshore environment the metazooplankton appeared to be able to ingest repeatedly near 100% of the phytoplankton standing stock whereas offshore it was only a fraction of that.

Late March - early April 1994. During this study period cold shelf water characterized the northernmost transect and warm South Atlantic Bight water occurred over the southernmost transect (Fig. 7). Concentrations of copepods, in response to the earlier spring bloom, were very high at 36°30′ N, surpassing 20,000 *Oithona* $\cdot m^{-3}$, and 5,000 *Centropages* $\cdot m^{-3}$ across much of the shelf (Figures 8 and 9). Concentrations were much lower in the warmer water at 35°30′ N (Figures 8 and 9) which had considerable abundances of subtropical zooplankton including salps and doliolids (Fig. 10). The inner and middle part of the transect at 36°00′ N was dominated by water from the Chesapeake Bay. Overall, the copepod genera *Pseudocalanus* and *Temora* contributed considerably to the high abundance of copepods at 36°00′ N and 36°30′ N, surpassing abundances observed during early May 1993. Estimates of overall phytoplankton consumption will be made after size distributions of copepods have been completed. It is likely, however, that the high abundances of copepods which repeatedly surpassed 40,000 $\cdot m^{-3}$, resulted in a significant consumption of phytoplankton carbon.

Late June 1994. By now the water column was stratified and dominated by Gulf Stream and South Atlantic Bight water. A qualitative analysis of the metazooplankton samples showed a dominance of subtropical taxa including tunicates (doliolids and salps) and copepods. These samples will be analyzed during the remaining months of Phase I.

In addition to determining abundance and distribution of metazooplankton, we quantified with advanced methodology the consumption of various types of food particles by abundant calanoid copepods. Copepods were collected in tows lasting less than 3 minutes while the ship was hardly moving. After diluting the codend sample experimental animals, being mostly late copepodid stages, were separated within 15

minutes and placed in the same type of water in which they would be later incubated. This water originated from the depth stratum where particle concentrations were highest. Usually between 6 to 12 copepodid stages IV of *Eucalanus pileatus* · l^{-1} were placed in 1900 ml experimental jars filled with the abovementioned water and ~ 2 µg · at · l^{-1} of nutrients. Incubation took place for 22 - 24 h in a light-dark cycle on a Ferris Wheel at 0.2 r.p.m. kept at the environmental temperature of 21.5°C. Two control jars (no metazoa) were treated the same way. Samples for analysis of food abundance were taken at the beginning and end of the experimental period, and analyzed, as presented earlier (Biomass of Phytoplankton and Protozoan Zooplankton), for various taxonomic and function groups of autotrophic and heterotrophic cells. Prior to the start of each experiment, the separated copepods, which had been in experimental water, were briefly checked for gut contents and appendage damage. At the end of each experiment, we first took samples for food concentration and then enumerated the surviving copepods, checked their escape performance and gut fullness coarsely, measured their cephalothorax length and then dried them at 60°C, to eventually determine their ash-free dry weight. In only one of 12 experiments did one copepod die. Nearly all animals escaped vigorously.

In an experimental series on 27-28 June 1994, copepodid stages IV and V of *Eucalanus pileatus* ingested between 55 and 61% of their body carbon per day (Table 2). From 30 June to 1 July 1994 copepodid stages III to V ingested between 67 and 82% of their body carbon per day at 21.5°C. At similar carbon concentrations and taxonomical composition of food particles the slightly younger copepodids ingested a higher fraction of their body carbon than their older relatives. This had been previously observed for other calanoid species (Paffenhöfer 1971, 1984). Several advantages of these feeding experiments should be mentioned:

(a) The composition of living potential food particles and the food particles removed by the metazooplankton is thoroughly specified, which permits quantification of omnivorous feeding, as compared to previously used methods which could only be utilized to quantify herbivory (gut fluorescence method, Mackas and Bohrer, 1976; isotope methods, White and Roman, 1991).

(b) The composition and condition of abundant zooplankton is readily determined; numerous feeding experiments can be set up within 60 to 90 minutes, allowing for variability estimates between samples and for inclusion of several species of zooplankton in those experiments.

(c) The procedures of collecting zooplankton, particularly copepods, are gentle and do not damage the copepods at winds ≤ 15 kn.

These results have been submitted recently as a manuscript (Appendices).

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	Living POC (µgC/*l*)	
Plankton	Expt 2 (6/27-28)	Expt 4 (6/30-7/1)
Autotrophs		
Pnano	32	40
Pdino	3	2
Ceratia	12	8
Diatoms	78	70
Total	125	120
Heterotrophs		
Bacteria	28	33
Hnano	28	24
Hdino	7	4
Ciliates	14	19
Total	77	80
Grand Total	202	200
Resp (μMO_2/kg/hr)	0.77	0.78

Table 1. The mean carbon content of the major plankton groups in the three control bottles from two grazing/predation experiments conducted at Cape Hatteras aboard R/V Columbus Iselin. See text for details of experimental design. Samples represent the mean concentrations during the incubations, determined by image analysis of initial and final samples. Respiration rates were measured by Winkler titration and represent the mean changes in duplicate control bottles during the 10 hour (6/27-28) and 11 hour (6/30-7/1) dark period of incubation.

Food Type	Cell Volume (μm^3)	Mean Cell Concentration ($\mu g\ C \cdot l^{-1}$)	Ingestion Rate ($\mu gC \cdot l \cdot h^{-1}$) Experiment A	B	C
Bacteria	1.0×10^{-2}		--	--	--
Pnano	2.1×10^{2}		--	--	--
Hnano	1.6×10^{2}		--	--	--
Heterotrophic Dinoflagellates	4.6×10^{2}	7	0.14	0.20	0.09
Ciliates	1.3×10^{3}	14	0.62	0.80	0.42
Ceratia	1.6×10^{4}	12	--	--	--
Diatoms	1.8×10^{4}	78	2.24	3.11	1.62
Total		111	3.00	4.11	2.13
Number and stage of *E. pileatus*			17 CIV/V	24 CIV/V	11 CIV/V
Total copepod carbon per jar (μg)			248.2	304.8	162.8
Consumption ($\mu g\ C \cdot \mu g\ C^{-1}$ of Copepod $\cdot 24\ h^{-1}$)			0.55	0.61	0.59

Table 2. Feeding experiments with *Eucalanus pileatus* from 27 to 28 June 1994 at 21.5°C.

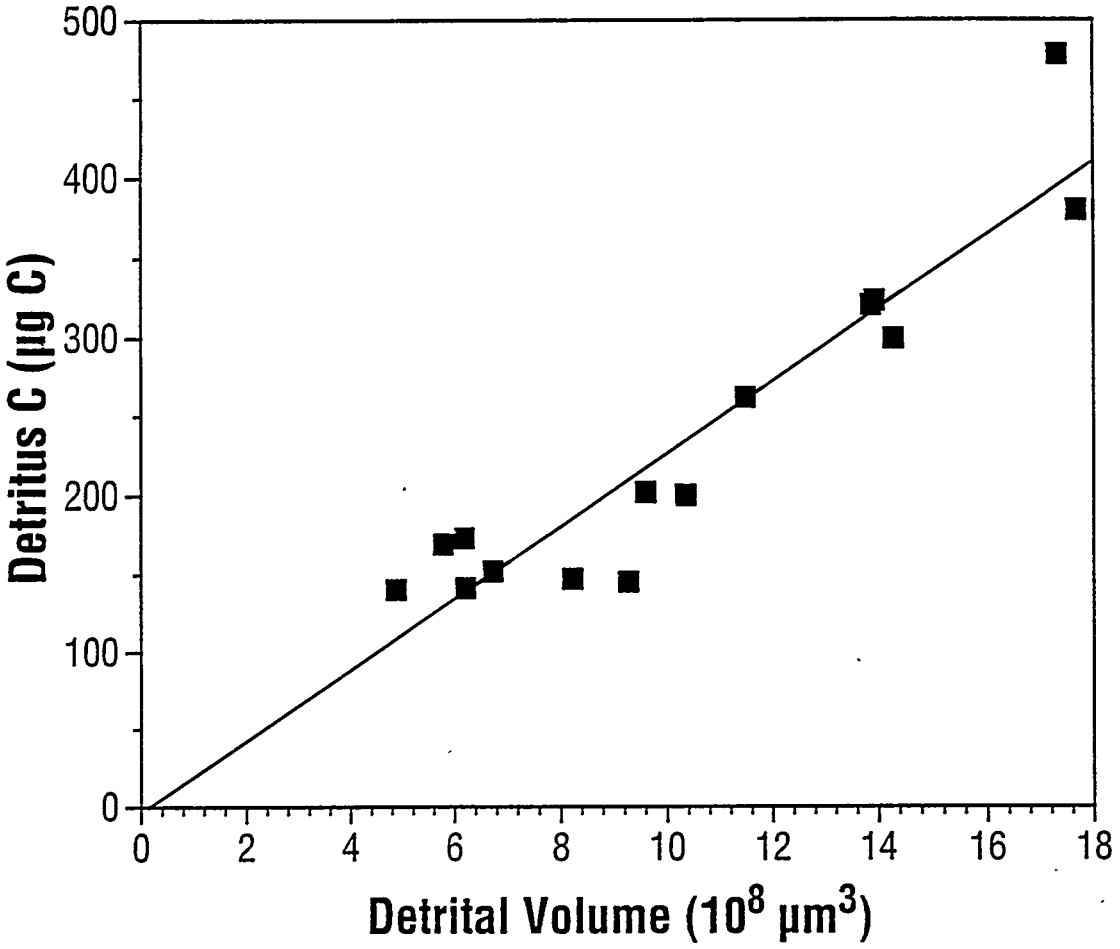

Figure 1. Relationship between volume of detrital particles measured in 3D and carbon content of detritus estimated from chemical and microscopic measurements. Samples were from two vertical profiles in continental shelf waters off Cape Hatteras in May 1993. Model I linear regression (y=ax+b): detrital carbon (µg/l) = 23.0 (detrital volume, 10^6 µm^3) - 3.6, $r^2 = 0.87$, n=15.

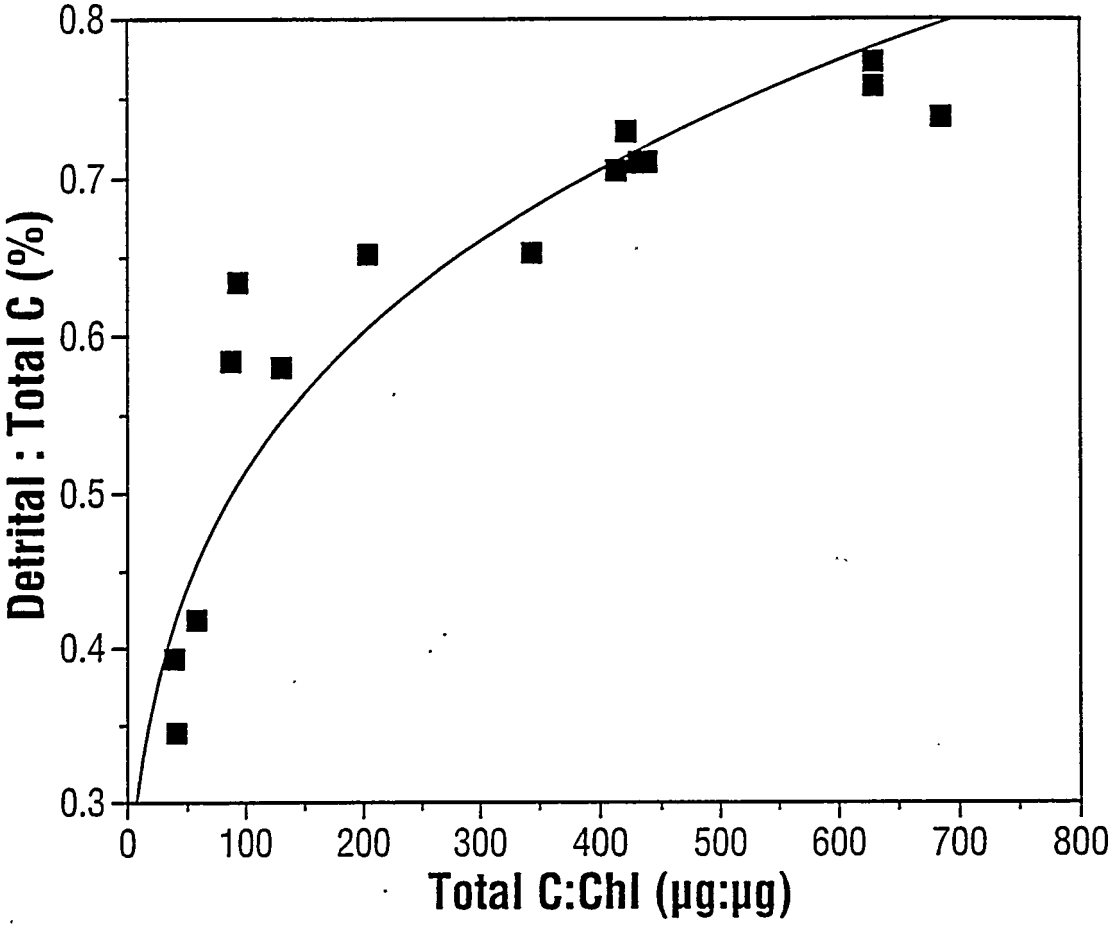

Figure 2. Relationship between detrital carbon as a percent of total POC, and the ratio of total POC to chl a, in the samples decribed in Fig. 2. Detrital:Total Carbon (%) = 0.29 Log (POC:Chl a, ug:ug) - 0.005, r^2 = 0.89, n = 15.

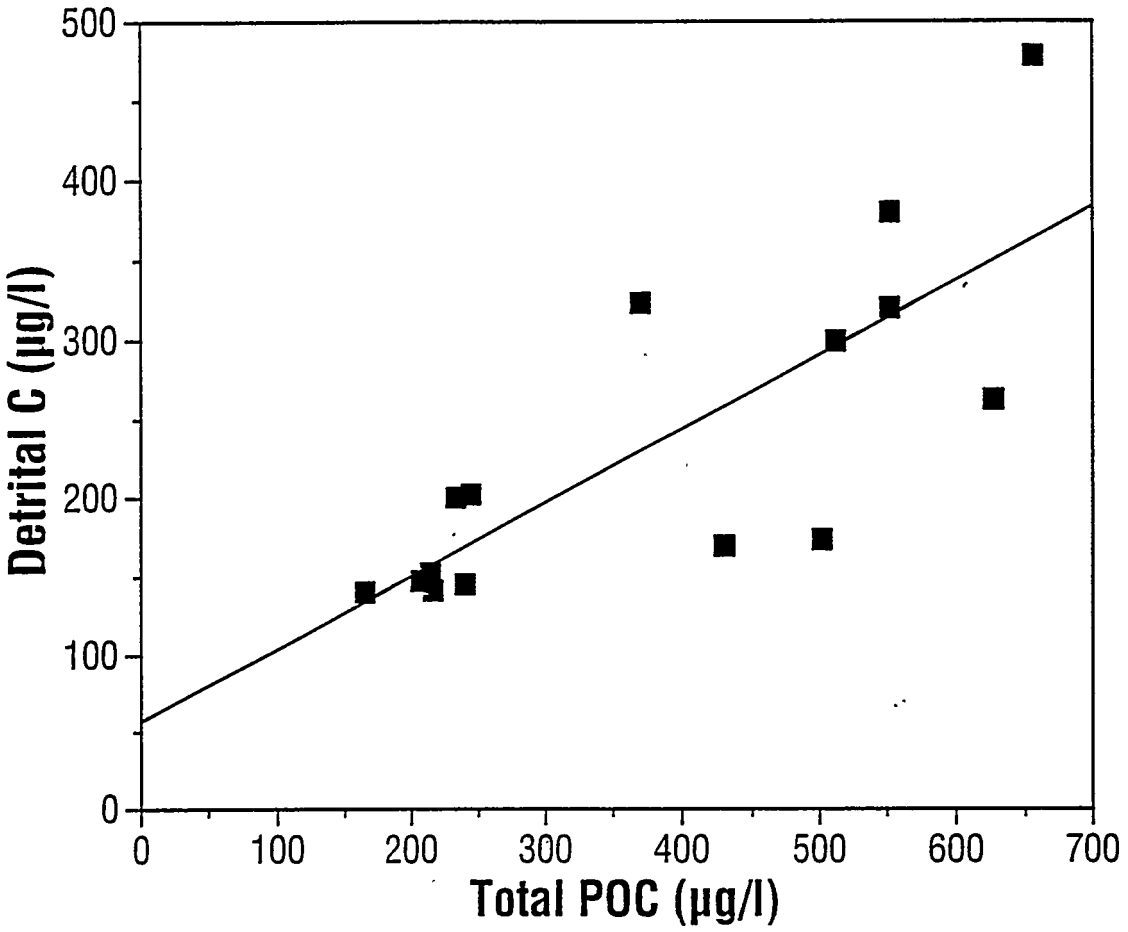

Figure 3. Relationship between detrital carbon and total POC for samples described in Fig. 2. Model I linear regression: detrital carbon (µg/l) = 0.47 (total POC, µg/l) + 57.1, $r^2 = 0.61$, n = 15.

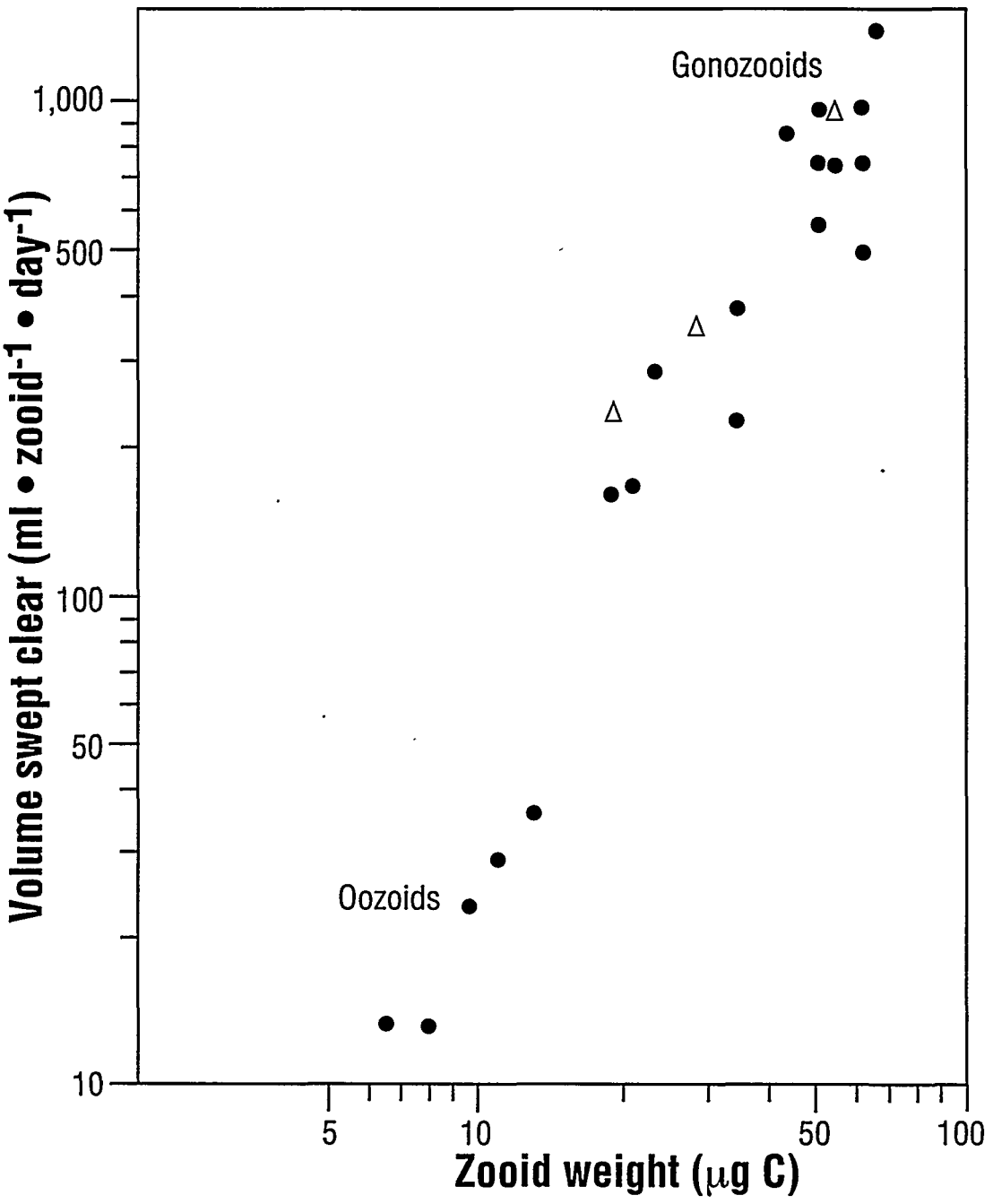

Figure 4. Clearance rates of oozooids and gonozooids of *Dolioletta gegenbauri* (Tunicata, Thaliacea) feeding on the diatom *Thalassiosira weissflogii* at ~ 20 µg C • l^{-1} at 20° C.

APPENDIX

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Rates of ingestion and their variability between individual calanoid copepods: direct observations

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Abstract. The goals of this study were to determine rates of ingestion and fecal pellet release, and their variability, for individual planktonic copepods over extended periods of time (>20 min). Ingestions and rejections of individual cells of the diatom *Thalassiosira eccentrica* by adult females of the calanoid *Paracalanus aculeatus* were directly quantified by observing individual copepods continuously at cell concentrations ranging from 0.1 to 1.2 $mm^3 l^{-1}$. Average ingestion rates increased with increasing food concentration, but were not significantly different between 0.3 and 1.0 $mm^3 l^{-1}$ (9.8 and 32.7 $\mu g C l^{-1}$) of *T.eccentrica*. Rates of cell rejections were low and similar at 0.1 and 0.3, but were significantly higher at 1.0 $mm^3 l^{-1}$. The coefficients of variation for average ingestion rates of individual copepods hardly differed between food concentrations, ranging from 17 to 22%, and were close to those for average fecal pellet release intervals which ranged from 15 to 21%. A comparison between individuals at each food concentration found no significant differences at 1.0; at 0.1 and 0.3 $mm^3 l^{-1}$, respectively, ingestion rates of four out of five females did not differ significantly from each other. Average intervals between fecal pellet releases were similar at 0.3 and 1.0 $mm^3 l^{-1}$ of *T.eccentrica*, but significantly longer at 0.1 $mm^3 l^{-1}$. Fecal pellet release intervals between individuals were significantly different at each food concentration; these significant differences were attributed to rather narrow ranges of pellet release intervals of each individual female. Potential sources/causes of variability in the sizes and rates of copepods in the ocean are evaluated.

Introduction

To exist and persist in an almost continuously food-limited and hostile environment, copepods have to find and capture food as well as avoid predators. These topics have been addressed repeatedly over decades in numerous publications (for references, see Ohman, 1988; Price, 1988). Despite these efforts, our understanding of the breadth of copepod behavioral ranges, the degrees of variability, and their causes, has advanced at a modest pace. This can partly be explained by the scarcity of interdisciplinary research, by the emphasis on larger scale (spatial and temporal) studies in the ocean, and by the lack of faith that behavioral observations would result in major advances in knowledge of copepod performances, i.e. emphasizing holistic and de-emphasizing mechanistic approaches.

The significance of observing freely moving zooplankters over time in larger volumes of water and eventually *in situ* has already been pointed out (e.g. Omori and Hamner, 1982; Marine Zooplankton Colloquium 1, 1989) and the importance of observations on a copepod's temporal and spatial scales has been demonstrated (e.g. Price *et al.*, 1983). After short-term observations (several seconds) of restrained and free-swimming copepods (Strickler, 1982; Paffenhöfer and Lewis, 1989), we took the next step by following free-swimming copepods continuously for extended periods on their activity scales of micrometers and milliseconds. We

intended to quantify ingestion and rejection of food particles by individuals as well as fecal pellet release frequency in relation to food concentration, and to improve our understanding of the interindividual variability of those variables.

Method

Adult fertilized females of the calanoid copepod *Paracalanus aculeatus*, which occurs abundantly on the middle and outer shelf off the southeastern USA (Bowman, 1971), were observed at 20°C in darkness. These animals were reared from hatching to adulthood at 0.1–0.3 $mm^3\ l^{-1}$ each of the flagellate *Rhodomonas* sp. (~300 μm^3 cell volume, ~174 $\mu g\ C\ mm^{-3}$) and the dinoflagellate *Gymnodinium nelsoni* (~25 000–45 000 μm^3, ~130 $\mu g\ C\ mm^{-3}$) in volumes of 960 and 1920 ml on a Ferris wheel rotating at 0.2 r.p.m. at 14 h light/10 h darkness. Within 1–2 days of reaching adulthood and being fertilized, the females were placed in 1920 ml jars and pre-conditioned at the respective food concentrations near 0.1, 0.3 and 1.0 $mm^3\ l^{-1}$ (3.3, 9.8 and 32.7 $\mu g\ C\ l^{-1}$, respectively; Strathmann, 1967) of the diatom *Thalassiosira eccentrica* (~20 000–35 000 μm^3) for 24–48 h. One to three females were then gently placed into cuvettes filled with 1000 or 3000 ml of copepod preconditioned filtered seawater with the respective concentration of *T.eccentrica.* The activity of individual females was videotaped on S-VHS (60 frames s^{-1}) by following each copepod with a motorized three-dimensional stage made by DAEDAL, Harrison City, PA, on which a laser diode (830 mm) and a Pulnix TM-745 camera were mounted with their respective optics. This instrument resembled closely the Critter Cam® designed by J.R.Strickler, as shown in Strickler (1985). The field of observation was ~16 × 10 mm, at which a resolution of ~15 μm was attained, allowing us to follow individual diatom cells as they were displaced towards the copepod, captured and at times rejected. The entire 21–73 min of observations of each copepod were analyzed. If a cell was captured and not rejected during the following seconds, it was assumed to have been ingested. Active rejection of a cell usually occurred within less than one to several seconds after capture. Also, fecal pellet releases were recorded. Analyses of the times of cell captures and rejections, and pellet releases, were made with motion-analysis software by Peak Performance, Inc., Boulder, CO.

Results

Comparison of average rates

We visually observed the ingestion of individual cells of *T.eccentrica* by five females at 0.10–0.14 $mm^3\ l^{-1}$, by five females at 0.30–0.34 $mm^3\ l^{-1}$ and by six females at 1.0–1.2 $mm^3\ l^{-1}$. Individual copepods were observed over a period of 21–73 min and each animal's mean ingestion rate (cells ingested min^{-1}) was calculated (Figure 1). The mean ingestion rates increased from 0.1 to 0.3 [significant at $P < 0.01$, Kruskal–Wallis Test, and Multiple Comparison Test (Conover, 1980)] and were not significantly different between 0.3 and 1.0 $mm^3\ l^{-1}$ (Figure 1). Maximum ingestion rates were reached near 0.3 $mm^3\ l^{-1}$ of *T.eccentrica* which equals 9.8 $\mu g\ C\ l^{-1}$. At this food level, a *P.aculeatus* female ingested daily 52.6% of its body carbon. The clearance rates decreased non-linearly with increasing food concentration (Figure

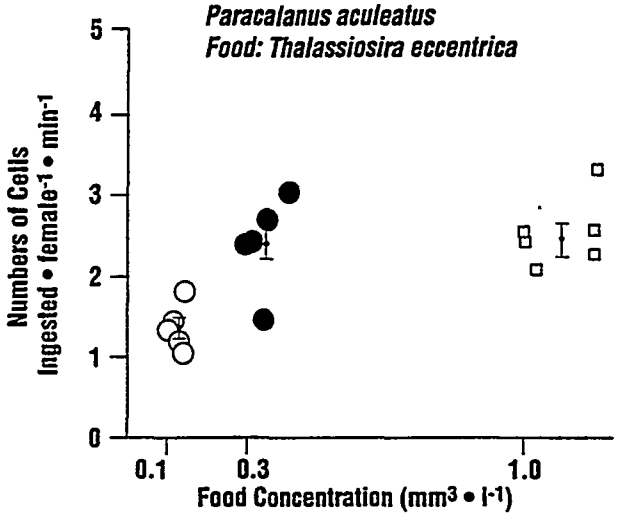

Fig. 1. Average ingestion rates of individual adult females of *P.aculeatus* at different concentrations of the diatom *T.eccentrica*. Vertical bars represent ± 1 SE.

2). The average number of actively rejected cells, i.e. captured and then rejected, did not differ between 0.1 and 0.3 mm³ l⁻¹, but was significantly higher at 1.0 mm³ l⁻¹ ($P < 0.01$; Figure 3). The percentage of gathered cells which were ingested was 85.7 at 0.1 mm³ l⁻¹, 90.3% at 0.3 mm³ l⁻¹ and 39.1% near 1.0 mm³ l⁻¹.

We also included in our observations the average intervals at which fecal pellets were released because they (i) indicate whether an animal is feeding and (ii) can serve as a coarse indicator of feeding activity (Paffenhöfer, 1994). The results showed significant differences in release intervals between 0.1 and 0.3 mm³ l⁻¹ ($P < 0.05$), but not between 0.3 and 1.0 mm³ l⁻¹ (Figure 4). The coefficients of variation between averages of ingestion rates and fecal pellet release rates were similar (Table I).

Comparison of rates of individuals

Next we conducted an analysis of variance of rates among individual females at each of the three food concentrations, again using the Kruskal–Wallis Test and, when needed, a Multiple Comparison Test (Conover, 1980). The number of cells ingested was quantified for each period between pellet releases and then normalized to cells ingested min⁻¹. For ingestion rates, the null hypothesis that the rates were identical was rejected for 0.1 and 0.3 mm³ l⁻¹ ($P < 0.05$), but not for 1.0 mm³ l⁻¹.

Table I. Individual *P.aculeatus* females feeding on *T.eccentrica*: coefficients of variability between averages (%)

	Food concentration (mm³ l⁻¹)		
	0.1	0.3	1.0
Ingestion rate	19.4	22.2	17.0
Pellet release rate	15.1	21.2	21.0

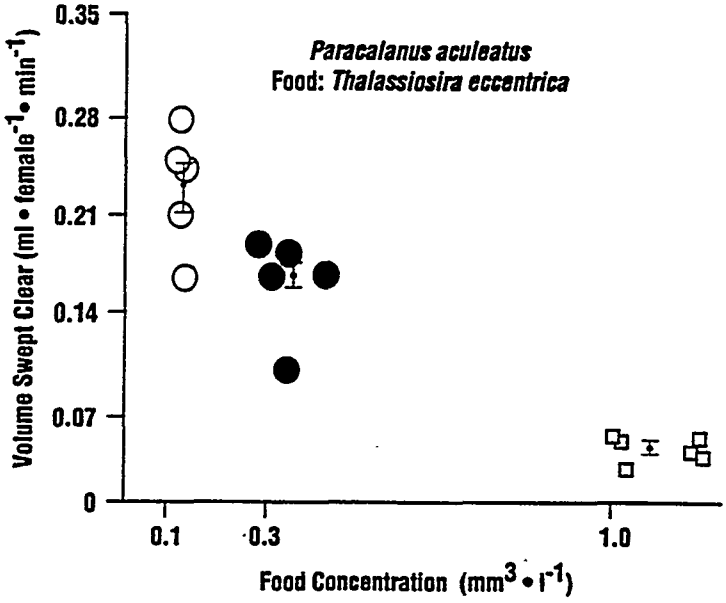

Fig. 2. Average clearance rates of individual adult females of *P.aculeatus*.

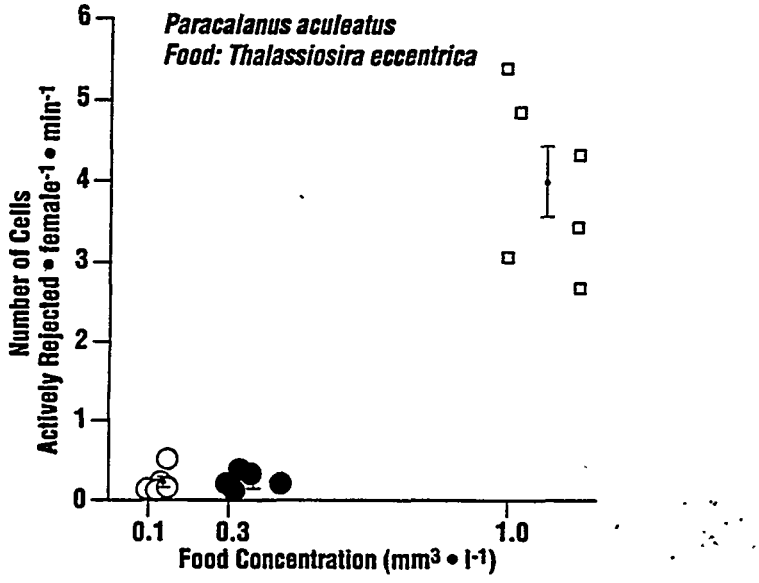

Fig. 3. Average food particle rejection rates of individual adult females of *P.aculeatus*.

At 0.1 mm^3 l^{-1}, the highest value was significantly different from the two lowest values ($P < 0.01$; Figure 1), and at 0.3 mm^3 l^{-1} the lowest value (1.42 cells ingested min^{-1}) was significantly different from the other four values ($P < 0.05$). The ranges of the coefficients of variation were similar at all three food levels and ranged from 23 to 36% (0.1), 20 to 42% (0.3), and 22 to 28% (1.0 mm^3 l^{-1}). Comparing the intervals between pellet releases at each food concentration resulted in rejection of the null hypothesis that the intervals were identical (Kruskal–Wallis Test). We present these data in detail to illustrate the differences (Tables II–IV). The Multiple Comparison Test revealed significant differences between the three highest and two lowest values at 0.1 mm^3 l^{-1} ($P < 0.05$; Table II). At 0.3, only two females had similar intervals (Table III) and at 1.0 mm^3 l^{-1} only females 1 and 2, and 4 and 5

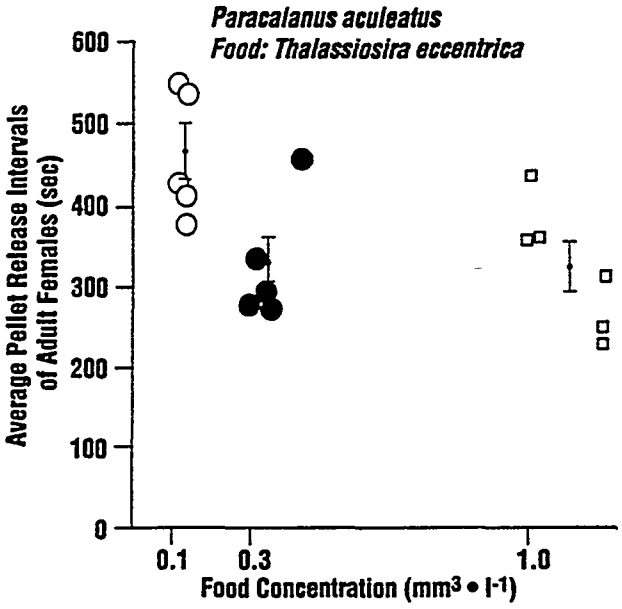

Fig. 4. Average fecal pellet release intervals of individual adult females of *P.aculeatus*.

Table II. Intervals between releases of fecal pellet(s) by individual adult females of *P.aculeatus* at 0.1 $mm^3 l^{-1}$ of *T.eccentrica*

	Female no.				
	1	2	3	4	5
Mean	375	413	427	534	550
n	8	7	7	6	7
± SD	31	20	83	119	59
± SE	11	7.4	31	49	22
CV (%)	8.3	4.7	19.5	22.3	10.7
Range	330–419	385–440	322–598	366–691	446–647

n = number of intervals.
SD = standard deviation.
SE = standard error.
CV = coefficient of variability.

(Table IV). The coefficients of variation for the pellet release intervals were low, particularly at 0.3 and 1.0 $mm^3 l^{-1}$, and may explain the significant differences found (see Discussion).

We did not compare rejection rates because they occurred only occasionally at 0.1 and 0.3 $mm^3 l^{-1}$. To illustrate the course of events (ingestion, rejection and pellet release), we display for one female at 0.3 $mm^3 l^{-1}$ (Figure 5, for 3200 s i.e. ~53 min) and another female at 1.0 $mm^3 l^{-1}$ (Figure 6, 3600 s i.e. ~60 min) when ingestions,

Table III. Intervals between releases of fecal pellet(s) by individual adult females of *P.aculeatus* at 0.3 $mm^3 l^{-1}$ of *T.eccentrica*

	Female no.				
	1	2	3	4	5
Mean	271	278	290	338	447
n	12	6	13	8	7
± SD	19	22	28	24	68
± SE	5.6	9	7.8	8.6	26
CV (%)	7.1	8.0	9.7	7.2	15.2
Range	243–321	247–304	220–331	299–375	381–578

n = number of intervals.
SD = standard deviation.
SE = standard error.
CV = coefficient of variability.

Table IV. Intervals between releases of fecal pellet(s) by individual adult females of *P.aculeatus* at 1.0 $mm^3 l^{-1}$ of *T.eccentrica*

	Female no.					
	1	2	3	4	5	6
Mean	232	250	313	353	354	433
n	6	9	8	8	10	8
± SD	27	30	25	15	31	50
± SE	11	10	8.8	52	9.9	18
CV (%)	11.6	12.1	7.9	4.1	8.8	11.5
Range	178–265	182–281	286–359	332–380	311–408	369–532

n = number of intervals.
SD = standard deviation.
SE = standard error.
CV = coefficient of variability.

rejections and pellet releases occurred. The *x*-axis represents time and the *y*-axis each of the four periods which make up the entire observational period. When ingestions or rejections were close to each other, one or two of the data points were positioned above/below the respective line to separate each event from the following one. At 0.3 and 1.00 $mm^3 l^{-1}$, ingestions sometimes occurred at irregular, at other times at fairly regular, intervals: e.g. at 0.3 between 1600 and 1800 s (Figure 5), at 1.0 from ~2000 to 2200 s (Figure 6). At 0.3 $mm^3 l^{-1}$, rejections were occasional and at irregular intervals, and pellet releases were at near regular intervals during the first 1200 s, becoming longer later. At 1.0 $mm^3 l^{-1}$, rejections occurred

frequently, and could not be related to preceding ingestions or pellet releases. Pellets were released at fairly regular intervals (Figure 6). The rejections presented are active rejections, i.e. the representative copepod had captured a cell and released/rejected it within ~1 s or less.

Discussion

Direct visual and continuous observations

This study was aimed at determining ingestion rates of individual copepods and the variables related to ingestion rates by using direct and continuous observations. This type of observation provides, in comparison to indirect observations, unequivocal evidence of what and how much has been eaten. Previously, almost all zooplankton feeding studies were based on quantifications integrated over time using a beginning and end point, e.g. (i) particle removal from a suspension, (ii) particle accumulation (e.g. radioisotopes) or (iii) decrease (e.g. gut fluorescence) in an animal's body. What actually happened between beginning and end points was unknown. Each of these methodologies was utilized assuming that constant conditions prevailed. Continuous uninterrupted coverage of individual copepods results in continuous observations of the process of ingestion, and increased statistical confidence in the data. Since each copepod was treated in a specific and

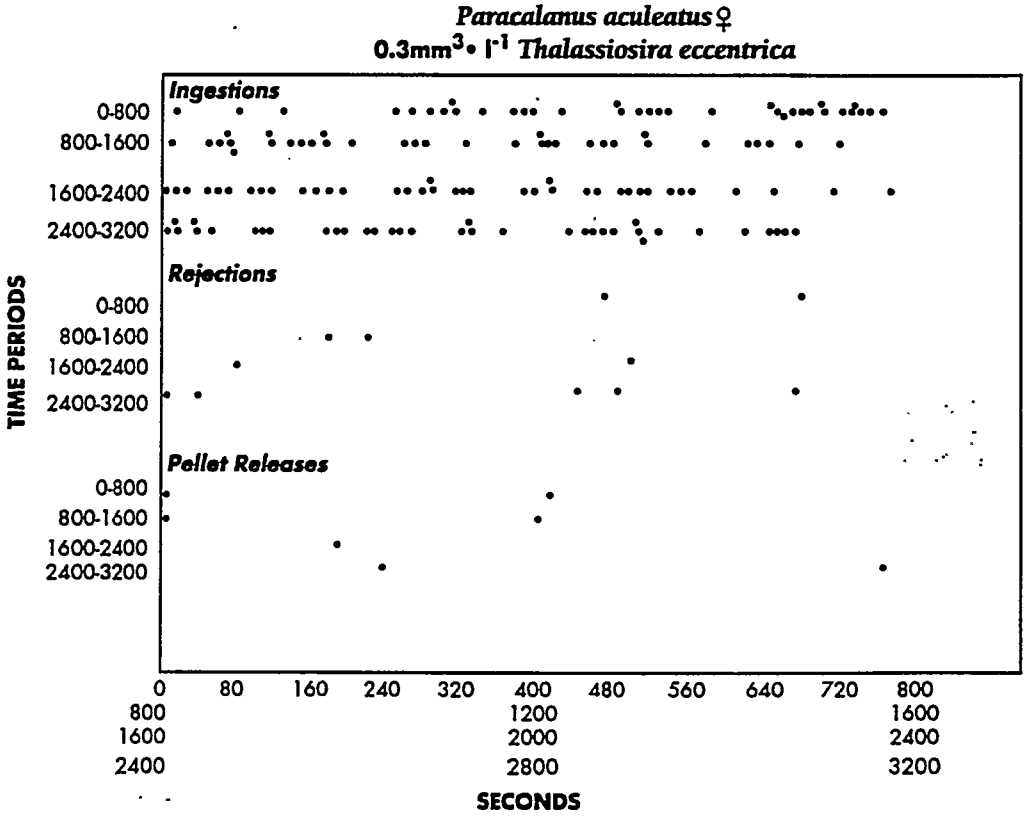

Fig. 5. Time course of ingestions and rejections of cells of *T.eccentrica* at a concentration of 0.3 $mm^3 l^{-1}$ by a female of *P.aculeatus* over a period of 3200 s. When ingestions or rejections were close to each other, the preceding or following one was positioned slightly above or below.

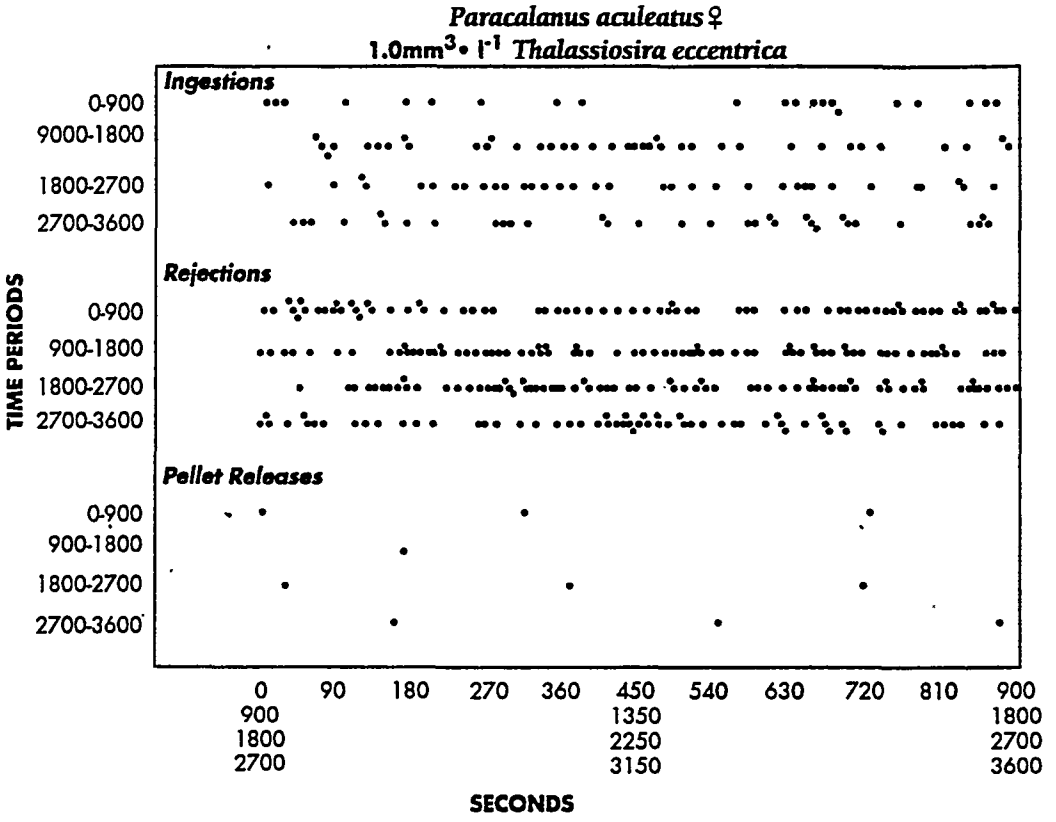

Fig. 6. As Figure 5, but for a different female at 1.0 mm^3 l^{-1} over 3600 s.

reproducible manner, observations on individual variability are strengthened statistically.

Paracalanus aculeatus is an offshore calanoid which usually encounters relatively low levels of particulate matter on the outer shelf and at the shelf break of the southeastern USA (e.g. Paffenhöfer and Lee, 1987). This species is found in other parts of the world's tropical and subtropical oceans (e.g. Gonzalez and Bowman 1965; Madhupratap and Haridas, 1986; Sameoto, 1986). Ingestion rates increased with increasing food concentration and reached a maximum near 0.3 mm^3 l^{-1} of *T.eccentrica*, which amounted to a daily rate of 52.6% of its body carbon. These rates are relatively low when compared to those of adult females of *Paracalanus parvus/quasimodo* which ingested daily a maximum of nearly 160% of its body weight at 20°C feeding on the diatom *Thalassiosira weissflogii* at 80 μg C l^{-1} (Paffenhöfer, 1984). The relatively low level of satiation concentration for females of *P.aculeatus* may be characteristic of small offshore copepods which have evolved to exist at very low, hardly varying food concentrations. It may also be that diatoms are not this species' preferred food (Kleppel *et al.*, 1988a). Therefore, our observations should be considered as an initial effort to quantify ingestion rates of free-swimming individuals.

Clearance rates increased non-linearly with decreasing food concentration (Figure 2) as observed in numerous previous studies (e.g. Frost, 1980). These rates may change over a period of 24 h, and may differ with different types of food. Recalculating our data, the average clearance rate of a *P.aculeatus* female (~0.9 mm

cephalothorax length) per 24 h would have been 318 ml at 0.1 $mm^3 l^{-1}$ (3.3 $\mu g C l^{-1}$) and 228 ml at 0.3 $mm^3 l^{-1}$ of *T.eccentrica* (9.8 $\mu g C l^{-1}$). The latter rate is similar to that of females of *P.parvus*, feeding on large cells at 18°C, which was near 240 ml $female^{-1}$ per 24 h at ~10 $\mu g C l^{-1}$ (Bartram, 1981; his Figure 9). *Paracalanus* sp. females (most likely *P.quasimodo*, ~0.7 mm cephalothorax length) removed the small diatom *T.weissflogii* (12 μm diameter) at average concentrations near 7 $\mu g C l^{-1}$ at rates between 181 and 234 ml $female^{-1}$ per 24 h in the presence of ~2 $\mu g C l^{-1}$ of *Rhizosolenia alata* and ~20 $\mu g C l^{-1}$ of *Isochrysis galbana* (personal observation).

Our continuous observations not only provided rates, but a mechanism by which an individual copepod modifies its clearance rates: at both low concentrations ~10% of the cells captured were actively rejected, whereas at 1.2 $mm^3 l^{-1}$ almost 60% were rejected (Figure 3). From these observations, one could assume that at low food levels nearly all cells perceived at a distance by *P.aculeatus* females were ingested, but not at higher food levels. The selection of cells for ingestion seemingly occurs at the mouth, i.e. by gustation (Paffenhöfer *et al.*, 1982). If such decisions indeed occur at the mouth, this could explain the overall lower ingestion rates, at similar food concentrations, when *Paracalanus* spp. was offered several phytoplankton species simultaneously as compared to feeding on only one species (Paffenhöfer, 1984). Decision making at the mouth with subsequent rejections requires time and effort, whereas deciding by olfaction, i.e. long distance, does not. For *Paracalanus* females, coarse decisions concerning food particles are probably made long distance, and fine-tuned ones at the mouth.

Variability

Our study was also designed to determine interindividual variability in the rates of feeding and fecal pellet release by changing food concentration, using animals of identical history. Here we will: (i) relate our findings to previous observations on feeding variability; (ii) evaluate possible causes for variability in copepod body size and physiological condition; and (iii) discuss the possibility of integrating environmental variability by copepods.

(i) The extent of individual variability among zooplankton and its causes have been addressed earlier, including feeding rates (e.g. Bamstedt, 1988; Paffenhöfer, 1994). We consider here only studies of actual cell ingestion or fecal pellet releases, and we do not include those of mouthpart activity because mouthpart activity cannot be used as an unequivocal indicator of feeding, as shown by our results on rejection and ingestion (0.3 versus 1.0 $mm^3 l^{-1}$; Figures 1 and 3), with all adult females moving their mouthparts nearly continuously. Earlier obtained measurements of indices of feeding of individual copepods had coefficients of variation (CV) from 35 to 65% (Rodriguez and Durbin, 1992) and 60 to 170% (Kleppel *et al.*, 1988b). These data represent one-time measures of the gut contents of copepods of the genus *Acartia*. The authors attributed the variability to non-synchronous feeding of individuals. Intermittent, temporally enhanced or reduced feeding cannot be excluded as copepods *in situ* may (i) modify feeding rates over periods of several hours, as could be assumed from the observations of Stearns *et al.* (1989) whose results on changes in gut fullness of female *Acartia tonsa* suggest that these animals may have altered their feeding activity during their main feeding period, i.e. night-

time (their Figure 1). This could be similar to dielly migrating *Calanus pacificus* whose ingestion rates decrease over hours after encountering high food levels (McAllister, 1970); (ii) reside only intermittently in thin vertical layers of abundant food (Cowles and Desiderio, 1993); and (iii) rapidly respond to changing food abundances by increasing or decreasing their mouthpart activity (female *Eucalanus hyalinus*; Price and Paffenhöfer, 1986).

CV between averages of ingestion rates and pellet release intervals of adult females of *P.aculeatus* were similar between food concentrations (Table I). These CV were close to those on average pellet release rates of tethered adult females of oceanic origin of the calanoid copepod *Eucalanus pileatus* which were 23.5, 27.5 and 24.2%, feeding on the diatom *T.weissflogii* at 0.1, 0.3 and 1.0 $mm^3 l^{-1}$, respectively (Paffenhöfer, 1994).

We also compared rates of individuals to determine interindividual differences. Significant differences were few for ingestion rates, and pronounced for pellet release intervals (see Results). Why would that be? Our observations on cell ingestions and active rejections over time (Figures 5 and 6) suggest that the distribution of cells of *T.eccentrica* in the experimental vessels (which we considered to be even) and the swimming routes taken by each copepod determined cell captures. Sometimes several cells were close to each other, resulting in many captures in e.g. 60 s; at other times, individual cells were farther apart from each other. This led to considerable differences in the number of ingested cells from one period to the next (feeding period = time between pellet releases, normalized to cells ingested min^{-1}) as seen from the rather high CV for individual females. All females of *P.aculeatus* which we observed were moving continuously and at rather even velocities, seemingly creating a feeding current all the time. Therefore, differences in other activities should not be considered for causing the high variability for ingestion rates in this study. Our findings suggest that encounter rates/long-distance perception governed ingestion rates at 0.1 and 0.3 $mm^3 l^{-1}$, but that at 1.0 $mm^3 l^{-1}$ satiation through selection behavior at the mouth determined the rate of food consumption.

CV for pellet release intervals of individual *P.aculeatus* were relatively low (Tables II–IV), particularly at 0.3 and 1.0 $mm^3 l^{-1}$. Variability of ingestion over several minutes will not immediately manifest itself in similar variability of pellet release rates. Rather, the latter should somehow integrate variability of ingestion frequency (Donaghay, 1988), resulting in lower variability of release intervals. As each animal, particularly at 0.3 and 1.0 $mm^3 l^{-1}$, had a relatively narrow range of release intervals, the probability of significant differences was high. Certain females had a specific longer (females 4 and 5 at 0.3 $mm^3 l^{-1}$) or shorter release period (females 1 and 2 at 1.0 $mm^3 l^{-1}$) which we could not relate to lower or higher feeding rates. Each animal appears to possess its specific release rate at saturating food levels. Higher or lower release intervals could also be related to smaller or larger pellet size, respectively.

(ii) How can our findings on the variability of feeding between individual copepods be related to the influence of the variability of environmental variables on zooplankton feeding in the ocean? The history of a copepod will translate itself into size, weight and physiological condition. Among diverse variables, intermittent scarcity and lower quality of food during juvenile life will affect later stages.

Variability in body weight of individual copepods, as observed by Bamstedt (1988) and Bamstedt *et al.* (1992), can be partly explained by the fact that not all juveniles of an identical stage of a species operate in the same vertical layer: e.g. field studies showed that a large percentage of copepodid stages I (CI) of *Temora turbinata* occupied the warm upper mixed layer (28°C) at low food abundance (with few diatoms), whereas another large fraction occurred in the cool (17–20°C) diatom-rich deep layer, and a third fraction was found in the thermocline (Paffenhöfer and Lee, 1987; Paffenhöfer *et al.*, 1987). In comparison, the vast majority of juveniles and adult females of *E.pileatus* remained in the cool, food-rich deep layer where food was usually evenly distributed (Paffenhöfer, 1983). Similarly, the vast majority of *Oncaea* spp. early copepodids remained together in the upper mixed layer, whereas the late copepodids and adults were almost exclusively found in the cold deep layer (Paffenhöfer, 1983). The latter two observations should result in small interindividual variability of feeding and therefore size, as was found for length of females of *E.pileatus* in summer (Paffenhöfer, 1994). If, however, a copepod shows a wide vertical distribution for each stage and, in addition, an extended juvenile life, then variability of size and physiological condition between individuals due to life history should be enhanced. The longer the juvenile life, the larger the number of environmental changes an individual will encounter, e.g. due to meteorological disturbances.

(iii) Copepods are thought to be able to integrate environmental variability through physiological and behavioral mechanisms (Donaghay, 1988). Here are some possible mechanisms. Decreases in food concentration are physiologically countered by increased perceptive ability, i.e. a decrease in food concentration from ~0.3 to ~0.1 $mm^3 l^{-1}$ of cells of *T.weissflogii* will result in only a small decrease of ingestion rate of adult females of *E.pileatus* (Paffenhöfer and Lewis, 1990). For early juvenile copepods which satiate at rather low food levels (e.g. Harris and Paffenhöfer, 1976; Ambler, 1986), wide variability in food concentrations should not be that important unless they reach levels at which these animals cannot cover their requirements for metabolism and slow growth. Older stages encountering low food levels can compensate not only physiologically (Paffenhöfer and Lewis, 1990), but behaviorally, by migrating vertically to find different food conditions. Females of the large calanoid *E.hyalinus* respond to decreasing food levels by increasing periods of inactivity between feeding bouts (Price and Paffenhöfer, 1986). Overall, copepods seem to have evolved behavioral mechanisms to limit the effects of varying food concentrations.

Our results for *P.aculeatus* females showed feeding rates similar to those found for congeneric females at low food levels. They revealed that above the satiation food level many cells are captured and soon afterwards rejected, indicating pronounced selectivity and energy expenditure when food is abundant. Considerable intraindividual variability of ingestion rates resulted in significant differences between only a few individuals at 0.1 and 0.3, and none at 1.0 $mm^3 l^{-1}$. However, females at the two high food levels had rather narrow, individual-specific pellet release rates which resulted in significant differences between numerous individuals. These observations are an attempt to improve our understanding of the feeding behavior of calanoid copepods, particularly the variability between

individuals, and to some extent within an animal. Our study was an initial effort using controlled conditions. We hope and expect that it will spawn wider-ranging efforts, revealing diel and short-term differences for different species and stages, leading to an understanding of previously environmentally observed variability. We feel that, ultimately, *in situ* observations on zooplankton individuals will be an essential component to comprehend how a pelagic community functions (U.S. GLOBEC, in press).

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Composition and biomass of plankton in spring on the Cape Hatteras shelf, with implications for carbon flux

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Abstract—

INTRODUCTION

Ocean margins are highly productive ecosystems. Continental shelf and adjacent slope waters represent 10–20% of the surface area of the global oceans, but contribute 25–50% of total oceanic primary production (Walsh, 1988). Biological productivity in these coastal oceans has been stimulated by increased anthropogenic nutrient loadings during the 20th century (Peierls *et al.*, 1991), with resulting increases in phytoplankton biomass and, likely, primary production (Walsh, 1988). The magnitude and fate of this new production is unknown. It has been argued that this enhancement is sufficient to affect the fate of anthropogenic carbon dioxide (Walsh, 1991).

Cape Hatteras lies at the confluence of the Middle and South Atlantic Bights, a watershed impacted by the activities of 100 million people. Productive shelf waters from north (Falkowski *et al.*, 1988) and south (Pietrafesa *et al.*, 1985; Verity *et al.*, 1993a) exit at Cape Hatteras, and the particles they contain enter the interior ocean or slope sediments. In the Middle Atlantic Bight, it has been argued that considerable shelf production is

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exported and stored in slope sediments (Walsh *et al.*, 1985), though this is debatable (Rowe *et al.*, 1986; Biscaye *et al.*, 1988). At Cape Hatteras, carbon fluxes to the sediments (Schaff *et al.*, 1992) and carbon concentrations within the sediments (Hathaway, 1971; Walsh *et al.*, 1985) are among the highest in the western North Atlantic Ocean.

The Ocean Margins Program of the U.S. Department of Energy, focused at Cape Hatteras, is studying how coastal oceans process carbon, the principal mechanisms of carbon transformations and fluxes, and patterns in their variability. As part of preliminary scoping studies, a cruise to the shelf was conducted in May 1993. This paper reports on the structure, composition, and biomass of autotrophic and heterotrophic plankton, and draws inferences concerning the production and fate of particulate organic carbon during this period.

MATERIALS AND METHOD

Sampling was conducted during 10–16 May 1993 aboard the R.V. *Gyre*, cruise 93-G6. Three transects across the shelf just north of Cape Hatteras were run sequentially, with 30 nautical miles (*ca* 50 km) between transects (Fig. 1). Each transect required *ca* 24h to complete. Twelve stations were spaced on each transect at intervals of six nautical miles (*ca* 10 km), with the innermost station coinciding approximately with the 20 m isobath and the outer stations exceeding the 500 m isobath. Samples for vertical profiles were collected using CTD rosettes, with typically 5 m between samples at stations < 30 m deep, 10 m between samples at stations 30–60 m deep, and 15 m between samples at stations > 60 m deep.

The following measurements were included in virtually all of the above samples: conductivity, temperature, and pressure (depth) via CTD; phytoplankton chl *a* via acetone-extracted fluorometry (Yentsch and Menzel, 1963); O_2 concentration via Winkler titration to meet WOCE (1991) standards (Wallace *et al.*, ~~in press~~); and plankton composition, abundance, size distribution, and biomass via microscopy. Oxygen saturation was calculated from the solubility data of Benson and Krause (1984). At selected stations, primary production was measured via C14 incubations (Falkowski *et al.*, 1988); meso- and macrozooplankton abundance and biomass via stratified net sampling; and bacterial abundance and bacterivory via uptake of fluorescently-labelled prey. The details of plankton biomass measurements are described below.

Plankton community composition and biomass was determined from samples prepared at sea and analyzed ashore, using a color image analysis system. Phytoplankton included photosynthetic nanoplankton, diatoms, dinoflagellates, cyanobacteria, and obligate photosynthetic ciliates (*Mesodinium*). Nano- and microzooplankton included heterotrophic nanoplankton, dinoflagellates, and plastidic and aplastidic ciliates (crustacean zooplankton are described below). Samples for these organisms were prepared and measured as described in Verity and Sieracki (1993). Briefly, samples were preserved immediately in glutaraldehyde to a final concentration of 0.3%, stained with 3-6-diaminoacridine hemisulfate (proflavin) for 1 min (5 μg/ml final concentration) and 4'6-diamindino-2-phenylindole (DAPI) for 4 min (5 μg/ml final concentration). Samples were then filtered onto an 0.2 μm black Nuclepore filter. In order to achieve an even distribution for counting and measurement purposes, black filters were placed on top of pre-wetted 0.45 μm Millipore backing filters. After staining, filters were rinsed with distilled water, concentrated under low vacuum, and the damp filter placed on a slide. A drop of low fluorescence

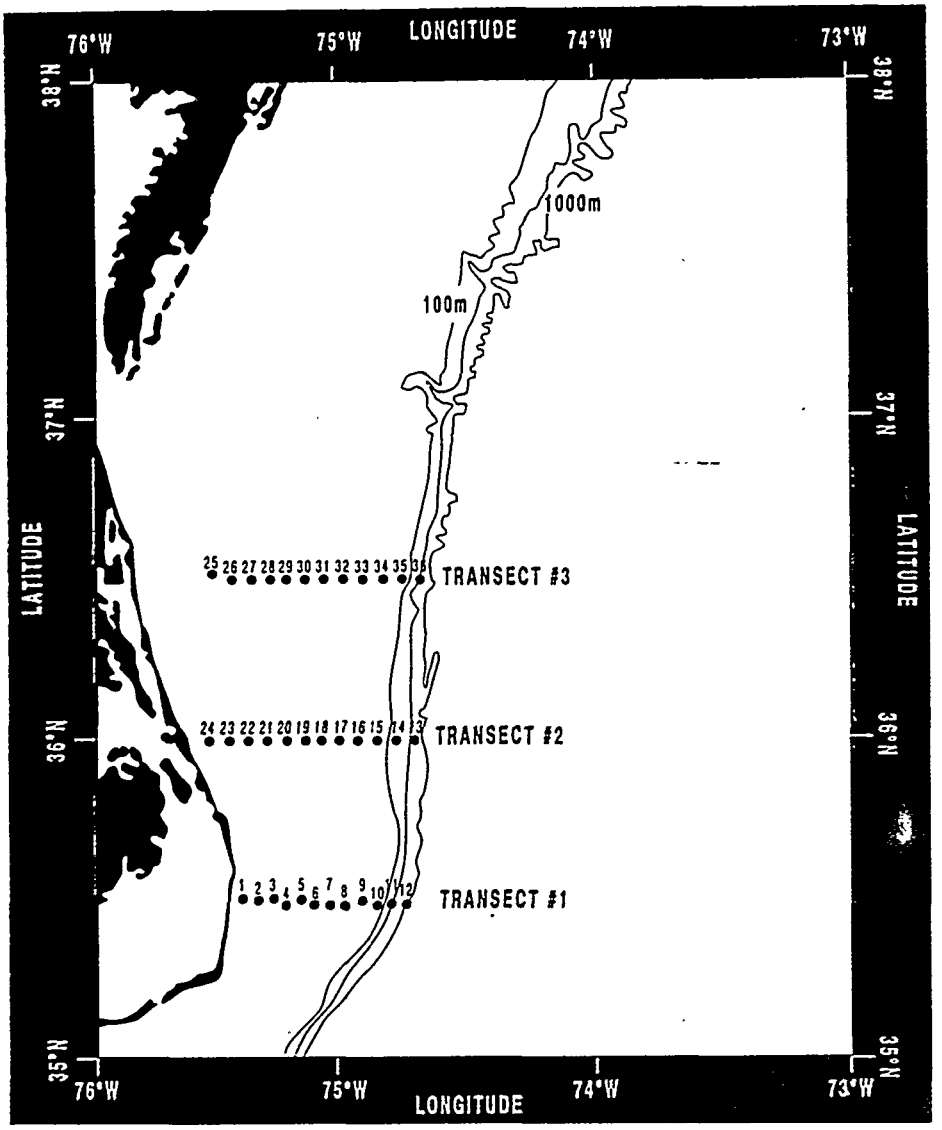

Fig. 1. Station arrays and positions for the three transects conducted in May 1993.

immersion oil was placed on top of the filter, which was covered with a cover slip and frozen at −20°C.

An Olympus BH-2 microscope equipped with an HBO-100 epifluorescence illuminator and the appropriate exciter/barrier filter sets for UV (330–385 nm), blue (420–480 nm), and green (510–550 nm) excitation was used for visualizing cells. Cell dimensions and biovolumes were determined using a true color image analysis system (Verity *et al.*, 1993a,b; Verity and Sieracki, 1993). Typically, 200 cells were measured in each sample. While sample volume was set to optimize cell density on the filters for image analysis, two approaches were used depending upon final cell density. For numerous cells of relatively

uniform brightness, randomly selected whole field images were analyzed automatically. In most cases, transects of the slide were scanned and individual cells were isolated by the operator interactively. Cell volumes were converted to cell carbon according to Verity *et al.* (1992: eucaryotic and procaryotic photosynthetic nanoplankton), Strathmann (1967: diatoms), Borsheim and Bratbak (1987: heterotrophic nanoplankton), Lessard (1991: heterotrophic dinoflagellates), and Putt and Stoecker (1989: ciliates).

Bacteria were enumerated via epifluorescence microscopy, using the acridine orange direct count (AODC) method (Hobbie *et al.*, 1977; Kirchman *et al.*, 1982). Bacterial carbon was estimated from bacterial abundance using a factor of 20 fgC cell^{-1} (Lee and Fuhrman, 1987). Rates of bacterivory were estimated using the fluorescently labelled bacteria (FLB) uptake method (Sherr *et al.*, 1987). FLB were prepared from a cultured marine bacterial isolate starved to decrease average cell size to 0.13 μm^3 (Gonzalez *et al.*, 1993). FLB were added to 130 ml samples of whole seawater in sterile tissue culture flasks to yield a final concentration of 0.3–0.5 $\times$ 10^6 FLB ml^{-1}; flasks were incubated in the dark at the temperature of surface seawater. Five depths in the euphotic zone were sampled at Stations 1, 4, 7, 12, 14, 16, 23, and 25, and four depths at Station 28 (Fig. 1). At each station, surface water samples were subsampled at 15, 30, and 45 min after addition of FLB, and samples from other depths were subsampled at 45 min only. Individual tissue culture flasks were used for each time point sampled. Samples were preserved with the Lugol-formalin decoloration technique (Sherr and Sherr, 1993).

Duplicate 30 ml aliquotes from each flask were stained with DAPI and filtered onto 0.8 μm black polycarbonate filters. Separate samples were taken for AODC counts of total bacteria, and for unstained counts of FLB abundance, and filtered onto 0.2 μm polycarbonate filters. Filters were mounted onto glass slides and stored at −20°C until returned to the laboratory for microscopic inspection (Sherr and Sherr, 1993). Time course of cell-specific rate of FLB ingestion was determined from the three-time point experiment carried out at each station: the period of linear uptake of FLB was determined from this experiment (Sherr *et al.*, 1987). Per-cell clearance rates of bacterivorous protists were calculated by dividing cell-specific uptake rates of FLB by the concentration of FLB (Sherr *et al.*, 1987). Rates of community clearance of bacteria were then calculated by multiplying the per-cell clearance rates by the total abundance of bacteria per ml and by the abundance of bacterivorous protists in the samples (Sherr *et al.*, 1987). Volumetric rates of bacterivory were converted to daily flux of bacterial carbon m^{-2} using a conversion factor of 20 fgC cell^{-1} and integrating the depth profiles of bacterivory over the total depth sampled at each station (20–85 m).

Metazoan zooplankton were collected using a custom Multiple Net System conceptually similar to a MOCNESS. At various stations across the shelf, consecutive strata from near bottom to near surface were sampled using 73 μm mesh nets. Contents of codends were preserved in 2% buffered formaldehyde and subsequently transferred into phenoxetol. Subsamples were enumerated until typically 30, usually >50, specimens of each taxa were counted. Abundances were converted to biomasses from length measurements using an ocular micrometer, or by stage-specific weights from literature data: *Paracalanus* (Paffenhöfer, 1984), *Temora* (Harris and Paffenhöfer, 1976), *Calanus* (Paffenhöfer, 1971), *Oithona* (Drits and Semenora, 1984), and *Centropages* (Paffenhöfer, unpubl. data).

Carbon ingestion by the major groups of zooplankton was estimated from their biomass and literature data on metabolic rates at temperatures and food concentrations similar to those observed in Cape Hatteras waters. For heterotrophic nanoplankton (Hnano)

ingestion was calculated by assuming only Hnano >500 μm^3 were herbivorous. This value was conservatively estimated from the median cell size of phototrophic nanoplankton (3–9 μm), their potential prey, and a volumetric ratio of predator:prey of 6:1 (Goldman and Caron, 1985). Using a less conservative assumption that all Hnano >5 μm in diameter are herbivorous (Sherr and Sherr, 1991) would increase herbivorous Hnano community biomass by *ca* 25%. Hnano growth was assumed to be 1.0 · day^{-1} and gross growth efficiency was 40% (Parslow *et al.*, 1986; Goldman and Dennett, 1990). For heterotrophic dinoflagellates (Hdino), ingestion was calculated by assuming growth was 0.4 · day^{-1} and gross growth efficiency was 20% (Strom, 1991). For ciliates, ingestion was estimated as 0.08 · h^{-1} (Verity, 1985, 1991). For metazoan zooplankton, ingestion was estimated from Ambler (1986) and Paffenhöfer (1984 and unpubl.).

RESULTS

Hydrography and plankton distributions

Winds during the study period were predominantly southwesterly. Shelf waters were strongly stratified and showed evidence of contributions from several sources (Figs 2–3). On the northern transect, water temperatures decreased from 16–18°C at the surface to 9–10°C over the bottom. This cold bottom water, which typically occurs over the outer portion of the shelf, is relict winter bottom water often termed the "cold pool". Salinities in the upper 5 m were <30 PSU apparently representing warmer, fresher Chesapeake Bay outflow overriding shelf water, as suggested by CTD profiles conducted seaward from the mouth of the bay (data not shown). On the mid transect, there was also evidence of the bay plume but salinities were a little higher and stratification less intense. The southern transect was considerably more complex: the plume occurred only over the inner 10 km, lenses of warm saline water trapped along the bottom at midshelf, and Gulf Stream water was overriding shelf water at the surface.

Chl *a* concentrations were highest in the northern transect, attaining peaks of 10–13 μg/l, compared to 5–7 μg/l in the middle and southern transects (Fig. 4). Maximum levels typically were observed at midshelf and depths of 20–25 m. A near-surface peak occurred at midshelf to the north. In the northern and middle transects, elevated phytoplankton pigments extended at least 10–20 km beyond the shelfbreak. The abundance of diatoms (Fig. 5) mirrored that of chl *a*, being greatest in northern waters and at depths of 20–30 m. Highest concentrations were 4–5 × 10^2 cells/ml, and were composed primarily of relatively large (20–30 μm) chain-forming taxa including *Rhizosolenia*, *Chaetoceros*, *Odontella*, *Leptocylindrus*, and *Cerataulina*.

The most abundant phytoplankton were eukaryotic photosynthetic nanoplankton (Fig. 6). Highest concentrations, reaching 6–7 × 10^3 cells/ml, typically occurred in surface waters and decreased with depth. A strong north–south gradient similar to that in diatoms was not observed for nanoplankton. Common taxa included *Cryptomonas*, *Calicomonas*, *Pyramimonas*, and unidentified chlorophytes and cryptomonads. Most cells were 3–9 μm ESD.

Two relatively large phytoplankton taxa were also notable in their spatial distributions. *Mesodinium* sp., a ciliate with a vestigial oral cavity which is obligately photoautotrophic, occurred in isolated patches at intermediate depths (Fig. 7). Patches were observed at midshelf in the northern and southern transects, but were found beyond the shelfbreak in

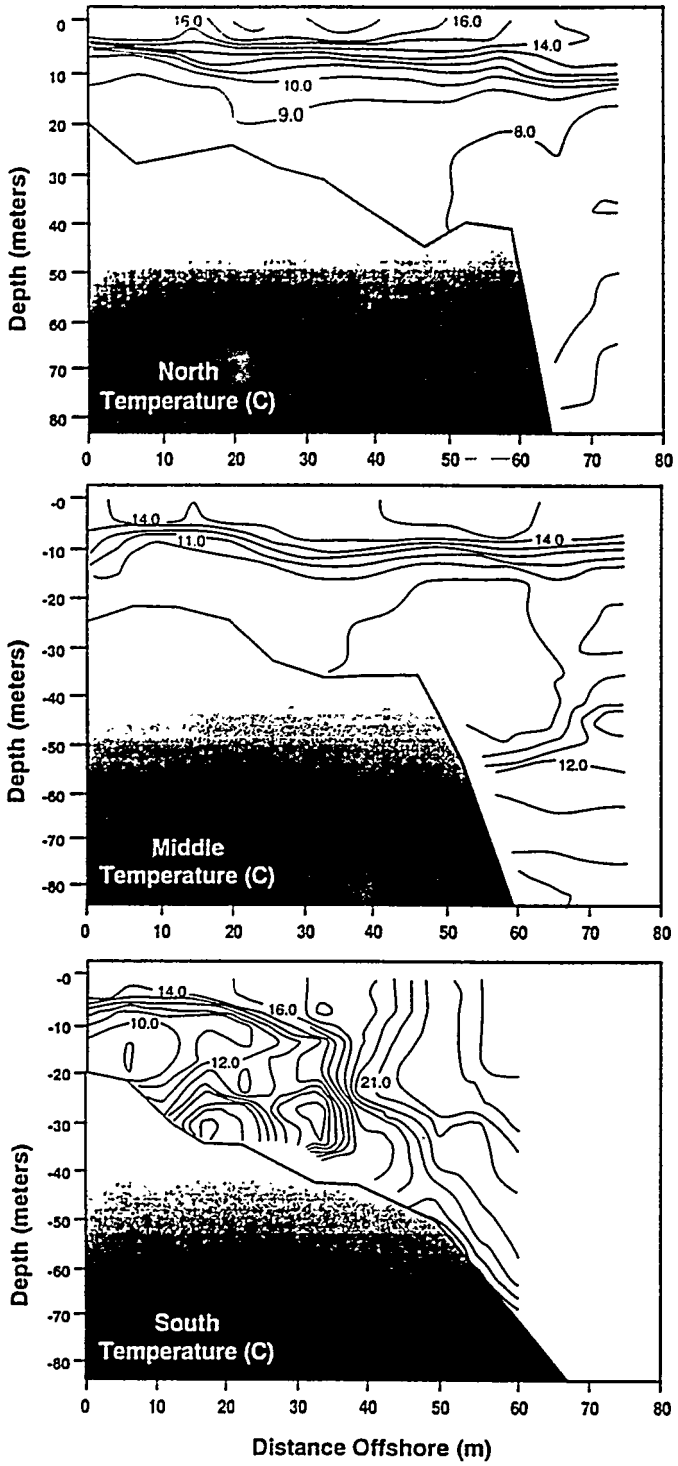

Fig. 2. Cross-shelf contour plots of temperature (C). A: Transect 3 (north). B: Transect 2 (mid). C: Transect 1 (south).

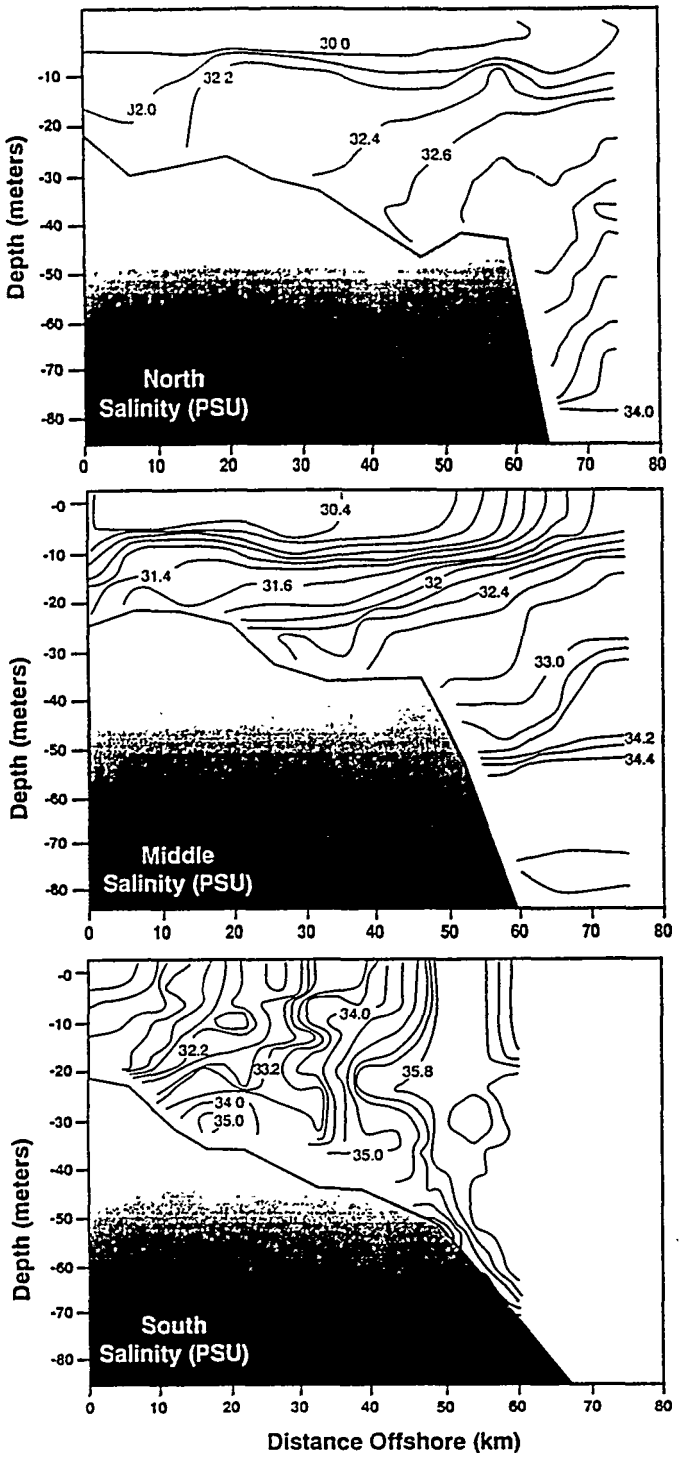

Fig. 3. Cross-shelf contour plots of salinity (‰). Panels as in Fig. 2.

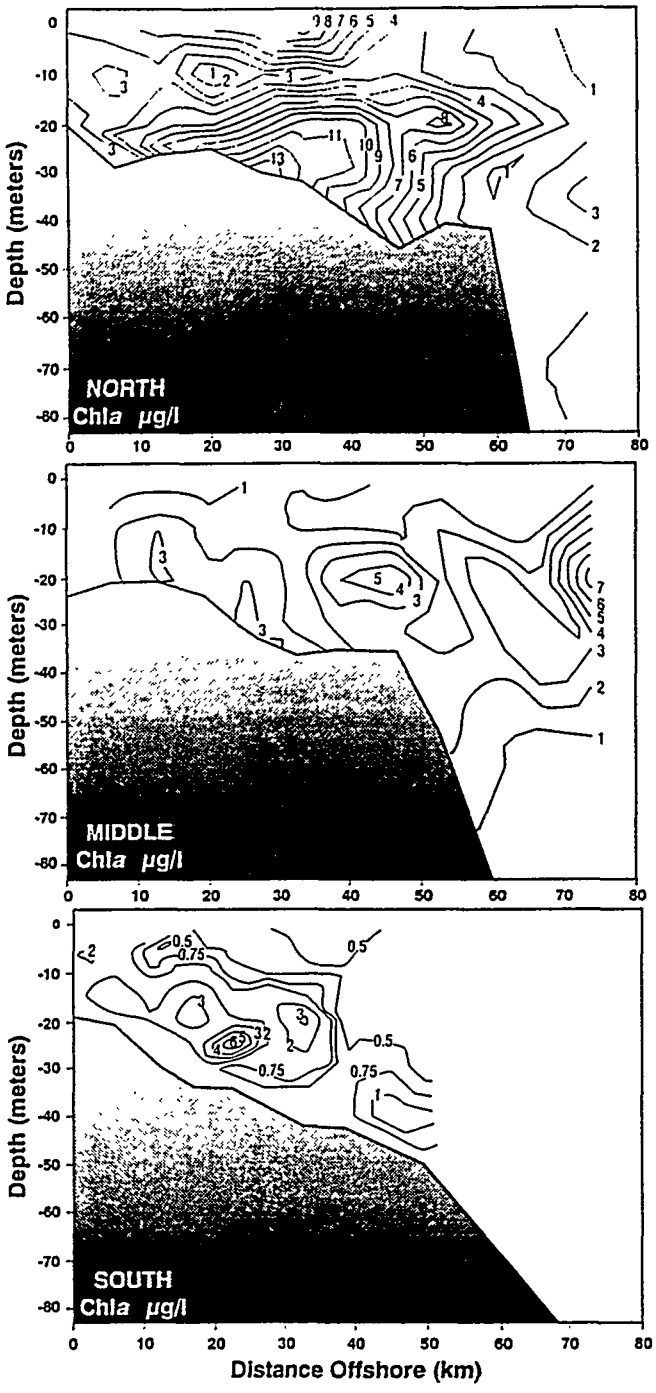

Fig. 4. Cross-shelf contour plots of chl *a* concentration (µg/l). Panels as in Fig. 2.

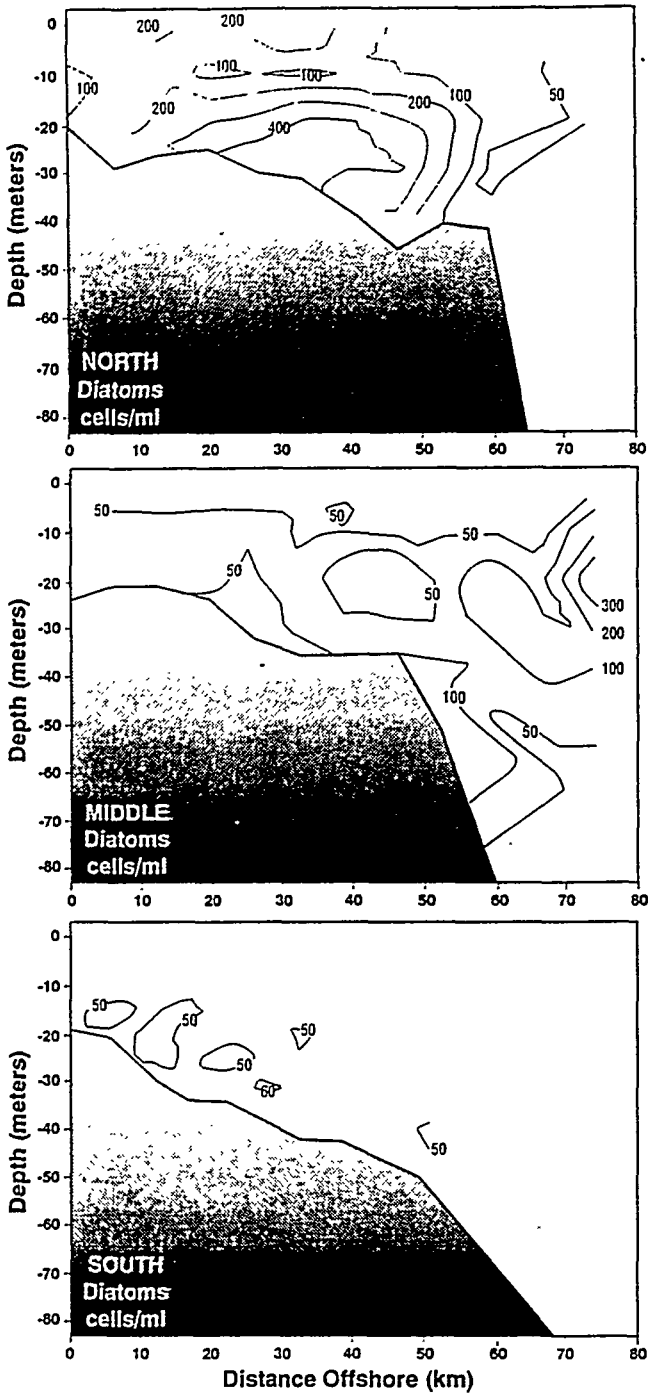

Fig. 5. Cross-shelf contour plots of abundance of diatoms (cells/ml). Panels as in Fig. 2.

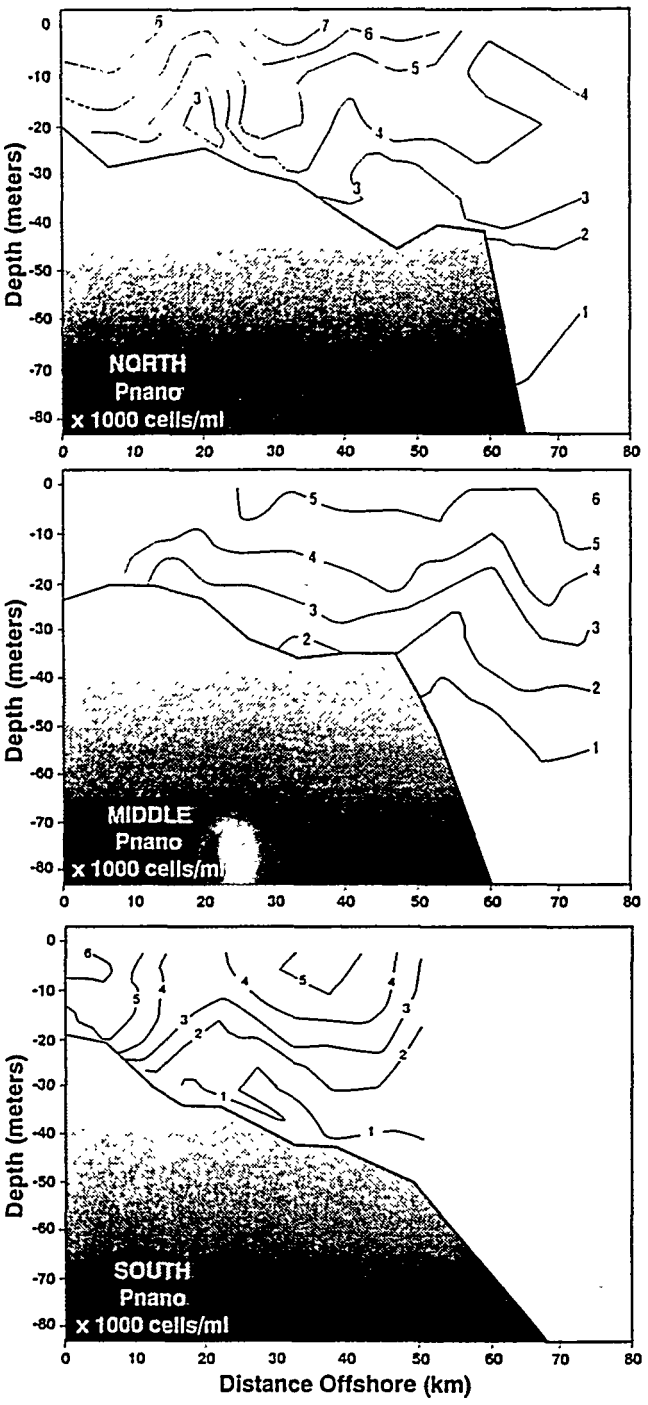

Fig. 6. Cross-shelf contour plots of abundance of photosynthetic nanoplankton (10^3 cells/ml). Panels as in Fig. 2.

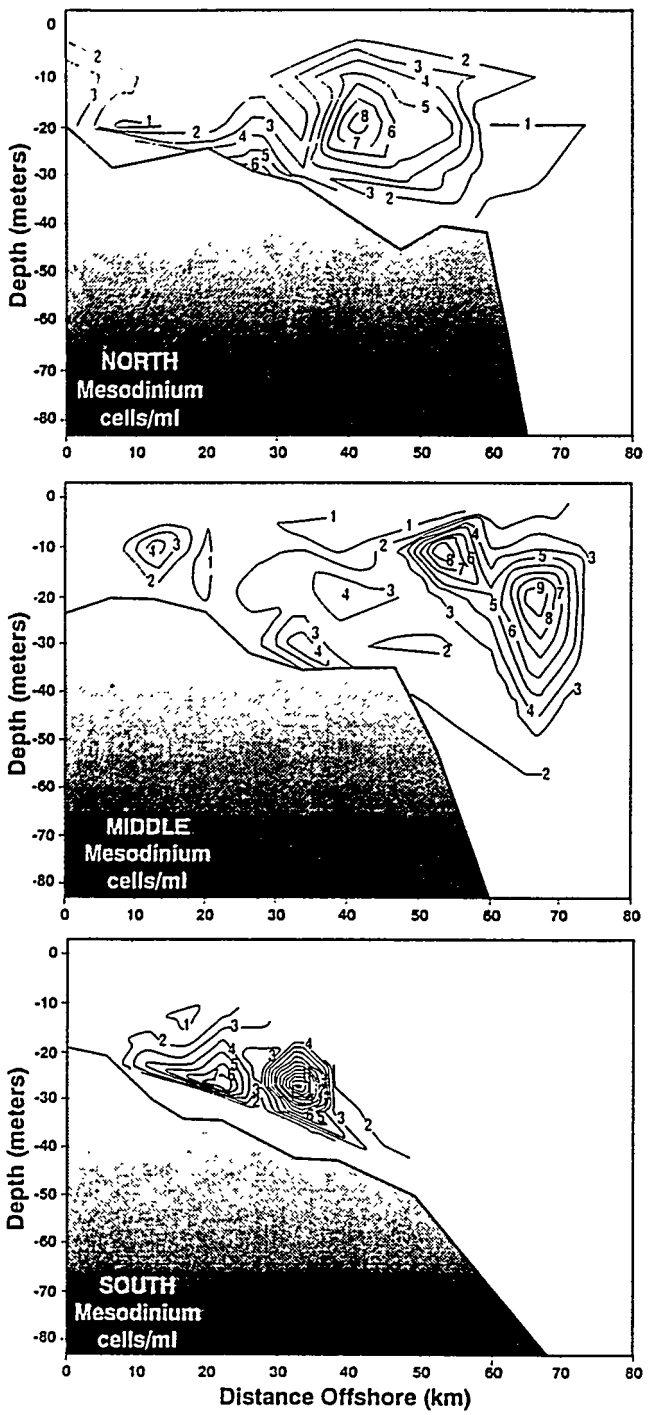

Fig. 7. Cross-shelf contour plots of abundance of *Mesodinium* (cells/ml). Panels as in Fig. 2.

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the middle transect. Provocatively, several species of the photosynthetic dinoflagellate *Ceratium* (*C. furca*, *C. tripos*, *C. lineatum*) exhibited a very similar horizontal distribution pattern (Fig. 8) to that of *Mesodinium*, with midshelf peaks in abundance to the north and south, and offshore peaks in the middle. Highest abundances of both taxa (10–15 cells/ml) occurred at intermediate depths, but their respective maxima did not always overlap: *Ceratium* often occurred 5–10 m deeper than *Mesodinium*. Other large dinoflagellates, including species of *Dinophysis* and *Prorocentrum*, were sporadically abundant and generally coincided with *Ceratium*.

Cyanobacteria occurred across the shelf at $1–6 \times 10^3$ cells/ml (data not shown), but these concentrations are an order of magnitude lower than those which typically occur in near-surface oceanic waters (e.g. Sieracki *et al.*, 1993). Due to their small size (1–2 μm), their biomass on the Cape Hatteras shelf/slope was only 5–10% that of eucaryotic nanoplankton.

Among protozoan zooplankton, heterotrophic (aplastidic) nanoplankton were the most numerous (Fig. 9), typically present at $1–3 \times 10^3$ cells/ml. They were relatively ubiquitous, occurring throughout the study region and only declining slightly with depth in the upper 65 m. Numerous taxa were present, including cells which appeared, based upon morphology to be species of *Leucocryptos*, *Bodo*, *Paraphysomonas*, *Cafeteria* and various choanoflagellates. Many cells were 6–10 μm.

Heterotrophic (aplastidic) dinoflagellates were also numerous, with maximum abundances of 60–80 cells/ml, which typically occurred in inner shelf waters (Fig. 10). Abundances increased north to south on the inner shelf, whereas outer shelf populations were relatively constant and depauperate. The dominant morphotypes included small spindle-shaped athecate taxa, with typical dimensions of 3–5 μm × 6–10 μm.

Planktonic ciliates were less abundant than nanoflagellates and dinoflagellates (Fig. 11), typically occurring at 1–3 cells/ml. Common genera included *Strombidium* (15–25 μm), *Strobilidium* (30–50 μm), and *Lohmanniella* (20–40 μm). Their spatial distribution was rather unremarkable, with maxima generally occurring at depths of 20–30 m. These latter peaks, however, were invariably due to patches of the large (60–80 μm) plastidic species, *Laboea strobila*, which coincided very closely with the spatial distributions of *Ceratium* and *Mesodinium*. Plastidic taxa (excluding *Mesodinium*) contributed a mean of 38% of total planktonic ciliates (range: 17–74%).

Sampling with 73 μm mesh implied that copepodid stages and adults of all copepod species were quantitatively collected, and also a large percentage of the nauplii. The latter amounted to near $100 \cdot l^{-1}$ (St. 4) or more at the four innermost stations on Transect #1 at 35°30′N, and were an order of magnitude or less offshore (St. 8, Fig. 12). Small copepods dominated in abundance and biomass nearshore and offshore. The most abundant genera were the calanoids *Paracalanus*, *Centropages* and *Temora*, and the cyclopoid *Oithona* (Fig. 12). Within the four nearshore stations concentrations of all four genera decreased slightly towards offshore, and then sharply towards Station 8. Whereas concentrations of all four genera decreased with depth of Station 8, only those of *Paracalanus* and *Centropages* did so at the innermost stations. These four genera were similar in abundance on the inner shelf at 36°00′ and 36°30′N.

Correlations among biological variables

Chl *a* concentrations were poorly correlated with abundance (Fig. 13) and biomass (data not shown) of photosynthetic nanoplankton. In contrast, chl *a* was positively related to the

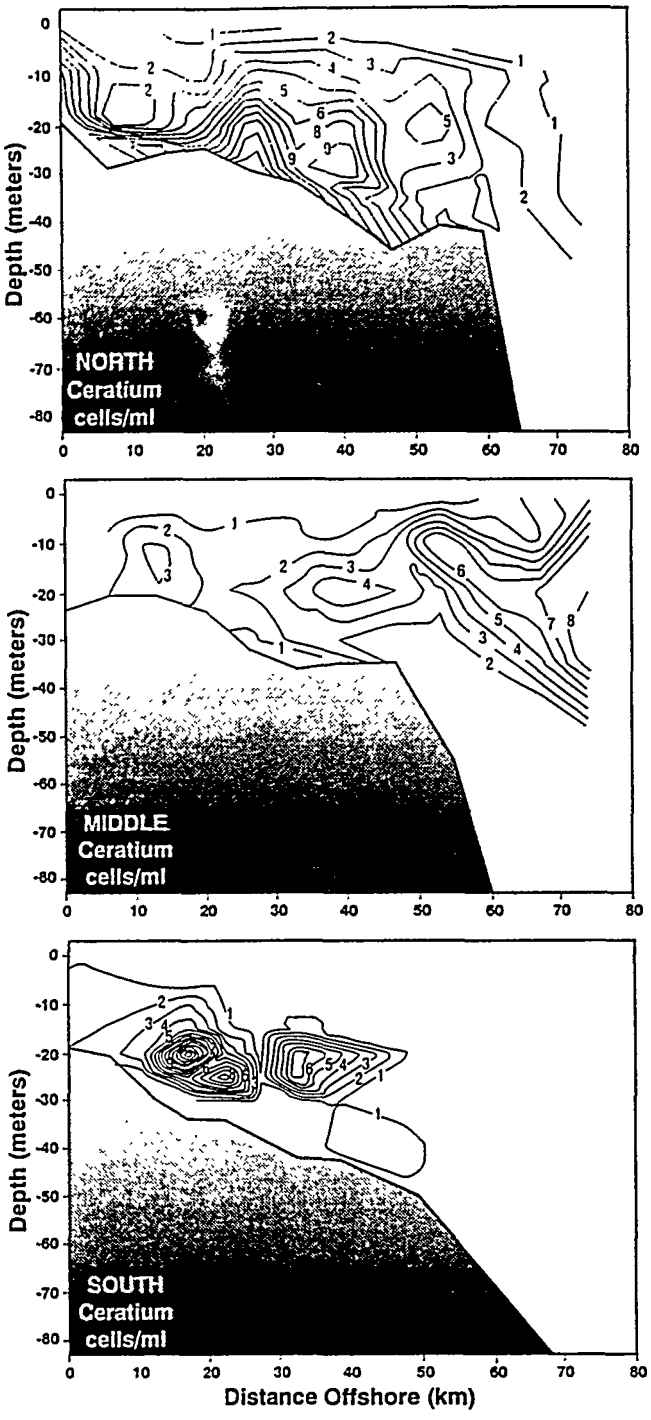

Fig. 8. Cross-shelf contour plots of abundance of *Ceratium* (cells/ml). Panels as in Fig. 2.

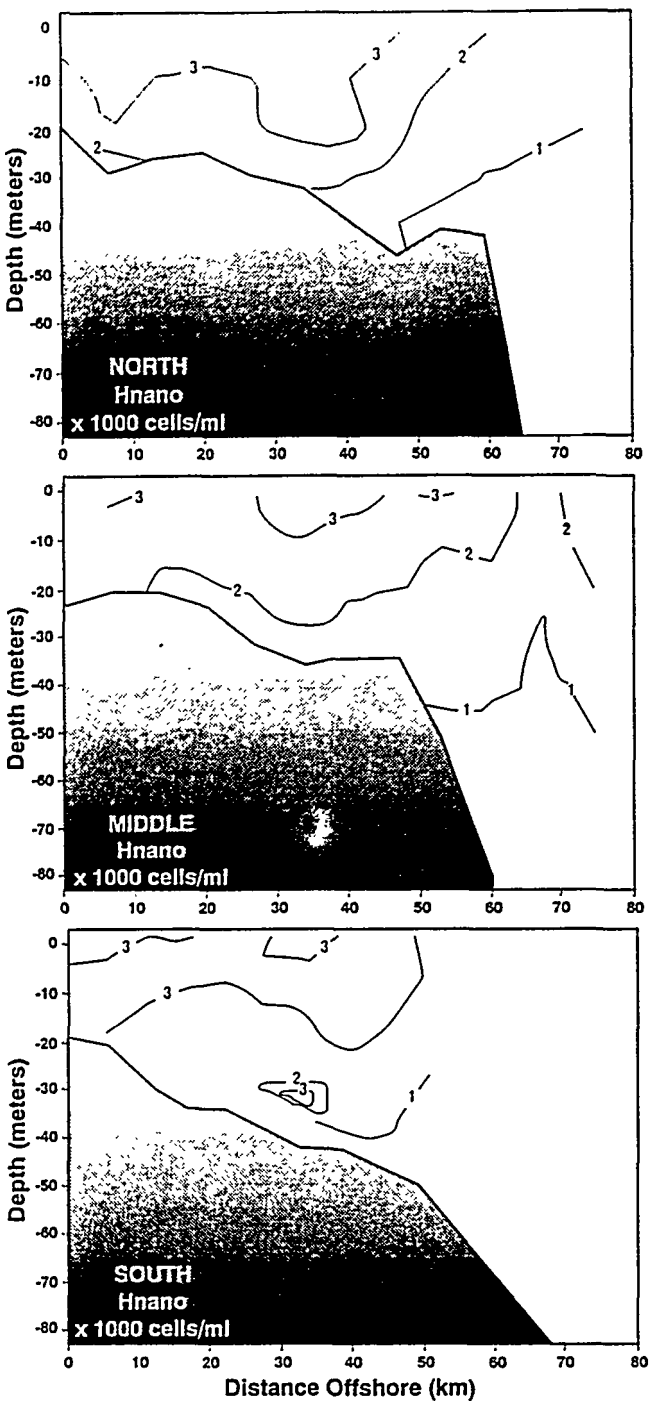

Fig. 9. Cross-shelf contour plots of abundance of heterotrophic nanoplankton (10^3 cells/ml). Panels as in Fig. 2.

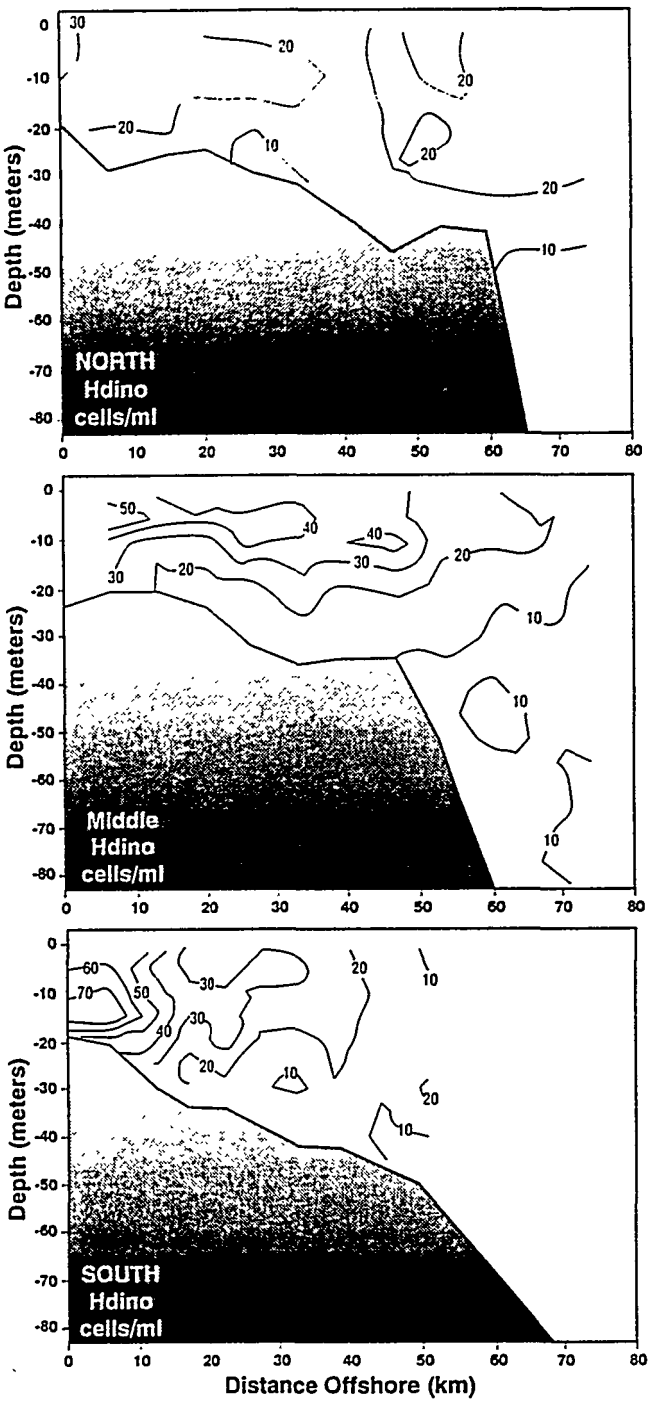

Fig. 10. Cross-shelf contour plots of abundance of heterotrophic dinoflagellates (cells/ml). Panels as in Fig. 2.

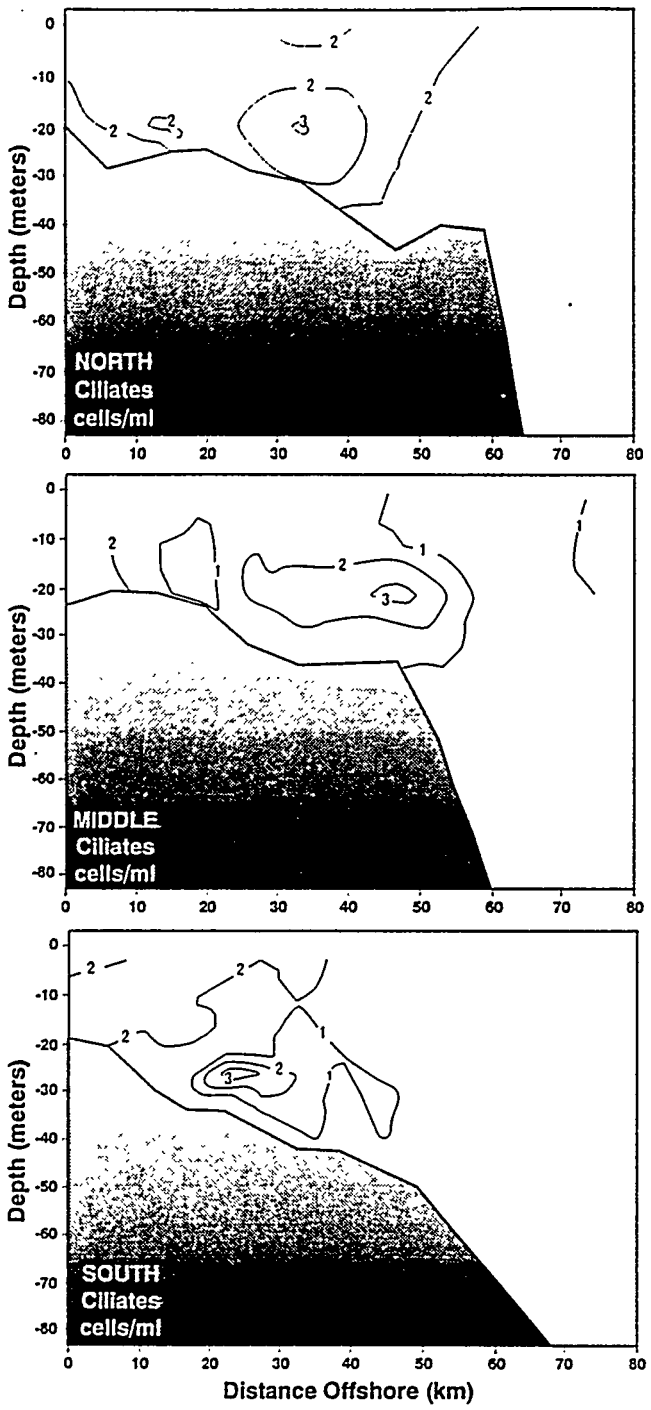

Fig. 11. Cross-shelf contour plots of abundance of ciliates (cells/ml). Panels as in Fig. 2.

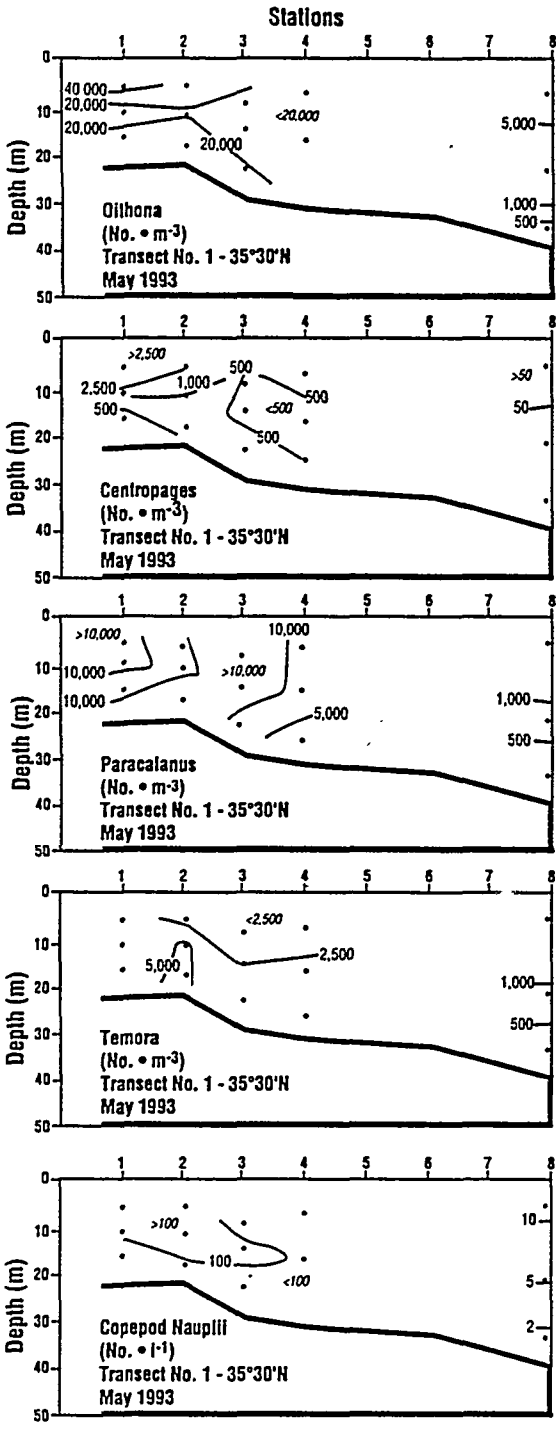

Fig. 12. Abundance of four copepod genera (No. · m^{-3}) and total copepod nauplii (No. · l^{-1}).

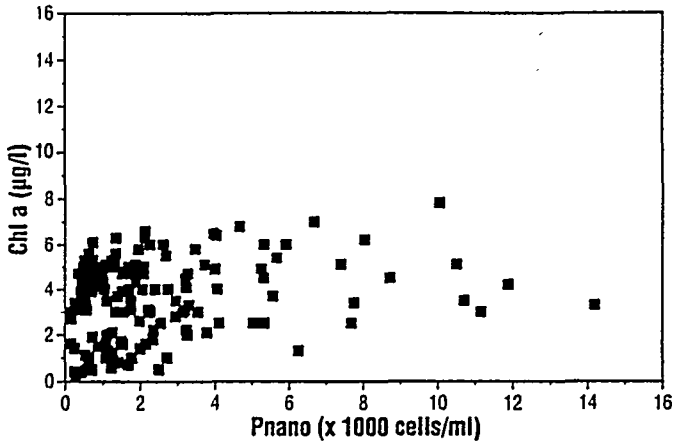

Fig. 13. Chlorophyll *a* concentration (µg/l) versus abundance of photosynthetic nanoplankton (10^3 cells/ml) in the same samples. Data from all stations and depths.

occurrence of diatoms (Fig. 14), suggesting that diatoms were the major contributors to primary production. However, this was apparently not the case. Oxygen saturation, which was significantly correlated with rates of primary production measured by ^{14}C uptake ($r^2 = 0.49$, $n = 29$, $p < 0.01$), was highest in surface waters and declined with depth (Fig. 15). This depth dependence was not simply a function of oxygen solubility, as oxygen saturation was not correlated with temperature ($r^2 = 0.003$, $n = 149$, $p > 0.001$). Diatom abundance was not obviously related to oxygen saturation (Fig. 16) whereas photosynthetic nanoplankton were strongly correlated ($r^2 = 0.58$, $n = 149$, $p < 0.001$) with oxygen saturation (Fig. 17).

The photosynthetic nanoplankton provided the basis for an abundant food web. They were positively correlated ($r^2 = 0.61$, $n = 141$, $p < 0.001$) with heterotrophic nanoplankton over all stations and depths (Fig. 18). Abundances of the larger heterotrophic dinoflagellates (Fig. 19) and ciliates (Fig. 20) were positively correlated with the combined

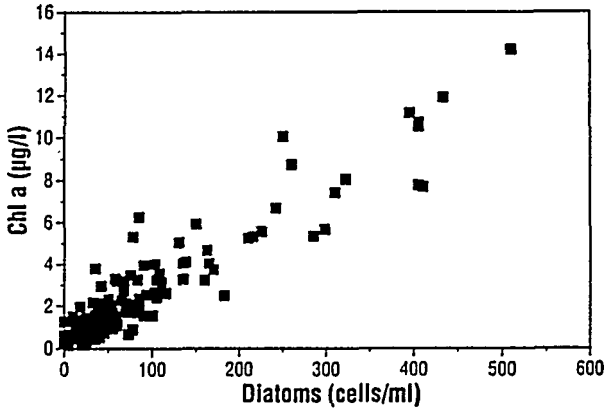

Fig. 14. Chlorophyll *a* concentration (µg/l) versus abundance of diatoms (cells/ml). Data from all stations and depths.

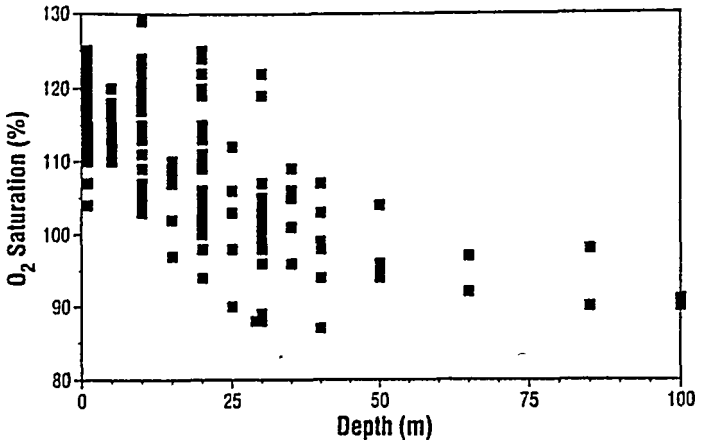

Fig. 15. Depth profile from all three transects of oxygen saturation (%) in the upper 100 m.

abundances of their potential prey, photosynthetic and heterotrophic nanoplankton and cyanobacteria.

Rates of bacterivory estimated via FLB uptake were highly variable from station to station, and with depth at each station sampled. Protists containing ingested bacteria were colorless flagellates at the smaller size range of the total community: they were primarily < 6 μm in ESD, and included a variety of morphological types of monads and choanoflagellates. At Station 14, 25 m depth, < 20 μm choreotrichous ciliates were sufficiently abundant (0.6 cells ml^{-1}) to warrant a separate estimation of their contribution to bacterivory. Ciliates were found to be responsible for 17% of the total rate of bacterial ingestion by the protistan community in this sample; in all other samples ciliate contribution was negligible. Cell-specific clearance rates averaged 1.7 ± 1.4 nl flagellate^{-1} h^{-1} (range of 0.4–7.0 nl flagellate^{-1} h^{-1}). The highest cell-specific clearance rate, as well as highest percentage of bacterial biomass cleared day^{-1}, occurred at Station 16, 20 m depth, where the Hnano community was dominated by 3–5 μm choanoflagellates. Community rates of bacterivory ranged from 0.0 to 1.5×10^7 bacteria grazed l^{-1} day^{-1}; bacterivory as a

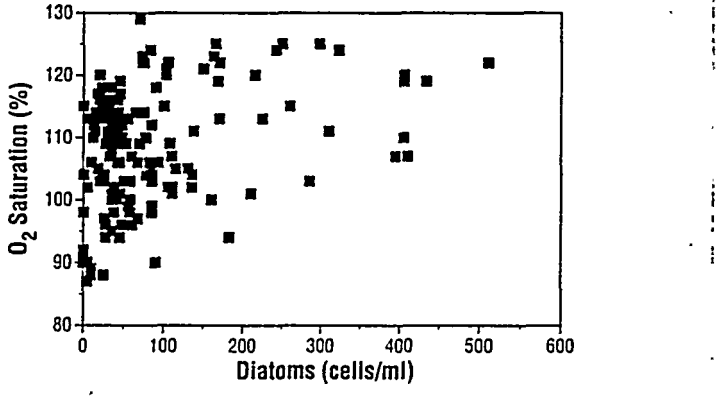

Fig. 16. Oxygen saturation (%) in the upper 100 m versus abundance of diatoms (cells/ml).

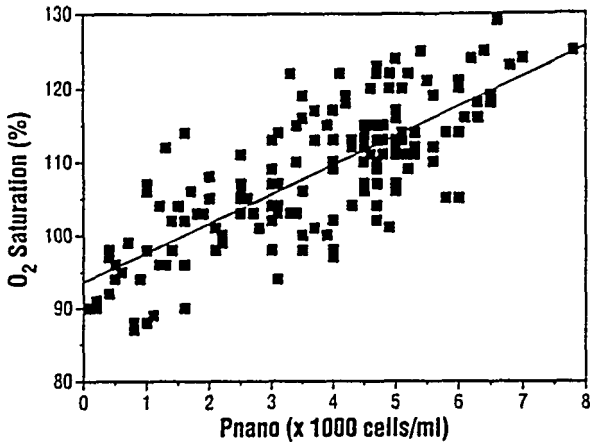

Fig. 17. Oxygen saturation (%) in the upper 100 m versus abundance of photosynthetic nanoplankton (10^3 cells/ml). Linear regression: O_2 saturation = (4.01)(Pnano) + 93.6, $r^2 = 0.58$.

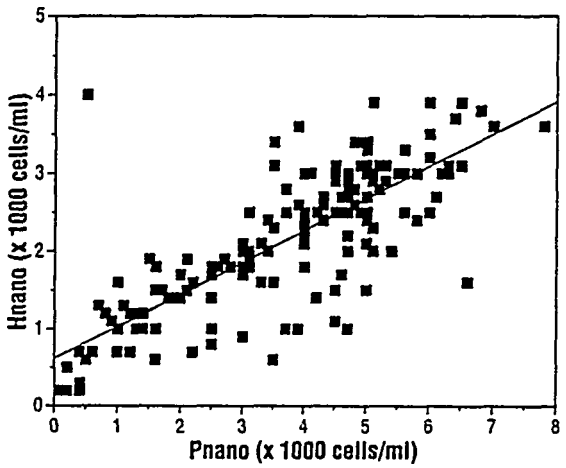

Fig. 18. Abundance of heterotrophic nanoplankton (10^3 cells/ml) versus abundance of phototrophic nanoplankton (10^3 cells/ml). Linear regression: Hnano = (0.41)(Pnano) + 0.64.

fraction of total bacterial standing stock ranged from 0.2% to 23.3% removal day^{-1} (Table 1). Integrated carbon flux from bacteria to bacterivores averaged 40, 98, and 70 mgC grazed m^{-2} day^{-1} at inner, mid, and outer stations respectively (Table 1).

DISCUSSION

The spring phytoplankton bloom in the Mid Atlantic Bight occurs in March–April (Falkowski *et al.*, 1988; Walsh *et al.*, 1988). Given that Cape Hatteras lies *ca* 400 km further south, thermal stratification and the spring bloom is expected to begin somewhat earlier there. In the present study, the upper water column of the Hatteras shelf was influenced by

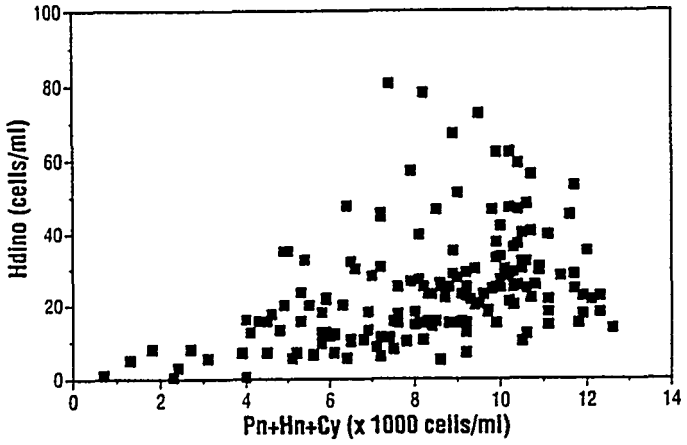

Fig. 19. Abundance of heterotrophic dinoflagellates (cells/ml) versus combined abundance of phototrophic nanoplankton, heterotrophic nanoplankton, and cyanobacteria (10^3 cells/ml).

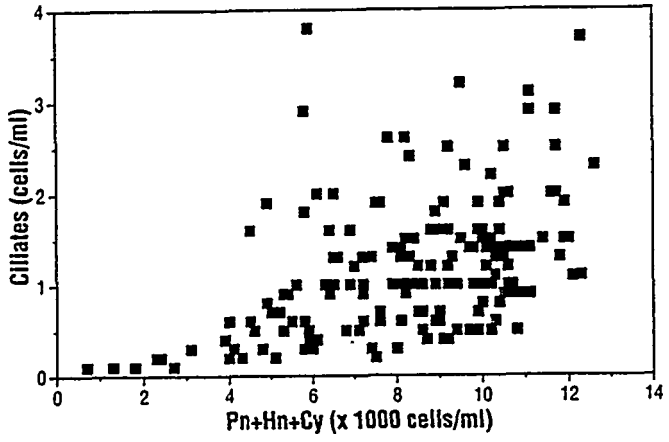

Fig. 20. Abundance of planktonic ciliates (cells/ml) versus combined abundance of phototrophic nanoplankton, heterotrophic nanoplankton, and cyanobacteria (10^3 cells/ml). Ciliates include plastidic and aplastidic taxa, excluding *Mesodinium*.

Table 1. *Average (± one standard deviation) of integrated bacterial biomass standing stock (mgC/m^2) and of rate of bacterivory estimated via FCB uptake (mgC grazed/m^2/d) for three regions of the shelf. Percentage of bacterial standing stock grazed per day was calculated from the first two parameters*

Region (Stations)	Bacterial biomass (mg C m^{-2})	Bacterivory (mg C grazed m^{-2} day^{-1})	% Biomass (Grazed day^{-1})
Inner (1, 23, 25, 28) (to 20–25 m)	703 ± 217	40 ± 13	5.7%
Mid (4, 7, 16, 19) (to 25 m)	965 ± 127	98 ± 35	10.2%
Outer (12, 14) (to 85 m)	1398 ± 126	70 ± 3.7	5.0%

Chesapeake Bay outflow to the north, which apparently was transported offshore at the surface by strong southwest winds and overrode shelf and cold pool waters. To the south, a Gulf Stream filament had moved onto the shelf, mixing with or displacing shelf water. Despite this complexity, nutrient distributions exhibited similar patterns on the three transects, with undetectable ammonium and nitrate concentrations of $<0.5\ \mu M$ down to 20 m, and a nutricline at 30–50 m on the outer shelf, shoaling to 20 m at midshelf (Wallace, unpubl.). Therefore a substantial drawdown of macronutrients had occurred prior to the cruise.

Diatoms were abundant at depths of 20–30 m, but not in shallower or deeper waters. Thus, they were found in or adjacent to the nutricline, and were likely light-limited below these depths and nutrient-stressed above. Irrespective of diatom physiology these deeper waters were not the sites of high primary production. Rather, the surface and near-surface waters dominated by small photosynthetic nanoplankton were apparently responsible for the major uptake of CO_2 and conversion into organic carbon during this time. The spring bloom in continental shelf waters is usually characterized as a "diatom event" (Malone *et al.*, 1983; Walsh *et al.*, 1988). While diatoms are important contributors to spring productivity, the evidence here suggests that smaller nanoplankton must be considered as well. Similar observations of co-occurrence of diatoms and nanoplankton in spring phytoplankton communities, or succession of diatoms by nanoplankton during the spring bloom, have been reported elsewhere (Sieracki *et al.*, 1993; Verity *et al.*, 1993). These data support the notion that the microbial or nanoplankton-based food web may be the rule rather than the exception in many planktonic ecosystems, with diatom blooms occasionally superimposed on this infrastructure (Smetacek *et al.*, 1990; Lenz, 1992; and citations therein). The general observation that episodic variations in phytoplankton biomass are often attributable to diatoms may partially reflect close coupling between smaller nanoplankton and their grazers. The joint positive correlations between photosynthetic nanoplankton and both oxygen saturation (implying significant net production) and micrograzers (implying substantial consumption) suggest that zooplankton dampened temporal variations in nanoplankton by responding quickly with increases in their own abundance, but were unable to maintain high biomass in the absence of elevated food concentrations.

Diatoms are traditionally associated with large herbivores, short food chains, and mass sedimentation, whereas nanoplankton are considered to support longer food chains with small grazers and extensive remineralization in the water column. The detailed analysis of plankton composition in the present study permits comparison of spatial trends in autotrophic and heterotrophic communities across and along the shelf.

Stations from the middle transect were selected to contrast communities on the inner and outer shelf (Table 2). Data from adjacent stations were combined because all plankton measurements were not collected at all stations. On the inner shelf, chl *a* integrated over 0–25 m (surface-bottom) was 87 mg/m^2. Phytoplankton biomass totalled 3.7 gC/m^2 and was comprised of *ca* 1/2 diatoms, 1/4 dinoflagellates, and negligible contributions by photosynthetic ciliates and cyanobacteria. On the outer shelf, chl *a* was 68 mg/m^2 integrated over 0–35 m (this range was chosen because it corresponded to that over which metazooplankton net tows were made; integration to the bottom of the euphotic zone would result in chl *a* concentrations equivalent to those in the shallower inner shelf). Diatoms retained their predominance and nanoplankton increased nearly two-fold in relative importance, while dinoflagellates decreased substantially. Carbon biomass of cyanobacteria, while relatively

Table 2. Budget of carbon biomasses of phytoplankton and heterotrophs at inner shelf and outer shelf stations on the middle transect. Data were integrated over 0–25 m and 0–35 m, respectively. See text for details

	Mid Transect Inner Shelf St. 25–26 (0–25 m) Chl*a* = 87 mg · m^{-2}		Mid Transect Outer Shelf St. 34–36 (0–35 m) Chl*a* = 68 mg · m^{-2}	
Phototrophs	mgC · m^{-2}	% Biomass	mgC · m^{-2}	% Biomass
Pnanos	810	22	991	38
Dinos	813	22	210	8
Diatoms	1825	48	1281	49
Mesodinium	225	6	63	2
Mixotrophic Ciliates	38	1	10	1
Cyanobacteria	30	1	60	2
Σ Phototrophs	3741	100	2615	100
C:Chl*a*	43		38	
Heterotrophs				
Bacteria	691	15	560	23
Hnanos	573	12	353	15
Hdinos	86	2	97	4
Ciliates	139	3	91	4
Nauplii	287	6	125	5
Metazooplankton	2857	62	1188	49
Σ Heterotrophs	4633	100	2414	100
H/P (%)	124		92	

insignificant, was twice as high on the outer shelf. Comparison of independently measured chl *a* to phytoplankton community carbon yielded C:Chl *a* ratios of 38–42 for inner-outer shelf communities. These ratios are similar to those of nutrient replete phytoplankton (Riemann *et al.*, 1989), including diatoms whose growth is light-limited (Yoder, 1979; Verity, 1981), suggesting that Hatteras communities in May were in healthy physiological state, and that the estimates of phytoplankton carbon were not wildly inaccurate.

Heterotrophic carbon biomass on the inner shelf, which totalled 4.6 gC/m^2, was dominated (62%) by juvenile and adult metazooplankton (Table 2). These were primarily small copepod genera, especially *Oithona* (Paffenhöfer ~~*et al., in prep*~~). Bacteria (15%) and heterotrophic nanoplankton (12%) were of secondary importance, while ciliates, dinoflagellates, and nauplii contributed relatively little carbon. On the outer shelf, metazooplankton decreased in relative contribution (49%) to heterotrophic carbon, while bacteria (23%) and nanoplankton (15%) increased in importance. Total heterotrophic carbon on the outer shelf (2.4 gC/m^2) was *ca* 1/2 that on the inner shelf. Comparison of total heterotrophic carbon to phototrophic carbon (H/P) yielded values of 92–124% on the middle transect.

A comparison of midshelf stations from the northern and southern transects is given in Table 3. Chl *a* integrated over 0–35 m was 285 mg/m^2 (north) vs 45 mg/m^2 (south). This large difference reflected the enormous contribution by diatoms (5 gC/m^2 = 70% of phytoplankton standing stocks) at northern stations. Other algal groups were comparatively unimportant. In contrast, to the south, diatoms decreased by nearly one order of

Table 3. Budget of carbon biomasses of phytoplankton and heterotrophs at midshelf stations on the northern and southern transects. Data were integrated over 0–25 m and 0–35 m, respectively. See text for details

	North Transect Midshelf St. 31 (0–35 m) Chl*a* = 285 mg · m^{-2}		South Transect Midshelf St. 7–8 (0–35 m) Chl*a* = 45 mg · m^{-2}	
Phototrophs	mgC · m^{-2}	% Biomass	mgC · m^{-2}	% Biomass
Pnanos	872	11	664	31
Dinos	847	11	315	15
Diatoms	5538	70	614	28
Mesodinium	511	6	465	22
Mixotrophic Ciliates	138	2	54	2
Cyanobacteria	18	0	38	2
Σ Phototrophs	7924	100	2150	100
C:Chl*a*	28		48	
Heterotrophs				
Bacteria	1400	46	911	31
Hnanos	541	18	969	33
Hdinos	55	2	260	9
Ciliates	257	9	344	12
Nauplii	122	4	15	0
Metazooplankton	652	21	452	15
Σ Heterotrophs	3027	100	2951	100
H/P (%)	38		137	

magnitude. Other algal groups except cyanobacteria also declined, but much less so, such that carbon biomass of photosynthetic nanoplankton (31%) was similar to that of diatoms (28%); plastidic ciliates (24%) and dinoflagellates (15%) were also important. Overall, phytoplankton carbon decreased four-fold over 110 km in the alongshore direction. The phytoplankton C:Chl *a* ratio increased from *ca* 30 to 50 over this distance and, interestingly, bracketed the estimates of 38–43 for the middle transect.

Heterotrophic carbon at northern stations (Table 3) was dominated by bacteria (46%). The most significant form of zooplankton carbon was adult and juvenile metazooplankton (21%) and heterotrophic nanoplankton (18%). In contrast, to the south bacterial carbon decreased by half. Among zooplankton, metazoans also declined while heterotrophic nanoplankton, dinoflagellates, and ciliates maintained or increased their relative and absolute importance. The ratio of heterotrophic:phototrophic carbon increased from 38% on the northern transect to 137% to the south, which, like the C:Chl *a* ratios, bracketed the values on the mid transect (92–124%).

Thus, algal carbon decreased from *ca* 8 gC/m^2 on the north transect to 3–4 gC/m^2 (mid) to 2 gC/m^2 on the southern transect, while the diatom-dominated algal community was replaced by photosynthetic nanoplankton. Heterotrophic carbon remained comparatively constant at 3–5 gC/m^2 from north to south. Metazooplankton declined in importance from inshore to offshore and from north to south, while nano- and microzooplankton increased over the same spatial domain. Net watermass transport on the shelf from Georges Bank to

Table 4. Estimated carbon biomass and ingestion by zooplankton from inner and outer shelf stations on the middle transect. See Method for details of calculations

	Mid Transect Inner Shelf		Mid Transect Outer Shelf	
Grazer	Biomass (mgC/m^2)	Ingestion ($mgC/m^2/d$)	Biomass (mgC/m^2)	I ($mgC/mg^2/d$)
Hnano	298	1117	212	794
Hdino	86	248	97	280
Ciliates	139	385	91	252
Nauplii	287	144	125	53
Metazooplankton	2857	2261	1188	358
Total	3667	4155	1713	1737

Cape Hatteras is southwesterly, with an areal mean alongshelf current of 6 cm/sec (Falkowski *et al.*, 1988). Little cross-shelf transport typically occurs in the Mid-Atlantic Bight, such that 50–60% of the along shelf transport is believed to exit at Hatteras. The distance between the northern and southern transects in the present study was *ca* 110 km which, at 6 cm/sec, would require 21 days to complete. Observations in enclosed water masses elsewhere at similar temperatures indicate that this is more than sufficient time for a four-fold decline in phytoplankton biomass and a conversion from a diatom-dominated community to one composed primarily of phytoflagellates. Thus, while this study represents but a snapshot in time over broad spatial scales, these data are consistent with the notion that, as shelf waters advected from north to south, phytoplankton and zooplankton communities changed to smaller cells, and phytoplankton carbon was converted to heterotrophic biomass.

From these biomass data and literature measurements of feeding rates at similar concentrations of phytoplankton carbon, first order constraints can be placed upon the relative contribution of the various zooplankton groups to total carbon ingestion (Tables 4 and 5). To the north (Table 5), zooplankton community ingestion of POC was estimated at 3.1 gC/m^2/day, with protozoans, particularly nanoplankton (39%) and ciliates (23%), apparently being responsible for much grazing. Despite higher biomass than protozoans,

Table 5. Estimated carbon biomass and ingestion by zooplankton from midshelf stations on the northern and southern transects. See Method for details of calculations

	Midshelf north		Midshelf south	
Grazer	Biomass (mgC/m^2)	Ingestion ($mgC/m^2/d$)	Biomass (mgC/m^2)	I ($mgC/m^2/d$)
Hnano	325	1217	395	1480
Hdino	55	159	260	750
Ciliates	257	712	344	954
Nauplii	122	62	15	5
Metazooplankton	652	933	452	136
Total	1411	3083	1466	3325

copepods were estimated to contribute only *ca* 30% due to their relatively low specific ingestion. This pattern may account for the persistence of large, presumably slowly-growing diatoms at depth in northern waters. On the middle transect (Table 4), however, juvenile and adult metazooplankton may have been the dominant grazers (54%) in inner shelf waters, while protozoans replaced them offshore. The importance of crustacean zooplankton in inner shelf waters (Fig. 12) coincided with decreases in diatom compared to northern waters. In southern midshelf stations, apparent metazooplankton grazing (4%) declined concurrent with the nine-fold decrease in diatom carbon (Table 3), leaving protozoans as the likely major herbivores.

The high percentage contribution of phagotrophic protists to overall rates of zooplankton herbivory is not surprising in light of recent theoretical and empirical evidence regarding the grazing impact of microzooplankton on phytoplankton. Phagotrophic protists are ubiquitous in marine systems, can utilize all size ranges of phytoplankton, and can grow as fast as, or faster than, their prey (reviewed in Sherr and Sherr, 1991, 1994). During the past decade, microzooplankton grazing rates, determined via the dilution method of Landry and Hassett (1982), have been evaluated at a number of sites in the world ocean (reviewed in Sherr and Sherr, 1994). Even in a coastal upwelling system dominated by chain-forming diatoms, protists, including extracellular-feeding armored dinoflagellates, consumed 26–50% of daily production (Neuer and Cowles, 1994). During the spring bloom in the North Atlantic, protists were estimated to graze 39–115% of daily phytoplankton production, while copepods consumed only about 1–2% of production (Burkill *et al.*, 1993; Verity *et al.*, 1993). The data presented here confirm the overall importance of protists, including $< 20\ \mu m$ flagellates, to herbivory in marine food webs.

Given the inadequate spatial resolution of the biomass estimates, detailed comparison of the calculated ingestion of each zooplankton group with the standing stocks of potential prey items seems unwarranted, especially since many of these zooplankton can be omnivorous. Provocatively, however, comparison of estimated zooplankton community ingestion to phytoplankton carbon indicates a trend from 39% in the north to 66–114% on the mid transect to 155% in the south. This spatial pattern in estimated ingestion is consistent with conclusions drawn from measurements of autotrophic and heterotrophic community composition and biomass, namely that phytoplankton carbon was converted to zooplankton carbon as shelf waters presumably advected south.

Although both bacterioplankton and Hnano were a significant fraction of total heterotrophic biomass (Tables 2–3), rates of consumption of bacteria by Hnano were relatively low, equivalent to an average turnover rate of standing stock bacterial biomass of 6% day^{-1} at inner stations, 10% day^{-1} at mid stations, and 5% day^{-1} at outer stations (Table 1). If bacterial standing stock abundances were in steady state during the sampling period, these rates of bacterivory may be considered a lower-limit estimate of rates of bacterial biomass production. Preliminary comparison of these data with empirical estimates of bacterioplankton cell production rates carried out on the same water samples indicated that the rates of bacterivory were in fact in the general range of 50–100% of rates of bacterial production (Kemp, personal communication). Calculated rates of carbon flux due to Hnano bacterivory (Table 1) were more than an order of magnitude less than those estimated due to Hnano herbivory (Tables 4–5). These results support the idea that heterotrophic flagellates $< 20\ \mu m$ in size are likely to have a quantitatively more important role as grazers of phytoplankton than as consumers of bacteria in marine food webs (Sherr and Sherr, 1991, 1992).

SUMMARY

Carbon budgets for Cape Hatteras in May indicated that inner shelf waters contained *ca* 50% more living POC than outer shelf waters. The relative importance of large phytoplankton and grazers decreased with distance offshore, and they were replaced by photosynthetic nanoplankton and nano/microzooplankton. Even greater changes in living POC occurred in the alongshore direction than cross-shelf, primarily due to the dramatic reduction in large phytoplankton in southern waters. The ratio of heterotrophic:autotrophic POC increased from 38% in northern waters to 137% in southern waters, suggesting that phytoplankton carbon was being converted into consumer carbon as shelf waters advected south. Calculated removal of plankton POC by zooplankton grazing was *ca* 2–4 $gC/m^2/day$, and the patterns of relative importance of large and small zooplankton were consistent with changes in the size structure of the phytoplankton community.

To the extent that declines in diatoms were due to grazing by adult crustacean zooplankton, whose fecal material typically sediments rapidly to depth (Paffenhöfer and Knowles, 1979) then considerable diatom carbon may have remained on shelf/slope sediments. However, the dominant primary producers on all three transects were apparently small photosynthetic nanoplankton, which supported an active protozooplankton community, whose fecal products generally remain in suspension (Stoecker, 1984). Moreover, fecal pellets formed by copepods feeding on nanoplankton sink significantly more slowly than those of copepods feeding on diatoms (Beinfang, 1980). Since most of the metazooplankton biomass on the Hatteras shelf in May was in the form of small copepods (e.g. *Oithona*: Paffenhöfer *et al.*, in prep.) which can feed upon nanoplankton (Uchima and Hirano, 1986; Paffenhöfer, 1993) and whose fecal pellets also remain in suspension in shelf environments (Paffenhöfer and Knowles, 1979; Small *et al.*, 1979) much of the non-diatomaceous POC may have been exported with shelf waters as they were entrained offshore at Cape Hatteras.

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Organism Life Cycles, Predation, and the Structure of Marine Pelagic Ecosystems

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ABSTRACT: This paper explores the notion that the theoretical basis for contemporary research concerning the structure and function of marine pelagic ecosystems is self-limiting. While some findings such as the microbial food web have extended our knowledge of the biological components of the upper water column and their relationships to fluxes of materials and energy, they have not advanced our understanding of why specific pelagic forms occur in time and space, and why only some attain dominant status and contribute the bulk of biogenic fluxes emanating from the mixed layer. It is argued here that a major impediment to improved conceptual models is the historic focus on resource-driven or "bottom-up" factors as being the dominant variables structuring planktonic ecosystems. Evidence is presented that predation or "top-down" trophic effects may be equally important in specifying the occurrence of particular taxa, the biomass within adjacent trophic levels, and the morphology of dominant herbivores and carnivores. It is suggested that key species, because of unique combinations of life history strategies, metabolic demands, and physiological performance, may exert a dominant role in the extent to which predatory interactions cascade through pelagic food webs. There is considerable evidence of evolution of predation avoidance strategies among phytoplankton and zooplankton. It is proposed that future research might profitably be directed towards the question of how the pelagic environment selects for life histories and morphologies of organisms under conditions when resource availability and predation are both significant structural buttresses. Methodological approaches should include detailed studies of dominant key taxa from different environments, with the goal of identifying the critical aspects of life history, behavior, or morphology which account for their success.

KEY WORDS: Morphology · Life History · Plankton · Pelagic · Top-down · Trophic Cascade · Predation · Ecosystem Structure · Bottom-up

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Conceptual Overview

Quantitative understanding of marine processes has increased rapidly in the past decades driven by the development of new methods and tools enabling measurement, mapping and modelling of the fluxes and cycling of biogenic elements, at scales extending from molecular diffusion to global ocean circulation. However, these substantial advances in quantification and delineation of biogeochemical processes and provinces have not been accompanied by commensurate progress in understanding of the mechanisms that structure pelagic ecosystems. Thus, the distribution patterns of dominant pelagic species are fairly well established, yet we do not know why they occur when and where they do. What is lacking is knowledge of the nature of properties that gear given species to specific environments, and which are responsible for their occurrence or persistence in given water masses.

For example, we do not clearly understand why only a few diatom genera dominate oceanic new production, or why calanoid copepods contribute the bulk of marine zooplankton biomass. These are important questions because the respective diatoms and copepods each impact the fate of organic matter produced or processed by them in unique ways: the silica frustules of diatoms and the compact fecal pellets of copepods both enhance vertical flux of organic matter. In contrast, the gelatinous colonies of *Phaeocystis*, which dominate new production in some environments, often contribute less to vertical flux than the equivalent biomass of diatoms (Wassmann 1994). Further, *Daphnia* - the dominant zooplankter in many lakes - produces loose fecal matter that disintegrates following release (Lampert 1987) and hence does not contribute to vertical flux. Thus, neither of these properties - silica frustules and fecal pellets - can presently be related to the dominant role diatoms and copepods play in the marine pelagial.

The above examples illustrate the extent to which biological properties of dominant organisms determine patterns of biogeochemical cycling in today's ocean, but they also demonstrate the substantial gaps that still exist in our understanding of marine ecological processes and which seriously hamper further progress. The traditional approach to the study of marine pelagic ecology is biased towards factors regulating growth of organisms, i.e. resource acquisition (bottom-up forces) is accorded more importance than predation (top-down forces) in regulating the structure and functioning of marine pelagic systems. We argue that this approach is now self-limiting as it has not provided an adequate framework to advance our understanding of how natural selection works in the marine pelagial.

We advocate here that predation and resource availability act through morphologies and life history strategies of organisms to structure pelagic ecosystems, and hence to drive biogeochemical cycles. The potential significance of the biological pump (organism-mediated incorporation of inorganic carbon into organic carbon, and its sequestration into the interior ocean via downward flux of biogenic particles), and our uncertainty in resolving that role (Longhurst & Harrison 1989), speaks volumes for the need of an improved mechanistic understanding of the linkages between cause and effect in plankton dynamics. For example, changes in food web structure have been associated with altered patterns of vertical flux in Lake Michigan (Kitchell & Carpenter 1987) and the North Atlantic Ocean (Sieracki et al. 1993). In other words, changes in structural components of the pump lead to changes in how the pump works. But what are the linkages, and how do the biological components direct one another? The ideas which follow are by no mean designed to promote one architectural principle over another (e.g. resource limiation vs. predation), but to emphasize that it may be

more valuable to understand why carbon flows where it does, rather than merely how much.

The Role of Organism Life Cycles and Morphologies

The bulk of large-scale biogenic cycling and production of fish food is dependent on relatively few taxa and morphotypes. In the following we discuss some examples of key species and their morphologies to illustrate how specific knowledge of individual biologies will greatly improve our understanding of the forces shaping ecosystem structure and driving biogeochemical fluxes. This information holds considerable promise for development of a predictive framework.

Fragilariopsis (Nitzschia) kerguelensis: This is a medium-sized (15-30μm long) pennate diatom that lives in ribbon-shaped chains of 20-100 cells and forms almost monospecific blooms in the Antarctic Polar Frontal Zone (Bathmann et al. 1994). Its global significance is bestowed by its inordinately robust siliceous cell walls which contribute the bulk of the silica deposits in the underlying sediments (Burckle & Cirilli 1987). Indeed, deposition of its frustules has continued unabated through glacial and interglacial cycles. This siliceous girdle around the continent is reputed to accumulate fully 75% of the biogenic silica being deposited in today's ocean (Ledford-Hoffman et al. 1986) although only 20% of global biogenic silica production occurs in these waters (Treguer et al. 1995). It is tempting to speculate that the thick cell wall of *F. kerguelensis* is somehow selected for as a grazer deterrent in these waters, inducing blooms and mass sedimentation. It follows that these factors, be they sinking and seeding strategy (sensu Smetacek 1985) or grazer protection or both, indirectly shape the ocean silica budget. But what are these factors?

Emiliania huxleyi: This is a small (<10μm) coccolithophorid that forms blooms in many different regions of the world oceans in both high and low latitudes (Brown & Yoder 1994) but also from oceanic to coastal regimes (Berge 1962). Its sedimentary record indicates that this amazing ability to grow to bloom proportions under such a wide variety of conditions has developed only since the Holocene. Prior to the last Ice Age, another small species *(Gephyrocapsa),* now of minor importance, dominated coccolithophorid blooms. Circumstantial evidence from mesocosm studies implicates viral attack rather than nutrient exhaustion in demise of two *Emiliania* blooms studied in successive years (Bratbak et al. 1993). Indeed Emiliani (1993) himself suggested that viral infections were a significant factor in driving species extinctions in marine plankton. Although biogeochemical fluxes (DMS and carbonate) mediated by this species are only important on a regional scale (Brown & Yoder 1994), its extraordinary ecological flexibility cries out for elucidation. Molecular genetic studies indicate that open ocean and coastal forms belong to the same species (Barker et al. 1994); however, when clonal cultures from the same mesocosm experiment were compared with one another, a surprising degree of genetic variability within the bloom population came to light. *Emiliania* is indeed a tantalizing species.

Phaeocystis: Phaeocystis is a polymorphic phytoplankton taxon whose life cycle includes, but may not be limited to, solitary flagellated cells of 3-8 μm and gelatinous colonies of non-flagellated cells up to 2 cm in diameter (Kornmann 1955, Verity et al. 1988, Rousseau et al. 1994). While some uncertainty remains about taxonomic identity, details of its life cycle, and transitions therein (Baumann et al. 1994, Cariou et al. 1994, Medlin et al. 1994), *Phaeocystis* is well known for prodigious blooms of the colonial phase. These occur

primarily in boreal and polar waters of both hemispheres, but occasionally in temperate and tropical waters (Kashkin 1963, Baumann et al. 1994). These blooms are important regionally and seasonally to primary production, and their significance to global CO_2 sinks has also been promoted (Smith et al. 1991). There is evidence that changes in the size structure of *Phaeocystis* blooms can alter the zooplankton community (Hansen et al. 1994), and that omnivorous zooplankton in the presence of *Phaeocystis* prey instead on protozoans, thereby releasing *Phaeocystis* from herbivory (Hansen et al. 1993). In the absence of substantial grazing pressure, sedimentation of colonies occurs in some environments (Wassmann 1994), although emigration of cells out of the colonies might minimize vertical export of cellular carbon (Verity et al. 1988). *Phaeocystis* is also a copious producer of dimethylsulfide (DMS) and its precursor in seawater, DMSP (Stefels & van Boekel 1993), so that *Phaeocystis* may be important to global sulfur balances, acidification of atmospheric aerosols and rain, and climate change (see Liss et al. 1994 for review). Thus factors influencing its productivity and fate may significantly and interactively affect biogeochemical cycling. However, the fate of *Phaeocystis* colony blooms seems intrinsically associated with its life cycle and the associated match or mismatch with herbivores.

Euphausia superba: Antarctic krill is *the* key species of the Southern Ocean as it maintains an enormous biomass (still in excess of *Homo sapiens*) in an area only twice the size of the USA. Its average density, using conservative stock estimates, is at least 10-30 individuals $\cdot$ m^2 equivalent to 1-3 gC $\cdot m^2$! None of the other species of pelagic euphausiids are as prominent in their respective habitats as krill in the Southern Ocean; therefore, the unique status of krill has to be explained by unique features of its biology and/or its habitat.

Apart from its larger size, krill is very similar to other euphausiids, hence any unique ability is not reflected in its morphology. Productivity of the krill habitat - the region bounded by the winter extent of sea ice around Antarctica - is rather low and the composition and biomass of plankton, contrary to earlier belief, is similar to that of other high-latitude oceans (Smetacek et al. 1990). Krill utilizes a wide range of food sources: phytoplankton, ice algae (Marschall 1988) and copepods (Huntley et al. 1994) and can easily survive long periods of starvation (Ikeda and Dixon 1982). Clearly, versatility is necessary if such a large portion of the annual primary production is to be sequestered in a single population, and Smetacek et al (1990) argue that it is the way in which *E. superba* has geared its life cycle to the seasonality of sea ice that enables it to maintain such large stocks. However, a notable feature of krill is the long life-span and consensus is now emerging that individuals live for at least five or six years (Knox 1994) which is very unusual among pelagic crustacea and implies a slower turnover rate of the population than previously believed. It follows that the exceptional stock size of krill when compared to euphausiid populations of other oceans cannot be explained by a faster growth rate but is more likely due to a lower mortality rate, as discussed below.

The unique feature of the krill habitat is the presence of the seasonal sea-ice cover which is utilized by krill as a food source but which also offers protection particularly during winter, from the air-breathing, visual predators that are the chief predators of krill. Another unique feature of the krill habitat is the relative absence of clupeid-type fish swarms. Although a clupeid morphotype has evolved in the Antarctic *(Pleurogramma antarcticus)*, its predation pressure on local euphausiids is negligible compared to that of capelin, herring, sardines etc. in their respective habitats. We speculate that this is due to the stress that hypoosmotic

teleosts would experience in continuous, close contact with sea-ice. Whatever the reason, the fact remains that their relative absence reduces predation pressure not only on krill but also on their alternative food - copepods - which are hence available to krill. Indeed, the actively foraging krill swarms encountered in open water during summer are themselves reminiscent of clupeid fish. It follows that the presence of sea ice, by changing predation patterns, leads to a biomass bulge in the euphausiid group, which in turn, has considerable ramifications for structure and population dynamics of the predators. The implications of the krill example for our conceptual view of factors constraining marine zooplankton populations are discussed next.

The organisms described above represent but a few examples of species whose morphologies or life history strategies are sufficiently successful that they dominate their respective environments in time and space. *They may hold space because they sequester resources (bottom-up structuring) better than their rivals, or because they avoid or inhibit predators (top-down).* The former strategy has generally attracted the most attention in marine plankton research. We consider below some conceptual arguments why equal attention should be focussed upon the significance of predation, and the potential selective pressure of avoiding being eaten, in conceptual models of food web structure and biogeochemical fluxes in the marine pelagial.

Predation Constraints on Zooplankton Morphology

Oceanic zooplankton are the most wide-spread form of animal life on earth with the longest history of evolutionary continuity. Yet the number of taxa is orders of magnitude

smaller than that of the marine benthos. Furthermore, the bulk of the biomass is present within only a few morphotypes represented by dominant species of copepods, euphausiids, pteropods, and chaetognaths. Among holoplankton, the same basic body shape is maintained through 10-100x variation in body volume in many cases. The special case of gelatinous zooplankton is discussed further below. The perceived similarity within the groups mentioned above is all the more striking when compared with morphological variation within the major benthic groups. Here we discuss the respective roles of food acquisition and predator avoidance as constraints on morphology using the most ubiquitous form - the epipelagic copepods - as an example of a highly successful body shape.

The similar morphology of calanoid copepods which is retained through about two orders of magnitude range in length contrasts strikingly with the degree of genetic variation (Bucklin et al. 1995) suggesting strong environmental constraints on morphology. The constraints do not appear to be dictated by food acquisition strategies because copepods as a group are known to feed on a wide variety of foods ranging from small suspended particles to zooplankton of equal size. Indeed, gut contents and the actual process of feeding need to be examined if one is to find out what constitutes the major food source of the various species (Paffenhöfer 1988; Turner 1991). It follows that food acquisition (bottom-up control) is not a major determinant of general body shape of epipelagic copepods.

Life history patterns also vary widely, ranging from an apparent absence of seasonality, even in polar waters, to presence of diapausing eggs in coastal species and diapausing sub-adults in many oceanic copepods (Smith & Schnack-Schiel 1990). Again, a particular life history does not select the morphology of the various life cycle stages that all copepods

undergo, although geographical distribution of the various species is probably constrained by this factor. Differences in reproductive behavior are manifested in relatively minor details such as morphology of appendages involved in transfer of sperm, although females of some species carry conspicuous egg sacs while others release them as they are produced. We are left with predation as the major constraint on copepod morphology and several arguments can be broached in support of this.

Firstly, all copepods throughout their respective ranges share the same types of predators - active hunters such as chaetognaths and fish, and passive trappers such as jellies and foraminifera. Most copepod predators are too large to be deterred by defenses such as spines or thick carapaces formed in limnetic cladocerans and none of these have been developed by copepods. Camouflage from visual hunters is effected by small size and transparency but also by avoiding the epipelagial during the day using diel vertical migration (DVM) and both these properties are well developed in copepods. Hays et al. (1994), in a comprehensive study of DVM behavior in copepod species across the North Atlantic, found that large and pigmented species migrated more. They conclude that "the risk of predation from visual predators (is) the most important factor influencing taxonomic differences in DVM."

Upon detection escape from attacking predators is by flight and, to our knowledge, all copepods can "jump" considerable distances when threatened. Hardy (1956) stated "if it *(Calanus)* makes a sudden spurt the first antennae fold back to offer the least resistance", propulsion being effected by the "oar-like thoracic limbs". The much shorter antennae of those copepods with well-developed eyes (e.g. *Corycaeus*) suggest a sensory (mechano-tactile) function of these appendages. Experimental studies indicate that the first antennae are

environmental sensors (Gill & Crisp 1985, Bundy & Paffenhöfer 1993), which could function to perceive food (Paffenhöfer & Lewis 1990) or predators. Support for the latter comes from those species in which adult males cease feeding and reduce their mouth parts whereby the first antennae and the basic morphology, however, are retained, although locating females by e.g. pheromones will also be an important function. Indeed, in some male copepods one of the antennae is hinged and used to embrace the female during mating (Hardy, 1956). Apart from this minor modification, male and female antennae are so similar that one would expect the same function in both sexes. Escaping predation is, of course, equally important in males as in females. Short-range predator recognition would be worthless were it not followed by an effective escape response which is a common feature of copepods. Indeed, about two-thirds of the body is occupied by the segments carrying the thoracic limbs responsible for flight. Vertical migration and horizontal swimming is also effected by these limbs, although slower speeds require correspondingly less investment in muscle. In the absence of evidence to the contrary, we suggest that the characteristic shape of the cephalothorax of copepods is selected more by flight ability from predators (top-down) than foraging efficiency (bottom up).

That genetic constraints on morphotype are not involved is amply demonstrated by the diverse grotesque morphologies of parasitic copepods that attach themselves to much larger hosts. These aberrant forms belong to the Poecilostomatoida and cyclopoid groups which in general exhibit much greater morphological plasticity than the more common calanoids. Steinberg et al. (1994) found that copepods were the most numerous animals living on discarded larvacean houses at midwater depths (100-500m) and that many of these species "possess benthic-like morphology and feeding strategy". This example also indicates that

morphology can well be modified to improve food acquisition. However, these species are only common below the depth where visual predation is efficient. The dominant cyclopoid genus in the epipelagial, *Oithona*, has a morphology remarkably similar to that of the common calanoids. Thus a substantial case can be argued for predation as a major factor constraining copepod morphology. In the past, feeding behavior of marine copepods has received much more attention than mechanisms of predator avoidance (see Kerfoot & Sih 1987, for freshwater zooplankton). This imbalance now needs to be rectified. We dwell next on some implications for pelagic food web structure.

We have argued that the characteristic copepod morphology is an effective way of avoiding predation in their respective size class. Further up the size spectrum, the euphausiid morphology, also shared by mysids and decapod shrimps, appears to be a better way of avoiding predators and maintaining large stock size. In this morphological group, the large muscular abdomen is used for the typical tail-flip flight mode which differs strikingly from the "normal" swimming pattern. The large investment in body tissue solely for flight is very evident here. The stream-lined form of chaetognaths and juvenile fish will be equally effective in capturing motile prey and escaping predators, however each uses a different strategy. Both rely on transparency to disguise themselves from predator and prey. The typical motionless hunting behavior of chaetognaths, interrupted by short distance darts for prey capture, further contributes to their invisibility. However, while fish larvae are also comparatively transparent, their well-developed pigmented eyes are not; this undoubtably increases both their detection of prey and their perception by predators. In contrast, chaetognaths have almost completely eliminated pigmented organs and instead rely upon

sensory hairs to detect prey and predator (R.L. Hopcroft pers. comm.).

So far we have only considered the non-gelatinous zooplankton that provide the bulk of the food for the commercially important fish stocks. The biomass of gelatinous zooplankton (hereafter jellies) e.g. coelenterates, ctenophores, salps and doliolids, does not flow up the economically important food chain and, because they eat the same food as the other zooplankters, they have long been considered a trophic dead-end, perhaps unjustly since over 100 fish species are reported to occasionally or regularly ingest jellies (G. R. Harbison, pers. comm.). Perhaps freed from the need to escape from attacking predators, jellies exhibit a much wider range of shapes than their more muscular counterparts. For example, three ctenophore genera *(Pleurobrachia, Beroe,* and *Mnemiopsis)* have strikingly different morphologies, each associated with a specific food acquisition strategy. In coelenterates, too, the range in body shape is wider than e.g. among free-living copepods and feeding behavior more easily apparent. Furthermore, the barrel-shaped body plan of salps and doliolids can be readily explained solely on the basis of food acquisition.

The common feature of jellies is their high volume/plasma ratio, which is widely believed to deter predators because of the low nutritional value. However, the constraints on eating jellies must be more subtle as there are enough examples of non-gelatinous predators of jellies (e.g. pelagic turtles, moon-fish, stromateoid fishes) to show that vertebrates can indeed make a living off jellies. But turtles and moon-fish are morphologically very different from the standard pelagic fish shape (exemplified by clupeids), and the stromateoid fish have a crop in their digestive system (Bühler, 1930) presumably to facilitate feeding on jellies (G. R. Harbison, pers. comm.). The question is, why are jellies not a principal dietary component of

commercially important fish. The answer may reflect the sleek, streamlined form of the dominant epipelagic fish which, as in the case of the zooplankters dealt with above, is maintained over several orders of magnitude (from anchovies to tuna). The bodies of these fish consist largely of flight muscle: 80-95% of the swimming musculature is white muscle which enables the high velocities necessary to avoid predators or to capture prey, with a correspondingly lesser investment in red muscle dedicated to 'normal' swimming (Bone 1978, Driedzic & Hochachka 1978). As in the case of copepods discussed above, fish body form deviates, often grossly, from the streamlined form wherever the environment offers protection from visual predators. This is exemplified by the grotesque shapes of deep-sea fish but also surface-living *Sargassum* fish that are models of effective camouflage. While streamlining may facilitate rapid motions to capture prey (Kils 1990), the above considerations imply that escape from predators may be equally or more important than resource acquisition in constraining morphotype of epipelagic fishes [Among lizards, for example, speed is used for escape from predation rather than food capture (Hertz et al. 1988)]. This streamlining may also be the selective factor primarily responsible for the development of gape limitation in pelagic systems (Hairston & Hairston 1993). In contrast, a diet composed solely of jellies inevitably requires large gut volume, and a "belly full of jelly", by producing drag, will critically reduce escape speed. It follows that exclusive jelly feeders will not be able to maintain the body form and muscle necessary for survival in the epipelagial, and we suggest that this may be the underlying reason for the dichotomy between the muscular and the gelatinous food chains. As always in nature exceptions occur, such as streamlined mackerel actively preying on small medusae in the laboratory (Runge et al. 1987) and on salps in

nature (Konchina 1991), however such events are unlikely to reflect the principal diets of these and similar-bodied fish given the highly episodic nature of abundant distributions of gelatinous planktonic organisms. Conversely, perhaps the potential ability of a school of predatory fish to nearly exterminate a patch of jellies favored another life history strategy: the very high rates of population growth characteristic of smaller gelatinous forms, e.g. salps, doliolids.

Predation Avoidance and the Arms Race

It is a delicate balance to find sufficient food while avoiding being eaten and there are several hypotheses describing the interdependent nature of organisms in evolution. It is generally recognized that terrestrial plants coevolve with their insect herbivores (Ehrlich & Raven 1964, Strong et al. 1984), with attendant costs, e.g. plant growth rates are inversely proportional to their chemical defenses (Coley et al. 1985). Another theory, recently termed escalation (Vermeij 1994), proposes that enemies are the primary agents of natural selection among organisms, and that predation-induced adaptations direct long-term behavioral and morphological traits. This theory, described elsewhere as an "arms race" (Dawkins 1987), proposes that as predators evolve more effective weapons systems, prey match these advances with equally successful antidotes. These arms races do not escalate out of control, or evolve into perfect capturing and escaping machines, because biological weaponry (e.g. speed to capture prey or to escape predators) must be bought at some other escalating cost, e.g. decreasing reproductive success.

Signor & Vermeij (1994) hypothesize that herbivorous and carnivorous plankton originally evolved as refuges from predation by marine benthic organisms. *The present paper argues that the concept of the arms race is currently applicable to the marine pelagial where, if predation is so important, selection for predator avoidance mechanisms should be evident.* Among plankton, predator avoidance strategies appear well developed, though perhaps not broadly distributed, and fall into four general categories: chemical, morphological, behavioral, and life history defenses. A few of these are described below; the reader is directed towards detailed reviews elsewhere (Havel 1987, Sih 1987). Predation avoidance has also recently been evaluated as a selection pressure acting on bacteria (Jürgens & Güde 1994).

Chemical Defenses. Several classes of phytoplankton are known to include taxa which are inhibitory or toxic to herbivores. Examples include the presumed raphidophyte *Olisthodiscus luteus/Heterosigma akashiwo* (Verity & Stoecker 1982, Van Alstyne 1986); several dinoflagellates (Huntley et al. 1986, Taniguchi & Takeda 1988); and the freshwater cyanophytes *Microcystis* and *Aphanizomenon* (Fulton & Paerl 1987, Jungmann et al. 1991). The active agents are generally unknown but presumed to be secondary metabolites sequestered within the cells (Faulkner 1984, Carmichael 1986). Some diatoms apparently contain or excrete chemical compounds which interfere or inhibit grazing by copepods (Malej & Harris 1993) and reduce egg production and hatching success (Poulet et al. 1994). While it cannot be certain that such compounds evolved in response to predation, i.e. allelochemical interactions within a trophic level may also be important (Lewis 1986), they are widely recognized for their role in deterring herbivory in terrestrial plants (Schultz 1988) and macroalgae (Hay & Fenical 1988). Lowered nutritional quality, independent of toxins or

compounds which reduce digestibility, also can affect susceptibility to predation (Butler et al. 1989). Induction of chemical defenses in plankton are inadequately understood.

Morphological Defenses. These include both fixed and inducible morphologies (Havel 1987). Among defensive changes in prey architecture which have been experimentally verified are: formation of colonies by previously single-celled *Scenedesmus* when exposed to herbivorous zooplankton (Hessen & Van Donk 1993; Lampert et al. 1994); growth of neck spines by *Daphnia* when exposed to kairomones of invertebrate predators, a process which is stage- and concentration-dependent (Tollrian 1993); development of extended wings and ridges by grazing ciliates when exposed to larger predatory ciliates (Kuhlmann & Heckmann 1985); and growth of spines by rotifers to deter predation by larger zooplankton (Stemberger & Gilbert 1987). In at least two of these cases, the prey apparently perceived the presence of the predator through polypeptides released by the predator. Several phytoplankton taxa with fixed morphological protuberances have been speculated to function as anti-predation devices, e.g. *Ceratium* (Hargrave & Geen 1970, but see Nielsen 1991). Rigid chitan threads, extending from cells of the diatom family Thalassiosiraceae, were shown to reduce their susceptibility to grazing by proto- (Verity & Villareal 1986) and metazooplankton (Gifford et al. 1981). While effective, some morphologies may not have evolved in response to predation, e.g. mucous sheaths in rotifers (Stemberger & Gilbert 1987).

Behavioral Defenses. Diel vertical migration, whereby zooplankton descend to depth during daylight hours and ascend again to feed during darkness, is often attributed to a behavioral response to predators which hunt by sight (Zaret & Suffern 1976, Ohman et al. 1983). Direct support for this notion comes from experiments in which the amplitude of

migration varied in proportion to predator abundance (Gliwicz 1986) and predation pressure (Bollens & Frost 1989). The freshwater cladoceran *Daphnia* also avoids predatory invertebrates by migrating upwards in their presence (Dodson 1988, Ramcharan et al. 1992), while the invertebrates themselves remain at depth during daylight to avoid predation by visually feeding fish (Roth 1968). Provocatively, both marine and freshwater crustacean zooplankton can reverse their migrations in response to surface feeding predators (Dodson 1988, Ohman 1990), indicating behavioral flexibility rather than strict genetic programming. As observed for the inducible morphological defenses, the behavioral responses appear to be triggered by chemical signals released by feeding predators (Dodson 1988, but see Bollens et al. 1994, for marine copepods). Such antipredator defenses may have costs in terms of reduced nutrition when feeding and defense are both dependent upon motility (Werner & Hall 1988, Ramcharan et al. 1992). For example, among marine zooplankton, those taxa which are especially visible to fish predators feed primarily at night (Hobson & Chess 1976).

Behavior such as DVM apparently evolved to minimize encounter with potential predators. Copepods (and presumably other metazooplankton) also have sensors which detect variations in mechanical stimuli, irradiance, and chemical gradients, and these provide defense mechanisms to reduce the probability of capture after encounter. Copepods, especially, are capable of rapid accelerations resulting in short-term swimming speeds of many body lengths per second (Strickler 1975). Their combined perceptive abilities and escape responses function to avoid potential predators which are stationary (Haury et al. 1980), drifting above (Buskey et al. 1986), in pursuit (Singarajah 1969), or are attempting to entrain prey in their flow fields (Trager et al. 1994). Developmental stages as well as adults are capable of escape

responses (Yen & Fields 1992), although the vulnerability to capture varies with prey taxa (Drenner et al. 1978), size and morphology (Confer & Blades 1975), and developmental stage (Greene & Landry 1985). Likewise, fish vary in their ability to capture evasive and nonevasive zooplankton (McComas & Drenner 1982, Vinyard 1982), and some can learn to improve capture efficiency for more evasive prey (Coughlin & Strickler 1990). Fish and large zooplankton also exhibit complex behaviorally-mediated predator avoidance mechanisms, e.g. swarm formation.

Life history strategies. In the enigmatic phytoplankton *Phaeocystis*, as described earlier, cells form colonies, colonies can increase or decrease in size, and cells can emigrate from colonies (Verity et al. 1988, Cariou et al. 1994), altering the size of *Phaeocystis* by volume factors of 10^6–10^9. While no direct evidence ties life cycle changes to grazing pressure, it is clear that the life history stages of *Phaeocystis* vary in their susceptibility to grazers and potentially structure the herbivore community (Hansen et al. 1994). Another approach is that of bioluminescent dinoflagellates, whose mechanically stimulated flashes reduce their consumption by copepods (White 1979) and increase predation by fish on the grazing copepods (Abrahams & Townsend 1993), irrespective of whether bioluminescence actually evolved as an antipredatory device. A different antipredation strategy is exhibited by the copepod *Diaptomus*, which switches from laying subitaneous eggs to diapause eggs coincident with seasonal peaks in predation pressure (Hairston 1987). A further example is the cladoceran *Daphnia*: in the presence of visually feeding planktivorous fish, which are especially effective at removing larger daphnids, *Daphnia* increases its intrinsic rate of population growth (Dodson 1989), reduces juvenile length increments, matures earlier, and

lays larger clutches of smaller eggs (Machacek 1993); the latter responses are induced by chemicals associated with the predatory fish. In this regard, it may be that trophic cascades are more likely to occur in the freshwater pelagial because *Daphnia* is so often the major herbivore: through parthenogenesis it can reproduce as rapidly as its prey and therefore control their stocks, but its swimming behavior leaves *Daphnia* particularly susceptible to population decimation by invertebrate and vertebrate predators (Allan 1976, Lynch 1980, Jürgens 1994).

Predation and Trophic Cascades as Structural Principles

It is argued above that the morphologies and life history strategies of plankton have evolved in relation to their environments. Traditionally, that "environment" has been considered to be a physical or chemical one, e.g. Margalef (1978) proposed that the functional morphology of phytoplankton is selected by the decay of mechanical energy of water, or turbulence. Kiørboe (1993) argued eloquently that pelagic food web structure is mediated by turbulence and phytoplankton cell size, and that general patterns of mesoscale plankton distributions are consistent with the theoretical bases for such arguments. However, this bottom-up or biogeochemical approach has not proven successful in predicting the timing, magnitude, or temporal dynamics of plankton communities. For example, considerations of fluid dynamics and cell physiology are consistent with the notion that small size is less advantageous to phytoplankton in turbulent waters, but they do not explain the comparative dominance of large cells in such environments, much less the typical dominance of large cells in blooms. Kiørboe (1993) proposed instead that large size provides a refuge from predation,

for two reasons: the relative abundance of predator to prey decreases as size increases; and the generation times of predators increase more rapidly than those of their prey as size increases, yielding longer lags between phytoplankton growth and zooplankton response. Thus the risk of being eaten is diminished for larger cells. Banse (1994), too, argued that food web structure cannot be fathomed without equal consideration being given to top-down control.

While the extent of predation between adjacent trophic levels has been the subject of considerable interest, e.g. Sih et al (1985), here we explore whether the consequences of predation cascade into more distant trophic levels, and the characteristics of predators which are associated with such multi-trophic relationships. *This discussion also has implications for the historically different approaches to limnology and oceanography.* While biology in the marine pelagial has been pre-occupied with biogeochemical fluxes and stock exploitation, freshwater efforts have been quietly dedicated to deconvolving how lakes and streams work. Hence the following includes examples from both disciplines, and identifies both similarities and differences between them where evidence warrants.

Although it has been common knowledge among farmers and hunters that both resources and predation are important in structuring plant and animal communities, most ecologists prior to 1960 viewed populations as being entirely resource-driven. In that year, Hairston, Smith, and Slobodkin published their famous "the world is green" hypothesis. They began with the observation that excessive or widespread destruction of vegetation by grazers is rare, and thus there was an abundant food source available to herbivores. If the grazers were not food-limited, then their abundance must be restricted by predators, preventing the herbivores from depleting their plant resources. Furthermore, since grazers must be predator-limited, their

predators must be food-limited. These ideas were developed for terrestrial ecosystems, while aquatic ecosystems were omitted because they are not always "green" (N.G. Hairston, Jr. pers. comm.), but the concepts were later adapted to aquatic habitats (Hrbacek 1962, Brooks & Dodson 1965, Smith 1969, Persson et al. 1992).

The generic form of these hypotheses is that organisms at the top of the food chain are food-limited, and at successively lower levels they are alternately predator- and then food-limited. Changes in food chains with strong trophic links may then "cascade" from top to bottom (Carpenter et al. 1987, McQueen et al. 1986 1989). The trophic cascade model predicts that, by their presence or absence, higher trophic levels will determine whether or not conspicuous accumulations of plant biomass will occur at the base of the system. The largest body of evidence supporting the trophic cascade model derives from freshwater habitats [Strong 1992, see McLaren & Peterson (1994) for a recent terrestrial example]. One reason why trophic cascades may be more likely to occur in aquatic than terrestrial habitats is because water is so much denser than air, that plants can be physically supported without having to put the vast majority of their primary production into relatively inedible structural components, as do terrestrial plants. Being small and of high nutritious quality, they can be eaten in their entirety by small grazers, for which prey capture is typically gape-limited (Zaret 1980), leading to a size-structured food chain. Thus there is little cheating in plankton-based food webs, because predators have to be big enough to eat their prey in its entirety (Hambright et al. 1991), unlike browsing herbivores in terrestrial ecosystems. Hairston & Hairston (1993) developed this idea further, suggesting that predator:prey size ratios in pelagic systems [where they may be size-dependent: Longhurst 1991] are likely to be substantially

larger than those typical of terrestrial ecosystems.

In the marine pelagial, little attention was given for many years to the possibility that top-down forces might be important. In part, this reflected the biased view of phytoplankton ecologists that production of algal biomass was limited by availability of nutrients or irradiance, and that accumulation of biomass depended only upon the extent of production. This bottom-up or resource interpretation was indirectly supported by the traditional view of marine food chains as diatoms → copepods → fish (see Kleppel 1993, for origins), because estimates of grazing impact by copepods typically were ca. 10-50% of primary production (e.g. Mullin 1969, Kiørboe 1991). This apparent inability to keep pace with algal growth implied that phytoplankton were not grazer-limited and that copepods were not food-limited (Huntley & Boyd 1984). Studies showing strong dependence of copepod growth upon temperature (Huntley & Lopez 1992), also implied that food was not limiting.

Since that time, contemporary thinking about plankton community structure has undergone a revolutionary reversal, a change too significant to be merely labelled a new paradigm. In one generation, the hundred-year old view that diatoms were the pastures of the sea has been replaced with the notion that much smaller cells are the workhorses in the euphotic zone (Pomeroy 1974), with occasional diatom blooms superimposed on their productivity. It is this microbial food web which tracks changes in controlling physical and chemical processes; diatoms leave and enter the system according to their own unique biology (Smetacek et al. 1990, Lenz 1992). With the recognition of pico- and nano-sized autotrophs and mixotrophs came the understanding that small zooplankton were often the dominant herbivores (references in Pierce & Turner 1992), and estimates of their grazing plus that of

larger zooplankton can equal or exceed that of primary production (e.g. Lenz et al. 1993 and Verity et al. 1993). The possibility of predator (grazer) control of prey became more distinct, especially given the capabilities for prey selection evidenced by both proto- and metazooplankton (Cowles et al. 1988, Verity 1991).

However, there are relatively few instances of actual trophic cascades in marine waters across three or more levels (Table 1). Of these, some are direct experimental manipulations, some are merely interpretations of gross patterns in field communities, and some include models of laboratory or field studies. The most compelling evidence suggests that certain plankton foodwebs may show coupled population oscillations with trophic levels limited alternately by predation and resource availability (e.g. Horsted et al. 1988, Dolan & Gallegos 1991). Provocatively, fully three quarters of the cascades in Table 1 were initiated by gelatinous predators, which suggests that their ability to rapidly increase population biomass, their high weight-specific ingestion, and/or their large predator-prey size ratios, may be key characteristics in pelagic organisms involved in trophic cascades. It has been argued that trophic cascades are unlikely in the absence of keystone predators (Strong 1992). Alternatively, the common denominator may be moderate-to-strong predation pressure rather than a true keystone predator (Menge et al. 1994). For example, the occurrence of gelatinous carnivores is not always associated with obvious trophic cascades (Purcell et al. 1994).

Only two regional assessments of the potential importance of trophic cascades in marine pelagic ecosystems have apparently been published. In both the Baltic Sea and Chesapeake Bay (Verity 1987, Rudstam et al. 1994), limited evidence suggested that trophic cascades may be seasonally or historically important, but convincing documentation was not available. It

Table 1. Examples of trophic cascades influencing the structure of marine pelagic communities.

Location	Cascade	Data Source	Citation
Roskildefjord	Sticklebacks → MesoZ → Phytopl	Enclosure	Horsted et al. (1988)
Limfjord	Mussels→Meso/MicroZ→NanoZ→Bacteria	Enclosure	Riemann et al. (1990)
Loch Ewe	Ctenophore → MesoZ → Phytopl	Enclosure	Gamble et al. (1977)
Narragansett Bay	Ctenophores → MesoZ → Phytopl	Enclosure/Field	Deason & Smayda (1982)
Rhode River	Rotifers → NanoZ → Bacteria	Enclosure/Model	Dolan & Gallegos (1991)
Vancouver Fjord	Medusae → MesoZ → Phytopl	Field Obs.	Huntley & Hobson (1978)
Kiel Bight	Medusae → MesoZ → Phytopl	Field Obs.	Moller (1979)
North Sea	Euphausids → Copepods → Phytopl	Field Obs.	Roff et al. (1988)
Gullmar Fjord	Medusae → MesoZ → Phytopl	Field Obs.	Lindahl & Hernroth (1983)
Helgoland Bight	Ctenophore → Ctenophore → MesoZ	Field Obs.	Greve & Reiners (1980)
Cheseapeake Bay	Cteno/Medusae → MesoZ → Phytopl	Field/Model	Baird & Ulanowicz (1989)

has also been argued that trophic cascades may be responsible for patterns in plankton biomass in HNLC regimes such as the subarctic Pacific (Frost 1991), and that recent accidental introductions of ctenophores into the Black Sea have caused significant structural changes (Kideys 1994).

The apparent importance of gelatinous predators in the few reported marine trophic cascades may also reflect on the role of fish in this capacity. While density-dependent competition for food is a general feature of larval and juvenile fish (Valiela 1984), fishery ecologists generally hold that stocks of adult fish are not limited by their resources and thus cannot limit them (Cushing 1975). This conclusion is supported by several typical features of fish populations including: the lack of a density-dependent stock-recruitment relationship, the occurrence of strong yearclasses developing from weak spawning events, and the huge variation in fish recruitment among years with no evidence of similar variance in prey production. All of these support the notion that populations of adult fish in the oceans are seldom likely to control the abundance of their prey, in contrast to the evidence from freshwater systems. It has been speculated that fish in the oceans are 10-1000 times less abundant than their freshwater counterparts (Horn 1972), perhaps accounting for their apparent lack of predatory impact. If this comparative dilution of fish in the sea is correct, the mechanism would seem to be the huge mortality losses applied primarily during embryonic and larval development.

It may be instructive to consider why trophic cascades appear so well developed in freshwater compared to marine pelagic ecosystems. An important distinction is that taxonomic diversity of the freshwater pelagial is simplified compared to marine waters

(Valiela 1984). For example, missing are a variety of herbi/omnivores (Lehman 1988) and carnivores (Greene 1985), including the near-absence of planktonic ambush predators and clupeid-like fishes (Banse, 1990). Moreover, feeding at more than one (lower) trophic level may be more extensive in saltwater because of the common importance of mixotrophic ciliates there (Stoecker et al. 1989, Putt 1990); mixotrophic flagellates are well developed in both fresh- and saltwater (Sanders 1991). In general, mixotrophy appears more important in oligotrophic vs. eutrophic waters (Arenovski 1994). If omnivory and generalist feeding are common in marine waters, and if such trophic diversity reduces the likelihood that a, e.g. climatically-induced, strong yearclass of predators would decimate only a single trophic level, then trophic cascades may be minimized there or difficult to unequivocably validate. However, although the success of trophic level manipulation experiments in freshwaters implies that omnivory there is not sufficient to obscure distinctions between trophic levels (Hairston & Hairston 1993), the converse (that extensive omnivory precludes trophic cascades) is not necessarily true. If cascades are less likely in oligotrophic environments, due to strong substrate control, then the better documentation of cascades in freshwater may reflect the generally more productive trophic status there compared to marine waters (Valiela 1984). Also relevant may be the life history of *Daphnia*, whose reproductive strategy is designed for rapid colonization and for export to new environments (e.g. by duck's feet), but whose motility behavior prevents it from holding space in the presence of visual planktivores. It is indeed intriguing that the predators most common in marine cascades (jellies) are essentially absent from freshwater, where cascades have been more commonly described, while the freshwater herbivore typically linked to cascades (cladocerans) are unimportant in marine waters.

The Role of Physics and Implications for Design of Future Studies

Ecological communities and physical processes vary in time and space across a broad range of scales (Marine Science Colloquium 1989, Powell 1989). In the ocean, the space/time scales of biology and physics are nearly coincident, so it is likely that their interactions are closely coupled (Steele 1991a). If so, positive correlations might be expected between the variabilities in physical and biological processes (Walsh 1977), although correspondence does not establish cause and effect. Physics and biology are best integrated at mesoscales where biology can be budgeted, e.g. as energy or mass, analogous to the physical laws of conservation of energy, mass, and momentum (Denman & Powell 1984). At these scales there is good evidence for strong influences by the physics of ocean circulation on biology of fish and plankton. One example is the close correlation between ocean currents and fish life cycles such as herring, driven by climatic variations (Steele 1991b). At the population level, this is explained by links between mesoscale mixing and reproductive processes. However, changes at the community level, such as switching between dominance by pelagic versus benthic fish, have not been explained. On the other hand, there is also no indication that dramatic changes in fish stocks (abundance or composition) in such areas as Georges Bank or the North Sea, have cascaded down to impact zooplankton (Koslow 1983; J.H. Steele pers. comm.).

The present paper addresses mechanisms whereby individual species, their characteristics, and their interactions contribute to ecosystem structure. Thus the central question in the context of physics is at what spatial and temporal scales do community parameters become less significant than species attributes (behavior, morphology, life cycles) in determining

apparent success, i.e. standing stocks (Steele 1991a)? While the larger scale physical processes discussed previously also cascade down to the level of the individual, e.g. turbulence effects upon encounter rates (MacKenzie & Leggett 1991), "conservation laws for mass, energy, and momentum ... are not sufficient to understand ... competition, predation, species succession and diversity, etc." (Denman & Powell 1984, p. 127). Although at larger scales the "concept of ecological succession ... does not seem appropriate to the changes in marine systems where we explain changing community patterns in a physical context" (Steele 1991a, p. 433), it can also be argued that organisms, especially successful key species, have evolved to take advantage of the higher frequency variability at smaller scales.

Another aspect of physics is relevant to evaluating the role of predation and key species in structuring pelagic ecosystems, and that is advection, dispersal, and patchiness. As discussed in the previous sections, there is sufficient theoretical and experimental evidence to believe that predation, morphology, and key species are crucial in structuring marine pelagic communities. However, much of the experimental support derives from contained populations or from descriptive field data, so that the significance of predation-driven forces in open waters remains uncertain. Both supportive and disputive data are available. The major weakness of descriptive data from open waters is that absence of evidence is not evidence of absence. The problem with containment, however, is that enclosures must be sufficiently large to mimic the real world. For example, if they offer no refuges or lack natural feeding guilds to utilize changing resources, then discrepancies will occur between theory and experimental results. Thus perhaps the ideal environment in which to test these hypotheses is an enclosed body of water with naturally restricted exchange with adjacent waters, e.g. Landry (1978). The ctenophore-zooplankton-phytoplankton cascade observed in Narragansett Bay

(Deason and Smayda 1982), which has a turnover time of ca. 26 days (Pilson 1985), is perhaps the best example to date.

A related issue is that patchiness, dispersal, advection cause significant sampling artifacts in marine ecosystems. The latter problems are minimized in small lakes, perhaps contributing to better documentation of trophic cascades there, or perhaps simply making cascades more likely to occur; large lakes are more complex (Schelske & Stoermer 1994). Thus it is not so much whether predation or trophic cascades structure marine plankton communities, but whether we can provide definitive approaches and methodologies to test competing hypotheses. Top-down forces may not cascade in the pelagial because advective processes act to separate predator and prey before cascades can be complete, i.e. disequilibria, or because seasonal weather patterns "reset" the system, e.g. Landry (1977). Or perhaps top-down forces may be operating but it cannot be confirmed because advection separates us in space from processes in different stages of temporal equilibrium. These sampling issues have long hindered pelagic science, whether chemistry or biology, but in trying to determine the forces structuring an ecosystem, there is an additional formidable obstacle: trophic identifications for each major taxonomic group are also necessary. Some techniques have been developed to sample over large spatial and temporal scales, to minimize problems of sampling statistics. For example, acoustics can be used for fish stock assessment over large scales; but can the return echoes identify a planktivore from a primary carnivore from a secondary consumer? The crux of the problem is the extent to which we are comfortable trading realism for analytical accuracy: our conceptual models and scientific method must necessarily be "a judiciously chosen set of lies, or perhaps more accurately put, partial truths about reality, which have been chosen to permit us to reason more effectively about some issue than we

otherwise could" (Baumol 1984).

Let us assume for the moment that one has carefully designed an experimental test, and sampling was spatially and temporally complete. Do complications exist which could impede interpretation of the data? Evidence from both freshwater and marine habitats suggests that several factors may obfuscate data analysis. For example, predator-induced effects may occur but may not be evident as changes in population abundance or as bulk removal. Rather, the affected populations may alter patterns in vertical distribution or migration (Rudstam et al. in press), the taxonomic composition of a trophic level may change (McCauley & Briand 1979), or its size structure (Hrbacek 1962). As such, food webs may not collapse clearly into food chains. Moreover, "tangles in trophic architecture" (Strong 1992), i.e. omnivory, mixotrophy, substitutability of species, compensation among taxa for functions of others, inedibility, mobility, defenses, may prevent the occurrence of key species upon which sustained consumption can more clearly take its toll. Top-down and bottom-up regulation can be additive, for example starving fish larvae may be more susceptible to predation, or both forces can be (differentially) influenced by the same abiotic factors, e.g. increased irradiance stimulates both primary production and visual predation (Aksnes & Giske 1993). Conversely, relationships between trophic levels may be non-linear (Dwyer & Perez 1983), non-additive (Stoecker & Evans 1985) or exhibit threshold dynamics: predation impact upon a prey population may be sustainable up to a certain point, beyond which drastic declines in prey abundance occur. If ontogenetic changes (eg dietary) occur, or time lags between prey consumption and predator reproduction, top-down effects may be obscured (Power 1992). Finally, physical processes on long time scales may dominate shorter frequency biological interactions (Aebischer et al. 1990), so that any top-down effects are muted or invisible.

Closing Thoughts

Theory is rather explicit about the conditions under which resource availability and predation should dominate trophic interactions. On the other hand, the real world is seldom as simple as theoretical boundary conditions (Pimm 1992). Research during the past 50 years has shown that not all plants are equally edible, not all herbivores are equally effective, not all predators are equally proficient, and not all environments are equally hospitable (Hunter & Price 1992). The considerations in this paper illustrate that it can be simpler to construct pragmatic post hoc explanations for observed patterns in trophic structure, than it can be to devise sound theoretical underpinning.

We propose that biogeochemical fluxes are spearheaded by key taxa, perhaps even "keystone species" (sensu Bond 1993) whose environmental adaptations, whether through behavior, morphology, physiology, or life history, are so strong that they direct trophic relations. Some specific examples were postulated in the section on life histories and morphologies. We have argued that dominant species in the marine pelagial attain their status as much by reducing mortality due to predation as by increasing growth or reproductive rates. While it can still be instructive to ask whether it is top-down or bottom-up forces which regulate community structure, because the regulatory mechanisms are distinctly different, we suggest a new synthetic focus: "*How does the pelagic environment select for life histories, morphologies, and behaviors of organisms under conditions when food availability and predation both affect trophic interactions?*" In our rush to get into explanatory modelling, planktologists have trodden on the species and stuffed them into trophic boxes, pyramids or size fraction relationships. The underlying assumption has been that the categories comprise

homogeneous units. Recent work is leading to differentiation but also expansion of our knowledge of pelagic system structure and its shaping forces: picoplankton and protozoa of the 1980's are now extended into viruses and parasitoids. At the same time, group-specific differences in behavior are coming to light that make it necessary to discriminate smaller constituents within the tidy boxes: compare Longhurst's (1991) box model with that of Steele (1974). We now advocate opening the boxes, taking out the dominant species and studying them at their relevant levels of selection be they predator avoidance, life cycle strategy etc. and not just growth physiology (ICES 1994).

This proposal has implications for contemporary ocean science, which is being increasingly focused on large-scale, interdisciplinary programs. Some of these are designed to derive horizontal or vertical fluxes of elements which have anthropogenic or geochemical significance, e.g. the carbon-based Joint Global Ocean Flux Study (JGOFS). Others concentrate on establishing the linkages between predator and prey, and their relation to physics, in order to describe ecosystem function, e.g. Global Ocean Ecosystem Dynamics (GLOBEC). The paths are distinctly different yet the long-term objectives are identical: an improved mechanistic understanding of ocean dynamics and the ability to predict future changes. *The inescapable conclusion from the present analysis is that attempts to identify the forces structuring the marine pelagial must be designed specifically to address that question, i.e. organism-organism and organism-environment interactions, and not to measure carbon fluxes.* This is simply because adaptation and selection operate at the species level. But the connection between organism- and element-driven oceanography is that the transfer of elements or energy through the system depends upon trophic structure and activity (Hairston & Hairston 1993): the composition of the biological pump determines its efficiency of

operation. One can no more predict carbon fluxes without a mechanistic understanding of ecosystem structure, than one can deconvolve the mechanisms from flux measurements alone: flux estimates cannot predict whether sardines vs. anchovies, or herring vs. mackerel, will dominate, but evidence DOES suggest that species determine fluxes. A study could be envisioned where both approaches are applied concurrently: thus, the thermodynamic constraints on the system (e.g. light field) could be derived from measurement of total community metabolism or throughput. The deviation of *in situ* instantaneous rates from theoretical maxima indicate the "state" of the system and would be necessary to define the units in structural terms; i.e. how much of the energy is diverted to deterrents or flight muscle and how much to reproduction of DNA supporting living plasma. Only by combining the two approaches will we ever understand how pelagic environments select their inhabitants and how the inhabitants determine energy flow and material fluxes.

Banse (1994) argued insightfully that "in regard to more realistic modeling of food webs .... we are short on explicit theory, not only for marine and freshwater systems, but also for ecology in general." He, too, proposed that research should focus on the biology of key species, but does not believe that scientists and funding agencies will support endless autecological investigations. The avenues we suggest here are not to concentrate so much on metabolic assessments or to search for minutiae, which are unlikely to differ much among species, but to identify critical aspects of life history, morphology, or behavior. These should be examined in the context of rigorous testing of explicit hypotheses. For too long we have shied from playing Pandora, that is, harnessing courage to curiosity. By studying individual species in greater detail, we will be able to understand the shaping forces of the abiotic environment on the one hand and biotic interactions, including competition and predation, on

the other. In answering this question we can truly integrate ecological concepts with predictive models, and make significant purchase in our climb toward understanding of pelagic processes.

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A New Staining Technique for Dual Identification of Plankton and Detritus in Seawater

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Abstract

A new method is described that employs dual staining and epifluorescence microscopy for the identification of plankton and detritus in seawater. The staining technique uses the fluorochromes propidium iodide (PI) and 4′, 6-diamidino-2-phenylindole (DAPI) in conjunction with UV excitation. PI/DAPI staining identifies living matter distinct from non-living matter by selectively staining detritus a deep orange color while cells are either blue-white or pink, depending upon stain and cell type. Comparison of this technique with existing epifluorescence techniques and staining reagents indicates that PI/DAPI offers an improved approach for staining detritus and plankton when evaluation of both is desired. This step is a prerequisite for quantitation of the volumes of detritus and plankton, preliminary to independent estimates of their carbon and nitrogen pools in aquatic ecosystems.

Keywords:

detritus, plankton, propidium iodide, DAPI, epifluorescence microscopy

A New Staining Technique for Dual Identification of Plankton and Detritus in Seawater

Introduction

Traditional concepts of marine planktonic food webs as being dominated by short linear food chains, with the dominant process being ingestion of relatively large phytoplankton by crustacean zooplankton, have been revised to include a significant protozoan component. The foundation for this food web is the picoplankton and small nanoplankton, a diverse taxonomic assemblage consisting primarily of bacteria, cyanobacteria, and small eucaryotic phytoplankton (Pomeroy, 1974; Iturriaga and Mitchell, 1986). These cells are too small to be efficiently collected or are poorly assimilated by copepods (Johnson *et al.*, 1982). Although the filtering mechanisms of larvaceans (King *et al.*, 1980), salps (Harbison and McAlister, 1979), and cladocerans (Turner *et al.*, 1988) can retain pico- and nanoplankton, these metazoans do not appear capable of influencing their dynamics except in unusually large swarms (Paffenhöfer *et al.*, 1995). Rather, the significant grazers appear to be a variety of phagotrophic protozoans, primarily heterotrophic nanoplankton, ciliates, and dinoflagellates (e.g. Verity *et al.*, 1993 and many others).

Currently, the method of choice for evaluating these small organisms is fluorescence microscopy, which has several advantages: (1) a specific type of macromolecule or cellular component can be selectively visualized in the presence of an excess of other species; (2) low concentrations can be quantitatively rendered due to the sensitivity associated with the emission of fluorochromes; and (3) low excitation intensities may be used to elicit a fluorescence signal, thereby specimen viability is preserved under lengthy observations

(Arndt-Jovin *et al.*, 1985).

Köhler (1904), who first demonstrated the principle of fluorescence, developed a method that employed ultra-violet light as the source of illumination of specimens, yielding greater resolution with any given combination of objectives (Smith, 1994). The arrival of fluorochromes (Haitinger and Hamperl, 1933), which yield secondary fluorescence by binding to specific compounds by various techniques (i.e. absorption, electrostatic forces, and intercalation) (Klut, 1988), assisted in the development of fluorescence microscopy. Fluorescence of the labeled structures permits qualitative and quantitative studies of organisms and their internal structure.

Wood (1955) was apparently the first to use fluorescence microscopy in plankton investigations as a means of discriminating between photosynthetic and non-photosynthetic plankton. Photosynthetic plankton were distinguished by the autofluorescence of their chlorophylls. To discriminate photosynthetic organisms from non-photosynthetic organisms, cells were stained with the fluorochrome, acridine orange. However, there were some issues that needed to be addressed: (1) the metachromatic behavior of acridine orange which caused dual fluorescence when bound to both single and double stranded DNA (Darzynkiewicz, 1979), and (2) the loss of flagella from smaller flagellates caused by acridine orange.

Hobbie *et al.*, (1977) amended this method by utilizing Nuclepore filters for counting bacteria, and described the metachromatic behavior of acridine orange in bacterial cells. Acridine orange fluoresces red when it is associated with single stranded nucleic acids and green when it is associated with double stranded nucleic acids. This additional red fluorescence obscures the red autofluorescence of chlorophyll *a* in phytoplankton, confounding separation of photosynthetic and non-photosynthetic organisms, thus diminishing the utility of

acridine orange in the staining of plankton cells.

In the last several decades, quantitative and qualitative studies have been performed using various other fluorochromes (Porter and Feig, 1980; Haas, 1982; Caron, 1983; Hara and Tanoue, 1989), fixatives (Tsuji and Yangita, 1981), and concentration devices (Hobbie and Daley, 1977; Geider, 1987) to visualize and enumerate pico- and nanoplankton populations. Epifluorescence microscopy has been applied to other studies of plankton including determination of the frequency of dividing cells as an indicator of instantaneous growth rate (Hagström *et al.*, 1979; Newell and Christian, 1981), and the use of fluorescent antibodies in the detection of specific bacterial types (Campbell *et al.*, 1983; Reed and Dugan, 1979).

Currently, the most sophisticated approach used to quantify plankton cells is dual staining with 4′,6-diamidino-2-phenylindole (DAPI) and proflavin, with measurement of cell volumes via image analysis, and subsequent conversion to cell carbon and nitrogen (Sieracki *et al.*, 1989a, b; Verity *et al.*, 1992; Verity and Sieracki, 1993). Proflavin stains the cytoplasm of plankton cells an apple-green color under blue excitation while chloroplasts emit a neon red to orange color, depending upon accessory pigment composition. Thus entirely green cells depend upon heterotrophic nutrition, while red cells are photosynthetic. Mixotrophic cells, which perform photosynthesis but may also ingest other cells or utilize dissolved organic substrates, cannot be distinguished by this technique unless the plankton community is incubated with a labeled substrate, (e.g. fluorescently labelled bacteria) (Sherr *et al.*, 1987) prior to staining. DAPI, which stains DNA (especially double stranded DNA)(Porter and Feig, 1980), fluoresces a brilliant blue-white under UV excitation. It is used to identify bacteria and the unique condensed nuclei of heterotrophic (=aplastidic) dinoflagellates. This staining approach is robust and the samples remain functional for up to one year if stored frozen (Verity and Sieracki,

1993). However, one glaring deficiency of all fluorescent techniques presently in use is that they do not selectively identify non-living particulate organic matter or detritus.

Considerable research (*e.g.* Pomeroy, 1980; Siebert and Naiman, 1980) has shown that detritus is an integral part of aquatic ecosystems, but the ability to quantify it distinct from plankton cells has hindered evaluation of its various roles. Interest in understanding the dynamics of both living and non-living organic carbon pools prompted us to establish a technique that would selectively stain non-living particulate organic matter distinct from living particulate matter. Here we report tests of various fluorochromes and a proposed dual staining technique for selectively delineating non-living particulate matter from living particulate matter. This procedure, coupled with visualization and quantitation of the volume of detritus and plankton, permits estimation of their carbon and nitrogen contents (Verity *et al.*, in prep.).

Materials and Methods

Estuarine water samples (10ml) from the dock of the Skidaway Institute of Oceanography, in Savannah, Georgia, USA, were preserved with glutaraldehyde to a final concentration of 0.3%, and filtered onto 0.8μm black Nuclepore filters (Verity and Sieracki, 1993). To achieve an even distribution for counting and measurement purposes, black filters were placed on top of prewetted 0.45 μm Millipore polycarbonate filters. Fluorochrome stock solutions were prepared with solvents listed in Table 1 and stored in darkness at 4° Celsius until required. The above staining agents were selected according to their known staining properties, and their presumed compatibility with the biochemical constituents of detritus and plankton cells. Samples were then stained using concentrations and staining times indicated in Table 1, and filtered under low vacuum. The filter was placed face up on a glass slide. A drop of low fluorescence

immersion oil was placed on the filter, and covered with a coverslip.

The stained samples were then viewed with an Olympus BH-2 epifluorescence microscope equipped with: a 100W mercury burner; objectives ranging from 10x-100x; a blue filter set (Olympus B-L0910) with exciter filter BP490, barrier filter O-515, and dichroic mirror DM500; a UV filter set (Olympus B-L0913) exciter filter UG1, barrier filter L-420, and dichroic mirror DM400; and a green filter set (Olympus B-L0911) with exciter filter BP545, barrier filter O-590 and dichroic mirror DM580. These filter cubes were mounted into an Olympus triple slider for rapid sequential excitation of samples.

Results

In samples stained with alcian blue, which is specific for negatively charged polysaccharides (Decho, 1990), detritus and plankton cells stained a dark blue. Detritus is composed primarily of carbohydrates (Gordon, 1970), making alcian blue a potentially effective choice for such staining; however some of the larger apparent detrital aggregates were not stained by alcian blue. Logan *et al.*, (1994) also observed unstained aggregates in lake and seawater samples stained with alcian blue, which may occur because of the general absence of polysaccharides, or the specific absence of negatively charged polysaccharides. The major drawback with alcian blue in the present study is that it stains both detritus and plankton cells dark blue, making it difficult to colorimetrically distinguish plankton cells from detritus using brightfield microscopy.

The fluorescent stain, calcofluor white, readily binds to cellulose and β-linked glucans (Hughs and McCully, 1975). In fixed samples stained with calcofluor white, both detritus and plankton have a transparent appearance with illumination of the periphery of both. In

dinoflagellates, the thecal plates and pusules fluoresce bright blue under UV excitation and bright green under blue excitation. Thus, calcofluor white was useful in staining the thecal plates of armored dinoflagellates which are cellulosic in nature (Fritz and Triemer, 1985), but ineffective in the staining of both detritus and plankton cells, for they were not clearly discernable from one another.

Dansyl lysine is specific for membrane integrity. It is able to identify dead or dying mammalian cells, for it is impermeant to the membranes of live cells and will only stain those cells which are dead or have compromised membranes (Haugland, 1992). In the present study, there was no indication that dansyl lysine stained glutaraldehyde-fixed detritus or plankton cells. It was concluded that detritus and plankton may be resistant to dansyl lysine staining, and since detritus has no unified membrane structure, dansyl lysine is not effective for selectively staining detritus and plankton.

Fluorescent lectins are versatile probes for detecting cell surface glycoconjugates in histochemical and flow cytometric applications (Haugland, 1992). FITC-Concanavalin A (Con-A) is specific to carbohydrates (Furlan *et al.*, 1979). Here, Con-A stained detritus and heterotrophs a bright neon green under blue excitation and a faint green under UV excitation. The chlorophylls of autotrophic dinoflagellates exhibit a bright orange-red color under blue and UV excitation. The thecal plates of dinoflagellates were blue under UV excitation. There was some indication that Con-A stains the thecal plates of dinoflagellates a dim neon green under blue excitation in cells with quenched pigment autofluorescence. Green fluorescence of thecal plates in fixed dinoflagellate cells that contain chlorophylls was not observed, apparently because the brilliant autofluorescence of chlorophylls swamped the effects of Con-A. Con-A was effective in staining dinoflagellates, but is not useful in selectively staining detritus

distinct from plankton cells, for both detritus and fixed heterotrophic cells fluoresce a neon green color under blue excitation. Con-A also requires a 1 hour incubation period.

Primulin, a UV-excitable stain, was originally introduced as the stain of choice for staining nanoplankton due to low nonspecific binding and staining of polysacharrides such as those found on the outer layers of all plankton cells (Brock, 1977). This stain was not widely accepted until it was reintroduced by Caron (1983), who proposed that primulin was a much better stain than proflavin, because its emission spectra differs substantially from that of chlorophyll *a*, permitting detection of fluorochrome or chlorophyll *a* fluorescence. Under UV excitation, primulin stained the cytoplasm of cells bluish-white. The color of detritus (green) under blue excitation, and (yellowish-gold) under UV excitation was visually similar to that of the cell walls (yellow) of plankton. Primulin stained the thecal plates of dinoflagellates green under blue excitation and bluish-yellow under UV excitation. The autofluorescence of algal pigments was brilliant red under blue and UV excitation yet the color of the cell walls was not distinct from that of detritus.

The optimal staining technique for staining detritus and plankton cells proved to be propidium iodide (PI) followed by 4′,6 diamidino-2-phenylindole (DAPI), in conjunction with wideband UV excitation filters. Under wideband UV excitation, PI in the presence of glutaraldehyde stains detritus red, while DAPI stains it yellow; combined they produce a deep orange color (Figure 1). The brilliant blue-white fluorescence of DNA associated with bacteria and nuclei of larger plankton, and induced by DAPI, is spectrally distinct and much brighter than detritus. PI stains cytoplasm of plankton cells a light pink, very similar to the color associated with the autofluorescence of chlorophyll under UV excitation, so that autotrophic, heterotrophic, and mixotrophic cells cannot be discriminated from one another, but they are all

distinct from detritus. The color difference between glutaraldehyde-fixed previously-living eucaryotic plankton, which are light pink, and glutaraldehyde-fixed non-living detritus, which is deep orange, is apparently due to increased (yellow) staining by DAPI of macromolecules other than nucleotides in detritus. We have also confirmed that, subsequent to cell death (defined as disappearance of chlorophyll autofluorescence in phytoplankton), eucaryotic cytoplasm shifts to a yellowish-orange color similar to that of detritus from natural seawater samples. Considerable uncertainty presently exists concerning the physiological state or viability of bacteria when stained with DAPI (Kepner and Pratt, 1994; Zweifel and Hagström, 1995). The protocol described here is presently being modified to explore this issue.

Samples stained with PI/DAPI and concentrated onto filters remain functional for at least 6 months if stored frozen. Our studies indicate that samples stained with PI/DAPI do not quench quickly during moderate exposure to UV-excitation; however, long exposures (>1 hour) of UV cause fading of algal pigments during lengthy observations.

Discussion

A revolution in understanding the pelagic ecosystems occurred with the proposal that it is the microbial food web (MFW), not the traditional phytoplankton-zooplankton-fish food chain that dominates plankton dynamics (Pomeroy, 1974). What is seldom acknowledged is that Pomeroy hypothesized that detritus was the energy for the MFW. Detritus consists of several types of particles, including amorphous flakes, fragments, small aggregates, and unclassified particles (Gordon, 1970). These particles are generally reported to be 5-50μm in size (Hobson, 1967; Gordon, 1970), although particles as small as 1μm occur (Lenz, 1977); therefore small detrital

particles overlap with the operational definition of transparent exopolymer particles or TEP (<3μm: Passow et al., 1994).

Detritus can be categorized into two functional groups: primary detritus derived from plant material and which has not been ingested by heterotrophs; and secondary detritus which is a product of consumption processes including fecal pellets, sloppy feeding, zooplankton molts, and appendicularian houses (Pomeroy, 1979, 1980). Detritus derived from higher plants and macrophytes is composed of higher fiber, lower nitrogen, and is more refractory than that derived from phytoplankton, which is typically more nutritious and labile (Benner *et al.*, 1984; Mann, 1988). The latter contains 69-85% carbohydrates, 12-18% protein, and 2-3% lipid by weight (Mel'nikov, 1974). In addition to being formed directly from senescence, death, or egestion of POM, detritus is thought to be derived from DOC by microbial scavenging (Pearl, 1978; Pomeroy, 1980) and abiotic conversion (Decho, 1990; Alldredge *et al.*, 1993). The latter process, in which exopolymers excreted by bacteria and phytoplankton coalesce into larger microfibrils, colloids, and eventually microscopically recognizable (>1μm) particles, may be especially significant (Passow et al., 1994). The chemical composition of these small detrital particles is primarily polysaccharides, but also includes a variety of non-carbohydrate components: amino acids, glycoproteins, phosphates, pyruvates, RNA, and DNA (Decho, 1990). The latter exogenous nucleotides, which may result from grazing, cell lysis, or active secretion (Stewart and Carlson, 1986), are also reported from larger detritus particles (Holm-Hansen *et al.*, 1968).

Generally two approaches have been taken in the investigation of detritus: chemical analysis of filtered matter, and microscopic investigations of filtered particulate and aggregate matter. The latter technique has flourished, and various studies were performed on detritus

using multiple stains, with many of the investigations employing histochemical dyes such as alcian blue (Parker and Diboll, 1966); acid fuchsin, light green, α-naphtol, Millon's reagent, Sudan black B, and orange yellow (Nemoto and Ishikawa, 1969); and acid fuchsin, acridine orange, and Nile blue (Mel'kinov, 1974). All of the above studies satisfactorily stained detritus, but they focused on investigating the morphology or specific biochemical components (e.g. carbohydrates) of nonliving organic particulate matter. Thus, the present staining technique with PI/DAPI is the first method to allow for distinct identification of both living and nonliving particulate organic matter, preparatory to quantitation of both pools (Verity *et al.*, in prep.).

Propidium iodide is often considered primarily as a nuclear stain because it strongly stains DNA and RNA and is excluded by living membranes; it is therefore used as a marker for dead cells in laser-based flow cytometry studies. Because of this, the excitation maxima of PI is widely thought to be at 530nm, but it also absorbs very strongly at short UV wavelengths and less strongly at long UV wavelengths (Taylor and Lappi, 1975); a microscope UV filter set can be selected to take advantage of this effect. Thus, while PI-stained material fluoresces strongly under green excitation, it fluoresces moderately under UV excitation, and emits at wavelengths different than that of DAPI. PI is from the family of noncovalent intercalating compounds which inserts itself indiscriminately between nucleotide base pairs, but which also electrostatically binds itself to various macromolecules including proteins, polysaccharides, glycosaminoglycans, and membranes (Arndt-Jovin and Jovin, 1989). DAPI covalently binds preferentially to double-stranded DNA, but also binds to polyphosphates, glycoproteins, polysaccharides, various sulfates, sarcoplasmic reticulum, tubulin, and microtubules (Haugland, 1992). As discussed above, these are all common components of detritus, hence the efficacy of dual PI/DAPI staining. Proflavin can also be used as a third stain, which has the advantage of

identifying photosynthetic and non-photosynthetic cells from one another, as well as from non-living POC, on the same samples (using sequential blue and UV excitation), but the stain time must be watched closely in order to avoid degrading the PI/DAPI effect on detritus. For routine use, we prefer to use PI/DAPI for determination of detritus and plankton, and separate slides of proflavin-stained sample to distinguish autotrophic from heterotrophic cells.

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Figure Legend

Figure 1. Water samples stained with propidium iodide/4',6 diamidino-2-phenylindole (PI/DAPI) and examined with an Olympus BH-2 epifluorescence microscope with wideband UV filters.

(a) Pennate diatom, note brightly stained nucleus, cytoplasm, and cell wall. Red autofluorescence of pigments indicate cell was living at time of sample preparation. Scale bar=10μm

(b) Dinoflagellate, with a brightly stained bluish-white nucleus and cell wall; the latter fluorescence may be stain-induced or autofluorescent (Carpenter *et al.*, 1991). Scale bar=10μm

(c) Diatom cells, dead before sample preparation (defined by the lack of chlorophyll autofluorescence), stained a yellowish-orange color similar to the color of detritus. Scale bar=10μm

(d) Centric diatom with a distinct cell wall and brightly stained nucleus. The bright cell wall may reflect staining of the organic matrix, or refraction of fluorescence from the bright nucleus. Scale bar=10μm

(e) Flagellate cell, stained pink by PI/DAPI, but distinct from surrounding (deep orange) detritus. Scale bar=10μm

(f) Bacteria stained bright blue by DAPI, and associated with detrital matter. Scale bar=10μm

(g) Regional view of stained detrital matter (deep orange) and living matter (blue-white). Scale bar=20μm

Table 1. Staining agents tested.

Chemical Designation[a]	Chemical Supplier	Stock Concentration[b]	Staining Concentration[c] (µl/10ml) Incubation period (min)	Fluorescence Wavelength[d] (ex;em in nm)	Specificity	Literature References
Alcian Blue	Sigma	0.5% (DW)	150µl (2)	N/A	polysaccharides	Parker and Diboll (1966)
Calcofluor White	Sigma	0.01% (DW)	150µl (5)	blue (437;490)	cellulose	Darken (1962); Fritz and Triemer (1985)
Dansyl lysine	Molecular Probes	0.1% (PBS)	150µl (5)	blue (365;545)	membrane specificity	Humphries and Lovejoy (1984) Humphries and Lovejoy (1983)
4',6-diamidino-2-phenylindole (DAPI)	Sigma	5mg/ml (DW)	300µl (3)	blue (437;490)	DNA	Porter and Feig (1980)
FITC-concanavalin A	Sigma	0.01% (PBS)	750µl (60)	green (460;515)	carbohydrates	Furlan et al. (1979); Shoham and Sachs (1972)
Primulin	Sigma	0.01% (DW)	150µl (5)	blue (365;425)	polysaccharides	Caron (1983)
Propidium iodide	Sigma	1mg/ml (PBS)	450µl (5)	red (505;615)	nucleic acids	Krishan (1975)

[a]The chemical names of the staining agents are listed in the corresponding literature references shown.

[b]Abbreviations in parentheses denote solvents used: DW, distilled water; PBS, phosphate buffered saline, 10^{-3} M, ph 7.2.

[c]The staining concentrations used and the appropriate incubation period for each.

[d]Ex, excitation peak wavelength; em, emission peak wavelength; NA, not applicable.

a
b
c
d

Visualization and quantitation of plankton and detritus using digital confocal microscopy

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ABSTRACT: The application of digital confocal microscopy to studies of plankton and detritus is described. Plankton cells are fluorescently stained using DAPI and proflavin according to established procedures, while detritus is uniquely stained with DAPI and propridium iodide using a recently published companion protocol. Stacks of digitized images of plankton and detritus are acquired in three dimensions (3D) using an integrating color charge-coupled device (CCD) mounted atop a fluorescence microscope. A Pentium PC drives a z-axis motorized controller to optically section plankton cells and detritus at very fine intervals (as small as 1 μm or less). Out-of-focus haze associated with each optical slice is removed via a nearest neighbor algorithm, in a process termed deconvolution. Image sets containing these stacks of deconvolved optical slices are subsequently displayed in 3D by volume-rendering software operating aboard a dedicated graphics engine. Since the exact x/y/z dimensions of each 3D picture element, or voxel, are known, the volume of plankton or detritus can be calculated. A procedure is described whereby the volume of detritus can be converted to units of carbon and nitrogen. This approach, combined with more traditional 2D image analysis of plankton communities, offers the first opportunity to separately quantify the pool sizes of plankton and detritus in aquatic ecosystems.

KEY WORDS: plankton, detritus, propidium iodide, DAPI, image analysis, confocal microscopy

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INTRODUCTION

Microscopy has been an invaluable tool for the study of plankton, both to visualize the morphology and behavior of such tiny creatures, as well as to quantify their abundance and biomass. While such approaches are sometimes denigrated as being primarily descriptive in nature compared to e.g. biochemical assays such as chlorophyll or rate measurements such as ^{14}C uptake, it is also argued that improved conceptual models of euphotic zone dynamics are dependent upon keener assessments of the population biology of key plankton (Verity & Smetacek accepted). Rather than a battleground over the superiority of biogeochemical science versus community ecology, progress would be better served by new techniques which reunite these experimental approaches; fluxes through a black box are, after all, mediated by individual organisms.

Several microscopic techniques are available for the study of plankton, including brightfield/darkfield, fluorescence, and electron microscopy. Typically these are used for one or another of two applications, to visualize (e.g. Friedman & Strickler 1975) or to measure (e.g. Verity & Sieracki 1993) plankton. While combinations of both visualization and quantitation have occasionally been accomplished (Taniguchi & Takeda 1988), generally the ability to display information which relates to taxonomy or morphology, while resolving details which relate to abundance or biomass, are logistically in conflict. A relatively new tool called confocal microscopy, developed primarily for biomedical sciences, holds considerable promise for simultaneous visualization and measurement with greater resolution (Arndt-Jovin 1991, Bundy & Paffenhofer 1993); here we describe digital confocal microscopy for smaller single-celled plankton.

Whether the approach is biogeochemical or organismal, the focus is typically on the living components because of the primary role of plants in the transformation of dissolved inorganic carbon into organic carbon, and because plankton provide the basis for most food webs in the sea. However, the estimated biomass in the sea of non-living particulate organic matter (POM), or detritus, typically exceeds that of plankton (Smetacek & Hendricksen 1979, Gassmann & Gillbricht 1982, Andersson & Rudehall 1993), and may approach the generally considered value of being 10x greater than living POM (Pomeroy 1980). The size of this non-living pool has long been recognized as a problem in phytoplankton research, because detrital carbon "contaminates" estimates of phytoplankton carbon in field samples from total particulate organic carbon (POC), and relatively "pure" measures of phytoplankton biomass such as chlorophyll *a* cannot be used to estimate phytoplankton carbon because the algal C:Chl *a* ratio varies with algal physiological state and recent nutrient and light history (Banse 1977).

Models of carbon flow indicate that perhaps as much as half of the primary production on continental shelves could pass through detritus and still support all the major trophic groups, albeit with transfer efficiencies of 20-30% instead of 10% (Pomeroy 1979). Moreover, pools of dissolved organic carbon (DOC) are generally considered to be 10x greater than those of detritus, and transformations between DOC and detritus occur via microbial exopolymers (Decho 1990). Detritus, therefore, interacts actively with both living POC stocks and DOC pools, and it would seem difficult to quantify or predict carbon (or nitrogen) fluxes without detailed knowledge of detritus biomass and dynamics.

To date, independent direct estimates of detrital biomass have been generally unsuccessful and fraught with contamination (Gordon 1970, Reiswig 1972). Direct measurement of detritus would not only allow more accurate estimation of phytoplankton C:Chl *a*, but also provide

data on repository sizes of a POC component which is considered to be a major conduit between living POC and DOC. "It would be desireable to obtain a better supported conversion factor from .. organic volume to carbon content .... new field studies should focus on (microscopically derived detrital carbon)" (Banse 1977, pp. 209-210).

If the desired goal is a chemical quantitation of detritus, two approaches are theoretically possible: (1) physically isolate detritus and measure its composition directly, and (2) measure a proxy of detritus within a whole water sample and subsequently convert to composition. The first problem has to date proven impregnable, simply due to the large size range of detrital particles: centrifugation or size fractionation is not feasible, if only because bacteria and plankton overlap with the sizes of detrital particles. The second approach has been historically difficult for several reasons. While detritus stains well with alcian blue, which is specific for polysaccharides (Parker & Diboll 1966), and can be visualized under brightfield microscopy, plankton cells are also stained blue and cannot be distinguished from detritus. With the advent of fluorescence microscopy, plankton could be visually recognized, but two problems remained: (a) how to selectively stain detritus distinct from plankton, and (b) how to quantify an amorphous three dimensional (3D) structure using optical techniques which produce a two dimensional (2D) image, i.e. standard microscopes? We pursued these questions by expanding upon techniques and experience gained from years of analysis of natural plankton communities using imaging cytometry. The methods for staining plankton and detritus are described by Williams et al. (in press); here we present a procedure to render stained plankton cells and detritus in three dimensions and to quantify their volumes and carbon/nitrogen contents.

MATERIALS AND METHODS

Sample Collection

The samples which were quantitatively analyzed for their carbon pool sizes of plankton and detritus, using the procedures described below, were derived from Niskin bottle vertical profiles at two stations in Virginia shelf waters in May 1993. The R/V Gyre provided water from eight depths at station 8 (35.50 °N, 74.99 °W, depth = 42m) and seven depths at station 31 (36.50 °N, 75.31 °W, depth = 39m). These two stations, which were ca. 100km distant and were sampled two days apart, were selected for analysis because they contained very different algal communities: one dominated by diatoms with high chl *a* (4-12 μg/l) and the other by photosynthetic nanoplankton with low chl *a* (<1 μg/l) (Verity et al. in press).

The steps involved in optically "capturing" and displaying plankton cells and detritus are identical, and as described below they are interchangeable. The procedures, however, differ for plankton and detritus in those steps prior to display and also in converting volume measurements to units of biogeochemical significance, e.g. carbon or nitrogen. (1) Plankton cells may be stained with single or multiple stains, to highlight external morphological features or subcellular components, and visualized using light or fluorescence microscopy, whereas detritus is stained to fluoresce a single color uniquely different from all plankton. (2) Procedures for measurements of the volume of plankton cells and detritus are unique to each; conversion of volume to carbon/nitrogen for plankton is derived from empirical measurements using laboratory cultures, whereas detrital volume to carbon is derived from natural suspended material, and thus must be corrected for the separate contributions of detritus and plankton.

To facilitate understanding of these related analyses, the procedures are described as

follows: separate staining of plankton and detritus; "capture" and display of images of both components; and separate analyses of object volume and conversion to carbon/nitrogen.

Sample Staining

Plankton. Samples in the present study were simply stained with dual fluorescent dyes according to generally used procedures for identifying autotrophic cells as distinct from heterotrophic or mixotrophic cells (Verity & Sieracki 1993). Samples were initially fixed with glutaraldehyde (0.3% final concentration), stained with DAPI (10 µg/ml final concentration) for 4 minutes, then momentarily stained with proflavine (1.4 µg/ml final concentration), and finally collected on black 0.4 µm Nuclepore filters. Filters were covered with a small drop of low-fluorescence immersion oil and a coverslip, and slides were used immediately or stored frozen. Proflavine and DAPI are ideal stains for image analysis of plankton due to their bright fluorescence. Depending upon external or internal features of interest, these stains could be supplanted by others or by molecular probes.

Detritus. Our approach was to selectively label detritus and plankton in a water sample using sequential application of glutaraldehyde and dual fluorescent stains, and then concentrate the detritus on 0.4um black Nuclepore filters as is typically done for plankton enumeration. We tested the efficacy of dozens of combinations of stains, excitation sources, and filter sets (Williams et al. in press.). The optimal combination proved to be propidium iodide (PI) followed by DAPI, in conjunction with UV filters. Under wideband UV excitation, PI in the presence of glutaraldehyde stains detritus red, while DAPI stains it yellow; combined they produce a deep orange color. The brilliant blue/white fluorescence of DNA associated with bacteria and the nuclei of larger plankton, and induced by DAPI, is spectrally distinct and much brighter than detritus. PI stains cytoplasm of plankton cells a light pink, very similar to

the color associated with the autofluorescence of chlorophyll under UV excitation, so that autotrophic, heterotrophic, and mixotrophic cells cannot be discriminated from one another, but they are all distinct from detritus. The color difference between glutaraldehyde-fixed previously living plankton, which are light pink, and glutaraldehyde-fixed nonliving detritus, which is deep orange, is apparently due to increased (yellow) staining by DAPI of macromolecules other than nucleotides in detritus (see Discussion). We have also confirmed that, subsequent to cell death (defined as disappearance of chlorophyll autofluorescence in phytoplankton), cytoplasm of eucaryotic plankton shifts to the orange color of detritus in natural seawater samples. Living and non-living bacteria cannot be distinguished by this PI/DAPI technique, as reported elsewhere for DAPI (Zweifel & Hagstrom 1995); we are developing a separate procedure using RNA probes to distinguish metabolically active from inactive bacteria (S. Williams, M. Frischer, and P. Verity, unpubl.).

Image Capture and Volume Rendering

The heart of the imaging system used here is that described by Verity & Sieracki (1993), with contemporary upgrades and features required for digital confocal microscopy. Basically, a powerful desktop computer (Pentium 100 mHz with 32 MB RAM and 1 GB hard drive) houses several integrated software packages which operate microscope-mounted hardware and additionally perform image processing functions. An Olympus BX-60 fluorescence microscope, which is also capable of brightfield, darkfield, phase contrast, and differential interference contrast illumination, provides a high quality initial view of the sample, and a range of objectives (10-100x) optimizes sample display. An Optronics DEI-470 integrating 2/3" CCD captures an analog RGB color image at variable frame rates from 1/10,000 to 2 minutes. An on-board frame grabber continuously displays full-frame images, with integration

times up to 2 minutes producing a minimum sensitivity of 0.0025 lux. An integrated imaging software package, Image Pro Plus (IPP) for Windows (Media Cybernetics, Inc.) controls image capture, enhancement, measurement, analysis, and output. IPP directs an electronic shutter mounted in-line in the microscope lightpath so that the sample is exposed to excitation for only as long as the camera shutter is open, thus minimizing photobleaching. Images are digitized via an ImaGraph ImaScan/Chroma PCI video capture board, and displayed on a Nokia 17" RGB monitor. Through customized macros, IPP also controls the z-axis motorized stage on the BX-60 and associated modular automation controller (described below).

Plankton or detritus samples were stained and collected on filters as described above. The microscope operator interacted with IPP to set the upper and lower focal planes which comprise the top and bottom optical slices of the object of interest, and to set the distance between slices, which decreases with increasing numerical aperture of the microscope objective. The BX-60 has twice the vertical resolution of its predecessors and the interslice distance for a 60x 1.4 NA oil objective is 0.5 μm (see Discussion). IPP then drove the z-axis motorized stage so that the microscope automatically focused on the top of the object, the shutter opened electronically (i.e., no vibration), the CCD grabbed an image (= an "optical slice") during the period when the camera shutter is open, (e.g.,1/30 sec), the electronic shutter closed, the motorized microscope stage moved down an exact prescribed amount so that a new "slice" was in focus, and the procedure was repeated until the bottom of the object was reached.

The next step was to mathematically remove the out-of-focus haze from the set of image slices using a software program called Micro-Tome (Vaytek, Inc.). Nearest neighbor algorithms were applied to individual images or to the entire image set, in a process termed

deconvolution, to remove out-of-focus blur; these algorithms have the same effect as pinhole apertures in laser scanning confocal microscopes (see Discussion).

The resulting sharply focussed optical slices were fed into a sophisticated graphics computer (Silicon Graphics Iris R4000 Indigo 2, 50 mHz, 64 MB RAM, 1 GB hard drive, 19" monitor), running a high-end volume rendering software package, VoxelView Ultra (Vital Images, Inc.). VoxelView's volume rendering technique represents objects as collections of 3D picture elements, called volume elements or voxels. Each voxel, which is a sample of the original volume and is identified by x-, y-, and z-coordinates, has the value of some measured property of the original object like intensity or color. Provided with the x-, y-, and z-axis dimensions, determined by microscope calibration using IPP, VoxelView interpolated between the image planes and rendered each imageset as an object in three dimensions. One of the significant features of VoxelView is that it can calculate the number of voxels in a 3D volume, or any subvolume thereof, which have corresponding properties. A typical 640x480 pixel image containing 20 slices is composed of a total of 6.1 million voxels. Since the exact size of each voxel in x-, y-, and z-dimensions was known (e.g. 0.26 x 0.21 x 0.50um at 600x magnification), the volume in μm^3 corresponding to those voxels could be calculated, e.g. the volume of chloroplasts within a diatom, or of a bolus of detritus. The computational speed and design of VoxelView Ultra running on an R4000 SGI rendered 6 million voxels in ca. 1 second, so that smooth animation sequences were composed to visualize the object from any elevation, azimuth, proximity, contrast, or transparency.

Carbon Content

Plankton. The objective in the present study of using digital confocal microscopy in plankton research is the insight offered by visualization in three dimensions. For rapid

quantitation of carbon content of small plankton cells, a better procedure is measurement in two dimensions, with calculation of the third dimension and subsequently of cell volume (Verity & Sieracki 1993, and below). Carbon content can then be estimated directly using empirically derived equations (Verity et al. 1992, Montagnes et al. 1994).

Detritus. Once the volume of detritus in a sample was determined using the confocal microscope approach described above, four steps were necessary to calculate the carbon/nitrogen content. The total amount of particulate organic carbon (POC) in a water sample was measured chemically, the amount of plankton carbon/nitrogen was measured microscopically, the amount of detrital carbon/nitrogen was calculated by difference, and detritus volume was regressed against detritus carbon/nitrogen to develop a predictive regression. These steps are described in sequence.

(1) Total POC and PON was measured by combustion in a commercial CHN analyzer according to standard methods (Sharp 1974). Basically, samples were filtered at low vacuum pressure onto pre-combusted (450 °C for 4h) 25mm GF/F filters, avoiding obvious sources of contamination. These were transferred onto similarly pre-combusted foil storage sheets, and stored frozen (-70 °C) until analyzed. After freeze-drying, they were combusted in a CHN analyzer using acetanilide standards, and corrected for equal volume media blanks.

(2) The methods used to determine the biovolume of plankton cells via image analysis techniques, and subsequent conversion to cell carbon and nitrogen have already been developed and tested (Sieracki et al. 1989a,b; Verity et al. 1992, Verity & Sieracki 1993), and were described above under "Sample Staining". Plankton cells were analyzed using an imaging cytometry system which is a subset of the one described for confocal research. For quantitative enumeration and sizing of plankton, an image was located, either randomly or on

a transect (see below), and focused on the video monitor visually. It was then rapidly digitized into the computer, avoiding significant fluorescence fading. The color video system used real-time frame averaging to reduce noise. At this point, the image was edited if necessary, e.g. to separate adjoining cells. Once the image was judged to be adequate either the whole image was analyzed or individual cell sub-images were saved for later analysis. Two approaches were used depending upon the density on the slide of the particular cell population being analyzed. For cells which are numerous per field and relatively uniform in brightness in a given sample (e.g. cyanobacteria and some flagellates), randomly chosen whole fields were analyzed. In this case all the cells in a field were segmented (identified in the image) and measured automatically. This approach required that a single threshold be used for all cells in the image, but it was faster than automatically finding individual cell thresholds. Analysis of an image with 40 cells takes about 4 s.

For more rare cells, transects of the slide were scanned and individual cells were isolated and identified by the operator interactively. In this way densities of rare cells were calculated per volume of sample. By interfacing the motorized stage and associated modular automation controller with the epifluorescence microscope, the operator can scan transects of variable length across the plankton slides while the computer records the fraction of the surface area of the slide which has been examined. For abundant populations, the computer can randomly select individual locations on the slide. The driver software has recently been modified so that the entire process (moving to a given location, focussing, opening an electronic shutter, grabbing an image, closing the shutter, and moving to a new location) is automated and computer-controlled. Sub-images containing the individual cells were temporarily stored and analyzed automatically overnight. The analysis procedure for these sub-images involved

automatic threshold determination using the second derivative method (Sieracki et al. 1989a), segmentation and cell measurement. Cell measurement data and example images were archived on tape or floppy disk for later analysis. Individual cell biovolume measurements (Sieracki et al. 1989b) were converted to carbon/nitrogen biomass using conversion factors based on literature values of carbon/nitrogen density or C:N ratios (Borsheim & Bratbak 1987, Bratbak & Dundas 1984, Kroer 1994, Lessard 1991, Putt & Stoecker 1989, Verity & Langdon 1984; Verity et al. 1992). The abundance, size, and biomass of crustacean zooplankton was measured according to Verity et al. (in press).

(3) The carbon/nitrogen content of detritus was calculated as the difference between total combusted POC/PON and that contained in bacteria, eucaryotic and procaryotic phytoplankton, protozoan and metazoan zooplankton. Most bacteria in field samples, where they typically comprise <5-10% of total POC in shelf environments (Smetacek & Hendrickson 1979; Verity et al. in press), pass GF/F filters (effective pore size ca. 1μm), so their contribution to combusted POC/PON is minimal. However, they are captured on 0.4 μm Nuclepore filters and thus contributed to estimated plankton carbon and nitrogen. This was corrected for by enumerating and sizing bacteria via image analysis in whole water samples prior to filtering the POC/PON samples, and again in the filtrate.

(4) The calculated detrital carbon/nitrogen from each sample was then compared to the measured volume of detritus in that sample to determine the detritus carbon:volume and nitrogen:volume conversion factors.

RESULTS

Plankton.

The first test of the ability of digital confocal microscopy was to confirm that it accurately represented the size and shape of known objects. Latex microspheres of various diameters were imaged as described in the methods, and 3D volumes were compared to those calculated from diameter dimensions applied to mensuration equations (Table 1). [The coefficient of variation of the diameter of latex microspheres decreases with increasing bead size, and is typically 1% or less for beads 10μm or larger (Polysciences, Inc., Warrington, PA, USA)]. For beads spanning the sizes of many unicellular plankton (6-20 μm), beads rendered by VoxelView showed only minor deviations from sphericity, and the two methods yielded similar estimates of volume. For beads 45 μm and larger, volumes derived by 3D imaging were larger than those calculated from measured diameters. Visual inspection of the rendered volumes of larger beads showed shape distortions in the lower portion of the bead, specifically that the lower portion of the bead was not spherical but rather was ellipto-cylindrical. This effect was apparently caused by the thickness or opacity of these larger specimens and the inherently lower resolution in the z-axis dimension (see Discussion, and Russ, 1995, Chap. 9).

Plankton cells in the size range of ca. 5-20 μm, fluorescently stained with DAPI and proflavin, were faithfully rendered in 3D; an example of *Thalassiosira* is illustrated in Figure 1. The cell was imaged under blue excitation using an infinity-corrected 60x Plan Apochromat oil immersion objective, N.A. 1.40. The valve diameter of this cell was measured as 21.4 μm. It was then optically sectioned at 0.50 μm intervals, resulting in 43 "slices". After the image set was acquired, the coverslip on the oil embedded filter holding the plankton cell was moved

laterally, displacing the *Thalassiosira* cell into girdle view. Cell thickness was measured at 6.9 μm. The image set was rendered by VoxelView, with one interpolated slice inserted between each measured slice to account for the 50% lower z-axis resolution. The resulting 3D image of the cell (Figure 1) was 7.2 μm thick. The cell volume calculated from direct linear dimensions and assuming a right circular cylinder shape [volume = $(\pi)(r^2)(h)$] was 2482 μm^3. The volume calculated by VoxelView was 2589 μm^3, or 4% greater than that calculated from length and depth. The volume of the cell which corresponded to the autofluorescence of chloroplasts was 596 μm^3, or 17% of the total cell volume; cytoplasm and nucleus comprised 50% and 7% of total cell volume, respectively.

The sequence of images in Figure 1 are individual 3D images taken from an animation of *Thalassiosira* created by VoxelView, and serve to illustrate the power and options of visualization. The initial view, which is at a slight angle to the z-axis plane, shows a brilliant white/yellow nucleus stained by DAPI, orange/pink autofluorescence of chloroplasts, and blue/green cytoplasm stained by proflavin, viewed against a black Nuclepore background. In subsequent images, voxels corresponding to the nucleus have been removed for clarity. The presence of black within the cell signifies the absence of any fluorescing cellular material, and presumably corresponds to the diatom vacuole. The frustule is not visible in this stained preparation, but can be simultaneously visualized by combining minimal darkfield illumination with blue wavelength fluorescence excitation. As the animation proceeds from left to right, the cell gradually rotates in that direction through 360°. Concurrently, VoxelView was instructed to gradually "peel away" those voxels corresponding to the blue/green cytoplasm, in order to reveal the number, size, and arrangement of the chloroplasts. Interestingly, the chloroplasts appear first at the girdle region, suggesting that the cytoplasm was thinnest there.

Upon removal of most of the cytoplasm, it is evident that the chloroplasts were concentrated around the peripheral edge of the cell, with two concentric rings of chloroplasts between the outer ring and the central nucleus.

Detritus.

Natural detritus from continental shelf waters concentrated on Nuclepore filters typically collapses down to thicknesses of 5-10μm on various sections of the filter. With intervals of 0.5μm between slices, only 10-20 optical slices are needed to measure all the detrital particles, compared to often 60-80 slices taken at higher magnification when larger (thicker) plankton cells are volume-rendered. The standard protocol described in the methods can be specifically modified for analysis of detritus. Since the boluses of detritus do not need to be visualized, but only quantified, one can simply measure the number of voxels corresponding to the uniquely-stained detritus in each optical slice and, knowing the exact depth of each slice, can directly calculate detrital volume. This approach avoids the need to render the object in 3D yet gives identical estimates of volume, which saves time and the need for an expensive SGI graphics workstation. Subroutines were composed in Visual Basic language to accomplish these measurements on-the-fly as each slice was imaged.

The number of optical fields of detritus which must be analyzed varies with the concentration of detritus collected on the filter, but is typically 20 fields. Fewer fields are required than when enumerating plankton cells because so much more detritus occurs per unit surface area of filter. It is best to adjust sample volume to reduce the number of fields required, by previewing initial samples in the microscope.

Measured volumes of detritus from the samples collected in Virginia shelf waters were

regressed against calculated detrital carbon and nitrogen (Fig. 2). The linear regression models suggested that detrital carbon was estimated from detrital volume with high confidence as 0.23 pg C per cubic micrometer of detrital volume. Detrital nitrogen was estimated from volume as 0.011 pg C per cubic micrometer, with greater variance. Total particulate organic carbon (POC) and nitrogen (PON), and those fractions corresponding to living plankton were also measured in the same samples (Table 1). Plankton POC ranged from 25 to 365 μgC/liter, while detrital POC was 140-478 μgC/liter. Plankton PON was 4-57 μgN/liter and detrital PON was 5-32 μgN/liter. Detrital carbon and nitrogen were not significantly correlated if all data points were included, but exhibited a significant ($P<0.05$) linear relationship if the highest carbon data point was excluded from analysis (Figure 3). Comparing % detrital POC to the total POC:Chl *a* ratio suggested that detritus was the most important carbon depot in high C:Chl *a* waters (Figure 4). Although preliminary, these data suggest that it may be possible to eventually derive predictive regression models to estimate detrital carbon from total POC (Figure 5), or estimate plankton carbon from C:Chl *a* or from Chl *a* directly (Figure 6).

DISCUSSION

In medicine, 3D visualization is uniquely valuable in the form of cat-scans or tomography, typically done on objects the size of the human body. With plankton, however, the object to be viewed is the size of a red blood cell within the human body, so there is a tremendous scaling problem. The CCD viewing plankton on the microscope does not use adjustable focussing lenses. By analogy, it would be as though your eyes only focussed on objects which were a constant distance away from you: as if everything closer or further away was blurry or out-of-focus. The solution is to move the microscope stage which holds the

specimen closer or further from the CCD in order to focus on it. However, the plane of focus is very thin, e.g. 0.3µm using blue excitation and a 60x 1.4 NA objective, and the other sections of the cell (above and below the plane in focus) are blurry.

To obtain the detailed 2D image slices necessary for high resolution rendered volumes, that out-of-focus light must be removed, and two types of methods are available. One is to allow only the light (= image) which is in focus to reach the camera, by forcing it to pass through very tiny pinholes or slits which can be set to stop passage of the out-of-focus light. The detriment to this approach is a tremendous (e.g. 95%) reduction in light intensity coming from the object, so that a substantial excitation light source is required. This is typically a laser, which has three undesirable attributes: (1) it emits very high energy which quenches fluorescence; (2) the illuminating beam and return light aperture must be precisely aligned; and (3) it is therefore comparatively quite expensive. Laser-based systems can compensate for intensity reduction by scanning the sample multiple times; they are also generally better for thicker or more opaque specimens (Agard et al. 1989, Rigaut et al. 1992).

The other approach described here, digital confocal microscopy, depends upon the theoretical distribution of light energy around a point source, which is theoretically and mathematically well understood (Gibson & Lanni 1990, Hiraoki et al. 1990). The out-of-focus blur or smear, which is a natural consequence of the optics of a light microscope, is added to the image in a precise and predictable manner by the point spread function (PSF) of the microscope (Young 1989). The software solution used in the present study calculates the PSF using diffraction theory and knowledge of the excitation wavelengths, the numerical aperture of the objective, the x/y dimensions of pixels within each image plane, and the z-axis distance between images. Using these data, the digital confocal method uses a nearest neighbor

algorithm (Agard et al. 1989) to computationally remove the out-of-focus haze, obviating the need for lasers and allowing samples to be imaged on modified standard microscopes. However either approach, laser scanning or digital confocal microscopy, will work, and the latter can be used to improve laser-derived images.

Plankton. Confocal microscopy provides several advantages over other methods of visualization of plankton. Because of their extremely narrow focal planes, a light microscope presents a 2D image to the eye or any other detector, hence the three dimensional structure of larger thicker specimens cannot be seen at any one time. Various techniques with light microscopes (e.g., phase contrast, shadow casting, differential interference contrast) provide some depth perception, but not 3D visualization. Electron microscopy combines superior resolution with depth perception, but it is comparatively costly and cannot resolve internal structure. Only confocal microscopy combines light- or laser-based micrometer-scale resolution with the ability to visualize the plankton cell in 3D. Two particular attributes are salient. One described here is the ability to quantify volumes or subvolumes thereof, e.g. chloroplasts or any other cellular feature which can be uniquely identified. This capability seems particularly promising with the development of specific molecular probes which can be conjugated to a broad color range of fluorescent stains. Another powerful tool is the ability to section a cell in any orthogonal direction, and then visually or quantitatively contrast internal features between and among sections.

While confocal microscopy is particularly well-suited for resolving and displaying internal structural features (e.g. Bundy & Paffenhöfer 1993), it is not presently the optimum tool for rapid estimation of the size or biomass of numerous plankton cells. It requires a skilled operator approximately 1 hour to acquire, deconvolve, and render a plankton cell in

3D. In the same amount of time, hundreds of plankton cells can be measured in 2D and their volumes estimated from knowledge of the third dimension (Sieracki et al. 1989b, Verity & Sieracki 1993).

Detritus. Detritus can be categorized into two functional types: relatively large, fast-sinking particles or aggregates, and smaller suspended particles. Because of interest in fluxes to the seafloor, methods for analyzing sinking aggregates have been developed (Alldredge & Gotschalk 1989, Walsh & Gardner 1992); these and related studies have confirmed the significance of aggregates to vertical fluxes and as microzones for enhanced plankton activity (Michaels & Silver 1988). Small detrital particles, however, are equally important, perhaps more so in the context of upper water column dynamics. It is the small well-suspended particles which are captured in Niskin sampling bottles, which end up on GF/F filters, and analysis of which indicates that non-plankton carbon often exceeds the contributions of plankton to total POC, e.g. Eppley et al. (1992). Thus, the working definition of detritus for the purposes of this study, derived from Lenz (1977) and Passow et al. (1994), is non-plankton POC captured in Niskin bottles and collected on 0.4μm polycarbonate filters.

There are essentially three steps in the method described here to measure detritus: (1) staining detritus distinct from other inorganic and organic material; (2) measuring the volume of detrital particles; and (3) estimating chemical content from volume. Each of these are discussed below.

Propidium iodide (PI) is generally considered to be a nuclear stain because it strongly stains DNA and RNA and is excluded by living membranes; it is therefore used as a marker for dead cells in laser-based flow cytometry studies. The excitation maxima of PI for such applications is 530nm, but PI also absorbs at short, intermediate, and long UV wavelengths

(Taylor & Lappi 1975). Thus, while PI-stained material fluoresces strongly under green excitation, it fluoresces moderately under wideband UV excitation, and emits at wavelengths different than that of DAPI. As a noncovalent intercalating compound, PI inserts itself indiscriminately between nucleotide base pairs, but also electrostatically binds to various macromolecules including proteins, polysaccharides, glycosaminoglycans, and membranes (Arndt-Jovin & Jovin 1989). While DAPI is most appreciated for binding preferentially to double-stranded DNA, DAPI also binds to polyphosphates, glycoproteins, polysaccharides, various sulfates, sarcoplasmic reticulum, tubulin, and microtubules (Haugland 1992). These are common components of detritus (Mel'nikov 1974, Decho 1990), hence the efficacy of dual PI/DAPI staining (Williams et al. in press). Proflavin can also be added as a third stain, which has the advantage of allowing measurement of nonliving POC and discrimination among types of plankton POC on the same samples (using both blue and UV excitation), but the stain time must be watched closely in order to avoid degrading the PI/DAPI effect on detritus. For routine use, it is preferable to make one preparation to distinguish detritus from plankton, and a separate slide to distinguish among the various types of plankton. At present, the operator manually distinguishes detrital particles by color distinction compared to plankton (Williams et al. in press), but we are developing an automated color separation algorithm as a more objective protocol to minimize inter-operator variance.

Once particles of interest (detrital or otherwise) can be distinguished from others, confocal microscopy provides a unique solution to their quantitation; laser scanning or digital confocal microscopy both are appropriate. The data illustrated here (Table 1, Figure 1) indicate that digital confocal microscopy captures and renders the volumes of nano- and small microplankton accurately; for detritus, the need to render it in 3D is secondary to mensuration

of volume. The volume measurements require knowledge of x/y dimensions of the pixels that comprise a 2D image, which are calibrated directly using a stage micrometer, and the z-axis distance between slices. The best possible z-axis resolution is defined as the theoretical Rayleigh minimum, which is inversely related to the resolving power of the objective in use according to $D_{min} = 1.22(\lambda)/2(NA)$, where lamda is the peak wavelength and NA is the numerical aperture of the objective (Shotton, 1989): using a 1.40NA oil objective under 0.5 μm illumination, the minimum resolution is 0.25 μm. The ideal z-axis spacing between image planes, according to Nyquist sampling theorem, should be twice the spatial resolution of the optical system (Castleman 1979), or 0.5 μm for the 1.4NA oil objective. Thus the x/y/z dimensions of each voxel corresponding to detritus are known, and with sufficient subsamples of each filter, the volume of detrital particles in a sample can be estimated.

The entire procedure is only as accurate as the conversion factor from volume to carbon or nitrogen. This factor (a) may have errors associated with its calculation, and (b) may vary naturally. As described here, the concentrations of detrital carbon and nitrogen were derived by subtracting microscopically measured plankton carbon/nitrogen from total POC/PON determined by high temperature combustion. The latter method for total POC/PON is well known. The comparative ease and accuracy of 2D imaging cytometry to estimate the carbon biomass of mixed plankton communities has been known for some time (Sieracki et al. 1993, Verity et al. 1992, 1993). C:Chl *a* ratios of phytoplankton derived from these measurements fall within the ranges derived from cultures with similar nutrient and light histories (Verity et al. in press), implying that the estimates of carbon biomass are not wildly inaccurate. Estimates of nitrogen biomass, however, may suffer from reduced accuracy of cell volume to cell nitrogen conversion factors, which are more poorly known than those for carbon.

Since total POC/PON and plankton POC/PON can be measured with acceptable accuracy, then detrital carbon/nitrogen can be estimated by difference. However, if the mass of detritus is quantitatively related to its volume, then detrital carbon and nitrogen can be estimated directly from volume, rather than having to measure both total and plankton POC/PON, and calculate by difference. The field data illustrated here imply that conversion from detrital volume to carbon, and to a lesser extent nitrogen, may be robust over mesoscale distances, at least during brief periods in time. However, the net effect of the various processes which produce and decompose material contained in detritus is that the ratio of POC and PON to particle volume (C:V, N:V) in detritus may or may not be constant. For example, the C:V ratio in detritus may decrease as bacteria and their associated microbial food web disrupt the integrity of the detrital material, enhancing leaching (Biddanda & Pomeroy 1988): typical degradation rates are 2-4%/day (Biddanda 1988; Seiki et al. 1991, Lee & Fisher 1992). Alternatively, the C:V ratio may increase as DOC adsorbs onto detritus via exopolymers and is cemented by microbial activities (Paerl 1974); there is experimental evidence for a constant flow of polysaccharides and proteins into detritus at rates of 3% per day (Khaylov & Finenko 1968). Thus the concurrent processes of production and consumption of detritus, termed the "detritosphere" concept by Biddanda & Pomeroy (1988), may act to minimize changes in the C:V ratio. It is also worth noting that the direction and magnitude of nitrogen flow in detritus may not be the same as that of carbon (Biddanda & Riemann 1992), which may contribute to the lower regression coefficients observed here for nitrogen vs. carbon (Figure 2), and the scatter in the relationship between detrital carbon and nitrogen (Figure 3). The variance in C:V and N:V of detritus, and its causes, is currently under investigation.

The capability to quantify both plankton and detritus is potentially significant from

several perspectives. First, the presence of detritus confounds attempts to estimate phytoplankton carbon in natural plankton communities: in fact, high total POC:Chl *a* ratios *in situ* are considered indicative of contamination by detrital carbon. The ability to independently quantify detritus would yield more accurate estimates of phytoplankton carbon, an outstanding problem in phytoplankton ecology (Banse 1977). In this regard, it should also improve algorithms to convert satellite data into ocean pigment budgets (Garver et al. 1994) and estimates of phytoplankton growth rates, e.g. "specific growth rates of phytoplankton cannot be accurately estimated from beam attenuation until the relative contributions of phytoplankton, microheterotrophs, and detritus are resolved" (Cullen et al. 1992). Second, detrital carbon, while generally comprising lower C:N ratios than phytoplankton, can enhance survival and reproduction of zooplankton (Heinle & Flemer 1975; Roman 1984). In the Peruvian upwelling zone, for example, detrital carbon was estimated to provide 40% of the diet of heterotrophs (Lebedeva et al. 1982). Third, because detritus is non-living and therefore lacks behavioral and physiological responses to environmental stimuli, its carbon pool may not follow the same flux or transformation routes as does plankton carbon, e.g. phytoplankton ingestion by zooplankton, mass sedimentation of diatoms. For example, living phytoplankton were estimated to contribute only 1% of offshore POC flux during the Shelf Edge Exchange Process (SEEP) studies (Falkowski et al. 1994). Thus, budgeting detrital carbon as distinct from plankton carbon may yield valuable insights into the structure and operation of planktonic ecosystems.

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FIGURE LEGEND

Figure 1.

A sequence of images from a computer animation of a single cell of *Thalassiosira* sp. Blue/green voxels are those portions of the cell stained by proflavin, i.e. cytoplasm. Pink/orange voxels are those portions of the cell which autofluoresce, i.e. chloroplasts. The intense white/yellow voxels in the cell center, which represent the DAPI-stained nucleus, were removed in images after the initial one, to illustrate the ability to selectively identify subregions of an object of interest. Proceeding from the upper left panel (A) to the lower right panel (I), the cell rotates through nearly 360 degrees. During the animation sequence, the volume rendering software was instructed to gradually peel away those voxels corresponding to cell cytoplasm, in order to visualize the size, number, and arrangement of chloroplasts. The cell was visualized using infinity-corrected optics and a 60x, 1.4NA objective, and optically sliced using an integrating color CCD. The 2D slices were deconvolved to remove out-of-focus blur, and the stack of 2D images were rendered as a 3D volume on a Silicon Graphics Indigo2 computer. The rendered volume and animation were created using VoxelView. See text for details.

Figure 2.

(A) Relationship between volume of detrital particles measured in 3D and carbon content of detritus estimated from chemical and microscopic measurements. Samples were from two vertical profiles in continental shelf waters off Cape Hatteras in May 1993. Model I linear regression (y=ax+b): detrital carbon (μg/l) = 23.0 (detrital volume, 10^6 μm^3) - 3.6,

$r^2 = 0.87$, n=15. (B) As in 2A, but for detrital nitrogen. Model I linear regression: detrital nitrogen (µg/l) = 1.1 (detrital volume, 10^6 μm^3) + 3.5, $r^2 = 0.37$, n=15.

Figure 3.

Relationship between estimated detrital carbon and nitrogen for samples in Fig. 2. The Dixon test for outliers (Taylor, 1990) supported the elimination (at the 95% confidence interval) of one data point from the linear regression model: detrital carbon (µg/l) = 6.0 (detrital nitrogen, µg/l) + 125.3, $r^2 = 0.35$, n = 14.

Figure 4.

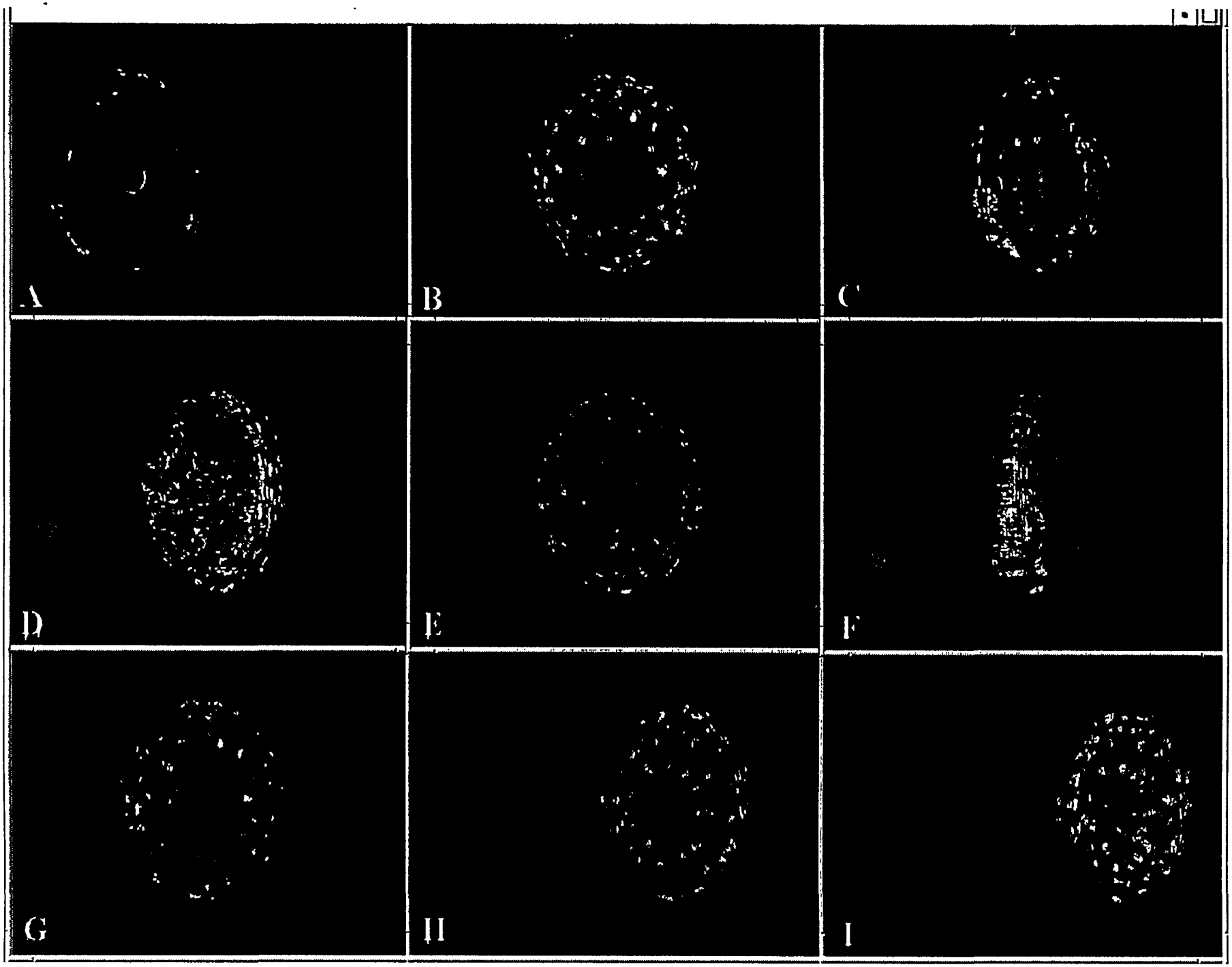
Relationship between detrital carbon as a percent of total POC, and the ratio of total POC to chl a, in the samples decribed in Fig. 2. Detrital:Total Carbon (%) = 0.29 Log (POC:Chl a, ug:ug) - 0.005, $r^2 = 0.89$, n = 15.

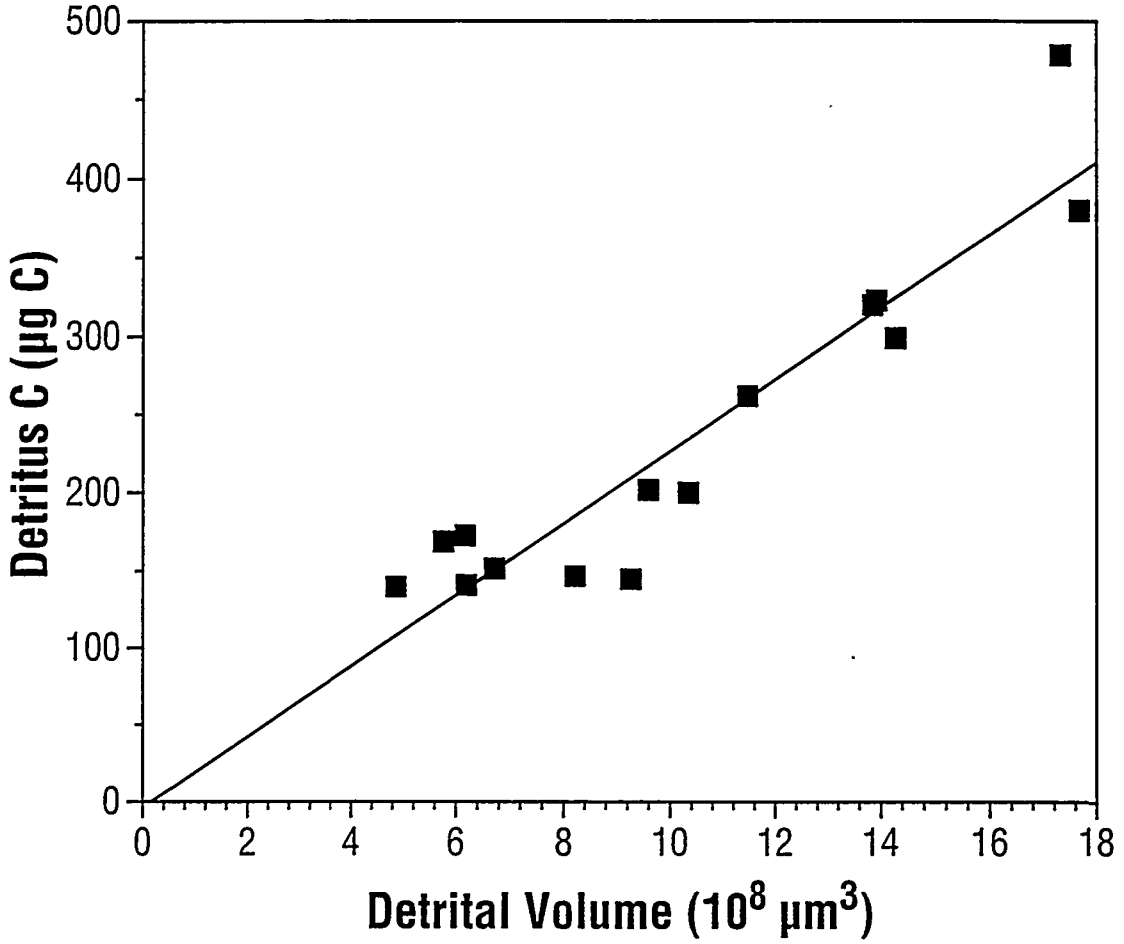
Figure 5.

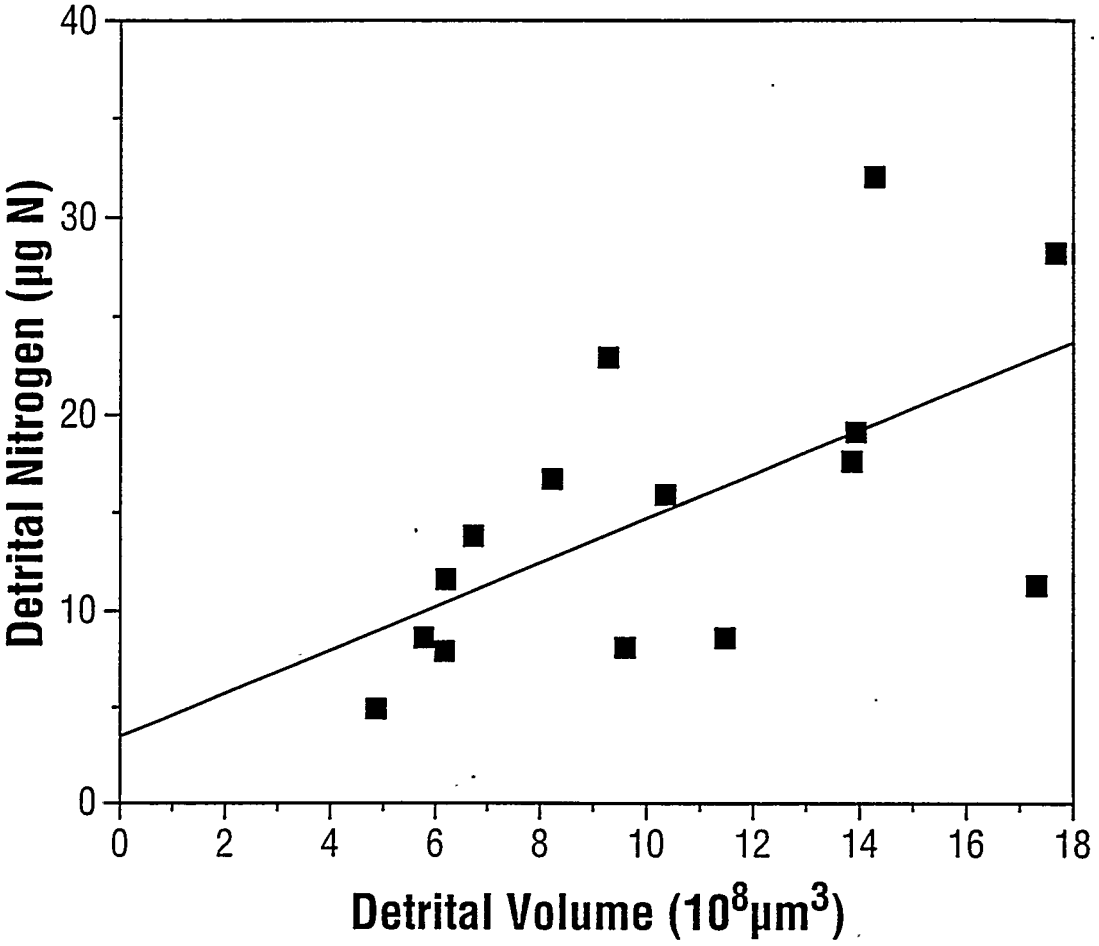
Relationship between detrital carbon and total POC for samples described in Fig. 2. Model I linear regression: detrital carbon (µg/l) = 0.47 (total POC, µg/l) + 57.1, r^2 = 0.61, n = 15.

Figure 6.

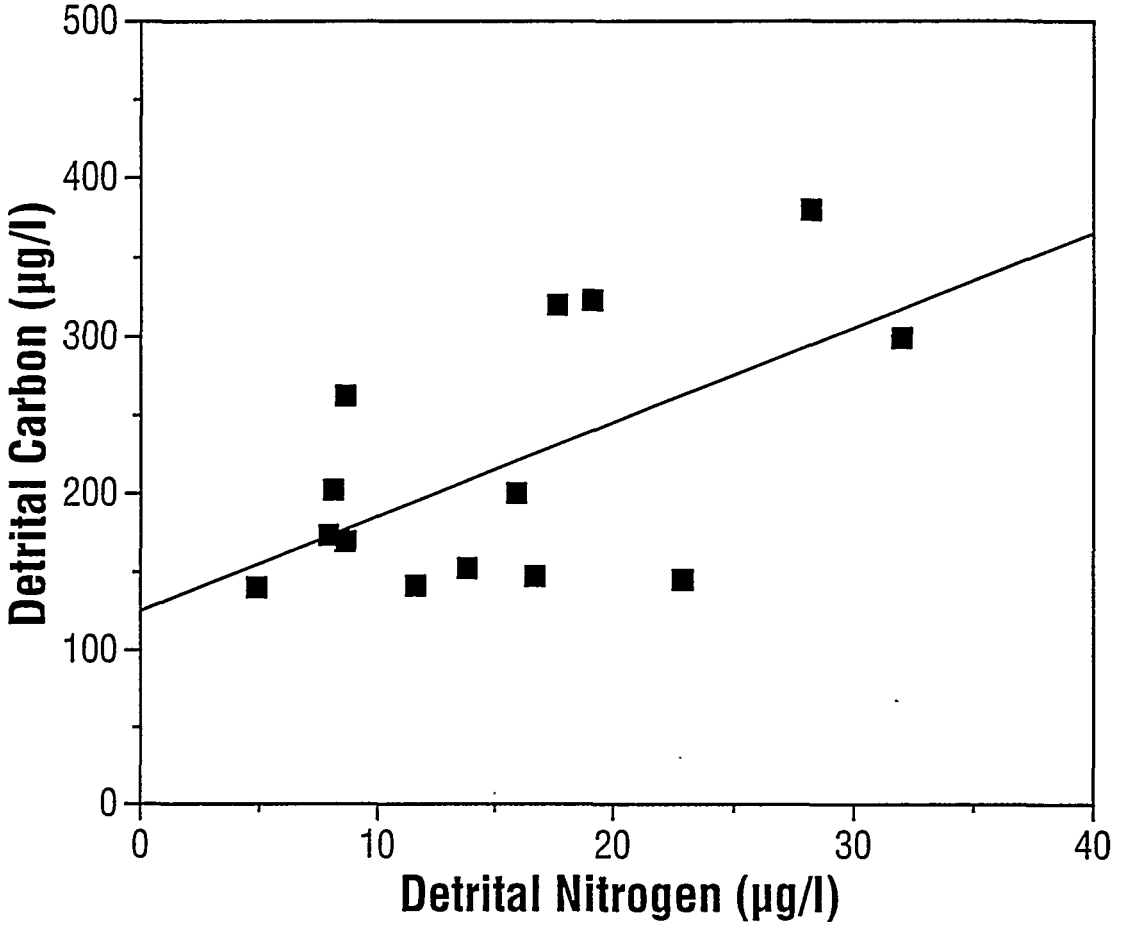
Relationship between plankton carbon measured by 2D image analysis and fluorometrically determined chl *a* in the samples described in Fig. 2. Plankton carbon (µg/l) = 172.6 Log (chl *a*, µg/l) + 108.4, $r^2 = 0.91$, n = 15.

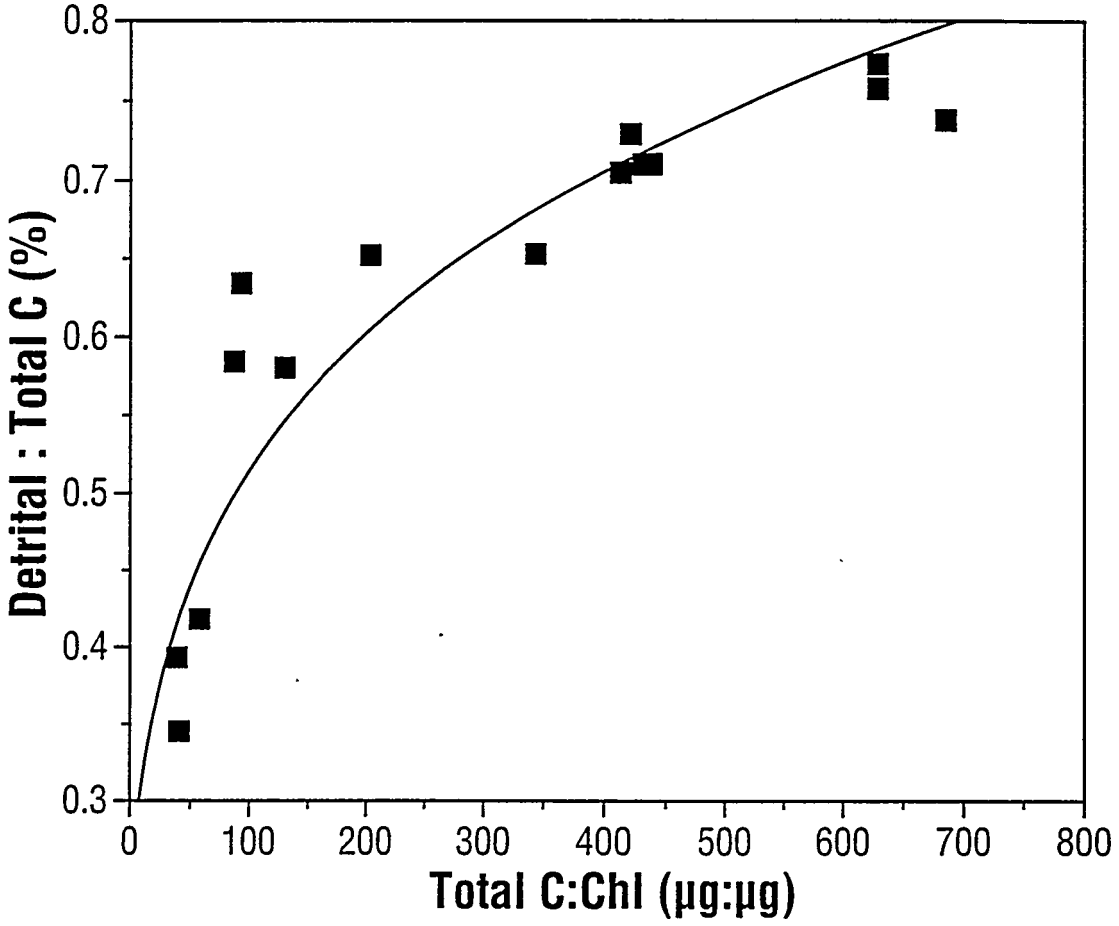

A
B
C
D
E
F
G
H
I

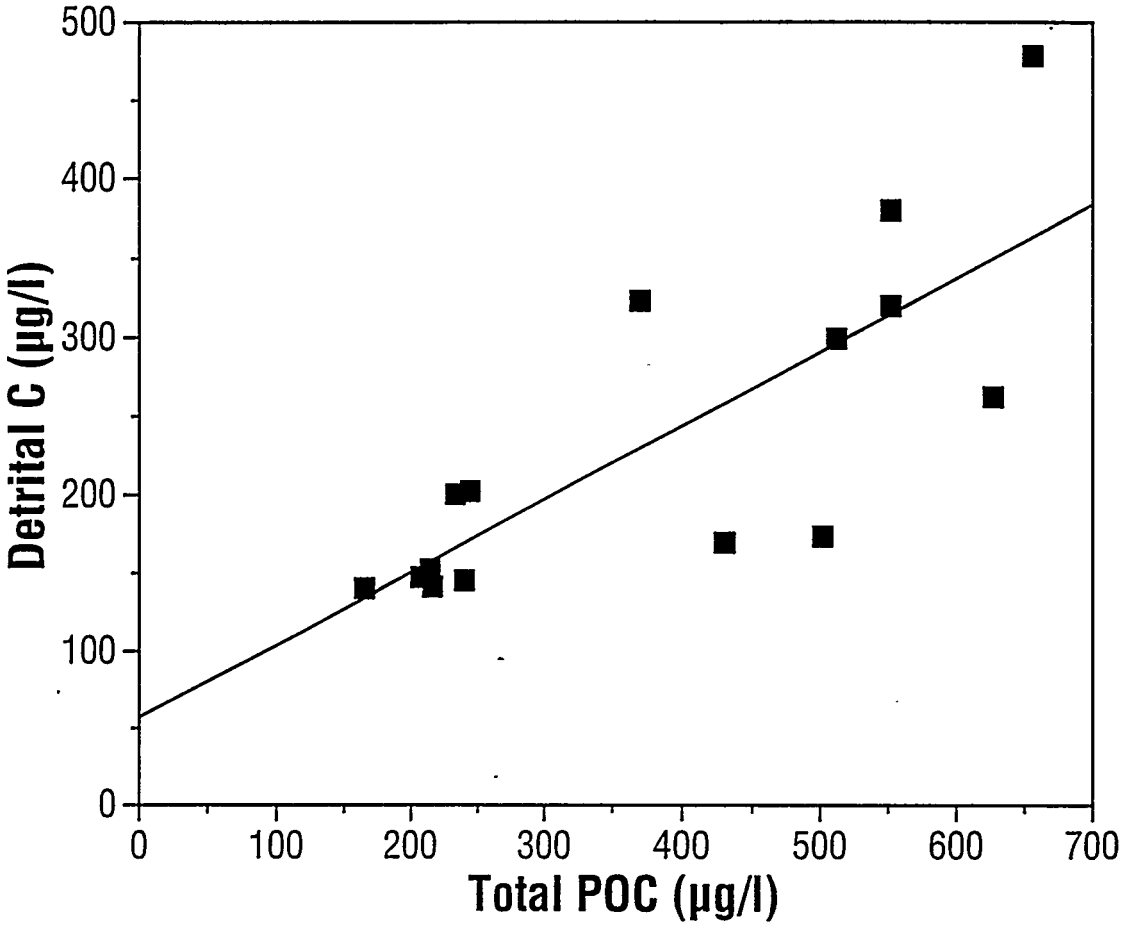

Detritus C (µg C)
500
400
300
200
100
0
0
2
4
6
8
10
12
14
16
18
Detrital Volume (10^8 µm^3)

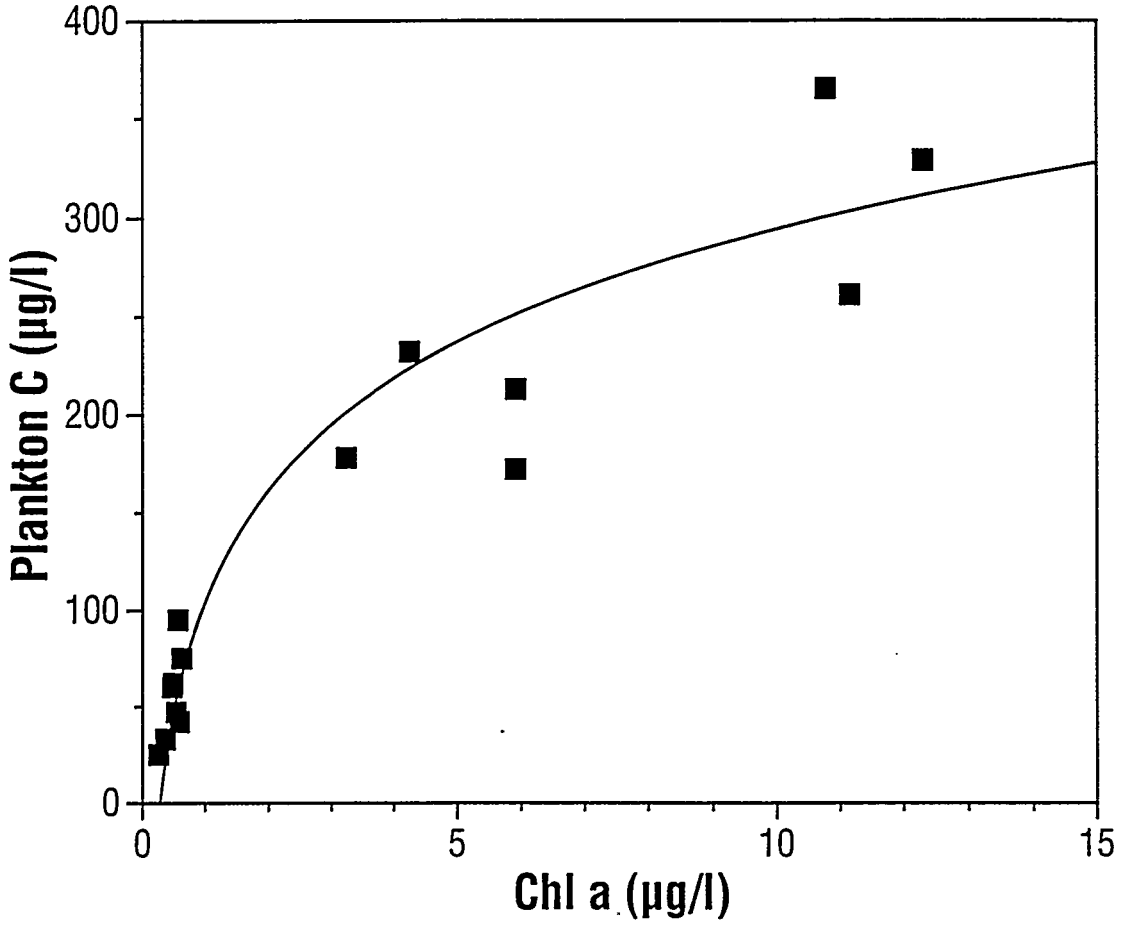

Detrital Nitrogen (µg N)
40
30
20
10
0
0
2
4
6
8
10
12
14
16
18
Detrital Volume (10^8µm^3)

2B


Detrital Carbon (µg/l)
500
400
300
200
100
0
0
10
20
30
40
Detrital Nitrogen (µg/l)


0.8
0.7
0.6
0.5
0.4
0.3
Detrital : Total C (%)
0
100
200
300
400
500
600
700
800
Total C:Chl (µg:µg)


Detrital C (µg/l)
Total POC (µg/l)
0
100
200
300
400
500
600
700


400
300
200
100
0
Plankton C (µg/l)
0
5
10
15
Chl a (µg/l)

Bead Diameter (μm)	Calculated Volume (μm^3)	Rendered Volume (μm^3)
6.0	1.13×10^2	1.05×10^2
10.0	5.24×10^2	5.39×10^2
20.0	4.19×10^3	4.06×10^3
45.0	4.77×10^4	5.41×10^4
90.0	3.82×10^5	4.49×10^5

Table 1. Volumes of fluorescent latex microspheres calculated directly from diameters and from images rendered as three dimensional objects.

Station	Depth (m)	Total POC	Total PON	Plankton POC	Plankton PON	Detrital POC	Detrital PON
8	5	216	24	75	12	141	12
	7	214	24	62	11	152	14
	11	207	27	60	10	147	17
	16	165	9	25	4	140	5
	21	233	21	33	5	200	16
	30	370	27	47	8	323	19
	35	240	39	95	16	145	23
	40	244	15	42	7	202	8
31	3	552	57	172	29	280	28
	6	552	55	232	37	320	18
	10	656	40	178	29	478	11
	15	512	66	213	34	299	32
	20	627	65	365	57	262	9
	25	502	59	329	51	173	8
	31	430	49	261	40	169	9

Table 2. Total particulate carbon and nitrogen, and those portions corresponding to living plankton and nonliving detritus. All units in $\mu g \cdot l^{-1}$.

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On Quantification of Feeding Rates of Zooplankton

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ABSTRACT

The objective of this study was to quantify the consumption of photosynthetic and heterotrophic cells by an abundant calanoid copepod species feeding on natural plankton communities. Quantification of food consumption was accomplished with an advanced image analysis method. Late copepodid stages of *Eucalanus pileatus* did not ingest in quantifiable amounts bacteria, photosynthetic and heterotrophic nanoplankton, nor the abundant *Ceratium* spp. Although diatoms were by far the most abundant cells (in terms of POC $\cdot$ l^{-1}), the copepods ingested a higher percentage of ciliates in relation to their abundance than of diatoms and small heterotrophic dinoflagellates in the first experiment, and ingested a higher percentage of dinoflagellates and ciliates compared to diatoms in the second experiment. Our studies yielded the following general results:

(1) Heterotrophic cells were repeatedly preferred by *E. pileatus* over autotrophs of similar or larger size;

(2) Among the cells which could be individually perceived by this calanoid, larger were not preferred over smaller cells, implying that food quality can be more significant than size;

(3) Feeding by copepods may be underestimated if ingestion of any part of heterotrophic food organisms is not quantified.

INTRODUCTION

Research during the past seven decades has shown that numerous zooplankton taxa are omnivorous, particularly copepods. This conclusion applies to all feeding stages of copepods from early juveniles (e.g. Stoecker and Egloff, 1987, for nauplii of *Acartia tonsa*) to adults of temperate (e.g. Marshall 1924) and tropical species (e.g. Mullin 1966). It includes taxa of estuarine (e.g. Landry 1978, Gifford and Dagg 1988), neritic (e.g. Turner 1987a, on *Centropages velificatus*), and open ocean waters (Mullin 1966). Other omnivorous zooplankton are thaliacea (e.g. Hopkins and Torres 1989, for *Salpa thompsoni*), protozoa (Petipa et al. 1970, Vinogradov et al. 1977, citing cannibalism among protozoa of the Equatorial Pacific), and cyclopoid copepods (e.g. Petipa et al. 1970).

Although it has been known that almost all calanoid species can be herbi-, carni- and/or detritivorous, studies on their feeding performance and feeding impact on their environment have infrequently reflected that knowledge. Some scientists, however, were aware of the copepods' range of feeding potential and included it in their measurements (e.g. Petipa et al. 1970, Petipa 1978, Landry 1978). Some of these quantifications revealed species- and stage-specific differences in the extent and degree of omnivory, i.e. some were weakly and others strongly carnivorous (e.g. Mullin and Brooks 1967, Barnett 1974, Paffenhöfer and Knowles 1980).

Among the objectives in the Department of Energy's (DOE) Ocean Margin Program (OMP) is the quantification of food consumption by proto- and metazooplankton. Here we report initial observations of the feeding of late copepodid stages of the subtropical calanoid *Eucalanus pileatus* on natural particle assemblages.

MATERIALS and METHODS

Among the abundant calanoid copepods in subtropical neritic waters is *Eucalanus pileatus* (Bowman 1971, Binet 1977, Valentin 1984, Turner 1987b, Madhupratap and Haridas 1986). During the latter part of June 1994, *E. pileatus* occurred abundantly between 35° 30' and 36° 30' on the continental shelf north of Cape Hatteras. After determining their depth of abundance, the copepods were collected in tows lasting 3 to 4 minutes with a net of 202 micron mesh and a 4-liter codend, while the ship was drifting. The *E. pileatus* were undamaged and each had a visible oil globule; within 15 minutes they were transferred into a 4-liter jar containing a natural suspension of particles from the depth of maximum occurrence of *E. pileatus*. Within the next 60 minutes each experiment was started in 1900ml jars after adding f-medium resulting in 3µmol of nitrate. A Ferris Wheel rotated at approximately 0.2 r.p.m. in a water bath maintained close to temperatures at which the copepods had been collected. Experiments were run over 24h in a natural light-dark cycle (15:9h).

At the beginning and end of each experiment, 100 ml samples were drawn from each jar for composition, enumeration, and biomass measurements of plankton using a color imaging cytometry system. The methods used to enumerate and determine the biovolume of plankton cells via image analysis techniques, and subsequent conversion to cell carbon and nitrogen, have already been developed (Sieracki et al., 1989a,b; Verity et al., 1992; Verity and Sieracki, 1993). They have been used to quantify plankton communities in diverse environments such as the Atlantic Ocean (Verity

et al., 1993a), Pacific Ocean (Verity et al., subm.), and Norwegian fjords (Hansen et al., 1994). Recent improvements to the imaging cytometry system include interfacing a motorized stage and associated modular automation controller with the epifluorescence microscope. This permits the operator to scan transects of variable length across the plankton slides while the computer records the fraction of the surface area of the slide which has been examined. For abundant populations, the computer can randomly select individual locations on the slide. The driver software has been modified so that the entire process (moving to a given location, focusing, opening an electronic shutter, grabbing an image, closing the shutter, and moving to a new location) is automated and computer-controlled.

Since the amount of food removed by a late copepodid stage of *E. pileatus* could only be approximated, not knowing composition and abundance of potential food particles, a series of concentrations was chosen ranging from 12 to 24 CIII/IV per 1900ml jar on 27 June, and of 1CIII/15CIV and 7CIII/7CIV on 30 June 1994 (Table 1). Their stage was estimated from size without a dissecting microscope based on experience (G.-A. Paffenhöfer). Since the age of collected adult female copepods cannot be accurately determined, and copepodids far outnumbered females, we decided to utilize the former for experiments. At the end of each experiment, the surviving copepods were removed from each jar, and their cephalothorax length (to the nearest 0.02mm) and stage was determined. Animals which molted to males were not included in the feeding rate calculations. They were immediately dried at 60°C and upon return to the laboratory the combined copepod weight from each jar was determined to the nearest 0.1µg with a Cahn-Balance. Ash-free dry weight was multiplied with a factor of 0.45 to obtain their organic carbon content (pers. obs. G.-A.P.).

RESULTS

For initial studies on quantifying grazing of metazooplankton on natural particle assemblages, we chose the calanoid copepod *Eucalanus pileatus* which occurred abundantly as nauplii and copepodid stages on the inner and middle shelf north of Cape Hatteras (unpubl. obs.). The water mass in which they occurred was characteristic of the southeastern continental shelf during late spring and summer because of its phyto- and zooplankton species composition (doliolids, *Temora turbinata, T. stylifera*).

During each 24h study most copepodids molted to the next stage (Table 1). Mortality occurred once (Exp. 30 June, A) which we assume was due to losing one copepod when closing or opening the respective jar. In one case, we had one more copepod at the end than at the start (Exp. 27 June, C) when a late nauplius molted to CI. Copepodid stages of any calanoid genus and nauplii of *E. pileatus* were removed from each control and experimental jar prior to addition of experimental animals.

We quantified particle removal by *E. pileatus* on 7 different groups of potential food organisms of which the bacteria are not shown (Tables 2 and 3). Cell volumes represent means of triplicate measurements of ≥ 100 cells from the two experiments. Absolute and relative abundances of the food organisms on both dates were similar, and their cell volumes differed only slightly (Tables 2 and 3). The phytoplankton community included diatoms (*Rhizosolenia alata*, *R. stolterfothii*, *Chaetoceros* spp., *Skeletonema costatum*, *Cerataulina* sp., *Leptocylindrus danicus*), dinoflagellates (*Ceratium tripos*, *C. lineatur*, *C. furca*, *Prorocentrum* spp.), and small photosynthetic nanoplankton (cryptomonads, prasinophytes, coccolithophorids). Heterotrophic (= apastidic) nanoplankton included species of *Paraphysomonas*, *Bodo*, *Leucocryptos*, and choanoflagellates. Heterotrophic (= aplastidic) dinoflagellates were represented by both thecate and athecate taxa, especially spindle-shaped morphotypes of the latter. Ciliates included both plastidic and aplastidic taxa, with the common genera being *Strombidium*, *Lohmanniella*, and *Strobilidium*.

During the first experiment clearance rates were highest on ciliates and lowest on the dinoflagellates which were also the smallest of the cells eaten in abundance (Table 2). During the second experiment, however, dinoflagellates and ciliates were both cleared at higher rates than diatoms (Table 3). Although being on average one order of magnitude smaller than the diatoms, ciliates were cleared at higher rates in both experiments. Bacteria, photosynthetic and heterotrophic nanoplankton, and Ceratia were not eaten. Copepod nauplii which also can serve as prey, were not accounted for because they occurred in insufficient quantities to be adequately accounted for by our method. Since they can perceive, avoid or escape from predators, their contribution as food organism is expected to be small except for strongly carnivorous copepods like *Centropages velificatus* (Paffenhöfer and Knowles 1980).

On 27-28 June, the copepods ingested daily between 55 and 61% of their body weight, and on 30 June - 1 July ingested 67 and 82%. When comparing the relative contribution of auto- and

heterotrophs to ingested carbon, the latter (ciliates and heterotrophic dinoflagellates) amounted to 24.5% in the first study although they represented only 18.9% (ciliates and Hdinos) of the available cell standing stock (Table 4). In the second study 36.1% of the ingested food were heterotrophs which contributed here 22.8% to the available food carbon (Table 4). In each individual experiment of the two dates the copepods ingested a higher percentage of heterotrophic than autotrophic food particles in relation to those available.

DISCUSSION

Studies on food consumption of zooplankton have been conducted for more than 60 years with the general goal of determining the amount and type of food consumed by respective stages, species, or groups, preferably simulating *in situ* (food) conditions. Hundreds of papers have been published on zooplankton feeding using various methodologies (Paffenhöfer 1988). An evaluation of these previous studies aimed at determining what zooplankton in general, and copepods specifically, consumed *in situ* suggests that in any improved protocol, all potential food particles are considered and can be quantified, and the behavior of the grazers are considered when interpreting the data.

It is now apparent that the vast majority of copepods, including nauplii, can ingest a variety of foods, i.e. they do not usually depend on one general food source, but several (e.g. Mullin 1966, Petipa 1978). In the ocean hetero- and autotrophic cells co-occur: their species composition, size and abundance determines to a large extent which ones are perceived, gathered and eventually ingested by a respective copepod stage of a certain species. Various studies have shown that copepods make decisions about what and how much they eat (e.g. Price 1988, Vanderploeg et al. 1988). The present results illustrate that late copepodid stages of *E. pileatus* ingest more heterotrophs than autotrophs in relation to their abundance, and that cell size is not necessarily the dominant variable influencing ingestion but rather the type of food organism. While *E. pileatus* may not represent all calanoids, larger protozoa (>10μm length or width) are ingested at high rates by various stages and species of calanoid copepods otherwise known to be herbivorous (e.g. Petipa 1978, Stoecker and Egloff 1987, Gifford 1993, Fessenden and Cowles 1994, Ohman and Runge 1994). For example, at similar abundances of larger phytoplankton and microzooplankton the percentage of animals (protozoa and metazoa) in gut contents of *Calanus pacificus* ranged from 23

to 99%, and of *Clausocalanus* sp. from 55 to 98% (Kleppel et al. 1988). These observations were based on pigment identification; the advantage of the approach used here is that information on sizes and specific taxonomic groups are simultaneously available.

Others used inverted microscope observations for quantifying ingestion of protists. *Calanus pacificus* females did not ingest measurable amounts of phytoplankton when cell sizes were small (<20μm, 170μgC·l^{-1}) but grazed heavily on ciliates (>10μm, 11μgC·l , Fessenden and Cowles 1994); *C. pacificus* copepodid Stage v ingested phytoplankton (mostly diatoms) when it occurred at high concentrations and was of large size (>20μm, 2230μgC·l^{-1}) and no measurable amounts of ciliates (15μgC·l^{-1}). Here sizes and abundances seem to have affected grazing rates on phytoplankton and ciliates. Feeding performances of adult females of *Calanus finmarchicus* from 2 different environments in the Gulf of St. Lawrence (Ohman and Runge 1994) yielded the following results: clearance rates on ciliates which were always much larger than diatoms and dinoflagellates were always higher than on the other food organisms, even when the diatoms amounted to 1460μgC·l^{-1} and the ciliates to only 3.1μgC·l^{-1} (Station 1). Ciliates were represented by *Lohmanniella* and *Strombidium* the latter ranging from ~10,000 to 30,000μ^3. Dinoflagellates were cleared at rates similar to those on diatoms. Both of these studies (Fessenden and Cowles 1994; Ohman and Runge 1994) show that the genus *Calanus* has highest clearance rates on ciliates when these are clearly larger than the accompanying food organisms.

In our case with late copepodids of *E. pileatus* the findings are that ciliates, being 5 to 10 times less voluminous than diatoms, are cleared at a far higher rate than the latter (Tables 2 and 3). In the second experiment (Table 3) clearance rates on heterotrophic dinoflagellates surpassed those on diatoms although the formers' individual cell volume was only 5% of that of the diatoms. It is obvious from the absolute values of clearance rates that all 3 types of ingested food were actively selected by *E. pileatus*. Why would *E. pileatus* select a smaller size over a larger size? Previous observations have shown that calanoids can be rather selective for quality when food is abundant (e.g. Paffenhöfer 1984, Vanderploeg et al. 1988), and more for size when food is scarce (e.g. Bartram 1981). As food abundance increased, late copepodids and adult females of *Paracalanus* sp. ingested increasing percentages of *Thalassiosia weissflogii* whereas the relative ingested amount of *Rhizosolenia alata* decreased (Paffenhöfer 1984, Figs. 8, 9, 10), i.e. the small *T. weissflogii* (1.2 x 10^{3}μ^3 was selected over the large *R. alata* (~6.3 x 10^4 μ^3 cell volume). In essence, among those

cells which a copepod would perceive by long distance, size was not the governing variable. Similar results were obtained in the present study during which food which could be readily perceived by *E. pileatus* was abundant (Tables 2, 3). How could it happen that large, easily perceivable and also ingestible food particles were not preferably ingested? Calanoids which create a feeding current use it to perceive food particles at a distance. Here the first decision is being made whether a particle is worth to be ingested ("coarse or preliminary evaluation"). After gathering the particle and displacing it to the mouth, the sensors there ("close or fine-tuned examination") let the copepod decide whether to ingest or reject this particle. In summary, when food is scarce long-distance perception is the dominant process concerning food ingestion (Paffenhöfer and Lewis 1990) i.e. food quantity appears to be more important than quality; when food is abundant, as in our study here, tasting at the mouth is more important, i.e. food quality is the deciding factor (e.g. Vanderploeg et al. 1988, Paffenhöfer et al. in press).

The clearance rates presented here for diatoms (70-78 $\mu gC \cdot l^{-1}$) ranged from 70 to 95 $ml \cdot CIV/V^{-1} \cdot 24h^{-1}$, as compared to 150 - 200ml by the same species and stages feeding on larger diatoms ($63 \mu gC \cdot l^{-1}$) in the laboratory, which is equivalent to 94% of their body weight ingested per day (Paffenhöfer and Knowles 1978). This value is about twice that of those found in our present studies on diatoms of similar size and concentration. In natural communities, different potential food types include dead and elongated algae, detritus, fecal pellets and aggregates, many of which would be gathered but not ingested. This would contribute to lower clearance and ingestion rates when compared to studies with a single or few food types because, during handling of a unwieldily or subsequently unacceptable particle (e.g. Ceratium sp.), ingestions of other (high-quality) particles are difficult to accomplish. *E. pileatus* CV often tasted individual fecal pellets of *Paracalanus* sp. for > 1 sec before deciding on ingestion or rejection (G.-A. Paffenhöfer unpubl. obs.). *Paracalanus* juveniles ingested far more when offered one food species (*Thalassiosira weissflogii*) in comparison to being offered three (Paffenhöfer 1984). Direct visual observations of the entire feeding process over time (Paffenhöfer et al. in press) would reveal whether this interpretation of ours would occur i.e. that an increasing number of different food particles would reduce the overall ingestion rate.

To fully assess the impact of metazooplankton on prey communities knowledge of the potential and actual food organisms is essential. The method described here is another step in that direction. It is somewhat laborious but a reliable, replicable, and data-intensive method which is not

burdened by assumptions. It can be used over endpoint incubations as done here or in time-series experiments by collecting samples at regular intervals. Given the diversity and different functional roles of potential prey organisms, this approach when applied to the abundant stages and species of zooplankton will improve our understanding of the size- and species-dependent nature of interactions between zooplankton and their prey (Vinogradov et al. 1977).

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Table 1. Feeding experiments with late copepodid stages of *Eucalanus pileatus*

	Exp. 27 - 28 June 1994			Exp. 30 June - 1 July, 1994	
Collection Site Isobath *in situ* Temperatures Exp. Temperature	36°00'N, 75° 25' W 21m 22.5 to 24.5° C in upper 18m 21.7			36°10'N, 75°16'W 30m 22°C in upper 14m 21.5	
	A	B	C	A	B
Estimated Number and stages of copepods at start	18 CIII/IV	24 CIII/IV	12 CIII/IV	1 CIII 15 CIV	7 CIII 7 CIV
At end of experiment	1♂, 1♀, 1CIII, 4CIV, 11CV	2♂, 2 CIII, 4CIV, 16CV	2♂, 1CI, 3CIV, 7CV	1♂, 1♀, 15 CIV, 8CV	2 CIII, 8CIV, 4CV

Table 2. Ingestion and clearance rates of *Eucalanus pileatus* on 27-28 June 1994

Food Taxon	Cell Volume (μm^3)	Mean Cell Concen-tration ($\mu gC \cdot l^{-1}$)	Ingestion Rates ($ng \cdot Copepod^{-1} \cdot h^{-1}$) For			Clearance Rates ($ml \cdot Copepod^{-1} \cdot h^{-1}$) For		
			A	B	C	A	B	C
Phototrophic Nanoplankton	$2.1x10^2$	32	0	0	0	0	0	0
Heterotrophic Nanoplankton	$1.6x10^2$	28	0	0	0	0	0	0
Heterotrophic Dinoflagellates	$4.6x10^2$	7	19.0	15.8	15.5	2.7	2.3	2.2
Ciliates	$1.3x10^3$	14	84.1	63.3	72.5	6.0	4.5	5.6
Ceratia	$1.6x10^4$	12	0	0	0	0	0	0
Diatoms	$1.8x10^4$	78	304.0	246.2	279.8	3.9	3.2	3.6
Total		171	407.1	325.3	367.8	3.7	2.9	3.3
Weight-Specific Consumption ($\mu gC \cdot \mu gC$ of $Copepod^{-1} \cdot 24h^{-1}$)			0.55	0.61	0.59	---	---	---

Table 3. Ingestion and clearance rates of *Eucalanus pileatus* on 30 June - 1 July 1994

Food Taxon	Cell Volume (μm^3)	Mean Cell Concentration ($\mu gC \cdot l^{-1}$)	Ingestion Rates ($ngC \cdot Copepod^{-1} \cdot h^{-1}$)		Clearance Rates ($ml \cdot Copepod^{-1} \cdot h^{-1}$)	
			A	B	A	B
Phototrophic Nanoplankton	$2.6x10^2$	40	0	0	0	0
Heterotrophic Nanoplankton	$2.3x10^2$	24	0	0	0	0
Heterotrophic Dinoflagellates	$6.5x10^2$	4	25.7	20.4	6.8	5.1
Ciliates	$2.4x10^3$	19	109.9	88.2	6.1	4.6
Ceratia	$1.4x10^4$	8	0	0	0	0
Diatoms	$1.3x10^4$	70	226.6	203.6	3.3	2.9
Total		165	361.2	312.2	3.6	3.1
Weight-Specific Consumption ($\mu gC \cdot \mu gC$ of $Copepod^{-1} \cdot 24h^{-1}$)			0.67	0.82	---	---

Table 4. Mean cell concentrations and ingestion rates as percent of total cell concentration and ingestion

Food Type	Exp. 27 - 28 June, 1994				Exp. 30 June - 1 July, 1994		
	Mean Cell Concentration	A	B	C	Mean Cell Concentraton	A	B
Heterotrophic Dinoflagellates	6.3	4.7	4.8	4.2	4.0	7.1	6.5
Ciliates	12.6	20.7	19.5	19.7	18.8	30.3	28.3
Ceratia	10.8	0	0	0	7.9	0	0
Diatoms	70.3	74.6	75.7	76.1	69.3	62.6	65.2
Total	100%	100%	100%	100%	100%	100%	100%