UC San Diego

UC San Diego Previously Published Works

Title

Size fractionation of phytoplankton as an estimate of food available to herbivores

Permalink

<https://escholarship.org/uc/item/0qt32791>

Journal

Limnology and Oceanography, 27(3)

ISSN

0024-3590

Authors

Runge, JA
Ohman, MD

Publication Date

1982

DOI

10.4319/lo.1982.27.3.0570

Peer reviewed

Size fractionation of phytoplankton as an estimate of food available to herbivores¹

Abstract—Eight species of phytoplankton with sizes and shapes common in temperate oceans were partitioned into categories with six or seven sizes of screens and filters. Analysis of chlorophyll *a*, particle numbers, and volume in successive size fractions showed clear cell or colony breakage for only one species. The size fractionation technique thus seems to be an informative way to partition phytoplankton into size classes available to suspension-feeding zooplankton. The technique does not always separate elongate species on the basis of their longest axial dimension, as some species seem more likely than others to orient in the direction of the flow.

Understanding growth processes of suspension-feeding zooplankton in their natural environment requires evaluation of what, of the many possibilities for shape, size, and nutritional quality in the suspended material, is actually available as food. Steele and Frost (1977) developed the argument that the size structure of the phytoplankton is the resource axis most important to consider. Particularly important is the ability to resolve size differences within the nanoplankton fraction ($<20\ \mu\text{m}$; Sieburth et al. 1978). For example, the minimum size of particles effectively captured by adult *Calanus pacificus* is about 8–10 μm (Frost 1972; Nival and Frost unpubl.), by *Pseudocalanus* sp. is about 3 μm (Corkett and McLaren 1978), and by the larvacean *Oikopleura dioica* is about 0.3 μm (Flood 1978).

Electronic particle counters are obviously useful for determining the particle volume distributions of natural suspensions (Parsons and LeBrasseur 1970). As Harbison and McAlister (1980) emphasized, however, sensing-zone counters

measure the volumes of particles and not their linear dimensions, a fact which complicates the interpretation of what is available to suspension feeders. Fractionation of phytoplankton into various size classes by screens and filters may be a relatively quick and informative supplement to, or substitute for, particle counting and also can readily be performed in the field. Screens remove particles from the water on the basis of their linear dimensions and not on the basis of their volumes (Harbison and McAlister 1980), and when such particles are analyzed for chlorophyll concentration, detrital material is not included. Although size fractionation techniques are frequently used in phytoplankton research (e.g. Malone 1971; Malone and Neale 1981; McCarthy et al. 1974; Durbin et al. 1975), few workers (Mullin 1965; Nival and Nival 1973; Checkley 1980) have used them explicitly with the herbivore in mind.

One of the uncertainties about the size fractionation approach concerns the possibility of breakage of cells or colonies during filtration (Mullin 1965). We describe here experiments designed to examine the accuracy of the technique. In previous studies (Sheldon and Sutcliffe 1969; Sheldon 1972; Malone et al. 1979), the retention characteristics of certain screens and filters have been investigated with respect to natural particle distributions. Our experiments extend these results by providing specific information about the effects of screening on individual phytoplankton species of known size and shape.

We thank B. W. Frost for encouragement, C. J. Lorenzen for exposing us to size fractionation of pigments, and B. Booth for help in phytoplankton culture. J. Fournier and J. Herstad assisted. We also thank C. M. Boyd and G. R. Harbison for reviewing the manuscript. The order

¹ Contribution 1256 of the School of Oceanography, University of Washington. Research supported by NSF grant OCE 78-18263 to B. W. Frost.

Table 1. Means (SD; range) of algal dimensions along two axes determined from measurements of 70–100 cells or chains under a compound microscope. Mean equivalent spherical diameter (ESD) determined with a model TAIL Coulter Counter; ESD at channel boundaries enclosing species shown in parentheses.

	Long axis (μm)	Short axis (μm)	ESD (μm)
Chlorophyceae			
<i>Platymonas suecica</i>	12.1 (1.3; 10–15)	7.6 (1.0; 6–10)	9.3 (6.5–12.5)
<i>Dunaliella tertiolecta</i>	7.9 (1.1; 6–10)	6.6 (1.1; 5–8.5)	7.1 (5.0–10.0)
Bacillariophyceae			
A. Unicellular diatoms			
<i>Thalassiosira angatii</i>	34.7 (3.2; 28–39)	22.7 (1.9; 20–26)	35.0 (25.5–51.0)
<i>Thalassiosira weissflogii</i>	12.9 (1.6; 9–14.5)	10.8 (1.2; 10–17)	13.6 (10.0–20.0)
B. Filamentous diatoms			
<i>Chaetoceros debile</i>	73.4 (28.7; 30–143)	103.0* (27.1; 60–164)	15.0 (10.0–20.0)
<i>Skeletonema costatum</i>	73.7 (31.7; 18–164)	3.7 (0.7; 2.5–5)	9.6 (6.5–12.5)
Dinophyceae			
<i>Ceratium fusus</i>	274.5 (11.7; 257–296)	22.2 (2.0; 19–27.5)	36.8 (25.5–51.0)
<i>Amphidinium carterae</i>	11.9 (1.6; 8–14.5)	8.6 (1.2; 7–12.5)	10.5 (8.0–16.0)

* Inclusive of setae.

of our names at the end of the text was determined by tossing a coin.

Species were selected to represent four groups of phytoplankton common in temperate oceans: unicellular diatoms, filamentous diatoms, dinoflagellates, and green flagellates. The eight species chosen cover a broad spectrum of sizes and morphologies (Table 1). Each was cultured under continuous light at 15°C in sterile, glass-fiber-filtered medium [f/2 for diatoms and "Ceratium" medium (Chan 1978) for other species]. A culture growing exponentially was diluted with twice glass-fiber-filtered seawater to a concentration between 5 and 10 μg Chl $a \cdot \text{liter}^{-1}$, and replicate 270-ml aliquots were filtered through Gelman A/E glass-fiber filters, 2- μm Nuclepore filters, and 5-, 10-, and 20- μm mesh Nitex screens; larger species were also fractionated with 44- and 73- μm mesh Nitex screens. Some species were filtered through 5- and 10- μm Nuclepore filters. Each aliquot was passed through a single screen or filter

(25-mm diam) mounted in a Gelman filter holder and subject to a low vacuum (<10 cm Hg). Pigments extracted from the screen or filter in 90% acetone were analyzed using the fluorometric technique of Holm-Hansen et al. (1965) as modified by Lorenzen (1966). MgCO_3 and a filtered seawater rinse were omitted from all treatments to avoid alteration of filter or screen characteristics. The filtrate was analyzed for particle number and size distribution with a Coulter Counter model TAIL, fitted with a 100-, 200-, or 400- μm aperture tube and calibrated with plastic spheres from Particle Information Service, Inc. Except for the flagellates, samples were preserved with Lugol's solution for microscopic measurement of cell or chain dimensions. Flagellates were anesthetized with MS-222 and measured live. As the chain-forming diatoms did not retain their quaternary structure on preservation, we measured living specimens from the same clone at a later date.

Fig. 1. Retention efficiency for chlorophyll *a* (solid lines, ●), and particle number (dashed lines, ○), for eight algal species. 2- μm size is Nuclepore filter; sizes $>2 \mu\text{m}$ are Nitex screens. Retention efficiencies of 5- μm Nuclepore filters also shown (unconnected circles) where different from 5- μm Nitex screens. Points represent averages of duplicate observations. Species illustrations not to scale.

Results are presented in Fig. 1. Retention efficiencies calculated from chlorophyll data represent the quantity of pigment retained on the particular screen or filter relative to the amount measured on

the glass-fiber filter. Retention efficiencies calculated from particle numbers came from the difference in cell counts between filtrates and unscreened controls. The two methods of analysis agree

closely on the performance of the screens and filters. Discrepancies between the two methods in the analyses of *Skeletonema costatum* and *Platymonas suecica* are discussed below.

Of the six species fractionated with Nuclepore filters (all but the two *Thalassiosira* species), retention efficiencies differed from results with Nitex screens only for *P. suecica* and *Amphidinium carterae* with the 5- μ m filter. For these two species, the Nuclepore filter was more effective than the Nitex screen in retaining algal cells. Whereas effective pore size (the size at which 50% of the particles are retained) of the 5- μ m Nuclepore filter is equivalent to its nominal pore size, effective pore size of the 5- μ m Nitex screen is actually closer to 7–8 μ m (Fig. 2); the latter probably reflects a manufacturing difficulty in weaving small meshes and shows that the 5- μ m Nuclepore filter more accurately fractionates phytoplankton cells.

The volume frequency distribution in the filtrate, coupled with data on retention efficiencies and the actual cell or chain dimensions (Table 1), allows us to examine each species closely for evidence of breakage during size fractionation. If breakage occurs, then particle volume in the lower Coulter Counter channels should increase relative to the untreated control. Figure 3A shows particle volume distributions in the filtrate after fractionation of *Thalassiosira weissflogii* and illustrates the pattern found in seven of the eight species. No particle production is apparent in the size range measured by the Coulter Counter. Figure 3B presents the pattern for *Dunaliella tertiolecta* and shows the one treatment (fractionation with the 2- μ m Nuclepore filter) in which cells seem to have been disrupted. Whether the particles produced are cell fragments or intact cells of reduced volume is unclear, although it is difficult to imagine how a 5–10- μ m cell can survive passage through an opening of 2 μ m.

Analysis of particle volume distribution may not have revealed all the possible cases of cell breakage. In the

Fig. 2. Retention efficiency for particles by 5- μ m Nitex screens and 5- μ m Nuclepore filters, as a function of particle size. Analysis includes channels in which sufficient numbers of detrital particles were present to obtain reproducible counts. Nominal median retention efficiency (+) shown for reference.

experiments with *S. costatum*, the difference in retention efficiencies between Chl *a* and particle numbers on the 5-, 10-, and 20- μ m mesh screens (Fig. 1) suggests breakage of the few chains that do go into the filtrate. The smaller chain units that were produced apparently remain in the size range for the species on the Coulter Counter and were therefore counted, resulting in an artificially high concentration in the filtrate and consequently a lower retention efficiency. This is a relatively minor source of error, however, that involves <10% of the total concentration (by pigment analysis) and is hidden in the volume distribution (Fig.

Fig. 3. Mean volume distributions in filtrates (z-axis: ppm by volume; x-axis: Coulter Counter ESD) showing effects of size fractionation for representative species. A—*T. weissflogii*; B—*D. tertiolecta*; C—*S. costatum*. Symbol C on y-axis indicates untreated control; other y-axis labels are mesh sizes, identifying treatments.

3C). Hallegraeff (1981) found a lower retention efficiency of 10- μ m screens for *S. costatum* chains than we did, perhaps due to the stronger vacuum he used. In two other instances in our investigation, fragmentation may have produced particles which have a volume less than the minimum channel size on the Coulter Counter. When *P. suecica* was fractionated with 2- and 5- μ m Nuclepore filters, 5% of the cells were found in the filtrate even though analysis of pigment retained on the filter indicated that 35–40% of the cells had passed through, suggesting that some cells lysed, losing their pigments to the dissolved fraction of the filtrate. The other case involves passage of most of *Chaetoceros debile* through the 44- μ m mesh screen despite the dimensions of the chain (Table 1). Setae make up about 95% of the width of this species, however; either the spines bend but remain intact during passage, or the tips break off but have a volume less than the lower limit of detection of the Coulter Counter.

Except for *D. tertiolecta*, the size fractionation technique accurately categorized the nearly spherical species according to their cellular diameter. The two centric diatoms, *Thalassiosira angustii* and *T. weissflogii*, fractionated into the 20–44- and the 10–20- μ m categories and *P. suecica* and *A. carterae* fell into the 5–10- μ m class when the Nuclepore filter was used. In our clone of *T. weissflogii*, extremely fine filaments, 1–2 cell diameters long, project from the valves of each cell, a factor which has recently been shown to enhance the probability of capture of *T. weissflogii* by a suspension-feeding copepod (Gifford et al. 1981). These filaments apparently did not influence the diatom's retention characteristics on screens, however.

The morphology of the other three species, *C. debile*, *S. costatum*, and *Ceratum fusus*, makes prediction of the appropriate size class from microscopic dimensions difficult. Screens remove *S. costatum* and *C. debile* approximately on the basis of their longest axial dimension, but remove the fusiform *C. fusus* more on the basis of its shorter axial dimen-

sion. Apparently *C. fusus* orients in the direction of flow, whereas the chain-forming diatoms do not. Further investigation of the manner in which particles of various shapes orient in a flow field may be useful in predicting retention characteristics of nonspherical phytoplankton. At present, however, supplementary analysis with a microscope seems to be the best recourse for accurately resolving the distribution of elongate particles in the larger size fractions.

The simple capture of phytoplankton with screens is unquestionably an imprecise indicator of the sophisticated feeding response by zooplankton to algae of different sizes, shapes, and nutritional qualities. Nevertheless, because the error due to cell or colony breakage seems to be limited, size fractionation is worth considering as an approach to estimating food availability. The technique provides a rapid way of partitioning suspended matter into size categories potentially edible by different planktonic suspension feeders, based on linear dimensions rather than on volume. It appears especially useful for partitioning phytoplankton smaller than 20 μm —information which may otherwise be difficult to obtain.

Jeffrey A. Runge²
Mark D. Ohman

School of Oceanography WB-10
University of Washington
Seattle 98195

References

- CHAN, A. T. 1978. Comparative physiological study of marine diatoms and dinoflagellates in relation to irradiance and cell size. I. Growth under continuous light. *J. Phycol.* **14**: 396–402.
- CHECKLEY, D. M., JR. 1980. Food limitation of egg production by a marine planktonic copepod in the sea off southern California. *Limnol. Oceanogr.* **25**: 991–998.
- CORKETT, C. J., AND I. A. MCLAREN. 1978. The biology of *Pseudocalanus*. *Adv. Mar. Biol.* **15**: 1–231.
- DURBIN, E. G., R. W. KRAWIEC, AND T. J. SMAYDA. 1975. Seasonal studies on the relative importance of different size fractions of phytoplankton in Narragansett Bay (USA). *Mar. Biol.* **32**: 271–289.
- FLOOD, P. R. 1978. Filter characteristics of appendicularian food catching nets. *Experientia* **34**: 173–175.
- FROST, B. W. 1972. Effects of size and concentration of food particles on the feeding behavior of the marine planktonic copepod *Calanus pacificus*. *Limnol. Oceanogr.* **17**: 805–815.
- GIFFORD, D. J., R. N. BOHRER, AND C. M. BOYD. 1981. Spines on diatoms: Do copepods care? *Limnol. Oceanogr.* **26**: 1057–1061.
- HALLEGRAEFF, G. M. 1981. Seasonal study of phytoplankton pigments and species at a coastal station off Sydney: Importance of diatoms and the nanoplankton. *Mar. Biol.* **61**: 107–118.
- HARBISON, G. R., AND V. L. MCALISTER. 1980. Fact and artifact in copepod feeding experiments. *Limnol. Oceanogr.* **25**: 971–981.
- HOLM-HANSEN, O., C. J. LORENZEN, R. W. HOLMES, AND J. D. STRICKLAND. 1965. Fluorometric determination of chlorophyll. *J. Cons. Cons. Int. Explor. Mer* **30**: 3–15.
- LORENZEN, C. J. 1966. A method for the continuous measurement of in vivo chlorophyll concentration. *Deep-Sea Res.* **13**: 223–227.
- MCCARTHY, J. J., W. R. TAYLOR, AND M. E. LOFTUS. 1974. Significance of nanoplankton in the Chesapeake Bay Estuary and problems associated with the measurement of nanoplankton productivity. *Mar. Biol.* **24**: 7–16.
- MALONE, T. C. 1971. The relative importance of nanoplankton and netplankton as primary producers in tropical oceanic and neritic phytoplankton communities. *Limnol. Oceanogr.* **16**: 633–639.
- , M. B. CHERVIN, AND D. C. BOARDMAN. 1979. Effects of 22- μm screens on size-frequency distributions of suspended particles and biomass estimates of phytoplankton size fractions. *Limnol. Oceanogr.* **24**: 956–960.
- , AND P. J. NEALE. 1981. Parameters of light-dependent photosynthesis for phytoplankton size fractions in temperate estuarine and coastal environments. *Mar. Biol.* **61**: 289–298.
- MULLIN, M. M. 1965. Size fractionation of particulate organic carbon in the surface waters of the western Indian Ocean. *Limnol. Oceanogr.* **10**: 459–462.
- NIVAL, P., AND S. NIVAL. 1973. Efficacité de filtration des copépodes planctoniques. *Ann. Inst. Oceanogr. Paris* **49**: 135–144.
- PARSONS, T. R., AND R. J. LEBRASSEUR. 1970. The availability of food to different trophic levels in the marine food chain, p. 325–343. *In* J. H. Steele [ed.], *Marine food chains*. Univ. Calif.
- SHELDON, R. W. 1972. Size separation of marine seston by membrane and glass-fiber filters. *Limnol. Oceanogr.* **17**: 494–498.
- , AND W. H. SUTCLIFFE. 1969. Retention of marine particles by screens and filters. *Limnol. Oceanogr.* **14**: 441–444.

² Present address: Department of Oceanography, Dalhousie University, Halifax, Nova Scotia B3H 4J1.

SIEBURTH, J. MCN., V. SMETACEK, AND J. LENZ. 1978. Pelagic ecosystem structure: Heterotrophic compartments of the plankton and their relationship to plankton size fractions. *Limnol. Oceanogr.* **23**: 1256-1263.

STEELE, J. H., AND B. W. FROST. 1977. The struc-

ture of plankton communities. *Phil. Trans. R. Soc. Lond.* **280**: 485-534.

Submitted: 17 December 1980

Accepted: 19 October 1981

Limnol. Oceanogr., 27(3), 1982, 576-584

© 1982, by the American Society of Limnology and Oceanography, Inc.

A transportable microprocessor-controlled precise Winkler titration suitable for field station and shipboard use¹

Abstract—An automatic Winkler titration based on a photometric end-point detector is described. The titration is controlled by a system based on a RCA 1800 series microprocessor. It takes about 3-4 min and in normal use has a coefficient of variation in the range 0.03-0.1%. The system is transportable and has been used at sea.

Oxygen is probably the most useful readily measurable biochemical feature of aquatic environments; it is a product and reactant of the two fundamental biological processes, photosynthesis and respiration. Early workers (Gaarder and Gran 1927; Riley 1939, 1941) used changes in dissolved oxygen, determined by the Winkler technique, to measure plankton photosynthesis and respiration. The approach had its limitations, stemming mainly from the relatively long incubation periods (3-4 days) needed to determine changes in oxygen concentration with sufficient precision. For the measurement of plankton photosynthesis, this approach was largely replaced by the more sensitive ¹⁴C method (Steemann Nielsen 1952). However, the use of ¹⁴C isotopes as a general means of studying plankton metabolism has certain shortcomings: no method has been devised to

measure total plankton respiration or overall heterotrophy with ¹⁴C and the well established ¹⁴CO₂ method of measuring photosynthesis is not without its problems (*see e.g.* Sournia 1971; Voors 1979; Peterson 1980).

The conventional Winkler titration (*e.g. see* Strickland and Parsons 1968) is attributed a coefficient of variation of about 0.5%. Despite claims to the contrary (*e.g.* Czaplewski and Parker 1973) oxygen electrodes cannot attain the accuracy of the Winkler titration. With careful attention to the titration it is possible to achieve a coefficient of variation of 0.1% or better on a routine basis (*see* Bryan et al. 1976). This has offered new research possibilities into whole plankton respiration and photosynthesis. For example we can make direct measurements of planktonic respiration and photosynthesis in oligotrophic ocean waters. However, the analytical method, although simple and relatively rapid, is tedious. Fieldwork tends to generate large numbers of samples and many hours are spent titrating, resulting in an inevitable falloff in the quality of the results.

This prompted us to consider an automatic version of the titration. We made a simple modification to the end-point detection system and produced a signal of sufficient quality to make automation feasible. Computer-controlled titrations have been described (*see* Almgren et al. 1977); however, we needed a transportable, self-contained system for field station and shipboard use. This was an ideal application for microprocessor control. We will not here describe the system in great

¹ Development and field use of the equipment was supported by the University of Southampton, Committee for Advanced Studies Research Award (S155/78/AR); the Royal Society, NSF (grant OCE 77-27906 awarded to the CEPEX program), NRC-Nuffield Foundation, and the Bigelow Laboratory for Ocean Sciences also helped. Roth Scientific gave valuable advice and assistance during development of the system.