Vol. 63: 27–35, 1990 | MARINE ECOLOGY PROGRESS SERIES
Mar. Ecol. Prog. Ser. | Published May 5

Microbial autotrophic and heterotrophic eucaryotes in Antarctic waters: relationships between biomass and chlorophyll, adenosine triphosphate and particulate organic carbon

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ABSTRACT: Microscopical examination of near-surface eucaryotic microbial populations in circumcontinental waters of Antarctica indicated that nanoplankton (< 20 μm diameter) dominated in regions with low chlorophyll concentrations (< 1 μg l^{-1}). About 30 % of the mean nanoplankton carbon consisted of heterotrophic flagellates. Heterotrophic microplankton carbon (> 20 μm diameter) was generally less significant. The variation in phytoplankton biomass was the result primarily of changes in cell density of pennate diatoms in the East Wind Drift, and of centric diatoms in the Weddell Sea and the Scotia Ridge region. Autotrophic and heterotrophic carbon as determined by microscopical analysis were compared with data for total particulate carbon, chlorophyll *a*, and adenosine triphosphate. Estimates for the C:chl ratio of autotrophs increased with decreasing concentrations of chlorophyll *a*, with mean values of 46 in bloom waters and 144 in 'blue water'. A C:ATP ratio for heterotrophic nanoplankton was estimated to be about 100, while that for heterotrophic microplankton may be lower. Algorithms, incorporating concentrations of chlorophyll *a* and ATP, are described which allow estimates of autotrophic and heterotrophic microbial biomass.

INTRODUCTION

The paradigm for the microbial food web in natural waters has undergone much change in recent years. It is now recognized that natural waters frequently contain sizable stocks of autotrophic picoplankton (< 2 μm) and nanoplankton (2 to 20 μm) eucaryotic organisms relative to that of the microplankton (20 to 200 μm) (Waterbury et al. 1979, Malone 1980, Johnson & Sieburth 1982). These size categories also include a variety of microbial predators (Sieburth et al. 1978, Sorokin 1981, Fenchel 1982). From this knowledge, new conceptual models for the predator/prey relationships within microbial systems have been formulated (e.g. Azam et al. 1983, Hewes et al. 1985). However, our comprehension of microbial food webs is hindered because biomass (as carbon), differentiated in terms of trophic mode, cannot be determined with precision.

This paper deals with estimation of autotrophic and heterotrophic biomass on the basis of measurements of C:chl and C:ATP ratios. Autotrophic and heterotrophic biomass are thus defined as organisms with and without chlorophyll *a*, respectively. Admittedly, this definition neglects the existence of trophic modes such as dasmotrophy (Estep & MacIntyre 1989).

It is generally recognized that there is no single 'best method' to estimate biomass for oceanic microbial populations. Microscopical methods are slow and tedious, and often not suited for the large number of samples taken during the course of expeditions. In field studies, it is more common to estimate 'living' carbon as biomass by applying conversion factors to specific extracted biochemical components. Three such commonly used determinants of biomass are adenosine triphosphate (ATP), chlorophyll (chl) and particulate organic carbon (POC). Measurements of particulate organic carbon include variable amounts of detrital contamination so the biologically active constituents may be preferred.

Conversion factors which relate microbial carbon to

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chl, ATP or POC exhibit variation due to species composition and/or environmentally induced changes in physiological properties (Sakshaug & Holm-Hansen 1977). The C:chl ratio of phytoplankton ranges from about 20 (Sullivan et al. 1983, Kiefer 1984, Geider et al. 1986) to greater than 100 (Redalje & Laws 1981, Landry et al. 1984, Sakshaug et al. 1989). Both nutrient limitation and the light regime can cause the C:chl ratio to vary considerably (Shuter 1979, Kiefer & Mitchell 1983, Laws et al. 1985, Geider & Platt 1986, Sakshaug et al. 1989). For most natural populations, the influence of these factors upon the C:chl ratio is not known. This problem is particularly pronounced in the Southern Ocean where, although nutrients are thought not to be limiting, the light regime is extremely variable, and a wide range of C:chl values has been reported (Sakshaug 1989).

Estimates of microbial carbon based on ATP measurement usually invoke a C:ATP factor of 250, which appears to be adequate for multispecies communities of natural phytoplankton (Holm-Hansen 1970, Sakshaug & Holm-Hansen 1977, Karl 1980). However, marine zooplankton may have lower C:ATP ratios (Karl 1980, Skjoldal 1981, Verity & Langdon 1984). Thus total microbial carbon may be overestimated if the ratio of 250 is used (Eppley et al. 1978). Measurements of POC set an upper limit for living biomass and may commonly represent a gross overestimation.

Microscopical analysis can yield detailed information on both autotrophic and heterotrophic carbon. Because ATP and chl are used extensively to estimate microbial carbon, it is important to evaluate the variation in such estimates against the independent estimates provided by microscopical analysis to find out if simple algorithms based on commonly used parameters can be generated for partitioning of microbial carbon. The present work pertains to the biomass structure for populations of microbial eucaryotes found during summer in the Southern Ocean pelagial; the general methodological approach should be relevant to marine waters in general.

MATERIALS AND METHODS

Data were obtained during 2 expeditions to the Antarctic: the VULCAN 6 expedition with RV 'Melville' during January 1981 (Holm-Hansen & Foster 1981) and the ACDA expedition with USCGC 'Polar Star' during January–February 1983 (Holm-Hansen & Chapman 1983). The VULCAN 6 expedition was near the Scotia Ridge between the South Orkney Islands and South Georgia. Samples were obtained at 0, 5, and/or 10 m by Niskin bottles. The ACDA expedition skirted the Antarctic continental shelf westward from

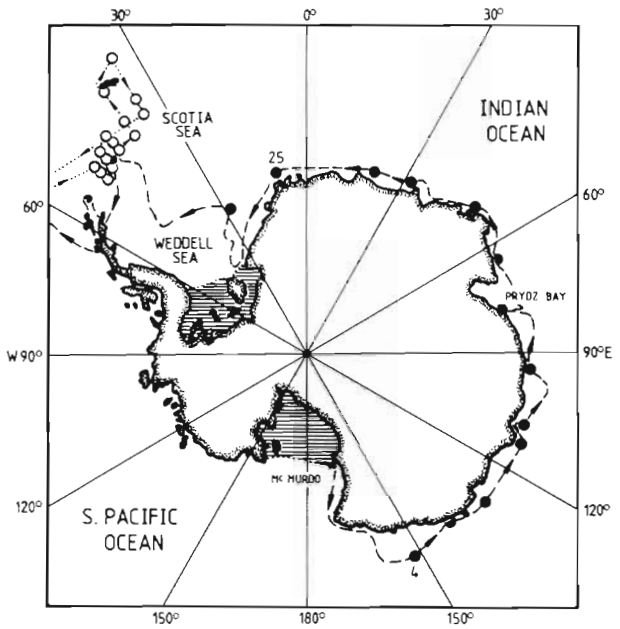

Fig. 1. Regions around the Antarctic continent which were covered by the VULCAN expedition, 1981 (dotted lines), and the ACDA expedition, 1983 (dashed lines). (o) VULCAN stations, (•) ACDA stations, from which data discussed in this paper were obtained

McMurdo Station to Palmer Station (Fig. 1). Water samples were obtained from the surface by bucket while the ship was under way. The data sets presented here are restricted to those which include microscopical analysis and all chemical analyses. Our study thus characterizes microbial communities in the upper euphotic zone over a large segment of the continental shelf of Antarctica in addition to some deep water areas of the Southern Ocean.

Chl *a* was measured fluorometrically in 100 % methanol extracts (Holm-Hansen et al. 1965, Holm-Hansen & Riemann 1978). ATP was extracted in boiling Tris buffer and determined by bioluminescence (Holm-Hansen & Booth 1966). POC was determined with a Hewlett Packard elemental analyzer (Sharp 1974).

All samples were screened by a 202 µm Nitex mesh to remove net-zooplankton. During the ACDA expedition, half of each sample was also screened by a 20 µm Nitex mesh to remove microplankton. All estimates for concentrations contained in the microplankton fraction were obtained by subtracting that of the < 20 µm fraction from the < 202 µm fraction. Samples for chemical analysis were filtered onto Whatman GF/C glass fiber filters.

Independent tests on GF/C and GF/F filters in replicate water samples showed that 95 to 100 % of the chl was retained by the GF/C filters. However, we assume that bacterial contamination of samples on GF/C filters was insignificant, based on earlier tests (Azam & Hod-

son 1977, Fuhrman & Azam 1980) and the knowledge that the contribution of ATP from free-living bacteria in Antarctic waters may be about 10 % of the total microbial ATP (Hodson et al. 1981).

Microscopical analysis was carried out using 2 different methods: (1) the Filter-Transfer-Freeze (FTF) technique (Hewes & Holm-Hansen 1983, Hewes et al. 1984), and (2) the inverted microscope technique of Utermöhl (1958) as applied by Reid (1983). Nanoplankton samples for FTF were fixed with 0.5 % glutaraldehyde (final dilution) for 5 to 10 min, washed with 0.2 µm filtered seawater, and stained with 4', 6-diamidino-2-phenylindole-2-HCl (DAPI). After removal of the Nuclepore filter, the preparations were stored in the dark at less than −10 °C until examination (within 24 h). For examination, the samples were embedded in gelatin, thawed, and examined for both DAPI-stained nuclei and chlorophyll using combined epifluorescence/phase contrast microscopical technique (see Hewes et al. 1984).

Replicate samples of the <202 µm fraction were preserved with 4 % formalin for inverted microscope analysis. This size fraction was also examined using the FTF technique (above) for determination of the trophic mode of organisms not observed in the <20 µm fraction. The relative abundance and taxonomic description obtained from this FTF analysis was used to extrapolate trophic mode of microplankton of the formalin-preserved material. Microscopical analysis of water samples from the VULCAN expedition relied on the inverted microscope technique as the FTF technique had not yet been developed. For these, cells were assumed to be autotrophic if the trophic mode could not be taxonomically determined.

Nanoplankton biomass for the ACDA expedition was estimated by calculating plasma volumes (Strathmann 1967) from the dimensions and shapes of cells and vacuoles as observed with phase contrast microscopy (FTF). The conversion factor used (pg C = $0.11 \times \mu m^3$ plasma volume) is reasonable because low concentrations of glutaraldehyde and brief fixation periods should not significantly shrink cytoplasm (Børsheim & Bratbak 1987). In contrast, biomass was estimated from the formalin-preserved water samples (all VULCAN samples and the microplankton of the ACDA samples) by a combination of equations described by Strathmann (1967; diatom and naked flagellate) and Beers et al. (1975; protozoan). Although this method of estimating cell carbon from formalin-fixed material appears appropriate (Reid 1983, Verity & Langdon 1984, Børsheim & Bratbak 1987), the density of cells less than 5 µm diameter will be underestimated (Hewes et al. 1984). It was also difficult to distinguish between autotrophic and heterotrophic flagellates in these formalin-preserved samples. As unidentified flagellates of the VULCAN samples were considered 'autotrophic', this compensated somewhat for underestimated densities of the photosynthetic forms, but did result in low estimates of heterotrophic nanoplankton.

RESULTS

In general, waters containing <1 µg chl l^{-1} ('blue water') were dominated by nanoplankton (i.e. >50 % of the autotrophic biomass), while waters containing >2 µg chl l^{-1} were dominated by microplankton (Table 1). Phycoerythrin-containing procaryotes were not observed during the ACDA expedition, but a small number of picoplankton eucaryotes (1 to 2 µm diameter, well-defined nuclei) were observed. Elevated chlorophyll concentrations resulted from increases of diatom biomass (Table 1). In the Scotia Sea, these increases were mainly due to centric diatoms; in the East-Wind Drift (ACDA Stns 4 to 25, Fig. 1) they were due to pennate diatoms. Autotrophic flagellates also appeared as a more important component of the VULCAN samples than of the ACDA samples. In spite of different microscopical methods to obtain microbial biomass, estimates of autotrophic carbon relative to ranges of chlorophyll concentration were similar for both ACDA and VULCAN expeditions.

Data in Tables 2, 3, and 4 represent mean values of biomass carbon and biomass determinants (chl, ATP, and POC). In Table 2, the blue water data from the East-Wind Drift is categorized with respect to the nanoplankton and microplankton size fractions. In Table 3, the values for all <202 µm samples are presented. In Table 4, the data of Table 3 have been partitioned into groups according to chlorophyll concentration.

In the East-Wind Drift, all but 2 stations had chlorophyll concentrations less than 1 µg l^{-1}. A small (1 to 2 × 3 to 4 µm) pennate diatom, *Nitzschia pseudonana* Hasle, dominated in terms of carbon, a finding similar to that described by Hasle (1969). For these stations, we found that on average 86 % of the POC, 78 % of the total microbial carbon (C_t), 76 % of the chl, and 61 % of the ATP (ATP_t) passed through a 20 µm screen (Table 2). More than 90 % of the <20 µm chl and ATP fractions passed through a 10 µm screen (data not presented). The highest chl concentration along the East-Wind Drift route was found in Prydz Bay (2.32 µg l^{-1}). At this station, *N. pseudonana* dominated the phytoplankton population, contributing 42 % of the autotrophic nanoplankton carbon. This station was near shore, and is thus not considered in the data presented in the tables or discussion.

Heterotrophic carbon (C_h) represented on average 30 µg l^{-1} (44 % of total eucaryotic microbial carbon) in the East Wind Drift, and 84 % of the eucaryotic hetero-

Table 1 Average microbial biomass classified to autotrophic and heterotrophic cells and taxonomic description as found for the VULCAN expedition (<202 µm water samples) and for the ACDA expedition (<20 µm and 20 to 202 µm screened water samples). Total values represent sums of the averages. These values have been grouped into ranges of chl concentration to show trends in the transformation of trophic structure from low to high chl values

	ACDA			VULCAN			
	Range of chlorophyll (µg l^{-1})						
	<0.5	0.5–1	1–2	<0.5	0.5–1	1–2	>2.0
No. of samples	10	3	1	4	4	4	3
µg chl l^{-1}							
<20 µm	0.2	0.4	0.4				
>20 µm	0.1	0.3	1.0				
Total	0.3	0.7	1.5	0.3	0.6	1.2	4.3
% Chl <20 µm	78	62	30				
Taxonomic description	Biomass estimated (µg C l^{-1})						
Autotrophic	Nanoplankton			<202 µm			
Diatoms pennate	12	41	55				
centric	<1	<1	<1				
Dinoflagellates	2	<1	<1				
Flagellate	14	3	<1	13	41	26	24
Other				1	1	2	1
(subtotal)	(28)	(44)	(57)				
	Microplankton						
Diatoms pennate	2	8	22	3	12	16	32
centric	8	16	34	4	18	40	123
Dinos. non-thecate	<1	<1	<1	4	7	5	5
thecate	<1	<1	<1	<1	<1	2	6
Other	<1	9	<1	1	1	2	1
(subtotal)	(9)	(28)	(57)				
Total autotrophic biomass	38	72	113	26	80	93	192
Heterotrophic	Nanoplankton			<202 µm			
Flagellates	10	11	5	1	1	<1	<1
choanoflag.	2	2	<1	<1	2	10	4
Dinoflagellates	7	16	2				
(subtotal)	(18)	(28)	(8)				
	Microplankton						
Dinos. non-thecate	<1	2	<1	2	4	4	3
thecate	<1	<1	<1	<1	<1	2	<1
Ciliates	2	11	14	2	11	13	12
(subtotal)	(3)	(13)	(14)				
Total heterotrophic biomass	21	41	22	6	18	29	19

trophic microbial biomass was nanoplankton (Table 2). The average protozoan biomass for both the ACDA and VULCAN expeditions was 23 % of the entire microbial population (Table 3). The most common ciliate was *Laboea conica* Lohmann, and most common dinoflagellates were *Amphidinium hadai* Balech, and *Gyrodinium lachryma* (Meunier) Kofoid & Swezy. In our samples, these dinoflagellates lacked chloroplasts, although they have been described as autotrophic (Balech 1976).

Autotrophic carbon (C_a) and total microbial carbon (C_t) estimates determined by microscopical analysis were less than, but covaried with fluctuations in POC (Tables 2 to 4). We therefore conclude that microscopical analysis yielded reasonable values for biomass. As can be obtained from Table 2, mean C:chl values differed little between the nanoplankton (114) and the microplankton (122). The mean C_a:chl ratio for the entire ACDA expedition was 137, in contrast to 88 for the Vulcan expedition (Table 3).

In general, data presented for both of the ACDA size fractions (Table 2) and total microbial values (Table 3) indicate that biomass derived from a C:ATP ratio of 250 (C_{atp}) was considerably higher than C_t, and in fact, often exceeded the POC values. For the ACDA nanoplankton, the mean C_{atp} ratio was 85 % of the mean

Table 2. Mean particulate chemical concentrations and microbial biomasses of nanoplankton and microplankton obtained by size fractioning found for 12 'blue water' stations along the East Wind Drift (Stns 4 to 23; Fig. 1) during the ACDA expedition. Biomass (μg C l^{-1}) was estimated through microscopical methods and differentiated as autotrophic (C_a), heterotrophic (C_h) and total (C_t). Particulate organic carbon (POC), chlorophyll (chl), and adenosine triphosphate (ATP) are in μg l^{-1}. Total microbial biomass in μg C l^{-1} as from ATP data (C_{atp} = 250 × ATP) and from Eq. (5) ($estC_t$). The ratios in Tables 2 to 4 represent the mean ratio of the weights, and cannot be derived by ratioing the mean values listed in the tables

Parameter	Nanoplankton	Microplankton
C_a	32 ± 22	11 ± 10
C_h	26 ± 11	4 ± 3
C_t	58 ± 21	15 ± 11
POC	95 ± 25	15 ± 12
Chl	0.28 ± 0.14	0.09 ± 0.10
ATP	0.33 ± 0.10	0.21 ± 0.21
C_{atp}	81 ± 24	52 ± 54
$estC_t$	52 ± 16	27 ± 26
Chl : ATP	0.83 ± 0.24	0.54 ± 0.59
$C_a : C_t$	0.58 ± 0.20	0.72 ± 0.15

Table 3. Mean values of total microbial biomass, particulate chemical composition, and derived ratios from both the VULCAN and ADCA expeditions. Autotrophic biomass as estimated from chl by the non-linear C:chl relationship, $estC_a$: chl, in Eq. (3) is shown as $estC_a$. All other units and terms as described in the caption for Table 2

Parameter	ACDA	VULCAN	ACDA plus VULCAN
Number of samples	14	15	29
C_a	51 ± 28	101 ± 76	78 ± 64
C_h	30 ± 16	17 ± 17	23 ± 18
C_t	81 ± 29	119 ± 79	101 ± 64
POC	118 ± 37	197 ± 142	160 ± 114
Chl	0.45 ± 0.35	1.82 ± 2.32	1.18 ± 1.84
ATP	0.55 ± 0.29	0.92 ± 0.73	0.75 ± 0.60
C_{atp}	137 ± 72	231 ± 183	187 ± 150
$estC_t$	83 ± 38	133 ± 97	119 ± 95
$estC_a$	47 ± 20	98 ± 74	74 ± 61
chl : ATP	0.82 ± 0.35	1.53 ± 0.76	1.20 ± 0.70
C_a : chl	137 ± 63	88 ± 35	111 ± 55
$estC_a$: chl	123 ± 28	91 ± 41	106 ± 39

Table 4. Mean values for the < 202 μm microbial fraction of the combined VULCAN and ACDA expeditions (last column, Table 3) categorized into ranges of chl concentration. The mean values of $estC_a$: chl and C_a : chl for each range in chl concentration are given. For all terms and units, see captions of Tables 2 and 3

Parameter	Range in chlorophyll concentration, μ l^{-1}			
	Chl < 0.5	0.5 < Chl < 1.0	1.0 < Chl < 2.0	Chl > 2.0
No. of samples	14	7	5	3
C_a	34 ± 12	77 ± 29	102 ± 26	190 ± 79
C_h	21 ± 16	28 ± 20	27 ± 19	19 ± 10
C_t	55 ± 20	105 ± 36	129 ± 24	209 ± 110
$estC_a$	36 ± 8	62 ± 8	93 ± 6	186 ± 57
$estC_t$	57 ± 14	118 ± 29	136 ± 16	287 ± 105
POC	94 ± 27	129 ± 25	187 ± 38	373 ± 84
Chl	0.27 ± 0.10	0.65 ± 0.15	1.28 ± 0.13	4.29 ± 2.19
ATP	0.36 ± 0.12	0.81 ± 0.26	0.80 ± 0.13	1.76 ± 0.72
$estC_a$: chl	136 ± 57	116 ± 36	79 ± 19	48 ± 5
C_a : chl	144 ± 22	96 ± 8	73 ± 3	46 ± 5
Chl : ATP	0.82 ± 0.38	0.87 ± 0.21	1.62 ± 0.16	2.42 ± 0.44
$C_a : C_t$	0.65 ± 0.17	0.74 ± 0.19	0.79 ± 0.13	0.89 ± 0.09

POC (a difference of 14 μg l^{-1}), while C_{atp} of the combined Vulcan and ACDA data was 117 % of the mean total POC (27 μg l^{-1} higher). Thus, although the mean C_{atp} of the nanoplankton tended to give values slightly less than mean POC, total C_{atp} overestimated POC and this is clearly unreasonable. In contrast, the mean C_t was 54 % of the POC for the ACDA nanoplankton and 67 % of the POC for the ACDA total microbial stocks.

The cellular C:ATP ratio of 250 appears realistic for natural phytoplankton communities (Holm-Hansen 1970, Sakshaug & Holm-Hansen 1986), especially for conditions existing in the Antarctic (Karl 1980). However, the heterotrophic biomass found for the ACDA samples averaged 33 % of the total nanoplankton biomass (14 stations). Therefore a higher concentration of ATP for heterotrophs would make a large difference in total microbial biomass estimates based on ATP (Banse 1980, Karl 1980, Skjoldal 1981, Verity & Langdon

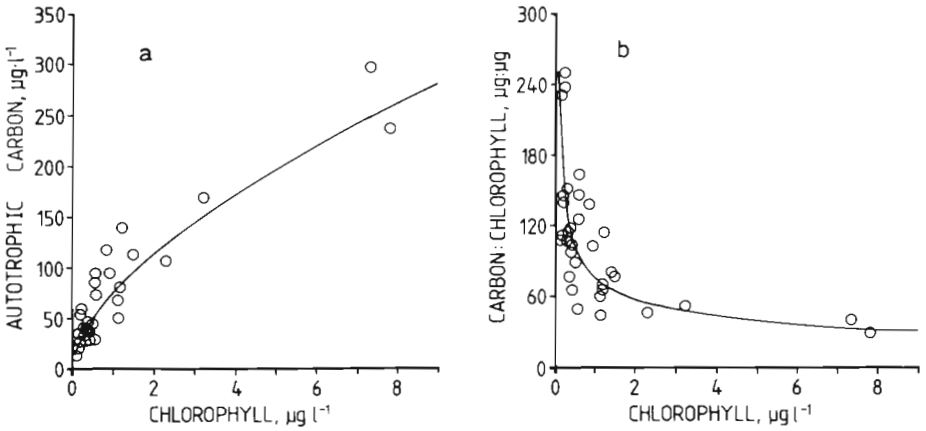

Fig. 2. Relationship between chlorophyll *a* and phytoplankton carbon in the Southern Ocean. (a) Relationship between autotrophic carbon (µg C l^{-1}, determined microscopically) and chlorophyll (µg chl l^{-1}), as described in the text. (b) Same regression as in (a), but autotrophic carbon divided by chlorophyll to demonstrate the non-linear relationship between C:chl and chl

1984). We used the following equation to estimate the C:ATP ratio for heterotrophic nanoplankton (C_{hn}:ATP_{hn}) in our material:

$$C_{hn}:ATP_{hn} = C_{hn}/[ATP_n-(C_{an}/250)] \quad (1)$$

where ATP_n = total nanoplankton concentration, given as µg l^{-1}; C_{an} and C_{hn} = autotrophic and heterotrophic nanoplankton biomass in µg C l^{-1} based on FTF analysis. This yielded a mean value of about 100 (n = 14, C_{hn}:ATP_{hn} = 106 ± 55). This is similar to values for marine zooplankton (Skjoldal & Båmstedt 1977, Karl et al. 1978, Skjoldal 1981), although higher than that reported for tintinnids (Verity & Langdon 1984).

It is evident that phytoplankton biomass (C_a) and chl concentration did not have a linear relationship (Table 4). Furthermore, the small variation in the C_a:C_t ratio implies that the contribution of heterotrophic ATP cannot entirely explain the large variation found in the chl:ATP ratio over the range of chlorophyll concentrations we examined (Table 4). The relationship between autotrophic carbon and chl concentration can be described as an empirical function:

$$C_a = 80\ chl^{0.6} \quad (2)$$

(n = 33, r^2 = 0.8; Fig. 2a). Values of autotrophic biomass as estimated in Eq. (2) (*est*C_a) are presented in Tables 3 and 4 where they may be compared with the other biomass determinants. It follows that the C:chl ratio (*est*C_a:chl) is related to chlorophyll concentration by the function:

$$estC_a:chl = 80\ chl^{-0.4} \quad (3)$$

as demonstrated in Fig. 2b.

The mean C:chl ratios (both C_a:chl and *est*C_a:chl) for various ranges of chlorophyll concentration are presented in Table 4. The non-linearity of *est*C_a:chl is also implied by comparing POC:ATP and POC:chl ratios (which can be estimated from the data presented in Table 4) for different concentrations of chl. The POC:ATP ratio is about the same for the lowest and highest concentrations of chl (POC:ATP about 240), whereas the POC:chl ratio varies from 87 to 348.

DISCUSSION

Many important answers related to pelagic microbial systems depend upon realistically differentiated estimates of microbial biomass. Traditionally, biomass has been estimated by assuming constant either C:chl or C:ATP ratios (Eppley 1972, Holm-Hansen et al. 1977). Constant ratios may lead to erroneous biomass estimates. We have demonstrated that both ratios vary, but in a fairly predictable fashion. From our data, algorithms may be derived to provide improvements over previous methods for the estimation of microbial carbon, at least for the conditions which exist during summer in the Antarctic.

The heterotrophic C:ATP ratio of 100 was estimated from biomass and ATP of nanoplankton obtained during the ACDA expedition. Our estimates of nanoplankton carbon were based on plasma volumes derived by microscopical analysis, and therefore this ratio would resemble 'cytoplasmic' values. Our ratio is in the range of values generally ascribed to multicellular marine zooplankton (26 to 125; see Karl 1980, Skjoldal 1981, references cited therein), but is higher than that of about 65 obtained for tintinnids (Verity & Langdon 1984). These data support Skjoldal's (1981) data, which implied that 'animal' (including protozoan) C:ATP values for various groups of zooplankton should vary within similar ranges, because the ratio is independent of the size of the organism.

It should be emphasized, however, that our data suggest a C:ATP value for some unicellular heterotrophic eucaryotes of less than 100. Comparison of C_a:C_t and chl:ATP ratios of size-fractioned samples (Table 2) indicates that, since the C:chl ratios were nearly the same, the heterotrophic microplankton had a much lower C:ATP ratio than the nanoplankton.

Ratios of C:ATP for the entire heterotrophic microbial population ranged from 33 (VULCAN) to 87 (ACDA), and thus were more similar to the value of 65 found for tintinnids (Verity & Langdon 1984). We place more confidence, however, in our estimate of C_h:ATP_h for nanoplankton from the ACDA expedition than for the formalin-preserved samples, because the method used to estimate carbon was more precise.

The differences in the C:ATP ratio for algae (250 to 286), bacteria (250 to 500) and zooplankton (40 to 125) may reflect the amounts of storage and structural carbon in the cells (Karl 1980). It is also likely that the C:ATP value for microbial heterotrophs of different size may vary with physiological status and the amount of structural carbon (e.g. scales, thecae or loricae).

The C:chl ratio of Antarctic blue waters (mean *est*C_a:chl = 91 to 123) is similar to values reported for other oligotrophic regions (Redalje & Laws 1981, Landry et al. 1984). This ratio approaches, however, about 30 in offshore Antarctic waters with high chl concentrations (Fig. 2b), and is similar to that reported for Antarctic ice algae (Sullivan et al. 1983) and coastal Antarctic blooms (Sakshaug 1989). The non-linearity of the relationship between the C:chl ratio and the chl concentration may reflect adaptation of phytoplankton to different light regimes which may result from differences in the rate and depth of vertical mixing, and/or different photoadaptational strategies of communities (El-Sayed & Mandelli 1965, Sakshaug & Holm-Hansen 1984, 1986). It is also possible that variations in the C:chl ratio may arise from the asymmetry of the photoadaptational response (i.e. from light to shade vs from shade to light) in deeply mixed water columns and from self-shading (Lewis et al. 1984, Geider & Platt 1986, Cullen & Lewis 1988).

Heterotrophic and autotrophic carbon may be estimated from data for chl and ATP concentrations. By rearrangement of Eq. (1) and using a value of 100 for the C:ATP ratio of heterotrophs, heterotrophic biomass may be estimated as:

$$estC_h = [ATP_t - C_a/250] \times 100 \quad (4)$$

Since autotrophic biomass can be estimated from chlorophyll concentration (Eq. 2), total microbial biomass can be estimated:

$$estC_t = 80\ chl^{0.6} + [ATP_t - (80\ chl^{0.6}/250)] \times 100 \quad (5)$$

where *est*C_t, ATP_t, and chl = concentrations in $\mu g\ l^{-1}$; and the constants of 250 and 100 = C:ATP ratios of autotrophic and heterotrophic eucaryotes, respectively. Table 2 indicates that *est*C_t is reasonably close to nanoplankton carbon, but does overestimate microplankton carbon slightly.

It has been suggested that the ratio between chl and adenylate pools might index the trophic structure of microbial communities (Chiaudani & Pagnotta 1978, Campbell et al. 1979). Our study indicates, however, that the chl:ATP ratio is a complex function not only of variation in the proportion of autotrophic biomass and changes in the cellular chl concentration of the phytoplankton, but also possible differences in size-fractioned heterotrophic C:ATP ratios (micro < nano < bacteria).

Estimations of algal biomass, whether on the basis of chl or ATP multiplied by certain factors, or on the basis of algorithms such as those presented here, represent a grossly simplified view of the pelagic ecosystem; species composition and differences in strategies between species are neglected. Yet simple algorithms are necessary for modeling of planktonic ecosystems on a regional or global scale. Presently-used methods for estimation of biomass carbon suffer for a variety of well-published reasons. The present algorithms for determination of autotrophic and heterotrophic carbon should in principle represent an improvement relative to earlier approaches while still being simple. The coefficients presented here are, however, specified only for a given region and time of the year. The ratios between microbial carbon, chlorophyll and ATP *do* vary, but the degree to which this occurs may be a function of environmental conditions. Variations in the C:chl ratio are, however, covered by the present algorithms, and to some extent variation in some ratios may be smoothed out by the presence of several species, for instance the C:ATP ratio of algae. Algorithms such as the present ones might therefore relate physical events occurring in the euphotic zone to biochemical and physiological characteristics of the microbial plankton.

Acknowledgements. This work was supported by US National Science Foundation grants DPP 79-21 295 and DPP 82-12 362, the Norwegian Research Council for Science and the Humanities, and the Norwegian Polar Institute. The authors thank J. R. Beers for helpful discussions and I. Dundas, F. Thingstad, and K. Y Børsheim for reviewing the manuscript.

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This article was presented by Dr H. R. Skjoldal, Bergen, Norway

Manuscript first received: January 7, 1988
Revised version accepted: February 20, 1990