REVIEW

Microzooplankton grazing in the oceans: impacts, data variability, knowledge gaps and future directions

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Here we review all published data on phytoplankton growth and microzooplankton grazing using the dilution technique to better understand the role of this group of grazers in different regions of the oceans, and to identify the knowledge gaps that require future efforts. A total of 1525 data points assimilated from 110 studies were included and grouped using the biogeographic subsets defined by Longhurst *et al.* [(1995) An estimate of global primary production in the ocean from satellite radiometer data. *J. Plankton Res.*, **17**, 1245–1271]. Total median phytoplankton growth rates in each of the subsets varied between 0.15 (Polar Southern) and 0.83 day^{-1} (Trades Atlantic), with the corresponding microzooplankton grazing rates ranging between 0.07 (Polar Southern) and 0.48 day^{-1} (Trades Indian). The median percentage of primary production (PP) grazed by microzooplankton was relatively constant among the regions and ranged from 49

to 77%, with the lowest percentage found in the Westerlies Southern and the highest in the Coastal Indian. Despite strong evidence that microzooplankton grazing impacts were highly variable, no global drivers for this variability (temperature, chlorophyll concentration) were identified in the analysis. As a global carbon budget, the overall consumption of phytoplankton for all regions was 31.3 Gt C year^{-1}, which accounted for 62.4% of the total PP grazed daily. The amount of carbon ingested by micro- and mesozooplankton varied proportionally to the PP and the consumption was five times higher for micro- than for mesozooplankton. As concluding remarks of the study, we present some suggestions that may improve the quality of the data obtained with the dilution technique.

INTRODUCTION

Microzooplankton are major grazers of phytoplankton in most marine habitats (e.g. Sherr and Sherr, 2002; Calbet and Landry, 2004). Most of the knowledge about the relevance of microzooplankton in marine food webs has been obtained quite recently following the establishment of the grazing dilution technique (Landry and Hassett, 1982). The technique provides simultaneous estimates of phytoplankton growth (μ) and mortality (m) rates, the latter attributable to microzooplankton (*sensu lato*) grazing. The method is based on the reduction of encounter rates between predator and prey by progressive dilution of natural communities with particle free water, and it involves three assumptions: (i) Growth of individual phytoplankton is not directly affected by the presence or absence of other phytoplankton *per se*. The implication of this assumption is that a reduction in the density of cells in natural seawater will not cause a direct change in the growth rate of the remaining cells. To fulfil this assumption, nutrients are routinely added to the incubation bottles, with the precaution of leaving some undiluted bottles without nutrients, which serve as controls for the natural growth rates of the phytoplankton. (ii) The probability of a phytoplankton cell being consumed is a direct function of the rate of encounter of consumers with prey cells. This implies that consumers are not food-satiated at natural prey densities, and that the number of prey ingested by a given consumer is linearly related to prey density. (iii) Changes in the density of phytoplankton (P) over time can be represented by the exponential equation $P_t = P_0 e^{(\mu - m)t}$, where μ and m are instantaneous coefficients of population growth and grazing mortality, respectively, and t is the incubation time. The phytoplankton mortality rate is determined as the slope of the regression line of a series of dilutions. The instantaneous growth rate of the

natural phytoplankton community (μ_0) cannot be directly obtained by the intercept of the equation because that would correspond to the nutrient-amended community. Instead, the term is derived by adding the absolute mortality rates to the net growth rates (k_0) of the phytoplankton in the unamended bottles (i.e. $\mu_0 = k_0 + m$).

The dilution approach is not free of criticism. For instance, because the regression analysis is commonly conducted with a small number of values (i.e. 8–10 bottles), low grazing rates are at times masked by variability and difficult to detect (e.g. non-significant slopes). This problem is complicated by non-linear responses wherever grazing is saturated at the highest prey concentrations (Gallegos, 1989; Evans and Paranjape, 1992). Additionally, non-proportional changes in the grazer concentration during incubations could result in inaccurate estimates of grazing (Dolan *et al.*, 2000; Agis *et al.*, 2007). Trophic cascades can also modify the outcome of dilution grazing experiments (Calbet *et al.*, 2008, 2011a,b; Calbet and Saiz, submitted). Mixotrophs, as they act simultaneously as grazers and prey, can confound and bias the grazing estimates based on chlorophyll (Calbet *et al.*, 2012). Other problems arise when the assumptions of the method are not met. For instance, nutrient limitation during incubations can result in incorrect estimations of grazing because the slope of the equation between dilution factor and phytoplankton net growth rates is affected by a differential instantaneous phytoplankton grazing in the bottles.

Perhaps, the most important criticism of the technique is that many of these artefacts are not easily detectable because the experiment will always provide a grazing estimate (even if it is incorrect). Nevertheless, dilution grazing experiments have been widely conducted since the establishment of the technique and it is today, without doubt, the most common method to estimate

microzooplankton grazing on planktonic primary producers.

Calbet and Landry (Calbet and Landry, 2004) reviewed all available data regarding microzooplankton grazing rates obtained with the dilution technique. Their results revealed that microzooplankton were important grazers in all marine habitats considered. Moreover, it was concluded that the mean impact of microzooplankton grazing activity (obtained as $m:\mu*100$) varied discreetly among marine regions, the data ranging between 59 and 74% of phytoplankton primary production (PP) consumed daily across systems, despite seasonality, trophic status, latitude or salinity (Calbet and Landry, 2004). There were 788 data points available at that time, impressive for any ecological study on plankton, but limited in its description of the dynamics of phytoplankton and microzooplankton in particular habitat types. Therefore, their integration of the data were restricted to very few domains: oceanic, coastal and estuarine, regarding geographic location and to polar, temperate and tropical regarding climatology.

We believe, given the extensive use of the technique, that the information is nowadays (three decades after the description of the technique) enough to extend the analysis of Calbet and Landry (Calbet and Landry, 2004). Therefore, we have collected all the published data on phytoplankton growth and microzooplankton grazing using the dilution technique to present a more detailed picture of the role of microzooplankton as grazers of primary producers in different marine environments. We have focused our study on the biogeographical provinces defined by Longhurst *et al.* (Longhurst *et al.*, 1995). Our intention is 2-fold; first to contribute to the understanding of the functioning of the different biogeochemical provinces, and secondly to identify the knowledge gaps in particular areas that require future efforts.

THE DATASET

The database used in this work consisted of 1525 values obtained from a literature search of all studies in which phytoplankton growth and microzooplankton grazing rates were estimated using the dilution technique (Landry and Hassett, 1982). It includes the whole dataset of the 788 experiments analysed by Calbet and Landry (Calbet and Landry, 2004) plus those studies published since 2004 (Supplementary data, Appendix Table SI). They were obtained from a total of 110 works. Data from iron-addition experiments in open ocean and data from mesocosm studies were excluded from the analysis because they do not represent natural

Table I: Number of data of phytoplankton growth and mortality rates in the literature survey

	μ_n	μ_0	k_0	μ	m	Indx.
Coastal Atlantic	74	32	27	37	109	1.42
Coastal Indian	19	7	0	13	51	0.59
Coastal Pacific	125	54	28	174	211	1.21
Polar Arctic, Atlantic, Pacific	87	29	13	31	115	1.00
Polar Southern	7	12	0	75	86	0.56
Trades Atlantic	124	25	0	8	124	0.19
Trades Indian	54	54	0	19	73	0.06
Trades Pacific	104	61	0	97	202	0.17
Westerlies Atlantic	165	124	69	119	308	1.39
Westerlies Pacific	6	6	0	50	56	0.09
Westerlies Southern	13	9	0	78	100	0.15

Here μ_n denotes nutrient-amended growth rates, μ_0 *in situ* growth rates, k_0 net growth rates, μ growth rates in no nutrient-amended experiments, m phytoplankton mortality rates by microzooplankton grazing and Indx the index calculated by dividing the number of studies by area (10^6 km^2) of each subset.

communities. We used the biogeographic subsets defined by Longhurst *et al.* (Longhurst *et al.*, 1995) to group the data into 12 subsets (according Table I in the original paper of Longhurst *et al.*, 1995; see Fig. 1): Coastal Atlantic (CA), Coastal Indian (CI), Coastal Pacific (CP), Polar Arctic (PAr), Polar Atlantic (PAt), Polar Southern (PS), Westerlies Atlantic (WA), Westerlies Pacific (WP), Westerlies Southern (WS), Trades Atlantic (TA), Trades Indian (TI) and Trades Pacific (TP). Few data points from the Polar Arctic, Polar Atlantic and Polar Pacific were available (<50 data points each) and, as such, were grouped into a common Polar Arctic (PA) region.

Following the strategy of Calbet and Landry (Calbet and Landry, 2004), some modest modifications were needed to facilitate calculations. To avoid dividing by zero or a negative number, a total of 76 negative estimates of phytoplankton growth rate were corrected to $+0.01$ day^{-1}. Likewise, negative rates for microzooplankton grazing were equalled to zero. This affected a total of 34 estimates. We used the $m:\mu$ ratio as a reasonable estimation of the proportion of PP grazed by microzooplankton (Calbet and Landry, 2004). Those values unrealistically higher than 5 (65 estimates in total) were considered extremes and were equalled to 1 (100% PP grazed per day). Median values and box plots were used to investigate the possible variations of phytoplankton growth and microzooplankton grazing rates among the different biogeographical areas. The total percentage of PP grazed (Gt C $year^{-1}$) by microzooplankton in the global ocean was estimated in each subset by multiplying the PP by the median of the $m:\mu$ ratio in each subset.
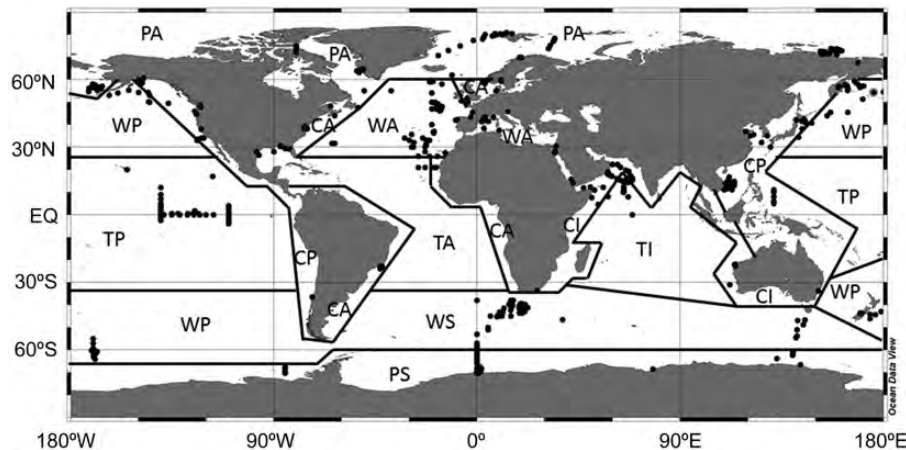
Fig. 1. Schematic map showing the approximate situation of the biogeographic subsets defined by Longhurst *et al.* (Longhurst *et al.*, 1995) grouping the data into 11 subsets. This figure also includes the geographical positions of the dilution experiments included in the review.

DATA DISTRIBUTION AND ROBUSTNESS

The analysis of the geographical distribution of the data revealed that there are limited data available in very extensive areas of the oceans, and that data pairs are not homogeneously distributed among the different biogeographical subsets. For instance, Westerlies Atlantic, Coastal Pacific and Trades Pacific together contained 50% of the total microzooplankton grazing rate estimates (21.5, 14.7 and 14.1%, respectively; Table 1, Fig. 1). The lack of data were particularly evident for the subtropical gyres (TP, WP, WA, TA), which together covered 57.7% of the total oceanic surface. The lowest indices of the number of studies per area of each subset were found in TI, WP, TP and TA (Table I), which indicated that these subsets are the less studied proportionately to their surface. Among all the studies collected from the literature, 46.2% of the data were from oceanic regions, 38.5% from coastal regions and 15.3% were from estuarine regions. Data on microzooplankton grazing and phytoplankton growth rates have increased since the Calbet and Landry (Calbet and Landry, 2004) study with the majority of the new research concentrated on coastal areas within the Northern hemisphere (China Sea or North-West Pacific Ocean). Only few studies have been conducted in the open ocean, e.g. in the Equatorial Pacific (Landry *et al.*, 2011), North Subtropical Atlantic (Gutiérrez-Rodríguez *et al.*, 2011) or in the Southern Ocean (Safi *et al.*, 2007).

There was also a disparity in the variables presented in each study. As described in the introduction, the original dilution technique (Landry and Hassett, 1982) is based on the assumption that phytoplankton growth is saturated and uncoupled from the dilution level.

Therefore, not only nutrient additions in those systems that are presumably nutrient limited, but also controls for natural phytoplankton growth (undiluted bottles without nutrient addition) are needed. It was, therefore, surprising that 51% of the studies only reported growth rates with nutrient-amended treatments (μ_n), and 47% of the studies gave growth rates from experiments without nutrient addition (μ). Furthermore, only 26.7% of the studies presented *in situ* growth rates (μ_0; as described in Landry and Hassett, 1982), and only 8.9% of the studies presented estimates of net growth rates in the nutrient-unamended bottles (k_0; Table I). As discussed in Calbet *et al.* (Calbet *et al.*, 2011a), net growth rates may be used as a proxy for the actual trophic state of the system being investigated.

GLOBAL DRIVERS OF VARIABILITY IN PHYTOPLANKTON GROWTH AND MICROZOOPLANKTON GRAZING RATES

Chlorophyll *a* median concentrations ranged between 0.70 and 5.40 μg L^{-1} among the different areas. The highest value was found in the Trades Atlantic, which included six studies, of which five were conducted in highly productive estuaries (Murrel *et al.*, 2002; Juhl and Murrel, 2005; First *et al.*, 2007; Putland and Iverson, 2007; Quinlan *et al.*, 2009), leading to higher chlorophyll *a* values (Table II). Total median phytoplankton growth rates varied between 0.15 and 0.83 day^{-1} among the different biogeographical subsets. The lowest median was found in the Polar Southern and the highest

Table II: Median values for each biogeographical subset of chlorophyll a*: phytoplankton growth rate (*μ*, day^{-1}), grazing mortality rate (*m*, day^{-1}), % primary production grazed day^{-1} (%PP), PP estimated in the study by Longhurst* et al. *(Longhurst* et al., *1995) (PP, Gt C year^{-1}) and the corresponding amount of carbon ingested computed from our study (Grazing; Gt C year^{-1})*

	Chl *a* (μg L^{-1})	μ (day^{-1})	*m* (day^{-1})	%PP grazed	PP (Gt C year^{-1})	Grazing (Gt C year^{-1})
CA	1.50 (0.42−4.19)	0.46 (0.26−0.77)	0.33 (0.15−0.54)	67.0 (35.5−100.0)	5.64	3.78
CI	0.36 (0.22−0.77)	0.40 (0.14−0.94)	0.39 (0.13−0.69)	77.0 (47.0−122.0)	3.60	2.27
CP	1.43 (0.50−3.95)	0.53 (0.24−0.89)	0.36 (0.11−0.65)	55.0 (18.0−100.0)	5.15	2.83
PA	1.21 (0.57−2.50)	0.28 (0.10−0.48)	0.14 (0.05−0.27)	57.0 (20.0−100.0)	4.20	2.39
PS	0.53 (0.19−1.42)	0.15 (0.03−0.58)	0.07 (0.03−0.27)	53.0 (17.0−100.0)	2.24	1.19
TA	5.40 (0.43−12.30)	0.83 (0.39−1.23)	0.49 (0.25−0.91)	70.0 (37.5−104.5)	4.60	3.22
TI	0.36 (0.21−0.48)	0.68 (0.44−0.94)	0.48 (0.32−0.65)	67.0 (50.0−100.0)	2.86	2.26
TP	0.27 (0.21−0.43)	0.44 (0.29−0.61)	0.29 (0.18−0.45)	67.0 (46.0−99.0)	5.54	3.71
WA	0.70 (0.31−1.66)	0.69 (0.37−1.06)	0.42 (0.24−0.69)	57.0 (37.0−100.0)	2.80	1.60
WP	0.44 (0.24−0.69)	0.47 (0.28−0.62)	0.24 (0.14−0.39)	67.5 (41.0−102.5)	7.62	5.14
WS	0.44 (0.25−0.59)	0.45 (0.21−0.81)	0.18 (0.08−0.33)	49.0 (19.0−73.0)	5.92	2.90
Total					50.17	31.29

Values in parentheses represent the 25 and 75% quartiles of the median.
CA, Coastal Atlantic; CI, Coastal Indian; CP, Coastal Pacific; PA, combined data for Polar Arctic, Polar Atlantic, and Polar Pacific; PS, Polar Southern; TA, Trades Atlantic; TI, Trades Indian; TP, Trades Pacific; WA, Westerlies Atlantic stands; WP, Westerlies Pacific; WS, Westerlies Southern.

median was found in the Trades Atlantic (Table II, Fig. 2A). Microzooplankton grazing rates ranged between 0.07 in the Polar Southern to 0.48 day^{-1} in the Trades Indian (Table II, Fig. 2B). The median percentage of PP grazed by microzooplankton ranged between 49% in the Westerlies Southern to 77% in the Coastal Indian. The PP grazed was significantly different among regions (non-parametric analysis, K−W test, $P < 0.001$), with 50% of the data ranging between 17 and 122%. The Trades Pacific was the less variable region, and the most variable was the Polar Southern (Table II, Fig. 2C). For comparative purposes with the original paper by Calbet and Landry (Calbet and Landry, 2004), we have calculated the percentage of PP grazed by microzooplankton among coastal, estuarine and oceanic zones. We found that there was a significant difference in the percentage of PP grazed by microzooplankton between the three groups (non-parametric analysis, K−W test, $P < 0.001$), being 52, 64 and 68%, respectively.

We investigated if there were any significant relationships between the variables considered in the study (temperature and chlorophyll concentration) and the different rates estimated. Significant linear relationships were found between chlorophyll and total μ ($P < 0.01$), and *m* ($P < 0.001$), and between temperature and μ and *m* (both $P < 0.001$). However, all the regressions had very low r^2 (always <0.2; Table III). The next step was to build a multiple regression model that allowed for interactions between chlorophyll and temperature to explain the microzooplankton grazing rates and/or the

m:μ relationship. The model was not significant for *m*:μ; however, it was significant for m ($P < 0.001$; $r^2 = 0.14$):

$$m = 0.19 + 0.014T + 0.005\text{Chl} + 0.0005T * \text{Chl},$$

where T is the temperature in °C and Chl chlorophyll in μg L^{-1}. However, despite the model producing significant relationships between the variables, its explanatory power was low.

It is not surprising that any effort failed to identify major oceanic trends between temperature and grazing. Similarly, no direct significant effect of temperature was found on copepod grazing in the oceans (Saiz and Calbet, 2007, 2011). There may be several reasons for the lack of a significant effect of temperature on copepod grazing. For instance, when considering the global ocean, it is evident that the inverse relationship between inorganic nutrients and water temperature is broken near the equator, where the upwelling of nutrient-rich waters fertilizes a warm area of the oceans. Coastal upwelling may also introduce variability in the temperature−nutrient inverse relationship by mixing cold nutrient-rich waters with warm nutrient-depleted ones in the same area. The variability in chlorophyll concentration is not explained by the balance between phytoplankton growth and microzooplankton grazing. Nonetheless, in the simplistic scenario of only microzooplankton grazing on phytoplankton, one should be aware that the snapshot of the rates driving a community, as provided by dilution grazing
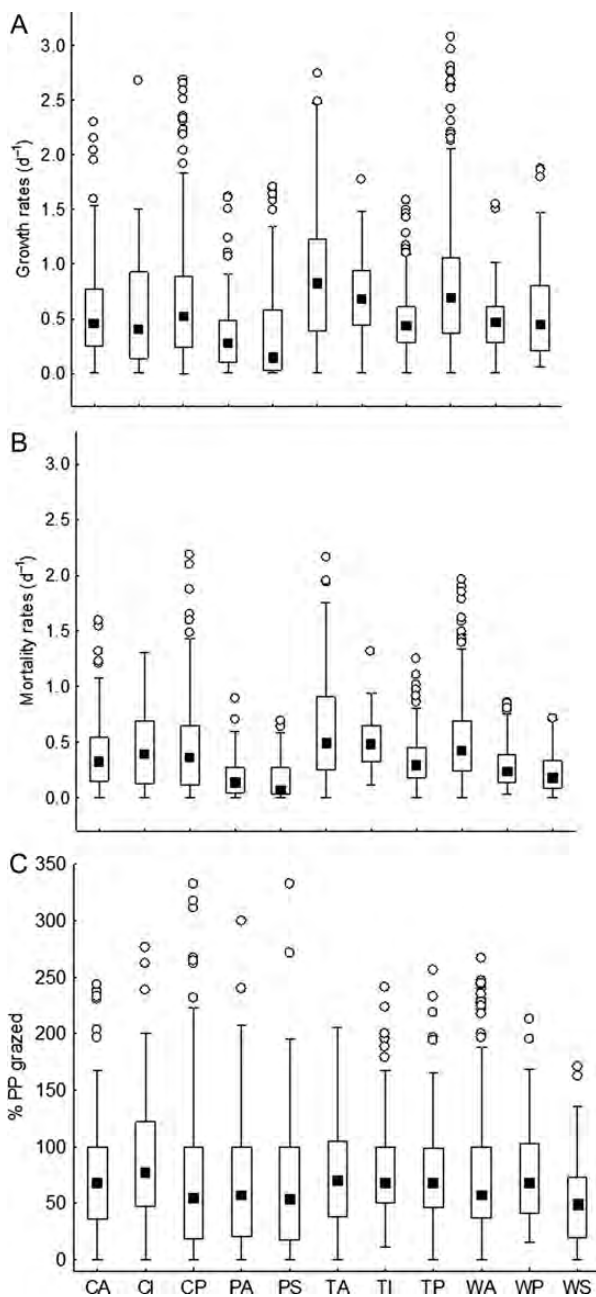
Fig. 2. Box-plot of (**A**) growth rates (μ, day^{-1}), (**B**) mortality rates (m, day^{-1}), (**C**) % primary production grazed daily[1] (%PP) by each biogeographic subset as defined by Longhurst (Longhurst *et al.*, 1995). Squares: median; Circles: outliers; Rectangles: quartiles of the median (25–75%); Vertical bars: non-outlier ranges. CA, Coastal Atlantic; CI, Coastal Indian; CP, Coastal Pacific; PA, combined data for Polar Arctic, Polar Atlantic and Polar Pacific; PS, Polar Southern; TA, Trades Atlantic; TI, Trades Indian; TP, Trades Pacific; WA, Westerlies Atlantic stands; WP, Westerlies Pacific; WS, Westerlies Southern.

Table III: Linear regressions between chlorophyll a *and temperature, and different rates obtained in this study: phytoplankton growth rate (*μ*, day^{-1}), grazing mortality rate (*m*, day^{-1}) and % of primary production grazed daily (%PP)*

	Chl *a*		Temp.	
	r^2	P	r^2	P
μ	0.005	0.009	0.180	<0.001
m	0.023	<0.001	0.112	<0.001
%PP	0.004	0.027	0.000	0.996

SEASONAL AND CLIMATIC INFLUENCES IN THE DIFFERENT BIOGEOGRAPHIC REGIONS

Polar and sub-polar regions are highly productive and dependent on a strong seasonal signature. They are, paradoxically, also the zones with the lowest phytoplankton growth rates, which implies that the mortality losses are even lower to allow for the establishment of phytoplankton blooms. There, the microzooplankton grazing rates, as well as the percentage of PP lost daily by grazing, were among the lowest of our analysis. In the Polar regions, the mean percentage of PP grazed per day ranged from 53 to 57%, although the variability in the regions was high. For instance, in the high Western Arctic Ocean, Sherr *et al.* (Sherr *et al.*, 2009) described a low control of microzooplankton grazing on primary producers (average 22%). Calbet *et al.* (Calbet *et al.*, 2011a), also in high Arctic waters, found that during the summer the grazing impact on phytoplankton was significant in only 6 out of 16 experiments, which resulted in 8% of the standing stock being consumed on average. In the Antarctic marginal ice-zone of the Bellinghausen Sea, Burkill *et al.* (Burkill *et al.*, 1995) found that 3–40% of phytoplankton was grazed daily, being positively related to sea temperature, phytoplankton and microzooplankton concentrations. Froneman and Perissinotto (Froneman and Perissinotto, 1996) and Froneman *et al.* (Froneman *et al.*, 1997) also found a low percentage of PP grazed, between 0 and 40% and between 9 and 25%, respectively.

Several potential hypotheses could explain the occurrence of such uncoupling. The most accepted cause would be the low-temperature constraints on protozoan growth, which would result in the imbalance between phytoplankton and microzooplankton growth rates (Rose and Caron, 2007). Other recent studies that have also indicated a low grazing impact of microzooplankton on PP in the Arctic Ocean (Strom *et al.*, 2007;

experiments, cannot explain steady-state variables, such as chlorophyll biomass, unless the different loss and gain terms are in equilibrium for a long time (Calbet *et al.*, 1996). This seldom occurs in natural systems.

Sherr *et al.*, 2009; Calbet *et al.*, 2011a,b) suggested alternative hypotheses. Most of these studies were conducted in spring and summer, during or at the end of a phytoplankton bloom (e.g. *Emiliania huxleyi* in Olson and Strom, 2002; *Phaeocystis pouchetii* bloom in Calbet *et al.*, 2011a). In these works, grazing rates were low even at high chlorophyll concentrations. Sherr *et al.* (Sherr *et al.*, 2009) suggested that these low rates may be explained by the poor nutritional quality of the phytoplankton cells at the end of the bloom period, which would reduce microzooplankton grazing. An alternative explanation of the low grazing rates of microzooplankton may be due to the strong top-down control by Arctic copepods (Sherr *et al.*, 2009), and perhaps by Antarctic Krill as well (Wickham and Berninger, 2007). However, there are exceptions to the above, and even if on average the microzooplankton impacts are low, few other studies found a strong control on PP, even during post-bloom conditions (e.g. 37–88% of the PP daily grazed, Paranjape, 1987; 104%, Tsuda and Kawaguchi, 1997; 55–68%, Archer *et al.*, 2000; 22–117%, Gaul and Antia, 2001; >100%, Pearce *et al.*, 2009).

The *temperate regions* are also characterized by a high seasonality. Sixty per cent of the studies analysed here have been conducted in temperate areas (65 of the 110 works collected in this study), covering oceanic, coastal and estuarine zones and nine of the biogeographical subsets. This large dataset (850 data) did not cover the four seasons of the year equally as 115 data were obtained in fall, 288 in spring, 303 in summer and only 113 in winter. Most of the data were obtained during oceanographic cruises which cover short periods of time and space. Because of this disparity in geographic areas and seasons, estimations of microzooplankton grazing and phytoplankton growth rates were very variable and global patterns are difficult to establish. For instance, within estuaries of the North Atlantic Ocean the values of PP grazed by microzooplankton ranged from 50% in the Rhode River Estuary during fall (Dolan *et al.*, 2000) to 99% in the North Atlantic Ocean in spring (Sautour *et al.*, 2000). For the coastal zone, in the North Atlantic Ocean (Westerlies Atlantic), Fileman *et al*. (Fileman *et al.*, 2002) showed that ca. 60% of the chlorophyll *a* biomass was grazed daily by microzooplankton during summer. In spring, also in the North Atlantic Ocean but in Trades Atlantic, First *et al*. (First *et al.*, 2009) found that on average 110% of the PP was grazed. Finally, within the open ocean, Fileman and Burkill (Fileman and Burkill, 2001) found that 40–85% of the PP was grazed by microzooplankton in the North Atlantic Ocean during summer, whereas, during spring, these percentages ranged from 38 to 154% (Fileman and Leakey, 2005).

In recent years, however, a few studies covering an annual cycle have been conducted (e.g. Collos *et al.*, 2005; Calbet *et al.*, 2008, Gutiérrez-Rodríguez *et al.*, 2011; Lawrence and Menden-Deuer, 2012), which have enabled the assessment of temporal patterns. For instance, in Narragansett Bay, USA, Lawrence and Menden-Deuer (Lawrence and Menden-Deuer, 2012) found that net phytoplankton growth rates were negative sometimes in both winter and spring, with peaks in growth rates recorded in summer. Also, in a seasonal study in coastal Mediterranean waters, it was found that all phytoplankton production was removed daily by microbial grazers in July, and only a quarter of it was accounted for microzooplankton in January, both periods displaying similar biomass of phytoplankton and grazers (Calbet *et al.*, 2008).

The seasonal changes in microzooplankton grazing rates are usually associated with changes in the planktonic community composition. Several authors pointed out that this factor, together with temperature, drives most of the growth and grazing rate variations (Rose and Caron, 2007; Calbet *et al.*, 2008; Chen *et al.*, 2012; Lawrence and Menden-Deuer, 2012).

Tropical waters are the end point relating seasonality; they are characterized by a considerable stability over the annual cycle, with high temperatures and, usually, low nutrient availability. As such, subtropical gyres are considered the most oligotrophic areas of the oceans with the lowest values of PP and phytoplankton biomass. Subtropical gyre regions are the least productive regions, but also the areas where microzooplankton grazing is higher. The combination of both processes results in most of the PP being grazed by microzooplankton in these regions.

A possible explanation for the higher grazing control in subtropical zones would be the lower biomass of mesozooplankton in warm areas (Hernández-León and Ikeda, 2005) promoting high microzooplankton abundance. Given that microzooplankton have a rapid growth and high specific ingestion rates, they might be able to control the small-sized phytoplankton standing stock of these blue waters. Thus, mesozooplankton have to feed on second-level producers, which results in an important loss of energy, and low biomass.

The highest grazing rates were found in the Trades Indian, which contains only two provinces, and covers 10.2% of the total oceanic area. Even though it is such a large oceanic surface, only two studies have been conducted in this region, and both in the same tropical province (Indian Ocean Monsoon Gyres, MONS), one during the monsoon forcing (Landry *et al.*, 1998), and the other after the monsoon period (Edwards *et al.*, 1999). Because of the monsoon winds, this region (even

if tropical) shows seasonality. The monsoon allows the formation of a seasonal upwelling in the northwest region, and the Arabian Sea becomes more eutrophic and the PP increases. At the end of the monsoon period, the normal oligotrophic situation is restored (Edwards *et al.*, 1999). In a study by Edwards *et al.* (Edwards *et al.*, 1999), it was found that the proportion of PP grazed by microzooplankton was higher during the inter-monsoon than during the southwest monsoon, most likely due to the presence of smaller phytoplankton cells. However, Landry *et al.* (Landry *et al.*, 1998) studied the dynamics of phytoplankton growth and microzooplankton grazing under different conditions of physical forcing and observed similar rates.

The coastal Indian domain had the highest percentage of PP grazed by microzooplankton. This domain contains both tropical (AUSW, REDS, MONS and ARAB regions) and temperate (AEFR) areas, but out of the eight studies conducted in these regions (AUSW, Ayukai and Miller, 1998; ARAB, Caron and Denett, 1999; REDS, Berninger and Wickham, 2005; MONS, Edwards *et al.*, 1999; EAFR, Froneman *et al.*, 1997; MONS, Landry *et al.*, 1998; AUSW, Paterson *et al.*, 2007; ARAB, Reckermann and Vedhuis, 1997), only one was from the temperate area (EAFR, Froneman *et al.*, 1997). The Red Sea is an ultra-oligotrophic marine system characterized by low planktonic abundance and dominated by small-sized organisms. Berninger and Wickham (Berninger and Wickham, 2005) could only find a significant control of grazing on bacteria from heterotrophic nanoflagellates, even when their concentrations were low. In the Arabian Sea, during the monsoon, Reckermann and Veldhuis (Reckermann and Veldhuis, 1997) found that phytoplankton growth rates were usually higher than microzooplankton grazing rates and that 67% of the PP was grazed daily.

On the coast of Western Australia, Ayukai and Miller (Ayukai and Miller, 1998) found that the proportion of potential PP grazed by microzooplankton ranged from 79 to 155%, resulting in higher losses by grazing than phytoplankton production. Finally, also in the Western Australia, Paterson *et al.* (Paterson *et al.*, 2007) found that more than 100% of the PP could be grazed by microzooplankton.

PHYTOPLANKTON BLOOM STABILITY AND MICROZOOPLANKTON GRAZING IMPACT

One of the key findings of Calbet and Landry (Calbet and Landry, 2004) was the unanticipated importance of microzooplankton grazing on the phytoplankton of highly productive areas. This finding questioned the well-established paradigm of a higher relevance of a diatom–copepod–fish (classic) based food chain for upwellings and other productive systems. The mechanisms that allow microzooplankton to deal with a high abundance of large autotrophs have been already discussed in other studies (Saito *et al.*, 2006; Calbet, 2008). However, we found that following the analysis of the available data (beyond simple averages), the coupling between phytoplankton growth rates and the microzooplankton grazing breaks down when approaching productive areas (e.g. Olson and Strom, 2002; Landry *et al.*, 2008; Calbet *et al.*, 2011b).

From an ecological perspective, systems with higher stability would allow a closer coupling between grazers and prey. Stability occurs in any system when there is an adequate time frame for populations to grow and reach equilibrium. For example, stable states typically occur within rich estuaries and oligotrophic areas, where, under low predatory pressure, microzooplankton typically play a key role in the control of phytoplankton populations (Juhl and Murrel, 2005; Stoecker *et al.*, 2008; Sanderson *et al.*, 2012). On the other hand, seasonal and discrete blooms do not conform to these conditions because they usually occur for short periods of time (Cushing, 1990). Microzooplankton have similar generation times as phytoplankton, in contrast to other grazers of phytoplankton, such as copepods. Therefore, within short time frames, microzooplankton should catch up with the fast-growing phytoplankton community.

The coupling between phytoplankton and microzooplankton during bloom episodes should weaken at higher latitudes due to low-temperature constraints on protozoan growth (Rose and Caron, 2007). In these systems, the trade-off between the permanence of nutrients in the water and the time required by the microzooplankton community to reach a critical biomass defines the duration of the bloom. On most of the occasions, this trade-off favours the establishment of long-lasting blooms, such as the ones typically found at high latitudes, in which microzooplankton grazing may, at times, approach the growth of the algae, even if not entirely consuming the bloom (Verity *et al.*, 1993, 2002; Landry *et al.*, 2002; Pearce *et al.*, 2010). However, there are examples of blooms being rapidly over taken by microzooplankton. In an iron fertilization experiment in the Subarctic Pacific, Saito *et al.* (Saito *et al.*, 2006) reported the astonishing decay of an artificially generated phytoplankton bloom in a few days because the grazing activity of a heterotrophic dinoflagellate. It is worth mentioning that when the experiment was

repeated a few years later the bloom did not develop. The reason for such disparate results was not due to the grazing by microzooplankton, but to the feeding activity of a well-established community of copepods (Tsuda *et al.*, 2007). As a matter of fact, the feeding of copepods may actually supress the impact of microzooplankton by top-down controls on their biomass growth, which in turn allows the settling of phytoplankton blooms (e.g. eutrophic areas such as Chesapeake Bay; Stoecker *et al.*, 2008). This copepod–microzooplankton interaction will be the core of the following section.

THE ROLE OF HIGHER TROPHIC LEVEL GRAZERS

In the introduction, we stated the major problems and artefacts of the dilution technique (Landry and Hassett, 1982). We do not expand further on this subject given that there are studies devoted to this (Dolan *et al.*, 2000; Agis *et al.*, 2007). However, we want to stress an additional artefact seldom considered in the literature as a result of this water confinement. The technique was developed to estimate the feeding impact of microzooplankton on phytoplankton. To accomplish this, the food web needs to be truncated above the microzooplankton, which means that on most occasions the consumers of microzooplankton are not well represented inside the experimental bottles. Therefore, the rates obtained with the dilution technique are maximum *potential* grazing rates. We may assume that, besides intra-guild predation within the microzooplankton, copepods are the major predators of this group in natural systems (Calbet and Saiz, 2005; Saiz and Calbet, 2011). Certainly, the initial biomass of microzooplankton in the experimental bottles is the result of this feeding impact by copepods (Table IV); thus, the artefact should only apply to the effect of predators on protozoan net growth rates during the incubation time. Consequently, as it occurs in field copepod grazing experiments, where parallel incubations of microzooplankton grazing on phytoplankton are advised (Nejstgaard *et al.*, 2001), we propose additional copepod grazing bottles, together with good estimates of copepod abundance and microzooplankton growth rates. With all this information in hand, a mechanistic model should be developed to better approximate the actual grazing impacts of microzooplankton on phytoplankton, likely overestimated in those systems with a strong control of microzooplankton by copepods.

We anticipate this effect will be more important in boreal/anti-boreal and polar waters, where the biomass of mesozooplankton is higher (Hernández-León and Ikeda, 2005), although detailed analysis in each location should be conducted to better parametrize any possible correction factor.

GLOBAL CARBON BUDGET: MICROZOOPLANKTON VS. MESOZOOPLANKTON GRAZING

In Table II, we computed the grazing by microzooplankton in the global ocean by using the estimates of PP provided by Longhurst *et al.* (Longhurst *et al.*, 1995) together with our grazing impacts in each biogeographic subset. This calculation will more accurately assess the global carbon flux through microzooplankton in the oceans. The overall consumption of phytoplankton for all regions was 31.3 Gt C year^{-1}, which accounted for 62.4% of the total PP grazed daily (Table II). We extracted data of mesozooplankton grazing rates from the review by Calbet (Calbet, 2001) and compared the data with microzooplankton grazing rates computed in this study as a function of the PP estimated in Longhurst *et al.* (Longhurst *et al.*, 1995). The amount of carbon ingested by both groups varied proportionally to the PP, although the slopes of the linear relationships were significantly different (0.64 and 0.12 for micro- and mesozooplankton, respectively; analysis of covariance $P < 0.001$; Fig. 3), indicating a ca. five times higher consumption by microzooplankton than by mesozooplankton.

With the information obtained in this review, combined with that of previous studies, we can approximate a global budget for the circulation of carbon through the different major compartments of the food web (Fig. 4). We based our calculations on a conservative total particulate PP of 50 Gt C year^{-1} (Longhurst *et al.*, 1995), and we assumed microzooplankton and mesozooplankton to be the major sources of phytoplankton mortality. We acknowledge, however, that other phytoplankton mortality factors may also play significant roles (e.g. viruses, Suttle 2007; apoptosis, Franklin *et al.*, 2006; allelopathy, Sukenik *et al.*, 2002). Unfortunately, for most of them, we lack global estimates on the relevance of these factors. We could assume, however, an average rough estimate of 6%, from the 2–10% of the phytoplankton production lost by viral lysis in steady-state pelagic systems (Jumars *et al.*, 1989; revised in Wilhelm and Suttle, 1999).

The estimates of microzooplankton consumption by mesozooplankton are not so straightforward. Calbet and Saiz (Calbet and Saiz, 2005) suggested, after correction for several artefacts, 2.8 Gt C year^{-1} should be circulating through the ciliate–copepod link, and a

Table IV: Literature review of the percentage of standing stock (%SS) and production (%P) of microzooplankton consumed daily by mesozooplankton

Area	Date	%SS	%P	Prey	Grazer	Reference
Chesapeake Bay	April–August 1987	35–200		Ciliates	Cop. assemb.	Dolan (1991)
Oregon Coast	January–August 1991	25–45		Ciliates	Cop. assemb.	Fessenden and Cowles (1994)
North South Georgia, Southern Ocean	February 1994	6		Ciliates	Cop. assemb.	Atkinson (1995)
NE Atlantic; NW Spain coast	August 1998	1–3	2–50	Protozooplankton	Cop. assemb.	Batten et al. (2001)
Alboran Sea (Mediterranean)	September 1999	1.5		Ciliates + Dinoflagellates	Cop. assemb.	Calbet et al. (2002)
New Zealand Subtropical Frontal Zone	October–November 1997, August 1998	6	21	Ciliates	Cop. assemb.	Zeldis et al. (2002)
Coastal NW Mediterranean	Seasonal cycle 1999–2000	2%		Ciliates	Cop. assemb.	Broglio et al. (2004)
Mejillones Bay, Chile	February 2001	40	29	Ciliates	Cop. assemb.	Vargas and González (2004)
Mejillones Bay, Chile	February 2001	9	8	Dinoflagellates	Cop. assemb.	
Mejillones Bay, Chile	August 2001	4	3	Ciliates	Cop. assemb.	
Mejillones Bay, Chile	August 2001	0.7	1	Dinoflagellates	Cop. assemb.	
Mejillones Bay, Chile	October 2001	3	2	Ciliates	Cop. assemb.	
Mejillones Bay, Chile	October 2001	14	13	Dinoflagellates	Cop. assemb.	
NW Mediterranean Coastal	July–September 2002, 2003, 2004	<10		Protozoo	Dominant species	Atienza et al. (2006)
Gerlache Strait and Bellingshausen Sea (Antarctica)	December 2002	<0.1		Protozoo	Calanoides acutus	Calbet et al. (2006)
Greenland Sea	June 1999		20–110	Protozoo	Small + large copepods	Møller et al. (2006)
Greenland Sea	August 1999		100–240	Protozoo	Small + large copepods	Møller et al. (2006)
Celtic Sea, phytoplankton bloom	April 2002	2–10		Protozoo	Cop. assemb.	Fileman et al. (2007)
Celtic Sea, no bloom	April 2002	12–17		Protozoo	Cop. assemb.	Fileman et al. (2007)
Aegean Sea, non-frontal	April 2000	72	134	Ciliates	Small + large copepods	Zervoudaki et al. (2007)
Aegean Sea, frontal	April 2000	88	165	Ciliates	Small + large copepods	Zervoudaki et al. (2007)
Irminger Sea	April–August 2002	1–1.3		Ciliates + Dinoflagellates	Dominant species	Castellani et al. (2008)

Cop. assemb., copepod assemblage; Protozoo, protozooplankton.

roughly equivalent amount should be added when considering other microzooplankton groups; i.e. 5.6 Gt C year^{-1} in total. That would leave ca. 20% of the PP (10 Gt C year^{-1}) for sedimentation, with the understanding that this would not be homogeneous across the entire ocean, but directly related to the values of PP (Wassmann, 1998). To this 'ready-to-settle production' we should add that processed by mesozooplankton and exported as faecal pellets (we consider that all the material voided by microzooplankton is recycled within the photic layer; González et al., 2000). Assuming an assimilation efficiency of 70% (Ikeda and Motoda, 1978; Kiørboe et al., 1985), ca. 13.5 Gt C (27% of PP) are available to settle. This value should be considered an upper boundary because a proportion of the settling material may be recycled before leaving the photic zone. It is interesting to note that the available carbon for settling, obtained from

our calculations, is quite similar to estimates based on the relationship of total and exported production, and monthly mean total production maps (16 Gt C year^{-1}; Falkowski et al., 1998).

Among the phytoplankton carbon ingested by the zooplankton, part will be respired and returned as CO_2. Assuming a median respiratory loss of about 33% (Bougis, 1974; Kiørboe et al., 1985; Pagano et al., 1993; Schmoker et al., 2011; Calbet et al., in press), the derived results indicate that 24% of planktonic PP is respired daily (i.e. 12 Gt C year^{-1}). To this number we should add the respiratory losses as a result of the ingestion of heterotrophic prey, which, overall provide evidence for a net heterotrophic ocean (del Giorgio et al., 1997; Duarte and Agustí, 1998; del Giorgio and Duarte, 2002), and question the capacity of the ocean to mitigate the atmospheric excess of CO_2.
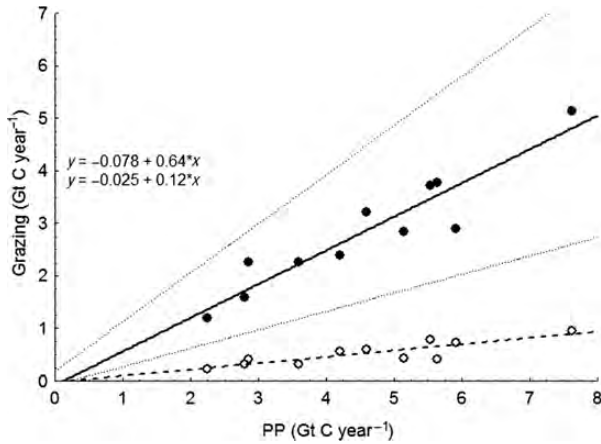
Fig. 3. Relationships between the amounts of carbon ingested by microzooplankton (full dots) computed from our study and by mesozooplankton (empty dots), computed by Calbet (Calbet, 2001) (Grazing, Gt C year^{-1}), and the PP (Gt C year^{-1}) as defined by Longhurst *et al.* (Longhurst *et al.*, 1995). Full dots correspond to microzooplankton grazing and empty dots correspond to mesozooplankton grazing (Linear regressions: $y = -0.078 + 0.64 * x$; $r^2 = 0.88$ and $y = -0.025 + 0.12 * x$; $r^2 = 0.73$ for micro- and mesozooplankton, respectively). The upper and lower dash lines are, respectively, the 25 and 75% quartiles of the microzooplankton grazing median.

GENERAL CONCLUSIONS, MAJOR GAPS AND FUTURE DIRECTIONS

It is evident from the data summarized here that microzooplankton are major grazers of phytoplankton in marine systems, typically outcompeting mesozooplankton. It is also clear that microzooplankton feeding impacts are highly variable, even if the median rates are quite similar for the different regions of the ocean considered. Major knowledge gaps have been identified during this study. The first refers to the lack of data in very extensive areas of the oceans (Fig. 1). Therefore, we recommend focussing future efforts in certain areas, such as TA, TI, TP, WP. Moreover, after the analysis of the data, it was also evident that most of the studies were conducted in coastal regions, data on open oceans being scarce. Obviously, the logistics required for coastal zone studies are not as great as the ones required for an oceanic cruise. However, the effort should be made if we want to complete our understanding of the global marine system. Also, some effort should be put into convincing policy makers about the need of funding
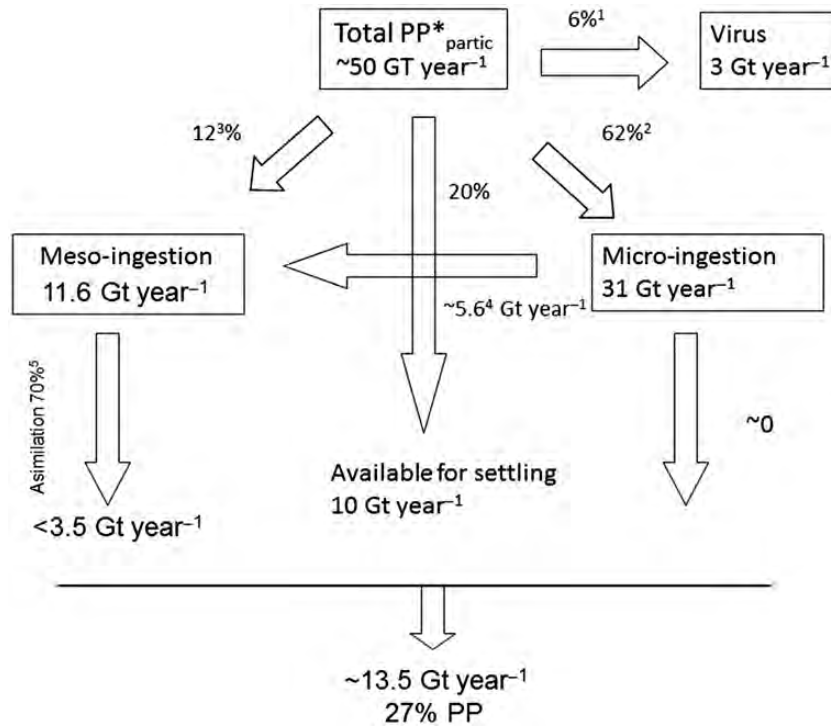
Fig. 4. Carbon flux diagram for the global ocean. Values in boxes are absolute Gt C either consumed or produced. Arrows represent the flux of carbon in percentage. The values below the horizontal line at the bottom of the figure correspond to the carbon available for settling. See text for details. *Data for primary production (PP) from (Longhurst *et al.*, 1995). Data for the rates from: 1 (Jumars et al., 1989; revised in Wilhelm and Suttle, 1999), 2 (this study), 3 (Calbet, 2001), 4 (Calbet and Saiz, 2005), 5 (Ikeda and Motoda, 1978; Kiørboe *et al.*, 1985).

long-term time series, which would facilitate knowledge on periods of the year (mostly winter) where data are less abundant.

Finally, it is also important to shed some light on the vertical distribution of microzooplankton grazing impacts, and the differences between day and night. Given the relevance of the vertical structure of marine communities, it seems rather simplistic to focus on one, at maximum two, depths, and to integrate the values for the entire water column.

Additional problems relate to the accuracy and consistency of the data when applying the Landry and Hassett (Landry and Hassett, 1982) dilution technique in the different studies. At times, when nutrients are added, controls without nutrients are lacking; at others, no nutrients are supplemented. There is, therefore, a need for comprehensive studies on the bias produced by nutrient manipulation in dilution series (Lawrence and Menden-Deuer, 2012), as well as for an agreement on the inorganic nutrients used and the concentrations required in each study. Certain studies add nitrate as a nitrogen source, others add ammonium; silicate and trace metals are mostly neglected even if the first is required by diatoms and the second for all algae. Perhaps a starting point would be to agree to add enough nutrients to allow a couple of doublings of the initial chlorophyll within 24 h. This could be easily calculated with knowledge of the C:chlorophyll quotient and using the Redfield ratio.

An additional aspect not discussed here, but important for the interpretation of the data, is that only a few studies provide proof of there being no photo-adaptation during incubations (e.g. Landry et al., 2009; Gutiérrez-Rodríguez et al., 2011; Calbet et al., 2012). Photo-adaptation can result in an incorrect estimation of μ and, therefore, a biased interpretation of the $m:\mu$ ratio. This is a complicated issue, because even on those occasions where light intensity and spectrum are carefully mimicked for a certain depth, as at sea the organisms are subjected to currents, internal waves and other water vertical displacements, some errors would be unavoidable. The solution to this artefact is not trivial because it would involve the use of density-dependent floating devices for the incubations, and a precise knowledge of the water circulation patterns in the study area. Nevertheless, there is a more simple way to approach this problem, which could be included as routine in the grazing experiments. This would be the counting of the major groups of phytoplankton in certain bottles for further comparison of the growth rates obtained from cell counts with those based on chlorophyll.

There are other intrinsic problems with the method that have not been well addressed so far. For instance, mixotrophy and trophic cascades are not adequately dealt with during dilution grazing experiments. This is actually a very critical issue, given that these factors are the probable reasons for the positive slopes found in dilution grazing experiments and may introduce other artefacts (Calbet et al., 2008, 2012). It is evident that a more robust theoretical framework (mechanistic model) is needed for dealing with the possible trophic interactions occurring during dilution grazing experiments in complicated food-webs, and with the already mentioned effects of removing mesozooplankton from the incubations. This modelling effort should be validated experimentally. Perhaps, by the use of artificial food webs (culture based), or possibly by the parallel assessment of microzooplankton grazing, using alternative methods, such as the suppression of heterotrophic grazers (inhibition of the respiratory electron transport system with, for example, rotenone; Svensson and Stenson, 1991).

We should also be aware that all the variability in the microzooplankton grazing rates presented here contrasts with the fact that both grazers and prey have been dealt here as combined groups. Diversity should be integrated somehow in our estimates of microzooplankton grazing. Some studies already consider different groups of species within the prey field (Yang et al., 2004; Chen et al., 2009). However, very few contemplate the functional diversity of grazers. A first approach in this respect could focus on different size-fractions (Reckermann and Veldhuis, 1997; Lessard and Murrel, 1998; Calbet et al., 2008), which would improve our understanding of food web dynamics, although the results of a size-truncated food web are not easy to interpret in a dilution experiment. Calbet et al. (Calbet et al., 2012) suggested additional experiments to identify the major grazers by the use of vital stains. In the case of Calbet et al. (Calbet et al., 2012) the technique was used to quantify mixotrophy, but it could be used to track major microbial grazers in a system. Why is this important? In addition to the mere scientific value of identifying major grazers of a system, it is of paramount relevance to correctly parametrize food-web models and to identify the initial point of entrance of the organic matter into the food chain. Energy loss and recycling terms should considerably differ if the main point of autotrophic carbon entering the heterotrophic web is at the level of small flagellates, large ciliates and dinoflagellates, or copepod nauplii.

Altogether, it seems that we have a long way to go in the understanding of marine planktonic food webs. However, since the establishment of the dilution

technique, we have at least gained a great deal of knowledge on the interaction between phytoplankton and their grazers in the oceans.

SUPPLEMENTARY DATA

Supplementary data can be found online at http://plankt.oxfordjournals.org.

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