



Marine heterotrophic bacteria, protozoan and metazoan zooplankton may experience protein N or mineral P limitation in coastal waters

Yngvar Olsen^{1,*}, Tom Andersen², Ingrid Gismervik², Olav Vadstein³

¹Trondhjem Biological Station, Department of Biology, Norwegian University of Science and Technology, 7491 Trondheim, Norway

²Department of Biology, University of Oslo, PO Box 1066 Blindern, 0316 Oslo, Norway

³Department of Biotechnology, Norwegian University of Science and Technology, 7491 Trondheim, Norway

ABSTRACT: The objective of the present study was to examine how N and P availability interact with C metabolism in marine heterotrophic plankton and whether or not heterotrophic groups are likely to be subjected to essential nutrient limitations in natural coastal waters. The nutrients studied were mineral P and N; the latter is a proxy for essential amino acids. We present a general theoretical framework and criteria for evaluating essential nutrient limitations in heterotrophs. Data were derived from a comprehensive mesocosm experiment, where food-web flows were estimated using inverse modelling. Bacteria of the mesocosm communities were severely P-deficient throughout. The heterotrophic nanoplankton was most likely limited by another essential nutrient or by food C availability. Ciliates were most likely P-limited, whereas copepods might experience P limitation, but were more likely limited by another essential nutrient or food C in the present experiments. The N contents of the food were close to the theoretical requirements for copepods and ciliates. All planktonic groups released dissolved inorganic nitrogen (DIN), and all except bacteria released dissolved inorganic phosphorus (DIP). Our results suggested that P limitation might be readily experienced by bacteria, ciliates and perhaps also by copepods, but not by heterotrophic nanoplankton in natural North East Atlantic coastal waters. A predator must balance its energy metabolism and growth under the variable availabilities of essential nutrients, and we propose that C growth efficiency is a dynamic variable mainly dependent on the availability of the most limiting nutrient. We support the view that C availability alone cannot be used as a proxy for the food limitation of bacterial and zooplankton growth. Specific essential nutrients should be regarded as potential limiting factors, as for phytoplankton. The variable nutritional requirements of heterotrophic predators will then represent a major driver of heterotrophic species diversity, allowing a broad diversity of heterotrophic species in plankton communities.

KEY WORDS: Planktonic N and P nutrition · Essential nutrients limitation · Conceptual model · Inverse modelling · CNP flow networks · Nutrient release · Growth efficiency · Elemental CNP stoichiometry

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INTRODUCTION

The common current understanding of planktonic nutrition is that the availability of mineral nutrients, like nitrate and phosphate, limits phytoplankton

growth and production yields in lakes and oceans; that planktonic bacterial growth mainly depends on the supply of dissolved organic carbon (DOC); and that the growth and reproduction of protozoan and metazoan grazers depends on the availability of food

*Email: yngvar.olsen@bio.ntnu.no

quantified as, for example, C for their anabolic processes. During the last few decades this general view has been challenged for planktonic bacteria (Thingstad & Rassoulzadegan 1995, Vadstein 2000), and also frequently for zooplankton (e.g. Olsen et al. 1986, Sterner & Elser 2002, Boersma et al. 2008, Müller-Navarra 2008). A gradually increasing number of studies are now also questioning the general concept of C control of growth and reproduction for zooplankton, resulting in a growing understanding of the idea that the availability of essential nutrients in food can be a key factor for anabolic processes, i.e. growth and reproduction, of zooplankton (e.g. Jónasdóttir 1994, Jónasdóttir & Kiørboe 1996, Sterner & Elser 2002, Helland et al. 2003, Evjemo et al. 2008, Kainz et al. 2009). Additional evidence has been provided by nutritional studies of cultured fish larvae and their live zooplankton feed (e.g. Makridis & Olsen 1999, Glencross 2009, Tocher 2010). The failure to relate zooplankton food conversion or growth efficiency to food C availability (e.g. Huntley & Lopez 1992, Straile 1997) calls for a revision of the conceptual understanding and interpretation of such data, and ultimately for the modelling of food webs.

From a wide range of nutritional studies of fish and other animals we know that the balance between essential nutrients in food is paramount for growth and the food conversion efficiency of domesticated animals, including marine larval stages of cultured fish species classified as carnivorous natural zooplankton (e.g. De Silva & Anderson 1995, Shields 2001, Glencross 2009, Tocher 2010). It is therefore most likely that nutrition is also paramount for natural zooplankton. Clear indications for this have been obtained in recent studies, and an excellent conceptual scientific basis was synthesised by Sterner & Elser (2002) in their book on ecological stoichiometry. C or energy is still important, but it can no longer be used to represent the availability of essential nutrients for the growth and reproduction of animals (Sterner & Elser 2002). C can represent energy, and energy or food amount can be the most important factor controlling growth if all essential nutrients are in excess and balanced relative to the actual requirements of the zooplankton species.

The main problem with nutritional studies of zooplankton is the high complexity and dynamic state of the planktonic food web together with the high number of potentially limiting essential nutrients and the fact that plankton are small organisms that cannot easily be individually handled like, for example, fish (Olsen 1999). The most advanced nutritional studies of marine plankton are probably those made during

efforts to cultivate marine fish larvae (e.g. Moksness et al. 2004). Even in a controlled hatchery environment for marine fish larvae, the supply of adequate amounts of long-chain highly unsaturated n-3 fatty acids (n-3 HUFA) in the diets of many fish larvae (e.g. Tocher 2010) can be a major problem. Other essential components may also be important for natural planktonic populations, but marine and freshwater ecologists can nevertheless learn many lessons from the nutritional studies of cultured marine fish larvae (Olsen 1999).

The terminology of nutritional sciences expresses nutrient contents, or nutritional value, as the mass of nutrients per food mass, which is the inverse of Redfield ratios and compatible with N:C and P:C ratios (P:N:C stoichiometry). Efficient communication and interactions between ecological and nutritional sciences require that ecologists accept the more logical terms used to express nutritional values in nutrition. This should present no major problem, and a pioneer in ecological stoichiometry (Droop 1973) emphasised the greater relevance of using P:N:C stoichiometry for expressing the nutritional state of phytoplankton. The methods and data obtained in classical nutritional and ecological studies are still quite compatible if the nutritional value is expressed as the mass of nutrients per food mass.

The objective of the present paper was to examine whether or not common groups of marine zooplankton and bacteria are likely to experience N or mineral P limitations in growth during variable rates of nutrient input into the ecosystem. Nitrogen is a proxy for the essential amino acids in proteins. The data were obtained from a comprehensive mesocosm experiment carried out in NE Atlantic coastal waters. We used an established inverse mathematical method to estimate the complete flow networks of C, N and P in the planktonic food web (Vézina & Platt 1988, Olsen et al. 2006, 2007). C flow networks and the development of zooplankton groups and species in the experiments were published by Olsen et al. (2007) and Gismervik et al. (2002), respectively.

MATERIALS AND METHODS

Method for analysing limitations

The method used to analyse the potential interactions between N and mineral P nutrition with the C supply of the heterotrophic plankton groups was originally introduced and used for freshwater cladocerans (Olsen & Østgaard 1985, Olsen et al. 1986,

Vadstein et al. 1995). The method states that the content of an essential nutrient in the food (Q , defined as the nutrient content of the food) is the main variable affecting ingestion and release of the nutrient (Olsen et al. 1986). The solid line in Fig. 1A illustrates the relationship between nutrient release per unit of food C ingested (Q_R) and Q :

$$Q_R = r_X i_C^{-1} = Q - Q_{CM} \quad (1)$$

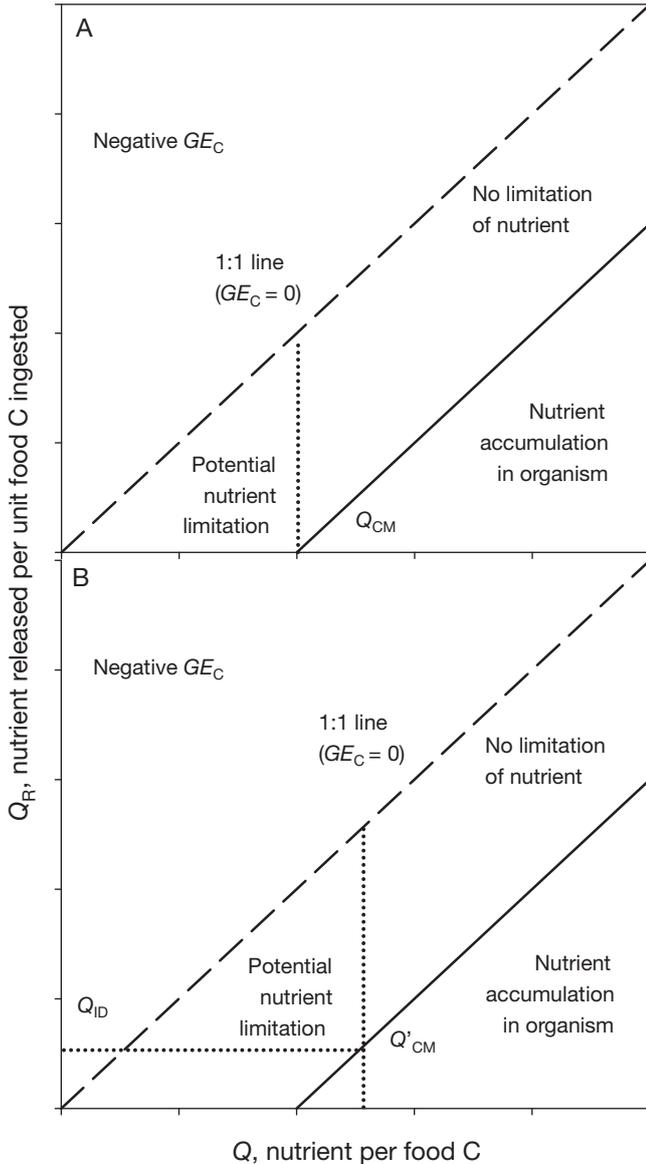


Fig. 1. Conceptual scheme for evaluating the quantitative requirements of essential nutrients and the metabolic interactions between essential nutrients and C (energy) resources in bacteria and zooplankton, assuming (A) that all nutrients in the food are digested and assimilated in the tissues of the organisms, or (B) that a certain fraction of the essential nutrients in the food is indigestible (Q_{ID}) and therefore egested. See Table 1 for other abbreviations

(from Olsen et al. 1986), where: r_X is the release rate of nutrient X per litre and day, i_C is the ingestion rate of C per litre and day, and Q_{CM} is the maximum critical nutrient content of food below which nutrient limitation of a predator may occur (see definitions in Table 1). Eq. (1) is based on a simple assumption of mass balance; nutrient ingestion must be balanced by nutrient release and nutrient allocation for growth and reproduction (Olsen et al. 1986).

Q_{CM} (Eq. 1, Fig. 1A) is a function of the maximum nutrient content of the consuming organism or the predator (Q_{ZM}) and its maximum C growth efficiency (GE_{CM}):

$$Q_{CM} = Q_{ZM} \times GE_{CM} \quad (2)$$

If $Q = Q_{CM}$ and Q_R of the predator is zero (intersection of the solid line and the Q axis), this will imply a maximum carbon growth efficiency (GE_{CM}) and a maximum nutrient content of the predator (Q_{ZM}). For a given species, Q_{CM} can be assumed to be constant, reflecting its demands for the essential nutrient.

Both Q_{ZM} and GE_{CM} represent the maximum value of the respective variables, and the general equation expressing the variable critical food concentration for the suboptimal values of Q_Z and GE_C (Q_C) is:

$$Q_C = Q_Z \times GE_C \quad (3)$$

Q_Z will vary between Q_{ZM} and a lower value, the lowest nutrient content of the predator that can support sustained growth (Q_{Z0}). The range of variation in Q_Z will depend on the degree of homeostasis, and may be relatively constant for metazoans (Andersen & Hessen 1991, Shimizu & Urabe 2008). With strict homeostasis, Q_Z will be constant and equal to Q_{ZM} . The value of GE_C will vary between zero and GE_{CM} . The dynamic states of Q_Z and GE_C represent physiological buffer mechanisms for mitigating an imbalance in the supply of essential nutrients relative to the C or energy supply, and GE_C is accordingly a function of Q , the content of the limiting nutrient in the food.

The specific areas in Fig. 1A express 4 different states of nutrition in heterotrophic organisms. The solid line describes when nutrient intake balances growth and egestion under optimal stoichiometry of the food (Eq. 1). The dashed line (1:1 line) expresses Eq. (1) for $Q = Q_R$ characterised by a GE_C of zero (Eq. 3). Sustained growth cannot take place in the space above the dashed line because it implies a negative GE_C , or C exhaustion of the organism. The area below the solid line expresses states where nutrients accumulate in the predator, which is also

Table 1. Definition of terms

Term	Definition	Units in present study
μ	Specific growth rate	d^{-1}
i_X	Ingestion rate, subscript X for C, N and P	$\mu g X l^{-1} d^{-1}$
r_X	Nutrient release rate, subscript X for N and P	$\mu g X l^{-1} d^{-1}$
g_X	Growth and reproduction, subscript X for C, N and P	$\mu g X l^{-1} d^{-1}$
GE_X	Growth efficiency ($g_X i_X^{-1} \times 100\%$), subscript X for C, N, and P	%
GE_{XM}	Maximal growth efficiency for species or group, subscript X for C, N and P	%
Q	Mass of nutrient per mass of food, termed nutrient content of food (Q_N for N; Q_P for P)	$\mu g mg C^{-1}$
Q_Z	Nutrient per biomass for heterotrophic predator (Q_{ZN} for N; Q_{ZP} for P)	–
Q_0 and Q_{Z0}	Subsistence quota, the lowest Q for food organisms and lowest Q_Z for predator organism that allows net growth. Positive net growth is only possible for $Q > Q_0$ ($Q_Z > Q_{Z0}$) (Q_{0N} and Q_{0P} for N; Q_{0Z} and Q_{0Z} for P)	$\mu g mg C^{-1}$
Q_M and Q_{ZM}	Maximum Q for food organisms and maximum Q_Z for predator organisms (Q_{MN} and Q_{ZMN} for N; Q_{MP} and Q_{ZMP} for P)	$\mu g mg C^{-1}$
Q_R	Nutrient released (r_X) per C ingested (i_X), X is N or P (Q_{RN} for N; Q_{RP} for P)	$\mu g mg C^{-1}$
Q_{ID}	Indigestible fraction of Q (Q_{IDN} for N; Q_{IDP} for P)	$\mu g mg C^{-1}$
Q_C	Theoretical food content yielding zero nutrient release (Q_{CN} for N; Q_{CP} for P)	$\mu g mg C^{-1}$
Q_{CM}	Theoretical content of a nutrient in food yielding zero nutrient release at the maximal growth efficiency of C (GE_{CM}) (Q_{CMN} for N; Q_{CMP} for P)	$\mu g mg C^{-1}$
Q'_{CM}	Q_{CM} corrected for the indigestible nutrient in the food	$\mu g mg C^{-1}$

impossible over time. The space between the solid and the dashed lines represents a sustained balance between C and nutrient metabolism in the organism. Nutrient limitation will never occur if $Q > Q_{CM}$, but it may become a reality for a specific nutrient X if $Q_X < Q_{XCM}$ only if no other essential nutrients are supplied in even lower amounts. Therefore, specific limitations should be justified by including metabolic indicators.

So far it has been assumed that the predator is capable of assimilating all nutrient molecules from the feed. However, this is not likely because there will always be an indigestible fraction of the nutrients, as for C (Olsen et al. 2007). The introduction of an indigestible fraction of a nutrient will slightly affect the picture in Fig. 1A, as indicated by the dotted lines in Fig. 1B. The horizontal dotted line indicates the lowest attainable nutrient release per unit C ingested (Q_{ID}), and the value is determined by the indigestible content of the nutrient in food, which is directly related to the assimilation efficiency of the nutrient. For degradable nutrients (e.g. essential fatty acids), Q_{ID} can also include losses through catabolism, but this is not studied further here.

The intersection between the horizontal dotted line ($Q_R = Q_{ID}$) and the solid line (Eq. 1) indicates a revised Q_{CM} value (Q'_{CM}), which is the maximum critical nutrient content of the food below which nutrient limitation of a predator may occur, now

corrected for the indigestibility of a fraction of the nutrient:

$$Q'_{CM} = Q_{CM} + Q_{ID} \quad (4)$$

The Q'_{CM} takes the maximum assimilation capabilities of food into account, expressing the 'true' digestible content of the nutrient in the food below which nutrient limitation may occur. For a given predator species and food quality, Q'_{CM} is assumed to be constant, reflecting its nutrient demands for a given food source and rate of food supply. The assimilation efficiency is inversely related to the rate of food supply (Olsen et al. 2007).

The fundamental metabolic regulatory mechanisms of heterotrophic organisms will determine the reaction pattern following changes in Q . The organism may react to a reduced Q by reducing GE_C , Q_Z or Q_R , but Q_Z cannot be reduced in the case of strict homeostasis. The detailed response of variations in these variables following a reduction in Q cannot be easily deduced. Upon decreasing $Q > Q'_{CM}$, an organism is expected to primarily react by reducing nutrient losses (reducing Q_R), but moderate reductions in GE_C and Q_Z cannot be ruled out, especially when Q_R approaches Q_{ID} . A reduction in Q_R alone while GE_C and Q_Z remain at their maximum values means that an organism remains nutrient and carbon sufficient (value on solid curve). Upon decreasing $Q < Q'_{CM}$, the balance must be obtained by reducing

GE_C or Q_Z , but a reduction in GE_C is probably the most efficient means to mitigate imbalance in the nutrient and carbon supply under strict homeostasis. The Q_R cannot be reduced by very much to achieve balance if Q_R approaches Q_{ID} .

Whatever physiological response and trajectory of the Q versus Q_R value following a reduction or increase in food Q , both C and nutrient metabolism will be simultaneously affected in the entire space between the solid and dashed lines in Fig. 1B. In fact, C metabolism only remains unaffected at maximum C growth efficiency for Q_R versus Q values on the solid line. The organism will be potentially nutrient limited when $Q < Q'_{CM}$. We emphasise 'potentially limiting' because another essential nutrient or severe food C or energy limitation may result in an even more pronounced reduction of GE_C than that caused by sub-optimal nutrient contents (e.g. Sterner & Elser 2002).

The essential nutrients examined in the present paper were mineral P and N—the latter as a proxy for essential amino acids—and we analysed the potential interactions between N and mineral P nutrition and C supply. Based on the scheme in Fig. 1B, we used the indicators below to evaluate the potential or real limitations of specific essential nutrients for the functional groups of heterotrophic plankton:

(1) A specific nutrient is potentially limiting for a predator only when $Q < Q'_{CM}$, implying that $GE_C < GE_{CM}$ for strict homeostatic organisms.

(2) The limitation of a specific nutrient may not be expressed if GE_C is even more depressed by the sub-optimal availability of a second essential nutrient, including C.

(3) The nutrient limitation of a specific nutrient is most likely real if Q_R is close to Q_{ID} for the species and nutrient, meaning that nutrient assimilation efficiency is at a maximum level.

It follows that C or energy will only be limiting or potentially limiting if all essential nutrients are less limiting, and certainly if $Q > Q_{CMX}$ for all other essential nutrients in the food, which appears to be an unlikely situation.

Experimental design

Data for the estimation of plankton biomass and major C, N and P flows under variable levels of nutrient input were obtained in a mesocosm experiment undertaken in a tidal-driven pristine lagoon system in Central Norway (Hopavågen, 63° 36' N, 9° 33' E) (chlorophyll *a* concentration: 1 to 3 $\mu\text{g l}^{-1}$; water residence time: 7 to 10 d). The upper 10 m of the meso-

cosm, enclosed by reinforced plastic, was cylindrical ($D = 2.3$ m) and the bottom 2 m conical ($V = 38$ m³). Seven mesocosms were mounted in individual cylindrical floats with a plastic tube ($D = 15$ cm) to ensure stability and stable working conditions. Weights in the bottom of the cone (12 m) and in the bottom of the circular part (10 m) ensured a stable vertical position of each mesocosm. The upper pelagic waters of the lagoon were enclosed by sinking and carefully lifting the mesocosms from a 12 m depth, enclosing water from a 12 to 0 m depth, before adjusting the final volume and attaching them to the floats. All mesocosms were filled within <3 h. The mesocosms were individually anchored to ensure similar physical conditions. Mixing was accomplished by wave action, and the water columns did not stratify during the experiment.

The temperature and salinity during the experiment were 15 to 16°C and 31 ± 1 , respectively. The experiment was a single-factor experiment (variable nutrient dose, balanced N:P:Si) run for 18 d (in August and September 1997). The nutrient doses added formed an exponential series of loadings from 0 to 30.2 mg N m⁻³ d⁻¹ (Si:N:P = 14.5:7.2:1, see Table 1 in Olsen et al. 2007). Freshly prepared nutrient mixtures made from stock solutions were added daily after sampling by lowering an open-ended 10 m plastic tube down towards the cone of the mesocosms, filling the tube with the mixed nutrient solution, with a volume equal to that of the tube, and then emptying the tube by lifting it to the surface. This method ensured immediate and efficient distribution and mixing of the nutrients.

Sampling and analytical methods

Integrated water samples (0 to 10 m) were collected daily using a Ramberg sampler (2 m length tube sampler with automatic opening and closing valves, $V = 4.2$ l). Water samples were collected in 25 l light-protected containers in the early morning (06:00 to 08:30 h). Samples of plankton and the chemical components were taken from these containers. Separate integrated mesozooplankton samples were collected from the mesocosms in the afternoon on every second day. The zooplankton was concentrated in a 35 μm net before further treatment. Methods of preservation, counting and the estimation of C biomass of the planktonic organisms assigned to the functional groups are reviewed in Table 2 (details in Olsen et al. 2007).

Water used for the analysis of particulate C, N and P was screened through a 200 μm nylon screen by reverse filtration to remove larger organisms.

Table 2. Principal methods for C biomass estimation for species assigned to the different food web components (functional groups) and the mean N:C and P:C ratios of the components. Samples were taken every second day. Q values represent the nutrient content of the food (Q_N and Q_P); in other cases, for the nutrient content of the predator organism, they must be interpreted as Q_Z values, as defined in Table 1. FNC: flow network construction; BB: biovolume-based C estimate; LB: length-based C estimate; MS: obtained from microscope or epifluorescence microscope analysis; IA: obtained by an image analysing system (Macintosh computer and an image processing program, IP Lab); HNF: heterotrophic nanoflagellates; fc: final concentration

Food web component (functional group)	Sedation, preserving and staining of samples	Dominant taxonomic groups and method used for biomass determination	Q used for FNC ($\mu\text{g mg C}^{-1}$)
APP Pico-autotrophs	0.5 mmol EDTA l^{-1} (fc) Glutaraldehyde (1% fc)	Picocyanobacteria, <2 μm , BB-IA, 0.21 $\text{pg C } \mu\text{m}^{-3}$ (Booth 1993)	N:C 250 P:C 20
ANP Nano-autotrophs	Acid Lugol's (1% fc)	Diatoms <20 μm , autotrophic flagellates, small dino-flagellates <20 μm , BB-MS, group-specific regressions (Strathmann 1967)	N:C 170 P:C 15
AMP Micro-autotrophs	Acid Lugol's (1% fc)	Diatom colonies, dinoflagellates, autotrophic ciliates, BB-MS, group-specific regressions (Strathmann 1967)	N:C 150 P:C 15
BAC Pico-heterotrophs	0.5 mmol EDTA l^{-1} (fc) Glutaraldehyde (1% fc) Staining by DAPI (Verity & Sieracki 1993)	Bacteria (and Archaea), <1 μm , BB-IA, 0.16 $\text{pg C } \mu\text{m}^{-3}$ (Vadstein & Olsen 1989)	N:C 250 P:C 75
HNP Nano-heterotrophs	0.5 mmol EDTA l^{-1} (fc) Glutaraldehyde (1% fc) Staining by DAPI (Verity & Sieracki 1993)	Flagellates (HNF, 2–8 μm), bacterivorous ciliates, appendicularia, BB-IA, 0.22 $\text{pg C } \mu\text{m}^{-3}$ (Børshiem & Bratbak 1987, Gismervik et al. 2002)	N:C 180 P:C 21
CIL Micro-heterotrophs	Acid Lugol's (2% fc)	Ciliates, 20–50 μm , BB-MS, 0.20 $\text{pg C } \mu\text{m}^{-3}$ (Putt & Stoecker 1989)	N:C 230 P:C 31
COP Meso-heterotrophs	Acid Lugol's (1% fc)	Calanoid and cyclopoid copepods, Cladocera, LB-MS, group-specific regressions (Gismervik et al. 2002)	N:C 180 P:C 34

Approximately 6 l of the <200 μm fraction was then successively size-fractionated by reverse filtration through 20 and 1 μm Nuclepore filters. The fractions were harvested on pre-combusted (450°C, 4 h), acid-washed (5% H_2SO_4) GF/F filters, and frozen (18°C) for later analysis. Particulate C and N in the fractions (200, 20, and 1 μm) was measured by a CHN analyser, and particulate P was measured according to the method of Grasshoff et al. (1983). Inorganic and dissolved organic nutrients were measured in water taken from the container and filtered by acid-washed GF/F filters (25 mm, 5% H_2SO_4). Inorganic nutrients (nitrate, ammonium, phosphate and silica) and dissolved organic N (DON) and P (DOP) were measured according to Grasshoff et al. (1983). The DOC in filtered (GF/F) and unfiltered water was analysed using catalytic high-temperature combustion and infrared detection. Detrital C, N and P was calculated as the difference between measured total particulate organic C, N and P (<200 μm) and the concentration of C, N and P in the biotic components <200 μm .

The estimation of N and P contents in the biomass of the food-web components was based on measurements of C, N and P in the particulate fractions (<200 μm , 20 μm , 1 μm), a principle of mass balance,

and assumptions of Redfield stoichiometry for organisms that are generally believed to have relatively constant C:N:P ratios and for groups that were present in low concentrations. The estimated N:C and P:C values used are shown in Table 2. The Q_P value for bacteria was mainly estimated based on the P concentration in the 1 μm fraction, with P in picocyanobacteria (APP) removed. The Q_P values of heterotrophic bacteria (BAC) were 2.5 times higher than the subsistence quota for bacteria and lower than the saturation level (Vadstein 2000).

Flow rate measurements

Samples for the measurement of primary and bacterial production were taken from the container immediately after sampling. Primary production was measured by the ^{14}C method. Radioactive bicarbonate ($\text{H}^{14}\text{CO}_3^-$, 3.6 $\mu\text{Ci bottle}^{-1}$, The International Agency for ^{14}C determination) was added to the mixed sample (0 to 10 m) from each mesocosm after transfer to replicate transparent bottles (63 ml Nunc) and 1 dark bottle per mesocosm. The samples were incubated at 2 m depth *in situ* for 4 h. After termina-

tion, the water samples were successively fractionated (20–200 μm , 1–20 μm and 0.2–1 μm) using a vacuum filter unit. The radioactivity was measured in a scintillation counter (Pacard Tri-Carb 1900).

Bacterial production was measured by adding 20 nM of methyl-(^3H)-thymidine to replicate samples (20 ml); 1% formaldehyde was immediately added to the control samples. Incubation (1 h, *in situ* temperature) was stopped by the addition of formaldehyde (final concentration 1%). All samples were filtered on a 0.2 μm cellulose acetate membrane and macromolecules were precipitated by adding 3 volumes of 5 ml ice-cold TCA (10%). ^3H -activity was measured by liquid scintillation counting (see previous paragraph), corrected for the background in pre-fixed samples.

The sedimentation rate of C and N was estimated based on the mass balance of P in each mesocosm combined with the measurements of C:N:P in sedimented matter collected in sedimentation traps suspended at a 9 m depth and harvested every fourth day. Sedimented P was estimated as the difference between the accumulated daily added P (Table 1 in Olsen et al. 2007) and the measured total P that accumulated in the water (Grasshoff et al. 1983). This means that added P could either accumulate in the water or in the sediments. The corresponding sedimentation rates of C and N were then estimated based on the sedimentation rates of P and the elemental P:C and N:C ratios, respectively, of the sedimented material. The sedimentation rates directly obtained from the traps agreed quite well with those estimated by mass balance, but these values were more scattered for the mesocosms because sedimenting material tended to concentrate along the walls of the mesocosms.

Flow network characteristics and construction

The methods of inverse modelling used are presented and discussed by Olsen et al. (2006; 2007), and other authors have reported previous appli-

cations of similar methods for the flow network construction of planktonic food webs (Jackson & Eldridge 1992, Stone et al. 1993, Vézina & Pace 1994, Lyche et al. 1996). Our simplified planktonic food web, which formed a framework for flow network construction in the mesocosm communities (Fig. 2), included 3 functional autotrophic components and 4 heterotrophic and 30 inter-compartmental C flows

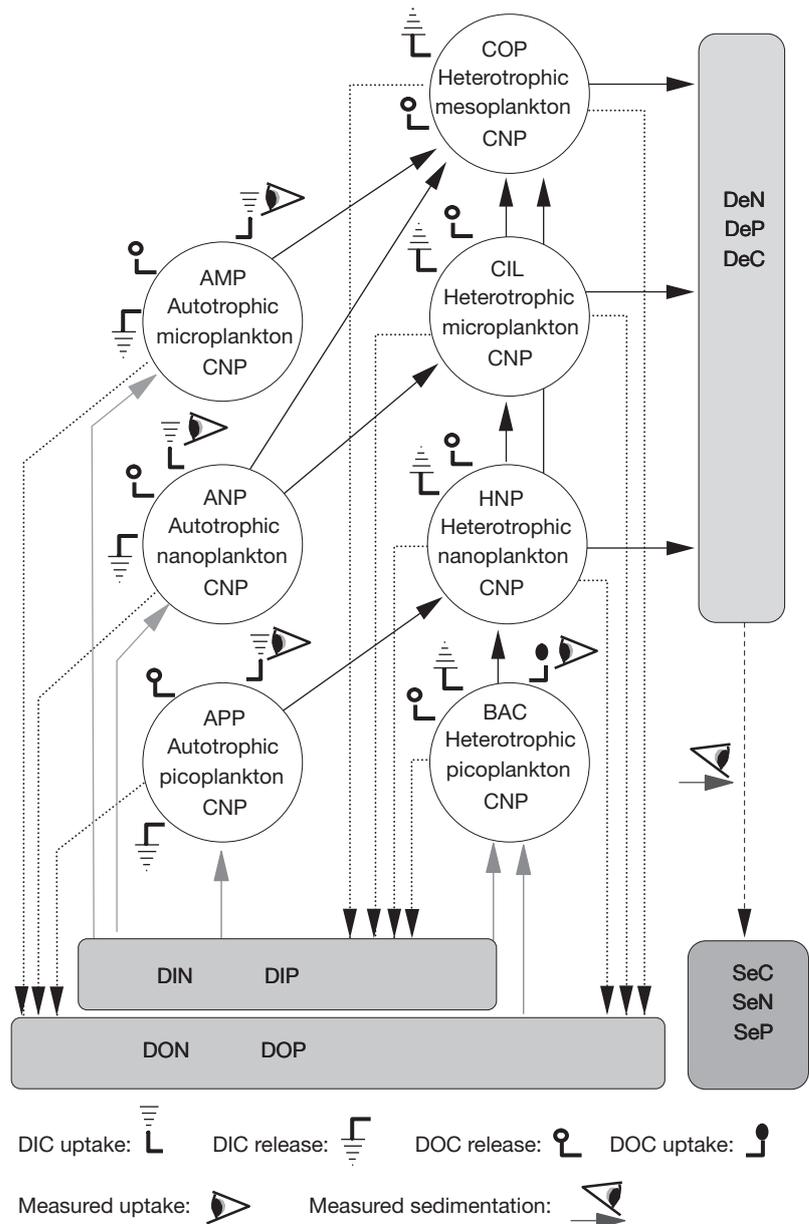


Fig. 2. Flow network including the biomass of the defined food web components (functional groups) and the included flows forming a framework for food web C, N and P biomass determination and flow estimates by inverse modelling (see Table 3 for constraints). DeC, DeN, DeP: detrital particulate C, N and P, respectively; DIC, DIN and DIP: dissolved inorganic C, N and P; DOC, DON, DOP: dissolved organic C, N and P, respectively; SeC, SeN and SeP: sedimentation rate of C, N and P, respectively. See Table 2 for other abbreviations

(see Olsen et al. 2007). All components and flows had analogous N and P terms (Fig. 2). A higher number of flows could have been considered, but this would have deteriorated the robustness of the results due to the limited information available for constraining the estimates. The input data for flow network construction included: time-series data (7 mesocosms, 10 sampling days) of the C biomass of the 7 functional groups; the corresponding N and P in the biomass; the concentrations of inorganic N and P; the concentrations of DOC, DON and DOP; the concentrations of detrital particulate C, N and P; primary production in the 3 fractions; bacterial production; and the sedimentation rate of C, N and P (Fig. 2). Accordingly, this included a total of 4410 measured or estimated concentrations and 490 measured flows.

The flow network construction process generated a complete set of food web flows representing mean flows for the 18 d period of each mesocosm; each unit was treated independently. The uptake (or ingestion) of N and P by heterotrophs was linked to the carbon uptake flows. The constraining factors involved no other strong links between the carbon flows and N or P. The estimated flows were means of 1000 'only C' Monte Carlo simulations, 1000 'C and N' simulations, and 1000 'C and P' simulations. The Monte Carlo simulations included all of the random variability measured for the input data (concentrations, biomasses, flow measurements) and allowed an estimation of the confidence limits for the estimated flows. The estimated C flows were almost identical with and without N and P linked to C ($r^2 = 0.9999$), and full rank solutions were obtained in all mesocosms. Other aspects of more systematic uncertainty were related to the relevance of the basic structure of the flow network, the quality of the input data, and the relevance

of the constraining windows (Table 3) (see critical discussion of C flow determinations in Olsen et al. 2007).

RESULTS

Biomass and food concentration

The BAC group included heterotrophic bacteria (and Archaea) and showed 1 main peak in biomass on Days 11 to 13 in all fertilised mesocosms (Fig. 3A, mean and range of variation for all mesocosms), with higher values being found for high nutrient input. The DOC concentration (BAC substrate) showed a slight increase with time after the first week in all units (Fig. 3B), but the increase in BAC substrates was not significantly related to the loading rates of the nutrients ($p > 0.05$, data not shown). The mean biomass for the individual mesocosms was positively correlated with their respective N loading rate (L_N , $p < 0.01$; Fig. 3C).

The heterotrophic nanoplankton (HNP) group mainly included small unpigmented nanoflagellates (2 to 8 μm ; 62 % heterotrophic nanoflagellates [HNF]), but also small bacterivorous ciliates (11 %), Craspedophyceae (13 %), and larger appendicularia (*Oikopleura dioica*, 14 %), which were only present in high densities at the start (Gismervik et al. 2002). The HNP biomass showed 1 main peak in biomass on Days 7 to 9 (Fig. 3D), apparent in most mesocosms, and 1 towards the end, especially in units that received the highest nutrient input. The first biomass peak of HNP developed 2 to 4 d after a maximum in their food concentration (Fig. 3E), whereas the last peak, which was very clear under a high nutrient input, could not be related to food concentration. The mean biomass of

Table 3. Rules used to constrain the N and P flows in inverse modelling. The constraints of the C flows are identical to those in Olsen et al. (2007). See Table 2 and Fig. 2 for abbreviations

Flow	Component	Constraint or rule (reference)
N and P ingestion or uptake	BAC, HNP, CIL, COP	N:C and P:C intake equal to food (Table 2)
Released DON:DOC and DOP:DOC	AMP, ANP, APP, HNP, CIL, COP	N:C biomass > DON:DOC > N:C _{min} P:C biomass > DON:DOC > P:C _{min}
Released DeN:DeC and DeP:DeC	HNP, CIL, COP	N:C biomass > DeN:DeC > N:C _{min} P:C biomass > DeN:DeC > P:C _{min}
N:C _{min}	DON:DOC	50 $\mu\text{g N mg C}^{-1}$; 5th percentile of measured DON:DOC and DeN:DeC ratios
P:C _{min}	DOP:DOC	3.3 $\mu\text{g P mg C}^{-1}$; 5th percentile of measured DOP:DOC and DeP:DeC ratios
N, P release and metabolic flows	BAC	No constraints

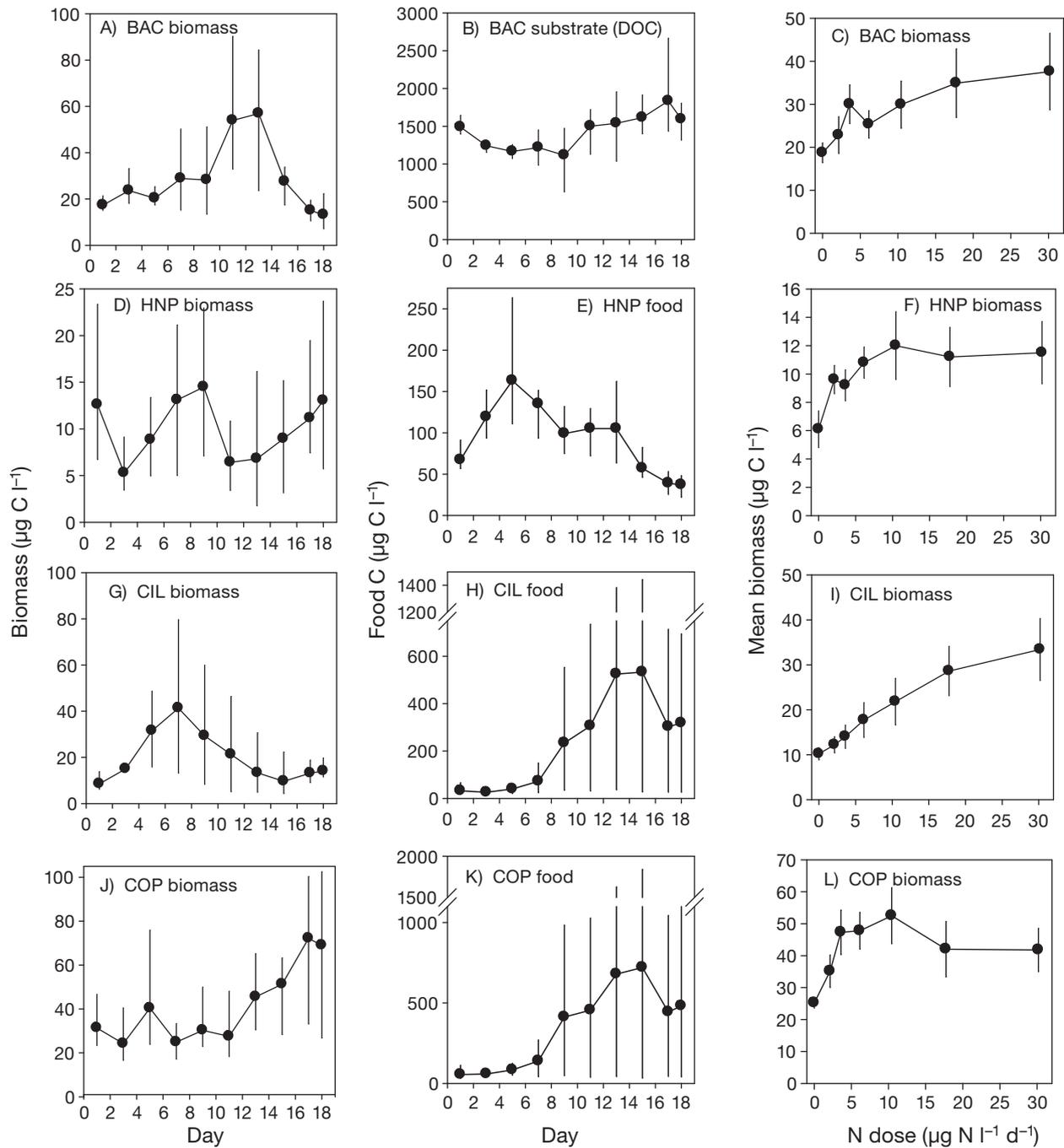


Fig. 3. (A,D,G,J) Mean biomass of heterotrophic plankton groups in all mesocosms versus time (bars indicate minimum-maximum range for the 7 mesocosms); (B,E,H,K) their mean food concentrations in all mesocosms versus time (bars indicate minimum-maximum range for the 7 mesocosms); and (C,F,I,L) mean biomass of individual mesocosms for the period versus the N loading rate (error bars show 1 SE, $n = 10$). See Fig. 2 and Table 2 for abbreviations and further information

HNP significantly increased with increasing L_N ($p < 0.05$), but a positive relationship was only apparent in the lower range of the nutrient input rates (Fig. 3F). The HNP food constituted picocyanobacteria (*Synechococcus* spp.), minor amounts of eukaryotic picoplankton, and heterotrophic bacteria.

The CIL group mainly constituted herbivorous ciliates (Strombidids and Strobilids, 20 to 50 μm ; Gismervik et al. 2002). The CIL showed 1 clear biomass peak on Day 7 (Fig. 3G). The food concentration of CIL showed an almost inverse relationship to CIL biomass, and responded very strongly after Day 7

(Fig. 3H). Both CIL biomass and CIL food concentration were significantly and positively related to L_N ($p < 0.001$ for both, biomass shown in Fig. 3I). The CIL food was comprised of diatoms, dinoflagellates and other small heterotrophic and autotrophic flagellates (cell width: 2 to 20 μm). The diatom *Rhizosolenia fragilissima* became totally dominant at the end of the high nutrient input period.

The COP group included a variety of calanoid copepods, of which *Centropages* spp. and *Acartia* spp. responded most strongly to increased nutrient input levels (Gismervik et al. 2002). The COP biomass did not respond to increased nutrient inputs until Day 11 (Fig. 3J), after which it increased with time in most units except those with the lowest amounts of added nutrients. This response corresponded well with the slight increase in food concentration of the COP group from Day 5 (Fig. 3K), because of the response of the CIL biomass. The increase in food concentration was much faster after Day 7. The main response in COP biomass was observed 6 d after the first increase in food concentration and 4 d after the strong increase. There was no general positive relationship between the mean COP biomass and L_N ($p > 0.05$; Fig. 3L). The COP food was comprised of diatoms (mainly *Rhizosolenia fragilissima* and colonies of *Skeletonema costatum*), *Rhodomonas* sp., unidentified pigmented flagellates, small thecates and larger (20 to 200 μm) dinoflagellates, and CIL biomass (Fig. 2).

Nutrient concentrations and stoichiometry

The mean inorganic nutrient (dissolved inorganic N [DIN] and P [DIP]; Fig. 4A,B) concentrations of the mesocosm significantly increased with the loading rates of the respective nutrients ($p < 0.0001$). The concentration of DON remained constant ($p > 0.05$; Fig. 4C), whereas DOP exhibited a slight but significant increase ($p < 0.01$; Fig. 4D). Particulate organic nutrients (<200 μm ; particulate organic N [PON] and P [POP]; Fig. 4E,F) significantly increased with the loading rates of the respective nutrients ($p < 0.0001$). Both N and P primarily accumulated in the particu-

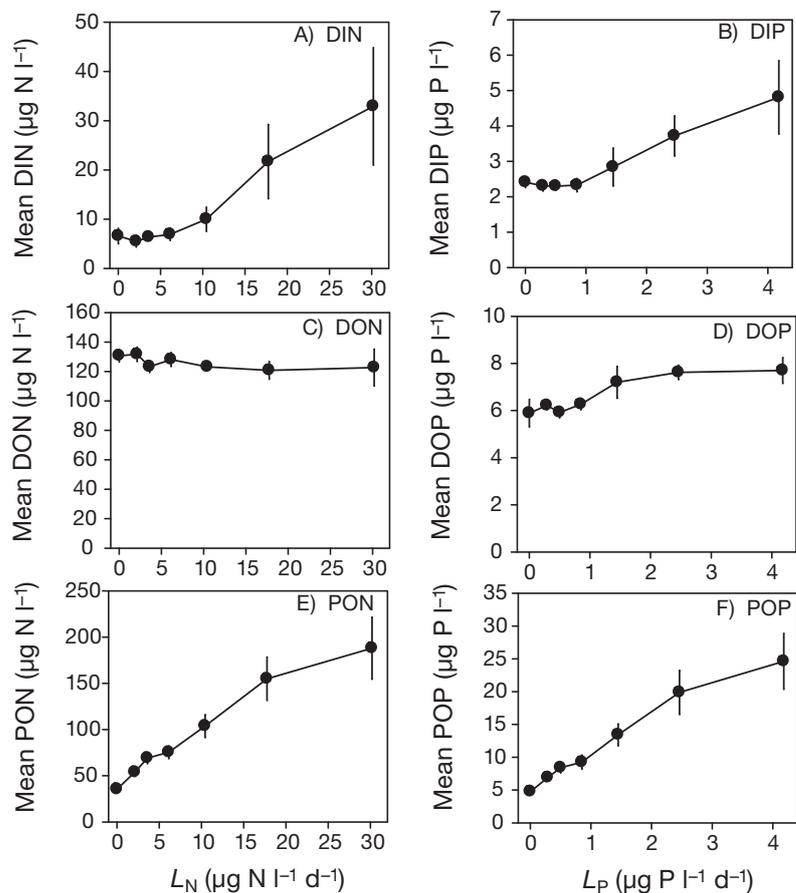


Fig. 4. Mean nutrient concentrations for individual mesocosms during the experimental period as a function of the respective nutrient loading rates. (A) DIN and (B) DIP: dissolved inorganic N and P; (C) DON and (D) DOP: dissolved organic N and P; and (E) PON and (F) POP: particulate organic N and P, respectively. Error bars show 1 SE ($n = 10$)

late organic fraction. The concentrations of inorganic nutrients only increased temporarily, showing higher values after around 1 wk and background concentrations again after Day 10 (cf. Olsen et al. 2007).

Both N:C and P:C ratios of the particulate matter <200 μm showed maxima around Days 5 to 7 and a slight increase during the high nutrient input at the end (Fig. 5A,B). The N:C and P:C ratios moderately increased by factors of 1.5 and 2, respectively, during the first week, and returned to the initial values around Days 9 to 10. It was notable that the mean N:C and P:C values of all size fractions were only moderately affected by L_N and L_P , respectively (Fig. 5C,D), suggesting that particulate C, N and P showed a similar pattern of variation in all mesocosms. Therefore, the mean ratios for all fractions remained constant and independent of the nutrient loading rate ($p > 0.05$; Table 4). The pronounced variability in the 20–200 μm size fractions was most likely a result of a low concentration of particulate matter (biomass).

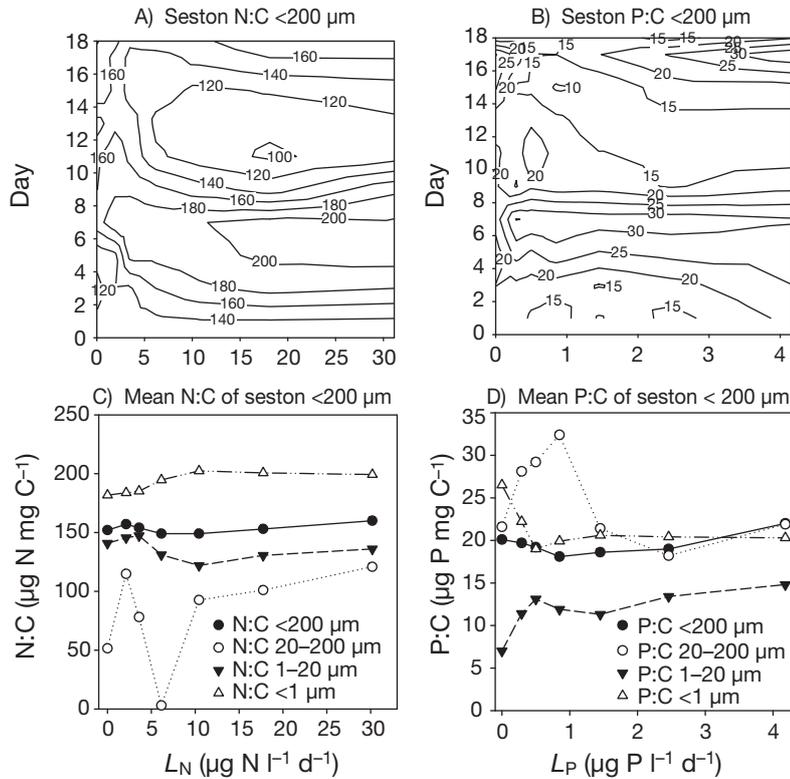


Fig. 5. Elemental compositions of seston fractions. (A) N:C and (B) P:C ratios of seston $<200 \mu\text{m}$ as functions of respective N and P nutrient loading rates and time of development; mean (C) N:C and (D) P:C ratios of seston fractions as functions of respective N and P loading rates

Table 4. Regression coefficients for N:C and P:C ratios of seston fractions as functions of N and P doses, respectively

Relationship	Fraction (μm)	Slope \pm 1 SE (l d) (mg C^{-1})	r^2	p
Mean N:C ratio of seston fractions for mesocosms vs. N dose (Table 1)	<200	0.185 ± 0.147	0.241	0.264
	$20\text{--}200$	1.92 ± 1.50	0.246	0.258
	$1\text{--}20$	-0.327 ± 0.350	0.149	0.393
	<1	0.611 ± 0.244	0.557	0.054
Mean P:C ratio of seston fractions for mesocosms vs. P dose (Table 1)	<200	0.483 ± 0.319	0.315	0.190
	$20\text{--}200$	-1.71 ± 1.37	0.239	0.265
	$1\text{--}20$	1.21 ± 0.51	0.527	0.065
	<1	-0.700 ± 0.686	0.172	0.353

Flow network construction

The input data for flow network construction by inverse modelling involved the C, N and P concentrations of 8 components and 5 flows (Fig. 2). The values were organised into dose-day-measurement matrices. The sedimentation rates were given as mean flows for the individual mesocosms over the experimental period (dose-measurement matrices).

We obtained full-rank flow solutions in all mesocosms. Estimated C flows were, on average, slightly lower than the corresponding measured input flows of C (slope: 0.972 ± 0.014 ; $p < 0.0001$; $r^2 = 0.993$; Olsen et al. 2007). The coefficient of variation of the flow estimates for N and P, derived from 3000 Monte Carlo simulations, was 0.1 to 7%, with the highest relative variability for small flows (Fig. 6). The confidence limits were small; therefore, they are not indicated in the flow estimates presented below. Instead, the variability and uncertainty are illustrated through the relationship between the independently generated flows of the 7 individual mesocosms along the gradient of increasing nutrient addition.

Metabolic nutrient flows and growth efficiencies

The estimated N and P release flows of the heterotrophic groups are summarised in Fig. 7. Particulate organic (in the form of detrital particulate N [DeN] and P [DeP]) and dissolved inorganic nutrients were the main N and P components released by the zooplankton groups, with particulate nutrients becoming gradually more important for high nutrient-loading rates. HNP was a major contributor of DIN and DIP release under low and moderate nutrient inputs, but their release rate did not respond very much to the increased nutrient input. Contrary to this, COP and particularly CIL released very little P and N under low nutrient-input conditions. Their release of all components increased in the higher nutrient-input range, especially for DeN and DeP.

Zooplankton may generate particulate nutrients through sloppy feeding, defecation and mortality, but our method did not distinguish between these mechanisms.

The BAC group exhibited a high release rate of DON in all mesocosms (Fig. 7A). The N:C ratio of DOC released from BAC was $85 \mu\text{g N mg C}^{-1}$ (range: 70 to $97 \mu\text{g N mg C}^{-1}$), which was, in fact, higher than in the substrates taken up ($66 \mu\text{g N mg C}^{-1}$, range: 49

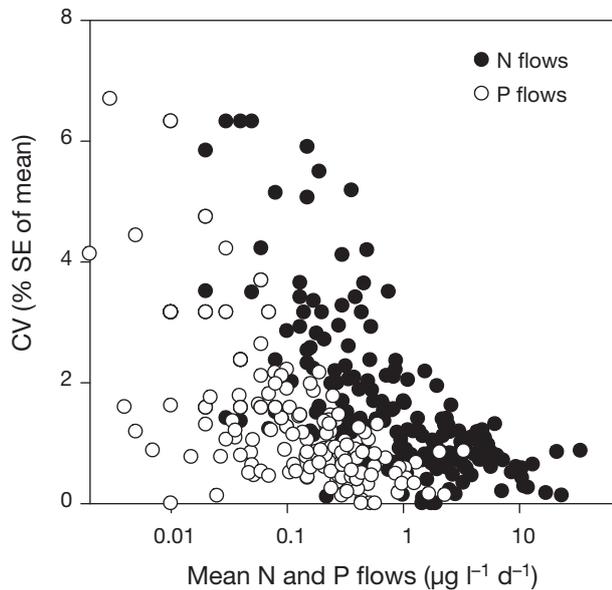


Fig. 6. Characteristics of estimated N and P flows expressed by the coefficient of variation ($SE/mean \times 100$, mean of 3000 Monte Carlo simulations for the flow rate estimates) as a function of the respective mean flow rates ($n = 3000$)

to $75 \mu\text{g N mg C}^{-1}$). The release of P from BAC was very low, and only DOP was released (Fig. 7B). The P:C ratio of released DOC was $3.4 \mu\text{g P mg C}^{-1}$ (range: 3.2 to $3.5 \mu\text{g P mg C}^{-1}$), which was always lower than that of the substrates taken up ($9.7 \mu\text{g P mg C}^{-1}$, range: 7.6 to $14 \mu\text{g P mg C}^{-1}$).

The N- and P-based growth efficiencies (GE_N and GE_P ; see Table 1) showed optimum values in Mesocosm 2 for all groups, with reduced GE values during severe nutrient starvation when no nutrients were added and gradually reduced values as the loading rate of N and P increased (Fig. 8A,B). The BAC and CIL groups exhibited higher growth efficiencies for N and P than HNP and COP throughout, and it is notable that the GE was very high for P; in fact, it was $>80\%$ throughout for the BAC group. The values for CIL were in the range of 60 to 90% for mesocosms that received nutrients close to the natural rates (Mesocosms 1 to 4). High GE values imply that few nutrients are released through defecation and excretion, i.e. meaning that a high fraction is assimilated and incorporated into the biomass.

Fig. 8C,D show N- and P-based growth efficiencies of the heterotrophic plankton groups as functions of GE_C , the growth efficiency based on C, which exhibited the same overall pattern of variation as the N- and P-based values (Olsen et al. 2007). The GE_N and GE_P for COP were equal or slightly higher than the GE_C . For BAC, both were much higher throughout

than GE_C , and GE_P was higher than GE_N . The values for CIL showed a similar pattern of variation, but were generally closer to GE_C than BAC. The opposite pattern was apparent for HNP, with GE_C values equal or slightly higher than both GE_N and GE_P .

The contents of N and P in the food (Q_N and Q_P , respectively; Table 1) of the heterotrophic groups was hardly affected by the nutrient loading rates of N and P, respectively (Fig. 9A,B), in agreement with the relatively constant N:C and P:C ratios of particulate matter (Fig. 5C,D). The Q_N and Q_P were estimated as the ratio between the ingestion rates (uptake flows for BAC) of nutrients and C. The HNP consumed the food with the highest Q_N and Q_P , whereas the BAC substrates, which included inorganic and organic dissolved nutrients, were relatively poor in both N and P. The CIL and COP consumed food with Q_N and Q_P values similar to, although slightly higher than, the respective N:C and P:C ratios of crude particulate matter.

In most cases the total N and P released by the zooplankton groups per unit of C ingested (Q_{RN} and Q_{RP} , respectively; Eq. 1) showed no or a moderate increase with increasing nutrient input rate (Fig. 9C,D). However, the zooplankton groups systematically exhibited different Q_R values that were generally opposite of the GE values (Fig. 8), with the highest Q_{RN} and Q_{RP} values for HNP and lowest for BAC. For the groups showing intermediate release values, the COP showed higher release rates than CIL. This means that there was a group-specific pattern in the amount of N and P released per consumed unit of food C (HNP $>$ COP $>$ CIL $>$ BAC), which was fairly independent of the nutrient loading rate.

These systematic differences in the Q_R values of the heterotrophic groups were related to the respective nutrient contents of the food (Q_N and Q_P , respectively; $p < 0.0001$ for both; Fig. 9E,F). The HNP group ingested the most N- and P-rich food, and, as a result, released more nutrients per unit of C ingested than any other group. The CIL and COP groups fed on similar food sources, but the COP released slightly more N and P per carbon consumed than CIL. The substrates taken up by BAC were relatively poor in both N and P, and their release of nutrients per C taken up was low, especially for P. All values except for one (N release for COP in Mesocosm 7) were below the 1:1 line, which is needed for maintaining a positive carbon growth efficiency (Fig. 1). Some of the variability in Q_R was caused by variability in GE_C (and Q_Z ; Eqs. 1 to 3), but the variability in Q_N and Q_P explained as much as 83 and 91% of the variability in Q_{RN} and Q_{RP} , respectively, for the complete data set.

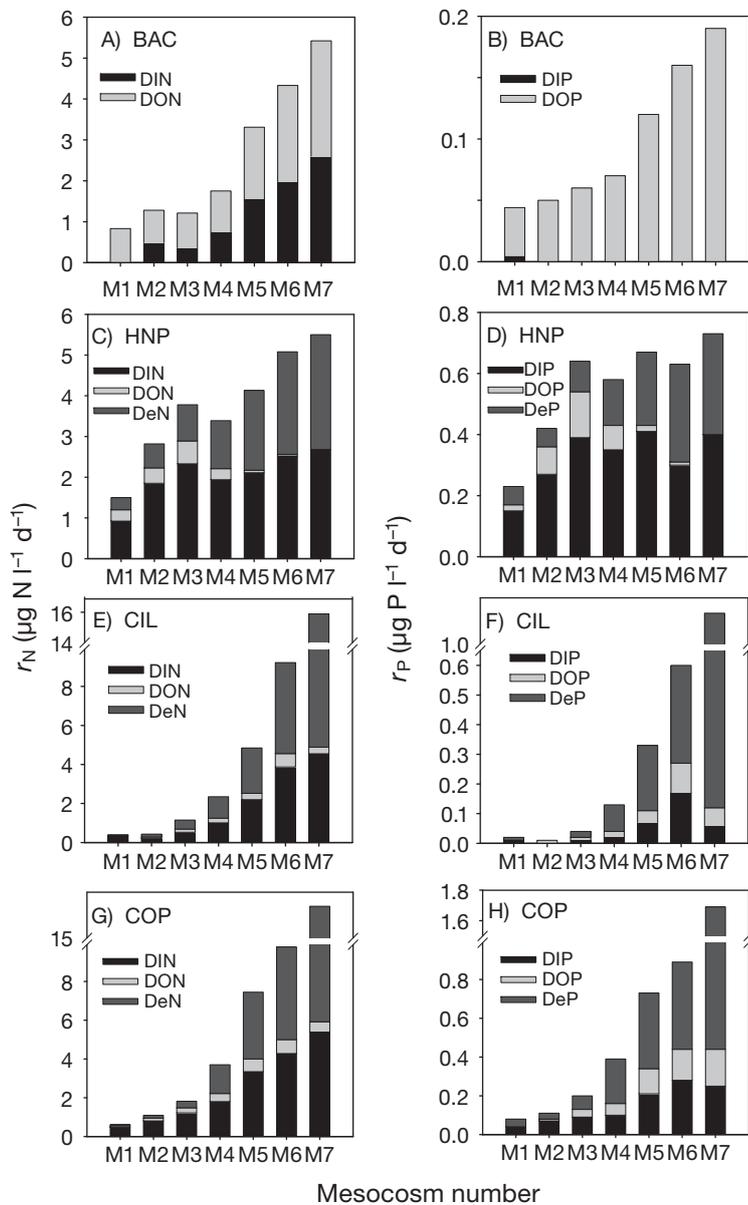


Fig. 7. Nutrient release rates: dissolved inorganic N (DIN) and P (DIP), dissolved organic N (DON) and P (DOP), and particulate organic nutrients as detrital particulate N (DeN) and P (DeP) by the heterotrophic plankton groups (A,B) BAC, (C,D) HNP, (E,F) CIL, and (G,H) COP in each of the 7 mesocosms (M1 to M7). See Table 2 for other abbreviations

A further evaluation of the nutritional state of the heterotrophic groups required estimates of GE_{CM} (Eq. 2) and the indigestible fractions of N and P in the food (Q_{IDN} and Q_{IDP} , respectively; Eq. 4), or the assimilation efficiency of the plankton. Such values are generally not available, but the results and the literature allowed some useful speculations. The results from Mesocosms 1 to 4 were used in the next exercise to derive such values. The average values of these meso-

cosms were assumed to be representative for the natural summer situation with respect to the nutrient loading rate of the actual plankton community (see Olsen et al. 2007). The mean values for the growth efficiencies and the contents of N and P in the food for these mesocosms are given in Table 5.

The GE_{CM} of zooplankton is difficult to measure, but such values must logically exist (Straile 1997). We have based our rough values of 60% for BAC and CIL and 50% for HNP and COP on the values and discussion reported by Straile (1997) and Fenchel et al. (1998) (our Table 5). The indigestible fractions of nutrients in the food (Q_{IDN} ; Table 5) were estimated by assuming a maximum assimilation efficiency of 80% for the N and P components in the food of HNP, CIL and COP (Straile 1997), and 90% for BAC (based on present data). This corresponds to an indigestible fraction of 20% of Q for the zooplankton groups and 10% for BAC. The consequences of all assumptions are discussed in 'Discussion — evaluation of nutrient limitation'.

The above estimates allowed Q_C , Q_{CM} and Q'_{CM} values to be calculated for N and P, according to Eqs. (2) to (4) above (Table 5). The Q'_{CM} values for N and P reflect the critical nutrient contents of the food below which nutrient limitation of a predator could occur, corrected for digestibility. The Q'_{CM} can be understood as the species- or group-specific lower critical nutrient content below which nutrient limitation could theoretically occur. This limitation will only become expressed when all other essential nutrients are supplied in higher amounts, or, more specifically, when the supply of other essential nutrients allow a higher growth efficiency of C.

DISCUSSION

Our experiment demonstrated a highly dynamic response of many nutrient pools and organisms to an increased nutrient supply rate, and nutrients mainly accumulated in the biomass of autotrophic plankton. In our approach, the nutrient content of the food (Q) was the most important factor for the quantitative nutrient intake in heterotrophic organisms. This was a consequence of assuming mass balance and homeostasis of

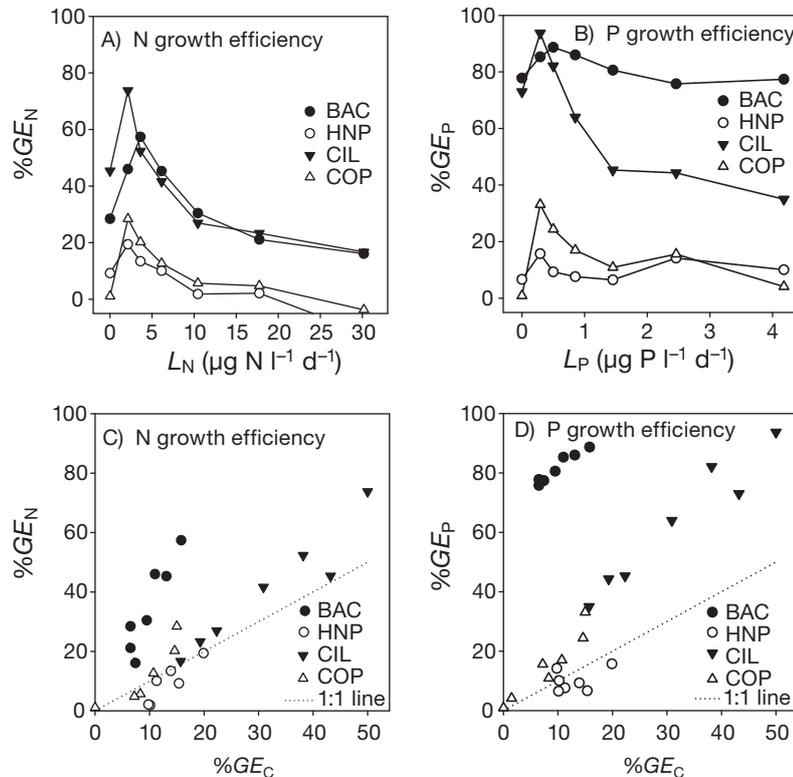


Fig. 8. Estimated C-, N- and P-based growth efficiencies (GE) of the plankton groups. (A) GE_N and (B) GE_P as a function of the respective nutrient loading rates (L); (C) GE_N and (D) GE_P as a function of GE_C . See Tables 1 & 2 for other abbreviations

nutrient contents, and these assumptions are not controversial for conservative, non-catabolised essential nutrients such as minerals (e.g. Sterner & Elser 2002). For nutrients that are metabolised, for example essential amino acids and fatty acids, there may be additional losses associated with catabolism that then become a component of Q_R , but this aspect was not covered here. The concentration of food affects the carbon ingestion rate, and, accordingly, also nutrient intake, but a reduced food concentration will not necessarily affect the metabolic balance of CNP too much because the nutrient proportions remain constant.

Criteria for evaluating nutrient limitations

Our framework for analysing the balance of C, N and P in the heterotrophic groups is fully compatible with the mass balance equations used during flow network construction. The fundamental relationship shows that the ingestion of nutrients is equal to the sum of their combined egestion and metabolic losses and their use for growth (definitions in Table 1):

$$i_X = g_X + r_X \quad (5)$$

Eq. (5) is equivalent to Eq. (1). Fig. 10 shows the data derived for the heterotrophic groups inserted in the framework of Fig. 1. The solid lines are described by:

$$\begin{aligned} Q_R &= Q - Q_{CM} = \\ Q - (Q'_{CM} - Q_{ID}) &= \\ Q - [Q_{ZM} \times GE_{CM}] & \end{aligned} \quad (6)$$

(definitions in Table 1). Eq. (6) expresses the total N or P released per unit of C ingested as a function of the food nutrient content Q for the situation of maximal carbon growth efficiency (GE_{CM}). The solid curve intersects the horizontal dotted curve ($Q_R = Q_{ID}$) in Q'_{CM} . The vertical dotted line expresses the Q value below which nutrient limitation may theoretically exist ($Q = Q'_{CM}$; see Fig. 1). The stippled curve expresses Eq. (1) for $GE_C = 0$. Accordingly, limitation cannot occur for Q values higher than that indicated by the vertical dotted line. Limitation may occur for lower Q values within the triangular space restricted by the dotted and stippled lines.

According to the indicators set to evaluate specific nutrient limitations of the predator, we asked the following 3 questions:

- (1) Is $Q < Q'_{CM}$ for N and P?
- (2) Are there any indications that C, or another essential nutrient, is more limiting than the one being considered?
- (3) Are the released nutrients per unit of ingested C (Q_R) close to the minimum (Q_{ID})?

The above considerations and indicators demonstrated that nutrient limitation will only occur if the nutrient contents of the food are lower than Q'_{CM} , the species- or group-specific critically lower nutrient level in the food for experiencing nutrient limitation. Nutrients that are available at higher concentrations than Q'_{CM} cannot be limiting. Those which are available in lower quantities are potentially limiting, and they are limiting unless another essential resource, including C, is available at even lower quantities. The individual essential nutrients and C, closely related to energy, are best judged as different resources.

When Q_R approaches the minimum Q_{ID} value, the organism can no longer respond to a lower Q by re-

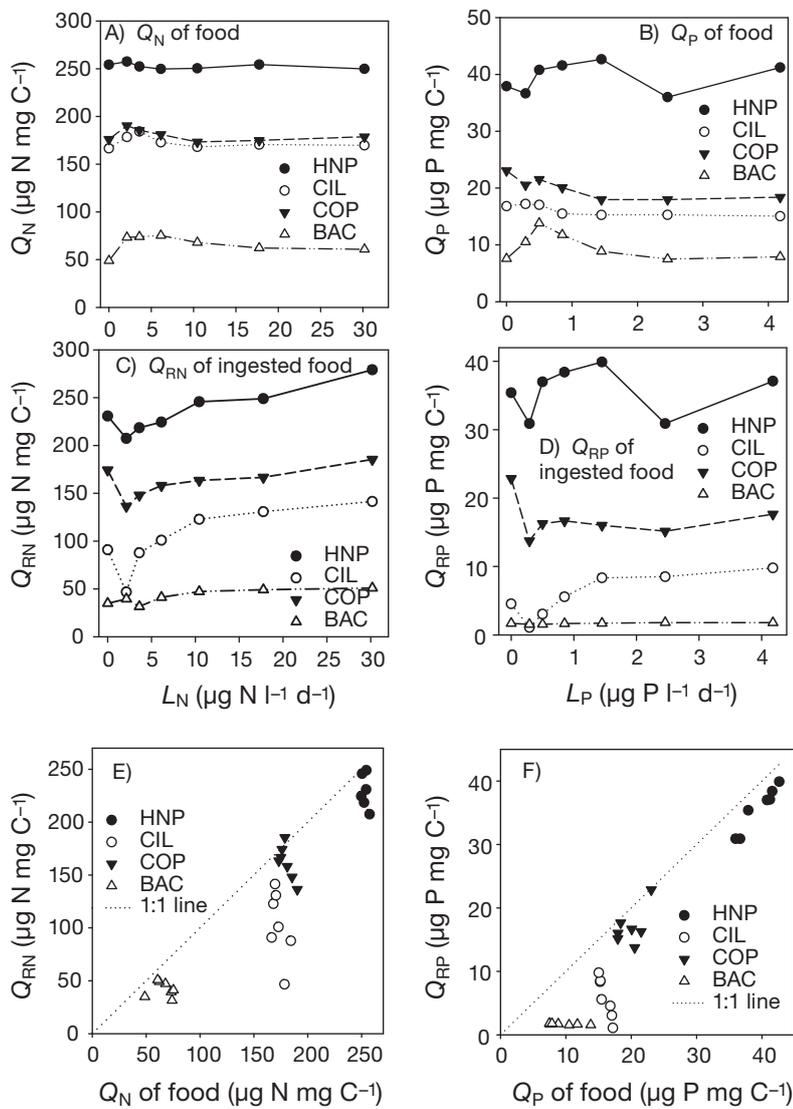


Fig. 9. Characteristics of Q and Q_R for N and P. (A,B) Mean estimated Q_N (N:C) and Q_P (P:C) of the ingested food, estimated as $i_N i_C^{-1}$ and $i_P i_C^{-1}$ (Table 1), respectively, as functions of respective N and P loading rates (L). (C,D) Estimated nutrient release rate per unit of food C ingested (Q_{RN} and Q_{RP}) of the plankton groups as a function of the respective nutrient loading rates. (E,F) Q_{RN} and Q_{RP} as functions of Q_N and Q_P of the food, respectively. Bacterial substrates (food) include both inorganic and organic dissolved components. See Tables 1 & 2 for abbreviations

ducing the losses of that nutrient. They are then left to reduce their internal body content of the nutrient or the growth efficiency of C. This situation increases the probability of expressed nutrient limitation.

Evaluation of nutrient limitation

An important and quite surprising result was that both the N and P contents of the particulate matter

were only temporarily and moderately affected by the rate of nutrient addition (Fig. 5). The direct measurements made in different fractions of particulate matter revealed constant mean N:C and N:P ratios of seston fractions independent of the nutrient input rate (Fig. 5C,D; $p > 0.05$ for all fractions; Table 4). These results led us to assume constant mean Q values for the individual autotrophic functional groups and bacteria as an input to the flow network construction (see 'Materials and methods' and Table 2).

For the situation studied, our data revealed that BAC substrates contained insufficient amounts of both N and P for maintaining GE_{CM} (Fig. 10). Contrary to this, HNP always consumed food with a surplus of N and P, and this group should therefore be capable of maintaining GE_{CM} when the food supply is adequate. We further deduced that CIL consumed a diet that could closely balance its N or protein requirements at GE_{CM} , whereas the mineral P content of the food was potentially limiting. The COP group consumed food with adequate N or protein contents and a slightly suboptimal P content for sustaining GE_{CM} . A moderate decrease in GE_{CM} (Table 5) caused by other essential nutrients being supplied in suboptimal amounts will reduce the probability of potential P limitation for COP. Major changes in GE_{CM} are, however, needed to affect the above conclusions for BAC and HNP, and for a potential mineral P limitation of CIL. A reduction of GE_{CM} from 60% to <20% is needed to affect the conclusions made for P limitations of BAC, at which point their N supply in the substrates would become sufficient.

A moderate change in Q_{ID} compared to those in Table 5, within normal biological limitations (e.g. range of 5 to 30% indigestible nutrient fraction), would not affect the above conclusions.

The next question to be addressed was whether or not the food C availability of the potentially nutrient-limited groups was very low. The CIL biomass was inversely related to CIL food C (Fig. 3). Moreover, the ciliate bloom in mesocosms with high amounts of added nutrients (CIL; Fig. 3G) was in fact initiated

Table 5. Mean N and P contents of food and growth efficiencies estimated for Mesocosms 1 to 4 together with estimates of the variables that are decisive for evaluating nutrient limitation (see 'Materials and methods'). Q_{ID} is the indigestible nutrient content of N and P in the food, calculated from Q_N and Q_P in the food (Fig. 9A,B) and the estimated indigestible fraction, assumed to be 10% for BAC and 20% for HNP, CIL and COP (this table); Q_{CN} and Q_{CP} were estimated (Eq. 3) using mean GE_C (this table) and the respective Q values in Table 2; Q_{CMN} and Q_{CMP} were estimated using Eq. (2), with GE_{CM} from this table and Q values for BAC, HNP, CIL and COP from Table 2 (assuming homeostasis, $Q = Q_{ZM}$); Q'_{CMN} and Q'_{CMP} were estimated using Eq. (4) based on Q_{CM} and Q_{ID} values in this table. See Tables 1 and 2 for other abbreviations

Mean for Mesocosms 1 to 4	BAC	HNP	CIL	COP
Food Q_N ($\mu\text{g N mg C}^{-1}$)	67.8 \pm 3.2	253 \pm 2	176 \pm 4	183 \pm 3
Food Q_P ($\mu\text{g P mg C}^{-1}$)	10.9 \pm 0.7	39.2 \pm 1.2	16.6 \pm 0.4	21.3 \pm 0.7
GE_C ($\text{g}_C \text{ i}_C^{-1}$), %	11.6 \pm 1.0 (6.6–15.8)	15.1 \pm 1.8 (11.3–19.9)	40.6 \pm 4.0 (30.9–50.0)	10.1 \pm 3.5 (0–15.0)
GE_N ($\text{g}_N \text{ i}_N^{-1}$), %	44.3 \pm 3.0 (28.5–57.4)	13.0 \pm 2.3 (9.2–19.4)	53.3 \pm 7.2 (41.6–73.8)	15.6 \pm 5.8 (1.1–28.4)
GE_P ($\text{g}_P \text{ i}_P^{-1}$), %	84.4 \pm 1.2 (77.8–88.7)	9.8 \pm 2.1 (6.7–15.8)	78.2 \pm 6.4 (64.0–93.7)	18.9 \pm 6.8 (0.9–33.1)
GE_{CM} , maximum GE_C value, %	60	50	60	50
Indigestible fraction, %	10	20	20	20
Q_{IDN} ($\mu\text{g N mg C}^{-1}$)	6.6	51	35	36
Q_{IDP} ($\mu\text{g P mg C}^{-1}$)	1.0	7.9	3.3	4.0
Q_{CN} ($\mu\text{g N mg C}^{-1}$)	29.0	27.2	93.3	18.1
Q_{CP} ($\mu\text{g P mg C}^{-1}$)	8.70	3.17	12.5	3.43
Q_{CMN} ($\mu\text{g N mg C}^{-1}$)	150	90	138	90
Q_{CMP} ($\mu\text{g P mg C}^{-1}$)	45.0	10.5	18.6	17.0
Q'_{CMN} ($\mu\text{g N mg C}^{-1}$)	157	141	173	126
Q'_{CMP} ($\mu\text{g P mg C}^{-1}$)	46	18	22	21

before the response in primary production and the biomass of nano-autotrophs, which is their main food (data not shown, responded after Day 5, and Fig. 3H, respectively). This suggests that the ciliates directly responded to the increased inorganic nutrient input or to the increase in N:C or P:C contents of their food, similar to the situation previously observed by Thingstad et al. (2005) for the ultraoligotrophic Eastern Mediterranean. The response in nutrient uptake is always faster than the response in growth. In the present case, the response times of P:C and N:C were 5 to 7 d until the maximum (Fig. 5A,B), which was comparable to the response time of the ciliate biomass (Fig. 3G), whereas the response of ciliate food took place after Day 7 (Days 7 to 13) (Fig. 3H). Thus, there were no indications of a C limitation for CIL, and this conclusion was also supported by their higher retention of nutrients (GE_N and GE_P) than C (GE_C ; Fig. 8, Table 5).

The copepod community (COP) exhibited N and P growth efficiencies that were only slightly higher than GE_C (Fig. 8, Table 5). Unlike the ciliates, their biomass increased to some extent along with their food concentration, but the COP biomass showed a maximum for intermediate nutrient input. This result, together with the fact that the Q_N and Q_P requirements for the copepods were closer to Q'_{CMN} and Q'_{CMP} than for ciliates (Fig. 10), suggests that in the present experiments the copepods were either C-

or energy-limited or limited by another essential nutrient, such as long-chain essential n-3 fatty acids (Evjemo et al. 2008).

The bacteria (BAC) exhibited very low GE_C values (7.4 to 16%; Fig. 8), suggesting other limitations of growth than C or energy. In fact, the BAC group released as much as 44 to 54% of the DOC taken up (data not shown), which may be interpreted as a sign of severe nutrient deficiency. It is important to note that the inverse method did not produce full-rank solutions unless the release of C, N and P was allowed for BAC in the flow network construction (Fig. 2).

The final question addressed was whether or not the released nutrients per unit of ingested C (Q_R) were close to the minimum (Q_{ID}) for the potentially nutrient-limited plankton groups. This would be expressed through a Q_R close to its minimum value (Q_{ID}) and high nutrient growth efficiency. Both BAC and CIL released minimal amounts of P (Fig. 10), thus exhibiting a Q_{RP} comparable to the Q_{IDP} values. The BAC also released N only slightly faster than their minimum (Q_{ID} ; Table 5, Fig. 10). A nutrient release rate per unit of C ingested that was well above the lower level was also apparent for P and N in COP, suggesting that although the P content of the COP food was too low to sustain GE_{CM} , C or energy availability or another essential nutrient were more likely to be limiting than mineral P in the present case.

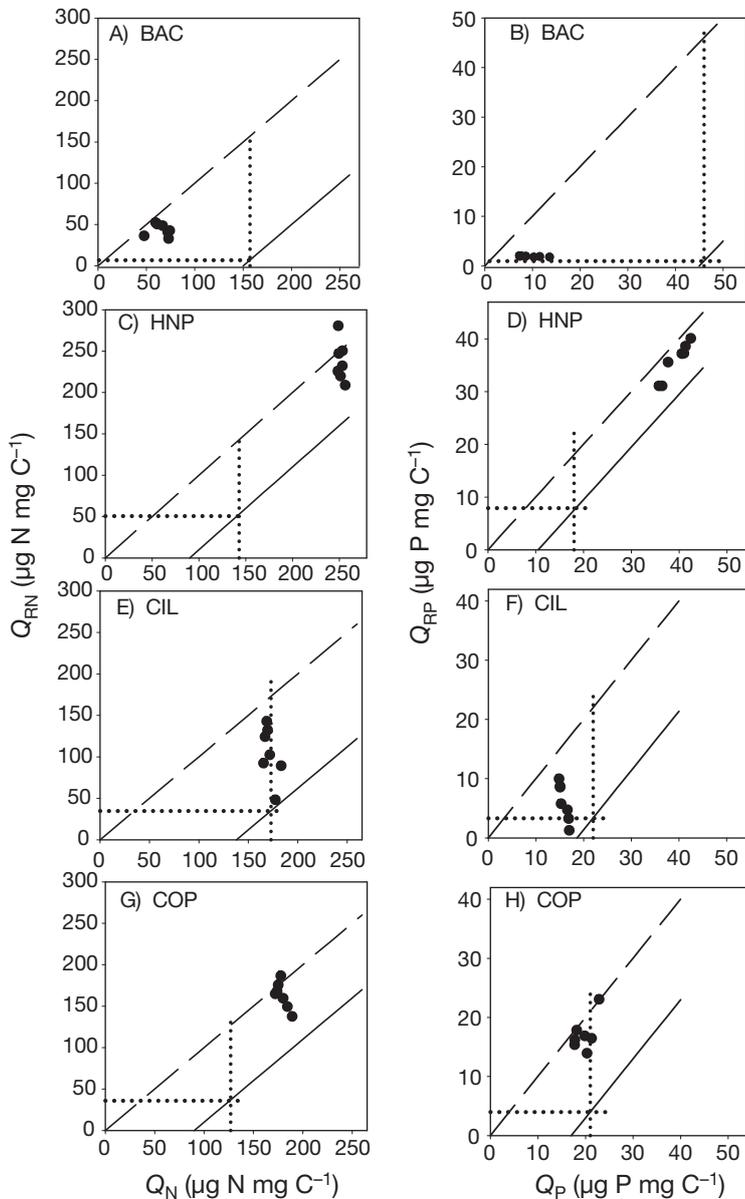


Fig. 10. Data for Q versus Q_R obtained for nutrients N and P for the heterotrophic groups (A,B) BAC, (C,D) HNP, (E,F) CIL, and (G,H) COP inserted in the conceptual Q_R - Q space for evaluating quantitative N and P requirements and the metabolic interactions of P, N and C (cf. Fig. 1B, values in Table 5). The solid line illustrates Eq. (1) ($GE_C = GE_{CM}$) and the dashed line illustrates Eq. (1) for $Q = Q_R$ ($GE_C = 0$). A stable, balanced state of growth can only occur in the Q - Q_R space between the solid and dashed lines. The dotted horizontal line expresses the indigestible content of the nutrient in the food ($Q_R = Q_{ID}$, Eq. 4), and the vertical dotted line describes $Q = Q_{CM}$. Limitation of the specific essential nutrient cannot occur for $Q > Q_{CM}$. Nutrient limitation may occur in the Q - Q_R space between the dotted and dashed lines (cf. Fig. 1B). See Tables 1 & 2 for abbreviations

Based on the evaluation of Questions (1) to (3) above within the conceptual framework presented (Fig. 1), our overall conclusions are:

(1) The HNF that dominated HNP were C- or energy-limited and did not experience N or P limi-

tation ($Q > Q'_{CM}$ for both N and P). C or energy limitation was realised as low specific C ingestion rates (1.1 to 1.9 d^{-1}), low specific growth rates (0.14 to 0.28 d^{-1} ; Olsen et al. 2007), and carbon growth efficiencies that were higher than those of N and P (Fig. 8) throughout. This conclusion was still valid when the Q_P of their food was reduced to the P-subsistence level of bacteria (Q_{0P} ; Vadstein 2000), and agreed with the low response in HNP food following the increased nutrient input (Fig. 3D).

(2) The heterotrophic bacteria were severely P-deficient and this was reflected by the very low GE_C value and the low rate of P release per unit of C consumed (Fig. 8).

(3) The CIL group, dominated by ciliates, also most likely experienced mineral P limitation in the lower range of the nutrient supply rates (Table 5). Important conditions forming the basis for this conclusion were that the CIL biomass responded far faster than the concentration of their food (Fig. 3), and that a low Q_{ZP} was used for the ciliates in our estimation of Q'_{CMP} (31 $\mu g P mg C^{-1}$; Table 2). Our value was lower than the only published values that we are aware of for ciliates (55 to 76 $\mu g P mg C^{-1}$; Wanlian & Xinzhou 1985). Accordingly, the literature provides little information on the Q_{ZP} of ciliates.

(4) Finally, we tentatively conclude that the copepods were most likely limited by an essential nutrient other than N or P, although they were potentially limited by mineral P ($Q_P < Q'_{CMP}$). This was supported by a high Q_{RP} , which makes P limitation more unlikely.

General considerations of the nutrient limitations of heterotrophic plankton

Our inverted Redfield ratios showed values comparable to the cellular contents of N and P in nutrient-saturated phytoplankton (Geider & La Roche 2002). Although

the Redfield C:N:P ratios of 106:16:1 have recently been questioned (Sterner et al. 2008), typical nutrient saturation values for Q_N and Q_P are around 0.17 mg N $mg C^{-1}$ and 24 $\mu g P mg C^{-1}$, respectively, in nutrient-saturated phytoplankton. The lower extreme val-

ues of Q_N and Q_P , reflecting the subsistence quotas (Q_0 ; Table 1), are typically 40% and 20% of the saturated Q_N and Q_P values in nutrient-saturated phytoplankton, respectively (giving $Q_{0N} \sim 70 \mu\text{g N mg C}^{-1}$; $Q_{0P} \sim 5 \mu\text{g P mg C}^{-1}$). The saturation values are only slightly higher than the respective requirements of ciliates and copepods (Fig. 10), whereas the lower Q_0 values are much lower. This means that even moderately nutrient-limited phytoplankton of coastal NE Atlantic water will not necessarily meet the N and P requirements of ciliates and copepods.

We hypothesise that zooplankton feeding on eukaryotic algae in general may experience N, but more likely P, limitation during the summer season. This is particularly the case for ciliates. Both ciliates and copepods may, however, counteract optional P limitation through a selective feeding behaviour, which may contribute to increasing the P intake beyond that obtained from feeding on phytoplankton. Selective feeding on ciliates by copepods is well documented (e.g. Gismervik 2006, Calbet et al. 2007) and can increase the P intake of the copepods. Moreover, studies have indeed suggested that the reproduction rates of the dominant copepods can be limited by the docosahexaenoic acid (22:6 n-3, DHA) content of food algae during the summer season (see Evjemo et al. 2008). Ciliates may consume minor fractions of heterotrophic bacteria (Lawrence & Snyder 1998, Mari et al. 2004). This could mitigate P limitation, but it is an open question whether large herbivorous ciliates (20 to 50 μm) are able to consume significant amounts of bacteria (linkage not represented in Fig. 2).

For marine heterotrophic planktonic bacteria, a significant uptake of inorganic P is the only way to cover their mineral P requirements because dissolved organic matter and potential substrates are far poorer in P (Vadstein 2000) than the Q'_{CMP} estimate for bacteria (Table 5). A fast and efficient uptake of phosphate by bacteria has indeed been documented in many studies, and the importance of P for regulating bacterial growth in both lakes and marine waters has been emphasised by several authors (e.g. Thingstad et al. 1996, Vadstein 2000). The need to take up inorganic N is not that clear because the Q'_{CMN} for bacteria was within the Q_N range of other organisms. The DIN was not taken up by bacteria in the present experiments, but a significant DIN uptake would have been needed to sustain a GE_{CM} of 60%. Our evaluations of heterotrophic bacteria agree with the conclusions of Smith & Prairie (2004). Based on a completely different approach, they found a positive relationship between GE_C and the total P of lake

systems. They found GE_C values in the range of 10 to 52%, with the higher values within the range found in P-rich lake systems and the lower values in P-poor lakes. Their conclusion agrees well with those of our findings, where a reduced GE_C appears to be a consequence of a severe limitation in an essential nutrient.

Carbon versus essential nutrient limitation

The main conclusion of the present paper is that the growth and reproduction of zooplankton is regulated by a balance in the availability of C (energy) and essential nutrients. Only a fairly optimal composition of the supply of these can support the maximal food conversion and growth of a given species. The confusion regarding the highly variable carbon growth efficiencies of heterotrophic plankton (Straille 1997) should be turned around; GE_C is a dependent variable expressing the C utilisation efficiency that can be maintained for the actual availability of the most limiting essential nutrient for growth. A highly dynamic GE_C can then be understood as an organism's ability to continuously balance its energy metabolism and anabolic processes according to the availability of the limiting resource. The ability of microorganisms to adapt their N:P:C stoichiometry during growth under steady-state conditions in continuous cultures under variable extents of nutrient limitation is but one illustration of the ability organisms have to adjust C metabolism in relation to their supply of N and P.

Sterner & Elser (2002) emphasised the fact that C cannot represent the bulk of the potentially limiting essential nutrients that can limit zooplankton growth. We agree, and add that the fundamental physiological mechanisms regulating metabolism and growth in zooplankton are unlikely to be different from those in fish, as revealed from nutritional studies (e.g. DeSilva & Anderson 1995). The essential non-substitutable nutrients of heterotrophic organisms would be better regarded as individual essential resources. This means that the nutritional characteristics of autotrophic and heterotrophic prey organisms of zooplankton will represent a major driving force of species diversity at their trophic level, giving room for a broad diversity of heterotrophic species in plankton communities. The same mechanism has, over the last few decades, been shown to shape phytoplankton communities and remains to be demonstrated for heterotrophic bacteria.

Acknowledgements. This experimental work was undertaken by the Norwegian partners in COMWEB, a project of the ELOISE Programme that was funded by the EC MAST Programme (MAST3-CT96-0052). The Research Council of Norway contributed to the final scientific treatment and publishing. The comprehensive base of the results derived is a result of the hard work of all of the enthusiastic project participants involved in the experiments carried out in Hopavågen, Central Norway, in August and September 1997, and in particular the enthusiastic and hard-working staff at Trondhjem Biological Station. Thanks to IMEDEA (Mallorca, Spain) and our colleagues there for kindly allowing Y.O. to work on this paper in a peaceful environment.

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Editorial responsibility: Matthias Seaman, Oldendorf/Luhe, Germany

*Submitted: March 29, 2011; Accepted: June 15, 2011
Proofs received from author(s): August 22, 2011*