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Quantification of the phosphorus released by zooplankton in an oligotrophic lake (La Caldera, Spain): regulating factors and adjustment to theoretical models

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Abstract. Using an *in situ* approach, we have evaluated the phosphorus inputs from zooplankton in a high-mountain oligotrophic lake. Values of the specific gross release rate (SGRR) fluctuated between 0.2 and 2.9 μg P mg^{-1} dry weight h^{-1}, and were higher when the nauplii of *Mixodiaptomus laciniatus* dominated the zooplankton community. The rate of P recycling by the zooplankton was high, reaching 1.6 μg P l^{-1} day^{-1}, and showed a highly consistent seasonal pattern from one year to the next, with maxima in midsummer. Zooplankton size accounted for as much as 85% of the variance obtained in the measurements of the specific rate of P release, while other factors, such as the quality or quantity of food, did not significantly influence the SGRR changes. Among the models tested, only the one proposed by Peters (*Int. Ver. Theor. Angew. Limnol. Verh.*, **19**, 273–279, 1975) was useful for predictions in this system. The stoichiometric model of Hessen and Andersen (*Arch. Hydrobiol.*, **35**, 111–120, 1992), applied in this oligotrophic system, adequately predicted the phyto- and zooplankton dynamics, whereas the values of P release estimated using this model were far higher than the excretion rates obtained experimentally. These differences were related to the type of egestion (formation of faecal pellets) of metazooplankton and to the relative importance of the food resistance to digestion. We believe that in communities where copepods constitute a substantial percentage of the zooplankton, an evaluation of the P release which is readily available [soluble reactive phosphate (SRP), total dissolved phosphate (TDP)] to algae and bacteria would not fit the predictions of general models of mass balance; under these circumstances, assimilation efficiency proves to be the key parameter for predicting the readily available P (excreted P).

Introduction

The central role of herbivores in the energy flow through the food chain is currently being explored from a new perspective (Hessen, 1992; Sterner and Hessen, 1994). From a biochemical standpoint, zooplankton act as a transformer of material from primary producers (with a relative elemental composition essentially different from that of the consumers) to higher trophic levels. Linked to this transformation process, and due to the differential assimilation of nutrients [carbon (C):nitrogen (N):phosphorus (P)], zooplankton regulate the relative quantity of nutrients released via excretion and thereby change the turnover rate at the lower trophic levels (Elser *et al.*, 1988; Urabe, 1993). Therefore, a precise understanding of the functioning of aquatic ecosystems requires an accurate quantification of the indirect effects mediated by the zooplankton, particularly those expressed in the liberation of limiting nutrients for autotrophic and bacterial heterotrophic production.

In freshwater ecosystems, the importance of the regenerating effect of zooplankton has traditionally been linked to the trophic status of the system. Peters (1975) and Axler *et al.* (1981) have demonstrated the intensity reached by this regeneration process in oligotrophic systems. Nevertheless, more recently, McQueen *et al.* (1986) and Dawidowicz and Gliwicz (1987) have analysed the relative importance of the processes of consumption versus regeneration, and have

proposed that the recycling of nutrients by zooplankton is more intense in eutrophic systems.

The P-release rate from zooplankton is affected by various factors: organism size, ambient temperature (Peters and Rigler, 1973; Scavia and Gardner, 1982; Ejsmont-Karabin, 1984), and food quantity and quality (Lehman and Naumoski, 1984; Olsen *et al.*, 1986). More recently, it has been proposed that, superimposed on these factors, the stoichiometry of the zooplankton may regulate the amount of P released by herbivores (Sterner, 1990). The dominance of *Daphnia* (with a low C:P ratio) would result in low rates of P release, increasing its important suppressive effect (by grazing) on the algal community. On the other hand, calanoids have a high C:P ratio, and would release proportionally more P (Hessen and Andersen, 1992), counterbalancing the negative effect of consumption (Cruz-Pizarro and Carrillo, 1991).

Andersen and Hessen (1991) and Hessen and Lyche (1991), studying different systems under different trophic conditions, showed that the elemental composition of zooplankton has strong interspecific, but only slight intraspecific, variation. These results support the hypothesis of zooplankton homeostasis (Sterner, 1990), which states that the proportion of nutrients released is regulated more by a particular species stoichiometry than by other factors.

The effects of factors regulating P release, such as size (Taylor, 1984) and temperature (Ejsmont-Karabin, 1984), have been well documented, while other factors, such as quality and quantity of food and elemental composition of the zooplankton, have not been completely elucidated. The overall analysis of the effects of these factors, and the relative importance of each, has been carried out only by Urabe (1993), for a eutrophic lake.

The release or excretion rate of P by zooplankton is difficult to quantify, especially for natural populations where P-limited algae or bacteria may immediately consume the released P (Vadstein and Olsen, 1989). For this reason, it is useful to construct models for predicting the quantity of P released. For variables, some models use size and temperature (Peters, 1975), food quality and quantity (Olsen and Ostgaard, 1985) or the relationship between the quality of the food and the elemental composition of the zooplankton (Sterner, 1990; Hessen and Andersen, 1992); predictions of this latter model have only recently been validated by results from Urabe (1993) and Urabe *et al.* (1995).

The primary goal of this study was to measure the *in situ* P-excretion rates during the ice-free period in an oligotrophic lake (La Caldera, Spain). We analysed the effects of endogenous and exogenous factors on the rate of P release. The factors tested were body size of the zooplankton, food concentration, and elemental composition and temperature. Finally, the values predicted from the different existing models proposed were compared to the experimental measurements obtained.

Method

Study site

La Caldera is a small, ultraoligotrophic high-mountain lake located in the Sierra Nevada (southern Spain) at 3050 m a.s.l., in a glacial cirque, on siliceous bedrock.

The lake is fishless, lacks littoral vegetation, is highly transparent (visibility reaching to the bottom for almost 90% of the ice-free period), has no visible surface inlets or outlets and during the time of this study had a maximum depth of 6 m, which diminished to only 3 m by the end of the summer of 1993.

The ice-free growing season usually extends from late June to September, when the lake becomes covered by ~2–4 m of ice and snow. During summer, the lake does not stratify. The water termperature ranges from 4°C after the thaw to a maximum of 15°C in mid-August. With the thaw, a nutrient enrichment (mainly P and ammonium) takes place (Carrillo *et al.*, 1990a), supporting rapid development of the algal populations.

Soluble reactive phosphate was below the detection limit and total P varied between 3 and 10 μg P l^{-1}, while primary production ranged from 1 to 3 mg C m^{-3} h^{-1}. The total dissolved nitrogen (TDN) concentration was from 0.144 to 0.549 mg l^{-1}, mostly as nitrates. Values of the DIN:TP ratio (dissolved inorganic nitrogen: total phosphorus) of >12 were registered throughout the ice-free period, strongly indicating a strict P limitation (Morris and Lewis, 1988). In terms of carbon, the bacterial fraction represents twice (maximum values 47.9 and 56 μg C l^{-1} for 1992 and 1993, respectively) the biomass of the algal fraction (maximum values 38 and 19 μg C l^{-1} for 1992 and 1993, respectively; Reche *et al.*, in press). Such proportions are common in oligotrophic systems (Simon *et al.*, 1992).

Experimental studies

Phosphorus excretion and ingestion rates by zooplankton were determined in seven *in situ* experiments during the summers of 1992 and 1993, requiring a manipulation of zooplankton and/or seston (algae and bacteria). Three treatments were performed in triplicate: zooplankton removed by 40 μm size mesh (treatment C), zooplankton added (treatment Z) and zooplankton added plus non-living $seston_{<40\ \mu m}$ (treatment E, see below; Figure 1).

Each experimental period was characterized by a specific composition, size structure and abundance of zooplankton, which were well documented through previous studies on zooplankton community dynamics (Cruz-Pizarro, 1981; Carrillo *et al.*, 1995).

To determine gross release rates, we carried out treatment E, consisting of 20 l of autoclaved non-filtered lake water to prevent either osmotic changes (which would affect P release of zooplankton) or the P assimilation by algae and bacteria (fraction <40 μm in this lake). To a container, we added zooplankton concentrated from the lake. The zooplankton were sampled at midday at the deepest layers (0.5 m above the lake bottom) because zooplankton in La Caldera undergo pronounced direct diel vertical migration (Cruz-Pizarro, 1978; Carrillo *et al.*, 1991). The zooplankton were collected with a Schindler–Patalas sampler of 32 l equipped with a screen of 40 μm pore size to trap the whole zooplankton assemblage in the lake (rotifers and crustaceans) without adding algae (size <20 μm).

The concentration of zooplankton, which depended on abundance in the lake, fluctuated 10–15 times; in any case, no overcrowding problems arose and the

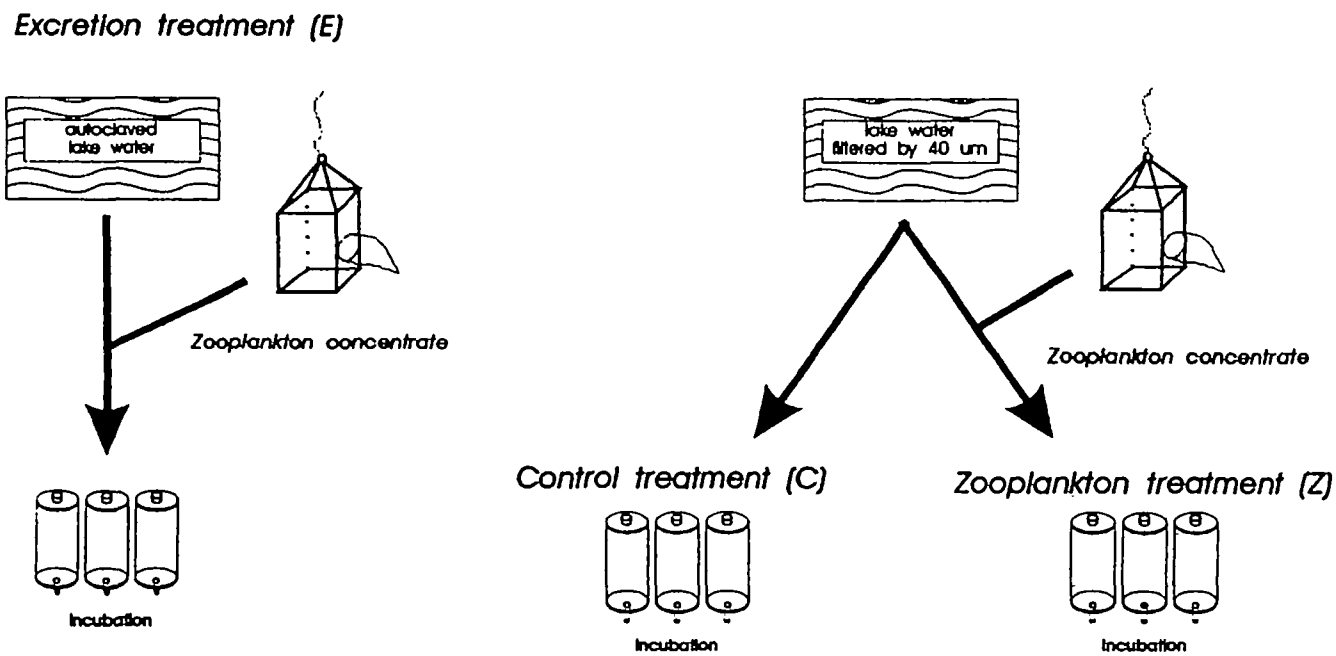

Fig. 1. Diagrammatic summary of treatments in the experimental design.

quantity of excreted P could be measured by the analytical methods used. The zooplankton concentrate was checked for viability and washed to remove accumulated excreted nutrients by replacing the water in which it was suspended. Once the zooplankton were placed in the container (20 l), and after stirring to homogenize the water volume, three transparent polyethylene containers (5 l capacity) were immediately (<3 min) filled.

To evaluate the quantity and quality of food ingested by zooplankton, we used two groups of experimental bottles (three C bottles and three Z bottles). The bottles representing treatment C were prepared by collecting lake water (using a Van Dorn sampler) from the same depth as the zooplankton, pooling the water in large plastic containers after screening through a 40 μm nitex net to remove all zooplankters and filling 5 l transparent polyethylene containers (three replicates). The treatment with zooplankton (Z) followed the same procedure as for the control bottles and in the end we added the zooplankton concentrate following the same procedure as for the excretion bottles (E). From these bottles (Z), we quantified the apparent P-release rates.

The remaining water in each case was used to determine the initial concentration [TP, $TP_{<40\,\mu m}$, total dissolved phosphate (TDP), soluble reactive phosphate (SRP)] of the P as well as the algal and bacterial concentrations (C and Z treatments, only). The bottles were incubated in the water at the depth where the zooplankton were collected. The incubation time (1 h) was determined from previous experiments, in which the quantity of SRP released was related to time, at a constant zooplankton biomass. This incubation time never exceeded the gut passage time, as recommended by Hartmann (1987), and thus the P released corresponded to that which was ingested in the feeding period preceding the experiment. This experimental approach can offer a realistic estimate of the excreted P (SRP and TDP) which is easily attainable by the algae and bacteria. The incubation was stopped by filtering the water through a 40 μm mesh (Z and E treatments). The density and biomass of the zooplankton, the final concentration of total and dissolved P (in all treatments), and algal and bacteria abundance (in C and Z treatment), were measured.

The specific gross release rate (SGRR) or the apparent release rate (SARR) were obtained using the following expression:

$$\text{SGRR or SARR } [\mu g\ P\ mg^{-1}\ \text{dry weight (dw)}\ h^{-1}] = P_t - P_0/W\,t$$

where P_0 and P_t are the concentrations of dissolved nutrients (SRP or TDP) in the excretion (SGRR) or zooplankton bottles (SARR) at the beginning and end of the incubation period (μg $P\ l^{-1}$), W is the dry weight of the zooplankton added (mg l^{-1}) to the excretion and zooplankton bottles, and t is the incubation time (h).

The ingestion rate of zooplankton on seston was estimated as

$$I = \frac{gVC_{z0}e^{(k-g)t} - 1}{N(k-g)t}$$

where k is the net growth rate of seston calculated as:

$$k = \ln(C_t/C_0)/t$$

and g is the grazing coefficient of zooplankton:

$$g = k - \ln(C_{zt}/C_{z0})/t$$

V is the volume of water in the Z bottles (ml), N is the number of animals in the zooplankton bottles, C_{z0} and C_{zt} are the initial and final particle concentrations in the zooplankton bottles (cell $5ml^{-1}$), respectively, and C_0 and C_t are the initial and final particle concentrations in the control bottles (C) (cells ml^{-1}).

This expression is appropriate when the zooplankton feeding rate is a linear function of the food concentration at food densities below a saturation level (the incipient limiting level or ILL), reflecting constant feeding behaviour that increases food intake as concentrations increase. In this case, the ingestion rate is dependent on the ambient food concentration at each moment of the experiment (Tack and Van de Vrie, 1985).

Chemical and biotic analysis

For the determination of zooplankton density and biomass, the samples were identified and counted under an inverted microscope and measured by image analysis. Twenty adults of each species and 20 individuals at each developmental stage (six naupliar and five copepodite instars) of *Mixodiaptomus laciniatus* were measured from every sample. The biomass of crustaceans and rotifers was estimated using standard length–mass relationships (Botrell *et al.*, 1976), or relationships developed specifically for certain species from La Caldera lake (Cruz-Pizarro, 1983; Morales-Baquero *et al.*, 1988). The carbon content in the zooplankton was calculated as 50% of the dry weight (Hessen and Lyche, 1991).

A 50 ml aliquot from phytoplankton samples was sedimented for 48 h in a compound chamber (2 cm in diameter), and cells were counted in 100 randomly selected fields of view at 100× magnification under an inverted microscope, as recommended by Sandgren and Robinson (1984). For every sample, 20 cells of each species were measured by image analysis (Leica Quantimet 500) to estimate the cell volume according to a corresponding geometrical shape. The biovolume density ($\mu m^3\ ml^{-1}$) for each taxon was determined by multiplying the mean cell volume by the population density. Cell volume was converted to carbon using a conversion factor of 0.15 pg C μm^{-3} (Vadstein *et al.*, 1988), which embraces the range of variations published for the phytoplankton species in this system (see Rocha and Duncan, 1985).

Bacterial abundance was determined by epifluorescence microscopy from bacterial subsamples stained with DAPI and filtered onto 0.2 μm black Nuclepore filters (Porter and Feig, 1980). At least 400 cells were counted for each sample. Preparations from the samples were photographed by Kodak T-max film (400 ASA at 30 s exposure time). Bacterial diameters were measured by image analysis (Leica Quantimet 500) on digitized negatives, while cell volumes were calculated by approximating the cell shape. Bacterial biomass (μg C $cell^{-1}$) was obtained using a

conversion factor dependent on the cell volume (Simon and Azam, 1989). The analysis of these filters showed the scarcity of detrital particulate matter and therefore we believe that using the estimates of seston carbon (<40 μm) as the total of bacterial and algal carbon may be adequate in this clear-water system; because of the small size of the bacteria, the use of GF/F filters leads to pronounced underestimates of the bacterial carbon content (Sondergaard and Middelboe, 1993).

To determine the SRP and TDP concentration, we filtered aliquots of the water sample onto a 0.45 μm Sartorius filter after removing zooplankton with 40 μm mesh. The SRP was determined by the ascorbic acid method of Murphy and Riley (1962), using a cuvette with a 10 cm optic range, the detection limit being 0.03 $\mu M\ l^{-1}$. The TP and TDP were determined as SRP after digestion with a mixture of potassium persulphate and boric acid at 120°C for 30 min.

The P in the seston was estimated as the difference between TP and TDP in the control bottles, and as the difference between $TP_{<40\ \mu m}$ and TDP in the Z bottles. Owing to the marked presence of naked cells (Chrysophyceae: *Chromulina nevadensis*), the phytoplankton are highly sensitive to the filtration process, especially since limited abundance of autotrophs requires the filtration of large volumes of sample, accentuating the filtration effects (cell breakage); this procedure proved more appropriate than direct measurement at filters. The P in the zooplankton was obtained as the difference between TP and $TP_{<40\ \mu m}$ in the Z and E bottles.

Data analysis

To assess the relative importance of selected factors (size, food quality and quantity, and temperature) affecting the excretion rate of phosphorus by zooplankton (Y), we carried out a stepwise multiple-regression analysis.

Models tested

Peters' model (1975). This provides the following expression:

$$0.032e^{0.039T}W^{-0.38} \leq SRR \leq 0.079e^{0.039T}W^{-0.38}$$

where SRR is the specific excretion rate of phosphorus ($\mu g\ P\ mg^{-1}\ dw\ h^{-1}$), T is the temperature (°C) and W is the mean individual dry weight (mg), obtained from the mean length measured for each replicate.

The model of Olsen and Ostgaard (1985). The specific release rate was calculated from the data on the quantity and quality of food obtained in the zooplankton treatment, according to the following expression:

$$SRR = \frac{1}{Wt}\left[P_{dzt} - P_{dz0} + \frac{P_{seston\,t}C_{z0} - P_{seston\,0}C_{zt}}{C_{zt} - C_{z0}} \cdot \ln\left(\frac{C_{zt}}{C_{z0}}\right)\right] + \left[K_z \cdot \frac{(P_{seston\,t} + P_{seston\,0})}{2\,W}\right]$$

where W is the zooplankton biomass (mg l^{-1}), t is the incubation time (h), P_{dzt} and P_{dz0} (μg P l^{-1}) are the SRP at the end and the beginning of the experiment, respectively, $P_{seston\ t}$ is the particulate phosphate <40 μm after the incubation, $P_{seston\ 0}$ is the particulate phosphorus <40 μm at the beginning, C_{z0} and C_{zt} are the concentration of food (μg C l^{-1}) at the beginning and end, and K_z is the growth of the organisms which constitute the food (h^{-1}).

Hessen and Andersen model (1992). To estimate the release rate according to this model, we also used zooplankton bottles. In this model, the elemental compositions of the seston and of the zooplankton were related by the following expressions:

$$SRR = \begin{cases} [(1 - K^*)\ Q_z]I_c, \text{ for } Q_s < Q_z \\ [Q_s - K^*\ Q_z]I_c, \text{ for } Q_s > Q_z \end{cases}$$

where K^* is the efficiency of zooplankton growth that we calculated from the values for the production/biomass (day^{-1}) of the *M.laciniatus* population (Cruz-Pizarro, 1983) divided by the specific ingestion of carbon (day^{-1}) (I_c), which considers changes due to the ontogenetic development of *M.laciniatus* (Table V). This growth efficiency appears to be representative of the community as a whole because of the predominance, in this study, of the *M. laciniatus* population. In the equations, Q_s is the P:C ratio of the seston <40 μm (μg P mg^{-1} C) and Q_z is the P:C ratio of the zooplankton (μg P mg^{-1} C).

Results

The zooplankton community was rather simple, with a calanoid copepod (*M.laciniatus*) comprising almost 85% of the total zooplankton density. Rotifers (*Hexarthra bulgarica*) and cladocerans (*Daphnia pulicaria*) were scarcely represented. The maximum density of this community was reached in midsummer (August) for both years (Figure 2A). The dry biomass changed from 2 to 96 μg C l^{-1} and from 5 to 56 μg C l^{-1} in 1992 and 1993, respectively.

The mean individual size was largest in August and September, when the zooplankton assemblage was comprised of mainly copepodites (instars 4 and 5) of *M.laciniatus*, but after the thaw, the reproduction of the first adults that had overwintered at copepodite stages 3 and 4 (Cruz-Pizarro, 1981) caused the mean individual size to decrease, as nauplii predominated (Figure 2B). The scarcity of adults appears to be related to the severe food limitation of this population (Carrillo, 1989; Reche, 1995).

The C:P ratio (by weight) of the zooplankton assemblage showed a quite similar pattern of variation in both years (Figure 2C). The lowest C:P values were measured when the nauplii predominated (July). In August and September, such values were close to the Redfield ratio (between 40 and 50). Values of the herbivore C:P ratio in this calanoid-dominated assemblage were low and related to the high P content (as a percentage of dry weight) of these organisms, particularly when nauplii were present (Figure 2D). In this period, values of the C:P ratio may

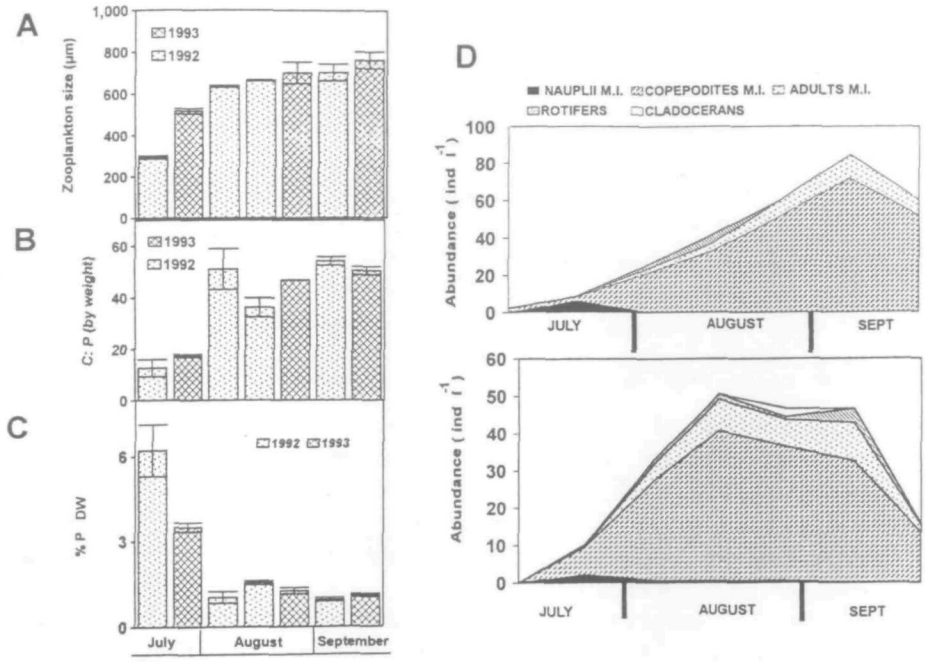

Fig. 2. Changes in the individual mean size of zooplankton (**A**), C:P ratio of zooplankton (**B**), % P of dry weight (**C**), and the abundance and composition of zooplankton during the 2 years studied (**D**).

even be an overestimate if, as Christoffersen *et al.* (1990) proposed, nauplii have a carbon content of around 0.35 µg C ind^{-1}.

The carbon concentration of seston changed from 13.6 to 57.2 µg C l^{-1}. The small size of the algal community (<20 µm) qualified the seston as edible for the zooplankton. Some authors have pointed out that calanoids do not feed on bacteria; however, experimental results (Pedrós-Alió and Brock, 1983), including ours (Reche *et al.*, submitted), confirm that the zooplankton ingest the fraction <1 µm. The quantity of food was below the ILL, and thus the calculation of the ingestion rate assumes that ingestion varies in relation to the quantity of food available. Nevertheless, the relationship established was not linear (Figure 3), suggesting that at these low food levels more complex functions are possible (Porter *et al.*, 1982). The fact that the concentration of seston carbon at the end of the incubation period, in some experiments, was higher than the initial concentration indicates that any suppression through grazing by zooplankton was balanced by stimulation through their P release.

The elemental content of the fraction lower than 40 µm—which included primarily algae and bacteria, given the absence of microfiltering organisms (ciliates) and the scarcity of detritus in this clear water lake—was analysed in the C and Z bottles at the beginning of the experiments. No significant differences (Table I) were found between the two experimental groups, indicating that the addition of zooplankton never increased the seston biomass. The elemental C:P ratio (Table I) of the seston was lower than the C:P ratio of the zooplankton. This result reflects

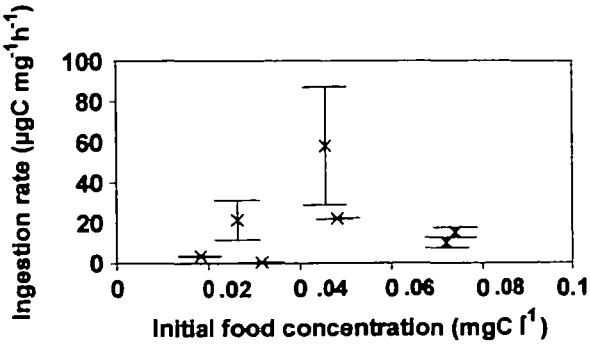

Fig. 3. Specific C ingestion rates versus food C content. Vertical bars show mean SE.

the importance of the bacterial fraction within the seston and because of their high specific content of P (determined by means of the analysis of surplus P in both the algal and bacterial fractions; data in preparation).

The gross release rate of zooplankton per unit of dry body mass changed markedly between dates and ranged from 0.2 to 2.9 µg P mg^{-1} h^{-1}, although a similar pattern persisted between years, with higher values at the beginning of the ice-free period and decreasing steadily to the end of this period (Table IV). The zooplankton released P primarily as SRP, representing (in most of the experiments) >75% of the total dissolved P released. The values of the non-reactive fraction reached 50% (Table II) only in the September 1993 experiment.

Figure 4 compares the apparent P-release rate measured in the zooplankton bottles (seston–zooplankton assemblages) with gross values of the P-release rate. Both values follow a highly consistent relationship between years, and after

Table I. Values for the initial C:P ratio (by weight) of seston in the control and zooplankton treatments, and for the C:P ratio by weight of zooplankton (±SE)

Date	Seston <40 µm		Zooplankton
	Control treatment	Zooplankton treatment	Zooplankton treatment
July 1992	10.8 ± 1.3	12.6 ± 0.7	12.6 ± 3.3
August 1992	23.7 ± 3.1	20.5 ± 2.4	51.0 ± 7.9
August 1992	14.0 ± 1.0	11.6 ± 1.2	36.1 ± 3.7
September 1992	11.1 ± 0.7	10.0 ± 0.2	54.1 ± 1.6
July 1993	8.2 ± 0.2	7.9 ± 0.4	17.3 ± 0.6
August 1993	8.9 ± 0.9	11.7 ± 1.3	46.8 ± 0.0
September 1993	2.1 ± 0.3	2.4 ± 0.2	50.4 ± 1.6

Table II. Zooplankton specific release rate of TDP and of SRP expressed in µm P mg^{-1} l^{-1}

	13 July 1992	8 Aug. 1992	11 Aug. 1992	14 Sept. 1992	11 July 1993	11 Aug. 1993	13 Sept. 1993
TDP	3.9	1.7	0.6	0.3	1.8	0.3	0.8
SRP	2.9	1.5	0.6	0.2	2.3	0.3	0.3

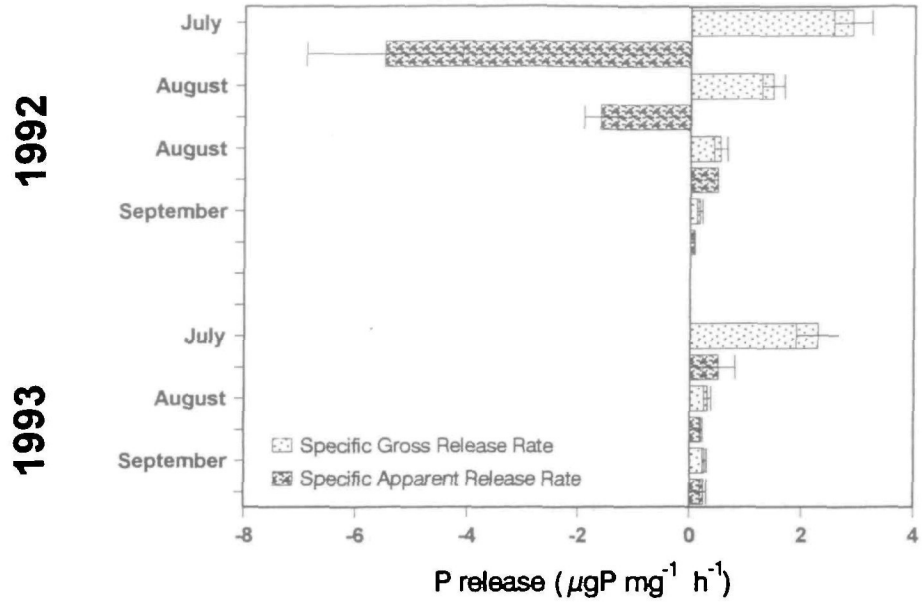

Fig. 4. Comparison between gross and apparent P-release rates in La Caldera lake.

ice-melt the apparent release rate shows lower (even negative) values, implying a high seston assimilation of released P. However, at the end of the ice-free period, the apparent and gross rates show no significant differences, revealing that in this period the seston fraction is probably P saturated, and in agreement with the lower C:P ratio measured for the seston in this experiment (Table I). The extremely low values of the C:P ratio in September 1993 are apparently related to the solubilization of the P near the sediment. This process may take place due to strong winds that thoroughly mixed the water column, which during this period does not exceed 3 m in depth.

The P released by the zooplankton (μg P m^{-3} h^{-1}), available for other trophic levels (phytoplankton and bacteria), could be considered from a trophic perspective as being more important than the specific rates themselves. The general pattern (Figure 5) showed the lowest values (10–20 μg P m^{-3} h^{-1}) in July and the highest (36–66 μg P m^{3} h^{-1}) in August, when the zooplankton biomass is highest. These P inputs did not reflect the values of reactive soluble P measured in the water, thus suggesting rapid algal and/or bacterial uptake.

The results of the stepwise regression analysis performed to determine the relative importance of the different factors with the rate of P release are shown in Table III. More than 85% of the variance in the release rates can be explained by zooplankton body size. Food quantity was not statistically significant. Walz (1987) and Conde-Porcuna *et al.* (1994) have pointed out that the effect of food quantity on different demographic parameters is better reflected by the ratio of food concentration to zooplankton biomass. Nevertheless, a second analysis (Table III), which includes this new variable, did not improve the result. Figure 6 shows the

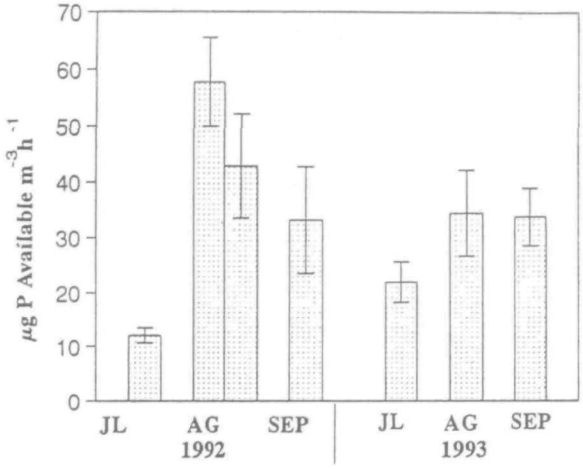

Fig. 5. Inputs of P to the lake from zooplankton excretion.

function which was established in this system between the size and specific rate of excretion of the zooplankton over the ice-free period.

Table IV presents the results of both the P-release rate obtained experimentally and those predicted by the models of Peters (1975), Olsen and Ostgaard (1985), and Hessen and Andersen (1992). The values estimated by these latter two models are significantly different from those obtained in our experiments, even though the growth efficiency of the zooplankton used in the Hessen and Andersen model was calculated specifically for the dominant population (*M.laciniatus*; Table V).

Nevertheless, as might be expected, due to the important effect of size on the variability of the specific excretion rates, our results fall within the limits established by the application of the Peters model. It is noteworthy that over the ice-free period, and in July of both years, the SGRR was closer to the upper limit predicted by the model, while at the end of the ice-free period the values were closer to the lower limit.

Discussion

C:P ratio in seston and zooplankton. Phosphorus excretion rates

The values of the sestonic C:P ratio in La Caldera lake approach the lower limit described in the literature (Hecky *et al.*, 1993; Urabe, 1993). A likely explanation may relate to the fact that most comparable studies so far carried out

Table III. Results of the stepwise regression analysis for the zooplankton P specific release rate (in bold considering the food quantity:zooplankton biomass ratio). R^2 is the multiple coefficient of determination and F is the variance ratio of the multiple regression

Variables	Multiple R^2		$F_{(4,16)}$	
Size	0.797		74.6***	
Temperature	0.805		0.7	
Food quantity (FQ/ZB)	0.819	**0.829**	1.3	**2.4**
Food quality	0.819	0.829	0.0	0.0

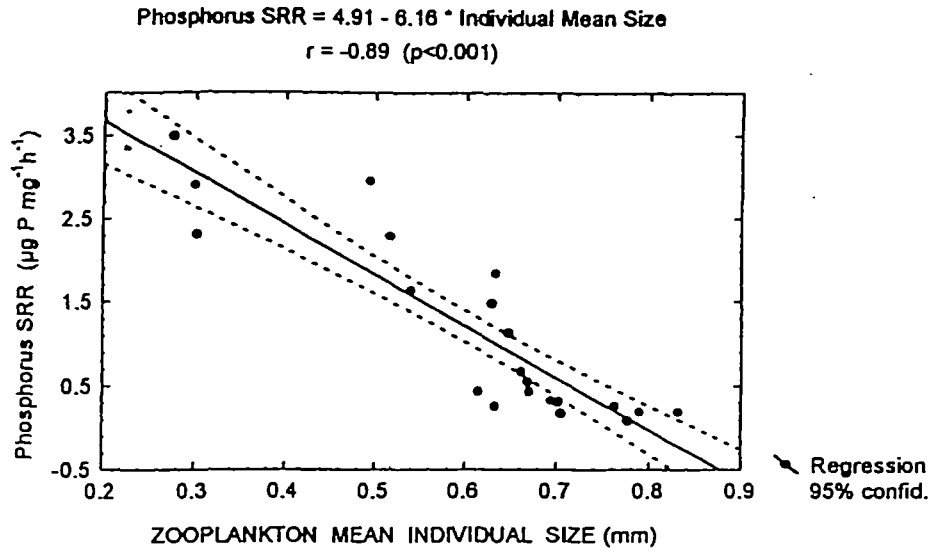

Fig. 6. Relationship between specific P-release rate and zooplankton individual mean size.

underestimate the bacterial fraction of the seston (because bacteria pass through GF/F filters) and thus underestimate the P content (because of the low C:P ratios). Despite this, our results are similar to those obtained by Hessen and Lyche (1991) for certain periods in the annual cycle.

Table IV. Experimental and model-derived estimated values of the P specific release rate

Date	Experimental (µg P mg^{-1} h^{-1})	Peters (1975)		Olsen and Ostgaard (1985)	Hessen and Andersen (1992)
		Upper limit	Lower limit		
13 July 1992	2.9 ± 0.3	3.98	0.016	11.8 ± 2.9	5.02 ± 2.54
8 Aug. 1992	1.5 ± 0.2	1.81	0.43	−1.5 ± 0.4	5.54 ± 1.35
11 Aug. 1992	0.6 ± 0.1	1.65	0.41	−0.5 ± 0.5	17.6 ± 4.16
14 Sept. 1992	0.2 ± 0.1	1.4	0.37	0.1 ± 0.1	4.74 ± 6.45
11 July 1993	2.3 ± 0.4	2.26	0.59	5.0 ± 1.1	22.0 ± 9.8
11 Aug. 1993	0.3 ± 0.1	1.54	0.39	0.1 ± 0.1	13.7 ± 2.34
13 Sept. 1993	0.3 ± 0.0	1.1	0.33	<0.001	149.0 ± 15.0

Table V. Values of the growth efficiency (K) of *M.laciniatus* during the ice-free period of 1992 and 1993. P/B is the production/biomass ratio from Cruz-Pizarro (1981) and I_c (specific ingestion of carbon) is from this study

Date	I_c (day^{-1})	P/B (day^{-1})	K
July 1992	3.27	1.60	0.49
August 1992	3.65	1.46	0.40
August 1992	5.19	0.49	0.09
September 1992	1.32	0.07	0.05
July 1993	5.64	1.50	0.27
August 1993	4.64	1.31	0.32
September 1993	18.98	0.08	0.004

The values of the zooplankton C:P ratio are also lower than expected for a community dominated by a calanoid copepod (see Andersen and Hessen, 1991; Hessen and Lyche, 1991; Urabe, 1993). We believe that this is due primarily to the high P content of the organisms in this system (especially in July when nauplii dominate), which may be related to an accumulation in lipids in membrane, probably as an adaptation to photodamage through UV and near-UV stress (Roberfroid and Buc-Calderon, 1994).

The values obtained for the rates of excretion in the present work fit very closely with those established for zooplankton groups dominated by calanoids and oligotrophic systems in experiments similar to ours (LaRow *et al.*, 1975; Korstad, 1983). Our results are also quite close to those of Peters and Rigler (1973), Peters (1975), Lehman (1980), Naumoski and Lehman (1984), Ejsmont-Karabin (1984) and Hartmann (1987), despite different experimental approaches (radiotracers or nutrient enrichment) and taxonomic differences (cladocerans).

The zooplankton in this lake released P primarily as SRP, as other authors (Peters and Lean, 1973; Ferrante, 1976) have already found. Phosphorus was released in organic form (TDP) only in September 1993, coinciding with a reduction of the mean individual size of the bacteria (to 0.41 ± 0.03 μm). This TDP increase in the products released may be related to the P content of bacteria which passed through the filter (0.45 μm mesh size).

Factors which determine the P excretion rates

Previous authors have suggested that the P-release rate is affected by different factors (Peters and Rigler, 1973; Scavia and Gardner, 1982; Ejsmont-Karabin, 1984; Lehman and Naumoski, 1984; Olsen *et al.*, 1986), but the relative importance of these remained unclear. Urabe (1993) has shown that the food quality (under conditions not limited by carbon) accounts for most of the variability shown by the P-release rates. Our results demonstrate that under conditions of food limitation for zooplankton, the release rates are affected mainly by zooplankton size, and that the other factors considered were not identified as significant in the determination of the P-release rates.

The effect of body size on P release is not related to taxonomic changes, but rather to changes in the demographic status of the dominant population (*M.laciniatus*). The slight effect on the release rates exerted by the other factors analysed may be due primarily to the narrow margin of variation in these parameters. The limited carbon availability caused feeding rates to show a non-linear relationship with seston carbon, as might be expected when the zooplankton feeding is below some threshold (Porter *et al.*, 1982). The functional response of zooplankton seems to be more complex, since the direct and indirect effects of the zooplankton on the seston prove to be strongly linked (Carrillo *et al.*, 1990b, 1991, 1995). Except in the July 1992 experiment, the C:P ratio of seston was lower than the elemental ratios of the zooplankton. We believe that, in these low-productivity systems (Reche *et al.*, in press), a robust interaction is established between the zooplankton and its food so that the seston, strongly limited by P availability (DIN:TP > 12), captured (bacterial strategy) and/or stored (algal

strategy) a high proportion of P recycled by the calanoids and, therefore, showed a low C:P ratio.

Finally, it is known that the relationship between the P-excretion rates and temperature is exponential within a temperature range of 10 and 35°C (Ejsmont-Karabin, 1984). Nevertheless, given the narrow range of temperature variation (6°C) in these experiments, this variable does not significantly explain any of the variability in excretion rates.

Adjustment to theoretical models

Of the models tested, the only one that includes the results obtained experimentally was the Peters model (1975). Undoubtedly, this predictability is linked to the close relationship between size and specific release rate (Figure 5). It is noteworthy that our experimental results approach the lower limit just when, as proposed by Peters (1975), the zooplankton are under conditions of severe food shortage, and approach the upper limit when food availability in relation to predator biomass is highest in La Caldera lake (July).

The validity and robustness of the Peters model determined its wide application in evaluating the P release, both during the substitution of zooplankton species induced by changes at upper trophic levels (e.g. fish: Bartell, 1981; Siegfried, 1987) as well as in relation to the differential migratory movements of different metazooplankton species over the water column (Dini *et al.*, 1987). Similarly, the evaluation of the P released into the system, estimated using the Peters model (Carrillo *et al.*, 1995) and that experimentally determined in the present work, fully coincide.

The lack of predictability and limited applicability of the model proposed by Olsen and Ostgaard (1985) appear to be the result of the model being formulated on a series of restrictions which are difficult to fulfil in nature. That is, the zooplankton must feed on concentrations of food above the incipient threshold of limitation, and the growth of the fraction constituting the food should be insignificant or constant. Therefore, this model is difficult to apply, especially in oligotrophic systems, where the quantity of food generally controls the zooplankton production (Lampert, 1988).

The model of Hessen and Andersen (1992) establishes a relationship between the C:P ratio of the seston and that of the zooplankton, although, within the seston fraction, only the algal fraction is effectively considered. It is possible that the differences between the values for P release estimated from this model and those obtained experimentally are due to the fact that our seston fraction includes bacterial P. We believe that it is correct to consider the bacteria, because these can be an important food source for the zooplankton (Hessen and Andersen, 1990) and, moreover, these organisms have a high specific P content (Tezuka, 1990). To include the bacteria in the seston fraction changes the relationship between the P:C ratio of food and that of the zooplankton, and does not suggest that zooplankton are limited by P. On this point, we propose that, since the P availability in the food is high, its use by the zooplankton may not be efficient. Traditionally, it has been believed that this fraction, when less than 1 μm, is not consumed by calanoid copepods. Nevertheless, recent work (Bremmer and Overbeck, 1994)

and our experimental data (Reche *et al.*, submitted) show strong grazing pressure on this group, which in terms of carbon represents almost 75% of the available carbon (Reche, 1995) and, given that zooplankton are strongly limited by this nutrient, the consumption of bacteria appears to be an alternative route.

On the other hand, Hessen and Andersen (1992) have specified that this model should work in communities dominated by cladocerans (which should be expected in an oligotrophic system without fish) and that the scarcity of data on the constancy of the C:P ratio in calanoids causes the predictions of the model to be uncertain in communities dominated by these organisms. To correct the possible differences linked to variations of the growth efficiency (K) for this community, we have estimated the K for *M.laciniatus*, which often differs from the value of 0.6 obtained by Olsen *et al.* (1986) for *Daphnia* populations. Despite these considerations, this model and our results still differ. The differences may originate from the variability of the C:P ratio in this community over the ice-free period.

It is paradoxical that the estimates for released P from Hessen and Andersen's model differed so significantly from those measured experimentally, although their predictions, in relation to the composition and dynamics of phyto- and zooplankton communities, coincided with those in the lake. Hessen and Andersen (1992) have proposed that in systems limited by P, the dominant community is composed of calanoids with a high N:P ratio, implying a high degree of P recycling. This process leads to the establishment of a phytoplankton community with a low N:P ratio, as occurs with the Cyanophyceae which dominate the phytoplankton in midsummer in La Caldera lake (Carrillo *et al.*, 1995). Similarly, the values of the ratio for released P:ingested C (dependent variable), in relation to µg P in the food/mg C in the food (independent variable) remain within the predictions of the model, except for the results of September 1993.

It is notable that the values obtained experimentally are significantly lower than those estimated from the Hessen and Andersen model. This fact is understandable from the perspective that the available P in the food is ingested, but not digested, and is released within the organic structures (bacteria and algae), possibly trapped in faecal pellets, which require a long period to return completely to the dissolved phase. In this sense, on a short-term scale, the faecal pellets would act as a sink, taking P from the water column. However, the particulate organic matter (POM) constituting the faecal pellets in this lake includes numerous live cyanophycean cells which take advantage of their passage through the gut to enrich themselves on nutrients and increase either in size after defecation (Carrillo, 1989; Reche, 1995) or in abundance over a greater time scale (Carrillo *et al.*, 1995). A mutualist strategy of this type could be followed by some bacteria (Vaque, personal communication).

In view of all the above, the model proposed by Hessen and Andersen (1992) appears not to be suitable. These authors assume that ingested P is incorporated into growth or remineralized (faeces, excretion products) and that the liberation of undigested POM is insignificant. This assumption is not appropriate for metazooplankton in general and less so when the copepods constitute a significant fraction (marine systems and freshwater during certain periods), due to the prolific production of faecal pellets even from the developmental phase N 3 (Green *et al.*,

1992). It is known that, in faecal pellets, there is a high proportion of refractory P (Sterner, 1990) which is not immediately available for algal or bacterial uptake.

The models of Hessen and Andersen (1992) and of Sterner (1990), based upon P availability in the food and upon the elemental ratio of the zooplankton, overestimated the released P in these cases. Therefore, we believe that it may be more appropriate to calculate the rate of nutrient excretion (on the basis of an elemental balance), assuming that the material ingested is assimilated by the organisms and afterwards used for growth and metabolism. The models can be rewritten for a particular element like P:

$$E_p = AE_p \times I_p - G_p \quad (1)$$

where AE_p is the assimilation efficiency of P (0–1), I_p is the P ingestion rate and G_p is the growth rate in terms of P.

The P ingestion rate is the product of I_c (carbon ingestion rate, mg C mg^{-1} dw day^{-1}) and the P:C ratio of food:

$$I_p = I_c \times P{:}C_{seston} \quad (2)$$

and G_p is calculated as the product of carbon growth rate (mg C mg^{-1} dw day^{-1}) (G_c) and P:C ratio of zooplankton:

$$G_p = G_c \times P{:}C_{zooplankton} \quad (3)$$

Substituting in equation (1):

$$E_p = AE_p \times I_c \times P{:}C_{seston} - G_c \times P{:}C_{zooplankton} \quad (4)$$

In our opinion, these considerations are also valid for communities in which cladocerans constitute the majority fraction of the metazooplankton, given that, as Urabe (1993) shows, the values of released nutrients obtained from the Sterner model (1990) are closer to the experimental ones when related to the eliminated N:P ratio than when related to the N:P ratio in seston in the Funada-like pond of eutrophic systems dominated by cladocerans. Urabe (1993) has shown that the elimination rate is close to the assimilation rate when the faeces persist as particles for a long time, and further shows that the models of Sterner (1990) and of Hessen and Andersen (1992) take into account only the soluble P fraction, which is not always the majority one.

Our results for P release largely approach the excretion rates in the strict sense, and this idea is supported by the high predictability of the Peters model, which is formulated expressly to quantify this fraction of P release.

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