Field evidence for stoichiometric relationships between zooplankton and N and P availability in a shallow calcareous lake

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SUMMARY

1. According to stoichiometric theory, zooplankters have a species-specific elemental composition. Daphniids have a relatively high phosphorus concentration in their tissues and copepods high nitrogen. Daphniids should, therefore, be more sensitive to phosphorus limitation and copepods more sensitive to nitrogen. A 2-year study of a shallow marl lake in the west of Ireland investigated whether population fluctuations of the two dominant taxa, *Daphnia* spp. and the calanoid *Eudiaptomus gracilis*, were associated with the availability of phosphorus and nitrogen.

2. In accordance with stoichiometric predictions, *Daphnia* and *Eudiaptomus* reproduction had contrasting relationships with dietary phosphorus and nitrogen availability. Egg production by *Daphnia* was negatively associated with the ratio of dissolved inorganic nitrogen (DIN) : total phosphorus (TP) and the ratio of light to TP which was used as an indirect index for seston carbon (C) : phosphorus (P). Conversely calanoid egg production had a positive relationship with the DIN : TP ratio and was unrelated to the estimated C : P (light : TP) ratio.

3. *Daphnia* biomass was not, however, correlated with phosphorus availability, and neither was calanoid biomass correlated with nitrogen. The high ratio of DIN : TP when *Daphnia* dominated the zooplankton biomass and the low ratio when calanoids dominated, is consistent with *Daphnia* acting as a sink for phosphorus and calanoids as a sink for nitrogen and suggests consumer-driven nutrient recycling.

Keywords: consumer-driven nutrient recycling, *Daphnia*, *Eudiaptomus*, nutrient limitation, stoichiometry

Introduction

Stoichiometric theory predicts contrasting response among zooplankton species to nutrient limitation, because there are large inter-species differences in cellular carbon (C) : nitrogen (N) : phosphorus (P) ratio (Sterner & Hessen, 1994). These differences affect competition among species (Schulz & Sterner, 1999; Conde-Porcuna, 2000) and relative rates of nutrient regeneration (Olsen *et al.*, 1986; Elser *et al.*, 1988).

Some taxa, such as daphniids (Andersen & Hessen, 1991) and rotifers (Conde-Porcuna, 2000; Jensen & Verschoor, 2004), are more susceptible to P limitation, owing to their high cellular P content, than taxa with lower cellular P contents, such as *Bosmina* (Urabe & Watanabe, 1992; Schulz & Sterner, 1999). Copepods, which have high cellular N : P ratio (Sterner & Hessen, 1994; Sterner, 1998), are more likely to be sensitive to deficiencies in dietary N (Kiørboe, 1989; Hessen, 1992; Van Nieuwerburgh, Wänstrand & Snoeijs, 2004).

Several laboratory studies have shown that deficiencies in N and, particularly in P, reduce the quality of algal food for *Daphnia* (Sterner *et al.*, 1993;

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Weers & Gulati, 1997; Urabe & Sterner, 2001), *Bosmina* (Schulz & Sterner, 1999) and rotifers (Conde-Porcuna, 2000; Jensen & Verschoor, 2004). Direct addition of phosphorus to P-limited food can stimulate somatic growth of *Daphnia* (Boersma, 2000; DeMott, Gulati & Van Donk, 2001; Boersma & Kreutzer, 2002) and isotopic analysis has shown a high assimilation efficiency of P in *Daphnia* grazing on P-deficient food (DeMott, Gulati & Siewertsen, 1998). Poor food quality has also been associated with a reduction in the content of some essential highly unsaturated fatty acids in algal cells (HUFAs; Müller-Navarra *et al.*, 2000; Ferrão-Filho *et al.*, 2003) and structural changes, such as thickened cell walls of algae, resulting in decreased digestibility (Lürling & Van Donk, 1997; Van Donk *et al.*, 1997). DeMott (1998) showed that the growth and reproduction of daphniids increased substantially on the addition of *Synechococcus* (high P, low fatty acid content) to *Scenedesmus* (low P, low fatty acid content) food. Consequently, direct mineral limitation is likely to be of substantial importance for the growth of zooplankton herbivores.

Previous work on nutrient limitation of the zooplankton has focussed on individual growth, with fewer investigations of the relationship between nutrient limitation of resources and zooplankton community composition and population dynamics (Gulati & DeMott, 1997). Most field investigations that have analysed the effects of nutrient ratios on zooplankton populations (Hessen, 1992; Hassett *et al.*, 1997; Sterner *et al.*, 1997; Brett, Müller-Navarra & Park, 2000) have provided evidence for nutrient limitation, particularly of P, in natural zooplankton communities. A threshold of food C : P ratio calculated for *Daphnia* growth and reproduction (Urabe & Watanabe, 1992; Sterner & Hessen, 1994; Brett *et al.*, 2000; Anderson & Hessen, 2005) is usually considered to occur in the region of 200–350 M; above which daphniid production is believed to be P-limited, provided that other limiting factors are not involved. Nevertheless, the interplay between individual growth and seasonal trends in zooplankton community structure is unclear. Investigations examining the relationship between seasonal nutrient availability and zooplankton community structure and fecundity are scarce (DeMott *et al.*, 2001; Conde-Porcuna, Ramos-Rodríguez & Pérez-Martínez, 2002; Scheuerell *et al.*, 2002).

Seasonal patterns in the quantity and quality of phytoplankton affect fecundity and growth of zooplankton (Brett *et al.*, 2000), with at least some zooplankton (e.g. *Daphnia*) capable of rapid responses to fluctuating food supply (George & Reynolds, 1997). Temporal variations in the supply of nutrients, as well as alterations in the physical environment such as temperature and day length (Sterner *et al.*, 1997), alter nutrient ratios in algae, while top-down effects of predation (Brooks & Dodson, 1965) operate concurrently to influence the overall structure and composition of the zooplankton.

Here we assess whether, in accordance with stoichiometric theory, *Daphnia* is more sensitive than the calanoid copepod *Eudiaptomus gracilis* (Sars) to fluctuations in dietary P. Correlation and stepwise regression analyses were used to examine the relationship between seasonal fluctuations in the physical, chemical and biotic environment and zooplankton community structure, biomass and fecundity.

Methods

Study area

Lough Carra (Fig. 1) is a marl lake in the west of Ireland (53°42′N, 09°15′W), with a 114 km² catchment underlain by carboniferous limestone. It has a surface area of approximately 16.1 km², a mean depth of 1.75 m, a maximum depth of 18 m and a volume of 25.2×10^6 m³. It is a well-mixed lake with no evidence of thermal stratification during summer. The lake consists of three well-defined basins; to the north the Castleburke Basin and to the south the Twin Island Basin. Between these lies the Castlecarra Basin. One sampling site was located in each of the three basins, referred to as the North, South and Mid sites, respectively. Sampling took place fortnightly between April 2001 and October 2002, and then monthly until July 2003.

The catchment is dominated by grassland used for grazing both sheep and cattle. The lake is managed as a brown trout (*Salmo trutta* L.) fishery and other important fish include pike (*Esox lucius* L.), perch (*Perca fluviatilis* L.), eels (*Anguilla anguilla* L.), minnows (*Phoximus phoximus* L.), three-spined sticklebacks (*Gasterosteus aculeatus* L.) and ten-spined sticklebacks (*Pygosteus pungitius* L.) (McGarrigle & Champ, 1999).
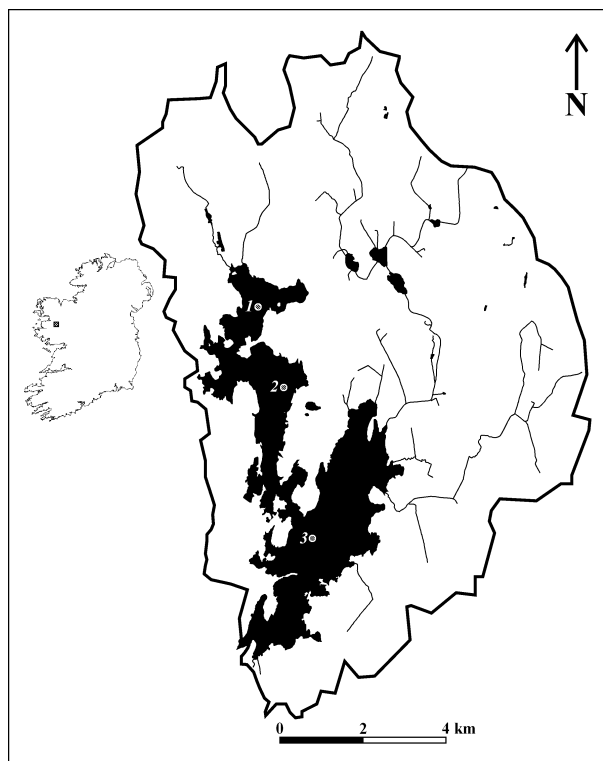
Fig. 1 Lough Carra and surrounding catchment, showing the three sampling sites; Castleburke = North (1), Castlecarra = Mid (2), Twin Island basin = South (3).

Limnological data

A geographical positioning system (GPS; Garmin® GPS 12, Garmin International Inc., Kansas, KS, U.S.A.) and an Echo Sounder (Scubapro® PDS-2, Scubapro-UWATEC, Hampshire, U.K.) were used to locate sampling sites and estimate the depth from which a zooplankton vertical haul was taken. Vertically integrated samples of the water column were taken using a plastic tube 5 cm in diameter and 6 m in length. Conductivity, pH and temperature were measured using WTW® meters (WTW, Weilheim, Germany). Oxygen and temperature profiles were taken during the summer months with a YSI® oximeter (Model 52; YSI Inc., Yellow Springs, OH, U.S.A.). Secchi depth was also recorded.

Dissolved nutrient analyses [soluble reactive phosphorus (SRP), total dissolved phosphorus (TDP) and dissolved inorganic nitrogen (DIN) were made on water filtered through 0.45 μm Whatman® membrane filters (Whatman plc., Middlesex, U.K.). Total phosphorus (TP), TDP and total nitrogen (TN) were analysed after digestion under pressure with potassium peroxide sulphate ($K_2S_2O_8$). Phosphorus concentrations were determined using spectrophotometer absorbance at 882 nm following Eisenreich, Bannerman & Armstrong (1975). Nitrogen was analysed using a continuous flow auto-analyser (AutoAnalyzer 3, Bran+Luebbe, Hamburg, Germany), following Grasshoff, Kleming & Ehrhardt (1999). Particulate phosphorus (PP) and particulate nitrogen (PN) were estimated as the difference between total P or N and the dissolved fractions of P or N. Quality control samples of known concentration were used in all the above analyses and were within acceptable ranges (±3%) in each case.

Phosphorus availability for zooplankton in the lake was assessed using an estimation of the seston C : P ratio, obtained by approximating the ratio of light : TP in the water column. Changes in the ratio of the availability of light to nutrient supply is thought to control nutrient use efficiency in autotrophs. Increased light results in greater C fixation, thereby increasing the amount of C relative to P in algae (Sterner *et al.*, 1997). The ratio of light : TP, as an index for the C : P ratio, has previously been applied to the prediction of zooplankton dynamics by Scheuerell *et al.* (2002), and involved estimating the mean light intensity in the mixed layer as a fraction of the surface light. As Lough Carra is a shallow lake that rarely if ever stratifies, the mixed layer was estimated as the depth of the water column at the time of sampling. On four occasions between April and September 2005, the C, N and P content of seston and zooplankton from the North basin of Lough Carra was analysed. The samples for seston nutrient content determination were collected using the 6 m integrated tube and passed through a 53 μm mesh to remove zooplankton. Subsamples were then filtered through precombusted (550 °C) acid-washed Whatman® GF/F filters (Whatman plc., Middlesex, U.K.). Filters were dried at 60 °C and weighed. Carbon and nitrogen were measured in triplicate using a LECO® – CNS analyser (LECO Corporation, St. Joseph, MI, U.S.A.). Phosphorus analyses was carried out in triplicate and determined spectrophotometrically by the ascorbate-reduced molybdenum-blue method following combustion at 550 °C for 2 h and digestion with potassium peroxide sulphate ($K_2S_2O_8$) under pressure (Eisenreich, Bannerman & Armstrong, 1975). Zooplankton was collected using a zooplankton net with a 53 μm mesh size and

analysed for C, N and P content following the same procedures as those described above for seston. All measurements for C, N and P were carried out using controls lacking zooplankton or seston.

Chlorophyll *a* analyses were carried out on 1 L triplicate samples from each integrated water sample, filtered through Whatman® GF/C filters and extracted with methanol (Standing Committee of Analysts, 1980), with absorbance read in a spectrophotometer at 665 and 750 nm in a 4 cm cell. Alkalinity was analysed on a 50 mL unfiltered sample of lake water by titration according to Mackereth, Heron & Talling (1978). Suspended particulate organic matter was obtained following Allen (1989). True colour (measured after filtration through Whatman® GF/C filters) and turbidity were measured using a Hach® DR2000 spectrometer (Hach Company, Loveland, CO, U.S.A.).

Single 120-mL subsamples of the well-mixed integrated water samples were taken and preserved with Lugol's Iodine for phytoplankton analysis. Phytoplankton samples from the North and South basins, collected from July 2002 to July 2003, were counted and identified across a transect of a circular chamber (25 mL) using an inverted microscope.

Zooplankton

Zooplankton was collected using a 53 µm mesh net (diameter 0.25 m) with a flow meter attached. The net was lowered to a depth of 10 m and drawn vertically through the water column at an approximate speed of 1 m s^{-1}. Until April 2002 a single haul was taken from each of the three sampling sites and, thereafter, four hauls were taken at each site. The zooplankton was narcotised with chloroform water, to prevent ejection of eggs, prior to being stored in industrial methylated spirits. An estimate of the distance towed through the water (*D*) was obtained using a precalibrated flow meter, and the volume of water (*V*) filtered was calculated from $V = D\pi r^2$, where *r* is the radius of the opening of the net.

Zooplankton species were identified following Scourfield & Harding (1966), Harding & Smith (1974), Ruttner-Kolisko (1974), Pontin (1978), Reddy (1994), Dussart & Defaye (1995) and Einsle (1996), and counted in a 5 mL grooved circular perspex disc (Jones, 1979). The mean of four well-mixed 5 mL subsamples taken from a known volume using a wide-bore pipette (Bottrell *et al.*, 1976) was used to estimate zooplankton abundance. A further subsample, of sufficient volume to obtain a suitable count size, was removed from each of the four replicates and pooled. Lengths of the first 50 rotifers encountered in the pooled samples and the lengths of crustaceans including copepod nauplii, up to a minimum of 50 post-nauplier individuals, were measured using a calibrated ocular micrometer. Copepod body-length was measured from the top of the head to the base of the furci rami. Cladocerans were measured from the top of the head to the tip of the abdomen, not including spines or projections. Calanoid and *Daphnia* abundance was converted to biomass using published regression formulae relating length to weight calculated for individuals from Lough Carra in May 2001 (de Eyto & Irvine, 2005). For other crustacean taxa, formulae were taken from Bottrell *et al.* (1976), Rosen (1981) and Dumont, Van de Velde & Dumont (1975). Rotifer abundance was converted to biomass by converting volume to fresh weight assuming a specific gravity of 1, following Ruttner-Kolisko (1977). Fresh weight was converted to dry weight using values calculated by Pauli (1989). If the specific value was unavailable, a ratio of 0.10 was assumed.

On each sampling date from January 2002 to January 2003, a minimum of 50 individuals of both *Daphnia* and *Eudiaptomus* were scored for length and number of eggs. The length of the smallest egg-bearing female was used as an estimate of the minimum size at maturity and all individuals of an equal or greater size were assumed to be mature. The number of eggs per mature female was calculated for both taxa. As this parameter in *Daphnia* depends directly on carapace length, on each sampling date a regression line was calculated to define the relationship between the number of eggs and the body length of egg bearing females according to Vijverberg, Koelewijn & van Densen (2005). The resulting regression equations were used to calculate the standard egg number (SEN) on each sample date, defined as the clutch size for a female of standard length (1.5 mm).

Statistical analyses

Pearson's product-moment and Spearman's rank correlations and multiple regression analyses were carried out using SPSS® Version 11 (SPSS Inc., Chicago, IL, U.S.A.). Pearson's product moment correlation

analysis was carried out on parametric data. Highly skewed data were normalised using logarithmic transformations. Spearman's rank correlation analysis was preformed when at least one nonparametric variable was involved. Multiple regression was performed using a forward stepwise procedure to compensate for covariance (Quinn & Keough, 2002). Variables with a *P*-value >0.05 were selected and only those independent variables which were correlated significantly with the dependent variable were included in the model. Separate analyses carried out for each basin indicated, in most cases, similar relationships among variables. For clarity only the results of the correlation analyses for the entire lake are presented.

Results

Abiotic and nutrient dynamics

Lough Carra had high pH ranging from 7.6 to 8.7, with Secchi depth ranging from 1.6 to 7.2 m. There was low mean colour (±SE) of 13.0 ± 1 mg L^{-1} PtCo ($n = 49$) and turbidity of 5.4 ± 0.6 FTU Turbidity Units ($n = 49$) with no consistent seasonal fluctuations. Mean alkalinity (±SE) of 139 ± 3 mg L^{-1} CaCO$_3$ ($n = 118$) and mean conductivity (±SE) of 321 ± 5 μS cm^{-1} ($n = 102$) followed similar seasonal patterns, with minima in late summer and autumn, most likely a result of CaCO$_3$ precipitation associated with summer photosynthesis.

Total nitrogen and DIN concentrations were strongly seasonal, with higher concentrations in winter (December to February) and spring (March to May), declining through summer (June to August) to an autumn minimum (September to November). DIN, in particular, was very low in autumn, ranging from 0.004 to 0.36 mg L^{-1}, which is close to the threshold suggested as an indicator of N-limitation (Hochstädter, 2000). There was no distinct seasonal pattern in either SRP or TP concentrations over the study period, apart from large peaks of TP, reaching approximately 35 μg L^{-1}, in all basins during winter. TP concentrations fluctuated throughout the rest of the year, with a mean (±SE) for the entire lake of 11.6 ± 0.59 μg L^{-1} ($n = 135$). SRP concentrations increased between November 2001 and April 2002 in all basins; otherwise the concentrations remained low, with a mean (±SE) of 2.4 ± 0.16 μg L^{-1} ($n = 135$).

The DIN : TP ratio was calculated to provide an estimate of the limiting nutrient for phytoplankton as recommended by Morris & Lewis (1988), where values >27 ᴍ were taken as an indicator of P-limitation, and values below 2 ᴍ an approximate indicator of N-limitation. In Lough Carra, the ratio of DIN : TP indicated probable N-limitation for most of the period between August and October and ranged from 0.32 to 23 (52% of the values within this range were ≤2) and mostly P-limitation throughout the rest of the year with values ranging from 12 to 197 (88% of the values within this range were ≥27). In June, July and November the DIN : TP ratio was generally between the two thresholds of limitation (ranging from 3 to 61). Approximations of the mean seston C : P (light : TP) ratio (±SE) in Lough Carra, estimated from Sterner *et al.* (1997), was 308 ± 2.6 ($n = 132$) and varied between 252 and 420. There was no significant association between the ratio of DIN : TP and the predicted C : P ratio (Pearson's $r = 0.09$, $P \geq 0.05$, $n = 125$). The mean C : P ratio (±SE) measured from samples taken between April and September 2005 was 223 ± 84 ($n = 4$). There was generally good agreement between these values and the estimated C : P (light : TP) ratio, with the exception of a low value of 75 recorded in July 2005 (Table 1). This was also the only date in which the C : P ratio of the zooplankton was higher than that of the seston and was coincident with calanoid dominated zooplankton biomass. The N : P ratios of seston and zooplankton measured in 2005 indicate that P was the most deficient element in the seston relative to the elemental contents of the zooplankton, apart from July where the seston N : P was only 9 compared with a N : P ratio of 77 recorded for zooplankton. In July nitrogen was, therefore, the most deficient nutrient in the seston relative to the nutrient content of the zooplankton and indicates

Table 1 Ratio of C : P and N : P in zooplankton and seston in the North basin of Lough Carra

	April 2005	May 2005	July 2005	September 2005
C : P				
Zooplankton	101	95	420	308
Seston	157	249	75	412
N : P				
Zooplankton	19	15	77	30
Seston	24	31	9	43

severe nitrogen limitation for the zooplankton (Table 1).

A similar seasonal trend in chlorophyll *a* concentration occurred in each of the three basins, with lowest values in spring. The mean chlorophyll *a* concentration (±SE) for all three basins of Lough Carra was 2.5 ± 0.1 µg L^{-1} ($n = 141$) and ranged from 0.3 to 8.5 µg L^{-1}. While there were no significant associations between chlorophyll *a* and either TP or TN, there was a negative correlation between chlorophyll *a* and DIN (Pearson's $r = -0.31$, $P \leq 0.001$, $n = 128$), and a significant positive association between chlorophyll *a* and SRP (Pearson's $r = 0.28$, $P \leq 0.001$, $n = 135$).

Phytoplankton community structure

Diatoms dominated the phytoplankton community in Lough Carra, comprising about 50% of cells throughout much of the year. Chlorophytes were the second most abundant taxon, followed closely by cryptophytes. In both the South and North basins, cyanophytes usually comprised only about 8% of the total community. In the North basin they dominated briefly during late July 2002, however, accounting for approximately 69% of phytoplankton cells and again from March to June 2003, comprising between 10% and 40% of the phytoplankton.

Zooplankton community structure

Seventeen species of Cladocera were recorded in Lough Carra over the sampling period. Copepods comprised principally *Eudiaptomus gracilis* (Sars) and cyclopoid copepods, mainly *Cyclops* spp. Rotifers, dominated by *Keratella cochlearis* (Gosse) and *Kellicottia longispina* (Kellicott), were the most diverse and abundant component of the community. Three species of predatory cladoceran, *Bythotrephes longimanus* (Leydig), *Leptodora kindti* (Focke) and *Polyphemus pediculus* (L.), were present, and most abundant between June and November of both 2001 and 2002. The total estimated biomass of zooplankton in Lough Carra ranged from 0.4 to 5455 µg DW L^{-1}. Maximum biomass occurred in spring 2002. Zooplankton biomass was dominated by *Daphnia hyalina* (Leydig), *Daphnia cucullata* (Sars) and the calanoid copepod, *E. gracilis*. The mean biomass of *Daphnia* and *Eudiaptomus* (±SE) was, respectively,

73 ± 43 µg DW L^{-1} and 44 ± 7 µg DW L^{-1} ($n = 129$). Together, these two genera accounted for approximately 98% of mean zooplankton biomass, with seasonally fluctuating dominance by *Daphnia* and *Eudiaptomus* (Fig. 2).

Maximum *Daphnia* biomass occurred in the spring of both years, declining to low values in summer and autumn. In both 2001 and 2002, *Daphnia* biomass was very low during autumn, ranging between 0 and 0.012 µg DW L^{-1}. *Eudiaptomus* biomass ranged from 0.10 to 671 µg L^{-1}, and was greatest during spring of 2002 in the North basin, but no consistent seasonal pattern of calanoid biomass was evident.
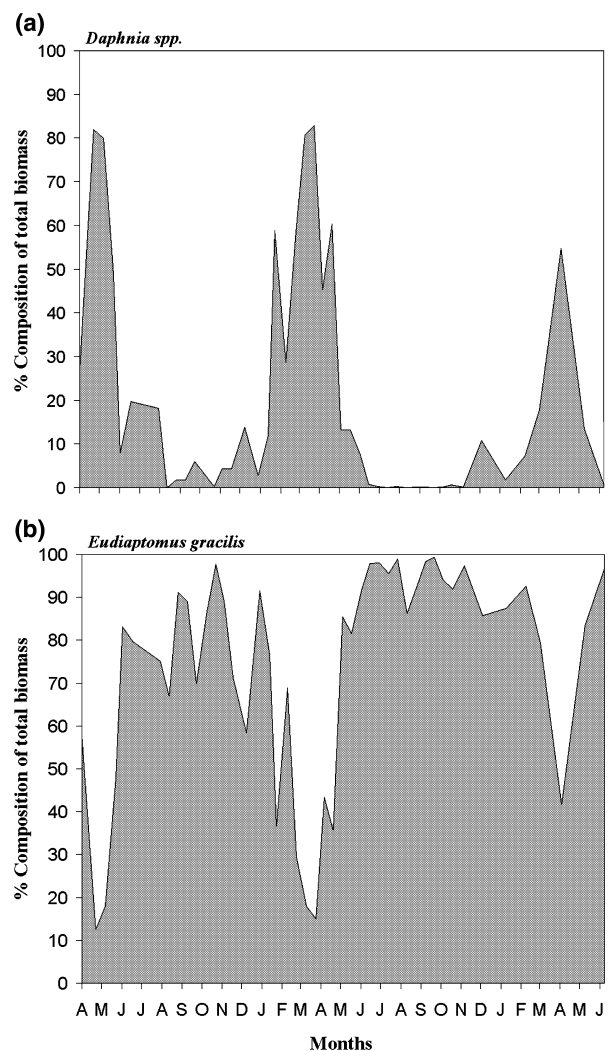

Fig. 2 Mean percentage contribution of *Daphnia* spp. and postnauplier *Eudiaptomus gracilis* to total zooplankton biomass for the three basins of Lough Carra, April 2001 to June 2003.

Table 2 Pearson's product-moment correlation coefficients (and sample size) for in-lake physical and chemical variables and various zooplankton biomass (µg DW L^{-1}) categories for Lough Carra, April 2001 to June 2003

	Total Zooplankton	Rotifers	*Daphnia*	Other cladoceran herbivores	*Eudiaptomus gracilis*	Calanoid nauplii	*Cyclops* spp.	Cyclopoid nauplii
SRP	−0.03 (126)	−0.12 (126)	−0.04 (99)	−0.10 (39)	−0.07 (126)	0.08 (124)	−0.12 (49)	0.15 (115)
TP	−0.01 (126)	−0.12 (126)	−0.17 (99)	0.10 (39)	−0.06 (126)	0.19 (124)*	−0.46 (49)***	−0.08 (115)
PP	−0.09 (125)	0.00 (98)	−0.01 (98)	0.07 (39)	−0.12 (125)	−0.04 (123)	−0.39 (49)**	0.02 (114)
TDP	−0.05 (111)	−0.08 (111)	−0.16 (85)	−0.03 (34)	−0.05 (111)	0.17 (109)	−0.18 (37)	0.04 (100)
DIN	0.31 (119)***	0.22 (119)*	0.66 (93)***	0.31 (38)*	0.13 (119)	0.15 (117)	0.28 (43)	0.61 (108)***
TN	0.24 (112)**	−0.02 (112)	0.40 (86)***	0.20 (34)	0.13 (112)	0.07 (105)	−0.40 (37)	0.40 (101)***
PN	−0.07 (112)	−0.23 (112)*	−0.28 (86)*	0.02 (34)	0.07 (112)	−0.07 (110)	−0.38 (37)*	−0.24 (101)*
Suspended particulate organic matter	−0.24 (122)**	0.09 (122)	−0.21 (95)*	−0.53 (39)***	−0.17 (122)	−0.15 (120)	−0.07 (48)	−0.15 (111)
Chlorophyll *a*	−0.36 (126)***	−0.17 (126)	−0.37 (99)***	−0.26 (39)	−0.27 (126)**	−0.07 (124)	−0.32 (49)*	−0.10 (115)

$*P \leq 0.05$; $**P \leq 0.01$; $***P \leq 0.001$.

SRP, soluble reactive phosphorus; TP, total phosphorus; PP, particulate phosphorus; TDP, total dissolved phosphorus; DIN, dissolved inorganic nitrogen; TN, total nitrogen; PN, particulate nitrogen.

Relationships of the zooplankton assemblage to nutrient availability

There was evidence that zooplankton herbivores suppressed algal biomass (Table 2), with maximum *Daphnia* biomass during spring 2001, 2002 and 2003 associated with minimum chlorophyll *a* concentrations (Fig. 3). Peaks of other cladoceran herbivores also occurred in spring in all three basins. Additionally, chlorophyll *a* correlated negatively with calanoid biomass (Table 2), and this relationship was particularly evident during periods of low *Daphnia* biomass (Fig. 3).

Zooplankton biomass (Table 2) correlated positively with DIN and TN, but not with TP or SRP, and these relationships were particularly strong for *Daphnia*. The very low autumn concentrations of DIN and TN corresponded with low *Daphnia* biomass in the autumn of both 2001 and 2002. Maximum recorded DIN concentration occurred in March 2002 in all basins (South: 0.88 mg L^{-1}, Mid: 0.64 mg L^{-1} and North: 0.53 mg L^{-1}) and concurred with large increases in *Daphnia* biomass. Stepwise multiple regression suggested DIN as the most significant variable (highest partial correlation) associated with *Daphnia* biomass ($r = 0.72$, Adjusted $r^2 = 0.55$, $P \leq 0.001$). *Daphnia* biomass, however, correlated negatively with PN. There was no significant relationship between *Daphnia* biomass and any of TP, SRP or PP. Where there was a significant relationship between phosphorus and other zooplankton taxa, this relationship tended to be negative, apart from calanoid nauplii biomass, which correlated positively with TP (Table 2).

The ratio of DIN : TP correlated positively with zooplankton biomass (Table 3) and particularly with *Daphnia*. The calanoids were the only zooplankton group which did not show a significant relationship with DIN : TP. There was also a positive association between *Daphnia* and the predicted ratio of C : P (light : TP), but no significant relationship with calanoid biomass.

Zooplankton fecundity

The fecundity of *Daphnia* spp. followed similar patterns in all three basins. The mean number of eggs per female was lowest between mid-April and May in each of the three basins, with the greatest increase occurring between July and August (Fig. 4). A similar seasonal trend in *Daphnia* SEN was evident, which ranged from 0 to 1.7 eggs per female of standard size. There was a tendency towards greater calanoid egg production over the winter and spring months, declining through summer and autumn. A particularly evident reduction in clutch size occurred between June and September, increasing again in late October (Fig. 4).

Both the number of eggs per female and the SEN of *Daphnia* correlated positively with both TP and chlorophyll *a*, but the number of eggs per female was
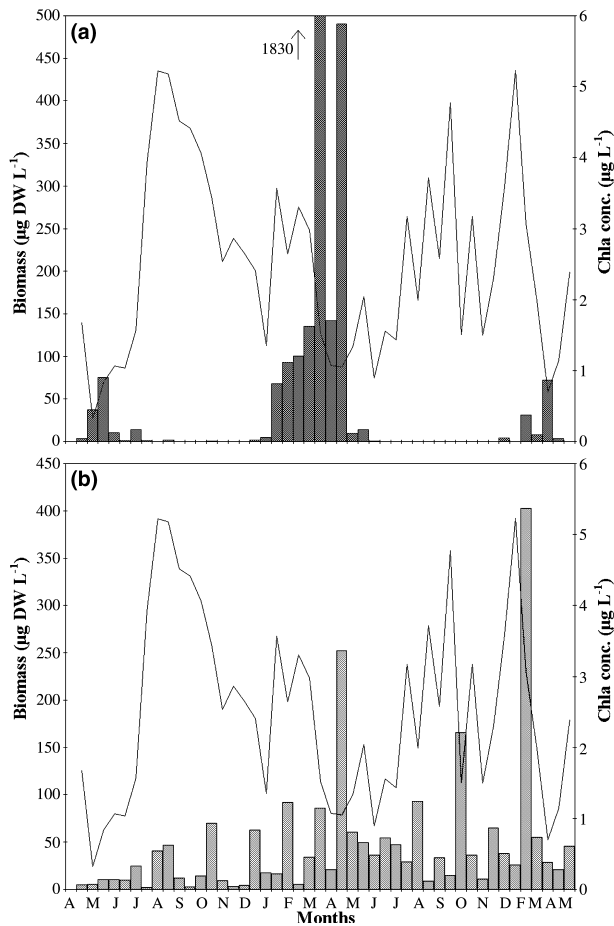
Fig. 3 (a) Mean biomass of *Daphnia* spp. (μg DW L^{-1}) and mean chlorophyll *a* concentration (μg L^{-1}) and (b) mean biomass of postnauplier *Eudiaptomus graclis* (μg DW L^{-1}) and mean chlorophyll *a* concentration (μg L^{-1}) for the three basins of Lough Carra from April 2001 to June 2003. ■ *Daphnia* spp. ▦ *Eudiaptomus gracilis* — chlorophyll *a*.

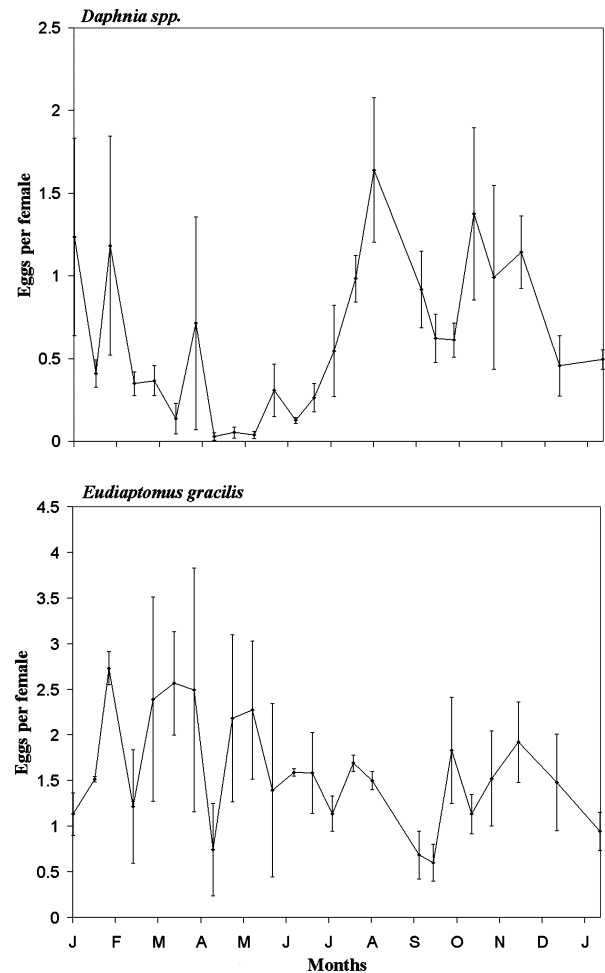**Fig. 4** Mean number of eggs per *Daphnia* spp. and *Eudiaptomus gracilis* female (±SE) for the three basins of Lough Carra, January 2002 to January 2003.

negatively related to DIN (Table 4). There was a positive relationship between DIN and the number of eggs per calanoid female (Table 4). The ratio of DIN : TP was correlated negatively with both meas-

ures of *Daphnia* fecundity and positively with calanoid egg production. There was also a negative relationship between the predicted ratio of C : P and the number of eggs per *Daphnia* female, but no significant relationship with calanoid fecundity (Table 5; Fig. 5). The

Table 3 Spearman-rank correlation coefficients (and sample size) for; DIN : TP and predicted C : P ratios and various zooplankton biomass (μg DW L^{-1}) categories, for Lough Carra, April 2001 to June 2003

	Total Zooplankton	Rotifers	*Daphnia*	Other cladoceran herbivores	*Eudiaptomus gracilis*	Calanoid nauplii	*Cyclops* spp.	Cyclopoid nauplii
DIN : TP	0.34 (119)***	0.25 (119)***	0.63 (119)***	0.37 (119)***	0.11 (119)	0.10 (119)	0.44 (119)***	0.58 (123)***
Predicted C : P (light : TP)	0.11 (124)	0.29 (124)***	0.21 (124)*	0.13 (124)	0.07 (124)	−0.12 (124)	0.06 (124)	−0.11 (127)

*$P \leq 0.05$; ***$P \leq 0.001$.
DIN, dissolved inorganic nitrogen; TP, total phosphorus; C, carbon; P, phosphorus.

Table 4 Pearson's product–moment correlation coefficients (and sample size) for in-lake physical and chemical variables and measures of *Daphnia* spp. and *Eudiaptomus gracilis* fecundity for Lough Carra, January 2002 to January 2003

	Daphnia spp. eggs : females	*Daphnia* spp. SEN	*Eudiaptomus gracilis* eggs : females
SRP	0.22 (65)	0.02 (51)	0.08 (69)
TP	0.35 (65)**	0.29 (51)*	−0.05 (69)
TDP	0.23 (62)*	0.21 (48)	−0.10 (68)
PP	0.23 (64)	0.17 (50)	0.05 (68)
DIN	−0.36 (65)*	−0.25 (51)	0.28 (69)*
TN	−0.05 (65)	−0.03 (51)	0.17 (69)
PN	0.29 (64)*	0.24 (51)	−0.10 (69)
Chlorophyll *a*	0.41 (65)***	0.35 (51)*	−0.12 (69)

*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$.
SRP, soluble reactive phosphorus; TP, total phosphorus; PP, particulate phosphorus; TDP, total dissolved phosphorus; DIN, dissolved inorganic nitrogen; TN, total nitrogen; PN, particulate nitrogen.

lowest number of eggs per *Daphnia* female was recorded between March and May 2002. This coincided with increasing *Daphnia* biomass and declining chlorophyll *a* concentration. Nevertheless, during periods of very low *Daphnia* biomass with relatively high chlorophyll *a* concentration, particularly from mid-June onwards, the negative relationship between the number of eggs per *Daphnia* female and the estimated seston C : P ratio (Spearman rank $r = -0.37$, $P \leq 0.05$, $n = 39$) and DIN : TP ratio (Spearman rank $r = -0.38$, $P \leq 0.05$, $n = 39$) persisted, whereas the correlation between *Daphnia* egg production and chlorophyll *a* was not significant (Pearson's $r = 0.23$, $P \geq 0.05$, $n = 38$). To compare the relative reproductive output between calanoid and *Daphnia*, the difference between the mean number of eggs per calanoid female and the mean number of eggs per *Daphnia* female for the three basins of Lough Carra was calculated. Increases in calanoid egg production relative to *Daphnia* egg production showed a positive association with both the mean approximated ratio of

C : P (Spearman rank $r = 0.45$, $P \leq 0.05$, $n = 24$; Fig. 6) and the mean DIN : TP ratio (Spearman rank $r = 0.66$, $P \leq 0.001$, $n = 24$; Fig. 6).

Discussion

Zooplankton fecundity

According to stoichiometric theory, the relative requirements for C, N and P differ among zooplankton taxa because of interspecific variation in the elemental content of their cells. Relative to copepods, the cellular ratio of N : P in *Daphnia* is lower, and as a result they are considered to have comparatively high requirements for P relative to N in their diets. Our results indicate that, in accordance with stoichiometric predictions, *Daphnia* and calanoid reproduction had opposite relationships to the availability of dietary phosphorus. In Lough Carra, there was a greater number of calanoid eggs per female relative to the number of *Daphnia* eggs per female during periods of high DIN : TP and estimated C : P (light : TP) ratios. This suggests that *Daphnia* egg production in Lough Carra was sensitive to P-limitation, whereas calanoid egg production increased with the availability of N. Although correlations must be interpreted with caution, especially under complex field conditions, the opposite fecundity response between the two taxa is further supported by the negative associations between the number of eggs per *Daphnia* female and the ratios of DIN : TP and approximated C : P; and the lack of a significant relationship between the estimated ratio of C : P and calanoid fecundity, coupled with a positive relationship with the DIN : TP ratio.

Reduction in the number of females carrying eggs can be symptomatic of low quantity, as well as quality, of food supply (Sterner & Schulz, 1998). Although the greatest reduction in *Daphnia* egg production in Lough Carra occurred during a period

Table 5 Spearman-rank correlation coefficients (and sample size) for DIN : TP and predicted C : P ratios and measures of *Daphnia* spp. and *Diaptomus gracilis* fecundity for Lough Carra, January 2002 to January 2003

	Daphnia spp. eggs : females	*Daphnia* spp. SEN	*Eudiaptomus gracilis* eggs : females
DIN : TP	−0.45 (70)***	−0.42 (55)***	0.31 (70)***
Predicted C : P (light : TP)	−0.30 (70)*	−0.27 (55)	0.04 (70)

*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$.
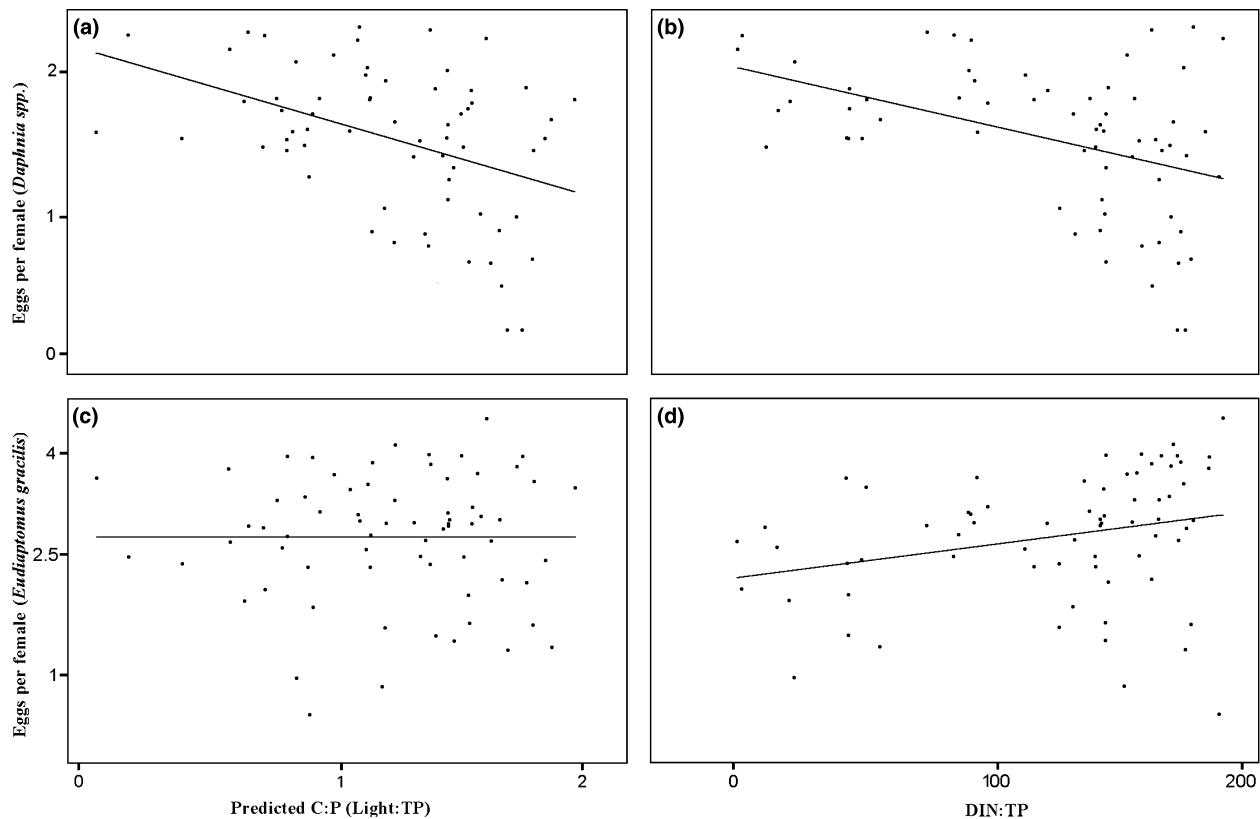DIN, dissolved inorganic nitrogen; TP, total phosphorus; C, carbon; P, phosphorus.

Fig. 5 (a) The relationship between the number of eggs per *Daphnia* female and the predicted ratio of C : P (light : TP) ($r^2 = 0.17$, $P \leq 0.001$, $n = 65$), (b) the relationship between the number of eggs per *Daphnia* female and the ratio of DIN : TP ($r^2 = 0.18$, $P \leq 0.001$, $n = 65$), (c) the relationship between the number of eggs per *Eudiaptomus gracilis* female and the predicted ratio of C : P (light : TP) ($r^2 = 0$, $P \geq 0.05$, $n = 69$) and (d) the relationship between the number of eggs per *E. gracilis* female and the ratio of DIN : TP ($r^2 = 0.08$, $P \leq 0.05$, $n = 69$) in Lough Carra, January 2002 to January 2003. All variables were logarithmically transformed.

of low phytoplankton biomass and high *Daphnia* biomass between April and June 2002, which is consistent with other work (e.g. Tessier & Consolatti, 1991; Lampert, 1993; Boersma, 1995), the importance of phosphorus limitation is suggested by the negative relationships between *Daphnia* fecundity and the ratios of both DIN : TP and predicted C : P that were maintained even after spring and early summer sampling dates were eliminated from correlation analysis. Between July 2002 and January 2003, *Daphnia* biomass was very low, chlorophyll *a* concentration high and estimations of the ratio of C : P often above a frequently suggested C : P-limitation threshold of between 200 and 300 (Urabe & Watanabe, 1992; Brett *et al.*, 2000; Anderson & Hessen, 2005). These periods of high C : P ratio and chlorophyll *a* concentration indicate low quality food, although quantitatively sufficient and generally corresponded to periods of low *Daphnia* fecundity. In contrast, increased *Daphnia*

egg production was associated with decreased modelled C : P ratio. This provides evidence that *Daphnia* fecundity was positively associated with the availability of dietary P and supports previous laboratory studies which have observed reduced clutch size in *Daphnia* fed P-deficient food (Sterner *et al.*, 1993; Lürling & Van Donk, 1997; Schulz & Sterner, 1999; DeMott *et al.*, 2001; Ferrão-Filho *et al.*, 2003).

Higher P content, relative to that of the adult, has been recorded in *Daphnia* neonates (DeMott *et al.*, 1998; DeMott, 2003; Færøvig & Hessen, 2003). Phosphorus content decreases rapidly at specific life stage and its reduction is particularly apparent with the development of ovaries (DeMott, 2003). Juveniles assign all available P to somatic growth but, once ovary development begins, an increasing proportion of P is invested in egg production (Færøvig & Hessen, 2003). A constant allocation of P to eggs, regardless of the availability of dietary P, was also observed
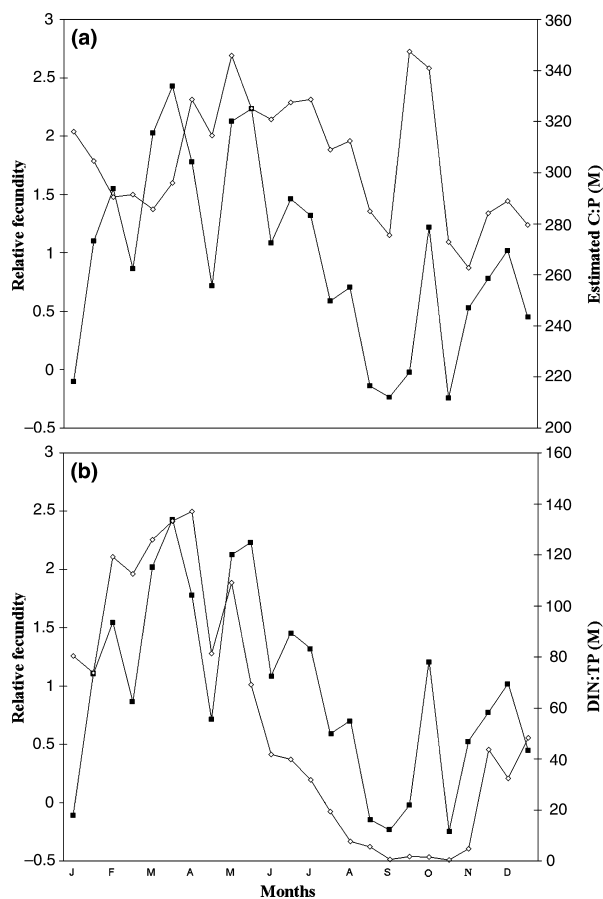
Fig. 6 (a) Relative fecundities of *Eudiaptomus gracilis* compared with *Daphnia* (the mean number of eggs per *E. gracilis* female minus the mean number of eggs per *Daphnia* female) and the estimated C : P ratio of the seston and (b) relative fecundities of *E. gracilis* compared with *Daphnia* and the ratio of DIN : TP of the seston in Lough Carra, January 2002 to January 2003. (a) ■, relative fecundity; ◇, estimated C : P. (b) ■, relative fecundity; ◇, DIN : TP.

by Becker & Boersma (2005). Consequently, egg production in *Daphnia* could certainly be sensitive to low P.

The reduced calanoid egg production associated with lower N availability concurs with observations by Kiørboe (1989) of copepod egg production declining with algal C : N ratio. Carrillo, Reche & Cruz-Pizarro (1996) found a high portion of N in copepod nauplii, declining rapidly with age and at a greater rate than P. This suggests a high allocation of nitrogen by gravid female calanoids to eggs and a reduction in clutch size under low nitrogen availability. This also provides an explanation for the observations that calanoids in food-limited environments invest in

larger, but fewer, eggs that provide reserves for naupliar development (Hart, Irvine & Waya, 1995).

Zooplankton community structure

The work in Lough Carra indicates that P-limitation operates through an impact on fecundity, but with no relationship between P availability and *Daphnia* biomass. This is contrary to other field studies that report a positive relationship between the abundance of *Daphnia* and seston phosphorus (Hessen, 1992; Hassett *et al.*, 1997; DeMott & Gulati, 1999; Brett *et al.*, 2000). In our study, whenever a significant relationship between zooplankton biomass and phosphorus occurred, this was negative, even for *Daphnia*, and irrespective of high DIN : TP ratios recorded that indicated P-limitation throughout much of the year. Conde-Porcuna *et al.* (2002) also did not find a significant correlation between phosphorus and daphniid biomass in a P-limited reservoir and Sterner (1998) failed to relate mean *Daphnia* birth rate and seston C : P in a lake expected to be P-limited. It may be that a clear demonstration of P-limitation on *Daphnia* is only likely across large ranges of C : P ratio, such as in the study of Makino *et al.* (2002) who found effects of P-limitation on a *Daphnia* population when C : P ranged from 100 to 600.

The greatest increase in biomass of both *Daphnia* and calanoids occurred in the spring of 2002, which coincided with a dramatic increase in DIN concentrations. Conversely, extremely low *Daphnia* biomass during summer and autumn corresponded to almost undetectable levels of DIN. Although nitrogen is required for protein synthesis, it is unlikely that *Daphnia* would be affected more than *Eudiaptomus* by nitrogen limitation, given the latter's low C : N content. Consequently, it is difficult to account for the apparent relationship between nitrogen and *Daphnia* in Lough Carra. It is unlikely that this relationship is causal. The seasonal reduction in DIN during the summer growing period is probably the result of increased autotroph growth and utilisation. Seasonal fluctuations in *Daphnia* biomass, however, are more difficult to explain. A combination of size-selective predation and density dependent resource limitation is the most likely explanation for the decline of *Daphnia* in early spring, but it is uncertain what maintained the continuously low numbers during summer and autumn. In accordance with expectations

of size-selective predation (Brooks & Dodson, 1965) body-size of the crustacean community in Lough Carra decreased from spring to late autumn, including fewer large *Daphnia* in summer and autumn compared with spring. There was, however, no overall shift towards dominance of smaller genera such as *Bosmina*. The principal fish in the lake is brown trout (*S. trutta*) whose diet shifts from the pelagic to benthic as they grow in size (Jonsson, 1989). The invertebrate predators *Bythotrephes* and *Leptodora* are in the lake, but were not sufficiently numerous to have a major impact on *Daphnia* (Barbiero et al., 2004; Wojtal et al., 2004), although *Bythotrephes* abundance was highly patchy. There was also evidence of adequate phytoplankton for pelagic herbivores during summer and autumn. Phytoplankton chlorophyll *a* increased during this period and was associated with relatively high *Daphnia* egg production, despite low *Daphnia* biomass, suggesting sufficient availability of food.

Low nitrogen availability, which can result in dominance by cyanophytes which may be inedible to *Daphnia* (Van Donk et al., 1997; Ghadouani, Pinel-Alloul & Prepas, 2003), might be of some importance in explaining overall plankton dynamics. In the North basin cyanophytes (comprising mainly *Anabaena* spp., *Synechococcus* spp. and *Mycrocystis* spp.) dominated briefly in July 2002, but nevertheless accounted for <8% of the phytoplankton community in the south basin, and diatoms and chlorophytes were prevalent throughout summer and autumn in both basins. Other food quality factors may be involved which were not measured here, such as deficiencies in essential fatty acids (Müller-Navarra et al., 2000; Ferrão-Filho et al., 2003), which may become particularly important at low algal C : P ratio when P is not limiting (Boersma, 2000), or digestion resistance such as chemical deterrents, or restrictions on ingestion owing to particle size or shape (Van Donk et al., 1997). Large colonial chlorophyte cells which are imbedded in a gelatinous sheath might be consumed by zooplankton, but they are poorly digested, resulting in reduced zooplankton growth rates (Porter, 1976). DeMott & Tessier (2002) and DeMott, Edington & Tessier (2004) found evidence for only weak and sporadic *Daphnia* P-limitation in lakes, despite a negative correlation between *Daphnia* growth and seston C : P. Growth assays and assimilation experiments revealed that food limitation was, in fact, more strongly associated with an increase in the percentage of digestion-resistant algae.

Stoichiometric retention of nutrients in zooplankton biomass

If a nutrient is in short supply in the food relative to demand, the animal should discriminately accumulate that element into the body tissue to maximise growth and reproduction, while eliminating nutrients in excess (Urabe, 1995). During summer and autumn calanoids dominated the total zooplankton biomass in Lough Carra. While *Eudiaptomus* abundance did not show marked summer increases in response to the declines of *Daphnia*, however, it is plausible that nitrogen limitation for primary production was accentuated by persistence of the calanoids in the DIN limited environment, facilitated by the ability of calanoids to feed discriminately based on food nutrient status (Butler, Suttle & Neill, 1989). Owing to their high N content, calanoids could differentially accumulate N in their biomass and recycle P at a relatively high rate. Elser et al. (1988) found that, under conditions of *Daphnia* dominance, phytoplankton growth was P-limited while, in situations where calanoids dominated, the phytoplankton were N-limited. Brett et al. (1994) found that *Diaptomus* markedly increased SRP relative to DIN, resulting in a low DIN : SRP ratio. In contrast, *Daphnia* increased the DIN : SRP ratio. Similar patterns were evident in Lough Carra. The vast difference between zooplankton and seston N : P contents in July 2005 further demonstrates that, during the summer, a considerable quantity of N relative to P was retained in the calanoid dominated zooplankton. At the same time the high zooplankton C : P ratio relative to the seston suggests a disproportionate excretion of P relative to N. Andersen (1997) suggested that the differential recycling of nutrients by zooplankton grazers is most likely to influence phytoplankton nutrient limitation in lakes where the N : P ratio of external nutrient loading is <40 : 1. Using TN and TP as indices of nutrient loading, Elser & Urabe (1999) suggested that approximately 25% of lakes had TN : TP <40. The TN : TP ratio in Lough Carra was <40 during the summer months, consistent with calanoid mediated N-limitation to the plankton. Variable release ratios are produced when consumers retain the element in least

supply and discard elements that are in excess (Elser & Urabe, 1999). When *Daphnia* act as a significant sink for P, a positive correlation should be expected between *Daphnia* abundance and the seston C : P ratio (DeMott & Gulati, 1999). In Lough Carra, during spring, *Daphnia* may have acted as a sink of P, but a source of N, accounting, therefore, for the substantial increase in the ratio of DIN : TP during this period.

In conclusion, this study provides a field test of the effect of the availability of dietary N and P on patterns of dominance between high P *Daphnia* and low P calanoids. Phosphorus availability was positively associated with *Daphnia* egg production, but was not related to calanoid fecundity. The ratio of DIN : TP was high when *Daphnia* dominated zooplankton biomass and low when calanoids dominated. The direct effect of elemental composition of algae on zooplankton communities can be confounded by interactions between herbivore and resource, through grazing effects and nutrient regeneration (Urabe, 1995; Urabe *et al.*, 2002). Suppression of P through stoichiometric retention in *Daphnia* during spring can account for the positive associations between the ratios of DIN : TP and estimated C : P and *Daphnia* biomass. Conversely, retention of N in calanoids during the summer may have accentuated N-limitation of the phytoplankton.

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(Manuscript accepted 20 May 2006)