Unsaturated fatty acid content in seston and tropho-dynamic coupling in lakes

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Determining the factors that control food web interactions is a key issue in ecology[1,2]. The empirical relationship between nutrient loading (total phosphorus) and phytoplankton standing stock (chlorophyll *a*) in lakes was described about 30 years ago[3] and is central for managing surface water quality. The efficiency with which biomass and energy are transferred through the food web and sustain the production of higher trophic levels (such as fish) declines with nutrient loading and system productivity[4,5], but the underlying mechanisms are poorly understood. Here we show that in seston (fine particles in water) during summer, specific ω3-polyunsaturated fatty acids (ω3-PUFAs), which are important for zooplankton[6–10], are significantly correlated to the trophic status of the lake. The ω3-PUFAs octadecatetraenoic acid, eicosapentaenoic acid (EPA) and docosahexaenoic acid, but not α-linolenic acid, decrease on a double-logarithmic scale with increasing total phosphorus. By combining the empirical relationship between EPA-to-carbon content and total phosphorus with functional models relating EPA-to-carbon content to the growth and egg production of daphnids[8], we predict secondary production for this key consumer. Thus, the decreasing efficiency in energy transfer with increasing lake productivity can be explained by differences in ω3-PUFA-associated food quality at the plant–animal interface.

The efficiency with which biomass and energy are transferred across the plant–animal interface is highly variable[11,12], and these trophic levels can even become uncoupled in very productive lakes[5,13]. This is especially true during the summer season, when food limitation for zooplankton is most prevalent[14,15] and when the effects of eutrophication are most obvious—for example, noxious phytoplankton blooms are most likely to occur in summer[15,16]. So far, there is no clear understanding of what determines the transfer efficiency from phytoplankton to zooplankton, which in turn can influence the zooplankton's capacity to suppress phytoplankton biomass. There is, however, a broad consensus that food quality for zooplankton is a pivotal factor[2,14,17,18]. In addition to differences in digestibility[17], pronounced differences in elemental stoichiometry[18,19] and the biochemical make-up[2,6–9] of plants and animals can be key components that determine food quality for zooplankton.

Studies suggest that the highly unsaturated fatty acids (ω3-HUFAs) EPA and docosahexaenoic acid (DHA) are important biochemical constituents in the natural diet of zooplankton because the prevalence of these fatty acids in seston is a strong predictor of zooplankton growth[6–8] and may be crucial in shaping food web pyramids[10]. To test whether the decrease in trophic interaction strength with increasing nutrient (total phosphorus; TP) loading can be associated with food quality for the primary producer measured as ω3-PUFA content, we sampled and analysed seston from lakes differing in food web structure and encompassing the natural range of productivity from ultra-oligotrophic to hyper-eutrophic states.

Several lake productivity measures showed the expected positive trend with increasing lake TP (ref. 3). For example, chlorophyll *a* (Chl-*a*; $\log(\text{Chl-}a) = 1.05 \times \text{TP} + 4.8$; $P < 0.005$; correlation coefficient squared, $r^2 = 0.56$) and particulate organic carbon (POC; $\ln(\text{POC}) = 0.55 \times \text{TP} - 2.25$; $P < 0.01$; $r^2 = 0.45$) increased, whereas water transparency (Secchi depth) decreased with increasing TP in these lakes (see Supplementary Information). Our estimates for the slope and intercept of the relationships agree with previously published values[5].

In contrast to Chl-*a* and POC as measures of zooplankton food

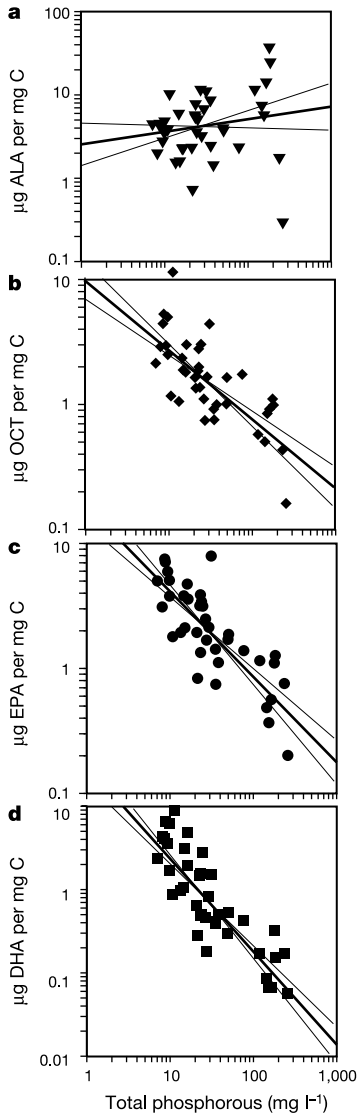

Figure 1 Regressions between lake TP concentration and sestonic ω3-PUFA-to-C contents. **a**, For ALA (18:3ω3), ln[ALA (µg per mg C)] = 0.15 × ln(TP) + 0.94; $P = 0.0531$; $r^2 = 0.03$. **b**, For OCT (18:4ω3), ln[OCT (µg per mg C)] = −0.69 × ln(TP) + 3.01; $P < 0.0001$; $r^2 = 0.62$, residual error 0.50. **c**, For EPA (20:5ω3), ln[EPA (µg per mg C)] = −0.55 × ln(TP) + 2.25; $P < 0.0001$; $r^2 = 0.55$; residual error 0.47. **d**, For DHA (22:6ω3), ln[DHA (µg per mg C)] = −1.11 × ln(TP) + 3.42; $P < 0.0001$; $r^2 = 0.73$; residual error 0.66. Each point represents one observation. Note that the *y* axis is tenfold larger in **a** than in **b–d**.

quantity, food quality of the summer seston assemblages, measured as the content of the ω3-HUFAs EPA and DHA relative to the carbon (C) content, was strongly negatively correlated to the trophic status of the lake across the whole naturally occurring gradient of productivity (Figs 1c, d, and 2). This finding can elucidate the observed trend that trophic transfer efficiency and food web strength decrease with increasing trophic status of the lake[4,5,13]. The border between oligotropy and eutrophy has been defined[3] as 20 μg of TP per litre, which corresponds to 2 μg EPA per mg C and about 3 μg of DHA per mg C (Fig. 2). Notably, a difference between the food quality derived from ω3-HUFA-to-C content and that derived from the C:P ratio is expected in oligotrophic lakes, where the C:P hypothesis predicts low food quality owing to a high seston C:P ratio under high light-to-nutrient ratios[20], and the sestonic ω3-HUFA-to-C content predicts high food quality of seston.

The ln–ln negative relationship (that is, the linear decrease on a double logarithmic scale) implies that seston food quality is not distributed equally over the range of TP concentrations (Figs 1 and 2). A decrease in sestonic ω3-HUFA-associated food quality is very sensitive to TP changes at low TP concentrations, but seems to be almost insensitive at high concentrations of TP. Thus, deviations from the regression curve have a much larger effect at low TP than at high TP. For example, small-scale TP perturbations may result in considerable deviations from the relationship between mean ω3-HUFA-to-C content and TP at low TP. The degree of non-steady-state between TP and ω3-HUFA-to-C content at a single time may be inferred from the difference in explained variance of the regressions between single (Fig. 1) and mean (Fig. 2) lake values.

The distribution of ω3-HUFA-related food quality for zooplankton corroborates observations that responses of phytoplankton to food web alterations (such as different fish stocks) are stronger at low than at high concentrations of TP, and that biomanipulation used as a lake restoration tool is more likely to fail at high TP concentrations[21], at which ironically it is needed most. By using the empirical relationship between TP and the mean EPA-to-C content in seston (Fig. 2), in conjunction with published zooplankton growth responses to seston EPA-to-C content[8], we estimated *Daphnia* growth rates and egg production from lake TP concentrations under conditions of non-limiting carbon (Fig. 3). These

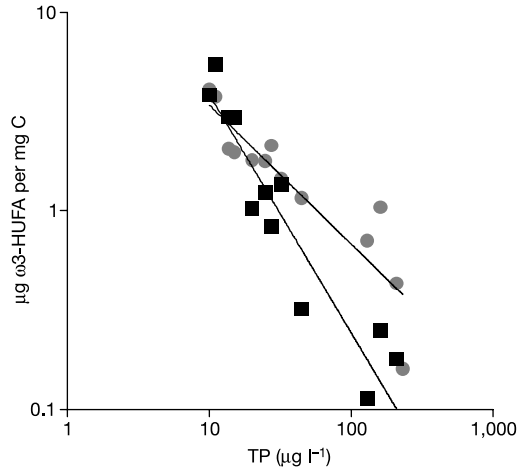

Figure 2 Regressions between lake TP concentration and sestonic EPA and DHA. Grey circles indicate EPA (20:5ω3): ln[EPA (μg per mg C)] = −0.69 × ln(TP) + 2.78; $P < 0.0001$; $r^2 = 0.82$; residual error 0.35. Black squares indicate DHA (22:6ω3): ln [DHA (μg per mg C)] = −1.07 × ln(TP) + 4.4; $P < 0.0001$; $r^2 = 0.89$; residual error 0.17. Each point represents the mean value of each lake sampled. Given our sample size ($n = 13$), a regression between TP and a dependent variable would have to have a value of $r^2 \geq 0.30$ to be statistically significant at an α-level of 0.005.

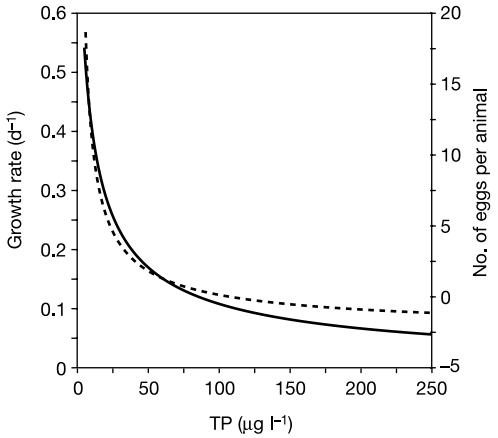

Figure 3 Calculation of the growth rates and egg production of *Daphnia magna*. Growth rates (unbroken line) and egg production (broken line) were calculated from the regression of EPA-to-C content versus TP concentration (Fig. 2), in conjunction with the functional response determined previously (Fig. 3 in ref. 8 for growth rate, $g = 0.74 \times (1 - e^{(-0.25 \times EPA/C + 0.01)})$, and Fig. 4 in ref. 8 for egg production, eggs = 4 × EPA/C − 2.5).

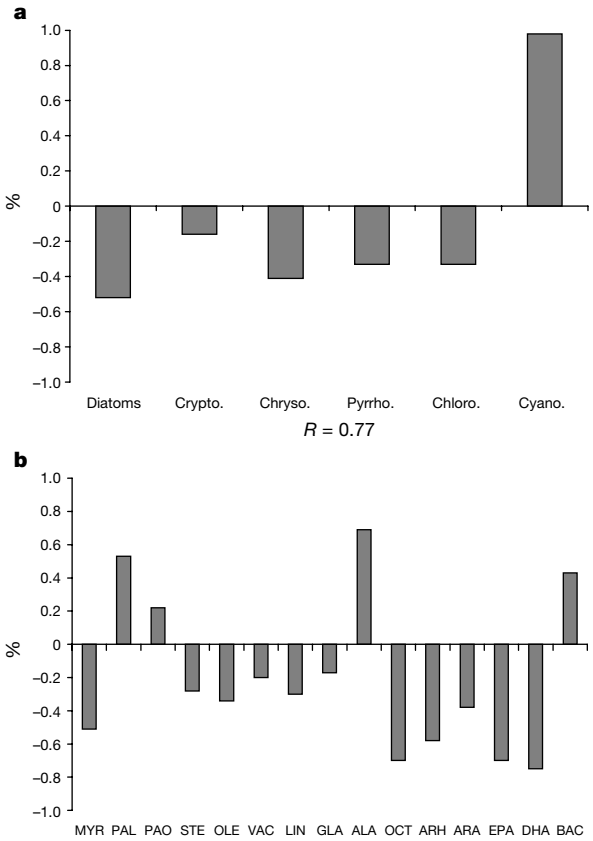

Figure 4 First canonical pattern. **a**, Phytoplankton groups: diatoms, Bacillariophycea; Crypto., Cryptophyceae; Chryso., Crysophyceae; Pyrrho., Pyrrhopyceae; Chloro., Chlorophyceae; Cyano., Cyanobacteria. **b**, Fatty acids: MYR, 14:0; PAL, 16:0; PAO, 16:1ω7; STE, 18:0; OLE, 18:1ω9; VAC, 18:1ω7; LIN, 18:2ω6; GLA, 18:3ω6; ARH, 20:0; ARA, 20:4ω6; BAC, bacterial fatty acids; other fatty acids are defined in the text. See Methods for an explanation of the statistical analysis.

estimates show that differences in food quality determined by ω3-HUFA content can have a great effect on the growth rate and egg production of this key grazer. As growth conditions also have an effect on the susceptibility of zooplankton to predation, these effects on food quality might be crucial to promoting grazer populations, thereby influencing the strength of trophic cascades.

By using canonical correlation analysis (CCA), a significantly strong interaction (coefficient of multiple correlation, $R = 0.77$) between phytoplankton group canonical pattern and seston fatty acid canonical pattern was detected, with ω3-PUFAs dominating the fatty acid pattern (Fig. 4). However, the first canonical pattern explained only 27% of the variance in the phytoplankton data, and only 26% of that in the fatty acid data, suggesting that other factors (such as the physiological state of the phytoplankton and the contribution of detritus and heterotrophic organisms to seston biomass) may have been important in determining the fatty acid composition of seston.

The phytoplankton pattern was dominated by the variation of cyanobacteria, which was in the opposite direction to the variation of all other phytoplankton groups (Fig. 4a). The variation of cyanobacteria covaried with α-linolenic acid (ALA), whereas all other phytoplankton groups covaried with the other ω3-PUFAs. A high concentration of TP favours cyanobacteria, which can have ALA but have little or none of the other ω3-PUFAs. In contrast to phytoplankton assemblages dominated by diatoms, chrysophytes and cryptophytes, cyanobacteria can proliferate and build up high standing stocks (blooms) but, owing to low food quality[22,23], cannot support higher trophic level (zooplankton) production even at high concentrations of carbon. In addition, as the ω3-HUFA content of seston is conservatively transferred up the food web into fish, understanding the dynamics of ω3-HUFA at the base of the food web is a key to their distribution in fish[24], which are strongly tied to human nutrition and health[25].

The relationships reported here imply that feeding and growth of zooplankton are not independent of, but are coupled to, the nutrient supply to phytoplankton. On the one hand, because of their high ω3-HUFA content phytoplankton species are more nutritious at low TP and thus can be more easily exploited by zooplankton. On the other hand, phytoplankton species in lakes with high TP will often have a low ω3-HUFA content and thus are less nutritious, rendering grazers (top-down control) less effective. In contrast to the general view that phytoplankton biomass is mainly controlled from the bottom up, our findings therefore suggest that both bottom-up and top-down processes contribute to the shape of the TP versus Chl-*a* relationship. □

Methods

Sampling

Between mid-June and the beginning of September of 1997–2000, we sampled 13 lakes (1–6 times each) in California, Oregon and Washington, USA (see Supplementary Information). Special attention was paid to ensure that the lakes included various types of lake distributed over a wide gradient of trophic states—which was between 8 and 230 μg of TP per litre. Both natural and artificial lakes were selected. The lakes sampled were close to a laboratory facility to ensure fast sample processing and proper storage, which are important for the otherwise easily degradable PUFAs. For each lake, we recorded the Secchi depth transparency and collected water samples from the upper mixed layer, generally from a depth of 1 m. For the subalpine lakes (Castle Lake and Lake Tahoe), water was taken from the subsurface to reduce ultraviolet inhibition[26]. At Lake Tahoe, we collected water from 10 m. At Castle Lake, water was taken from the Chl-*a* maximum (~15 m).

Sample analyses

TP analyses were conducted from raw water samples[27]. Water for phytoplankton species identification and enumeration was preserved in Lugol's solution, and phytoplankton was counted using an inverted microscope. After filtration onto glass-fibre filters (Whatman GF/C), suspended matter was analysed for Chl-*a*[28] with a 10AU fluorometer (Turner), and for POC by either a Model 2400 CHN analyser (Perkin-Elmer) for Stonegate Pond and Lake Tahoe, or a Hydra 20/20 continuous flow isotopic ratio mass spectrometer (PDZ Europa Scientific) for the other lakes. We verified analytical consistency between the latter two instruments. Fatty acids were analysed after extraction and methylation[29] with an HP6890 gas chromatograph (Hewlett Packard) equipped with a programmable temperature vaporizer injector, a fused silica DB-WAX capillary column (J&W Scientific) and a flame ionization detector. ALA, octadecatetraenoic acid (OCT), EPA and DHA are all ω3-PUFAs, whereas only EPA and DHA are ω3-HUFAs.

Statistical analyses

The relative abundances of phytoplankton groups and fatty acid compositions were related to each other by the multivariate statistical CCA method[30]. CCA identifies optimal linearly coupled pattern in two data sets[30]. Hence for the coupled pattern, the phytoplankton group and fatty acid pattern have the highest possible correlation. In the calculations we used a sample size of 26, which included at least one sample from each lake studied. Data were normalized to their standard deviation. Empirical orthogonal functions (EOFs) were calculated for both the phytoplankton and the fatty acid anomalies (deviation from the mean). The first three EOFs for both the phytoplankton and the fatty acid pattern were used as vectors in the CCA analysis. Explained variability of the phytoplankton EOFs was 28% for the first, 22% for the second and 19% for the third pattern. Thus, 69% of the variability could be captured by the first three EOF functions. Explained variability of the fatty acid EOFs was 29% for the first, 14% for the second and 12% for the third pattern, explaining 55% of the variability with the first three EOF patterns. Both phytoplankton and fatty acid canonical patterns were very similar to their respective EOFs.

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SOS response promotes horizontal dissemination of antibiotic resistance genes

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Mobile genetic elements have a crucial role in spreading antibiotic resistance genes among bacterial populations. Environmental and genetic factors that regulate conjugative transfer of antibiotic resistance genes in bacterial populations are largely unknown[1]. Integrating conjugative elements (ICEs) are a diverse group of mobile elements that are transferred by means of cell–cell contact and integrate into the chromosome of the new host[2]. SXT is a ~100-kilobase ICE derived from *Vibrio cholerae* that encodes genes that confer resistance to chloramphenicol, sulphamethoxazole, trimethoprim and streptomycin[3]. SXT-related elements were not detected in *V. cholerae* before 1993 but are now present in almost all clinical *V. cholerae* isolates from Asia[4]. ICEs related to SXT are also present in several other bacterial species and encode a variety of antibiotic and heavy metal resistance genes[4–7]. Here we show that SetR, an SXT encoded repressor, represses the expression of activators of SXT transfer. The 'SOS response' to DNA damage alleviates this repression, increasing the expression of genes necessary for SXT transfer and hence the frequency of transfer. SOS is induced by a variety of environmental factors and antibiotics, for example ciprofloxacin, and we show that ciprofloxacin induces SXT transfer as well. Thus, we present a mechanism by which therapeutic agents can promote the spread of antibiotic resistance genes.

SXT transfer requires *recA* in donor cells, but the molecular basis for this requirement was unclear[3]. We identified genes at the 3′ end of the integrated SXT that regulate SXT transfer[8]. Two loci, *setC* and *setD*, encode transcriptional activators required for SXT excision and transfer[8]. Overexpression of these activators was toxic to cells that harboured SXT but not in cells lacking SXT. *setR*, the 3′-most gene in integrated SXT, is similar to the λ bacteriophage CI repressor and, like this repressor, is predicted to contain both a helix–turn–helix DNA-binding motif and a protease motif (see Supplementary Information). *setR* cannot be deleted from SXT unless the mutation is complemented *in trans* from a plasmid[8], indicating that the removal of SetR repression of some SXT-encoded factor(s) might be deleterious to cell growth. Because the overexpression of *setC* and *setD* is toxic, we hypothesized that SetR represses these SXT transcriptional activators; we found that *setR* could be deleted in a *setCD* deletion strain.

To measure *setC* and *setD* gene expression, we replaced *setC* and *setD* with a promoterless *lacZ* reporter gene (*setCD*::*lacZ*). β-Galactosidase activity from this reporter was relatively low (15 Miller units) in the wild-type background; in contrast, the activity was 1,870 Miller units in the *setR* deletion background (Table 1, rows 1 and 2). Introduction of a plasmid carrying *setR* restored the repression of *setCD*::*lacZ* in the *setR* deletion background (data not shown), confirming that SetR represses *setC* and *setD* expression.

The similarity of SetR to the λ-phage repressor CI suggested that the regulation of SXT transfer might be similar to the regulation of λ lysogeny. In λ lysogens, CI represses prophage gene expression. After DNA damage and the induction of the SOS response, the co-protease activity of RecA is stimulated and promotes the autoproteolysis of CI, alleviating CI-mediated repression and beginning the phage lytic cycle[9]. Because SXT transfer requires *recA* in donor cells[3] and SetR is similar to CI, triggering the SOS response might result in a RecA-dependent cleavage and inactivation of SetR, increasing *setC* and *setD* expression and enhancing

Table 1 **Mitomycin C activates expression of SXT conjugation-associated loci**

Site of fusion (background)	β-Galactosidase activity	
	Without mitomycin C	With mitomycin C
Δ*setCD*::*lacZ*	15	171
Δ*setCD*::*lacZ*(Δ*setR*)	1,870	1,710
Δ*s003*::*lacZ*	11	73
Δ*traG*::*lacZ*	17	70
Δ*floR*::*lacZ*	34	33
Δ*setCD*::*lacZ* (*setR*^{G94E})	112	124

All strains were derivatives of *E. coli* MG1655 harbouring SXT. Mutations in *setR* are indicated in parentheses. The values presented are the means of at least three experiments; standard deviations were less than 10%.

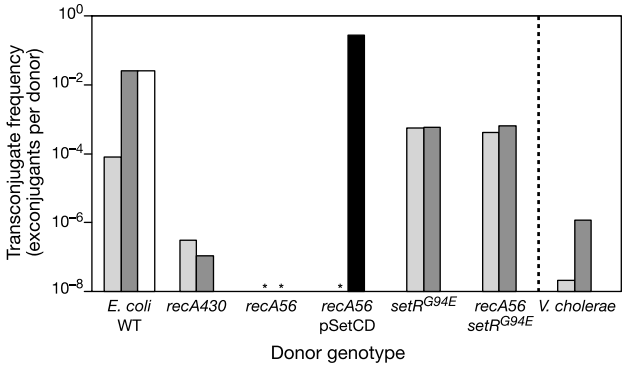

Figure 1 SOS-inducing agents activate SXT transfer. Transconjugate frequency was calculated as transconjugants observed per donor cell as described[3]. The wild type (WT) and *setR*^{G94E} donor strains are derivatives of *E. coli* strain BW25113 (MG1655 *lacI*^{q} *rrnB*$_{T14}$ Δ*lacZ*$_{WJ16}$ *hsdR514* Δ*araBAD*$_{AH33}$ Δ*rhaBAD*$_{LD78}$)[20] containing SXT. The *recA56* and *recA430* donor strains are derivatives of MG1655. *V. cholerae* transfer experiments used MO10 as a donor[3]. In all cases the recipient was CAG18439 (ref. 21). pSetDC contains *setDC* under the control of an arabinose-inducible promoter[8]. The different growth conditions used for the donor cells before their use in the conjugation assay are represented by light grey bars for growth in LB, dark grey bars for growth in LB containing mitomycin C, white bars for growth in LB containing ciprofloxacin, and black bars for growth in LB containing arabinose. Asterisks represent data that were below the detection limits of the assay (~10^{-8}). Values presented are the averages of three independent assays; standard deviations were less than 30%.