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The functional role of planktonic mixotrophs in altering seston stoichiometry

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ABSTRACT: Mixotrophic protists are widespread and relevant primary producers and consumers in planktonic food webs. Given their dual mode of nutrition, mixotrophs face different constraints in allocating resources to cellular structures compared to strict photoautotrophs. However, little is known about their stoichiometric requirements and their flexibility in nutrient content and thus food quality, or how this affects consumer performance and nutrient recycling. In the present study, we tested for systematic differences in elemental composition between photoautotrophic and mixotrophic protists. We compiled intracellular nutrient ratios of mixotrophic and phototrophic species from culture experiments and from 2 lake surveys. Overall, both laboratory and field data indicated that mixotrophy has a stabilizing effect on the nutrient stoichiometry of primary producers under changing nutrient supply. In laboratory experiments, mixotrophs showed a lower variability in intracellular N:P ratios compared to strict phototrophs and were more stable in their elemental composition in response to a gradient of dissolved N:P availability. With increasing contributions of mixotrophic phytoplankton taxa to total lake phytoplankton, both the mean and variance in seston C:P ratios decreased, i.e. communities with higher proportion of mixotrophs overall exhibited more constrained seston stoichiometry. Our results show that mixotrophy may have direct implications for nutrient cycling and secondary production through regulation of seston stoichiometry, buffering stoichiometric constraints for herbivores and enabling a more stable secondary production compared to systems dominated by phototrophic specialists.

KEY WORDS: Mixotrophy · Stoichiometry · Nutrients · Food quality · Trophic transfer · Trophic efficiency · Plankton food web

INTRODUCTION

Mixotrophic planktonic protists, which are able to combine heterotrophic and phototrophic modes of nutrition within a single cell, have increasingly been recognized and documented in aquatic systems in the past decades (e.g. Sanders & Porter 1988, Caron 2000, Stickney et al. 2000, Unrein et al. 2007, Hartmann et al. 2012). The combination of using photosynthesis and phagotrophic feeding enables mixotrophs to function on multiple trophic levels within a food web and to use particulate and dissolved nutrient pools, thus augmenting their nutrition in terms of energy, macronutrients and micronutrients including vitamins and trace metals (Sanders et al. 1990, Caron et al. 1993, Maranger et al. 1998, Jones 2000). These advantages may lend mixotrophs a competitive advantage over strict phototrophs and heterotrophs (Bockstahler & Coats 1993a,b) and seem to outweigh increased metabolic costs of maintaining both modes of nutrition (Raven 1997).

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Mixotrophy, here defined as the combination of photosynthetic carbon fixation and phagotrophic feeding, occurs in a variety of marine and freshwater systems and in various phylogenetic groups and size classes, including algal groups such as chrysophytes, dinoflagellates, haptophytes, raphidophytes and cryptophytes (e.g. Sanders 1991, Unrein et al. 2007, Jeong et al. 2010, Caron 2016). Phagotrophic phytoflagellates in the nanoplankton (2 to 20 µm) size class can constitute a substantial fraction of the phytoplankton community and can be major bacterivores, particularly in oligotrophic systems (Zubkov & Tarran 2008, Hartmann et al. 2012, Unrein et al. 2014). A number of bloom-forming harmful algae such as dinoflagellates, haptophytes and raphidophytes are also able to consume prey organisms, which is assumed to facilitate their dominance and bloom formation in phytoplankton communities (Burkholder et al. 2008).

Recent modeling efforts indicate dramatic increases in cumulative carbon fixation and/or trophic transfer efficiency when mixotrophy is included (Mitra et al. 2016, Ward & Follows 2016). In spite of the diversity and wide distribution of mixotrophic organisms and their potential contribution to these ecosystem level processes, they have largely been ignored or marginalized by many plankton ecologists, biological oceanographers and modellers (Mitra et al. 2014; but see Caron 2016 and references therein). There are many systems for which the contribution of mixotrophs to community production and thus also their influence on global biogeochemical cycles remain largely unknown. This becomes especially obvious when addressing coupled elemental cycles and stoichiometry, which has been studied almost exclusively using pure photoautotrophs. Depending on the relative availability and algal demand, algae take up different dissolved nutrients such as nitrogen (N) and phosphorus (P) and thus link the cycle of different elements. The stoichiometry of phytoplankton composition is therefore an important component of global and regional biogeochemical models and has received broad attention in the literature (Sterner & Elser 2002, Hillebrand et al. 2013 and references therein). Microalgae usually show a broad plasticity in elemental composition depending on nutrient supply ratios, as nutrient assimilation and photosynthetic carbon fixation are physiologically separated; fluctuations in resource supplies and their ratios are therefore partly reflected in a phototroph's elemental composition (Ågren 2004, Hillebrand et al. 2013). In contrast, metazoan consumers generally have a more confined stoichiometry and higher nutrient content compared to phototrophs (Elser et al. 2000, Persson et al. 2010).

The concept of ecological stoichiometry (ES) acknowledges the importance of nutrient imbalances between consumers and their prey, which particularly affect herbivore–phototroph interactions. Altered stoichiometry of primary producers alters food quality for herbivores, which strongly influences trophic interactions and food web dynamics, constraining consumer grazing and growth rates, as well as nutrient recycling (e.g. Sterner & Elser 2002, Iwabuchi & Urabe 2012). ES has successfully been applied to explain consumer food uptake rate, assimilation and growth efficiency (Cross et al. 2003, Fagan & Denno 2004, Frost et al. 2006), competition between consumer species (Hall 2004, Loladze et al. 2004) as well as the effects of consumers on prey nutrient composition (Daufresne & Loreau 2001, Hillebrand et al. 2009b).

Even though the relevance of phytoplankton stoichiometry for the transfer of matter and energy to higher trophic levels has been recognized and acknowledged in numerous studies, very few studies have addressed the stoichiometry of mixotrophic organisms that are able to pursue alternative production pathways and can supplement their uptake of inorganic dissolved nutrients and photosynthetic carbon fixation by feeding on prey. While fluctuations in dissolved inorganic nutrients strongly affect intracellular C:nutrient ratios in purely photosynthetic organisms, intracellular nutrient concentrations and ratios should be more stable in mixotrophic organisms, since phagotrophic feeding on nutrient rich prey can potentially compensate for inorganic nutrient limitation. In bacterial prey, for instance, concentrations of potentially limiting nutrients are often several orders of magnitude higher compared to the dissolved phase (e.g. Vadstein 2000). Examining the stoichiometry of mixotrophic organisms thus has important implications for plankton food web dynamics and the cycling of matter and energy, especially in the face of anthropogenic nutrient loadings to aquatic systems (Vitousek et al. 1997, Rockström et al. 2009).

Several studies have investigated concentrations of particular nutrients in mixotrophic flagellates, but most of them focused more on the effect of light, temperature and dissolved nutrient conditions on phagotrophic feeding and its contribution to C and nutrient budgets (Caron et al. 1993, Li et al. 2000, Skovgaard et al. 2000, 2003, Smalley et al. 2003, Carvalho & Granéli 2010, Simonds et al. 2010, Brutemark & Granéli 2011, Wilken et al. 2014, Johnson 2015) or on the relationship of internal nutrient concentrations and toxin production in different harmful dinoflagel-

lates (Johnsen et al. 1999, John & Flynn 2000, Van de Waal et al. 2013, Pérez Blanco et al. 2015). We are aware of only 2 empirical studies that have specifically addressed the balance of intracellular nutrients in particular mixotrophs and its dependence on environmental factors and potential differences in their nutrient stoichiometry compared to purely phototrophic or heterotrophic organisms. Chrzanowski et al. (2010) investigated the elemental stoichiometry of the mixotrophic flagellate *Ochromonas danica* in response to varied bacterial resource composition. They found the molar C:N:P composition in *O. danica* to be variable depending on the C:N:P of the food source, indicating that this flagellate only weakly regulated its element composition compared to a purely heterotrophic species. However, the authors did not consider stoichiometric variation or comparison of the mixotroph to purely phototrophic algae. The first experimental data suggesting that intracellular nutrient concentrations and ratios are more stable (less variable) in mixotrophs compared to pure phototrophs were provided by Katechakis et al. (2005). Their study compared stoichiometry and biomass production of a phototrophic specialist alga (*Scenedesmus*) and 2 mixotrophic nanoflagellates (*Cryptomonas* sp. and *Ochromonas tuberculata*) under different light and P supply and compared their relative food quality for zooplankton. In accordance with the light:nutrient hypothesis (Sterner et al. 1997), biomass and nutrient stoichiometry of the phototrophic specialist were very variable and strongly depended on light and nutrient supply. Growth and fecundity of the zooplankton consumer *Daphnia magna* fed with *Scenedesmus* was limited by food quantity at low light intensities and by food quality (high C:nutrient ratios of prey) at high light intensities. In contrast, biomass and nutrient stoichiometry of the mixotrophs were hardly affected by different nutrient and light supply, as they compensated for light and P deficiency by feeding on bacteria. *O. tuberculata* was toxic to *D. magna* and not considered further by Katechakis et al. (2005), but presence of the mixotroph *Cryptomonas* resulted in higher and more stable secondary production at most light:nutrient supply ratios compared to the purely phototrophic *Scenedesmus*. This suggests that mixotrophs indeed may have a balancing effect on food webs under variations of light and dissolved nutrient supply, potentially increasing the energy transfer efficiency to higher trophic levels. Despite its potential relevance for food web dynamics and nutrient cycling in a changing environment, the specific role of mixotrophs for seston stoichiometry has been largely ignored.

In the present study we therefore aimed for a better understanding of mixotroph stoichiometry by comparing intracellular nutrient data (N:P) from laboratory studies on mixotrophic species (nanoflagellates, dinoflagellates and ciliates) growing at different concentrations of dissolved N and P. We compared this information to equivalent laboratory data from purely photosynthetic organisms across marine and freshwater systems spanning different taxonomic groups (dataset derived from Hillebrand et al. 2013). In addition, we analyzed seston C:P ratios from natural phytoplankton communities as a function of the contribution of potentially mixotrophic species to the total phytoplankton community. We utilized data from 2 lake surveys that were carried out during summer stratification in 2004 (Striebel et al. 2009a, 2009b) and 2012 (Horváth et al. 2017).

We used these data to address 2 hypotheses: (1) purely phototrophic species are more variable in their intracellular N:P ratios than mixotrophic species and show a stronger response to varying dissolved N:P ratios in the external medium compared to mixotrophic species because mixotrophs are able to compensate for limiting of dissolved nutrients by also exploiting particulate organic nutrient sources, i.e. feeding on prey; and (2) with increasing contribution of potentially mixotrophic species to the total phytoplankton community, seston C:P ratios and the variability of these ratios decrease because mixotrophs are able to buffer inorganic nutrient limitation by phagotrophic feeding on prey.

METHODS

Laboratory cultures

For the purely phototrophic species, we used a composite dataset of algal N:P compiled by Hillebrand et al. (2013). This composite dataset includes 56 datasets comprising 590 N:P ratios from 29 publications, including only cyanobacteria and diatoms, i.e. taxonomic groups in which no potential mixotrophs occur, as well as chlorophytes which have few marine and no known freshwater mixotrophs.

For mixotrophic species, we searched the Web of Science by the Institute for Scientific Information for studies reporting intracellular particulate nutrient concentrations. We used the search terms 'mixotroph*' and ('nutrient*' or 'stoichiometr*'), as well as 'dinoflagellate*' and ('nutrient*' or 'stoichiometr*'). From the studies we found, we included those into our analysis that contained particulate N and P data

of laboratory cultures of mixotrophic species to calculate intracellular N:P ratios. In many cases, a single publication yielded multiple datasets that were included if independent experiments were performed by growing the same mixotrophic species under different conditions (e.g. different nutrient or light conditions (see Figs. S1 & S2 in the Supplement at www.int-res.com/articles/suppl/a079p235_supp.pdf) or if more than one species were investigated in a single study. In total, we collected 212 N:P ratios for 17 mixotrophic species from 16 publications and 2 so far unpublished studies, comprising 3 ciliate species, 8 dinoflagellate species, 3 chrysophyte, 2 haptophyte and 1 cryptophyte species (Table 1), feeding on either bacterial or microalgal prey. For our analysis, we pooled all the data from different taxonomic groups, as data were too scarce to distinguish between different groups of mixotrophs. Overall, the studies included were quite inconsistent in the data they provided. Some of them stated intracellular nutrient concentrations of mixotrophs after growing with prey organisms (we excluded studies where particulate nutrient data presented combined results for the stoichiometry of mixotrophs and their prey), while other studies provided data for known mixotrophs without enrichment with specific prey organisms (especially dinoflagellate studies), and some studies provided both kinds of data. However, none of the mixotrophic cultures were axenic, i.e. all of them contained potential bacterial prey, even if protistan prey was not present. Therefore, we can assume that mixotrophic cultures that were grown without specific prey additions might have ingested bacterial prey and were thus also growing mixotrophically. All of the included laboratory studies provided N:P availability in the medium of the cultures, most of the studies also provided light intensities (see Figs. S1 & S2); other environmental parameters, however, were provided too inconsistently to be included in our analyses.

We compared the median N:P ratio of purely phototrophic species to that of mixotrophic species across the entire data set, transforming all data to molar N:P ratios. Thereby, we determined the range and variability of N:P ratios for both groups and tested potential differences of the N:P distributions with a Kolmogorov-Smirnov (KS) test. We then analyzed the correlation between the internal N:P ratios to the supplied N:P ratios for phototrophs and mixotrophs respectively. We used a general linear model (glm) with organism identity (mixotroph versus phototroph) as categorical binary variable and ln-transformed N:P availability as a continuous variable. A significant interaction ($p < 0.05$) between organism identity and ln-transformed N:P availability suggests a significant difference between slopes.

Table 1. Publications providing data on intracellular N:P ratios in mixotrophic organisms

Taxonomic group	Species	Publication
Freshwater		
Chrysophyte	*Dinobryon cylindricum*	Caron et al. (1993)
Chrysophyte	*Ochromonas danica*	Chrzanowski et al. (2010)
Chrysophyte	*Ochromonas danica*	Simonds et al. (2010)
Chrysophyte	*Ochromonas danica*	Wilken et al. (2014)
Chrysophyte	*Ochromonas tuberculata*	Katechakis et al. (2005)
Cryptophyte	*Cryptomonas* sp.	Katechakis et al. (2005)
Ciliate	*Euplotes daidaleos*	S. D. Moorthi (unpubl. data)
Ciliate	*Coleps* sp.	S. D. Moorthi (unpubl. data)
Marine		
Dinoflagellate	*Alexandrium catenella*	M. Busch et al. (unpubl. data)
Dinoflagellate	*Lingulodinium polyedrum*	M. Busch et al. (unpubl. data)
Dinoflagellate	*Alexandrium tamarense*	Van de Waal et al. (2013)
Dinoflagellate	*Ceratium furca*	Smalley & Coats (2002)
Dinoflagellate	*Ceratium furca*	Smalley et al. (2003)
Dinoflagellate	*Dinophysis norvegica*	Gisselson & Granéli (2001)
Dinoflagellate	*Fragilidium subglobosum*	Skovgaard et al. (2000)
Dinoflagellate	*Gyrodinium galatheanum*	Li et al. (2000)
Dinoflagellate	*Prorocentrum minimum*	M. Johnson (2015)
Haptophyte	*Chrysochromulina leadbeateri*	Johnsen et al. (1999)
Haptophyte	*Prymnesium parvum*	Carvalho & Granéli (2010)
Haptophyte	*Prymnesium parvum*	Skovgaard et al. (2003)
Haptophyte	*Prymnesium parvum*	Brutemark & Granéli (2011)
Ciliate	*Mesodinium rubrum*	Brutemark & Granéli (2011)

Field data

We used seston stoichiometry (C:P) data from 2 lake surveys that were carried out in Germany (Bavaria) and Austria in September 2004 (Survey A; Striebel et al. 2009a,b) and August to September 2012 (Survey B; Horváth et al. 2017). These particular surveys were selected because both studies had reliable microscopic biovolume estimates and used highly comparable methods in terms of sampling procedure etc. (Striebel et al. 2009a,b, Horváth et al. 2017). In these surveys, altogether 76 samples were collected from 61 different lakes in south-eastern Germany and Austria during summer stratification (July to early

September). Survey A used 42 samples from 36 lakes and Survey B used 34 samples from 34 lakes; 9 lakes were sampled in both surveys. Integrated epilimnetic water samples were taken from boats in both cases. Species were identified and quantified by light microscopy, and species-specific biovolumes were estimated by approximation to simple geometrical bodies. At least 20 cells were measured for each abundant species in each sample. In an initial analysis, we checked whether microscopic estimates of algal biovolume scaled with sestonic particulate organic carbon (POC) (Fig. S3). For details on sampling procedure and microscopic analysis see Striebel et al. (2009a) and Horváth et al. (2017). Particulate fractions of C and P were analyzed as outlined in Striebel et al. (2009a). Data from both lake surveys were pooled, and the percentage of mixotrophs was estimated by pooling the biovolume of all taxa with potential phagotrophic capacity (chrysophytes excluding Synurophyta, Cryptophyta, Dinophyta and Haptophyta) and dividing it by the total phytoplankton biovolume. Ciliates and heterotrophic protists (especially heterotrophic dinoflagellates and cryptophytes) were not considered in this analysis. In order to limit the variability of total P as confounding factor, eutrophic samples (total P >30 µg l^{-1}) were excluded from the analysis. Within the selected data, C:P is uncorrelated with total P (Spearman's ρ = 0.053; Fig. S3).

In order to examine the dependence of seston C:P ratios on mixotrophs, we performed a regression of molar C:P on the proportion of mixotrophs (% mixotrophs) in the total phytoplankton community for each sample, using generalized additive models for location scale and shape (GAMLSS; Rigby & Stasinopoulos 2005). Non-constant error variance was fit along with the mean, as the pattern suggested heteroscedasticity along the % mixotrophs gradient. Model selection was performed by first fitting a minimal model (trend of the mean) and subsequently testing whether the model was significantly improved when taking non-constant error variance into account (AIC criterion). In order to test for the robustness of a trend of C:P with % mixotrophs, we also calculated a rank correlation.

RESULTS

Laboratory cultures

The distributions of molar cellular N:P ratios differed significantly between mixotrophs and phototrophs (KS test, D = 0.196, p < 0.001). The median molar N:P of phototroph biomass in the laboratory studies examined here was 15.00 (interquartiles from 8.53 to 31.37), whereas for mixotrophs the median was slightly higher (19.7) and the interquartiles were less widespread (12.32 to 25.40) (Fig. 1). With increasing N:P of available dissolved nutrients, both mixotrophs and phototrophs increased their internal N:P ratios. Both main factors, available N:P and organism identity (mixotroph versus phototroph), significantly affected cellular N:P ratios (p < 0.001 for both factors), explaining 54% of the variation in the glm (adjusted R^2 = 0.538). Also the interaction of both factors was significant (p < 0.001), suggesting a significant difference between the slopes of cellular versus dissolved N:P in ln–ln space (Fig. 2). These data indicate that, as the relative availability of dissolved P decreased (available N:P increased), more P was retained in mixotroph biomass compared to phototroph biomass.

Overall, the range of N:P ratios provided for different groups in different studies was broader for phototrophs than for mixotrophs (Fig. S1), which also might have contributed to more variable N:P ratios in phototrophs. The range of light intensities used in

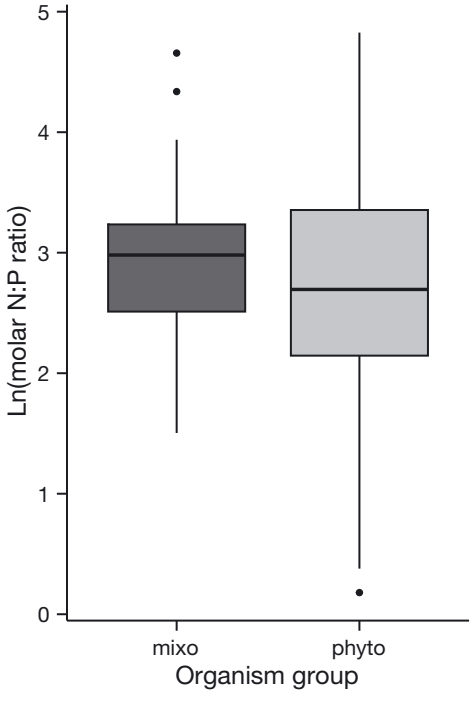

Fig. 1. Molar N:P ratios (ln) of mixotrophic (mixo, n = 212) and strictly photoautotrophic (phyto, n = 590) planktonic protists, based on data pooled from laboratory studies (Table 1; Hillebrand et al. 2013). Median (horizontal line), interquartiles (boxes) and ranges (vertical lines) are shown as well as outliers (>1.5 times interquartile range)

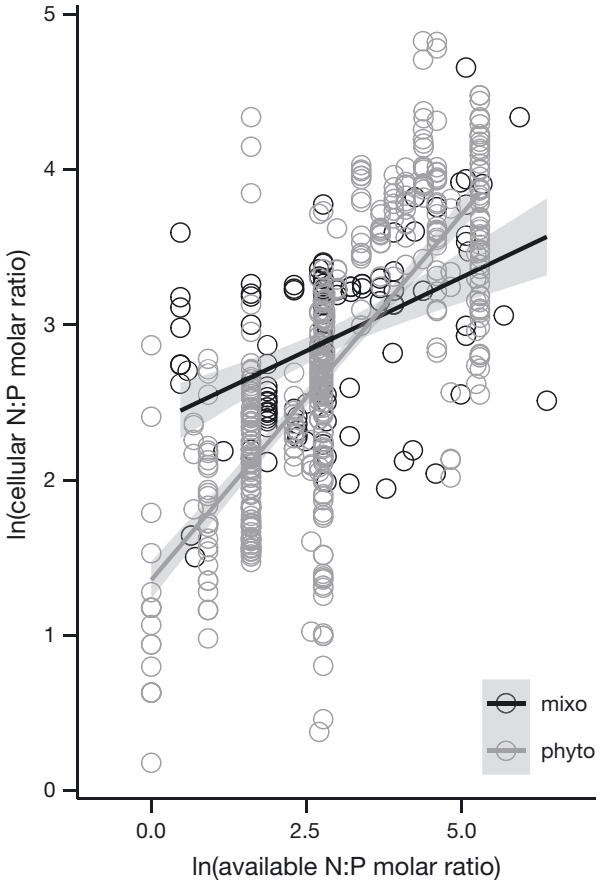

Fig. 2. Molar N:P ratios (ln) of mixotrophic (mixo) and strictly photoautotrophic (phyto) planktonic protists along a gradient of available N:P ratios in the external medium. Lines are linear regressions with the standard error of the slope as shaded area

different studies, however, was much broader for phototrophs than for mixotrophs; we can thus assume that light intensities did not play a role as a biasing parameter confounding the patterns observed in our study (Fig. S2).

Field data

A negative correlation between proportion of mixotroph biomass in a community and sestonic C:P was evident from a rank correlation (Spearman's $\rho = -0.29$, $p = 0.01$). As the data indicated non-constant error variance with increasing fraction of mixotrophs, we fitted a GAMLSS model, which allows fitting trends in mean and error variance simultaneously. Model selection clearly supported a negative relationship between sestonic C:P ratios and the relative contribution of mixotroph biomass to the total phytoplankton community (fraction of mixotrophs; Fig. 3). Furthermore, variation of this relationship decreased with increasing proportion of mixotrophs on total phytoplankton abundance in the lake surveys: the predictability of seston stoichiometry was higher in samples with high proportion of mixotrophs (heteroscedastic relationship; Fig. 3).

DISCUSSION

Both laboratory and field data indicated that mixotrophic organisms have a balancing effect on the nutrient stoichiometry of primary producers under changing environmental conditions that potentially alter algal nutrient content and thus food quality. Supporting Hypothesis 1, in laboratory cultures, mixotrophs showed a lower variability in their intracellular N:P ratios compared to strict photoautotrophs and were less responsive to the N:P availability gradient, i.e. showed a less pronounced increase in internal N:P with increasing N:P ratios in the external medium. Investigation of seston stoichiometry in the Austrian and Bavarian lakes revealed that with increasing contribution of potentially mixotrophic phytoplankton taxa, seston C:P ratios decreased, as did the variance of C:P, thus supporting Hypothesis 2.

The 2 lake datasets show systematic deviation in their seston C:P ratio. C:P ratios in the 2 surveys differ most at low % mixotrophs, while they are very similar towards high % mixotrophs. A negative relationship between the fraction of mixotrophs and C:P ratio is especially evident in Survey B, while Survey A only shows a decrease in variation of C:P ratio with increasing fraction of mixotrophs (Fig. 3). At the same time, the 2 datasets show very similar scaling relationships between total P, algal biovolume and POC (Fig. S3). This confirms that methods for biovolume estimation and sampling procedures are generally comparable. We therefore suggest that the deviation between the 2 surveys reflects different environmental conditions in terms of lakes and climatic conditions (stratification pattern and availability of free resources).

Given the ubiquitous occurrence of mixotrophs in most aquatic environments (Caron 2016), our findings have major implications for trophic transfer and nutrient cycling in plankton food webs. Supporting the initial findings of Katechakis et al. (2005), whose results provided empirical evidence for the hypotheses tested in our study, our findings encompassing a breadth of studies indicate that mixotrophy may indeed enhance the transfer of energy and nutrients to higher trophic levels, ensuring more stable second-

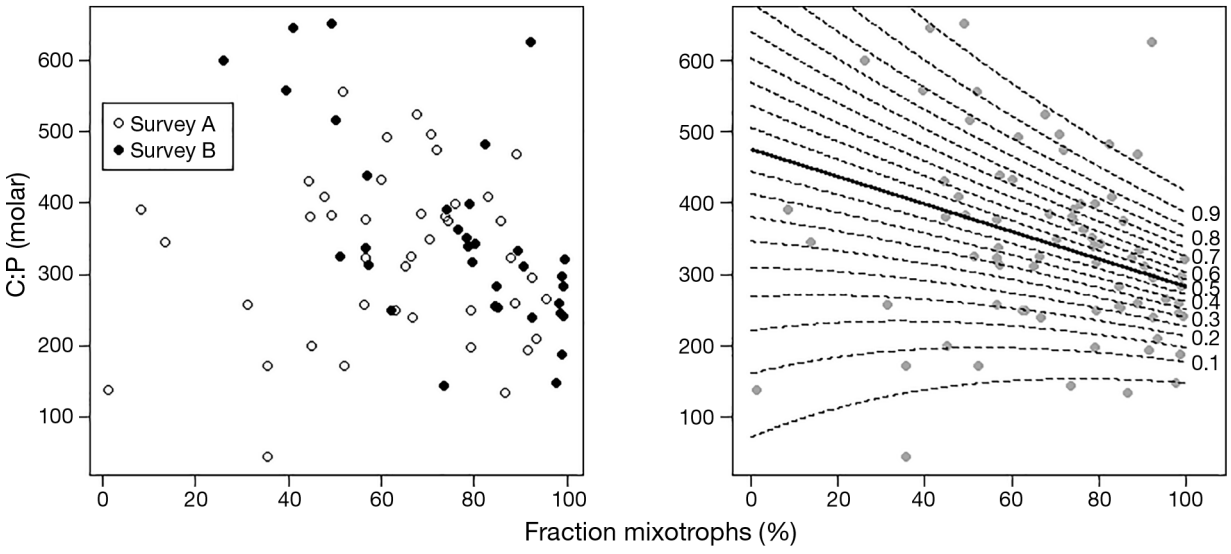

Fig. 3. Sestonic C:P ratio as a function of fraction of mixotrophs in samples from natural lakes. Left panel shows the raw data from 2 surveys: Survey A in 2004 (Striebel et al. 2009a) and Survey B in 2012 (Horváth et al. 2017). The right panel shows all data together with results of analysis using generalized additive models for location scale and shape (GAMLSS), fitting the mean trend to the trend in variation along the *x*-axis. The bold line gives the trend of the mean, dashed lines indicate the percentiles of error variance in steps of 5% along the predictor (i.e. the area below the lowest line comprises 5% of the error variance, the next line 10% etc.). The estimates for the mean (±SD) slope and trend in variance are −1.92 ± 0.877 and −0.011 ± 0.0035, p = 0.003, respectively, for 76 observations with 72 residual degrees of freedom

ary production in plankton food webs. The large variability in cellular elemental ratios of strict photoautotrophic species can have severe consequences for consumers that generally show a more confined stoichiometry, which can lead to an elemental mismatch of the consumers' nutrient demands and the relatively plastic nutrient balance in their algal prey (e.g. Elser et al. 2000, Persson et al. 2010). Such mismatches alter consumption rates on low quality prey (high C:nutrient), either resulting in prey avoidance, or in compensatory feeding at the consumer individual level (e.g. Hillebrand et al. 2009a). Eventually, feeding on low quality food may lower consumer performance and growth efficiency, leading to reduced consumer population size and trophic transfer (Cross et al. 2003, Fagan & Denno 2004, Frost et al. 2006, Hillebrand et al. 2009a, Moorthi et al. 2016). Thus, stoichiometric constraints of herbivores may even propagate through the food web to secondary consumers (e.g. Malzahn et al. 2007) and have far-reaching consequences for the community structure and productivity of the entire food web.

Anthropogenic nutrient input into the biosphere has almost doubled in the past decades (Vitousek et al. 1997, Rockström et al. 2009), leading to shifting N:P ratios in many freshwater and coastal marine systems. Atmospheric N deposition has already induced a secondary P limitation (Elser et al. 2009), potentially leading to constraints in energy and nutrient flow through consumer–resource interactions and thus biogeochemical cycling in ecosystems (Cherif & Loreau 2013, Glibert et al. 2013). The results of the present study indicate that the stoichiometric composition of mixotrophs is less affected by changes in nutrient supply than the stoichiometry of strictly photoautotrophic organisms, based on the mixotrophs' ability to use both particulate and dissolved nutrient sources. Potentially limiting nutrients such as P are often much more concentrated in the prey organisms of mixotrophs, such as bacteria, compared to the dissolved phase (e.g. Vadstein 2000). Bacteria are known to have relatively low and constrained C:P and C:N ratios (Sterner & Elser 2002, Makino et al. 2003), enabling bacterivorous mixotrophs to maintain low C:nutrient ratios even when dissolved nutrient concentrations are low, potentially making them a nutrient-rich food source for higher trophic levels in nutrient depleted environments. Mixotrophy may thus enhance the transfer of energy and nutrients to higher trophic levels, resulting in more stable secondary production in plankton food webs, as was demonstrated by Katechakis et al. (2005). In a marine study involving N-limited planktonic assemblages, Ptacnik et al. (2004) found that the presence of the mixotrophic flagellate *Chrysochromulina* led to increasing seston C:N ratios and enhanced copepod reproduc-

tion. The results by Ptacnik et al. (2004) show that mixotrophy does not necessarily reduce the C content of seston biomass. Especially in oligotrophic marine systems, bacteria contain a larger fraction of the total amount of nutrients. Here the net effect of bacterivorous mixotrophs may be an enhancement of phytoplankton biomass at the expense of bacteria, resulting in higher sestonic C content (see also Thingstad et al. 1996 for ambivalent effects of mixotrophs on phototrophic algae).

Chrzanowski et al. (2010) demonstrated that the mixotrophic flagellate *Ochromonas danica* was more variable in element stoichiometry (C:N, C:P and N:P) in response to different bacterial resource composition compared to purely heterotrophic flagellates. Taken together, the experimental observations of Chrzanowski et al. (2010), Katechakis et al. (2005) and our study suggest that mixotrophic organisms are intermediate in their homeostatic abilities, being more variable in their stoichiometry compared to strict heterotrophs, but less variable compared to strict photoautotrophs. However, more data and studies on the stoichiometry of heterotrophic and mixotrophic protists are required to verify this assumption. The various nutritional strategies that mixotrophic protists have evolved as well as the variable contribution of the 2 nutritional modes to the overall nutrition of the organism substantially complicate the description of food web structure in aquatic systems. The contribution of mixotrophs to net community production on different trophic levels is difficult to quantify, and its integrated impact on global biogeochemical cycles still remains largely unknown. The prevailing nutritional mode of a mixotrophic species strongly determines its influence on trophic dynamics, regarding grazing control, nutrient uptake and regeneration (e.g. Rothhaupt 1997, Fischer et al. 2016, 2017), and very likely also intracellular stoichiometry.

Here, we presented an effort to resolve the effect of mixotrophic organisms on nutrient stoichiometry in plankton food webs. Our study indicates that mixotrophs might enhance food quality for herbivores by constraining stoichiometric variation in the phytoplankton, resulting in a more stable transfer of nutrients and energy to higher trophic levels. However, further studies are necessary to validate this finding and unravel the relationship of different mixotrophic feeding strategies and their influence on nutrient dynamics across trophic levels. Due to the scarcity of data on the stoichiometry of mixotrophs, we could only include a few mixotrophic species that are frequently used in laboratory experiments, and were not able to distinguish between different groups pursuing different nutritional strategies, such as primarily phototrophic flagellates capable of phagotrophy and primarily heterotrophic protozoa that have gained photosynthetic capacity through kleptoplastidy or symbiotic algae (Caron 2016). Not much is known about protozoa that have kleptoplastidic or symbiotic associations with microalgae, such as ciliates and many species of Rhizaria (Foraminifera, Acantharea, Radiolaria). Rhizaria are particularly common in tropical and subtropical oceans (Caron 2016) and their importance has been grossly underestimated in the world ocean (de Vargas et al. 2015).

There are a few caveats to our investigation. Some studies in our analysis included potential mixotrophs that are known to ingest bacteria grown in non-axenic cultures, but in which phagotrophic feeding by mixotrophs was not quantified. We assumed that bacteria were ingested and might have potentially buffered nutrient limitation by dissolved nutrients. Furthermore, the laboratory cultures examined in our study were growing under different environmental conditions, such as different light and temperature conditions. The variability of light conditions used in different studies was higher for mixotrophs than for phototrophs and did not play a role as confounding factor for our observations (Fig. S2). However, there was a broader range of nutrient availability (dissolved N:P) for phototrophs than for mixotrophs, which might have contributed to the increased intracellular N:P variability observed in phototrophs compared to mixotrophs.

Temperature data were not consistently provided for different studies and could therefore not be analyzed, although this factor is known to alter the stoichiometry in algae. With increasing temperature, algal C:nutrient ratios have been shown to increase, either due to enhanced carbon fixation per unit resource (e.g. Moorthi et al. 2016) or due to decreasing intracellular N and P concentrations based on enhanced efficiency of RNA (requiring P) and proteins (requiring N) in biochemical reactions (Woods et al. 2003). Also, the contribution of photosynthesis and phagotrophy varies with temperature in mixotrophs (Wilken et al. 2013, Princiotta et al. 2016), which presumably also alters intracellular nutrient stoichiometry. Our lake data analysis also needs to be considered with some care, as we included all potentially mixotrophic taxonomic groups, but of course cannot be sure whether the dominant organisms in these groups were employing phagotrophic feeding at the time of sampling. Nevertheless, both lab and field data in our study implicate a significant role of mixotrophic organisms in balancing stoichiometric con-

straints for herbivorous zooplankton, which so far have been neglected in many empirical and theoretical studies focusing on trophic dynamics and nutrient cycling in plankton food webs. An integrated approach combining targeted empirical studies disentangling the effects of different mixotrophic strategies will substantially improve our understanding of the relevance of mixotrophy for ecological stoichiometry and trophic transfer in plankton food webs.

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