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Seasonal dynamics of zooplankton resource use revealed by carbon amino acid stable isotope values

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ABSTRACT: Reliable tracer techniques are fundamental to retrieving accurate information on consumer dietary resource use in dynamic ecosystems. Analysis of $\delta^{13}C$ values in essential amino acids has shown great promise in effectively capturing consumer food sources, since essential amino acids are not synthesized by heterotrophic organisms but instead routed directly from dietary sources to consumers, resulting in negligible ^{13}C trophic discrimination. We assessed seasonal feeding patterns of a widespread key copepod species (*Acartia* spp.) in the northern Baltic proper using complementary approaches: bulk $\delta^{13}C$ and $\delta^{15}N$ values, $\delta^{13}C$ values of essential amino acids, and quantitative phytoplankton taxonomic data. Our results showed distinct differences between *Acartia* and seston $\delta^{13}C$ essential amino acid values measured at weekly to monthly sampling intervals, which indicated that *Acartia* preferentially utilized specific dietary resources that comprised only parts of the total phytoplankton composition (varying from 19.7% to 81.4% during the season). Results also indicated that care should be taken when inferring trophic position from bulk stable isotopes when consumers are highly selective, since isotope values of seston may not accurately reflect consumer specific diet resource uptake. Analysis of $\delta^{13}C$ values in essential amino acids was shown to be a promising tool to accurately trace consumer resource use in complex natural systems.

KEY WORDS: Stable isotopes · Amino acids · *Acartia* · Zooplankton · Phytoplankton · Food webs

INTRODUCTION

Understanding consumer feeding dynamics is critical to accurately estimate and predict flows of energy in complex networks, such as aquatic food webs. This is especially important in coastal ecosystems where phytoplankton community composition and abundance is highly variable due to changes in accessible elemental nutrients (Cloern 1996). Altered phytoplankton composition in turn leads to temporal variability in both quantity and quality of available dietary sources for consumers, such as zooplankton (Walve & Larsson 1999, Winder et al. 2012). Zooplankton occupy an important intermediate trophic niche, as they graze on phytoplankton and protists, while themselves being a primary food source for many fish larvae and small pelagic fishes. The trophic linkage between phytoplankton and zooplankton is especially critical for energy and nutritional fluxes to upper trophic levels since heterotrophs have limited capability in synthesizing certain macromolecules needed for reproduction and growth (Brett & Müller-Navarra 1997, Müller-Navarra 2008). Many zooplankton species alternate among food sources and level of omnivory (Stoecker & Egloff 1987, Ptacnik et al. 2004), which results in variable trophic positions (Peters et al. 2013) and resource use (Kürten et al. 2013). An ongoing challenge in marine systems is therefore to estimate how shifts in dietary resources at the base of the food web propagate to consumer organisms.

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Natural tracers, such as stable isotopes of nitrogen (N) and carbon (C), are valuable tools for depicting both species trophic information and nutritional flows in ecosystems, due to predictable ^{13}C and ^{15}N trophic discrimination (i.e. change in isotopic value from diet to consumer; Peterson & Fry 1987, Post 2002). Amino acid (AA) compound-specific C stable isotope analysis (AA-CSIA) is an additional tool to elucidate dietary C sources (Larsen et al. 2009). $\delta^{13}C$ values of essential AA (E-AA) provide particular promising dietary tracers since E-AAs cannot be synthesized de-novo by heterotrophic organisms and are instead routed directly from dietary sources to consumers, resulting in negligible ^{13}C trophic discrimination of E-AAs (Howland et al. 2003, Jim et al. 2006, Larsen et al. 2009, McMahon et al. 2010).

Accurate tracing of zooplankton dietary sources, however, also requires separation of isotope composition among major primary producer taxa. While differentiation between $\delta^{13}C$ E-AA values of phytoplankton taxa has yet to be fully resolved, Larsen et al. (2013) have recently shown clear separation in relative difference of E-AA $\delta^{13}C$ values between major primary producers of bacteria, fungi, terrestrial plants and phytoplankton, which likely result from differences in AA syntheses pathways between different types of primary producers (McCarthy et al. 2004, Larsen et al. 2013). Differences in $\delta^{13}C$ E-AA values of aquatic invertebrate consumers seem to accurately distinguish between resource use of marine, terrestrial (Ellis et al. 2014, Vokhshoori et al. 2014) or marsh primary producers (Fantle et al. 1999). The fact that different dietary components are visibly encoded in consumer isotope composition makes $\delta^{13}C$ E-AA values good proxies for understanding organism diet uptake.

In addition to different dietary resources, variation in consumer isotope values can be affected by the isotope composition at the base of the food web (i.e. isotopic baseline), and/or change in trophic position (Michener et al. 2007), though the latter is assumed negligible for $\delta^{13}C$ E-AA values (McMahon et al. 2010). Acquiring precise knowledge of the isotopic baseline needed for accurately estimating consumer trophic information (Vander Zanden & Rasmussen 2001, Post 2002) can be challenging, since consumers may selectively assimilate certain dietary components, which can be difficult to retrieve in the field. Seston can be used as a proxy for the isotopic baseline, though seston is a variable mixture of C sources, comprising autotrophic, heterotrophic and detrital material with individually distinct stable isotope values. Despite the apparent uncertainties accompanied with inferring isotope baseline information from pooled seston material, analysis of bulk isotope composition of aquatic consumers relative to seston has provided insight into seasonal variation in dietary source contribution (Grey et al. 2001, El-Sabaawi et al. 2010) and change in trophic position (Peters et al. 2013). Zooplankton bulk isotope values may also vary due to different nutritional quality of prey (Matthews & Mazumder 2005, Ventura & Catalan 2010), while different dietary macromolecules can also vary in isotope composition (Degens 1969, Kürten et al. 2013).

The myriad of factors potentially influencing bulk isotope values may make assessment of consumer feeding patterns difficult. Complimenting bulk stable isotope analysis with AA-CSIA could disentangle some of the uncertainties when applying bulk stable isotope analysis to retrieve information in complex food webs, and in turn also give valuable new information on the powerful but still novel application of $\delta^{13}C$ E-AA values in aquatic organisms. Here, we used a combination of bulk $\delta^{13}C$ and $\delta^{15}N$ as well as $\delta^{13}C$ E-AA values to trace the seasonal feeding patterns of a widespread key copepod species, *Acartia* spp., in the northern Baltic proper. Specifically, we hypothesize that our complimentary analysis of both bulk and C E-AA isotope composition will provide information on zooplankton consumer's ability to selectively forage on specific prey resources throughout the growing season. To effectively capture changes of dynamic species with relatively short life cycles, such as phytoplankton and zooplankton, we employed a sampling scheme with a high temporal resolution.

MATERIALS AND METHODS

Sampling and isotope analysis

We sampled zooplankton and seston at weekly to monthly intervals between April and December 2012 at a coastal station ('B1', 58° 49′ N, 17° 36′ E) in the northern Baltic proper. Sampling dates are presented as day of year, which denotes the number of days since the start of the year 2012. The study site has a maximum depth of approx. 40 m, salinity is around 6–7, and a thermocline at around 20 m depth during most of the productive period. Sampling of zooplankton was done with a vertical tow from 0 to 20 m using a 90 µm mesh size plankton net. Immediately after sampling, adult *Acartia* species (*A. bifilosa, A. tonsa, A. longiremis*) were sorted and 70 ind. (~0.5 mg) were pooled per isotope sample analysis. Water for

seston samples (~4 l) was collected uniformly from 0 to 20 m, pre-screened through 100 µm mesh to exclude zooplankton and filtered onto 47 mm precombusted GF/F (0.7 µm pore size) filters. All samples were immediately stored at −20°C and subsequently freeze-dried before isotope analyses.

Acartia and seston samples of bulk $\delta^{13}C$ and $\delta^{15}N$ values, and $\delta^{13}C$ values of the E-AAs were analysed at the University of California (Davis) stable isotope facility. E-AAs of Isoleucine (Ile), Leucine (Leu), Lysine (Lys), Phenylalanine (Phe) and Valine (Val) were considered in this study, as these E-AAs were consistently and reliably measurable. $\delta^{13}C$ AA-CSIA was done using GC-C-IRMS according to the method described in detail by Walsh et al. (2014). Briefly, AAs of the prepared samples were separated from proteins during acid hydrolysis, followed by derivatization with methyl chloroformate. Derivatives of AAs were then converted to gaseous CO_2 and provisional δ-values were calculated based on reference CO_2 values, and subsequently corrected relative to; an internal standard of norleucine, kinetic isotope effects and carbon atoms added during the derivatization process (Walsh et al. 2014). Stable isotope ratios are described as:

$$\delta(‰) = [(R_{sample}/R_{standard}) - 1] \times 1000 \quad (1)$$

where R = $^{13}C/^{12}C$ or $^{15}N/^{14}N$ of AA or bulk tissue. $R_{standard}$ is an international standard of atmospheric N_2 for N or Pee Dee belemnite for C. Our field analysis did not provide replicate samples for AA-CSIA analysis; however, 3 replicates of cultured red algae *Rhodomonas salina* were included in the AA-CSIA analysis to assess analytical reproducibility. Standard errors (SE) for *R. salina* ranged from 0.1 to 1.1 for all $\delta^{13}C$ AA—except for Ile, which had a SE of 1.6 (data not shown).

Quantitative data for phytoplankton taxonomic groups (calculated in % based on C biovolume) from the same station at the same dates was obtained from the Swedish Meteorological and Hydrological Institute database SHARK (Svenskt HavsARKiv, www.smhi.se, accessed 8 Jun 2013; see Table 2). These data are based on an integrated water sample from 0 to 20 m and subsequently analysed to species level (see Olenina et al. 2006 for description of analytical procedure).

Statistical analysis

Correlation coefficients of C and N bulk isotope values of *Acartia* and seston were calculated using nonparametric Spearman's rank correlations. Autocorrelation was low for all variables and did not significantly influence any statistical outcomes. Due to expected short isotope incorporation time in zooplankton and phytoplankton we considered comparison of sampling dates with no difference in lag time the best option, as sampling dates were usually separated by 2 wk or more. However, it is acknowledged that differences in lag time between zooplankton and phytoplankton likely introduce some variability.

Hierarchical clustering analyses using Ward's minimum variance method (Legendre & Legendre 2012) was applied to differentiate major seasonal clusters of *Acartia* and seston $\delta^{13}C$ E-AA values, respectively. Clusters of seasonal groups were tested for significance using permutational multivariate ANOVA (PERMANOVA, Euclidian distance, 999 permutations; Anderson 2001, Anderson & Walsh 2013).

Dynamic factor analysis (DFA), a suitable technique for evaluating common trend(s) in shorter multiple time series (Zuur et al. 2003a) was used to assess seasonal variation in $\delta^{13}C$ E-AAs values for *Acartia* and seston separately. DFA models time series as a linear combination of common trends, explanatory variables, a constant level parameter and a noise component (see Zuur et al. 2003b for complete details of DFA). Factor loadings infer if and to what extent the time series of $\delta^{13}C$ values from a specific E-AA follow the pattern of the common trend(s) in a positive or negative fashion. Prior to analysis, each time series of E-AA $\delta^{13}C$ values were logged and then standardized by subtracting the mean and dividing by the standard deviation (Zuur et al. 2003b). No *a priori* assumptions were made regarding the time series error component (i.e. whether there is shared or unique variance or covariance among $\delta^{13}C$ E-AA values). For all matrix structures in the DFA models we followed the recommendations of Holmes et al. (2013). All model combinations including 1 to 3 common trends and all possible time series error structures were fitted using the MARSS package v. 3.9 (Holmes et al. 2012). The corrected Akaike information criterion (AICc, Burnham & Anderson 2002) was used to identify the most parsimonious model.

To assess common patterns of correlation among all E-AA $\delta^{13}C$ values and phytoplankton taxonomic composition (see Table 2), we constructed redundancy analysis (RDA) models based on Euclidean distances (Zuur et al. 2007), for either *Acartia* or seston E-AA $\delta^{13}C$ values as the response variable and the phytoplankton composition as explanatory variables. RDA is a statistical method which can be considered an extension of PCA, used to summarise variation in a set of response variables (e.g. $\delta^{13}C$ E-

Table 1. *Acartia* and seston essential amino acid $\delta^{13}C$ values of Isoleucine (Ile), Leucine (Leu), Phenylalanine (Phe), and Valine (Val), and Lysine (Lys) for *Acartia* only, sampled from the northern Baltic proper. 'Day' signifies day of year in 2012. '*Acartia* cluster' denotes significantly different clusters as partitioned by the Hierarchical cluster analysis (PERMANOVA, model *F*: 28.99, $p < 0.01$, df = 18). – denotes no measurement available

Day	*Acartia* cluster	*Acartia* essential amino acid $\delta^{13}C$ values					Seston essential amino acid $\delta^{13}C$ values			
		Ile	Leu	Lys	Phe	Val	Ile	Leu	Phe	Val
116	1	–25.58	–35.73	–27.25	–31.78	–31.14	–30.26	–37.30	–28.29	–31.17
137	1	–26.27	–34.25	–25.30	–32.26	–29.31	–27.52	–36.06	–25.11	–30.06
145	1	–24.72	–31.98	–24.95	–31.91	–29.07	–26.02	–35.03	–26.11	–29.16
150	1	–24.45	–33.91	–25.36	–33.88	–29.13	–27.90	–36.54	–26.77	–33.50
161	1	–26.68	–32.53	–28.11	–32.20	–28.64	–27.54	–35.72	–27.00	–23.86
172	1	–23.45	–30.34	–27.92	–33.32	–29.84	–26.32	–34.25	–25.68	–30.52
186	2	–27.23	–35.00	–21.44	–31.97	–31.46	–26.99	–34.39	–26.83	–35.63
199	2	–26.41	–32.67	–21.87	–31.70	–33.09	–27.42	–33.84	–24.41	–33.45
214	2	–27.99	–34.43	–24.60	–32.56	–35.10	–25.28	–33.46	–26.24	–31.23
228	2	–29.65	–36.20	–23.26	–35.86	–36.43	–26.24	–32.24	–24.54	–29.94
242	2	–25.96	–33.04	–22.60	–30.95	–33.76	–25.12	–33.38	–26.01	–30.44
248	2	–28.40	–35.72	–25.57	–31.47	–34.19	–24.02	–33.32	–26.20	–29.86
256	2	–28.42	–35.73	–24.00	–33.14	–34.99	–26.42	–34.60	–26.07	–31.36
270	3	–21.76	–29.42	–21.36	–25.47	–26.22	–24.83	–33.42	–25.71	–30.28
285	3	–24.67	–32.31	–21.22	–28.24	–27.48	–28.13	–35.46	–26.87	–30.84
298	3	–21.84	–29.65	–20.93	–25.77	–26.82	–25.87	–33.32	–25.51	–30.99
312	3	–23.90	–31.58	–20.63	–26.41	–26.26	–27.18	–32.99	–25.76	–30.11
318	3	–22.45	–30.53	–21.48	–25.10	–26.81	–25.21	–31.88	–24.76	–27.89
340	3	–22.04	–30.21	–18.45	–26.89	–26.85	–25.97	–33.71	–	–30.29

AA values) that can be explained by a set of explanatory variables (e.g. phytoplankton composition) using multiple linear combinations to find the best ordination of the multivariate data (Zuur et al. 2007). The most parsimonious RDA model was chosen based on forward selection and 999 permutations. The reported *F*-value was derived from the permutation procedure. For hierarchical clustering analyses, DFA and RDA we included $\delta^{13}C$ values of Ile, Leu, Phe and Val for analysis of seston, and additionally $\delta^{13}C$ Lys values for *Acartia*. For RDA and PERMANOVA analyses of seston, Day 340 was not included since the $\delta^{13}C$ Phe value was not measured. The vegan package 2.0-10 was applied for hierarchical cluster analysis, PERMANOVA and RDA (Oksanen et al. 2013) and for all statistical analysis R CRAN v. 3.1 with R-studio v. 0.98 interface was used (R Development Core Team 2014).

RESULTS

Bulk $\delta^{13}C$ and $\delta^{15}N$ values and phytoplankton community composition

Acartia $\delta^{13}C$ bulk values (–26.4‰) were lowest in early summer followed by a late summer increase (–20.6‰) before declining again in fall to around –26.2‰ (Fig. 1a). Bulk C isotope composition of seston showed a similar cycle to *Acartia* $\delta^{13}C$ values, peaking in summer with values of –22.9‰ before declining to –26.9 ± 0.06‰ (SD) in fall (Fig. 1a). *Acartia* $\delta^{13}C$ correlated significantly with seston $\delta^{13}C$ values at lag zero (Spearman's rho = 0.52, $p < 0.01$, df = 15). However, changes in *Acartia* $\delta^{13}C$ bulk values did not closely reflect those of seston $\delta^{13}C$ bulk values as relative differences varied seasonally between –1.7 and 3.9‰ (Fig. 1c).

Acartia bulk $\delta^{15}N$ values varied between 5.5 and 10.0‰, changing at similar times but in the opposite direction to $\delta^{13}C$ bulk values, with lowest values in late summer (Fig. 1b). Similar seasonal fluctuations were present in seston N isotope composition, varying from 2.3 ± 0.14‰ to 6.4 ± 0.09‰ (SD) (Fig. 1b). Seston and *Acartia* $\delta^{15}N$ values significantly correlated (Spearman's rho = 0.62, $p < 0.01$, df = 15) with relative differences between $\delta^{15}N$ values of *Acartia* and seston varying between 1.7 and 4.7‰, increasing from Day 161 to late fall (Fig. 1c).

The phytoplankton taxonomic composition (expressed as % C) varied temporally during the season, with diatoms abundant in spring followed by an increase of the photosynthetic ciliate *Mesodinium rubrum* and dinoflagellates (Table 2). In summer a bloom of cyanobacteria, mainly *Nodularia spumegina* and *Aphanizomenon* spp. was followed by a

Table 2. Percentage of major phytoplankton taxa based on carbon biomass throughout the sampling period in the northern Baltic Proper (day of year [Day] 116 to 340). Taxa abbreviations: Cryp (cryptophytes), Cyan (cyanobacteria), Diat (diatoms), Dino (dinoflagellates), Meso (*Mesodinium rubrum*), Pras (prasinophytes), Prym (prymnesiophytes) and Other (comprised of chlorophytes, chrysophytes, euglenophytes and unidentified species)

Day	Phytoplankton carbon composition (%)							
	Cryp	Cyan	Diat	Dino	Meso	Pras	Prym	Other
116	5.6	0.0	10.7	43.6	14.6	21.5	0.0	4.0
137	1.2	1.2	1.7	16.8	64.1	0.6	0.0	14.3
145	3.0	0.2	1.2	26.6	47.6	8.4	0.2	13.0
150	2.0	0.5	0.7	25.2	33.2	6.6	19.8	12.0
161	1.0	0.9	0.2	23.8	18.8	4.9	39.4	11.1
172	1.2	13.2	0.2	27.1	11.4	1.6	40.6	4.8
186	1.9	40.8	0.4	33.6	14.1	4.4	1.6	3.4
199	10.8	10.4	0.0	55.2	4.3	5.0	5.6	8.8
214	6.8	17.7	0.9	36.7	3.7	3.1	20.2	11.1
228	7.4	27.5	1.2	19.6	5.6	12.2	22.6	4.0
242	20.5	2.8	2.0	37.4	10.0	6.5	12.1	8.7
248	22.3	1.6	1.4	39.4	11.9	8.1	6.2	9.1
256	24.1	0.3	0.9	41.3	13.9	9.8	0.3	9.4
270	17.2	0.3	14.6	16.7	20.3	4.2	1.1	25.7
285	21.7	0.1	1.5	14.7	13.1	6.8	35.9	6.1
298	14.3	1.8	15.8	14.5	17.6	6.4	27.0	2.6
312	13.1	0.9	9.4	8.3	35.0	4.6	17.0	11.8
318	11.9	0.0	3.0	2.0	52.3	2.8	7.0	21.0
340	26.1	0.3	3.9	15.2	31.2	1.5	6.8	15.1

second increase of dinoflagellates. In autumn dinoflagellates decreased while prymnesiophytes and *M. rubrum* increased. Throughout the annual cycle a mixture of chlorophytes, chrysophytes, euglenophytes and unidentified species persisted (grouped as 'other algae'), contributing between 3 and 26% of total phytoplankton.

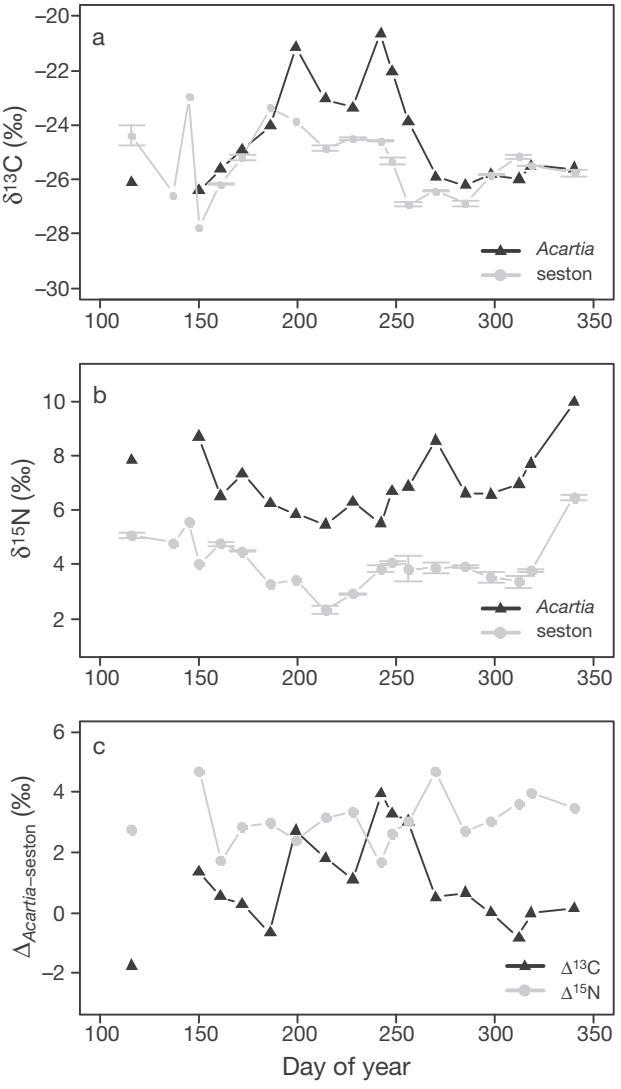

Fig. 1. Seasonal dynamics of (a) *Acartia* and seston δ^{13}C and (b) δ^{15}N bulk values, and (c) Δ^{13}C and Δ^{15}N (differences between C and N isotope composition of *Acartia* and seston) for day of year 116 to 340 sampled from the northern Baltic Proper

Seasonal changes in δ^{13}C essential amino acid values

Acartia δ^{13}C E-AA values separated into 3 significant seasonal clusters (PERMANOVA, model *F*: 28.99, $p < 0.01$, df = 18): early summer (Days 116–172), late summer (Days 186–256) and fall (Days 270–340) (Table 1). In contrast, no significant seasonal clusters were observed for seston δ^{13}C E-AA values (PERMANOVA, model *F*: 2.37, $p > 0.05$, df = 17).

Difference in absolute values between individual *Acartia* E-AAs δ^{13}C values and seston E-AAs δ^{13}C values of Ile (ranging from –4.38 to 4.68‰), Leu (–3.97 to 4.00‰) and Val (–6.49 to 4.37‰) showed 3 seasonal phases, with high values in early summer, lower values in late summer and increased values in fall (Fig. 2), similar to the cluster analysis. Differences in *Acartia* and seston δ^{13}C Phe values (–11.31 to 0.24‰) were relatively constant around –5‰ though with a low value of –11‰ at Day 228, before increasing to values of approx. 0‰ at Day 270. The 3 seasonal phases were caused primarily by the *Acartia* δ^{13}C E-AA values, while seston δ^{13}C E-AA values instead showed a more or less continuous seasonal increase (Table 1), patterns that supported our hypothesis of selective feeding in *Acartia*.

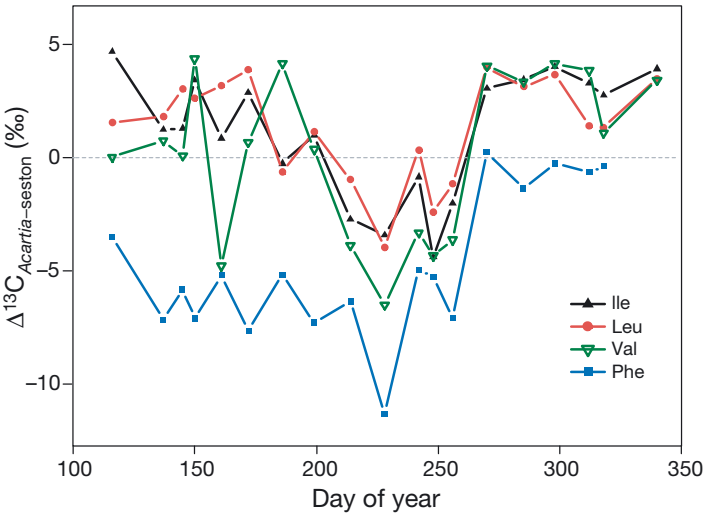

Fig. 2. Differences between *Acartia* and seston $\delta^{13}C$ E-AA values of Isoleucine (Ile, black), Leucine (Leu, red), Phenylalanine (Phe, blue) and Valine (Val, green) for day of year 116 to 340 sampled from the northern Baltic Proper

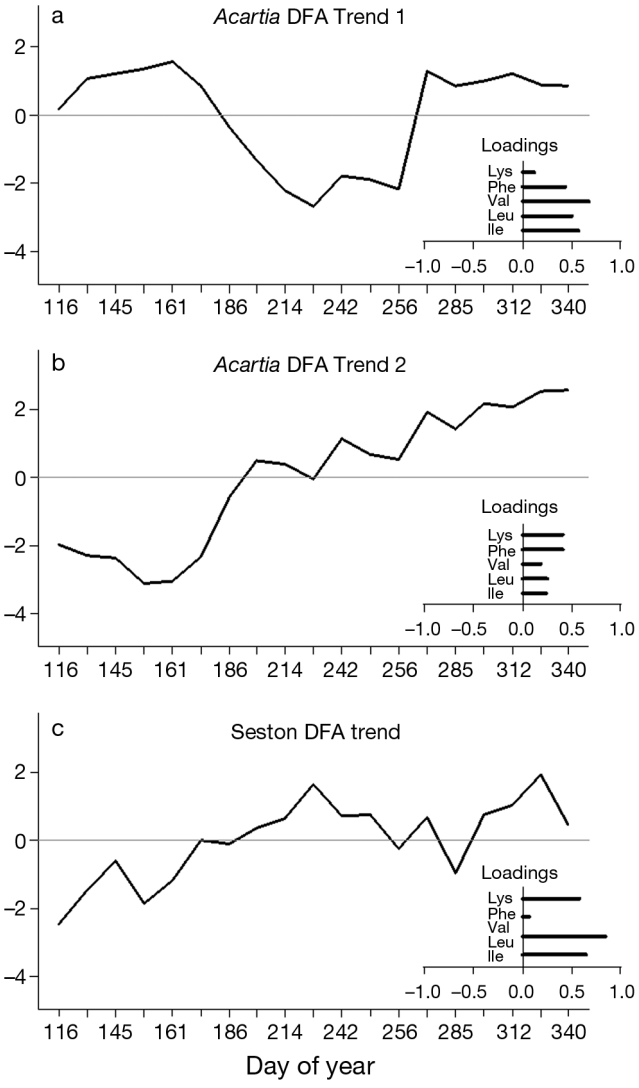

Fig. 3. Common trends of $\delta^{13}C$ E-AA values and the individual E-AA loadings onto the trend (insets) from dynamic factor analysis (DFA) for day of year 116 to 340 sampled from the northern Baltic Proper. (a) The 1st *Acartia* trend, (b) the 2nd *Acartia* trend, and (c) the 1st and only seston trend. Factor loadings in each plot denote the fit of individual $\delta^{13}C$ E-AA values relative to the common trend. DFA models of *Acartia* and seston were estimated from $\delta^{13}C$ values of Isoleucine (Ile), Leucine (Leu), Phenylalanine (Phe) and Valine (Val). For *Acartia* $\delta^{13}C$ Lysine (Lys) values were also included

Dynamic factor analysis of *Acartia* and seston $\delta^{13}C$ essential amino acid values

Acartia and seston DFA models showed distinct differences in seasonal patterns. The most parsimonious *Acartia* DFA model included 2 common trends and a diagonal and unequal variance-covariance matrix (Fig. 3a,b). All time series of the $\delta^{13}C$ AA values were explained well by the final *Acartia* DFA model (r^2 = 0.73–0.99). The first trend showed 3 clear phases during the seasonal cycle, with positive values in early summer (Days 116–172), followed by negative values in late summer (Days 186–256) and again high values in fall (Days 186–256). The first trend was driven positively by factor loadings of Ile, Leu, Phe and Val $\delta^{13}C$ values. The second trend, showing a continuous seasonal increase, was driven mainly by Lys and Phe. There were only small differences in model fits (ΔAICc = 1.29) between the 3 most parsimonious *Acartia* models. Importantly, all these models had the same 2 similar trends, and only differed in their time series error structure (diagonal and unequal variance-covariance matrix, diagonal and equal variance-covariance matrix and equal variance-covariance matrix). *Acartia* DFA results were not affected by removal of the $\delta^{13}C$ Lys isotope composition time series (and thus directly comparable to the seston DFA).

The most parsimonious seston DFA model included one common trend and a diagonal and unequal variance-covariance matrix (Fig. 3c). The common seston trend increased positively, except for a slight decrease from Day 210 to Day 285 with positive factor loadings of Ile, Leu and Phe. $\delta^{13}C$ values of Ile, Leu and Phe all fitted well with the final seston DFA model (r^2 = 0.50–0.99). In contrast, Val showed only little relationship with the common trend (r^2 = 0.01), indicating that variation in the isotope composition of Val was poorly described by the seston DFA model. The inclusion of a diagonal and unequal variance-

covariance matrix, present for both *Acartia* and seston models, indicated that the $\delta^{13}C$ E-AA values have unique error variances.

Linking $\delta^{13}C$ essential amino acid values to phytoplankton composition

The most parsimonious RDA model used to associate common variation in phytoplankton composition with the E-AA $\delta^{13}C$ values showed a strong fit, with the phytoplankton explaining 73.6% of the total variance of the *Acartia* E-AA $\delta^{13}C$ values (adj. $r^2 = 0.64$, $F_{1,18} = 7.26$, $p < 0.01$, Fig. 4a). The first and second RDA axes significantly ($p < 0.01$) explained 59.4% and 12.3% of the constrained variance, respectively. The *Acartia* RDA model separated the data in the same 3 distinct groups as partitioned by the hierarchal cluster analysis. The final model included 5 phytoplankton groups as predictor variables with dinoflagellates explaining 35% of the constrained variance (adj. r^2: 0.30, $p < 0.01$), while 13% of the variance was explained by diatoms (adj. r^2: 0.12, $p = 0.01$), 9.5% by cryptophytes (adj. r^2: 0.08, $p = 0.01$), 8.4% by prasinophytes (adj. r^2: 0.07, $p = 0.03$) and 7.5% by cyanobacteria (adj. r^2: 0.06, $p = 0.04$). The 5 significant phytoplankton dietary sources seasonally comprised between 19.7% and 81.4% of the total phytoplankton composition (Table 2), supporting our hypothesis of selective feeding. The unconstrained component of the model (i.e. variation not explained by the phytoplankton composition) accounted for only 26.4% of the total variance.

The final RDA model for the seston E-AA $\delta^{13}C$ values and phytoplankton composition included groups of cyanobacteria, cryptophytes and prasinophytes and explained only 35% of the total variance (adj. r^2: 0.21, $F_{1,17} = 2.47$, $p = 0.04$, Fig. 4b), with a significant first RDA axis explaining 23.4% of the variance ($p < 0.05$). Even though the 3 phytoplankton groups were included in the most parsimonious model, no individual phytoplankton group significantly explained enough variance of the constrained component for seston (9.7–13.3%, r^2: 0.06–0.08, $p = 0.08$–0.15). These 3 phytoplankton groups comprised between 3.1 and 47.1% of the total phytoplankton composition throughout the year (Table 2). Instead, the unconstrained component in the RDA model explained 65% of the total variance, indicating that the majority of the total variation in the seston E-AA $\delta^{13}C$ values is accredited to other factors than the phytoplankton composition.

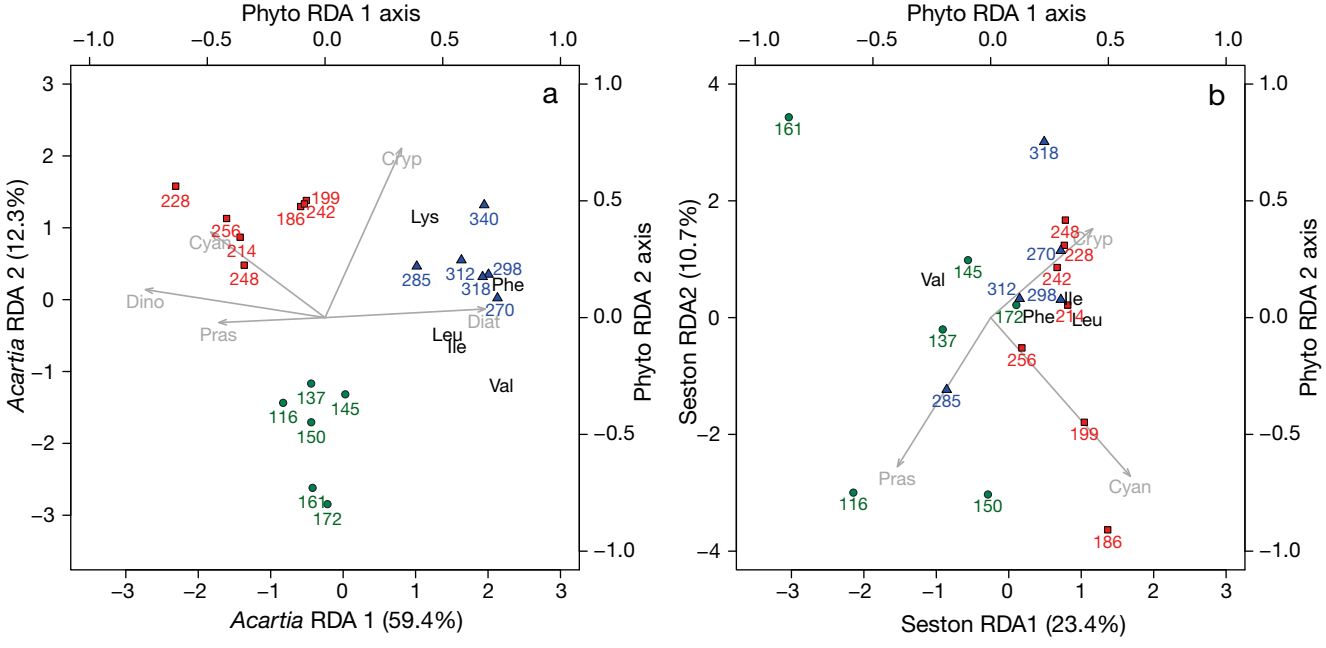

Fig. 4. Redundancy analysis (RDA) using $\delta^{13}C$ E-AA values for day of the year as response variables and phytoplankton composition as predictor variables for (a) *Acartia* and (b) seston sampled from the northern Baltic Proper. Colours in both *Acartia* and seston plots denote seasonal groups of early summer (green), late summer (red) and fall (blue), as partitioned from the *Acartia* hierarchical cluster analysis (PERMANOVA, model *F*: 28.99, $p < 0.01$, df = 18). Predictor variables of phytoplankton groups included in the final model are denoted by grey arrows, while scores for each E-AA's $\delta^{13}C$ values are shown in black. Abbreviations of AAs and phytoplankton groups as in Tables 1 & 2

DISCUSSION

The distinct differences between *Acartia* and seston $\delta^{13}C$ E-AA values indicated that *Acartia* does not simply feed opportunistically on all phytoplankton taxa, but instead preferentially assimilates certain dietary sources. The dual results achieved by complementing AA-CSIA with conventional bulk stable isotopes allowed for better estimation of isotope baseline accuracy, which will lead to improved trophic position calculation inferred from bulk N isotope composition. Low summer $\delta^{15}N$ values in both *Acartia* and seston as a result of high diazotroph cyanobacteria abundance coincided with changes in *Acartia* $\delta^{13}C$ E-AA values, indicating the incorporation of atmospheric fixed N into the Baltic Sea pelagic food web by zooplankton consumers. As expected, our analysis showed the capacity of $\delta^{13}C$ values of E-AAs to accurately trace consumer resource use in pelagic food webs.

Acartia and seston $\delta^{13}C$ essential amino acid values

Seasonal variation in consumer isotope values may reflect either change in assimilated dietary sources or change in the isotope composition at the base of the food web (Post 2002, Michener & Kaufman 2007). Specifically, *Acartia* $\delta^{13}C$ E-AA values may vary due to (1) alterations in dietary source assimilation, assuming each dietary component has a distinct isotopic composition, (2) change in the isotopic composition of nutrients or components of organic material at the base of the food web (here inferred as seston), and/or (3) variable isotope trophic discrimination between consumer and diet, something assumed negligible for $\delta^{13}C$ E-AA values (Howland et al. 2003, Larsen et al. 2009, McMahon et al. 2010). Our results showed that *Acartia* and seston $\delta^{13}C$ E-AA values varied substantially throughout the season, but in clear contrasting patterns. *Acartia* $\delta^{13}C$ E-AA values showed 3 distinct phases as visible in the first *Acartia* DFA trend (Fig. 3a) and absolute values (Table 1), while the only seston DFA trend contrarily showed a more or less gradual seasonal increase (Fig. 3c). The seasonal deviations between *Acartia* and seston $\delta^{13}C$ E-AA values (Fig. 2) indicate that *Acartia* does not feed opportunistically on all components from the seston pool but instead preferentially selects and assimilates certain diet items, similar to other calanoid copepod species (Donaghay & Small 1979, Wiadnyana & Rassoulzadegan 1989, Kürten et al. 2013).

Redundancy analyses (RDA, shown in Fig. 4b) of seston $\delta^{13}C$ E-AA values and phytoplankton composition indicated that the majority of variance was explained by the unconstrained component (i.e. other sources than the phytoplankton composition). This suggests no clear relationship between the seston $\delta^{13}C$ E-AA values and the proportional change in the phytoplankton composition. It is still not well known how the various C sources of autotrophic, heterotrophic and detrital material influence the seston C E-AA isotope composition. In the ocean, reworked heterotrophic material and dissolved organic material from autotrophic sources differ in $\delta^{13}C$ AA values (McCarthy et al. 2004), while different primary producers also vary in C AA isotope composition due to differing AA syntheses pathways (Macko et al. 1987, McCarthy et al. 2004, Larsen et al. 2013). The lack of a consistent change in the seston C E-AA isotope composition with altering phytoplankton taxonomic composition is likely due to phytoplankton not being the sole component of the seston material.

In algae cells, the balance between respiration and photosynthesis influences the $\delta^{13}C$ values of dissolved inorganic carbon (Quay et al. 1986), though changes in the ratio between HCO_3^- and CO_2 uptake as well as differences between phytoplankton taxa may alter the C isotope composition (Fogel & Cifuentes 1993, de Kluijver et al. 2014). We speculate that the higher C isotope composition of the seston pool during spring and summer is due to increased photosynthetic rates and higher ecosystem production, which may explain the apparent seasonal increase of seston $\delta^{13}C$ E-AA values until late July (Day 210) (Fig. 3c, Table 1). The continuous increase of the one and only seston DFA trend until late summer was also visible in the second *Acartia* DFA trend (Fig. 3b). This concurrence suggests that the changing C isotope composition of the community seston pool is also, to some extent, incorporated in dietary sources subsequently taken up by *Acartia*. Nonetheless, the seasonal deviations between *Acartia* and seston $\delta^{13}C$ E-AA values indicate that the majority of variation in *Acartia* $\delta^{13}C$ E-AA values stem from assimilation of selected dietary components.

Acartia dietary resources

The *Acartia* RDA model showed a significant relationship between *Acartia* E-AA $\delta^{13}C$ values and presence of a range of taxonomic groups (i.e. dinoflagellates, cryptophytes, diatoms, prasinophytes and cyanobacteria), suggesting that these phytoplankton

groups make up significant *Acartia* E-AA dietary contributions. Furthermore, the constrained component in the *Acartia* RDA model (Fig. 4a) explained approx. 75% of the variance, indicating that phytoplankton resources contribute to the majority of the variation in *Acartia* $\delta^{13}C$ E-AA values. Dinoflagellates seemed to be a major contributing dietary source, especially in summer when relative abundance is high. Dinoflagellates are generally considered a good-quality food source due to high levels of polyunsaturated fatty acids and proteins (Brown 1991, Brown et al. 1997), and are known to be an important dietary source for calanoid copepods in both the Baltic Sea (Peters et al. 2006) and other marine ecosystems (Kleppel 1993, El-Sabaawi et al. 2010). Other dietary sources such as diatoms and cryptophytes also showed significant correlations with *Acartia* E-AA values. Based on our results, it is difficult to evaluate the exact dietary importance of diatoms for *Acartia* because sampling only started in April, after the diatom-dominated spring bloom.

Interestingly, we observed a significant relationship between *Acartia* $\delta^{13}C$ E-AA values and the higher cyanobacteria abundance in the summer months, which also coincided with both lower *Acartia* and seston bulk $\delta^{15}N$ values. Low bulk $\delta^{15}N$ values have previously been accredited to the influence of summer N-fixing cyanobacteria blooms on ecosystem production in the Baltic Sea (Rolff 2000). *Acartia* can assimilate cyanobacteria directly (Motwani & Gorokhova 2013), though secondary utilization through microbial reworking or nutrient leaking by N fixating cyanobacteria subsequently utilized by other phytoplankton taxa may also occur (Larsson et al. 2001). However, estimating the precise dietary importance and pathway of cyanobacteria may be difficult since they comprise a range of isotopically diverse species (Vuorio et al. 2006), from picocyanobacteria to large filamentous cells (Stal et al. 2003). Nonetheless, the co-occurring change found in *Acartia* and seston bulk N isotope composition support the information encoded in the *Acartia* $\delta^{13}C$ E-AA values that summer cyanobacteria production plays an important role for the nutrient dynamics across the phytoplankton-zooplankton interface in the Baltic Sea.

Microzooplankton such as ciliates may also be a contributing food source for zooplankton in the Baltic Sea, especially after the spring bloom and throughout the summer period (Johansson et al. 2004). Unfortunately, no data on ciliates other than mixotrophic *M. rubrum* were available in this study. *M. rubrum* were not a significant component in the *Acartia* RDA model; however, we found a significant relationship between *M. rubrum* and single *Acartia* $\delta^{13}C$ values of Val and Ile (Spearman's rho = 0.52 to 0.72, $p < 0.05$, df: 17). Ciliates such as *M. rubrum* are known to ingest and utilize cryptophyte chloroplasts (Gustafson et al. 2000), which should also result in incorporation of the isotope composition of cryptophytes, a group that was significant in our RDA model. Lack of trophic discrimination in $\delta^{13}C$ E-AA values (Howland et al. 2003, Larsen et al. 2009, McMahon et al. 2010) likely makes distinction between relative dietary contributions of cryptophytes and *M. rubrum* in zooplankton difficult, something that may also be the case for other mixotrophic species. While *Acartia* seemingly utilize several diet resources, highlighted by the 5 significant phytoplankton groups inferred by the *Acartia* RDA model (i.e. dinoflagellates, cryptophytes, diatoms, prasinophytes and cyanobacteria), these groups comprised only parts of the total phytoplankton composition (19.7 to 81.4% throughout the season). This supports our hypothesis that *Acartia* selectively feed on specific prey items and not broadly on all available phytoplankton sources. The degree of selective feeding by *Acartia* is likely greater during high availability of various dietary sources, compared to periods of more opportunistic feeding during food scarcity.

Factors influencing *Acartia* stable isotope composition

Different macromolecules vary in isotope composition (Degens 1969). For example, lipids usually have depleted $\delta^{13}C$ values relative to both carbohydrates and proteins (Degens 1969), something known to influence zooplankton bulk $\delta^{13}C$ values (Matthews & Mazumder 2005). In fact, zooplankton $\delta^{13}C$ isotope values of lipids seasonally vary and may be both higher or lower than bulk C isotope composition (Kürten et al. 2013). We suspect that our results of *Acartia* bulk C isotope composition, which synchronized in opposite direction to that of AA-CSIA, are due to seasonal variation in the isotope composition of lipids and/or changes of lipid content in *Acartia*.

Change in consumer macromolecule composition is not expected to directly influence $\delta^{13}C$ E-AA values, though synthesis of additional E-AAs during digestion of nutritionally low-quality diet by gut microbes can result in altered $\delta^{13}C$ E-AA values of consumers relative to their food source (Newsome et al. 2011, 2014). To what extent this kind of AA synthesis occurs in *Acartia* and potentially varies seasonally is not known but previous studies on cladocerans

suggest that microbial AA synthesis is likely (Larsen et al. 2013). However, it is doubtful that increased E-AA synthesis by gut microbes during ingestion of poor quality foods can alone explain the substantial seasonal differences between seston and *Acartia* δ^{13}C E-AA values.

The rate at which the isotope composition of prey sources is manifested into a consumer varies temporally with growth, type of tissue and ambient abiotic factors (Perga & Gerdeaux 2005). Isotopic bulk turnover time in zooplankton is assumed to be relatively short; i.e. days (Aberle & Malzahn 2007) to weeks (Kürten et al. 2013), though this is at present still poorly studied (El-Sabaawi et al. 2010). Because of the expected fast turnover time in *Acartia* we considered a relatively high frequency sampling scheme and comparison between consumer and seston with no lag time the best option, despite expected differences in isotope incorporation times between phytoplankton and *Acartia.* Nonetheless, offsets in incorporation times likely introduced some variability in the comparison between consumer and diet isotope values. Interestingly, incorporation rates in δ^{13}C values likely varies between individual AAs as shown for δ^{15}N AA values (Bradley et al. 2014, Downs et al. 2014). At present little information is available on individual incorporation rates of consumer AA δ^{13}C values, and future experiments measuring such turnover times will provide essential information on consumer physiology including AA uptake and assimilation rates.

Conclusions

Acartia δ^{13}C E-AA values encoded seasonal variation from a range of putative dietary sources, supporting our hypothesis that application of δ^{13}C E-AA values are a suitable technique to study consumer resource use in aquatic systems (Larsen et al. 2013, Vokhshoori et al. 2014). Selective feeding by consumers, as shown here for *Acartia*, stresses the importance of critically evaluating isotope baseline information prior to inferring organismal trophic position. The seasonal increase in differences between *Acartia* and seston δ^{15}N values could indicate elevated zooplankton trophic position, for example through increased feeding on microzooplankton (Stoecker & Egloff 1987, Mitra et al. 2013) or alternatively change in ^{15}N discrimination of the various N pools (Montoya 2007). Seasonal changes in the seston δ^{15}N are may also be influenced by diazotrophic N_2 fixation or ammonium uptake, while the relatively high oxygen levels most of the year likely make denitrification at our sampling site less influential (Eilola et al. 2009). However, the preferential feeding pattern apparent in the δ^{13}C E-AA values indicate that using seston to infer isotope baseline information may be misleading, something also noted in bulk isotope studies of other preferential zooplankton feeders (El-Sabaawi et al. 2010, Kürten et al. 2013). Improving baseline information, for example through the use of AA-CSIA methods, is therefore a key component in advancing stable isotope studies of food webs. Differentiation of the various components of the seston pool and their associated isotope values is an important first step, and while measures of isotope composition of individual species or taxonomic groups may not always be feasible in natural systems, improved baseline information may be possible from size fraction approaches, as shown by Rolff (2000). Understanding the degree of potential consumer food selectivity is equally important. Researchers wishing to retrieve detailed information on predator–prey interactions may consider coupling bulk stable isotope measurements with other dietary measures such as AA-CSIA (as shown in this study), fatty acids analysis (Dalsgaard et al. 2003), or molecular tools (Corse et al. 2010, Pompanon et al. 2012).

Feeding on different food sources can also result in switching between prey items of different nutritional quality both in terms of elemental stoichiometry (Walve & Larsson 2010) and important macromolecules (Peters et al. 2013), which in turn influence consumer biochemical composition, growth and reproduction (Müller-Navarra et al. 2004, Veloza et al. 2006, Ventura & Catalan 2010). Seasonal changes in the nutritional quality of zooplankton as diet resources may therefore propagate to higher trophic level predators (Möllmann et al. 2004) and consequently change nutritional flows across aquatic food webs (Winder & Jassby 2011). Realizing that energy uptake in zooplankton consumers can be a highly selective process, which is not temporally or spatially static, should motivate future work on measuring zooplankton resource assimilation in order to retrieve accurate information on ecosystem energy dynamics.

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