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RESEARCH

Comparisons of fatty acid accumulation patterns of two filter feeders, *Branchinella kugenumaensis* and *Daphnia magna* in a controlled environment

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Background: Filter-feeding zooplankton has limited food resources owing to their habitat. Consequently, it is crucial for them to acquire all essential compounds, such as fatty acids (FAs) and amino acids, from confined diets. To elucidate the trophic transfer of FAs to filter feeders, the primary consumers in freshwater ecosystems, we compared the FA accumulation patterns of two species of filter-feeding zooplankton, *Daphnia magna* and *Branchinella kugenumaensis*, in a laboratory experiment. Experimental neonates and nauplii preyed on a single phytoplankton species (*Selenastrum capricornutum*) for three days after hatching prior to diet switching. Five replicates per feeding group in each species were fed on six different types of mixed phytoplankton diet for 10 days after diet switching. Subsequently, the consumers and diets were harvested and FAs were extracted.
Results: Principal component analysis showed that the FA profiles of zooplankton were well-grouped by species and diet. Although diet affects the FA profiles of consumers, they exhibit different FA accumulation patterns. *D. magna* had a higher 18C-ω3 content and ω3/ω6 ratio than did *B. kugenumaensis*. In contrast, *B. kugenumaensis* had higher contents of 18:1ω7 and 20:5ω3 (eicosapentaenoic acid), 22:6ω3 (docosahexaenoic acid), and a higher ratio of ∑18C monounsaturated FAs to ∑18C-ω3 polyunsaturated FAs than did *D. magna*.
Conclusions: This study showed that two primary consumers, *D. magna* and *B. kugenumaensis*, fed the same diet had different assimilation patterns of FAs under controlled environments. Specific FA accumulation patterns in filter feeders can provide information on the transfer process of various FAs to high-trophic organisms.

Keywords: 18C MUFAs, 18C-ω3 polyunsaturated fatty acids, *Branchinella kugenumaensis*, *Daphnia magna*, Fatty acid, Filter feeder

Introduction

The diet of filter-feeding zooplankton is limited because they consume seston, which is a suspended particulate matter found in aquatic ecosystems. Therefore, they are assumed to have adaptive strategies to accumulate essential compounds, such as fatty acids (FAs) and amino acids, from their constricted diet in order to survive (Müller-Navarra 2008). Notably, animals cannot synthesize ω3 polyunsaturated FAs (PUFA), and the conversion rates of C18ω3 PUFA to highly unsaturated FAs are low (Cook and McMaster 2002). Thus, consumers should obtain essential FAs from their diets, and their FA profiles should reflect those of their diets (Brett et al. 2006). Many laboratory and field studies have been conducted to elucidate trophic transfers between consumers and their diets in aquatic ecosystems (Burns et al. 2011; Kainz et al. 2009).

Previous studies have examined the transfer rates and accumulation patterns of dietary compounds such as FAs in clams (Caers et al. 1999), cladocerans (Brett et al. 2006; Masclaux et al. 2012; Taipale et al. 2011), and fairy shrimp (Mura et al. 1994, 1997a; Yang et al. 2016). Moreover, most studies have focused on cladocerans and copepods, comparing the FA compositions of diets and consumers in laboratories (Brett et al. 2006; Masclaux et al. 2012; Taipale et al. 2011; Weers et al. 1997) and pelagic lakes (Persson and Vrede 2006; Smyntek et al. 2008) to understand their feeding strategies and metabolic needs. However, few studies

have experimentally compared the feeding traits of cladocerans and other filter-feeding zooplankton species, such as anostracans (e.g., fairy shrimp, *Branchinella kugenumaensis*) (Yang et al. 2017) and have not focused on the transferred metabolic compounds of the cladoceran and anostracan species.

In this study, we used two filter feeders—a freshwater anostracan, *B. kugenumaensis*, and a cladoceran, *Daphnia magna*—in a feeding experiment to compare their FA accumulation patterns. Daphnids and *B. kugenumaensis* coexist in temporary wetlands, particularly in rice paddy fields in South Korea, during short periods of water filling. Although *D. magna* has been reported in rice paddy fields (Han et al. 2007), its presence has not been confirmed by adequate illustrations or by original findings in South Korea (Jeong et al. 2014). For this laboratory experiment, we employed *D. magna*, one of the most commonly studied species in biological research (Ebert 2022), representing other daphnids in temporary bodies of water. Daphnids and fairy shrimps co-occur with other crustacean species in ephemeral pools and rice paddy fields (Han et al. 2007; King et al. 1996). They are influenced by the limited resources and short-lived environments for survival and reproduction (King et al. 1996). Therefore, they may have characteristic survival strategies, such as accumulation of essential compounds under severe conditions.

The purpose of this study was to compare the accumulation patterns of FAs between filter feeders, cladocerans, and anostracans through indoor experiments and field samples. Our hypothesis was that these filter-feeding zooplankton would exhibit species-specific FA compositions, even though both were fed the same diet or under the same environment. To test this, we conducted a feeding experiment in which two filter feeders (*D. magna* and *B. kugenumaensis*) were fed mixed cultured phytoplankton representing a wide range of taxa (e.g., cryptophytes, bacillariophytes, chlorophytes, and cyanophytes), and the FA profiles of the diet and body of the filter feeder were analyzed after the feeding experiment. Moreover, we compared the accumulation patterns of FAs derived from the feeding experiment with those of daphnids and fairy shrimp in the field.

Materials and Methods

Phytoplankton cultures

Representative strains of phytoplankton were obtained from the Culture Collection of Algae at the University of Texas at Austin, USA (UTEX) and the Korea Marine Microalgae Culture Center (KMMCC): *Cryptomonas ovata* (UTEX LB 2783) for Cryptophyceae, *Nitzschia apiculata* (KMMCC 1209) for Bacillariophyceae, *Selenastrum capricornutum* (UTEX 1648) and *Scenedesmus obliquus* (UTEX 383) for Chlorophyceae, and *Microcystis aeruginosa* (UTEX 2385, which is known to produce microcystins) for Cyanophyceae. *Selenastrum carpricornutum* and *C. ovata* were cultured in Bold 1NV medium. *Scenedesmus obliquus* and *M. aeruginosa* were cultured using modified Bold 3N according to the instructions from UTEX, whereas *N. apiculata* was cultured using synthetic growth medium L16 (Lindström 1983) supplemented with vitamin B. All phytoplankton species were cultivated in a growth chamber at a constant temperature (25°C) and a 16:8 hour light:dark cycle.

Laboratory feeding experiment

The feeding experiment began by using monoclonal cohorts of *D. magna* and *B. kugenumaensis* and feeding them *S. capricornutum* (UTEX 1648) using L16 medium (Lindström 1983) in a 2 L container for the first 3 days after hatching. Five individuals of *D. magna* and three individuals of *B. kugenumaensis* were transferred to a 250 mL beaker containing 100 mL of a mixed phytoplankton diet. The number of individuals per species was considered for survival and the amount of FA in the extract for limits of detection in gas chromatography (GC) after completion of the experiment. Five replicates were used for each mixed diet specifically prepared to constitute one or two phytoplankton species in abundance compared to the other species used in this experiment (abundant species for Diet 1, *C. ovata*; Diet 2, *M. aeruginosa*; Diet 3, *C. ovata* and *M. aeruginosa*; Diet 4, *N. apiculata*; Diet 5, *S. obliquus*; Diet 6, *N. apiculata* and *S. obliquus*). A mixed diet representing a single abundant species (Diets 1, 2, 4, and 5) consisted of 75% of the species added in abundance and was made up to 100% using the other three species, as confirmed by chlorophyll *a* concentration levels determined using a spectrometer. Diets 3 and 6 were mixed with these two species in equal proportions. Notably, these mixed diets have different concentrations of different types of FAs based on the FA composition of the cultured phytoplankton (Yang et al. 2016). We considered these dynamic feeding conditions in aquatic environments and the diverse FA compositions. Diets with a fixed species composition and concentration were provided daily. The animals were grown in a growth chamber at a constant temperature (25°C) and a light:dark cycle (16:8 hour) during the experiment (Fig. 1). After 10 days of feeding on the mixed diets, *D. magna* and *B. kugenumaensis* were kept in the medium with no diet for 1 day to clear their gut contents. Neonates of *D. magna* that hatched during the experiment were immediately collected and placed in a beaker containing L16 medium for 1 day. Some animals in replicates could not be collected because they died within 10 days. Harvested animals were kept in a deep freezer at −80°C prior to the extraction of FAs. Mixed phytoplankton diets were filtered using a pre-combusted glass fiber filter GF/C from Whatman (Maidstone, UK) in the laboratory. The filtered diets were then stored at −80°C until analysis.

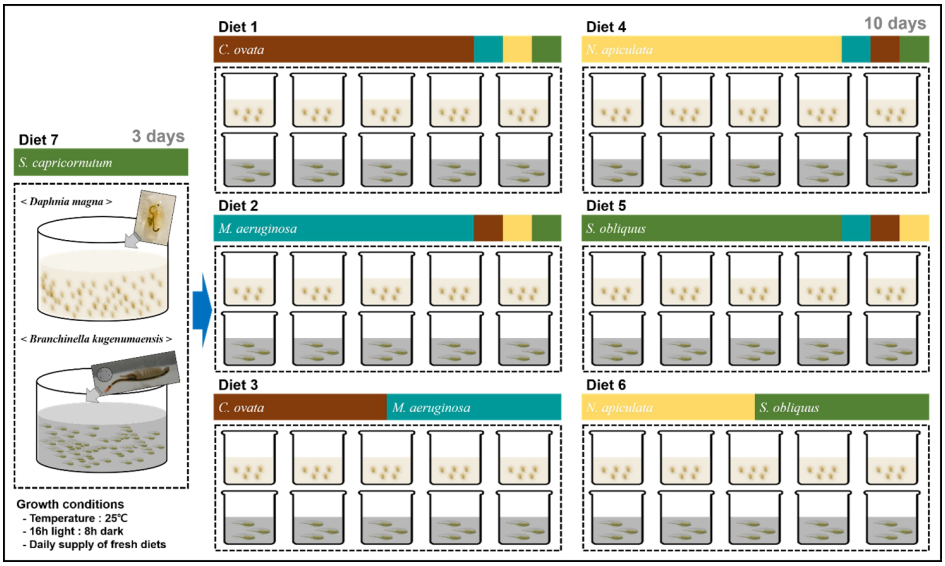

Fig. 1 Schematic of the feeding experiment in this study. Color bars show concentration ratio of taxonomic groups in the mixed diets.

Field sampling

Daphnids (not including *D. magna*) and *B. kugenumaensis* were collected from irrigated rice paddy fields under conventional and organic management located in Hongseong-gun, Chungcheongnam-do, South Korea, during the summers of 2011–2012. Wild consumers were sampled using a hand-net and transported to a laboratory in an ice box with ice packs. Subsequently, the sorted samples were rinsed with distilled water and stored at −80°C until analysis.

Fatty acid analysis

The frozen samples were dried using a freeze dryer (Heto Model FD2.5; Heto Lab Equipment, Allerød, Denmark). Whole samples of *B. kugenumaensis* guts were removed for FA extraction and cleaned before harvesting for experimental samples. Extraction and methylation were performed according to the method described by Kattner and Fricke (1986). Heneicosanoic acid (21:0) was used as an internal standard and added to freeze-dried samples immediately prior to the extraction process. The extracted FA samples were analyzed using GC (Hewlett Packard 5890) and quadruple mass spectrometry detector (MSD) (Hewlett Packard, 5972A MSD). FAs analyzed using GC-mass spectrometry were quantified by comparing the area ratios of the samples to the internal standard. Commercial FA methyl ester (FAME) standards, 37 components FAME mixture (Supelco® 37 Component FAME MIX; Sigma Aldrich, St. Louis, MO, USA) and PUFAs from marine source (PUFA No.1; Sigma Aldrich) were used as a reference to identify FAs based on retention time.

Principal component analysis and other statistical analysis

The percentage values of each FA type with respect to the total FA amount were compiled to produce a data matrix. We used log transformation (log [x + 1]) to ensure the homogeneity of variance (Poerschmann et al. 2004). A value of zero was assigned if there was no matching peak. Log-transformed data were subtracted from the mean of each FA variable and divided by the square root of their standard deviation (Pareto scaling) prior to principal component analysis (PCA) (Van den Berg et al. 2006).

PCA was performed using covariance data matrices to reduce dimensionality. We examined the variance of each mode using several selection criteria, including the scree test (Cattell 1966), Kaiser's criterion, and rule N (Overland and Preisendorfer 1982; Termonia 2001), and chose the subspace dimension (m) (Jassby 2000). The loading factors were rotated using varimax rotation after PCA (Everitt, 2006). We checked for normality of the data sets using the Kolmogorov–Smirnov test. Statistical analyses, including PCA and analysis of variance (ANOVA), were performed using S-Plus 6 for Windows (Insightful Corp., Seattle, WA, USA). Partial least square discriminant analysis (PLS-DA) was conducted using R with the PLS package (http://cran.r-project.org/web/packages/pls/index.html) (Mevik and Wehrens 2007) to compare the FA profiles of *D. magna* and *B. kugenumaensis* after the feeding experiment. An S-plot was drawn using loading values and correlation loading values from PLS-DA using a self-written R script. High loading values indicate significant contributions of variables (peaks), whereas high correlation loadings indicate strong correlations with patterns, regardless of the magnitudes of the variables (size of peaks) (Wiklund et al. 2008). In addition, t-tests or Wilcoxon rank tests were performed to compare the concentrations of essential FAs among the filter feeder species based on the results of the Shapiro-Wilk normality test using R software (R Core Team 2018).

Results

FA composition of the mixed phytoplankton diets

The mixed phytoplankton diets had distinct FA profiles based on the dominant phytoplankton species related to their taxonomic groups (Table. 1). Diet 1 was dominated by 16:0 and 18:4ω3 FA contents and had higher contents of 20:5ω3 (docosahexaenoic acid, DHA) and 22:6ω3 (eicosapentaenoic acid, EPA) than the other diets. The FA composition of Diet 2 was dominated by 16:0, 18:0, and 18:3ω6. Diet 4 had remarkable FA content of 16:1ω7 and Diet 5 was dominated by 16:0, 16:1ω7 and 18:3ω3 (ALA). Moreover, in the two-species mixed phytoplankton diet, Diet 3 contained high proportions of 16:0 and 18:4ω3, whereas Diet 6 had high ALA and 16:0. The initial diet of *S. capricornutum* was Diet 7, which was fed for 3 days before commencing the experiment and showed the highest ALA content among the FA profiles.

FA accumulation patterns in the filter feeders

The PCA of the FA profiles of the two filter feeders, *D. magna* and *B. kugenumaensis*, showed considerable changes in FA composition during the feeding experiment (Fig. 2). The first (PC1) and second principal components (PC2) explained 48.5% of the total variance (28.1% and 20.4% for PC1 and PC2, respectively). PCA scores showed that the FA compositions of *D. magna* and *B. kugenumaensis* were similar to those of their diets after 10 days of feeding. The scores were grouped by species along PC1 and by diet along PC2. After a 10-day feeding experiment, the FA profiles were well separated by species (*D. magna* vs. *B. kugenumaensis*) along the first component of PLS-DA (Fig. 3A). The animals were also separated into two groups, regardless of species, along with component 2. The groups divided along component 2 were clustered by diet with and without a high amount of *S. obliquus.*

The S-plot based on PLS-DA results suggested that ALA and 16:1ω7 were abundant in *D. magna,* whereas 18:0 appeared to be a marker for *B. kugenumaensis* (Fig. 3B).

Two-way ANOVA revealed that the content of several FAs, such as 18:0, 18:1ω7, and 18:4ω3, was significantly different between consumers fed the same diet and those fed different diets of the same species ($p < 0.001$) (Table 2). The variance explained by species was higher than that explained by diets for 18:0 and 18:1ω7, whereas diets explained more of the variance in FA composition than species for ALA and EPA. Other FAs, such as 16:1ω7, 18:1ω9, 18:4ω3, 20:4ω6 (arachidonic acid, ARA), and ω3/ω6 FA ratios, were explained by both species and diets.

The 18:0 content was significantly higher in *B. kugenumaensis* than in *D. magna*, whereas the ALA content was significantly higher in *D. magna* fed mixed diets for 10 days (Fig. 4). Both EPA and DHA contents were significantly higher in fairy shrimp than in *Daphnia*, despite a

Table 1 Fatty acid content (%) of mixed diets and an initial diet in the feeding experiment

	Diet 1 (*Cryptomonas ovata*)	Diet 2 (*Microcystis aeruginosa*)	Diet 3 (*C. ovata, M. aeruginosa*)	Diet 4 (*Nitzschia apiculate*)	Diet 5 (*Scenedesmus obliquus*)	Diet 6 (*N. apiculate, S. obliquus*)	Diet 7 (initial; *Selenastrum capricornutum*, sole species)
Fatty acid							
14:0 (myristic acid)	2.3	1.7	2.0	4.3	3.8	2.2	2.8
16:0 (palmitic acid)	17.4	30.9	23.2	24.5	21.0	19.2	17.0
16:1ω7 (palmitoleic acid)	4.3	6.0	2.2	28.2	19.9	5.3	0.5
18:0 (stearic acid)	9.1	13.9	10.7	10.8	11.1	11.3	0.4
18:1ω9 (oleic acid)	3.1	4.2	2.9	4.2	5.6	6.4	14.5
18:1ω7 (vaccenic acid)	2.2	2.8	2.1	1.4	2.1	2.8	1.2
18:2ω6 (linoleic acid)	9.2	7.2	8.9	2.9	3.2	6.3	12.6
18:3ω6 (γ-linolenic acid)	1.5	12.2	6.9	2.0	0.9	2.2	1.1
18:3ω3 (α-linolenic acid)	12.5	7.6	9.2	5.6	19.1	26.5	32.2
18:4ω3 (stearidonic acid)	16.4	5.4	13.3	3.6	4.4	7.7	8.4
20:4ω6 (Arachidonic acid)	ND	ND	ND	1.2	0.7	0.7	ND
20:5ω3 (Eicosapentaenoic acid)	13.6	3.3	10.4	7.1	3.9	2.9	ND
22:6ω3 (Docosahexaenoic acid)	2.8	0.7	2.1	0.9	0.1	0.5	ND

Given phytoplankton species indicate the abundant species in each diet group.
ND: not detected.

few *Daphnia* having low DHA content. Furthermore, ω3-PUFA content was higher in *Daphnia* than in fairy shrimp, and ω3/ω6 ratios were higher in *D. magna*.

Discussion

The present study showed that the FA compositions of the two filter feeder species, *B. kugenumaensis* and *D. magna*, differed even after feeding on the same diets, corroborating previous studies that showed that filter feeders (e.g., *Daphnia*, *Ceriodaphnia*, and *Bosmina* species) could maintain species-specific FA accumulation patterns (Burns et al. 2011; Kainz et al. 2004; Masclaux et al. 2012; Mura et al. 1998; Persson and Vrede 2006; Smyntek et al. 2008).

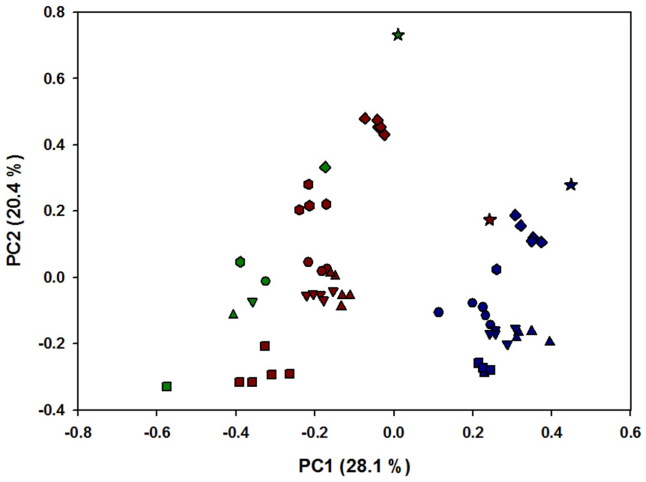

Fig. 2 PCA scores based on fatty acid profiles of *Branchinella kugenumaensis* and *Daphnia magna* and their diets in the feeding experiment. Diets were composed of a deliberately chosen abundant species and other species. The dominant species with indicated symbol in parenthesis; Diet 1 (circle): *Cryptomonas ovata*, Diet 2 (up triangle): *Microcystis aeruginosa*, Diet 3 (down triangle): *C. ovata* and *M. aeruginosa*, Diet 4 (square): *Nitzschia apiculata*, Diet 5 (diamond): *Scenedesmus obliquus*, Diet 6 (hexagon): *N. apiculata* and *S. obliquus*, Diet 7 (star): *Selenastrum carpricornutum* (initial diet with one species of algae). Green symbol indicates the diets. Red and blue symbols indicate *D. magna* and *B. kugenumaensis* fed with the diets. Diet 1 to Diet 6 were fed for 10 d after diet-switching and Diet 7 for 3 day after hatching. PCA: principal component analysis.

Our results highlighted that, while the FA profiles of the two filter feeders were similar to those of their diets after being fed mixed phytoplankton diets, the FA profiles of *D. magna* were closer to those of their diets than those of *B. kugenumaensis* on the PCA score plot, suggesting that the FA profiles of *D. magna* are more reflective of their diets than those of *B. kugenumaensis* (Fig. 2). The FA compositions of cladocerans, especially *Daphnia* and copepods, matched well with those of monocultured diets and seston in laboratory experiments and field surveys (Brett et al. 2006; Ravet et al. 2010; Taipale et al. 2011). In addition, *D. magna* and *B. kugenumaensis* exhibited rapid FA accumulation. Taipale et al. (2011) reported that *D. magna* replaced more than 50% FA composition and adapted to a new diet after only 2 days from diet-switching. In contrast, *B. kugenumaensis*-fed *M. aeruginosa* had a significantly higher FA content of 18:3ω6, which represents an FA marker of *M. aeruginosa*, compared to those fed *S. capricornutum* after three days of feeding from diet-switching in a feeding experiment (Yang et al. 2016).

Although *D. magna* showed fast uptake of FAs from their diets, juvenile *Daphnia* fed the initial diet (Diet 7) for three days after hatching were at a distance from the other *Daphnia* diet-switching groups on the PCA score plot (Fig. 2). Although it was only one sample (not one individual), it might have been influenced by maternal diet. The FA composition of the neonates (parthenogenetic daughters) of *D. magna* that were not fed was similar to that of the mothers

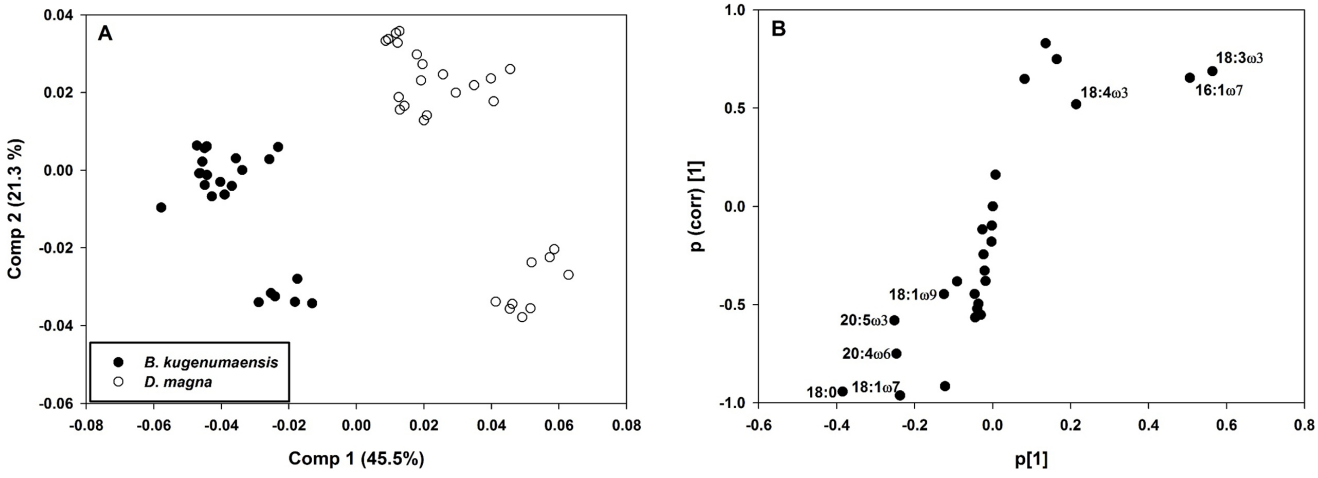

Fig. 3 (**A**) PLS–DA scores based on fatty acid profiles of *Branchinella kugenumaensis* and *Daphnia magna* after a feeding experiment. (**B**) S-Plot of loadings (p [1]) and correlation loadings (p(corr) [1]). Important variables of fatty acids are indicated on plot. Symbols indicated in the PLS-DA score plot; closed circle: *B. kugenumaensis*, open circle: *D. magna*. PLS-DA: partial least square discriminant analysis.

Table 2 Results of two-way analysis of variance for the essential fatty acid categories of *Branchinella kugenumaensis* and *Daphnia magna* after the 10-day feeding experiment

Source	df	SS	*p*	Variance explained (%)	Source	df	SS	*p*	Variance explained (%)
16:1ω7					18:4ω3				
Species	1	887.7	< 0.001	33.1	Species	1	296.5	< 0.001	45.7
Diet	5	1,428.9	< 0.001	53.3	Diet	5	260.9	< 0.001	40.2
Species × Diet	5	327.8	< 0.001	12.2	Species × Diet	5	82.9	< 0.001	12.8
Error	40	38.0		1.4	Error	40	8.7		1.3
18:0					20:5ω3 (Eicosapentaenoic acid)*				
Species	1	559.5	< 0.001	88.2	Species	1	118.9	< 0.001	15.7
Diet	5	25.6	0.001	4.0	Diet	5	585.9	< 0.001	77.1
Species x Diet	5	12.7	0.030	2.0	Species × Diet	5	27.8	< 0.001	3.7
Error	40	36.6		5.8	Error	40	27.1		3.6
18:1ω9					20:4ω6 (Arachidonic acid)				
Species	1	136.9	< 0.001	45.2	Species	1	162.7	< 0.001	39.3
Diet	5	135.4	< 0.001	44.7	Diet	5	204.5	< 0.001	49.4
Species × Diet	5	17.5	< 0.001	5.8	Species × Diet	5	20.8	< 0.001	5.0
Error	40	13.1		4.3	Error	40	26.0		6.3
18:1ω7					ω3 /ω6 fatty acid ratio				
Species	1	185.9	< 0.001	81.7	Species	1	19.1	< 0.001	39.0
Diet	5	30.0	< 0.001	13.2	Diet	5	25.0	< 0.001	51.0
Species × Diet	5	2.6	0.058	1.2	Species × Diet	5	3.8	< 0.001	7.8
Error	40	8.9		3.9	Error	40	1.1		2.2
18:3ω3 (α-linolenic acid)									
Species	1	708.4	< 0.001	22.8					
Diet	5	2,124.0	< 0.001	68.3					
Species × Diet	5	228.0	< 0.001	7.3					
Error	40	50.2		1.6					

Percent variance is the percent of the total sum of squares explained by that term.
df: degree of freedom; SS: sum of squares.
*The sum of the percentage does not equal 100% because of rounding.

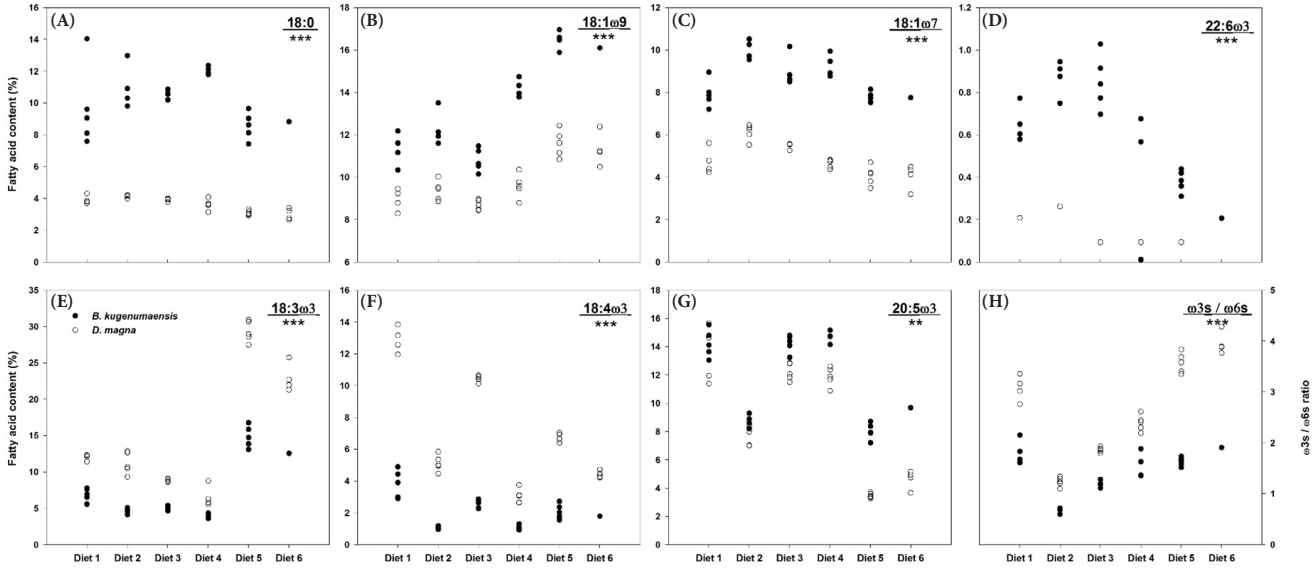

Fig. 4 Comparisons of essential fatty acid content and ratio (**A**) 18:0, (**B**) 18:1ω9, (**C**) 18:1ω7, (**D**) 22:6ω3, (**E**) 18:3ω3, (**F**) 18:4ω3, (**G**) 20:5ω3, and (**H**) ω3s/ω6s in *Branchinella kugenumaensis* and *Daphnia magna* fed mixed phytoplankton diets for 10 days. Closed circle: *B. kugenumaensis*, Open circle: *D. magna*. Asterisks indicate statistical differences between species in each fatty acid content or ratio (t-test or Wilcoxon rank test according to a result of normality test: $^{**}p < 0.01$, $^{***}p < 0.001$). Values of 22: 6ω3 not detected in *D. magna* are not marked.

(data not shown). The mothers of the initial individuals consumed a mixed diet of *S. capricornutum* and *C. ovata* before the feeding experiment in this study. Moreover, neonates have lower food uptake rates than adults, which is related to their filter mesh size (Gophen and Geller 1984). Therefore, it might retain the maternal FA composition and only slowly reflect the dietary FA. However, *B. kugenumaensis* hatched from dormant eggs and was used in the present study. Both the initial diet-fed individuals and diet-switching fairy shrimps reflected the FA composition of their diets along the PC2 axis (Fig. 2). With respect to the fairy shrimp *Chirocephalus kerkyrensis*, different FA profiles were observed between their dormant eggs and adult individuals under wild conditions (Mura et al. 2000). The FA composition further varied with season in relation to the abiotic environment. Thus, the nauplius and juvenile *B. kugenumaensis* might not have had any influence on maternal effects in this study.

Although affected by feeding diets, especially diets dominated by *S. obliquus* with a high ALA content (Table 1), PLS-DA and S-plot showed that *D. magna* had higher 18C-ω3s (ALA and 18:4ω3) and 16:1ω7, whereas *B. kugenumaensis* had higher 18:0, 18:1ω9, and 18:1ω7, despite being fed the same diets (Fig. 3). Herbivorous cladocerans such as *Daphnia* and *Holopedium* had the most abundant ALA content among FA collected from lakes in northern United States (Smyntek et al. 2008). This indicates that daphnids can synthesize EPA from dietary ALA (Müller-Navarra 2006) and directly assimilate EPA from their diet (Wacker and von Elert 2001). EPA levels are related to somatic growth and reproduction in cladocerans (Müller-Navarra et al. 2000; Von Elert 2002). Both *D. magna* and *B. kugenumaensis* showed high EPA levels in the filter feeders, except for the Diet 5 and 6 feeding groups in this study (Fig. 4). *D. magna* females had eggs or neonates, regardless of diet, during the experiment, becoming mature adults earlier than *B. kugenumaensis*. *Daphnia magna* can rapidly accumulate highly unsaturated FAs from their diet in their tissues (Taipale et al. 2011). Our previous feeding experiment showed that *B. kugenumaensis* females had a higher ALA content than males (unpublished data). In addition, *B. kugenumaensis* had a higher ALA content than their associated diets, which are deficient in ALA (*M. aeruginosa*). Other studies have shown that the lipid content of *B. kugenumaensis* in females is slightly higher than that in males (Bernice 1972). The high content of ALA and EPA in *D. magna* and females of *B. kugenumaensis* seems to be related to short generation times (Smyntek et al. 2008) with severe environmental conditions.

Furthermore, the present study shows that few *D. magna* have detectable levels of 22:6ω3 (DHA), with a very low DHA content (average 0.19%, n = 3 of 29). Studies have shown that cladocerans, such as daphnids, have a limited ability to store or accumulate 22:6ω3 (DHA), even when their diets contain PUFA, as observed in field studies (Kainz et al 2009; Persoon and Vrede 2006; Smyntek et al. 2008) and in laboratory supplementation experiments (Brett et al. 2009; Masclaux et al. 2012; Taipale et al. 2011; Von Elert 2002; Weers et al. 1997). In contrast, fairy shrimp are known to contain DHA (Mura et al. 1994, 1997a), suggesting that it may be related to the nervous system. The high concentration of DHA in copepods could be explained by the highly developed nervous system used for tracking, mating, and detecting and escaping predators

Table 3 Ratios of ∑18C MUFA (18:1ω7 and 18:1ω9) to ∑18C-ω3 PUFA (18:3ω3 and 18:4ω3) contents in anostracan and cladoceran species on this study and references

Anostracan					
Feeding experiment					
Species	Diet (Dominant* or Single)	Taxonomic group	n	∑18C MUFA/ ∑18C-ω3 PUFA	Reference
Branchinella kugenumaensis	*Cryptomonas ovata**	Cryptophyceae	5	1.85±0.4	this study
	*Microcystis aeruginosa**	Cyanophyceae	4	3.90±0.59	
	*Cryptomonas ovata** *Microcystis aeruginosa**		5	2.59±0.14	
	*Scenedesmus obliquus**	Chlorophyceae	5	1.45±0.17	
	*Nitzschia apiculata**	Bacillariophyceae	4	4.71±0.45	
	*Scenedesmus obliquus** *Nitzschia apiculata**		1	1.66	
B. kugenumaensis	*Selenastrum capricornutum*	Chlorophyceae	6	2.75±1.00	Yang et al. 2016
	Microcystis aeruginosa	Cyanophyceae	5	13.37±3.76	
Branchipus pasai	*Selenastrum capricornutum*	Chlorophyceae	-	2.29	Mura et al. 1997a
	Saccharomyces cerevisiae	Yeast/Saccharomycetes	-	14.07	
	HUFA enriched dried yeast		-	10.20	
Chirocephalus kerkyrensis	*Selenastrum capricornutum*	Chlorophyceae	-	1.71	
	Saccharomyces cerevisiae	yeast	-	29.49	
	HUFA enriched dried yeast		-	11.50	
Artemia salina	*Isochrysis galbana*	Prymnesiophyceae	-	1.69	Zhukova et al. 1998
	Phaeodactylum tricornutum	Bacillariophyceae	-	12.31	
	Nannochloropsis oculata	Eustigmatophyceae	-	7.00	
	Yeast		-	10.15	
Field study					
Species	Sampling site		n	∑18C MUFA/ ∑18C-ω3 PUFA	Reference
B. kugenumaensis	Rice paddy fields		6	3.16±1.09	this study
C. kerkyrensis	Temporary plain pools		-	3.57	Mura et al. 1997b
	Astatic pools		-	2.40	Mura et al. 1994
Chirocephalus diaphanus	Temporary plain pools		-	2.09	Mura et al. 1997b
	Astatic pools		-	0.57	Mura et al. 1994
Chirocephalus marchesoni	Astatic high-level lake		-	0.42	Mura et al. 1997b
Chirocephalus ruffoi	Mountain pool		-	5.31	
Chirocephalus salinus	A volcanic plateau		-	2.75	

(Persson and Vrede 2006). Male fairy shrimps tracked and grasped females using an enlarged second antennae for mating. They can also instantaneously change their direction (Wiman 1981).

Several studies have asserted that zooplankton require DHA for overwintering (Farkas et al. 1984, Smyntek et al. 2008). In natural waters, *B. kugenumaensis* cannot survive in winter, although some fairy shrimp species live at low temperatures (Moriya 1985) and do not require DHA for overwintering. In this study, *B. kugenumaensis* fed a DHA-deficient diet consisting of plentiful *S. capricornutum* and *M. aeruginosa* had similar DHA compositions. Especially, the group fed with a *M. aeruginosa*-dominated diet (Diet 2) had higher content of DHA than those of the diets. Notably, our previous study showed that the filtering rates of *M. aeruginosa by B. kugenumaensis* were higher than those by *S. capricornutum* (unpublished data). Another study showed that the gut contents of fairy shrimp have a higher proportion of Cyanophyceae (e.g., *M. aeruginosa* and *Anabena flos-aquae*) than any other taxonomic groups (Selvarani 2009). In addition, *B. kugenumaensis* consumed more toxic and non-toxic *M. aeruginosa* diets than did *D. magna* in a previous feeding experiment (Yang et al. 2017). Collectively, it may be inferred that *B. kugenumaensis* readily takes up the essential FAs from dietary sources that contain DHA and accumulates different FAs from other phytoplankton taxa, even though fairy shrimp have high filtering rates of DHA-deficient diets such as cyanobacteria. Otherwise, they may synthesize DHA from precursor FAs, such as copepods, to meet their metabolic needs (Persson and Vrede 2006).

This study showed that *B. kugenumaensis* had higher 18C-saturated FA (SAFA) (18:0) and 18C-monounsaturated FAs (MUFAs) (18:1ω9 and 18:1ω7) contents than *D. magna* (Fig. 4). A previous study reported that some species of the genus *Chirocephaus* collected from fields have the highest

Table 3 Continued

Cladoceran					
Feeding experiment					
Species	Diet (Dominant* or Single)	Taxonomic group	n	∑18C MUFA/ ∑18C-ω3 PUFA	Reference
Daphnia magna	*Cryptomonas ovata**	Cryptophyceae	4	0.55±0.07	this study
	*Microcystis aeruginosa**	Cyanophyceae	5	0.96±0.16	
	*Cryptomonas ovata** *Microcystis aeruginosa**		5	0.74±0.01	
	*Scenedesmus obliquus**	Chlorophyceae	5	0.44±0.05	
	*Nitzschia apiculata**	Bacillariophyceae	5	1.54±0.27	
	*Scenedesmus obliquus** *Nitzschia apiculata**		4	0.57±0.08	
Daphnia galeata	*Scenedesmus obliquus*	Chlorophyceae	-	0.60	Müller-Navarra 2006
	Cryptomonas erosa	Cryptophyceae	-	0.31	
	Nitzschia palea	Bacillariophyceae	-	0.96	
Field study					
Species	Sampling site		n	∑18C MUFA/ ∑18C-ω3 PUFA	Reference
Daphnids	rice paddy fields		10	1.66±0.74	this study
Daphnia spp.	sub-alpine oligotrophic lakes		-	0.72	Persson and Vrede 2006
Bosmina coregoni s.l.	astatic pools		-	0.96	
Holopedium gibberum	temporary plain pools		-	0.92	
Bythotrephes longimanus	astatic pools		-	1.73	

We calculated the ratios using mean values of fatty acid contents from Mura et al. (1994) and Persson and Vrede (2006). 18:1ω9 content from Persson and Vrede (2006) included 18:1ω6 content.
MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid; -: not available.

18:1ω9 content among FAs (Mura et al. 1997b), whereas the 18:1ω7 content in animals fed different diets in a feeding experiment was higher than that in their diets (Mura et al. 1997a). Furthermore, the 18C SAFA and 18C MUFAs levels of *B. kugenumaensis* were high regardless of the diet used in this study.

Although the ALA content in animals was more influenced by diet than by species in this study, we selected species-specific FAs, such as 18C-MUFAs (18:1ω9 and 18:1ω7), in high concentrations in *B. kugenumaensis*, and 18C-ω3 PUFAs (ALA and 18:4ω3), in high concentrations in *D. magna*, based on the results of a two-way ANOVA (Table 2). Based on these results, we compared the ratios of 18C-MUFAs and 18C-ω3 PUFAs between anostracan and cladoceran species in laboratory and field studies (Table 3). The ratios for anostracan species were mostly higher than those for cladocerans in both the laboratory and field studies. Moreover, the 18C-MUFAs:18C-ω3 PUFAs ratios for *B. kugenumaensis* were higher than those of daphnids coexisting in rice paddy fields during the summer season (Table 3). Daphnids and most anostracans are generally known to be non-selective filter feeders (Brendonck 1993; Hessen 1985). Among the anostracans, some *Branchinecta* species (*B. gigas* and *B. raptor*) show carnivorous feeding (Rogers et al. 2006) and *Branchinecta orientalis* appears to shift from herbivorous to carnivorous with increasing inorganic turbidity in feeding experiments and a field study (Lukić et al. 2020). Coexisting daphnids and *B. kugenumaensis* might compete for diet or have different trophic niches in natural ecosystems owing to environmental factors (e.g., turbidity). However, herein, we showed that field samples had consistent accumulation patterns of FAs with controlled samples, regardless of their trophic niche. Overall, our results support the findings of previous feeding experiments and field studies that targeted anostracan, including brine shrimp (*Artemia*) found in saltwater bodies, and cladoceran families. These differences might be due to their ability to synthesize FAs through biosynthesis (Weers et al. 1997; Zhukova et al. 1998) and their physiological requirements for survival, growth, and reproduction.

Conclusions

In conclusion, this study showed that two primary consumers, *D. magna* and *B. kugenumaensis*, fed on the same diet could have different assimilation patterns of FAs under controlled environments. In particular, 18C MUFAs such as oleic acid (18:1ω9) and vaccenic acid (18:1ω7) were higher in *B. kugenumaensis*, whereas 18C-ω3 PUFAs such as ALA and stearidonic acid (18:4ω3) were higher in *D. magna* after feeding. Moreover, the species-specific accumulation pattern derived from our results is supported by previous laboratory and field studies on filter-feeding anostracan and cladoceran species.

Abbreviations

ALA: α-linolenic acid
ANOVA: Analysis of Variance
ARA: Arachidonic acid
DHA: Docosahexaenoic acid
EPA: Eicosapentaenoic acid
FA: Fatty acid
FAME: Fatty acid methyl ester
GC: Gas chromatography
KMMCC: Korea Marine Microalgae Culture Center
MSD: Mass spectrometry detector
MUFA: Monounsaturated fatty acid
ND: Not detected
PCA: Principal component analysis
PLS-DA: Partial least square discriminant analysis
PUFA: Polyunsaturated fatty acid
SAFA: Saturated fatty acid
UTEX: University of Texas at Austin

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Authors' contribution

DY performed the experiment and the analysis, and wrote the manuscript. SJ and JK maintained cultured phytoplankton strains and zooplankton for the experiment. SP planned this study and edited the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interest

The authors declare that they have no competing interests.

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