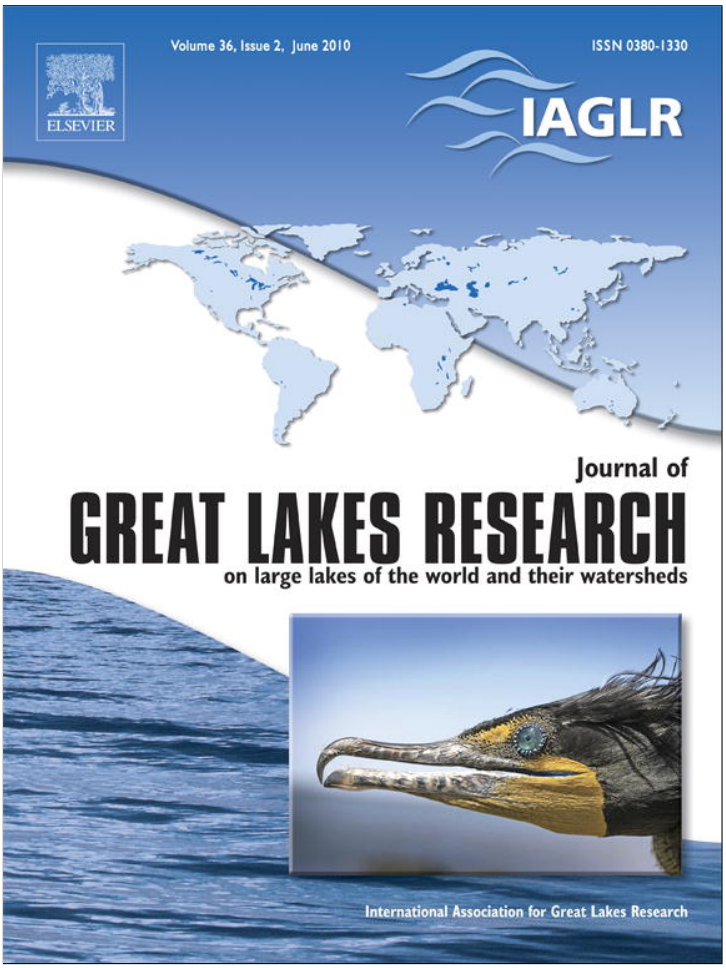

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Diet effects on lipid composition, somatic growth potential, and survival of the benthic amphipod *Diporeia* spp.

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ABSTRACT

The benthic amphipod *Diporeia* represents a crucial trophic link that conveys vital nutrients and energy to predators at higher trophic levels. The current decline of *Diporeia* populations, mostly in the North American Great Lakes, may, in part, be related to concurrent declines in food quantity and/or quality. We hypothesized that somatic growth and survival of *Diporeia* would be positively related to dietary supply and subsequent retention of polyunsaturated fatty acids (PUFA); a class of chemicals known to affect diet quality. We examined how different algal PUFA concentrations in; a) *Ankistrodesmus falcatus* (Chlorophyta), b) a naturally occurring diatom assemblage from Lake Ontario, c) a non-toxic strain of *Microcystis aeruginosa* (Cyanophyta), and, d) fasting for 30 d, affected PUFA concentrations, somatic growth, and survival of *Diporeia*. Total PUFA concentrations were significantly higher in *A. falcatus* than in diatoms and *Microcystis*, but only diatoms contained considerable amounts of eicosapentaenoic (EPA) and docosahexaenoic acid (DHA). EPA, DHA, and arachidonic acid (ARA) were highly retained in *Diporeia* even in the absence of dietary supply with ARA being the most efficiently bioaccumulated PUFA. Survivorship of *Diporeia* ranged from 60% (diatom-fed), 68% (*A. falcatus*-fed), to 70% (fasting treatment), but was 0% in the *M. aeruginosa* diet treatment. Nucleic acid ratios (RNA:DNA), commonly used as proxies for somatic growth potential, were highest in *Diporeia* feeding on diatoms and lowest in fasting animals. We conclude that overall condition of *Diporeia* improved with dietary access to EPA and DHA, but survival was not related to this food quantity and/or quality.

Introduction

Odum (1953) suggested that the number of pathways through which energy can flow through a food web is a qualitative measure of food web stability. MacArthur (1955) added to this classic hypothesis by postulating that community stability increases as the number of links increases mostly due to patterns of interaction among the species in the community. Subsequent studies have examined and elaborated the many ecological factors that affect community stability in aquatic food webs. For example, changes in biodiversity (Dunne et al., 2002; Duffy, 2002), energy transfer efficiency (Pazzia et al., 2002), and/or species invasions (Vander Zanden et al., 1999) are now all known to affect food web stability.

There is mounting evidence that the stability of food webs in the Laurentian Great Lakes of North America has weakened in the past 20 years by the invasion of exotic species such as zebra and quagga mussels (*Dreissena* spp.), predatory cladocerans (*Bythotrephes longimanus*, and *Cercopagus pengoi*), and the round goby (*Neogobius melanostomus*) (e.g. Madenjian et al., 2002; Mills et al., 2003). Dreissenids have had particularly strong impacts on Great Lakes food webs, through both direct and indirect impacts. They are also associated with drastic population declines of the native benthic burrowing amphipod *Diporeia* spp. (hereafter *Diporeia*). Nalepa (1991) calculated that *Diporeia* was the dominant benthic invertebrate in profundal habitats of the Lakes Superior, Michigan, and Ontario, accounting for 69–91% of total benthic biomass. In recent years, however, *Diporeia* biomass has declined (by 90% in some areas) in Lake Michigan (Nalepa et al., 2006, 2009), and similar population declines have been observed in Lakes Ontario (Dermott and Kerec, 1997; Lozano et al., 2003), Erie (Dermott, 2001), and Huron (Nalepa et al., 2003). Many consumers at higher trophic levels that previously preyed on *Diporeia* cannot consume dreissenids effectively. This results in a severe reduction in energy supply to higher trophic levels (e.g., Pothoven et al., 2006; Nalepa et al., 2006; Mills et al., 2003; Johannsson et al., 2000); for example, changes in populations and condition of lake whitefish (*Coregonus clupeaformis*), the most economically important commercial fish species in the Great Lakes, have been linked to variation in diet, prey energy content, and prey abundance. Although it is clear that the recent decline of *Diporeia*

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biomass weakens dietary nutrient and energy transfer to consumers and thus native food web stability, the causal mechanisms underlying the *Diporeia* population decline are still not well understood.

Diporeia are rich in lipids (Gardner et al., 1985) and historically attained high population densities and supplied high amounts of energy to their consumers. Nalepa et al. (2000) reported significant differences in *Diporeia* total lipid contents at different sampling depths and sites in Lake Michigan. They found densities and lipid contents to be lower in *Diporeia* at sites with higher abundances of zebra mussels (*Dreissena polymorpha*) and suggested that regional differences in densities and lipid contents of *Diporeia* may have resulted from competition with *D. polymorpha* for food. Moreover, these authors argued that the low lipid content (mean 16.6% dry weight) they observed may represent the minimum for successful reproduction in *Diporeia*. In a subsequent field study, Nalepa et al. (2006) noticed high lipid levels in *Diporeia* as the population disappeared, indicating that more detailed information about the biochemical composition of lipids are required to better understand the relationship between potential lipid deficiencies and survival.

There is growing evidence from field and laboratory studies that some lipids (especially those that are highly retained) are essential for aquatic invertebrates because they enhance somatic growth and reproduction (Kainz et al., 2004; Persson and Vrede, 2006). There is experimental evidence that polyunsaturated fatty acids (PUFA) increase somatic growth rates in freshwater zooplankton (e.g., Sundbom and Vrede, 1997; Müller-Navarra et al., 2000; Wacker and von Elert, 2001) and the midge *Chironomus riparius* (Goedkoop et al., 2007). For fish, it is now known that PUFA, in particular the long-chain omega (n)−3 and n−6 PUFA, are crucial for somatic growth and reproduction (Izquierdo et al., 2000; Tocher, 2003). Moreover, Tocher (2003) concluded that the enzymatic conversion from C_{18} to C_{20} and C_{22} PUFA is poorly developed in many fish species. Thus, fish depend on obtaining the proper dietary amounts and ratios (Ahlgren et al., 2009) of long-chain n−3 and n−6 PUFA to attain healthy physiological status. There is also evidence that membrane competency and immune response of fish is related to PUFA availability in the diet (reviewed by Arts and Kohler, 2009). So far, very little is known about the role of dietary lipids and their fatty acids (FA) for somatic growth, condition, and survival in *Diporeia*; an organism which feeds on organic material (e.g. algae and bacteria) settling to the bottom of lakes (Gardner et al., 1990; Quigley and Vanderploeg, 1991).

Such uncertainties led us to pose the following question: what is the role of dietary FA composition, as a measure of biochemical diet quality, for somatic growth and survival of *Diporeia*? To investigate this, we designed laboratory experiments to test the effects of different diets on; a) FA composition, b) somatic growth potential (as assessed by nucleic acid ratios; RNA:DNA), and c) survival of *Diporeia*. We hypothesized that somatic growth and survival of *Diporeia* would be positively related to dietary PUFA concentrations.

Materials and methods

Because of their scarcity in Lake Ontario, *Diporeia* were collected (May 2006) from the sediment–water interface of Charleston Lake (N44.50; W76.03), Ontario, Canada, using an Ekman sediment grab. Surficial sediments containing *Diporeia*, along with the overlaying lake water, were kept at 4 °C in containers during transport to the laboratory at the Canada Centre for Inland Waters (CCIW), Burlington, Ontario. For total lipid, fatty acid and nucleic acid quantification, fresh *Diporeia* were immediately removed from sediments in the field and stored at −80 °C until further analysis (see below). Time from collection to freezing was <10 min; well below the time interval before large-scale changes in lipids or nucleic acid concentrations are normally observed (for nucleic acids; see Schlechtriem et al., 2008a,b). For lab experiments, *Diporeia* were kept with the lake sediments for 3 d in a dark, temperature-controlled (4 °C) room until the start of experiments. For the experimental design, sediment from the sediment–water interface was dried at 85 °C for 48 h causing thermal-induced degradation of labile organic matter including PUFA. Homogenized sediments (ca. 1 cm^3) were put into pre-combusted (400 °C) glass vials (15 mL) and rehydrated with filtered (0.22 μm) lake water from Charleston Lake. Three days after collecting the *Diporeia* from Charleston Lake, organisms of similar size and body weights (959 ± 27 mg per individual DW; Table 1) were haphazardly placed into these culture vials, one per vial. Visual inspection confirmed that they could all burrow into the prepared sediments. All organisms fasted for 48 h prior to the addition of food at the start of the different experiments.

To test the effect of different diets and fasting on FA composition, somatic growth potential, and survival of *Diporeia*, we used 3 different diets that differed in their FA profiles: a) the green alga *Ankistrodesmus falcatus*, b) a natural diatom assemblage consisting mostly of *Stephanodiscus niagarae*, collected from Lake Ontario in May when diatoms were dominant, c) the cyanobacterium *Microcystis aeruginosa* (non-toxic strain 632; University of Toronto, phytoplankton culture collection), and, d) no food (fasting). *A. falcatus* was cultured in CHU-10 medium, and *M. aeruginosa* in Bold's Basal Medium (BBM; for both media, see Stein, 1973). *Diporeia* spp. were fed *A. falcatus*, diatoms, and *M. aeruginosa*, *ad libitum* (i.e., ~500 μg d^{-1}; Dermott and Corning, 1988), for the duration of the 30 d experiment. Each treatment contained 40 *Diporeia* placed individually in separate vials. Water in the test vials was changed weekly with filtered (0.45 μm) Charleston Lake water to ensure that test conditions did not turn anoxic. Oxygen levels were tested regularly at the sediment–water interface (Thermo Orion Model 805Aplus) to confirm that the water overlying the sediments remained oxic (>2 mg L^{-1}) in all treatments throughout the experiments.

Diporeia survivorship was monitored daily and dead organisms were removed. At the end of the experiment, organisms were removed from the culture vessels, verified to be alive or dead, and stored at −80 °C. *Diporeia* for lipid analysis were subsequently freeze-dried and stored again at −80 °C until further analysis.

Lipid and fatty acid analysis

All freeze-dried samples were weighed prior to FA analysis. Fatty acid methyl esters (FAME) of; a) organic material in the sediments used during the experiments, b) *A. falcatus*, c) lake diatom-dominated food, d) *M. aeruginosa*, and, e) *Diporeia* (pooled; 3–7 mg dry weight) were obtained in a three-step process: extraction, derivatization, and quantification on a gas chromatograph (GC). Samples were extracted 3 times by grinding freeze-dried tissues in (2:1 v/v) chloroform: methanol (Bligh and Dyer, 1959) followed by centrifugation at 3300 r.p.m. to remove non-lipid material (e.g., exoskeletons). Extracted lipids were brought to a final volume of 2 mL. Duplicate 200-μL aliquots were dispensed into pre-weighed tin cups which were dried and reweighed on a Sartorius (Model ME5) microbalance with 1 μg

Table 1
Diporeia weights and total lipid concentrations (both on a DW basis). Dry weight (mean ± SD) of *Diporeia* before (field samples) and after the different diet treatments, and of algae (diet); n.a. = not applicable.

Treatment	*Diporeia* weight (μg individual^{-1})	*Diporeia* (total lipids; mg g^{-1})	Algae (total lipids; mg g^{-1})
Lake *Diporeia*[a]	959 ± 27	135 ± 17	n.a.
A. falcatus	1010 ± 18[b]	162 ± 18[b]	230 ± 8
Diatom diet[c]	1053 ± 206[b]	96 ± 26[b]	70 ± 3
M. aeruginosa	n.a.[d]	n.a.[d]	46 ± 6
Fasting	744 ± 161[b]	55 ± 25[b]	n.a.

[a] Field samples from Charleston Lake (day 0), subsequently used for experiments.
[b] At the end of experiments (30 d).
[c] Diatom assemblage of Lake Ontario, consisting of mostly *Stephanodiscus niagarae*.
[d] Not measured because of 100% mortality of *Diporeia*.

precision to provide a quantitative measure of total lipid content. Lipid content (%) was reported on a dry weight basis. The remaining extract was then transferred into a Shimadzu vial (Sigma 27319U) and evaporated to dryness using nitrogen gas and stored at −80 °C until derivatization.

For FAME formation, aliquots of the lipid extract were evaporated to near dryness under nitrogen in a pre-cleaned vial. Sulphuric acid in methanol (1:100 mixture used as methylation reagent) and toluene were added to the vials, the headspace was flushed with nitrogen, the vial was vortexed and then incubated (12 h) at 50 °C in a water bath. After the samples were cooled, potassium hydrogen carbonate, isohexane:diethyl ether (1:1 v/v), and butylated hydroxy toluene (0.01% w/v) were added, and the vials were vortexed and centrifuged. The upper organic layer was transferred to another labeled centrifuge tube; isohexane:diethyl ether (1:1 v/v) was added to the original tube which was then shaken, vortexed, and centrifuged. All FAME containing layers were pooled and concentrated under nitrogen.

FAME were separated using a Hewlett Packard 6890 GC with the following configuration: splitless injection; column = Supelco (SP-2560 column) 100 m × 0.25 mm ID × 0.20 μm thick film; oven = 140 °C (hold for 5 min) to 240 °C at 4 °C min^{-1}, hold for 12 min; carrier gas = helium, 1.2 mL min^{-1}; detector = FID at 260 °C; injector = 260 °C; total run time = 42 min per sample. A 37-component FAME standard (Supelco 47885-U) was used to identify FAME in the samples by comparing their retention times to those of the FAME standard. Quantification of individual FAME components was calculated on the basis of known amounts of injected standard dilutions (2000, 1000, 500, 250, and 1.25 ng μL^{-1}). The following PUFAs were considered essential to the somatic growth and survival of *Diporeia* because they are integral parts of cell membranes: linoleic acid (LIN; 18:2*n*−6), α-linolenic acid (ALA; 18:3*n*−3), arachidonic acid (ARA; 20:4*n*−6), eicosapentaenoic acid (EPA; 20:5*n*−3), and docosahexaenoic acid (DHA; 22:6*n*−3). LIN and ALA cannot be synthesized *de novo* by animals (Cook and McMaster, 2002).

Nucleic acid and protein analyses

For the investigation of somatic growth potential, we analyzed nucleic acids following the methods described in Schlechtriem et al. (2008b). Nucleic acid concentrations and ratios are a good proxy for somatic growth and development as the amount of RNA in the cell is directly proportional to the amount of protein synthesis, while the amount of DNA is quasi-constant in somatic tissues and thus a good correlate of cell number (Dortch et al., 1983; Wagner et al., 1998). In brief, freeze-dried, individual *Diporeia* were weighed and homogenized in 0.2 M perchloric acid (PCA) with a Polytron® homogenizer. Samples were kept on ice to avoid nucleic acid degradation. The PCA soluble fraction containing free amino acids and nucleotides was separated from insoluble components as protein and nucleic acids by centrifugation (10,000 × *g*, 4 °C, 8 min). All pellets were washed twice with fresh 0.2 M PCA to ensure a complete removal of soluble compounds. The insoluble homogenates were finally dissolved in 0.2 M NaOH and incubated (1 h at 37 °C). The NaOH solutions were used to determine the concentration of RNA, DNA and protein in all individual animals. Total protein concentrations were determined (Lowry et al., 1951) using bovine serum albumin (BSA, 50–250 μg mL^{-1}) as standard protein. RNA was extracted from the NaOH solutions by precipitation of proteins and DNA using 20% PCA. RNA concentrations were determined in the supernatant after centrifugation (10,000 × *g*, 4 °C, 8 min) by a modification of the Schmidt–Thanhauser dual absorbance (260 and 232 nm) method (Munro and Fleck, 1966). DNA was extracted from the precipitated material obtained during the RNA analysis. The pellet was washed 2× with 2% PCA to remove protein. The remaining DNA fraction was re-suspended in 0.6 N PCA and incubated (30 min at 70 °C). DNA concentrations were determined again by the dual absorbance technique (Munro and Fleck, 1966).

Results

Oven-dried (85 °C for 48 h) lake sediment samples contained less total FAME (0.4 ± 0.0 mg g^{-1}) than freeze-dried sediments preserved directly from the lake (1.6 ± 0.4 mg g^{-1}). Both consisted mostly of long-chain (>C_{20}) saturated fatty acids (SAFA), however, both oven-dried and freeze-dried sediments did not contain PUFA (at least within the constraints of our detection limits).

There was no significant difference among *Diporeia* dry body weights of wild-caught animals (before the start of the experiments), animals subjected to the different diet treatments, and fasting animals (ANOVA, $p<0.2$; Table 1). Survivorship of *Diporeia* ranged narrowly between 60% and 70% (i.e. 60%, 68%, or 70% in the diatom-fed, *A. falcatus*-fed or fasted *Diporeia*, respectively) but was 0% in the *M. aeruginosa* diet treatment. Because of concerns over potentially rapidly occurring changes of labile fatty and nucleic acids in dead animals, no *M. aeruginosa*-fed *Diporeia* were analyzed for lipids or nucleic acids.

Concentrations of total lipids and FA differed among the three algal diets; *A. falcatus*, diatoms, and *M. aeruginosa*, as well as among the *Diporeia* fed these different treatments for 30 d (Tables 1 and 2). Total lipid concentrations differed significantly (ANOVA; $F = 2271.5$; $p<0.001$) among the three diets (Tukey's post-hoc test: $p<0.01$) and were highest in *A. falcatus*, lower in natural lake diatoms, and lowest in *M. aeruginosa* (Table 1). In *Diporeia*, total lipid concentrations were significantly different (ANOVA, $F = 10.2$; $p<0.05$) with highest concentrations in *A. falcatus*-fed *Diporeia* (162 ± 18 mg g^{-1} DW) and the lowest in fasting *Diporeia* (55 ± 25 mg g^{-1} DW; Table 2). However, results from pair-wise multiple comparisons show that total lipid concentrations were not significantly different between the diatom and *A. falcatus* diets ($p = 0.1$) nor between the diatom and fasting treatments ($p = 0.3$).

Table 2
Concentrations (mg g^{-1} DW; mean ± SD) of total fatty acids, summary of fatty acid indices (SAFA, MUFA, and PUFA) and selected individual PUFA compounds in diet sources and *Diporeia* exposed to the diet treatment for 30 d. *Diporeia* preserved directly upon collection (lake *Diporeia*) are presented for comparison. DW = dry weight; FAME = fatty acid methyl esters; SAFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; and individual PUFA compounds: LIN = linoleic acid; ALA = α-linolenic acid; ARA = arachidonic acid; EPA = eicosapentaenoic acid; DHA = docosapentaenoic acid; *tr* = trace.

	A. falcatus treatment		Diatom treatment		*M. aeruginosa*	Fasting	Lake *Diporeia*
	Diet	*Diporeia*	Diet	*Diporeia*		*Diporeia*	
FAME	108.1 ± 7.2	119.0 ± 5.7	31.5 ± 0.4	58.7 ± 4.2	13.0 ± 2.9	35.6 ± 2.1	94.6 ± 8.1
SAFA	36.2 ± 2	30.7 ± 1.4	7.9 ± 0.1	13.5 ± 1.1	9.2 ± 2.1	7.0 ± 1.5	21.8 ± 2.3
MUFA	39.3 ± 4	48.1 ± 0.4	6.2 ± 0.1	18.9 ± 0.5	2.3 ± 0.6	10.4 ± 1.1	30.4 ± 1.8
PUFA	32.6 ± 2	39.6 ± 4.0	17.3 ± 0.1	25.9 ± 2.4	1.4 ± 0.2	18.2 ± 0.5	42.4 ± 4.1
LIN	8.0 ± 0.2	8.1 ± 0.1	2.4 ± 0.0	2.4 ± 0.0	0.4 ± 0.1	1.6 ± 0.0	5.1 ± 0.5
ALA	23.2 ± 1.9	8.2 ± 0.4	1.6 ± 0.0	1.5 ± 0.1	0.3 ± 0.1	0.6 ± 0.1	3.1 ± 0.4
ARA	*tr*	1.6 ± 0.2	*tr*	1.5 ± 0.1	*tr*	1.7 ± 0.0	2.7 ± 0.3
EPA	*tr*	8.2 ± 1.4	8.7 ± 0.0	8.9 ± 1.1	*tr*	6.0 ± 0.1	12.1 ± 0.3
DHA	*tr*	12.2 ± 2.9	3.4 ± 0.0	10.7 ± 1.0	*tr*	8.0 ± 0.2	18.3 ± 2.6

Total FAME concentrations differed significantly among the three diets (Table 2; $F=236$; $p<0.001$) and were highest in *A. falcatus*, lower in diatoms, and lowest in *M. aeruginosa* (Tukey's post-hoc test; $p<0.05$). Total FAME concentrations were also significantly different in *Diporeia* ($F=230$; $p<0.001$), the highest being in those fed *A. falcatus*, and the lowest in fasted animals (Tukey's post-hoc test; $p<0.01$). Similarly, PUFA concentrations differed significantly among the diet types ($F=432$; $p<0.001$; Tukey's post-hoc test; $p<0.01$) and among the *Diporeia* in the different diet treatments ($F=32$; $p<0.01$; Tukey's post-hoc test; $p<0.05$). *Diporeia* had the highest PUFA concentrations after being fed *A. falcatus* (40 ± 4 mg g^{-1} DW) and the lowest PUFA concentrations after fasting (18 ± 1 mg g^{-1} DW). Total PUFA concentrations of *Diporeia* were significantly higher than their dietary PUFA concentrations (*A. falcatus*: $F=19.8$; $p<0.05$, and diatoms: $F=25.2$; $p<0.05$).

Fresh-caught *Diporeia* from Charleston Lake had the following concentration 'hierarchy' of FA groups (i.e., SAFA; monounsaturated fatty acids, MUFA; and PUFA) and selected individual PUFA compounds (Table 2):

- for FA classes: PUFA>MUFA>SAFA; and
- for individual PUFAs: DHA>EPA>LIN>ALA≥ARA.

Although FA concentrations decreased after 30 d of fasting, the FA distribution patterns observed as in lake *Diporeia* after collection were similar; i.e., total PUFA concentrations were higher than MUFA and SAFA concentrations, and DHA concentrations were higher than the other individual PUFAs.

In an effort to understand how retention of FA groups and individual PUFAs differed during fasting, we calculated losses of FAs after 30 d of fasting (in mg g^{-1} DW) between lake *Diporeia* (field samples) and fasted *Diporeia* (Fig. 1). We suggest that these FA losses provide a measure of preferential use and retention of FA groups and individual PUFA compounds in the absence of dietary inputs. We found significant decreases in SAFAs, MUFAs, and PUFAs between fresh-caught *Diporeia* and those measured after fasting ($F=9.47$, $p<0.01$). On average, fasted *Diporeia* lost 68% SAFA, 66% MUFA, and 57% PUFA. Similarly, significantly different retention patterns of individual PUFA were detected after fasting ($F=49.36$, $p<0.001$). The largest concentration differences between fresh-caught and fasted *Diporeia* were detected for the C_{18} PUFA, i.e., 81% less ALA and 69% less LIN, followed by 57% less DHA, 50% less EPA, and 37% less ARA.

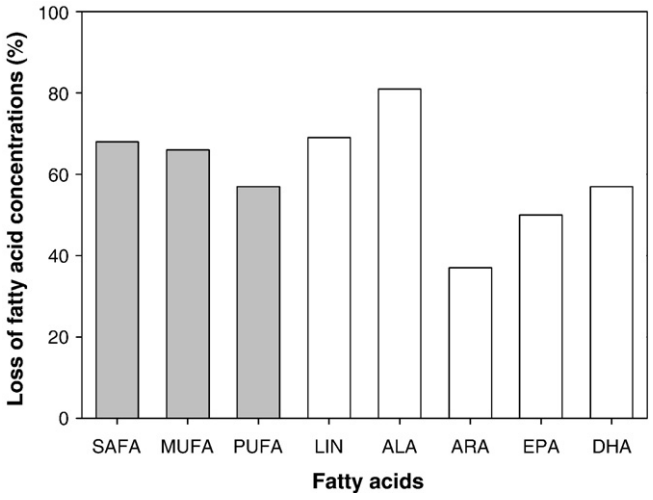

Fig. 1. Loss (%) of fatty acid concentrations per unit dry weight (mg g^{-1}) in *Diporeia* after fasting for 30 d. SAFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids. Individual PUFA compounds: LIN = linoleic acid; ALA = α-linolenic acid; ARA = arachidonic acid; EPA = eicosapentaenoic acid; DHA = docosapentaenoic acid.

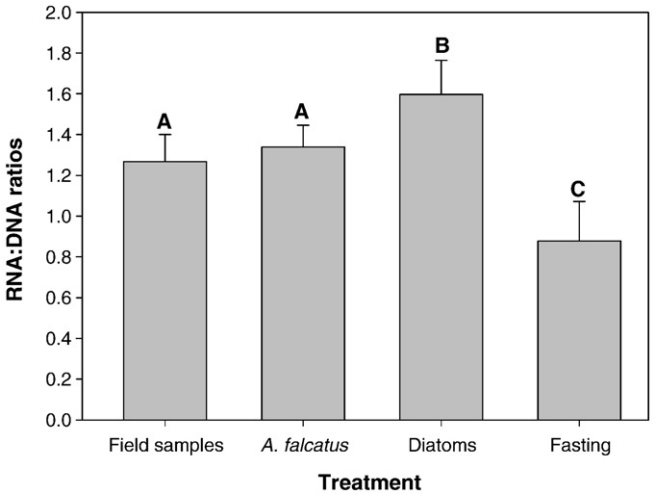

Fig. 2. RNA:DNA ratios (means ± SD) of *Diporeia* collected in the field, after 30 d raised on *A. falcatus* or diatoms and fasting. Bars labeled with the same letters are not significantly different (Tukey's HSD following ANOVA).

Nucleic acid ratios (RNA:DNA; Fig. 2), used as a 'snap-shot' index of the cell's metabolic intensity (i.e. a measurement of recent growth), were significantly different ($F=50.07$; $p<0.001$) among the treatments. RNA:DNA ratios were highest in the diatom-fed treatment (1.60 ± 0.17) and lowest, as expected, in the fasting amphipods (0.87 ± 0.19). Interestingly, there was no significant difference in nucleic acid ratios between *Diporeia* fed *A. falcatus* and fresh-caught *Diporeia* from Charleston Lake (Tukey's post-hoc test: $p=0.7$).

Discussion

We examined the effect of different diet treatments on the retention of lipids and their constituent FA, somatic growth potential, and survival of the benthic amphipod *Diporeia*. *Diporeia*'s potential for growth, as assessed by RNA:DNA ratios, is associated with the dietary supply of long carbon chain, $n-3$ PUFA, in particular EPA and DHA. *Diporeia* survival was, however, not affected by diet quantity or total lipid quantity and quality within the 30 d time frame of our experiments.

Total lipid content of *Diporeia* from Charleston Lake was 13.5% DW, and *Diporeia* fed *ad libitum* ranged from 10% DW (diatom-fed) to 16% DW (*A. falcatus*-fed). Despite the difference in total lipid content of these *Diporeia*, their survivorship in the diatom vs *A. falcatus*-fed feeding experiments was similar (60% and 68%, respectively). Fasting *Diporeia* reached a total lipid level of 6% DW at the end of the experiment without a significant increase in mortality, compared with the fed groups.

The lipids contained in *Diporeia* are crucial for providing energy to its consumers. For example, Pothoven et al. (2006) found that decreased body condition and growth of lake whitefish (*Coregonus clupeaformis*) was associated with the loss of *Diporeia* as a high-energy prey resource. In addition, membrane competency and immune response of fish is related to dietary PUFA availability (reviewed by Arts and Kohler, 2009). Among dietary PUFA, DHA may be of particular importance in this regard because its length, coupled with its high degree of unsaturation, makes this molecule particularly flexible, perhaps contributing to increased fluidity of cold-challenged cell membranes. Consequently, dietary DHA is likely to enhance the ability of animals (e.g benthic dwelling/feeding fish, such as lake whitefish, slimy sculpin (*Cottus cognatus*) and deepwater sculpin (*Myoxocephalus thompsoni*), and invertebrates, such as *Diporeia* and *Mysis*) to adapt to cold water conditions experienced year-round in Great Lakes hypolimnetic, benthic habitats.

Of the individual $n-3$ and -6 FA examined here, DHA concentrations (on a per mg DW basis) were highest in *Diporeia* from all

experimental treatments and also in wild-caught *Diporeia* from Charleston Lake. The PUFA profile of *Diporeia* is similar to that of freshwater copepods (Persson and Vrede, 2006; Kainz et al., 2009), *Mysis diluviana*, which contain high concentrations of both EPA and DHA (Schlechtriem et al., 2008a), and fish (e.g., *Coregonus* spp.; Ahlgren et al., 1994). Thus, in the context of DHA supply in the Great Lakes, both *Diporeia* and mysids are excellent sources of DHA with mysids being the dominant organism in the deep profundal (>100 m) and *Diporeia* in the shallower (<100 m) regions of the hypolimnion. High dietary DHA concentrations are important for fish as this or other essential FA cannot always be biosynthesized *de novo* or at rates sufficient to meet physiological requirements (Tocher, 2003). When compared to other PUFA, DHA also seems to be highly required for *Diporeia* even during fasting as DHA concentrations, per unit biomass, remain higher than other PUFA concentrations. As is the case for fish (see above; Arts and Kohler, 2009), it is likely that *Diporeia* requires high DHA concentrations to keep its cell membrane fluid at low temperatures (4 °C experimental temperature: in deep waters their natural benthic habitat is 4 °C or colder. Temperatures may be higher in the shallower regions of their distribution).

PUFA requirements in fasting *Diporeia* seem to differ among the different FAs as concentrations of individual PUFAs decreased differently. The loss of arachidonic acid (ARA; 20:4*n*−6) concentrations between fresh-caught and fasted *Diporeia* was lowest when compared with other PUFAs, indicating that this *n*−6 PUFA is most probably highly required. To date, little is known about the physiological role of ARA in *Diporeia* or in many other aquatic organisms, but it is known that ARA clearly contributes to the structural integrity of membranes (Cook and McMaster, 2002) and is also important in the immune response (eicosanoid production; Arts and Kohler, 2009). The least retained PUFAs were the two C_{18} ALA and LIN. Although we did not investigate specific enzymatic processes in this study, it is possible that *Diporeia* converted ALA to EPA and DHA, and LIN to ARA, a common, albeit inefficient, process in mammalian cells (Cook and McMaster, 2002), to keep ARA, EPA, and DHA concentrations as high as possible during periods of food shortage. The similar ARA, EPA and DHA concentrations in the *A. falcatus* and diatom-fed *Diporeia* (levels that were higher than in the fasted *Diporeia*) support this hypothesis as *A. falcatus* contains only trace amounts of ARA, EPA and DHA but high concentrations of the precursors LIN and ALA. Finally, there were significant differences in the retention of total SAFA, MUFA, and PUFA between *Diporeia* under natural (lake) and fasting conditions. Total PUFAs were retained more efficiently than SAFA and MUFA, which suggests preferential metabolism of these latter FA groups during fasting.

The three diet treatments (*A. falcatus*, lake diatoms, and *M. aeruginosa*) provided different FA profiles to *Diporeia*. The cyanobacterium *M. aeruginosa* contained significantly less MUFA and PUFA than *A. falcatus* and lake diatoms. The 100% mortality of *Diporeia* cannot be explained by dietary total lipids or FA alone because fasting *Diporeia* had the highest survival rate during these experiments. However, we did not measure potentially toxic compounds, including peptides or other lipids, such as polyunsaturated aldehydes (e.g., Watson et al., 2009) or lipids of the dead *Diporeia* fed *M. aeruginosa*, thus it is so far not possible to state how the dietary composition of this presumably non-toxic strain of *M. aeruginosa* may have contributed to the death of *Diporeia*. The effect of phyto-toxins warrants further research to better understand how they may exert negative effects on *Diporeia* survival.

Dietary PUFA affected the retention of individual PUFA compounds in *Diporeia* differently. LIN showed no significant concentration differences between *Diporeia* and their respective diet, suggesting that dietary LIN supply greatly affects LIN concentrations in this amphipod. However, it should be noted that LIN was still retained after fasting, which indicates that some base concentrations of this *n*−6 PUFA are required for *Diporeia* even in the absence of dietary LIN.

Unlike LIN, dietary supply of ARA, another *n*−6 PUFA, was scarce. Both, *A. falcatus* and diatoms contained only traces of ARA (i.e., 0.1 and 0.25 mg g^{-1} DW, respectively); however, ARA concentrations of *Diporeia* were 12× and 6× higher than in their respective diet sources. Retention of ARA in the absence of dietary supply in all three treatments suggests that ARA is highly required, most likely as a precursor for eicosanoid synthesis and as a constituent of the membrane phospholipids involved in signal transduction (Smith and Fitzpatrick, 1996).

Concentrations of ALA in *Diporeia* showed the highest range of PUFA examined, i.e., from 1 (fasting) to 8 (*A. falcatus* diet) mg g^{-1} DW, indicating that the retention of dietary ALA is highly dynamic and dependent on dietary contribution. Similar to LIN, ALA increased in *Diporeia* when being fed *A. falcatus* that contains high ALA concentrations. The absence of dietary ALA supply resulted in rapid reduction of ALA concentrations (see fasting treatment). However, the results of our fasting trials indicate that ALA concentrations can be drawn down to at least 1 mg g^{-1} DW in *Diporeia* without causing immediate death.

Diporeia feeding on *A. falcatus* and diatoms had significantly higher DHA and EPA concentrations than amphipods which had fasted, although *A. falcatus* provided no measurable EPA or DHA to *Diporeia*, and diatoms only low levels of DHA, yet sufficient dietary EPA. This suggests that *Diporeia* selectively retain these key PUFAs and/or have the enzymatic ability to convert other precursor FA, such as ALA, to the longer chain *n*−3 PUFAs EPA and DHA. The finding that ALA in fasting *Diporeia* was the least retained PUFA lends support to the latter argument.

We used RNA:DNA ratios as an index of the growth potential of *Diporeia* cells. The lowest RNA:DNA ratios were found in fasting *Diporeia*, indicating a poor somatic growth potential of *Diporeia* due to lack of food. RNA:DNA ratios were significantly higher in *Diporeia* after feeding on diatoms. Both *A. falcatus* and diatoms were supplied *ad libitum* and thus, supposedly, at higher concentrations than under natural feeding conditions in lakes. In addition to dietary elemental compositions (C:N:P ratios) and/or other dietary nutrients, we suggest that the highest RNA:DNA ratios in diatom-fed *Diporeia* may be related to the dietary supply of EPA and DHA, two *n*−3 PUFAs that only occurred in the diatom feeding treatment. Moreover, it is clear that the two *n*−6 PUFAs are not related to the different patterns of RNA:DNA ratios because LIN concentrations were significantly higher in the *A. falcatus* than in the diatom treatment, and ARA concentrations of *Diporeia* did not significantly differ among the *A. falcatus*, diatom, and fasting treatments. Finally, the somatic condition of fasting *Diporeia* was significantly lower than in organisms feeding on diatoms, yet this condition index and feeding difference did not affect survival of *Diporeia* as animals of both treatments had similar survivorship after 30 d.

This is the first study showing that *Diporeia* selectively retain certain PUFA compounds. Because profundal lake sediments as well as the processed sediments used in this study did not contain any detectable PUFA, we show that sediments alone are not, or a very poor source of dietary PUFA and argue that the required PUFA supply for *Diporeia* depends on the settling of PUFA produced in pelagic and littoral zones. We demonstrate that EPA and DHA are highly retained in the absence of dietary supply and that ARA was the most bioaccumulated PUFA in this benthic amphipod. The measured FA profiles are similar to those of many freshwater fish (Ahlgren et al., 1994), providing further evidence that *Diporeia* supply highly favorable dietary FA to upper trophic levels. The similar survivorship between *ad libitum* fed and fasting *Diporeia* indicates that short-term fasting (i.e., 30 d) may not immediately challenge the near-term survival of individuals, but low RNA:DNA ratios indicate that they were not growing. Investigating the role of changing diet quantity and quality to assess how seasonal changes of food supply affect somatic development, reproduction, and survival of this key benthic amphipod should be an important objective for future research.

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References

Ahlgren, G., Blomqvist, P., Boberg, M., Gustafsson, I.B., 1994. Fatty acid content of the dorsal muscle — an indicator of fat quality in freshwater fish. J Fish Biol 45, 131–157.

Ahlgren, G., Vrede, T., Goedkoop, W., 2009. Fatty acid ratios in freshwater fish, zooplankton and zoobenthos — are there specific optima? In: Arts, M.T., Brett, M.T., Kainz, M.J. (Eds.), Lipids in aquatic ecosystems. Springer-Verlag, New York, pp. 147–178.

Arts, M.T., Kohler, C.C., 2009. Health and condition in fish: the influence of lipids on membrane competency and immune response. In: Arts, M.T., Brett, M.T., Kainz, M.J. (Eds.), Lipids in aquatic ecosystems. Springer, New York, pp. 237–256.

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37, 911–917.

Cook, H.W., McMaster, C.R., 2002. Fatty acid desaturation and chain elongation in eukaryotes, In: Vance, D.E., Vance, J.E. (Eds.), Biochemistry of lipids, lipoproteins and membranes, 4th ed. Elsevier, Amsterdam, pp. 181–204.

Dermott, R., 2001. Sudden disappearance of the amphipod *Diporeia* from Eastern Lake Ontario, 1993–1995. J Great Lakes Res 27, 423–433.

Dermott, R., Corning, K., 1988. Seasonal ingestion rates of *Pontoporeia hoyi* (Amphipoda) in Lake Ontario. Can J Fish Aquat Sci 45, 1886–1895.

Dermott, R., Kerec, D., 1997. Changes to the deepwater benthos of eastern Lake Erie since the invasion of *Dreissena*: 1979–1993. Can J Fish Aquat Sci 54, 922–930.

Dortch, Q., Roberts, T.L., Clayton, J.R., Ahmed, S.I., 1983. RNA/DNA ratios and DNA concentrations as indicators of growth rate and biomass in planktonic marine organisms. Mar Ecol Prog Ser 13, 61–71.

Duffy, J.E., 2002. Biodiversity and ecosystem function: the consumer connection. Oikos 99, 201–219.

Dunne, J.A., Williams, R.J., Martinez, N.D., 2002. Network structure and biodiversity loss in food webs: robustness increases with connectance. Ecol Lett 5, 558–567.

Gardner, W.S., Frez, W.A., Cichocki, E.A., Parrish, C.C., 1985. Micromethod for lipids in aquatic invertebrates. Limnol Oceanogr 30, 1099–1105.

Gardner, W.S., Quigley, M.A., Fahnenstiel, G.L., Scavia, D., Frez, W.A., 1990. *Pontoporeia hoyi* — a direct trophic link between spring diatoms and fish in Lake Michigan. In: Tilzer, M.M., Serruya, C. (Eds.), Large lakes: ecological structure and function. Springer Verlag, New York, NY, pp. 632–644.

Goedkoop, W., Demandt, M., Ahlgren, G., 2007. Interactions between food quantity and quality (long-chain polyunsaturated fatty acid concentrations) effects on growth and development of *Chironomus riparius*. Can J Fish Aquat Sci 64, 425–436.

Izquierdo, M.S., Socorro, J., Arantzamendi, L., Hernandez-Cruz, C.M., 2000. Recent advances in lipid nutrition in fish larvae. Fish Physiol Biochem 22, 97–107.

Johannsson, O.E., Dermott, R., Graham, D.M., Dahl, J.A., Millard, E.S., Myles, D.D., et al., 2000. Benthic and pelagic secondary production in Lake Erie after the invasion of *Dreissena* spp. with implications for fish production. J Great Lakes Res 26, 31–54.

Kainz, M., Arts, M.T., Mazumder, A., 2004. Essential fatty acids within the planktonic food web and its ecological role for higher trophic levels. Limnol Oceanogr 49, 1784–1793.

Kainz, M., Perga, M.-E., Arts, M.T., Mazumder, A., 2009. Essential fatty acid concentrations of different seston sizes and zooplankton: a field study of monomictic coastal lakes. J Plankton Res 31, 635–645.

Lowry, O.H., Osenbrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with Polin phenol reagent. J Biol Chem 193, 265–275.

Lozano, S.J., Gedeon, M.L., Landrum, P.F., 2003. The effects of temperature and organism size on the feeding rate and modeled chemical accumulation in *Diporeia* spp. for Lake Michigan sediments. J Great Lakes Res 29, 79–88.

MacArthur, R.H., 1955. Fluctuations of animal populations and a measure of community stability. Ecology 36, 533–536.

Madenjian, C.P., Fahnenstiel, G.L., Johengen, T.H., Nalepa, T.F., Vanderploeg, H.A., Fleischer, G.W., et al., 2002. Dynamics of the Lake Michigan food web, 1970–2000. Can J Fish Aquat Sci 59, 736–753.

Mills, E.L., Casselman, J.M., Dermott, R., Fitzsimons, J.D., Gal, G., Holeck, K.T., et al., 2003. Lake Ontario: food web dynamics in a changing ecosystem (1970–2000). Can J Fish Aquat Sci 60, 471–490.

Müller-Navarra, D.C., Brett, M.T., Liston, A.M., Goldman, C.R., 2000. A highly unsaturated fatty acid predicts carbon transfer between primary producers and consumers. Nature 403, 74–77.

Munro, H.N., Fleck, A., 1966. The determination of nucleic acids. Methods Biochem Anal 14, 113–176.

Nalepa, T.F., 1991. Status and trends of the Lake Ontario macrobenthos. Can J Fish Aquat Sci 48, 1558–1567.

Nalepa, T.F., Hartson, D.J., Buchanan, J., Cavaletto, J.F., Lang, G.A., Lozano, S.J., 2000. Spatial variation in density, mean size and physiological condition of the holarctic amphipod *Diporeia* spp. in Lake Michigan. Freshw Biol 43, 107–119.

Nalepa, T.F., Fanslow, D.L., Lansing, M.B., Lang, G.A., 2003. Trends in the benthic macroinvertebrate community of Saginaw Bay, Lake Huron, 1987 to 1996: responses to phosphorus abatement and the zebra mussel, *Dreissena polymorpha*. J Great Lakes Res 29, 14–33.

Nalepa, T.F., Fanslow, D.L., Foley, A.J.I., Lang, G.A., Eadie, B.J., Quigley, M.A., 2006. Continued disappearance of the benthic amphipod *Diporeia* spp. in Lake Michigan: is there evidence for food limitation? Can J Fish Aquat Sci 63, 872–890.

Nalepa, T.F., Fanslow, D.L., Lang, G.A., 2009. Transformation of the offshore benthic community in Lake Michigan: recent shift from the native amphipod *Diporeia* spp. to the invasive mussel *Dreissena rostriformis bugensis*. Freshw Biol 54, 466–479.

Odum, E.P., 1953. Fundamentals of ecology, III. Saunders, Philadelphia.

Pazzia, I., Trudel, M., Ridgway, M., Rasmussen, J.B., 2002. Influence of food web structure on the growth and bioenergetics of lake trout (*Salvelinus namaycush*). Can J Fish Aquat Sci 59, 1593–1605.

Persson, J., Vrede, T., 2006. Polyunsaturated fatty acids in zooplankton: variation due to taxonomy and trophic position. Freshw Biol 51, 887–900.

Pothoven, S.A., Nalepa, T.F., Madenjian, C.P., Rediske, R.R., Schneeberger, P.J., He, J.X., 2006. Energy density of lake whitefish *Coregonus clupeaformis* in Lakes Huron and Michigan. Environ Biol Fish 76, 151–158.

Quigley, M.A., Vanderploeg, H.A., 1991. Ingestion of live filamentous diatoms by the Great Lakes amphipod, *Diporeia* sp.: a case study of the limited value of gut contents analysis. Hydrobiologia 223, 141–148.

Schlechtriem, C., Arts, M.T., Johannsson, O.E., 2008a. Effect of long-term fasting on the use of fatty acids as trophic markers in the opossum shrimp *Mysis relicta* — a laboratory study. J Great Lakes Res 34, 143–152.

Schlechtriem, C., Johannsson, O.E., Bowen, K.L., Smith, R.W., Arts, M.T., 2008b. Effect of fasting under different temperature conditions on nucleic acid ratios in the opossum shrimp *Mysis relicta*: a calibration approach. J Great Lakes Res 34, 461–471.

Smith, W.L., Fitzpatrick, F.A., 1996. The eicosanoids: cyclooxygenase, lipoxygenase, and epoxygenase pathways. In: Vance, D.E., Vance, J.E. (Eds.), Biochemistry of lipids, lipoproteins and membranes. Elsevier, Amsterdam, pp. 283–308.

Stein, J.R., 1973. Handbook of phycological methods: culture methods and growth measurements. Cambridge University Press, Cambridge.

Sundbom, M., Vrede, T., 1997. Effects of fatty acid and phosphorus content of food on the growth, survival and reproduction of *Daphnia*. Freshw Biol 38, 665–674.

Tocher, D.R., 2003. Metabolism and functions of lipids and fatty acids in teleost fish. Rev Fish Sci 11, 107–184.

Vander Zanden, M.J., Casselman, J.M., Rasmussen, J.B., 1999. Stable isotope evidence for the food web consequences of species invasions in lakes. Nature 401, 464–467.

Wacker, A., von Elert, E., 2001. Polyunsaturated fatty acids: evidence for non-substitutable biochemical resources in *Daphnia galeata*. Ecology 82, 2507–2520.

Wagner, M., Durbin, E., Buckley, L., 1998. RNA:DNA ratios as indicators of nutritional condition in the copepod *Calanus finmarchicus*. Mar Ecol Prog Ser 162, 173–181.

Watson, S.B., Caldwell, G., Pohnert, G., 2009. Fatty acids and oxylipins as semiochemicals. In: Arts, M.T., Brett, M.T., Kainz, M. (Eds.), Lipids in aquatic ecosystems. Springer, New York, pp. 65–92.