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Eicosapentaenoic acid limitation decreases weight and fecundity of the invading predator *Bythotrephes longimanus*

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Bythotrephes longimanus is an invasive predatory cladoceran that is negatively impacting North American zooplankton in the Laurentian Great Lakes and on the Canadian Shield. Concurrently, algal community composition, which affects zooplankton food quality, is changing in many lakes of the Canadian Shield. The *n*-3 fatty acid eicosapentaenoic acid (EPA) is highly retained in *Bythotrephes*, but the effects of EPA limitation on *Bythotrephes*' population dynamics are unknown. To test the hypothesis that EPA limitation results in decreased weight and fecundity of *Bythotrephes*, the green alga *Scenedesmus obliquus* was cultured in the laboratory, split into EPA-enriched lines and un-enriched controls, then fed to *Daphnia ambigua*, which were in turn offered to juvenile *Bythotrephes*. *Bythotrephes* consuming EPA-enriched daphniids were heavier and had larger clutch sizes than those consuming control daphniids. Both diets supported ontogenesis, but not brood release, of *Bythotrephes*. To understand why laboratory-reared *Bythotrephes* did not release broods, we compared their fatty acid profiles with those of field-collected specimens, and found that they were EPA impoverished compared with field-collected conspecifics. Our results suggest that EPA availability influences *Bythotrephes* population dynamics, establishment success and impacts in nature.

KEYWORDS: aquatic invasive species; *Bythotrephes*; *Daphnia*; EPA; fatty acids; prey quality

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INTRODUCTION

The nutrient-poor soft-water lakes of the Canadian Shield in Ontario, Canada, are currently in a state of flux due to various environmental stressors (Yan *et al.*, 2008) and their interactions (Palmer and Yan, 2013). The zooplankton communities in these lakes have responded to a host of natural and human-induced abiotic and biotic factors including, but not limited to, climate warming (Rusak *et al.*, 2008), decreasing total phosphorus concentrations (Quinlan *et al.*, 2008), changing phytoplankton assemblages (Paterson *et al.*, 2000), and the introduction and spread of the Ponto-Caspian zooplanktivore, *Bythotrephes longimanus* Leydig (1860; herein "*Bythotrephes*").

Bythotrephes was likely introduced to North America from a source population in Lake Ladoga, Russia, as a consequence of shipping activities (Berg *et al.*, 2002). Initially detected in Lake Ontario in the early 1980s (Johannsson *et al.*, 1991), *Bythotrephes* soon spread to the remaining Laurentian Great Lakes (Bur *et al.*, 1986; Lehman, 1987; Jin and Sprules, 1990). In 1989 and the early 1990s, secondary invasions occurred inland in the District of Muskoka, north of Toronto, Ontario, Canada (Yan *et al.*, 1992). Its present distribution encompasses at least 170 lakes from south central to northwestern Ontario, as well as southern Manitoba, Canada and the northeastern USA (Kerfoot *et al.*, 2011; Strecker *et al.*, 2011; J. Shead, Manitoba Ministry of the Environment, personal communication). The ecological repercussions of *Bythotrephes* introductions on native pelagic food webs have been far reaching. Documented impacts on indigenous zooplankton include declines in species diversity and richness, changes in abundance and shifts in community size structure (Yan *et al.*, 2001; Boudreau and Yan, 2003; Strecker *et al.*, 2011). Additionally, *Bythotrephes* appears to be displacing the native predatory macroinvertebrate *Leptodora kindtii* through competitive interactions (Branstrator, 1995; Foster and Sprules, 2009; Weisz and Yan, 2011). The rapid spread of *Bythotrephes* is attributable mainly to human-induced factors that affect propagule pressure (Weisz and Yan, 2010). Population establishment, however, requires a minimum number and sufficient quality of resting eggs to guarantee persistence beyond initial colonist survival and reproduction (Wittmann *et al.*, 2011). This persistence is contingent on positive population growth.

Bythotrephes population growth is regulated by prey abundance (Young *et al.*, 2011), but whether or not the quality of their prey affects population growth has not yet been examined. Two key aspects of food quality limit growth and reproduction of zooplankton, mineral and lipid requirements. Burkhardt and Lehman (Burkhardt and Lehman, 1994) examined the phosphorus requirements for *Bythotrephes* to complete one parthenogenic life cycle, and found them to be equivalent to ingesting 14 *Daphnia* daily. Unlike daphniids (Jeziorski and Yan, 2006), aqueous calcium requirements of *Bythotrephes* are minimal, as they can reproduce in the laboratory when cultured in 0.1 mg Ca L^{-1} water (Kim *et al.*, 2012).

If daphniid prey are readily available and minerals are not limiting for *Bythotrephes*, how might lipid availability affect *Bythotrephes*' growth and reproduction? This question has not yet been examined. Lipids take various forms, but here we focus on long-chain polyunsaturated fatty acids (LC-PUFAs; that is, PUFAs containing 20 or 22 carbon atoms). Zooplankton lipid profiles mirror those of their diets (Brett *et al.*, 2006). In *Daphnia*, for example, ~98% of their lipids are derived from their algal diets rather than being synthesized *de novo* (Goulden and Place, 1990). Since the retention of LC-PUFAs generally increases with the trophic level (Kainz *et al.*, 2004), it is likely that *Bythotrephes* will be impacted by differences in algal LC-PUFA contents, benefiting if the quality and quantity of dietary LC-PUFA supplies are adequate, and suffering if they are not.

Eicosapentaenoic acid (EPA, 20:5n-3) is the most highly retained *n*-3 LC-PUFA in freshwater cladocerans (Kainz *et al.*, 2004; Persson and Vrede, 2006; Ravet *et al.*, 2010), and this includes *Bythotrephes*. Most of the EPA is found in the phospholipids of cell membranes where, in combination with monounsaturated fatty acids (MUFAs; Arts and Kohler, 2009), it is thought to help maintain fluidity. EPA also serves as a precursor for eicosanoids, which are physiologically important as signaling molecules. EPA availability increases somatic growth and fecundity of *Daphnia* in both field and laboratory settings (Muller-Navarra *et al.*, 2000; von Elert, 2002; Becker and Boersma, 2003). *Daphnia magna* incorporates 2.4 times more EPA into its subitaneous eggs than into somatic tissues (Wacker and Martin-Creuzburg, 2007), which ensures that offspring will have adequate resources for initial growth and development. We currently do not know if EPA plays similar roles in the biology of *Bythotrephes*.

Bythotrephes accumulates high proportions of EPA relative to other FAs. Smyntek *et al.* (Smyntek *et al.*, 2008) found that EPA was the most abundant PUFA in *Bythotrephes* collected from the Great Lakes, comprising 38-μg EPA mg organic C^{-1}. In its native range, late summer *Bythotrephes* have been found to contain 11–16% EPA (in a Russian reservoir; Bychek and Gushina, 2001) and up to 23% EPA in the total lipid [total lipid (TL); in oligotrophic, northwestern Swedish lakes; Persson and Vrede, 2006]. Thus, *Bythotrephes* probably has strong EPA requirements. As a predator, it is moreover likely that *Bythotrephes* is dependent

almost entirely on the fatty acid (FA) contents of its prey to meet its needs (Brett *et al.*, 2009).

Here we clarify the role of EPA on *Bythotrephes* growth and reproduction, in an attempt to examine the potential effects of EPA limitation on population growth. Experiments were conducted at 21°C under controlled laboratory conditions using a food-chain approach. Our main hypothesis was that EPA supplementation, from algae to prey, would result in increased *Bythotrephes* weight and fecundity. We predict that daphniids reared on algae supplemented with EPA would be larger than those fed algae without EPA addition. Finally, we also predict that EPA would be the most highly retained FA in *Bythotrephes*.

METHOD

Algal culture and enrichment with EPA

We batch-cultured the chlorophyte *Scenedesmus obliquus* (Canadian Phycological Culture Centre, Waterloo, Canada; herein "*Scenedesmus*") in autoclaved Bold's Basal Medium (BBM; modified from Stein, 1973; recipe: http://www.phycol.ca/system/files/BBM) at 21°C under 100 μM m^{-2} s^{-1} continuous lighting. Cell counts were performed daily using a hemacytometer (Bright-Line™, Cambridge Instruments, Inc.), and algae were harvested in the log phase of growth. The algae were well suspended in BBM, then poured into 50-mL centrifuge tubes. We randomly selected half of the tubes from each batch of algae (five batches total) to enrich with EPA (99% purity; Matreya, Pleasant Gap, USA via http://biolynx.ca in Canada, catalog # MT1167) using a modified version of von Elert's (von Elert, 2002) protocol.

Scenedesmus treated with an EPA–bovine serum albumin (BSA; product number A7030; Sigma-Aldrich Co., Oakville, Canada) complex is denoted the "EPA-enriched" algae. "Control" algae were subjected to the same procedures but treated with BSA only (no significant effects of BSA incubation on altering the FA profiles of *Scenedesmus* have been observed; von Elert, 2002).

Our modified steps for EPA enrichment were as follows. Algae were centrifuged to form a pellet at 1500 rpm for 7 min. From each tube of algae, we removed 30 mL of the overlying BBM solution. We then mixed 5 mL of BSA stock solution (3.5 g L^{-1}) together with 400 μL of an EPA–EtOH stock solution (2.5 mg mL^{-1}) in a clean glass beaker. We added this EPA–BSA complex to each tube of algae, which was then capped and inverted five times to mix the contents. We placed the resulting suspensions on a rotary shaker set at 100 rpm for 7 h under ~100 μM m^{-2} s^{-1} cool white fluorescent lighting at room temperature (20–22°C). After the incubation period, we concentrated the algal cells by centrifugation (1500 rpm for 7 min) and discarded the supernatant. To remove excess BSA and EPA–EtOH, we washed the cells twice in 20 mL of sterile BBM. Prior to feeding the daphniids we re-suspended the algae in FLAMES medium, the soft-water culture medium (Celis-Salgado *et al.*, 2008) used to culture all zooplankton in this study. Following each enrichment episode, we checked the integrity of the algal cells qualitatively by inspecting them using a compound microscope.

Prey cultures

All prey (*Daphnia ambigua* and *Bosmina freyii*; herein "*Daphnia*" and "*Bosmina*") were reared in FLAMES medium and housed in Conviron E2/7 growth chambers at 21°C under 100 μM m^{-2} s^{-1} cool white fluorescent lighting and a 14:10 h light:dark photoperiod. Cultures were fed every other day, transferred to clean FLAMES medium once a week and covered with Parafilm® to prevent evaporation and contamination. *Daphnia* cultures were kept in 2 L beakers and fed a unialgal diet of *Scenedesmus ad libitum* until 1 month prior to assays with *Bythotrephes*. At this time, half of the cultures were switched to a diet of EPA-enriched *Scenedesmus*. These daphniids are referred to as the "EPA-enriched" daphniid prey, whereas the cultures fed the control *Scenedesmus* are denoted the "control" daphniid prey. We assume that variations in any of the parameters examined for the prey can be attributed to the differences in food quality. *Bosmina* cultures (originating from local softwater lakes) were maintained in 1-L straight-sided borosilicate glass containers and fed EPA-free *Scenedesmus ad libitum* during the study; *Bosmina* acted as a supplemental food source for juvenile *Bythotrephes*, as parthenogenic *Bythotrephes* are difficult to maintain on a mono-prey diet throughout all of their three instar stages (Kim and Yan, 2010).

Length measurements

Daphniids were measured under a dissecting microscope with the aid of the NIS-Elements D 3.0 software (Nikon Instruments, Inc., Melville, USA) by making "squash" preparations (one drop of water on a glass slide with a coverslip on top). For each experimental treatment, *Daphnia* were sub-sampled via pipette from each culture container, collected in a large Petri dish, gently stirred and left to settle for a few minutes. From this pool, specimens were randomly selected until at least 180 barren daphniids had been measured. Daphniids were measured from the top of the head just above the eye to the base of the tailspine.

Bythotrephes culture and assay conditions

The maternal generation of *Bythotrephes* ($n = 143$) bearing parthenogenic offspring used in experiments was collected from Fletcher Lake (45.3°N, 78.8°W) near Dorset, Ontario, Canada on 12 September 2010 as in Kim and Yan (Kim and Yan, 2010). *Bythotrephes* were brought back to the nearby Field Laboratory for the Assessment of Multiple Ecological Stressors (FLAMES), located at the Ontario Ministry of the Environment's Dorset Environmental Science Centre in Dorset. The tubes containing individual third-instar *Bythotrephes* were placed in a growth chamber set at 21°C and left to acclimate overnight. The following day, each *Bythotrephes* was transferred to its own 200-mL capacity glass mason jar filled with 175 mL of FLAMES medium. Control *Daphnia* and unenriched *Bosmina* were offered to the *Bythotrephes* at rates of 30 *Daphnia* day^{-1} and 10 *Bosmina* day^{-1} for 4 days.

On the fourth day following the collection of mothers, 40 F_1 parthenogenically produced *Bythotrephes* offpsring (i.e. <24 h old) were attained, placed in individual jars and immediately transferred to food treatments ($n = 20$ neonates treatment^{-1}). Treatments were the control and EPA-enriched diets, which consisted of 30 mid-size *Daphnia* (control or EPA-enriched) and 10 *Bosmina* (reared on EPA-free *Scenedesmus*) daily. To minimize variability between treatments from maternal effects only two of neonates from the same *Bythotrephes* mother were retained and assigned to different food treatments (control or EPA-enriched daphniid prey). Each day, *Bythotrephes* were visually assessed and survivors were transferred to fresh FLAMES medium with new prey. *Bythotrephes* that were immobile for ≥10 s were classified as dead. After the first 24 h of the predator being incubated with the prey, contents of the jars previously housing *Bythotrephes* were examined thoroughly under a Leica MZ 12(5) dissecting microscope to determine juvenile predation rates for *Bythotrephes* <48 h old on daphniids and bosminids.

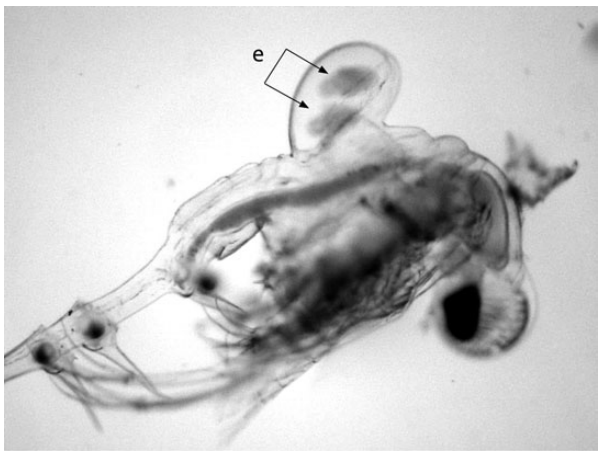

Fig. 1. Laboratory-reared, parthenogenic, 11-day-old *Bythotrephes* female with two non-viable embryos ('e') visible through the broodsac. This particular female had been fed EPA-enriched daphniids.

We terminated this study on the 11th day, as the majority of the *Bythotrephes*' broodsacs began to shrink noticeably and embryos began "resorbing" (as described and illustrated by Rivier, 1998) (Fig. 1). Previous experiments indicate that parthenogenic *Bythotrephes* normally release offspring at 10 or 11 days of age at 21°C, when they are not limited by food quantity or quality (Kim and Yan, 2010). To assess clutch sizes, the broodsacs of mature females were examined using a dissecting microscope and the numbers of distinct embryos being resorbed were counted. The premature termination of the experiment did not affect our ability to test our hypotheses.

Fatty acid analyses and weighing of *Bythotrephes*

Algal, daphniid and *Bythotrephes* tissues were prepared for FA analyses as follows. For every batch of *Scenedesmus* fed to the daphniids, we randomly selected four tubes of algae per treatment to concentrate into a pellet and analyze for FAs. Following the experiment with *Bythotrephes* (experiment duration: 11 days), *Daphnia* (of mixed age and reproductive status) were transferred into clean FLAMES medium without algae for 24 h (based on the observations for *Daphnia magna*; Fuschino, 2010); this depuration time was verified in a preliminary trial to also be sufficient for the gut clearance of *D. ambigua*. Daphniids from three to five culture containers were combined to provide sufficiently large sample masses for lipid analyses. Daphniids were concentrated onto a 113-μm Nitex mesh filter, gently rinsed with FLAMES medium and transferred to 2-mL plastic cryovials ($n = 5$ subsamples treatment^{-1}). *Bythotrephes* were starved for a few hours, rinsed with FLAMES medium and then placed individually into cryovials. All samples were frozen at −85°C until the time of FA analyses.

Prior to extraction, tissue samples were freeze dried and weighed on a microbalance. TLs were extracted from *Scenedesmus*, *Daphnia* and *Bythotrephes* by grinding the samples in 2 mL of chloroform/methanol (2:1) using a homogenizer with a Teflon pestle. TL was measured gravimetrically using a modified version of Folch *et al.*'s (Folch *et al.*, 1957) methods (see Iverson *et al.*, 2001, for modifications). Methylation of lipid samples was performed using a methanol/sulfuric acid methylation protocol from D. Tocher (University of Stirling, Scotland), which was modified from Christie (Christie, 2003). Fatty acid methyl esters (FAMEs) were separated and quantified by Agilent 6890 gas chromatography (GC) with a 100 m × 0.25 mm × 0.2 μm capillary

column (Supelco SP-2560) and flame ionization detector. Helium was used as the carrier gas. The GC oven temperature ramping program went from 70 to 140°C at 20°C min^{-1}, held at 140°C for 5 min, increased to 170°C at 4°C min^{-1}, then to 240°C at 2°C min^{-1} and held again for 10 min. The total run-time was 62 min. Individual FAMEs (reported as proportions of total quantified FAME) were identified by comparison with the Supelco 37-component fatty acid standard (catalog #47885-U).

To analyze the FA profiles of wild *Bythotrephes*, second and third instar *Bythotrephes* with unpigmented broodsacs (Yurista, 1992) were collected from nearby Mary Lake (45.3°N, 79.2°W) in Huntsville, Ontario; a lake that has harbored thriving *Bythotrephes* populations since 1990 (Yan *et al.*, 1992). Collections were made on 8 July 2010 using an 80-μm conical zooplankton net and specimens were pipetted into 50-mL tubes filled with 20-μm filtered lake water. The *Bythotrephes* were then transported to the Canadian Centre for Inland Waters in Burlington, Ontario, Canada where they were freeze dried and analyzed for FA as per the above procedure.

Statistical analyses

Results are expressed as means plus or minus 1 standard error (SE) unless otherwise indicated. One-tailed *t*-tests were used to detect the differences in the daphniid size, along with *Bythotrephes*' weight and clutch sizes between treatments ($\alpha = 0.05$). A two-tailed *t*-test was used to compare the differences in juvenile *Bythotrephes* predation rates among treatments ($\alpha = 0.05$). Predation rate and clutch size data were square-root transformed prior to statistical testing to meet normality assumptions. Because we report FAs extracted from *Scenedesmus*, *Daphnia* and *Bythotrephes* on a proportional basis, we applied the logit transformation (Warton and Hui, 2011) prior to statistical analyses to meet normality assumptions. The treatment means for each FA, except EPA, were assessed using two-tailed *t*-tests. One-tailed *t*-tests were used to compare the EPA content in algae, daphniids and *Bythotrephes*. All statistical analyses were performed in JMP 9 (SAS Institute, Inc., Cary, NC, 1989–2009).

RESULTS

Impacts of EPA on *Daphnia* and *Bythotrephes*

Dietary EPA impacted non-primiparous daphniid size and juvenile *Bythotrephes* feeding rate. *Daphnia* reared on EPA-enriched *Scenedesmus* were significantly larger (0.98 ± 0.012 mm, $n = 192$) than those reared on control *Scenedesmus* (0.94 ± 0.012 mm, $n = 187$; one-tailed $P = 0.008$). The mean predation rate of juvenile *Bythotrephes* raised on EPA-enriched *Daphnia* (12.5 ± 1.53 ind. day^{-1}, $n = 15$ observations) was significantly lower than those consuming control daphniids (21.5 ± 1.09 ind. day^{-1}, $n = 15$ observations; two-tailed $P < 0.001$) over the first 24 h of the experiment. *Bythotrephes* consumed *Bosmina* at equal rates regardless of food treatment (8.2 ± 0.56 and 7.9 ± 0.37 *Bosmina* day^{-1} for the control and EPA-enriched treatments, respectively); thus, any treatment effects may be attributed solely to the difference in daphniid prey quality.

Adult *Bythotrephes* (i.e. 11 day old) reared on EPA-enriched daphniids were significantly heavier than those reared on control daphniids (112.0 ± 3.30 μg vs. 92.3 ± 4.50 μg as dry mass; $n = 2$ samples of seven to eight pooled *Bythotrephes* per treatment; one-tailed $P = 0.001$; see also Fig. 2). *Bythotrephes* mortality by the

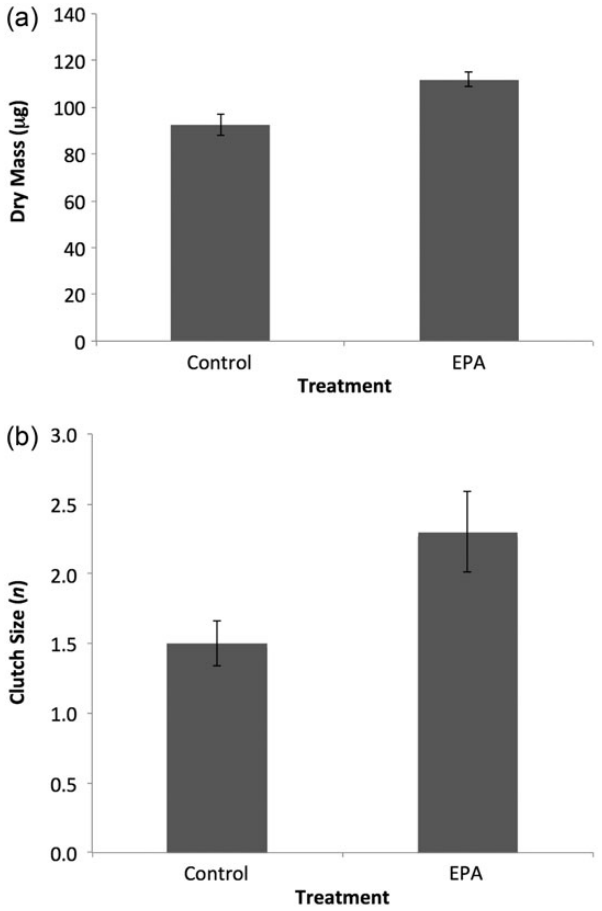

Fig. 2. (**a**) Mean dry masses and (**b**) clutch sizes of laboratory-reared *Bythotrephes* fed control and EPA-enriched *Daphnia*. Means of dry mass were calculated from two replicates of 7–8 *Bythotrephes*. Mean clutch sizes were calculated from $n = 11$ *Bythotrephes* (control treatment) and $n = 9$ *Bythotrephes* (EPA treatment). Error bars represent SE.

11th day was 4 out of 20 animals in the control treatment and 5 out of 20 animals in the EPA treatment.

Finally, adult *Bythotrephes* reared on the EPA-enriched diet had 53% more embryos in their broodsacs (2.3 ± 0.29, $n = 9$) than those maintained on the control diet (1.5 ± 0.16, $n = 11$; one-tailed $P = 0.008$).

Fatty acid patterns in Scenedesmus, Daphnia, and Bythotrephes

As anticipated, there are higher proportions of EPA in the EPA-enriched *Scenedesmus* relative to control *Scenedesmus* (12% compared with non-detectable levels; Table I), indicating our procedures were successful. The PUFA 18:3n-3 (α-linolenic acid, ALA) was the most abundant FA in all *Scenedesmus* samples, regardless of enrichment status.

There was a significantly higher proportion of EPA in *Daphnia* fed the EPA-enriched *Scenedesmus* than in daphniids fed control *Scenedesmus* (6.1 vs. 3.0% EPA as FA; one-tailed $P < 0.0001$; Table II). *Daphnia* demonstrated a limited capacity to synthesize EPA, as those fed EPA-free algae were found to contain EPA.

In all laboratory-reared *Bythotrephes*, the most abundant FA was the saturated fatty acid (SAFA) 16:0 (20.8% as FA regardless of treatment), whereas in wild-caught *Bythotrephes* from Mary Lake and Swedish lakes, the most abundant FA was EPA (23.3 and 23.0%, respectively; Table III). However, there was a significantly higher relative proportion of EPA measured in the *Bythotrephes* fed the EPA-enriched daphniids (7.6% EPA as FA) compared with those fed control daphniids (4.8% EPA as FA; one-tailed $P = 0.031$, $n = 2$ samples of seven to eight pooled animals per treatment). Additionally, there were no significant effects of diet on the major FA groups of laboratory-reared *Bythotrephes*. Wild-caught *Bythotrephes* had overall higher relative proportions of EPA as FA, higher $\sum n\text{-}3{:}\sum n\text{-}6$, less $\sum$SAFA and more $\sum$MUFA than laboratory-reared *Bythotrephes*. Wild-caught Swedish *Bythotrephes* had the highest $\sum n\text{-}3{:}\sum n\text{-}6$ at 2.6, followed by Mary Lake *Bythotrephes* at 1.6, and then all laboratory-reared *Bythotrephes* at 1.2.

Table I: Mean proportions (as % of total FAs) of selected FAs in Scenedesmus *without and with EPA addition at 21°C ± SD (n.d. = not detected).*

Fatty acid	Common name	Control (%)	EPA enriched (%)
16:0	Palmitic acid	17.4 ± 1.65*	14.8 ± 1.13*
16:1*n*-7	Palmitoleic acid	1.0 ± 0.21	0.9 ± 0.27
18:0	Stearic acid	1.9 ± 0.60	1.9 ± 0.73
18:1*n*-9	Oleic acid	7.5 ± 0.97	6.7 ± 0.84
18:1*n*-7	(No common name)	4.4 ± 0.88	4.2 ± 0.36
18:2*n*-6 (LIN)	Linoleic acid	8.2 ± 1.07	7.2 ± 0.93
18:3*n*-6 (GLA)	γ-Linolenic acid	0.8 ± 0.13	0.7 ± 0.18
18:3*n*-3 (ALA)	α-Linolenic acid	45.3 ± 3.34	39.4 ± 4.82
18:4*n*-3 (SDA)	Stearidonic acid	7.3 ± 0.26	6.3 ± 0.67
24:0	Lignoceric acid	1.1 ± 0.37	1.0 ± 0.23
20:5*n*-3 (EPA)	Eicosapentaenoic acid	n.d.	11.9 ± 4.09

There were five sample replicates for each treatment.
*Indicates significantly different treatment means ($\alpha = 0.05$).

DISCUSSION

Adult *Bythotrephes* reared on EPA-enriched daphniid prey were heavier than those receiving less EPA in their diet, indicating that dietary EPA content affects *Bythotrephes*' growth, which in turn may impact fecundity. Higher EPA availability also conferred benefits to *Daphnia* in terms of increased body length. Von Elert (Von Elert, 2002) demonstrated improved growth of juvenile *Daphnia galeata* when reared on EPA-enriched *Scenedesmus* (as well as *Scenedesmus* supplemented with either ALA or DHA; *D. galeata* appears to have at least some ability to convert these FAs, as well as C_{16}-PUFAs to EPA). The *D. ambigua* in our study also demonstrated the ability to synthesize EPA from precursor FAs, as control daphniids had 3% EPA as TL.

Because larger daphniids are known to be faster swimmers (Dodson and Ramcharan, 1991), increases in the daphniid size and swimming speed most likely account

Table II: Mean proportions (as % of total FAs) of selected FAs in Daphnia *fed the chlorophyte* Scenedesmus *treated without and with EPA at 21°C ± SD (n.d. = not detected).*

Fatty acid	Common name	Algal diet	
		Control (%)	EPA enriched (%)
14:0	Myristic acid	1.9 ± 0.44	1.9 ± 0.19
16:0	Palmitic acid	15.8 ± 0.43	15.9 ± 0.26
16:1*n*-7	Palmitoleic acid	2.7 ± 0.12*	2.5 ± 0.11*
17:0	Margaric acid	1.0 ± 0.08*	0.9 ± 0.03*
18:0	Stearic acid	7.3 ± 0.36	7.1 ± 0.14
18:1*n*-9	Oleic acid	12.5 ± 0.79	12.3 ± 0.35
18:1*n*-7	(No common name)	8.6 ± 0.30*	9.3 ± 0.18*
18:2*n*-6 (LIN)	Linoleic acid	10.6 ± 0.65*	8.5 ± 0.20*
18:3*n*-6 (GLA)	γ-Linolenic acid	1.3 ± 0.19*	1.0 ± 0.11*
18:3*n*-3 (ALA)	α-Linolenic acid	17.1 ± 0.63*	18.9 ± 0.45*
18:4*n*-3 (SDA)	Stearidonic acid	3.2 ± 0.39	2.7 ± 0.36
20:4*n*-6 (ARA)	Arachidonic acid	9.5 ± 0.61*	6.7 ± 0.39*
20:5*n*-3 (EPA)	Eicosapentaenoic acid	3.0 ± 0.19*	6.1 ± 0.31*
22:5*n*-3 (DPA)	*n*-3-Docosapentaenoic acid	1.3 ± 1.29	1.0 ± 0.39

For each treatment, $n = 5$.
*Indicates significantly different treatment means ($\alpha = 0.05$).

Table III: Mean proportions of selected FAs and FA functional groups (as % of FAs) in Bythotrephes *reared without and with EPA addition at 21°C, ± 1 SD (n.d. = not detected)*

Fatty acid	Lab-reared *Bythotrephes*: control diet (%)	Lab-reared *Bythotrephes*: EPA-enriched diet (%)	Wild-caught *Bythotrephes*: Mary Lake (%)[a]	Wild-caught *Bythotrephes*: Swedish lakes, $n = 4$ lakes(%)[a,b]
14:0	2.7 ± 0.53	3.2 ± 1.18	2.4 ± 0.41	4.4 ± 0.7
16:0	20.8 ± 2.05	20.8 ± 1.01	17.0 ± 1.78	19.1 ± 1.5
16:1*n*-7	1.9 ± 0.03	2.4 ± 0.41	1.4 ± 0.09	2.9 ± 0.9
17:0	1.0 ± 0.16	0.9 ± 0.08	1.7 ± 0.16	0.9 ± 0.1
18:0	10.8 ± 1.28	9.7 ± 0.59	8.3 ± 2.37	4.7 ± 0.7
18:1*n*-9t	0.2 ± 0.28	0.4 ± 0.07	1.2 ± 0.59	(Included with FA below)
18:1*n*-9c	10.2 ± 0.57	11.1 ± 0.04	10.6 ± 1.36	10.3 ± 1.4
18:1*n*-7	5.1 ± 0.35	5.7 ± 0.24	6.3 ± 0.62	6.0 ± 0.5
18:2*n*-6 (LIN)	6.5 ± 0.69	6.4 ± 0.42	3.0 ± 0.44	3.6 ± 0.6
18:3*n*-3 (ALA)	10.4 ± 0.85	10.3 ± 0.62	3.4 ± 0.54	5.3 ± 1.7
18:4*n*-3	1.7 ± 0.10	1.7 ± 0.16	1.7 ± 0.36	4.1 ± 1.5
20:4*n*-6 (ARA)	11.5 ± 1.14	10.4 ± 0.89	13.0 ± 1.98	9.3 ± 1.4
20:5*n*-3 (EPA)	4.8 ± 0.60*	7.6 ± 0.38*	23.3 ± 3.04	23.0 ± 1.4
22:5*n*-3 (DPA)	9.0 ± 9.39	5.4 ± 4.37	1.1 ± 0.40	n.d.
22:6*n*-3 (DHA)	n.d.	0.3 ± 0.45	1.5 ± 0.32	2.1 ± 1.2
∑*n*-3 (%)	25.7 ± 0.81	25.2 ± 0.20	26.3 ± 0.66	34.4 ± 2.0
∑*n*-6 (%)	18.6 ± 0.06	17.5 ± 0.04	17.0 ± 0.53	13.5 ± 1.0
∑*n*-3:∑*n*-6	1.2 ± 0.27	1.2 ± 0.16	1.6 ± 0.04	2.6 ± 0.3
∑SAFA	37.2 ± 0.21	36.6 ± 0.09	26.3 ± 0.60	30.8 ± 1.8
∑MUFA	18.3 ± 0.04	20.7 ± 0.01	30.4 ± 0.61	19.2 ± 1.1
∑PUFA	44.5 ± 0.36	42.8 ± 0.06	43.4 ± 1.03	47.9 ± 1.0

[a]Wild-caught *Bythotrephes* were excluded from statistical analyses.

[b]Data from Persson and Vrede (Persson and Vrede, 2006); percentages of individual FAs do not sum exactly 100% because there was ~2% unidentified, and traces (<1%) of FAs are not included here.

*Indicate significantly different treatment means ($\alpha = 0.05$). Also shown are mean proportions of FAs in *Bythotrephes* from Mary Lake, Huntsville, Ontario, Canada. There were two sample replicates of lab-reared *Bythotrephes* consisting of seven to eight individuals each, and five sample replicates of Mary Lake-caught *Bythotrephes*, consisting of 10–19 individuals each. Also included for reference are data from Persson and Vrede (Persson and Vrede, 2006) for *Bythotrephes* collected from four oligotrophic lakes in northwestern Sweden ($n = 4$).

for juvenile *Bythotrephes*' decreased predation rates on the EPA-enriched daphniids. Larger daphniids probably escaped more frequently from the predator, as *Daphnia magna* do from *Triops cancriformis* Bosc (Rabus and Laforsch, 2011). Moreover, the effects of EPA supplementation on *Bythotrephes* observed in our study may have been more pronounced if EPA-enriched *Daphnia* had been ingested at similar rates with those of the control *Bythotrephes*. Thus, a diet that includes abundant and easily caught prey may be critical for juvenile *Bythotrephes*' predation success. Provided that *Bythotrephes* is well fed early on, it is plausible that its ability to capture and consume larger and faster EPA-rich prey increases as it nears reproduction.

The finding that EPA limitation depresses *Bythotrephes* parthenogenic clutch sizes is important, given that lower clutch sizes can suppress population growth rates. For *Bythotrephes* reared in the laboratory with a more varied diet that included *Artemia* nauplii (~50 nauplii day^{-1}), clutch sizes averaged 2.2 offspring ($n = 6$; Kim *et al.*, 2012). In this experiment, the mean clutch size of EPA-enriched *Bythotrephes* was 2.3 offspring ($n = 9$; on par with previous results), whereas that of the control *Bythotrephes* (1.5 offspring, $n = 11$) was closer to what was observed when *Bythotrephes* lacked sufficient food in a previous study (1.7 offspring; $n = 3$; Kim and Yan, 2013). A clutch size of 2 offspring is typical for *Bythotrephes* in our study region in late summer (Young, 2008). Although *Artemia* nauplii are not particularly EPA rich, they likely provided a vital food source for *Bythotrephes* that went on to release multiple broods in previous studies (Kim *et al.*, 2012; Kim and Yan 2013). In addition, energy expenditure of *Bythotrephes* in the present study was probably greater than that of *Bythotrephes* in our past studies, due to the absence of slow-moving *Artemia* nauplii as back-up prey (i.e. if daphniids evaded capture). In this respect, both food quantity and quality may impact *Bythotrephes* growth, fecundity, and in turn, population growth in the field.

Despite the observed increase in the clutch size with EPA supplementation (i.e. *Daphnia* with 6.1% EPA), the proportions of EPA as TL in our laboratory raised *Bythotrephes* were much lower than that of wild-caught *Bythotrephes*. We did not develop a range of EPA concentrations with which to specifically discover the true threshold for EPA contents sufficient to allow *Bythotrephes* to reproduce, but we suggest that EPA availability, at the level we managed to achieve (i.e. *Bythotrephes* with 7.6% EPA), may be one explanation for *Bythotrephes*' consistent loss of parthenogenic embryos. Rivier (Rivier, 1998)

describes the phenomenon of embryo loss and possible "resorption" for *Bythotrephes* populations in the Rybinsk Reservoir in Russia. Late summer embryo loss was observed in 36% of all fecund females, with a mean inferred clutch size of 3 (Rivier, 1998). Approximately, half of the embryos were resorbed in specimens mentioned by Rivier (Rivier, 1998), with loss and resorption taking place at the stage of cephalic segment delimitation and the beginning of swimming antennae development. The reasons for resorption and loss were unclear but Rivier (Rivier, 1998) suggested that eutrophication and chemical contamination might be two explanations.

An alternative explanation is that all *Bythotrephes* were infected by brood parasites that prevented the further development of embryos. This is a phenomenon that is common and well documented in *Daphnia*. It has been observed that daphniid mothers cannot produce viable offspring from infected clutches, although the majority of them go on to release the infected brood during molting and produce healthy offspring (Tellenbach *et al.*, 2007). Further investigation of whether brood parasite infection is also a frequent occurrence in *Bythotrephes* is warranted.

Bythotrephes' population growth and establishment success will likely be enhanced in lakes with EPA-retentive prey that are easily caught. *Bythotrephes* depletes lipids to promote rapid juvenile and young adult growth and later allocates lipids to its developing embryos, as *Bythotrephes* embryos are nourished entirely from their mother's hemolymph and grow in broodsacs devoid of yolk (after Yurista, 1992; Bilkovic and Lehman, 1997). Past work with *Bythotrephes* in the laboratory revealed that *Artemia* nauplii are preferentially selected (Kim and Yan, 2010). *Artemia* nauplii contain some EPA (~2%) and high amounts of ALA (~27%) in TL (Furuita *et al.*, 1996). *Artemia* nauplii are clearly not a naturally encountered prey of *Bythotrephes*, but other small and easily caught prey, such as copepod nauplii (which *Bythotrephes* readily consumes; Vanderploeg *et al.*, 1993) and bosminids, are present *in situ* and may be particularly good food sources when they are able to graze on EPA-rich phytoplankton. Freshwater copepods are known to contain high amounts of LC-PUFAs, with high *n*-3:*n*-6 ratios ranging from 4.2 to 5.2 (Brett *et al.*, 2009, and references therein). *Bythotrephes* are also thought to feed directly on phytoplankton such as the dinoflagellate *Peridinium* and diatoms (both of which contain high levels of EPA as well as DHA; Ahlgren *et al.*, 1990; Ahlgren *et al.*, 1992; Grigorovich *et al.*, 1998). While it is widely considered an obligate carnivore, *Bythotrephes* may consume small amounts of phytoplankton directly and indirectly via the gut contents of its prey.

Bythotrephes' population persistence, and hence, establishment success, should thus be affected by EPA availability. Owing to the sexually reproductive phase in its life cycle, *Bythotrephes* must maintain sufficient numbers for populations to persist (Wittmann *et al.*, 2011). *Bythotrephes* relies on the establishment of a viable resting egg bank for inter-annual persistence. Brown and Branstrator (Brown and Branstrator, 2011) found that heavier (and presumably resource-rich) *Bythotrephes* mothers were able to manufacture resting eggs that had an increased likelihood of hatching the next spring. For *Daphnia pulicaria*-fed *Scenedesmus* (without and with EPA additions) as well as EPA-rich *Cryptomonas*, Abrusan *et al.* (Abrusan *et al.*, 2007) found that resting egg production was highly positively influenced by EPA availability, and that mothers invested much more EPA into resting than subitaneous eggs. Sperfeld and Wacker (Sperfeld and Wacker, 2012) also observed a greater investment of PUFAs in eggs relative to somatic tissue, and found increased hatching success of *D. magna* eggs when the diet of mothers were supplemented with EPA compared with when they were not. Our results suggest that this may also be true for *Bythotrephes*.

EPA is clearly important for cladocerans, but this biochemical may become increasingly limiting in the future due to climate warming. Warming waters can cause changes in phytoplankton community composition and affect algal *n*-3 PUFA contents. Of the freshwater phytoplankton groups, diatoms, dinoflagellates and cryptophytes typically contain the highest amounts of EPA, whereas chlorophytes (including *Scenedesmus*) and cyanobacteria produce little to no EPA (Ahlgren *et al.*, 1990; Ahlgren *et al.*, 1992; Gulati and DeMott, 2003). Since increases in water temperature promote cyanobacterial growth (Kosten *et al.*, 2012), less EPA would be available to *Bythotrephes* and other zooplankton. As *Bythotrephes* is highly sensitive to temperatures approaching above 25°C (Kim and Yan, 2010), warm waters dominated by cyanobacteria may present unfavorable conditions for *Bythotrephes* population growth and establishment. Moreover, Fuschino *et al.* (Fuschino *et al.*, 2011) showed that increased temperature decreases the amount of ALA (precursor of EPA) in *Scenedesmus*. It has been repeatedly observed that *Bythotrephes* abundances decrease or that populations disappear altogether in European lakes that become eutrophic (reviewed in Therriault *et al.*, 2002; see references therein). Although other factors likely contribute (e.g. planktivory; Jeppesen *et al.*, 1996), we propose that EPA limitation due to changes in phytoplankton communities may play a role. This may partly explain why *Bythotrephes* tends to be absent from very productive systems in its native Norwegian range, unlike *L. kindtii* (Hessen *et al.*, 2011), which appears to have much lower EPA requirements (Bychek and Gushina, 2001).

In conclusion, an EPA-rich diet will likely enhance the fitness, and thus the damaging impacts, of *Bythotrephes*.

Conversely, an EPA-poor diet may hinder *Bythotrephes* establishment. More work is needed to identify the critical EPA thresholds in the diet of *Bythotrephes* that might limit or promote its establishment and spread, and on how a changing climate might alter these biochemical dynamics.

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