Journal of Great Lakes Research 38 (2012) 561–568

Contents lists available at SciVerse ScienceDirect

Journal of Great Lakes Research

journal homepage: www.elsevier.com/locate/jglr

Lipid content of *Mysis diluviana* in the offshore region of southeastern Lake Michigan in 2009–2010

Steven A. Pothoven [a,*], David L. Fanslow [b,1], Gary L. Fahnenstiel [a,2]

[a] National Oceanic and Atmospheric Administration, Great Lakes Environmental Research Laboratory, 1431 Beach Street, Muskegon, MI 49441, USA
[b] National Oceanic and Atmospheric Administration, Great Lakes Environmental Research Laboratory, 4840 S. State Rd., Ann Arbor, MI 48108, USA

ARTICLE INFO

Article history:
Received 28 February 2012
Accepted 10 May 2012
Available online 13 June 2012

Communicated by Michael Sierszen

Keywords:
Great Lakes
Condition indices
Mysid
Food web

ABSTRACT

The lipid content of *Mysis diluviana* in the offshore region of southeastern Lake Michigan was determined in 2009 and 2010. Total lipids (mg) increased with *Mysis* length, but percent lipids (% dry weight) did not. Increases in lipids for juveniles (<10 mm) were related to increases in structural lipids (i.e., phospholipids and sterols), whereas increases in total lipids for adults were related to increases in storage lipids (i.e., triacylglycerols). Percent lipids varied seasonally, and were in general, highest in spring or late fall for juveniles and small adults (<15 mm). For the largest adults (16+ mm), % lipid content did not vary much over the year in 2009, but in 2010, % lipids peaked in July. The rate of lipid retention differed among cohorts of juveniles, but it did not differ among cohorts of adults. In 2009 and 2010 respectively, 14% and 40% of *Mysis* >13 mm had lipid content <14%, a value associated with a high percentage of docosahexaenoic acid that may reflect starvation for this size of *Mysis*. Food availability (chl *a* levels or zooplankton biomass) was not correlated with *Mysis* lipid content for any size group of *Mysis*. Seasonal gradients in food availability might not be large enough to elicit strong responses in lipid content of *Mysis*.
Published by Elsevier B.V. on behalf of International Association for Great Lakes Research.

Introduction

The opossum shrimp, *Mysis diluviana* (formerly *M. relicta*, see Audzijonyte and Väinolä, 2005; hereafter *Mysis*) inhabits freshwater lakes of the once-glaciated regions of North America, including the Laurentian Great Lakes. *Mysis* play a key role in the food web linking primary production and fish production, as well as benthic and pelagic food webs in the deep regions of these lakes, including Lake Michigan (Bowers and Grossnickle, 1978; Wells, 1980).

Mysis have long been an important component of fish diets in the Great Lakes (Anderson and Smith, 1971; Wells, 1980). However, recent declines of another glacial relict, the amphipod *Diporeia*, have shifted additional predation pressure onto *Mysis* (Pothoven and Madenjian, 2008; Pothoven et al., 2011; Walsh et al., 2008). Furthermore, this increased fish predation has occurred at a time when lake productivity has been declining, a change attributed to filtering by the invasive quagga mussel *Dreissena rostriformis bugensis* in Lake Michigan (Fahnenstiel et al., 2010). In turn, with their intermediate position in the food web and both top down and bottom up constraints increasing, *Mysis* numbers have declined by as much as 70% in the offshore region since the 1990s in Lakes Michigan (Pothoven et al., 2010) and biomass has declined by up to 45% in Lake Ontario (Johannsson et al., 2011).

Lipid content can provide insight into the ecology and health of an organism. Many factors may contribute to variation in lipid content, including availability of food resources, season, life stage, and reproductive state (Arts et al., 1992; Cavaletto and Gardner, 1999; Gardner et al., 1985). Lipid content provides information on the feeding ecology of organisms, especially organisms such as *Mysis* that live in lakes with seasonal variation in food supply (Gardner et al., 1985). For example, increased lipids in the marine *Mysis mixta* were linked with the appearance of the spring phytoplankton bloom (Richoux et al., 2004a). Monitoring lipids also provides a means to understand how changes in trophic state may affect an organism. For example, recent work has suggested that adult *Mysis* in Lake Huron may be starving based on lipid content (Mida Hinderer et al., 2012).

Despite the importance of *Mysis* in the food web, little work has been done to examine seasonal or size specific trends of their lipid content in the Laurentian Great Lakes. We hypothesized that *Mysis* lipid content and composition in Lake Michigan would vary ontogenetically as mysids grew and matured. We also hypothesized that *Mysis* lipid content, lipid composition, and lipid retention rates would vary seasonally in response to seasonal changes in food resources that are characteristic of Lake Michigan (Bowers and Grossnickle, 1978; Branstrator et al., 2000; Gardner et al., 1985). Finally, we compared *Mysis* lipid content to laboratory derived measures to evaluate whether adult *Mysis* might be experiencing starvation conditions in the lake (Adare and Lasenby, 1994; Schlechtriem et al., 2008).

* Corresponding author. Tel.: +1 231 759 9035.
E-mail addresses: steve.pothoven@noaa.gov (S.A. Pothoven), david.fanslow@noaa.gov (D.L. Fanslow), gary.fahnenstiel@noaa.gov (G.L. Fahnenstiel).
[1] Tel.: +1 734 741 2353.
[2] Tel.: +1 231 759 7824.

0380-1330/$ – see front matter. Published by Elsevier B.V. on behalf of International Association for Great Lakes Research.
doi:10.1016/j.jglr.2012.05.003

Methods

Mysis were collected from southeastern Lake Michigan at a 110-m station (43°11.99'N, 086°34.19'W) during April–November 2009 and March–December 2010. Collections were scheduled monthly but some sample periods were missed because of adverse weather or logistical constraints. Samples were collected with a 1-m diameter plankton net (1000-μm mesh; 1:3 mouth-to-length ratio) towed vertically from 1 to 3 m above bottom to the surface at speeds of approximately 0.5 $m \cdot s^{-1}$. Mysids were rinsed into a bottle and held at 4 °C during transport to shore. Additional replicate samples of *Mysis* were collected for size frequency and abundance determinations. These mysids were anesthetized with carbonated water and preserved with sugar-buffered formalin to form a final 3% solution. All samples were taken at least 1 h after sunset or 1 h prior to sunrise.

To collect data on the availability of potential prey, we used data collected as part of a long-term lower food web study at the same site as *Mysis* were collected (see Fahnenstiel et al., 2010). A Seabird Conductivity–Temperature–Depth (CTD) profiler with an attached fluorometer was cast at the site to determine water temperature profiles. Discrete water samples were collected with a Niskin bottle to determine chlorophyll *a* (chl *a*) levels as a proxy for phytoplankton biomass (see Fahnenstiel et al., 2010). Samples of zooplankton were collected with replicate whole water column tows with a 153-μm mesh net and preserved with sugar-buffered formalin.

Water for chl *a* analysis was filtered onto Whatman GF/F filters, extracted with N, N-dimethylformamide (Speziale et al., 1984) and analyzed fluorometrically. Chlorophyll *a* is reported as the mean water column value (isothermal period) or the mean zone weighted value for the meta- and hypolimnion combined (stratified period). This region was determined using temperature profiles and was chosen to represent available food in the stratified period because *Mysis* would generally not be found in the warm epilimnion (Boscarino et al., 2007). We also determined the deep chlorophyll layer (DCL) defined as the subsurface region where chl *a* concentrations were $>2\ mg \cdot m^{-3}$ (Fahnenstiel et al., 2010). Regressions were developed between extracted chl *a* at a given depth and fluorometric values at the same depth to determine the size of the DCL. These regressions were used to convert fluorescence values to chl *a*, and integral chl *a* concentration in the DCL was then determined using an image analysis system.

To determine zooplankton biomass, an aliquot was taken from a known sample volume with a Hensen–Stempel pipette so that a minimum of 600 zooplankters were counted and identified to species for each sample. To determine zooplankton biomass, length measurements were made on up to 25 individuals of taxa that represented at least 8% of the sample density using Image Pro Plus analysis software (Media Cybernetics, Silver Spring, MD, U.S.A.) and biomass was determined using published weight–length regressions (Culver et al., 1985). For zooplankton taxa that were <8% of the sample, a mean default weight was used (Hawkins and Evans, 1979).

Mysis for lipid determinations were sorted and measured within 8 h of collection. In the laboratory all mysids were counted, and body length, sex, and eggs-per-female were recorded. Body length was measured from the tip of rostrum to the base of the telson. To track cohorts, length frequency distribution plots were constructed by combining all replicates for each respective date. The lipid content from the most populated size classes of a cohort (mode and two adjacent size classes) was used as a measure of lipid content for that cohort. Not all individuals could be placed into a cohort. Sex was only determined for animals longer than 10 mm because sex for smaller individuals was undistinguishable. The brood size for females larger than 10 mm was determined as the number of eggs or embryos. *Mysis* for lipid determinations were then placed in vials and held at −80 °C. Lipid content of broods (eggs or embryos) was analyzed separately from *Mysis*.

For lipid determinations, whole *Mysis* were freeze-dried and weighed (nearest 0.001 mg). Total lipids (TL) were determined by the Phosphovanillin Colorimetric method (Van Handel, 1985) on whole body homogenates extracted in 2:1 chloroform:methanol and subjected to a Folch purification (Folch et al., 1957; Lu et al., 2008). Following extraction, a duplicate lipid extract sample was removed for use in lipid class analysis (2010 only) and sealed under nitrogen in a 100 μl capillary pipette. The lipid classes for 2010 *Mysis* were determined by thin layer chromatography with Iatroscan Flame ionization analysis (TLC–FID) with all samples run in triplicate (Lu et al., 2008; Parrish, 1986, 1987). This procedure allowed for the separation of five lipid class peaks (i.e., triacylglycerides (TAG), free fatty acids (FFA), alcohols (AL), sterols (ST), and phospholipids (PL)). Individual lipid classes were measured with an Iatroscan Mark IV (Iatron Laboratories, Tokyo, Japan) connected to a windows-based computer running Peak Simple Chromatography software (Shell USA, Fredericksburg, Virginia).

Total lipids were determined as total lipid weight (TL mg) and as percent of total *Mysis* dry weight (%DW). Lipid classes were reported as a percentage of the total lipids. In general, lipid determinations were made on individual *Mysis* with a couple exceptions. First, for smaller *Mysis* (<6 mm), several *Mysis* were combined to provide a large enough sample for determinations. Second, in 2009, a number of "composite" samples of *Mysis* from 5-mm size groups (i.e., 0–5 mm, 6–10 mm, etc.) were used (27% of samples run) in addition to individual *Mysis*. When multiple individuals were used for determinations, the total lipid in a sample was divided by the number of individuals to determine individual TL mg for the composite sample except in a few cases, when the number of individuals was not known and only %DW was determined. The average length of individuals in a composite was used in analyses requiring *Mysis* length.

To examine seasonal lipid trends for a given size of *Mysis*, we separated *Mysis* into four size groups (i.e., 0–5 mm, 6-10 mm, 11–15 mm, 16+ mm). Percent lipids were compared across dates for each size groups using ANOVA after transforming proportion lipid content (arcsin of the square root), and a Tukey's HSD test was used to examine pair-wise differences among dates for each size group. To examine retention rates of lipids, lipid content (TL mg) was plotted as a function of day of year (DOY) and slopes of regression lines were examined for each cohort. We standardized the start date for each cohort to zero and used a general linear model to determine if there was a significant interaction between day and cohort to determine if lipid retention rates differed among cohorts of juveniles or adults, respectively. We examined the proportion of *Mysis* that could be classified as starving based on %DW values reported from previous laboratory studies (Adare and Lasenby, 1994; Schlechtriem et al., 2008). Finally, we examined Pearson correlations between lipid content (%DW) of each size group of *Mysis* and food resources (i.e., chl *a* or zooplankton biomass).

Results

Overall abundance of *Mysis* was similar between years, averaging $106 \cdot m^{-2}$ in 2009 and $107 \cdot m^{-2}$ in 2010 across all sampling dates. Six cohorts of individuals could be followed to some degree. These included cohort 1, a 2008 cohort that were adults in 2009. Cohorts 2 and 3 could be followed from hatching in spring or late summer 2009, respectively, into 2010 as adults. Cohorts 4, 5, and 6 could be followed during 2010 as juveniles from their appearance in spring, early summer, or late summer, respectively. In 2009, the highest percentages of females with broods were found in April–June in 2009 (16–29%), with few females with broods thereafter (<9%); in 2010 a relatively high percentage of females had broods in March (21%) as well as October–December (8–18%), but <6% of females had broods May–September.

Total lipids (%DW) for *Mysis* ranged from 7.5 to 43.8% (mean = 16.60 ± 0.32 (SE)) for juveniles and 6.5–43.3% (mean = 16.95 ± 0.31)

for adults at our study site in 2009–2010. Total lipids (mg) increased with *Mysis* length for both years, but TL (%DW) did not follow the same pattern, especially in 2010 (Table 1). We did not find a difference in TL (mg) for females with and without broods for a given size group (paired *t*-test, $p>0.12$), or between females and males for a given size group (paired *t*-test, $p>0.20$), so in general, all adults were combined for subsequent analyses.

For 2009, total lipids (%DW) differed among dates ($p<0.001$) except for the largest size class (≥16 mm). The temporal differences were largely due to relatively high lipid content in the early spring (April) for the two juvenile size groups (<11 mm) and small adults (11–15 mm) (Fig. 1). For 2010, total lipids (%DW) differed among dates for all size groups ($p<0.02$). Temporal patterns however, differed somewhat from those in 2009. For the two juvenile size groups, lipid content was relatively high in spring (May or June), but also in December, which was not sampled in 2009 (Fig. 1). Lipid content for small adults (11–15 mm) was low in March and high in December. For larger adults (≥16 mm), % lipids were highest in July (Fig. 1).

In 2009, 14% of *Mysis* ≥13 mm had lipid content (%DW) <14%, a value associated with a high percentage of docosahexaenoic acid that may reflect starvation for this size of *Mysis* (Schlechtriem et al., 2008). The largest percentage of *Mysis* with lipids <14% in 2009 was in early spring and mid-summer. In 2010, 40% of *Mysis* ≥13 mm had lipid content <14%. The highest percentage of *Mysis* with lipids<14% in 2010 was in March (71%), and values ranged from 17 to 58% over the rest of the year. However when comparing lipid content to the 7% (% LFDW basis) starvation threshold determined by Adare and Lasenby (1994), we found that after converting our lipid content to a LFDW % basis, no adult *Mysis*≥13 mm fell below a 7% threshold in 2009 and only 1% of *Mysis* in 2010.

To examine the rate of lipid retention, lipid data from December 2010 were excluded because of an unusually high rate of retention between November and December. There was a significant interaction between day (standardized to start at zero) and juvenile *Mysis* cohort ($p<0.001$). The rate of lipid retention for juveniles ranged from 0.00047 $mg \cdot d^{-1}$ (cohort 3) to 0.0009 $mg \cdot d^{-1}$ (cohort 6) (Fig. 2), but there was no consistent trend in slopes between early or late cohorts. Lipid content (%DW) for juvenile cohorts was generally highest at their first appearance, then decreased to relatively low levels (<15%), before increasing in the fall (Fig. 2). This trend was more apparent for cohorts that appeared in spring or early summer (i.e., cohorts 2, 4, and 5) than for cohorts that appeared later in the year (i.e., cohorts 3 and 6). The rate of non-lipid weight accumulation did not differ among cohorts and ranged from 0.00280 $mg \cdot d^{-1}$ (cohort 6) to 0.00561 $mg \cdot d^{-1}$ (cohort 5).

There was no significant interaction between day (standardized to start at zero) and adult *Mysis* cohort ($p=0.60$) (Fig. 2). The rate of lipid retention ($mg \cdot d^{-1}$) did not differ between adult male and female *Mysis* for any cohort. For cohort 2, analyses were restricted to 2010 to allow for consistent comparison with the time frame observed for cohort 1 and so that overwinter changes in lipid content did not affect analysis. The temporal pattern of total lipids (%DW) for each cohort of adult *Mysis* appeared to differ among adult cohorts (Fig. 2). The rate of non-lipid weight accumulation did not differ among adult cohorts, ranging from 0.01837 $mg \cdot d^{-1}$ (cohort 1) to 0.02907 $mg \cdot d^{-1}$ (cohort 2).

Cohorts 2 and 3 could be tracked over winter from November 2009 to March 2010. On average, individuals from both cohorts grew in length overwinter (0.02 $mm \cdot d^{-1}$) and accumulated lipids overwinter (0.00078 $mg \cdot d^{-1}$). On the other hand, lipid content (%DW) decreased overwinter for both cohorts (33–44% decrease). Both cohorts had considerably less total lipids (mg and %DW) in spring 2010 than cohort 1 had in the spring 2009. Storage lipids (TAG) were quite low in both cohort 2 (8% of TL) and cohort 3 (3% of TL) in March 2010.

Total lipids (%DW) of broods for 2009–2010 combined ranged from 26 to 48% and averaged 34 ± 2% (n = 12 composites). Based on 2010 data, lipid composition of broods was mainly TAG (71%) and PL (25%).

The amount of TAG (mg) and FFA increased with *Mysis* size once they reached about 10 mm, but the amounts of TAG or FFA/mysid were quite variable even for the largest individuals (Fig. 3). Further, much of the variation in FFA/mysid was related to relatively high values observed for a given sized *Mysis* in December (DOY 337). Despite this relatively high amount of FFA in December, we did not believe it represented samples which had experienced oxidation, because amounts of all lipid classes increased during December. The two other major lipid classes, ST and PL began increasing as soon as *Mysis* emerged and were less variable than TAG and FFA. For ST, as with FFA, much of the variability was related to relatively high values for a given sized individual in December. Alcohols were a minor component (<1%) and not analyzed further.

Overall, the importance of TAG (as a % of TL) increased with *Mysis* size, and the importance of ST and PL decreased with size (Table 2). For juvenile size groups, %TAG was generally low (<5%) over the year, with a couple exceptions (Fig. 4). The %ST and %PL were pretty consistent over the year. For small adults (11–15 mm), %TAG was highest in spring and early summer, before decreasing in late summer, and then increasing again in late fall (Fig. 4). Values for %TAG ranged from 10 to 33% of TL. As with juveniles, the %ST and PL were fairly consistent over the year, but %FFA increased in the fall. For the largest *Mysis* (≥16 mm), %TAG ranged from 23 to 57% (Fig. 4). There was little variation over time for %FFA and ST in this largest size group, and variation in %PL varied inversely to the temporal trends in TAG.

For juvenile *Mysis* (<10 mm), higher ST and PL (% of *Mysis* total DW) were both associated with higher % lipid content ($r^2=0.65$ and 0.71, respectively). On the other hand, TAG and FFA (% of *Mysis* DW) were poor predictors of TL (%DW) for juveniles ($r^2<0.38$). In contrast, TAG (% of *Mysis* DW) was strongly related to TL (%DW) for adult *Mysis* ($r^2=0.80$), but other lipid classes were not ($r^2<0.10$).

Although mean available chl *a* (i.e., whole water column if isothermal or metalimnion and hypolimnion if stratified) differed somewhat among years, the patterns for both years had some similarities; i.e., low chl *a* levels in the spring followed by increases into the mid-summer, then declines in the fall (Fig. 5). A couple exceptions to this pattern for 2010 were relatively high values of chl *a* in March and the late fall. The DCL averaged 5 $mg \cdot m^{-2}$ in 2009, peaking at 25 $mg \cdot m^{-2}$. In 2010, the DCL averaged 3 $mg \cdot m^{-2}$, peaking at 9 $mg \cdot m^{-2}$. There was no relationship between chl *a* levels and mean *Mysis* lipid content (%DW) for any size group of *Mysis* ($p>0.09$, $r^2<0.27$).

Biomass of zooplankton was generally lowest in the spring for both years, increasing somewhat in summer, and then reaching the highest levels in fall (Fig. 5). In general, zooplankton abundance appeared relatively similar between years in spring, but somewhat higher in summer and fall 2010 than in 2009. There was no

Table 1
Median lipid content (%DW) (range) and lipid weight (mg) (range) of various size groups of *Mysis* averaged across all sampling dates for 2009 and 2010. n = number of *Mysis* (or composites) analyzed in each group. Some composite samples were not included in lipid weight determinations, so sample sizes differ between lipids (%) and lipids (mg) in 2009.

Year	Size group (mm)	Lipids (%)	n	Lipid (mg)	n
2009	<6	15.1 (10.7–35.6)	30	0.03 (0.02–0.07)	13
2009	6 to 10	15.4 (9.5–40.3)	74	0.15 (0.04–0.68)	41
2009	11 to 15	21.0 (8.9–43.3)	96	0.97 (0.26–2.26)	82
2009	≥16	18.7 (8.9–37.6)	32	1.44 (1.47–3.98)	32
2010	<6	18.7 (9.9–43.8)	47	0.03 (0.01–0.12)	47
2010	6 to 10	13.9 (7.5–26.9)	212	0.10 (0.03–0.69)	212
2010	11 to 15	13.6 (6.5–30.3)	166	0.47 (0.07–2.47)	166
2010	≥16	16.6 (6.5–34.0)	67	1.26 (0.09–2.75)	67

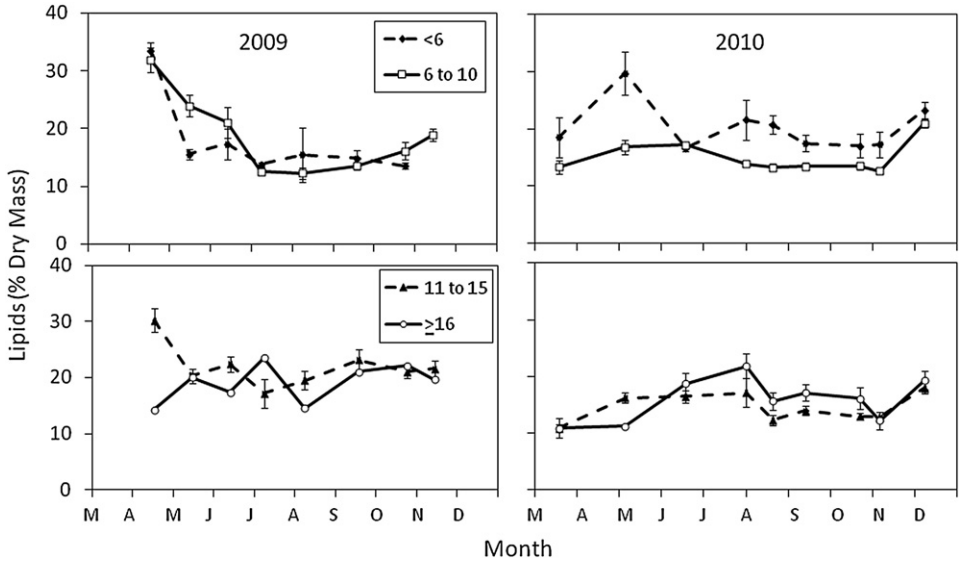

Fig. 1. Total lipids (%DW) of 4 size groups (<6 mm, 6–10 mm, 11–15 mm, ≥16 mm) of *Mysis* in Lake Michigan during 2009 (left panels) and 2010 (right panels).

relationship between zooplankton biomass and mean *Mysis* lipid content (%DW) for any size group of *Mysis* ($p > 0.24$, $r^2 < 0.09$).

Discussion

This study demonstrates the ontogenetic and seasonal variation in growth and resource allocation in *Mysis* within Lake Michigan. Lipid content and composition indicate that juvenile *Mysis* focused mainly on growth while adults accumulated storage lipids for reproduction. Variation in lipid content did not appear strongly linked to pelagic food supply in Lake Michigan, and there was some evidence of resource limitations for *Mysis* in the lake.

Although *Mysis* accumulate lipids as they grow, TL (%DW) remained relatively constant with *Mysis* size. A study in inland lakes indicated that juvenile *Mysis* allocate energy reserves toward somatic growth (Chess and Stanford, 1998). This is consistent with our results that as juvenile *Mysis* grew, lipids accumulated at a relatively slow rate compared to larger *Mysis*. However, our results contrast with previous work for *Mysis* in other inland lakes, where % lipid content increased with *Mysis* size and was one of the most distinguishing characteristics of the populations studied (Adare and Lasenby, 1994). Similarly, lipid content for *M. mixta* increased on both a total (mg) and a percentage basis as the mysids grew (Richoux et al., 2004a). Adare and Lasenby (1994) suggested that decreased metabolic demands relative to food intake could result in an energetic

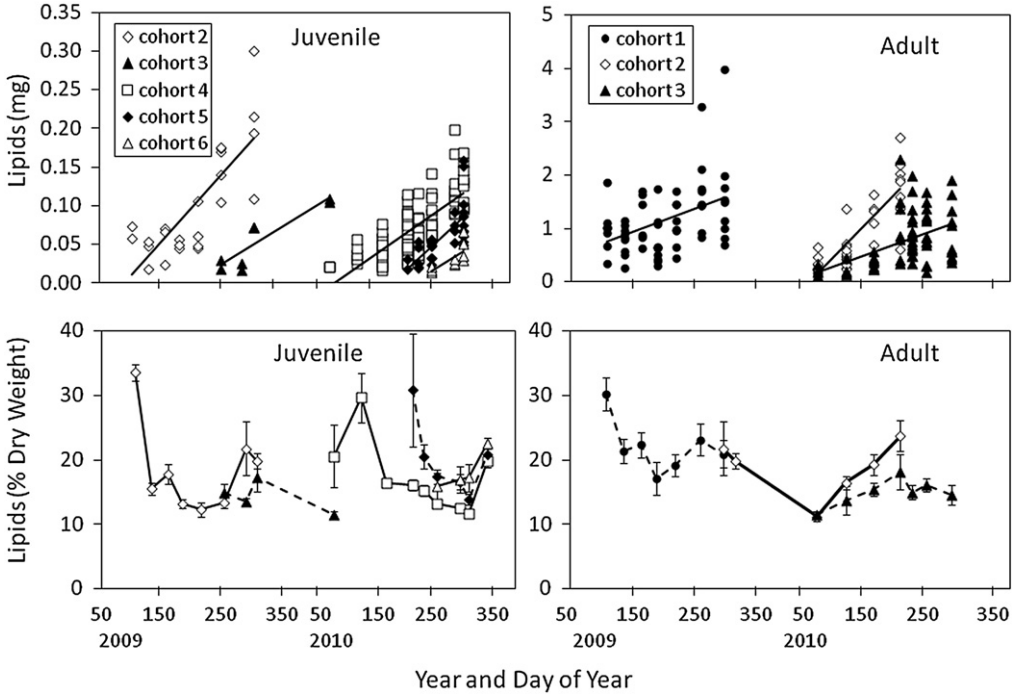

Fig. 2. Total lipids (mg) (top panels) and lipid content (%DW) (±1 SE) (bottom panels) of juvenile and adult *Mysis* for various chorts during 2009 and 2010. Slopes (i.e., lipid retention rates) differed among juvenile cohorts (2 = 0.00080 mg·d^{-1}; 3 = 0.00047 mg·d^{-1}, 4 = 0.00053 mg·d^{-1}; 5 = 0.00072 mg·d^{-1}, 6 = 0.00090 mg·d^{-1}), but not among adult cohorts (1 = 0.0044 mg·d^{-1}; 2 = 0.0120 mg·d^{-1}; 3 = 0.0042 mg·d^{-1}).

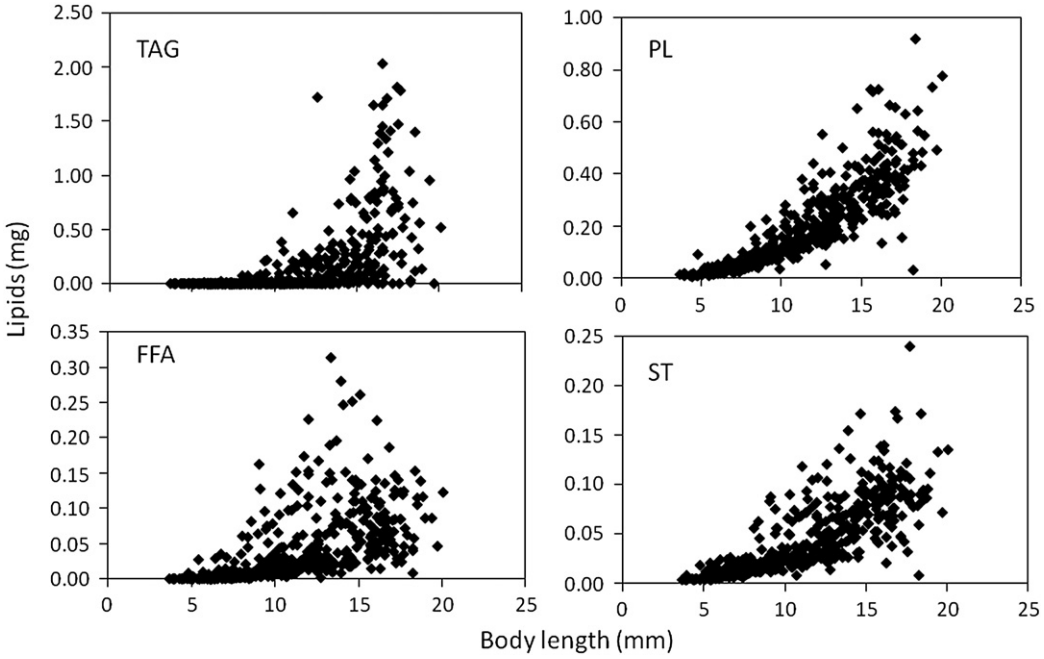

Fig. 3. Amount of triacylglycerides (TAG), phospholipids (PL), fatty acids (FFA), and sterols (ST) as a function of *Mysis* length in Lake Michigan in 2010.

advantage for larger mysids. Additionally, larger mysids may be able to take advantage of higher quality food resources such as zooplankton while smaller *Mysis* consume mainly phytoplankton (Branstrator et al., 2000). However, because adult *Mysis* accumulated both non-lipid weight as well as lipid weight at a faster rate than juveniles, the diet shift and increased metabolic efficiencies for adults do not appear to have resulted in any increased lipid content on a percentage basis.

An examination of the lipid class composition provides further evidence that juveniles were allocating energy toward somatic growth rather than lipid storage. Increases in juvenile lipids were related to increases in structural lipids (i.e., PL and ST), whereas increases in total lipids for adults were related to increases in storage lipids (i.e., TAG). Although juvenile *Mysis* focused more on growth than lipid retention, lipids still play an important role in their development. For example, sterols play a structural role for building and maintaining cell membranes, so they could be particularly important for growing juveniles (Martin-Creuzburg and von Elert, 2009). Further, sterols can't be synthesized by crustaceans, so food web disruption could impact dietary sources of sterols (Martin-Creuzburg and von Elert, 2009; Sánchez-Paz et al., 2006). Sterols are obtained through carnivory or from phytosterols that can be converted to useable sterols (e.g., cholesterol), with differing algal types having varying amounts of sterols available to crustaceans (Martin-Creuzburg and von Elert, 2009). Although the importance of sterols decreases as adults invest energy in reproduction and energy storage, they are still needed for egg production (Martin-Creuzburg and von Elert, 2009; Sánchez-Paz et al., 2006).

Lipid content varied seasonally for *Mysis* in Lake Michigan, but the patterns varied for different size groups and years. Lipid content (%DW) of juveniles was generally highest in spring (April), and then declined through the summer. Lipid content of small adults had a similar pattern to that seen in juveniles in 2009, but in 2010, % lipid content did not vary much over the year except for a late season increase in December. For the largest adults, % lipid content did not vary much over the year in 2009, but in 2010, % lipid content peaked in late July. Previous work has shown that adult *Mysis* % lipid content in Lake Michigan increased between spring and summer in 2008 (Mida Hinderer et al., 2012), which is consistent with our results for the largest adults in 2010, but not for 2009 or for smaller adults in both years. Gardner et al. (1985) also found that lipids increased between spring and early summer for *Mysis* of unreported sizes in Lake Michigan in 1984, and attributed the increase in part to the appearance of the spring diatom bloom. Similarly, lipid content for the marine species *M. mixta* generally increased following the spring phytoplankton bloom and subsequent sedimentation in Conception Bay, Newfoundland (Richoux et al., 2004a). However, in a study of *Mysis* in inland lakes, Adare and Lasenby (1994) found that although % lipid content of adult *Mysis* increased slightly from spring into summer, seasonal variation in lipid content was relatively low. They attributed this lack of a strong seasonal pattern to omnivory and diel vertical migrations which may dampen seasonal patterns even when faced with a fluctuating food supply.

Although *Mysis* grew in length and accumulated lipids overwinter, lipid content (%DW) decreased overwinter between 2009 and 2010. The %TAG (i.e., storage lipids) in *Mysis* was also relatively low in the spring of 2010. Thus, it appears that any lipid retention in the winter was generally allocated toward somatic growth or reproduction, rather than toward storage. Reduced lipids overwinter is consistent with results from two inland lakes, where % lipids decreased overwinter (Chess and Stanford, 1998). The relative decrease in % lipids in the Chess and Stanford (1998) study appeared to differ among lakes in part due to varying rates of consumption during winter. Thus, food supply in winter and early spring could influence the rate of lipid depletion on a percentage basis during the winter.

Although changes in food supply can rapidly influence lipid levels in mysids and other crustaceans (Parrish et al., 2005; Richoux et al., 2004a), seasonal changes in available phytoplankton (i.e., chl *a*) or zooplankton did not appear to provide much insight into variation in lipid content of *Mysis* in our study. In fact, *Mysis* lipids often were

Table 2
Mean lipid class composition (% of total lipids) of various size groups of *Mysis* averaged across all sampling dates for 2010. TAG = triacylglycerides, FFA = fatty acids, ST = sterols, PL = phospholipids.

Size group (mm)	TAG	FFA	ST	PL
<6	4.2	7.0	22.1	66.6
6 to 10	5.4	8.7	18.4	67.3
11 to 15	20.7	10.5	12.0	56.8
≥16	41.8	7.0	8.2	42.9

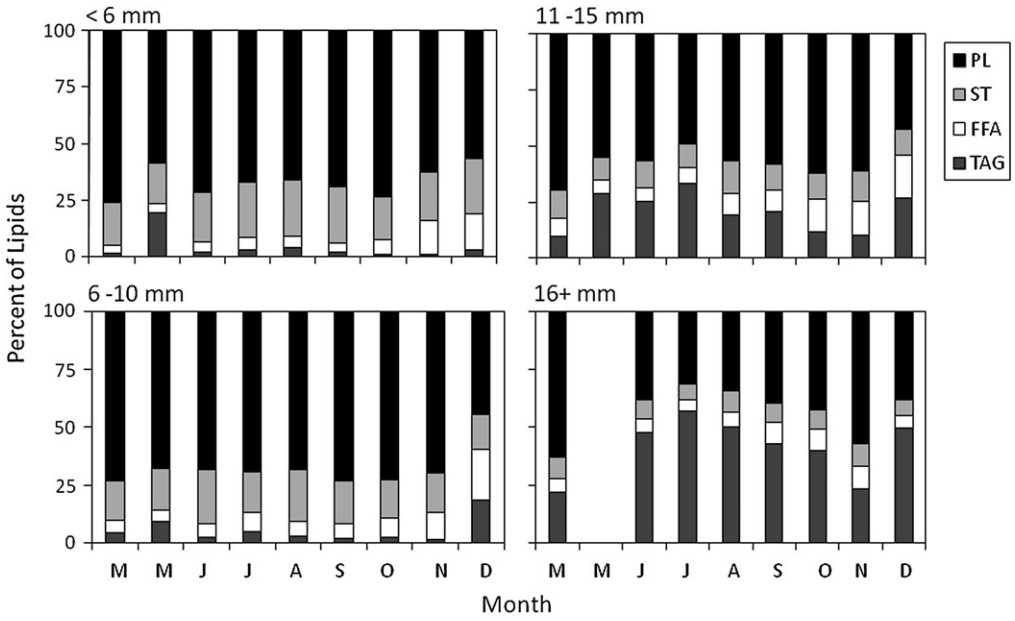

Fig. 4. Lipid class percent composition (% of total lipids) for 4 size groups of *Mysis* in Lake Michigan in 2010. Lipid class abbreviations are as in Fig. 3.

lowest when chl *a* levels were highest. Similarly, patterns in lipid content did not follow patterns in zooplankton biomass. Food sources that we did not consider such as detritus and benthos could also have been important in adult *Mysis* diets (Johannsson et al., 2001; Sierszen et al., 2011). However, considering the nearly complete loss of *Diporeia* in Lake Michigan, benthos might not be an important source of energy compared to Lake Superior where *Diporeia* persist (Sierszen et al., 2011).

It is possible, given the recent changes in the food web, that the seasonal gradients in food availability are no longer large enough to elicit strong responses in lipid content of *Mysis*. Since the 1980s, the chl *a* levels during the spring have decreased 74%, with concurrent 78% declines in primary production (Fahnenstiel et al., 2010). Spring bloom chl *a* values (April or May) in the 1980s and 1990s exceeded 3 $mg \cdot m^{-2}$ (Fahnenstiel et al., 2010) compared to <1 $mg \cdot m^{-3}$ observed in 2009–2010. Similarly, the DCL may also be declining, as average areal chl *a* concentrations in the DCL in 2009–2010 were <5 $mg \cdot m^{-2}$, much lower than values reported in the 1980s and 1990s (13–47 $mg \cdot m^{-2}$) (Fahnenstiel et al., 2010). Both the spring phytoplankton bloom and the DCL have been reported as important sources of food for *Mysis* in Lake Michigan in the past (Bowers and Grossnickle, 1978; Branstrator et al., 2000; Gardner et al., 1985) and would have provided strong seasonal inputs of food that may have elicited stronger seasonal patterns in *Mysis* lipid retention. In comparison, lipids of *M. mixta* appeared to respond rapidly to the spring bloom in Conception Bay, Newfoundland that was characterized by increases from 0.36 to 0.43 $mg \cdot m^{-3}$ to 2.22 to 5.27 $mg \cdot m^{-3}$ (Richoux et al., 2004a, 2004b).

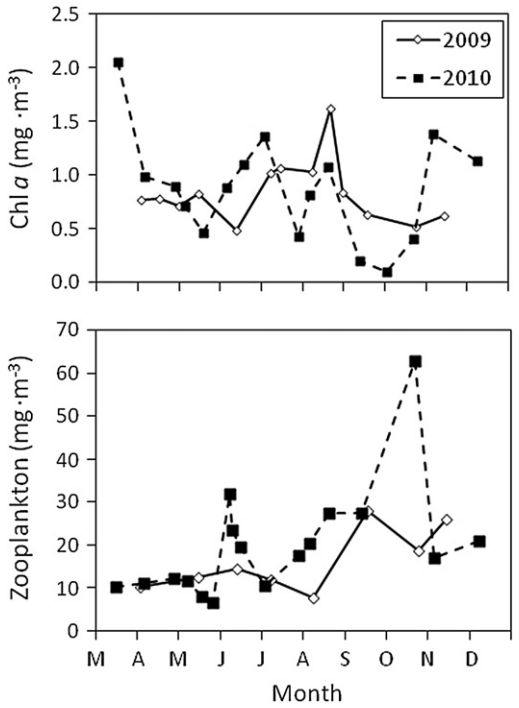

Fig. 5. Mean chl *a* for whole water column (isothermal) or metalimnion and hypolimnion combined (stratified) (top panel) and whole water column zooplankton biomass (bottom panel) for 2009 and 2010 at an offshore site in Lake Michigan.

Our data on food availability may also be too coarse to correspond with lipid patterns of *Mysis*. Similarly, Adare and Lasenby (1994) suggested that trophic status may indicate an increase in phytoplankton, but not an increase in food quality or quantity. For example, Chess and Stanford (1998) demonstrated how total energy differed among two populations of *Mysis* in oligotrophic lakes with relatively similar prey densities. These differences in energy were attributed to the prey actually eaten, spatial overlap with that prey, and to predation efficiency (Chess and Stanford, 1998). Feeding rates of *Mysis* may depend on phytoplankton size structure (Bowers and Grossnickle, 1978) and perhaps species composition. The types of prey eaten by *Mysis* may vary seasonally (Johannsson et al., 2001), and therefore total prey available may not reflect what *Mysis* are actually eating. Finally, energy content of prey may vary seasonally. For example, increases in lipid content of *Mysis* in the fall could correspond with concurrent seasonal increases in lipid content of prey such as *Limnocalanus* (Vanderploeg et al., 1998). Further research on *Mysis* diets, the spatial connection between *Mysis* and their food, and patterns in lipid content of *Mysis* prey would be valuable for understanding the ecology of this species in a changing food web.

Given the large range of lipid content we found for *Mysis* (6.5 to 43.8%DW), our values overlap with most reported values for *M. diluviana* in the Laurentian Great Lakes (Cavaletto and Gardner, 1999; Gardner et al., 1985; Mida Hinderer et al., 2012; Schlechtriem

et al., 2008), inland lakes (Adare and Lasenby, 1994; Chess and Stanford, 1998), and even for marine species (*Mysis mixta*; Richoux et al., 2004a). We did find evidence that food limitation could be affecting *Mysis* in Lake Michigan though. We noted that 14% and 40% of *Mysis* ≥13 mm had lipid content <14% in 2009 and 2010, respectively. The starvation threshold of 14% was determined for adult *Mysis* that were starved for 42 d in a laboratory study (Schlechtriem et al., 2008). This starvation level of total lipids however only resulted in 22–23% mortality, suggesting that it is not a lower limit of lipids for survival. This relatively high survival despite starvation conditions points to the adaptability of *Mysis* to food shortage and possibly to food web disruption (Schlechtriem et al., 2008). Further evidence for food limitation in our study is the relatively low storage lipids (i.e., %TAG) for adult *Mysis* in June 2010 (32%) compared to levels reported for June 1987 (72%) (Cavaletto and Gardner, 1999). It should not be surprising that *Mysis* could be starving in Lake Michigan considering the recent declines in primary productivity within the lake (Fahnenstiel et al., 2010). Declining lipid content of *Mysis* would have direct impacts on the lipid content of planktivores that consume *Mysis*.

The evidence for starvation conditions in Lake Michigan is not totally conclusive however. For example, Adare and Lasenby (1994) reported that lipid content of adult *Mysis* that were starved for 35 d declined from 30% to 7% (LFDW basis) and remained constant thereafter through 45 d. After converting our lipid content to a LFDW % basis, we found that almost no *Mysis* fell below this 7% threshold. Further, a study that used estimated lipids on a dry weight basis found no evidence that lipid content for adult *Mysis* in Lake Michigan in 2008 fell below starvation thresholds of 14% TL and another indicator, (% docosahexaenoic acid; DHA), was well below a starvation threshold (Mida Hinderer et al., 2012).

The available threshold criteria for assessing starvation were determined for adult *Mysis* (Adare and Lasenby, 1994; Schlechtriem et al., 2008). Juveniles had similar lipid content on a %DW basis as adults in our study, and in 2010, 32% of the juveniles had TL content <14%DW. Further, TL (mg) were much lower for juveniles than for adults. Considering the low levels of storage lipid in juvenile *Mysis*, their higher metabolic demands and their allocation of energy toward growth (Adare and Lasenby, 1994; Chess and Stanford, 1998), it would not be surprising if juvenile *Mysis* in Lake Michigan were also food limited to some degree. Recent research in both Lake Michigan and Ontario has suggested that changes in food availability may be affecting survival of juvenile *Mysis* (Johannsson et al., 2011; Pothoven et al., 2010). Further research could shed light on lipid thresholds for starvation in juvenile *Mysis* as well as thresholds for survival for all sizes of *Mysis*.

Given the high lipid content (34%) and %TAG (71%) of broods, it appears they represent a substantial energy investment on the part of *Mysis*. The importance of storage lipids in reproduction is evidenced by relatively low %TAG in adult *Mysis* (with broods removed) in spring and fall when the highest percentage of females had broods and storage lipids were apparently allocated to broods. By contrast, %TAG increased in late spring through summer as adult *Mysis* apparently built up storage lipid reserves prior to fall reproduction. However, total lipid content of adult *Mysis* did not appear to be strongly linked to periods of high reproduction. Although some crustaceans, including *Mysis*, might require reaching a certain lipid content to reproduce (Chess and Stanford, 1998; Hill et al., 1992), that did not appear to be the case in this study of *Mysis* in Lake Michigan. Lipid content of female *Mysis* with broods was as low as 7%, a value that could indicate a state of starvation. Although small adults generally had high % TL in spring (2009) or late fall (2010) corresponding to periods of reproduction, lipid content for larger adults did not vary with time for 2009 and was highest in summer in 2010. At least some female *Mysis* with broods were found in most months, and the presence of small mysids confirms some reproduction did occur year round, which could mask linkages between lipid content and reproduction to some degree in this study.

A study in Conception Bay demonstrated that although most *M. mixta* accumulate lipids before reproducing, some early reproducing females had low lipid levels, suggesting that *M. mixta* may require lipids for storage, but high lipids may not be required to reproduce (Richoux et al., 2004a). The *M. mixta* that spawned later did have larger broods than the early spawning females in this study, suggesting some advantage to lipid storage and later reproduction. Similarly, although *Mysis* as small as 11 mm were found with broods (S. Pothoven, unpubl. data), brood size for *Mysis* in Lake Michigan increases with *Mysis* size (Pothoven et al., 2010). On the other hand, Adare and Lasenby (1994) indicated that body size and lipid content (mg) were more important thresholds for reproduction than lipid content (%).

This paper provides a starting point for evaluating the condition of *Mysis* in the changing ecosystem of Lake Michigan. Our results indicate that food web modelers may need to consider *Mysis* ontogeny and season when examining flow of energy through *Mysis* in the food web. Furthermore, future work on *Mysis* diets, dietary lipids and the spatial coupling of *Mysis* and their prey will be useful to help evaluate the changing ecosystem in Lake Michigan and other Great Lakes.

Acknowledgements

Assistance in the field was provided by the R/V Laurentian. A. Flood provided assistance in the laboratory. GLERL contribution 1630.

References

Adare, K.I., Lasenby, D.C., 1994. Seasonal changes in the total lipid content of the opossum shrimp, *Mysis relicta* (Malacostraca: Mysidacea). Can. J. Fish. Aquat. Sci. 51, 1935–1941.

Anderson, E.D., Smith, L.L., 1971. A synoptic study of food habits of 30 fish species from western Lake Superior. Univ. Minn. Agric. Exp. Stn. Tech. Bull. 279.

Arts, M.T., Evans, M.S., Robarts, R.D., 1992. Seasonal patterns of total and energy reserves of lipids of dominant zooplanktonic crustaceans from a hyper-eutrophic lake. Oecologia 90, 560–571.

Audzijonyte, A., Väinolä, R., 2005. Diversity and distributions of circumpolar fresh- and brackish-water *Mysis* (Crustacea: Mysida): descriptions of *M. relicta* Lovén, 1862, *M. salemaai* n. sp., *M. segerstralei* n. sp., and *M. diluviana* n. sp., based on molecular and morphological characters. Hydrobiologia 544, 89–141.

Boscarino, B.T., Rudstam, L.G., Mata, S., Gal, G., Johannsson, O.E., Mills, E.L., 2007. The effects of temperature and predator–prey interactions on the migration behavior and vertical distribution of *Mysis relicta*. Limnol. Oceanogr. 52, 1599–1613.

Bowers, J.A., Grossnickle, N.E., 1978. The herbivorous habits of Mysis relicta in Lake Michigan. Limnol. Oceanogr. 23, 767–776.

Branstrator, D.K., Cabana, G., Mazumder, A., Rasmussen, J.B., 2000. Measuring life history omnivory in the opossum shrimp, *Mysis relicta*, with stable nitrogen isotopes. Limnol. Oceanogr. 45, 463–467.

Cavaletto, J.F., Gardner, W.S., 1999. Seasonal dynamics of lipids in freshwater benthic invertebrates. In: Arts, M.T., Wainman, B. (Eds.), Lipids in Freshwater Ecosystems. Springer, New York, pp. 109–131.

Chess, D.W., Stanford, J.A., 1998. Comparative energetic and life cycle of the opossum shrimp (*Mysis relicta*) in native and non-native environments. Freshw. Biol. 40, 783–794.

Culver, D.A., Boucerle, M.M., Bean, D.J., Fletcher, J.W., 1985. Biomass of freshwater crustacean zooplankton from length weight regressions. Can. J. Fish. Aquat. Sci. 42, 1380–1390.

Fahnenstiel, G.L., Pothoven, S.A., Nalepa, T.F., Vanderploeg, H.A., Klarer, D., McCormick, M., Scavia, D., 2010. Recent changes in primary production and phytoplankton in the offshore region of southeastern Lake Michigan. J. Great Lakes Res. 36 (S3), 20–29.

Folch, J., Lees, M., Sloane Stanley, G.H., 1957. A simple method for the isolation and purification of total lipides from animal tissues. J. Biol. Chem. 226, 497–509.

Gardner, W.G., Nalepa, T.F., Frez, W.A., Cichocki, E.A., Landrum, P.F., 1985. Seasonal patterns in lipid content of Lake Michigan invertebrates. Can. J. Fish. Aquat. Sci. 42, 1827–1832.

Hawkins, B.E., Evans, M.E., 1979. Seasonal cycles of zooplankton biomass in southeastern Lake Michigan. J. Great Lakes Res. 5, 256–263.

Hill, C., Quigley, M.A., Cavaletto, J.F., Gordon, W., 1992. Seasonal changes in lipid content and composition in the benthic amphipods *Monoporeia affins* and *Pontoporeia femorata*. Limnol. Oceanogr. 37, 1280–1289.

Johannsson, O.E., Leggett, M.F., Rudstam, L.G., Servos, M.R., Mohammadian, M.A., Gal, G., Dermott, R.M., Hesslein, R.H., 2001. Diet of *Mysis relicta* in Lake Ontario as revealed by stable isotope and gut content analysis. Can. J. Fish. Aquat. Sci. 58, 1975–1986.

Johannsson, O.E., Bowen, K.L., Holeck, K.T., Walsh, M.G., 2011. *Mysis diluviana* population and cohort dynamics in Lake Ontario before and after the establishment of *Dreissena* spp., *Cercopagis pengoi*, and *Bythotrephes longimanus*. Can. J. Fish. Aquat. Sci. 68, 795–811.

Lu, Y., Ludsin, S.A., Fanslow, D.L., Pothoven, S.A., 2008. Comparison of three microquantity techniques for measuring total lipids in fish. Can. J. Fish. Aquat. Sci. 65, 2233–2241.

Martin-Creuzburg, D., von Elert, E., 2009. Ecological significance of sterols in aquatic food webs. In: Arts, M.T., Brett, M.T., Kainz, M.J. (Eds.), Lipids in Aquatic Ecosystems. Springer, New York, pp. 43–64.

Mida Hinderer, J.L., Jude, D.J., Schaeffer, J.S., Warner, D.M., Scavia, D., 2012. Lipids and fatty acids of *Mysis diluviana* in lakes Michigan and Huron. J. Great Lakes Res. 38 (S2), 93–97, http://dx.doi.org/10.1016/j.jglr.2011.07.001.

Parrish, C.C., 1986. Dissolved and particulate lipid classes in the aquatic environment. Ph.D. thesis, Department of Oceanography, Dalhousie University, Halifax, Nova Scotia, Canada.

Parrish, C.C., 1987. Separation of aquatic lipid classes by Chromarod thin-layer chromatography with measurement by Iatroscan flame ionization detection. Can. J. Fish. Aquat. Sci. 44, 722–731.

Parrish, C.C., Thompson, R.J., Deibel, D., 2005. Lipid classes and fatty acids in plankton and settling matter during the spring bloom in a cold ocean coastal environment. Mar. Ecol. Prog. Ser. 286, 57–68.

Pothoven, S.A., Madenjian, C.P., 2008. Changes in consumption by alewives and lake whitefish after dreissenid mussel invasions in Lakes Michigan and Huron. North Am. J. Fish. Manag. 28, 308–320.

Pothoven, S.A., Fahnenstiel, G.L., Vanderploeg, H.A., 2010. Temporal trends in *Mysis relicta* abundance, production, and life-history characteristics in southeastern Lake Michigan. J. Great Lakes Res. 36, 60–64.

Pothoven, S.A., Hondorp, D.W., Nalepa, T.F., 2011. Declines in deepwater sculpin *Myoxocephalus thompsonii* energy density associated with the disappearance of *Diporeia* spp. in lakes Huron and Michigan. Ecol. Freshw. Fish 20, 14–22.

Richoux, N.B., Deibel, D., Thompson, R.J., Parrish, C.C., 2004a. Seasonal changes in the lipids of *Mysis mixta* (Mysidacea) from the hyperbenthos of a cold-ocean environment (Conception Bay, Newfoundland). Can. J. Fish. Aquat. Sci. 61, 1940–1983.

Richoux, N.B., Deibel, D., Thompson, R.J., Parrish, C.C., 2004b. Population biology of hyperbenthic crustacean in a cold water environment (Conception Bay, Newfoundland). I. *Mysis mixta* (Mysidacea). Mar. Biol. 144, 881–894.

Sánchez-Paz, A., García-Carreño, F., Muhlia-Almazán, A., Peregrino-Uriarte, P.B., Hernández-López, J., Yepiz-Plascencia, G., 2006. Usage of energy reserves in crustaceans during starvation: status and future directions. Insect Biochem. Mol. Biol. 36, 241–249.

Schlechtriem, C., Arts, M.T., Johannsson, O.E., 2008. 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.

Sierszen, M.E., Kelly, J.R., Corry, T.D., Scharold, J.V., Yurista, P.M., 2011. Benthic and pelagic contributions to *Mysis* nutrition across Lake Superior. Can. J. Fish. Aquat. Sci. 68, 1051–1063.

Speziale, B.J., Schreiner, S.P., Giammatteo, P.A., Schindler, J.E., 1984. Comparison of N, N-dimethylformamide, dimethyl sulfoxide, and acetone for extraction of phytoplankton chlorophyll. Can. J. Fish. Aquat. Sci. 41, 1519–1522.

Van Handel, E., 1985. Rapid determination of total lipids in mosquitoes. J. Am. Mosq. Control. Assoc. 1, 302–304.

Vanderploeg, H.A., Cavaletto, J.F., Liebig, J.R., Gardner, W.S., 1998. *Limnocalanus macrurus* (Copepoda: Calanoida) retains a marine arctic lipid and life cycle strategy in Lake Michigan. J. Plankton Res. 20, 1581–1597.

Walsh, M.G., O'Gorman, R., Strang, T., Edwards, W.H., Rudstam, L.G., 2008. Fall diets of alewife, rainbow smelt, and slimy sculpin in the profundal zone of southern Lake Ontario during 1994–2005 with an emphasis on occurrence of *Mysis relicta*. Aquat. Ecosyst. Health Manage. 11, 368–376.

Wells, L., 1980. Food of alewives, yellow perch, spottail shiners, trout-perch, and slimy and fourhourn sculpins in southeastern Lake Michigan. U. S. Fish Wildl. Serv. Tech. Bull. 98.