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Temporal variation of energy reserves in mayfly nymphs (*Hexagenia* spp.) from Lake St Clair and western Lake Erie

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SUMMARY

1. We analysed changes in energy reserves (lipid and glycogen) and length–weight relationships of burrowing mayflies (*Hexagenia* spp.) in 1997–99 to compare an established population in Lake St Clair with a recovering population in western Lake Erie of the Laurentian Great Lakes. In addition, we measured changes in water temperature and potential food in both water columns and sediments.
2. Although overall mean values of lipid and glycogen levels of *Hexagenia* nymphs from Lake St Clair and western Lake Erie were not significantly different, there were differences in seasonal patterns between the two lakes. In Lake St Clair, levels were highest in early spring, declined throughout the year, and reached their lowest levels in fall during all 3 years of study. In contrast, levels in western Lake Erie were lower in spring, increased to a maximum in summer, then declined in fall. Seasonal patterns in length–weight relationships were similar to those for lipid and glycogen.
3. Total lipid as a percentage of dry weight did not increase with developmental stage of nymphs until just prior to metamorphosis and emergence from water. However, the major reserve lipid, triacylglycerols, increased systematically with development stage. In the final stage of development, triacylglycerols declined, probably as a result of energy consumption and its conversion to other biochemical components for metamorphosis and reproduction.
4. Indicators of potential food (algal fluorescence in the water column and chlorophyll *a* and chlorophyll *a*/phaeophytin ratio in sediments) suggest that *Hexagenia* in Lake St Clair have a food source that is benthic based, especially in early spring, whereas in western Lake Erie nymphs have a food source that is water column based and settles to the lake bottom during late spring and summer.

Keywords: benthic food sources, glycogen, Great Lakes macroinvertebrates, *Hexagenia* spp., lipid

Introduction

Burrowing mayflies, *Hexagenia* spp., can be abundant in shallow bays, basins and connecting channels of the Laurentian Great Lakes. Two regions where they have been abundant historically are Lake St Clair and western Lake Erie (Britt, 1955; Beeton, 1969; Edsall, Madenjian & Manny, 1999; Edsall, 2001). These two lake areas are characterised by conditions favoured by *Hexagenia*; that is, both are relatively shallow, have extensive soft-bottom regions and are mesotrophic. Because of increasing anthropogenic pollution, *Hexagenia* disappeared from western Lake Erie in the

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1950s and 1960s (Carr & Hiltunen, 1965). In contrast, populations in Lake St Clair remained abundant throughout the 1950s and 1960s and remain so (Schloesser *et al*., 1991). After a 40-year absence, *Hexagenia* began to return to western Lake Erie in the early 1990s (Krieger *et al*., 1996), and it is now re-established throughout the western basin (Madenjian, Schloesser & Krieger, 1998; Schloesser *et al*., 2000). The return of *Hexagenia* to Lake Erie presented an opportunity to compare energy reserves of individuals from a recovering population with those from an established, stable population found in Lake St Clair. Over the 1997–99 period, energy reserves (glycogen and lipid) and length–weight relationships of *Hexagenia* nymphs in Lake St Clair were compared with those of nymphs in western Lake Erie. Glycogen is the compound for short-term storage of glucose that is metabolised quickly (minutes to hours), whereas lipids (mainly triacylglycerols) are long-term storage compounds that are metabolised over long periods of time (days to months).

Lipids are undoubtedly important for *Hexagenia* to complete its metamorphosis from aquatic nymph to adult, as has been found for terrestrial and other aquatic insects (Hanson *et al*., 1983; Beenakkers, Van der Horst & Van Marrewijk, 1985; Cargill *et al*., 1985; Arrese *et al*., 2001). For example, in the caddisfly, *Clistoronia magnifica*, a critical level of lipid is needed for successful metamorphosis to the adult stage, and this level was related to the quality of food eaten during larval stages (Cargill *et al*., 1985). Energy reserves, mainly lipid, have been determined in some mayfly species, but seasonal changes have not been examined extensively (Landrum & Poore, 1988; Meyer, 1990; Bell, Ghioni & Sargent, 1994; Ghioni, Bell & Sargent, 1996; Ruffieux, Elouard & Sartori, 1998; Meier, Meyer & Meyns, 2000).

As energy reserves are generally a function of the food available to aquatic insects (Hanson *et al*., 1983; Cargill *et al*., 1985; Goedkoop *et al*., 1998), we also measured seasonal changes in potential food as indicated by levels of algal fluorescence in the water column, and chlorophyll *a*, phaeophytin and particulate organic carbon (POC) in the sediments.

Methods

Hexagenia nymphs were collected from one site each in Lake St Clair (Site 1; 42°25′00″N, 82°45′00″W) and in western Lake Erie (Site 7M; 41°44′00″N, 83°17′50″W) on a monthly basis from April 1997 to November 1999 (Fig. 1, Table 1). Water depth at each site was 6 m, and sediments consisted of silt with some clay. These sites were chosen for comparison because of similar habitat conditions and nymphal densities (Schloesser & Nalepa, 2001). Nymphs were also collected at Sites 5B, 7L and 8D in western Lake Erie in the spring of 1998 and Site 7L in the spring of 1999 (Fig. 1). These sites were chosen, including 7M, because they have been previously named and sampled (Carr & Hiltunen, 1965) and relative densities are known (Schloesser *et al*., 2000). To compare Site 1 with a site located closer to shore, Site 188 was sampled in Lake St Clair in June and July 1999 (Fig. 1). Nymphs were collected with a grab sampler, washed through a 500-μm screen, and transported in coolers back to the laboratory. Usually, nine grabs produced enough nymphs in various size classes for lipid and glycogen analysis. In addition to nymphs, subimagos were collected for glycogen and lipid analysis in July 1997 and late June/early July 1998. Individuals were collected at night (attracted with lights) at each site during emergence. In 1998, eggs were extracted from subimagos at both sites for lipid analysis only.

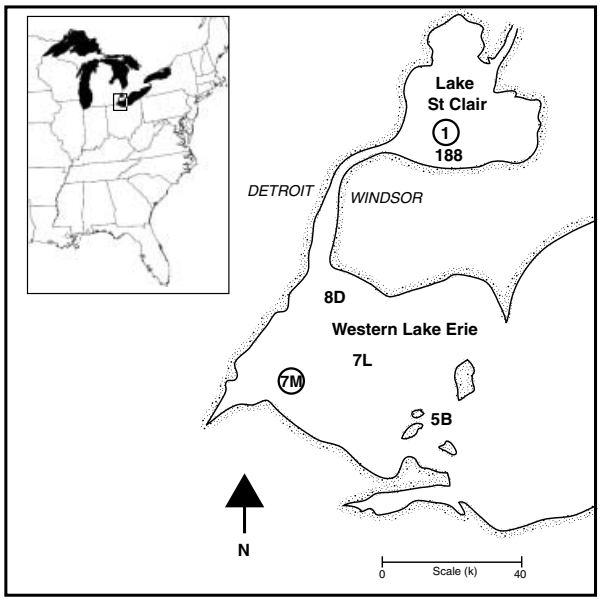

Fig. 1 Locations of *Hexagenia* collection sites in western Lake Erie and Lake St Clair. Monthly collections were made at Sites 1 and 7M (circled numbers), whereas occasional collections were made at the other four sites.

Table 1 Sizes of *Hexagenia* used for lipid and glycogen analysis. Listed are the ranges of lengths, and mean (±SE) of lengths and dry weights for monthly collections. Dry weights used for glycogen analysis were calculated from length–weight regressions from animals used for lipid analysis

	Lake Erie					Lake St Clair				
	Lipid			Glycogen		Lipid			Glycogen	
Date	Range (mm)	Length (mm)	Dry weight (mg)	Range (mm)	Length (mm)	Range (mm)	Length (mm)	Dry weight (mg)	Range (mm)	Length (mm)
1997										
20–23 April	6–27	16.3 (1.4)	15.1 (2.8)	18–25	21.7 (0.6)	3.5–24	10.8 (1.0)	5.5 (1.4)	11–15	12.4 (0.5)
27–28 May	5–26	14.6 (1.4)	11.3 (2.9)	21–27	24.2 (0.9)	4–24	12.7 (1.1)	9.7 (2.6)	13–15	14.3 (0.6)
23–24 June	7–28	16.4 (1.6)	12.6 (2.9)	21–28	23.7 (1.0)	7–27	15.2 (1.1)	11.9 (2.6)	14–21	17.4 (0.6)
24 July	8–20	15.0 (0.8)	8.6 (1.3)	16–21	18.0 (0.6)	8–26	16.4 (1.0)	12.0 (2.3)	16–23	19.7 (0.5)
28 August	9–25	16.9 (0.8)	10.2 (1.3)	17–25	20.1 (0.8)	15–25	18.5 (0.6)	11.5 (1.5)	18–23	20.4 (0.6)
22 September	11–22	15.9 (0.7)	8.6 (1.2)	17–23	19.1 (0.4)	12–23	17.6 (0.6)	12.9 (1.6)	18–23	20.2 (0.6)
23 October	6–26	16.9 (0.9)	8.5 (1.3)	17–24	20.8 (0.9)	14–23	18.1 (0.6)	10.0 (0.4)	16–24	19.6 (0.8)
1998										
24 March	5–24	16.1 (0.9)	6.6 (0.9)	17–25	20.6 (1.0)	15–25	19.1 (0.6)	19.7 (2.9)	18–23	20.2 (0.5)
20 April	12–23	17.0 (0.8)	10.5 (1.5)	16–25	20.5 (1.0)	18–25	21.1 (1.0)	23.5 (1.8)	19–23	21.2 (0.4)
27 May	10–25	18.2 (0.8)	12.9 (1.3)	19–27	22.3 (0.8)	20–27	24.1 (0.7)	37.0 (3.9)	21–27	24.9 (1.0)
15 June	9–25	17.0 (0.8)	10.7 (1.5)	17–26	20.8 (1.2)	18–25	21.9 (0.6)	24.5 (2.2)	21–28	23.2 (0.7)
16 July	13–24	17.2 (0.8)	13.9 (1.4)	nd	nd	10–23	15.5 (1.1)	9.0 (1.8)	Nd	nd
26 August	5–24	13.9 (1.2)	9.2 (2.2)	nd	nd	4–9	5.8 (0.3)	0.5 (0.1)	Nd	nd
23 September	9–27	16.8 (0.8)	11.6 (1.7)	nd	nd	7–22	12.9 (0.7)	4.8 (0.9)	Nd	nd
15 October	9–29	17.3 (1.0)	11.2 (2.0)	17–27	21.6 (0.9)	7–20	15.5 (0.8)	6.3 (0.7)	18–20	18.8 (0.2)
18 November	10–21	15.6 (0.8)	7.4 (1.0)	19–23	20.9 (0.3)	9–26	15.2 (0.9)	6.4 (1.0)	17–23	19.3 (0.6)
1999										
8 April	10–25	16.5 (0.9)	10.6 (1.5)	18–27	20.9 (1.0)	10–24	15.4 (0.7)	8.9 (1.1)	17–20	18.0 (0.3)
3 May	8–29	16.8 (1.2)	12.5 (2.4)	18–25	22.4 (0.8)	9–23	15.1 (0.9)	9.1 (1.3)	17–21	19.0 (0.4)
9 June	9–26	16.8 (0.9)	11.3 (1.6)	17–23	20.3 (0.6)	10–25	17.6 (1.1)	14.0 (2.4)	19–24	22.3 (0.8)
7 July	12–25	19.0 (0.9)	17.0 (2.1)	19–25	21.6 (0.8)	12–24	17.7 (1.0)	10.3 (0.7)	19–25	21.3 (0.7)
11 August	13–26	20.2 (0.7)	19.3 (1.9)	18–27	22.0 (0.9)	13–25	17.7 (0.8)	10.5 (1.2)	18–22	20.1 (0.3)
31 August	9–25	16.6 (1.1)	11.4 (1.9)	20–27	23.5 (1.0)	13–24	18.1 (0.6)	11.2 (1.0)	17–23	20.0 (0.6)
12 October	9–28	17.6 (1.2)	11.4 (1.9)	21–28	23.5 (0.8)	nd	nd	nd	nd	nd

On each sampling date at Sites 1 and 7M, we measured vertical profiles of temperature and algal fluorescence with a conductivity, temperature, and depth (CTD) instrument equipped with a fluorometer. In a previous study the same instrument was used to measure *in situ* algal fluorescence and was compared with the direct extraction and measurement of chlorophyll *a* in the water column (Nalepa *et al.*, 1996a). The results yielded about a one-to-one, linear relationship between the two variables ($P < 0.001$; $r^2 = 0.663$). However, as we did not measure water column chlorophyll directly, we refer to the CTD measured fluorescence as algal fluorescence. Algal fluorescence is expressed as $\mu g\ L^{-1}$ based on the internal calibration of the instrument.

In 1998 and 1999, we measured water column secchi depth and also collected sediment samples for measurement of chlorophyll *a* concentration, chlorophyll *a*/phaeophytin ratios, and POC. Sediments were collected by scraping 1 cm of sediment off the top of a Ponar grab sample. Sediment samples were immediately freeze-dried and kept frozen until analysis. Chlorophyll *a* was extracted with absolute ethanol and analysed fluorometrically following extraction and centrifugation. Chlorophyll *a* concentration was corrected for the presence of phaeophytin, and the chlorophyll *a*/phaeophytin ratio was determined (Hansson, 1988; Goedkoop & Johnson, 1996). Particulate organic carbon was determined on a portion of the freeze-dried sediment with a C : H : N elemental analyser. Analytical duplicates were determined for sediment chlorophyll *a* and POC.

Within 24 h of collection, 20–25 nymphs were selected for lipid analysis from the total number found in the samples. Individuals represented the complete size range of nymphs found in samples for

that particular date and lake station (Table 1). Selected nymphs were measured (tip of the mentum to caudal process), sexed, identified to species when possible, and placed in one of six developmental stages based on wing pad development (Heise, Flannagan & Galloway, 1987). Nymphs in Stage 1 are in the earliest stage of development, while nymphs in Stage 6 have black wing pads and are in the final stage of development just prior to emergence from the water as winged subimagos. Nymphs were placed individually into preweighed glass test tubes (6 × 50 mm), dried under nitrogen for 48 h at 60 °C, stored in a desiccator under vacuum and kept frozen until analysis. Dry weights of individual nymphs or subimagos were determined with a microbalance to the nearest 0.001 mg prior to lipid analysis. Total lipid was extracted from individual nymphs using chloroform : methanol (2 : 1 v/v) and quantified gravimetrically (Gardner *et al*., 1985). A subsample of the total lipid extract was saved under nitrogen and frozen for later lipid class analysis. Lipid classes were separated with thin layer chromatography with flame ionization detection (TLC-FID) by spotting a portion of the lipid extract onto silica-coated Chromarods SIII (Raytest/Shell-USA, Inc., Fredericksburg, VA, USA) and developing them sequentially in solvent systems of increasing polarity. Chromarods were scanned after each group separation in an Iatroscan Mark IV (Iatron Labs, Tokyo, Japan). A mixed lipid standard, containing one compound from each of the following lipid classes – hydrocarbon, sterol ester, triacylglycerol, free fatty acid, alcohol (aliphatic), sterol (alicyclic), and phospholipids – was used for TLC-FID calibration and quantification. Calibration curves were determined over a range of 0.15–30 μg for each standard compound (Parrish, 1987). Lipid classes were examined only in 1997 and 1998 on a subset of individuals that were analysed for total lipid (number of individual nymphs analysed are listed in Figs 4 and 6).

For glycogen analysis, five to 15 large nymphs were randomly selected from the total number found in the monthly samples, measured, placed immediately into individual cryogenic vials, and stored in liquid nitrogen. Large nymphs were chosen because we presumed that they would provide the maximum level of nymphal glycogen for each lake and date. In addition, the large size allowed for analysis of individual nymphs as was the case for lipid analysis. Glycogen levels were determined spectrophotometrically, after converting glycogen to glucose using the enzyme degradation method (Roehrig & Allred, 1974). As glycogen analysis was carried out on freshly thawed animals, dry weights were determined from length–weight regressions derived from nymphs used for lipid analysis. Purified glycogen from the marine mussel *Mytilus edulis* was used as a standard.

Two species of *Hexagenia* were identified in the two lakes: *H. limbata* and *H. rigida*. Both species have near identical life cycle and habitat preferences. However, it has been noted, mostly from laboratory reared nymphs (Neave, 1932; Giberson & Rosenberg, 1994), that *H. limbata* reaches a greater maximum length and has a higher fecundity than *H. rigida*. In Lake Erie, proportions of the two species emerging as adults alternate between years, with a higher proportion of one species emerging one year and a higher proportion of the other species emerging the next (L.D. Corkum, University of Windsor, personal communication). However, as only male nymphs can be identified to species with confidence (McCafferty, 1975), we refer to the two species collectively as *Hexagenia*. Of male nymphs selected for both lipid and glycogen analysis and identified to species, 83% in Lake St Clair ($n = 374$) and 53% in western Lake Erie ($n = 319$) were *H. rigida*.

Statistical comparisons of energy reserves (lipid, glycogen and triacylglycerols) were made using SYSTAT V.8 and performed on arcsine transformed data. Transformed data met the assumptions of variance homogeneity.

Results

Environmental variables

Mean water temperature was significantly higher at Site 7M in western Lake Erie than Site 1 in Lake St Clair (paired *t*-test, $P < 0.001$). On all 22 sampling dates when both sites were sampled between 1997 and 1999, temperatures were higher at the Lake Erie site, the mean difference being 1.9 °C (Table 2). Maximum water temperature occurred in July during all 3 years at both sites. Water transparency, as measured by secchi depth, was significantly greater (paired *t*-test, $P < 0.001$) in Lake St Clair than in Lake Erie (Table 2).

Algal fluorescence in the water column and chlorophyll *a*, phaeophytin (degradation pigments of

Table 2 Physical and chemical variables from Site 7M western Lake Erie (LE) and Site 1 Lake St Clair (LSC) 1997–99

	Temperature (°C)		AFWC (μg L^{-1})		Secchi depth (m)		SChl (μg g^{-1})		SChl/phaeo ratio		POC (percentage dry weight)	
Date	LE	LSC	LE	LSC	LE	LSC	LE	LSC	LE	LSC	LE	LSC
1997												
20–23 April	6.5	6.0	2.2	0.9								
27–28 May	14.5	10.9	13.0	1.4								
23–24 June	19.9	15.7	5.0	1.3								
24 July	23.5	21.2	6.8	1.7								
2 August	20.6	19.6	13.3	1.5								
22 September	19.2	18.2	3.1	1.5								
23 October	11.6	11.4	2.5	1.8								
1998												
24 March	2.2	1.7	3.5	1.9	0.2	0.6	2.0	11.6	0.2	2.0	2.4	1.4
20 April	10.8	8.3	6.4	1.5	0.1	2.1	1.4	8.5	0.2	1.2	1.9	1.5
27 May	21.6	17.3	2.7	1.0	0.3	3.3	3.6	13.5	0.6	2.3	2.5	1.8
15 June	18.9	16.7	7.7	1.3	1.2	2.1	2.9	10.3	0.4	2.2	2.0	1.3
16 July	24.0	23.2			1.6	2.4	2.8	17.3	0.4	2.6	1.9	1.7
26 August	21.2	19.0	7.0	1.7	0.3	1.2	2.6	13.9	0.3	2.7	1.8	1.6
23 September	20.5	15.4	7.0	1.5	0.2	2.0	3.5	17.8	0.2	1.6	1.7	1.5
15 October	13.6	13.5			1.0	1.1	2.0	7.3	0.3	1.6	1.8	1.4
18 November	9.6	9.0			0.2	2.5	2.4	7.4	0.3	1.2	1.8	1.3
1999												
8 April	8.9	6.5	5.0	1.4	0.2	1.3	2.1	11.1	0.3	2.1		
3 May	12.2	9.9	4.5	1.0	0.3	2.0	1.9	10.8	0.3	2.4	2.0	1.2
9 June	17.4	16.2	10.3	1.5	1.0	3.0	1.8	10.2	0.3	2.4	1.4	1.1
7 July	25.4	21.6	6.2	1.6	1.3	2.8	6.8	4.6	1.1	3.5	1.1	1.3
11August	23.1	21.8	8.3	1.6	0.1	1.1	2.8	4.3	0.5	1.9	1.8	1.3
31 August	21.4	21.2	6.4	1.9	0.1	1.3	1.9	5.2	0.4	1.7	1.7	1.3
12 October	14.0		3.8		0.2		1.6		0.3		1.6	

AFWC, algal fluorescence in the water column; SChl, sediment chlorophyll *a* of upper 1 cm; SChl/phaeo ratio, ratio between sediment chlorophyll *a* and sediment phaeophytin pigments; POC, particulate organic carbon content of upper 1 cm of sediment.
Differences between lake sites were significant (paired *t*-test, $P < 0.001$) for all variables.

chlorophyll), and POC in the sediments provided a general measure of algal/detrital biomass and potential food available to *Hexagenia* nymphs. Over the 3-year sampling period, algal fluorescence in the water column was significantly higher in western Lake Erie than in Lake St Clair, whereas sediment chlorophyll *a* was significantly higher in Lake St Clair (paired *t*-test, $P < 0.001$; Table 2). In Lake Erie, algal fluorescence ranged between 2 and 13 μg L^{-1} and was highest in summer months; in Lake St Clair, values ranged between 0.9 and 1.9 μg L^{-1}, and seasonal changes were not apparent. Sediment chlorophyll *a* averaged about five times higher in Lake St Clair than Lake Erie; values were higher in Lake St Clair on all but one of 15 sampling dates. Values ranged from 4.3 to 17.3 μg g^{-1} in Lake St Clair and from 1.4 to 6.8 μg g^{-1} in Lake Erie. Consistent seasonal patterns in sediment chlorophyll *a* were not apparent in either lake. In Lake St Clair, a pattern of higher sediment chlorophyll *a* occurred in spring than in summer and fall in 1999, but higher spring values were not apparent in 1998. Chlorophyll *a* concentrations in sediment at Sites 5B, 7L and 8D in Lake Erie in April 1998 were 2.4, 2.8 and 8.0 μg g^{-1}, respectively.

The ratios of chlorophyll *a*/phaeophytin in sediments were also determined and ranged between 0.2 and 1.1 in Lake Erie (mean ± SE = 0.4 ± 0.06) and 1.2 and 3.5 in Lake St Clair (mean ± SE = 2.1 ± 0.16). The ratios were consistently lower in western Lake Erie than in Lake St Clair on all sampling dates (Table 2). Chlorophyll *a*/phaeophytin ratios at other Lake Erie sites in April 1998 were 0.2, 0.3 and 1.1, at 5B, 7L and 8D, respectively. The characteristics of both chlorophyll *a* concentration and chlorophyll *a*/phaeophytin ratio in sediments at Site 8D in Lake Erie were more similar to those from Lake St Clair than the main

sampling site in Lake Erie. In addition, sediment POC was significantly higher in Lake Erie than in Lake St Clair (paired *t*-test, $P < 0.001$; Table 2). However, seasonal trends were not apparent.

Seasonal patterns in glycogen and lipids

Seasonal patterns in mean glycogen levels differed between *Hexagenia* nymphs from Lake St Clair and western Lake Erie (Fig. 2). In Lake St Clair, glycogen levels of nymphs were highest in early spring (March and April) and then declined to reach their lowest levels in summer or fall. This seasonal pattern was consistent in each of the 3 years sampled. In contrast, glycogen levels in nymphs from western Lake Erie were low in spring, increased in summer and then declined in late summer or fall. In addition, monthly glycogen levels were more variable from year-to-year in nymphs from Lake Erie, and maximum levels were not as high as in nymphs from Lake St Clair. The maximum monthly mean glycogen level measured in nymphs was about 16% dry weight in Lake St Clair, but only 10% in western Lake Erie (Fig. 2). Although seasonal patterns differed, overall mean glycogen levels were not significantly different (ANOVA, $P = 0.09$; Table 3); mean glycogen values over all 3 years were 5.8% of dry weight ($n = 150$) and 4.0% of dry weight ($n = 161$) in Lake St Clair and Lake Erie, respectively.

Seasonal patterns of mean lipid levels were similar to glycogen patterns in the two lakes (Fig. 3). Lipid levels in nymphs from Lake St Clair were highest in early spring (April and May) and then declined throughout the year to reach lowest levels in late summer or fall, and this trend was similar in all 3 years. In contrast, levels in nymphs from western Lake Erie were lower in spring, increased in summer (June–August), and declined in fall. In addition, year-to-year differences were more apparent in western Lake Erie than Lake St Clair. A strong summer peak did not occur in 1997 as in 1998 and 1999, and overall lipid levels were significantly higher in 1999 than in the other 2 years (ANOVA, $P < 0.01$). Despite differences in seasonal patterns over the 3-year study period, overall mean lipid levels in *Hexagenia* nymphs from Site 1 in Lake St Clair and Site 7M in western Lake Erie were not significantly different (ANOVA, $P = 0.20$; Table 3); mean values were 9.7 % of dry weight ($n = 517$) and 9.1% of dry weight ($n = 615$) at the two sites, respectively.

Table 3 Results of two-way ANOVA of glycogen, total lipids (proportion dry weight) and triacylglycerols (proportion of total lipids) between lakes and developmental stages (six stages of nymphs and subimagos) of *Hexagenia* at Site 1 in Lake St Clair and Site 7M in Lake Erie

Source	SS	d.f.	MS	*F*-ratio	*P*
Glycogen					
Lake	76.7	1	76.7	2.8	0.09
Stage	1189.8	3	396.6	14.7	<0.01
Lake × stage	429.1	3	143.0	5.3	<0.01
Total Lipids					
Lake	38.0	1	38.0	1.6	0.20
Stage	3640.4	5	728.1	31.1	<0.01
Lake × stage	183.6	5	36.7	1.6	0.17
Triacylglycerols					
Lake	962.5	1	962.5	4.7	0.03
Stage	40770.8	5	8154.2	39.8	<0.01
Lake × stage	455.9	5	91.2	0.4	0.82

All values are arcsine transformed.

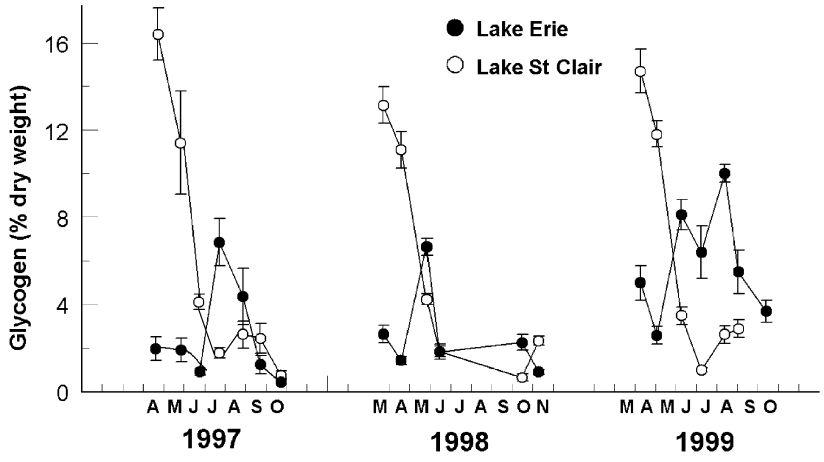

Fig. 2 Mean glycogen content (percentage dry weight ± SE) of *Hexagenia* nymphs from western Lake Erie and Lake St Clair from 1997 to 1999.

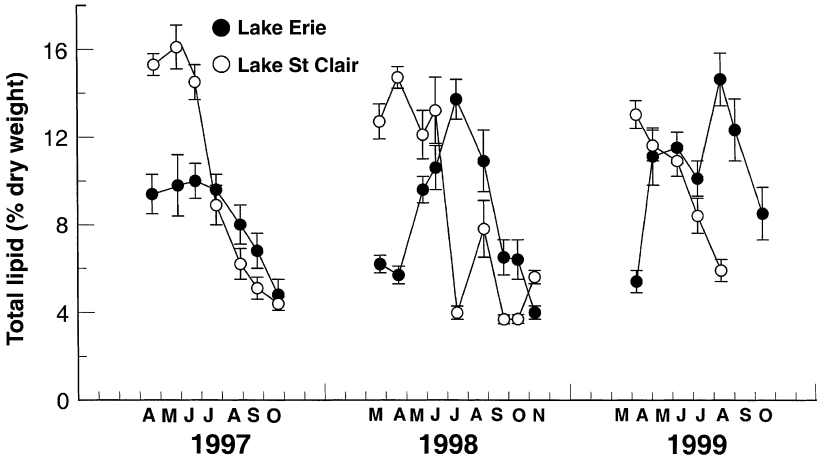

Fig. 3 Mean total lipid content (percentage dry weight ± SE) of *Hexagenia* nymphs from western Lake Erie and Lake St Clair from 1997 to 1999.

Additional sites sampled in both lakes on a few dates provided a measure of within-lake variability in lipid levels. In early May 1998, lipid levels were measured in nymphs collected at three additional sites in western Lake Erie and compared with lipids at Site 7M. Mean lipid levels at Sites 5B, 7L and 8D, were 7.0, 13.4 and 7.2% of dry weight, respectively. By comparison, mean lipid levels at Site 7M were 5.9% in mid-April and 9.7% of dry weight in late May 1998. Whereas lipid values at Sites 5B, 8D and 7M were comparable, values at Site 7L were significantly higher than at the other three sites (ANOVA, $P < 0.01$). In early May 1999, mean lipid level at Site 7L was 14.2% compared with 11.1 % of dry weight at site 7M, and this difference was significant (*t*-test, $P < 0.05$). In Lake St Clair, lipid levels at Site 1 were not significantly different (*t*-test, $P > 0.05$) from levels at a shallower site, Site 188, on each of two dates in 1999 (June 9 and July 8).

Energy reserves and developmental stage

Total lipid levels did not increase incrementally with nymphal development stage (Fig. 4a). Mean lipid levels in Stages 1–4 ranged from 6 to 10% of dry weight, but increased abruptly to 12–13% of dry weight in Stages 5 and 6. Levels in Stages 1–4 were not significantly different from each other, but all were significantly lower than in Stages 5 and 6. (ANOVA, Tukey's LSD, $P < 0.05$). Mean lipid levels in subimagos (13%) were similar to those in Stages 5 and 6. Although lipid levels over all stages were not significantly different between lakes, lipid level per stage was generally higher in Lake St Clair than in western Lake Erie (Fig. 4a). Regardless of nymphal

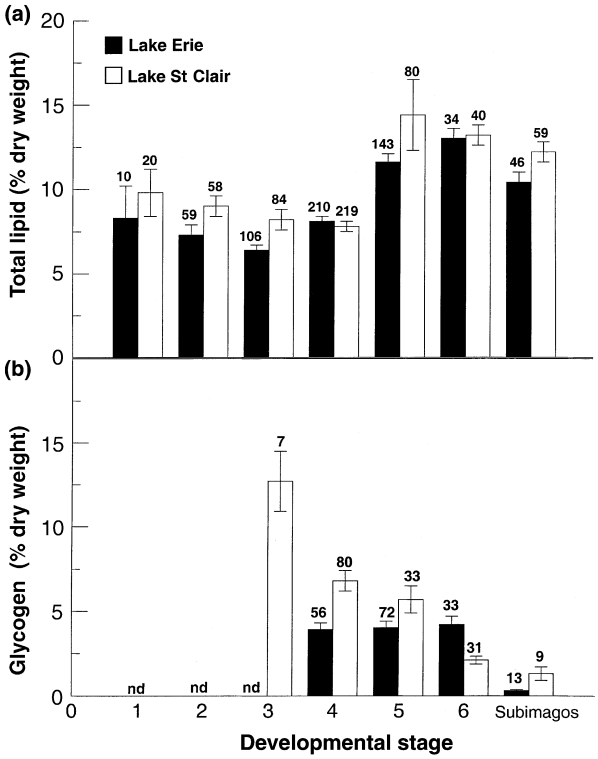

Fig. 4 Mean total lipid (a) and glycogen (b) content (percentage dry weight ± SE) of *Hexagenia* nymphs at developmental Stages 1–6 and subimagos from western Lake Erie and Lake St Clair. Above each bar are the numbers of individuals analysed.

development stage, total lipid levels in nymphs generally reflected the seasonal patterns of each lake where levels were usually higher in the spring in Lake St Clair than in Lake Erie (Fig. 5).

While patterns in lipid levels relative to developmental stages were similar for the two lakes, patterns in glycogen levels were different (Table 3; Fig. 4b). In

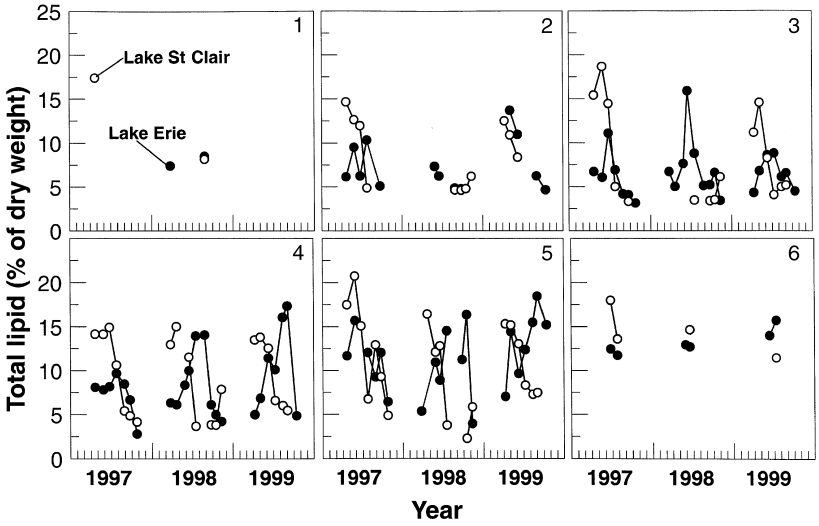

Fig. 5 Monthly mean total lipid content (percentage dry weight) of *Hexagenia* at nymphal development Stages 1–6 from western Lake Erie and Lake St Clair from 1997 to 1999.

Lake St Clair, glycogen levels in nymphs were highest in Stage 3 and then declined steadily to Stage 6 and subimagos. In Lake Erie, glycogen levels in nymphs remained the same in Stages 4, 5 and 6 and then declined after metamorphosis into subimagos (Fig. 4b).

Triacylglycerols (energy storage lipids) and phospholipids (membrane component lipids) were the most abundant lipid classes found in nymphs from both lakes, and together these two classes represented at least 75% of total lipids. When total lipids were high, triacylglycerols dominated the lipid class profile, and when total lipids were low, phospholipids dominated the lipid profile. Other lipid classes were present in minor amounts. In contrast to total lipids, there was a systematic change in lipid composition with nymphal development. In both lakes, the percentage of triacylglycerols in total lipids increased to a peak at Stage 5 and then decreased, whereas the percentage of phospholipids declined up to Stage 5 and then increased (Table 3, Fig. 6a,b). For both lakes, the mean percentage of triacylglycerols in Stages 2, 3, 4, 5 and 6 was 25.1, 39.4, 61.6, 73.2 and 56.3% of total lipids, and the mean percentage of phospholipids was 47.5, 35.8, 16.8, 9.0 and 13% of total lipids.

For the two lakes over all years, there were no significant differences ($P > 0.05$) in total lipids, triaclyglycerol, or phospholipids between male and female nymphs over all developmental stages. As subimagos were collected only once a year (during emergence) from each lake, differences between lakes and sexes were examined separately from the other stages. In both years, total lipids were higher in males

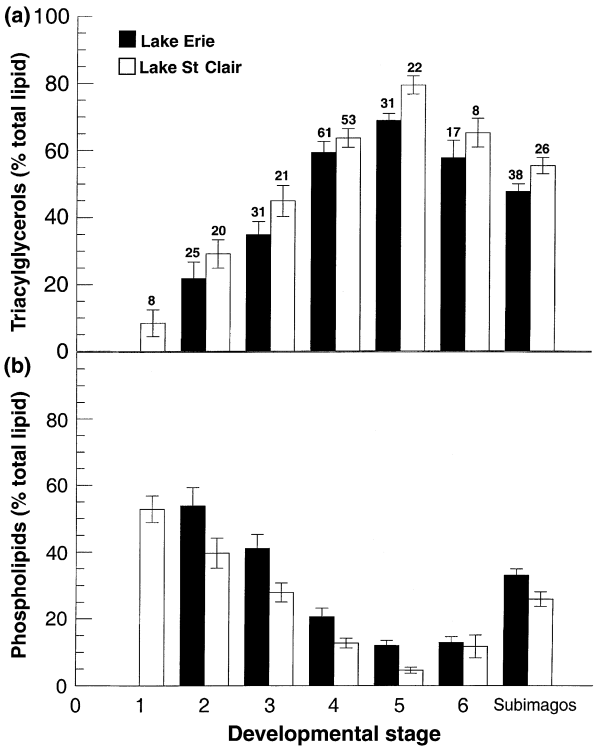

Fig. 6 Mean triacylglycerol (a) and phospholipid (b) content (percentage total lipids ± SE) of *Hexagenia* nymphs at developmental Stages 1–6 and subimagos from western Lake Erie and Lake St Clair. Above each bar are the numbers of individuals analysed for both a and b.

than females regardless of lake (Fig. 7). The difference was significant in 1997 (two-way ANOVA, $P < 0.01$) but not in 1998 ($P = 0.06$). Lipid class composition was similar in the two sexes ($P > 0.05$). In both lakes,

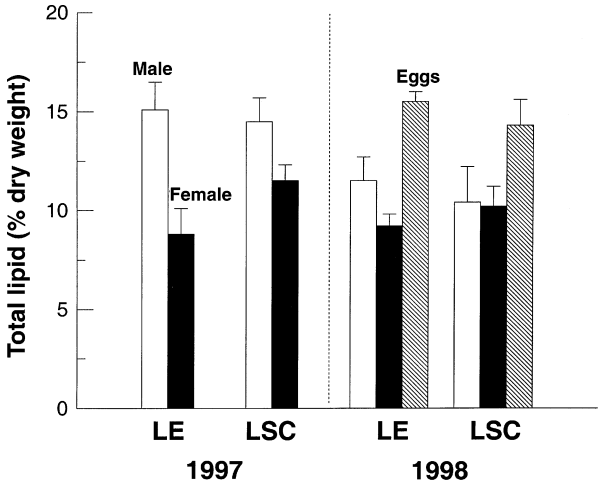

Fig. 7 Mean total lipid content (percentage dry weight ± SE) of male and female *Hexagenia* subimagos, and eggs from 1997 and 1998.

total lipid levels of eggs were higher than in female subimagos (Fig. 7).

Furthermore, there were no significant differences in lipid and glycogen content between male *H. rigida* and *H. limbata* regardless of lake (two-way ANOVA, $P > 0.05$). The differences were non-significant for each developmental stage and for all stages combined.

Seasonal patterns in length–weight relationships

From length–weight regressions determined on each sampling date, the dry weight of a standard 20-mm nymph was determined for each of the two sites sampled (Fig. 8). Length–dry weight relationship of nymphs from Lake St Clair was ln(dry weight) = −5.779 + 2.844 ln(length) ($n = 511$; $r^2 = 0.932$) whereas the relationship for nymphs from Lake Erie was −5.890 + 2.862 ln(length) ($n = 574$; $r^2 = 0.938$). Seasonal patterns of dry weights were similar to patterns observed for lipid levels. Weight of a standard 20-mm nymph from Lake St Clair was highest in spring and declined during summer and fall during all 3 years. In Lake Erie, the weight of nymphs was lower in spring than those from Lake St Clair; however, in summer, weights remained lower than animals from Lake St Clair in 1997, but were higher than Lake St Clair in 1998 and 1999. There was no significant difference in length–weight relationships between the two lakes over all years combined (ANCOVA, $P > 0.05$).

Discussion

Seasonal patterns of energy reserves (lipid and glycogen) and length–weight relationships of *Hexagenia* nymphs were consistently different between Lake St Clair and western Lake Erie from 1997 to 1999. In Lake St Clair, energy reserves and length–weight relationships were highest in early spring and subsequently declined, whereas in western Lake Erie, energy reserves and length–weight relationships peaked in mid-summer and then declined. These different patterns in energy reserves during the year may be related to sources of food and water temperatures (Rosillon, 1988).

Hexagenia burrows in the upper sediments and ingests organic material settled from the water column or produced on the sediment surface (Zimmerman & Wissing, 1978; Charbonneau & Hare, 1998).

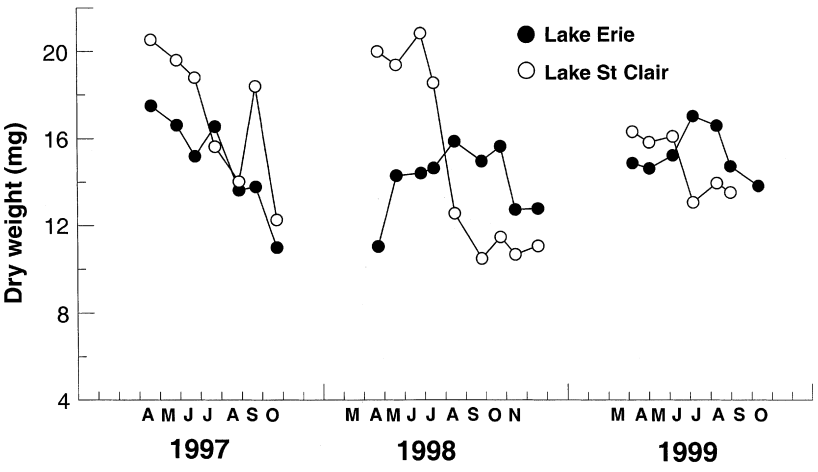

Fig. 8 Dry weight of a standard 20-mm *Hexagenia* nymph from western Lake Erie and Lake St Clair. Weights were derived from a length–weight regression determined for each sampling date.

Relatively high sediment chlorophyll *a* concentrations and chlorophyll *a*/phaeophytin ratios in the spring in Lake St Clair compared with western Lake Erie suggests that more benthic algae/periphyton would be available as fresh food over winter/early spring, leading to relatively high levels of energy reserves in nymphs in Lake St Clair during this period. Good light penetration through the water column, as measured by secchi depth, may provide better conditions for benthic algae/periphyton growth in Lake St Clair than in western Lake Erie. High glycogen levels measured each spring in nymphs from Lake St Clair are especially indicative of available food, otherwise this energy reserve, that can be mobilised quickly, would not be present at the levels found in the nymphs. In addition, it is conceivable that *Hexagenia* in Lake St Clair were consuming settled diatoms that originated in Lake Huron during the spring bloom. Phytoplankton biomass and composition in Lake St Clair are generally similar to those of Lake Huron (Munawar, Munawar & Sprules, 1991). The rapid flow of water from Lake Huron via the St Clair River flushes the lake on average every 9 days (Leach, 1991) and would renew the phytoplankton community more often than in western Lake Erie. Laboratory studies indicate that the growth and reproduction of some species of mayflies is enhanced on an algal/diatom (periphyton) based diet when compared with mayfly nymphs fed an aged leaf or detritus diet (Bird & Kaushik, 1984; Webb & Merrit, 1987; Rosillon, 1988; Gupta, Michael & Gupta, 1993).

In Lake Erie, energy reserves and length–weight relationships of nymphs peaked in summer. Thus, nymphs in this lake may depend more on material settled from the water column in late spring. Relatively higher algal fluorescence was observed in the water column of Lake Erie than in Lake St Clair in April or late May/early June. However, the low chlorophyll *a*/phaeophytin ratios found in Lake Erie sediments suggest that food available to *Hexagenia* nymphs may be more detrital in nature than the food in Lake St Clair (Table 2). One potential reason for this may be the filtering activity of zebra mussels (*Dreissena polymorpha*) that results in the processing of water column algae into faeces and pseudofaeces. In western Lake Erie, Klerks, Fraleigh & Lawniczak (1996) found that zebra mussels increased settling rate of particulate material from the water column to sediments and probably increased sediment organic content. The influence of mussels is likely to be greatest in late spring when filtering rates tend to peak (Fanslow, Nalepa & Lang, 1995). Nymphs may have to consume more detritus- or zebra mussel-processed material to achieve the same nutritional value as the relatively fresh benthic algae/periphyton found in Lake St Clair (Gupta *et al*., 1993). Although zebra mussels are also abundant in Lake St Clair (Nalepa *et al*., 1996b), the rapid flushing rate of the lake may prevent their faeces and pseudofaeces from accumulating as readily in the sediments as in western Lake Erie.

The different water temperature at Site 1 in Lake St Clair and Site 7M in western Lake Erie may partially explain differences in seasonal energy reserve patterns of *Hexagenia* at the two sites. Water temperature affects rates of activity, feeding and growth of aquatic insects (Sweeney, 1984; Giberson & Rosenberg, 1992). In Lake Erie, during spring, higher water temperatures may have led to increased feeding and food assimilation rates that were followed by increased glycogen and lipid accumulation in nymphs. Therefore, the water temperature in Lake Erie may have allowed the nymphs to accumulate energy reserves to the level necessary for their metamorphosis in late June/early July, although their food appeared to be available later in spring and of a more decomposed nature than food available to nymphs in Lake St Clair. In Lake St Clair, it is possible that energy derived from food available to *Hexagenia* in the winter/early spring period was stored as glycogen and lipid while water temperature was low and used later for growth when water temperature increased (Rosillon, 1988).

Density can affect nymphal growth through competition for food and burrowing sites (Rosillon, 1988; Hanes & Ciborowski, 1992), and therefore could also affect energy reserves. Densities were highest in both lakes in 1997 (Schloesser & Nalepa, 2001). Mean annual densities in 1997, 1998 and 1999 were 1190, 442 and 540 m^{-2} in western Lake Erie and 720, 518 and 438 m^{-2} in Lake St Clair (ibid). In 1997, the relatively high density of nymphs in Lake Erie may have had negative effects on their ability to accumulate energy reserves. Negative impacts on *Hexagenia* growth have been observed at densities over 1000 m^{-2} under laboratory conditions (Hanes & Ciborowski, 1992). Generally, lipids in *Hexagenia* from Lake Erie were lowest when the density was highest in 1997. In Lake St Clair, during 1997, densities were lower than in

Lake Erie and possibly at a level that would not cause negative impacts. In 1998 and 1999, nymphal densities declined and were similar in both lakes with some seasonal differences (Schloesser & Nalepa, 2001). As maximum lipids in 1998 and 1999 were similar in both lakes (although they occurred at different times of the year), perhaps densities in these 2 years were more at equilibrium with available food resources.

Although seasonal differences in lipid stores occurred regardless of developmental stage, there were distinct trends associated with nymph growth. In the early stages of development, when growth is rapid (Meyer, 1990), total lipids and the energy storage lipid, triacylglycerol, were relatively low. Thus, it appears that other biochemical components (i.e. proteins and carbohydrates) and cell membrane lipids (i.e. phospholipids) may be more important to nymphs at this time. Highest lipid stores were found in the later two stages of development (Stages 5 and 6). During these developmental stages, stored lipid is probably metabolised for energy and then converted to other biochemical components for metamorphosis into subimagos and the development of gonads (Meyer, 1990). As subimagos do not feed, further stored energy will be used for flying and for the final moult to fully reproductive imagos. In subimagos, lipids were generally higher in males than females, and lipids in eggs were higher than in both males and females. More lipid reserves in males may be beneficial as a source of energy during swarming flights in search of mates (Sartori *et al*., 1992). In contrast, females need to support developing eggs and, therefore, may transfer stored lipid as well as yolk protein to eggs that is converted from lipids. In support of this, a study of body compounds in the stream mayfly *Epeorus sylvicola* found that females had higher protein levels as well as lower lipid levels than males in the later nymphal stage when eggs were beginning to develop (Meyer, 1990).

In conclusion, the different seasonal patterns in glycogen and lipids in *Hexagenia* from Lake St Clair and western Lake Erie demonstrate an adaptive ability towards the use of differently timed food resources in two lakes of close proximity. This ability to optimise energy reserves to adjust to food resources may play a role in the ability of *Hexagenia* to inhabit a wide range in the temperate zone of North America from southern United States to the near arctic (McCafferty, 1975; Giberson & Rosenberg, 1994).

References

Arrese E.L., Canavoso L.E., Jouni Z.E., Pennington J.E., Tsuchida K. & Wells M.A. (2001) Lipid storage and mobilization in insects: current status and future directions. *Insect Biochemistry and Molecular Biology*, **31**, 7–17.

Beenakkers A.M.Th., Van der Horst D.J. & Van Marrewijk W.J.A. (1985) Insect lipids and lipoproteins, and their role in physiological processes. *Progress in Lipid Research*, **24**, 19–67.

Beeton A.M. (1969) Changes in the environment and biota of the Great Lakes. In: *Eutrophication: Causes, Consequences, Correctives*, pp. 150–187. *Proceedings of a Symposium of the National Academy of Sciences*, Washington, DC, 661 pp.

Bell J.G., Ghioni C. & Sargent J.R. (1994) Fatty acid compositions of 10 freshwater invertebrates which are natural food organisms of Atlantic salmon parr (*Salmo salar*): a comparison with commercial diets. *Aquaculture*, **128**, 301–313.

Bird G.A. & Kaushik N.K. (1984) Survival and growth of early-instar nymphs of *Ephemerella subvaria* fed various diets. *Hydrobiologia*, **119**, 227–233.

Britt N.W. (1955) *Hexagenia* (Ephemeroptera) population recovery in western Lake Erie following the 1953 catastrophe. *Ecology*, **36**, 520–522.

Cargill A.S., Cummins K.W., Hanson B.J. & Lowry R.P. (1985) The role of lipids, fungi, and temperature in the nutrition of a shredder caddisfly, *Clistoronia magnifica*. *Freshwater Invertebrate Biology*, **4**, 64–78.

Carr J.F. & Hiltunen J.K. (1965) Changes in the bottom fauna of Lake Erie from 1930 to 1961. *Limnology and Oceanography*, **10**, 551–569.

Charbonneau P. & Hare L. (1998) Burrowing behavior and biogenic structures of mud-dwelling insects. *Journal of the North American Benthological Society*, **17**, 239–249.

Edsall T.A. (2001) Burrowing mayflies (*Hexagenia*) as indicators of ecosystem health. *Aquatic Ecosystem Health and Management Society*, **4**, 283–292.

Edsall T.A., Madenjian C.P. & Manny B.A. (1999) Burrowing mayflies in Lake Erie – a review. In: *State of Lake Erie (SOLE): Past, Present and Future* (Ed. M. Munawar), pp. 210–231. Backhuys, Leiden, The Netherlands.

Fanslow D.L., Nalepa T.F. & Lang G.A. (1995) Filtration rates of the zebra mussel (*Dreissena polymorpha*) on natural seston from Saginaw Bay, Lake Huron. *Journal of Great Lakes Research*, **21**, 489–500.

Gardner W.S., Frez W.A., Cichocki E.A. & Parrish C.C. (1985) Micromethod for lipids in aquatic invertebrates. *Limnology Oceanography*, **30**, 1100–1105.

Ghioni C., Bell J.G. & Sargent J.R. (1996) Polyunsaturated fatty acids in neutral lipids and phospholipids of some freshwater insects. *Comparative Biochemistry and Physiology*, **114**, 161–170.

Giberson D.J. & Rosenberg D.M. (1992) Effects of temperature, food quality, and nymphal rearing density on life-history traits of a northern population of Hexagenia (Ephemeroptera: Ephemeridae). *Journal of the North American Benthological Society*, **11**, 181–193.

Giberson D.J. & Rosenberg D.M. (1994) Life histories of burrowing mayflies (*Hexagenia limbata* and *H. rigida*, Ephemeroptera: Ephemeridae) in a northern Canadian reservoir. *Freshwater Biology*, **32**, 501–518.

Goedkoop W. & Johnson R.K. (1996) Pelagic-benthic coupling: profundal benthic community response to spring diatom deposition in mesotrophic Lake Erken. *Limnology and Oceanography*, **41**, 636–647.

Goedkoop W., Sonesten L., Markensten H. & Ahlgren G. (1998) Fatty acid biomarkers show dietary differences between dominant chironomid taxa in Lake Erken. *Freshwater Biology*, **40**, 135–143.

Gupta S., Michael R.G. & Gupta A. (1993) Influence of diet on growth, food retention time, and gill ventilation rate of nymphs of *Cloeon* sp. (Ephemeroptera: Baetidae). *Hydrobiologia*, **271**, 41–44.

Hanes E.C. & Ciborowski J.J.H. (1992) Effects of density and food limitation on size and mortality of larval *Hexagenia rigida* (Ephemeroptera: Ephemeridae). *Canadian Journal of Zoology*, **70**, 1824–1832.

Hanson B.J., Cummins K.W., Cargill A.S. & Lowry R.R. (1983) Dietary effects on lipid and fatty acid composition of *Clistoronia magnifica* (Tricoptera: Limnophilidae). *Freshwater Invertebrate Biology*, **2**, 2–15.

Hansson L.-A. (1988) Chlorophyll *a* determination of periphyton on sediments: identification of problems and recommendation of method. *Freshwater Biology*, **20**, 347–352.

Heise B.A., Flannagan J.F. & Galloway T.D. (1987) Life histories of *Hexagenia limbata* and *Ephemera simulans* in Dauphin Lake Manitoba. *Journal of the North American Benthological Society*, **6**, 230–240.

Klerks P.L., Fraleigh P.C. & Lawniczak J.E. (1996) Effects of zebra mussels (*Dreissena polymorpha*) on seston levels and sediment deposition in western Lake Erie. *Canadian Journal of Fisheries and Aquatic Sciences*, **53**, 2284–2291.

Krieger K.A., Schloesser D.W., Manny B.A., Trisler C.E., Heady S.E., Ciborowski J.J.H. & Muth K.M. (1996) Recovery of burrowing mayflies (Ephemeroptera: Ephemeridae: *Hexagenia*) in western Lake Erie. *Journal of Great Lakes Research*, **22**, 254–263.

Landrum P.F. & Poore R. (1988) Toxicokinetics of selected xenobiotics in *Hexagenia limbata*. *Journal of Great Lakes Research*, **14**, 427–437.

Leach J.H. (1991) Biota of Lake St Clair: habitat evaluation and environmental assessment. *Hydrobiologia*, **219**, 187–202.

Madenjian C.P., Schloesser D.W. & Krieger K.A. (1998) Population models of burrowing mayfly recolonization in western Lake Erie. *Ecological Applications*, **8**, 1206–1212.

McCafferty W.P. (1975) The burrowing mayflies of the United States (Ephemeroptera: Ephemeridae). *Transactions of the American Entomology Society*, **101**, 447–504.

Meier G.M., Meyer E.I. & Meyns S. (2000) Lipid content of stream macroinvertebrates. *Archiv fur Hydrobiologie*, **147**, 447–463.

Meyer E. (1990) Levels of major body compounds in nymphs of the stream mayfly *Epeorus sylvicola* (PICT.) (Ephemeroptera: Heptageniidae). *Archiv fur Hydrobiologie*, **117**, 497–510.

Munawar M., Munawar I.F. & Sprules W.G. (1991) The plankton ecology of Lake St Clair, 1984. *Hydrobiologia*, **219**, 203–227.

Nalepa T.F., Fahnenstiel G.F., McCormick M.J., Johengen T.H., Lang G.A., Cavaletto J.F. & Goudy G. (1996a) Physical and chemical variables of Saginaw Bay, Lake Huron in 1991–1993. *National Oceanic and Atmospheric Administration Technical Memorandum ERL GLERL-91*, p. 78.

Nalepa T.F., Hartson D.J., Gostenik G.W., Fanslow D.L. & Lang G.A. (1996b) Changes in the freshwater mussel community of Lake St Clair: from Unionidae to *Dreissena polymorpha* in eight years. *Journal of Great Lakes Research*, **22**, 354–369.

Neave F. (1932) A study of the may flies (*Hexagenia*) of Lake Winnipeg. *Contributions to Canadian Biology and Fisheries* **7**, 177–201.

Parrish C.C. (1987) Separation of aquatic lipid classes by chromarod thin-layer chromatography with measurement by Iatroscan flame ionization detection. *Canadian Journal of Fish Aquatic Sciences*, **44**, 350–356.

Roehrig K.L. & Allred J.B. (1974) Direct enzymatic procedure for the determination of liver glycogen. *Analytical Biochemistry*, **58**, 414–421.

Rosillon D. (1988) Food preference and relative influence of temperature and food quality on life history characteristics of a grazing mayfly, *Ephemerella ignita* (Poda). *Canadian Journal of Zoology*, **66**, 1474–1481.

Ruffieux L., Elouard J.M. & Sartori M. (1998) Flightlessness in mayflies and its relevance to hypotheses on the origin of insect flight. *Proceedings of the Royal Society of London*, **265**, 2135–2140.

Sartori M., Keller L., Thomas A.G.B. & Passera L. (1992) Flight energetics in relation to sexual differences in the mating behaviour of a mayfly, *Siphlonurus aestivalis*. *Oecologia*, **92**, 172–176.

Schloesser D.W. & Nalepa T.F. (2001) Changing abundance of *Hexagenia* mayfly nymphs in western Lake Erie of the Laurentian Great Lakes: impediments to assessment of lake recovery? *International Review of Hydrobiology*, **86,** 87–103.

Schloesser D.W., Edsall T.A., Manny B.A. & Nichols S.J. (1991) Distribution of *Hexagenia* nymphs and visible oil in the sediments of the upper Great Lakes connecting channels. *Hydrobiologia*, **219**, 345–352.

Schloesser D.W., Krieger K.A., Ciborowski J.J.H. & Corkum L.D. (2000) Recolonization and possible recovery of burrowing mayflies (Ephemeroptera: Ephemeridae: *Hexagenia* spp.) in Lake Erie of the Laurentian Great Lakes. *Journal of Aquatic Ecosystem Stress and Recovery*, **8**, 125–141.

Sweeney B.W. (1984) Factors influencing life history patterns of aquatic insects. In: *The Ecology of Aquatic Insects* (Eds V.H. Resh & D.M. Rosenberg), pp. 56–100. Praeger, New York.

Webb K.M. & Merrit R.W. (1987) The influence of diet on the growth of *Stenonema vicarium* (Walker) (Ephemeroptera: Heptoageniidae). *Hydrobiologia*, **153**, 253–259.

Zimmerman M.C. & Wissing T.E. (1978) Effects of temperature on gut-loading and gut-clearing times of the burrowing mayfly, *Hexagenia limbata*. *Freshwater Biology*, **8**, 269–277.

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