

Use of Physiological Parameters for Determining Condition
of Burrowing Mayflies (*Hexagenia* spp.)

A Final Report to the Lake Erie Protection Fund
for project SG17-95

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Currently, much interest has been generated by the recolonization of western Lake Erie sediments by burrowing mayflies of the genus *Hexagenia* (Krieger et al. 1996). Furthermore, recovery of these mayflies has been proposed as a benthic invertebrate indicator of the re-establishment of mesotrophic conditions in regions of the Great Lakes that have suffered cultural eutrophication (Reynoldson et al. 1989). Studies of freshwater benthic invertebrates usually measure density of target organisms, but physiological measures of condition are less routinely employed. Often, changes in physiological condition of organisms is a more sensitive indicator of stress than is a decrease in density and changes in condition allow prediction of changes in density (Haag et al. 1993). Furthermore, knowledge of condition allows one to infer the mechanism driving changes in density. Use of physiological measures of condition provides an alternative to simple density measurements for management agencies seeking to assess the quality of habitat and forage for benthic-feeding sport fishes such as yellow perch.

Objectives

We proposed three objectives for this project.

- Refine spectrophotometric techniques for measurement of total glycogen and total lipids in *Hexagenia*.
- Conduct a laboratory experiment measuring effects of resource (food) levels on condition (weight-specific total lipid or glycogen content).
- Measure total glycogen and total lipid content in *Hexagenia* from five sites across western Lake Erie.

All three objectives were met or exceeded over the course of the project.

Methods

We were able to utilize spectrophotometric methods from the published scientific literature, with very few modifications, for analysis of total glycogen and lipid content in *Hexagenia* nymphs. We used a phenol-sulfuric acid method for glycogen analyses (Naimo et al. 1998) and a vanillin-phosphoric acid assay for total lipids (Van Handel 1985).

A laboratory experiment was used to assess the effects of food level on weight-specific glycogen and lipid content in *Hexagenia* nymphs. *Hexagenia* eggs were obtained from Jan Ciborowski of the University of Windsor. Eggs were allowed to hatch at room temperature and nymphs were placed in oxygenated, 120 ml cups (1 nymph per cup) that contained 6 cm of Portage River sediments and 60 ml of dechlorinated tap water. Fifty nymphs were assigned to each of three food treatments: low (0.5 X), medium (X), and high (5X). These food levels were based on standard protocols for laboratory rearing of *Hexagenia* (Ciborowski et al. 1992) and represented an order of magnitude difference between low and high levels. Nymphs were raised at these food levels for approximately three months. At the end of the experiment, individuals were flash frozen in liquid nitrogen and stored at -80°C until analyzed. For each treatment, 50% of the individuals were analyzed for glycogen and 50% for lipids. Total length, head capsule width, and wet weight were measured before analysis.

Hexagenia nymphs were collected from six sites in the western basin of Lake Erie in May-June, 1997 (Figure 1). Nymphs were collected with an Ekman dredge and flash frozen in liquid nitrogen and stored at -80°C until analyzed. For each site, approximately 50% of the individuals were analyzed for glycogen and 50% for lipids. Total length, head capsule width, and wet weight were measured before analysis.

Results

Laboratory Experiment: Survival was inversely proportional to food level, with 62% of low food, 52% of medium food, and 20% of high food treatment animals surviving. Total wet mass was significantly different among food treatments (1-way ANOVA, $F_{2, 63} = 122.65$, $p < 0.0001$), with high food animals being largest and low food animals smallest (Table 1). Similar results were seen for total length and head capsule width. Weight-specific glycogen levels were also significantly different among treatments (1-way ANOVA, $F_{2, 32} = 16.01$, $p < 0.0001$), with glycogen level being directly proportional to food level (Table 2; Bonferroni / Dunn multiple comparison test with experimentwise error rate of $\alpha = 0.05$). Weight-specific lipid levels were not significantly different among treatments (Table 3; 1-way ANOVA, $F_{2, 28} = 1.06$, $p = 0.3607$).

Field survey: Geographic variation in glycogen and lipid levels in western Lake Erie was assessed by measuring concentrations in nymphs from six sites. Glycogen levels varied significantly across sites (1-way ANOVA, $F_{5, 142} = 14.87$, $p < 0.0001$), with

average glycogen content ranging from 7.1% to 11.6% (Table 4). Lipid levels were not significantly different across sites (1-way ANOVA, $F_{5, 148} = 1.56$, $p = 0.1761$), with mean values between 0.40% and 0.75% (Table 5).

Discussion

The results of the experiment confirm that glycogen levels do respond to food levels in the environment and thus, glycogen is a good indicator for measuring the response of *Hexagenia* to variations in food availability in the environment. Because glycogen is the primary means for storage of carbohydrates (and thus, energy) in insects, high glycogen levels represent high fitness of individuals. Thus, glycogen levels can be used to measure the health of *Hexagenia* in response to environmental conditions.

The lack of a relationship between weight-specific lipid levels and food levels indicates that total lipid concentration is not a good tool for measuring the response of *Hexagenia* to environmental conditions such as food availability. It is quite likely that many lipids in *Hexagenia* are structural rather than energy storage molecules and as such, will not change concentration in response to changing food levels. In addition, weight-specific lipid concentrations had significantly higher coefficients of variation than did weight-specific glycogen concentrations in both the experiment (Mann-Whitney test, $U = 0$, $p = 0.0495$, $n = 6$) and the field survey (M-W, $U = 1$, $p = 0.0065$, $n = 12$). Thus, lipid levels were highly variable within experimental treatments and within survey sites. Because eggs contain large amounts of lipid, and female nymphs contain eggs well before emergence, lipid concentration would also reflect gender and reproductive condition of nymphs.

Our results are similar to those found in a study of the response of freshwater mussels (Unionidae) to biofouling by *Dreissena* (Haag et al. 1993). When fouled by zebra mussels, native mussels had lower glycogen levels than did unfouled controls. However, lipid levels were not related to fouling by zebra mussels. The authors reached a conclusion similar to ours: glycogen concentration is a useful indicator of fitness that represents response of an organism to environmental conditions, while lipid concentration does not appear to change with variation in environmental conditions.

Glycogen levels are a useful tool for measuring condition of *Hexagenia*. Such a tool can be used to 1) determine the relative conditions of populations from various locations in Lake Erie; 2) based on condition of individuals, predict which populations are most likely to continue to expand; 3) relate condition to food availability, sediment type and other ecological factors; 4) provide management agencies and other researchers with a tool that allows rapid assessment of condition of recolonizing *Hexagenia* populations. Use of this physiological measure of condition will provide an alternative to simple density measurements for management agencies seeking to assess the quality of habitat and forage for benthic-feeding sport fishes such as yellow perch.

Project Outputs

Results generated by this project have been presented at the 1998 annual meetings of the International Association for Great Lakes Research and the Ohio Lake Management Society. These results will be incorporated into a manuscript that will be submitted to a peer-reviewed scientific journal within the next year. In addition, this work served as a pilot project that resulted in the awarding of a \$50,367 grant by the Ohio Sea Grant College Program for the study of interactions between *Hexagenia* and zebra mussels. A critical factor in the awarding of the Sea Grant was the use of physiological indicators of fitness developed by this project.

Literature Cited

- Ciborowski, J. J. H., E. C. Hanes, & L. D. Corkum. 1992. Standardized rearing materials and procedures for *Hexagenia*, a benthic aquatic bioassay organism. Semiannual report prepared for the Research Advisory Committee, Ontario Ministry of the Environment, Toronto, Ontario.
- Haag, W. R., D. J. Berg, D. W. Garton, & J. L. Farris. 1993. Reduced survival and fitness of native bivalves in response to fouling by the introduced zebra mussel (*Dreissena polymorpha*) in western Lake Erie. *Canadian Journal of Fisheries and Aquatic Sciences* 50: 13-19.
- Krieger, K. A., D. W. Schloesser, B. A. Manny, C. E. Trisler, S. E. Heady, J. J. H. Ciborowski, & K. M. Muth. 1996. Recovery of burrowing mayflies (Ephemeroptera: Ephemeridae: *Hexagenia*) in western Lake Erie. *J. Great Lakes Res.* 22: 254-263.
- Naimo, T. J., E. D. Damschen, R. G. Rada, & E. M. Monroe. 1998. Nonlethal evaluation of the physiological health of unionid mussels: methods for biopsy and glycogen analysis. *J. N. Am. Benthol. Soc.* 17: 121-128.
- Reynoldson, T. B., D. W. Schloesser, & B. A. Manny. 1989. Development of a benthic invertebrate objective for mesotrophic Great Lakes waters. *J. Great Lakes Res.* 15: 669-686.
- Van Handel, E. 1985. Rapid determination of total lipids in mosquitoes. *J. Am. Mosq. Control Assoc.* 1: 302-304.

Table 1. Wet weight of *Hexagenia* nymphs among three food treatments. Means are significantly different among treatments.

Food Level	n	mean (g)	std. error
Low	30	0.011	0.001
Medium	26	0.035	0.002
High	10	0.095	0.01

Table 2. Percent glycogen concentration of *Hexagenia* nymphs among three food treatments. Means are significantly different among treatments.

Food Level	n	mean (%)	std. error
Low	16	7.98	0.60
Medium	14	11.65	0.78
High	5	15.21	1.34

Table 3. Percent lipid concentration of *Hexagenia* nymphs among three food treatments. Means are not significantly different among treatments.

Food Level	n	mean (%)	std. error
Low	14	0.51	0.17
Medium	12	0.25	0.03
High	5	0.47	0.08

Table 4. Percent glycogen concentration of *Hexagenia* nymphs among from western basin sites. Significant geographic variation exists.

Site	n	mean (%)	std. error
1a	25	7.73	0.28
1b	25	9.04	0.27
2	24	11.65	0.63
3	26	7.09	0.46
4	22	9.62	0.76
5	26	11.00	0.29

Table 5. Percent lipid concentration of *Hexagenia* nymphs among from western basin sites. Sites are not significantly different.

Site	n	mean (%)	std. error
1a	19	0.40	0.03
1b	27	0.61	0.06
2	28	0.58	0.05
3	25	0.53	0.05
4	25	0.75	0.19
5	30	0.50	0.04

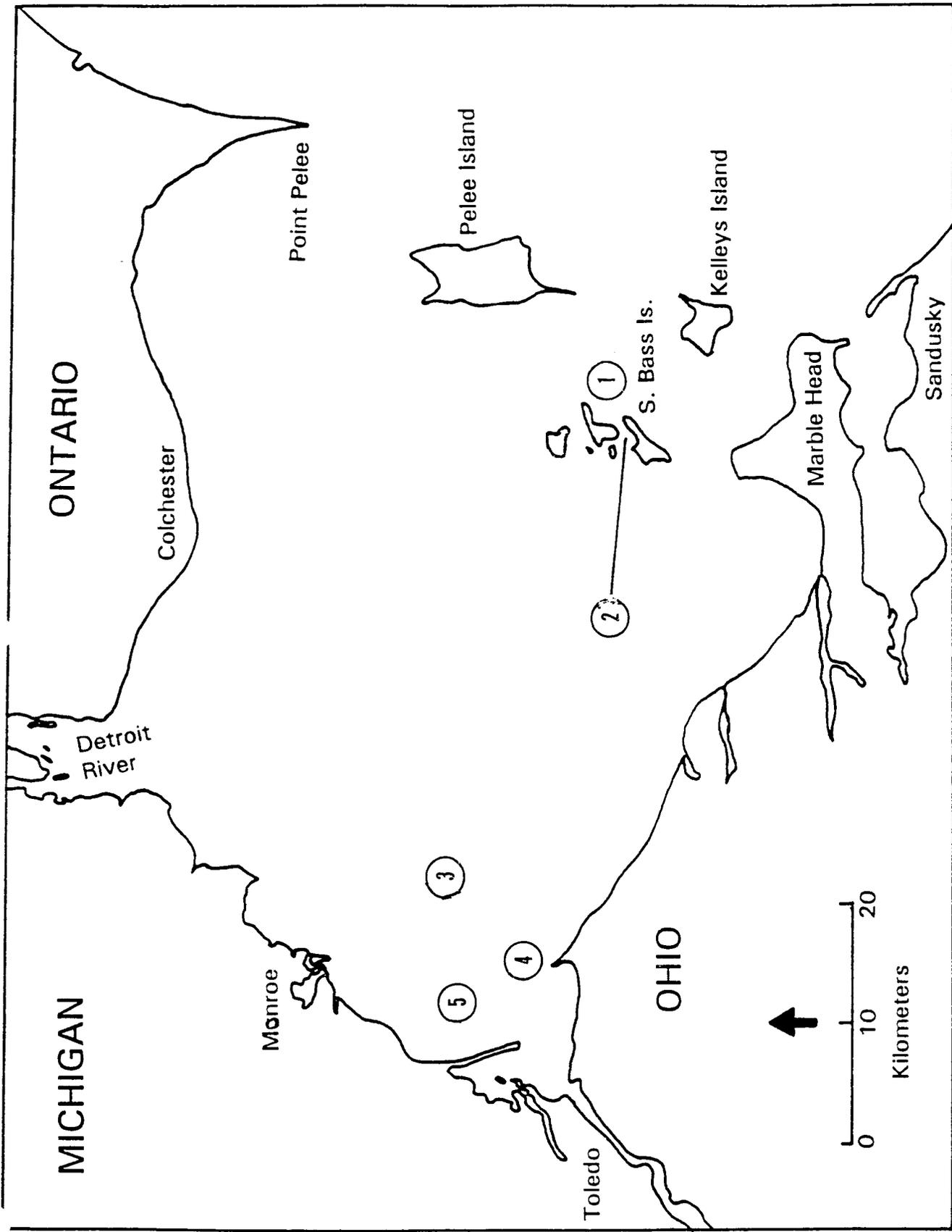


Figure 1. Sites in western Lake Erie sampled during the field survey. "Site 1" identifies two sites, 1a and 1b.

Final Lake Erie Protection Fund Small Grant Accounting Sheet

	Budgeted Amount	Amount Expended	Amount Not Expended
A. Salaries & Wages	\$ <u>1,530.00</u>	\$ <u>1,123.70</u>	\$ <u>406.30</u>
B. Fringe Benefits	\$ <u>46.00</u>	\$ <u>34.98</u>	\$ <u>11.02</u>
TOTAL PERSONNEL	\$ <u>1,576.00</u>	\$ <u>1,158.68</u>	\$ <u>417.32</u>
C. Permanent Equipment	\$ _____	\$ _____	\$ _____
D. Expendable Supplies & Equipment	\$ <u>1,750.40</u>	\$ <u>2,003.77</u>	\$ <u>(253.37)</u>
E. Travel	\$ <u>660.00</u>	\$ <u>725.34</u>	\$ <u>(65.34)</u>
F. Publications & Presentations	\$ _____	\$ _____	\$ _____
G. Other Costs	\$ _____	\$ <u>98.61</u>	\$ <u>(98.61)</u>
TOTAL DIRECT COSTS (A through G)	\$ <u>2,410.40</u>	\$ <u>2,827.72</u>	\$ <u>(417.32)</u>
INDIRECT COSTS	\$ <u>996.60</u>	\$ <u>996.60</u>	\$ <u>0.00</u>
TOTAL COSTS	\$ <u>4,983.00</u>	\$ <u>4,983.00</u>	\$ <u>0.00</u>

CERTIFICATION

I certify that the grant expenditures listed and the description of the charges are true and accurate to the best of my knowledge. These expenditures represent approved grant costs that have been previously paid and for which complete documentation is on file.

PROJECT DIRECTOR: David J. Len DATE: June 26, 1998
 AUTHORIZING OFFICER: Harold Gibbons DATE: June 26, 1998
 FISCAL AGENT: Harold Gibbons DATE: June 26, 1998