Refining and Implementing the Mayfly *(Hexagenia)* Metric of the Lake Erie Quality Index

Proceedings of a Workshop
Held 8-9 February 2002 at Heidelberg College, Tiffin, Ohio

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Summary and Recommendations

Mayfly and fisheries biologists and managers convened at Heidelberg College on 8 and 9 February 2002 to address specific issues related to the refinement of the Hexagenia *mayfly metric of the Lake Erie Quality Index as it is presented in the 1998* State of the Lake Report *of the Ohio Lake Erie Commission. The participants are actively engaged in research or surveys on the ecology, distribution, and abundance of* Hexagenia *nymphs and adults, as well as fisheries surveys in the western and central basins of Lake Erie. The sessions began by addressing conceptual foundations linking mayfly ecology to management objectives for Lake Erie, followed by presentations of (1) ongoing Lake Erie monitoring programs being conducted by U.S. and Canadian entities; and (2) appropriate sampling methods, with special attention to reaching consensus on a common methodology.*

Although biologists historically have documented the distribution and densities of nymphs, it was suggested that productivity of nymphs or estimates of winged stages on shore may be better measures of ecological status. A repeated theme was that too few years of data are available since Hexagenia *began to repopulate the western basin in the 1990s to know the extent of natural annual variations in density or biomass or to know if the carrying capacity of the basin has been reached yet. Thus, though a target of 500 nymphs per square meter of sediment was originally established for the LEQI, it may be too soon to state what the target number of nymphs should be. Therefore, it may only be possible to establish a correct target number via continued annual monitoring.*

The thought was expressed that rather than a maximum number, "sustainability" of a given population density should be the goal. Furthermore, no single index value can be applied to all three basins of the lake, as the productivity and habitat conditions are different in each. Jan Ciborowski has divided the western basin into five zones that presently appear to differ from each other in annual densities, and these zones might serve as the basis for studying consistent differences in densities that have been observed annually within the basin. Although there seems to be no solid rationale for selecting specific index (reference) stations at this time, it might be realistic to select one or a few stations within each zone in place of the entire grid of stations across the basin.

The primary factor that controls the survival and growth of Hexagenia *apparently is oxygen depletion. The question was raised as to whether* Hexagenia *densities are really tied to phosphorus loading to the lake, and the general consensus was that indirectly they are. It appears unlikely that lake management will be aimed at sustaining a target density of* Hexagenia*, but rather at maintaining other lake qualities, such as high water clarity, lack of visible algal blooms, and sustainable yields of walleye and yellow perch.*

The topics for which it was hoped a consensus would be reached at this workshop, and the outcomes of the associated presentations and discussions, are summarized in Table 1.

Table 1. Consensus reached or outcome of discussions on workshop topics.

	Topic	Consensus or Outcome
1	**Reference ("master") stations for annual *Hexagenia* surveys**	**Not practical at this time because of large relative variations annually between stations; representative stations within "zones" of the western basin may be best approach**
2	**Interval for repetition of basin-wide sampling**	**Annually, at least until additional years of data reveal extent of annual fluctuations in densities**
3	**Common methodology for conducting annual *Hexagenia* surveys**	**Sampling design and sampler dependent on study objectives and historical sampling methods; no consensus among workshop participants**
4	**Consortial arrangement and coordination among ongoing monitoring programs**	**Will continue informal cooperation among independent investigators, with probable continued overlapping of efforts**
5	**Concurrent physicochemical measurements during mayfly sampling**	**No consensus reached; dissolved oxygen and temperature near bottom appear to be universally accepted**
6	**Physicochemical measurements throughout the year**	**Not addressed**
7	**Statistics to represent mayfly densities**	**Moving average pre-emergence density (3 or 4 years); annual biomass production of cohorts (SOLEC) potentially practical**
8	**Ranges of *Hexagenia* densities representative of different trophic states of Lake Erie; a "target" density**	**Indeterminate at this time; highest lake quality may be represented by an intermediate density rather than maximum density**

Introduction

A workshop was held to address specific issues related to the refinement of the *Hexagenia* mayfly metric of Ohio's Lake Erie Quality Index as it is presented in the 1998 *State of the Lake Report* (Ohio Lake Erie Commission 1998, pp. 44-46). The workshop was attended by most of the biologists and managers who are conducting research or surveys on the ecology, distribution, and abundance of *Hexagenia* nymphs and adults in Lake Erie, as well as fisheries biologists representing State and federal field offices which regularly survey the fish communities of the western and central basins. The participants and their affiliations are listed in **Appendix A**, the agenda in **Appendix B**, and a glossary of acronyms in **Appendix C**.

The Friday agenda included the following topics, in order.

A. Conceptual foundations
1. Linkages between management objectives and management policies
2. The conceptual basis for a SOLEC indicator comprised of *Hexagenia*
3. *Hexagenia* densities and biomass as a reflection of lake quality and productivity
4. Ranges of *Hexagenia* representative of excellent lake quality

B. Current Lake Erie monitoring programs of the U.S. and Canada
1. University of Windsor
2. Great Lakes Science Center, USGS
3. Pennsylvania State University Erie
4. Ohio Department of Natural Resources at Painesville and Sandusky
5. The Ohio State University Franz Theodore Stone Laboratory
6. US EPA Great Lakes National Program Office

C. Sampling protocols
1. Selection of reference ("master") stations for annual *Hexagenia* surveys
2. Appropriate sampling designs for organisms with a patchy distribution
3. Common methodology for conducting annual *Hexagenia* surveys
4. Consortial arrangement and coordination among ongoing monitoring programs
5. Concurrent physicochemical measurements during mayfly sampling
6. Physicochemical measurements throughout the year

D. Analytical approaches
1. Selection of one or more statistics to represent mayfly densities
2. Interval for repetition of basin-wide sampling

The Saturday agenda began with a summary and discussion of the consensus and conclusions reached on Friday followed by presentations of special research efforts. The workshop concluded with a discussion of opportunities for collaboration and funding. The following sections of this report summarize the progress made on the topics listed above. No attempt has been made to restate the details of each individual presentation made during the two days of the workshop. Special thanks are extended to Dr. Jan Ciborowski for recording extensive notes throughout the workshop and to several speakers who provided a summary or entrusted me with their PowerPoint files or other notes to assist me in compiling these proceedings. All participants received a draft of this document for review, and their suggestions and corrections are incorporated herein.

Conceptual Foundations

Role of *Hexagenia* in Ohio's Management Objectives and Policies

Roger Thoma (OEPA): The Ohio EPA (OEPA) has had longstanding interest in measuring the "biological integrity" of waters of the State. Biological integrity is defined as "the ability of an aquatic community to support and maintain a structural and functional performance comparable to the natural habitats of a region" (Karr and Dudley 1981). To assess biological integrity, OEPA uses indicator assemblages of fish (Modified Index of well-being and Index of Biotic Integrity) and macroinvertebrates (Invertebrate Community Index) in assessing the water quality of streams and lakes (Ohio EPA 1989, Thoma 1999). That information is coupled to assessments of the physical habitat, chemical quality of sediments and water, fish contaminants, and biomarkers. In recent years OEPA has extended its aquatic bioassessments to the Lake Erie nearshore zone and estuaries (the flooded lower reaches and adjacent floodplains of tributaries) or lacustuaries (Thoma 1999). The TMDL (total maximum daily load) concept requires biological information in order to set the load values. As part of the macroinvertebrate assemblages of streams and lakes, mayflies (*Hexagenia* and others) are used by OEPA in NPS (nonpoint source) pollution assessment, CSO enforcement/litigation support, water quality standards/criteria and use designations, and habitat modifications (404 permits).

Use of indicators strengthens surface water programs by developing concerns and priorities based on environmental feedback and enabling policymakers to make informed value judgments. *Hexagenia* has been incorporated as an indicator into the Lake Erie LaMP through the Ecosystem Objectives and Beneficial Use

Impairment components, especially from the perspectives of nutrient enrichment and habitat destruction. The benthic fish community in Lake Erie appears to be recovering, and fishes that historically fed upon *Hexagenia*, such as silver chubs (*Hybopsis storeriana*), are increasing in numbers.

The Lake Erie Quality Index (LEQI) is to be reviewed by the Lake Erie Commission every five years beginning in 2003. There is a need to calibrate the LEQI – for example, how many *Hexagenia* should the western basin support?

Paul Bertram (US EPA, Great Lakes National Program Office): Great Lakes Indicators are used to link environmental status to management actions. There are three groups of activities related to indicators: (1) research – e.g., ecosystem function, indicator development; (2) monitoring – implementing and reporting indicators; (3) management – decisions and activities based on information provided by indicators. Research activities ask how the ecosystem works, what to monitor, and how to monitor. Monitoring of status and trends determines if management intervention is needed and tracks the results of management actions. Environmental management should be responsive to ecosystem objectives and goals, and environmental managers decide what should be done as well as actually taking action.

The overall purpose of the Great Lakes Water Quality Agreement (GLWQA) between the U.S. and Canada, as amended in 1987, is ". . . to restore and maintain the chemical, physical, and biological integrity of the waters of the Great Lakes Basin Ecosystem." The GLWQA calls for the establishment of Lakewide Management Plans (LaMPs), Remedial Action Plans (RAPs; reports at www.great-lakes.net/places/aoc/erieaoc.html) for 43 designated Areas of Concern (AoCs), Beneficial Use Impairments (14 total), Ecosystem Objectives, Indicators of progress toward the objectives, and biennial reporting on the status and progress made toward the objectives for each of the Laurentian Great Lakes and connecting channels.

SOLEC (State of the Lakes Ecosystem Conferences) is the biennial reporting mechanism. The SOLEC objectives are to (1) assess the state of the Great Lakes ecosystem based on accepted indicators, (2) strengthen decision making and management, (3) inform local decision makers of Great Lakes environmental issues, and (4) provide a forum for communication and networking among all stakeholders. The SOLEC audience includes environmental managers, local decision makers, upper administration and the public, with an applied rather than academic focus. A suite of indicators has been developed to provide the assessment, to strengthen decision making about the Great Lakes, and to inform local decision makers of Great Lakes environmental issues. From ~850 potential indicators originally proposed, SOLEC indicator groups have been organized that contain specific indicators for nearshore and open waters, coastal wetlands, nearshore terrestrial habitats, human health, land use, societal issues, and "unbounded" topics (covering several categories), from which 80 indicators have been selected for development and monitoring. Indicators were adopted in 1998, and the first reports were published in 2001. *Hexagenia*, one of the indicators for nearshore and open waters, indicates that lake conditions are improving. By contrast, the unexplained recent, nearly total disappearance of the crustacean *Diporeia*, another indicator, provides evidence of worsening conditions in Lake Erie.

Indices need to be developed that aggregate the indicators so that 10 to 20 indices are reported rather than 80 individual indicators. This is an area of ongoing work. The Great Lakes National Program Office (GLNPO) proposes that all index values have a range between 0 and 1.0 (0-0.2 Poor, >0.2-0.4 Mixed Declining, >0.4-0.6 Mixed, >0.6-0.8 Mixed Improving, >0.8-<1.0 Good, 1.0 Excellent). Furthermore, a polygon that connects all the individual indicator values within an indicator group is proposed as an effective visual reporting tool. The points along each scale are joined to provide a "realized niche" within a "fundamental potential condition". This presentation approach has drawn criticism on the grounds that indices lose too much information while others argue that the approach is a good way to distill the data. The concept of an index is to compile the information for a different audience rather than to lose information.

Challenges facing the SOLEC process include (1) maintaining linkages between research, monitoring, and management actions; (2) respecting that the data needed by managers are not necessarily those needed by researchers; (3) maintaining visibility and value to managers and agencies in order to attract resources to implement the indicators; and (4) keeping the monitoring systems relevant to the issues of concern to the public.

Additional information about SOLEC efforts and results is available at www.on.ec.gc.ca/solec/, www.epa.gov/glnpo.solec/, and www.binational.net. A CD-ROM is available from GLNPO that contains the 2001 SOLEC report and the list of indicators.

The Conceptual Basis for Using *Hexagenia* as a SOLEC Indicator

Thomas Edsall and Carol Edsall (USGS, Great Lakes Science Center): The SOLEC indicators will be used to report to the International Joint Commission and the public on progress made in restoring the chemical, physical, and biological integrity of the Great Lakes, as called for in the GLWQA. Two defining characters of indicators are that they quantify information so its significance is more readily apparent, and they simplify information about complex phenomena thereby improving communication. SOLEC criteria require that the indicator be scientifically based, a reasonably objective representation of environmental conditions, and easily understood by non-technical audiences. As pointed out above, indicators can be aggregated to form indices, such as the Index of Biotic Integrity and the Lake Erie Quality Index. Three widely recognized types of indicators are state (measures the current state or condition of some ecosystem element), pressure (measures factors believed to be responsible for state or condition), and response (measures what managers or society is attempting to do to improve a state or condition). The discussion below addresses only state indicators.

The choice of an indicator metric is important to indicate the state correctly. Although there is considerable information available on the numerical abundance of *Hexagenia* in some Great Lakes habitats, the Edsalls chose not to base indicator development on abundance as it is traditionally measured because *Hexagenia* nymphs vary in length from about 0.8 to 30 mm and length-frequency distribution of the nymphs in samples can vary widely from site to site and seasonally at a site. Thus, numerical abundance (count data) alone does not adequately describe the trophic value of the population unless the length composition of the population is also known.

To avoid this problem, they chose mean annual biomass (*B*, dry weight) and annual production (*P*) as the primary metrics for indicator development. Biomass was chosen because it can be used directly as an indicator and is used to estimate *P*, and *P* was chosen because it integrates recruitment, growth, and mortality, and when considered with *B* provides a measure of turnover (P/B), which can be substantial in *Hexagenia* populations. The size-frequency method was used for estimating *P* because it permits direct comparison of results with most other *P* studies, does not require the identification of cohorts, and can be applied to samples containing more than one *Hexagenia* species if they have the same life span and maximum body size.

The Edsalls studied *B* and *P* of *Hexagenia* in Michigan waters of Lake St. Clair in 1995-96. Three replicate sediment samples were collected with a Ponar grab at 16 stations each month during the ice-free portion of that 12-month period, and each sample was washed on a 0.65 mm screen. In the laboratory the nymphs' body lengths were measured to the nearest 0.5 mm. High B and P values at most stations reflected a healthy environment. *P* and *B* were strongly correlated (r^2=0.94) whereas density was not strongly correlated with either *P* (r^2=0.49) or *B* (r^2=0.25). These same relationships are seen for all other *Hexagenia* data from North America. Thus, the size-frequency method gives good results, but is labor intensive, requiring monthly sampling during the ice-free period, prolonged sampling washing and extraction of nymphs, and measurements on large numbers of nymphs.

To reduce the amount of labor required, a cohort-based approach was explored. This appeared to be reasonable because in early summer most of the biomass resided among nymphs >16 mm long that were about to emerge as winged sub-adults, and the nymphal biomass peaked in June just before the onset of emergence. Regression analysis showed that the biomass of the emergent cohort in June (*BE*) could be used to estimate *B* (r^2=0.792) and *P* (r^2=0.786). The cohort approach was tested using a coarse sieve made of standard 1/8-inch (3.2 mm) hardware cloth. The small nymphs, sediment, and plant detritus in samples passed quickly through the coarse screen and the 1998 cohort that was about to emerge as well as the largest nymphs of the 1999 cohort that would have emerged the following year were retained by the screen. The method greatly reduced processing time and the cohort-based method could be applied to the nymphs that were retained.

The cohort-based method was applied in the spring of 2001 to *Hexagenia* populations in several areas of the Great Lakes, but the length-frequency data did not permit separation of the two cohorts. As an interim approach, both cohorts were used in the computations. In western Lake Erie, the densities showed the same spatial pattern as biomass (r^2=0.81). Therefore, similar data can be used as metrics to provide status and trend information for *Hexagenia* populations in the Great Lakes. Furthermore, if the two cohorts present in these samples can be reliably separated, existing equations can be used to estimate *B* and *P*; however, if separation is not feasible, either density (*D*) or *B* can be used as the metric. If *D* is used, the nymphs on the screen can simply be counted in the field, the count recorded, and the sample discarded without further processing.

Discussion: Jan Ciborowski pointed out that early emergence resulting from warmer-than-normal temperatures may result in a low estimate of *B* if sampling is not done until June. Various participants noted that May is often a stormy month and may force postponement of sampling until June, although sampling in May has been successful most years. Jan also noted that if investigators continue to use other field and lab sorting procedures with finer mesh sieves, the samples could still be passed through the coarse mesh sieve in the laboratory to obtain numbers consistent with the Edsalls' approach.

Hexagenia Densities as a Reflection of Lake Quality and Productivity

Ken Krieger (Heidelberg College): The LEQI sets a target density for *Hexagenia* in western basin sediments at 500 nymphs/m^2, with a rating of "excellent" attained at 450 nymphs/m^2. Densities of that magnitude in the twentieth century in the western basin, however, were probably artificially high as a result of decades of nutrient enrichment. Therefore, a lower target density should be set. Moderate enrichment, or eutrophication, yields the maximum density, oligotrophication reduces the density, and anoxia under high enrichment severely reduces density or even eliminates the mayflies altogether. Crowding at high densities may also limit the carrying capacity.

The basis for setting a target density or range of densities that would indicate "excellent" or "good" conditions in the western basin of Lake Erie or other parts of the Great Lakes has not been established. The carrying capacity for *Hexagenia* nymphs may be dependent on the overall productivity of the lake, which is governed by fisheries management (e.g. for walleye and yellow perch) rather than management for invertebrates. Therefore, the density of *Hexagenia* that can be sustained in Lake Erie is correlated with the measures used to sustain fish populations at a given level.

The density range of each lake condition rating class (excellent, good, fair, poor) and the width (difference between the highest and lowest values) of each rating class, remains to be determined. Relatively high densities probably indicate less than excellent conditions, and the highest densities may indicate only "good" or "fair" conditions. Therefore, a scale is recommended in which excellent conditions are indicated when the mayfly density is intermediate between low and high densities, and as the density increases progressively above the range of "excellent", conditions of progressive degradation by over-enrichment of the lake would be indicated. Furthermore, density should be reported in terms of a multiple-year moving average in addition to single-year averages because moving averages smooth out the natural variation in annual densities.

Discussion: Paul Bertram responded that sustainability is the goal that should govern the managed density of *Hexagenia*, while Don Schloesser and Bill Kovalak suggested that the maximum productivity level that will avoid dissolved oxygen depletion should be the deciding factor. Bill also suggested that no single index can be universal for all three basins of Lake Erie. Jan suggested that years of reduced populations may be related to dry years when there is little flooding and import of phosphorus from the

Maumee River. Subsequent to the workshop, Jan provided data from Dave Dolan as reported to US EPA in December 2001. Years with low loading are low-water years, and most of the variation is due to the Maumee and Sandusky rivers. The phosphorus loads (metric tons per year) were estimated as: 1996, 10,000; 1997, 13,400; 1998, 13,200; 1999, 10,000; 2000, 7,000.

Don Schloesser suggested that phosphorus is the controlling nutrient although it does not directly affect *Hexagenia* densities; rather, the mayflies respond to anoxia from too much BOD stemming from settling organic particles. Thus, we need to know what triggers the anoxia.

Current Lake Erie Monitoring Programs of the U.S. and Canada

University of Windsor

Jan Ciborowski: Jan established a grid of sampling stations throughout the western basin in the mid-1990s at which he collects five replicate sediment samples with a Petite Ponar grab in May annually. His annual average densities agree closely with the estimates produced by the samples at other stations collected by the USGS Great Lakes Science Center (Don Schloesser) using a Ponar grab. As of 1999 and 2000, *Hexagenia* still has not reappeared in the northeastern part of the western basin. Study of the sediments has revealed that *Hexagenia* eggs are being laid, and other studies have shown that at least a small percentage of the eggs will survive in the sediments for up to four years. It is not known if the eggs are not hatching in that area, or if the nymphs die shortly after hatching.

It is questionable whether *Hexagenia* will colonize the deeper areas of the central basin. Eggs will not hatch, nor will the nymphs grow, below 10°C. Thus, if the temperature of the hypolimnion remains below 10°C, no hatching or growth will take place. Multiplication of growing degree days by the growth rate yields a prediction of emergence time. The calculation predicts that *Hexagenia* will not occur in the central basin because of the cold temperatures.

Jan's density data from the western basin show that it can be divided into five zones. It is apparent that the sediment plume from the Detroit River, when visible, follows a path that coincides with a region toward the center of the basin where the densities of nymphs are very low. This may indicate an impact of organics such as PCBs.

Lynda Corkum: Adult *Hexagenia* serve as useful indicators of the status of nymphs because there is a significant relationship between the number of nymphs in the sediment and the number of adults onshore ($r^2 = 0.8$ in 1997). Low numbers of *Hexagenia* nymphs and adults in the northeastern part of the western basin indicate that either the habitat is sub-optimal (e.g., coarse-grained sediments) or that there are occasional intrusions of water low in dissolved oxygen from the central basin. Biological interactions (competition or predation) also may account for the reduced density of *Hexagenia* in some areas of the basin.

The size of male imagoes does not change over the emergence period, whereas the size of females declines (a function of decreasing fecundity and a trade-off between growth and emergence). Adults from island sites and Erie, PA, are smaller than adults elsewhere, but the factors responsible for that are unknown. However, the deep cool, oligotrophic waters of both the eastern basin and the open water sites in the western basin compared with the shallow, warm, mesotrophic waters in the shoreline sites of the western basin may account for the size difference in adults collected in these areas. The ratio of the two species as adults varies from year to year. A 6-year data base reveals that *H. rigida* are more abundant than *H. limbata* in "odd" calendar years. *Hexagenia limbata* are more abundant than *H. rigida* in even calendar years.

Kovats *et al.* (1996) showed that the mean dispersal distance for *Hexagenia* adults is 1.2 km. Most adults were collected within 150 m from shore. A small proportion of adults exhibit wind assistance dispersal for long distances (>5 km). Recent field studies by Lynda confirm the limited dispersal of *Hexagenia*. Limited dispersal by winged adults is beneficial because it enables researchers to use adult collections for comparisons among nearby sampling locations. The density of adults varies widely among sites on the same night and during an entire emergence season, and this is related to the local abundance of nymphs and ultimately to the environmental conditions in the lake and surrounding drainage basin.

Mating swarms form between 8:00 and 8:30 p.m. Huge swarms are visible over nearshore vegetation (tall crops, shrubs or trees) about an hour before sunset. In 1997, there was a peak swarm at 10:00 p.m. for imagoes but a delayed peak for subimagoes at 10:15 p.m. or later. Females appear earlier than males, and there is no difference in periodicity of the two species.

There are several advantages in the use of adult *Hexagenia* in water quality monitoring programs: 1. The adults can be collected cheaply (sampling is conducted on land and so expense and safety issues of using boats are eliminated). 2. Sampling and processing of aerial insects is fast. The adults need not be separated from debris or from other invertebrates (sorting and identifying nymphs collected from sediment is costly and time-consuming). 3. The adults disperse for short distances so that their abundance reflects local conditions. 4. Size of adult male *Hexagenia* does not change over the extended emergence at a given site, enabling comparisons of potential productivity among sites.

Great Lakes Science Center, USGS

Don Schloesser: Monitoring of mayfly nymphs in the western basin goes back to 1929 and 1930, when it was already recognized that pollution was beginning to impact the western basin (Wright 1955). Wright's transects were designed to detect sources of pollution and the samples were collected June through September – following the peak abundance of nymphs (i.e., after emergence had begun). Wright estimated average densities at 171 nymphs/m^2 in 1929 and 152/m^2 in 1930, but the numbers were probably closer to 500/m^2 when a correction for his use of the Petersen grab is made. The US Fish & Wildlife Service resampled many of Wright's stations using a Ponar grab in 1961 and again in 1982 and 1993. Collection devices, methods, and dates have varied over the past 70 years, confounding efforts to understand changes in *Hexagenia* abundance. Consistent methods, and the best methods, should be used in current and future studies within and across organizations.

Don is presently collecting 150-200 nymphs at each of six western basin stations (including 7M and 5B) to determine cohort sizes, and 7M is collected monthly for a life history study. Reynoldson *et al.* (1989) listed three characteristics that a bioindicator organism should possess: "it should provide a suitable end-point that represents a stable ecosystem; it should have characteristics that allow progress toward the desired endpoint to be measured; and, it should be achievable if recovery actions are taken" (Reynoldson *et al.* 1989, p. 672). Burrowing mayfly densities could provide an appropriate objective (endpoint); however, the nymphs do not satisfy all three criteria. Further, in order to rate the quality of a station, an external reference (e.g., a station in Lake St. Clair) is needed. For example, in 1997 a year class was lost in Lake Erie, but it was also lost in Lake St. Clair.

Don recommends looking at the total zoobenthos on a regular basis. The target number of nymphs can be determined only by continued monitoring because of the wide variability in average densities in the western basin, and at individual sites from year to year. Sediment oxygen demand (SOD), which Don measures during his collections, may yield clues about the geographic variability in densities. Related, ongoing studies Don is conducting with *Hexagenia* include sampler efficiency comparisons, lipid levels as an indicator of health of nymphs, life history, annual recruitment, incidence of parasites, and paleoecological indicators of historical *Hexagenia* distributions.

Pennsylvania State University Erie

Ed Masteller and Chris Felege: They and associates have employed SCUBA diving in the Presque Isle, PA, nearshore area as an effective means of locating pockets of favorable *Hexagenia* habitat. Potential habitat was distributed among beds of *Dreissena* druses, sand, and shale bedrock. The diver forced a 4-inch X 18-inch PVC tube horizontally through the mud just below the substrate-water interface to collect a known quantity of clay. The tube was lifted vertically from the mud, capped on one end, and pulled to the surface, where technicians on board screened the mud through a 2 mm-mesh sieve. Nymphs were found at a station north of Presque Isle (1999) and two stations several km east of Presque Isle (2000, 2001), but not at a station immediately east of the mouth of Presque Isle Bay (2000). The nymphs comprised three possible size classes, but it was not clear if the different sizes were due to year classes or sexes. The deepest sample, collected at 46 ft (14 m), contained *Hexagenia* nymphs.

Ohio Department of Natural Resources

John Deller (Fairport Harbor): Trawl and lower trophic sample sites from ODNR-Fairport: Bottom trawling is conducted monthly May through August and in October at 16 randomly selected stations within four depth strata (5-10 m, 10-15 m, 15-20 m, and >20 m) at established transects in each district (Perry and Chagrin). Additional transects are established every 20 km from Berlin Heights to the Pennsylvania state line (District 2: Berlin Heights, Vermilion, Lorain, Avon, Cleveland, Chagrin; District 3: Perry, Ashtabula, and Conneaut). Trawling is conducted at two stations per depth stratum per transect before the thermocline sets up (June), during stratification (August), and after fall turnover (October). Secchi depth and temperature/dissolved oxygen profiles are recorded at each depth stratum per transect. Bottom trawling includes six fixed index stations in District 2 and three fixed stations in District 3 that have been sampled in October since 1969. A 10-minute tow is conducted at each site using a Yankee two-seam bottom trawl with a 10.4 m headrope, 25 mm bar mesh in the cod end, 13 mm stretched mesh liner, and 25.4 cm roller gear.

Lower trophic sample sites from ODNR-Fairport: The protocol developed for the lower trophic sampling calls for each station to be sampled every two weeks from May through September. There are eight stations, one in each depth stratum west (Chagrin) and east (Perry) of the Grand River, Fairport Harbor, Ohio. Data from two (10m and 20m west stations) of the eight stations are recorded in the Lake Erie Committee's Forage Task Group lower trophic data set. At each station two zooplankton samples are collected with a vertical haul (net configuration is 0.5 m, 64 µm conical net with flow meter), sampling on both the ascent and descent ("down-and-up", Ohio State Univ., Dr. David Culver). Water samples for chlorophyll *a* and phytoplankton are collected and temperature/dissolved oxygen profiles and secchi readings are recorded. Additional water samples are collected at the western 10 m and 20 m sites for total phosphorus analysis (Forage Task Group lower trophic sampling sites).

Jeff Tyson (Sandusky): Trawling in the western basin is done at 41 randomly selected stations during the third week of each month from May through September. Information is collected on the abundance and lengths of young-of-year percids and forage fishes, diet and consumption of yellow and white perch at selected locations, bottom and surface temperature, bottom D.O., and secchi depth (water transparency).

Samples are also collected at four stations in the western end and four stations in the eastern end of the basin every two weeks from May through September to obtain information on phytoplankton and zooplankton species composition, abundance, and size. Concurrent samples are collected for chlorophyll *a*, phosphorus, surface and bottom D.O. and temperature, and Secchi depth. Dr. David Culver of Ohio State Univ. processes all plankton samples.

The Sandusky office also collects samples for the Interagency Lower Trophic Level Assessment Program sample sites. Seven agencies represented on the Forage Task Group conduct coordinated sampling of Lake Erie. Samples are collected every two weeks, May through October, at 20 stations. Information gathered includes phytoplankton and zooplankton species composition, abundance, and size, zoobenthos (using a Petite Ponar spring and fall only), total phosphorus, chlorophyll *a*, surface and bottom D.O. and temperature, and secchi depth.

Beginning in 1989, ODNR has applied a "Yellow Perch Index of Invertebrate Abundance" that uses diet data from stomach contents to assess the availability of zoobenthos as forage. The time series of data includes seasonal variability. The index does not account for selectivity but does indicate presence/absence well and possibly relative community composition as it relates to forage availability. For example, in 1989 chironomids comprised a high preponderance of the yellow perch diet, but beginning in 1995 *Hexagenia* began to replace midges in the diet and as of 1999 comprised a very high proportion of the diet.

Discussion: Roger Thoma asked whether gobies are feeding on mayflies. Mike Bur responded that mayflies are found in goby stomachs in the western basin, but their relative abundance is low, perhaps because gobies spend more time on hard substrates than on *Hexagenia* habitat.

Franz Theodore Stone Laboratory, The Ohio State University

Matt Thomas: Monitoring is conducted by Stone Laboratory staff primarily in the context of aquatic workshops and summer courses. The lab's workshop program caters primarily to sixth through ninth grade students. A typical two-hour boat cruise during each part-day or whole-day workshop includes plankton and zoobenthos sampling, as well as a 10-minute trawl and a dissolved oxygen-temperature vertical profile. Sampling is done at eight stations close to Middle Bass, South Bass, and Rattlesnake islands. Benthic samples, though not retained, could form a backbone for sampling if an additional staff person went on the cruises. Sampling equipment being used by Stone Lab staff includes a YSI 6600 multiparameter sonde that measures depth, dissolved oxygen, temperature, chlorophyll *a*, turbidity, pH, specific conductance, PAR (photosynthetically active radiation), and ORP (oxidation reduction potential). It is also used as a real-time sampler. The sonde has been set out under ice one meter off the bottom and has recorded D.O. loss during winter. The summer of 2001 was marked by calm, hot periods. D.O. at 1 m off the bottom was observed to decline to as low as 2.2 mg/L in one or more locations during the time of peak mayfly emergence. The laboratory also operates a Deep Ocean Engineering XTL remotely operated vehicle (ROV) that is used at workshops and for research in the island area. Its tether provides a 350 ft range; it has automatic depth control and full color zoom video, and can carry a lightweight payload. Mayfly burrows are seen in the camera on every excursion. Laboratory research space and room and board are available to agencies and individual researchers.

Discussion: Jeff Tyson noted that he saw a similar dissolved oxygen decline down to 2.2 mg/L at 1 m above the bottom in early July at West Sister Island. After a few storm events D.O. measurements showed that the profile was completely disrupted. Ken Krieger stated that he is most interested in the D.O. profile within the lowest half-meter above the sediment-water interface because the D.O. concentration will be lowest and ecologically most important in that region. Tom Edsall said he had measured hypolimnetic water in the Bass Islands area in the late 1950s.

U.S. Environmental Protection Agency, Great Lakes National Program Office

Paul Bertram: US EPA's Great Lakes monitoring program encompasses water, aquatic life, sediments, and air. A water quality survey is accomplished aboard the *R/V Lake Guardian* on all five lakes in April and August each year. During those cruises, plankton and nutrients are sampled in offshore waters (>60 m except Lake Erie). There are a few benthic stations (started in 1980) that do not include *Hexagenia* habitat. There is also an annual study of oxygen depletion rates at ten locations in the central basin of Lake Erie. Details of the monitoring program, including sampling stations, sampling frequency, and recent results, are posted at http://www.epa.gov/glnpo/monitoring/.

Sampling Protocols

Selection of Index Stations for Annual *Hexagenia* Surveys

Ken Krieger: In order to monitor the density of *Hexagenia* nymphs in the western basin more efficiently, it is desirable to reduce the number of stations sampled each year from the present number of approximately 30 to a few, perhaps 5 to 10, "index" or "master" stations. The index stations must yield statistically the same average density as that obtained by sampling the larger number of stations. Initial exploration of annual survey data from 1995 through 2001 reveals that index stations may not exist that consistently represent the average density of the whole western basin or regions within it. Densities fluctuate dramatically at individual stations from year to year, and correlation analyses show that the five of 22 stations that were strongly correlated ($p<0.05$) with the basin-wide mean or median density over six years (1995-2000) follow the annual fluctuations in basin-wide density but may have very different absolute densities from the basin-wide value. A number of stations were strongly correlated with each other and thus only one station from each correlated group might be selected to reduce the number of stations that need to be sampled. Contour plots of station densities revealed that the regions of the basin possessing the greatest densities of nymphs have shifted over time, further complicating the selection of a few representative stations that could replace the larger set of stations over the long term. Cluster analysis (complete linkage, Euclidean distance, unstandardized data) failed to show any consistent relationships among stations from year to year.

Discussion: Jeff Tyson suggested that cluster analysis using standardized data might yield stronger results. Jan Ciborowski suggested using Ward's method after log-transforming the data.

Appropriate Sampling Designs for Organisms with a Patchy Distribution

Jan Ciborowski: Sampling design depends on the goal of the research program. Three types of goals are (1a) to delineate "effect", (1b) to delineate distribution, and (2) to estimate population size. Sampling for effect (1a), e.g., a test site versus a reference site, requires within-site replication for comparison between/among sites. Analysis is most powerful when a paired-comparison or Dunnett's test (ANOVA) is used. The benefits of this approach are the powerful tests for effects and the limitation of site selection to areas of concern. This approach is limited in that population estimates cannot be spatially extrapolated. Sampling for distribution (1b) requires lattice or grid sampling and permits documentation of change in distribution through time. Within-site replication is necessary only for comparisons among sites within sampling periods. Analysis is performed with repeated-measures/randomized block ANOVA. Benefits include a powerful test to detect temporal change, accommodation of disparate sampling techniques among sites across time, and facilitation of mapping and spatial interpolation. Geostatistical interpolation improves precision. Limitations include only being able to interpolate an approximate true population mean because estimates are biased unless the ecosystem is assumed to be spatially homogeneous. The benefit of establishing index stations is that the sites are localized to areas of concern, and pair-matching provides high power to detect temporal change. The limitations are that population estimates are limited to the areas sampled unless geostatistics are applied to spatially interpolate among multiple index stations.

Sampling to estimate population size (Goal 2) relies on an equal probability of sampling any area within the basin (i.e., random sampling). This can be accomplished by developing a grid of stations, randomly selecting and sampling at the required number of stations, estimating the mean and standard error of the population, and extrapolating the estimate to the entire area of the ecosystem. The benefits are that the population estimate applies to the entire sampling universe (the basis of US EPA's EMAP and REMAP approaches). The station is the unit of replication, so only one sample is required per station. The approach is limited in that environmental heterogeneity yields low precision and thus requires more samples, and consistent (or compatible) sampling methods must be used at all stations.

The number of stations required depends on the desired level of precision, the density of nymphs, and the variability of that density (e.g., coefficient of variation (CV)). For the years 1991 through 2001, estimates of the mean density of *Hexagenia* in western Lake Erie (derived from five Petite Ponars or three Ekmans at each of 9 to 47 stations) had a median CV of 144%. Given that amount of variation, in order to achieve $\pm$50% precision when there are 250 *Hexagenia*/m^2, 33 stations would need to be sampled.

The locations of the stations are determined by defining and numbering N possible sampling locations ($n_1 - n_N$), selecting n random numbers ($r_1, r_2 \ldots r_i$), and sampling at stations $n_{r1}, n_{r2} \ldots . n_{ri}$. New random numbers must be selected for each sampling period. In a homogeneous sampling area, one can use completely random allocation (or lattice sampling). In a heterogeneous sampling area, partition the area into subareas and allocate resources (stations) among the subareas to improve overall precision. Sampling should be concentrated in the strata that are biggest, most variable, and have the lowest sampling cost. Jan divided the western basin into five zones: western, northern, channel, northeast, and southeast island area. The 33 stations determined above are allocated among the five zones according to the proportional area and relative CV of each zone.

The number of replicates per station depends on the project goals. When sampling for effect, within-site replication is critical if using an index station design for among-site comparisons. The number of replicates depends on the amount of within-site variability (s^2) and the size of the effect that is deemed to be biologically important (i.e., reference mean minus test mean). When sampling for distribution or population size (among years), if using a lattice or randomized block design, the best use of resources is one sample per station at many sites (minimizes pseudoreplication). Replication at stations is important to provide insurance against loss of single samples (especially if extra samples are efficient to collect) and to increase sensitivity of sampling. If densities are high relative to the sampler size, one replicate at a greater number of stations is best; if densities are low, increasing the number of replicates per station will increase the probability of capture of nymphs.

Recommendations for central basin sampling, where *Hexagenia* nymphs are sparse: Sample according to depth strata (e.g., 2-5 m, 5-10 m, 10-20 m zones), allocate stations according to random procedures rather than using transects, except for complementary index transects, and adjust replication effort within stations to the previous year's density estimates (7 samples/station if density <5 nymphs/m^2).

Selection of a Common Methodology for Conducting Annual *Hexagenia* Surveys

Don Schloesser: Variable sampling methodology has presented difficulties for historical comparisons of benthic invertebrate surveys. Most data prior to 1960 is suspect because samplers (e.g., Petersen grab) were only semi-quantitative. Review of equipment catalogs and standard methods descriptions indicated that, when empty, the large Ekman sampler is lighter than the Petite Ponar, which is lighter than the Ponar. All can be operated without a winch, but the Ponar (27 kg when full) is especially physically demanding, whereas a large Ekman (19 kg full) and a Petite Ponar (12 kg full) can be hand-hauled all day by two people. A large Ekman typically fills to about 12 cm depth, whereas a Petite Ponar is full at 10 cm and a Ponar at 16 cm. Samples collected by Don and Tom Nalepa (NOAA, Great Lakes Environmental Research Laboratory, Ann Arbor, MI) revealed that a Ponar almost always obtained more nymphs than the other samplers, but the difference was not statistically significant because of high inter-replicate variability. The Ekman samples more tiny nymphs than the Ponar, but the Ponar collects more large nymphs. This difference is believed to be caused by a hydraulic shock wave promulgated by the settling Ponar, which is screened on top, that washes away the upper millimeters of sediment; the Ekman sampler, with an open top, has less tendency to produce a shock wave upon descent. The amount of sediment disturbance is speed-dependent, resulting in a bias dependent on the technician and/or winch. The Ponar consistently samples more sediment than the Ekman (presumably compresses the sediment more). Extra weights can be added to the Ekman, but the added weight defeats one advantage of the Ekman. The Petersen grab, with no opening on top, produces a greater shock wave disturbance than the Ponar. Don calculated the following ranges of conversion factors (for number of *Hexagenia* nymphs obtained) based on his sample comparisons: Orange peel grab to Ponar, 1.1-1.7; Petersen to Ponar, 0.9-1.6; Petite Ponar to Ponar, 1.1-2.2; large Ekman to Ponar, 1 – 4.4. Conversion factors vary considerably among sampling sites, probably a result of differing substrate firmness. Decisions as to which sampler to use must consider relative costs of the different samplers, including purchase and installation of winch. A bigger sampler requires more laboratory processing but provides more accurate density estimates.

General Discussion (Friday afternoon)

Common Methodology: As Jan Ciborowski noted in his presentation, different objectives require different methodologies. The Lake Erie Quality Index (LEQI) requires one estimate of *Hexagenia* density for the entire western basin; therefore, random sampling across entire basin with one sample per station would be adequate; stratified random sampling may be a better approach, if consistent density zones become evident over the years.

For the LEQI, one average nymph density per year for the western basin based on the same stations sampled the last seven years might be best for continuity. However, Jan questioned whether we should sample the same stations every year.

Lynda Corkum felt that winged *Hexagenia* are easier and cheaper to collect and less weather dependent for collections than nymphal collections. The numbers of adults can be indicators of nymph densities. Also, the adults congregate at the shorelines, so that the estimates spatially integrate emergence from large areas of the lake. Male imago size does not decline over the season, but female size does. Size differences between sexes reflect a decline in female fecundity over time and a trade-off between growth and emergence.

Don Schloesser noted that the sampling method is not as important as a consistent method – year to year and agency to agency. Jan observed that conversion factors can be applied between methods. Don reiterated that his data show that the conversion factor differs from site to site.

Season and Frequency of Sampling: Most participants agreed that if nymphs are to be sampled only once per year, the best time is immediately before emergence; otherwise fall might be added to estimate the size of the new cohort. Lynda asked why it is necessary to sample every station every year? Don responded that yearly sampling detects failed year classes, which might be of importance to the LEQI. Less than yearly sampling might not provide as good, or timely, an indication of trends in *Hexagenia* numbers.

Choice of Sampler: The Ponar, Petite Ponar and Ekman were the samplers recommended. The consensus was that it is likely that each investigator will continue using the same one as before, that investigators are not likely to agree on a uniform sampler. However, a single type of sampler must be used for the LEQI if multiple agencies are going to collect the data each year.

Coordination and Sharing of Sampling Efforts: Don Schloesser and Jan Ciborowski both plan to continue to sample nymphs in the western basin in May in future years. Don will use a Ponar, Jan a Petite Ponar. Ken Krieger will continue to sample the central basin nearshore region in Ohio in June (pre-emergence) and is shifting from an Ekman to a Ponar this year. He will make collections with both samplers in order to compare sampler efficiencies.

General Discussion (Saturday morning)

(Paraphrased from Jan Ciborowski's notes)

Ken Krieger summarized major points of the Friday sessions. Question 1: What is an appropriate density of *Hexagenia* to rate as "excellent" in the western basin?

Tom Edsall: Production is a better measure than density. With overlapping cohorts, we cannot use only density; but *P:B* seems to correlate highly with density immediately before emergence, if one uses a coarse screen to examine just the large mayflies.

Ken: So if we only used the large mayflies, we could use Tom's regression equation to estimate the productivity numbers.

Tom Edsall: We should do a size-frequency productivity study to confirm the data.

Ken: We have too few years of data yet to have a good idea of the annual variability in density; density has fluctuated strongly.

Jack Kramer: In terms of a density to settle on, we still are looking at a moving target; i.e., the carrying capacity of the lake is changing.

Ed Masteller: It is better to suggest a range as Ken suggested rather than a number.

Tom Edsall: We need statistics and a trend line to show where densities are now and follow changes through the future to see how trajectories are changing. There is a lot of variation among lakes and we don't know where Lake Erie really belongs on that graph. Even going back to Trefor Reynoldson's tusk data (Reynoldson and Hamilton 1993), there were times of stability, but draining the Black Swamp caused a rise, then a return to lower densities of nymphs, then another rise during the 1940s.

Ken: But then that indicates that *Hexagenia* is responsive. We treat *Hexagenia* as a sensitive indicator, but it really is resilient. All we know is that it persists when there's enough oxygen; we don't know much beyond presence/absence, so we don't know how good an indicator of intermediate conditions density is. Tom is correct that we have to look at the trend line.

Dave Berg: Recall that in trying to restore "integrity" we are doing it against a changing background as a result of incorporation of all the invaders and a new trend to steady state.

Jeff Tyson: If it ever does reach a steady state. Consider climate as well. We don't know the details of Trefor's sediment record for tusks.

Ed: Someone should track important disruptions, e.g. sewage spills, that could create local effects but whose impact is lost from the record of benthic data as a cause of anomalies.

Ken: An example is the *Microcystis* bloom of September 1998; a large study was conducted to find the cause, and the bloom didn't recur; *Cladophora* is another possible example. Maybe we do need a changing target.

Tom Edsall: It would be useful to have a Web page where people could keep track of records of disruptions like that.

Ken: At a December 2001 meeting at GLNPO in Chicago, interest was expressed in discharges of phosphorus from the Detroit sewage treatment plant, and apparently no comprehensive data sets are available to detect or measure those. Ken and Don have debated whether toxics are keeping *Hexagenia* out of the lake; only Jan's data seem to indicate any real evidence apart from anecdotal evidence. Several people have successfully reared *Hexagenia* in the laboratory in contaminated sediments from Lake Erie; maybe it's a synergistic effect with oxygen.

Paul Bertram: Sustainability is the key in an indicator; he is not sure how to tackle that except for a range of density.

Bill Kovalak: Most universal indices vary from lake to lake, so don't look for a single magic number. The number of *Hexagenia* is tied indirectly to the amount of phosphorus in the lake, so we should keep track of phosphorus. The question of index stations remains evasive. We are not justified in picking a single index station. Perhaps we should keep track of zones and develop a number for each zone. The public may wish to know about mayfly effects in different areas; that approach would be more informative than a single number.

Mike Bur: If we came up with a number for different zones we could weight the zones to come up with a single number.

Jan Ciborowski: Do we want to sample the same stations forever or should we do random sampling? We have to know our question before we decide. We discussed this well yesterday; scientists have individual scientific objectives and have to apply appropriate sampling methods, but we have to separately consider the LEQI in terms of sampling design. In terms of methodology, we haven't reached any good conclusions on that.

Don: The specific sampling methods used are not as important as consistency of methodology.

Ken: Yes, especially if we know the biases of our different sampling approaches (e.g., time of year). If we know what conversion factors to use for different samplers, we can perhaps equate the numbers that different people are producing. For the LEQI, whoever is collecting the data should use the same sampler.

Don: The sampler conversion factor is site dependent, depending on sediment characteristics.

Ken: We should be careful in the field that if the sampler isn't to a sufficient depth, it is discarded.

Ed: Don is going to come and have a diver observe the sampling process. Using radar can tell the difference between soft and hard substrate based on frequency.

Jan: Mark Blouin (USGS, Sault Ste. Marie) has important diver-made observations on effects of sampling on sediment compression and *Hexagenia* collection; he presented a poster at NABS in Keystone in 2000. Despite biases, the differences due to sampler type are probably relatively minor in comparison to spatial and temporal variability.

Ed: Hydroacoustics might be able to detect emerging nymphs on the screen.

Ken: Does ODNR have the time to add some index stations? Given time limitations, even adding five or six new stations may be too much of a burden. We have not talked about what concurrent data to collect when we do go out: D.O. and temperature just above the bottom? Certainly notes should be made of sediment characteristics, e.g., zebra mussel distribution.

Ed: The Stone Lab staff is taking information off the bottom without disturbing it. That information is very important.

Tom Diggins: One idea would be to piggyback *Hexagenia* sampling with analysis of oligochaete and chironomid communities.

Ken: Several labs have saved all sample organisms collected over a number of years, so that could be done.

Jan: Dan Breneman (NRRI - UMN Duluth) uses a sieve bag technique. USGS (Ann Arbor) has a special bucket with screen sides as well as a screen base. Jan has full benthic data from 1996; he has been using a 250 µm mesh bucket since 1999 to retain fine benthos.

Lynda: Remember the adults.

Ken: As Lynda showed, adults can be indicators of nymphal density and collections are less weather dependent.

Lynda: The public might be able to relate better to the adults.

Ken: This discussion has motivated him to think about the volunteer monitoring approach. He agrees that adults are what the public sees and that they have advantages.

Isaac: The public does see adults only; but nymphs are a more immediate indicator of effects. Adults are more visual to relate to the public, but in terms of immediacy of measurement of water quality, they may not be as useful as nymphs.

Lynda: She favors a combined program; but the adults do not feed and are so short-lived that they can be analyzed for contaminants. The contaminant burden of the adult provides a lifetime integration of the exposure of the nymph over its life time.

Ken: That's right, and documented very well (Corkum *et al.* 1997). Home in on nymphs because it's easier to quantify nymph density; it is more difficult for adults, although Lynda's data show some progress on this. We have to have a change in thoughts of what we're measuring on shore.

Tom Diggins: Adults would be a good first indicator of recovery in areas that didn't previously have nymphs. The public in Buffalo now sees adults, even though nymphs are still very rare. Adults may be a great first indicator of the return of *Hexagenia*, because adults are much easier to see.

Tom Edsall: He documented one swarm two years ago in Saginaw Bay. Michigan DNR (Bill Bryant) stocked the bay with nymphs many years ago and the location of the swarm corresponds with where stocking was done.

Current Research on *Hexagenia* in the Laurentian Great Lakes

André Bachteram and Jan Ciborowski (University of Windsor) "Bioturbation of the Burrowing Mayfly *Hexagenia* spp. (Ephemeroptera: Ephemeridae): Effects of Size, Density and Temperature"

The relative importance in the western basin of Lake Erie of bioturbation (mixing of lake sediments by invertebrates) compared to wave resuspension of bottom sediments is not known. The authors predicted that sediment flux in western Lake Erie will increase as burrowing *Hexagenia* larvae return to historic densities. To investigate this, they designed a three-factor (5 larval densities, 5 larval sizes, and 5 water temperatures) laboratory experiment. They predicted increased sediment flux in areas with higher larval densities, larger larvae, and/or higher temperatures. Lake sediment from north of Middle Sister Island was passed through a 1-mm sieve and placed 3.5 cm deep in wide-mouth jars; the jars were filled to capacity with a depth of 8.5 cm of water. Turbidity rose during initial burrow construction followed by a slight decline to maintenance values. Total suspended solids increased linearly with increasing size, but temperature was very important. Although adding food during the experiment was expected to increase turbidity, turbidity dropped after feeding. The effect of feeding was most evident with the large larvae. Preliminary results revealed a positive relationship of total suspended solids with larval size, larval density, and water temperature. It appeared that food limitation may affect bioturbation at high larval densities. Additional work will incorporate more experimental replicates, field verification of the laboratory findings (enclosures as well as water samples collected from sites with high and low larval densities), and modeling a basin-wide budget for *Hexagenia* bioturbation.

Discussion: Dave Berg asked if the decline in turbidity with regard to food varied according to temperature; the largest decline seemed to be at a high temperature. Ken stated he thought that *Hexagenia* ingest sediment when they feed, but these data suggest that they are filtering rather than ingesting sediment. Could they be filtering with their gills? André replied that they are probably filtering with their legs and extracting the suspended sediment. Ken wondered how rapidly *Hexagenia* filter relative to zebra and quagga mussels. The experimental sediment depth is only 3.5 cm; in the field, the nymphs burrow down to about 10 cm. It is possible that in the experiment they continuously attempt to burrow deeper and thereby cause more bioturbation than they would create in nature.

Wes Plant (University of Windsor): "Ingestion of *Hexagenia* Eggs by *Chironomus* Larvae"

Although *Hexagenia* nymphs have colonized the soft sediments in most of the western basin of Lake Erie, certain regions continue to have few or no nymphs. Biotic interactions may play a role in influencing the distribution of nymphs. This study is investigating the interactions between *Hexagenia* mayflies and *Chironomus* midges in western Lake Erie. Both taxa spend most of their lives in sediments; both emerge and oviposit eggs on the water surface, which then sink to the mud. Both taxa also display a checkerboard pattern of distribution in the western basin. Generally, their distributions in Lake Erie do not overlap, which is consistent with competition. Maps of the distribution of *Hexagenia* and *Chironomus* in the western basin for each year from 1996 through 2000, show regions of high *Hexagenia* density, high *Chironomus* density, and high densities of both. Although there was no evidence of competitive interactions for space, it was hypothesized that *Chironomus* larvae may eat *Hexagenia* eggs and thereby prevent them from colonizing areas inhabited by *Chironomus*. Mass emergence of *Chironomus*, largely prior to the emergence of *Hexagenia* each year, results in large numbers of eggs added to the sediment. Because *Chironomus* are generalist feeders and consume organic material, they may feed on *Hexagenia* eggs when those are deposited. A laboratory experiment was performed using 45 core tubes (66 mm diameter) that each contained 4 cm natural Lake Erie sediment. The overlying water was aerated with capillary tubing. Three treatments consisted of the control, one *Hexagenia* present, and one *Chironomus* present. Each treatment had three densities of *Hexagenia* eggs: 50 eggs/core (14,615/m^2), 30 eggs/core (8,769/m^2), and 10 eggs/core (2,923/m^2). Five replicates of each case were run for 48 hours and no food was added. Eggs were retrieved by washing the sediment through a 90 µm sieve, adding lignin pink to stain the eggs, and observing eggs through a dissecting microscope. At all three egg densities, there was a significant reduction in the number of eggs remaining after 48 hours in the *Chironomus* cores, but no difference was seen between the controls and the cores containing a *Hexagenia* nymph. It was concluded that *Chironomus* feed on *Hexagenia* eggs but that *Hexagenia* do not. Future work will attempt to determine the importance of egg consumption by *Chironomus* in affecting the distribution and abundance of *Hexagenia* nymphs. The experiment should be repeated using a larger number of *Hexagenia* nymphs to rule out the Allee effect and also using realistic egg and *Chironomus* densities.

<u>Discussion</u>: Tom Edsall wondered if eggs are recognizable inside the gut of *Chironomus*. If so, this experiment could be related to field collections. Ken asked how Wes tested for competitive interaction for space. Wes said he did lab experiments between large and small larval mayflies and chironomids and never found evidence of mortality or size reduction. Wes showed slides of the results of his competition experiment. He also examined priority effects. He started with *Chironomus* added at various periods of time before the *Hexagenia* were added; no priority effects were observed. Ken stated that given those results, the experimental evidence contradicts any suggestion that the checkerboard pattern is due to competition. Dave asked, if feeding of midges on mayfly eggs accounts for the absence of *Hexagenia* from high-*Chironomus* areas, what is the evidence for the contrary pattern (absence of *Chironomus* in high-mayfly areas)? Wes thought the mayflies may exhibit competitive dominance and thus may exclude *Chironomus*. Lynda observed that it would be interesting to know if *Hexagenia* eat *Chironomus* eggs. Ken suggested that *Hexagenia* may be a selective feeder. Jan thought that maybe the eggs are being buried by *Hexagenia* burrowing activity.

Isaac Hagenbuch and Ed Masteller (Pennsylvania State University at Erie): "Monitoring *Hexagenia* Mayfly Emergence from Lake Erie using Doppler Radar in Erie, PA"

Radar images were recorded at WJET TV-24 from 25 June to 25 July 2001 with assistance from meteorologist Tom Atkins at intervals of 4 to 5 minutes, a radius of 88 km (55 miles), and a shore length of 134 km (82 miles). A series of images from 3 July 2001, 9:39 p.m. to 10:00 p.m., shows a *Hexagenia* swarm developing over the water offshore, increasing in density, and the shoreward edge of the swarm moving onto shore. Another series from 10 July 2001, 9:25 p.m. to 10:48 p.m., shows a similar development occurring simultaneously with the movement of thunderstorms over the area of emergence and the absence of the swarm following the storms. The swarms can form very quickly. It is difficult to estimate the height of swarms because the radar signal has an expanding cone of coverage with increasing distance. Further, the site had a ground shadow (50 m of elevation) that prevented estimation of the full extent of the swarm. The colors of the images indicate the intensity of reflection. An image on radar where there is no precipitation indicates the presence of a mayfly swarm. Each 5 dB increment corresponds to a level of precipitation but it is difficult to convert that into the number of insects. Among the questions that need to be resolved are: What is the altitude of the swarms? How can the number of mayflies in the swarms be measured? Are the swarms being carried by the wind? What causes the emergences to take place? Why have they found *H. rigida* but not *H. limbata*?

Discussion: Jan asked whether reference was being made to emergences (subimagoes coming out of the water) or to swarms (ovipositing imagoes). Ed Masteller responded that ground-truth collections indicate that most of the individuals are subimagoes. Furthermore, the insects move inland, so they are probably emergences and not mating swarms. Isaac stated that the storm fronts may be blowing new emergences in to shore and actually forming the visible swarms. The data are insufficient at this time. Jack Kramer noted that the radar images should permit determination of velocity. Isaac replied that a persistent point must be defined in order to do that; the swarms change shape and dimensions, so it is a difficult task.

Lynda Corkum and Jan Ciborowski (University of Windsor), David M. Dolan and Michael J. Roy (Univ. of Wisconsin at Green Bay): "Predicting Mass Emergence of *Hexagenia* Adults in Lake Erie"

Mayflies are good water quality indicators and important prey for fishes and birds, but their mass emergences near shore are a nuisance. The present study objective was to determine which abiotic factors are most useful in predicting the onshore density of male and female adult (subimago and imago stages) *Hexagenia* spp. Although degree days can be used to predict general emergence time (Giberson and Rosenberg 1994), they wanted to determine which factors best predict *peak* emergence. Lynda sampled at Colchester, Ontario, on 18 dates from 2 June through 18 July 2000 using five hula hoops (A = 0.65 m^2) (only three hoops on 3 dates), collecting from 9:30 to 11:00 p.m. EDT. Each hoop was placed over a white sheet. Meteorological variables recorded by a wireless weather monitor included air temperature, wind speed, wind direction, wind chill, barometric pressure, humidity, dew point, and rainfall. Moon phase was also recorded. A logistic regression equation based on the abiotic variables correctly predicted only 37% of the cases for total *Hexagenia*. However, when applied only to imagoes, it correctly predicted 76% of cases, and for subimagoes it predicted 67.5% of cases correctly, for females 76% , and for males 60%. Temperature and photoperiod provide a cue for emergence. The attainment of a water temperature of about 20°C is a cue for the first mass emergence. Wind chill (wind speed and temperature) predicts *peak* emergence of each adult stage for males and females.

Discussion: Tom asked whether wind chill was affecting emergence, i.e., the activity of the *Hexagenia*, or rather one's ability to catch the *Hexagenia*. Would a cooler temperature keep them from flying as far? Ken stated that, along the same line, he has wondered how weather above the water is affecting nymphs in the sediments; perhaps wave height and thus the amount of water movement near the sediment is a factor. Lynda relayed that Rasmussen and Rowan (1997) used wave height, shoreline slope, and fetch to predict the depositional boundary layer in a lake; i.e., they were able to predict the location of fine sediments and associated depositional fauna. Ed wondered if barometric pressure plays a role; his work on emergence traps in streams showed that insects are highly sensitive to barometric pressure changes, serving as predictors of weather changes; it is important to measure barometric pressure where the nymphs are, rather than at a distant buoy.

Opportunities for Collaboration and Funding

Data sets exist that are unknown to most researchers. A list of such data bases would be useful to mayfly researchers. As examples, Jeff Tyson's office has data on temperature, D.O., and secchi transparency on 41 (variable) stations monthly back to 1992, and temperature data monthly back to 1969. Ontario Ministry of Natural Resources has information on D.O. The Toledo and other water intake plants record water chemistry data. Mike Bur's staff collects temperature, D.O., and Secchi transparency. Jan mentioned the existence of a Web site containing a Lake Erie research inventory. The Great Lakes Forecasting System (http://superior.eng.ohio-state.edu/) updates and archives three-dimensional limnological conditions on each of the Great Lakes every six hours.

Opportunities for funding might include:

1. The national and state Sea Grant programs: they tend to fund projects addressing their lists of priorities and have specific application deadlines. The four Sea Grant directors for Lake Erie (Ohio, Michigan, New York, Pennsylvania) might be convinced to pool their resources for a collaborative study.
2. Ohio Lake Erie Commission: The agency operates funding programs through the Ohio Lake Erie Protection Fund (LEPF) similar to those of Sea Grant. Both LEPF and Sea Grant have separate large and small (developmental) funding programs.
3. Fisheries agencies: They might be willing to fund projects from the perspective of *Hexagenia* as a fish food resource.
4. Great Lakes Commission: Its lake committees issue RFPs for funding projects with small budgets.
5. GLNPO: This agency is providing funds to develop a *Hexagenia* indicator, and it appears receptive to funding new research directions.
6. US Fish & Wildlife Service Region 5: The agency does not award external funds but conducts surveys and may be willing to cooperate on projects (contact: Kofi Fynn-Aikins). The agency also has some funds to match in-kind work.

Ontario Ministry of the Environment will be willing to collect data from their new boat (contacts: Fred Fleischer, Todd Howell). GLNPO, USGS, Ohio DNR, and Stone Laboratory have vessel schedules that might be useful for cooperative sample collections for external projects.

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Appendix A

Participants and Affiliations

André Bachteram	Department of Biological Sciences, University of Windsor
Dave Berg	Department of Zoology, Miami University
Paul Bertram	U.S. EPA, Great Lakes National Program Office, Chicago
Mike Bur	US Geological Survey, Lake Erie Biological Station, Sandusky, Ohio
Jan Ciborowski	Department of Biological Sciences & Great Lakes Institute for Environmental Research, University of Windsor
Lynda Corkum	Department of Biological Sciences & Great Lakes Institute for Environmental Research, University of Windsor
John Deller	Ohio Department of Natural Resources, Fisheries Res. Unit, Fairport Harbor
Tom Diggins	Department of Biology, Hamilton College
Carol Edsall	US Geological Survey, Ann Arbor, Michigan
Tom Edsall	US Geological Survey, Ann Arbor, Michigan
Chris Felege	Penn State-Erie
Isaac Hagenbuch	Penn State-Erie
Bill Kovalak	Detroit Edison
Jack Kramer	Water Quality Laboratory, Heidelberg College
Ken Krieger	Water Quality Laboratory, Heidelberg College
Julie Letterhos	Ohio EPA, Division of Surface Water
Gary Longton	Detroit Edison
Chuck Madenjian	US Geological Survey, Ann Arbor, Michigan
Ed Masteller	Penn State University
Tom Nalepa	Great Lakes Environmental Research Laboratory, NOAA, Ann Arbor, MI
Wes Plant	Department of Biological Sciences, University of Windsor
Don Schloesser	US Geological Survey, Ann Arbor, Michigan
Fred Snyder	Ohio Sea Grant Extension (western basin)
Roger Thoma	Ohio EPA, Northeast District Office
Matt Thomas	Ohio State University, Stone Laboratory
Jeff Tyson	Ohio Department of Natural Resources, Fisheries Research Unit, Sandusky

Workshop Participants

<u>Standing, Left to Right</u>: Fred Snyder, Jeff Tyson, Chuck Madenjian, Tom Nalepa, Matt Thomas, Paul Bertram, John Deller, Julie Letterhos, Jan Ciborowski, Wes Plant, Lynda Corkum, Isaac Hagenbuch (back), André Bachteram, Gary Longton, Mike Bur, Tom Edsall, Chris Felege, Carol Edsall, Tom Diggins

<u>Seated</u>: Dave Berg, Ed Masteller, Ken Krieger, Don Schloesser, Bill Kovalak

<u>Not Shown</u>: Jack Kramer, Roger Thoma

Appendix B

Agenda

***Hexagenia* Workshop, 8-9 February 2002**

Pfleiderer Center, Herbster Chapel, Heidelberg College

"Refining and Implementing the Mayfly Metric of the Lake Erie Quality Index"

Sponsored by the Ohio Lake Erie Commission through Grant LEQI 01-03

FRIDAY, 8 February

10:00 a.m. **Welcome and Overview of Workshop Objectives**: Dean Kathryn Venema and Ken Krieger, Heidelberg WQL

10:10 a.m. **Conceptual Foundations**

Linkages between management objectives and management policies

Presenters: Roger Thoma, Ohio EPA
Paul Bertram, Great Lakes National Program Office

Conceptual basis for a SOLEC indicator

Presenter: Tom Edsall, Great Lakes Science Center, USGS

***Hexagenia* densities (and biomass?) as a reflection of lake quality and productivity (Discussion)**

Ranges of *Hexagenia* nymph densities that represent various degrees of lake quality

Presenter: Ken Krieger

11:15 a.m. **Current Lake Erie monitoring programs of the U.S. and Canada**

Presenters: Jan Ciborowski, U. Windsor
Lynda Corkum, U. Windsor
Don Schloesser, Great Lakes Science Center, USGS
Ed Masteller, U. Penn.-Erie

12:30 p.m. **Lunch** (available at Rock Creek Café; return to conference room for working lunch)

1:00 p.m. **Current Lake Erie monitoring programs of the U.S. and Canada (Continued)**

Presenters: John Deller, Ohio DNR
Jeff Tyson, Ohio DNR
Matt Thomas, Ohio State Univ. Stone Lab
Paul Bertram

2:00 p.m. **Sampling Protocols**

Appropriate sampling methods for organisms *(Hexagenia)* with a patchy distribution

Presenter: Jan Ciborowski

Selection of "representative" or "master" stations for annual *Hexagenia* surveys

Presenter: Ken Krieger

Common methodology for annual surveys throughout Lake Erie (Discussion)

Consortial arrangement and coordination with ongoing monitoring programs: Who will sample where? (Discussion)

Choice of sampler (Ponar vs. lighter sampler) to accommodate everyone sampling mayflies

Presenter: Don Schloesser

Concurrent physicochemical measurements during mayfly sampling (Discussion)

Linkage to Sporadic Events: Physicochemical measurements throughout the year (Discussion)

3:30 p.m. **Break**

3:45 p.m. **Analytical Approaches**

Selection of statistic to represent mayfly densities -- moving average or other approach

Presenter: Ken Krieger

Interval for repetition of basin-wide sampling (Discussion)

Which mayfly metrics to record (Discussion)

Archives of samples for later analyses (Discussion)

5:00 p.m. **Adjourn and Supper**

7:30 p.m. **Follow-up Discussion** (if needed)

SATURDAY, 9 February

8:30 a.m. **Summary of Friday Workshop**

Items needing action

8:45 a.m. **Current Research on *Hexagenia* in the Laurentian Great Lakes**

André Bachteram: Effects of sediment bioturbation by *Hexagenia*

Wes Plant: Ingestion of *Hexagenia* eggs by midge larvae

Ed Masteller and Chris Felege: "SCUBA Diving for *Hexagenia* in the Pennsylvania Waters of Lake Erie"

10:15 a.m. **Break**

10:30 a.m. **Current Research on *Hexagenia* in the Laurentian Great Lakes**

Lynda Corkum: "Predicting mass emergence of *Hexagenia* adults in Lake Erie"

Ed Masteller and Isaac Hagenbuch: "Monitoring *Hexagenia* mayfly emergence from Lake Erie using Doppler Radar in Erie, PA"

11:30 a.m. **Discussion of Potential Collaborations and Funding Possibilities**

12:30 p.m. **Workshop Wrap-up**

Appendix C

Glossary of Acronyms

CSO	combined sewer overflow (in reference to sewers whose flows exceed capacity when they carry urban storm runoff water as well as domestic sewage)
DNR	Department of Natural Resources
D.O.	dissolved oxygen
GLNPO	Great Lakes National Program Office of the US EPA, located in Chicago, IL
LaMP	Lake-wide Management Plan
LEQI	Lake Erie Quality Index
NABS	North American Benthological Society
NPS	nonpoint source (in reference to sources of pollution)
OEPA	Ohio Environmental Protection Agency
ORP	oxidation-reduction potential
PAR	photosynthetically active radiation
P:B	ratio of production to biomass
RFP	request for proposals
ROV	remotely operated vehicle
SOLEC	State of the Lakes Ecosystem Conferences
US EPA	United States Environmental Protection Agency