JGLR-01361; No. of pages: 15; 4C:

Journal of Great Lakes Research xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Journal of Great Lakes Research

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

Evaluation of lower Green Bay benthic fauna with emphasis on re-ecesis of *Hexagenia* mayfly nymphs

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ARTICLE INFO

Article history:
Received 1 December 2017
Accepted 5 June 2018
Available online xxxx

Communicated by Kevin Fermanich

Keywords:
Green Bay
Hexagenia
Benthos
Sediment
Diversity

ABSTRACT

The last historic *Hexagenia* specimen in lower Green Bay was officially recorded in 1955. Field surveys and *Hexagenia* viability studies were completed to determine if lower Green Bay could support *Hexagenia* re-ecesis and where in the bay egg stocking could best be accomplished. The invertebrate field data were compared with historical population data based on earlier published studies in the 1950s, 1970s and 1990s to determine the bay's ecological trajectory to better understand the re-ecesis success potential of *Hexagenia*. No native *Hexagenia* were observed during this study. Deep water invertebrate diversity within the upper lower bay appears to be improving, whereas the diversity along the lower mid-bay may be deteriorating. Shallower, nearshore samples indicated a better condition with *Caenis* mayflies sparsely present, amphipods, isopods, gilled snails, odonates, oligochaetes, chironomids, and meiofauna present. These results suggested improved conditions shoreward versus degraded conditions deeper. *Hexagenia* egg viability and neonate growth indicated *Hexagenia* could successfully inhabit in situ Green Bay nearshore (<2 m) substrates; however, deep substrates were generally inhospitable probably due to hypoxia and unstable fluid substrates. As an outcome of the field surveys and studies of *Hexagenia* viability in Green Bay mud, *Hexagenia* stocking began in 2014 with the first adults since 1955 emerging in 2016 at several lower bay nearshore locations. Improved water quality from remediation efforts in the watershed could facilitate the return of *Hexagenia* to deeper water.

Introduction

Throughout most of the past seventy-plus years, the bay of Green Bay, Lake Michigan, has experienced a chronic degradation in its water quality. Much of this degradation has been due to point and non-point land-use activity-derived sources discharged to the Fox River which feeds into the bay from the south and travels north primarily along the eastern coast of the bay (western Door County). Historically, the Fox River has been an effluent recipient for paper mills, various manufacturing companies, and intensive agricultural use. For example, when paper companies first started manufacturing specialized papers in 1954, PCBs used in their production were discarded into the river unregulated until 1971, when the use of PCBs was ended (Wisconsin Department of Natural Resources 2012, http://dnr.wi.gov/topic/greatlakes/documents/RAP-UpdateLGBFR2012final.pdf). In 1972, the U.S. and Canada implemented the Great Lakes Water Quality Agreement, and major efforts to clean up the lower Fox River and Green Bay were begun when the area was designated an "Area of Concern" (AOC).

The ecological degradation of Green Bay has framed the circumstances leading to vast changes in invertebrate populations, notably, the local extinction of the mayfly *Hexagenia* spp., (historically presuming *H. limbata*, *H. bilineata*, and *H. rigida*). The zoobenthos community provides a lens for temporally comparative analyses to understand the ecological trajectory of the bay that is important for determining if *Hexagenia* re-establishment through egg stocking might be timely. A burrowing benthic Ephemeroptera, *Hexagenia* spp. have long been known as an important indicator taxon for their intolerance of poor water quality (Fremling, 1989). Prior to 1939, *Hexagenia limbata*, once regionally coined the "Green Bay Fly", emerged synchronously in large masses on an annual basis (Schuette, 1928; Fremling, 1968). Since then, the population of the insect dropped to the point where the last *Hexagenia* was officially recorded in the southern bay in 1955 (Balch et al., 1956). Long-term remediation actions can prove successful in improving the quality of the environment to the benefit of *Hexagenia* re-ecesis. In western Lake Erie, the same mayfly similarly disappeared years ago due to poor water quality and hypoxia. Then, in the 90's, populations of *Hexagenia* returned to the lake after a 40-year disappearance (Bridgeman et al., 2006). This remarkable recovery was attributed to both remedial efforts and the invasion of zebra mussels (*Dreissena* sp.), which initially helped clear the water and reduce the effects of

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https://doi.org/10.1016/j.jglr.2018.06.006
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pelagic sedimentation. In 1991, several living *Hexagenia bilineata* (not *H. limbata*) were collected from the lower Fox River tributary to Green Bay (Cochran, 1992), an optimistic sign of natural recovery and/or effective remedial efforts in the Green Bay area.

Monitoring population dynamics of a stressed ecosystem over time can link the past, present, and future health of an environment. Because contaminants can accumulate and persist in the bottom sediment of lakes and rivers long after the water has been cleared of pollutants, population changes of benthic fauna are important for understanding the health of the habitat. It is suggested that macrobenthic invertebrates and meiofauna within Green Bay could be responding positively to remedial efforts, and possibly the dreissenid impact. This study describes the current population trends of benthic fauna juxtaposed with historical population studies to better understand the environmental state and ecological trajectory of Green Bay as referenced to re-establishment of *Hexagenia*. A comparison was made between our data (this study), Surber and Cooley (1952), Howmiller era data 1969–1977 (Mozley and Howmiller, 1977; Howmiller and Beeton, 1971), and Harris' survey data in 1978 (1998) to determine the directional shift of the benthos. Howmiller and Maas (1973) sampled southern Green Bay for benthic fauna in 1969 and in 1970, leading Howmiller and Beeton (1970) to make predictions about future changes in population dynamics assuming the continuation of the incidence of eutrophication and pollution at that time. Postulations were that populations of oligochaete worms would decrease near the mouth of the Fox River and in the southernmost area of the bay in general, and midge larva populations would decrease in the northern stations (Mozley and Howmiller, 1977). These changes may not have been the general association of these worms to degraded habitat (increased worms, e.g., *Limnodrilus* spp. signifying a degraded habitat), but rather related to a complex hypoxia situation (Klump et al., this issue) and inhospitable sediment changes resulting in decreased worms and severely degraded habitat. These relationships are closely tied to the ecological events that decimated the historical native *Hexagenia* population and to the future possibility for a restored *Hexagenia* population.

The consolidation of sediment became a topic of interest when sediment collected from some Green Bay sites with known historical *Hexagenia* populations appeared to have little cohesive structure. For burrowing nymphs of *Hexagenia*, highly fluidized substrate with little structure leads to the collapse of burrows making the substrate bioenergetically uninhabitable (Fremling, C. personal communication to Kaster, J.L., 1968). Sediment spike tests (Fremling, C. personal communication to Kaster, J.L., 1968) were conducted to rate the cohesive firmness of the sediment that could be crucial to the survival of obligate tube-forming organisms, including *Hexagenia* and certain tubificid oligochaetes. From 2011 to 2015 laboratory rearing of *Hexagenia* from eggs and nymphs was used to provide a window into the effects of fluidized sediment on burrow construction and general background pollution on the early life-stages of *Hexagenia* (Groff and Kaster, 2017). This suite of field and *Hexagenia* viability lab studies indicated that nymphs successfully hatched as adults in sediment collected from the southernmost portion of Green Bay, and it followed that in-situ field conditions may be able to support *Hexagenia* populations considering both field invertebrate studies and viability studies. Our overriding question was, is it time to facilitate re-establishment of *Hexagenia* in Green Bay through a stocking program? Armed with information from these studies, an aggressive 2014 *Hexagenia* stocking program was initiated in Green Bay. This study examines time-comparative field analyses of zoobenthos population ecology, enclosure *Hexagenia* viability studies, and *Hexagenia* stocking program in lower Green Bay.

Methods

The target *Hexagenia* stocking sites within the lower bay were: Sawyer Bay 421 ac (part of Sturgeon Bay); Little Sturgeon Bay 395 ac; Little Tail Point 1334 ac; and Long Tail Point 1248 ac, and Fox River De Pere impoundment 600 ac. The area of these sites total 1629 ha (3998 acres), an area that did not include nearby areas that had historical high abundances located in the middle of the AOC. Considering the mass swarming and dispersal behavior of adults, the project's area being stocked could serve as an inoculum source as the lower bay impact is mitigated.

Field studies

Macrobenthos

The bulk of benthic field samples were collected on June 21–June 23, 2011, in Green Bay aboard the R/V Neeskay and additional summer samples primarily collected from smaller craft from 2012 to 2016. In total 34 sampling stations were chosen from a grid-pattern arrangement of sites throughout the lower bay (Fig. 1). The open lake sites sampled included 0, 2 (Hx15), 3, 3A, 5, 6, 8, 9, 10, 12, 17, 18, 20, 21, 22, 24, 25, 26, 27, 42, 43, 44, 47, and H7, H7A, H7B and H8 in the nearshore south bay, S1, S2, S3 (H12), H12A, Hx11 in Sturgeon Bay, and HR9 and HR10 in the Oconto and Menominee River estuaries, respectively (Table 1). Emphasis on sampling the lower portion of the bay ensured that as many sampled sites as possible were in the proximity of historical sampling sites of Surber and Cooley (1952), Mozley and Howmiller (1977), and Harris (1998). Three standard sized Ponar grabs (~23 × 23 cm) were collected at each station, and three spike cohesive test replicates were taken for each location. At the first sampling sites, when a lack of cohesiveness was first observed, only one trial was completed, e.g., 0/1 or 1/1. Sediment debris and fauna samples were sieved through a 0.5 mm screen and the remaining detritus and animals from each grab were stored separately and preserved in 70% ethanol. It is important to note that practices of collection have varied over the years, e.g., Mozley and Howmiller (1977) used an Ekman grab to collect benthos, whereas Harris' 1978 study (1998) used a Ponar grab. A conversion adjustment between Ekman and Ponar was not applied in this study.

All sorted fauna from each grab sample were placed in a scintillation vial, and counted and identified as they were sorted from debris using dissection microscopes, compound microscopes when necessary, and a multiple-tally counter. Numbers of each taxon from the three Ponar grabs at each site were averaged and expressed on a m^{-2} basis. Samples from site 44 were not sorted or documented as they were predominated by fine manganese nodules with the observation of no living macroinvertebrates.

Data were converted into a series of maps using ArcGIS and basemaps obtained from the Wisconsin DNR, created in imitation of those presented in Mozley and Howmiller (1977) (e.g., Surber and Cooley, 1952) and Harris' thesis (1998). Size-graduated circle symbols were used at similar taxa resolution to show differences in densities of fauna between sites, consistent with the early Howmiller studies (Mozley and Howmiller, 1977). Unfortunately, the original Mozley and Howmiller data are not available (Mozley pers. com. to Kaster 2012). These maps were juxtaposed with historical maps to determine population changes from 1952 through 2011. Historical data sites from Harris' 1978 study were obtained from coordinates given by Harris (1998).

Diversity

Diversity indices were calculated using Shannon diversity (1948) and Simpson diversity (1949) and allied computer programs and methods of Krebs (1999). Diversity values were calculated using only taxa in Harris' thesis (Harris, 1998) to make a parallel comparison between Harris' sampling method and data, and the current data. Nematodes were present, but not quantified in Harris' thesis, so these values were omitted completely from the diversity calculation. The diversity index values of selected historical sites were contrasted with current sites. Statistical inference followed Elliott (1993).