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Burrowing mayfly populations in Chequamegon Bay, Wisconsin: 2002 and 2012

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Burrowing mayflies (Ephemeroptera: Ephemeridae) are sensitive to pollution and have been used as environmental indicators in the Great Lakes. *Hexagenia limbata* and *Ephemera simulans* population abundance and biomass estimates from Chequamegon Bay, Lake Superior, were compared between the years 2002 and 2012 as well as inside and outside the Northern States Power Lakefront Superfund site. Mean abundance was similar and mean biomass of Ephemeridae was slightly less in 2012 than in 2002, most likely due to the occurrence of *E. simulans* in 2012, a smaller species not collected in 2002. In 2012, mean ephemerid abundance and biomass outside the Superfund site was significantly higher than inside the Superfund site. Biomass was higher in clay, clay with sand, and sand with clay substrates than in fine sand, coarse sand, or wood debris substrates. Substrate in the Superfund site was predominantly wood debris. Future monitoring of ephemerid populations in Chequamegon Bay, and particularly in the Superfund site as clean up begins, would be valuable to establish long-term population trends for these two species and potentially shed light on the habitat requirements of *E. simulans*, an understudied species in the Great Lakes.

Keywords:: benthic invertebrates; water quality; Lake Superior; Chequamegon Bay; contaminants; Superfund

Introduction

Burrowing mayflies (Ephemeroptera: Ephemeridae) have been used as indicators of ecosystem condition throughout the Laurentian Great Lakes (Krieger et al. 1996; Kolar et al. 1997; Edsall 2001; Edsall et al. 2004, 2005). These organisms are appropriate indicators of ecosystem health because they were historically abundant in unpolluted areas with soft sediment across the Great Lakes (Clemens 1915; Burks 1953; Mozley & LaDronka 1988), they are intolerant of pollution (Rasmussen 1988) and their populations recover when harmful pollution levels subside (Krieger et al. 1996). The population status of burrowing mayflies is also of public interest in areas with records of highly visible mass emergences of adults (Fremling 1968).

Edsall et al. (2004) compared burrowing mayfly populations at two locations on Lake Superior in 2002 and found that human activities had negatively impacted the St. Louis River Estuary population but not the Chequamegon Bay population. In this study, we replicated the sampling of Edsall et al. (2004) in Chequamegon Bay but also added sampling stations in the Northern States Power Lakefront Superfund site (hereafter, the 'Superfund site') of Chequamegon Bay near Ashland, WI, USA. The Superfund site is contaminated

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by oils and tars from a manufactured gas plant that operated from 1885 to 1947 and wood debris and wood treatment pollutants from local sawmills that operated from 1880 to 1940. The main contaminants are volatile organic compounds including benzene, semi-volatile organic compounds, and polycyclic aromatic hydrocarbons attached to benthic accumulations of wood debris (United States Environmental Protection Agency [USEPA] 2009). The wood debris consists of slabs, chips, and smaller sized material that overlay the sediments in thickness from a few cm to 2 m. The Superfund site was added to the National Priority List in 2002, making it eligible for remediation under the Superfund program; however, no actions have been taken yet to address sediment contamination at the site.

Our objectives were to assess potential temporal changes in mayfly populations by comparing populations of burrowing mayflies between 2002 and 2012 in Chequamegon Bay at sampling stations outside the Superfund site and to assess the effect of pollutants on mayfly populations by comparing populations of burrowing mayflies between stations in the Superfund site and stations outside the Superfund site in 2012.

Methods

Study area

Chequamegon Bay is located in southwest Lake Superior (46° 40′ N 90° 50′ W). The bay is 19 km long and 8 km wide, has a surface area of 16,600 ha, a maximum depth of 24 m, and an average depth of 8.6 m. The south and east areas of the bay are shallower than the rest of the bay and are mostly less than 5 m deep. The bay is semi-isolated from Lake Superior by Long Island (Figure 1). The bay thermally stratifies from June to September with surface waters warming as high as 23 °C (Ragotzkie et al. 1969).

Experimental design and sampling

Burrowing mayflies were collected in Chequamegon Bay in July 2012 from the same 33 stations sampled by Edsall et al. (2004) in 2002 (Figure 1). Stations were randomly selected within 25 ha grid cells overlaid upon a map of Chequamegon Bay. A single sampling station was selected within each cell where the substrate type most closely matched the clay or clay–sand substrate preferred by burrowing mayflies in the Great Lakes (Edsall et al. 2004). In 2012, an additional nine collections were made at stations in the Superfund site that were approximately equidistantly spaced on three transects parallel to shore (Figure 1). Samples were confined to the western half of the Superfund site because of navigational hazards in the eastern half.

Methods were similar between years, and one author (L.M. Evrard) collected and processed the samples in both 2002 and 2012 studies. In both the studies, five 0.05 m^2 sediment samples were taken with a standard Ponar grab sampler at each sampling station. Ponar samples were washed with lake water on a 3.2-mm mesh sieve by use of a boat mounted water pump. This method effectively cleaned the samples and retained the largest cohort of Ephemeridae nymphs, which is where the population biomass is concentrated (Edsall 2001; Edsall et al. 2004). Burrowing mayflies were preserved in 90% ethanol. Prior to washing the sample, substrate type was described visually and by feel (clay, coarse sand, mostly clay with some sand, fine sand, mostly sand with some clay, or wood debris) and water depth (m; median 6.1 m, range 1.8–24.4 m) was recorded for each station.

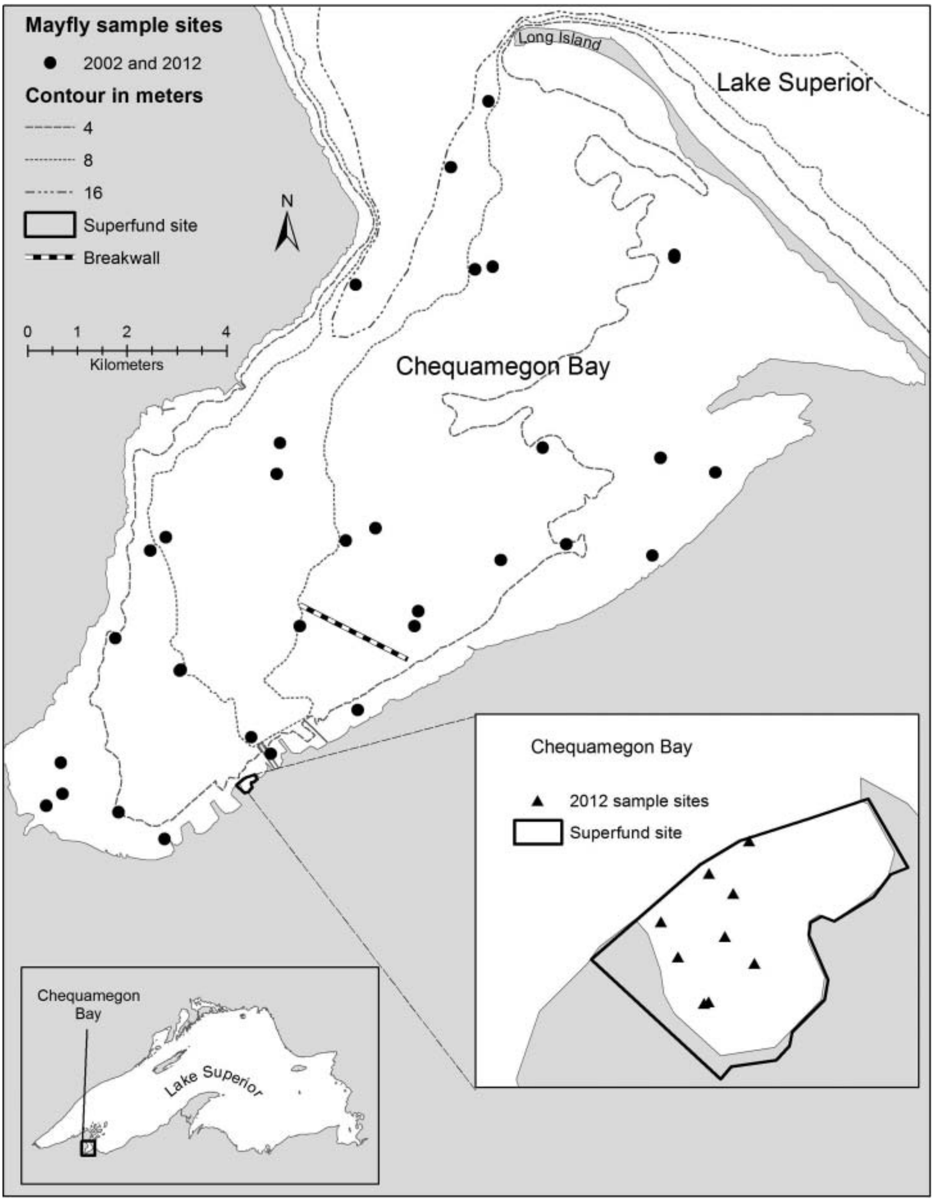

Figure 1. Locations of 33 sampling stations in Chequamegon Bay outside the Superfund site and, on the inset map, 9 sampling stations inside the Superfund site. Stations outside the Superfund site were sampled in both 2002 and 2012.

In the laboratory, mayflies were identified to species (McCafferty 1975) and a digital image was captured. The total length (TL; tip of frontal process to base of caudal filaments) and width of the head capsule (HW) were measured to the nearest 0.001 mm from the digital images using ImageJ software (Image Processing and Analysis in Java; Wayne Rasband; Bethesda, MD, USA). The TL of damaged individuals was estimated from the relationship between HW and TL computed from intact mayflies. Individual dry weight

(DW) was determined for each intact mayfly after being dried for one week at 65 °C. Dry weights of partial mayflies were estimated from the relationship between DW and TL computed from intact mayflies.

Statistical analysis

Mean dry weight biomass and abundance of ephemerid mayflies in Chequamegon Bay (excluding the Superfund site) were compared between 2002 and 2012 using a paired-sample *t*-test. Two temporal comparisons using all mayflies captured in 2012 and *Hexagenia limbata* captured in 2012 were used because Edsall et al. (2004) reported only *Hexagenia* (species unknown) in 2002 and we collected *H. limbata* and *Ephemera simulans* in 2012. Mean biomass and abundance for all mayflies in 2012 were compared between stations in Chequamegon Bay and stations in the Superfund site using a two-sample *t*-test. Mean biomass for all mayflies in 2012 was compared among substrate types using a one-way ANOVA followed by Tukey's multiple comparisons. A chi-square test was used to determine if any change in the proportional distribution of substrate types at stations outside the Superfund site occurred between 2002 and 2012. As depth differed between years and areas, we used simple linear regression to describe the relationships between biomass and abundance of all mayflies and water depth at sampling stations in 2012 as a possible explanation for potential differences in biomass and abundance. Abundance, biomass, and depth were log-transformed to stabilize variances among years, locations, and substrates and increase symmetry of the distribution of model residuals. A rejection criterion of <0.10, commonly used for large-scale ecological experiments (Scheiner 2001), was used to determine significance. All statistical analyses were computed using R$^{\mathrm{TM}}$ v3.0.2 (R Development Core Team 2013).

Results

In 2012, outside the Northern States Power Lakefront Superfund site, mean (±SD) *H. limbata* abundance and biomass, respectively, was 12.4 (±18.6) m^{-2} and 114.8 (±148.9) mg m^{-2} and mean *E. simulans* abundance and biomass was 2.5 (±5.5) m^{-2} and 11.7 (±24.2) mg m^{-2}. *Hexagenia limbata* was collected at 22 and *E. simulans* was collected at 9 of 33 stations outside the Superfund site. *Hexagenia limbata* was collected at two of nine stations inside the Superfund site. The mean abundance and biomass of all burrowing mayflies in 2012 outside the Superfund site was 14.9 (±18.9) m^{-2} and 126.4 (±147.8) mg m^{-2}, respectively. In 2002, at these same stations, mean abundance and biomass of *Hexagenia* spp. was 20.8 (±38.4) m^{-2} and 260.2 (±530.5) mg m^{-2}, respectively. Mean biomass was slightly higher in 2002 than in 2012 ($t = -1.79$, $p = 0.08$; Table 1) and mean abundance was similar between years ($t = -1.60$, $p = 0.12$; Table 1). However, when only *H. limbata* populations collected in 2012 were compared to *Hexagenia* populations collected in 2002, there were no differences in mean biomass ($t = -0.53$, $p = 0.603$) or mean abundance ($t = -0.32$, $p = 0.755$). In 2012, mean abundance and biomass of all burrowing mayflies was higher outside than inside the Superfund site (abundance: $t = 3.59$, $p = 0.001$; biomass: $t = 3.69$, $p = 0.001$; Table 1).

There was a significant difference in the distribution of the dominant substrate category at the same stations between years ($\chi^2 = 9.69$, $p = 0.046$). Unlike 2002, no stations were classified with coarse sand in 2012. In 2012, nearly twice as many stations were classified with fine sand and sand with clay than in 2002. Biomass ($p = 0.057$; $r^2 = 0.088$) but not abundance ($p = 0.112$; $r^2 = 0.062$) of all mayflies in 2012 was weakly,

Table 1. Number of stations (*N*) and mean (standard deviation) biomass (dry weight mg m^{-2}) and abundance (number m^{-2}) of Ephemeridae by location (Chequamegon Bay, outside the Superfund site and inside the Superfund site), year (2002 and 2012), and dominant substrate type within location. *Ephemera simulans* and *Hexagenia limbata* were pooled in 2012 and only *Hexagenia* (unknown species) were reported in 2002. All stations in the Superfund site had wood debris, whereas no stations outside the Superfund site had wood debris.

	N		Biomass		Abundance	
	2002	2012	2002	2012	2002	2012
Chequamegon Bay (outside Superfund site)						
All substrates	33	33	260.2 (530.5)	126.4 (147.8)	20.8 (38.4)	14.9 (18.9)
Clay	9	8	568.3 (853.8)	190.8 (142.1)	41.5 (59.1)	13.8 (18.5)
Coarse sand	7	0	0	0	0	0
Clay with sand	5	4	362.3 (297.1)	217.4 (249.3)	40.4 (34.4)	24.8 (26.5)
Fine sand	8	13	23.4 (52.4)	36.3 (45.3)	2.4 (4.5)	5.9 (8.0)
Sand with clay	4	8	368.0 (555.7)	163.1 (153.2)	22.9 (31.1)	25.8 (23.1)
Inside Superfund site						
Wood debris	–	9	–	4.2 (12.6)	–	0.4 (1.3)
All stations	–	42	–	100.3 (140.2)	–	11.8 (17.7)

positively related to water depth. However, the weak relationship between biomass and water depth is likely due to abundant wood debris at shallow stations with few mayflies. Mayflies were nearly absent from coarse sand and wood debris and these substrates were excluded from the next analysis. Mean biomass differed among the remaining four substrates ($F = 5.03$; $p = 0.004$), with a lower mean biomass (all $p < 0.04$) at stations dominated by fine sands as compared to stations with substrates partially composed of clay.

The mean (±SD) TL of 108 *H. limbata* captured in 2012 was 13.7 (±5.1) mm and the mean DW was 9.3 (±10.4) mg. Strong positive relationships existed between *H. limbata* TL and HW ($\log(TL) = 0.927 \times \log(HW) + 2.036$; $p < 0.001$; $r^2 = 0.88$) and DW and TL ($\log(DW) = 3.175 \times \log(TL) - 6.523$; $p < 0.001$; $r^2 = 0.93$). The mean TL of 22 *E. simulans* captured in 2012 was 10.7 (±3.7) mm and the mean DW was 4.6 (±4.4) mg. Strong positive relationships existed between *E. simulans* TL and HW ($\log(TL) = 1.059 \times \log(HW) + 2.017$; $p < 0.001$; $r^2 = 0.93$) and DW and TL ($\log(DW) = 2.929 \times \log(TL) - 5.705$; $p < 0.001$; $r^2 = 0.96$).

Discussion

The slight decrease in mean burrowing mayfly biomass between 2002 and 2012 appears due to the collection of *E. simulans* in addition to *H. limbata* in 2012. In 2002, only *Hexagenia* were reported, most likely *H. limbata*. Mature *E. simulans* larvae are smaller than *H. limbata*, so mean overall biomass values were less in 2012 when *E. simulans* were included in analyses. This finding is consistent with similar comparisons made by Heise et al. (1987) in Dauphin Lake, Manitoba, Canada, and Mozley and LaDronka (1988) in the Straits of Mackinaw between Lakes Huron and Michigan, USA. When we made comparisons solely between *Hexagenia* assemblages, we found no difference in biomass and abundance between time periods.

Though not reported in 2002 in Chequamegon Bay (Edsall et al. 2004), the occurrence of *E. simulans* is not surprising given that they are a common Great Lakes species (McCafferty 1975; Mozley & LaDronka 1988). However, in studies documenting recovery of burrowing mayfly populations in the Great Lakes, accounts of recolonization and recovery of *Hexagenia* (*H. limbata* and *H. rigida*) populations are widespread (e.g., Krieger et al. 1996; Kolar et al. 1997; Schloesser et al. 2000), whereas reports of recovery of *E. simulans* are lacking. Despite its widespread distribution, little work has been done on the life history of *E. simulans*, particularly in comparison to *H. limbata* (Heise et al. 1987). The dearth of information on the recovery of *E. simulans* (assuming no sampling bias) suggests differences in sensitivity to pollution or dissolved oxygen requirements between species. While the appearance of *E. simulans* in the 2012 collections could be due to differences in dominant substrate types between years (Mozley & LaDronka 1988), the presence of *E. simulans* indicates that sediment habitat quality has not further degraded since 2002 because *E. simulans* are believed to be less tolerant of low dissolved oxygen concentrations than *H. limbata* (Eriksen 1963).

The abundance and biomass of mayflies was significantly lower in the Superfund site than outside the Superfund site in Chequamegon Bay. Hypoxia and toxic sediments lead to increased mortality, slow growth, and reduced productivity of burrowing mayfly populations (Prater & Anderson 1977; Rasmussen 1988; Edsall et al. 1991; Kolar et al. 1997). Burrowing mayflies have a strong affinity for fine mineral (i.e., non-wood) substrates (Eriksen 1968). In our study, we cannot separate the influence of sediment toxicity from sediment physical characteristics, because we lack data on sediment toxicity. Wood debris occurred at all stations sampled in the Superfund site, but did not occur at any of the stations outside the Superfund site. Substrates dominated by wood debris appear to be a legacy of the many local sawmills that operated along the Ashland waterfront from 1880 to 1940.

The large differences in biomass and abundance between stations inside and outside the Superfund site in Chequamegon Bay coupled with only a slight difference in biomass but not abundance between 2002 and 2012 at stations outside the Superfund site suggests the contamination and non-native wood substrates inside the Superfund site depress ephemerid populations. The slight difference in mayfly populations between years outside the Superfund site further suggests that contaminants from the Superfund site do not appear to be negatively impacting burrowing mayfly populations outside the Superfund boundary to any great extent. The slight change in biomass between years could be natural variability or a response to unmeasured biological factors. For example, competition with Chironomidae (Diptera) larvae may have negatively impacted burrowing mayfly populations in Lake Erie (Kolar et al. 1997). Predation by fish may also play an important role in structuring mayfly populations (Clady & Hutchinson 1976; Hayward & Margraf 1987; Schaeffer et al. 2000). As the largest common benthic invertebrate in Chequamegon Bay, burrowing mayflies are readily consumed by common Chequamegon Bay fishes including yellow perch (*Perca flavescens*), walleye (*Sander vitreus*), smallmouth bass (*Micropterus dolomieu*), and lake whitefish (*Coregonus clupeaformis*; Devine et al. 2005). Population differences between sampling events can also be a phenological sampling artefact related to inter-annual variation in organism development and the timing of sampling (e.g., pre-emergence and post-emergence sampling events).

Northern States Power Lakefront Superfund site Phase II (Chequamegon Bay) clean-up activities are scheduled to begin in 2014. Previous Great Lakes studies have shown that if oxygen is high enough and if the substrate contains enough fine substrate for burrowing, then robust populations of burrowing mayflies are possible (Lyman 1956; Rasmussen 1988; Krieger et al. 1996; Kolar et al. 1997). The lack of reports of recovery of *E. simulans* populations throughout the Great Lakes over the past four decades suggests it may be less tolerant of pollution than *Hexagenia*. This aspect of burrowing mayfly ecology needs further study. Future monitoring of populations in the Chequamegon Bay, and particularly inside the Superfund site as clean up begins, would be valuable for establishing long-term trends in mayfly biomass in this bay of Lake Superior and perhaps shed light on the habitat requirements of *E. simulans*.

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