EPA Public Access

Author manuscript

J Great Lakes Res. Author manuscript; available in PMC 2019 January 30.

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Published in final edited form as:

J Great Lakes Res. 2017 ; 43(1): 108–120. doi:10.1016/j.jglr.2016.11.006.

A depth-adjusted ambient distribution approach for setting numeric removal targets for a Great Lakes Area of Concern beneficial use impairment: degraded benthos

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Abstract

We compiled macroinvertebrate data collected from 1995 to 2014 from the St. Louis River Area of Concern (AOC) of Lake Superior. Our objective was to define depth-adjusted cutoff values for benthos condition classes to provide an analytical tool for quantifying progress toward achieving removal targets for the degraded benthos beneficial use impairment. We used quantile regression to model the limiting effect of depth on selected benthos metrics, including taxa richness, percent non-oligochaete individuals, combined percent Ephemeroptera, Trichoptera, and Odonata individuals, and density of ephemerid mayfly nymphs (*Hexagenia*). We created a scaled trimetric index from the first three metrics. Metric values above the 75th percentile quantile regression model prediction were defined as being in relatively excellent condition in the context of the degraded beneficial use impairment for that depth. We set the cutoff between good and fair condition as the 50th percentile model prediction, and we set the cutoff between fair and poor condition as the 25th percentile model prediction. We examined sampler type, geographic zone, and substrate type for confounding effects. Based on these analyses we combined data across sampler types and created separate models for each of three geographic zone. We used the resulting condition-class cutoff values to determine the relative benthic condition for three adjacent habitat restoration project areas. The depth-limited pattern of ephemerid abundance we observed in the St. Louis River AOC also occurred elsewhere in the Great Lakes. We provide tabulated model predictions for application of our depth-adjusted condition class cutoff values to new sample data.

Keywords

Great Lakes; St. Louis River; Benthos; Area of Concern; Beneficial Use Impairment; *Hexagenia*

Introduction

Great Lakes Areas of Concern (AOC) were listed by the signatories of the United States-Canada Great Lakes Water quality Agreement (GLWQA, 1987 Amendment; <http://binational.net/annexes/a1/>; accessed 6 October 2016) as places where significant impairment of beneficial uses of the Laurentian Great Lakes or their tributaries has occurred due to local human activities (<https://www.epa.gov/great-lakes-aocs>; accessed 6 October 2016). Beneficial use impairments (BUIs) result from changes in the chemical, physical, or biological integrity of the system sufficient to cause impairment in structural or functional attributes directly or indirectly important to humans. Among the 14 established BUIs are restrictions on fish and wildlife consumption, degradation of aesthetics, degraded fish and wildlife populations, and degradation of benthos.

For an AOC in the U.S. to be “delisted,” all local BUIs must be removed through the achievement of local removal targets created by states, tribes, and local stakeholder groups in a manner consistent with guidance provided by the U.S. Environmental Protection Agency (United States Environmental Protection Agency [USEPA], 2001). BUI removal targets should be premised on local goals and be consistent with applicable federal and state regulations. Targets should be based on measureable indicators, be realistic and achievable (<https://www.epa.gov/great-lakes-aocs/beneficial-use-impairments>; accessed 6 October 2016).

Degradation of benthos (i.e., benthic macroinvertebrate assemblages) is a BUI at twenty-three of the remaining twenty-seven AOCs in the US Great Lakes, including the St. Louis River (SLR), a tributary of western Lake Superior. The rationale for the degraded benthos BUI for the SLR AOC was evidence that benthic abundance and diversity were “reduced” in areas of known habitat alteration or in proximity to known sediment contamination in the lower SLR (Minnesota Pollution Control Agency [MPCA], 2013). Assemblages in these areas were dominated by pollution-tolerant taxa and lacked diversity (MPCA, 2013). The narrative removal target for the SLR AOC specifies that this BUI may be considered for removal when:

The benthic community in historically degraded areas (e.g., chemically, biologically, or physically) of the AOC does not significantly differ from unimpacted sites of comparable characteristics within the AOC. Benthic communities’ characteristics including native species richness, diversity, and functional groups will be considered when comparing sites (MPCA, 2013).

“Comparable characteristics” means that physical habitat characteristics (e.g., depth, substrate), that are largely unrelated to the source of the impairment, are similar between historically degraded sites and restored sites or sites away from known historical degradation.

The wording “unimpacted sites” implies a reference expectation against which to assess the condition of sites. The reference condition approach for aquatic bioassessment is well developed (Bailey et al., 2004; Grapentine, 2009; Yates and Bailey, 2010). However, defining reference is challenging in historically and extensively impacted contiguous

ecosystems where sites are not independent, comparable unimpacted sites are rare, and reliable and sufficiently fine-scaled stressor gradient data are not available (Angradi et al. 2009b). Such is the case for the lower SLR. Other Great Lakes ecosystems with similar assessment challenges include the Lake Erie-Lake Huron Corridor (McPhedran and Drouillard, 2013) and the Upper St. Marys River. Because we could not define reference sites in the lower estuarine part of the SLR using stressor gradient data, we used an ambient distribution approach to define numeric condition-cutoff values (e.g., between impaired for the beneficial use and unimpaired for the beneficial use). In this approach, a priori percentiles are chosen representing particular condition cutoffs (e.g., poor, fair, good) which are applied to the distribution of available data for the resource (USEPA, 2000; Stoddard et al., 2006). We use the term “cutoff” instead of “threshold” to define condition classes to avoid confusion with the concept of ecological thresholds [Dodds et al., 2010]).

There are problems with using the ambient distribution to define reference expectations, especially with respect to assessing the condition of a population of sites, which are discussed below. However, the ambient approach allows the *relative* impairment of sites to be compared among sites with similar characteristics. The relative condition of a site can thus be quantified and cutoff values derived. BUI removal and AOC delisting decisions must occur despite the lack of ecological reference as traditionally defined for data rich systems. Our view is that numeric BUI criteria based on an imperfect method for deriving relative condition, are an improvement on the narrative removal target.

Methods for deriving condition-class cutoff values that account for variation associated with natural factors improve the sensitivity and reliability of the indicator (Davis and Simon, 1995; Reynoldson et al., 1997; Bailey et al. 2004). One version of this idea, illustrated in Fig. 1a, is that while there are multiple natural and anthropogenic sources of variation in the ambient distribution of benthos metrics, and assuming that not every sampled site is significantly impacted, a natural factor limits the maximum value of the metric (Terrell et al., 1996; Scharf et al., 1998). Values below the upper bound of the ambient distribution are limited by other factors, including anthropogenic disturbance. The upper bound of the distribution represents the extant least disturbed benthic conditions over the range of the limiting factor. A quantile function describing some upper bound of the data either as a straight line or as a peaked distribution can be used to define the reference condition for the metric in the local setting.

Preliminary analyses of selected benthos metrics from SLR samples showed that depth had a significant relationship with metric values (Fig. 2). This corroborates earlier studies in the lower SLR that showed an effect of depth on diversity and the relative abundance of many taxa (Swanson, 1999; Breneman et al., 2000; Trebitz et al., 2009). The distribution of the metrics was wedge-shaped with respect to depth (Fig. 2), suggesting a limiting effect of depth. Data included in Edsall et al. (2004) provide independent support for the existence of a depth-limited distribution for benthos in the lower SLR. In those data, there was a strongly wedge shaped relationship between depth and the density of *Hexagenia* nymphs (Fig. 1b). Samples with visible evidence of pollution were not near the upper limit of nymph density for any depth at which they collected nymphs.

There are several potential explanations for the association between depth and the benthos in the SLR. Because of the geomorphology of the lower river, there is an association between depth and exposure or fetch (Fig. 3a), a correlate of natural physical disturbance, especially over the shallow 0 to 4m depth range. Exposure increases with depth up to a depth of 2 to 3m, which is the dominant depth outside of navigation channels. Other Great Lakes studies have shown an effect of fetch on benthos (e.g., Cardinale et al., 1998; Burton et al., 2002, 2004). There is a strong relationship between depth and the probability of occurrence of submerged aquatic vegetation (SAV; Fig. 3b). The occurrence of SAV, which has a significant effect on benthos diversity in the lower SLR (Trebitz et al., 2009), declines rapidly with depth and is rarely present deeper than 2m (Angradi et al., 2013). The maximum organic matter content of the substrate, which also influences benthic assemblage composition (Cole and Weigmann, 1983), declines an order of magnitude over the depth range of the lower SLR (Fig. 3c). At the shallow end of the depth range, breaking wave-induced water velocity, which is a function of depth (Fig. 3d), has an effect on aquatic vegetation, sediment transport (Silander and Hall, 1997), and the littoral benthos (Winnell and Jude, 1984; Duffy et al., 1987; MacKenzie et al., 2004). Other factors, including water temperature, hypoxia (Reynoldson and Hamilton, 1993; Krieger et al., 1996), hydrostatic pressure (Cole and Weigmann, 1983), fish predation (Krieger et al., 2007), non-indigenous species (Trebitz et al., 2010), and littoral ice scour all likely vary with depth. Although outside the scope of this paper, a plausible hypothesis is that one of these depth-associated factors or the integration of multiple factors results in a limiting depth for the maximum abundance of many macroinvertebrate taxa, which is reflected in metric values.

Our objective was to develop quantile regression models to predict benthos condition indicators from depth for the SLR AOC. From these models, we derived condition-class cutoff values to be used as numeric criteria and to quantify the relative condition of potential benthic habitat restoration project areas, for tracking post-management change in condition (Grapentine 2009), and other for decision support applications. Our results are provided in a format easily transferable to local AOC practitioners. We also examined our findings for an important indicator taxon, *Hexagenia*, in the context of other published and unpublished information.

The St. Louis River AOC

The boundaries of the St Louis River AOC include the lower 63 km of the SLR, the Nemadji River Watershed, and part of Lake Superior (MPCA, 2013). In this paper, we address benthic condition in the estuarine part of the AOC downstream from Fond du Lac Dam (Fig. 4) and excluding Lake Superior. The lower SLR is a Great Lakes “rivermouth” estuary (Larson et al., 2013) formed by post-glacial isostatic rebound (Herdendorf, 1990). The 50 km² estuary lies at the terminus of the SLR Basin (9,250 km²), but also receives discharge from several tributaries, the largest of which is the Nemadji River (1,140 km² basin area). Land cover in the SLR watershed is 94% forest, wetland, and water, 4% agriculture, and 2% developed. Land use around the estuary is much more highly developed (Fig. 4).

The SLR estuary includes several geographic zones of different character. Tributaries to Allouez Bay and Pokegama Bay (Fig. 4) drain highly erodible clays deposited in proglacial

Lake Duluth (Magner and Brooks, 2008) and these bays are nearly always much more turbid than the rest of the estuary (see images in electronic supplementary materials [ESM] 10). Superior Bay is a lagoon formed behind a natural 16 km-long sandbar and is open to Lake Superior at its northwestern and southeastern ends. The bay contains the outer Duluth-Superior Harbor, a large commercial port, with extensive ship channels and industrial development. St. Louis Bay includes the inner harbor and is industrialized and channelized. It is shallower than Superior Bay and is less hydrologically influenced by Lake Superior. Spirit Lake, a large flooded backwater of the river, is shallower and the riparia is currently less developed than downriver area. Formerly, a large steel mill and coking operation was situated on the west shore of Spirit Lake (<https://www.pca.state.mn.us/waste/st-louis-river-us-steel-superfund-site>; accessed 6 October 2016). Above the Spirit Lake zone, the system is more riverine. Natural substrates in Spirit Lake and downriver are dominated by fine sand and silt (ESM 1). Natural coarse substrates are rare except in the upper, riverine portion of the estuary (Brenneman et al., 2000; Swanson, 1999). Submerged aquatic macrophyte beds dominated by American wild celery (*Vallisneria americana*, Michx.) were, until recently, widespread across shallow (< 2-m deep) areas of the estuary (Angradi et al. 2013). Since a large flood in 2012 (Czuba et al., 2012), aquatic macrophytes have been less abundant. The non-indigenous mussel, *Dreissena polymorpha*, was first detected in the SLR in 1989 followed by *D. bugensis* in 2005 (Trebitz et al., 2010). Prior to 1978, municipal and industrial wastewater was discharged to the river virtually untreated. The Western Lake Superior Sanitary District Treatment Facility, which began operating in 1978, dramatically improved water quality in the River (<http://wlssd.com/about-us/history/>; accessed 31 August 2016; Bellinger et al., 2016). Improvements in water quality since the mid 1980s have been less dramatic. See St. Louis River Citizens' Action Committee (2002) for a summary of the pre-and post-development human history of the SLR.

Methods

Data sources and processing

This study is based on benthic samples collected by multiple researchers and agencies from 1995 to 2014, between June and November. Only samples collected using a standard (0.052 m²) or petite Ponar sampler (0.023 m²) from the estuarine portion of the AOC and excluding the turbid bays (Allouez and Pokegama Bays) were included in the study (see ESM 2 for sample metadata). We excluded the turbid bays because they have water chemistry and substrate characteristics that are distinct within the SLR, have been infrequently sampled, and are not areas for which benthos-BUI-related habitat restoration projects are currently anticipated.

Most data were not collected using a randomized sample design. The available benthos data are therefore biased spatially, temporally, and by habitat. To control for seasonal and inter-annual variation we standardized benthos metrics to the mean year of sample collection (2010) and sample day (13 August) using regression residuals as described in ESM 3. Sample collecting and processing methods for some datasets are published (Brenneman et al., 2000; Trebitz et al., 2010). There was some variation in limiting mesh sizes used (ESM 2), but we did not attempt to correct for this source of error. Benthos metrics for all samples

were calculated using data expressed as number per sample or per m², standardized to the same operational taxonomic units, usually genus or family.

We selected four “positive” benthos metrics for analyses (the metrics are presumed to increase with improved benthic conditions): taxa richness, percent non-oligochaete individuals, percent of individuals in the insect orders Ephemeroptera, Trichoptera, and Odonata (ETO), and benthic abundance (density) of ephemerid nymphs. These metrics were selected from a large suite of candidate metrics based on their sensitivity to disturbance and because they are likely intuitive to managers not expert in benthos-based indicators.

We examined the sensitivity of these metrics to human disturbance in the SLR estuary by comparing metric values for samples collected adjacent to and more distant from riparian areas with current or historical industrial and commercial activity (see ESM 4 for details). We judged this characterization of disturbance suitable for screening of benthic metrics for biological responsiveness, but not as the basis of a general reference condition approach for the system because: 1) we know that contaminated sediment “hot spots” can occur well away from riparian areas; 2) the intensity of disturbance likely varied greatly among areas identified as disturbed based on riparian conditions; and 3) the boundaries of disturbed areas were highly subjective.

Advantages of the selected metrics are that they do not depend on autecological details (e.g., species’ traits such as feeding guild or habit) or numerical pollution tolerance values that may not be available for all taxa or may not apply to Great Lakes estuaries; they are easy to compute from abundance data, and they are based on taxa that likely vary in their optimal depth range. Oligochaetes are widely used as the basis of environmental assessment in the Great Lakes (e.g., Lauritsen et al., 1985; USEPA 2016). Although they vary in pollution tolerance, most oligochaete taxa are classified as relatively tolerant (Mandaville, 2002). The metric percent ETO individuals is a variant of the widely accepted metric, percent Ephemeroptera, Plecoptera and Trichoptera (EPT) individuals (e.g., Barbour et al. 1999; Flotemersch et al., 2014), with Odonata replacing Plecoptera, which is a rare taxon in the SLR estuary.

The mayfly family Ephemeridae includes the genus *Hexagenia*, a pollution-intolerant taxon (Edsall et al., 1991) the density of which is a recommended State of the [Great] Lakes Ecosystem Conference (SOLEC) indicator (International Joint Commission, 2014) with a long history of use as an indicator of ecosystem health in the Great Lakes (Reynoldson et al., 1989; Krieger et al., 1996; Edsall, 2001). Although not the case for the St. Louis River AOC, BUI removal targets may be expressed directly in terms of *Hexagenia* abundance. For example, both the Lower Green Bay/Fox River AOC and the Maumee AOC have a benthos BUI removal target of an average *Hexagenia* abundance of 100 to 400 nymphs/m² (Wisconsin Department of Natural Resources [WDNR], 2014; Ohio Environmental Protection Agency, 2014).

The density metric is based on Ephemeridae rather than *Hexagenia* because some ephemerids were not identified to genus, and we suspect there was confusion of *Hexagenia* and *Ephemera* in some datasets. The great majority of individuals were *Hexagenia*. To

examine if a depth limitation on this taxon is a widespread pattern, we compared our results for this metric with data collected in Chequamegon Bay of Lake Superior, the Lake Huron - Lake Erie connecting channel, including Lake St. Clair, and in the nearshore benthic habitat of the five Laurentian Great Lakes (metadata in ESM 2). We $\log_{10}(x+1)$ transformed highly skewed ephemerid density.

Correlations among metrics were generally low (ESM 5) suggesting that metrics do not necessarily reflect stressor effects in the same ways. We therefore created an integrative trimetric index (sensu Barbour et al., 1999) from the other metrics but excluding ephemerid density. We excluded ephemerid density to provide a second final assessment metric to be used in conjunction with the trimetric index. To create the trimetric index, we scaled each transformed and date-standardized metric to 0 to 1 by dividing the difference between each value and the minimum value by the difference between the maximum and minimum values. We then summed the scaled values and rescaled the metric as above. Information needed to calculate the index for new sample data is provided in ESM 6.

Analysis approach

An assumption of the limiting factor approach is that there is not systematic variation in the data associated with other important natural or methodological factors. In our case, the most important other natural factors is probably substrate composition. We compiled observational substrate data for most samples. Because they were not all classified by the same individuals using the same particle size categories, we did not attempt to pool substrate observations across datasets. By examining the influence of substrate type on mean metric value, we can assess the degree to which this factor confounds the inference.

A methodological factor related to the application of the results is sampler type used (i.e., standard versus petite Ponar sampler) which has been shown to influence benthos estimates (Barton, 1989; Schloesser and Nalepa, 2002). By examining the variation in metric means and quantile regression models between sampler types, we can determine if data can be combined or if the results are sampler-specific.

We fit two quantile regression functions for each metric, a straight line and the Ricker function, $y = axe^{-b}$, where a is the initial rate of increase to a peak at $1/b$. The Ricker function is a widely used phenomenological model for environmental variables that start at zero, increase to a peak, and decrease gradually back to zero (Bolker, 2008).

We defined the condition-class cutoffs as the straight line or Ricker function fit through the data for the 25th, 50th, and 75th percentile. We defined the relatively poor versus relatively fair condition cutoff as the linear function fit through the data for the 25th percentile (lower quartile); good condition was defined by the fit for the 50th percentile (median); Excellent condition was defined by the fit for the 75th percentile (upper quartile). For these distributions, 25, 50, and 75% of the metric values, respectively, are less than or equal to the specified quantile function of depth (Cade and Noon, 2003). These condition-class definitions are both arbitrary and a compromise. Other percentiles could be used to delineate classes. A trisection of the distribution at the 33rd and 66th percentile is common (e.g., Angradi et al. 2009a). We selected percentiles for defining conditions classed in consultation

with local AOC practitioners. In our view consistent application of the criteria across sites and over time is more important than the exact percentiles used.

We fit quantile regression models using the “quantreg” package in R (Koenker, 2015; R Core Team, 2015) using the “rq” and “nlrq” functions for the linear and nonlinear models respectively. We used the default bootstrapping option to calculate standard errors for parameter estimates. We used the significance of t for the test: slope = 0. For ephemeral density, for which there were many samples where this taxon was not present, we compared models with and without zeros included, found no difference, and developed the models for all data. Because the percentile quantile regression models depend on a subset of the data at the top of the distribution, high outliers that are not well distributed across the depth range can have a large effect on models. Based on plots of the data (Fig. 2) we omitted three outliers for ephemeral density clustered near a depth of 7 m. The depth range of the St. Louis River data was from near 0 to 14 m; however, there were only six observations from deeper than 11 m. We therefore only used samples where depth was ≥ 11 m in our models.

We separately examined the effect of sampler type (i.e., standard Ponar sampler versus petite Ponar sampler) and geographic zone (i.e., Superior Bay, St. Louis Bay, and Spirit Lake) on mean metric values and quantile models. The Ricker function for the 75th percentile did not fit the data better than the linear function for any metric based on the Akaike Information Criterion, and the Ricker function is not considered further here.

Results

Effects of sampler type and geographic zone on mean metric values

There was no effect of sampler type on mean metric value (Fig. 5, see also ESM 7). The effect of geographic zone was significant for nine of fifteen comparisons (Fig. 5). Consistently, the largest differences were between Superior Bay and Spirit Lake.

Relative to the mean, variation in the trimetric index between sampler types and among zones was generally less than variation in the constituent metrics (ESM 7). Variation in the log-transformed ephemeral density metric was about twice that of the trimetric index.

Effect of sampler type on metrics' relationship to depth

Quantile regression slopes for each sampler type were significant in most cases (regression results given in ESM 8). Predicted metric values were slightly higher in standard Ponar samples than in petite Ponar samples for two metrics, percent non-oligochaetes, and the trimetric index (Fig. 6). The relationship to depth (i.e., slope) was similar between sampler types for all metrics. We judged the difference in slopes and intercept between sampler types across metrics to be insufficiently large to warrant separate final models for each sampler type, which would complicate application of the results. We therefore combined data from standard and petite Ponar samples for subsequent analyses. Because more of the samples were collected using a petite Ponar sampler ($n = 419$) than a standard Ponar sampler ($n = 184$), the regression models for the combined data better reflect the sampling characteristics of the petite Ponar sampler.

Effect of geographic zone on metrics' relationship to depth

Regression slopes were significant for all metrics for the 75th percentile models (Table 1). Compared across metrics and zones, regression slopes were steeper for 75th and 50th percentile models (mean slope = -1.3 , from Table 1), than for 25th percentile models (mean slope = -1.0). The bottom of the generally wedge-shaped distribution was flat for all metrics (Fig. 7) suggesting that the effect of depth on benthic condition declined at more stressed sites. There were not fewer sites in poor condition at shallow sites than deep sites, implying that the dominant stressors were not strongly correlated with depth over the 11 m depth range.

Variation in slope and elevation of regressions was greater for geographic zones than for sampler types. At depths greater than 3m, metric values in Spirit Lake were much lower than elsewhere (Fig. 7). Model results for Spirit Lake reflect distinct local geomorphology and contamination history and are considered in more detail in the discussion.

Based on these findings, we used the linear models for each geographic zone (with data pooled across sampler types) for the 75th, 50th, and 25th percentiles to define condition cutoffs. Zone specific models for all condition-class cutoff values for the trimetric index and back-transformed ephemerid density are shown in Fig. 8. Tabulations of condition-class cutoff values are given in ESM 9.

Effects of substrate on mean metric values

Metrics were relatively insensitive to subjectively determined substrate type. For thirty comparisons between substrate types across four sample sets (Fig. 9; see ESM 2 for metadata), only five comparisons were significant (t -tests, $P < 0.05$). For three of the sample sets (MED EPA, NRRI, and 2005 and 2006 invasive species EPA) there was a trend of higher ephemerid density in finer substrates (silt/clay or clay versus sand), but the effect was only significant for one sample set (Fig. 9c). For NRRI samples, mean percent non-oligochaete individuals was significantly higher in sand than silt, and the mean trimetric index value was higher in clay than in silt (Fig. 9b). For invasive species samples collected in 2005 and 2006, the percent ETO individuals was higher in silt/clay than in sand (Fig. 9c).

Relationship of ephemerid density to depth across the Great Lakes.

In SLR estuary samples, the highest observed Ephemered density was shallow, < 2 m (excluding outliers, Figs. 2 and 10). In samples from a nearby Lake Superior embayment, Chequamegon Bay, and the Lake Huron – Lake Erie connecting channels, the highest sampled density of ephemerid nymphs was deeper, at ≈ 6 m (Fig. 10). Across the Great Lakes nearshore zone, the highest density was at ≈ 3 m. When the data from the SLR is combined with records from these deeper systems, the wedge-shaped distribution persists, suggesting this pattern may be general for Ephemeridae (i.e., *Hexagenia*). In all cases where a density of 1000 nymphs/m² was measured (density 3.0 in Fig. 10), depth was < 8 m.

Discussion

We used quantile regression *models* to derive condition-class cutoff values to be used as BUI removal targets for the St. Louis River AOC. The application of these models is potentially confounded by systematic variation in benthos indicators associated with natural or methodological factors unrelated to anthropogenic impacts. If present and unaddressed, this variation could result in “false positives” where methodological or natural limiting factors other than depth decrease the metric value and wrongly indicate impaired conditions. To minimize this potential for bias we have taken several precautions in analyzing and interpreting our data. We standardized the data to remove the effect of sample year (vintage) and sample day (season). Season has a strong effect on sampled assemblage composition in the SLR estuary (Swanson, 1999). Data vintage is a concern because of uncertainty about the colonization history of invasive species (Trebitz et al., 2010). We confirmed that sampler type had only a minor effect on metric values so we could justify combining data from both gear types.

Breneman et al. (2000) and Crane et al. (2005) found a strong longitudinal effect (i.e., distance downriver from Fond du Lac dam; Fig. 4) on benthos diversity and composition in the SLR. Our final models exclude data from the uppermost riverine section of the lower SLR, and are zone-specific which reduces the effect of longitudinal variation in benthos assemblages and in related physical habitat factors such as depth or exposure. We showed that variation in benthos metric values among the substrate types that dominate in the estuarine parts of the SLR (i.e., sand and silt), was minimal, with the exception of clay-dominated substrates, which usually support higher metric values than other substrates. Finally, we created a trimetric index to integrate the benthos response to anthropogenic disturbance for a more reliable assessment of condition at a site.

Although we did not detect strong or consistent substrate effects, substrate characteristics should be noted for all new benthos samples to identify non-typical conditions. Samples with substrates uncharacteristic of the geographic zone or of the estuary in general such as gravel or high clay content should be treated with circumspection to avoid misleading interpretation (e.g., false indication of impaired conditions). The same reasoning applies to samples from areas of more or less pure sand, which in the estuary are mostly restricted to areas near Lake Superior and behind the sand bar that forms the estuary. These substrates are probably insufficiently cohesive for burrow building by ephemerid mayflies, including *Hexagenia* (Edsall, 2001), and caution is recommended in interpreting the ephemerid density metric for samples from these areas. We also recommend that samples that contain substantial non-native substrate (e.g., taconite pellets, coarse or fine wood waste, coal waste) or that are redolent of hydrocarbon residues, not be considered to be in unimpacted condition regardless of the benthic metric values.

Because our condition class cutoffs are based on the ambient distribution rather than on reference conditions defined by traditional methods using independent data, it would not be appropriate to use the cutoff values we determined to assess the condition of the SLR AOC as a population of sites (e.g., Bellinger et al. 2016). The data on which the condition-class cutoff values are based are representative of extant biological conditions at the geographic

zone or estuary scale, date-standardized to mid-August of 2010. Barring large changes in estuary-wide condition, any near-future assessment with a spatial extent and depth range that overlaps this study will, if they use our cutoff values, necessarily conclude that $\approx 25\%$ of the benthos is in poor condition, $\approx 50\%$ is in at least good condition, etc. This would be a circular result – a consequence of defining condition class thresholds and assessing the population using essentially the same information. Our intent is that samples from individual sites or from restoration project areas can be evaluated to determine if they, in the words of the benthos BUI target “differ from unimpacted sites of comparable characteristics within the AOC (MPCA, 2013).” For example, sites within a project area can be evaluated *relative* to all the other sites in the geographic zone at the same depth using the tabulated cutoff values in ESM 9. Alternatively, a project area can be resampled post-project and change in conditions assessed using the tabulated values.

Application to management

We recommend that users of these findings focus on the trimetric index and ephemeral density. As described above, the trimetric index is probably more reliable than constituent metrics because any unknown taxonomic or methodological biases among the combined datasets are “smoothed out” to some degree in combining across the metrics. Because it is based on a single taxon with an inherently patchy distribution, ephemeral density is more variable than the trimetric index (ESM 7). This indicator has a long history in the Great Lakes (Edsall, 2001), however, and it is methodologically straightforward to determine, and likely to be readily perceived by managers as a useful indicator of benthic conditions. The trimetric index and ephemeral density were correlated, but not strongly so ($r = 0.59$), suggesting they are not redundant. They should be considered in combination: where both metrics indicate the same relative condition, confidence in the assessment of the site’s condition should be higher than when the metrics disagree.

Figure 11 shows an example application of these indicators. The plots show the condition class of sites based on our models for samples collected on lower St. Louis Bay including three proposed AOC habitat restoration project areas. The two indicators, the trimetric index and ephemeral density, mostly agree that benthic condition in the 21st Avenue West project area is poor compared to the other project areas. Within the 40th Avenue West and Grassy Point project areas, the indicators are in general agreement with regard to which areas within the project area are in each condition class, but there are sites where the indicators disagree, nearly always by just one condition class, suggesting a localized differential response to stress between the indicators. Plots of site condition at the project area scale (pie charts in Fig. 11) show that the indicators are in broad agreement with regard to benthic condition of each project area.

The Spirit Lake geographic zone is shallower than the other zones in the estuary (ESM 7). Depth range of Spirit Lake samples available for modeling was from near 0 to 8.5 m, but only five sites were deeper than 4 m. The deepest sites sampled in Spirit Lake were from a known-to-be contaminated dredged hole adjacent to the site of the former steel mill (Barr Engineering Inc., 2015). No uncontaminated deep locations were included in the available Spirit Lake data. This has the effect of depressing the expected benthic condition at depth.

Since we have no reason to suspect that deep sites that are in unimpacted condition are entirely absent from Spirit Lake, we recommend that the St. Louis Bay models be used to evaluate samples from Spirit Lake from locations deeper than 3 m.

We produced cutoff values for a specific depth that are greater than the value predicted by the 25th, 50th, and 75th percentile quantile regression line, where sites > the prediction from the 75th percentile were considered in excellent condition. In practice, AOC managers may want to consider sites in good or excellent (i.e., above the depth-specific cutoff defined by the 50th percentile regression line) to be in a condition justifying BUI removal, and to focus instead on addressing, through habitat restoration, sites in fair and poor condition. Conditions defined by the 75th percentile of the ambient distribution as excellent are probably more aspirational than widely attainable at the estuary or AOC scale, at least for the near term.

Ultimately, it will be necessary to define what “does not significantly differ” means for comparing between historically degraded sites and unimpacted or restored sites in the St. Louis River. One approach for habitat restoration project-area assessments could be to quantify the percentage of sampled sites within a prescribed area that are above the appropriate depth-adjusted cutoff value. This approach could be employed for locations resampled post-project, or for randomly or systematically located sites with a project area. A benchmark percentage of sites that needs to be at or above the cutoff could be established to assist decision-making.

In the SLR AOC and presumably elsewhere in the Great Lakes, AOC managers have limited resources for sampling and sample analyses, little institutional capacity to develop or adapt novel techniques for practical application, and short programmatic timelines. Managers need straightforward indicators, condition-class cutoffs, and other benchmarks that can be easily applied by agency staff to existing or new datasets to support multiple lines of evidence for habitat restoration, BUI removal, and AOC delisting decisions. This analysis was directly motivated by the language of the St. Louis River benthos BUI removal target with the goal of supporting the effort to remove all BUIs and achieve delisting of the St Louis River AOC by 2025 (MPCA, 2013). The removal target for the St. Louis River is not unique among AOCs. For example, the removal target for the Milwaukee Estuary AOC requires that “the benthic community... is statistically similar to a reference site with similar habitat and minimal sediment contamination (WDNR, 2013).” As stated above for the St. Louis River AOC, defining the condition justifying BUI removal (i.e., what percentile of the ambient distribution to use), always requires judgement. For the Milwaukee Estuary, “Reference sites, if used, must be in areas that are urban. Reference sites will [therefore] likely be degraded and the [delisting] target will need to take into consideration the achievability of targets (WDNR 2013).”

The approach and models described herein are explicitly designed for practical application; they constitute a practical biomonitoring tool managers can use to inform local decision-making. This study applies specifically to the estuarine part of the lower St. Louis River, but the compiled ephemeral density data suggests that depth-limiting relationships may be

general for some Great Lakes indicator taxa, and could be exploited elsewhere for refining assessments based on this taxon.

Finally, we urge AOC managers to base decisions, whenever possible, on the strongest lines of evidence, including reference conditions derived from sites in undisturbed or minimally disturbed areas (Grapentine, 2009). Our approach of deriving depth (or other biophysical parameter)-adjusted BUI removal criteria using an ambient distribution may be applicable at locations across the Great Lakes where traditional approaches to defining reference condition are not practical.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

Dan Breneman provided benthos data, inspiration, and pie. Julie Lietz assisted with data compilation. Susan SanRomani and Karen Blocksom calculated the benthos metrics. Christy Dolph and Jan Ciborowski, provided comments on the manuscript. The views expressed in this paper are the authors', and do not necessarily reflect views or policies of the U.S. Government. Mention of trade names or commercial products does not constitute endorsement or recommendation for use. This is contribution number xxxx of the Natural Resources Research Institute, University of Minnesota, Duluth.

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Figure 1.

Plot A: Hypothetical distribution of an indicator metric when a natural limiting factor is present. The population represented by all the points has a wedge-shaped distribution implying a linear limiting relationship wherein the maximum value of the metric occurs at a limiting factor value near 0. The population represented by the open symbols has a non-linear peaked relationship. In this case, the maximum value for the metric occurs at a factor value between 1 and 2. Plot B: Wedge-shaped distribution for the density of *Hexagenia* nymphs in the lower St. Louis River from data in Edsall et al. (2004). Filled symbols in plot B are samples that had visible evidence of pollution (e.g., sheen, coal, coal waste).

Figure 2.

Scatter plots of metric values with depth for the St. Louis River estuary. Filled symbols for ephemeropterid density are outliers excluded from quantile regression analysis (see text). The gap in ephemeropterid density is caused by the interval between the $\log_{10}(x+1)$ transformed value for 0 and 1 individuals in a Ponar sample. Regression slopes were significant in all cases, $P < 0.0001$.

Figure 3.

Natural biophysical variables with a relationship to depth that may contribute to the influence of depth on benthos indicators in the St. Louis River estuary. Plot A shows the empirical association between depth and relative exposure index (REI) in the St. Louis River. REI is an integrated measure of natural physical disturbance caused by wind (see Angradi et al. 2013). Plot B shows the empirical relationship between depth and probability of submerged aquatic vegetation (SAV) for benthic sample locations based on local models (Angradi et al., 2013). Plot C shows the decline with depth in the maximum organic matter

content of substrates in the St. Louis River estuary (see Bellinger et al., [2014] for methods). Plot D shows the theoretical maximum horizontal breaking wave water velocity by depth (after Silander and Hall, 1997). The line in plot A is a loess fit for a sampling proportion of 0.4.

Figure 4.

Area map for the lower St. Louis River. Bars delineate geographic zones defined for this study. Locations of three aquatic habitat restoration project areas mentioned in the paper are identified by capital letter. Darker shading of land represents impervious surfaces. Benthos sample locations are shown in ESM 10. Location of Sky Harbor Airport is latitude 46.72692 N, longitude 92.04693W.

Figure 5.

Effect of sampler type and geographic zone on distribution of benthos indicator values for the St. Louis River estuary. Geographic zones are Superior Bay (SB), St. Louis Bay (SLB), and Spirit Lake (SL). Whiskers show 5th and 95th percentiles. Ends of boxes are 25th and 75th percentiles. Solid and dashed line across boxes are median and mean, respectively. Asterisks indicate significant differences based on t-tests. For geographic zone comparisons, the middle asterisk is for the comparison between Superior Bay and Spirit Lake. Mean values, sample sizes, confidence intervals, and coefficients of variation given in ESM 7.

Figure 6.

Quantile regressions (75th percentile) for benthic indicators by sampler type for the St. Louis River estuary. Regression details are given in ESM 8.

Figure 7.

Quantile regressions (75th percentile) for benthic indicators by geographic zone for the St. Louis River estuary. Regression details given in Table 1.

Figure 8.

Geographic-zone-specific predictive models for the trimetric index and ephemeral density for the St. Louis River estuary. Lines in each plot are, from the top down, 75th, 50th, and 25th percentile regression models. Ephemeral density is back-transformed from log-transformed data. Density observations out of plotted y-axis range are not shown (symbols indicate the depth of these observations). Tabular results for models given in ESM 9.

Figure 9.

Plot A: Mean ($\pm 95\%$ confidence intervals) metric values for dominant substrate types for MED EPA 2010 samples (see ESM 2 for sample metadata) from St. Louis Bay. Five samples from infrequent substrate types (clay, gravel) are not represented. Plot B: Mean metric values for dominant substrate types for NRRI samples from St. Louis Bay ($n = 88$) and Spirit Lake ($n = 99$). Four samples from infrequent substrate types (detritus, wood chips) are not represented. Plot C: Mean metric values for dominant substrates for 2005 and 2006 invasive species samples from Superior Bay ($n = 67$), St Louis Bay ($n = 50$), and Spirit Lake ($n = 12$). Plot D: Mean metric values for dominant substrates for 2012 invasive species samples from Superior Bay ($n = 26$) and St Louis Bay ($n = 15$). Four samples from infrequent substrate types (debris, gravel, organic matter) are not represented. Asterisks indicate significant differences (t -test). Values in legends are sample sizes. Metrics: % non-

oligo = arcsine ($x^{0.5}$) transformed percent non-oligochaete individuals; % ETO = arcsine ($x^{0.5}$) transformed percent individuals of Ephemeroptera, Odonata, and Trichoptera; SLR TMI = St. Louis River trimetric index; Dens Ephr = $\log_{10}(x+1)$ transformed ephemerid density (number/m²). Right-hand vertical axis applies to all metrics except number of taxa.

Figure 10.

Ephemerid density plotted against depth for the St. Louis River estuary, Chequamegon Bay of Lake Superior, the Lake Huron - Lake Erie connecting channel (HECC), and the nearshore zone of the Laurentian Great Lakes. Sites where ephemerids (mostly *Hexagenia*) were not collected are not shown. For these data, the deepest sample collected was at 33 m. Metadata given in ESM 2.

Figure 11.

Relative benthic condition from two indicators for the lower reach of St. Louis Bay of the St. Louis River estuary, including three proposed AOC habitat restoration projects (outlined in yellow in the figure; also see Fig. 4). Condition for each sample location was determined using the depth-specific cutoff values tabulated in ESM 9. Values $\leq$ the tabular value for the 25th percentile are in poor condition. Values $>$ the tabular value for the 25th percentile and $\leq$ the tabular value for the 50th percentile are in relatively fair condition. Values $>$ the tabular value for the 50th percentile and $\leq$ the tabular value for the 75th percentile are in

relatively good condition. Values > the tabular value for the 75th percentile are in relatively excellent condition. Pie charts show the percent of sample locations from each project area in each relative condition class. Maps showing the entire SLR AOC are in ESM 10.

Note: Pie charts will appear to the right of the map panels in print.

Table 1.

Linear quantile regression models ($y = a + bx$, where y = the metric value and x = depth in m) for predicting benthos metrics from depth by geographic zone. Models were used to determine depth-adjusted condition-class cutoff values for benthos metrics which are tabulated in ESM 9. Sample sizes for taxa richness apply to other metrics unless specified.

Geographic zone	75 th percentile			50 th percentile			25 th percentile					
	<i>a</i>	<i>b</i> (SE)	<i>t_b</i>	<i>p</i> -value	<i>a</i>	<i>b</i> (SE)	<i>t_b</i>	<i>p</i> -value	<i>a</i>	<i>b</i> (SE)	<i>t_b</i>	<i>p</i> -value
Taxa richness												
Superior Bay (<i>n</i> = 153)	21.92	-0.45 (0.32)	-1.41	<0.05	15.47	-0.27 (0.20)	-1.36	0.18	11.68	-0.31(0.18)	-1.68	0.09
St. Louis Bay (<i>n</i> = 321)	25.76	-0.76 (0.21)	-3.61	<0.001	19.93	-0.58 (0.20)	-2.90	<0.01	15.23	-0.62 (0.17)	-3.68	<0.001
Spirit Lake (<i>n</i> = 129)	27.80	-2.55 (0.84)	-3.03	<0.001	24.39	-2.15 (0.60)	-3.59	<0.001	20.34	-1.70 (0.38)	-4.42	<0.001
Percent non-oligochaete individuals												
Superior Bay	72.29	-2.38 (0.57)	-4.21	<0.001	60.38	-2.94 (0.95)	-3.08	<0.01	33.64	-1.16 (0.69)	-1.69	<0.05
St. Louis Bay	82.57	-1.80 (0.65)	-2.79	<0.01	71.28	-2.67 (0.65)	-4.12	<0.001	55.90	-2.67 (0.68)	-3.90	<0.001
Spirit Lake	90.94	-8.67 (2.66)	-3.26	<0.01	84.80	-9.98 (2.16)	-4.62	<0.001	65.97	-7.76 (1.98)	-3.92	<0.001
Percent ETO individuals												
Superior Bay	10.40	-0.82 (0.26)	-3.19	<0.01	3.42	-0.11 (0.11)	-0.96	0.33	1.27	-0.01 (0.08)	-0.11	0.91
St. Louis Bay	8.04	-0.37 (0.18)	-2.03	<0.05	3.84	-0.27 (0.10)	-2.77	<0.01	1.06	-0.08 (0.04)	-1.95	0.05
Spirit Lake	7.72	-0.91 (0.37)	-2.43	<0.05	3.88	-0.46 (0.20)	-2.31	<0.05	1.18	-0.26 (0.23)	-1.14	0.26
Trimetric index												
Superior Bay	0.57	-0.02 (0.01)	-4.60	<0.001	0.43	-0.02 (0.01)	-3.88	<0.001	0.35	-0.02 (0.01)	-9.70	<0.001
St. Louis Bay	0.63	-0.02 (0.01)	-4.70	<0.001	0.51	-0.02 (0.01)	-4.98	<0.001	0.43	-0.02 (0.01)	-6.08	<0.001
Spirit Lake	0.66	-0.08 (0.02)	-4.23	<0.001	0.59	-0.07 (0.01)	-9.00	<0.001	0.51	-0.06 (0.01)	-5.47	<0.001
Log ₁₀ (<i>x</i> +1) transformed ephemeral density (number/m ²)												
Superior Bay (<i>n</i> = 151)	2.83	-0.25 (0.03)	-3.26	<0.001	2.09	-0.20 (0.03)	-7.29	<0.001	0.29	-0.01 (0.01)	-0.41	0.70
St. Louis Bay (<i>n</i> = 315)	2.25	-0.06 (0.02)	-2.85	<0.01	1.74	-0.17 (0.04)	-3.92	<0.001	0.13	0.01 (0.01)	0.24	0.81
Spirit Lake (<i>n</i> = 129)	2.50	-0.23 (0.12)	-1.98	<0.05	2.20	-0.26 (0.11)	-2.39	<0.05	0.00	0 (0.14)	0.00	1.00