Assessing condition of macroinvertebrate communities and sediment toxicity in the St. Lawrence River at Massena Area-of-Concern

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Abstract

In 1972, the United States and Canada agreed to restore the chemical, physical, and biological integrity of the Great Lakes ecosystem under the first Great Lakes Water Quality Agreement. In subsequent amendments, part of the St. Lawrence River at Massena, New York and segments of three tributaries, were designated as an Area of Concern (AOC) due to the effects of polychlorinated biphenyls (PCBs), lead and copper contamination, and habitat degradation and resulting impairment to several beneficial uses. Because sediments have been largely remediated, the present study was initiated to evaluate the current status of the benthic macroinvertebrate (benthos) Beneficial Use Impairment (BUI). Benthic macroinvertebrate communities and sediment toxicity tests using *Chironomus dilutus* were used to test the hypotheses that community condition and sediment toxicity at AOC sites were not significantly different than those of adjacent reference sites. Grain size was found to be the main driver of community composition and macroinvertebrate assemblages, and bioassessment metrics did not differ significantly between AOC and reference sites of the same sediment class. Median growth of *C. dilutus* and its survival in three of the four river systems did not differ significantly in sediments from AOC and reference sites. Comparable macroinvertebrate assemblages and general lack of toxicity across most AOC and reference sites suggest that the quality of sediments should not significantly impair benthic macroinvertebrate communities in most sites in the St. Lawrence River AOC.

Keywords: St. Lawrence, macroinvertebrate, *Chironomus dilutus*, bioassay, beneficial use impairment, AOC

Introduction

During the 1970s and 1980s, Canada and the United States committed to restore the physical, chemical, and biological integrity of Areas of Concern (AOC) throughout the Great Lakes region under the Great Lakes Water Quality Agreement (GLWQA) (<http://www.epa.gov/greatlakes/glwqa/1978/index.html>). An AOC is defined as “a geographic area that fails to meet the general or specific objectives of the Agreement where such failure has caused or is likely to cause impairment of beneficial uses or of the area’s ability to support aquatic life.” Part of the St. Lawrence River and lower reaches of the Grasse, St. Regis, and Raquette Rivers at Massena, New York (Fig 1) was designated as one of 43 AOCs with impairment to eight beneficial uses caused mainly by past industrial pollution (NYSDEC, 1990). The benthic macroinvertebrate community (benthos) beneficial use was designated impaired because data on its condition were unavailable or inconclusive. Limited data from the New York State Department of Environmental Conservation (NYSDEC) ambient surface water quality monitoring program indicate that the macroinvertebrate communities are not impaired in the St. Lawrence River and its tributaries in the Massena AOC (NYSDEC, 2009, unpublished data). Thus, the benthos Beneficial Use Impairment (BUI) may be outdated in the Massena AOC.

Each AOC has a Remedial Action Plan (RAP) to guide the remedial process towards the goal of attaining similar or better conditions than surrounding areas (USPC, 2001). The primary goal of the St. Lawrence River at Massena AOC (Massena AOC) RAP, developed by NYDEC, the Massena Citizen Advisory Committee (CAC), the Canadian government, the Cornwall Public Advisory Committee (PAC), and the Saint Regis Mohawk Tribe at Akwesasne (SRMT) is to “restore, protect, and maintain the chemical, physical, and biological integrity of the St. Lawrence River ecosystem and in particular the Akwesasne, Cornwall-Lake St. Francis, and

Massena AOC in accordance with the Great Lakes Water Quality Agreement, and other agency laws, regulations, and policies” (Hartig and Thomas, 1988; NYSDEC, 1990, 2006). The RAP established specific criteria in the Massena AOC for removing the “Degradation of Benthos” BUI. These criteria are: (1) “Benthic macroinvertebrate community structure does not significantly diverge from unimpacted control sites of comparable physical and chemical characteristics”, and (or) (2) “In the absence of community structure data, the toxicity of bed sediment-associated contaminants is not significantly higher than controls at unimpacted sites” (George and Boyd, 2007; IJC, 1991; NYSDEC, 2006). For the present study, the term “reference” is used for sites outside the AOC used to establish regional conditions needed to evaluate BUI removal criteria.

The use of regional reference sites of comparable physical condition as a standard for comparison provides a realistic and attainable benchmark for BUI assessment and is in line with RAP goals (USPC, 2001). This approach is recommended by the IJC (1991) in its guidelines for BUI criteria development as well as by Grapentine (2009) and has been used successfully for assessing the plankton BUI in the Massena AOC (Baldigo et al., 2012) and Rochester AOC (Baldigo et al., 2016). It also acknowledges the limited reach of the RAP and AOC program where impacts originating outside the AOC “should not impinge on the ability to delist an AOC” (USPC, 2001). Sources of impairment originating outside the AOC are addressed by Lakewide Management Plans, the Great Lakes Binational Toxics Strategy, and other state and federal programs.

More comprehensive information on the status of benthic communities and toxicity of bed sediments is needed to determine if delisting criteria for the benthos BUI have been achieved. In 2012, the NYSDEC, the U.S. Geological Survey (USGS), and the SRMT

investigated resident benthic macroinvertebrate communities and toxicity of bed sediments from across the region to determine the status of the benthos BUI. Benthic macroinvertebrate community data and bed sediment-toxicity test results were used to test two hypotheses that address the criteria for delisting the benthos BUI. The first is that benthic macroinvertebrate communities from sites in the AOC (in three tributaries and in the St. Lawrence River) are not significantly different or more impacted than the communities found at reference sites located outside the AOC. The second hypothesis is that bed sediments at selected locations in the AOC are no more toxic to macroinvertebrates than are the bed sediments from reference sites outside the AOC.

Testing these two hypotheses provided multiple lines of evidence that characterize the benthic macroinvertebrate community condition and the potential toxicity of bed sediments to representative macroinvertebrate species in the AOC. Additionally, inherent variability in biological assessments must be considered (Smith and Bode, 2004) and is an important limitation for removing BUIs (George and Boyd, 2007) because it often makes quantitative metrics, needed to characterize biological communities and to assess site-to-site differences, difficult to estimate precisely (Stemberger et al., 2001). Because sediment contamination is not necessarily an indication of benthic community degradation, the use of standardized toxicity tests to complement community assessment provides additional certainty in results and helps to define causality (Damásio et al., 2011). All results were used to evaluate whether conditions across the AOC meet the criteria for removing the benthos BUI.

Materials and Methods

Study design

Sediment samples were collected from 17 sites within and 13 sites outside the AOC between July 30 through August 1, 2012, (Fig 1; Table S1). Sites in the AOC were generally located downstream of one or more potential contaminant sources and often upstream and downstream of confluences for major tributaries in the St. Lawrence River. This arrangement maximized the likelihood of sampling contaminated bed sediments from sources in the three AOC tributaries and the St. Lawrence River. Reference sites were selected where fine sediments could be found. With the intent of minimizing watershed driven variability, two to three reference sites, upstream and outside the AOC, were sampled in each river, and two additional reference sites, downstream and outside the AOC, were sampled in the St. Lawrence River. Sample locations consisted of three reference and three AOC sites in the Grasse River, three reference and four AOC sites in the Raquette River, two reference and two AOC sites in the St. Regis River, and five reference and eight AOC sites in the St. Lawrence River (Fig 1; Electronic Supplementary Material (ESM) Table S1). Two sites, one in the power canal near the Alcoa Inc. plant (law-pc), and one in St. Lawrence Seaway Wiley-Dondero Canal between Eisenhower and Snell locks (law-sw), were considered St. Lawrence River AOC sites because their source is the main stem (Fig 1). Duplicate sediment samples were collected from three sites to assess the quality of data generated from the toxicity tests.

Field sampling

Latitude and longitude (ESM Table S1), water temperature, surface velocity, total depth, Secchi depth, date and time were measured and recorded at each study site. A water quality multi-probe meter (YSI 556) was used to characterize water temperature (temp), pH, specific conductance (cond), and dissolved oxygen (DO) profiles. Ten bed sediment samples were collected at each site using a petite Ponar (0.03m²) dredge to assess macroinvertebrate

community, sediment toxicity, and physical habitat. The organic material and organisms from five of the 10 samples were retained by sieving all material through a 500 µm mesh screen bottom bucket. The retained material was placed into individual 1 liter (L) wide-mouth bottles and preserved with 95 % ethanol. These five samples from each site were shipped to a contract taxonomic laboratory for specimen identification.

The material from the remaining five samples was composited in a bucket, mixed, subsampled, and placed into a 1 L polyethylene container. This composite sample was placed in a cooler, chilled with ice, and shipped to Aquatec Biological Sciences for receipt within 48 hours of collection for use in *C. dilutus* toxicity tests. Another subsample was collected from this composite, placed on ice, and shipped to a contract laboratory for analysis of grain size (ASTM, 2007) and total organic content (TOC) (Kahn, 1988). Grain size composition was characterized as clay (<0.005 mm), silt (0.005-0.075mm), fine (0.0750-0.25mm), medium (0.25-0.85mm), and coarse (0.85-2.0mm) sand, and fine gravel (2.0-4.75mm), and converted by modified classification to mean phi units for each site, as described in Cummins (1962), for comparison of substrates among sites. Phi units were used as a simplified grain size distribution because of their importance in New York State (NYS) impairment assessment criteria to determine habitat comparability to minimize physical differences between sites (Bode and Novak, 1995).

Macroinvertebrate community analyses

This study employed NYSDEC's multimetric index of biological integrity, the Biological Assessment Profile (BAP) for Ponar samples to assess benthic community condition (Simpson et al., 1986; Smith et al., 2012). A 100-organism subsample was sorted and identified to the lowest possible taxonomic level, usually genus or species. The Ponar BAP is calculated using species richness, Hilsenhoff Biotic Index (Hilsenhoff, 1987), Dominant-3 (Smith et al., 2012), Percent

Model Affinity (Novak and Bode, 1992), and Shannon-Weiner diversity, each of which is converted to a standardized 10-scale value. The mean of the five 10-scale metrics represents the Ponar BAP score, a single value for which a four-tiered scale of water quality impact (non-, slight, moderate, or severe) have been established (Smith et al., 2012). A BAP score of 5.0 or lower corresponds to moderate or severe impact tiers, designates impaired biological condition, and results in inclusion of the site on the 303(d) list of impaired waters for New York State (NYS) (NYSDEC, 2014).

While intent was to minimize watershed driven variability by comparing AOC and reference reaches within the same river, initial comparisons between benthic communities within rivers indicated a strong habitat driven influence on community composition and abundance. A principle components analysis (PCA) was used to identify habitat gradients in the data that could be related to the macroinvertebrate community. Water quality variables (temp, cond, pH, DO) were excluded from the following analyses because they are potential disturbance gradient indicators and the physical habitat gradient appeared strongest in the PCA. To visualize the biological relationship to physical habitat conditions, a non-metric multidimensional scaling (nMDS) bubble plot (site phi values) was produced from a $\log(x+1)$ transformed Bray-Curtis similarity matrix of combined site replicate taxa composition and abundance. The site distribution in the nMDS was correlated (Spearman rank) and overlaid with a euclidean distance matrix of physical habitat variables. The length of each vector represented the strength of the relationship of each habitat variable to the nMDS axes. The nMDS, along with Biota-Environmental Stepwise (BEST) analysis, suggested macroinvertebrate community composition was distributed across a gradient of grain size. The BEST analysis (Primer-6) uses Spearman rank correlation between a sample similarity matrix (taxa assemblage) and a normalized variable

matrix (physical habitat) that identifies the best combinations of variables explaining the similarity matrix (Clarke et al., 2008b). Significance ($P < 0.05$) of the BEST analysis is determined by comparing 1000 random variable permutations to the variable combinations with the highest correlation. Because grain size is well established as primary driver of sediment macroinvertebrate community composition (Breneman et al., 2000; Crane et al., 2005; Panis et al., 1995), a critical component in NYS biological impairment criteria assessment (Bode and Novak, 1995), and it explained a significant amount of the variance in the community distribution, it was used to reconfigure AOC and reference groupings into statistically significant sediment classes to minimize habitat driven community variance. A hierarchical cluster analysis with a similarity profile (SIMPROF) test on a resemblance matrix (Euclidean distance) of normalized grain size compositions was used to restructure AOC and reference sites into four significantly different sediment classes. The SIMPROF test looks for statistically significant multivariate patterns within nodes of a dendrogram by determining whether observed similarities between sites were greater than those expected by chance (Clarke et al., 2008a; Clarke and Gorley, 2006; Somerfield and Clarke, 2013). Significance ($P < 0.05$) was determined by generating the test statistic π between real cluster similarities and 1000 random variable permutations and comparing it to the null distribution.

Non-parametric Kruskal-Wallis tests were run on replicate BAP scores between pooled reference and pooled AOC sites for each river and sediment class to assess the relative macroinvertebrate community integrity. Additionally, analysis of Similarity (ANOSIM) (Clarke, 1993) of a $\log(x+1)$ transformed Bray-Curtis similarity matrix of taxa abundances was used to evaluate community structure separation between AOC and reference sites within each river and sediment class. ANOSIM is a non-parametric method used to compare and assess the

significance of difference in species abundance and composition among defined groups. ANOSIM computes a test statistic, R, between 0 (no difference) and 1 (complete separation) indicating group dissimilarity. According to Clarke and Gorley (2006), the R values should be considered the primary ANOSIM output because the P-value is largely a function of sample size, whereas the R value is not. An R value threshold of less than 0.25 indicates barely separable groups, a value of 0.25 to 0.5 indicates barely distinguishable but overlapping groups, an R value of 0.5 to 0.75 indicates separate but overlapping groups, and values greater than 0.75 indicate well separated groups (K.R. Clarke, 2016, personal communication) (Ramette, 2007). Lastly, the similarity percentage-species contributions (SIMPER) procedure (Clarke and Warwick, 2001), which breaks down the contribution of each taxon to the Bray-Curtis similarity matrix, was used to identify taxa driving dissimilarity between statistically different AOC and reference sites within sediment classes.

Chironomous dilutus bioassays

Aquatec Biological Sciences (Williston, VT) initiated all *C. dilutus* toxicity tests within 72 hours of sample collection using a modified U.S. Environmental Protection Agency (USEPA) chronic (50- to 65-d) life-cycle test for sediment-associated contaminants, Test Method 100.5 (USEPA, 2000). This method was abbreviated to 20-d to provide acute (survival) and chronic (growth) endpoints. Each test was started with eight replicates (modified from 16 as described in Test Method 100.5 (USEPA, 2000) of 12 first instar (less than 24-h old) larvae and continued through day 20. Each test chamber (300 mL) contained 100 mL of sediment and 175 mL of overlying clean (laboratory-control) water and is maintained at 23 °C with a 16-h light and an 8-h dark photoperiod (illuminance of 100 to 1,000 lux). The overlying water was renewed daily within each replicate by adding 350 mL of laboratory-control water (175 ml every 12h). The

larvae in each chamber were fed 1.5 mL of a 4-g/L Tetrafin[®] suspension daily. Survival and growth were measured after 20 days of exposure to site sediments. Survival at each site was determined by the mean number of surviving larvae divided by the original number of larvae used to initiate the test in each of eight replicates. Growth at each site was defined by the mean ash-free dry weight (AFDW) of all surviving larvae in each of the replicates at the end of the test, assuming they essentially weighed 0 mg at the start of each test. The quality of data generated by the toxicity tests was assured by (a) confirming that the sensitivity of test organisms was within normal ranges based on results using a standard control toxicant (SRT), (b) using laboratory-water controls to verify that test conditions and organism responses generally met minimally acceptable standards (Test Acceptability Criteria), and (c) including three sets of duplicate site-water tests to determine if the precision of test endpoints was acceptable or (USEPA, 2000).

Bioassay data were summarized and used in Kruskal-Wallis one-way analysis of variance tests to assess the statistical significance of differences between median *C. dilutus* survival and growth (toxicity endpoints) as determined at the end of respective exposures to sediments from AOC sites and reference sites. Because sediment grain size is not as critical to toxicity test results (USEPA, 2000), all analysis presented are between AOC and reference sites on the same river. Differences for all statistical tests were considered significant at $P \leq 0.05$. Any group of AOC sites found with median *C. dilutus* survival or growth endpoints that were significantly lower than that in a group of corresponding reference sites were considered adversely affected.

Results

Macroinvertebrates and habitat

Physical habitat (Table 1A) generally differed within and across rivers and could potentially confound analysis of community differences between AOC and reference sites. Sediment TOC was highly variable between AOC and reference sites in the tributaries, especially in the Grasse River, where TOC measured from AOC sites averaged over five times higher than respective reference sites. Differences in phi units and grain size composition in the Grasse River indicated that sediments were finer at AOC sites than reference sites. The sediments in both AOC and reference sites of the St. Lawrence River were relatively fine as indicated by high silt (50 and 53 %, respectively) and clay (14 and 7.5 %, respectively), as well as high phi values (5.5 and 4.7, respectively) (Table 1A). The PCA (Table 2) of environmental variables indicated silt was most correlated with principle component (PC) 1 and sand/gravel were most correlated with PC2 and both variables accounted for 56% of the variance. The nMDS of study sites overlaid with physical habitat vectors relating to the community composition illustrated the relationship between grain size, phi values, and community composition (Fig 2). In contrast, TOC appeared as a minor vector on the nMDS and was not a significant factor in the BEST analysis determining the macroinvertebrate community distribution in this study. The BEST analysis ($P = 0.01$) suggested clay and silt explained 36 percent of the variance in the community distribution while various other combinations of grain size explained 33 percent (Fig 2).

Because grain size composition has been found to be an important compositional driver of sediment dwelling macroinvertebrates in this (Fig 2) and other studies (Bond et al., 2014; Breneman et al., 2000; Crane et al., 2005; Panis et al., 1995; Simpson et al., 1986; Smit et al., 1995) and is a critical component in NYS biological impairment criteria assessment (Bode and Novak, 1995), it was used to reconfigure the AOC/reference groups into four statistically

significant sediment classes to control for physical habitat driven community differences (Fig 3; ESM Table S1). Sediment class 4 contained sites with the coarsest sediments and consisted of some of the most upstream sites in the three tributaries (reference – reg-04, raq-06, gra-06) along with one site located in the Massena Power Canal (AOC – law-pc). These sites were characterized by the coarsest and most heterogeneous sediment composition averaging over 10 % fine gravel and coarse sand (Table 1B). Sites in class 2 were slightly less coarse than those in class 4 but included only two sites located in the AOC on the Raquette River (raq-02 and raq-03) (Table 1B). Thus, AOC sites in class 2 had no comparable reference sites to test for differences in BAP scores and community composition. Sites in class 1 contained the highest percentage of fine sand (67.6-87.9 %) and included sites from the St. Lawrence River (reference – law-10, law-11; AOC – law-04) and all three tributaries (reference – reg-03, raq-05, raq-07, gra-04, gra-05; AOC – reg-01, reg-02, raq-01, raq-04) (Table 1B). The sites in class 3 represented the most depositional habitat as characterized by large amounts of silt (39.7-75.1 %) and clay (3.1-33.2 %) which was reflected in the high phi values relative to other sediment classes. Class 3 included St. Lawrence River (reference – law-02 and law-12; AOC – law-03, law-05, law-06, law-08, law-09, and law-sw) and the three most downstream Grasse River sites (AOC – gra-01, gra-02, and gra-03) (Table 1B).

Median BAP scores for the macroinvertebrate assemblages ranged from 3.21 to 8.74 (Fig S1; Table S2) across all study sites and indicated that the condition of local benthic communities varied from moderately impacted to non-impacted (Smith et al., 2012). Median scores ranged from 3.33 to 8.74 at all reference sites and from 3.21 to 7.93 at all AOC sites (ESM Fig S1; ESM Table S2). When analyzed by river system, Kruskal-Wallis tests indicated that BAP scores at AOC and reference sites differed significantly in the St. Regis, Raquette, and Grasse Rivers ($P =$

0.019, 0.023, <0.001, respectively) (ESM Table S3A; Fig 4A; ESM Fig S2). BAP scores did not differ between AOC and reference sites in the St. Lawrence River ($P = 0.426$) (ESM Table S3A; Fig 4A; ESM Fig S2). When controlling for grain size composition, except for sites in sediment class 4, the BAP scores were not significantly different between most AOC and reference sites within the same sediment class (ESM Table S3B; Fig 4B).

Results of ANOSIM conducted between AOC and reference sites within rivers suggest moderate differences between AOC and reference macroinvertebrate composition and abundances for the Grasse and St. Regis rivers (R statistic = 0.56 and 0.57, respectively) (Table 3A). When analyzed by sediment class, differences in macroinvertebrate composition and abundance indicate a large separation between AOC and reference sites of sediment class 4 (Table 3B). An examination of compositional differences between sediment classes indicated a few taxa were common among most sites, but that certain very abundant taxa were driving similarity within each class. The most ubiquitous and abundant taxon was the predatory midge, *Procladius* sp. Both *Polypedilum halterale* gr. and *Dubiraphia* sp. were also very common among all sediment classes. *Chironomus* sp. was most common in sediment class 3 along with *Sialis* sp. (Megaloptera: Sialidae). The burrowing mayfly, *Hexagenia* sp., was found primarily in the fine sediments of class 3. Oligochaeta were generally uncommon, contributing an average of 3.5 % to each class similarity (range 2.5–4.89 %) and an overall average community abundance of 4.5 % (range 0–49 %). SIMPER analysis indicated 86% dissimilarity between AOC and reference sites in sediment class 4 of which 26% was made up of *Procladius* sp., *Dubiraphia* sp., *Polypedilum halterale* gr., *Pseudochironomus* sp., and *Dicrotendipes modestus*. SIMPER identified Ceratopogonidae, Sphaeriidae, Tubificidae, *Chironomus* sp, and *Cladopelma* sp. as the dominant taxa at law-pc, the only AOC site in sediment class 4.

Sediment grain size varied widely and phi values were much higher at Grasse River AOC sites (5.6 to 6.2) than the upstream reference (3.3 to 3.8) portion of the river indicating much finer sediment in the AOC (ESM Table S2). There was an average of 3.3 EPT taxa at three reference sites, but only an average of 1.0 EPT taxon on at the three AOC sites in the Grasse River. The burrowing mayfly, *Hexagenia* sp., often abundant in depositional or lentic habitats (Merritt and Cummins, 1996), was the most common EPT taxon at Grass River AOC sites. *Hexagenia* sp. was also found at the furthest downstream AOC sites in the Raquette and St. Regis Rivers. *Centroptilum* sp. (Ephemeroptera: Baetidae) and caddisflies, mainly from the families Leptoceridae (*Mystacides* sp., *Oecetis* sp.) and Helicopsychidae (*Helicopsyche* sp.), typically found in a lotic environments (Merritt and Cummins, 1996), were most common at reference sites in the Grasse River. Lentic, vascular plant-associated *Callibaetis* sp. (Ephemeroptera: Baetidae) and two caddisflies of the family Hydroptilidae, *Agraylea* sp. and *Oxyethira* sp. (Merritt and Cummins, 1996), were also found in the reference reach of the Grasse River where habitat was shallow and macrophytes were evident.

In situ water chemistry measurements for each river suggested variable chemical habitat, particularly between AOC and reference sites in the Grasse, Raquette, and St. Regis Rivers. In the Grasse and Raquette Rivers, mean pH and DO increased substantially between the reference and AOC sites and DO decreased in the St. Regis AOC by 0.5 mg/L compared to the reference reach (Table 4A). In the Grasse River, specific conductance averaged 143 $\mu\text{S}/\text{cm}$ at AOC sites and 88 $\mu\text{S}/\text{cm}$ at reference sites. Except for temperature in the St. Lawrence, which was lower than in the tributaries, most chemical parameters did not differ significantly between AOC and reference sites (Table 4A). When restructured by sediment class, chemical habitat became more uniform between AOC and reference sites in the same class, except for sediment class 4 (Table

4B). The single AOC site in class 4, law-pc, differed from all other AOC and reference sites; it was the deepest site (5.0 m), was hypoxic at bottom (DO=1.2 mg/L), had a specific conductance of 452 $\mu\text{S}/\text{cm}$, and was stratified with a surface temperature of 25.3°C and a bottom temperature of 14.7°C.

Chironomus dilutus survival and growth

The median survival of *C. dilutus* at the end of 20-d exposures to sediments from all AOC and reference sites ranged from 41.7 to 87.5 % (Table S4). The median AFDW of surviving individuals in sediments from all AOC and reference sites ranged from 1.7 to 2.9 mg (ESM Table S4). The number of *C. dilutus* replicates assessed (n) and measures of variability (SD and SE) for survival and AFDW at the end of the 20-d exposures to sediments from each study site, and laboratory controls, are also summarized in ESM Table S4.

Results of Kruskal-Wallis tests for survival and AFDW in pooled AOC sites and pooled reference sites within each river system generally indicated that median survival of *C. dilutus* did not differ significantly between reference and AOC sites within each system (*P*-values ranged from 0.046 to 0.970) (Fig 5A; ESM Table S5). The one exception was in the Raquette River where median survival was significantly greater in the reference sites (66.7 %) than the AOC sites (58.3 %) (*P* = 0.046), mainly due to unusually high survival in sediments from one reference site – raq06 (Fig S2A). Median *C. dilutus* growth at the end of exposure periods did not differ significantly between the reference and AOC sites in any river system (*P*-values ranged from 0.105 to 0.826) (Fig 5B; ESM Table S5).

Quality assurance data from the sediment toxicity tests were generally within acceptable ranges and indicated that toxicity test data are valid. The 96-h LC50 value generated from an

SRT test done on August 21, 2012, was 5.30 g/L KCl for *C. dilutus* and fell within the acceptable range for quality control charts maintained by Aquatec Biological Sciences Inc. for this test organism. The mean AFDW for *C. dilutus* in both laboratory controls ranged from 1.4 to 1.6 mg (ESM Table S4), which surpassed the minimum growth criteria of 0.48 mg for 20-d old larvae used in life-cycle tests (USEPA, 2000). Though growth is the primary 20-d test-acceptability criteria according to Table 15.1 (USEPA, 2000), this document also recommends that average *C. dilutus* survival “in control sediment should be greater than or equal to 70 % on day 20 during long-term, life-cycle tests”. Survival of *C. dilutus* in both laboratory-control tests averaged 67.7 and 68.8 % (ESM Table S4), slightly less than the 70 % target. The tests were considered acceptable, however, because they were essentially at the threshold and resulted from unusually low survival (40 to 50%) in only one of the eight control replicates. Data from the three sets of duplicate samples indicated that the absolute Relative Percent Difference for *C. dilutus* survival and growth averaged 19.3 and 16.3 %, respectively, which met original data quality objectives.

Discussion

Sediment composition appears to be the main driver of macroinvertebrate community assemblages across the Massena AOC and at nearby reference sites. This finding is supported by the river continuum concept (Vannote et al., 1980) which predicts structural and functional shifts in biological communities occurring in response to the physico-chemical gradient from headwaters to the lowest reaches of a river system. Such shifts could be accelerated by physical alterations in the AOC created by the St. Lawrence River and Seaway that produce depositional habitat in the lower reaches of its tributaries compared to shallow, run of the river conditions immediately upstream. Variations in substrate composition meant that an unbiased evaluation of community condition between reference and AOC sites from the same river system was not

possible without first grouping sites by sediment class. Comparing macroinvertebrate community data from AOC and reference sites within the same sediment classes eliminated the longitudinal, habitat driven differences in the community composition and enabled unbiased evaluations by using reference sites of “comparable physical and chemical characteristics” (IJC, 1991; NYSDEC, 2006). Prior work also indicated that habitat was a main driver of benthic communities in the Massena AOC (USFWS, 1979) and noted the relationship between habitat alteration and macroinvertebrate populations in the St. Lawrence River (Patch et al., 1986). Other investigations have found that habitat comparability is a critical requirement for assessing sediment-dwelling macroinvertebrate communities (Bond et al., 2014; Breneman et al., 2000; Cummins and Lauff, 1969; Panis et al., 1995; Peeters et al., 2001; Simpson et al., 1986; Smit et al., 1995). Reinhold-Dudok van Heel and den Besten (1999) used a similar substrate-classification approach (grouped sites by grain size and organic matter) to control for sediment heterogeneity while evaluating the impacts of contamination on macroinvertebrate communities. Their approach yielded four grain size categories within which changes to the community could be attributed to stressors such as sediment contamination and toxicity. Grapentine (2009) and others suggest that by separating variability due to natural causes (i.e., grain size) the range of condition within reference sites can be better defined and the sensitivity of analysis between test and reference sites can be improved (Bailey et al., 1998; Reynoldson et al., 1997).

Acute and chronic toxicity results provide a second line of evidence that generally supported the conclusion that there were few differences between macroinvertebrate assemblages from sediments of the AOC and from the surrounding region. In the present study, toxicity results generally indicated no significant differences in endpoints at AOC and reference sites within the same rivers. Consistent with other studies, habitat was found to be the primary driver

of community composition where toxicity and community results differed. Additionally, when toxicity data from the present study sites were analyzed by sediment class (not shown), no differences in acute or chronic endpoints were found to be statistically significant. The pairing of toxicity data with the results of the community assessment to evaluate the removal criteria for the benthos BUI strengthens our conclusions and supports the concept that habitat, not toxic contamination, is the primary driver of the composition of benthic macroinvertebrate communities in the St. Lawrence River AOC.

Community and toxicity endpoints from individual AOC sites were also compared to corresponding reference site groups (river and sediment class) to ensure that any localized contamination at individual AOC sites was not overlooked (ESM Table S6). While this approach to AOC site assessment identified significant differences in toxicity endpoints at several AOC sites, there was little consistency between toxicity and community results, nor did these differences correspond to known areas of contamination (NYSDEC, 1990, 2006). This disconnect indicates that survival and growth results are not always reflected by community composition and, conversely, that community degradation does not always predict toxicity. Comparisons of data from pooled AOC and pooled reference sites may better reflect overall conditions across the AOC because it accounts for the normal biological variability (ter Braak and Verdonschot, 1995).

Without accounting for the effects of habitat, the present study may have concluded that sediment contamination from past industrial discharges still significantly affects macroinvertebrate communities in some parts of the AOC. Historical data collected by the NYSDEC Stream Biomonitoring Unit and others, however, indicate that benthic community structure was not necessarily correlated with the presence of PCBs (Novak et al., 1988; Peeters et

al., 2001). The Grasse River contains a 7.2-mile stretch of unremediated PCB-contaminated sediments originating from the ALCOA plant at the uppermost point in the AOC (<http://www.thegrasseriver.com/index.html>, accessed September 5, 2013). However, habitat in the AOC portion of the Grasse River is altered more strongly than it is in AOC reaches of other two tributaries. The most obvious modifications are caused by the 22-million gallon lockage releases from the St. Lawrence Seaway through the Snell Lock and physical alterations to the channel made to accommodate diverted flow through the now closed power canal. Reverse flows regularly occur in the downstream (AOC) portion of the Grasse River because of the Snell Lock releases. Downstream of the city of Massena, the Grasse River is generally five to eight meters deep and 120 to 180 m wide with steep banks, while the river upstream remains in a more natural state, and is generally one to two meters deep and 60 m wide with better flood plain access. In the absence of additional flow provided by the power canal, water velocities are slower and conditions are more lentic and depositional at most AOC sites (<http://www.thegrasseriver.com/index.html>, accessed September 5, 2013) compared to most upstream reference sites. Low velocities, inactive diversions, and flow reversals likely have a strong effect on sediment sizes and habitat quality in the downstream (AOC) portions of the Grasse River and could account for seemingly impaired (low BAP scores) resident macroinvertebrate assemblages. The St. Lawrence Seaway also creates similar depositional effects in lower AOC segments of the other two tributaries. The highly divergent physical conditions at sites in the upper and lower (AOC) segments in the Grasse River exacerbated differences in community composition more so than habitat differences did in the other two rivers. Dredging and remediation of contaminated AOC sections of the Grasse River is scheduled to be completed by 2020 (<http://www.thegrasseriver.com/index.html>, accessed September 5,

2013), but removal of contaminated sediments may not improve benthic community conditions because of flow and habitat issues described above.

While community and toxicity data generally indicate that conditions inside and outside the AOC are comparable after accounting for habitat differences, the results from several sites could not be explained (or assessed) by sediment composition alone. Only two AOC sites on the lower Raquette River (raq-02, raq-03) were classified in sediment class 2, therefore, results could not be compared to data from reference sites. Toxicity results at Raquette River sites provided some conflicting evidence for an AOC impact; survival in waters from the four Raquette River AOC sites were significantly lower than survival in the three upstream reference sites, but the comparison appeared to be influenced by an unusually high survival at one reference sites, raq06 (ESM Fig. S2A). In contrast, growth appears to have been enhanced at Raquette River AOC sites and others with the loss of individuals and increased food availability to the surviving organisms in the bioassay (ESM Fig S2A). The macroinvertebrate community at one AOC site located in the Massena Power Canal (law-pc), which formerly diverted water from the St. Lawrence River to the Grasse River for power generation, differed significantly from the reference sites in sediment class 4. The Power Canal now functions ecologically as a lentic habitat with deep, stratified, and anoxic conditions at the bottom. Although the site was physically comparable to the reference sites in sediment class 4, it was not chemically comparable to other AOC or reference sites in the same class due to greater depth (5.0 m), thermal stratification, and hypoxic dissolved oxygen levels (1.2 mg/L) at the bottom. Toxicity tests indicate sediments at this site were not toxic, therefore, hypoxic conditions appear to limit benthos communities at this AOC site.

Our findings have several important implications for biological assessments in this and other AOCs across the Great Lakes. First, our ensemble approach provided data which helped support and explain unusual results and thereby reduced uncertainty in our interpretations and conclusions. This approach is very effective and might benefit BUI assessments in other AOCs. Second, comparison of AOC results to regional reference sites of comparable habitat provides an unbiased assessment of the AOC within the broader context of watershed stressors. Given that the intent of the AOC remedial process is to assure that conditions within AOCs are comparable to surrounding (non-AOC) areas, comparisons between AOC and regional reference sites are an essential component of BUI-criteria assessments (Grapentine, 2009; USPC, 2001). Third, consistent with results from other studies, habitat should be a major consideration when designing or implementing benthos BUI assessments, especially in AOCs that border lakes and seaways. Fourth, additional community and toxicity assessments may be warranted in the lower Grasse River because of current contaminant and habitat issues and because sediment remediation efforts are planned for the near future. Lastly, even though uneven distribution of AOC and reference sites in sediment classes limit some community comparisons, the acute and chronic toxicity tests generally support the results of the benthic community assessment and indicate that both removal criteria for the benthos BUI have been met in the St. Lawrence River AOC.

Acknowledgements

The authors extend their appreciation to James Snyder and James Costello of the Saint Regis Mohawk Tribe at Akwesasne for field support. We also thank the Saint Regis Mohawk Tribe for permission to access their lands. Sampling locations and methods used in this manuscript were influenced by discussions with technical advisors from Federal and New York

State Government, the Saint Regis Mohawk Tribe, the St. Lawrence at Massena New York Area of Concern Remedial Action Program committee, and private consulting firms involved in sediment quality and biological assessments. This research was supported by funds from the New York State Department of Environmental Conservation, the U.S. Geological Survey, and the U.S. Environmental Protection Agency under the Great Lakes Restoration Initiative.

References

- ASTM, 2007. Standard Test Method for Particle-Size Analysis of Soils. ASTM International, West Conshohocken, PA, 2007, DOI: 10.1520 D422
- Bailey, R.C., Kennedy, M.G., Dervish, M.Z., 1998. Biological assessment of freshwater ecosystems using a reference condition approach: comparing predicted and actual benthic invertebrate communities in Yukon streams. *Freshwat Biol* 39, 765-774.
- Baldigo, B.P., Duffy, B.T., Nally, C.J., David, A.M., 2012. Toxicity of waters from the St. Lawrence River at Massena Area-of-Concern to the plankton species *Selenastrum capricornutum* and *Ceriodaphnia dubia*. *J Great Lakes Res* 38, 812-820.
- Baldigo, B.P., Duffy, B.T., Smith, A.J., George, S.D., 2016. Toxicity of waters from the Rochester Embayment Area of Concern to the plankton species *Pseudokirchneriella subcapitata* and *Ceriodaphnia dubia*. *J Great Lakes Res.*
- Bode, R.W., Novak, M.A., 1995. Development and Application of Biological Impairment Criteria for Rivers and Streams in New York State, in: Davis, W.S., Simon, T.P. (Eds.), *Biological Assessment and Criteria Tools for Water Resource Planning and Decision Making*. CRC Press, Boca Raton, FL, pp. 97-107.

- Bond, M., Rowan, D., Silke, R., Carr, J., 2014. Drivers of Abundance and Community Composition of Benthic Macroinvertebrates in Ottawa River Sediment near Chalk River Laboratories. *Nuclear Review* 2, 41-54.
- Breneman, D., Richards, C., Lozano, S., 2000. Environmental influences on benthic community structure in a Great Lakes embayment. *J Great Lakes Res* 26, 287-304.
- Clarke, A., Mac Nally, R., Bond, N., Lake, P., 2008a. Macroinvertebrate diversity in headwater streams: a review. *Freshwat Biol* 53, 1707-1721.
- Clarke, K.R., 1993. Non-parametric multivariate analyses of changes in community structure. *Aust J Ecol* 18, 117-143.
- Clarke, K.R., Gorley, R.N., 2006. *PRIMER v6: User Manual/Tutorial*. PRIMER-E, Plymouth, England.
- Clarke, K.R., Somerfield, P.J., Gorley, R.N., 2008b. Testing of null hypotheses in exploratory community analyses: similarity profiles and biota-environment linkage. *J Exp Mar Biol Ecol* 366, 56-69.
- Clarke, K.R., Warwick, R.M., 2001. *Change in marine communities: an approach to statistical analysis and interpretation*, 2nd Edition. PRIMER-E Ltd, Plymouth, UK.
- Crane, J.L., Richards, C., Breneman, D., Lozano, S., Schuldt, J.A., 2005. Evaluating methods for assessing sediment quality in a Great Lakes embayment. *Aquat Ecosyst Health Manage* 8, 323-349.
- Cummins, K.W., 1962. An evaluation of some techniques for the collection and analysis of benthic samples with a special emphasis on lotic waters. *Am Midl Nat* 67, 477-504.
- Cummins, K.W., Lauff, G.H., 1969. The influence of substrate particle size on the microdistribution of stream macrobenthos. *Hydrobiologia* 34, 145-181.

- Damásio, J., Fernández-Sanjuan, M., Sánchez-Avila, J., Lacorte, S., Prat, N., Rieradevall, M., Soares, A.M., Barata, C., 2011. Multi-biochemical responses of benthic macroinvertebrate species as a complementary tool to diagnose the cause of community impairment in polluted rivers. *Water Res* 45, 3599-3613.
- George, T.K., Boyd, D., 2007. Limitations on the development of quantitative monitoring plans to track the progress of beneficial use impairment restoration at Great Lakes Areas of Concern. *J Great Lakes Res* 33, 686-692.
- Grapentine, L.C., 2009. Determining degradation and restoration of benthic conditions for Great Lakes Areas of Concern. *J Great Lakes Res* 35, 36-44.
- Hartig, J.H., Thomas, R.L., 1988. Development of plans to restore degraded areas in the Great Lakes. *Environ Manage* 12, 327-347.
- Hilsenhoff, W.L., 1987. An improved biotic index of organic stream pollution. *Great Lakes Entomol* 20, 31-40.
- IJC, 1991. Commission approves list/delist criteria for Great Lakes areas of concern. Focus on International Joint Commission Activities, Volume 16, Issue 1, ISSN 0832-6673. International Joint Commission United States and Canada.
- Kahn, L., 1988. Determination of total organic carbon in sediment. United States Environmental Protection Agency, Region II, Edison, NJ.
- Merritt, R.W., Cummins, K.W., 1996. An introduction to the aquatic insects of North America. Kendall Hunt.
- Novak, M., Reilly, A., Jackling, S., 1988. Long-term monitoring of polychlorinated biphenyls in the Hudson River (New York) using caddisfly larvae and other macroinvertebrates. *Arch Environ Contam Toxicol* 17, 699-710.

- Novak, M.A., Bode, R.W., 1992. Percent model affinity - A new measure of macroinvertebrate community composition. *J N Am Benthol Soc* 11, 80-85.
- NYSDEC, 1990. St. Lawrence River at Massena Remedial Action Plan- Stage 1. New York State Department of Environmental Conservation, Great Lakes Section, Division of Water, Albany, NY, p. 233 pp.
- NYSDEC, 2006. St. Lawrence River at Massena, New York Remedial Action Plan Status Report: October 2006. New York State Department of Environmental Conservation and the St. Lawrence River at Massena Remedial Advisory Committee, Albany, NY, p. 138.
- NYSDEC, 2014. 2014 NYSDEC Section 303(d) List of Impaired Waters, Albany, NY.
- Panis, L.I., Goddeeris, B., Verheyen, R.F., 1995. On the reliability of Ponar grab samples for the quantitative study of benthic invertebrates in ponds. *Hydrobiologia* 312, 147-152.
- Patch, S.P., Busch, W.D.N., Fish, U.S., Office, W.S.C.F., District, U.S.A.C.o.E.B., 1986. A Biological Survey in the International Section of the St. Lawrence River: With Special Emphasis on Aquatic Macrophytes, Fish Spawning, and Macroinvertebrates. U.S. Department of the Interior, Fish and Wildlife Service, Cortland Field Office.
- Peeters, E.T., Dewitte, A., Koelmans, A.A., van der Velden, J.A., den Besten, P.J., 2001. Evaluation of bioassays versus contaminant concentrations in explaining the macroinvertebrate community structure in the Rhine-Meuse delta, The Netherlands. *Environ Toxicol Chem* 20, 2883-2891.
- Ramette, A., 2007. Multivariate analyses in microbial ecology. *FEMS Microbiol Ecol* 62, 142-160.

- Reinhold-Dudok van Heel, H.C., den Besten, P.J., 1999. The relation between macroinvertebrate assemblages in the Rhine-Meuse delta (The Netherlands) and sediment quality. *Aquat Ecosyst Health Manage* 2, 19-38.
- Reynoldson, T.B., Norris, R.H., Resh, V.H., Day, K.E., Rosenberg, D.M., 1997. The reference condition: A comparison of multimetric and multivariate approaches to assess water-quality impairment using benthic macroinvertebrates. *J N Am Benthol Soc* 16, 833-852.
- Simpson, K.W., Fagnani, J.P., Bode, R.W., DeNicola, D.M., Abele, L.E., 1986. Organism-substrate relationships in the main channel of the lower Hudson River. *J N Am Benthol Soc* 5, 41-57.
- Smit, H., Reinhold-Dudok van Heel, H.C., Wiersma, S.M., 1995. Sublittoral macrozoobenthic assemblages in the enclosed sediment-polluted Rhine-Meuse Delta; their relationship to environmental conditions. *Netherlands J Aquat Ecol* 29, 31-47.
- Smith, A.J., Bode, R.W., 2004. Analysis of variability in New York State benthic macroinvertebrate samples. New York State Department of Environmental Conservation, Albany, New York, p. 43.
- Smith, A.J., Heitzman, D.L., Lojpersberger, J.L., Duffy, B.T., Novak, M.A., 2012. Standard Operating Procedure: Biological Monitoring of Surface Waters in New York State. New York State Department of Environmental Conservation Albany, NY, p. 163.
<http://www.dec.ny.gov/chemical/23847.html>.
- Somerfield, P.J., Clarke, K.R., 2013. Inverse analysis in non-parametric multivariate analyses: distinguishing groups of associated species which covary coherently across samples. *J Exp Mar Biol Ecol* 449, 261-273.

- Stemberger, R.S., Larsen, D.P., Kincaid, T.M., 2001. Sensitivity of zooplankton for regional lake monitoring. *Can J Fish Aquat Sci* 58, 2222-2232.
- ter Braak, C.J., Verdonschot, P.F., 1995. Canonical correspondence analysis and related multivariate methods in aquatic ecology. *Aquat Sci* 57, 255-289.
- USEPA, 2000. Methods for measuring the toxicity and bioaccumulation of sediment associated contaminants with freshwater invertebrates. Second Edition. US Environmental Protection Agency, Office of Research and Development, Duluth, MN.
- USFWS, 1979. Biological along the St. Lawrence River for the St. Lawrence Seaway additional locks and other navigation improvements study, Cortland, NY, p. 574.
- USPC, 2001. Restoring United States Areas of Concern: Delisting Principles and Guidelines. United States Policy Committee.
- Vannote, R.L., Minshall, G.W., Cummins, K.W., Sedell, J.R., Cushing, C.E., 1980. The river continuum concept. *Can J Fish Aquat Sci* 37, 130-137.

Table 1. Number of bed sediment samples and mean concentration of total organic carbon (TOC), percent composition of six grain size categories, and phi units from AOC and reference sites for each (A) river and (B) sediment class. Insufficient sediment was collected at one reference site (law-01), note asterisk, to process grain size. [na = not applicable]

A.

System	Site type	n	TOC (mg/kg)	Percent composition						Phi units
				Fine gravel	Coarse sand	Medium sand	Fine sand	Silt	Clay	
Grasse	AOC	3	40167	0	0	3	29	53	15	5.7
	reference	3	7767	2	1	3	75	17	2	3.6
Raquette	AOC	4	16750	1	3	9	63	18	6	3.8
	reference	3	40033	3	2	6	69	15	6	3.7
St. Regis	AOC	2	8000	0	1	7	82	9	2	3.3
	reference	2	18400	4	5	9.3	61	17.7	5.4	3.7
St. Lawrence	AOC	8	23312	1	1.2	6	28	50	14	5.5
	reference	5	30620	0	1	1	55	35	7.5	4.7

B.

Sediment Class	Site type	n	TOC (mg/kg)	Percent composition						Phi units
				Fine gravel	Coarse sand	Medium sand	Fine sand	Silt	Clay	
1	AOC	5	5100	0.4	0.8	9.4	77.7	9.7	2.2	3.3
	reference	7	6700	0.2	0.7	0.8	80.9	14.7	3.2	3.7
2	AOC	2	27500	1.3	4.2	11.1	50.8	25.2	7.5	4.1
	reference	0	na	na	na	na	na	na	na	na
3	AOC	9	32789	0.0	0.2	3.5	21.5	58.7	16.0	6.0
	reference	2	19350	0.0	0.2	1.8	27.5	57.4	13.2	5.8
4	AOC	1	6800	4.9	7.4	17.4	37.9	20.0	12.4	4.0
	reference	3	11300	5.3	4.7	11.8	57.7	19.3	6.0	3.6

Table 2. Principle components analysis of physical and chemical variables collected from each of 30 sites assessed across the entire study area.

Variable	PC1	PC2	PC3
TOC (mg/kg)	0.16	0.09	-0.55
DO (mg/L)	0.30	-0.02	0.11
Depth (m)	0.17	0.21	0.13
Conductance (μ mhos)	0.34	0.11	0.43
pH	0.37	-0.07	0.30
Temp (C)	-0.20	0.13	-0.46
% Fine gravel	-0.25	0.43	0.14
% Coarse sand	-0.22	0.52	0.14
% Medium sand	-0.19	0.50	0.16
% Fine sand	-0.36	-0.35	0.17
% Silt	0.41	0.14	-0.19
% Clay	0.35	0.27	-0.24
Eigenvalues	4.55	2.13	1.84
Percent variation explained	38	18	15
Cumulative percent variation	38	56	71

Table 3. Results of Analysis of Similarities (ANOSIM) indicating the amount and significance of overlap in the abundance and composition of macroinvertebrate communities from all AOC and reference sites within (A) each river and (B) sediment class. The *R* statistic quantifies the degree of overlap (0 = 0 % similar; to 1 = 100 % similar) between AOC and reference sites. [*no reference sites in sediment class 2, na = not applicable]

A.

System	<i>R</i> statistic	<i>P</i> -value
Grasse	0.56	0.001
Raquette	-0.02	0.68
St. Regis	0.57	0.001
St. Lawrence	0.1	0.005

B.

Sediment class	<i>R</i> statistic	<i>P</i> -value
1	0.125	0.002
2*	na	na
3	0.115	0.057
4	0.983	0.001

Table 4. Mean *in situ* surface water chemistry measurements and depth for AOC and reference groups by (A) river and (B) sediment class. No profile data are included as law-pc, part of the St. Lawrence AOC group, was the only site to show substantial change. [^ bottom temperature was 14.7°C, *bottom DO was 1.2 mg/L]

A.

System	Site type	n	Temp (°C)	Cond (μS/cm)	pH	DO (mg/L)	Depth (m)
Grasse	AOC	3	27.0	143	8.2	8.4	2.8
	reference	3	26.0	88	7.0	6.0	1.1
Raquette	AOC	4	26.8	65	7.7	8.1	1.6
	reference	3	25.9	57	6.7	7.2	1.7
St. Regis	AOC	2	25.5	109	7.9	7.4	3.2
	reference	2	26.1	92	7.2	7.9	1.7
St. Lawrence	AOC	8	24.4	288	8.5	10.1	2.7
	reference	5	24.3	300	8.6	9.6	3.1

B.

Sediment Class	Site type	n	Temp (°C)	Cond (μS/cm)	pH	DO (mg/L)	Depth (m)
1	AOC	5	25.7	130	7.9	8.5	2.3
	reference	7	25.1	142	7.5	7.1	1.4
2	AOC	2	27.1	64	7.8	8.1	1.5
	reference	0	na	na	na	na	na
3	AOC	9	25.2	247	8.5	9.0	3.1
	reference	2	24.7	296	8.5	9.8	2.9
4	AOC	1	25.3^	452	8.2	6.4*	5.0
	reference	3	25.8	78	6.7	7.1	1.6

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falling beyond the whiskers. Asterisks indicate significant difference between AOC and reference sites.

Figure1

Base from U.S. Geological Survey Digital Data. Universal Transverse Mercator Projection, Zone 18N, NAD83

Figure2

2D Stress: 0.22

BEST Analysis
Global test $Rho = 0.364$, $P = 0.012$
Clay and Silt, $Rho = 0.364$
Clay, Medium Sand, and Silt, $Rho = 0.333$
Clay, Fine Sand, Medium Sand, Silt, $Rho = 0.332$

Figure3

Figure4

Figure5