Toxicity of waters from the Rochester Embayment Area of Concern to the plankton species
Pseudokirchneriella subcapitata and *Ceriodaphnia dubia*

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ABSTRACT

The lower Genesee River and Rochester Embayment of Lake Ontario are a designated Area of Concern (AOC) under the binational Great Lakes Water Quality Agreement. The “degradation of phytoplankton and zooplankton populations” or plankton Beneficial Use Impairment (BUI) was classified as unknown and in need of further assessment in this AOC because water quality data suggested plankton communities could be effected and community data were either unavailable or indicated impacts. The plankton BUI may now be obsolete because local contaminant sources have been largely eliminated. The present study was conducted between July 2013 and August 2014 to assess the BUI-removal criteria: "AOC plankton bioassays confirm that toxicity in ambient waters (i.e., no growth inhibition) is not significantly higher than comparable non-AOC controls". Acute and chronic toxicity of waters from 13 sites were quantified seasonally using standardized bioassays with the green alga *Pseudokirchneriella subcapitata* and water flea *Ceriodaphnia dubia* to test the hypothesis that toxicity of waters from AOC sites was not higher than that of waters from comparable non-AOC reference sites. Survival and reproduction of *C. dubia* did not differ significantly between site types, systems, or months. The growth of *P. subcapitata* did not differ between site types, but differed among systems and months. All results indicate that waters from AOC sites were no more toxic to both plankton species than waters from reference sites. Assuming test species represent natural plankton assemblages, water quality should not negatively affect survival and growth of resident plankton populations in the Rochester Embayment AOC.

KEYWORDS: Rochester Embayment, area of concern, plankton, bioassay, beneficial use impairment

INTRODUCTION

During the 1970s and 80s, the governments of Canada and the United States committed to restore the physical, chemical, and biological integrity of Areas of Concern (AOC) throughout the Great Lakes under the Great Lakes Water Quality Agreement (GLWQA) (<http://www.epa.gov/greatlakes/glwqa/1978/index.html>). An AOC is 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. The Rochester Embayment and 9.6 km of the lower reach of the Genesee River at Rochester, New York (Fig. 1) was designated as one of 43 AOCs with likely or known impairment to beneficial uses caused mainly by past municipal and industrial pollution (NYSDEC, 1993a, b). The "degradation of phytoplankton and zooplankton populations" or the plankton Beneficial Use Impairment (BUI) was designated as "unknown" and in need of further assessment in the Rochester Embayment AOC because water quality data suggested their communities were likely impacted and because plankton data were unavailable or indicated their communities were affected. Nutrients, polychlorinated biphenyls (PCBs), mirex, dioxin, pathogens, oil and grease, and silt/sediment were identified as the pollutants that impair aquatic life in the lower 18.8 km of the Genesee River (NYSDEC, 2003). This report noted that the significant silt and sediment load was natural and that anthropogenic contaminants originate from: (a) various industrial and municipal point sources and combined sewage overflows (CSOs) in the metropolitan Rochester area, (b) nonpoint urban and agricultural runoff, (c) contaminated sediments, and (d) inactive hazardous waste sites (NYSDEC, 2003). Although contaminated, sediments from the Genesee Harbor are dredged by the U.S. Army Corps of Engineers every other year and disposed in the Rochester Embayment about 3 km northeast of the river's mouth; re-suspended contaminants

could potentially impact water quality and plankton communities in the AOC (NYSDEC, 1993b). Other studies identified phenols and eutrophication as possible sources for reduced survival and growth of *C. dubia* in toxicity tests done during 1989-90 (NYSDEC, 1993b). Although they were not the focus of this investigation, contaminated sediments could also affect water quality (and toxicity) under some circumstances (e.g., flood flows). Concentrations of dichlorodiphenyltrichloroethane (DDT), dieldrin, mercury, and PCBs exceeded acceptable (standards or guidance) levels in some sediment samples collected from the Lower Genesee River or Rochester Embayment between 1973 and 1990 (NYSDEC, 1993b). A more recent investigation detected low levels of dieldrin, endrin, DDT, and methoxychlor and concentrations of cadmium and zinc that exceeded probable effect levels at several sites in the Lower Genesee River as well as high concentrations of cadmium, copper, nickel, zinc, DDT, and PCB 1260 in Braddock Bay (NYSDEC, 2007). Over the past three decades, declines in the quantity of waste water discharges and (or) decreases in contaminants from permitted wastewater dischargers (NYSDEC, 1993a, 2011) suggest that the quality of local waters has improved and that the plankton BUI may be outdated in this AOC.

[Figure 1 Near here.]

Since the 1980s, Remedial Action Plans (RAPs) were developed by local Remedial Action Committees to guide restoration efforts and remove existing BUIs from each of the 43 AOCs. The Rochester Embayment RAP was developed by the Monroe County Department of Health and includes an ecosystem approach to AOC assessment and remediation, recognizing that the entire Genesee River watershed drains into the Embayment (NYSDEC, 1993b). The RAP established specific criteria in the Rochester Embayment AOC for delisting (restoring and protecting) the “Degradation of Phytoplankton and Zooplankton Populations” BUI. These

criteria have been modified since their inception and most currently state that: (1) "AOC plankton bioassays confirm that toxicity in ambient waters (i.e., no growth inhibition) is not significantly higher than comparable non-AOC controls," or (2) "Ambient water samples of AOC waters comparable to non-AOC control sites cause no toxicity to zooplankton and phytoplankton" (Personal communication with Charles Knauf, 4/12/2013). Detailed background information on the Rochester Embayment AOC and the RAP can be found in the report "December 2011 Addendum to Stage I and II Remedial Action Plans" (NYSDEC, 1993a, 2011). Unfortunately, current information needed to assess water toxicity and the health of plankton communities are unavailable; thus, the status of the plankton BUI in this AOC remains unknown.

The U.S. Geological Survey, New York State Department of Environmental Conservation and American Aquatic Testing initiated a study in 2013 to evaluate the toxicity of ambient waters from across the Rochester Embayment AOC to the green alga *Selenastrum capricornutum* and water flea *Ceriodaphnia dubia* in order to determine if the plankton BUI should be removed in this AOC. *Pseudokirchneriella subcapitata* is the currently accepted taxonomic nomenclature for *S. capricornutum* (<http://www.fritschalgae.info/Selenastrum.html>) and is used for the remainder of the present paper. Both plankton species are very sensitive to toxins and widely distributed in ponds, marshes, and lakes across most of the United States and Canada (USEPA, 2002a; WDNR, 2004). Standardized toxicity tests (bioassays) have been developed for each species and are commonly used to assess the levels of nutrients or toxins in freshwater environments (USEPA, 2002b). Both species were exposed in the laboratory to seasonal (summer 2013, fall 2013, and spring 2014) water samples from 13 sites and monthly (July 2013 through August 2014) water samples from two sites to assess current toxicity at sites from inside and outside of the AOC. The main objective of the present paper is to determine if

the phytoplankton and zooplankton beneficial use is impaired or not impaired in part or in all of the Rochester Embayment AOC. Plankton toxicity data are summarized and evaluated specifically to test the hypothesis that waters at sample locations in the Genesee River and Rochester Embayment AOC are no more toxic to plankton than are waters from reference (control) sites located outside the AOC.

METHODS

Toxicity was assessed in waters collected from 7 AOC and 6 control sites located inside and outside the Genesee River and Rochester Embayment AOC (Fig. 1, Table 1). There were only two basic criteria for all study site locales; they had to be either within or outside the AOC (George and Boyd, 2007; IJC, 1991). All control sites (referred to as reference sites hereafter) were located outside of the AOC; they were intended to provide a gauge, not necessarily of pristine (or non-toxic) water-quality conditions, but of the prevailing water quality conditions (whether good or poor) in comparable sites from across the region. Because reference sites only needed to be outside the AOC, riverine sites were selected to capture the urban influence from Rochester and a more rural influence upstream from the city. Study sites in the Genesee River portion of the AOC were selected both upstream and downstream of likely point and non-point sources of contaminants. Because waters of the lower river mix well, and there are no major tributaries in the short (9.6 km) AOC river segment, the water quality from one site at the mouth (gen06) was expected to represent worst-case water quality (and toxicity) conditions within the entire AOC. A second AOC-river site (gen04), located upstream of the metropolitan area, was sampled to: (a) provide a second (replicate) AOC site and (b) evaluate potential spatial differences in water toxicity that may be linked to local contaminant sources (e.g., CSOs) in the

city proper. Two reference sites in the river were located about 5 km (gen03) and 10 km (gen01) upstream of gen04. The furthest downstream AOC site (gen06) and the furthest upstream non-AOC site (gen01) in the Genesee River were generally tested monthly to characterize temporal variations in toxicity at gen06 - the site most likely to be affected by high sediment loads, turbidity, and contaminants. Most AOC sites in the Rochester Embayment were situated 2.5 to 4.5 km offshore; two sites (bay05 and bay04) were located immediately east and west of the mouth of the Genesee River and two other sites (bay06 and bay03) were located midway between these sites and the east and west boundaries of the AOC (Fig. 1). The highly turbid plume of Genesee River likely affected water quality at AOC sites in the eastern Embayment due to the prevailing easterly winds and shoreline currents; however, it could also affect water quality at western Embayment AOC sites during infrequent deep-water upwelling or westward-moving storms (Makarewicz et al., 2012). Another AOC site (brad02) was situated inside Braddock Bay because high levels of metals, DDT, and PCB 1260 were recently detected there (NYSDEC, 2007). Like AOC sites, reference sites in Lake Ontario were generally 2.5-4.5 km offshore and located at various distances west (bay01 and bay02) or east (bay07 and bay08) of the AOC boundaries based mainly on their proximity to boat launches (Fig. 1).

A total of 69 water samples were collected from AOC and reference sites to assess seasonal (summer, fall, and spring) and monthly variations in water toxicity. Seasonal water samples were collected at all 13 sites during July and September 2013 and May 2014; with a few exceptions, monthly samples were collected at a reference site (gen01) and AOC site (gen06) from July 2013 to August 2014. The intent of these samples was to quantify any temporal variations in water toxicity at key sites which serve as surrogates for potential larger scale variations in toxicity at other sites. All samples were used to quantify potential whole-water

toxicity to the green alga species *P. subcapitata* and to the water flea *C. dubia*. Duplicate grab samples were collected from two study sites during each of the three seasons, and three more duplicates were obtained during monthly sample collections to assess the quality (precision) of data generated from the toxicity tests. In general, each sample was collected at mid-depth either (a) near the middle of the channel from a bridge, (b) away from shoreline off a boat dock in the Genesee River, or (c) from a boat in the Embayment, using clean Teflon® tubes and a 12-V DC pump. At each site, 2 L of water were placed into two 1-L polyethylene containers, stored on ice, and shipped to the testing laboratory (American Aquatic Testing) on the same day that they were collected. Upon arrival at the laboratory, all water samples were filtered to 0.45 µm (USEPA, 2002b) to remove organisms which could compete with test organisms and confound results. Sample information (site identification code, site name, depth, temperature, collection time, name of field person, and method) was recorded on a field log and chain-of-custody form (that accompanied sample shipments) when each sample was collected.

American Aquatic Testing initiated all toxicity tests within 24 h of collection following standardized U.S. Environmental Protection Agency (USEPA) Test Methods 1003.0 for *P. subcapitata* (Electronic Supplementary Material (ESM) Table S1) and 1002.0 for *C. dubia* (ESM Table S2) (USEPA, 2002b). Both analyses used chronic endpoints as primary indicators of any adverse response. For *P. subcapitata*, a four replicate, static, 96-h exposure was used for each sample (site) utilizing a 10,000 cells/mL initial loading and continuous illumination (86 µE/m²/s) at 25 °C for the test duration. At the end of the exposure period, cell counts were performed on all replicates from each site, and a single average was generated for comparison with the results from other sites. A rapid spectrophotometer technique was used to estimate (count) the number of cells and, indirectly *P. subcapitata* growth in each replicate over the 96-h exposure period.

The turbidity or percent absorbance for each replicate was measured at a wavelength of 750 nm, which determined the percentage of transmitted light that was absorbed by the sample. Cell densities (cells/mL) were estimated from absorbance (percentage reductions) using an internally developed calibration curve (regression equation) utilizing *P. subcapitata* and moderately hard laboratory water as recommended in USEPA (2002b). Any sample found with statistically fewer total cells at the end of the exposure period would be considered adversely impacted. Complete guidelines for maintaining *P. subcapitata* cultures, conducting growth tests, and interpreting results are available in Section 14 of USEPA Method 1003.0 (USEPA, 2002b) (ESM Table S1). For *C. dubia*, a 7-day (daily water renewal) exposure with 10 individuals (each in a separate chamber and treated as 10 replicates) was used for each sample. These tests utilized a 16-h light and 8-h dark cycle, daily renewal of food, and daily observations of survival and production of offspring. Any sample found with statistically fewer surviving adults or statistically fewer offspring (total number produced by all adults at the end of the exposure period) would be considered adversely impacted. The specific guidelines for handling organisms, conducting *C. dubia* tests, and interpreting reproduction and survival results are provided in Section 13 of USEPA Method 1002.0 (USEPA, 2002b) (ESM Table S2).

Bioassay data were summarized and analysis of variance (ANOVA) tests were used to evaluate the statistical significance of differences between mean *C. dubia* survival and offspring reproduction and density of *P. subcapitata* cells (toxicity endpoints) as determined at the end of respective exposures to waters from AOC sites and corresponding reference sites using the Statgraphics® centurion XVI software (StatPoint, 2010). The ANOVA tests were used to assess differences in acute and chronic toxicity of waters from all reference and AOC sites (site type) and to identify variations that could be associated with “system” (the river or bay) or sampling

“month”. The differences for all statistical tests were considered significant at $\alpha = 0.05$ ($P \leq 0.05$). In brief, Shapiro-Wilks tests were used to evaluate whether *C. dubia* survival and reproduction data and *P. subcapitata* cell density from exposures to water from each reference and AOC site were normally distributed. Because almost all data were normally distributed and corresponding Bartlett’s tests (using one-factor ANOVAs) confirmed variances within each site type were homogeneous, two-factor ANOVA tests were used to assess the significance of differences in mean responses between site type (AOC vs. reference) and system (river vs. bay) or between site type and month. The mean and standard errors for *C. dubia* reproduction and *P. subcapitata* densities for all (3 to 12) tests done at each site over the study were summarized graphically to illustrate differences due to site type, system, and month.

The quality of data generated by all toxicity tests was assured by: (a) confirming that the sensitivity of test organisms was within normal limits using standard reference toxicant (SRT) tests and quality-control charts; (b) utilizing laboratory-water controls to verify that test conditions and organism responses met minimally acceptable standards; and (c) assessing results from nine pair of duplicate samples to determine if the precision of test endpoints was acceptable (USEPA, 2002a, b).

During May 2013, pre-study dilution experiments were conducted to evaluate the potential effects of turbidity on acute and chronic endpoints for *C. dubia* and *P. subcapitata*. One 5-liter river-water sample was collected at gen01 on May 13, 2013 and shipped to American Aquatic Testing. Turbidity of this whole-water “test” sample averaged 344.3 Nephelometric Turbidity Ratio Units (NTRU). About half of the sample was centrifuged to remove suspended sediments and reduce turbidity down to 9 NTRU and used as a river control and for all dilutions. Dilutions of 100, 70, 50, 30, 10, 5, and 0 percent test water were prepared and used in one

toxicity test to characterize the turbidity-endpoint response gradient and (or) ascertain the highest turbidity no-effect threshold for endpoints for each plankton species following standard procedures (ESM Tables S1 and S2). If a threshold for turbidity effects was detected, it would be used to adjust or help interpret test results (growth, survival, or offspring production) from any site where turbidity levels exceeded the threshold during one or more sample-collection periods.

RESULTS

Turbidity experiment

The turbidity experiment evaluated the potential effects of different turbidity levels on acute and chronic endpoints for *C. dubia* and *P. subcapitata* presented herein. Turbidity from a water sample collected at gen01 on May 13, 2013 averaged 344.3 (NTRU), whereas, it averaged 9 NTRU in the centrifuged dilution water. Turbidity in the 100, 70, 50, 30, 10, 5, and 0 percent dilutions averaged 344, 134, 95, 67, 25, 21, and 9 NTRU, respectively (Table 2). Mean survival of *C. dubia* was 100 percent in all experiment dilutions; mean reproduction ranged from 22.4 to 26.4 young, and density of *P. subcapitata* ranged from 4.319×10^6 to 5.168×10^6 cells/mL at the end of test exposures (Table 2). The lowest turbidity-effect threshold for all three endpoints was undetermined, but had to be greater than 344 NTRU. The mean turbidity levels for test waters collected from all thirteen study sites during July and October 2013 and May 2014 and from sites gen01 and gen06 monthly from July 2013 to August 2014 were usually less than 344 NTRU (Table 3). Except possibly for high turbidity levels at Genesee River sites gen01, gen03, gen04, and gen06 (372.0, 327.0, 347.0, and 367.7 NTUR, respectively) during the May 2014 survey, turbidity could not have affected chronic or acute endpoints during all toxicity tests.

Ceriodaphnia dubia tests

Results for Shirpiro-Wilk's and Bartlett's tests (not summarized) generally show that data from all sites (and months) were normally distributed and variances were homogeneous; i.e., P -values were greater than 0.05. Thus, results from one- and two-factor ANOVAs were used to assess significance of differences in *C. dubia* survival and offspring production between site type, system, and month. The mean survival of *C. dubia* after 7 days of exposure to waters from all AOC and reference sites during 2013 and 2014 ranged from 90 to 100 percent (Table 3). The results from a one-factor ANOVA (not shown) indicate that mean survival of *C. dubia* did not differ significantly among all sites ($P = 0.4953$). The P -values for two-factor ANOVAs assessing difference in *C. dubia* survival between site type and system or between site type and month ranged from 0.4475 to 0.9651 and indicate that mean survival did not differ significantly in waters from AOC sites (99.7%) and reference sites (99.3%) and that system and month also had no significant effect on *C. dubia* survival. There were no significant interactions between site type, system, or month in both two-factor ANOVAs.

The mean number of offspring produced by female *C. dubia* (reproduction) in waters from all study sites (AOC and reference) during July and October 2013 and May 2014 and monthly at gen01 and gen06 ranged from 24.2 to 26.9; the number of offspring from all laboratory controls for these tests averaged 23.3 (Table 4). The results from a one-factor ANOVA (not shown) indicate that mean reproduction did not differ significantly among all sites ($P = 0.8470$). Results from two-factor ANOVAs indicate that the mean reproduction did not differ significantly between AOC sites (25.2 young) and reference (24.9) sites (P -values varied from 0.7746 to 0.9171) and between systems ($P = 0.9958$), but reproduction differed significantly among months ($P < 0.0001$). There were no significant interactions between site

type, system, or month in both two-factor ANOVAs. Although differences in mean reproduction between all AOC and reference sites were consistently small (Fig. 2A), reproduction at all sites, regardless of type, was lower during December (18.2 young), March (15.7 young), and April (18.6 young) than it was for all other months (mean reproduction ranged from 22.8 to 29.4 young) (Fig. 3A). [Figures 2 and 3 near here.]

Pseudokirchneriella subcapitata tests

The density of *P. subcapitata* cells averaged from 4.120×10^6 to 5.033×10^6 cells/mL in waters from all study sites (AOC and reference) during July and October 2013 and May 2014 and monthly at gen01 and gen06; the number of offspring from all laboratory controls (all tests) averaged 4.513×10^6 cells/mL (Table 5). The results from a one-factor ANOVA indicate that mean cell density did not differ significantly among sites ($P = 0.4931$). Results from two-factor ANOVAs indicate that the mean density at AOC sites (5.007×10^6 cells/mL) and reference sites (4.852×10^6 cells/mL) did not differ significantly (P -values ranged from 0.1989 to 0.2259), but density differed significantly between systems ($P = 0.0002$) and among months ($P < 0.0001$). There were no significant interactions between site type, system, or month in both two-factor ANOVAs. Cell density was slightly greater at most AOC sites than corresponding reference sites in the same system (Fig. 2B). Mean cell density, regardless of site type, was slightly higher at river sites (4.887×10^6 cells/mL) than at embayment sites (4.349×10^6 cells/mL). Mean cell density at all sites during July and October 2013 and May 2014 and at both sites that were tested monthly (gen01 and gen06) was significantly lower during October (3.944×10^6 cells/mL) than during eight other months (cell density ranged from 4.302×10^6 to 5.326×10^6 cells/mL) (Fig. 3B).

Quality assurance

The quality of data generated by all toxicity tests was assured by evaluating the sensitivity of test organisms and the results from laboratory-water control and duplicate samples. The mean 7-day 25% inhibition concentration (IC₂₅) from 11 SRT tests done with *C. dubia* between July 2013 and March 2014 was 309 ppm KCl (the IC₂₅ ranged from 243 to 356 ppm KCl); the mean 96-h IC₂₅ for 16 SRT tests done with *P. subcapitata* over the same period was 2,571 ppm KCl (the IC₂₅ ranged from 1,929 to 3,920 ppm KCl). All IC₂₅ values fell within the normal (acceptable) ranges in quality-control charts maintained by the laboratory for each species. For all laboratory controls, mean *C. dubia* survival was 100%; about 89% of *C. dubia* produced a third brood within 7 days; and an average of 23.3 offspring were produced by each surviving female - all of which exceeded minimum criteria for laboratory controls (USEPA, 2002a, b). In addition, the mean density of *P. subcapitata* in all control exposures (4.5×10^6 cells/mL) exceeded 1×10^6 cells/mL, and variability in cell density was less than 20% (CV = 12.3%) after 96 h, which also surpassed EPA criteria for acceptable tests (USEPA, 2002b). Analysis of data from the nine pairs of duplicate samples showed that the absolute Relative Percent Difference (RPD) for *C. dubia* survival, *C. dubia* offspring production, and *P. subcapitata* cell density averaged 0.0, 16.1, and 9.4%, respectively.

DISCUSSION

Plankton bioassay results from the present study demonstrate that ambient waters across the Genesee River and Rochester Embayment AOC were not acutely or chronically toxic to two test species. Results from the *C. dubia* tests proved that whole waters were not acutely toxic to

this zooplankton species at any AOC (or reference) site and that the production of *C. dubia* offspring in waters from all AOC sites was consistently comparable to reproduction observed in waters from corresponding reference sites. Similarly, the growth (cell density) of the green alga *P. subcapitata* in waters from all AOC sites was comparable to that in waters from corresponding reference sites. Though *P. subcapitata* growth and *C. dubia* reproduction sometimes differed significantly between systems or among months but confirmed that whole waters from all AOC sites were not acutely or chronically toxic to either species nor were they toxic during any month. These results provide strong evidence that the quality of surface waters across the Rochester Embayment AOC should not negatively affect the survival and growth of resident phytoplankton and zooplankton species.

The frequent use of terms such as restored, remediated, recovered, and improved in the Great Lakes Restoration Initiative program cause many to assume that targeted beneficial uses were once seriously degraded and that removing BUIs means that the: (a) underlying contaminant or habitat issues have been rectified, (b) beneficial use has returned to unimpacted conditions, and (c) conditions in the AOC are comparable to that in natural (pristine or unimpacted) reference sites. All of these assumptions are usually incorrect. The Rochester Embayment AOC study sites are (or were) potentially polluted (by wastewaters, contaminated sediments, etc.) and also affected by highly turbid conditions, which originate in the Genesee River watershed and are dispersed mainly down current (easterly) in the Rochester Embayment. The underlying assumption for removing the plankton BUI (or any BUI) is that the conditions (e.g., toxicity or community condition) inside the AOC are no different (or better) than the conditions at reference sites located outside of the AOC. Thus, three scenarios could produce a recommendation for removal of a BUI. First, high contaminant levels or degraded habitat that

occurred in an AOC over many decades may have improved or been ameliorated through passive (natural) or active (anthropogenic) remediation efforts. Second, the BUI may have never been impaired, but assumed to be impaired based on ancillary or circumstantial evidence. Third, the beneficial use was and is still impaired within the AOC, but the same use is also impaired in comparable nearby areas due to large (region-wide) stressors, which are not specific to the AOC. In all three scenarios, current data, which show water quality is either very good or even very poor at AOC sites, are generally not pertinent to the BUI-removal recommendation.

Investigations that prove water (or sediment) toxicity or community condition at sites inside the AOC are not significantly different (or better) than those found at sites outside the AOC can justify removing the BUI. The water quality, biotic community, and toxicity data at one or all AOC sites may be abysmal, but as long as they are not significantly different, or if they are “better” than those at corresponding reference sites, then the BUI is not justified.

The lack of toxicity or significant differences in plankton data from a few sites does not mean that the plankton communities in the Rochester Embayment AOC are totally unaffected by other stressors. For example, chlorophyll a levels at many embayment and nearshore sites along the southern shoreline of Lake Ontario were generally elevated, and mean total phosphorus (TP) concentrations in river (e.g., Genesee River), embayment, and nearshore sites were significantly higher than the limit of 10 µg P/L proposed by International Joint Commission for Lake Ontario and levels observed at sites located more than 4 km off shore (Makarewicz et al., 2012). Possibly more important, concentrations of phycocyanin (a pigment-protein complex found in Cyanobacteria), total phosphorus, and chlorophyll concentrations typically exceeded levels found in nearby offshore waters (Makarewicz and Lewis, 2015). The authors conclude that waters of the Genesee River and Rochester Embayment are moderately phosphorus limited and

may also be nitrogen limited, and that additional nutrients could stimulate growth of phytoplankton and blue-green algae (bacterium) such as photoautotrophic Cyanobacteria, known to produce harmful hepatotoxins. Excessive nutrients, occasional overabundant plankton populations, and tangible threat of harmful algal blooms in nearshore waters inside and outside the Rochester Embayment AOC cannot be denied, yet these issues are not germane to removal of the plankton (Degradation of Phytoplankton and Zooplankton) BUI for two reasons. First, as noted above, removal criteria for the plankton BUI only weigh differences, or the lack of differences, in plankton toxicity or community data from comparable AOC and non-AOC sites within the region. Whether plankton communities are overabundant or even reach nuisance levels is unimportant to the assessment as long as target metrics are comparable at nearshore sites located inside and nearshore sites outside the AOC. Second, eutrophication caused by excessive nutrients (enrichment) and resultant blooms of harmful algae are addressed in the Rochester Embayment AOC by a separate "Eutrophication or Undesirable Algae" BUI. The plankton BUI is obviously linked, at least partially, to this BUI, but specific actions required to mitigate underlying causes for each impairment, as well as the criteria needed to remove each BUI, differ substantially (NYSDEC, 1993a, b).

Although routine plankton surveys from the Rochester Embayment and surrounds are rare, some unpublished data support the extrapolation that the health of plankton communities in this AOC is not impaired. Community data from three nearshore sites sampled approximately 15 years ago showed that plankton communities were comparable at one site inside the AOC and two sites outside the AOC. Unpublished density data from standardized (<12 m depth) zooplankton tows collected biweekly between April and October of 1998-2000 averaged 17154 individuals/L at one nearshore Embayment site near Irondequoit Bay (inside the AOC) and

21836 and 13918 individuals/L at nearshore sites near Sodus Point and Oak Orchard Creek (both outside the AOC), respectively (K. Holeck, Cornell University, personal communication).

Zooplankton biomass averaged 28.6, 42.3, and 17.3 $\mu\text{g/L}$ during all surveys at the same three sites, respectively. Results of several two-factor ANOVAs using site type (AOC vs. non-AOC), month, and year determined that there were no significant differences in zooplankton density ($P = 0.4823$) or biomass ($P = 0.0960$) between site types, but indicated density differed significantly among months ($P = 0.0108$) and years ($P = 0.0364$). Though some plankton species may become overabundant due to periodic nutrient enrichment, this finding supports the inference that the health of plankton communities is not seriously affected by toxic contaminants in the AOC.

The potential effects of turbidity on acute and chronic endpoints for *C. dubia* and *P. subcapitata* were especially concerning because variations in turbidity were anticipated (especially in the Genesee River) and because associated effects (bias) on test endpoints were not known. Decreases in light penetration could reduce growth rates of *P. subcapitata* because all green algae require light for energy production, growth, and reproduction. The small volumes of water (25 mL) used in each 125-mL flask likely exclude this potentiality in our laboratory tests. Increases in turbidity might reduce *C. dubia* growth and reproduction in the wild, however, because low-light conditions could increase prey-search effort and suspended particles (that contribute to turbidity) could be ingested mistakenly for food. Because the effects of turbidity on growth and reproduction of *C. dubia* and *P. subcapitata* were unknown, a turbidity experiment was conducted to (a) define the lowest turbidity “effect” thresholds for both species, and (b) adjust or correct results from all toxicity tests where and when turbidity levels may have surpassed these effect thresholds. The highest turbidity measured in the undiluted Genesee River water collected at gen01 on May 13, 2013 averaged 344.3 (NTRU). This turbidity level, and all

lower dilutions, had no significant effect on acute and chronic endpoints for both species. Therefore the lowest turbidity-effect threshold for all three endpoints was greater than 344 NTRU, and turbidity should not have affected any test endpoint when or where measurements were less than 344 NTRU. Turbidity from samples collected at the four Genesee River sites in May 2014 ranged from 327 to 372 NTRU and did not significantly affect endpoints for any toxicity test; thus, the lowest turbidity-effect threshold was likely greater than 372 NTRU. Elevated turbidity was not a concern for waters used in any of our toxicity tests because turbidity levels did not surpass a minimum effect threshold.

Our study design and resultant toxicity dataset were necessarily limited in scope, which could potentially subject our findings (and conclusions) to certain criticisms. As discussed in Baldigo et al. (2012), the use of two test species, their ability to represent community responses, the spatial extent and number of sites, and the number of sampling periods (for all sites) were constrained by the number of toxicity tests that could be funded. To account for potential effects of seasonality, the design of the present study was enhanced to quantify acute and chronic toxicity monthly at one AOC and one reference site. The results identified significant temporal variations (differences) in chronic endpoints for both species (pooled results from all AOC and reference sites); however, the lack of significant differences between endpoints in waters from both sites (one AOC and one reference) proved that site type had no effect on toxicity. The temporal changes in *C. dubia* reproduction (the December and February-April declines and the January spike) were also somewhat unexpected but not overly problematic. These fluctuations may have been caused by a general decrease in river productivity during the colder winter and spring months. During the warm months, reproduction of *C. dubia* can exceed normal levels because larger amounts of particulate matter such as algae, bacteria, and detritus provide

supplemental nutrition (beyond that of standard feeding protocols) (Schulze, 1999; Stewart and Konetsky, 1998). Discharge data from a USGS gage at Ford Street in Rochester indicates a small runoff event occurred during January which could have increased river productivity and *C. dubia* reproduction. Growth of *P. subcapitata* also differed significantly by site type during September; however, it was higher in waters from the AOC site than in waters from the reference site. Despite study limitations, our results effectively characterized the spatial and temporal variability in water quality across the region and only identified nominal differences in toxicity (to plankton) of waters from reference sites and from sites across the Rochester Embayment AOC (including sites in the lower reaches of the Genesee River, also inside the AOC).

The use of bioassays to characterize the quality or toxicity of ambient waters across the Great Lakes is an effective tool for characterizing the capacity of ambient waters to sustain key ecological processes and beneficial uses. This is undoubtedly one reason that plankton bioassays, which confirm no significant toxicity, were identified as an optional criterion for removing plankton BUIs within several AOCs in the Great Lakes along the United States and Canadian border (George and Boyd, 2007). Accordingly, Baldigo et al. (2012) used results from acute (survival) and chronic (reproduction) *C. dubia* tests and chronic (growth) *P. subcapitata* tests to evaluate water toxicity and show that removal criteria for the plankton BUI were generally met in waters from the St. Lawrence River at Massena New York AOC. The results reported in the present paper are comparable but more straight forward than those from Baldigo et al. (2012), which were recently used to help justify the removal of the plankton BUI in the St. Lawrence River AOC. Toxicity tests generally avoid several drawbacks commonly associated with quantifying endemic biota and testing for significant differences among sites. Site-to-site differences in habitat and water quality; seasonal changes in temperature, daylight, and river

flows; and variable recruitment of lentic plankton from upstream sources can create large spatial and temporal fluctuations in density and biomass of plankton species and their communities (Basu et al., 2000; Hudon, 2000). These issues act to make precise characterization of endemic populations and communities at comparable sites, and statistical assessments problematic. Toxicity tests simply characterize toxicity, or the lack of toxicity, for waters collected at strategic points within a river network. The primary requirement for toxicity-test water is that it comes from sites situated either at potentially contaminated (AOC) locations or at non-affected reference locations. On the other hand, plankton community assessments require extensive characterizations of the physical and thermal habitat at reference and AOC sites to prove they are not dissimilar and that habitat variations could not account for site-to-site differences in community composition. Although acute and chronic toxicity results cannot irrefutably prove that the lower trophic levels of natural lake and river ecosystems are fully intact, healthy, or unaffected by nutrient enrichment, the information provides convincing evidence that both plankton species and the communities they represent should not be adversely affected by toxic contaminants at most locations inside the Genesee River and Rochester Embayment AOC. Comparable toxicity tests should be employed more widely to effectively evaluate plankton BUIs and other BUIs in AOCs across the Great Lakes Region.

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Table 1. Site identification (ID), type, orientation upstream or up current (up), or downstream or down current (down), and location (latitude and longitude - NAD83) for reference and AOC sites sampled in the Genesee River and Rochester Embayment AOC from July 2013 to August 2014.

Site ID	Site Type	Orientation	Latitude	Longitude
Lake Ontario				
bay08	reference	down	43.30315	-77.0127
bay07	reference	down	43.29834	-77.2763
bay06	AOC	down	43.26994	-77.4935
bay05	AOC	down	43.28052	-77.5691
bay04	AOC	up	43.29724	-77.6016
bay03	AOC	up	43.32017	-77.6734
bay02	reference	up	43.36742	-77.8712
bay01	reference	up	43.37850	-77.9264
Genesee River				
gen06 ^a	AOC	down	43.25253	-77.6093
gen04	AOC	down	43.20182	-77.6243
gen03	reference	up	43.14297	-77.6136
gen01 ^a	reference	up	42.09270	-77.6806
Braddock Bay				
brad02	AOC	down	43.31594	-77.7183

^a water samples generally collected monthly

Table 2. Mean turbidity levels, survival of *C. dubia*, number of *C. dubia* offspring, and density of *P. subcapitata* after standard exposures to laboratory control water, river control water, and dilutions of gen01 waters that were collected May 5, 2013. NTRU are Nephelometric Turbidity Ratio Units

Test water or dilution	Turbidity (NTRU)	<i>C. dubia</i> survival (%)	Number of <i>C. dubia</i> offspring	<i>P. subcapitata</i> density (cells/mL x 10 ⁶)
Lab control	0.3	100	22.4	3.346
River control	8.9	100	22.4	4.460
5% dilution	21.1	100	23.8	4.746
10% dilution	24.9	100	24.8	5.168
30% dilution	67.4	100	26.4	4.802
50% dilution	95.2	100	24.2	4.512
70% dilution	134.3	100	24.7	4.319
100% dilution	344.3	100	23.7	4.845

Table 3. The number of observations; mean, minimum, and maximum turbidity levels; and mean, minimum, and maximum survival for *C. dubia* after 10 days of exposure in waters collected (1) during July and October of 2013 and May of 2014 at all thirteen AOC and reference sites and (2) generally monthly from July 2013 to August 2014 at sites gen01 and gen06. [na = not applicable]

Site ID	<i>n</i>	Turbidity (NTRU)			<i>C. dubia</i> survival (percent)		
		Mean	Minimum	Maximum	Mean	Minimum	Maximum
control	3	na	na	na	100	100	100
bay01	3	1.3	0.4	3	96.7	90	100
bay02	3	1.7	0.6	3.6	100	100	100
bay03	3	0.9	0.6	1.1	100	100	100
bay04	3	1.5	0.6	2.6	100	100	100
bay05	3	18.5	0.7	52.8	100	100	100
bay06	3	14.2	0.6	39.2	100	100	100
bay07	3	2.2	0.4	5.3	100	100	100
bay08	3	1.6	0.5	3.3	100	100	100
gen01	12	72.1	5.4	372	100	100	100
gen03	3	118.6	10.5	327	99.2	90	100
gen04	3	125.6	11.5	347	100	100	100
gen06	12	75.3	9.9	367.7	96.7	90	100
brad02	3	8.3	5.2	11.8	100	100	100

Table 4. Mean percent survival and statistics for the number of offspring produced by *C. dubia* after 7 days of exposure to waters from (a) all AOC and reference (ref) sites sampled three times, July and October of 2013 and May of 2014, and (b) generally monthly at gen01 and gen06 between July 2013 and August 2014.

System	Site	Site	Mean	n	Statistics for offspring production			
	ID	type	survival		Mean	Median	SD	SE
Laboratory	control	lab	100	12	23.3	23.7	4.67	1.35
Embayment	bay01	ref	97	3	24.2	23.1	4.01	2.31
	bay02	ref	100	3	25.8	25.4	3.42	1.97
	bay07	ref	100	3	24.7	25.6	3.89	2.24
	bay08	ref	100	3	24.8	25.0	2.66	1.53
	bay03	AOC	100	3	25.5	25.0	4.03	2.32
	bay04	AOC	100	3	25.7	26.2	2.88	1.66
	bay05	AOC	100	3	24.5	22.5	4.82	2.78
	bay06	AOC	100	3	25.5	25.2	3.91	2.26
Genesee	gen01	ref	99	12	24.7	25.4	5.03	1.45
	gen03	ref	100	3	26.4	24.6	3.83	2.21
	gen04	AOC	97	3	26.4	24.7	3.99	2.31
	gen06	AOC	100	12	24.4	25.8	4.51	1.30
Braddock	brad02	AOC	100	3	26.9	27.0	1.25	0.72

Table 5. Statistics for cell density (cells/mL) of *P. subcapitata* after 4 days of exposure to waters from (a) all AOC and reference (ref) sites sampled three times, July and October of 2013 and May of 2014, and (b) generally monthly at gen01 and gen06 between July 2013 and August 2014.

System	Site	Site	n	Statistics for density (cells/mL x 10 ⁶)			
	ID	type		Mean	Median	SD	SE
Laboratory	control	lab	12	4.51263	4.60225	0.41578	0.12003
Embayment	bay01	ref	3	4.22958	4.44775	0.38853	0.22432
	bay02	ref	3	4.35775	4.27300	0.61030	0.35236
	bay07	ref	3	4.34625	4.43650	0.40891	0.23609
	bay08	ref	3	4.12033	4.04775	0.37173	0.21462
	bay03	AOC	3	4.16075	4.24800	0.57709	0.33318
	bay04	AOC	3	4.13992	4.20975	0.21661	0.12506
	bay05	AOC	3	4.49217	4.56050	0.43504	0.25117
	bay06	AOC	3	4.42017	4.62950	0.53491	0.30883
Genesee	gen01	ref	12	4.81688	4.84742	0.56277	0.16246
	gen03	ref	3	4.71708	4.94025	0.61230	0.35351
	gen04	AOC	3	4.75825	4.60425	0.98829	0.57059
	gen06	AOC	12	5.03267	5.16275	0.45183	0.13043
Braddock	brad02	AOC	3	4.87150	4.53400	0.60920	0.35172

List of Figures

1. Map of AOC and reference sites sampled in or near the Rochester Embayment and Genesee River AOC.

2. Mean and 95% confidence intervals for the (A) number of offspring produced by *C. dubia* and (B) density of *P. subcapitata* (cells/mL) after exposure to waters from all Rochester Embayment AOC and reference sites sampled three times, July and October 2013 and May 2014, and from gen01 and gen06 generally sampled monthly between July 2013 and August 2014.

3. Mean and 95% confidence intervals for the (A) number of offspring produced by *C. dubia* and (B) density of *P. subcapitata* (cells/mL) after exposure to waters from reference site gen01 and AOC site gen06, generally sampled monthly between July 2013 and August 2014.

Base from *The National Map*, Universal Transverse Mercator projection, zone 18, WGS84, 1:1,000,000

Figure 1.

Figure 2.

Figure 3.