

Microbeads in Sediment, *Dreissenid* Mussels, and Anurans in the Littoral Zone of the Upper St. Lawrence River, New York

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ABSTRACT: Global plastic production has exceeded 300 million tons per year (Plastics Europe, 2015). In the marine and freshwater environments, larger plastics abrade and photo-degrade resulting in persistent environmental microplastics that are not effectively removed by existing wastewater treatment plants (WWTPs). The ecological effects of microplastics on the marine environment are poorly understood, with even less attention to freshwater systems. To assess whether microplastics have infiltrated food webs of shallow nearshore ecosystems of the St. Lawrence River, we sampled four sites along the international section of the St. Lawrence River, from Alexandria Bay to Waddington, NY. Twelve sediment samples along with one hundred and forty-nine *Dreissenid* mussels (*Dreissena polymorpha* and *D. bugensis*) were collected from the littoral zone, and forty one road-killed anuran amphibian specimens were collected adjacent to the river. Sediment subsamples at two of four sediment sampling sites contained plastic micro-particles. No microbeads were detected within any of the *Dreissenid* mussels or anuran digestive tract samples. The *Dreissenids* were likely too small to ingest microbeads greater than 35 microns. Microplastics congregating in the littoral zone may pose a threat within the food web through potential ingestion, requiring further methodological development.

Keywords: Microplastic, trophic transfer, benthic invertebrate, amphibian

INTRODUCTION

Global plastic production has been growing and exceeded 300 million tons per year (Plastics Europe, 2015) with plastic considered to be the most abundant of “marine debris” (Moore et al., 2008, Thompson et al., 2004). Over the past 45 years, plastic particles have been found on shorelines, in open water, and in the deep sea in the northeast Atlantic and Indian Oceans. The global extent of microplastic pollution has become well documented (Browne et al., 2011; Cole et al., 2011; Cozar et al., 2014). Studies have found

small plastics in the littoral sediment (Browne et al., 2010; Thompson et al., 2004), the pelagic water column (Derraik, 2002; Cole et al 2011; Cozar et al., 2014), and in organisms (Derraik, 2002; Browne et al., 2008). However, less is known about the extent and prevalence of microplastics in freshwater systems (Eriksen et al., 2013; Zbyszewski et al., 2014). According to Driedger et al. (2015), current surveys illustrated that the concentration of plastic debris in aquatic systems like the Great Lakes is higher where human and industrial activity are higher.

Once in the environment, larger plastics, such as plastic bottles or bags, photo-

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degrade or break down through oxidation or mechanical weathering (Andrady, 2003) smaller secondary microplastics break off and float in the water, becoming readily available to organisms of various sizes (Moore, 2008). "Primary microplastics", typically smaller than 5mm diameter in size, are created intentionally for use in health care products or as raw materials used to generate larger plastic products (Eerkes-Medrano et al., 2015; Eriksen et al., 2013; Free et al., 2014; Wagner et al., 2014). Sources of plastics entering waterways range from drainage systems from households, sewage system overflow and wastewater treatment plant effluents (EPA, 2007, Browne et al., 2011), improper garbage disposal (Browne et al., 2010; Eriksen et al., 2013), runoff of degraded terrestrial pollution (Andrady, 2003; Lechner et al., 2014), and surface runoff from roadways during storm events (Peters & Bratton, 2016). Industrial sites such as textile laundering and sandblasting facilities have also been known to pollute marine environments with microplastic fibers and particles (Dreidger et al., 2015; Eriksen et al., 2013). Both primary and secondary microplastics are a pervasive environmental issue (Andrady, 2011; Dreidger et al., 2015) which are not fully removed in most existing wastewater treatment plants (WWTPs) (Browne et al., 2011; Dreidger et al., 2015).

Small plastic particles are a growing ecological concern due to their capacity to sorb persistent organic pollutants from the environment (Castañeda et al., 2014; Baldwin et al., 2016). Upon ingestion, contaminants may desorb from the plastic particles and be absorbed via the digestive tract resulting in decreased nutrient absorption and potential bio-accumulation (Teuten et al., 2007; Wright et al., 2013). Depending on the abundance and density, microplastics can reduce copepod and algal populations (Lee et al., 2013; Cole et al., 2011). Benthic studies have found that

invertebrates and demersal fish can ingest the microplastics and then pass micro-particles to the upper trophic levels posing a potential health risk to higher level consumers (Castañeda et al., 2014; Driedger et al., 2015). However, Koelmans, et al., 2016 concluded that hydrophobic organic contaminants bio-accumulated from natural food items were more important than those obtained from ingested plastics in most marine environments. Ingestion of microplastics may result in gut blockages (e.g. Shore Crab *Carinus maenas*, Watts et al., 2014) or have little effect on feeding capacity (e.g. Pacific oyster *Crassostrea gigas*, Cole & Galloway, 2015).

Microplastics have been found within both the pelagic and littoral regions of large lakes throughout North America and Europe (Zbyszewski & Corcoran, 2011; Erikson et al., 2013; Zbyszewski et al., 2014) including the Laurentian Great Lakes (Eriksen et al., 2013; Driedger et al 2015; Mason et al., 2016), their tributaries (Baldwin et al., 2016) and the St. Lawrence River (Castañeda et al., 2014). Freshwater systems should be investigated more thoroughly for microplastic presence due to their integral role in supplying water to oceans and seas (Law et al., 2010; Lechner et al., 2014; Moore et al., 2011). Our study focuses on the 120 km international section of the upper St. Lawrence River. We hypothesized that microplastics would be present in nearshore sediments of the St. Lawrence River where flow is slow and allows deposition. Furthermore, we hypothesized that microplastic presence in the water column and sediment could allow for their uptake and transfer through the lower trophic levels through consumption or accidental ingestion. Specific objectives included sampling to examine whether microplastics, particularly microbeads, were present in sediments, either of two *Dreissenid* mussels and various anuran amphibians common in these nearshore environments.

MATERIALS & METHODS

The St. Lawrence River is over 1223 kilometers long (CWCSNY) and drains an area of over 775,000 square kilometers of land at its most downstream location with 18,300 kilometers of freshwater river and streams from New York State alone entering its watershed (NYSDEC). The St. Lawrence River Basin is relatively unpopulated with habitat consisting primarily of boreal forest along with minimal development and agriculture (CWCSNY) compared to other North American river systems of similar size.

We sampled four sites along the St. Lawrence River, from south of Alexandria Bay to Waddington, NY (Figure 1). The four sites were chosen based on proximity to

human activity and settlement and low water velocity. The first sample site was near Grass Point in Orleans, NY at the beach access point. The second site was Oak Point in Hammond, NY at the public docks. The third and fourth sites were at Rockway Point in Lisbon, NY at a small inlet and along Rt 37 near Coles Creek in Waddington, NY at the beach access point, respectively. All collection sites had a small bay and depositional area. Sediment, mussels, and amphibian road mortality samples were collected. Additional mussels, but neither sediment nor anurans, were sampled from the public dock access at Cape Vincent, NY. The size of sediment particles, mussels, and amphibians depended on site and varied between subsamples.

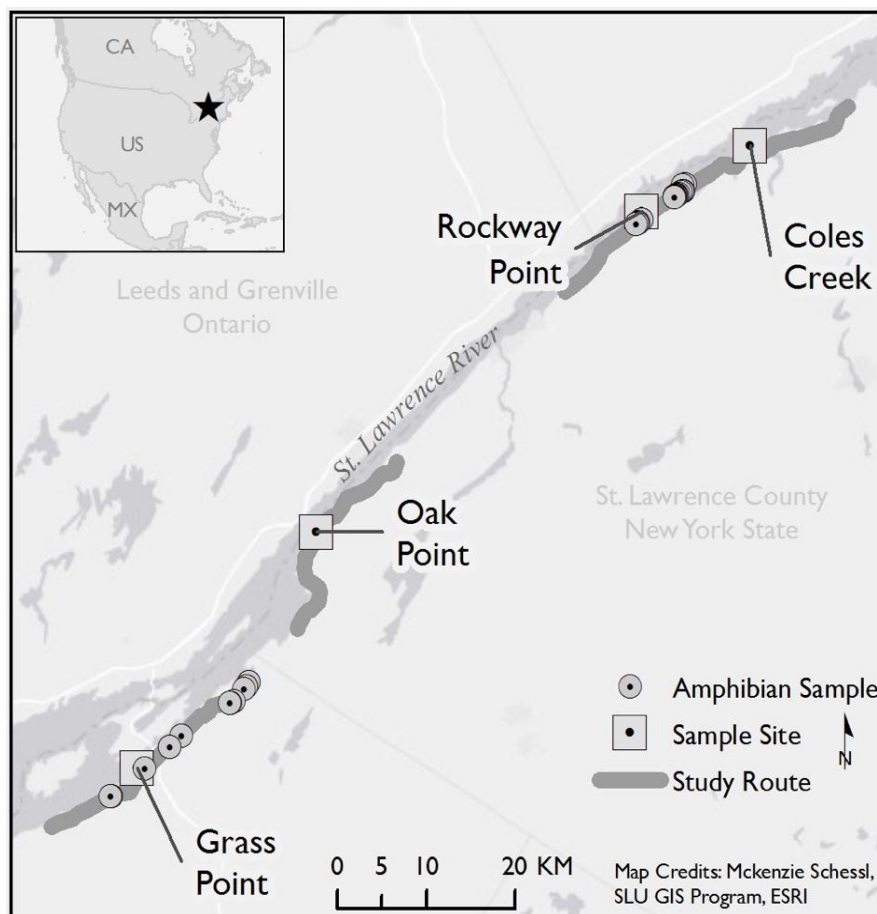


Fig. 1. International section of the upper St. Lawrence River showing the four collection site locations for sediments, mussels, and anurans (44.3359° N, 75.9177° W). Shaded study routes for each sample site indicate the road examined for anurans. Circles indicate locations of individual anuran samples.

Sediment samples were collected in triplicate from each of the four sample sites. Sediment sample sites were selected based on locations where decreased current velocity or low energy zones were evident as in coves or inlets (Vianello et al., 2013). Samples were collected at Grass Point on 5 September 2015. Oak Point, Rockway Point, and Coles Creek sediment samples were collected on 31 October 2015. All samples were taken at a water depth of 0.5 m using a metal hand scoop (355 mL). Three scoops were taken per sample to a depth of 7.5 cm beneath the surface sediment and combined to make one sample. Each scoopful was lifted gently through the water to minimize loss of fine sediment material. The samples were sieved to pass a 500 micron grade metal sieve (Castañeda et al., 2014) on site using river water. Each sample was placed in a clean plastic container with enough water to cover the sediment. Samples were transported and stored at 18 degrees Celsius until processing. Samples were sorted using forceps, removing larger rocks and debris ranging from 3 to 30 mm in diameter which were placed in a separate container. These contents were rinsed and visually inspected for microplastic particles (microbeads) based on spherical shape, color, and texture. Smaller sediments were visually inspected for the presence of microbeads by separating each sample further into petri dishes, using forceps and a dissecting microscope (Olympus SZ-ST, SZ30, SZ3060) at 2x magnification. Any microbeads found were removed and stored in a 20 mL scintillation vial with water.

Dreissenid mussels (*D. polymorpha* and *D. bugensis*) were collected at all four sediment sites through haphazard sampling of larger rocks. Mussels were gently removed, transported in buckets of river water, kept at 16 degrees Celsius for 48 hours, placed into labelled plastic bags, and then sealed and frozen until analysis. Specimens were removed from the freezer, identified to species, then their maximal

length was measured using digital calipers (Maxwell 150 mm). Once thawed, a scalpel was inserted between the valves to cut the attachment of mantle, separating the bivalve shells and the soft tissue was removed from the shell entirely (Ram et al., 1999; Johns, 2011). The shell and soft tissues of each individual mussel were placed in a petri dish. The soft tissues were cut open using a scalpel and viewed under a dissecting scope at 2x magnification to inspect for microbeads in the digestive tract or within the body cavity fluid.

Surveys for amphibians were conducted on paved roadways nearest each sampling site, in both up and downstream directions of sediment sampling sites with a total of 25 km surveyed for each site. Surveys were conducted on rainy nights that followed warm days, during peak to end of amphibian migration throughout the month of October 2015. This sampling period was selected due to climatic factors that increase movement of amphibian species which increased the likelihood of amphibian mortality on the road (Todd & Winne, 2006). The vehicle was driven at 8 km/h along paved roads while scouting for amphibians. Dead specimens found on the road were inspected to see if the digestive tract was intact and of large enough size to equate adult age for the species. Individuals were placed into a sealable plastic bag, Global Positioning System (GPS) coordinates were recorded using Garmin smartphone application (Garmin 2015, GPS app., version 1.8.3) and species identified. After transport to the lab on ice, specimen were frozen until further analysis. In the lab, samples were allowed 24 hours to thaw before analysis, any that were still frozen were left in the bag and placed in a container of room temperature water for 10 minutes to finish thawing. Once thawed, snout to vent length, and wet weight were recorded for each viable sample. When present, the digestive tract was dissected out and placed in the original bag and the rest of the sample discarded. These bags were then

refrozen until further analysis. Once thawed, the digestive tract was cut open and placed in 2.5 oz. glass jars and allowed to liquefy for 2.5 weeks at room temperature of 21 degrees Celsius. To speed up the liquefaction, the jars were agitated every two days. Liquefied digestive tracts were placed in petri dishes and examined for the presence of microbeads using a scalpel, forceps, and a dissecting microscope at 2x magnification within a fume hood.

RESULTS & DISCUSSION

Microbeads were found in sediment samples at two of our four sampling sites (Table 1). The color of the microbeads showed little variation. At Oak Point, one hundred eighty-eight white, opaque microbeads were found in sample 1, twenty in sample 2, and one hundred thirty-eight in sample 3. At Rockway Point two white, opaque microbeads and one black microbead were found in sample 1. At Rockway Point macro Styrofoam™ spheres were found floating at the surface of the water, on the shoreline, and amidst aquatic vegetation. A sample of this debris was collected for reference. The microbeads varied in size 0.0748 mm - 1.2264 mm with an average diameter of 0.8069 mm for Oak Point microbeads (20% of microbeads were measured) and 0.8040 mm - 1.0164 mm with an average of 0.9264

mm for those detected at Rockway point. No microbeads were found within the sediment subsamples at Grass Point or Coles Creek.

A total of one hundred and forty-nine *Dreissenid* mussels were collected. The size ranged from 10.59 - 22.9 mm with the smallest mussel found at Grass Point and the largest at Oak Point. The average length was 17.33 mm. At Grass Point eighteen zebra and twenty quagga individuals were collected. Forty quagga samples were collected at Oak Point, and thirty-six zebra mussels from Coles Creek. At Rockway Point, thirty-five individuals were collected, both zebra and quagga mussels. At Cape Vincent, fourteen mussels were collected and identified as zebra and quagga. No microbeads were found within any of the mussels regardless of site (Table 1). On adjacent roads, a total of forty-one roadkill anuran samples were collected (American Toad *Bufo* (*Anaxyrus*) *americanus*, American Bullfrog, Pickerel Frog *Lithobates* (*Rana*) *palustris*, Northern Leopard Frog *Lithobates* (*Rana*) *pipiens*, Mink Frog *Lithobates* *septentrionalis*, Green Frog *Lithobates* (*Rana*) *clamitans*; Table 2). In thirty-one of the samples, the digestive track or a partial digestive tract, due to vehicle mutilation, was present and examined. No microbeads were found in any of the amphibian samples (Table 1).

Table 1. The number of microbeads found in sediment, mussels collected (species, size, presence microbeads), and anuran samples by site (NA: Not available).

Site	Sediment Microbead Presence	Mussel Species	Number of mussels	Mussel Size (mm)	Mussel Microbead Presence	Anuran Microbead Presence
Oak Point	346	Quagga	40	12.31 - 22.90	0	NA
Rockway Point	3	Zebra and Quagga	35	12.88 - 17.68	0	0
Coles Creek	0	Zebra	36	15.76 - 22.78	0	0
Grass Point	0	Zebra	18	10.59 - 21.66	0	0
		Quagga	20	12.57 - 19.47	0	NA
Cape Vincent	NA	Zebra and Quagga	14	12.62 - 20.41	0	0

Table 2. The number (N) of anuran samples collected adjacent to site, species identification, stage of development, sex (FG: female gravid), and the range wet weight and snout-vent length (NA: Not available)

Site	Species	N	Stage	Sex	Range Wet Weight (g)	Range Snout-Vent Length (mm)
Coles Creek	American Toad	1	Adult	Male	+100	102
	Mink	1	Adult	Unknown	68	94
	American Bullfrog	1	Adult	Unknown	75	98
		1	Unknown	Unknown	62	75
	Northern Leopard	1	Adult	FG	NA	NA
Grass Point	Green Frog	2	Adult	Unknown	53 - 63	74 - 80
	American Bullfrog	1	Adult	FG	+100	111
		7	Adult	Unknown	60 --100	84 - 132
	Northern Leopard	1	Adult	FG	73	88
		2	Adult	Unknown	NA	NA
	Unknown	1	Juvenile	Unknown	13	NA
	Pickerel Frog	1	Adult	Unknown	47	80
Rockway Point	Green Frog	1	Juvenile	Unknown	NA	NA
	American Bullfrog	1	Adult	Unknown	43	69.7
		6	Adult	FG	37 - 74	70 - 78
	Northern Leopard	4	Adult	Unknown	30 - 55	60 - 71
		5	Juvenile	Unknown	22 - 29	61 - 64
		3	Unknown	Unknown	27 - 33	65
	No Samples Found	NA	NA	NA	NA	NA

This study provides evidence that sediment at selected near-shore littoral sites along the upper St. Lawrence River contain microplastic debris, however microplastic particles were not detected within either *Dreissenid* mussels or anurans in and around our sampling sites. The visual identification technique used to identify and remove microbeads from samples focused on particles of spherical shape, like those originating in cosmetic products. Visual technique likely excluded plastic

fragments that may have degraded from larger plastic objects like plastic bags (Castañeda et al., 2014). A challenge in researching microplastics is there is no set definition for “microplastic” (Cole et al., 2011) even though it is commonly referred to as a plastic particle smaller than 5 mm (Driedger et al., 2015). The lack of a standardized microbead/microplastic definition makes study and quantification somewhat subjective. Others have argued that standardization of sampling techniques

would enhance comparability among studies (Claessens et al., 2011, Costa et al., 2010, Twiss, 2016). Although multiple types of microplastics have been found in the Great Lakes, with fragments and fibers being most common (Baldwin et al., 2016; Mason et al., 2016), we focused on microbeads.

In our study microbeads were detected at two sites out of four. Low detection rates in this study could be influenced by river currents forcing microbeads and other debris to settle where currents converge (Driedger et al., 2015). Deposition of sediment in aquatic environments is influenced by energy flow within the site allowing for settling of fine particles like microbeads (Vianello et al., 2013). The international section of the St. Lawrence River is fast-flowing (mean flow of 7051 cm/s at the Moses-Saunders power dam at Massena, NY) with limited depositional areas except very near-shore. Thus, the lack of detection could be due to the low deposition at our sites not absence from the St. Lawrence River. Microplastics have been detected at multiple sediment sites downstream from our study area (Castaneda et al., 2014). Microplastics have been found throughout the Great Lakes upstream of our sites (Mason et al., 2016; Erikson et al., 2013, Baldwin et al., 2016) so the outflow of Lake Ontario could be a source to this section of the river.

The lack of microbeads detected within mussel and amphibian samples does not necessarily mean that microbeads are not transferred through trophic levels within the St. Lawrence River. We selected two ubiquitous filter feeders and several commonly occurring anurans to assess whether microbead ingestion and trophic transfer could be quickly detected. The largest mussel found at any sampling location was 22.9 mm long; they averaged 17.33 mm. Typically, these two species selectively ingest particles between 5-35 microns diameter (Sprung & Rose, 1988).

The microbeads sampled from Alexandria Bay to Waddington, NY were 74.8 to 1226 microns in size. Finding microbeads within mussels was unlikely due to physical constraints and feeding preferences. Feeding experiments using larger mussels and small microplastics would be useful to assess potential ingestion of minute microplastics, including fibers.

The majority of amphibian species with viable digestive tracts collected were American bullfrogs (*Rana catesbeiana*) and Northern leopard frogs (*Rana pipiens*) (Table 2). The diet of the American bullfrog includes small fish, crayfish, insects, and even other frogs and bugs and for the Northern leopard frog insects, leafhoppers, and spiders (Gibbs et al., 2007). Thus the possibility of finding microbeads within the bullfrogs exists, but is unlikely within the northern leopard because its diet consists primarily of insects. Further research using a variety of aquatic species would help assess whether microbeads are being transferred through trophic levels of the St. Lawrence River littoral areas. Larger, ubiquitous bottom feeding species such as the Round Goby (*Neogobius melanostomus*) or the Rusty Crayfish (*Orconectes rusticus*) may be viable monitoring species because their diets consist of larger aquatic organisms which could increase likelihood of accidental ingestion in addition to transfer from prey.

Potential for trophic transfer of plastics and sorbed contaminants remains insufficiently studied.

Many plastic beads and particles resemble macroinvertebrates, eggs, and organic debris that larger aquatic organisms feed on, making ingestion probable (Carpenter et al., 1972; Teuten et al., 2007). Sanchez et al. (2014) and Imhof et al. (2013) investigated the presence of microplastics in aquatic organisms such as the gudgeon (*Gobio gobio*) and annelids (*Lumbriculus variegatus*), crustaceans (*D. magna* and *Gammarus pulex*), ostracods (*Notodromas monacha*), and gastropods (*Potamopyrgus*

antipodarum), respectively, and found that aquatic organisms are potentially prone to microplastic ingestion. In France, twelve percent of 186 wild gudgeon (*Gobio gobio*), a freshwater species, contained microbeads in their guts (Sanchez et al., 2014). More research on the effects of ingested microplastics, the residual chemicals they may contain, the potential for bioaccumulation and negative effects is needed. Some studies suggest that the hydrophobic contaminants adsorbed onto plastic particles may facilitate bioaccumulation and adverse metabolic effects, as the chemicals desorb (Teuten et al., 2007; Betts, 2008; Besseling et al., 2013; Browne et al., 2013; Rochman et al., 2014; de Sa et al., 2015; Tanaka et al., 2015). Other recent studies have not found that microplastics enhance bio-accumulation, or adverse effects, of persistent organic pollutants after ingestion (Bakir et al., 2016; Herzke et al., 2016; Sleight et al., 2016).

WWTPs have been cited as major sources of microplastics to aquatic environments (Browne et al., 2007; Fendall et al., 2009) although more recent studies suggest their contribution is less important or variable (Baldwin et al., 2016). Our sites were chosen based on their proximity to towns and public access points like docks and beaches. Their distance from WWTPs was not a main factor in site location. On average one microplastic particle occurs in every liter of WWTP effluent (Browne et al., 2011); these include beads from cosmetic products and cleaners but also synthetic fibers from laundered clothing (Eerkes-Medrano et al., 2015). Sampling closer to WWTPs might have increased microbead detection in our study. However, most WWTPs locate their outfall pipes to maximize effluent dispersion in the main current of the river. And, most municipalities along our side of the river are small. Further research needs to explore the role WWTPs play, generally, as a source of microbead and microplastic

pollution in freshwater systems. Sampling more locations and use of a smaller sieve size would allow microbeads smaller than 333 microns to be collected (Moore et al., 2011; Eriksen et al., 2013; Baldwin et al., 2016). Sampling up and downstream of WWTPs would elucidate the extent of microbead release from these facilities. Collecting samples from WWTP effluent could provide information about the relative contribution of microplastics from local sources compared to the outflow of Lake Ontario, since 95 to 98% of the river flow in the international section of the St Lawrence comes from the lake (Colburn et al., 1990).

Another source of microplastic pollution is the spillage of industrial plastic pellets, the raw material used to create plastic, during transport (Derraik, 2002; Driedger et al., 2015). Secondary microplastic pollution includes the degradation of larger plastic particles within the environment (Cole et al., 2011; Eerkes-Medrano et al., 2015). According to Driedger et al. (2015), current surveys have found the concentration of plastic debris in aquatic systems like the Great Lakes is higher where human population density and industrial activity are higher. Discharges from large municipalities and major industries occur downstream of our study sites. Eight of ten sites sampled from the Moses-Saunders dam down to Quebec City contained microbeads greater than 500 microns in their sediments (Castaneda et al., 2014). These authors concluded that microplastics were ubiquitous in St. Lawrence River sediments and that they may have substantially underestimated their presence by using a sampling regime that excluded a smaller size fraction (<500 microns) as did our study. Baldwin et al. (2016) also suggested that their study of microplastics in Great Lakes' tributaries may have missed microbeads from cosmetics because most are thought to be smaller than 333 microns diameter.

Even though WWTPs are a point source of microbeads and other microplastic particle pollution (Cole et al., 2011; Driedger et al., 2015) there is currently no requirement in the United States to monitor plastics in influents and effluents of these facilities (Driedger et al., 2015). Removal of microbeads will require new filtration technology (Nalbone, 2014). Tertiary treatment can retain 77% of microbeads from wastewater (Kalčíková et al., 2017). Technologies such as rapid sand filters, dissolved air flotation, and membrane bioreactors could decrease microplastic release from WWTPs by 95% (Talvitie et al., 2017). However, communities along the New York side of the international section of the river are too small to afford these technologies. Only Canton, Potsdam, Ogdensburg and Massena have even secondary treatment facilities. Smaller communities along the river and its tributaries have only primary treatment. Preventative measures will be less expensive and more effective. The Microbead Free Waters Act of 2015, passed by the U.S. Congress and signed into law by President Obama in 2015 addresses the microbead problem, but not microplastics more generally, by outlawing the manufacture of microbeads effective July 1, 2017, banning manufacture of over-the-counter (OTC) drugs and cosmetic products containing microbeads as of July 1, 2018. And, it bans sale of OTC drugs containing microbeads as of July 1, 2019 in the United States (FDA, 2015). The full implementation of this ban should substantially reduce future microbead inputs.

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