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ARTICLE

Eastward Expansion of Round Goby in New York: Assessment of Detection Methods and Current Range

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Abstract

The Round Goby *Neogobius melanostomus* has spread rapidly around the Great Lakes region since its introduction to North America in 1990. In 2014, a specimen was captured in the New York State Canal System west of Utica, prompting concerns that Round Goby would soon reach the ecologically and economically valuable watersheds of Lake Champlain and the Hudson River estuary. The establishment of Round Goby populations elsewhere has been linked to a number of negative ecological consequences, yet methods for monitoring the invasion front of this species remain limited. The objectives of this study were to assess the current distribution of Round Goby in central New York and to determine the most effective methods for monitoring the invasion front. This was achieved by concurrently using benthic trawling, seining, minnow traps, and environmental DNA (eDNA) twice annually from 2016 to 2019 at 12 sites on the canal system between Oneida Lake and the Hudson River. Of the three traditional gear types, benthic trawling was the most effective method and captured Round Goby as far east as Utica by 2019. This finding suggests only minimal eastward expansion of Round Goby occurred between 2014 and 2019. Round Goby DNA was detected in water samples during all surveys in which individuals were captured with trawling, and the estimated concentration of DNA explained 69% of the variability in trawl catch. At multiple study sites, Round Goby DNA was identified during consecutive surveys before Round Goby were first captured with trawling. This suggests that in lotic waters, eDNA has the potential to forecast or serve as a sentinel for the expansion of Round Goby to new locations. Our results demonstrate the importance of using eDNA in a repeated sampling framework and supplementing eDNA sampling with some level of effort with traditional sampling methods.

The Round Goby *Neogobius melanostomus* has proven to be one of the most prolific and problematic invasive fishes in North America. First documented in the continent in 1990 (Jude et al. 1992), Round Goby spread to all five Great Lakes by approximately 1995 (Marsden et al. 1996) and by 2002 had reached an estimated population size of 9.9 billion in western Lake Erie alone (Johnson et al. 2005). Round Goby have become widespread throughout the Great Lakes region with confirmed captures in at least eight states (USGS 2020), and in 2018 the

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species was documented in the Illinois portion of the Mississippi River (Merry et al. 2019). This incredible invasion success over a period of just 30 years is attributed to a number of factors, including multiple introduction points (LaRue et al. 2011) and the ability of Round Goby to spawn repeatedly during the warmwater season and exploit dreissenid mussels, which are widely distributed throughout the Great Lakes, as a food source (Charlebois et al. 2001; Corkum et al. 2004; Kornis et al. 2012).

In New York, Round Goby have invaded east and south from their Lake Erie and Lake Ontario populations towards the interior of the state over the past 15 years (Figure 1). They were identified in Onondaga Lake (2010), Cayuga Lake (2011), and Oneida Lake (2013), and they are also widespread throughout the New York and Canadian portions of the St. Lawrence River. Additionally, a single Round Goby was found dead in the Susquehanna River near Binghamton in 2013 with signs of having been used as bait by an angler, but no evidence exists to date that a population has been established at this location (USGS 2020). In central New York, the complete colonization of Oneida Lake, the largest inland lake in the state, occurred in just 2 years. Round Goby were first reported in the west end of Oneida Lake in 2013 and had become the most abundant benthic fish in the lake by 2015 (Jackson et al. 2016). In 2014, a single Round Goby was captured by an angler in the New York State Canal System approximately 40 km east of Oneida Lake near Utica (New York State Museum, catalog #71439). This capture prompted immediate concern about the distribution of Round Goby in the interior of the state and the rate at which the species may be expanding to new waters.

The arrival of Round Goby in central New York is concerning for many reasons. Across the Great Lakes region, a number of negative ecological consequences have been observed when Round Goby colonize new habitats. Round Goby can outcompete and displace native benthic fish such as darters and sculpins (Janssen and Jude 2001; Lauer et al. 2004; Morissette et al. 2018), consume eggs of economically important game fish (Steinhart et al. 2004a), and mobilize contaminants into the food web through their consumption of dreissenid mussels (Kwon et al. 2006; Marentette et al. 2010). They can also carry the viral hemorrhagic septicemia virus, which has been linked to significant fish kills in parts of New York (Farrell et al. 2017; Getchell et al. 2019), and some evidence suggests Round Goby are an important vector in avian botulism outbreaks (Ruffing 2004; Hannett et al. 2011). However, positive effects of Round Goby introductions, such as increased growth of Smallmouth Bass *Micropterus dolomieu* (Steinhart et al. 2004b) and other predators, have also been observed. A more thorough summary of the ecological impacts of Round Goby invasion can be found in Kornis et al. (2012) and Corkum et al. (2004).

Round Goby are expected not only to affect ecosystems in central New York, but also to gain access to two iconic waterways in eastern New York, the Hudson River and Lake Champlain. Both of these water bodies can be reached through a series of locks and channels on the New York State Canal System. From Utica, Round Goby have a relatively unobstructed path eastward through the canal system, which runs together with the Mohawk River for much of the distance to the confluence with the Hudson River. This eastward route to the Hudson River is in a downstream direction, which should accelerate the rate of expansion associated with larval drift (Janáč et al. 2013; Ramler et al. 2016). Once in the Hudson River, Round Goby can move north through the Champlain Canal into Lake Champlain and parts of its 21,326 km^2-watershed spanning both New York and Vermont, and south into the 246-km Hudson River estuary. The lower 35–109 km of the estuary are brackish water, depending on flow conditions (de Vries and Weiss 2001), and salinity typically averages <20‰ for all but the downstream-most 5–10 km of this stretch (Ralston et al. 2008; HRECOS 2020). Round Goby are believed to have an oceanic salinity (NaCl) tolerance as high as 20–30‰ (Ellis and MacIsaac 2009; Karsiotis et al. 2012; Kornis et al. 2012; Behrens et al. 2017), suggesting salinity levels are suitable for colonization in the vast majority of the Hudson River estuary. Thus, the status of Round Goby in the Mohawk River portion of the New York State Canal System is of critical importance because this reach serves as the gateway through which Round Goby can invade these ecologically and economically valuable watersheds.

In response to the Round Goby invasion in central New York and the impending threat posed to important ecosystems in eastern New York, the U.S. Geological Survey (USGS) and the Mohawk River Basin Program of the New York State Department of Environmental Conservation partnered on a study of Round Goby in the New York State Canal System. The primary objectives of the project were to determine (1) the current distribution and rate of expansion of Round Goby and (2) the most effective methods to monitor the invasion front. These objectives were achieved by concurrently employing several sampling methodologies, including environmental DNA (eDNA) and traditional netting and trapping methods at the same locations. The performance of eDNA relative to traditional sampling methods was of particular interest because eDNA has shown great promise for the detection of species at low densities (Jerde et al. 2011; Lodge et al. 2012; Thomsen et al. 2012; Thomsen and Willerslev 2015). We hypothesized that eDNA would be a more sensitive method for tracking the invasion front of Round Goby than traditional fish sampling methods. However, we were also concerned about the potential for downstream transport of Round Goby DNA to saturate the study area, obscuring our ability to determine the specific locations where populations were present.

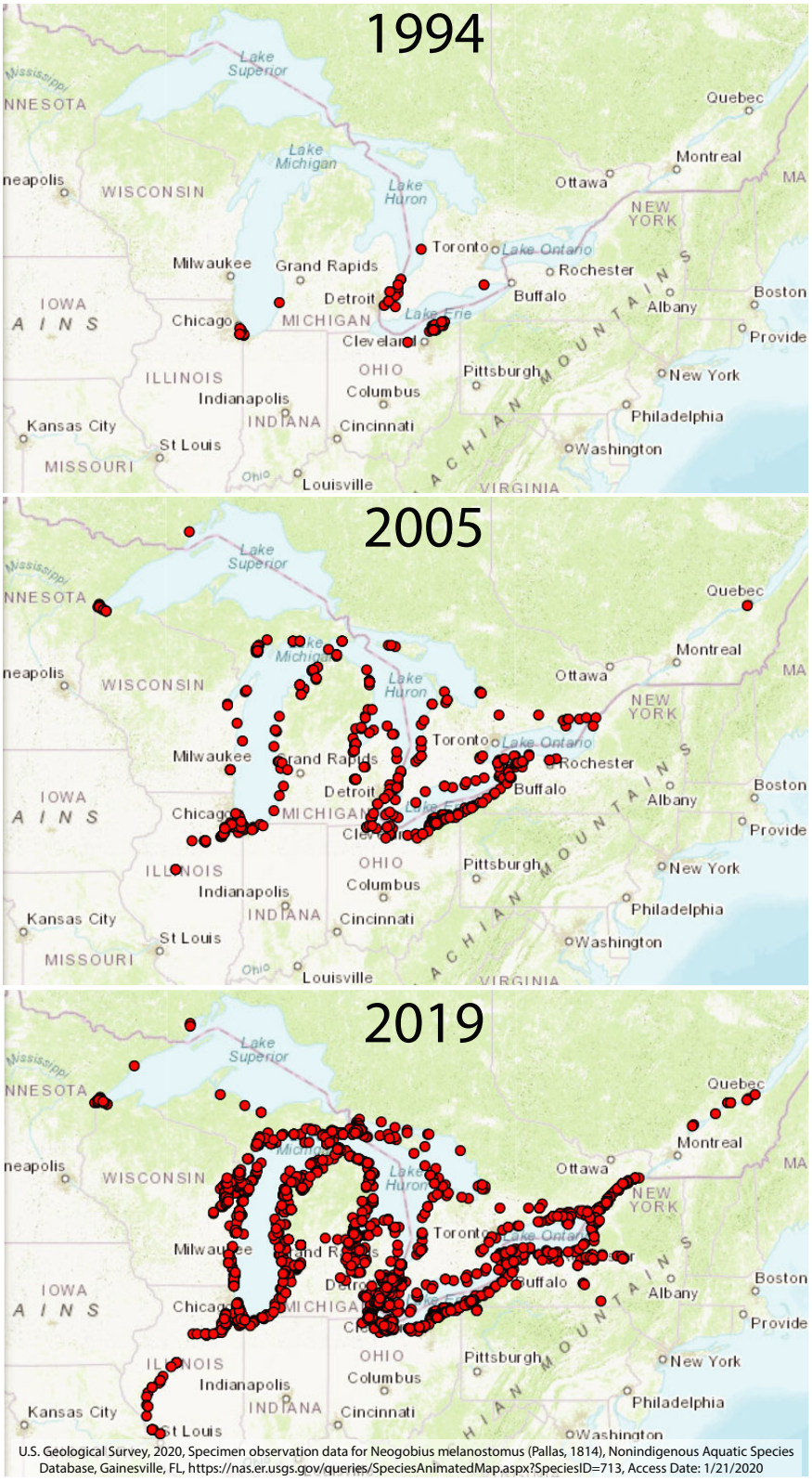

FIGURE 1. Locations of Round Goby captures in North America reported to the USGS Nonindigenous Aquatic Species Database from 1994, 2005, and 2019.

METHODS

Study area.—The New York State Canal System runs across the east–west axis of New York, connecting the Hudson River, Lake Erie, and other water bodies along its route. Its predecessor, the original Erie Canal, was completed in 1825 and created a water linkage between the Great Lakes and Hudson River drainages for the first time. Due to its limited shipping capacity, the Erie Canal was replaced by the larger New York State Barge Canal in 1918, which canalized a number of major rivers (including the Mohawk River) and incorporated them into the navigation route (McBride 1994). Known today as the New York State Canal System, this network of canals, dams, and locks connects most of the major waters in the state including Lake Erie, Lake Ontario, the Finger Lakes, the Hudson River, and Lake Champlain. This linkage of formerly separated watersheds has enabled the extensive movement of migratory and resident species into new habitats and has facilitated the spread of aquatic invasive species (Mills et al. 1996; Pimentel 2005).

The focus of this investigation was the section of the canal system between Sylvan Beach (at the east end of Oneida Lake) and the upstream terminus of the Hudson River estuary. The highest elevation in this section occurs in the Rome area, which is also the location where the Mohawk River enters the canal system from the north. From this point, the Mohawk River and canal waters east of Rome flow east towards the Hudson River. For most of this distance, the Mohawk River and the canal utilize the same channel, but in some areas they are separated as parallel channels (Figure 2). Canal habitats in the study area are generally greater than 60 m wide and have a maintained navigation channel with a depth of at least 4.3 m (George et al. 2016). Twelve study sites were distributed across the study area to be approximately equidistant (~20 km apart) while also taking advantage of public access points (Table 1; Figure 2). Eleven of the sites were

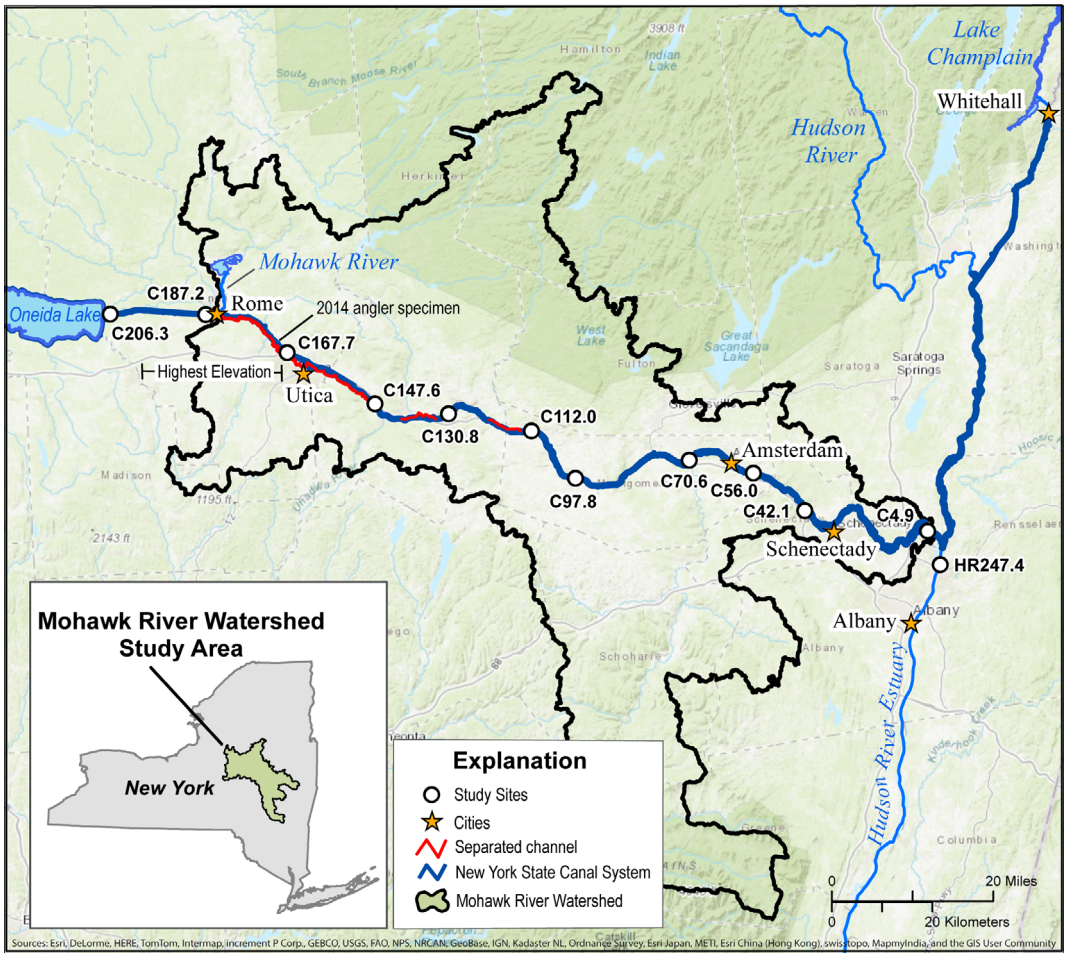

FIGURE 2. Location of 12 study sites on the New York State Canal system where Round Goby surveys were conducted twice annually from 2016 to 2019. Site ID numbers correspond to site descriptions given in Table 1.

TABLE 1. Site name, site identification code, and location of 12 study sites (ordered from west to east) where eDNA and fish surveys were conducted twice annually between 2016 and 2019 in the New York State Canal System. Coordinates are for the location where eDNA samples were collected, although trawling, seining, and trapping were generally conducted within 500 m. The NWIS ID refers to USGS National Water Information System (https://waterdata.usgs.gov/nwis) site identification number. Site IDs starting with "C" are interpreted as approximate number of river kilometers as measured from the mouth of the Mohawk River, while the site ID starting with "HR" is interpreted as the approximate number of river kilometers from mouth of the Hudson River.

Site description	Site ID	NWIS ID	Latitude (°)	Longitude (°)
Canal at Sylvan Beach	C206.3	431209075424401	43.20254	–75.71215
Canal at Rome	C187.2	431210075284001	43.20282	–75.47787
Canal west of Utica	C167.7	430806075163901	43.13489	–75.27763
Canal/Mohawk River at Frankfort	C147.6	01342702	43.04334	–75.06212
Canal/Mohawk River at Little Falls	C130.8	430132074525501	43.02554	–74.88200
Canal/Mohawk River at St. Johnsville	C112.0	01348065	42.99420	–74.67957
Canal/Mohawk River at Canajoharie	C97.8	425432074342101	42.90896	–74.57249
Canal/Mohawk River at Schoharie Ck Confluence	C70.6	0134953803	42.94022	–74.29420
Canal/Mohawk River downstream of Lock 10	C56.0	425456074081701	42.91568	–74.13808
Canal/Mohawk River at Rotterdam Kiwanis Park	C42.1	425050074005001	42.84726	–74.01385
Canal/Mohawk River upstream of Lock 6	C4.9	424828073425301	42.80774	–73.71460
Hudson River downstream of Troy Dam	HR247.4	424450073410701	42.74712	–73.68523

located on the canal west of the Hudson River and are labeled with their corresponding distance in river kilometers from the Hudson River confluence (e.g., "C4.9"), while one site (HR247.4) was located in the northernmost portion of Hudson River estuary.

Traditional fish sampling.— Benthic trawling, bag seining, and minnow trapping were used twice annually from 2016 to 2019 at the 12 study sites. Each year, surveys were conducted during daylight hours in spring (late May to early June) and again in summer (August) to obtain multiple data points and to account for seasonal differences in fish habitat usage. The intensity of sampling with each gear type was intentionally moderate to approximate a repeatable level of effort that managing agencies might be able to put forth on a large spatial scale in the future. Minnow traps and seines were used in shallow habitats near the banks while benthic trawling was conducted in deeper habitats that could not be sampled with the other techniques. Within a site, it was common to stagger the exact areas that were sampled by 500 m or occasionally more in order to find appropriate habitat for each gear type (i.e., wadable habitat for seining and habitat free of snags for trawling).

Benthic trawling was conducted using a Siamese Trawl (Innovative Net Systems) following methods suggested by Herzog et al. (2009). The Siamese Trawl is a slingshot balloon trawl similar to a mini-Missouri trawl (Herzog et al. 2009) and is 6.1 m long with a mesh size of 4 mm and a 2.4-m opening. The trawl was deployed from the bow of a 16-ft (4.9 m) jon boat with a 15- or 20-hp motor and pulled in a downstream direction (operating the engine in reverse) for 150 m at approximately 2.5–3.0 km/h. Three trawl pulls (replicates) were conducted at each site: one in the center of the navigation channel and one along the shoulders of each bank. Water depth was typically 5–8 m in the navigation channel and 1.5–6.0 m in the shoulders. Seining was conducted using a 9.1-m bag seine, rotating 180° around a fixed point on shore. Three replicate seine pulls were conducted at each site. Trapping was performed with standard cylindrical, wire-mesh minnow traps (3-cm opening) baited with cheese and dog food. Two traps were deployed at each site for a one-night soak. All fish captured using each method were identified to species, enumerated, and released, with the exception of some Round Goby that were archived as voucher specimens. The raw data from these surveys are available in a USGS data release in George et al. (2020).

Environmental DNA sample collection.— Samples for eDNA analysis were collected from the same 12 sites within 10 d of the spring and summer traditional sampling gear surveys. Sites were sampled from east to west during each survey as a precaution against field-based contamination, since the westernmost site was already known to have a Round Goby population at the start of the study. Samples were collected just below the water surface from littoral habitats by wading. Two replicate eDNA water samples were collected from each site during each survey. Upon arrival at a site, a 1-L Nalgene bottle, filter holder (stand and funnel), and forceps were decontaminated for 2–3 min in a solution of 10% bleach and 90% site water and then rinsed for 30–60 s in site water to remove the bleach solution. For each replicate, a hand pump was used to vacuum 2 L of river water through a 47-mm-diameter, glass-fiber filter (1.5-μm pore size). The filter was then

removed from the stand with forceps, folded (dirty side in), put into a labeled vial and an unused plastic bag, and immediately placed on ice. If the filter clogged before reaching the 2-L target, additional filters were used for the remaining volume and placed in the vial with the first filter. Beginning in the summer 2017 survey, a field blank was collected before sample collection at the first and last site sampled each day, which equated to 4–6 field blanks per 12 sites in each survey depending on the number of sampling days needed. Field blanks were collected by decontaminating equipment for 2–3 min in a solution of 10% bleach and 90% site water, rinsing with laboratory water to remove the bleach solution, and then filtering 2 L of laboratory water through a normal filter. Tap water was used for field blanks in 2017 and 2018, and deionized water was used for field blanks in 2019. All samples were frozen at –16°C at the USGS laboratory and were shipped on dry ice to the U.S. Fish and Wildlife Service Northeast Fishery Center in Lamar, Pennsylvania within 20 d of sample collection where they were stored at –80°C prior to DNA extraction.

Environmental DNA extractions.— Preserved filters were extracted using the Qiagen DNeasy Blood and Tissue Kit (Qiagen Corporation, Valencia, California) in a dedicated DNA extraction room with mechanical controls and laminar flow hoods to maintain a clean, contamination-free work environment. Several steps in the protocol were modified and followed that of the U.S. Fish and Wildlife Service eDNA Quality Assurance Project Plan for filter samples (USFWS 2019). All samples were eluted in 200 μL elution buffer. When multiple filters were collected from a single water sample, filters were extracted individually, eluted with 200 μL, then pooled into the same vial (i.e., if a sample had three filters, the final elution volume was approximately 600 μL). Extracted DNA was stored at –20°C until quantitative PCR (qPCR) analysis.

Tests for PCR inhibition.— The presence of PCR inhibition was evaluated for each eDNA sample by running triplicate PCR reactions with the TaqMan Exogenous Internal Positive Control reagents kit (Applied Biosystems, Waltham, Massachusetts). Reactions for internal positive control qPCR were run in 20-μL volumes and included 3 μL of DNA template. Cycling conditions were based on manufacturer's recommendations for 40 cycles. Inhibition of PCR in environmental samples was evaluated by comparing the mean cycle threshold (*Ct*) value of triplicate PCR reactions to the mean *Ct* of PCR reactions with double-distilled water added as template (control samples). Environmental samples that demonstrated a shift (delay) in *Ct* of ≥1 relative to control samples were deemed inhibited and required cleanup with an inhibitor removal column (Zymo Research) following the manufacturer's protocol. Cleaned samples were then retested for PCR inhibition.

Marker validation and qPCR analysis of environmental samples.— All samples were analyzed using two fluorescent qPCR probe-based markers designed to detect Round Goby eDNA. The first marker, *NeversCOI* (Nevers et al. 2018), targeted a 147 bp region of the Round Goby mitochondrial cytochrome oxidase I (COI) gene. The second marker (*ReesCOI*) targeted a 167-bp region of the same Round Goby gene and was designed at Northeast Fishery Center as part of this project. Extensive marker validation testing was conducted with both markers, including in silico comparisons with relevant sequencing data, in vitro specificity and sensitivity testing, and in situ testing with samples collected at sites known to contain Round Goby and sites where Round Goby were presumed absent. The *NeversCOI* marker, although used in previous eDNA work in the Great Lakes, had not been used outside of the Lake Michigan and Huron watersheds. Previous population genetics testing of Round Goby populations from the Great Lakes suggests multiple founding sources and nearly simultaneous introductions in different areas from different colonizing source populations (Dillon and Stepien 2001; Stepien and Tumeo 2006). Despite the recency of the Great Lakes introductions, Round Goby populations in Lake St. Clair and Lake Erie were shown to have significant divergence in two regions of mitochondrial DNA sequences (Dillon and Stepien 2001). Due to the uncertainty about the source population for Round Goby in central New York and the demonstrated genetic variation in mitochondrial DNA sequences of Great Lakes Round Goby populations, we opted for a two-marker approach to increase the chances for detection of Round Goby eDNA.

In silico (computer-simulated sequence comparison) testing was carried out using multiple sequence alignments of Round Goby COI sequences as well as COI sequences of locally extant nontarget species. In vitro specificity testing was conducted for each of the two Round Goby markers through PCR tests of fin clip DNA from both Round Goby specimens and nontarget specimens from individuals collected in the study area and Great Lakes waters. In vitro sensitivity tests were then accomplished using synthetic control DNA. Briefly, synthetic gBlock standard (Integrated DNA Technologies, Coralville, Iowa) containing the full amplicons of both markers (*NeversCOI* and *ReesCOI*) was used to serve as material for generation of standard curves. Prior to qPCR, the standard material was diluted into a 5× dilution series to create a set of gBlock standards with the following PCR reaction quantities: 31,250 copies, 6,250 copies, 1,250 copies, 250 copies, 50 copies, 10 copies, 2 copies, and 0.4 copies. Standard curves were then generated by running six PCR replicates of each gBlock standard quantity and were used to evaluate the sensitivity of the markers and to extrapolate copy estimates for any positive PCR reactions during qPCR

analysis of environmental samples. The gBlock standard was also used as positive control DNA during qPCR. Finally, in situ marker validation tests were conducted prior to analysis of samples from the study area to verify utility and accuracy of markers in amplifying Round Goby DNA from sites where Round Goby were known to be present. For these blind tests, positive source samples were collected in March 2016 from a location on the Oneida River west of Oneida Lake (43.27050°, –76.20564°), while presumed negative source samples were collected from two locations on the Mohawk River over 70 km east of the nearest known Round Goby population, (42.90753°, –74.57717° and 42.80286°, –73.84636°).

The *NeversCOI* and *ReesCOI* markers were evaluated extensively against Round Goby fin clips obtained from the study area, a museum specimen, and additional Round Goby fin clip samples obtained from throughout the Great Lakes basin. Both markers positively amplified Round Goby DNA from all Round Goby tissue samples while failing to amplify tissue-derived DNA from nontarget species collected throughout the Great Lakes. This included a failure to amplify Freshwater Tubenose Goby *Proterorhinus semilunaris* DNA from Lake Erie and the St. Lawrence River. Although Freshwater Tubenose Goby are not known to occur in the New York State Canal System, recent range expansion has been documented for this species within the Great Lakes (Kocovsky et al. 2011; Goretzke et al. 2019). This species represents the only other member of the Gobiidae family of fish present in the Great Lakes basin and is more closely related to Round Goby than other nontarget species within the basin. Both markers produced similar sensitivity metrics, including standard curves with high r^2 values and PCR efficiencies and acceptable metrics for *y*-intercept and slope. The *NeversCOI* marker had an r^2 of 0.993, PCR efficiency of 96.5%, *y*-intercept of 39.4, and slope of –3.41, while the *ReesCOI* marker had an r^2 of 0.985, PCR efficiency of 100.5%, *y*-intercept of 37.7, and slope of –3.31. Finally, in situ tests revealed that only samples collected from the expected positive location produced positive qPCR amplification, while samples from the two expected negative locations resulted in no detectable Round Goby DNA. As such, validation testing demonstrated both markers to be specific and highly sensitive in the detection of Round Goby DNA from a variety of sample sources.

Reaction conditions for qPCR analysis with the *NeversCOI* and *ReesCOI* markers were as follows: 500 nM of each primer, 125 nM of qPCR probe, and 1× concentration of TaqMan Environmental Master Mix 2.0 (Applied Biosystems). The qPCR probes were double-quenched ZEN (Integrated DNA Technologies) probes labeled on the 5′ end with 6-FAM and run as single-plex reactions (see Table 2 for primer and probe sequence information). The qPCR reactions were run in 20-μL volumes and included 17 μL of master mix/primer/probe mixture and 3 μL of DNA template. All qPCR reactions were analyzed on an ABI ViiA7 PCR thermal cycler (Applied Biosystems). Cycling conditions were based on manufacturer's recommendations and carried out for 45 cycles. For each environmental sample (field replicate) analyzed, eight PCR replicates were performed per marker. Each plate included replicated positive PCR controls (gBlock standard and/or Round Goby fin clip DNA) and replicated negative PCR controls (nuclease-free water). Following PCR, *Ct* values were calculated and used to produce estimates of DNA copies per liter of ambient river water using the standard curves. Any samples that had at least one PCR reaction (out of eight total replicates) resulting in a *Ct* value of ≤40 were considered positive for Round Goby DNA. The *Ct* values and copies per liter estimates for all PCR laboratory replicates are available in George et al. (2020).

Statistical analysis.— The relationship between DNA concentration and trawl catch rate was assessed using polynomial regression in R (R Core Team 2019). To avoid pseudoreplication, the catch rates from the three replicate trawl pulls were averaged to produce a single mean trawl catch rate (hereafter "trawl catch") for each site during each survey. Similarly, the estimated DNA concentration (copies/L) of each laboratory replicate was averaged across both field replicates (a total of 16 possible values) to produce a single mean estimate of DNA concentration (hereafter "DNA concentration") for each site during each survey. Only positive replicates (PCR reactions that produced a *Ct* value of ≤40) were included in

TABLE 2. Markers used for qPCR in eDNA surveillance for Round Goby in the New York State Canal system, including primer and probe sequences. The *NeversCOI* marker (Nevers et al. 2018) was modified slightly to include a ZEN double-quenched probe.

Marker	Assay component	Primer/probe name	Sequence (5′–3′)
NeversCOI	Forward primer	GobyCOI-F2d	CTTCTGGCCTCCTCTGGTGTTG
	Reverse primer	GobyCOI-R2d	CCCTAGAATTGAGGAAATGCCGG
	Probe	GobyCOI-Pr	6FAM-CAGGCAACT\ZEN\TGGCACATGCAG
ReesCOI	Forward primer	NM_COI_F	GAGCATCCGTCGACTTGACA
	Reverse primer	NM_COI_R	GAGTAGGACCGCCGTAATCAGA
	Probe	NM_COI_F_P	6FAM- CCCGCCGTC\ZEN\TCACAATACCARACCC

this calculation, although sites where all 16 laboratory replicates did not amplify were assigned a DNA concentration of 0 copies/L. The mean trawl catch rate and DNA concentration data were then $\log_{10}(x+1)$ transformed because each data set spanned multiple orders of magnitude while also containing zeros (von Ammon et al. 2019; Wiersma 2019). A likelihood ratio test was used to compare the fit of different model orders and select the most parsimonious model. Model order was increased until subsequent increases failed to provide a significantly improved fit to the data using a type I error rate of $\alpha = 0.05$. Following this procedure, a third-order polynomial function was selected to characterize the relationship between the transformed data sets because it produced a significantly better fit to the data than a first- or second-order model.

RESULTS

Traditional Fish Sampling

Round Goby were captured using traditional sampling methods only at the three westernmost study sites during the 4-year study. Round Goby were captured at C206.3, C187.2, and C167.7 in 7, 3, and 2 of the 8 surveys, respectively (Figure 3; Table 3). Benthic trawling was the most consistently successful capture method, while seining was less effective, and minnow trapping did not capture any Round Goby during the study. At the three sites where Round Goby were found during the study, trawling captured Round Goby in 50% of surveys, compared to 21% for seining and 0% for trapping. Catch rates with trawling and seining were also higher during the summer surveys than the spring surveys for almost all comparisons.

During 2016 and 2017, Round Goby were only captured at C206.3, the westernmost site in Sylvan Beach (Figure 3; Table 3). In 2018, only one Round Goby was captured at C206.3, but Round Goby were captured at the next site to the east (C187.2) for the first time. In 2019, Round Goby were captured at the three westernmost sites, which included the first captures at C167.7. Given the strong performance of trawling relative to seining and trapping, only the trawl catch data were retained for subsequent comparisons with eDNA results.

Trawling captured 35 fish species throughout the study, including at least five benthic species that may have niche overlap with Round Goby. These species include Tessellated Darter *Etheostoma olmstedi*, which was present at all sites with relative abundance as high as 147 fish/trawl; Logperch *Percina caprodes*, which was present at 10 sites and generally at low abundances; and Blackside Darter *P. maculata*, Brindled Madtom *Noturus miurus*, and Fantail Darter *E. flabellare*, which were only captured occasionally and always at low abundances (George et al. 2020). A preliminary assessment of the relative abundance of Tessellated Darter at the three sites where Round Goby were captured yields inconclusive but potentially concerning results. At site C206.3 where Round Goby were well established prior to the start of the study, the summer catch per trawl of Tessellated Darter was consistently low: 3.0, 2.3, 0.0, and 0.3 fish/trawl in 2016, 2017, 2018, and 2019, respectively. At site C187.2 where Round Goby were first captured in summer 2018, the summer catch per trawl of Tessellated Darter was 13.0, 2.3, 0.3, and 16.0 fish/trawl in 2016, 2017, 2018, and 2019, respectively. At site C167.7 where Round Goby were first captured in summer 2019, the summer catch per trawl of Tessellated Darter was 147.3, 88.0, 47.3, and 5.3 fish/trawl in 2016, 2017, 2018, and 2019, respectively. Although the data from all three sites are plagued with high interannual and intra-site (between individual trawl pulls) variability, the consistently low abundance of Tessellated Darter at C206.3 and the marked drop in abundance at C167.7 provide evidence of potential impacts of Round Goby on resident benthic fish populations.

Environmental DNA

Negative controls were analyzed from every step in the process, including field blanks, each batch of DNA extractions, and on each qPCR plate, and positive controls were included in each batch of DNA extractions and on all plates during qPCR analysis. Round Goby DNA was not detected with either the *NeversCOI* or *ReesCOI* marker in any negative controls throughout the duration of the study. In addition, all positive extraction and PCR controls positively amplified with both markers indicating field sampling, filtration, and laboratory processing protocols were suitable for avoiding cross contamination of samples. Inhibition testing found no evidence of inhibition in samples collected in 2016, 2018, summer 2017, or summer 2019. Evidence of inhibition was observed in two samples from the spring 2017 collection, which had a delay in *Ct* for internal positive control amplification of 4.7 cycles, and in six samples from the spring 2019 collection, which had delayed *Ct* values of 1 to 3.4 cycles. After treatment, signs of PCR inhibition were relieved in all inhibited samples.

Detection of Round Goby DNA in field samples was primarily confined to the westernmost study sites, although detections occurred occasionally at other sites. Round Goby DNA was detected at two sites, C206.3 and C167.7, with both markers during all eight surveys. During each of the first three surveys (spring 2016, summer 2016, and spring 2017), Round Goby DNA was only detected at those sites with the exception of a single positive replicate at C97.8 during summer 2016 with the *ReesCOI* marker (Table 4; Figure 3). In summer 2017, eDNA results were again strongly positive (16 of 16 laboratory

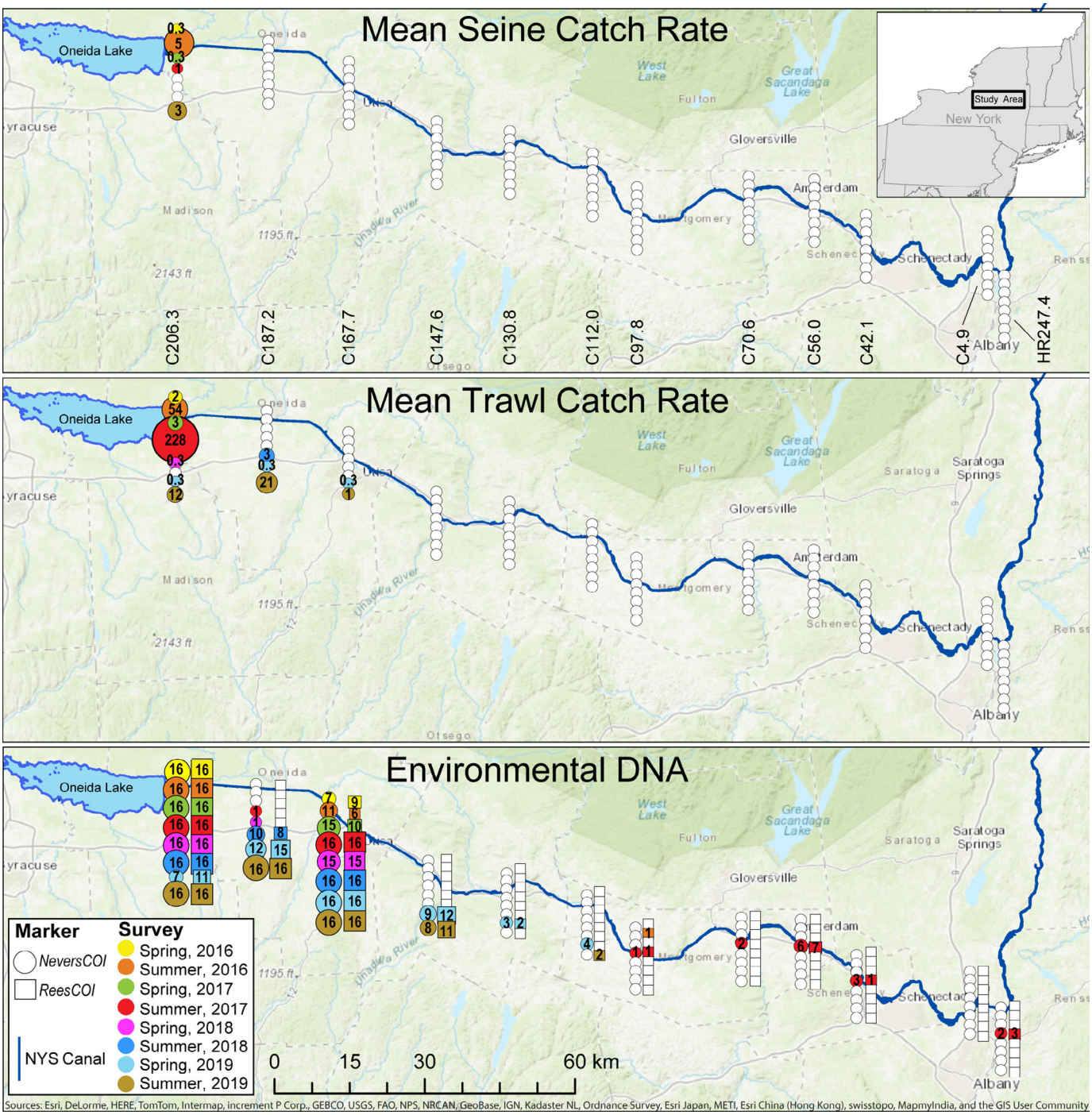

FIGURE 3. Mean number of Round Goby captured from three replicates via seining (top panel) and trawling (middle panel), and the number of PCR replicates positive for Round Goby DNA out of a possible 16 using two different markers (bottom panel) at 12 sites on the New York State Canal System. Points are sized proportionately and are color coded by survey. An empty white point indicates a value of zero.

PCR replicates) at sites C206.3 and C167.7, but weaker detections (<50% PCR replicates) also occurred at six additional sites, including some at the eastern end of the study area, such as the Hudson River site HR247.4. These positives at the eastern end of the study area were unexpected and were not consistent with existing knowledge of Round Goby distributions at that time. In 2018, results were more similar to those of the first three surveys with detections confined to the three westernmost study sites during both the spring and summer surveys. In 2019,

TABLE 3. Mean catch rates (number of fish per seine haul or trawl) of Round Goby captured with seining and benthic trawling in the New York State Canal system. Minnow trap catch rates are not shown because no Round Goby were captured with this methods; *n* is the number of replicates.

Site	Spring 2016	Summer 2016	Spring 2017	Summer 2017	Spring 2018	Summer 2018	Spring 2019	Summer 2019
Mean seine catch rate ($n = 3$)								
C206.3	0.3	5	0.3	1	0	0	0	3
All other sites	0	0	0	0	0	0	0	0
Mean trawl catch rate ($n = 3$)								
C206.3	2	54	3	228	0.3	0	0.3	12
C187.2	0	0	0	0	0	3	0.3	21
C167.7	0	0	0	0	0	0	0.3	1
All other sites	0	0	0	0	0	0	0	0

detections occurred at the six westernmost study sites during the spring survey (which occurred under elevated flows) and at the four westernmost sites (as well as a weak detection at C112.0 with the *ReesCOI* marker) during the summer survey. The concentration of DNA varied greatly among positive samples, ranging from 45 to 84,049 copies/L with the *NeversCOI* marker and from 34 to 69,394 copies/L with the *ReesCOI* marker (Table 4). For both markers, the concentration of DNA across all positive samples averaged approximately an order of magnitude greater during the summer surveys than the spring surveys.

A relationship was evident between trawl catch rates and eDNA detections and concentrations. There were no instances where Round Goby were captured with trawling but undetected with eDNA. At least 7 of 16 PCR replicates were positive for both eDNA markers from all surveys in which Round Goby were captured, and in 9 of 12 instances 100% of the replicates were positive. The estimated concentration of Round Goby DNA in canal waters was also positively related to the catch rate of Round Goby in trawls (Figure 4). Round Goby were not captured in any survey where the water contained DNA concentrations of less than 100 copies/L. At sites where DNA concentrations were 100–1,000 copies/L, Round Goby were either not captured or were captured at low relative abundances ($\leq$3 fish/trawl). At sites where DNA concentrations were greater than 1,000 copies/L, Round Goby were usually captured and relative abundance was often high, reaching up to 228 fish/trawl. Polynomial regression of the transformed data identified a highly significant positive relationship between DNA concentration and trawl catch for both the *NeversCOI* ($F = 69.23$; df = 3, 92; $P < 0.001$) and *ReesCOI* ($F = 69.48$; df = 3, 92; $P < 0.001$) markers (Figure 4). The estimated DNA concentration explained >69% of the variability in trawl catch using either marker. Although imprecise, this relationship suggests that the concentration of DNA in water samples can be used as a coarse predictor of the relative abundance of Round Goby in this system.

DISCUSSION

One of the most unexpected findings of this investigation is the minimal eastward expansion of Round Goby observed during the 4-year study. A single Round Goby was captured by an angler in 2014 less than 3 km west of study site C167.7, and yet no Round Goby were captured in our surveys at this site until spring 2019. At the conclusion of the study in 2019, Round Goby had not been captured at any site east of C167.7, and eDNA results only provided compelling evidence of expansion towards site C147.6 in 2019. The authors note, however, that during the publication process of this article a single Round Goby was captured at C147.6 in June 2020 (New York State Museum, catalog #84461). Expansion rates of Round Goby populations in an upstream direction on Great Lakes tributaries have been observed as low as 0.5 km/year (Bronnenhuber et al. 2011) and 1–4 km/year (Kornis et al. 2012). In a downstream direction, however, expansion rates have been estimated at 25 km/year in the Chicago area waterways (Steingraeber and Thiel 2000; Corkum et al. 2004), 20–30 km/year in the Illinois River (Merry et al. 2018, 2019), and >40 km/year in the St. Lawrence River (Olivier Morissette, Quebec Ministère des Forêts, de la Faune et des Parcs, personal communication). We expected that expansion rates in our study area would be more consistent with the downstream expansion rates documented elsewhere since Round Goby had already crossed the watershed divide in Rome, and further eastward expansion towards the Hudson River is in a downstream direction. Instead, expansion rates in the Utica area between 2014 and 2019 were so low they were difficult to measure and were likely $\leq$1 km/year. One possible explanation for the limited eastward expansion of Round Goby is that water velocities in this section of the

TABLE 4. The number of PCR replicates out of a possible 16 that were positive for Round Goby DNA (NumReps) in the New York State Canal System and the mean DNA concentration estimated from the positive replicates (copies/L) for two molecular markers during eight surveys. Sites are arranged from west to east.

	Spring 2016		Summer 2016		Spring 2017		Summer 2017		Spring 2018		Summer 2018		Spring 2019		Summer 2019	
Site	NumReps	Copies/L	NumReps	Copies/L	NumReps	Copies/L	NumReps	Copies/L	NumReps	Copies/L	NumReps	Copies/L	NumReps	Copies/L	NumReps	Copies/L
								***NeversCOI* marker**								
C206.3	16	287	16	2,685	16	3,395	16	84,049	16	851	16	6,711	7	343	16	4,182
C187.2	0		0		0		1	45	1	237	10	136	12	167	16	7,319
C167.7	7	154	11	192	15	151	16	504	15	383	16	1,955	16	898	16	6,174
C147.6	0		0		0		0		0		0		9	234	8	231
C130.8	0		0		0		0		0		0		3	218	0	
C112.0	0		0		0		0		0		0		4	177	0	
C97.8	0		0		0		1	71	0		0		0		0	
C70.6	0		0		0		2	265	0		0		0		0	
C56.0	0		0		0		6	150	0		0		0		0	
C42.1	0		0		0		3	91	0		0		0		0	
C4.9	0		0		0		0		0		0		0		0	
HR247.4	0		0		0		2	59	0		0		0		0	
								***ReesCOI* marker**								
C206.3	16	148	16	2,675	16	2,225	16	69,394	16	775	16	6,053	11	286	16	2,893
C187.2	0		0		0		0		0		8	110	15	152	16	6,036
C167.7	9	79	6	183	10	113	16	223	15	204	16	1,944	16	777	16	4,475
C147.6	0		0		0		0		0		0		12	125	11	208
C130.8	0		0		0		0		0		0		2	61	0	
C112.0	0		0		0		0		0		0		0		2	77
C97.8	0		1	409	0		1	34	0		0		0		0	
C70.6	0		0		0		0		0		0		0		0	
C56.0	0		0		0		7	82	0		0		0		0	
C42.1	0		0		0		1	101	0		0		0		0	
C4.9	0		0		0		0		0		0		0		0	
HR247.4	0		0		0		3	65	0		0		0		0	

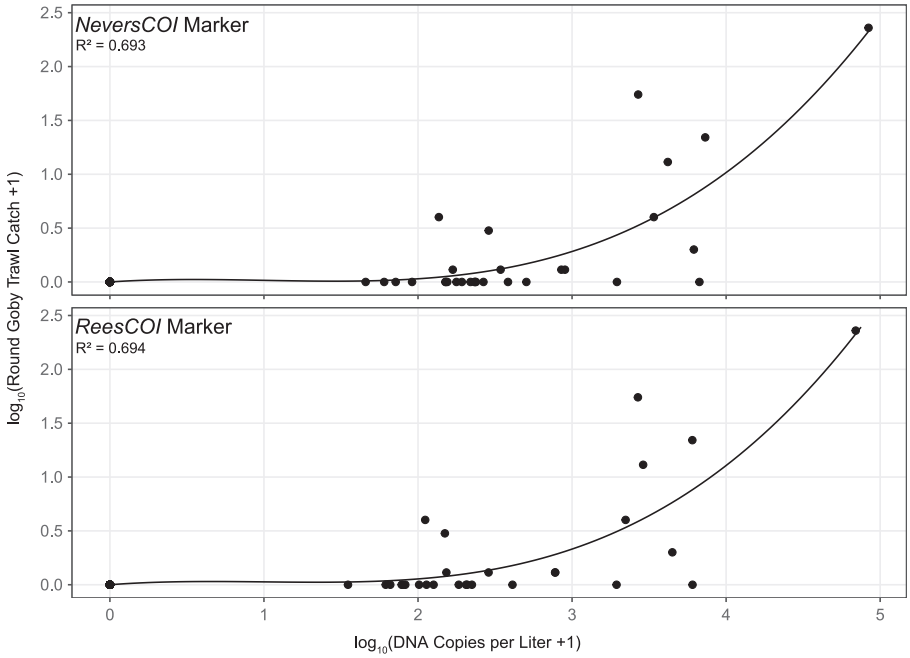

FIGURE 4. Relationship between Round Goby DNA concentration and the trawling catch rate (mean number of fish per trawl) of Round Goby for 12 sites on the New York State Canal System sampled twice annually between 2016 and 2019. The relationship is shown with each variable $\log_{10}(x+1)$ transformed and is fit with a third-order polynomial curve with the equation $y = 0.0400x^3 - 0.1203x^2 + 0.0953x + 0.00005$ for the *NeversCOI* marker and $y = 0.0415x^3 - 0.1242x^2 + 0.1092x + 0.00002$ for the *ReesCOI* marker.

canal are negligible because the Mohawk River runs parallel to (rather than within) the canal (Figure 2). The rate of expansion may be expected to accelerate in sections of the canal to the east where the Mohawk River and canal share the same channel and water velocities are greater. Another explanation for the slow rate of expansion is the possibility that the specimen captured near Utica in 2014 represented a new introduction point rather than a natural expansion from Oneida Lake, and therefore Round Goby were still colonizing the immediate area and had not reached levels that necessitated expansion. This would be similar to the Grand River in Ontario, Canada, where genetic analyses indicated that a separate introduction occurred beyond the invasion front (Bronnenhuber et al. 2011). At the onset of our study, it was widely assumed that the 2014 capture near Utica meant that Round Goby had colonized the entire 40-km reach of canal between Oneida Lake and that location. However, at site C187.2 in Rome, approximately halfway between Utica and Oneida Lake, eDNA was not detected until summer 2017 (absent from the first three surveys) and Round Goby were not captured until summer 2018. The initial absence of catchable Round Goby or detectable concentrations of Round Goby DNA at C187.2 provides some evidence that colonization of this 40-km reach may not have occurred in a linear fashion.

Of the three traditional sampling methods evaluated, benthic trawling was the most effective at capturing Round Goby. At the three sites where Round Goby were found during the study, trawling captured Round Goby in 50% of surveys, compared to 21% for seining and 0% for trapping. This finding may reflect habitat usage by Round Goby as trawling was conducted at depths of 1.5–8.0 m compared to <1.2 m for seining. It is unclear why minnow traps were so unsuccessful since other studies have had success using them to monitor Round Goby populations (Diana et al. 2006; Young et al. 2010; Bhagat et al. 2015). A proof-of-concept test using a high density of minnow traps was conducted at the Sylvan Beach site (C206.3) in June 2016 and found that traps baited with cheese and dog food, and those baited with beef liver, each captured Round Goby at a rate of 0.5 fish/trap. This indicated that our traps and bait were physically capable of capturing Round Goby, but the results of our 4-year study indicate they were simply ineffective at doing so. Other studies found that minnow traps (Diana et al. 2006), angling, trawling, and visual census (Johnson et al. 2005) were most effective at capturing Round Goby or describing their populations in lacustrine environments, while seining was most effective at detecting Round Goby in wadable streams (Nett et al. 2012). Less information is available on detecting Round Goby in canal or large river environments, especially along an invasion front where densities are low and Round Goby are absent from large areas. Our results are probably most comparable to those of Merry et al. (2018), who sampled the Illinois River and found that trawling was an effective method for sampling

small and dispersed Round Goby populations in large riverine habitats with soft substrate. Thus, our results and those of Merry et al. (2018) suggest that trawling may be the most effective traditional gear type for detecting Round Goby in canal habitats. However, we also note that trawling is more labor- and equipment-intensive than most alternative methods and is challenging in our study area where large wood and rocky bottoms frequently cause net damage.

Environmental DNA results were generally intuitive and improved our understanding of Round Goby distributions in the watershed. Round Goby DNA was detected in all instances where Round Goby were captured with the traditional sampling gear. Furthermore, there were three notable instances where eDNA results provided critical information that was not obtained from the traditional sampling gear until a year or more later. The traditional fish sampling gear did not capture a single Round Goby at C167.7 in 2016, 2017, or 2018, yet during that same period, Round Goby DNA was detected at this site during every survey using both markers with DNA concentrations ranging as high as 1,955 copies/L. In fall 2018, we investigated this discrepancy by exploring a number of locations immediately upstream of site C167.7 to determine the source of DNA. A high-density Round Goby population was discovered near Lock E20, approximately 1.5 km west of C167.7 and approximately 0.5 km east from the location of the angler-caught specimen in 2014. This dense but localized population would have been nearly impossible to locate with random sampling using the spatially limited traditional gear at the scale of the entire study area had the eDNA results not informed our sampling. In spring and summer 2019, Round Goby were finally captured at C167.7 via trawling. As another example, eDNA indicated the upstream or nearby presence of Round Goby at the Rome site (C187.2) during two consecutive surveys. In summer 2017 and spring 2018, a single qPCR replicate was positive for Round Goby DNA at C187.2 using the *NeversCOI* marker, and in summer 2018 a greater number of qPCR replicates were positive with both markers and Round Goby were captured at this location for the first time. Finally, both markers detected Round Goby DNA at C147.6 for the first time during the spring and summer surveys in 2019, and following the conclusion of this study, a Round Goby was captured at this location in 2020 (New York State Museum, catalog #84461). Together, these examples underscore the importance of obtaining repeated eDNA detections and suggest that eDNA has the potential to forecast the arrival of Round Goby at locations in this watershed by a year or more, making it a powerful monitoring tool. It is also worth noting that downstream transport of Round Goby DNA did not saturate the study area to the point that a single population caused multiple sites to test positive. For example, C167.7 tested positive for Round Goby DNA with both markers during every survey of the study, yet the next two sites downstream, C147.6 and C130.8, did not detect Round Goby DNA with either marker until spring 2019. This suggests that rates of DNA transport, degradation, and dilution in the canal system are such that Round Goby are generally not detectable at distances of >20 km (and potentially much less than that). This makes eDNA a practical screening tool for informing targeted surveys with traditional sampling gear.

In addition to providing a sensitive means to identify expansion of Round Goby into new locations, our data set suggests that eDNA results can be used to coarsely estimate the relative abundance of Round Goby. Despite the limited number of sites where Round Goby were captured, a strong relationship was evident between the quantity of Round Goby DNA in the water and the relative abundance of Round Goby captured by trawling. Round Goby were only captured with trawling when DNA concentrations exceeded 100 copies/L, and the quantity of DNA explained approximately 69% of the variability in the trawl catch data. These results are similar to those of Nevers et al. (2018), who found a strong relationship ($R^2 = 0.76$) between DNA concentration and the number of Round Goby captured in the Great Lakes. In both studies, it is important to note that the relationship between DNA quantity and Round Goby catch rate is inherently imprecise because the methods are not representative of the same spatial area. In the case of our study, the trawl catch rate is the average of three replicate 150-m pulls at the immediate study site, while the eDNA sample integrates the DNA signature of all individuals from an unknown distance upstream of the sampling location. For example, in the case of a weakly positive eDNA sample with a low DNA concentration, it is not clear if Round Goby are present in low density at that immediate sampling location or if the DNA is the result of a population some distance upstream of higher but unknown density. Despite these uncertainties, our data suggest that orders-of-magnitude differences in DNA concentration generally represent meaningful differences in the relative abundance of Round Goby.

Although the eDNA results show great promise for identifying the presence, and even relative abundance, of Round Goby, a number of inconsistencies were observed between the physical catch data and DNA detections. Round Goby were only captured in 40% and 43% of the surveys in which eDNA detections occurred with the *NeversCOI* and *ReesCOI* markers, respectively. Much of this discrepancy can be easily explained by the fact that eDNA and traditional methods are not representative of the same spatial area and that physical sampling methods may have been ineffective at detecting low-density populations or capturing early life stages of Round Goby. However, the anomalous summer 2017 results are problematic because

Round Goby DNA was detected at five unexpected locations, some of which were >100 km downstream from where the invasion front was believed to be at that time. The field blanks, negative extraction controls, and negative PCR laboratory controls from this survey did not detect Round Goby DNA, however, indicating that field- or laboratory-based contamination was not likely the cause of these detections. More likely these positive results were due to the widespread presence of Round Goby DNA in the water during this survey, yet the mechanisms responsible for this and the reason similar results were not obtained in other surveys are not clear. Anomalous or unexplained eDNA detections are problematic because they can create confusion or uncertainty amongst the public and natural resource managers (Darling and Mahon 2011; Jerde 2019). In our study, however, eDNA was utilized in a repeated sampling framework and was supported by results from companion trawling, seining, and trapping surveys at these sites. The traditional sampling methods did not capture Round Goby at any of the five sites during the summer 2017 survey, and subsequent eDNA surveys in 2018 and 2019 did not reproduce these detections. Together, these results suggested the summer 2017 eDNA results were anomalous and reduced concerns of widespread Round Goby colonization throughout the lower Mohawk River. For this reason, eDNA may be best used in a repeated sampling framework in which repeated positives are required to truly constitute a detection (Erickson et al. 2019; Mize et al. 2019) and complemented with some level of effort using traditional sampling gears (Rees et al. 2014; Hinlo et al. 2017).

Our results have important implications for monitoring Round Goby invasions and for the management of the Mohawk River, Hudson River, and Lake Champlain basins. Despite the surprisingly slow eastward expansion of Round Goby through the New York State Canal System since 2014, evidence is mounting that Round Goby are becoming more widely distributed and abundant in the 40-km reach between Oneida Lake and the Utica area. Capture and eDNA data indicate that the invasion front of Round Goby was most likely between Utica and Frankfort during summer 2019 and reached Frankfort in 2020. Thus, the threat to the Hudson River estuary and Lake Champlain ecosystems remains imminent. The potential for transport of Round Goby by anglers, such as that documented or suspected on the Susquehanna River (New York), Grand River (Ontario, Canada), and elsewhere in spite of existing state baitfish regulations, also increases the invasion potential of this species and further complicates expansion projections in the study area. Finally, eDNA proved to be a useful tool for understanding the distribution and relative abundance of Round Goby across a large area and informing targeted sampling with traditional gear types. The ability of this method to detect DNA from a short distance upstream of the sampling location is a substantial advantage over most traditional gear types, which are spatially limited. However, positive eDNA results can be challenging to interpret because the method does not identify the source of the DNA—it simply indicates that DNA from the target species is present. Therefore, using eDNA within a repeated sampling framework and supplementing eDNA sampling with traditional gear types is critical to obtain a more comprehensive understanding of the invasion status. Our results suggest that pairing eDNA with benthic trawling may be the most effective strategy for monitoring Round Goby invasions in navigable lotic waters.

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