Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Molecular, chemical, and conventional fisheries methods reveal early invasion dynamics and range expansion of an invasive fish in a large North American estuary

Richard Pendleton

richard.pendleton@dec.ny.gov

New York State Department of Environmental Conservation https://orcid.org/0000-0001-7172-7192

Kelsey Alvarez del Castillo

New York State Department of Environmental Conservation

Jessica Best

New York State Department of Environmental Conservation

Karin Limburg

SUNY-ESF: SUNY College of Environmental Science and Forestry

Chloe Moore

Cary Institute of Ecosystem Studies

Steven Pearson

New York State Department of Environmental Conservation

ElizaBeth Streifeneder

New York State Department of Environmental Conservation

Research Article

Keywords:

Posted Date: November 13th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11459855/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Title: Molecular, chemical, and conventional fisheries methods reveal early invasion dynamics and range expansion of an invasive fish in a large North American estuary

Authors: Richard Pendleton[1*], Kelsey Alvarez del Castillo[2], Jessica Best[1], Karin E. Limburg[3], Chloe Moore[4], Steven Pearson[2], and ElizaBeth Streifeneder[5]

Affiliations

[1]Cornell University in cooperation with New York State Department of Environmental Conservation, New Paltz, NY, USA

[2]New York State Department of Environmental Conservation, Albany, NY, USA

[3]College of Environmental Science and Forestry, State University of New York, Syracuse, New York, USA

[4]Cary Institute of Ecosystem Studies, Millbrook, NY, USA

[5]New York State Department of Environmental Conservation, New Paltz, NY, USA

Contact information

Richard Pendleton: richard.pendleton@dec.ny.gov

Kelsey Alvarez del Castillo: kelsey.alvarezdelcastillo@dec.ny.gov

Jessica Best: Jessica.best@dec.ny.gov

Karin E. Limburg: klimburg@esf.edu

Chloe Moore: moorec@caryinstitute.org

Steven Pearson: steven.pearson@dec.ny.gov

ElizaBeth Streifeneder: elizabeth.streifeneder@dec.ny.gov

Abstract

Round Goby (*Neogobius melanostomus*) have quickly expanded throughout the Hudson River Estuary since their initial detection in 2021, yet patterns of establishment, dispersal, and population status remain poorly understood. We studied Round Goby distribution, relative abundance, establishment, and natal origins in the Hudson River Estuary from 2022–2024 using environmental DNA (eDNA), beach seining, and otolith microchemistry. These approaches provided complementary information on spatial expansion and connectivity between the Hudson and Mohawk rivers, where hydrological and anthropogenic connections can influence aquatic organism movement across river networks. Concurrent eDNA sampling and seining showed agreement in detections, supporting eDNA as a complementary monitoring tool in large tidal rivers. Throughout the study period eDNA detections increased spatially and temporally in the lower Hudson River Estuary, with most positive detections in 2024 occurring across approximately 70 km of brackish habitat, indicating downstream range expansion and likely establishment in brackish waters. Despite continued range expansion, cumulative captures during 2022–2024 were lower than in 2021, suggesting the population has not undergone rapid exponential growth. Otolith microchemistry revealed overlapping but distinguishable natal signatures between Hudson and Mohawk River fish, demonstrating demographic connectivity and indicating that the expanding Hudson River Estuary population is supported by both local recruitment and downstream immigrants from the Mohawk River. Together, these results identify the Hudson–Mohawk connection, facilitated in part by the NYS Canal system, as an important invasion pathway and highlight the value of combining detection and provenance approaches to resolve invasion dynamics across connected freshwater and estuarine habitats.

Keywords: Round Goby, environmental DNA, otolith microchemistry, Hudson River Estuary

Statements and Declarations

Ethics approval and consent to participate - This research was conducted in accordance to Cornell University Institutional Animal Care and Use Committee's (IACUC) protocols and guidelines or working with live animals (Protocol #2023-0252).

Consent for publication - The authors confirm that the work described in this manuscript has not been published previously and is not under consideration for publication elsewhere. All authors have approved the manuscript

Availability of data and material - The data underlying this article will be shared on request to the corresponding author.

Competing interests - The authors have no competing interests to disclose.

Funding - The authors have no relevant financial interests to disclose. This study was a partnership project between the NYSDEC and Cornell University, with funding from the Environmental Protection Fund through the Hudson River Estuary Program and the Ocean and Great Lakes Program. Any use of trade, product, website, or firm names in this publication is for descriptive purposes only and does not imply endorsement by the New York state government. Part of this work was funded by a research grant from the Hudson River Foundation for Science and Environmental Research, Inc. The views expressed herein do not necessarily reflect the belief or opinions of the Foundation, which assumes no responsibility or liability for the contents or use of the information herein.

Authors' contributions - All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Richard Pendleton, Kelsey Alvarez del Castillo, Jessica Best, Karin E. Limburg, Chloe Moore, Steven Pearson, and ElizaBeth Streifeneder. The first draft of the manuscript was written by Richard Pendleton and all authors

commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Acknowledgements - We thank all past and present NYSDEC staff for their field work and data collection, Jack Philips (NYSDEC), Gregg Lemke (Cornell University) and Zach Kubsch for collecting, preparing and ageing otoliths, and Andrew Hayden (SUNY Albany) for Round Goby qPCR analysis.

Introduction

Aquatic invasive species (AIS) pose significant threats to aquatic ecosystems through the loss of biodiversity and restructuring of food webs while also imposing significant economic costs (Vitousek et al. 1996; Kernan 2015; Cuthbert et al. 2021; Wainright et al. 2021). AIS can drive ecological shifts if they exhibit certain life history traits, such as generalist feeding, high reproductive output, and a tolerance to broad environmental conditions (Havel et al. 2015). These life history traits in turn aid in rapid population growth and establishment, leading to a higher likelihood of harmful impacts (Pyšek & Richardson 2010).

Preventing the spread of AIS into aquatic ecosystems is challenging due to the inherent connectivity within watersheds. Additionally, many waterways have engineered connectivity and associated recreational and industrial vectors (e.g., boating, angling, ballast water) that can lead to species introductions within and among watersheds. Connectivity among waterbodies can increase opportunities for both natural dispersal and human-mediated secondary spread, making highly connected systems potential conduits for AIS expansion (Kao et al. 2021). Such connections can influence invasion dynamics because the opportunity for movement depends not only on physical connections but also on flow, habitat configuration, species behavior, and temporal variation in environmental conditions (Fullerton et al. 2010; Shao et al. 2019). Prevention of AIS tends to be costly, takes time to implement, and is most successful in smaller, isolated systems (Bailey 2015; Havel et al. 2015; Simberloff 2021; Vander Zanden et al. 2024), yet it is far more effective and cost efficient relative to post-introduction management. If prevention methods are not feasible or fail, then early detection is critical for increasing the success rate of rapid management actions (e.g. eradication, containment). Finding a species

during the early phase when invading populations are smaller can lessen the extent of damage and reduce overall costs (Lazur et al. 2006; Mehta et al. 2007; Vander Zanden et al. 2010).

Conventional fisheries sampling (e.g. electrofishing, netting) has been foundational in monitoring AIS, yet these techniques are time and labor intensive and may take years to detect AIS at initial low abundance (Curto et al. 2025). Environmental DNA (eDNA) is becoming a standard method for AIS monitoring to identify the presence of a species' DNA from the environment (e.g. water, soil, air). This molecular tool reduces the lag time between species introduction and detection and can be more sensitive at detecting rare and/or cryptic species relative to conventional sampling gear (Mehta et al. 2007; Sard et al. 2019; George et al. 2021; Curto et al. 2025).

While conventional sampling and eDNA are useful tools for detecting invasive fish species, these methodologies lack the ability to provide insight into the origin, movement, and migratory routes of an invading species. Otolith microchemistry can help fill the knowledge gap of invasion timelines and pathways as otoliths acquire the unique chemical signatures of the water that individual fish inhabit. Such signatures are routinely used in mixed-stock analysis for fisheries, but are also used to identify provenance of AIS (e.g., Akland et al. 2025). In connected river networks, identifying provenance is particularly useful because detection of an invasive species at a downstream location does not necessarily indicate local reproduction; individuals may instead arrive through immigration or other connected pathways. Distinguishing these processes can therefore improve understanding of how invasions progress through linked freshwater and estuarine habitats.

Despite improved detection and prevention strategies, AIS are still being introduced and are spreading rapidly throughout the United States, including the Round Goby (*Neogobius*

melanostomus). This small, benthic fish, native to the Ponto-Caspian region, was first introduced into the Laurentian Great Lakes in the 1990's in ballast water (Jude et al. 1992). Round Goby have since colonized all five of the Laurentian Great Lakes (Charlebois et al. 1997; Kornis et al. 2012), the Mississippi River (Harris et al. 2024), and the St. Lawrence River (Hickey & Fowlie 2005). In New York, Round Goby dispersed inland from Lake Erie and Lake Ontario using the New York State (NYS) Canal system, which links the historically separated watersheds of the Great Lakes, Mohawk River, Hudson River Estuary. The NYS Canal system has facilitated the transfer of other AIS with Round Goby being the most recent example and their establishment of the Hudson River Estuary in 2021 (Pimentel et al. 2005; George et al. 2021; Pendleton et al. 2022). This invasion history provides a clear example of how engineered connectivity can facilitate interbasin range expansion.

The Hudson River Estuary is home to ecologically and economically significant fish species, such as Atlantic Sturgeon (*Acipenser oxyrinchus*) and American Shad (*Alosa sapidissima*), that are potentially at risk if Round Goby establish and proliferate in the estuary as they have in other waterbodies. A suite of ecological consequences can follow Round Goby invasion, including but not limited to declines in macroinvertebrate assemblages (Lederer et al. 2008; Brooking et al. 2022; Astorg et al. 2022) and fish diversity (Janssen & Jude 2001; Lauer et al. 2004; Balshine et al. 2005), changes to food webs (Kipp & Ricciardi 2012, McAllister et al. 2022), and vectors of diseases (Haws et al. 2025). As a brackish-tolerant species in their natal and invaded European ranges (Kornis et al. 2012), managers are concerned about these potential impacts and the ability of Round Goby to spread and tolerate salinity levels found in the southern extent of the estuary. A recent study conducted by Alvarez del Castillo et al. (2025) found adult Round Goby taken from the Mohawk River system have a higher salinity tolerance than previously reported,

indicating adult Round Goby will likely survive throughout the entire Hudson River Estuary, at least temporarily. Because the lower Hudson River opens directly into New York Harbor, establishment farther downstream could increase the geographic extent of habitats accessible to Round Goby and potentially place adjacent coastal rivers at greater risk. Recent assessments of Round Goby salinity tolerance and invasion risk indicate that portions of New York Harbor, Long Island Sound, and adjacent coastal waters may provide suitable seasonal conditions, particularly during colder periods (Alvarez del Castillo et al. 2025, 2026). These findings identify potential connectivity pathways but do not demonstrate that Round Goby have established in those coastal systems.

The New York State Department of Environmental Conservation (NYSDEC) has continued to monitor the relative abundance of Round Goby annually through several long-term surveys. Here, we report the spread, distribution, and relative abundance of Round Goby since the initial establishment in 2021 (Pendleton et al. 2022). We then relate these findings to eDNA data collected concurrently with the fisheries surveys to assess the validity and feasibility of eDNA as a complementary monitoring tool in a large, tidal river. Lastly, we infer the establishment timeline and the natal origin of Round Goby collected in the Hudson River Estuary through otolith microchemistry using fish collected from the Hudson and Mohawk rivers. By integrating these approaches, we evaluate not only where Round Goby have established but also how connectivity between the Mohawk and Hudson rivers may contribute to invasion dynamics. This provides a basis for considering how the existing invasion could progress through the broader freshwater-to-estuarine network, including the NYS Canal system, New York Harbor, and potentially connected coastal rivers.

Methods

167 *Study System*

168 The tidal, estuarine section of the Hudson River stretches for 246 km from the Federal Dam in
169 Troy, New York, USA to the Battery in New York City (Figure 1). The location of the salt front
170 (100 mg/L Cl^-) fluctuates significantly based on freshwater outflow, but it is generally located at
171 or south of Newburgh, New York (Geyer & Chant 2006), delineating the brackish, lower river
172 (river kilometer [rkm] 0-97; hereafter referred as lower river) from the freshwater, upper river
173 (rkm 98- 246; hereafter referred as upper river). South of the Battery (rkm 0), the tidal Hudson
174 River opens to New York Harbor whereas to the east, the East River connects to Long Island
175 Sound. Upstream of the Federal Dam, the non-tidal Hudson River continues for another 8 km
176 north, where it meets the New York State Canal System. This system consists of a network of
177 canals and locks and dams that link the major waterways with many larger waterbodies including
178 Lakes Erie, Ontario, and Champlain.

179 Elemental chemistry of the tidal Hudson River is longitudinally variable, influenced by river
180 discharge from sub-basins contributing differential weathering of unique Pleistocene land
181 features and saltwater intrusion (Limburg and Siegel 2006). Generally, the tidal Hudson is low in
182 Barium (Ba) and Strontium (Sr), but higher concentrations of Sr, Calcium (Ca) and Magnesium
183 (Mg) can be found in the lower river corresponding to the salt front and brackish water. In
184 contrast, the Mohawk River tends to have slightly higher concentrations of Sr and Ba originating
185 from older Pleistocene rocks as well as higher concentrations of Manganese (Mn) released from
186 wetlands (Limburg and Siegel 2006).

187 *Beach seine surveys*

188 Annually, NYSDEC conducts three long-term, standardized beach seine surveys to monitor the
189 relative abundance of juvenile alosine species and Striped Bass ((*Morone saxatilis*): the Hudson

River alosid survey, Hudson River Striped Bass survey, and the Western Long Island Sound survey (Figure 1A).

The Hudson River alosid beach seine survey (1980–present) utilizes a 30.5-m seine with 0.64-cm bar mesh to sample 28 fixed sites between June and October from Albany (rkm 225) down to south of Newburgh (rkm 87). For the Hudson River Striped Bass beach seine survey (1985–present), an off-center 61-m seine (0.64-cm bar mesh) is deployed at 13 fixed sites from July to November from rkm 35 to rkm 63, spanning the Haverstraw Bay and Tappan Zee regions. Both Hudson surveys occur during daytime on a biweekly basis. The Western Long Island Sound survey uses a similar 61-m seine to the Hudson River Striped Bass beach seine, and occurs at 30 fixed stations that extend east from Jamaica Bay to Oyster Bay in Long Island Sound and primarily targets year-1 Striped Bass. In all surveys, personnel identify and enumerate all fishes to species and measure a subset for total length (TL). Although these surveys target specific migratory fishes, numerous other species are incidentally captured including Round Goby (Pendleton et al. 2022). Across the period of this study, all Round Goby captured were measured for TL (mm) and either stored on ice or in 95% ethanol until removal of the otoliths.

eDNA monitoring

To validate the ability to detect Round Goby DNA in a large, tidal river and test for the presence and distribution of Round Goby eDNA, water samples were collected from the mainstem of the Hudson River Estuary between the Federal Dam (rkm 246) and the George Washington Bridge (rkm 19), select tributaries, and locations along western Long Island Sound (Figure 1B). Sampling locations varied among years (2022-2024) based on the hypothesized downstream spread of Round Goby and the distribution of positive samples from the prior year of sampling. In general, eDNA locations were sampled two to three times per year. Water samples were

collected during an outgoing tide (i.e. ebb) to minimize the potential for Round Goby DNA to be detected from a downstream location which could more likely occur during an incoming tide (i.e. flood). This enabled standardization and the assumption that any positive detection of DNA occurred at that location or originated from upstream. Water samples from tributaries were collected below the head of tide and approximately within 1 km of the confluence with the Hudson River Estuary.

In 2022, 27 mainstem Hudson (rkm 245-35), 6 tributaries (rkm 180-135), and 3 Long Island Sound locations were sampled in late June/early July and early August (Table 1). Twenty-one of these sampling locations corresponded with beach seine sites in which eDNA sampling occurred immediately before seining to validate eDNA results to the number of Round Goby physically captured at the same location (Figure 1C). For subsequent years (2023-2024), documenting downstream expansion of Round Goby was prioritized over eDNA validation with beach seine captures, thus 7 of the 15 mainstem locations corresponded to beach seine sites in the lower river estuary only (i.e. Newburgh to George Washington Bridge; Figure 1C). In situ water quality data (i.e. temperature, salinity, conductivity, and pH) were also collected during the majority of eDNA sampling events using a YSI ProQuatro.

Sampling of eDNA involved collecting up to 2 L of river water at each site. In 2022-2023, samples were filtered through 47 mm glass-fiber filters (1.5 µm pore size) using a hand-held vacuum pump; in 2024, 5 µm Smith-Root self-preserving polyethersulfone (PES) filters were used with a Smith-Root backpack eDNA sampler. If filters clogged with < 2L, then the volume of filtered water was recorded. Glass-fiber filters were placed in vials, kept on ice, and then frozen until further lab preparation for eDNA extraction, while self-preserving Smith-Root filters were stored in boxes at room temperature until received by the lab and then frozen until

analyzed. A field control sample (500 mL) was collected at the beginning of each sampling day (consisting of ~5 to 6 environmental samples) using store-bought distilled water and the aforementioned filtering methods (USFWS 2023).

Laboratory analysis followed the methodology detailed in George et al. (2021) with the ReesCOI marker with the following modifications. In 2024, the Taqman Environmental Master Mix 2.0 was replaced with NEB LUNA Probe qPCR Master Mix, after comparison testing between the two probes indicated improved performance. No template controls (NTC) were run for every qPCR experiment to identify nonspecific amplification. An internal positive control (Promega GoTaq® Enviro IPC qPCR Control (Catalog Number: AM2030) was used on a subset of samples to determine if PCR inhibition was observed. All test samples were normalized to 3 ng of eDNA as input (lowest common amount present in suspected positive samples) and ran in 8 technical replicates per sample. All laboratory analyses occurred at the Center for Functional Genomics at the State University of New York, University at Albany. Data analysis included geographical mapping of positive detection sites and calculations of copies/liter. Copies/L were calculated as copies per reaction x (elution volume/reaction volume) / sample volume.

Microchemistry

The tidal Hudson and Mohawk rivers vary in water chemistry resulting in differential elemental uptake in fish otoliths (see study area; Limburg and Siegel 2006; Turner 2014). Chemical signatures incorporated in the otoliths of Round Goby collected at the leading edge of the invasion were used to infer the chronology of establishment in the Hudson River Estuary; recent, rapid colonization of Round Goby from the Mohawk River versus establishment of an undetected, reproductively mature population in the Hudson River Estuary prior to 2021 (Pendleton et al. 2022). To establish a baseline chemical profile for the Mohawk River, nine

Round Goby were collected during August 2022 from the Mohawk River at Peebles Island, ~ 1 km upstream of the Mohawk/Hudson confluence (N 42.7853605, W -73.6903295). For the Hudson River Estuary, a subset of Round Goby collected during beach seining in 2021 and 2022 were used for microchemistry analysis based on capture location and TL (Supplemental Material 1). Due to low Round Goby catches during the Hudson River alosid survey in 2022, additional Round Goby were collected during August 2022 through additional sampling at beach seine sites in Albany to increase sample size. Six Round Goby incidentally captured during 2022 at the Roseton Generating Station in Newburgh were also included for comparison as these individuals represented the southernmost physical capture of Round Goby.

Pairs of sagittal otoliths were extracted, cleaned, and stored dry until further preparation. Otoliths were independently examined by two experienced readers using a dissecting microscope and age estimates were assigned based on annuli count considered as the two-part zone with an opaque and translucent band corresponding to 1 year of growth (Florin et al. 2018). Age estimates indicated Round Goby collected in the Hudson River Estuary in 2021 were age-0 with the exception of one age-1. Likewise, Hudson River Round Goby collected in 2022 were estimated age-1 with the exception of one age-2. Round Goby collected from the Mohawk River in 2022 were age-0 with one age-1 individual.

Otoliths were analyzed by laser ablation inductively coupled mass spectrometry (LA-ICP-MS) at the Analytical and Technical Services Group at the State University of New York College of Environmental Science and Forestry (SUNY ESF). Prior to chemical analyses, one otolith was embedded in EpoFix (Struers) epoxy, then polished with fine grit sandpaper in the sagittal plane to expose the core. A Teledyne-Cetac (formerly Photon) Analyte Excite 193-nm excimer laser ablation unit was used with a Thermo Scientific iCAP TQ ICP-MS. Transects were

set up to start at one edge of the otolith, run through the core, and out to the edge on the opposite side or on an axis where the edge appeared to be intact. Pre-ablations were carried out at 50 µm/second using a 135-µm diameter spot to clean the sample surface. Transects were ablated with 85- or 110-µm diameter spots at 5 µm/second transit speed; laser energy on the surface (i.e., fluence) was 1.6 to 1.71 J/cm2. Calibrations and corrections for instrument drift were made with Micro-Analytical Phosphate Standards (MAPS) produced at the Mineral Resources Program, US Geological Survey, Denver, CO (Wilson 2017). Elements analyzed included Boron (B), Manganese (Mn), Copper (Cu), Strontium (Sr), Iodine (I), Barium (Ba), and Lead (Pb) and were expressed in parts per million (ppm) or as element:calcium ratios x 1000.

Following laser ablation, otoliths were photographed and laser transects and associated microchemistry data were inspected for abnormalities. Otoliths where the transect included epoxy or missed the core were excluded from analysis. Microchemistry transect data were then summarized to characterize the edge and core region for each individual as these regions were assumed to reflect the elemental chemistry of each individual's capture location and natal habitat (i.e. Hudson vs Mohawk), respectively. The otolith's 'edge' was defined as the outermost 150 µm for a given element:calcium ratio and the otolith's 'core' as the innermost 100 µm for a given element:calicum ratio. Microchemistry data were then grouped based on capture location, year, and estimated age (i.e. Hudson 2021, Hudson 2022, Mohawk 2022), and 'edge' and 'core' regions were compared using the mean of measurements for each elemental ratio. Estimated age structure indicated individuals within groups were primarily the same age; Hudson 2021 = age-0, Hudson 2022 = age-1, and Mohawk 2022, age-0, thus we did not include age or TL as separate variables in analyses.

To first assess whether Round Goby could be differentiated based on otolith chemical signatures acquired at the capture location, each element:calicum ratio in the 'edge' region was compared among Hudson and Mohawk individuals, regardless of capture year, using a Wilcoxon rank-sum test with continuity correction. We then performed a Kruskal-Wallis Rank Sum test to test for significant differences in the 'edge' region among capture location and year (Hudson 2021, Hudson 2022, Mohawk 2022) and used a pairwise Wilcoxon Rank Sum test to identify pairwise differences among the three groups. Elements found to be significantly different between capture location (Hudson, Mohawk) were included in multivariate comparisons.

Analysis of similarities (ANOSIM) using Euclidean distance and 9999 permutations was used to test for significant differences ($\alpha = 0.05$) in element:calicum ratios among groups (Hudson 2021 v. Hudson 2022 v. Mohawk 2022) for both the 'core' and 'edge' region. A pairwise ANOSIM was then used for the 'core' and 'edge' region using Euclidean distance and 9999 permutations to identify pairwise multivariate differences among capture location and year. Non-metric multidimensional scaling (NMDS) using Euclidean distance matrices calculated on two dimensions was then used to visualize relationships among groups to infer natal river origin (i.e. 'core' analysis) and establishment time (i.e., 'edge' analysis) for Round Goby collected in the Hudson River Estuary. Elemental ratios were standardized to a zero mean and unit variance prior to inclusion in the ANOSIM and NMDS and all statistical analyses were performed using R version 4.4.3 (R Core Team 2025), with vegan version 2.6.8 (Oksanen et al. 2024).

Results

Beach seine surveys

Between 2021 and 2024, 183 Round Goby were collected in the Hudson River Estuary during long-term beach seine monitoring. The highest number of individual Round Goby were

caught in 2021 (n = 112 & CPUE = 0.29) with fewer individuals collected in other study years (2022 = 12 & CPUE = 0.03; 2023 = 42 & CPUE = 0.11; 2024 = 17 & CPUE = 0.04 Table 2). Among years, the majority of Round Goby were collected in Albany (83% of catch) with lower catches occurring in the Poughkeepsie (4%) and Coxsackie (3%) regions (Supplement Material 1). No Round Goby were captured in the Newburgh, Haverstraw, Tappan Zee, or Long Island Sound regions. The size distribution of Round Goby was similar over time and among regions with mean TL of 51 mm among all years (minimum TL = 26 mm; maximum TL = 88 mm; Table 2).

eDNA monitoring

No PCR inhibition was observed, and field and laboratory controls were negative of Round Goby DNA throughout the duration of the study.

During 2022, Round Goby eDNA was positively detected at approximately half (19 of 27) of mainstem river locations with a demarcation between positive and negative detections occurring at Newburgh (Figure 2, Appendix 1). Positive eDNA detections occurred at most mainstem sites (19 of 20) during at least 1 sampling event from Troy to Newburgh, whereas Round Goby eDNA was not detected at any site (7 of 7) south of Newburgh for either sampling event in 2022 (Appendix 1). Mean concentrations of Round Goby eDNA ranged from 6.7 to 630.7 copies/L among sites and sampling events and eDNA concentrations generally decreased from Troy to Newburgh (Appendix 1). Among the six tributaries in the upper river estuary, Round Goby eDNA was detected (0.3 to 92.6 copies/L) in Catskill and Esopus creeks.

Of the 21 beach seine sites sampled, three Round Goby were captured at two sites where eDNA sampling occurred immediately before seining in 2022. Two Round Goby were collected in Albany and one in Poughkeepsie with corresponding eDNA concentrations of 22 and 9

copies/L, respectively. Positive eDNA detections (7 to 118 copies/L) also occurred at 10 beach seine sites between Troy and Newburgh; regions where Round Goby have previously been collected (Pendleton et al. 2022). No Round Goby eDNA was detected at the other 9 beach seine locations south of Newburgh and along western Long Island Sound (Figure 2, Appendix 1).

Based on the 2022 distribution of positive eDNA from Troy to Newburgh, the remainder of eDNA sampling (2023-2024) occurred from Newburgh to the George Washington Bridge with the exception of tributaries located in the upper river. In 2023, Round Goby eDNA was detected (42.0 to 210.7 copies/L) in Newburgh; no additional detections occurred farther south (Figure 2, Appendix 1).

Conversely, in 2024 Round Goby eDNA was detected at the majority (11 of 15) of southern locations with positive detections (65.1 to 572.9 copies/L) occurring from Newburgh to the George Washington Bridge (Figure 2, Appendix 1). No Round Goby were physically collected during 2023 or 2024 among the 9 beach seine locations south of Newburgh and along western Long Island Sound.

Among tributaries, Round Goby eDNA was present in Hannacroix and Rondout creeks (33.9 to 58.6 copies/L) during at least one sampling event in 2023, but eDNA was not detected in the remaining creeks (Catskill, Esopus, Sawkill, Enderkill, Black). No tributaries were sampled in 2024.

Microchemistry

Among the seven elements analyzed, Sr:Ca, Mn:Ca, and B:Ca concentrations in the 'edge' of the otoliths were significantly different between Round Goby captured in the Hudson and Mohawk rivers, with (i.e. Kruskal-Wallis Rank Sum) and without (i.e. Wilcoxon) accounting for capture year (Figure 3, Table 3). Significant post-hoc differences were observed for all three

373 elemental ratios between Hudson 2021 and Mohawk 2022 whereas Sr:Ca and Mn:Ca were
374 significantly different between Hudson 2022 and Mohawk 2022. Only B:Ca was significantly
375 different between Hudson 2021 and Hudson 2022 (Table 3). No significant differences were
376 observed between Hudson and Mohawk individuals for element:Ca ratios for Cu, I, Ba, and Pb,
377 both across and among capture years.
378 Using Sr, Mn, and B, ANOSIM identified significant differences ($p < 0.01$) among Round
379 Goby captured in the Hudson and Mohawk rivers for both the ‘core’ and ‘edge’ of the otoliths
380 (Figure 4; Table 4). Although R values were relatively low, the otolith 'edge' ($R = 0.32$)
381 distinguished Hudson from Mohawk groups more strongly than did the 'core' ($R =0.16$). For the
382 ‘core’, significant post-hoc differences were observed between both Hudson years (2021, 2022)
383 and Mohawk 2022, but no difference was observed between Hudson 2021 and Hudson 2022
384 Table 3). In general, Hudson 2021 and Hudson 2022 individuals tended to be similar within and
385 between Hudson groups, where Mohawk 2022 individuals had greater within group variability
386 and overlap with Hudson individuals (Figure 4). For the ‘edge’, all pairwise comparisons were
387 significantly different ($p > 0.02$) among Hudson 2021, Hudson 2022, and Mohawk 2022 with
388 greater support for group differences between Hudson 2021 and Mohawk 2022 ($R = 0.63$) and
389 Hudson 2022 and Mohawk 2022 ($R= 0.57$; Table 4). Similar to patterns observed in the ‘core’,
390 Hudson 2021 and 2022 individuals tended to be more similar within and between Hudson groups
391 for the otolith ‘edge’ whereas Mohawk 2022 individuals were less related to other Mohawk
392 individuals, as well as to less overlap with Round Goby captured in the Hudson in 2021 and
393 2022.
394 **Discussion**

The consistent collection of Round Goby combined with the presence of Round Goby DNA throughout most of the Hudson River Estuary reaffirms that this species has used connected and engineered pathways to successfully disperse, establish, and spread since 2021 (Pendleton et al. 2022). Results indicate the population of Round Goby has expanded downstream, and likely established in, the brackish, lower Hudson River Estuary as indicated by the increasing presence and downstream spread of Round Goby eDNA over time. Despite range expansion into brackish water, the population does not appear to have grown over time, with fewer Round Goby (n = 71 & mean CPUE = 0.06) cumulatively captured during the three-year study (2022-2024) compared to 112 Round Goby (CPUE = 0.29) captured in 2021. Microchemistry of Round Goby otoliths collected from the leading edge of the invasion suggests a reproductive population comprised of natal Hudson River individuals and recent Mohawk River immigrants. Thus, the Hudson River Estuary invasion appears to be shaped by connectivity at both local and inter-river scales: local reproduction is occurring within the estuary, while immigration from the Mohawk River continues to contribute individuals to the population. This pattern is consistent with the broader role of river-network connectivity in facilitating dispersal among habitats, although connectivity alone does not necessarily predict establishment or population growth (Fullerton et al. 2010; Shao et al. 2019). Furthermore, these results collectively demonstrate eDNA and otolith microchemistry are complementary fisheries monitoring tools in a large, tidal river and can provide valuable information on the early stages of a species invasion. More broadly, the results demonstrate the value of pairing detection with provenance approaches when evaluating invasions across connected riverine and estuarine habitats.

Concurrent eDNA monitoring and beach seining in 2022 produced intuitive results suggesting that eDNA can be an informative surrogate for identifying areas that have been

418 colonized by Round Goby. Positive eDNA detections with the physical capture of Round Goby
419 at two sites coupled with no eDNA detections and no Round Goby captured at nine sites in the
420 lower, brackish river demonstrate the feasibility and validity of eDNA as a complementary
421 fisheries monitoring tool in a large estuary. However, there were several sites where Round
422 Goby DNA was present, yet no Round Goby were collected during seining, indicating potential
423 false negative (i.e. goby present and not physically captured). Several studies have shown a
424 greater likelihood of Round Goby detections occurring with eDNA relative to fisheries gears
425 (Nevers et al. 2018, Przybyla-Kelly et al. 2023) and Millard-Martin et al. (2024) specifically
426 demonstrated eDNA sampling detects more species compared to beach seining, particularly
427 species inhabiting adjacent rocky habitat. Thus, we postulate Round Goby evaded capture or
428 were proximately present at the time of sampling based on the trends observed here,
429 inefficiencies of seines capturing benthic goby (George et al. 2021, Millard-Martin et al. 2024),
430 and the patchy, distribution of goby observed in Pendleton et al. (2022). This demonstrates the
431 importance of using both complementary monitoring methods to document the expansion of a
432 newly invading species that may still have a relatively small population.
433 The increasing presence and downstream detection of Round Goby DNA in brackish water
434 over time suggests the population spread to the lower estuary more gradually than to the rapid
435 establishment of ~ 120 km of river between the Federal Dam and Poughkeepsie in 2021
436 (Pendleton et al. 2022). All positive detections during 2022 (n = 31) and 2023 (n = 6) occurred in
437 freshwater conditions (<0.3 ppt) whereas all but two positive detections (n = 17) occurred in
438 brackish conditions (> 0.3 ppt) in 2024. These numerous within- and among-site positive
439 detections spanning ~ 70 km of river in June, August, and December 2024 provide evidence that
440 Round Goby have likely established in the lower, brackish Hudson River Estuary. However,

positive detections should also be considered cautiously: little is known about the release, transport, and degradation of Round Goby DNA. It is possible the downstream-most eDNA detections in our study were the result of DNA being transported from an upstream source and may not represent live Round Goby present at those locations.

Environmental factors such as tides, currents, and salinity can influence eDNA monitoring, although studies have reached differing conclusions regarding the magnitude and direction of these effects (Oka et al. 2024, Scriver et al. 2024, Van Driessche et al. 2024). An experimental study found that Round Goby DNA decayed rapidly, with a half-life of 12–15 hours, although DNA remained detectable for 7–15 days in aquaria following goby removal (Nevers et al. 2018). In lotic systems, eDNA can also be transported downstream, with a recent meta-analysis suggesting that DNA particles typically travel approximately 2 km under normal flow conditions (Jo & Yamanaka 2022). However, transport distances depend on hydrological conditions, particle characteristics, and DNA degradation. In the Hudson River Estuary, average summer discharge is approximately 180 $m^3 s^{-1}$ (Howarth et al. 2000), under which downstream transport from upstream sources is possible. Ralston and Geyer (2017) modeled water travel times from the head of tide near Troy to Poughkeepsie ranging from nearly two months at a discharge of 150 $m^3 s^{-1}$ to just over two days at 5,000 $m^3 s^{-1}$, demonstrating the strong influence of discharge on downstream transport. Although these water travel times do not directly estimate eDNA transport, they provide hydrological context for evaluating whether DNA could travel the approximately 100 km from known source locations near Poughkeepsie to the George Washington Bridge. Pont (2024) predicted approximate eDNA uptake distances of 26–38 km at a discharge of 100 $m^3 s^{-1}$; however, these model predictions were developed primarily for non-tidal lotic systems, including relatively small streams, and their applicability to a large, tidally

influenced estuary such as the Hudson is uncertain. Taken together, these findings suggest that, although upstream transport cannot be ruled out, detection at the George Washington Bridge is unlikely to be explained solely by eDNA transported from known sources near Poughkeepsie. This interpretation should nevertheless be considered provisional because neither model provides a direct, Hudson-specific estimate of eDNA transport under the conditions of sampling.

Notably, the southernmost eDNA detections occurred in December 2024, when mean water temperature was lowest (6.9°C). DNA degradation has been shown to slow as temperature declines (Joseph et al. 2022, Lamb et al. 2022), potentially increasing eDNA persistence and the likelihood of detection following downstream transport. Conversely, DNA degradation occurs more rapidly in marine environments than in freshwater (Lamb et al. 2022), suggesting that changes in salinity along the estuarine gradient may further influence DNA persistence. Although numerous eDNA studies have been conducted in brackish systems, the complex interactions among environmental factors, including current speed and direction, turbidity, and salinity, and biological factors, such as DNA transport and degradation rates and the relative abundance of source organisms, remain insufficiently understood to fully characterize their effects on detection (Sahu et al. 2025). These considerations highlight an important connectivity-related limitation of eDNA monitoring in tidal systems: detection can provide evidence of presence within a connected river reach, but may not, by itself, establish whether organisms are locally present or whether their DNA has been transported from elsewhere in the network. Incorporating river-network structure, discharge, and temporal variability into eDNA interpretation may therefore improve assessments of ecological connectivity while accounting for uncertainty in the spatial origin of detections (Fullerton et al. 2010).

The likely establishment of Round Goby in brackish water is further supported by similarity in positive eDNA concentrations between the lower river in 2024 (0.3-630 copies/L; $\mu = 96$ copies/L) and in the upper river in 2022 (51-573 copies/L; $\mu = 158$ copies/L) where goby have established and were collected annually throughout the duration of the study (Pendleton et al. 2022). Furthermore, positive eDNA concentrations in the lower, brackish river were within the eDNA concentration ranges of other established populations in the Erie Canal (0-6,771 copies/L; George et al. 2021) and Lakes Huron (0-1,332 copies/L) and Michigan (0-5,070 copies/L; Nevers et al. 2018). Lastly, recent experimental salinity trials using Round Goby sourced from the Mohawk River indicated goby did not exhibit signs of stress (i.e. change in appetite, coloration, reaction) until 18 ppt with mortality primarily beginning at 24 ppt (Alvarez del Castillo et al. 2025). Mortality was much lower in the cold-water 5°C treatment relative to 20°C and 26°C, with high survival at 33 ppt and the cessation of the 12-week salinity progression experiment. Mean salinity was 2.5 ppt and ranged from 0.17 to 7.55 ppt among the months and sites where positive Round Goby eDNA detections occurred in 2024. Based on experimental results, Round Goby in the Hudson River Estuary could survive at salinity concentrations observed at the time of eDNA collection (Alvarez del Castillo et al. 2025). As mentioned above, the two southernmost eDNA detections occurred in December 2024 when water temperature was 6.4-6.5°C and salinity was 6.3-6.4 ppt, supporting the suggestion that Round Goby range expansion may occur during winter months with coastal migration to adjacent river systems possible. If such seasonal movements occur, the lower Hudson River Estuary and New York Harbor could represent an important transition zone between the established Hudson population and other coastal or estuarine habitats. Recent assessments indicate that cooler seasonal conditions may increase the potential for Round Goby survival and expansion into portions of

connected coastal waters (Alvarez del Castillo et al. 2026). However, these results represent invasion potential rather than evidence of established coastal populations, and continued monitoring is needed to determine whether Round Goby actually move between the Hudson River Estuary, New York Harbor, and neighboring coastal rivers.

Methodological changes (i.e. sampling device, filter material/pore size, filter storage/preservation) did occur throughout the study which could have influenced eDNA results and conclusions drawn within. For instance, changing filter material/pore size between 2023 and 2024 could have led to higher proportions of positive eDNA detections observed in the lower, brackish portion of the river. However, switching from 1.2 µm to the larger 5 µm pore size in 2024 likely reduced the recovery Round Goby DNA leading to lower concentrations of detectable DNA as pore size is inversely related to DNA quantities (Lui et al. 2024, Stica et al. 2025). Furthermore, filter material does not appear to heavily influence DNA concentrations in fish (Stica et al 2025).

Unlike other populations that experienced short-term, exponential growth (Jackson et al. 2019, Brownscombe & Fox 2012), the Hudson River Estuary Round Goby population does not appear to have increased in the three years proceeding their establishment. In the Mississippi River basin, Round Goby experienced another canal-facilitated interbasin range expansion through the Chicago Area Waterway System from Lake Michigan to the Illinois River, yet the relative abundance of Round Goby has remained low over time (Irons et al. 2006; Merry et al. 2018). The Illinois and tidal Hudson rivers share similar chemical and physical characteristics with the upper section of the rivers characterized by harder substrates, lower turbidity, and narrow channel whereas the lower sections of the rivers are characterized by soft substrates, higher turbidity, and a wider channel and backwater habitat (Delong 2005, Jackson et al. 2005).

Despite the presence of hard substrate (the preferred habitat of Round Goby; Kornis et al. 2012) in the upper sections of the rivers, the majority of the substrate in both rivers are mud and silt (Delong 2005, Nitsche et al. 2007). Although these broad riverine similarities represent a small portion of factors influencing invasion success, they may help to limit the population size. Predation by Hudson River estuarine piscivores may also suppress population numbers. Striped Bass, Blue Crab (*Callinectes sapidus*), and American Eel (*Anguilla rostrata*) would likely consume Round Goby and would be novel predators. The relatively low abundance observed despite substantial latitudinal connectivity also suggests that connectivity alone does not guarantee rapid population growth. Rather, dispersal opportunities interact with local habitat conditions, environmental gradients, predation, recruitment, and other factors to determine whether movement results in successful establishment (Shao et al. 2019)

Elemental ratios in the 'edge' region of Round Goby otoliths collected from the Hudson River Estuary and Mohawk River were significantly different, demonstrating that otolith microchemistry can differentiate between the rivers in which Round Goby were collected. Differences between rivers were observed for boron, manganese, and strontium, although the greater variation in manganese concentrations in the Mohawk River (Figure 4) may reflect multiple sources within the river. For example, Mohawk River wetlands can be an important source of reduced Mn, depending on hydrologic conditions such as flushing rates associated with precipitation (Limburg & Siegel 2006). Significant differences between rivers were also observed in the 'inner' or natal region of the otoliths. Inner otolith signatures of Round Goby captured in the Hudson River Estuary in 2021 and 2022 were statistically similar to one another but differed from those of Round Goby captured in the Mohawk River in 2022. These patterns

554 indicate that otolith microchemistry can distinguish between natal environments and provide
555 evidence of different natal locations among Round Goby captured in the two rivers.
556 The similarity in inner otolith signatures between Hudson River Estuary Round Goby
557 collected in 2021 and 2022 suggests that natural reproduction has occurred within the Hudson
558 River Estuary, while the differences between Hudson and Mohawk River signatures indicate
559 distinct natal origins. However, several Mohawk River goby grouped more closely with Hudson
560 River goby in both years, suggesting that downstream immigration from the Mohawk River has
561 also likely contributed to the Hudson River Estuary population. Given the young age structure
562 observed in this study (i.e., age-1 and age-2 fish), these microchemistry data cannot determine
563 whether Round Goby were introduced prior to 2021. A plausible scenario is that smaller Round
564 Goby initially dispersed within the Hudson River Estuary, as observed in other systems
565 (Nogueira Tavares et al. 2020), followed by reproduction as sexually mature individuals
566 established in the river. Multiple spawning events throughout the season (Charlebois et al. 2001)
567 could have subsequently contributed to downstream dispersal of larvae and juveniles (Nogueira
568 Tavares et al. 2020). However, inferences regarding natal origin and population establishment
569 may have been more definitive if water chemistry between the rivers were more dissimilar or if
570 Round Goby had traveled greater distances within a river. For comparison, blueback herring
571 (*Alosa aestivalis*) otoliths exhibit more pronounced chemical patterns associated with their long
572 anadromous migrations (>300 km) across habitats with more variable water chemistry, including
573 the Atlantic Ocean, brackish/freshwater Hudson River Estuary, and freshwater Mohawk River
574 (Limburg 2001, Limburg & Siegel 2006, Turner 2014). In the present study, Round Goby were
575 captured within the upper freshwater portion of the Hudson River Estuary (~140 km) and the
576 adjacent freshwater Mohawk River at Peebles Island, approximately 4 km upstream. Thus, the

relatively short distance between sampling locations and similarity in freshwater environments may have limited the degree of chemical differentiation among fish. Additional longitudinal water-chemistry studies of the Hudson and Mohawk Rivers would improve understanding of seasonal and fine-scale spatial variation in elemental concentrations beyond the patterns previously documented (Limburg & Siegel 2006). Such work could also improve resolution of movement between the Mohawk and Hudson rivers and help determine whether additional sampling along the connected river and canal network can identify finer-scale invasion pathways. Because connectivity can vary through time with discharge and other environmental conditions, repeated sampling across seasons and along longitudinal gradients may be particularly informative for distinguishing persistent population connectivity from episodic transport (Fullerton et al. 2010).

This study demonstrates that Round Goby have successfully colonized and expanded throughout much of the tidal Hudson River, including the lower brackish reaches, while exhibiting a slower population growth trajectory than observed in many other invaded systems. The increasing downstream occurrence of eDNA detections, combined with experimentally supported salinity tolerances and eDNA concentrations comparable to established populations elsewhere, strongly suggests that Round Goby are now established in portions of the lower Hudson River Estuary. At the same time, otolith microchemistry indicates the invasion is being sustained through both local reproduction and continued immigration from the Mohawk River. Although uncertainties remain regarding eDNA transport and persistence in tidal, brackish environments, the integration of eDNA monitoring with conventional fisheries sampling and otolith analyses provided a robust framework for evaluating invasion dynamics across a large and complex river system. Importantly, the study also illustrates how an AIS invasion can unfold

600 across a connected freshwater-to-estuarine network: the NYS Canal system facilitated the initial
601 interbasin connection to the Mohawk and Hudson rivers, while downstream establishment in the
602 tidal Hudson creates potential connectivity with New York Harbor and adjacent coastal rivers
603 (George et al. 2021; Pendleton et al. 2022; Alvarez del Castillo et al. 2026). Monitoring across
604 these connected habitats, particularly at potential transition points among the canal system,
605 Mohawk River, Hudson River Estuary, New York Harbor, and coastal rivers, could help
606 distinguish continued population expansion from transient detections and identify emerging
607 pathways before populations become widely established. Network-based approaches to AIS
608 prevention similarly emphasize the value of identifying highly connected waterbodies and
609 pathways for targeted surveillance and management (Kao et al. 2021).

References

Akland, M. K., Limburg, K. E., Healy, B. D., & Pine, W. E. (2025). Mixed natal origins present management challenges for a non-native fish established throughout a modified river network. *Canadian Journal of Fisheries and Aquatic Sciences*, *82*, 1-13. https://doi.org/10.1139/cjfas-2024-0267

Alvarez del Castillo, K., Sethi, S. A., Won, E., Maniscalco, J., Pendleton, R., Ryan, E., & Rudstam, L. G. (2025). Salinity tolerance of Round Goby: Informing invasion potential in North American coastal watersheds. *PLoS ONE*, *20*, Article e0316327. https://doi. org/ 10. 1371/ journal. pone. 0316327

Alvarez del Castillo, K., Sethi, S. A., Won, E., Maniscalco, J., & Rudstam, L. G. (2026). Assessing Round Goby invasion risk in the Hudson River estuary and adjacent coastal waters. *Transactions of the American Fisheries Society*, *155*(3), 323-337.https://doi.org/10.1093/tafafs/vnag004

Astorg, L., Charette, C., Windle, M. J. S., & Derry, A. M. (2022). Two decades since first invasion: Revisiting Round Goby impacts on nearshore aquatic communities in the upper St. Lawrence River. *Journal of Great Lakes Research*, *48*, 581–592. https://doi. org/ 10. 1016/ j. jglr. 2022. 01. 017

Bailey, S. A. (2015). An overview of thirty years of research on ballast water as a vector for aquatic invasive species to freshwater and marine environments. *Aquatic Ecosystem Health & Management*, *18*(3), 261-268. https://doi.org/10.1080/14634988.2015.1027129

Balshine, S., Verma, A., Chant, V., & Theysmeyer, T. (2005). Competitive interactions between round gobies and logperch. *Journal of Great Lakes Research*, *31*(1), 68-77. https://doi.org/10.1016/S0380-1330(05)70238-0

633 Brooking, T. E., Rudstam, L. G., Jackson, J. R., VanDeValk, A. J., Holeck, K. T., Hotaling, C.
634 W., & Cooper, J. E. (2022). Effects of Round Goby on the benthic invertebrate com-
635 munity and on diets and growth of Yellow Perch and White Perch in Oneida Lake, New
636 York. *Transactions of the American Fisheries Society*, *151*, 641–654. https://doi. org/ 10.
637 1002/ tafs. 10377
638 Brownscombe, J. W., & Fox, M. G. (2012). Range expansion dynamics of the invasive round
639 goby (*Neogobius melanostomus*) in a river system. *Aquatic Ecology*, *46*(2), 175-189.
640 https://doi.org/10.1007/s10452-012-9390-3
641 Charlebois, P. M., Corkum, L. D., Jude, D. J., & Knight, C. (2001). The round goby (*Neogobius*
642 *melanostomus)* invasion: current research and future needs. *Journal of Great Lakes*
643 *Research*, *27*(3), 263-266. https://doi.org/10.1016/S0380-1330(01)70641-7
644 Charlebois, P. M., Marsden, J. E., Goettel, R. G., Wolfe, K., Jude, D. J., & Rudnika, S. (1997).
645 *The Round Goby N*eogobius melanostomus *(Pallas): A review of European and North*
646 *American literature* (Illinois Natural History Survey Special Publication 20). Illinois–
647 Indiana Sea Grant Program; Illinois Natural History Survey. https://repository. library.
648 noaa. gov/ view/ noaa/ 36828
649 Christensen, E. A. F., Norin, T., Tabak, I., van Deurs, M., & Behrens, J. W. (2021). Effects of
650 temperature on physiological performance and behavioral thermoregulation in an inva-
651 sive fish, the Round Goby. *Journal of Experimental Biology*, *224*, Article jeb237669.
652 https://doi. org/ 10. 1242/ jeb. 237669
653 Curto, M., Batista, S., Santos, C. D., Ribeiro, F., Nogueira, S., Ribeiro, D., ... & Gante, H. F.
654 (2025). Freshwater fish community assessment using eDNA metabarcoding vs. capture-
655 based methods: Differences in efficiency and resolution coupled to habitat and

656 ecology. *Environmental Research*, *274*, 121238.
657 https://doi.org/10.1016/j.envres.2025.121238
658 Cuthbert, R. N., Pattison, Z., Taylor, N. G., Verbrugge, L., Diagne, C., Ahmed, D. A., ... &
659 Courchamp, F. (2021). Global economic costs of aquatic invasive alien species. *Science*
660 *of the total environment*, *775*, 145238. https://doi.org/10.1016/j.scitotenv.2021.145238
661 Delong, M. D. 2005. Upper Mississippi River basin. Pages 327-374 *in* Benke, A. C. & Cushing,
662 C. E., editors. Rivers of North America. Elsevier Academic, Burlington, ME.
663 Florin, A. B., Hüssy, K., Blass, M., Oesterwind, D., Puntila, R., Ustups, D., ... & Odelström, A.
664 (2018). How old are you—Evaluation of age reading methods for the invasive round
665 goby (*Neogobius melanostomus*, Pallas 1814). *Journal of Applied Ichthyology*, *34*(3),
666 653-658. https://doi.org/10.1111/jai.13596
667 Fullerton, A. H., Burnett, K. M., Steel, E. A., Flitcroft, R. L., Pess, G. R., Feist, B. E., Torgersen,
668 C. E., Miller, D. J., & Sanderson, B. L. (2010). Hydrological connectivity for riverine
669 fish: Measurement challenges and research opportunities. Freshwater Biology, 55(11),
670 2215-2237. https://doi.org/10.1111/j.1365-2427.2010.02448.x
671 Geyer, W. R. & Chant R. 2006. The physical oceanography processes in the Hudson River
672 estuary. Pages 24–38 *in* Levinton, J. S. & Waldman, J. R., editors. The Hudson River
673 Estuary. New York: Cambridge University Press, Cambridge, United Kingdom.
674 George, S. D., Baldigo, B. P., Rees, C. B., Bartron, M. L., & Winterhalter, D. (2021). Eastward
675 expansion of Round Goby in New York: Assessment of detection methods and current
676 range. *Transactions of the American Fisheries Society*, *150*, 258–273. https://doi. org/ 10.
677 1002/ tafs. 10290

Harris, B. S., Spear, M. J., Whitten, A. L., Hine, E. C., Lenaerts, A. W., Mathis, A. T., ... & Lamer, J. T. (2024). Invasive round goby *Neogobius melanostomus* distribution, relative abundance, and establishment in pools of the Illinois Waterway following 30 years of invasion. *Journal of freshwater ecology*, *39*(1), 2301096. https://doi.org/10.1080/02705060.2023.2301096

Havel, J. E., Kovalenko, K. E., Thomaz, S. M., Amalfitano, S., & Kats, L. B. (2015). Aquatic invasive species: Challenges for the future. *Hydrobiologia*, *750*, 147–170. https://doi. org/ 10. 1007/ s10750- 014- 2166- 0

Haws, A. L., Getchell, R. G., & Farrell, J. M. (2025). Distribution of viral hemorrhagic septicemia virus in the nearshore fish community of the St. Lawrence River. *Journal of aquatic animal health*, *37*(3), 136-146. https://doi.org/10.1093/jahafs/vsaf011

Hickey, M. B. C., & Fowlie, A. R. (2005). First occurrence of the Round Goby, *Neogobius melanostomus*, in the St. Lawrence River at Cornwall, Ontario. *The Canadian Field-Naturalist*, *119*, 582–583. https://doi. org/ 10. 22621/ cfn. v119i4. 212

Howarth, R. W., Swaney, D. P., Butler, T. J., & Marino, R. (2000). Rapid communication: climatic control on eutrophication of the Hudson River Estuary. *Ecosystems*, *3*(2), 210-215. https://doi.org/10.1007/s100210000020

Irons, K. S., McClelland, M. A., & Pegg, M. A. (2006). Expansion of round goby in the Illinois waterway. *The American Midland Naturalist*, *156*(1), 198-200. https://doi.org/10.1674/0003-0031(2006)156[198:EORGIT]2.0.CO;2

Jackson, J. K., Huryn, A. D., Strayer, D. L., Courtemanch, D. L., & Sweeney, B. W. (2005). *Atlantic Coast rivers of the northeastern United States*. In A. C. Benke & C. E. Cushing

(Eds.), *Rivers of North America* (p. 21–71). Academic Press. https://doi.org/10.1016/B978-012088253-3/50005-5

Jackson J. R., VanDeValk, A. J., Brooking, T. E., Holeck, K. T., Hotaling, C., & Rudstam, L. G. 2019. The fisheries and limnology of Oneida Lake 2018. Bridgeport, New York: New York Federal Aid in Sport Fish Restoration, Job 2-2, Study 2, F-63-R

Janssen, J., & Jude, D. J. (2001). Recruitment failure of Mottled Sculpin *Cottus bairdi* in Calumet Harbor, southern Lake Michigan, induced by the newly introduced Round Goby *Neogobius melanostomus*. *Journal of Great Lakes Research*, *27*, 319–328. https://doi. org/ 10. 1016/ S0380- 1330(01)70647- 8

Jo, T., & Yamanaka, H. (2022). Meta-analyses of environmental DNA downstream transport and deposition in relation to hydrogeography in riverine environments. *Freshwater Biology, 67*:1333-1343.

Joseph, C., Faiq, M. E., Li, Z., & Chen, G. (2022). Persistence and degradation dynamics of eDNA affected by environmental factors in aquatic ecosystems. *Hydrobiologia*, *849*(19). https://doi.org/10.1007/s10750-022-04959-w

Jude, D. J., Reider, R. H., & Smith, G. R. (1992). Establishment of Gobiidae in the Great Lakes basin. *Canadian Journal of Fisheries and Aquatic Sciences: Journal Canadien des Sciences Halieutiques et Aquatiques*, *49*, 416–421. https://doi. org/ 10. 1139/ f92- 047

Kao, S.-Y. Z., Enns, E. A., Tomamichel, M., Doll, A., Escobar, L. E., Qiao, H., Craft, M. E., & Phelps, N. B. D. (2021). Network connectivity of Minnesota waterbodies and implications for aquatic invasive species prevention. Biological Invasions, 23, 3231-3242. https://doi.org/10.1007/s10530-021-02563-y

722 Kernan, M. (2015). Climate change and the impact of invasive species on aquatic
723 ecosystems. *Aquatic Ecosystem Health & Management*, *18*(3), 321-333.
724 https://doi.org/10.1080/14634988.2015.1027636
725 Kipp, R., & Ricciardi, A. (2012). Impacts of the Eurasian round goby (Neogobius
726 melanostomus) on benthic communities in the upper St. Lawrence River. *Canadian*
727 *Journal of Fisheries and Aquatic Sciences*, *69*(3), 469-486.
728 https://doi.org/10.1139/f2011-139
729 Kornis, M. S., Mercado- Silva, N., & Vander Zanden, M. J. (2012). Twenty years of invasion: A
730 review of Round Goby *Neogobius melanostomus* biology, spread and ecological
731 implications. *Journal of Fish Biology*, *80*, 235–285. https://doi. org/ 10. 1111/ j. 1095-
732 8649. 2011. 03157. X
733 Lamb, P. D., Fonseca, V. G., Maxwell, D. L., & Nnanatu, C. C. (2022). Systematic review and
734 meta-analysis: Water type and temperature affect environmental DNA decay. *Molecular*
735 *Ecology Resources*, *22*(7), 2494-2505. https://doi.org/10.1111/1755-0998.13627
736 Lauer, T., Allen, P., & McComish, T. (2004). Changes in Mottled Sculpin and Johnny Darter
737 trawl catches after the appearance of Round Gobies in the Indiana waters of Lake
738 Michigan. *Transactions of the American Fisheries Society*, *133*, 185–189. https://doi. org/
739 10. 1577/ T02- 123
740 Lazur, A., Early, S., & Jacobs, J. M. (2006). Acute toxicity of 5% rotenone to northern
741 snakeheads. *North American Journal of Fisheries Management*, *26*(3), 628-
742 630.https://doi.org/10.1577/M05-074.1
743 Lederer, A., Janssen, J., Reed, T., & Wolf, A. (2008). Impacts of the introduced Round Goby
744 (*Apollonia melanostoma*) on dreissenids (*Dreissena polymorpha* and *Dreissena bugensis*)

and on macroinvertebrate community between 2003 and 2006 in the littoral zone of Green Bay, Lake Michigan. *Journal of Great Lakes Research*, *34*, 690–697. https://doi.org/ 10. 1016/ S0380- 1330(08)71611- 3

Limburg, K. E. (1996). Growth and migration of 0-year American shad (*Alosa sapidissima*) in the Hudson River estuary: otolith microstructural analysis. *Canadian Journal of Fisheries and Aquatic Sciences, 53*:220-238.

Limburg, K. E., Blackburn, I., Schmidt, R., Lake, T., Hasse, J., Elfman, M., & Kristiansson, P. (2001). Otolith microchemistry indicates unexpected patterns of residency and anadromy in Blueback Herring, Alosa aestivalis, in the Hudson and Mohawk Rivers. *Bulletin Français de la Pêche et de la Pisciculture*, (362-363), 931-938. https://doi.org/10.1051/kmae:2001028

Limburg, K. E. & Siegel, D. I. (2006). The hydrogeochemistry of connected waterways, and the potential for tracing fish migrations. *Northeast Geol Environ Sci*, *28*, 254-265.

Liu Q, K. E., Wang, M., Xin, N., Qi, R., & Wang H (2024) Optimization of pore size and filter material for better enrichment of environmental DNA. Front. Environ. Sci. 12:1422269. doi: 10.3389/fenvs.2024.1422269

McAllister, K., Drake, D. A. R., & Power, M. (2022). Round Goby (Neogobius melanostomus) impacts on benthic fish communities in two tributaries of the Great Lakes. *Biological Invasions*, *24*(9), 2885-2903. https://doi.org/10.1007/s10530-022-02816-4

Mehta, S. V., Haight, R. G., Homans, F. R., Polasky, S., & Venette, R. C. (2007). Optimal detection and control strategies for invasive species management. *Ecological Economics*, *61*(2-3), 237-245. https://doi.org/10.1016/j.ecolecon.2006.10.024

767 Merry, J. L., Fritts, M. W., & Bloomfield, N. C. (2018). Invasive Round Goby (Neogobius
768 melanostomus) Nearing the Mississippi River. *The American Midland Naturalist*, *180*(2),
769 290-297. https://doi.org/10.1674/0003-0031-180.2.290
770 Millard-Martin, B., Sheridan, K., Morien, E., Lemay, M. A., Hessing-Lewis, M., Clemente-
771 Carvalho, R. B., & Sunday, J. M. (2024). Effect of environmental DNA sampling
772 resolution in detecting nearshore fish biodiversity compared to capture
773 surveys. *PeerJ*, *12*, e17967. https://doi.org/10.7717/peerj.17967
774 Nevers, M. B., Byappanahalli, M. N., Morris, C. C., Shively, D., Przybyla-Kelly, K., Spoljaric,
775 A. M., ... & Roseman, E. F. (2018). Environmental DNA (eDNA): A tool for quantifying
776 the abundant but elusive round goby (Neogobius melanostomus). *PloS one*, *13*(1),
777 e0191720. https://doi.org/10.1371/journal.pone.0191720
778 Nitsche, F. O., Ryan, W. B. F., Carbotte, S. M., Bell, R. E., Slagle, A., Bertinado, C., ... &
779 McHugh, C. (2007). Regional patterns and local variations of sediment distribution in the
780 Hudson River Estuary. *Estuarine, Coastal and Shelf Science*, *71*(1-2), 259-277.
781 https://doi.org/10.1016/j.ecss.2006.07.021
782 Nogueira Tavares, C., Brauns, M., Hille, S., Krenek, S., Borcherding, J., & Weitere, M. (2020).
783 Tracing the colonization process of non-native gobies into a large river: the relevance of
784 different dispersal modes. *Biological Invasions*, *22*(8), 2421-2429.
785 https://doi.org/10.1007/s10530-020-02281-x
786 Oka, S. I., Sado, T., Ushio, M., & Miya, M. (2024). Assessing the impact of tidal changes on fish
787 environmental DNA metabarcoding in temperate and tropical coastal regions of
788 Japan. *Metabarcoding and Metagenomics*, *8*, e135461.
789 https://doi.org/10.3897/mbmg.8.135461

Oksanen, J., Simpson, G., Blanchet, F., Kindt, R., Legendre, P., Minchin, P., O'Hara, R., Solymos, P., Stevens, M., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., De Cáceres, M., Durand, S., ... Weedon, J. (2024). *vegan* (Version 2.6-8) [R package]. Comprehensive R Archive Network (CRAN). https://CRAN.R-project.org/package=vegan

Pendleton, R., Berdan, R., George, S., Kenney, G., & Sethi, S. A. (2022). Round Goby captured in a North American estuary: Status and implications in the Hudson River. *Journal of Fish and Wildlife Management*, *13*, 524–533. https://doi. org/ 10. 3996/ JFWM- 22- 012

Pimentel, D., Zuniga, R., & Morrison, D. (2005). Aquatic nuisance species in the New York State Canal and Hudson River systems and the Great Lakes Basin: An economic and environmental assessment. Environmental Management, 35(5), 692-702. https://doi.org/10.1007/s00267-004-0214-7

Pont, D. (2024). Predicting downstream transport distance of fish eDNA in lotic environments. Molecular Ecology Resources, 24, e13934. https://doi.org/10.1111/1755-0998.13934

Przybyla-Kelly, K. J., Spoljaric, A. M., & Nevers, M. B. (2023). Round goby detection in lakes huron and michigan—An evaluation of eDNA and fish catches. *Fishes*, *8*(1), 41. https://doi.org/10.3390/fishes801004

Pyšek, P., & Richardson, D. M. (2010). Invasive species, environmental change and management, and health. *Annual review of environment and resources*, *35*, 25-55. https://doi.org/10.1146/annurev-environ-033009-095548

R Core Team (2025). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org.

812 Ralston, D. K., & Geyer, W.R. (2017). Sediment transport time scales and trapping efficiency in
813 a tidal river." Journal of Geophysical Research: Earth Surface 122:2042-2063.
814 Sahu, A., Singh, M., Amin, A., Malik, M. M., Qadri, S. N., Abubakr, A., ... & Ahmad, I. (2025).
815 A systematic review on environmental DNA (eDNA) Science: An eco-friendly survey
816 method for conservation and restoration of fragile ecosystems. *Ecological*
817 *Indicators*, *173*, 113441. https://doi.org/10.1016/j.ecolind.2025.113441
818 Sard, N. M., Herbst, S. J., Nathan, L., Uhrig, G., Kanefsky, J., Robinson, J. D., & Scribner, K. T.
819 (2019). Comparison of fish detections, community diversity, and relative abundance
820 using environmental DNA metabarcoding and traditional gears. *Environmental*
821 *DNA*, *1*(4), 368-384. https://doi.org/10.1002/edn3.38
822 Scriver, M., von Ammon, U., Pochon, X., Zirngibl, M., Audrezet, F., Leonard, K., ... & Zaiko,
823 A. (2024). Ebbs and flows of marine biodiversity: navigating spatiotemporal patterns of
824 environmental DNA in a coastal tidal ecosystem. *Environmental DNA*, *6*(6), e70039.
825 https://doi.org/10.1002/edn3.70039
826 Simberloff, D. (2021). Maintenance management and eradication of established aquatic
827 invaders. *Hydrobiologia*, *848*(9), 2399-2420. https://doi.org/10.1007/s10750-020-04352-
828 5
829 Shao, X., Fang, Y., Jawitz, J. W., Yan, J., & Cui, B. (2019). River network connectivity and fish
830 diversity. Science of the Total Environment, 689, 21-30
831 https://doi.org/10.1016/j.scitotenv.2019.06.340
832 Stica, P. V., Pie, M. R., Horodesky, A., Dal Pont, G., Cozer, N., Biernaski, …& Ostrensky.
833 (2025). Which filter should I use? A comparative study of environmental DNA

concentration methods for detection of freshwater species. *Aquat Sci* **87**, 96 https://doi.org/10.1007/s00027-025-01222-x

Turner, S. M. (2014). *Understanding river herring movement patterns at small and large spatial scales through geochemical markers*. Ph.D. dissertation, State University of New York College of Environmental Science and Forestry, Syracuse, NY.

USFWS (2023). *Protocol: Field Collection of Environmental DNA (eDNA) Water Samples.* USFWS Northeast Fishery Conservation Genetics Lab.

Van Driessche, C., Everts, T., Neyrinck, S., Halfmaerten, D., Verschelde, P., Breine, J., ... & Brys, R. (2024). Environmental DNA metabarcoding reflects spatiotemporal fish community shifts in the Scheldt estuary. *Science of the total environment*, *934*, 173242. https://doi.org/10.1016/j.scitotenv.2024.173242

Vander Zanden, M. J., Gorsky, A., Hansen, G. A., Johnson, P. J., Latzka, A. W., Mikulyuk, A., ... & Walsh, J. R. (2024). Nine lessons about aquatic invasive species from the North Temperate Lakes Long-Term Ecological Research (NTL-LTER) program. *BioScience*, *74*(8), 509-523.https://doi.org/10.1093/biosci/biae062

Vander Zanden, M. J., Hansen, G. J., Higgins, S. N., & Kornis, M. S. (2010). A pound of prevention, plus a pound of cure: early detection and eradication of invasive species in the Laurentian Great Lakes. *Journal of Great Lakes Research*, *36*(1), 199-205. https://doi.org/10.1016/j.jglr.2009.11.002

Vitousek, P. M., d'Antonio, C. M., Loope, L. L., & Westbrooks, R. (1996). Biological invasions as global environmental change. *American scientist*, *84*(5), 468-478.

Wainright, C. A., Muhlfeld, C. C., Elser, J. J., Bourret, S. L., & Devlin, S. P. (2021). Species invasion progressively disrupts the trophic structure of native food webs. *Proceedings of*

the National Academy of Sciences, *118*(45), e2102179118.
https://doi.org/10.1073/pnas.2102179118

Wilson, S. 2017. USGS Microanalytical Reference Materials (MRMs) Development. Microscopy and Microanalysis 23 (Suppl.1): 492-493. Available: https://web.archive.org/web/20180727114240id_/https://www.cambridge.org/core/services/aop-cambridge-core/content/view/6357188F5D9C659F7460D1147D3B05CA/S1431927617003142a.pdf/div-class-title-usgs-microanalytical-reference-materials-mrms-development-div.pdf Accessed 2/24/2025.

Table 1. Environmental DNA (eDNA) sampling locations, time of year (denoted by X), and number of samples collected for Round Goby qPCR analysis. Number of concurrent NYSDEC beach seine sites in paratheses.

Year	Location			Time of Year																			
	Mainstem	Tributary	Long Island	March			May			June			July			Aug			Dec				
2022	27 (21)	6	3									X	X			X							
2023	15 (7)	7	0		X		X									X							
2024	15 (7)	0	0								X						X		X				

869 Table 2. Total number (*n*), catch per unit effort (CPUE; number of fish per haul), and total length
870 (TL) characteristics of Round Goby collected during NYSDEC annual beach seining between
871 2021 and 2024 among all regions in the Hudson River Estuary.

Year	*n*	CPUE	Average TL (mm)	Minimum TL (mm)	Maximum TL (mm)
2021	112	0.29	49	26	87
2022	12	0.03	67	50	88
2023	42	0.11	51	35	76
2024	17	0.04	66	51	83

872

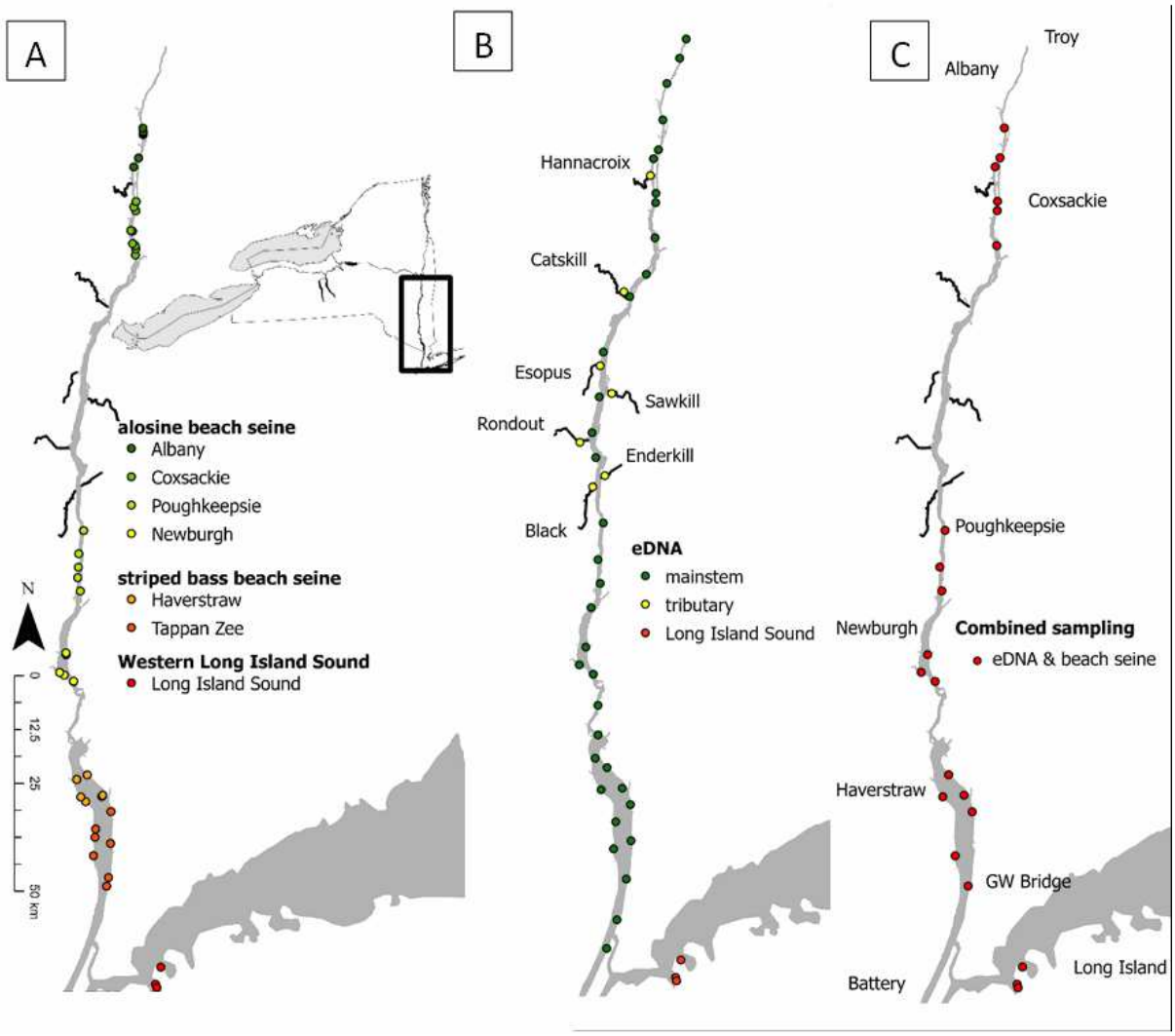
873 Table 3. Test statistics and p-values from comparisons in the mean element:calcium ratios in the
874 otolith edge region (outer 150 um measurements) for Round Goby collected in the Hudson and
875 Mohawk rivers during 2021 to 2022. Bold values indicate significant differences ($\alpha = 0.05$) in
876 the element:calcium ratios among all Hudson and Mohawk individuals (Wilcoxon test) and
877 significant differences among microchemistry groups (Kruskal-Wallis Rank Sum & pairwise
878 Wilcoxon Rank Sum test).

	Wilcoxon Test		Kruskal-Wallis Rank Sum		Pairwise Wilcoxon Rank Sum		
	Hudson, Mohawk		Hudson 2021, Hudson 2022, Mohawk 2022		Hudson 2021, Hudson 2022	Hudson 2021, Mohawk 2022	Hudson 2022, Mohawk 2022
Element:calcium	statistic	*p*-value	statistic	*p*-value	*p*-value	*p*-value	*p*-value
Sr:Ca x1000	35	**< 0.01**	12.0	**< 0.01**	0.98	**< 0.01**	**< 0.01**
Mn:Ca x1000	42	**< 0.01**	10.4	**< 0.01**	1	**0.021**	**< 0.01**
B:Ca x1000	49	**< 0.01**	15.2	**< 0.01**	**0.021**	**< 0.01**	0.13
I:Ca x1000	96	0.12	4.5	0.10	0.35	0.76	0.28
Cu:Ca x1000	107	0.22	7.4	**0.03**	0.05	0.10	1.00
Ba:Ca x1000	191	0.21	4.5	0.10	0.28	1.00	0.23

879

Table 4: R-statistics and p-values from Analysis of Similarities (ANOSIM) multivariate tests comparing mean element:calcium ratios (i.e. Sr, B, Mn) in the otolith core and edge region for Round Goby collected in the Hudson and Mohawk rivers during 2021 to 2022. Bold values indicate significant differences ($\alpha = 0.05$) among all Hudson and Mohawk individuals and significant post-hoc differences among microchemistry groups.

	Core		Edge	
	R statistic	p-value	R statistic	p-value
Hudson 2021, Hudson 2022, Mohawk 2022	0.16	**< 0.01**	0.32	**< 0.01**
Hudson 2021, Hudson 2022	0.03	0.14	0.08	**0.02**
Hudson 2021, Mohawk 2022	0.39	**< 0.01**	0.63	**< 0.01**
Hudson 2022, Mohawk 2022	0.28	**0.01**	0.57	**< 0.01**



887 **Figure 1.** [Inset] New York State and connected navigable waters of NYS Canal System with
888 black box encompassing the tidal Hudson River and Long Island Sound. A) Location of
889 NYSDEC annual beach seining sites colored by region and long-term, standardized survey; B)
890 locations of eDNA sampling conducted between 2022 and 2024 (see Methods as sample location
891 varied by year); C) NYSDEC beach seine sites where eDNA sampling was conducted
892 immediately before seining on selected dates.

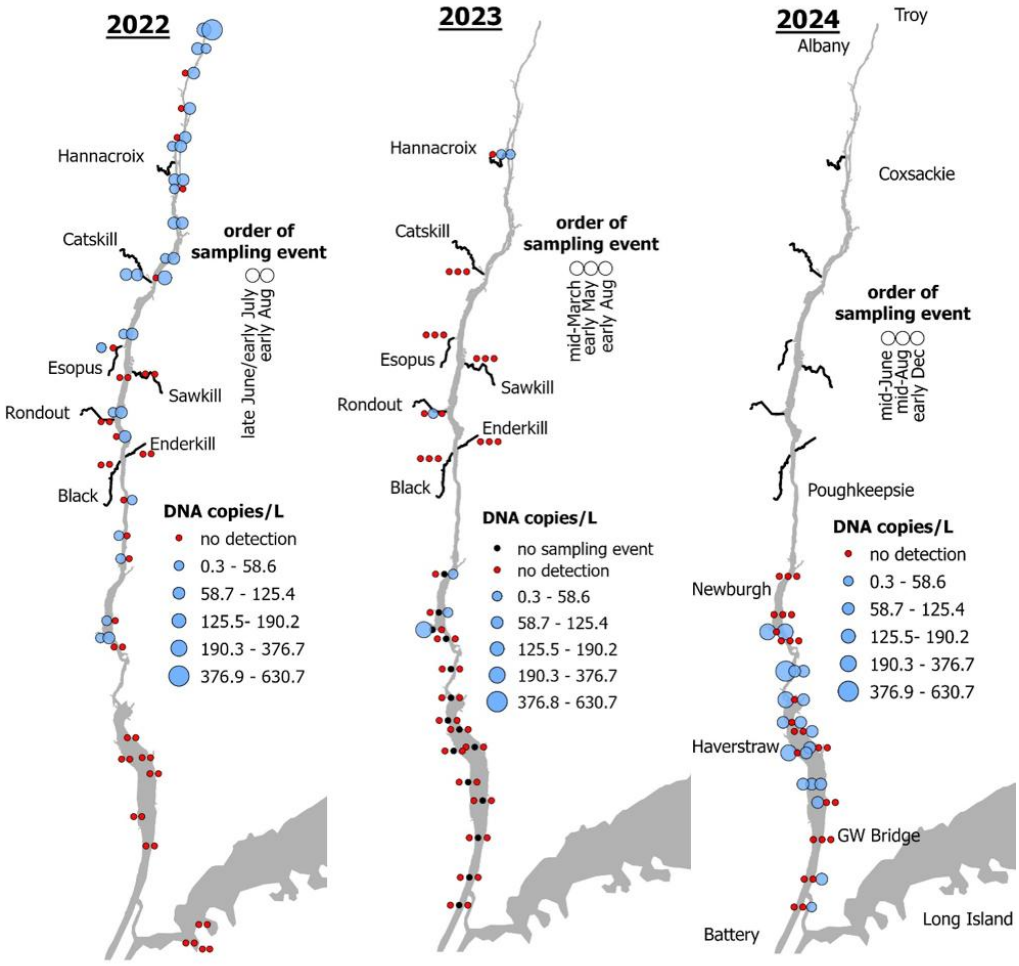


Figure 2. Mean quantity, derived from eight lab replicates, of Round Goby eDNA (copies/L) detected at sites (blue graduated circles) along the mainstem Hudson River Estuary, tributaries, and Long Island Sound from 2022 to 2024. Within each year, results are displayed horizontally by order of sampling event (see open circles and date range of sampling events). Red circles indicate Round Goby eDNA was not detected and black circles indicate sampling did not occur.

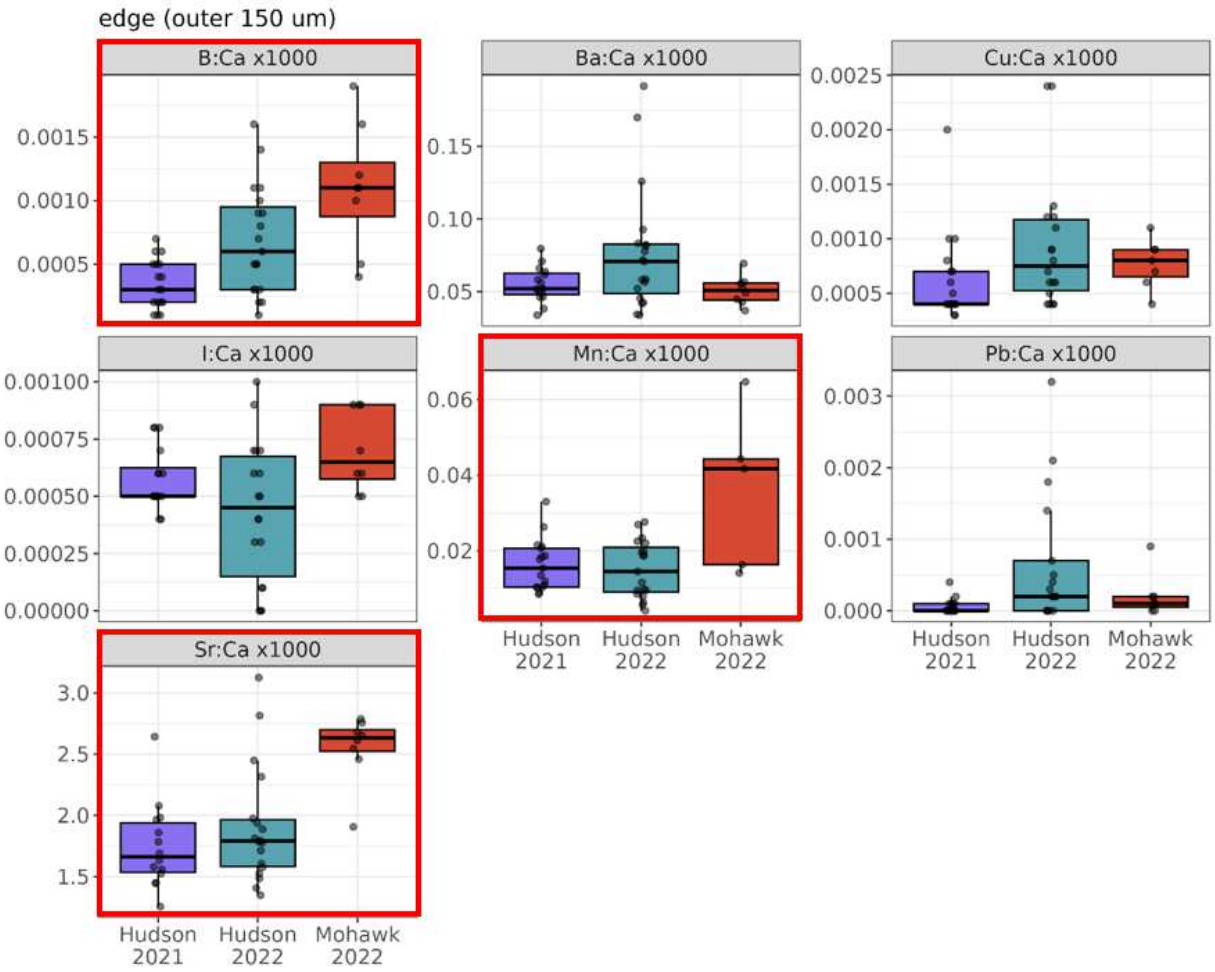


Figure 3. Boxplot comparison of mean element:calcium ratios in the otolith edge region (outer 150 µm measurements) for Round Goby collected in the Hudson and Mohawk rivers during 2021 to 2022. Microchemistry groups based on capture location, year, and estimated age are depicted by color (purple = Hudson 2021, teal = Hudson 2022, red = Mohawk 2022). Red boxes indicate significant differences ($p < 0.05$) in the element:calcium ratios between Hudson and Mohawk individuals based on Wilcoxon rank-sum test with continuity correction (see Table 3 for pairwise comparisons). Extreme outliers (values greater than 95th percentile, less than 5th percentile) were removed from the plot for visualization purposes.

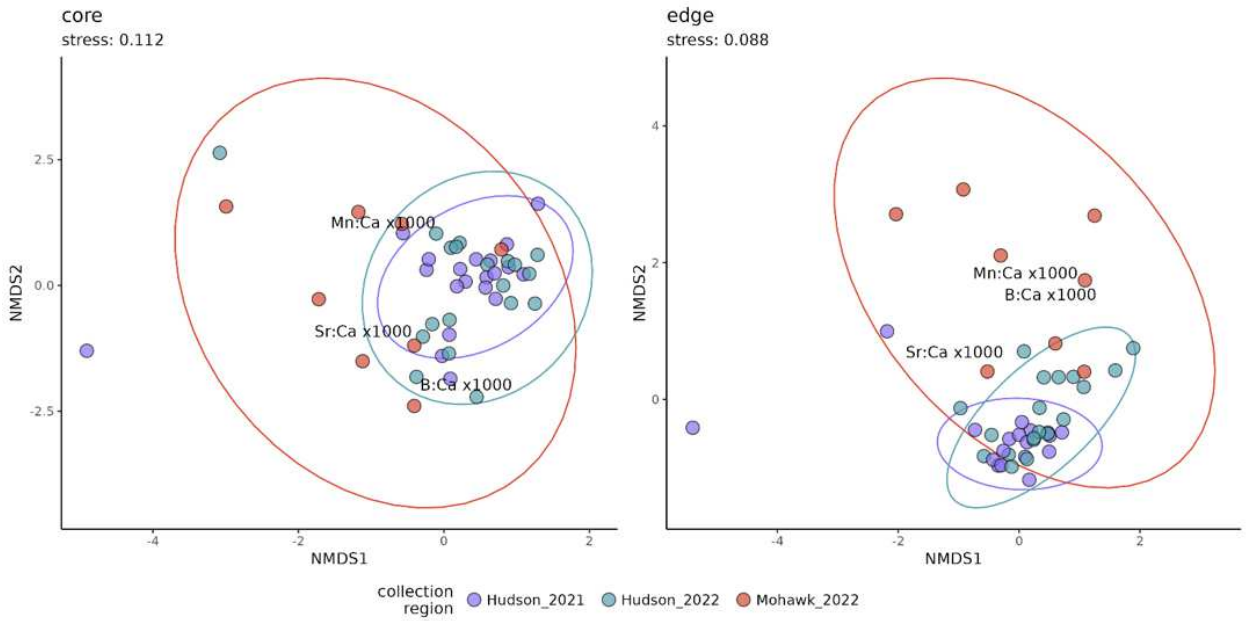


Figure 4. Non-metric multidimensional scaling (NMDS) plots of mean element:calcium ratios (i.e. Sr, B, Mn) in the otolith core (left) and edge (right) region for Round Goby collected in the Hudson and Mohawk rivers during 2021 to 2022. Microchemistry groups based on capture location, year, and estimated age are depicted by color (purple = Hudson 2021, teal = Hudson 2022, red = Mohawk 2022) and colored ellipses show 95% confidence level for a multivariate t-distribution

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Appendix1.pdf
- Supp1.pdf
- Supp2.pdf