



Freshwater Snail Inventory at Upper Delaware Scenic and Recreational River



Roland Wall, Executive Director of the Patrick Center for Environmental Studies at the Academy of Natural Sciences, kayaks on the Delaware River near the Callicoon Access Point in Damascus, Pennsylvania, June 15, 2022. (First day on the river; site scouting.)

NPS / TANYA DAPKEY

Freshwater snail inventory at Upper Delaware Scenic and Recreational River

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Contents

	Page
Figures.....	iv
Tables.....	v
Appendices.....	v
Abstract.....	vi
List of Terms or Acronyms.....	vii
Acknowledgments.....	viii
Introduction.....	1
Methods.....	2
Sampling Design	2
Site Establishment	5
Kick-net Sampling.....	7
Qualitative Sampling.....	7
eDNA Collection Methods.....	7
Variations in 2023 Sampling.....	8
Laboratory Procedures.....	8
Tissue Barcoding Methods.....	8
Analysis Methods.....	9
Results and Discussion	10
Presence and Distribution.....	10
Taxa List.....	10
Mystery snails.....	14
Distribution.....	16
Habitat Preferences.....	17
Density.....	20
Temperature.....	24
eDNA.....	27
Recommendations.....	28
Literature Cited	30

Figures

	Page
Figure 1. Map of 2022 and 2023 snail inventory sites.....	5
Figure 2. An example site structure for pebble counts along the transect.	6
Figure 3. 2022 Diversity of Freshwater Community by Family in the Upper Delaware River based on number of individuals.	13
Figure 4. 2023 Diversity of Freshwater Snail community by Family in the Upper Delaware River based on number of individuals.	14
Figure 5. Distribution map of <i>Cipangopaludina japonica</i> in UPDE from 2022 to 2024.	15
Figure 6. Bar graph of the mean catch per unit effort (number of individuals found in 30 mins; CPUE) per site type for <i>Cipangopaludina japonica</i>	16
Figure 7. Taxa ranges for 2022 and 2023.	17
Figure 8. Habitats sampled in 2022 and 2023.....	18
Figure 9. Number of individuals by family for the habitat types for 2022 and 2023.	20
Figure 10. Density (number of individuals per 0.25 square meter) by family for 2022 pool and riffle kick-net samples.....	21
Figure 11. Catch per unit effort (number of individuals per 30 minutes) by family for 2022 boulder, macrophyte and mud hand collected samples.....	22
Figure 12. Composite CPUE (number of individuals per 30 minutes) for all sampling sites as a function of habitat for both years of sampling.....	23
Figure 13. Density (number of individuals per 0.25 square meters) of pool and riffle/run habitats for both years of sampling.	24
Figure 14. The comparison of density for the two sections of river: cold-water (indicated with a blue outline) and warmwater (indicated with a red outline) zones for both pools and riffle/runs.....	25
Figure 15. Map of the mean catch per unit effort (CPUE) by site and the interpolated temperature of the river for both years.....	26
Figure 16. The most abundant bacteria found in eDNA samples using the 16S gene.....	39

Tables

	Page
Table 1. Snail inventory site list for both years, including latitude and longitude coordinates and site type.....	2
Table 2. Gastropoda of the Upper Delaware River taxa list.	11
Table 3. Taxa list by site with habitat data for 2022 and 2023 sampling.....	18
Table 4. Comparing findings from previous surveys and checklists.....	36
Table 5. Species not found in this survey but found in previous studies.	38
Table 6. List of mussel species found with eDNA metabarcoding of the CO1 and 16S gene.....	40
Table 7. List of fish species found with eDNA metabarcoding of the 12S gene.	42

Appendices

	Page
Appendix A: Programming Code and Script.....	34
Appendix B: Comparative Taxa List.....	36
Appendix C: eDNA Results.....	39

Abstract

The Upper Delaware Scenic and Recreational River (UPDE) unit of the National Park Service partnered with the Academy of Natural Sciences of Drexel University (ANS) to perform a freshwater snail inventory within the park. ANS and UPDE teams conducted field research in July and August of 2022 and 2023 using a combination of kick-net sampling and hand-picking methods to determine the abundance and diversity of freshwater snail populations. Fifty-four sites were surveyed along the 73 miles of the Delaware River within UPDE and included cold-water and warmwater fisheries zones. In 2022, field collections occurred at 30 sites along the river edge from Hancock, New York, to the No. 2 railroad bridge near Mill Rift, Pennsylvania. In 2023, fieldwork occurred at 24 sites in relatively disturbed areas like river access points and boat launches, and within unique river features that were not sampled in 2022 (i.e., backwater, channels, and islands). Snails were identified using traditional morphological taxonomy and DNA barcoding of the CO1 (Cytochrome c oxidase I) gene. Additional environmental DNA (eDNA) was analyzed from water collected at ten eDNA Atlas Sites. Based on genetic analysis, 27 species of gastropods were identified, one of which is a non-native species, the Japanese Mystery Snail (*Cipangopaludina japonica*); previous studies found 18 species and identified the mystery snail as the Chinese (*Cipangopaludina chinensis*) (Evans 2004, Evans and Ray 2008, Evans and Ray 2010). There were more snails below the Cannonsville Reservoir influence in the warmwater zone. A better understanding of the snail community and the ecosystem has been gained with the results of this inventory, which will inform effective and responsible river management plans.

List of Terms or Acronyms

ANS: Academy of Natural Sciences

UPDE: Upper Delaware Scenic and Recreational River

CO1: Cytochrome c oxidase I mitochondrial gene

USFS: United States Forest Service

CPUE: Catch per Unit Effort

BIN: Barcode Index Number

BOLD: Barcode of Life Database

ESV: Exact Sequence Variant

USGS: United States Geological Survey

Acknowledgments

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Introduction

The National Wild and Scenic Rivers System was created by Congress in 1968 to preserve certain rivers with outstanding natural, cultural, and recreational values in a free-flowing condition for the enjoyment of present and future generations. Upper Delaware Scenic and Recreational River (UPDE) is one of those rivers that was designated for preservation, comprising 73 miles of the Delaware River that forms the boundary between New York and Pennsylvania. There are no dams downriver to the ocean, therefore the river supports an array of freshwater species, including migratory fishes. This river area and its ecosystem are influenced by human activities, such as dam releases and recreational use. The Cannonsville Reservoir, upriver of UPDE, supplies New York's drinking water and releases water into the Delaware River, creating a cold-water zone upstream from Callicoon. Downstream of Callicoon, the water temperature returns to more regular warmwater zone where the temperature impacts from the Cannonsville Reservoir are no longer measurable. This temperature delineation is a major example of human influence on an abiotic factor of the UPDE ecosystem. Developing effective and responsible river management plans requires a thorough understanding of this historic region's ecosystem and a complete species list that includes both states.

Freshwater snails have been observed in high numbers in many parts of the Delaware River; however, species composition and distribution are relatively understudied. Due to their abundance and trophic level, they play a large role in nutrient cycling as well as community and habitat structure; snails can stabilize ecosystems via interactions with primary producers and are an important food source for other organisms (Mackie 2022; Lv, et al. 2022; Thorpe and Covich 2010). Few studies within the Delaware Basin have included snails as a major focus, and if snails are identified to species, they are limited in geographic range (Pennsylvania only) or habitat (riffles), and identifications could be invalid (Evans 2004, Evans and Ray 2008, Evans and Ray 2010, Silldorff and Limbeck 2009). To better understand the needs of the aquatic community and to gather more data on the gastropods in the UPDE the National Park Service (NPS) partnered with the Academy of Natural Sciences (ANS) to conduct a freshwater snail inventory.

The project goal was to conduct an inventory survey of freshwater snail species along the 73-mile length of the UPDE which included eDNA Atlas sites (areas previously surveyed for freshwater mussels). The project also collected covariate data, including habitat types (riffle, run, pool), substrate types (bedrock, cobble, sand, etc.), water depth at the survey site, water temperature, turbidity, and flow. Additionally, species richness, estimated abundance, species density, and habitat suitability of sites within the park for each species observed were determined and analyzed. A species list of all freshwater snails observed was developed, and the presence of non-native freshwater snails in survey areas was summarized. Species identifications were confirmed using genetic tissue barcoding, providing the most up-to-date and accurate species list.

Methods

To inventory the freshwater snail populations, ANS developed survey methods along the 73-mile length of the UPDE. Selected sites represented randomly selected eDNA Atlas sites, backwaters, access points, and an island. Methods were a mixture of semi-quantitative kick-net samples and qualitative hand collections to ensure all possible species were found. Covariate data were collected, including habitat types (riffle, run, pool), substrate types (bedrock, cobble, sand, etc.), water depth at the survey site, water temperature, turbidity, and flow. Freshwater snail samples were identified using traditional and genetic techniques.

Sampling Design

Sites were chosen along 73 miles of the river and included cold-water and warmwater fisheries zones—Table 1 shows the list of site codes with latitude and longitude coordinates while Figure 1 maps all the sites. These zones reflect temperature variations, generally with colder water upstream and warmer water downstream. In the first year of sampling, 2022, ANS collected specimens from 30 sites within UPDE from Hancock, NY, to the No. 2 railroad bridge near Mill Rift, PA. Sites were spaced evenly every 4 km on alternating sides of the river and when possible coincided with United States Forest Service (USFS) eDNA Atlas sites that were previously surveyed for freshwater mussels. The crews accessed sites using kayaks and canoes. During the 2022 field season, teams noticed that snails were often abundant at boat launches and other sites that had anthropogenic influences. Therefore, in the second year of sampling, 2023, ANS collected specimens within UPDE at 24 selected sites in more disturbed areas like river access points and boat launches, and sites that represented unique river features that were not well represented in the 2022 sampling approach (i.e., backwaters, channels, and islands). Crews were able to access most of these sites via driving and walking; canoes were used at only two sites for sampling. Environmental variables such as water temperature, flow velocity, substrate composition, pH, dissolved oxygen, and specific conductance were collected at all sites at the time of sampling. Global positioning system (GPS) coordinates were taken for each site.

Table 1. Snail inventory site list for both years, including latitude and longitude coordinates and site type.

Year	Site	Latitude	Longitude	Site Type
2022	O433-3-1PA ^A	41.926292	-75.276998	Riverbank
2022	O394-1-3NY	41.897257	-75.271958	Riverbank
2022	O336-3-5PA	41.864280	-75.261773	Riverbank
2022	O327-2-7NY	41.860733	-75.220578	Riverbank
2022	O359-1-9PA	41.862002	-75.190243	Riverbank
2022	O362-2-11NY	41.857918	-75.166908	Riverbank
2022	O342-1-13PA	41.849383	-75.137268	Riverbank
2022	O334-1-15NY	41.825583	-75.113958	Riverbank
2022	O292-7-17PA	41.815037	-75.076382	Riverbank

Table 1 (continued). Snail inventory site list for both years, including latitude and longitude coordinates and site type.

Year	Site	Latitude	Longitude	Site Type
2022	O292-3-19NY	41.793498	-75.094553	Riverbank
2022	O234-1-21PA	41.769292	-75.085383	Riverbank
2022	O222-2-23NY	41.747053	-75.052267	Riverbank
2022	O184-2-25PA	41.712732	-75.050585	Riverbank
2022	O165-1-27NY	41.686268	-75.052048	Riverbank
2022	O133-1-29PA	41.661217	-75.049680	Riverbank
2022	O91-1-31NY	41.629538	-75.046343	Riverbank
2022	O75-1-33PA	41.610472	-75.063817	Riverbank
2022	O63-1-35NY	41.593817	-75.061772	Riverbank
2022	O54-3-37PA	41.566157	-75.036375	Riverbank
2022	O30-1-39NY	41.541632	-75.020772	Riverbank
2022	O14-2-41PA	41.519812	-75.001897	Riverbank
2022	O6-1-43NY	41.495487	-74.982138	Riverbank
2022	O787-1-45PA	41.476150	-74.963813	Riverbank
2022	O818-1-47NY	41.480922	-74.929530	Riverbank
2022	O799-4-49PA	41.457262	-74.894357	Riverbank
2022	O754-3-51NY	41.439722	-74.878611	Riverbank
2022	O763-2-53PA	41.432480	-74.839077	Riverbank
2022	O775-1-55NY	41.441389	-74.804444	Riverbank
2022	O752-2-57PA	41.424303	-74.768767	Riverbank
2022	O776-1-59NY	41.430172	-74.737158	Riverbank
2023	AC-HAN-NY1	41.934427	-75.276892	Access Point
2023	AC-BUC-PA3	41.866502	-75.263318	Access Point
2023	AC-LOR-NY4	41.868427	-75.214287	Access Point
2023	AC-LON-NY5	41.848270	-75.133957	Access Point
2023	CH-L1-NY6	41.845812	-75.125113	Channel
2023	AC-CAL-NY8	41.771797	-75.073763	Access Point
2023	IS-R1-PA9	41.756070	-75.055265	Island
2023	BW-T1-PA10	41.738973	-75.055977	Backwater
2023	BW-T2-NY11	41.730543	-75.052328	Backwater
2023	CH-U1-NY12	41.720483	-75.050495	Channel
2023	CH-V1-PA13	41.713568	-75.066455	Channel
2023	AC-DAM-PA14	41.703472	-75.066018	Access Point
2023	AC-MIL-PA15	41.673070	-75.060245	Access Point
2023	AC-SKI-NY16	41.669112	-75.056950	Access Point

Table 1 (continued). Snail inventory site list for both years, including latitude and longitude coordinates and site type.

Year	Site	Latitude	Longitude	Site Type
2023	BW-Z1-PA17.R	41.642238	-75.050382	Backwater
2023	BW-Z1-PA17.B	41.642107	-75.050663	Backwater
2023	AC-NAR-NY18	41.611157	-75.058495	Access Point
2023	AC-TEN-NY19	41.553085	-75.018357	Access Point
2023	AC-WES-PA20	41.510463	-75.004408	Access Point
2023	AC-LAC-PA22	41.485325	-74.986382	Access Point
2023	AC-HIG-NY23	41.483912	-74.937268	Access Point
2023	AC-BAR-NY24	41.704082	-75.066787	Access Point
2023	BW-FF1-PA27	41.432382	-74.824238	Backwater
2023	AC-MON-NY28	41.424530	-74.756225	Access Point

^A The last section of the site name is used as an abbreviated label for the sites. In 2022, the state initials come after the site number. In 2023, the state initials come before the site number. For O433-3-1PA, the abbreviated label is 1PA.

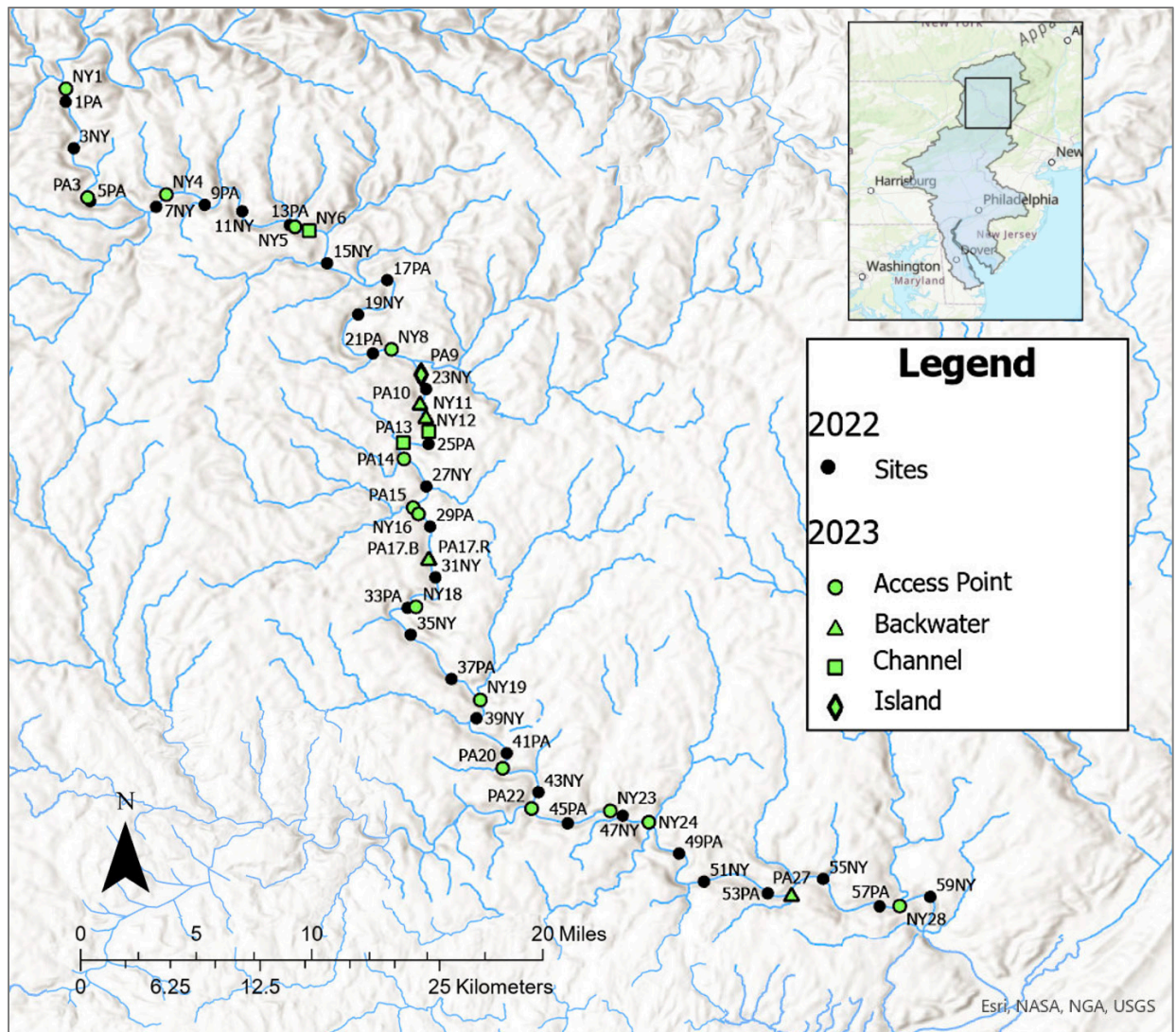


Figure 1. Map of 2022 and 2023 snail inventory sites. 2022 sites are indicated on the map with black dots, 2023 sites are indicated on the map with green dots while different shapes indicate the different habitat types.

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Site Establishment

Upon arriving at each site during both years of sampling, a visual survey was conducted to identify the different habitat types along the river. A habitat checklist was used to ensure that the sampling reach included a diversity of habitats. Once the sampling reach was established, a 100 m survey reach parallel to the bank was delineated. Selected reaches did not have any large tributaries entering the assessment area as they could impact the flow and temperature. eDNA water samples and water quality readings were taken upstream of the transect before collecting specimens to avoid disturbing the habitat. Gastropods were sampled from the main channel of the Delaware River. GPS coordinates and digital photos were taken at the beginning and end of the 100 m sampling reach transect. Parameters collected included water temperature, dissolved oxygen, specific conductance, and

turbidity. Physical habitat was characterized by using a modified version of the Habitat and Environmental Conditions survey performed in the Survey Protocol for Aquatic Mollusk Species: Preliminary Inventory & Presence/Absence Sampling (Duncan 2008). This assessment evaluated each 100 m site and quantified habitat qualities such as the types of macrophyte present, substrate types, and the overall state of the site.

Along each reach, substrate composition was determined at six transects within that reach to provide data via pebble count assessment (Figure 2); this method was based on the EPA Rapid Bioassessment protocols (Barbour et al. 1999). A pebble count was completed along 100 m transect every 20 m to assess the substrate of the river bottom. While traveling up the transect, a width into the river was determined by the crew for each site: if the crew was unable to wade into the river because of the speed or the depth of the river, 0 m was recorded and the bank substrate was recorded; the maximum wadable distance was capped at 50 m. Along the 100 m reach, width (from bank to wadable depth up to 50 m) was measured every 20 m, depth and substrate type were recorded at 0%, 25%, 50%, 75%, and 100% from the bank along the width of the transect and averaged.

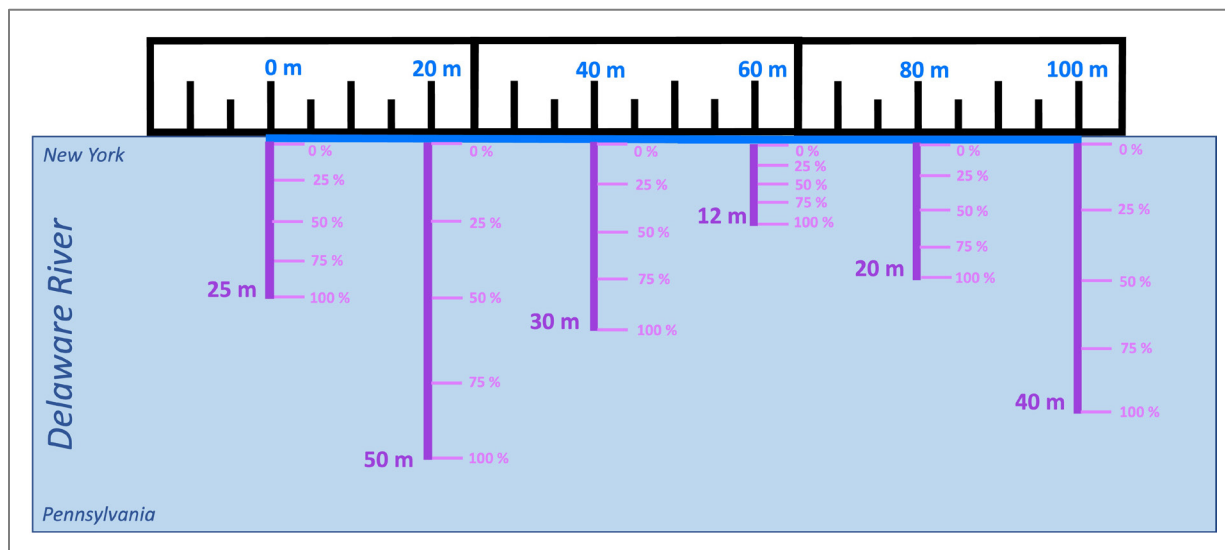


Figure 2. An example site structure for pebble counts along the transect. At each site, a 100 m transect parallel to the bank of the river (blue line along the ruler) was established. Substrate type was reported based on a percentage from the bank (See ANS Field Data Sheet Template 2022.pdf for Substrate List). The substrate at 0%, 25%, 50%, 75%, and 100% from the bank was reported every 20 m along the transect.

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A multihabitat sampling approach was followed. The aim was to collect representative samples, or a subset of the gastropod community that accurately reflected the nature of the larger community, from similar habitat types. Snails are known to reside in riffles, runs, pools, stream edges, submerged and emergent vegetation, and woody debris (Thorp and Covich 2010). As such, these habitats were identified at each site along the transect and sampled using the appropriate techniques (<https://irma.nps.gov/DataStore/Reference/Profile/2308142>). The number and types of habitats

differed across sites, and some sites lacked representation from all habitats. This sampling protocol included all possible habitat types (riffles, runs, pools, boulders, concrete, submerged emergent overhanging vegetation, organic matter, and woody debris), and each type of habitat present was sampled at a given site.

Kick-net Sampling

Kick-net sampling, a semi-quantitative technique, was utilized in riffles, pools, and deep runs/glides. The habitats were separated into two categories: lentic and lotic. Lotic referred to fast-moving water (riffles, runs, and glides), while lentic referred to slow or stagnant water (pools). Riffle habitat was defined as areas of broken water with rapid current that had some cobble or boulder substratum (Hose et al. 2004; Gillies 2009). For riffles and other lotic habitats, the sampling consisted of two 60-second timed kicks in the faster current. A “kick” involved disturbing the substrate of a riffle using feet and/or hands. The sample location was randomly selected by throwing the 0.25m² quadrat into the habitat. After the 60-second kicks, each rock was scrubbed into the kick net to ensure all gastropods, specifically limpets, were collected. All samples taken in lotic habitats were composited into one sample jar and preserved with 80% ethanol. To sample the lentic habitats, a timed sample was taken for 30–60 seconds. The sample location was randomly selected by throwing the 0.25m² quadrat into the habitat. All lentic samples were collected, and the nets were drained of as much water as possible without damaging the contents of the sample. Samples were then composited into a labeled jar and preserved with 80% ethanol.

Qualitative Sampling

Qualitative hand samples were taken by sieving, raking, and searching the different habitats along the riverbank. The habitats sampled were boulder/rock, macrophyte/coarse woody debris, and muck/mud. All habitats were sampled for 30 minutes. Boulders were defined as any large emergent and submerged rocks; there were many locations with large boulders that provided habitat for algae and diatoms. The plant materials included macrophytes, submerged riparian plants, leaf packs, and coarse woody debris. For the muck/mud habitat sampling, a 0.25m² quadrat was used to select the area to sample. Material was removed from within the quadrat and put into a sieve (500 µm) to remove fine material. The sieved material was placed into a jar along with detritus from that habitat. Once the entire quadrat was thoroughly searched and all snails were collected, the field crew member placed the quadrat in the same habitat type but in another random location along the transect. The number of quadrats used in 30 minutes was recorded.

eDNA Collection Methods

Water samples for environmental DNA (eDNA) were taken at 10 sites, every 7 miles from the northern end of UPDE in Hancock, New York, to the southern end in Millrift, Pennsylvania, in 2022. Jonah Ventures (2024) kits were used to collect and filter the water collected from the sampling location. Kits included a syringe filter (0.45 microns), 60 mL syringe, gloves, and barcoded sample bag. Water for eDNA samples was taken above the 0-meter mark of the transect prior to specimen collection and disturbance of the site. The person collecting the eDNA sample would put on the gloves and then proceed to collect and push water in the syringe and through the filter until the filter was clogged. The amount of water pushed through each filter was recorded. Triton TE buffer (0.5

mL) was used as a preservative for the filters. Filters were shipped to Jonah Ventures lab and analyzed using metabarcoding primers for mussels, bacteria and fish. Detailed methods for DNA extraction and sequencing can be found on the NPS DataStore (<https://irma.nps.gov/DataStore/Reference/Profile/2307735>). The 16S gene was amplified with the ITS primer to determine mussel and bacteria species; the CO1 gene was amplified with the PfaCOI2 Degen primer to determine mussel species; and the 12S gene was amplified with the MiFishU primer to determine fish species. Each sequence was represented by an exact sequence variant (ESV) and was generated from a denoising algorithm. ESVs were generated by examining the most abundant sequences and then removing sequences that had random differences in them. Those with consistent differences, even just one base difference, became their own ESV instead of being lumped together. The percentage of base pairs in the sequence were matched to the species in the library, and the number of species matching the sequence at a given level was used to reference the ESV file to see which species matched.

Variations in 2023 Sampling

2023 sampling occurred in river features that were not visited in 2022, such as river access points, backwaters, islands, channels, and other unique habitats along UPDE to determine if different snail species concentrated in those areas. Methodology was adjusted for these new sites, where transect delineation was determined on location at the time of sampling. Hand collection and kick-net sampling proceeded as in 2022. Human-made surfaces (e.g., concrete and metal) were categorized as "boulder" due to their similar surface character. Pebble counts were completed at sites that had one bank. Backwaters had two banks, and if viable, sampling occurred along both banks, with two 100 m transects starting at the apex, or the furthest reach into the backwater and continuing to the mouth of the backwater. Pebble count was not completed in backwaters as they were typically difficult to traverse with deep water. Water for eDNA analysis was not collected in 2023.

Laboratory Procedures

Snails were sorted from all the samples collected using a dissecting microscope (Leica EZ4 and Leica EZ4W, 8X to 35X zoom range). Snails were identified using taxonomic morphology to the lowest possible taxonomic level. Arthur Bogan, from the North Carolina Museum of Natural History, provided training to increase aquatic snail taxonomic knowledge. Dr. Bogan reviewed identifications to determine accuracy. Photographs of snails were taken with the Leica EZ4W microscope utilizing the LAS EZ 3.4 Imaging software for Windows OS and saved as .jpeg digital images.

Tissue Barcoding Methods

475 snail specimens collected in 2022 had tissue samples removed and sent to the University of Guelph for barcoding of the CO1 mitochondrial gene and BIN (Barcode Index Number) assignment. BINs are unique identifiers for clusters of sequences that are similar to species. Sequences from the snail tissue were assigned a BIN and matched with type specimens in the Barcode of Life Data (BOLD) System database (Ratnasingham et al. 2024).

Analysis Methods

Using both quantitative and qualitative data collected in both years, a species list of the observed freshwater snails was developed, and the presence and distribution of native and non-native freshwater snails observed along the 73-mile river section were summarized. For quantitative kick-net sampling (pools and riffle/run habitats), density was calculated as the total number of individuals found within 0.25m² collection area divided by the number of quadrats. For qualitative hand sampling (macrophytes, boulders, mud, metal), catch per unit effort (CPUE) was calculated by dividing the number of snails found within 30 minutes in the collection area by 30 minutes. Outlier data points that were more than 1.5 times higher or lower than the middle 50% of the data distribution (the interquartile range) were removed from summaries to facilitate visualization; further work should be done to investigate the source and potential meaning behind these outliers.

Water temperatures from the inverse distance weighting interpolation of 8 USGS gages (spanning from Hancock to Port Jervis) on the main stem of the Delaware were used to verify the temperature regime difference between the upstream cold-water and downstream warmwater fisheries zones. The interpolation took the average daily values from January of 2021 to December of 2023, trimmed to the June–July–August subset, then calculated average monthly values per site and were then spatially autocorrelated using inverse distance weighting (IDW; Appendix A).

Results and Discussion

Results for this project include gastropods that were collected in July and August of 2022 and 2023, from fifty-four sites along UPDE (see Figure 1 and Table 1).

Presence and Distribution

Taxa List

While surveying the freshwater snail community of Pennsylvania and New York is not a novel venture (Jokinen 1992, Evans 2004, Bilger et al. 2005, Evans and Ray 2008, Evans and Ray 2010), surveying one water body systematically and utilizing genetic analysis is unprecedented. Previous studies focused on one state, PA or NY, and while some species were found in the main stem of the Delaware, most of the species listed in these papers were located basin wide (Appendix B). The most recent survey by Evans and Ray (2010) identified 18 species of freshwater snails, whereas this study identified 27. This research represents an improvement in documenting the diversity of freshwater snails in the Delaware River Basin, particularly in the Upper Delaware River.

In total, 18,467 snails were collected during this survey and traditional morphological and novel tissue barcoding techniques were used to identify snails to the lowest taxonomic unit. Snails are generally difficult to identify to the species level based on physical features; often, genitalia or mouth parts determine the species which can be difficult to distinguish on preserved specimens (Mackie 2022). Based on traditional methods, there were 7 families and 9 species. There were three large groups that could not be delineated further: *Physella*, *Ancylinae*, and *Galba*. Tissue barcoding the CO1 gene of morphological types chosen from the 2022 collected specimens revealed 27 species (Table 2) and identified the species of mystery snail as *Cipangopaludina japonica*. Sixteen species had BINs that did not match any species in the BOLD database (these are indicated with an asterisk in Table 2, also see “Snails Barcoding Results” PDF found at <https://irma.nps.gov/DataStore/Reference/Profile/2307735>). This could have been due to two possible reasons: 1) there is no barcoded type specimen on the BOLD database, or 2) they are a new undescribed species. Taxonomists will need to examine the specimens and compare them to type specimens. More taxonomic work for gastropod specimens is needed globally due to the high amounts of morphological diversity inherent in snails (Williams et al. 2017).

Table 2. Gastropoda of the Upper Delaware River taxa list.

Order ^A	Family	Traditional ID ^B	Barcode ID ^C	BINS ^D
Littorinimorpha	Amnicolidae	<i>Amnicola limosus</i>	<i>Amnicola limosus</i>	BOLD: ADG1873
	Amnicolidae	<i>Amnicola limosus</i>	<i>Somatogyrus</i> ^E	BOLD: AFQ5690 ^F
Hygrophila	Lymnaeidae	<i>Galba</i>	<i>Galba</i> EG1	BOLD: AAY4012 ^F
	Lymnaeidae	<i>Galba</i>	<i>Galba humilis</i>	BOLD: AAG1584
	Lymnaeidae	<i>Galba</i>	<i>Ladislavella elodes</i>	BOLD: ADY1241
	Lymnaeidae	<i>Pseudosuccinea columella</i>	<i>Pseudosuccinea columella</i> EG1	BOLD: ADK1522 ^F
	Lymnaeidae	<i>Pseudosuccinea columella</i>	<i>Pseudosuccinea columella</i> EG2	BOLD: ADH1931 ^F
	Planorbidae	<i>Ancylinae</i>	<i>Ferrissia rivularis</i> EG1	BOLD: ACI9961 ^F
	Planorbidae	<i>Ancylinae</i>	<i>Ferrissia rivularis</i> EG2	BOLD: AAH3019 ^F
	Planorbidae	<i>Ancylinae</i>	<i>Laevapex fuscus</i>	BOLD: AAJ3057
	Planorbidae	<i>Ancylinae</i>	<i>Laevapex nipponica</i>	BOLD: AAE6642
	Planorbidae	<i>Gyraulus</i>	<i>Gyraulus</i> sp.	BOLD: AEN6379 ^F
	Planorbidae	<i>Helisoma anceps</i>	<i>Helisoma anceps</i>	BOLD: AAG1582
	Planorbidae	<i>Menetus dilatatus</i>	<i>Menetus dilatatus</i>	BOLD: AAX4717
	Planorbidae	<i>Planorbella trivolvis</i>	<i>Planorbella trivolvis</i> EG1	BOLD: AAV0531 ^F
	Planorbidae	<i>Planorbella trivolvis</i>	<i>Planorbella trivolvis</i> EG2	BOLD: AFR1232 ^F
	Pleuroceridae	<i>Elimia virginica</i>	<i>Elimia virginica</i>	BOLD: ADB2456
	Physidae	<i>Physella</i>	<i>Physella</i> EG1	BOLD: AAB3770 ^F
	Physidae	<i>Physella</i>	<i>Physella</i> EG2	BOLD: AGE9419 ^F
	Physidae	<i>Physella</i>	<i>Physella</i> EG3	BOLD: AEZ1104 ^F
	Physidae	<i>Physella</i>	<i>Physella</i> EG4	BOLD: AFQ6704 ^F
	Physidae	<i>Physella</i>	<i>Physella</i> EG5	BOLD: AAE4821 ^F
	Physidae	<i>Physella</i>	<i>Physella</i> EG6	BOLD: AFQ7210 ^F
	Physidae	<i>Physella</i>	<i>Physella</i> EG7	BOLD: AFQ7211 ^F

^A These are considered similar levels taxonomically however they are not all “Orders”: Hygrophila is a super order, Lower Heterobranchia is a Subclass.

^B Traditional identification is based on morphological dichotomous key from J. B. Burch.

^C Species names from barcoded gastropods from 2022. If the name ends in “EG#”, these species did not have a type specimen or are a new species. Taxonomic review is necessary to determine species.

^D BINs = Barcode Index Number.

^E Tissue barcoding revealed an additional genus, *Somatogyrus*. After reviewing the samples there does seem to be a morphological difference in the mantle tissue, however it has not been recorded before and will need to be reviewed by a taxonomist.

^F Novel BINs. In BOLD Database, these BINs have not been associated with a described species.

Table 2 (continued). Gastropoda of the Upper Delaware River taxa list.

Order ^A	Family	Traditional ID ^B	Barcode ID ^C	BINS ^D
Architaenioglossa	Viviparidae	<i>Campeloma decisum</i>	<i>Campeloma decisum</i>	BOLD: ACO0695
	Viviparidae	<i>Cipangopaludina japonica</i>	<i>Cipangopaludina japonica</i>	BOLD: AAR9203
Lower Heterobranchia	Valvatidae	<i>Valvata bicarinata</i>	<i>Valvata bicarinata</i>	BOLD: AFR1383

^A These are considered similar levels taxonomically however they are not all "Orders": Hygrophila is a super order, Lower Heterobranchia is a Subclass.

^B Traditional identification is based on morphological dichotomous key from J. B. Burch.

^C Species names from barcoded gastropods from 2022. If the name ends in "EG#", these species did not have a type specimen or are a new species. Taxonomic review is necessary to determine species.

^D BINs = Barcode Index Number.

^E Tissue barcoding revealed an additional genus, Somatogyrus. After reviewing the samples there does seem to be a morphological difference in the mantle tissue, however it has not been recorded before and will need to be reviewed by a taxonomist.

^F Novel BINs. In BOLD Database, these BINs have not been associated with a described species.

In 2022, 10,213 snails were found representing 7 families and 13 genera. Snails in the families Physidae and Amnicolidae were the most common, making up 44% and 39% respectively (Figure 3). Only 1 specimen was identified to the family Valvatidae. There were 3 unidentifiable individuals; they were excluded from the figure. 225 Physidae and 8 Amnicolidae (from Site 41PA Pool and Riffle/Run) were mislabeled, and their habitat information was unclear; they were excluded from analysis.

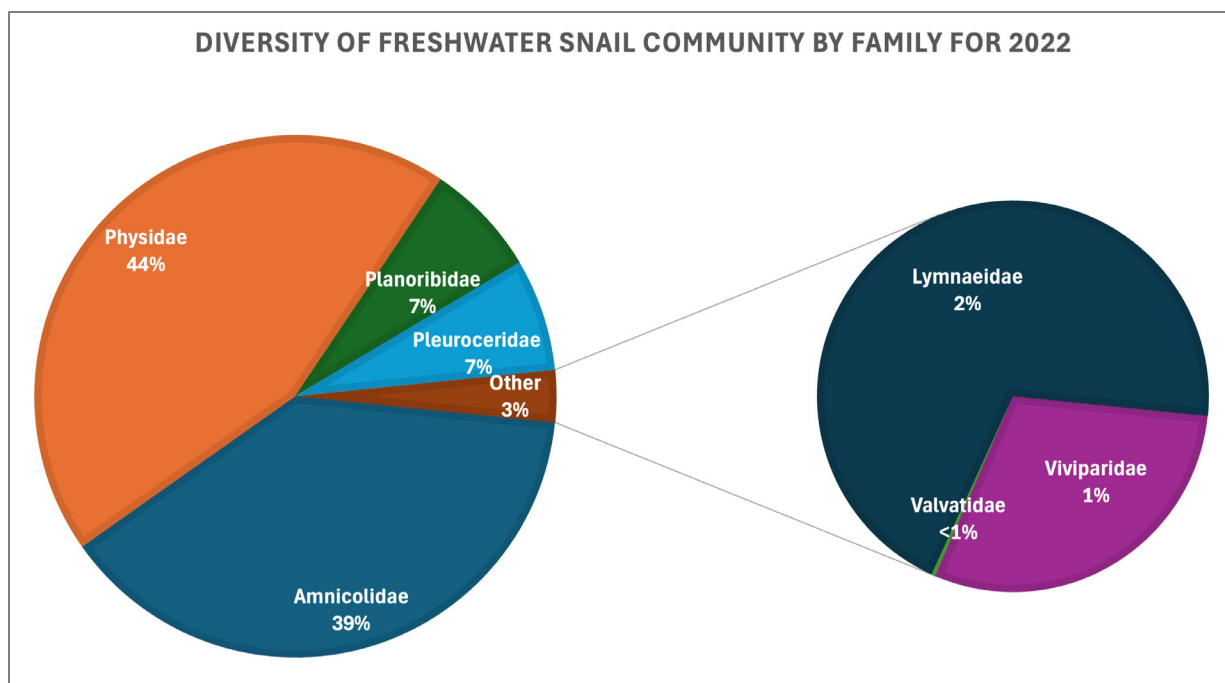


Figure 3. 2022 Diversity of Freshwater Community by Family in the Upper Delaware River based on number of individuals.

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In 2023, 8,254 snails representing 7 families and 12 genera were collected. Physidae was the most common family comprising 58% of the snails that were found (Figure 4). There were 4 unidentifiable individuals; they were excluded from this diagram.

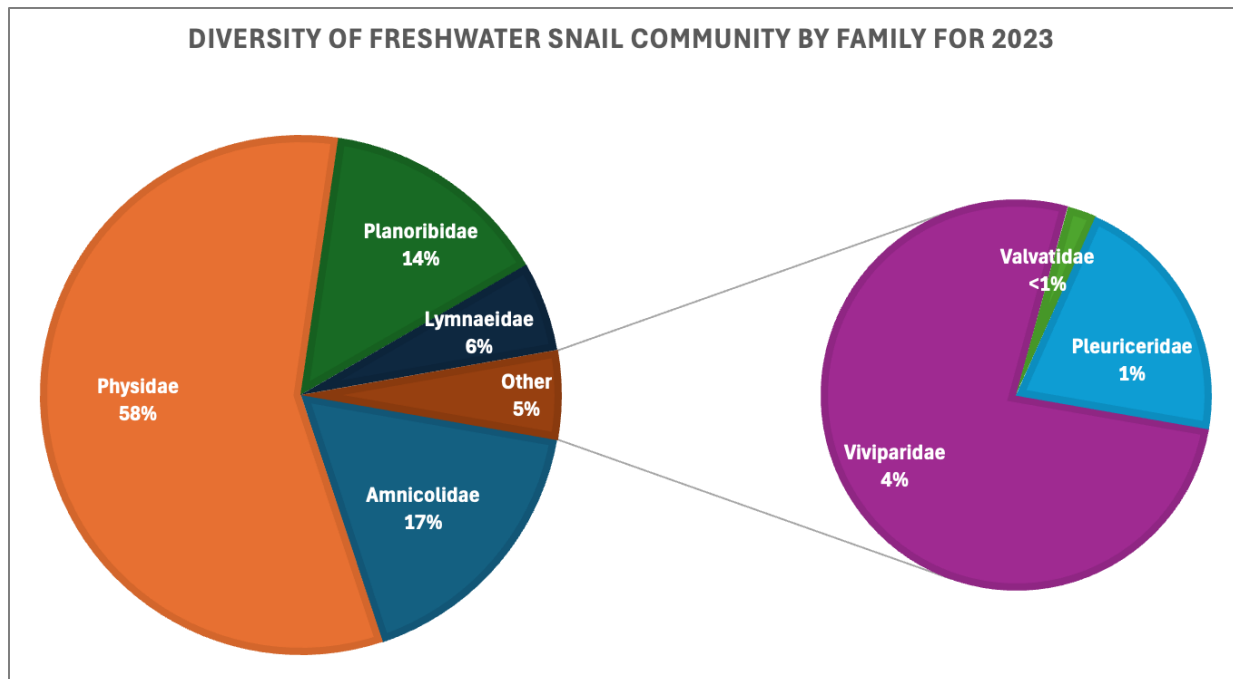


Figure 4. 2023 Diversity of Freshwater Snail community by Family in the Upper Delaware River based on number of individuals.

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Physidae had the highest number of individuals in both years, with a total of 9,369 individuals, comprising 50% of all snails collected (18,467). Evans (2004) and Evans and Ray (2008) also found a high number of Physidae, particularly *Physella acuta*, at every sampling location and within museum collections. The second most common aquatic snail was the species *Amnicola limosus*; this species was not observed in the field very often; due to their small size these snails revealed themselves during microscopic examination of the collected detritus. One site alone had 495 *Amnicola limosus* picked out of a riffle/run sample at site 55NY. After tissue barcoding, we found that this group delineates and includes the genus *Somatogyrus*; however, the exact number is yet to be determined due to difficulty separating the groups based on morphological traits.

Mystery snails

Tissue barcoding of the CO1 gene has confirmed that the mystery snails are *Cipangopaludina japonica*, the Japanese Mystery Snail, extending the known range of this species. *C. japonica* was discovered at 7 sites (Figure 5). Tissue barcoding of the CO1 gene matched the specimens collected to BIN BOLD: AAR9203 (*Cipangopaludina japonica*). In 2022, 48 mystery snails were collected at 3 sites and in 2023, 348 mystery snails were observed at 4 sites; our results show more non-native snails were found in disturbed locations (i.e. access points). NPS staff found *C. japonica* at two access points—Ten Mile River and Lackawaxen—in the summer of 2024 where they had not been observed in 2022 or 2023.

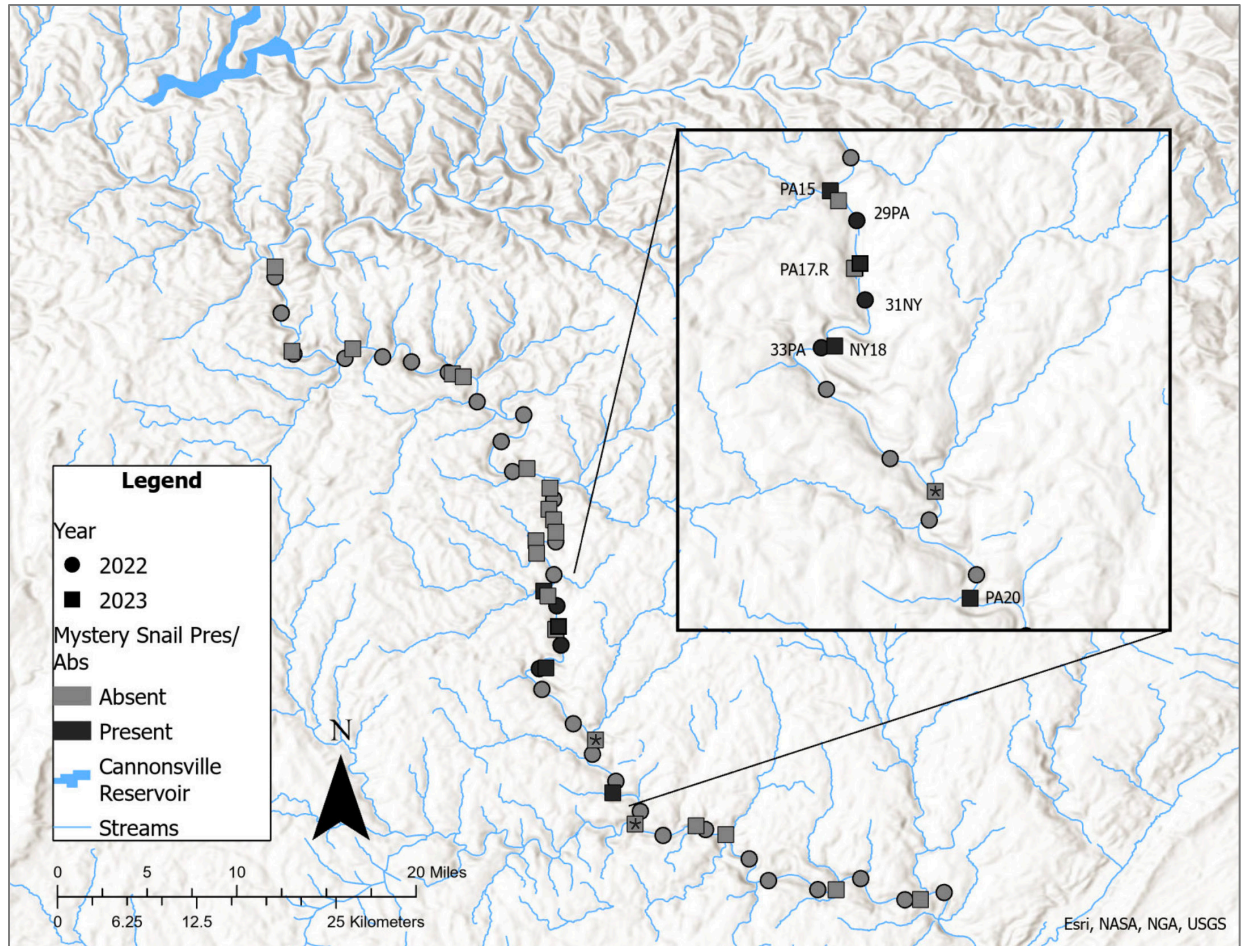


Figure 5. Distribution map of *Cipangopaludina japonica* in UPDE from 2022 to 2024. The asterisks within the shapes indicate the sightings of *C. japonica* in 2024.

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In general, we can see that the *C. japonica* populations were clustered to just seven sites in the center of UPDE (Figure 5). *C. japonica* were found only in the warmwater zone and in higher numbers at sites that had more anthropogenic influences, such as access points (Figure 6). For example, in 2023, 321 *C. japonica* individuals ($80.25 \text{ individuals}/30\text{min} \pm 34.83$) were found at the Narrowsburg Access (site NY18), while only 38 total (28 individuals or 7 individuals/ 0.25 m^2 in Riffle/Run, 10 individuals/30min on boulder) were found at site 29PA in 2022. The backwater site *C. japonica* CPUE was 2 individuals/30min.

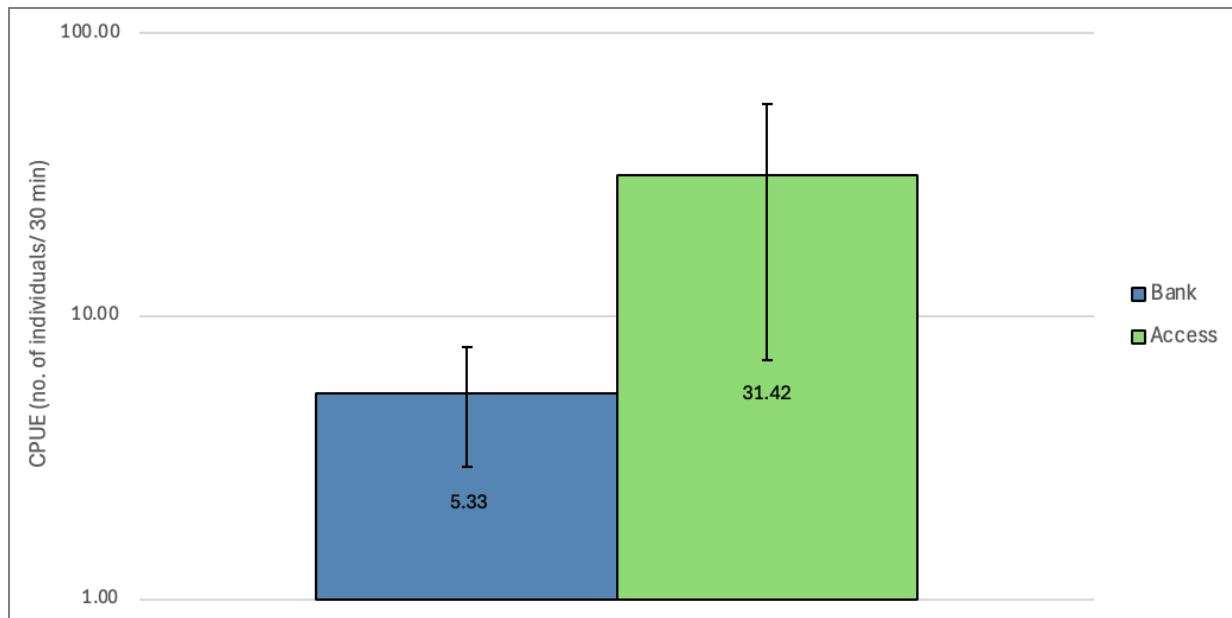


Figure 6. Bar graph of the mean catch per unit effort (number of individuals found in 30 mins; CPUE) per site type for *Cipangopaludina japonica*. The y axis is log scaled. The mean CPUE for Bank Sites was 5.33 individuals/30min $\pm$ 2.4 SE, number of bank sites = 3. The mean CPUE for access point sites was 31.42 individuals/30min $\pm$ 24.42 SE, number of access sites = 3. Access sites are from the 2023 sampling, while the bank sites are from 2022. The error bars show the standard error.

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Previous freshwater snail surveys in Pennsylvania did inventory a mystery snail; however, that snail is listed as the Chinese Mystery Snail and not the *C. japonica* (Evans 2004, Evans and Ray 2010). Mystery snails are very difficult to identify to species using traditional taxonomic characteristics, especially since shells can vary even within species (Mackie 2022).

It is interesting that *C. japonica* were isolated to 7 sites; suitable habitat combined with anthropogenic dispersal may be the cause (Mackie 2022). *C. japonica* are popular aquarium snails that are easily transported via boats and aquarium dumping. There is potential for these large introduced species to compete with native snails, and possibly other macroinvertebrates that fall into the same niche. This species, like most mystery snails, can outcompete other species and impact nutrient cycling (Mackie 2022). Management of this snail within UPDE is currently underway via a pilot project funded by the National Park Foundation.

Distribution

Snails were found at all 54 sites along the UPDE, but different taxa had different ranges (Figure 7). *Amnicola limosus*, *Physella*, and *Galba* were found throughout UPDE however they were not found at every site. *Planorbella trivolvis* was not found in the northernmost sites; they were increasingly found further downstream. *Gyraulus*, *Helisoma anceps*, and *Menetus dilatatus* were not found in the downstream-most bottom 4 sites and *Pseudosuccinea columella* was not found in the downstream-most 5 sites; *Gyraulus*, *H. anceps*, *M. dilatatus*, *P. columella* were found throughout the rest of the river, with *M. dilatatus* being the most sparse. *P. columella* occurred more frequently at the

upstream-most sites and were found sporadically downstream, to the end of UPDE. Ancylinae were found along much of the river, however they seemed to be found in clusters in the middle of UPDE and towards the end of UPDE. *Elimia virginica*, *Campeloma decisum*, *C. japonica*, and *Valvata bicarinata* were found in a smaller subset of sites, with *V. bicarinata* being found the least. *E. virginica* was observed near the confluence of the Lackawaxen River and found upstream of the Mongaup access point. This range is quite small, yet they were found in high numbers (Table 3). Notably, *C. japonica* was detected at more sites in 2023 than in 2022. *C. japonica* prefers more slow-moving water and more sites in 2023 were backwaters or access points which had eddies, stagnant water, and low flow.

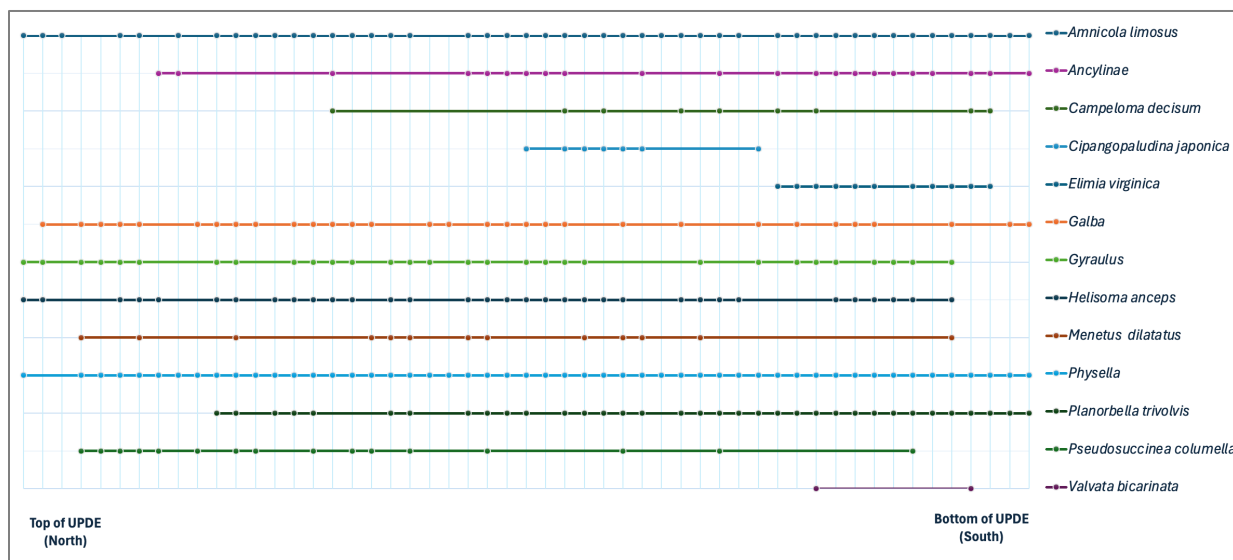


Figure 7. Taxa ranges for 2022 and 2023. The visualization shows all 54 sites from the top of UPDE to the bottom. The lowest taxonomic classification is on the y-axis in alphabetical order. The dots along the lines indicate the sites where snails were found.

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Habitat Preferences

The habitats sampled were based on what was present at each site. Most sites contained boulders (including concrete and metal features) and macrophytes (including woody debris) (Figure 8). The habitats most individuals preferred were boulders and macrophytes. Table 3 shows the taxa with the habitats in which they occurred for 2022 and 2023. Many taxa were found on multiple habitat types, but boulders and macrophytes hosted the most individuals. *Menetus dilatatus* was found only in macrophytes in 2022 but in 2023 this species was found in all five habitats. *Valvata bicarinata* was only found in mud. *Pseudosuccinea columella* was found only on boulders and macrophytes.

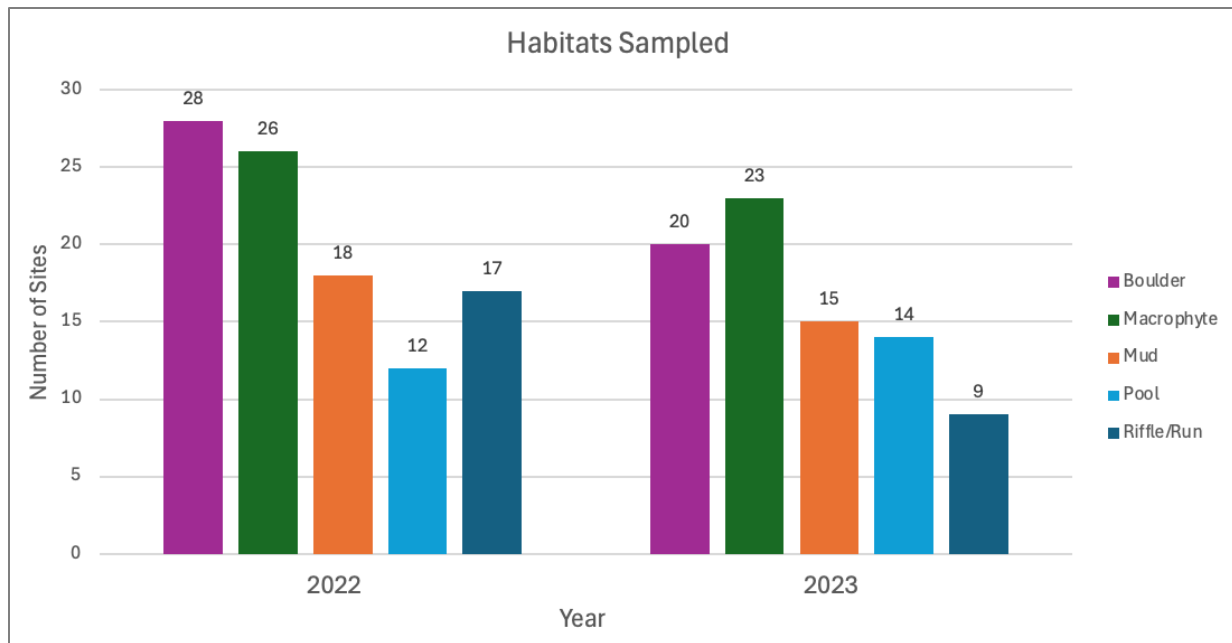


Figure 8. Habitats sampled in 2022 and 2023. Boulder, macrophyte, and mud were sampled using hand collection, while pools, riffles and runs utilized kick-net sampling. In 2022, sites were selected based on existing eDNA atlas sites, while 2023 sites were selected based on unique river features missed in the previous year's sampling.

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Table 3. Taxa list by site with habitat data for 2022 and 2023 sampling. The total number of individuals, the range of the sites where these individuals were found, and the habitats from most occurrence to least occurrence is also listed. The parentheses indicate the same number of occurrences for the habitats within.

Family	Lowest Classification	Year	No.	Site Range	Habitats Found (From most occurrences to least occurrences)
Amnicolidae	<i>Amnicola limosus</i>	2022	3853 ^A	1PA – 59NY	Macrophyte, (Boulder, Riffle/Run), Mud, Pool
	<i>Amnicola limosus</i>	2023	1415	NY1 – NY28	Macrophyte, Mud, Boulder, Pool, Riffle/Run
Lymnaeidae	<i>Galba</i>	2022	211	1PA – 59NY	Boulder, Macrophyte, Mud, Pool, Riffle/Run
	<i>Galba</i>	2023	427	PA3 – NY28	Boulder, Macrophyte, Mud, Riffle/Run, Pool
	<i>Pseudosuccinea columella</i>	2022	13	5PA – 51NY	Boulder, Macrophyte
	<i>Pseudosuccinea columella</i>	2023	32	PA3 – NY18	Macrophyte, Boulder

^A Adjusted from 3,852 snails to account for difference due to subsampling.

Table 3 (continued). Taxa list by site with habitat data for 2022 and 2023 sampling. The total number of individuals, the range of the sites where these individuals were found, and the habitats from most occurrence to least occurrence is also listed. The parentheses indicate the same number of occurrences for the habitats within.

Family	Lowest Classification	Year	No.	Site Range	Habitats Found (From most occurrences to least occurrences)
Physidae	<i>Physella</i>	2022	4408	5PA – 59NY	Boulder, Macrophyte, Riffle/Run, Mud, Pool
	<i>Physella</i>	2023	4736	NY1 – NY28	Macrophyte, Boulder, Mud, Pool, Riffle/Run
Planorbidae	<i>Ancylinae</i>	2022	235	9PA – 59NY	Boulder, Macrophyte, Riffle/Run, Mud, Pool
	<i>Ancylinae</i>	2023	41	PA13 – NY24	(Macrophyte, Boulder), (Mud, Pool)
	<i>Gyraulus</i>	2022	57	1PA – 51NY	Macrophyte, (Mud, Pool), Riffle/Run
	<i>Gyraulus</i>	2023	441	NY1 – PA27	Macrophyte, Pool, (Boulder, Mud, Riffle/Run)
	<i>Helisoma anceps</i>	2022	135	1PA – 51NY	Boulder, Macrophyte, (Mud, Pool) Riffle/Run
	<i>Helisoma anceps</i>	2023	213	NY1 – PA27	Mud, Boulder, (Macrophyte, Pool), Riffle/Run
	<i>Menetus dilatatus</i>	2022	3	23NY – 33PA	Macrophyte
	<i>Menetus dilatatus</i>	2023	47	PA3 – PA27	Macrophyte, Boulder, Pool, Mud, Riffle/Run
	<i>Planorbella trivolvis</i>	2022	270	7NY – 59NY	Boulder, Macrophyte, Riffle/Run, Mud, Pool
	<i>Planorbella trivolvis</i>	2023	443	NY5 – NY28	Macrophyte, Boulder, Mud, Pool, Riffle/Run
Pleuroceridae	<i>Elimia virginica</i>	2022	694	43NY – 57PA	Boulder, Riffle/Run, Mud, Macrophyte, Pool
	<i>Elimia virginica</i>	2023	96	PA22 – PA27	Boulder, (Macrophyte, Pool), Mud
Valvatidae	<i>Valvata bicarinata</i>	2022	1	57PA	Mud
	<i>Valvata bicarinata</i>	2023	11	NY23	Mud
Viviparidae	<i>Campeloma decisum</i>	2022	48	21PA – 57PA	Mud, (Boulder, Macrophyte), Riffle/Run
	<i>Cipangopaludina japonica</i>	2022	48	29PA – 33PA	Boulder, (Macrophyte, Riffle/Run)
	<i>Cipangopaludina japonica</i>	2023	348	PA15 – PA20	Boulder, Mud, Macrophyte, Pool

^A Adjusted from 3,852 snails to account for difference due to subsampling.

Figure 9 displays all habitat types and the number of individuals found by families of snails. The family Physidae was typically observed on boulder and macrophyte habitats. The second most common was the species *Amnicola limosus* (Amnicolidae), which was observed more in macrophytes.

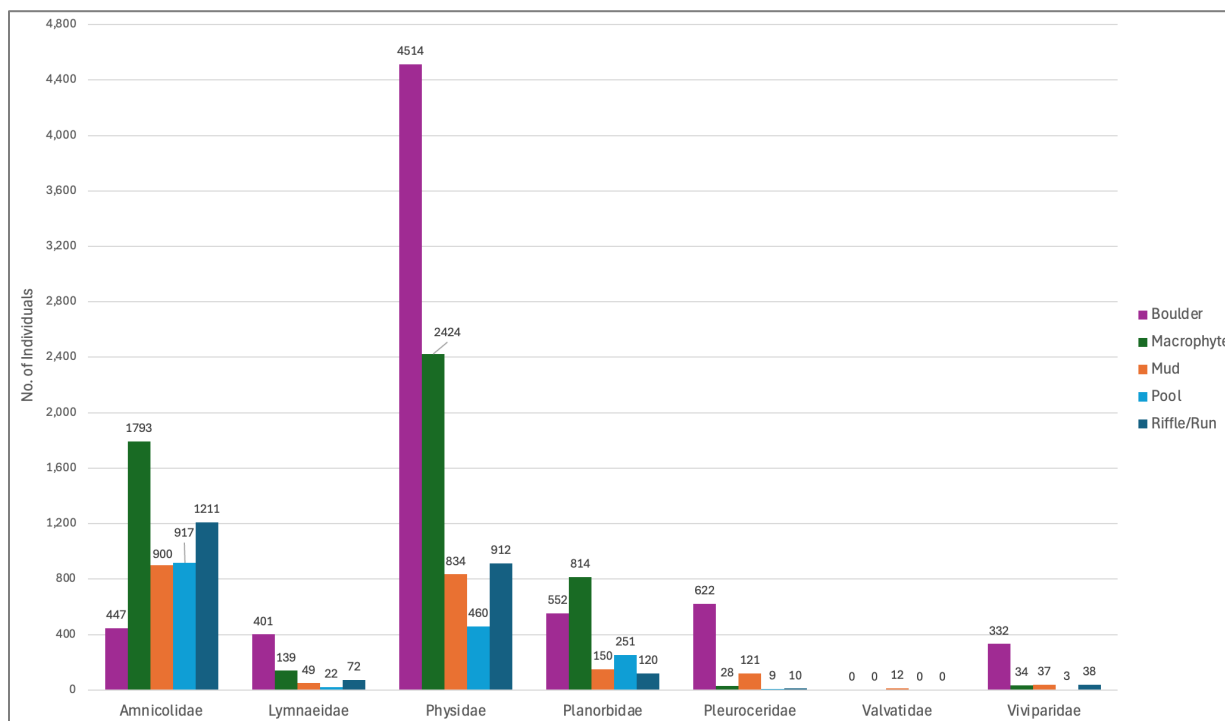


Figure 9. Number of individuals by family for the habitat types for 2022 and 2023.

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Density

In pools, the family Amnicolidae had the greatest mean density (10.75 individuals per 0.25 square meter, mean $\pm$ SE), followed by Physidae (9 individuals per 0.25 square meter; Figure 10). Amnicolidae had a higher mean density in riffle or runs than in pools. There were two Viviparidae occurrences in riffle or runs in 2022 and no occurrences in pools. Lymnaeidae and Pleuroceridae also had low densities and low occurrences. Outlier data points that were more than 1.5 times higher or lower than the middle 50% of the data distribution (the interquartile range) were removed from analysis; those values are 202 individuals/0.25m² for Amnicolidae in pools, 124.25 individuals/0.25m² for Amnicolidae in riffles/runs, 79 individuals/0.25m² for Physidae in pools, 22.33 and 23.33 individuals/0.25m² for Physidae in riffles, and 20.33 individuals/0.25m² for Planorbidae in riffles/runs. It should also be noted the small sample size: Lymnaeidae pool, n= 2; Lymnaeidae riffle, n=1; Pleuroceridae pool, n=1; Viviparidae riffle, n=2. Site 41PA is excluded from this graph due to a sorting error. Pools had a higher mean density than riffles or runs (6.26 individuals/0.25 square meter on average for all taxa versus 4.4, respectively).

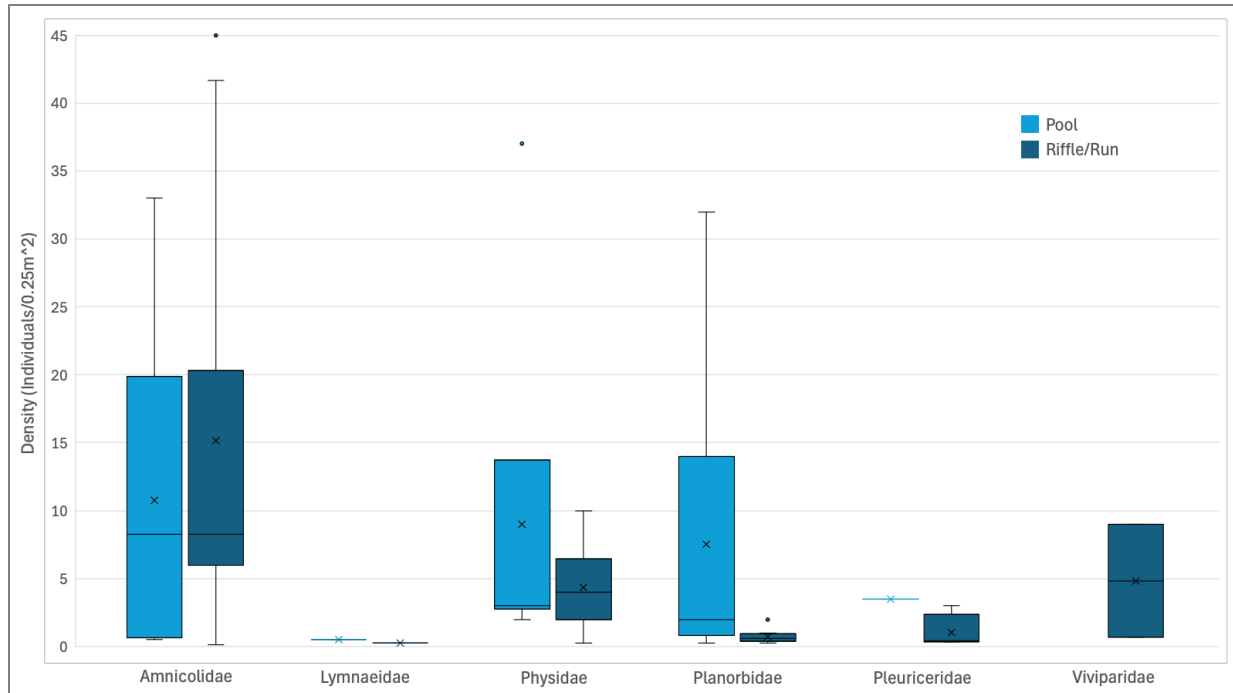


Figure 10. Density (number of individuals per 0.25 square meter) by family for 2022 pool and riffle kick-net samples. The “x” is the mean.

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The highest mean CPUE was Physidae (103.60 individuals per 30 minutes) and Pleuroceridae (46.50 individuals per 30 minutes) on boulders (Figure 11). Amnicolidae had a relatively low mean, however they had the most data removed from the analysis based on the criteria for outliers (9 data were removed, 3 of which were the highest CPUE: 355 and 343 individuals/30min in macrophyte, and 413 individuals/30min in mud). Outlier data points that were more than 1.5 times higher or lower than the middle 50% of the data distribution (the interquartile range) were removed from analysis; those values are 82, 77, and 29 individuals/30min for Amnicolidae in boulder; 355, 343, 166, and 124 individuals/30min for Amnicolidae in macrophyte; 413 and 109 individuals/30min for Amnicolidae in mud; 103 individuals/30min for Lymnaeidae in boulder; 3 individuals/30min for Lymnaeidae in macrophyte; 10 individuals/30min for Lymnaeidae in mud; 88 individuals/30min for Planorbidae in boulder; 39 individuals/30min for Planorbidae in macrophyte; 260 individuals/30min for Pleuriceridae in mud; and 12 individuals/30min for Viviparidae in mud. There was only one Valvatidae found in mud (it is not shown in figure 11). Pleuroceridae had low occurrences in macrophytes and mud (n=3 for both habitats) as well, Lymnaeidae had low occurrences in mud (n=4, one outlier removed). Viviparidae had low occurrences in macrophytes and boulders but higher occurrences on mud (n=4, n=5, and n=11 [one outlier removed] respectively).

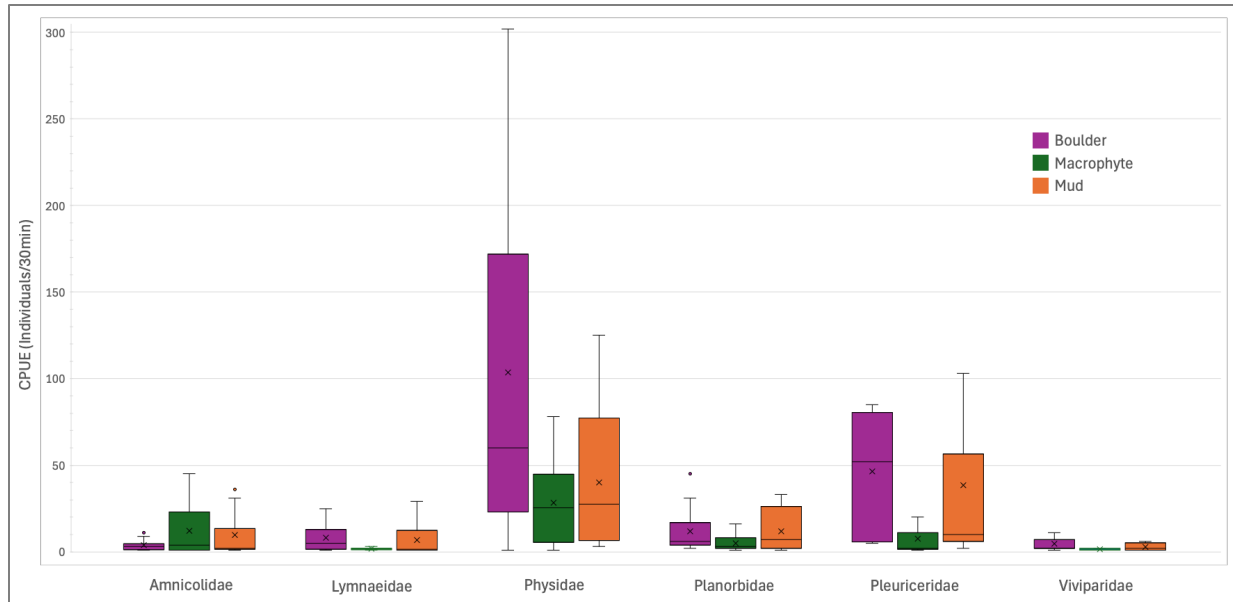


Figure 11. Catch per unit effort (number of individuals per 30 minutes) by family for 2022 boulder, macrophyte and mud hand collected samples. The “x” is the mean.

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Examining CPUE by habitat type shows that in both years, most individuals were found on boulders (Figure 12). Macrophytes were also popular habitat types, but more so in 2023 compared to 2022, potentially due to a higher number of macrophyte habitats encountered in backwaters and islands (Figure 12). Outlier data points that were more than 1.5 times higher or lower than the middle 50% of the data distribution (the interquartile range) were removed from analysis: 2022 macrophyte – 427, 404, 234 number of individuals/30min; 2022 mud – 485, 278, 240 number of individuals/30min; 2023 macrophyte – 693 number of individuals/30min; 2023 mud – 281 and 137 number of individuals/30min. In 2022, the largest value was 320 individuals/30min at site 43NY and in 2023 the largest value was 248.33 individuals/30min at site NY18. In 2022, CPUE on boulders ranged from 1 to 320 individuals/30min, with a mean of 138.17 individuals/30min. CPUE on macrophytes ranged from 3 to 156 individuals/30min (with three outliers of 427, 404, and 234 individuals/30min), with a mean of 44.13 individuals/30min. CPUE in mud ranged from 1 to 101 individuals/30min (with three outliers, 485, 278, 240 individuals/30min), with a mean of 24.07 individuals/30min. In 2023, CPUE on boulders ranged from 18.46 to 248.33 individuals/30min, with a mean of 140.81 individuals/30min. CPUE on macrophytes ranged from 7 to 305 individuals/30min (with one outlier, 693 individuals/30min), with a mean of 112.38 individuals/30min. CPUE in mud ranged from 4 to 55 individuals/30min (with two outliers, 281 and 137 individuals/30min), with a mean of 23.92 individuals/30min.

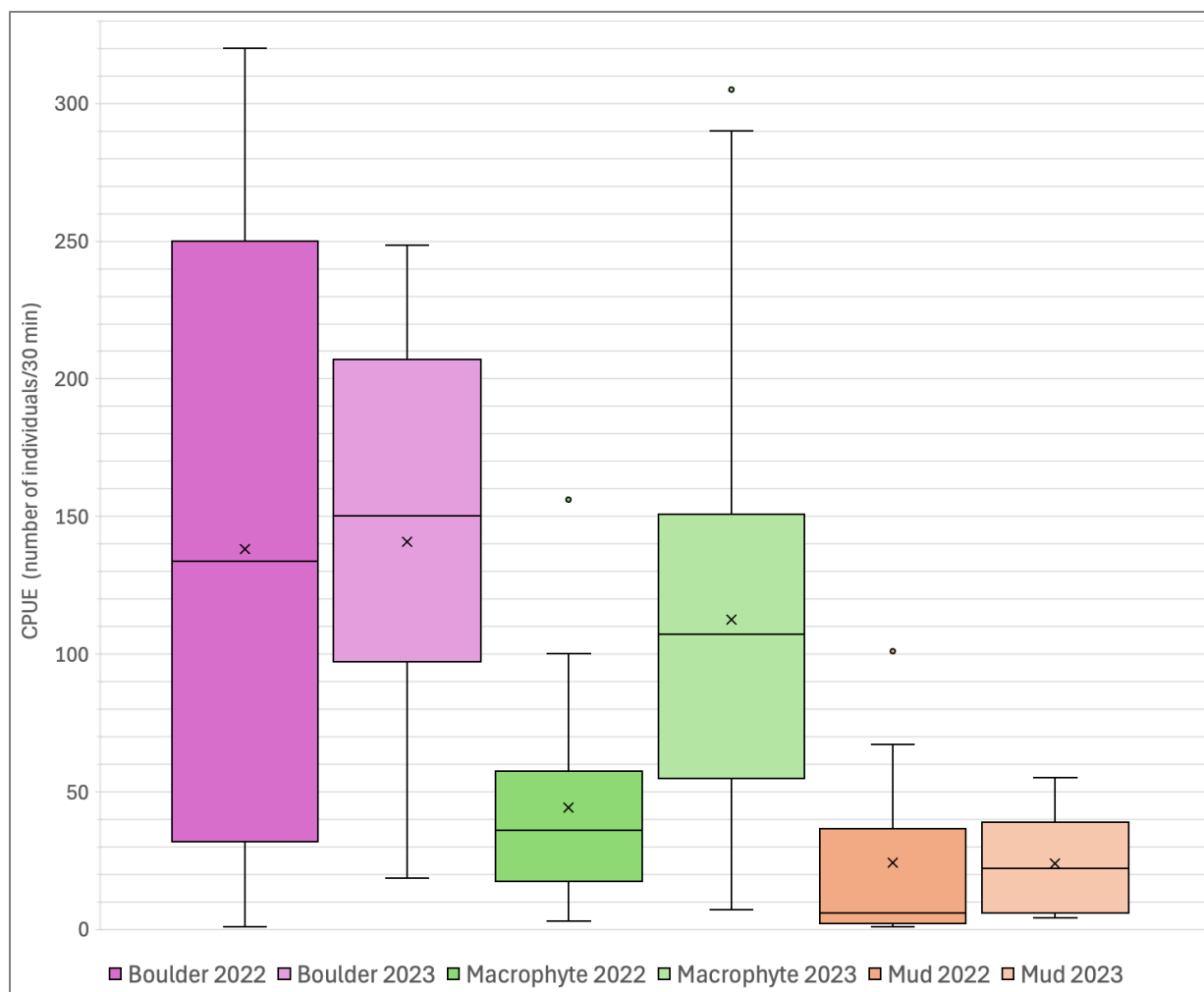


Figure 12. Composite CPUE (number of individuals per 30 minutes) for all sampling sites as a function of habitat for both years of sampling. The “x” is the mean.

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When density was examined, there is no significant difference between pool and riffle/run habitat (Figure 13). Outlier data points that were more than 1.5 times higher or lower than the middle 50% of the data distribution (the interquartile range) were removed from analysis. Outliers not observed in the figure include: 313 individuals/0.25m² in 2022 and 104.5 individuals/0.25m² in 2023, both in pools; and 129 individuals/0.25m² in 2022 in riffle/runs. Site 41PA was excluded from this graph due to a sorting error.

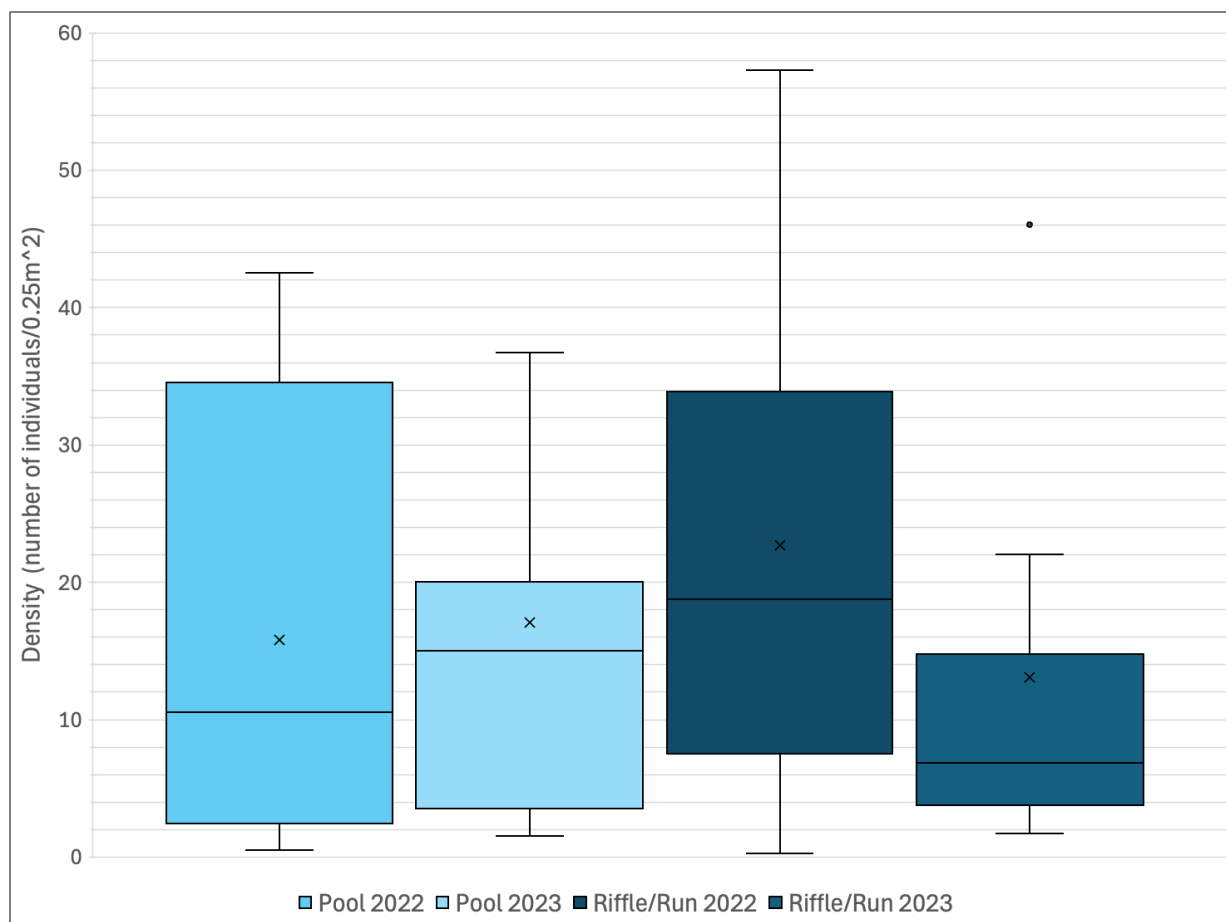


Figure 13. Density (number of individuals per 0.25 square meters) of pool and riffle/run habitats for both years of sampling. The “x” is the mean.

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Temperature

The UPDE temperature gradient is impacted by tributaries, surrounding reservoirs and hydropower plants. To verify this, water temperature data from USGS gages along the mainstem of the Delaware (see Analysis Methods) showed that the difference between the temperatures upstream and downstream of Callicoon are significantly different ($p = 1.929907e-13$). Even with larger tributaries further downstream entering the river at Lackawaxen and Mongaup, the temperatures stay consistent (USGS 2024).

Though the sample size is small (cold pool $n=4$, warm pool $n=5$, cold riffle/run $n=3$, warm riffle/run $n=12$), results suggest a pattern of higher snail density in the warmwater zone (below Callicoon) than in the cold-water zone for both pools and riffle/runs (Figure 14, Figure 15). Outlier data points that were more than 1.5 times higher or lower than the middle 50% of the data distribution (the interquartile range) were removed from analysis: site 49PA (pool, CPUE= 313 number of individuals/0.25m²); site 55NY (riffle/run, CPUE= 129 number of individuals/0.25m²); and site 21PA (riffle/run, CPUE= 33.5 number of individuals/0.25m²). Site 41PA is excluded from this graph due to a sorting error.

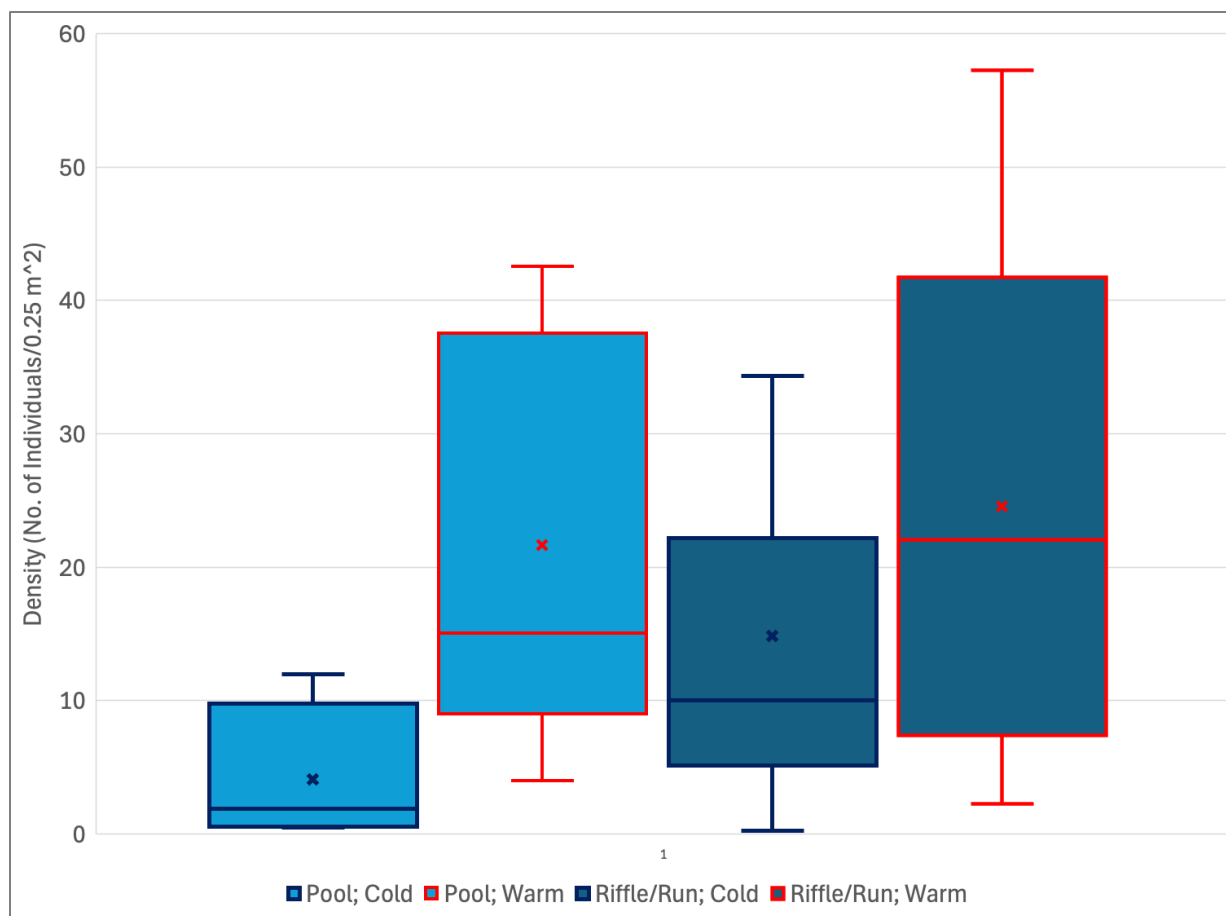


Figure 14. The comparison of density for the two sections of river: cold-water (indicated with a blue outline) and warmwater (indicated with a red outline) zones for both pools and riffle/runs. The “x” is the mean.

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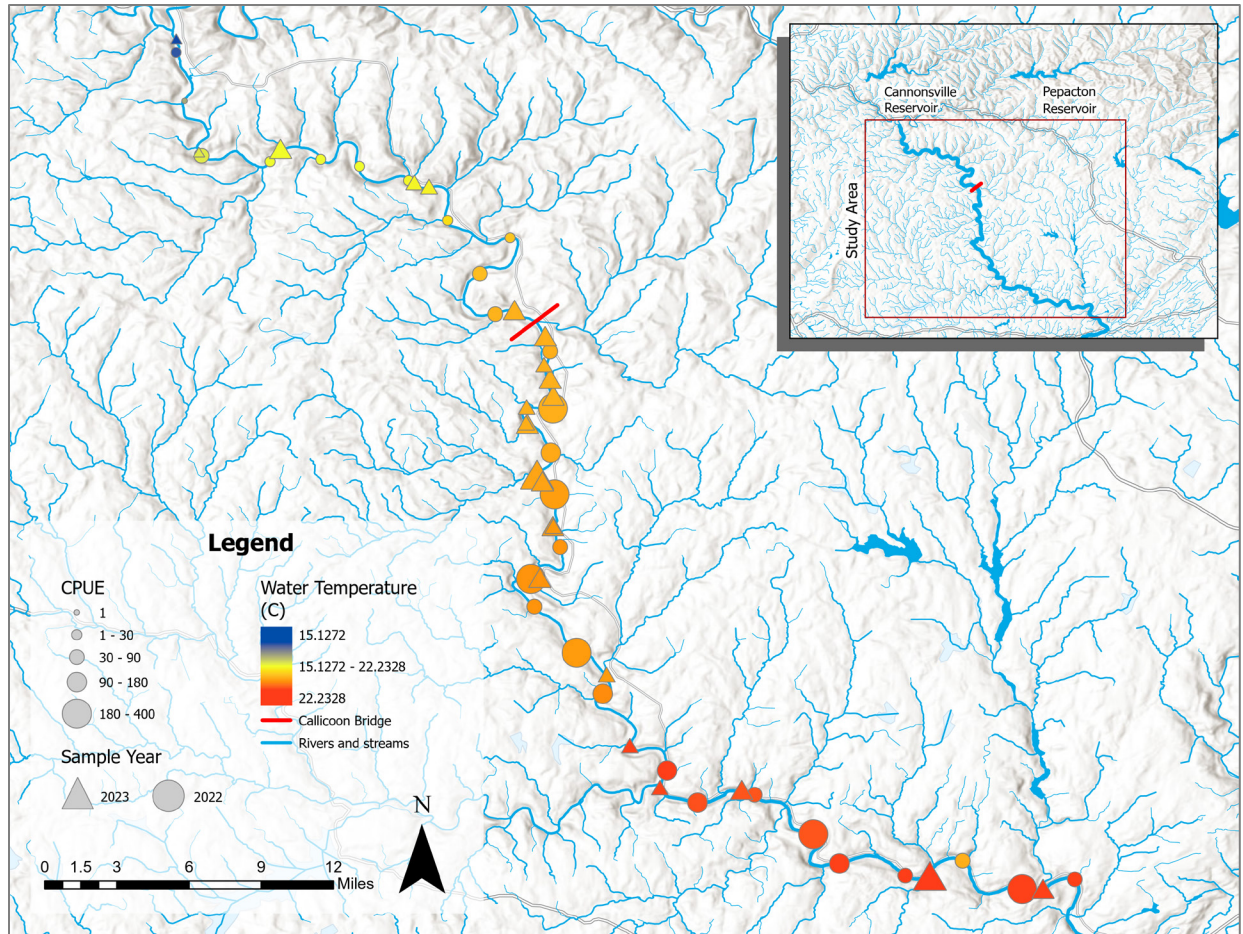


Figure 15. Map of the mean catch per unit effort (CPUE) by site and the interpolated temperature of the river for both years. The size of the shape indicates the CPUE: the larger the shape, the higher the number of individuals per collection time. Water temperature is indicated by color; lower temperatures appear blue while warmer temperatures appear orange to red. A red line across the sites indicates the cold and warm water delineation, which is at Callicoon Bridge.

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Our results suggest a correlation between snail abundance and stream temperature change at Callicoon. Silldorff and Limbeck (2009) also note this temperature change near Callicoon and attribute it to the inputs from the Cannonsville Reservoir on the west branch of the Delaware River. Additionally, others have found most freshwater snail species prefer warmer water and are more abundant in warmwater zones (Thorp and Covich 2010, Van der Schalie and Berry 1973). The Cannonsville Reservoir releases cold water from the bottom which decreases the temperatures downstream, thus creating a consistent temperature zone. The river transitions to warmer, natural temperatures around Callicoon, and so there are two zones within UPDE: the cold-water and warmwater zones.

It is interesting to consider the impact of more frequent dam releases that could change the temperature gradient of the river, possibly impacting the snail community. Climate change will create unpredictable changes; with this basic understanding of how snails respond to temperature better

forecasting impacts is now easier. Additionally, snails overwhelmingly preferred boulders, macrophytes and areas where the flow of the water is slower; dam releases are known to increase flow, and this increase could negatively impact snail distribution (Ferencz et al. 2019).

eDNA

eDNA results allow us to understand unseen members of the community at a specific moment in time, allowing future studies to compare community structure as climate change inevitably impacts the ecosystem. These results do show the diversity of the community; however, it's important to understand that eDNA results can detect species that are not physically in the river, but rather their DNA has been transported to the sampling location by other means (Goldberg et al. 2016). Since this section of the Delaware River has a significant recreational component, it's important to keep in mind that other organisms, local fisherman and people who recreate there could be bringing genetic material from nearby areas.

eDNA results for bacteria, mussels, and fish revealed 30 taxonomic classes of bacteria, 6 species of bivalves, and 24 species of fish are in Appendix C and accessible from <https://irma.nps.gov/DataStore/Reference/Profile/2307735>.

Recommendations

Freshwater snails in the UPDE are plentiful and diverse. While they are spread from Hancock to Mongaup, results suggest that there is a correlation between the water temperature regime and the distribution and abundance of freshwater snails in the UPDE. If snail communities are to be kept stable, it is recommended that there are no increases in the number of inputs that would decrease the temperature of the water (i.e., from reservoirs). Climate change will most likely increase annual average temperatures, so continuing long term temperature monitoring at gages ensures the ability to forecast impacts on the snail community.

Additionally, when considering the habitats snails occupied, they overwhelmingly prefer boulders and macrophytes. To ensure a stable snail community these types of habitats should be preserved, when possible, and management should focus on maintaining these areas.

The impact of *C. japonica* is generally unknown even though they've been in North America for around 100 years (Mackie 2022). While there has been more research on Chinese Mystery Snails, even their ecological impacts have proven indeterminate. In a study by Schuler et al. (2020), they found that an introduced species in the same family, Viviparidae (the Banded Mystery Snail), had a significant impact on increased nitrogen and phosphorus levels when in high-nutrient environments. While the Schuler study did not observe the *C. japonica*, it suggests that similar introduced species could impact ecosystem nutrient cycling. A future pilot study by ANS will investigate the impacts of mystery snails in urban parks and specifically the impacts on nutrient cycling. In July of 2025, ANS will once again partner with the NPS at UPDE to conduct a *C. japonica* removal project (funded by the National Park Foundation) and develop an outreach program to educate the public. The goal of this project is to determine effective methods for *C. japonica* removal and develop a citizen science outreach program that will continue to monitor and remove *C. japonica* in UPDE. Any future studies on *C. japonica* should include a budget for tissue barcoding to confirm the species when possible.

We recommend future studies that focus on the biota of the Delaware River continue to take water samples and analyze it for eDNA to create a long-term data set that can show changes over time. It is also recommended that some ground truthing be conducted of the eDNA results, in particular the *E. dariensis* and *P. grandis* mussels and the four species of fish that are not found in the basin (Lake sturgeon, Atlantic herring, Bluebreast darter, and Pink salmon). As discussed previously, eDNA can be a useful bioassessment tool in aquatic ecosystems, however there are limitations with initial data and further studies, both genetic and ecological, are needed to gain a full understanding of biodiversity. eDNA can continue to be used for biodiversity assessments but experts should be consulted for specific taxa groups (i.e., malacology).

Specimens that were tissue barcoded and whose BINs did match any type specimens should be examined by gastropod taxonomists and assigned names or describe them as new species. ANS is currently seeking funding to complete this work.

Evans (2004) found that dissolved oxygen and water hardness (mg/L of calcium and/or magnesium) had a significant impact on snail distribution, showing higher abundance where water had adequate calcium levels. Snails require calcium for egg development (Madsen 1987, Thorp and Covich 2010). Dissolved oxygen levels differentiate prosobranch (gill-breathing) gastropods, which need stable aquatic environments, from pulmonate (lung-breathing) gastropods, which can adapt to variable and temporary habitats (Thorp and Covich 2010). The Evans (2004) study found pulmonate snails in diverse, fluctuating environments like reservoirs, woodland pools, ditches, and road pools. Water hardness was not measured, nor did we examine the effects of dissolved oxygen, therefore it is recommended to add this additional layer of data for future studies to determine if it also has an impact on the freshwater snail community abundance and distribution. It is also recommended that future studies include the additional habitats of woodland pools, ditches, canals, and road pools to ensure this survey did not miss any additional species.

ANS will continue to seek funding opportunities to explore ideas about the influence from competition and co-occurrence with macroinvertebrates and impacts that predation from birds and fish have on the native versus introduced species.

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Appendix A: Programming Code and Script

The R script below was used to calculate the Inverse Distance Weighting (IDW) temperature interpolation.

```
library(sp)
library(gstat)
library(sf)
library(this.path)

gages <- read.csv(gsub("/", "\\\\",
file.path(dirname(this.path()), "gages.csv", fsep = "/")),
fileEncoding = "UTF-8-BOM")

sites <- read.csv(gsub("/", "\\\\",
file.path(dirname(this.path()), "sites.csv", fsep = "/")),
fileEncoding = "UTF-8-BOM")

coordinates(gages) <- ~lon + lat
proj4string(gages) <- CRS("+proj=longlat +datum=WGS84") # ensure
the correct projection

coordinates(sites) <- ~lon + lat
proj4string(sites) <- CRS("+proj=longlat +datum=WGS84")

# idw

idw_result <- gstat::idw(temp2021 ~ 1, gages, sites, idp = 2)

idw_temp <- as.data.frame(idw_result)
print(idw_temp)
write.csv(idw_temp, "idw interpolation.csv", row.names = FALSE)

# krig

vgm_model <- variogram(temp2022 ~ 1, gages)
```

```
vgm_fit <- fit.variogram(vgm_model, model = vgm("Gau"))

kriging_result <- krige(temp2022 ~ 1, gages, sites, model =
vgm_fit)
krig_temp <- as.data.frame(kriging_result)
write.csv(krig_temp, "krig interpolation.csv", row.names = FALSE)
```

Appendix B: Comparative Taxa List

Freshwater snail inventories have occurred throughout the Delaware Basin since 1992 (Jokinen); for a complete comparative list see Table 4. In Jokinen's 1992 survey, the aim was to create a species list for the state of New York. There were very few sites on the Delaware River, none of which were within the bound of UPDE.

Table 4. Comparing findings from previous surveys and checklists. Based on the results from this study's findings, specifically the 2022 tissue barcoding results, we compare and update the findings from four studies, Jokinen 1992, Evans 2004, Bilger et al. 2005, Evans and Ray 2008, Evans and Ray 2010. Since we are still analyzing the genetic results, some of the species are not defined; in those circumstances we will use the genus to generalize and compare. Therefore, when a previous survey has more than one species within that genus, they are represented by a second "X"

Species from 2022 Inventory	Tissue Barcode Results	Jokinen, 1992	Evans, 2004	Bilger. et al., 2005	Evans and Ray, 2010
<i>Amnicola limosus</i>	—	X	X ^{B, E}	X ^{B, C, E}	X
<i>Somatogyrus sp.</i>	—	—	—	—	—
<i>Galba EG1</i>	<i>Galba</i>	X ^E	—	—	—
<i>Galba humilis</i>	<i>Galba</i>	X ^E	—	—	—
<i>Ladislavella elodes</i>	—	—	—	—	—
<i>Pseudosuccinea columella EG1</i>	<i>P. columella</i>	X ^A	X ^B	—	X ^B
<i>Pseudosuccinea columella EG2</i>	<i>P. columella</i>	X ^A	X ^B	—	X ^B
<i>Ferrissia rivularis EG1</i>	<i>F. rivularis</i>	X ^A	X ^{B, E, F}	X ^{B, C}	X, X ^{B, E, F}
<i>Ferrissia rivularis EG2</i>	<i>F. rivularis</i>	X ^A	X ^{B, E, F}	X ^{B, C}	X, X ^{B, E, F}
<i>Laevapex fuscus</i>	—	—	—	X ^{B, D}	—
<i>Laevapex nipponica</i>	—	—	—	—	—
<i>Gyraulus sp.</i>	<i>Gyraulus</i>	X ^A	—	X ^{B, C} , X ^{B, D}	X ^B , X ^B
<i>Helisoma anceps</i>	—	X ^A	X ^B	—	X ^B
<i>Menetus dilatatus</i>	—	X ^A	—	—	X ^{B, E}

^A Found in the Delaware River but not in the UPDE

^B Found in the Delaware River Basin; the survey may not have specified the location. Those findings are included here because the UPDE is in the Delaware River Basin and sites were indicated on the maps provided.

^C From the Bilger et al., 2005, indicates this is from the survey completed by the New York State Department of Environmental Conservation.

^D From the Bilger et al., 2005, indicates this is from the survey completed by Pike County Conservation District. Pike County is in Pennsylvania and is bordered by the UPDE

^E Taxonomy has changed. The species name used in the survey is no longer valid, for the purpose of comparing between papers, the names have been updated here.

^F Species was combined. Since the survey was published, the species has been combined with another species.

Table 4 (continued). Comparing findings from previous surveys and checklists. Based on the results from this study's findings, specifically the 2022 tissue barcoding results, we compare and update the findings from four studies, Jokinen 1992, Evans 2004, Bilger et al. 2005, Evans and Ray 2008, Evans and Ray 2010. Since we are still analyzing the genetic results, some of the species are not defined; in those circumstances we will use the genus to generalize and compare. Therefore, when a previous survey has more than one species within that genus, they are represented by a second "X"

Species from 2022 Inventory	Tissue Barcode Results	Jokinen, 1992	Evans, 2004	Bilger. et al., 2005	Evans and Ray, 2010
<i>Planorbella trivolvis</i> EG1	<i>P. trivolvis</i>	X	X ^B	—	X ^B
<i>Planorbella trivolvis</i> EG2	<i>P. trivolvis</i>	X	X ^B	—	X ^B
<i>Elimia virginica</i>	—	—	—	—	X ^B
<i>Physella</i> EG1	<i>Physella</i>	X ^E , X ^{B, E, F}	X	X ^{B, C, E}	X ^{E, F} , X ^{B, E}
<i>Physella</i> EG2	<i>Physella</i>	X ^E , X ^{B, E, F}	X	X ^{B, C, E}	X ^{E, F} , X ^{B, E}
<i>Physella</i> EG3	<i>Physella</i>	X ^E , X ^{B, E, F}	X	X ^{B, C, E}	X ^{E, F} , X ^{B, E}
<i>Physella</i> EG4	<i>Physella</i>	X ^E , X ^{B, E, F}	X	X ^{B, C, E}	X ^{E, F} , X ^{B, E}
<i>Physella</i> EG5	<i>Physella</i>	X ^E , X ^{B, E, F}	X	X ^{B, C, E}	X ^{E, F} , X ^{B, E}
<i>Physella</i> EG6	<i>Physella</i>	X ^E , X ^{B, E, F}	X	X ^{B, C, E}	X ^{E, F} , X ^{B, E}
<i>Physella</i> EG7	<i>Physella</i>	X ^E , X ^{B, E, F}	X	X ^{B, C, E}	X ^{E, F} , X ^{B, E}
<i>Campeloma decisum</i>	—	—	X ^B	—	X ^B
<i>Cipangopaludina japonica</i>	—	—	—	—	—
<i>Valvata bicarinata</i>	—	—	—	—	—

^A Found in the Delaware River but not in the UPDE

^B Found in the Delaware River Basin; the survey may not have specified the location. Those findings are included here because the UPDE is in the Delaware River Basin and sites were indicated on the maps provided.

^C From the Bilger et al., 2005, indicates this is from the survey completed by the New York State Department of Environmental Conservation.

^D From the Bilger et al., 2005, indicates this is from the survey completed by Pike County Conservation District. Pike County is in Pennsylvania and is bordered by the UPDE

^E Taxonomy has changed. The species name used in the survey is no longer valid, for the purpose of comparing between papers, the names have been updated here.

^F Species was combined. Since the survey was published, the species has been combined with another species.

For the checklist by Bilger et al., 2005, taxa were found in different surveys completed by 4 surveying groups (Pennsylvania Department of Environmental Protection, Delaware River Basin Commission, New York State Department of Environmental Conservation, and Pike County Conservation District). However the exact location of the sites is not given so we cannot know what is exclusively in the UPDE. Also, two of the surveys compiled in this checklist keep the taxa to family or genus; Pike County Conservation District and New York State Department of Environmental Conservation identified to species.

The most recent freshwater snail surveys in the Delaware Basin sampled 398 sites across Pennsylvania, 6 sites on the main stem UPDE (one site found no snails, and one site is at the intersection of the Lackawaxen and the Delaware). They found 18 species, 5 species in the UPDE and an additional 2 species in the Lackawaxen, a tributary of the UPDE (Evans and Ray 2010).

The inventory by Evans looked specifically at freshwater snail community in Pennsylvania, sampling one site in UPDE where they found three *Physella acuta*. Two of those species found in the UPDE have since been combined (*Physa ancillaria* and *Physa gyrina* are now *Physella gyrina*) (Evans and Ray 2010).

The surveys and check lists offered some species that were not found in our survey (Table 5). This could be due to the large area of sampling in each survey (sampling the entire Delaware river basin) or possible species extirpation from the river. The only species we did not find in our extensive sampling of the UPDE but was reported by these older surveys to have occurred in the UPDE was *Lyogyrus granum* (family Amnicolidae). We did not find this species in our extensive surveying of the UPDE leading us to wonder if this species has been extirpated from the UPDE or if it was misidentified.

Using these inventories as a reference or species checklist (Bilger et al. 2005, Evans and Ray 2008), can be advisable, however they may not be complete, and they may use obsolete taxonomy.

Table 5. Species not found in this survey but found in previous studies. The report and species found are listed.

Report	Species not found in this Inventory
Jokinen, 1992	<i>Ferrissia walkeri</i>
	<i>Promenetus exacuus</i>
Evans, 2004	<i>Lasdislavella catascopium</i>
	<i>Cipangopaludina chinensis</i>
	<i>Leptoxia carinata</i>
	<i>Planorbella campanulata</i>
Bilger et al., 2005	<i>Bithynia tentaculata</i>
	<i>Planorbella campanulata</i>
Evans and Ray, 2010	<i>Lyogyrus granum</i>
	<i>Bellamya chinesis</i>
	<i>Planorbella campanulata</i>
	<i>Ferrissia californica</i>

Appendix C: eDNA Results

eDNA results allow us to see parts of the community at a moment in time, allowing future studies to compare community structure as climate change inevitably impacts the ecosystem. Our hope is that studies that focus on the Delaware River continue to take water samples and analyze it for eDNA to create a long-term data set that can show changes over time. Our results can show diversity of the community; however, we want to also emphasize that eDNA results can detect species that are not physically in the river, but rather their DNA has been transported to the sampling location by other means (Goldberg et al. 2016). Since this section of the Delaware River has a significant recreational component, it's important to keep in mind that local fisherman and other people who recreate there could be bringing genetic material from nearby areas.

Bacteria analysis shows that the bacterial load in the Delaware River is higher than the tributaries (Figure 16). Water samples from the headwater streams were taken the same year (2022) and were also processed by Jonah Ventures, which allows a comparison of the results. Bacteria within the mainstem of the Delaware River was more abundant and diverse than what was found in smaller headwater streams; this is unusual as the reverse is usually true (Richardson 2019). The bacteria class with the largest abundance belongs to the class Bacteroidia (dark green bands in the bars), the most commonly abundant bacteria found in rivers (Thomas et al. 2011). Actinobacteria and Gammaproteobacteria were moderately abundant and are both very common groups of bacteria found in freshwater ecosystems (van der Meij et al., 2017, Li et al., 2012). Cyanobacteria, also called blue-green algae, has a higher abundance in the first order stream Abe Lord Creek.

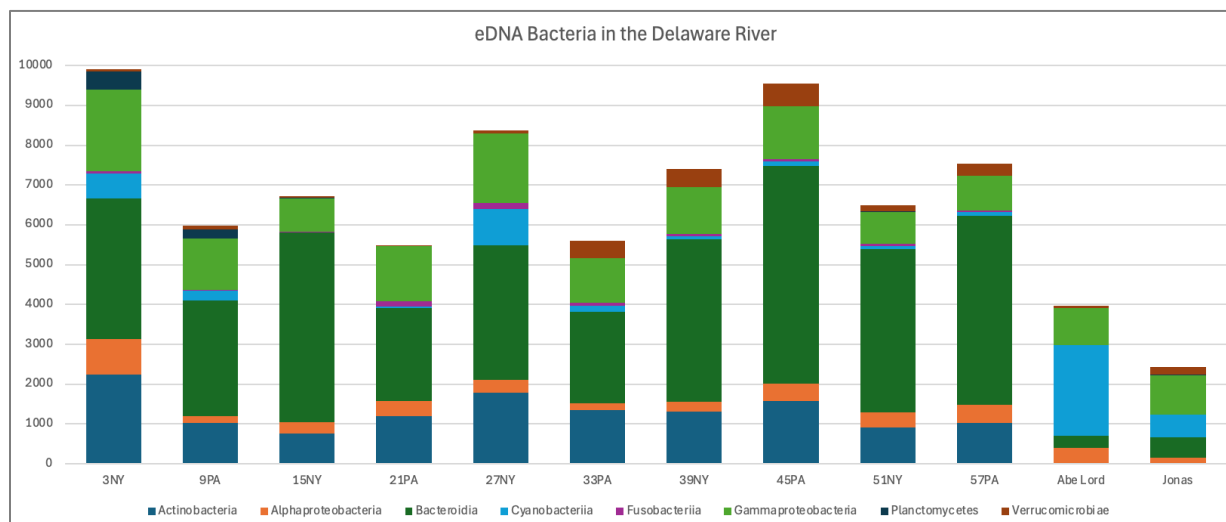


Figure 16. The most abundant bacteria found in eDNA samples using the 16S gene. The colors represent the class of bacteria, and the height of the bar indicates relative abundance of the eDNA. The bacteria from the Delaware River were compared to water samples taken from other headwater streams (Abe Lord Creek and Jonas Creek) within the basin. Sites are organized upstream (3NY) to downstream (57PA) moving from the left side of the graph to the right.

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Normally larger streams have a lower abundance and diversity of bacteria compared to smaller streams (Richardson 2019, Zhao et al. 2023). However, Snyder et al., 2023, discusses the impacts of biofilms, sunlight and transport time on the amount of eDNA that can be detected. Again, there could be additional inputs to the mainstem of the Delaware River from the high amount of recreational use and the reservoir inputs.

The mussel species that was detected most often was *Elliptio complanata*, the Eastern elliptio (Table 6). This species had a high relative abundance of eDNA detected at every sampling location; this high abundance was also detected during the 2014 mussel survey (Silldorff and Schwartz 2014). One other species was also found in relatively high abundance, *Elliptio dariensis*. This species is not commonly found in the northeastern United States. Consultation with malacology experts Arthur Bogan and Curt Elderkin (personal communication, Dec. 4, 2024, and Dec. 5, 2024) proposed that this species is most likely not *E. dariensis*. They suspected that the reference sequence was misidentified, or it is possibly just another sequence for *E. elliptio*. Further fieldwork is needed to visually confirm the presence of a possible new species as it was not recorded in the 2014 survey (Silldorff and Swartz 2014). eDNA results also detected *Pyganodon cataracta* and an *Anodonta* species (most likely *A. implicata*), but the species found with eDNA were found with a relative low abundance. Discussion with New Jersey Department of Environmental Protection Senior Zoologist Robert Somes confirms that the *Pyganodon grandis* has been found in the Hudson River in New York and in Somerset County, New Jersey; this species is native the Mississippi River, but has most likely been introduced to this area (Robert Somes, personal communication, Dec. 10, 2024). *Fusconaia flava* and *Elliptio complanata* were found at nearly every sampling location, while other species were found mainly in the warmwater zones. *Pyganodon grandis* was found in low abundance at two locations, one in the cold-water zone (3NY) and one in the warmwater zone (45PA).

Table 6. List of mussel species found with eDNA metabarcoding of the CO1 and 16S gene. Site codes are listed at the top where those denoted with superscript “A” (also shown in blue) are within the cold-water zones and those denoted with superscript “B” (also shown in orange) are in the warmwater zones. X indicates the species eDNA was detected at that site.

Species Name	3NY ^A	9PA ^A	15NY ^A	21PA ^A	27NY ^B	33PA ^B	39NY ^B	45PA ^B	51NY ^B	57PA ^B
<i>Elliptio complanata</i>	X	X	X	X	X	X	X	X	X	X
<i>Elliptio species</i> ^C	–	–	–	X	X	–	–	X	X	–
<i>Pyganodon cataracta</i>	–	–	–	–	–	–	X	–	–	–
<i>Fusconaia flava</i>	X	X	X	–	X	X	X	X	X	X
<i>Anodonta species</i>	–	–	–	–	–	X	–	–	–	X
<i>Pyganodon grandis</i>	X	–	–	–	–	–	–	X	–	–

^C This is the species that matched to the *E. dariensis* ESV, but that Dr. Bogan and Dr. Elderkin believe is not *E. dariensis*.

The mussel survey along the Delaware River in 2014 revealed 7 species (Silldorff and Schwartz 2014), however eDNA metabarcoding revealed 6 species. Arthur Bogan (personal communication, Dec 4, 2024) thinks that the *E. dariensis* species sequence listed was not correctly identified and is

not *E. dariensis*. In the 2017 revised mussel list for the United States, the genus *Elliptio* is the largest Unionid genus; furthermore the genus has a diversity that is not well understood and untested species concepts due to the variability within shell morphology (Williams et al. 2017). It's possible that our eDNA results are merely confirming the high species diversity within this genus and the difficulties in describing and naming species. Therefore, it's highly plausible that there are two species of *Elliptio* mussels in the Delaware River, one not identified. *Pyganodon grandis* has been verified in New Jersey and New York in watersheds adjacent to the Delaware; it's not beyond reason to believe this species has been introduced into the Delaware River (Robert Somes, personal communication, Dec. 10, 2024). Most of the mussel species found with eDNA were detected at low relative abundance; the number of reads of those sequences were below 50. (For comparison, *E. complanata* has reads of over 1000.) Recent research has discussed how genetic work in mussels can be difficult and this is most likely due to the issues with mussels shedding eDNA at low rates (Ruiz-Ramos et al. 2024).

Fish eDNA has become very common in freshwater surveys around the world and here in the United States, it's an easy way to detect diversity and abundance of fish species (Wang et al. 2024, Hagerty 2023). Our eDNA results were able to detect 28 species of freshwater fish; those species that do not occur in the basin and have issues are highlighted in blue (Table 7). Based on communication with Dr. David Keller of ANS, the lake sturgeon, Atlantic herring, bluebreast darter, and pink salmon do not occur in the basin. The reads for these four species were very low (8–23 reads) and all from one site. The ESV for pink salmon was also a 100% match however, it is more likely that this reflects rainbow trout as pink salmon are not stocked and do not occur in the basin. The ESV for Atlantic herring was only a 98.8% match; this is a marine species, and so it is more likely that this record reflects the presence of other clupeids known to occur in the area, most likely gizzard shad or the more closely related alewife. Lastly, the ESV for the bluebreast darter was a 97.6% match, and because this species is not known to occur in the basin, we believe this record is most likely contamination or a detection of the closely related and native Tessellated darter.

Table 7. List of fish species found with eDNA metabarcoding of the 12S gene. Site codes are listed at the top, where those denoted with superscript “A” (also shown in blue) are within the cold-water zones and those denoted with superscript “B” (also shown in orange) are in the warmwater zones. X indicates the species eDNA was detected at that site. Species denoted with superscript “C” are considered incorrect for the basin.

Species	3NY ^A	9PA ^A	15NY ^A	21PA ^A	27NY ^B	33PA ^B	39NY ^B	45PA ^B	51NY ^B	57PA ^B
<i>Acipenser fulvescens</i> ^C	–	–	–	–	–	–	–	X	–	–
<i>Alosa pseudoharengus</i>	–	X	–	–	–	–	–	–	–	–
<i>Ambloplites rupestris</i>	X	X	X	X	X	X	X	X	X	X
<i>Ameiurus natalis</i>	–	–	–	–	–	–	X	–	–	–
<i>Anguilla rostrata</i>	X	X	X	X	X	X	X	X	X	X
<i>Catostomus commersonii</i>	X	X	X	X	X	X	X	X	X	X
<i>Clupea harengus</i> ^C	–	–	–	–	–	–	–	X	–	–
<i>Cyprinella spiloptera</i>	–	–	–	–	–	X	–	–	–	–
<i>Dorosoma cepedianum</i>	X	X	X	–	X	X	X	X	X	X
<i>Etheostoma camurum</i> ^C	–	–	–	–	–	–	–	X	–	–
<i>Etheostoma olmstedii</i>	X	X	X	X	X	X	X	X	X	X
<i>Hypentelium nigricans</i>	X	X	X	X	X	–	X	X	X	X
<i>Ictalurus punctatus</i>	–	–	–	–	–	X	X	X	–	X
<i>Lepomis auritus</i>	X	–	X	X	X	X	X	X	X	X
<i>Lepomis cyanellus</i>	X	–	–	–	–	–	X	–	X	X
<i>Lepomis gibbosus</i>	X	–	–	X	X	X	X	X	X	X
<i>Lepomis macrochirus</i>	–	–	–	–	–	–	X	–	X	–
<i>Morone saxatilis</i>	X	–	–	–	–	X	–	–	X	X
<i>Notropis hudsonius</i>	X	–	X	–	X	X	X	–	–	X
<i>Oncorhynchus gorbuscha</i> ^C	–	–	–	–	–	–	–	X	–	–
<i>Perca flavescens</i>	X	–	–	–	X	X	X	–	–	–

Table 7 (continued). List of fish species found with eDNA metabarcoding of the 12S gene. Site codes are listed at the top, where those denoted with superscript “A” (also shown in blue) are within the cold-water zones and those denoted with superscript “B” (also shown in orange) are in the warmwater zones. X indicates the species eDNA was detected at that site. Species denoted with superscript “C” are considered incorrect for the basin.

Species	3NY ^A	9PA ^A	15NY ^A	21PA ^A	27NY ^B	33PA ^B	39NY ^B	45PA ^B	51NY ^B	57PA ^B
<i>Percina peltata</i>	X	X	X	X	X	X	X	X	X	X
<i>Petromyzon marinus</i>	X	–	–	–	–	–	–	–	–	–
<i>Rhinichthys atratulus</i>	X	X	–	–	–	–	–	–	–	–
<i>Rhinichthys cataractae</i>	X	X	X	X	X	X	X	X	X	X
<i>Salmo trutta</i>	X	X	X	–	–	–	–	X	–	–
<i>Sander vitreus</i>	X	–	X	–	X	–	X	X	X	X
<i>Semotilus corporalis</i>	X	X	X	X	X	X	X	X	X	X

Our eDNA results were able to detect 28 species of freshwater fish; however, based on communication with Dr. David Keller of ANS, 4 of those species should not be considered present in the basin. The species are: the lake sturgeon, Atlantic herring, bluebreast darter, and pink salmon. This data showed no obvious pattern in fish species occurrence in relation to the temperature gradient other than brown trout appearing to be more associated with the three upper most sites compared to occurring at only one lower site. Also of note, channel catfish were absent from the upper five sites and present in four of five lower sites. However, it is unclear if these observations are being driven by temperature or other habitat variables.

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