Utilizing environmental DNA to identify aquatic invasive species

Lisa Newton¹

INTRODUCTION

This project was designed to develop methods to assess the presence and extent of incursion of several selected aquatic invasive species in the Croton and Delaware watersheds for New York City. There is a need to determine techniques to assess whether invasive species are present and the level of invasion that are cost-effective, rapid and accurate, especially when the species are present in the reservoirs at low densities. Physical surveys and environmental DNA (eDNA) techniques will be used to identify the presence of invasive species in the Ashokan and Rondout reservoirs of the Delaware Watershed and the West Branch, New Croton and Kensico reservoirs of the Croton Watershed.

Environmental DNA is genomic DNA that is released into the environment by an organism in a variety of ways (Pilliod et al. 2013). These include epithelial tissue, feces, gametes and mucous, as well as cells that are released from dead organisms (Pilliod et al. 2013). The use of eDNA can allow a more efficient and relatively inexpensive manner to examine presence/absence of invasive species (Pilliod et al. 2013). Techniques involving eDNA may be especially useful for the early detection of unwanted non-native species, monitoring the levels of native species after invasion of non-native species, and determining the effectiveness of management techniques (Wilcox et al. 2013, Pilliod et al. 2013). A last important benefit of eDNA techniques is that they can replace the traditional survey methods used to assess the levels of invasive species in an area at a much lower cost and with a drastic decrease in overall expended effort required to determine the levels of invasive species (Wilcox et al. 2013).

While the use of environmental DNA has its advantages, it also possesses limitations. DNA obtained from the environment is often very dilute with the DNA of the target species contributing only a small percentage of the total DNA obtained from the eDNA sample, and the non-target DNA possibly being made up of closely related species (Wilcox et al. 2013). Species-specific molecular markers, or other means of differentiating the target species from genetically similar species that may be present, must therefore be developed.

The species selected for this project include Cipangopaludina chinesis, Corbicula fluminea, Dreissena polymorpha, Hydrilla verticillata, Orconectes rusticus and Myriophyllum spicatum. Each has been identified as a known invasive species of New York State and considered of interest to the NYC Department of Environmental Protection in order to determine their presence, as well as abundance, in the selected reservoirs.

Hydrilla verticillata is an aquatic plant of the family Hydrocharitacea. Hydrilla possess slim, branching stems that can grow up to 7.6 m long. Its leaves are small and narrow with a pointed tip, and grow around the stem in whorls, ranging from four to eight leaves in each whorl.

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(Ramey & Peichel 2001a). Its native range is thought to include India, Africa, Australia and portions of Asia. *Hydrilla verticulata* was most likely introduced to the United States through aquarium trade in Florida in the 1950s and 1960s, as well as through mail-order water lilies. Hydrilla forms dense stands of vegetation in almost any freshwater habitat (Ramey & Peichel, 2001a). This plant can grow in both lotic and lentic water, including lakes, rivers, marshes and ditches, and it can grow in a few inches of water or in water up to 6.1 m deep (Ramey & Peichel, 2001a). *Hydrilla verticillata* is also capable of thriving in a variety of conditions, ranging from eutrophic to oligotrophic, as well as being able to grow in a mere 1% of full sunlight, making it highly competitive (Ramey & Peichel 2001a).

*Myriophyllum spicatum*, also known as Eurasian water-milfoil, is a member of the Haloragaceae family (Ramey & Peichel 2001b). It has thin, branched stems that typically grow 1.8-2.7 m in length, although they can reach up to 6.1 m long (Ramey & Peichel 2001b). The leaves of *M. spicatum* are olive-green colored and feather-like in appearance (Ramey & Peichel 2001b). The leaves are arranged in whorls, with 3-6 leaves in each whorl, and each leaf less than 5 cm in length (Ramey & Peichel 2001b). Eurasian water-milfoil is thought to be native to Europe, Asia and northern Africa (NBII & IUCN/SSC ISSG 2006). It is suspected that this plant was introduced intentionally to the United States or was introduced in the 1800s through the release of ship ballast and was first documented in the U.S. in Washington, D.C. in 1942 (Ramey & Peichel 2001b). *Myriophyllum spicatum* prefers habitats which possess slow moving water, such as lakes, ponds and sluggish rivers (Ramey & Peichel 2001b). While its preference is for water movement that is slow, Eurasian water-milfoil can also grow in fast moving water (Ramey & Peichel 2001b). Like Hydrilla, *M. spicatum* can tolerate a wide range of habitat conditions. This plant can prosper in spring water, as well as brackish water (Ramey & Peichel 2001b). It also possess a great deal of temperature tolerance, being able to withstand the winter conditions of frozen lakes in northern areas of the U.S. and to flourish in over-heated bays in southern areas of the U.S. (Ramey & Peichel 2001b).

*Dreissena polymorpha*, commonly known as the zebra mussel, is a small, striped, shellfish that can grow up to 50 mm in size (Benson et al. 2013). *Dreissena polymorpha* will attach itself to any stable substrate in the water body, both natural and artificial, including rock, cement, boat props and other organisms living in the water. Zebra mussels are considered native to the Azov, Black and Caspian Seas (Benson et al. 2013). *Dreissena polymorpha* was initially documented in the United States in 1988, where it was found to be present in the Great Lakes (Benson et al. 2013). Introduction is thought to have occurred through the release of the larval stage of the zebra mussels from a ballast exchange of a commercial ship, which had travelled from the Black Sea to the Great Lakes (Benson et al. 2013). *Dreissena polymorpha* can withstand a variety of temperature variations and has been known to grow in temperatures ranging from 3-30˚C, with optimal growth occurring in the 20-25˚C range (Benson et al. 2013).

*Orconectes rusticus*, or the rusty crayfish, is a large crayfish characterized by an overall greenish blue color, large, tough claws and prominent rust colored spots on either side of its carapace (National Biological Information Infrastructure (NBII & ISSG 2010). The rusty crayfish has been known to grow up to 10 cm in length (NBII & ISSG 2010). *Orconectes rusticus* is native to portions of the U.S., including areas in Indiana, Illinois, Kentucky, Michigan and Ohio (NBII & ISSG 2010). Introduction of *Orconectes rusticus* to other areas in the United
States has occurred through the use of the crayfish as bait by anglers, leading to its classification as an invasive species in many areas (NBII & ISSG 2010). The rusty crayfish can be found in lakes, ponds and streams, failing to display a predilection for lotic or lentic environments (NBII & ISSG 2010). A variety of substrates are acceptable for habitation of *O. rusticus*, including sand, silt, clay and rock (NBII & ISSG 2010). One habitat requirement for the rusty crayfish are areas that contain a variety of debris, such as rocks and submerged wood fragments that can be used to cover and protection (NBII & ISSG 2010). Often, areas with large amounts of debris are found to be preferred by the crayfish. *Orconectes rusticus* is often found in water bodies that have a depth of less than 1 m, but they have also been sampled in water with depths up to almost 15 m (NBII & ISSG 2010). Water that contains high levels of oxygenation is preferred by *O. rusticus*, and it thrives in a temperature range of 20-25°C (NBII & ISSG 2010). While this range of temperature is preferred, the rusty crayfish can survive in temperatures as low as 0°C and as high as 39°C (NBII & ISSG 2010).

*Corbicula fluminea*, commonly known as the Asian clam, is a freshwater mollusk, with shell coloration ranging from a yellow-brown shade to black (National Biological Information Infrastructure (NBII) & IUCN/SSC Invasive Species Specialist Group (ISSG) 2005). The Asian clam can grow up to sizes of 50 mm in length and possesses uniformly spaced concentric rings on the surface of its shell (NBII & ISSG 2005). The clam is native to southeastern areas in China and Russia, as well as Korea and the Ussuri Basin (NBII & ISSG, 2005). *Corbicula fluminea* was first discovered in the United States in the Columbia River in Washington in 1938 (Foster et al. 2013). It was first thought to have entered the U.S. through Chinese immigrants importing the clam for consumption (Foster et al. 2013). The Asian clam is now present in at least 38 states and the District of Columbia (NBII & ISSG 2005). This magnitude of dispersal is thought to be aided by usage as bait, aquarium trade and release of ship ballast water (NBII & ISSG 2005). *Corbicula fluminea* can be found in lakes, streams and estuaries which can contain a range of substrates, including silt, sand and gravel (NBII & ISSG 2005). Its preference of substrate is fine clay or sand, and coarse sand that allows for burrowing (NBII & ISSG 2005). High levels of oxygenated water are required by Asian clams for suitable habitation and it can survive in temperatures that vary from 2-30°C (NBII & ISSG 2005).

*Cipangopaludina chinesis*, also called the Chinese mystery snail, is a freshwater mollusk that has a shell that can be brown, reddish or greenish brown or an olive green color and can grow up to 70 mm in length (National Biological Information Infrastructure (NBII) & IUCN/SSC Invasive Species Specialist Group (ISSG) 2011). The thick shell is convex and typically contains 6 to 7 whorls (NBII & ISSG 2011). *Cipangopaludina chinesis* is considered native to China, Korea, Japan, Java, Myanmar, and the Philippines (NBII & ISSG 2011). It is also native to Eastern Russia, Taiwan and Vietnam (NBII & ISSG 2011). Introduction to the United States is thought to have occurred through sale in the Chinese food market in the late 1800s in San Francisco (NBII & ISSG 2011). It was first documented in Boston in 1914 and was suspected of being released sometime between 1931 and 1942 in the Niagara River (Kipp et al. 2013). The Chinese mystery snail may also have been introduced to non-native areas in a variety of other methods, such as aquarium trade, attachment to recreational boat hulls, ornamental plants shipped into the U.S. and stocked as a food source for catfish in Lake Erie (NBII & ISSG 2011). *Cipangopaludina chinesis* inhabits both slow-moving lotic and lentic water systems that have a substrate consisting of mud or silt (NBII & ISSG 2011). Favorable habitats include
streams, lakes, rivers, canals and ditches (NBII & ISSG 2011). *Cipangopaludina chinesis* has been found to be highly resistant to desiccation, facilitating its distribution to other non-native areas through transport on boats and can also tolerate stagnant water conditions (NBII & ISSG 2011). Discovered at depths ranging from 0.2 to 3 m, the Chinese mystery snail can tolerate a temperature range of 0-30°C and a pH of 6.5-8.4 (NBII & ISSG 2011).

**METHODOLOGY**

This project will involve the utilization of environmental DNA to identify the six selected invasive species of New York through the collection of water samples from five different reservoirs of the Croton and Delaware watersheds. To complete this research, several steps must be accomplished to ensure accuracy and reliability of the techniques developed. DNA extraction directly from each target organism will occur to determine whether the techniques developed to identify each selected species will produce results. To determine whether the target species is present in the selected reservoir, species-specific primers will be utilized when they are obtainable. Other further techniques will be used to determine specific species when species-specific primers are not attainable. Following the direct DNA extraction, extraction of eDNA from an artificial aquatic environment containing the organism will occur. This artificial environment will consist of a tank containing a known quantity of water harboring the target species. Following this, serial dilutions of water from the artificial environments will occur to determine the lowest density of target species at which presence can be detected. Once these levels have been determined, the techniques will be tested on water collected from water bodies that are known to be inhabited by the target species. If successful, this process will then ultimately lead to the testing of the developed techniques on the selected NYC reservoirs.

Molecular identification of each species will occur using a variety of manners. *Cipangopaludina chinesis* and *Corbicula fluminea* will be identified through the development of species-specific microsatellite markers. Extracted DNA from both species will be sent to Cornell where it will be sequenced and microsatellite markers that are specific to each species will be identified. Family specific primers designed by Theriault et al. (2003) will be utilized for identification of *Dreissena polymorpha*. These primers amplify the 28S rRNA gene. One limitation of the use of these primers is that they fail to distinguish between zebra mussels and another member of the dreissenid family, quagga mussels. This is not considered to be significant due to the fact that both species are considered invasive to NYS. *Hydrilla verticillata* will be identified through the use of species-specific primers obtained from Rybicki et al. (in press). These primers will be able to distinguish Hydrilla from other morphologically similar waterweeds, including *Egeria densa*, *Elodea nuttallii*, and *Elodea canadensis*. *Orconectes rusticus* will be identified through the use of family-specific primers developed by Taylor & Knouft (2006) which amplify *Cytochrome Oxidase I*. The utilization of restriction fragment length polymorphisms (RFLPs) will assist in differentiating between the different species of the family cambaridae. RFLPs use restriction enzymes that cut at known locations on the DNA creating specific lengths of fragments based on the species. The restriction enzymes that were selected for *O. rusticus* include NsiI, BsaHI and BsrGI. Molecular identification of *Myriophyllum spicatum* will also involve the use of RFLPs. Moody & Les (2002) developed primers that were effective on *M. spicatum*, but also were effective on other members of the
Myriophyllum genus. To identify our target species, the restriction enzymes BsaHI and Hpy99I will be utilized.

REFERENCES


