




Northern hydrilla (*Hydrilla verticillata* ssp. *lithuanica*): discovery and establishment outside the Connecticut River

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Abstract

Hydrilla [*Hydrilla verticillata* (L. f.) Royle], an invasive aquatic weed, has had a rich introduction history into the United States, with multiple subspecies being introduced since the 1960s. The most recent occurred before 2016, when northern hydrilla (*Hydrilla verticillata* ssp. *lithuanica*) was discovered in the Connecticut River. By 2021, following a 3-yr survey from Agawam, MA, to the Long Island Sound by the Connecticut Agricultural Experiment Station Office of Aquatic Invasive Species, *H. verticillata* ssp. *lithuanica* was found in more than 113 km of the river, occupying 344 ha. Since this survey, there has been concern that *H. verticillata* ssp. *lithuanica* would spread to nearby waterbodies and have a significant negative impact. Here, we report the first documented spread and establishment of *H. verticillata* ssp. *lithuanica* from the Connecticut River to five waterbodies in Connecticut and one in Massachusetts. Of the eight sites where *H. verticillata* observations were made, 75% ($n=6$) were confirmed to be *H. verticillata* ssp. *lithuanica* and 25% ($n=2$) to be *Hydrilla verticillata* ssp. *peregrina* (wandering hydrilla). Except for one site, all six locations infested with *H. verticillata* ssp. *lithuanica* provide watercraft access through public or private boat ramps. The authors also postulate on the mechanisms facilitating the spread and establishment of this subspecies.

Introduction

The aquarium and ornamental trades have led to the introduction of numerous aquatic invasive species in the United States, including hydrilla [*Hydrilla verticillata* (L. f.) Royle] (Gettys and Enloe 2016; June-Wells et al. 2012; Padilla and Williams 2004). Before 2016, two subspecies within clade B (Tippery et al. 2020) were present in the United States: southern hydrilla (*Hydrilla verticillata* ssp. *verticillata*) and wandering hydrilla (*Hydrilla verticillata* ssp. *peregrina*). *Hydrilla verticillata* ssp. *verticillata* was discovered in Crystal River, FL, and in a canal near Miami, FL, in the United States in the 1960s (Blackburn et al. 1969), and *H. verticillata* ssp. *peregrina* was discovered in Delaware, USA, in the 1980s (True-Meadows et al. 2016; Steward et al. 1984; Tippery 2023). In Connecticut, the first report of *H. verticillata* was recorded in 1989 from a pond located in Mystic (Les et al. 1997). Initially, tests indicated that this population was *H. verticillata* ssp. *verticillata*, but subsequent examinations from the same pond confirmed that the plants were *H. verticillata* ssp. *peregrina*. (Madeira et al. 1997, 2000, 2004).

In 2016, scientists from the Connecticut Agricultural Experiment Station (CAES) Office of Aquatic Invasive Species (OAIS) discovered a third subspecies, northern hydrilla (*Hydrilla verticillata* ssp. *lithuanica*), in the Connecticut River in Glastonbury, CT (Tippery 2023; Tippery et al. 2020). Upon the initial recognition of this subspecies, it was determined that *H. verticillata* ssp. *lithuanica* was already widely distributed and prevalent throughout the river. From 2017 to 2019, CAES OAIS surveyed the Connecticut River to quantify the distribution and abundance of this new intruder. Survey data indicated that its presence extended across more than 344 ha, spanning from Agawam, MA, to approximately 9 km from the Long Island Sound, where it is believed to be restricted by a lack of salt tolerance (Steward and Van 1987). *Hydrilla verticillata* ssp. *lithuanica* was found in high abundances predominantly in shallow coves, shoals, shorelines, and adjoining tributaries at depths less than 3 m (Bugbee and Stebbins 2021, 2022).

While many biological attributes (reproductive biology, nutrient requirement, etc.) of this subspecies remain unknown, it exhibits phenological differences from the other two subspecies, most notably a lack of tubers, greater turion production, a greater number of leaves per whorl

Management Implications

The global reputation of *Hydrilla verticillata* (L. f.) Royle (hydrilla), an invasive submerged aquatic plant, is well established. Upon the discovery of *Hydrilla verticillata* ssp. *lithuanica* (northern hydrilla), a new subspecies in the United States, its distribution and abundance were quantified. It was found to cover more than 344 ha along a 113-km section of the Connecticut River, extending from Agawam, MA, to about 9 km north of the Long Island Sound, exhibiting high to very high levels of abundance. There was concern this subspecies would spread from the river to waterbodies throughout the Northeast. Here, we report the first documented spread from the Connecticut River to other waterbodies within the states of Connecticut and Massachusetts. Of the eight sites where *H. verticillata* observations were made, 75% ($n = 6$) were confirmed to be *H. verticillata* ssp. *lithuanica* and 25% ($n = 2$) to be *H. verticillata* ssp. *peregrina*. Except for one site, all six locations infested with this novel subspecies provide watercraft access through public or private boat ramps. Significantly, three of these sites are well regarded for their fishing opportunities and are frequented by fishing tournaments from the Connecticut River. To minimize the risk of *H. verticillata* ssp. *lithuanica* spread and establishment, agencies overseeing public boat ramps for aquatic invasive species should give priority to monitoring anglers and other watercraft with previous activity in the Connecticut River.

(5 to 11), and increased robustness (Tippery 2023). Nonetheless, based on morphology alone, distinguishing the three subspecies present in the United States from one another can still be challenging due to significant overlap in leaf length, leaf width, the ratio between leaf length and width, and the number of leaves per node (Tippery 2023).

Following establishment, *H. verticillata* quickly proliferates throughout the waterbody through the production of turions, tubers, drifting fragments, root crowns, and stoloniferous growth (Haller and Sutton 1975). *Hydrilla verticillata* infestations are challenging to manage and if left unchecked quickly lead to numerous ecological and economic concerns. *Hydrilla verticillata* ssp. *verticillata* forms dense canopies within the water column and, as a result, can hinder recreational activities such as boating, fishing, and swimming, potentially leading to diminished property values in the affected areas (Milon et al. 1986). Extensive populations of *H. verticillata* result in changes to the local ecology through the plant's ability to outcompete and displace native species (Balciunas et al. 2002). Ecological alterations following invasion of *H. verticillata* occur through changes in water chemistry and intensified competition among plants for space and nutrient resources. Additionally, it serves as a host to a potentially harmful epiphytic cyanobacterium (*Aetokthonos hydrillicola*) (Wilde et al. 2005, 2014). *Aetokthonos hydrillicola* produces a neurotoxin that has been implicated as a causal agent of avian vacuolar myelopathy, a fatal disease of waterfowl and bald eagles (*Haliaeetus leucocephalus*) (Wilde et al. 2014).

The discovery of *H. verticillata* ssp. *lithuanica* in the Connecticut River and the breadth of the infestation represent a significant ecological invasion event with potentially far-reaching implications. *Hydrilla verticillata* is considered one of the world's worst aquatic weed species (Gettys and Enloe 2016). In North America, the annual management cost is estimated to be more than \$2,471 ha⁻¹ (Langeland 1996). In Florida alone, the Florida

Wildlife Commission spends an estimated \$5 to \$15 million yr⁻¹ on *H. verticillata* management (Hiatt et al. 2019; Weber et al. 2020).

Once *H. verticillata* ssp. *lithuanica* was discovered in the Connecticut River, there was great concern this subspecies would spread outside the Connecticut River and degrade the native plant communities of previously uninvaded waterbodies. In 2023, the CAES OASIS received reports (OASIS, personal communication) of *H. verticillata* outside the Connecticut River in close proximity to boat ramps. Each site was visited and searched to document the extent of the infestation, collect samples for genetic analysis, and inform stakeholders (i.e., lake associations and marinas). In addition to receiving reports of potential *H. verticillata* ssp. *lithuanica* infestations, the authors also discovered apparent populations through regular invasive aquatic plant surveys on waterbodies throughout the state.

Materials and Methods

By October 2023, six individual reports of *H. verticillata* infestations in previously uninvaded waterbodies were received by CAES OASIS (Table 1). Amos Lake, a previously unidentified location, was found to have an *H. verticillata* infestation following routine aquatic plant surveys conducted by CAES OASIS. Additionally, Congamond Lake was identified as another *H. verticillata*-infested site due to suspicions raised by angling activity originating from the Connecticut River. Following reports or sightings, sites were visited to estimate the population density and collect specimens. Specimens were mounted and sub-sampled for genetic determination. Herbarium mounts are housed at the CAES OASIS and can also be found online at <https://portal.ct.gov/CAES/OASIS/Herbarium>.

At the University of Wisconsin–Whitewater, genomic DNA was extracted using a cetyl trimethylammonium bromide (CTAB) method (Doyle and Doyle 1987), modified to use an initial buffer volume of 600 µl of pure chloroform (without isoamyl alcohol) at the extraction step and ethanol (instead of isopropanol) at the DNA precipitation step. Polymerase chain reaction (PCR) was conducted to amplify the internal transcribed spacer (ITS) region using the p5F and p4R primers (Baldwin 1992; Cheng et al. 2016) and a 55 C annealing temperature, with the Phire Hot Start II DNA polymerase (Thermo Fisher Scientific, Waltham, MA). PCR products were cleaned using the Exonuclease and FastAP alkaline phosphatase enzymes (Thermo Fisher Scientific). Sanger sequencing (Sanger et al. 1977) was performed by Eurofins Genomics (Louisville, KY). DNA sequences were compared visually against previously published sequences for *H. verticillata* (Tippery et al. 2020) in the program Mesquite v. 3.81 (Maddison and Maddison 2023).

Results and Discussion

Once a species is introduced to an area outside its native range, it may experience a sharp population expansion within its new environment (e.g., a waterbody). However, even in such cases, there is typically a temporal gap or lag phase between the time when the species was introduced to the new area and the subsequent secondary expansion into areas outside its original establishment zone. The specimens sequenced for this study were exact matches from previously published sequences for each of the respective subspecies. Of the eight *H. verticillata* infestation reports received by or discoveries made by CAES OASIS, 75% ($n = 6$) were confirmed to be *H. verticillata* ssp. *lithuanica*, and the remaining

Table 1. Waterbody locations, including report and site visit dates, *Hydrilla verticillata* subspecies, distance from the Connecticut River to the site location, and the distance from the boat ramp to the infestation site.

Waterbody	City and state	Report date	Site visit	Subspecies	Distance from	
					Connecticut River to waterbody km	Boat ramp to infestation m
East Twin Lake	Salisbury, CT	June 27, 2023	June 29, 2023	<i>lithuanica</i>	62	13
Amos Lake	Preston, CT	N/A	August 14, 2023	<i>lithuanica</i>	37	7
Lake Lillinonah	Newtown, CT	August 17, 2023	September 3, 2023	<i>peregrina</i>	59	3,210
Middle Bolton Lake	Vernon, CT	September 4, 2023	September 8, 2023	<i>lithuanica</i>	17	24
Meckauer Park	Bethel, CT	July 21, 2023	July 31, 2023	<i>peregrina</i>	66	NA
Lake Pocotopaug	East Hampton, CT	September 4, 2023	October 13, 2023	<i>lithuanica</i>	6	400
Congamond Lakes	Southwick, MA	N/A	October 12, 2023	<i>lithuanica</i>	13	18
Pameacha Pond	Middletown, CT	October 7, 2023	October 19, 2023	<i>lithuanica</i>	1.6	NA

25% ($n=2$) to be *H. verticillata* ssp. *peregrina*. Before 2022, *H. verticillata* ssp. *lithuanica* had not been detected beyond the confines of the Connecticut River. However, by the end of 2023, there was a 6-fold increase in the number of locations where *H. verticillata* ssp. *lithuanica* infestations were observed compared with the preceding 7 yr, when this strain was initially identified in 2016 (Figure 1). The rate at which these discoveries are being made is cause for concern and is likely higher than the results presented here.

East Twin Lake

East Twin Lake was the first site reported to have *H. verticillata*. East Twin Lake is one of two lakes in the Twin Lakes area, in Salisbury, CT, located approximately 62 km from the Connecticut River, in the northwest corner of Connecticut (Figure 1). East Twin is one of the most managed and studied coldwater lakes in the state and spans 230 ha with a maximum depth of 25 m and a watershed covering 1,068 ha.

On June 27, 2023, Northeast Aquatic Research (NEAR), a private company specializing in aquatic plant surveys, identified populations of *H. verticillata* in the vicinity of the O'Hara's Landing private boat ramp. The East Twin Lakes Association and CAES OASIS were promptly notified of this discovery. Three days later, CAES OASIS carried out a site visit and collected plant samples for herbarium mounts and genetic analysis. The analysis confirmed that the *H. verticillata* strain found in East Twin Lake belonged to clade C and was *H. verticillata* ssp. *lithuanica*. Subsequent visits to the lake were conducted by CAES OASIS and NEAR to document the extent of the infestation.

East Twin was the first waterbody discovered to have *H. verticillata* ssp. *lithuanica* outside the Connecticut River. Altogether, a minimum of 19 separate populations were located in the vicinity of the boat ramp, marina, and an area 0.7 km to the north of the boat ramp where they were mixed in with emergent and submerged vegetation (Figure 1). After obtaining the necessary state herbicide application permits, efforts were made to address the infestations in and around the marina by employing florparyauxifen-benzyl (ProcellaCOR® SePRO, Carmel, IN 46032). The outcomes of this treatment are currently under assessment.

Amos Lake

Amos Lake, spanning 47 ha, is located in Preston, CT, and is positioned approximately 37 km east of the Connecticut River, in the central-southern region of eastern Connecticut. This lake

has a maximum depth of roughly 14 m and an average depth of approximately 6 m and features a public boat ramp on its western shoreline. Notably, Amos Lake is a popular recreational destination, highly regarded as a prime location for fishing enthusiasts seeking trophy bass and trout.

During standard aquatic plant assessments conducted by CAES OASIS, *H. verticillata* was detected on August 14, 2023. Genetic analysis was conducted on sampled populations, confirming the presence of *H. verticillata* ssp. *lithuanica*. The *H. verticillata* ssp. *lithuanica* population was found approximately 7 m away from the public boat ramp and was intertwined with emergent (e.g., swamp loosestrife [*Decodon verticillatus* (L.) Elliott], pickerelweed [*Pontederia cordata* L.], white water lily [*Nymphaea odorata* Aiton], and yellow water lily [*Nuphar variegata* Engelm. ex Durand]) and submerged (e.g., coontail [*Ceratophyllum demersum* L.], western waterweed [*Elodea nuttallii* (Planch.) H. St. John], and purple bladderwort [*Utricularia purpurea* Walter] native plants.

Middle Bolton Lake

Middle Bolton Lake is in Vernon, CT, approximately 17 km east of the Connecticut River, and spans 49 ha. With a maximum depth of 6 m and an average depth of 3.7 m, this lake offers a state-managed public boat ramp, a town beach, and a camp for recreational activities.

On September 4, 2023, a report issued by NEAR identified a population of *H. verticillata* near the northern end of the lake, approximately 24 m from the public boat ramp (Figure 1). The CAES OASIS visited the location on September 8, 2023, and gathered samples for genetic analysis that confirmed the presence of *H. verticillata* ssp. *lithuanica*. The population was relatively small and limited in distribution, with only a few mature plants found. Efforts were made to remove the population through manual removal via hand pulling, and monitoring is ongoing.

Lake Pocotopaug

Lake Pocotopaug, located in East Hampton, CT, is approximately 6 km east of the Connecticut River, spans over 207 ha, and has an average depth of 3.4 m and a maximum depth of 7.3 m. The town-owned boat ramp is located on the western side of the lake. On September 4, 2023, CAES OASIS received a report and a sample of *H. verticillata* from the lake. Following genetic analysis, *H. verticillata* ssp. *lithuanica* was confirmed. On October 13, 2023, CAES OASIS carried out a field visit to record the presence and density of *H. verticillata* ssp. *lithuanica* in the southern area of this lake (Figure 1). The northern and eastern shorelines of the lake

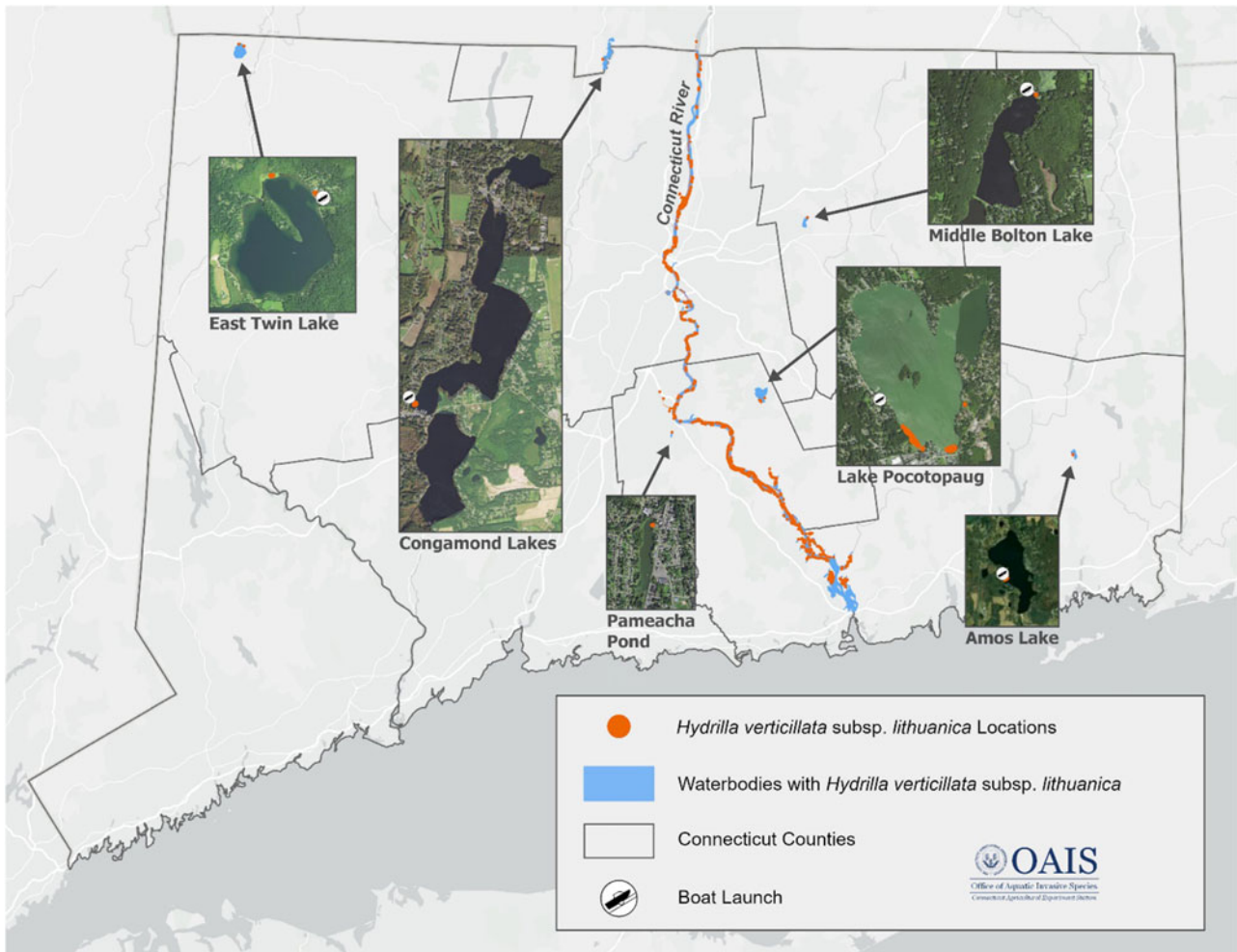


Figure 1. Distribution of *Hydrilla verticillata* ssp. *lithuanica* in the Connecticut River and the six new infestations in waterbodies within or bordering Connecticut, USA.

were not included in the survey. The infestation was identified approximately 400 m south of the town-owned boat ramp, covering an area of about 1 ha, representing the most significant known infestation outside the Connecticut River. Currently, we have no information regarding the extent, if any, of *H. verticillata* ssp. *lithuanica* infestations on the lake's northern and eastern sides. The Lake Pocotopaug Project was informed of the infestation, and discussions surrounding the best management approach are currently ongoing.

Congamond Lakes

The Congamond Lakes are a natural lake system in Southwick, MA, with part of the shoreline following the Massachusetts–Connecticut border (Figure 1). Congamond Lakes consist of three connected basins: Northern, Middle, and Southern, totaling 188 ha. Within this lake system are two boat ramps, both in the middle basin. The Congamond Lakes have gained a strong reputation among anglers due to their renowned fish populations, which include regular trout stocking and the annual organization of fishing tournaments. Upon the suspicion of *H. verticillata* ssp. *lithuanica* presence, prompted by the schedule of angling tournaments that had previously taken place in the Connecticut River, a survey of the site was conducted on October 12, 2023. *Hydrilla verticillata* populations were found within 18 m of the

southern boat ramp in the middle basin. Again, *H. verticillata* ssp. *lithuanica* was confirmed. Upon subspecies confirmation, the Massachusetts Department of Conservation and Recreation and related lake associations and authorities were notified. To date, the presence of *H. verticillata* ssp. *lithuanica* in the Congamond Lakes is the first and only known location in Massachusetts, outside the Connecticut River, to have this subspecies.

Pameacha Pond

Pameacha Pond is a relatively small lake (0.7 ha), 1.6 km west of the Connecticut River, in Middletown, CT. Pameacha Pond has limited recreational activity and does not have a public or private boat ramp. During pretreatment surveys of aquatic fauna by the Pond and Lake Connection on October 7, 2023, *H. verticillata* ssp. *lithuanica* was observed, sampled, and transported to CAES OAIS for genetic analysis. It was subsequently confirmed to be *H. verticillata* ssp. *lithuanica*. A follow-up site visit by CAES OAIS was also conducted on October 19, 2023, to determine the extent of the infestation. The *H. verticillata* population was limited in distribution and abundance and was located on the northeastern shore intermixed with dense populations of *C. demersum*.

The introduction of invasive plants and invertebrates into new aquatic environments is frequently linked to recreational activities such as boating (Johnson et al. 2006; Johnstone et al. 1985;

Rothlisberger et al. 2010). Understanding the importance of these transmission pathways provides valuable insights into how regulatory agencies and volunteers can collaborate to mitigate the rate of expansion (Haight et al. 2021). Notably, East Twin Lakes, Amos Lake, and Congamond Lakes serve as hosts for numerous annual angling tournaments, drawing participants from across the state. Of particular concern are tournaments that involve travel from infested waterbodies to noninfested ones.

Except for Pameacha Pond, all six *H. verticillata* ssp. *lithuanica*-infested sites offer watercraft access through public or private boat ramps. Given the variable distances of *H. verticillata* ssp. *lithuanica* infestation sites from the Connecticut River, the limited size of the infestations, and the distance from the boat access points to the locations of the infestations, avian dispersal seems unlikely. Consequently, the primary mechanism for the spread of *H. verticillata* ssp. *lithuanica* from the Connecticut River to these waterbodies appears to be inadequate adherence to decontamination protocols when transporting recreational equipment, especially watercraft (Mohit et al. 2021; Rothlisberger et al. 2010).

Further research should focus on assessing the relative contribution of angling tournaments to vectoring this highly invasive species from one waterbody to another. Understanding and addressing these vectors are critical for developing effective strategies to prevent and manage the spread of *H. verticillata* ssp. *lithuanica* in these diverse aquatic ecosystems.

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