Native Plant Trust

Wild Rice Restoration on the Sudbury River

USFWS Award #F15AC00380

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Wild Rice (*Zizania aquatica* and *Zizania palustris*)

Project Overview

The goal of this project is to restore native wild rice to riparian areas in the Sudbury, Assabet, and Concord (SuAsCo) River Watershed, to increase diversity of native flora and to benefit migratory waterfowl. The project consists of two phases: Phase 1 – Discovery and Research; and Phase 2 - Restoration. This report is the product of Phase 1 which included searches for existing occurrences, research of successful restoration techniques, implementation of small-scale experiments, development of a draft restoration plan, and selection of potential seed sources for future large-scale restoration. Future restoration efforts will target suitable waterfowl habitat where invasive species, such as water chestnut (*Trapa* natans) and American lotus (*Nelumbo lutea*) have been removed, primarily along the Sudbury River and Great Meadows NWR – Concord Impoundment.

Figure 1. *Zizania* sp. at early flowering stage, Concord Impoundments

About Wild Rice

Wild rice is a tall, aquatic, annual grass noted for producing large grains eaten by both humans and wildlife. In the northeast wild rice is represented by two species[1], *Zizania aquatica* L. var. *aquatica* and *Z. palustris* L. var. *palustris*[2] both of which occur in a variety of habitats including brackish or salt marshes and flats, fresh tidal marshes or flats, and banks of rivers, lakes or ponds (Haines, 2011). Both species produce a large showy panicle of flowers in August that develop into the protein-rich seeds (caryopsis) by mid-late September. The species are similar in appearance, but can be distinguished by the relatively open or restricted nature of their carpellate inflorescence as well as the presence and distribution of rough hairs (scabrules) on the fertile carpellate lemmas.

Both species have a unique life cycle. Seeds overwinter in sediment in near freezing temperatures (34º F) and begin to germinate in early spring when waters reach about 45 °F (April). Seeds develop into a distinctive aquatic, thread-leaved form during these early stages. As subsequent leaves emerge the plant eventually reaches the water's surface, entering the floating stage (June). During this critical development period plants are particularly vulnerable as they can be easily uprooted or drowned by flooding or wave action and are often consumed by geese and rodents. By late June, surviving plants become emergent and begin to form

flower structures that will open in July-August (Figure 1).

In the Sudbury-Assabet-Concord (SUASCO) River watershed, *Zizania* is historically known from three main locations in Concord: Great Meadows National Wildlife Refuge, Sudbury River, and Assabet River. A limited search of herbarium records include: *Z. palustris* - along the Sudbury River; (Robbins, S.D.; FC S s.n., 1930); *Z. aquatica* - Concord Great Meadows National Wildlife Refuge (Eaton, R.J. 1957); *Z. aquatica* - Assabet River, Concord, MA (LER, 1961); and *Z. aquatica* - Concord Great Meadows National Wildlife Refuge (Shea, K.; Blair, E. 1977).

Additional historical accounts included some 28 references of *Z. aquatica* in Thoreau's journals. He describes locations in Concord along the Concord River (1852) and along the Assabet River (1854), on the north side of the Concord River near the Holt at Great Meadows (1854), and on the Assabet River at Joseph Hosmer's Property near Derby Bridge (1854), presumably in Hudson, where he describes it as abundant. Hosmer, who studied much of the same area as Thoreau from the 1870s to the early 1900s, describes *Z. aquatica* as "common." Eaton, author of *A Flora of Concord* 1974, says it was formerly scattered along the riverbanks and locally abundant at Great Meadows by 1957. More recent observations of *Zizania* sp. include Brumback, Native Plant Trust (formerly New England Wild Flower Society) (1992) and Suzanne Flint, OARS (date unknown) along the Assabet River.

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[1] While Flora *Novae Angliae* (Haines 2011) distinguishes two species of *Zizania*, previous treatments have, in some cases, only included *Z. aquatica* with and distinguished forms as varieties. In those cases *Z. palustris* is referred to as *Z. aquatica* var. *angustifolia*. Nomenclatural changes as well as misidentification of *Z. palustris* as *Z. aquatica* have led to some taxonomic confusion and many historical records may be inaccurate. Refer to Fassett (1924) and Terrell (1997).

[2] *Z. palustris* is tracked as a "Watch Listed" species by Massachusetts Natural Heritage and Endangered Species Program and is ranked S1/S2, Uncommon.

Survey of Existing Populations

Methods

In an effort to understand current distribution and characteristic habitat for the two species of *Zizania*, Native Plant Trust (formerly New England Wild Flower Society) staff conducted surveys by canoe, kayak, or motorboat in the SUASCO watershed. Surveys were conducted in mid-late August, when plants are flowering and easily visible and targeted large stretches of Sudbury and Assabet Rivers, as well as adjacent impoundments or ponds. Surveys also included the Concord River from Egg Rock down to and including the impoundments at the Great Meadows National Wildlife Refuge. During these searches, surveyors collected data on number of plants, phenology, and habitat and documented locations using hand-held GPS units. Locations were also photographed using point-and-shoot digital cameras.

Results

Zizania sp. was found in approximately 128 locations, 30 of which contained stands of 25 or more individuals (Figure 2). Six locations contained stands of more than 100 individuals and are suitable for seed collection (Appendix A-F). Although specimens were not identified to the species level at each location due to time constraints, periodic in-the-field examination identified both southern (*Z. palustris*) and northern wild rice (*Z. aquatica*) on the Assabet River. Although it may be possible that both species are present, we are skeptical of this finding and need to revisit sites in order to collect specimens for in-office examination. For this reason we will not distinguish between *Zizania aquatica* and *Z. palustris* for the remainder of the report.

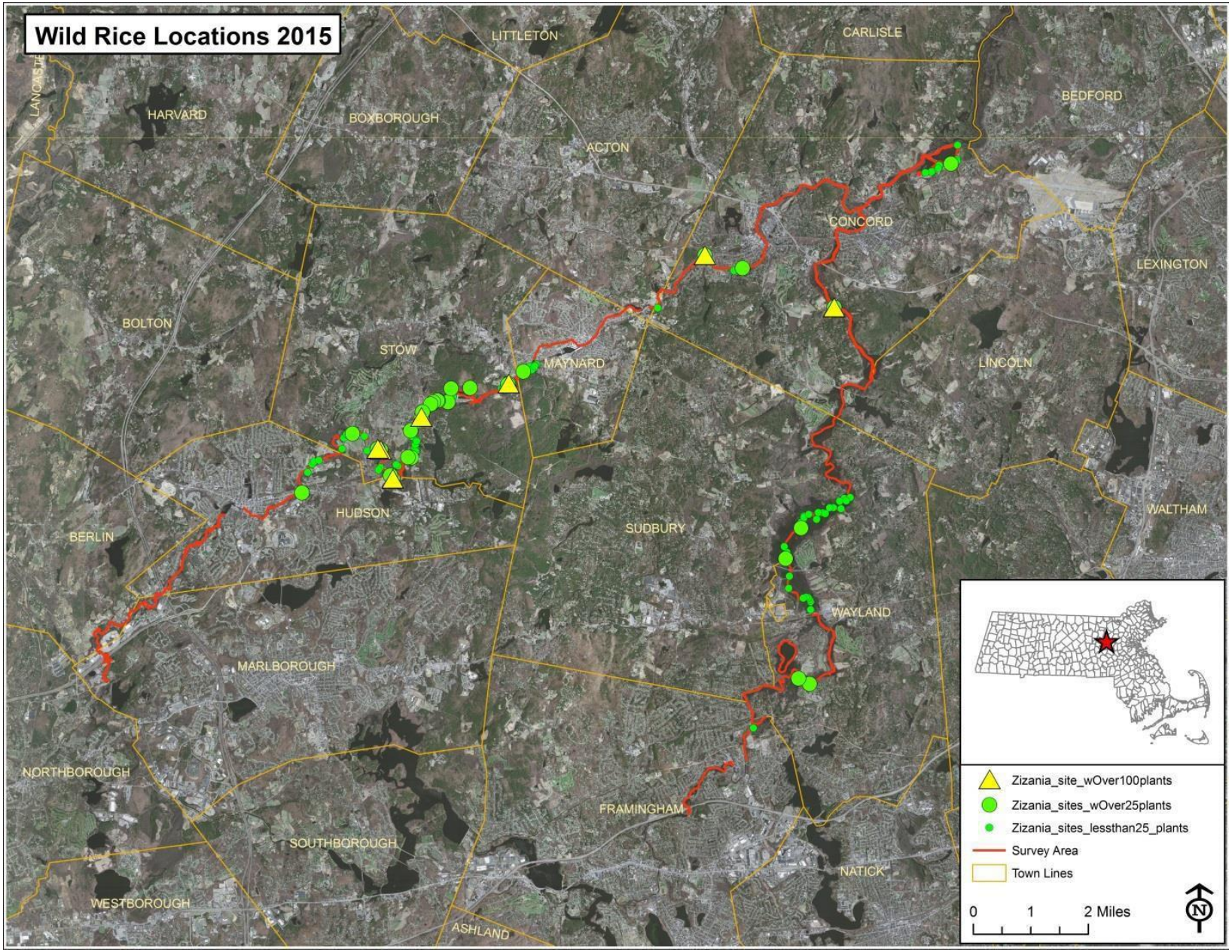


Figure 2. Survey area and *Zizania* locations with 0-24, 25-99, and 100+ plants

Large stands were primarily located on the Assabet River in open backwater areas or where tributaries joined the main stem of the river, often near conserved, open land. These large stands were strongly associated with beaver impoundments and protected, shallow (1-2 ft.), open- water areas in full sun. Stands along the main river channels were predominantly found in areas of slow moving water or muddy flats, often on the inside of a winding bend where plants are protected from fast moving, high- water events (Figure 3).

Figure 3. Large stand of *Zizania* sp. on the Assabet River, downstream of Elizabeth Brook

Overlaying spatial data with the state's wetlands layers indicate large stands of *Zizania* more frequently occur in habitats described as Shallow Marsh Meadow or Fen and only occasionally occur in Deep Marsh, Shrub Swamp, Deciduous Wooded Swamp, or Open Water. Similar comparisons with bedrock and soil data layers characterize sites as having muck, mucky silt loam, or silt loam on top of granite or diorite.

Associated emergent plants, in order or co-occurrence, included pickerelweed (*Pontederia cordata*), invasive reed canary grass (*Phalaris arundinacea*), bur-reed (*Sparganium* sp.), common arrowhead (*Sagittaria latifolia*), buttonbush (*Cephalanthus occidentalis*), aquatic smartweeds (*Persicaria punctata and Persicaria pensylvanica*), and invasive purple loosestrife (*Lythrum salicaria*).

Zizania also occasionally occurred with emergent species such as jewelweed (*Impatiens capensis*), bulrush (*Scirpus* sp.), bluejoint grass (*Calamagrostis canadensis*), swamp milkweed (*Asclepias incarnata*), swamp-loosestrife (*Decodon verticillatus*), broad-leaved cat-tail (*Typha latifolia*), as well as floating and submerged species such as invasive water chestnut (*Trapa natans*), pondweeds (*Potamogeton* spp.) duckweeds (*Lemna* spp.) and likely invasive water-milfoil, (*Myriophyllum* sp.).

Figure 4. *Zizania* sp. found amongst mats of Pennsylvania smartweed, Concord Impoundments

In the Concord impoundments, plants were scattered in low numbers, and predominantly occurred on floating mats of aquatic smartweeds (*Persicaria* spp.) (Figure 4). Plants occasionally occurred in openings between American lotus (*Nelumbo lutea*) leaves, but the dense foliage most likely prohibits large stand establishment.

The majority of invasive plant species occurred just beyond the open-water, mudflat habitat preferred by *Zizania* with the exception of water chestnut. The majority of water chestnut infestations were observed on the Sudbury River between Sherman Bridge and Fairhaven Bay. On the Assabet, water chestnut was generally not as abundant but observed in Hudson above the dam and where the river crosses under Cox Street.

Other invasives observed during the survey that may threaten *Zizania* include common reed (*Phragmites australis*), Japanese stiltgrass (*Microstegium vimineum*), and Brazilian water weed (*Egeria densa*). Common reed was found to be widespread in open marsh meadows through the survey area while stiltgrass and water weed were only observed in a few locations on the Sudbury River. Non-native species found on the Sudbury River like yellow flatsedge (*Cyperus flavescens*) and European water-clover (*Marsilea quadrifolia*) may likewise compete with *Zizania* for habitat (Figure 5).

Figure 5. European water-clover densely covering the banks of The Sudbury River, August 12th, 2015

Discussion

While wild rice was documented throughout a large portion of the survey area, the majority of stands contained fewer than 25 individuals and large stands of 100^{+} plants were predominantly found on the Assabet River.

These findings suggest there may be limited habitat available for large *Zizania* stands along the Sudbury, Assabet, and Concord Rivers. Findings also suggest the Assabet may provide more suitable conditions as compared to the Sudbury River and its associated ponds and wetlands. More frequent damming on the Assabet may foster suitable conditions and also prevent major hydrological fluxes which can be catastrophic to young wild rice seedlings (Figure 6). Populations also occurred more frequently within or near blocks of conserved land, suggesting protected areas may support normal hydrologic patterns and disturbance regimes conducive to *Zizania* establishment. And whereas the Assabet is a small river best navigated by kayak or canoe, the Concord and Sudbury Rivers are large enough to support navigation by motorboat in many sections which is known to cause of *Zizania* mortality (wave action and turbidity).

Competition with fast growing, invasive plants such as water chestnut and reed canary grass also contribute to *Zizania* habitat availability. Field observations confirm invasives are particularly

problematic on the Sudbury River and actively compete with *Zizania* stands. Reed canary grass, a wide spread perennial, may be the most problematic species. This species forms monotypic stands that stabilize areas which, without invasion, would be disturbed during flood events and provide good habitat for *Zizania*. Anecdotal observation also suggests its establishment may raise marsh elevation, interrupting hydrologic flow to backwater areas and consequently preventing *Zizania* establishment. Similarly, water chestnut competes with *Zizania* in riverine mudflat habitat by forming dense mats that prevent light from reaching developing plants. We also speculate that the plant's dense root systems may also interfere with *Zizania* seed dispersal.

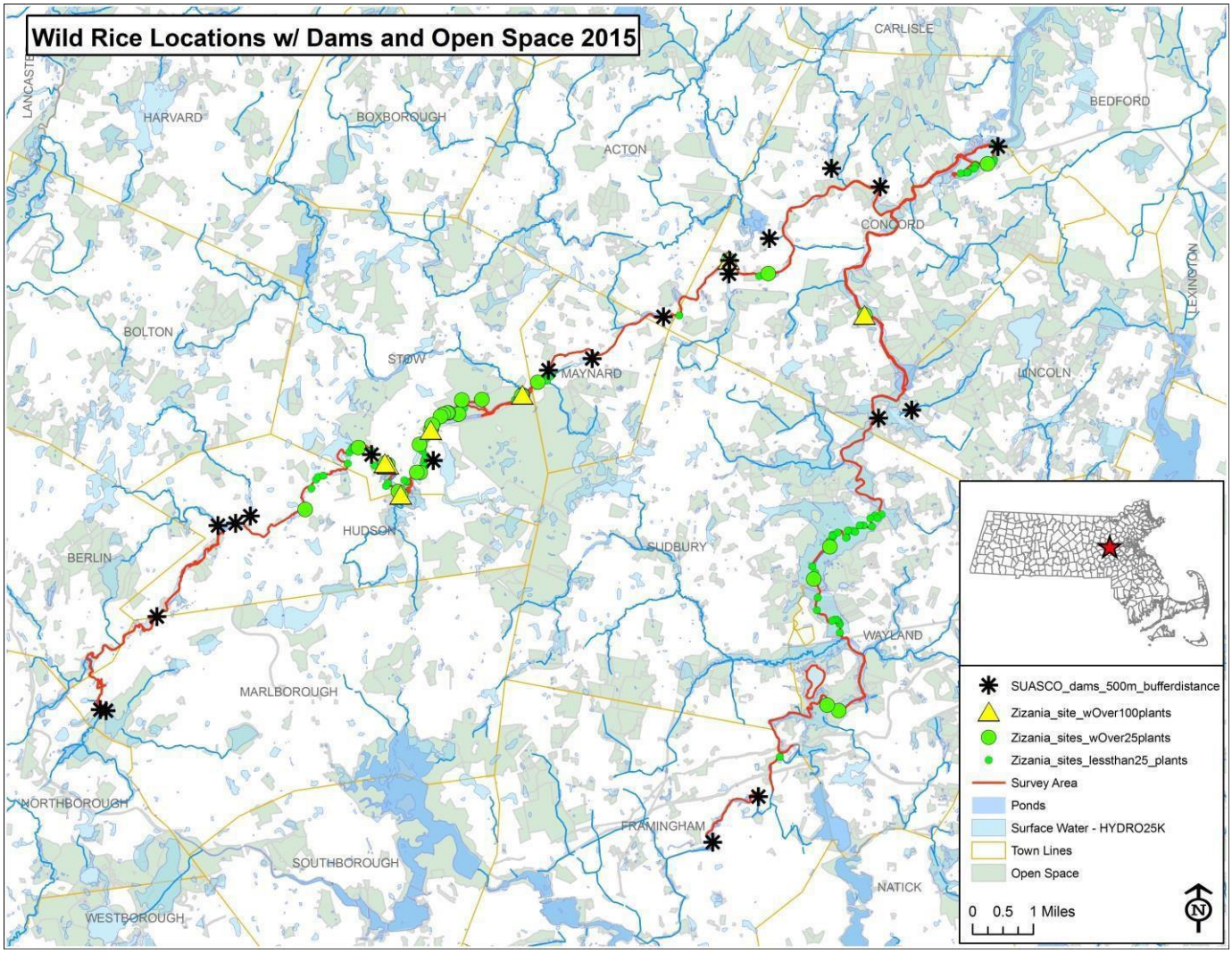


Figure 6. Locations of *Zizania* with open space and dams on the Sudbury, Assabet and Concord Rivers

In addition to invasives, monotypic stands of native and non-native species may also play a role in *Zizania* habitat availability. Cattail is one such species observed in abundance in Shallow Marsh Meadow or Fen habitat along the Sudbury River. Development of these monotypic stands is usually symptomatic of underlying ecosystem imbalances and is often associated with nutrient inflows from lawn, garden, and agricultural fertilizers (Zedler and Krecher 2004). Nutrient influxes can create imbalances in wetland microbial communities and foster conditions that favor species with broad environmental tolerances (i.e. invasives and other generalists).

Review of available literature as well as conversations with ecologists from the Great Lakes Region suggest other factors may contribute to establishment and success of *Zizania* stands. Such factors include herbivory by over abundant Asian Carp (Havranek 2011) or Canada geese (Haramis 2007) populations, both of which were observed in abundance during this study. This information provides important evidence as to *Zizania*'s sensitivity to herbivory and suggests that overpopulations of any given predatory species can impact immediate and long-term success of *Zizania.*

Disease and insect herbivory may also contribute to decline and even collapse of *Zizania* populations year to year. Literature from the Great lakes Region cites brown spot fungal disease and rice worms as commonly affecting the species. During our field work we observed an unidentified white cottony fungal disease on the seeds of many plants (20-60%). Infected plants flowered normally but seeds failed to form in all examined individuals.

Based on the finding of our field work, we believe further research is needed to identify primary factors affecting stand establishment and viability. Further research is also needed to determine the average size of historic stands. We suspect local conditions may have never supported stands with thousands to tens of thousands of individuals as seen in Maine and the Great Lakes Region, therefore restoration techniques from those locations may not be wholly transferable to this area.

Restoration on the Sudbury River

Experimental Seeding 2015

To better understand the logistics of *Zizania* seed collection and planting in this watershed, we attempted a small-scale restoration experiment in late-September following *Wild Rice Seeding Guidelines* (NRCS - Minnesota 2004). Conservation staff and one volunteer collected seed from two sites containing over 100 individuals: West Concord on Rt. 62 near Pond Lane and south of the Gleasondale dam in Stow (Appendices A and E). Seeds were collected either by boat or on foot and then placed in tubs of water in order to maintain seed-moisture during storage. Seeds were either "free-floating" in the tubs or submersed in cloth bags.

Figure 7. *Zizania* seed prior to sowing

The West Concord site was dried down at the time of collection therefore had to be collected on foot. The site's mucky conditions required the use of Mudder's Boot Supports and sections of lumber to prevent staff from sinking-in.

Collection was also complicated by diseased seeds which had to be avoided to prevent spread of infection. Overall, seed collection was time-consuming taking two staff members approximately four hours to collect only 3 lbs. of seed from the site.

Seeds were planted at four locations on the Sudbury River that appeared to have suitable habitat, but lacked *Zizania* (Figure 8). These locations were not selected as long-term restoration areas but as sites to evaluate planting techniques and possibly assess *Zizania* germination rates. Seeds were broadcast planted by hand shortly after collection (within 1-2 weeks) at a rate of 0.25-1.5 lbs. per site, depending on total area. Seeding itself was relatively easy and fast both on foot and by boat but seed stored in cloth bags became snagged in the fabric, which slowed the process. For this reason, we recommend only storing seeds in loose-weave synthetic cloth bags or "free-floating" in water-filed tubs. Late season, low water condition also made travel to sites via kayak difficult and time consuming and should be taken into consideration during logistics planning.

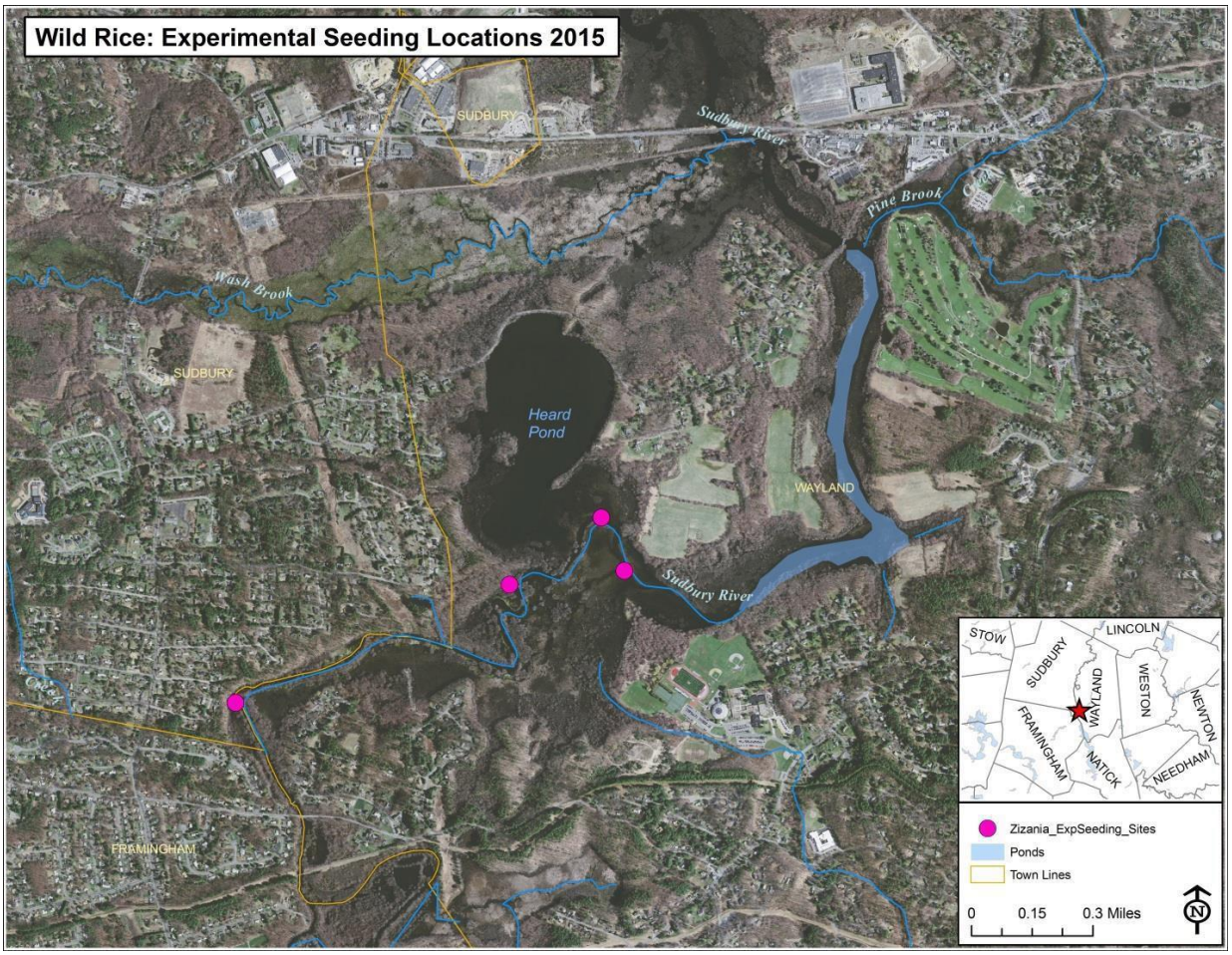


Figure 8. Locations of experimental *Zizania* seeding sites on the Sudbury River

Potential Restoration Sites

Historical Locations

According to literature from the Great Lakes Region, restoration is best conducted in locations where *Zizania* is absent 5 years or more (due to the soil-seed bank and seasonal population fluctuations) but has been previously observed. Historical site descriptions such as those of local botanists and herbarium records compared with current known locations provide a way to locate these historical stands. Unfortunately, records obtained for this watershed lack much of the locational information needed to complete such a comparison. Records also lack information regarding the size and density of populations necessary to determine if stands or populations are declining.

Sudbury River

Areas where invasive plants have been removed along the main-stem of the Sudbury River may provide suitable restoration or even augmentation locations for *Zizania* (Figure 9). We believe the best locations are those where water chestnut was found co-occurring with *Zizania* and occupying areas of suitable habitat. Restoration can be coordinated with ongoing water chestnut removal efforts and, if successful, may provide a means to resist reinvasion. Motorboat activity in these areas may threaten restoration success, and therefore may need to be restricted.

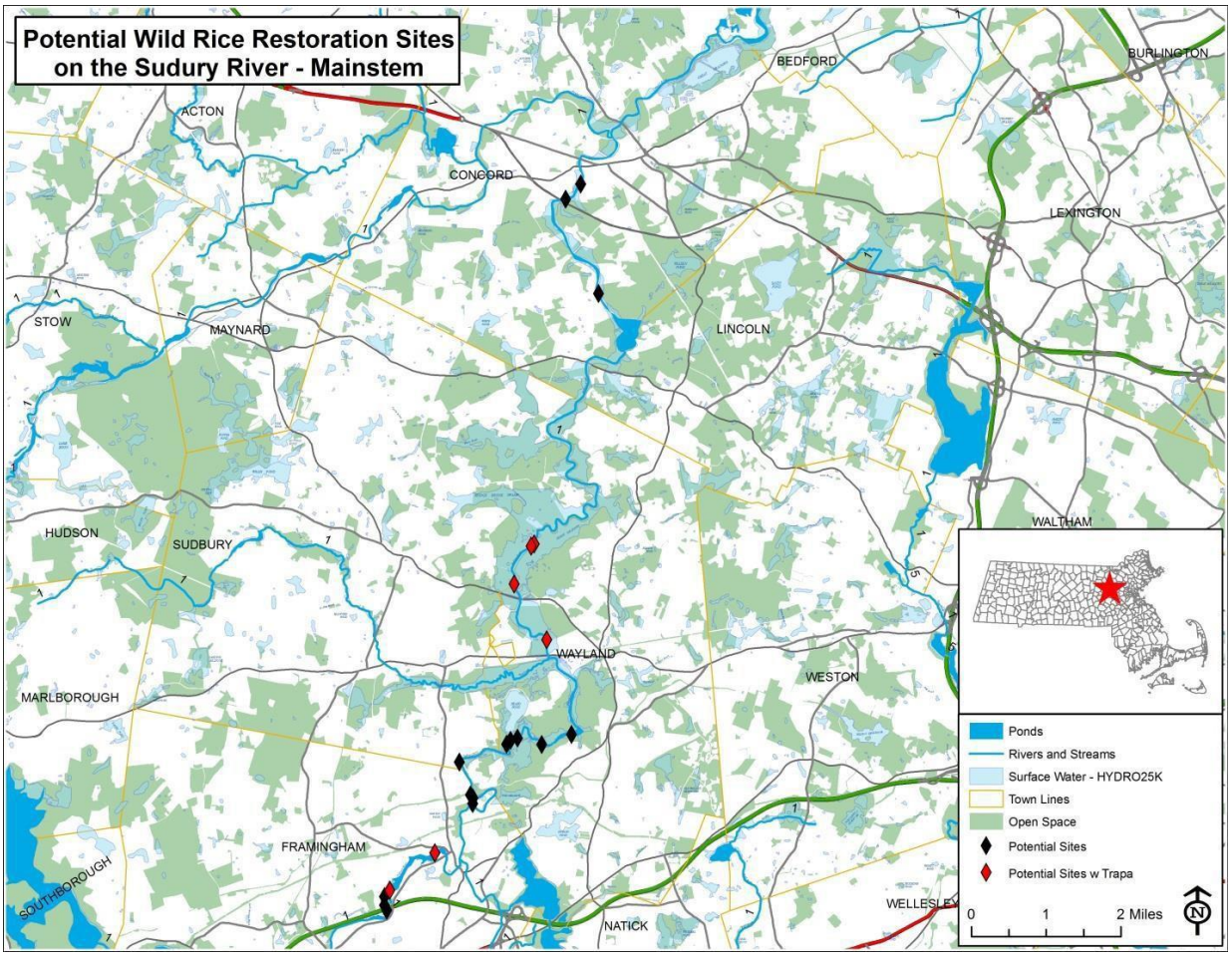


Figure 9. Potential Restoration Sites along the Sudbury River including sites infested with water chestnut.

Concord Impoundments

The Concord Impoundments may also provide a suitable restoration/augmentation location for *Zizania* (Figure 10). While *Zizania* was documented at the site, it was not found in any abundance despite the sites relative suitability. Waters are 0.5-3 ft. in depths with low turbidity and can be draw down periodically to prevent establishment of perennials which can outcompete *Zizania*. The site is in full sun with mucky soils, both of which are preferable to the species. We speculate removal of invasive plants (i.e., water chestnut and water-milfoil) and aggressive, non-native American lotus, would create conditions conducive to *Zizania* establishment. Obstacles for restoration, in addition to invasive management, include an abundance of Asian carp at the site which are known to feed on the seeds and young shoots of *Zizania*. For this reason we suggest researchers document the site's carp population, conduct exclosure studies to determine if carp are a primary predator of *Zizania* and, depending on study results, manage the population. Muskrat may also be problematic at the site but we speculate that once a large stand of rice has been established, browsing impacts will be lessened if not eliminated. Lastly, migrating waterfowl are known to be particularly abundant at the site in fall and spring and may consume a large portion of seed, therefore over seeding or temporary exclosures may be necessary.

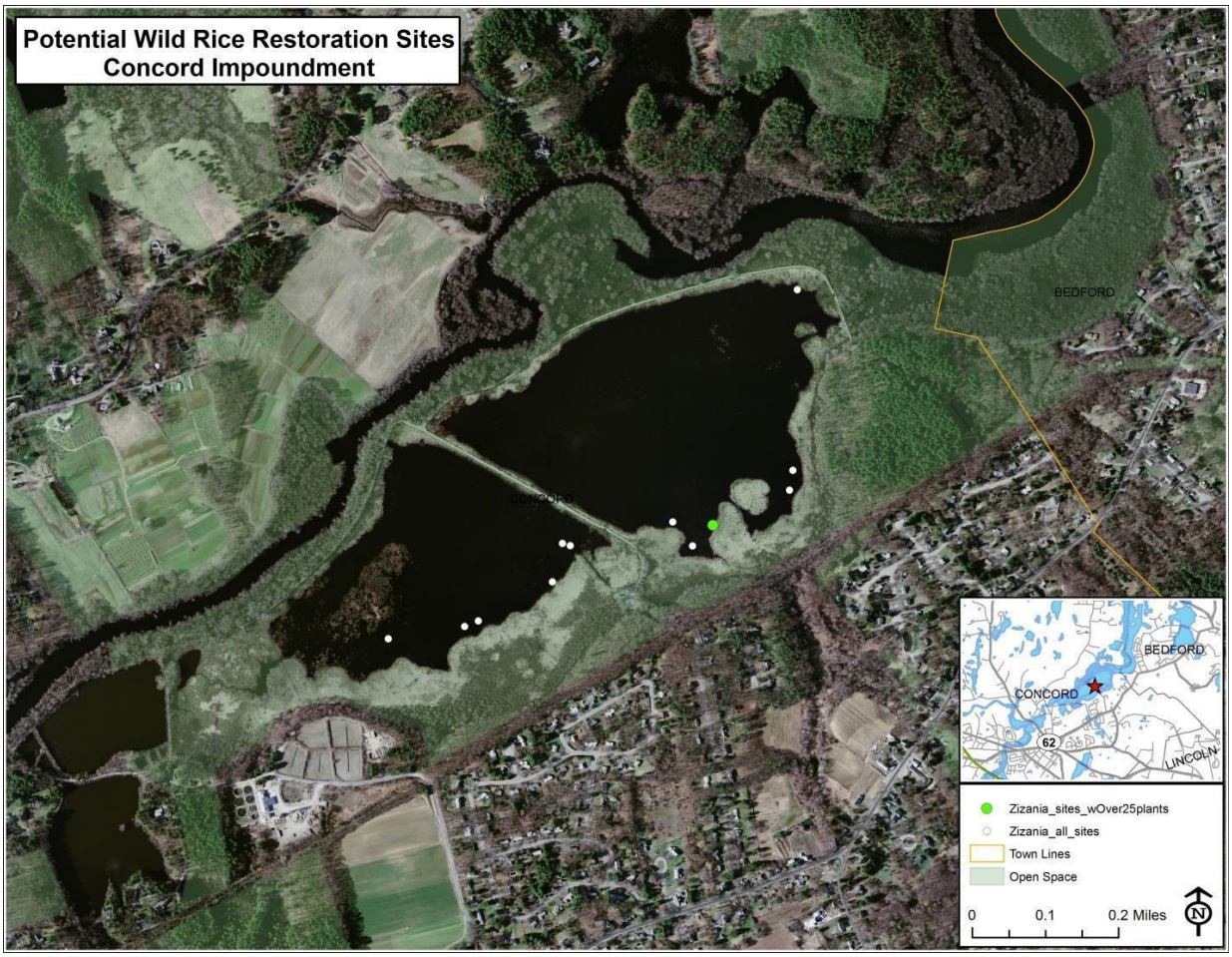


Figure 10. Locations of *Zizania* at the Concord Impoundments

Other Suitable Sites

Other possible sites for restoration sites include Shallow Marsh Meadow of Fen habitat on Sudbury and Assabet Rivers, along tributaries such as Pantry Book, Elizabeth Brook, Fort Meadow Brook and possibly nearby ponds like Farrar Pond and Warner Pond. These areas have yet to be surveys and will require assessment to determine site suitability.

Restoration on the Sudbury River

Plan for Experimental Restoration

Scope of Work: 2016-2019

To date there have been no comprehensive surveys or restoration efforts for *Zizania* in Massachusetts and possibly even in New England. Our survey results provide important baseline data for the species and a framework to explore restoration. Please see Wild Rice Restoration on the Sudbury River (Phase 1) for details (Native Plant Trust, February 2016). Using techniques developed in the Great Lakes Region as a framework, we conducted a 4-year experimental restoration program to further understand species habitat requirements and refine methods for application in this region.

We also revisited the four sites where we tested sowing wild rice in 2015 on the Sudbury River. No plants could be found at one site, two plants appeared at another, and there were hundreds of plants at the other two sites. It is not clear whether these large numbers were due to the success of the sowing by Native Plant Trust staff or the presence of nearby native stands seeding into the sites.

2. Experimental restoration

Native Plant Trust constructed a total of 20 exclosures to test sowing of wild rice. Each 1 x 1 meter square exclosure is 6 ft. tall. There are four PVC pipes, one at each corner, and hardware cloth was installed 2 ft. from the bottom of the exclosure. The design allows the exclosure to be driven into the mud until the hardware cloth reaches the bottom, blocking entry by fish and birds.

Seed was collected from two large stands of wild rice, one on the Assabet River and one on the Sudbury River. The seed is currently being held in buckets of water in a cool location. Per recommendation from wild rice experts, the water is changed every other day until sowing in experimental restorations, and this seed will also be used for germination tests.

Concord Impoundments

Native Plant Trust staff met with staff from the Great Meadows National Fish and Wildlife Refuge to determine best location for placement of exclosures at the impoundments. Bathymetric data and surveys of the impoundments yielded several possible locations. Native Plant Trust staff and three staff from the refuge used canoes to transport the exclosures and install them in the impoundments. Twelve exclosures (four exclosures at three different locations) were driven into the mucky bottom with rubber hammers.

Sudbury River

In consultation with Refuge staff, sites were chosen along the Sudbury River in areas were water chestnut control has occurred. Eight exclosures have been constructed for two sites along the river. These were installed in October 2015 in areas where water chestnut has been controlled.

Objective 2. Experimental Restoration

Introduction

In fall of 2017 we focused our efforts on assessing the effects of competing, invasive and non-native vegetation on the survival and fitness of southern wild rice (*Zizania aquatica*). Data collected from our 2016-2017 exclosure sites on the Sudbury River (SR) and Great Meadows NWR – Concord Impoundment (GMNWR-CI) to analyze effects of herbivory and predation by fish and waterfowl informed our work conducted during the fall of 2017. This work is ongoing at time of progress report submittal.

Fig. 11 - Wild rice flowering; water-chestnut in background

Initially, our experimental design concentrated on the effects of wildlife (fish, rodents and waterfowl) on the growth and survival of wild rice. We constructed exclosures (as discussed in detail in the previous reporting period) to limit access by fish (full exclosures) or rodent/avian predation with fish excluded (full exclosures, cut below water-line to allow fish passage). Preliminary data suggests wildlife herbivory on wild rice is not a significant factor inhibiting wild rice germination and survival within SR or GMNWR-CI sites. Our focus for this phase of experimental wild rice restoration was to evaluate the effect of invasive, non-native vegetation – water chestnut (*Trapa natans*) in SR, and American lotus (*Nelumbo lutea*) in GMNWR-CI.

Following our work detailed in the previous report period, 30 June 2016 – 30 September 2017, we collected seed from the Sudbury River on sections of the river owned by U.S. Fish & Wildlife Service (USFWS). On 20 September 2017 and 12 Oct 2017, Native Plant Trust staff collected a total of approximately 60 oz. of ripe seed by hand from >300 plants, and stored seed in a container of freshwater above freezing (5-16°C) in relative darkness for 2-3 weeks prior to use in quadrats or exclosures at either experimental restoration site. With seed collected, we began to scout viable habitat to conduct the second phase of our experimental research design.

Fig. 12 - Wild rice exclosures; open-top and quadrats shown in fall, GMNWR-CI.

A. Seed tests for wildlife predation

- **Exclosure Trials**

Native Plant Trust staff constructed total of eight (8) exclosures to test wildlife predation of wild rice, four exclosures, of two treatments at both SR and GMNWR-CI. Each 1 x1 meter square exclosure is 6 ft. tall, and there are four PVC pipes, one at each corner, and hardware cloth was installed 2 ft. from the bottom of the exclosure. The design allows the exclosure to be driven into the mud until the hardware cloth reaches

the bottom, blocking entry by fish and birds.

In this phase, two exclosures per site were capped with hardware cloth upon installation, while a duplicate pair at each location was left opened at the top. The intent being to restrict fish and mammalian access (full exclosures) or to allow waterfowl predation on wild rice seeds and plants (open-top exclosures).

Thus for this experiment, we constructed two types of exclosures at each location (SR, GMNWR-CI), and seed was sown in both types:

1. **Full Exclosures** (no openings) – Excludes all wildlife herbivory – seed sown
   - Included a control, where no seed was sown.
2. **Exclosures with the cap removed** (open-top) – Excludes mammalian and fish predation, not avian. – seed sown
   - Included a control, where no seed was sown.

- **Quadrats**

In addition, we added eight quadrats constructed of 1" diameter PVC pipe at both SR and GMNWR. These were simple structures, $1m^2$ with no wire hardware cloth to restrict wildlife predation. Four treatment types occurred at these quadrats. All quadrats were located in areas adjacent to invasive species.

Fig. 14 - One of many water-chestnut plants removed from quadrats.

Quadrats were built and installed 1-3 November 2017, with four quadrats installed at SR and four at GMNWR-CI. Each quadrat was placed in an area which met several primary criteria including:

- Proximal area of quadrat not under invasive species management
- Proximal area of quadrat contains wild rice habitat, though no wild rice observed within immediate quadrat
- Area not previously seeded with wild rice

During this visit (1-3 November 2017) 50 ripe seeds of wild rice were sown within each exclosure treatment, and within each of the quadrats.

Beginning in May 2018, we periodically removed invasive plant species by hand from four total quadrats within each location (4 quadrats at SR, 4 quadrats at GMNWR-CI) throughout the period of seed germination and during the reproductive stages of wild rice. Treatments of quadrats at both SR and GMNWR-CI include the following:

Qa. Quadrat Seeded & Invasive species removed
Qb. Quadrat Seeded & Invasive species unaltered
Qc. Quadrat – Not Seeded & Invasive plant species removed
Qd. Quadrat – Not Seeded & Invasive plant species unaltered

2018 Results

- Exclosure Trials

On June 20th, and June 26th, 2018, Native Plant Trust staff returned to the exclosure sites at both locations to record data on wild rice seed germination, and to assess any potential structural issues following overwintering and spring flooding. Exclosures without tops had germination and growth was recorded all sites. The type of treatment was randomized among the exclosures.

The following germination data was recorded on **June 20th, 2018** at the four exclosure sites and four quadrats at **Great Meadows NWR – Concord Impoundment:**

Fig. 15 - Wild rice germination in open quadrat, Sudbury River.

Full Exclosure (a)	Germination (amt/50)	% Germination Rate
Seeded	31	62
Not Seeded	0	0

Full Exclosure (b)	Germination (amt/50)	% Germination Rate
Seeded	28	56
Not Seeded	0	0

Open-top Exclosure (c)	Germination (amt/50)	% Germination Rate
Seeded	21	42
Not Seeded	0	0

Open-top Exclosure (d)	Germination (amt/50)	% Germination Rate
Seeded	14	28
Not Seeded	0	0

Quadrats (4)	Germination (amt/50)	% Germination Rate
Qa) Seeded / Invs. Removed	34	68
Qb) Seeded / Invs. Unaltered	10	20
Qc) Not Seeded / Invs. Removed	1	2
Qd) Not Seeded / Invs. Unaltered	0	0

The following germination data was recorded on **June 26th, 2018** at the four exclosure sites and four quadrats at **Sudbury River** (USFWS):

Full Exclosure (e)	Germination (amt/50)	% Germination Rate
Seeded	19	38
Not Seeded	0	0

Full Exclosure (f)	Germination (amt/50)	% Germination Rate
Seeded	30	60
Not Seeded	1	?

Open-top Exclosure (g)	Germination (amt/50)	% Germination Rate
Seeded	34	68
Not Seeded	0	0

Open-top Exclosure (h)	Germination (amt/50)	% Germination Rate
Seeded	19	38
Not Seeded	0	0

Quadrats (4)	Germination (amt/50)	% Germination Rate
Qe) Seeded / Invs. Removed	42	84
Qf) Seeded / Invs. Unaltered	25	50
Qh) Not Seeded / Invs.	3	?
Qi) Not Seeded / Invs.	0	0

On August 24th and August 25th, 2018, Native Plant Trust staff returned to the exclosure sites at both locations to record data on wild rice seed germination survivorship and general plant growth.

The following data was recorded on **August 24th, 2018** at four exclosure sites and four quadrats along the **Sudbury River (USFWS)**:

Fig. 5 - Wild rice growing through full-exclosure (top opened for photo); American lotus in background.

Full Exclosure (e)	Survival (amt/19)	% SurvivalR	Plant Height	#/Reproductive Stage
Seeded	9	47.4	1m	2 in flower, 7 veg.
Not Seeded	0	0	N/A	N/A

Full Exclosure (f)	Survival (amt/30	% Survival Rate	Plant Height (avg.)	#/Reproductive Stage
Seeded	12	40	1.4m	4 in flower, 8 veg.
Not Seeded	1	N/A	1m	1 veg.

Open-top Exclosure (g)	Survival (amt/34	% Survival Rate	Plant Height (avg.)	#/Reproductive Stage
Seeded	19	55.9	1.3m	2 in flower, 17 veg.
Not Seeded	0	0	N/A	N/A

Open-top Exclosure (h)	Survival (amt/19	% Survival Rate	Plant Height (avg.)	#/Reproductive Stage
Seeded	10	52.7	1m	1 in flower, 9 veg.
Not Seeded	0	0	N/A	N/A

Quadrats (4)	Survival	% Survival Rate	Plant Height (avg.)	#/Reproductive Stage
Qe) Seeded / Invs. Removed	36 (/42)	85.7	1.5m	9 in flower, 27 veg.
Qf) Seeded / Invs. Unaltered	10 (/25)	40	1m	1 in flower, 9 veg.
Qh) Not Seeded / Invs. Removed	1 (/3)	33	1.2m	1 veg.
Qi) Not Seeded / Invs. Unaltered	0	0	N/A	N/A

The following data was recorded on **August 25th, 2018** at four exclosure sites and four quadrats within the **Great Meadows NWR – Concord Impoundment**:

Full Exclosure (a)	Survival (amt/31	% Survival Rate	Plant Height (avg.)	#/Reproductiv e Stage
Seeded	21	67.7	0.8m	2 in flower, 19 veg.
Not Seeded	0	0	N/A	N/A

Full Exclosure (b)	Survival (amt/28	% Survival Rate	Plant Height (avg.)	#/Reproductive Stage
Seeded	13	46.4	1m	1 in flower, 12 veg.
Not Seeded	0	0	N/A	N/A

Open-top Exclosure (c)	Survival (amt/21	% Survival Rate	Plant Height (avg.)	#/Reproductive Stage
Seeded	10	47.6	0.8m	2 in flower, 8 veg.
Not Seeded	0	0	N/A	N/A

Open-top Exclosure (d)	Survival (amt/14	% Survival Rate	Plant Height (avg.)	#/Reproductive Stage
Seeded	1	7	1m	1 in flower, 9 veg.
Not Seeded	0	0	N/A	N/A

Quadrats (4)	Survival	% Survival Rate	Plant Height (avg.)	#/Reproductive Stage
Qa) Seeded / Invs. Removed	26 (/34)	76.5	1.5m	9 in flower, 17 veg.
Qb) Seeded / Invs. Unaltered	2 (/10)	20	1m	2 veg.
Qc) Not Seeded / Invs.	1	N/A	1.2m	1 veg.
Qd) Not Seeded / Invs.	0	0	N/A	N/A

- Germination Trials at Garden in the Woods

In addition to exclosure trials, *ex situ* germination trials were conducted at Garden in the Woods, Framingham, MA.

Seed was sown into Metro-Mix professional growing mix in 8, 5x4" propagation flats and floated in 3" of freshwater. Each flat contained 25 Wild rice seeds, and was allowed to overwinter and freeze while floating in (relatively muddy) water. This experimental germination trial required a total of 200 (~4 oz.) wild rice seeds. The remainder of seed materials was stored in a container of freshwater above freezing (5-16°C), and in relative darkness for the winter months.

Fig. 16 - Wild rice seeds

The following table summarizes these findings over the 33-week period data was collected on seed germination.

DATE	Flat	Germination Results (x/25)	% Germination	Avg. Germination	Total # Surviving (X/200)
11/20/2017					
	A-H	N/A	N/A		
4/23/2018					
	A	18	72		
	B	1	4		
	C	0	0		
	D	1	4		
	E	19	76		
	F	8	32		
	G	12	48		
	H	8	32		
			Avg. 33.5%	**8.4/25**	**67**
5/4/2018					
	A	3	12		
	B	1	4		
	C	0	0		
	D	1	4		
	E	0	0		
	F	6	24		
	G	1	4		
	H	5	20		
			Avg. 8.5%	**2.1/25**	**17**

5/18/2018					
	A	1	4		
	B	1	4		
	C	0	0		
	D	2	8		
	E	2	8		
	F	2	8		
	G	3	12		
	H	4	16		
			Avg. 7.5%	**1.9/25**	**15**
6/8/2018					
	A	0	0		
	B	0	0		
	C	0	0		
	D	1	4		
	E	0	0		
	F	4	16		
	G	1	4		
	H	0	0		
			Avg. 3%	**0.75/25**	**6**
7/5/2018					
	A-H	0	N/A	N/A	0

Discussion of 2017-2019 Results

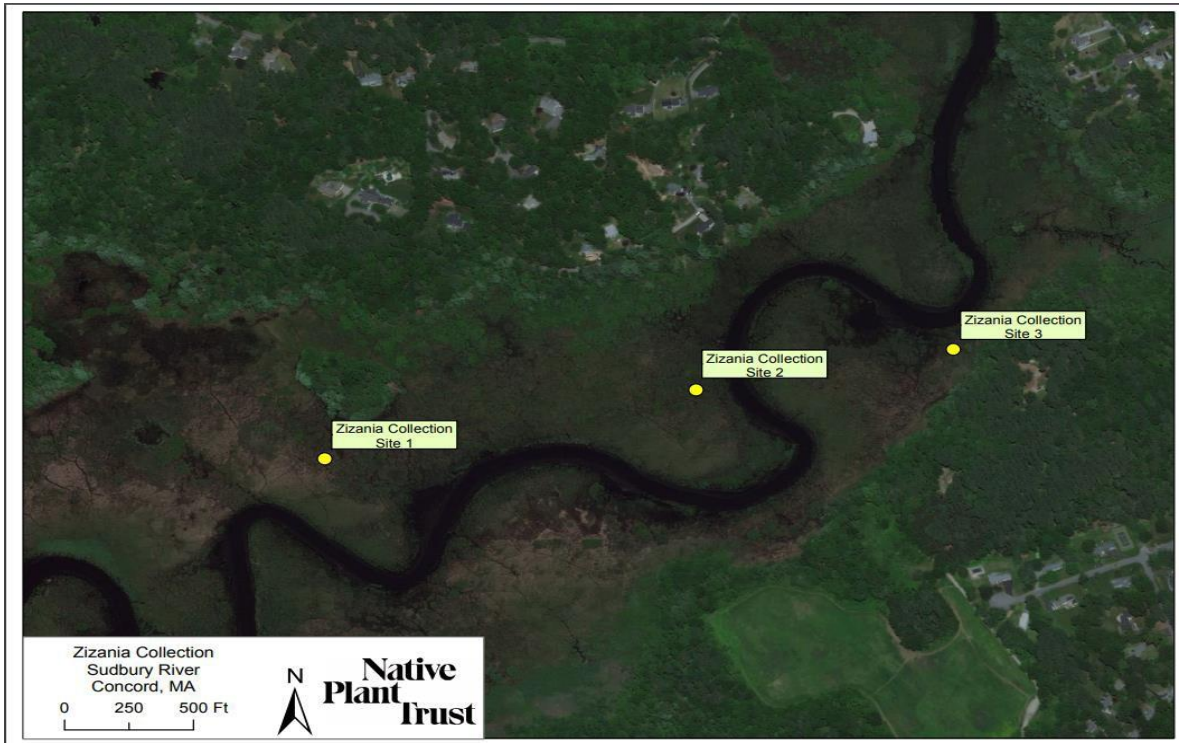
- Seeding:

Sowing seeds produced wild rice plants in every case, regardless of treatment type. We observed wildlife herbivory at both SR and GMNWR-CI, but none that appeared to impact wild rice populations to a significant degree. Where waterfowl cause damage appears to be in the early stages of vegetative development on wild rice (through consumption of shoot and leaf materials), which may inhibit growth, depending on time of grazing. Seeds broadcast within full exclosures (even in a relatively small amount of 50/exclosure) survived to flowering and seeding.

From September 23th to 26th, 2019, we collected seed from wild rice plants from areas within the study area on USFWS property along the Sudbury River. A total of approximately 220oz of wild rice seed was collected from 68 plants, with care taken to collect <20% of seed from each individual, and to distribute collection along approximately 0.8 river miles.

Following collection, seed was stored in water for 1 day (Sept 24th, 2019), before being filtered to exclude floating, presumably dead and inviable seeds.

Seed was broadcast from a canoe along the Sudbury River, in areas where ongoing management of *Trapa natans* is occurring, or has occurred since last reporting (see Conclusion for details on seed broadcasting 2019).



Map 1 – Collection areas of Zizania aquatica for broadcasting. Broadcast occurred primarily between these collection areas.

Fig. 17 – American lotus growing within full exclosure from pre-existing soil seedbank.

- Invasive Species:

One primary concern is that invasive plant species are the dominant competition for habitat (and likely, for nutrients). This was observed at both SR and GMNWR-CI evidenced by the highest survival rates where seed was sown and invasive plant species were removed by hand at quadrats Qa and Qe (76.5%, 85.7%, respectively). Further, where quadrats were sown but invasive plant species were not removed (Qb and Qf) exhibited <50% survivorship (20% and 40%, respectively) when compared with quadrats where invasive plant species were controlled. In addition, of the 12 total genets of wild rice observed within quadrats where invasive species were unaltered, only 1 genet was observed at reproductive stage. This is likely a result of seed germination, stem growth, and reproductive structures slowed due to competition with dense invasive vegetation.

a. Although not quantified, with each visit to any of the exclosures sites, we found the most significant negative impact to wild rice in these exclosures appeared when invasive plant species (primarily water-chestnut on Sudbury River and American lotus at Great Meadows) were able to enter the exclosures, either through pre-existing seeds in the soil seedbank below or possibly penetrating the exclosures from the openings. Anecdotally, some researchers think that invasive species are a major reason for the decline of wild rice, and understanding the dynamic between invasive plants and wild rice is important to our understanding of how to successfully restore wild rice on the landscape.

- Exclosures

While few incidental seeds did germinate in the exclosures that were not seeded (Full Exclosure F, Qc and Qh), the seeded exclosures and quadrats had many times more seeds exhibiting germination, and wild rice plants growing to reproductive stages of flowering and fruiting. Even in the most abundant case of incidental seeding (from seedbank, seed flotation, etc.) only 1 wild rice plant was observed. It appears from this preliminary evidence that broadcasting of seed on either site (GMNWR-CI or SR) may be possible as part of a largescale restoration, with greater germination and survivorship at SR than GMNWR, due to apparent habitat preference and less total area dominated by invasive vegetation. Broadcasting of wild rice seed should only be conducted following implementation of a comprehensive invasive species management regime or in areas where invasive species are not present. Further, wild rice habitat typically includes low-energy riparian edges with moderate fluctuation in water levels, oxbows, mudflats, and tributaries. These habitat types located within the Sudbury, Assabet, and Concord River Watershed would be ideal for future broadcasting of wild rice seed.

Fig. 18 – Wild rice in flower in exclosures with American lotus in foreground & background, GMNWR-CI.

- Germination Trials:

Germination was first recorded on April 23rd, 2018, and with a rate of 33.5%, with 67 genets recorded. Germination began to decline as data was recorded every 2-3 weeks, and all flats (with the exception of Flat C (which had no germination) continued to germinate new genets until the end of the trial on August 10th, 2017. The highest recorded number of surviving wild rice plants was on May 23rd, 2018 with 67 genets, with many plants reaching 1-2ft in height.

Survivorship declined during May 2018, with only 6 genets surviving into June. The increase in mortality of surviving plants is likely due to high water levels caused by major rain events in early June, and flooding of the flats in an artificial environment.

This trial indicates an average germination percentage of 13% over the course of the germination trial, far less than observed *in situ*. Further germination trials would be better conducted in an area of more natural hydrology, typical of the habitat where wild rice occurs in the region.

Conclusion

Fig. 19 – Water-chestnut removed from Sudbury River quadrats.

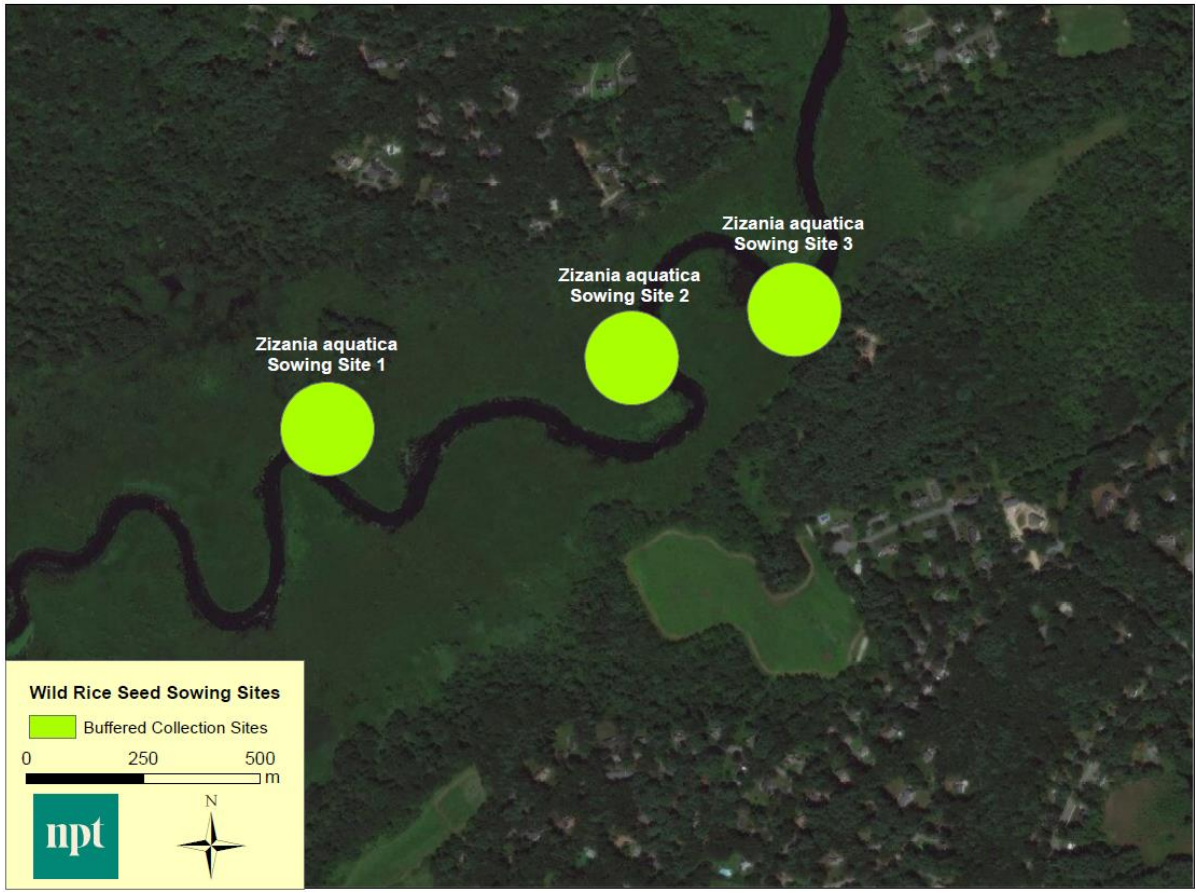
From the data collected in 2016-18, we are beginning to better understand the potential of wild rice restoration at both Great Meadows NWR– Concord Impoundment and Sudbury River**.** Regardless of this research, it appears much of the potential habitat suitable to wild rice proliferation has been compromised at GMNWR-CI. This is due primarily to the complete domination of surface water from American lotus. Low-energy riparian edges found on the SR are more ideal for future broadcasting of wild rice seed. It appears the exclosures have a positive effect on the growth and survivorship of *Zizania aquatica* in these environs, allowing it to flourish in areas otherwise vulnerable to invasive species proliferation. However, predation by waterfowl and wildlife do not seem to be the immediate stressor on growth of wild rice. From our preliminary data, we recognize the proliferation of invasive plant species to be the primary limiting factor to wild rice growth. We were able to further assess this hypothesis by collecting data within our opened quadrats, which did not limit wildlife from preying upon wild rice at either site. From this preliminary data, we strongly recommend future research focuses on the relationship of invasive plant species (in particular, water-chestnut [*Trapa natans*] at SR and American lotus [*Nelumbo lutea*] at GMNWR-CI) with that of wild rice and its habitat. Further research may also factor in the effects of water chemistry, nutrient loading, and climate-related events (such as storm surges, extensive flooding, drought, etc.) on wild rice and its habitat.

Fig. 20 – Bumblebee pollinating wild rice flowers, Sudbury River.

In the fall of 2018 and spring of 2019, we removed all existing exclosures and quadrats from GMNWR-CI and SR, and disposed of invasive species plant materials collected during this phase of our research. In addition, we broadcasted seed of wild rice in areas of the Sudbury River where we believe suitable habitat remains unaltered by invasive species and will support wild rice growth going forward. In September 2019, we broadcast sowed approximately 220oz of wild rice seed in areas where ongoing invasive species management has taken place, specifically, for *Trapa natans* or water chestnut.

In 2019-2020, we compiled all data collected during this project into this cohesive final report.

2019 Zizania aquatica Seed Sowing Locations – Great Meadows NWR / Sudbury River



Zizania aquatica Sowing Site (Sudbury River)	Lat.	Long.
Site 1	42.3898	-71.3715
Site 2	42.3908	-71.3656
Site 3	42.3915	-71.3625

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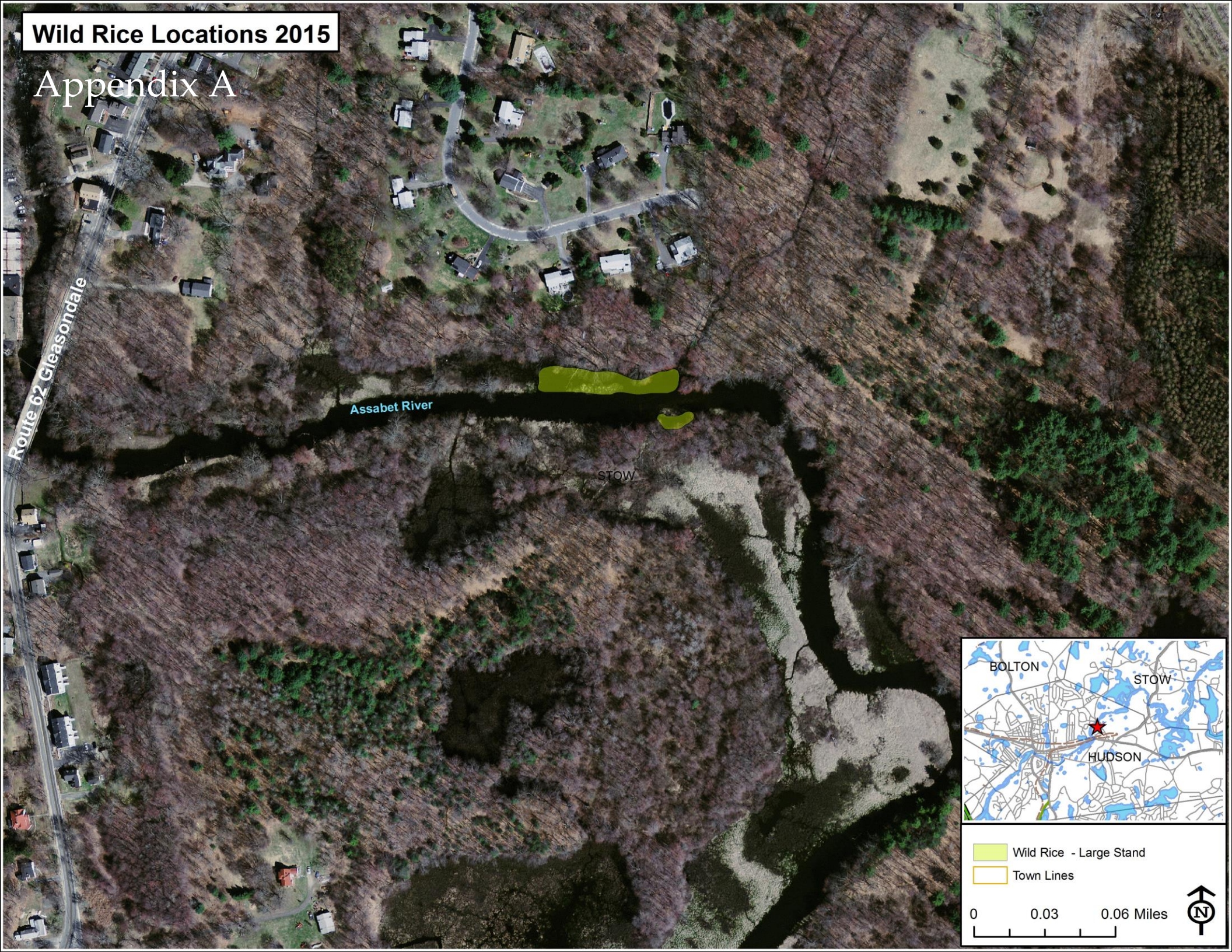
Wild Rice Locations 2015

Appendix A

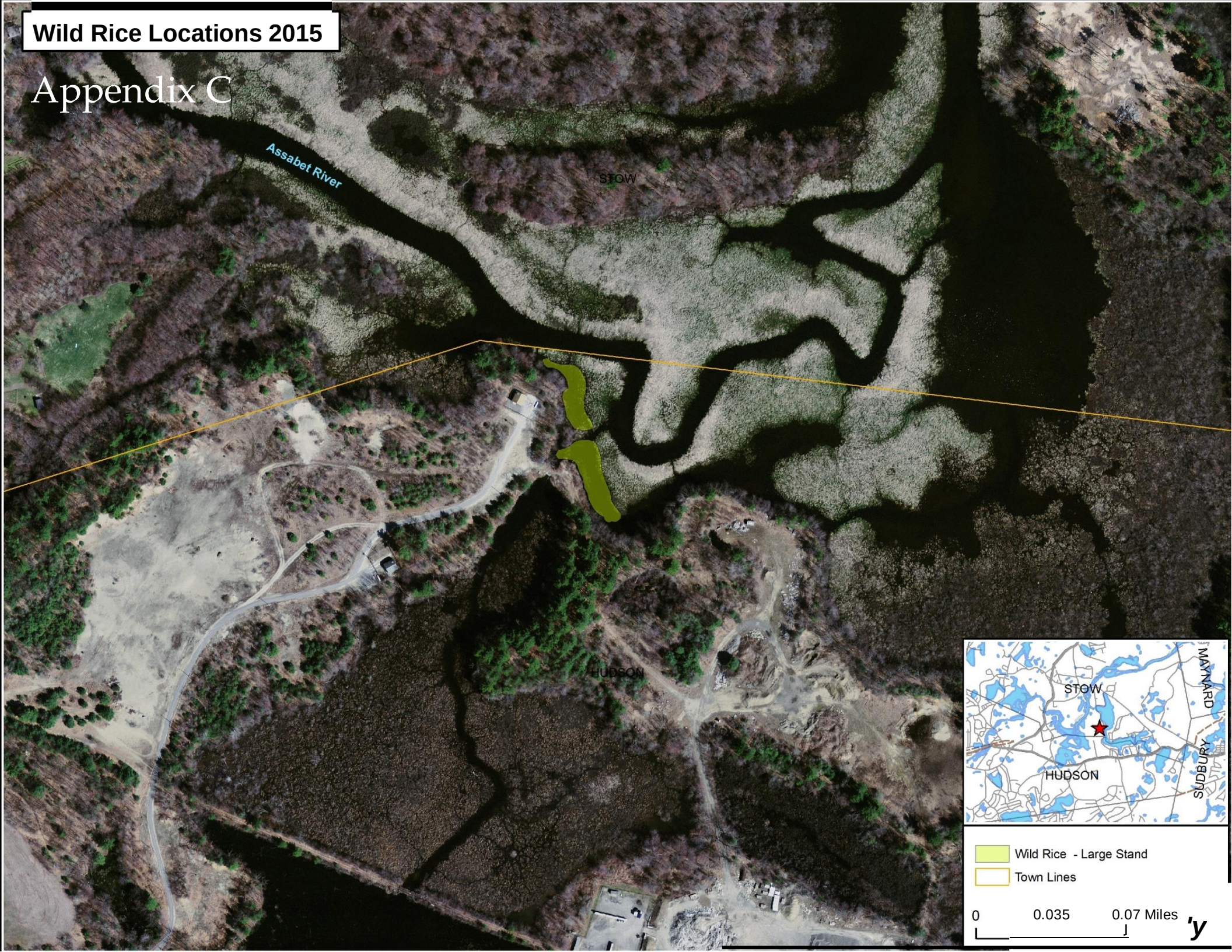


Wild Rice Locations 2015

Appendix B

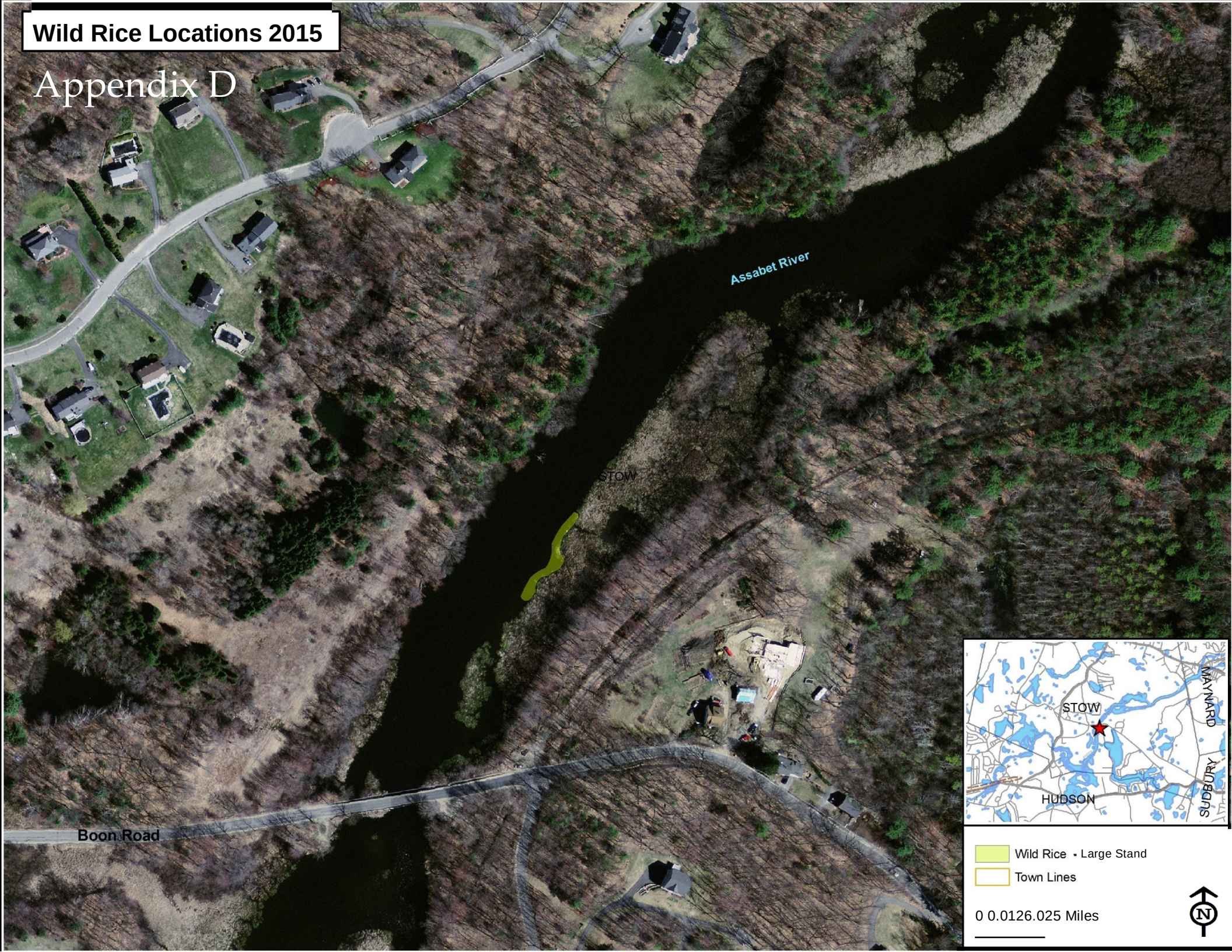
Wild Rice Locations 2015

Appendix C



Wild Rice Locations 2015

Appendix D



Wild Rice Locations 2015

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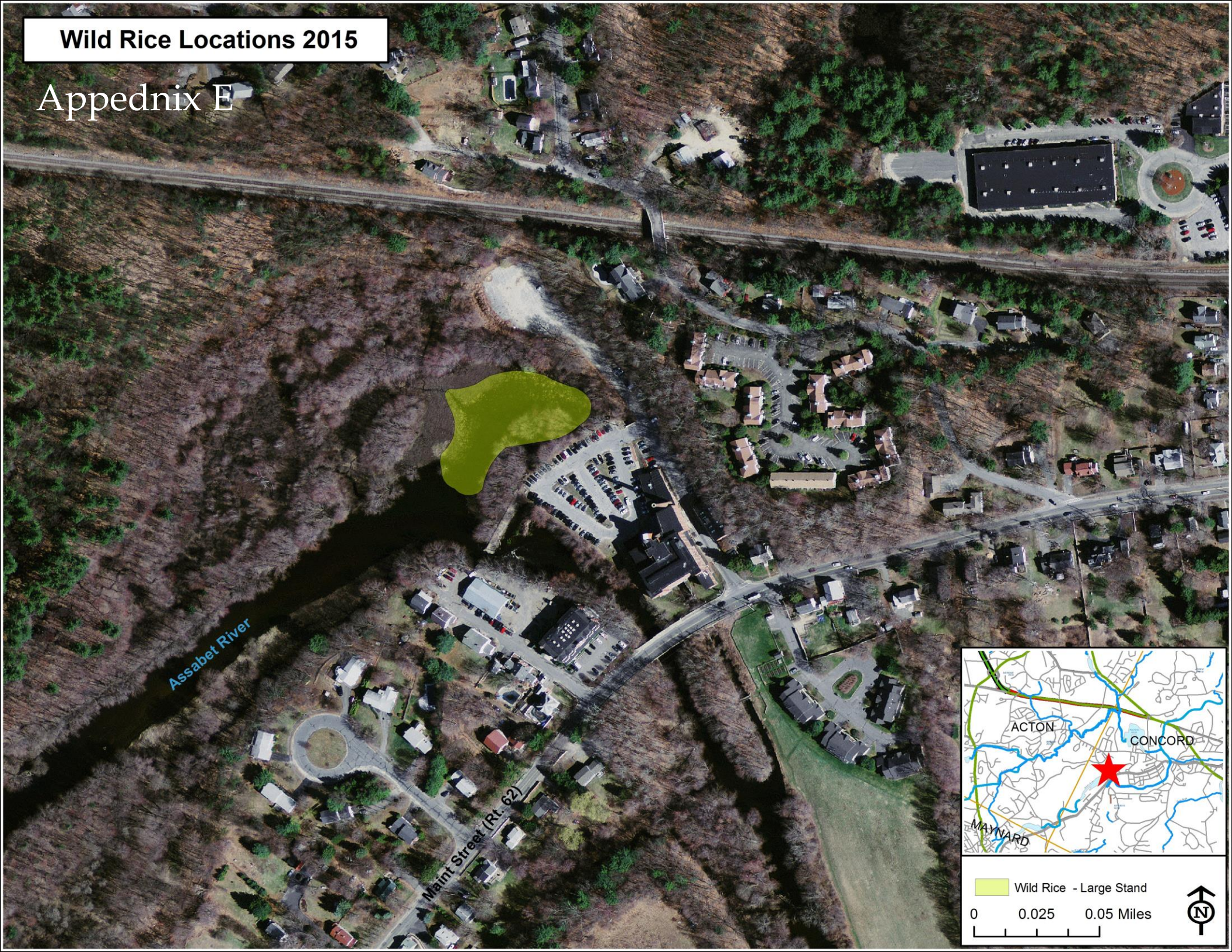

Wild Rice Locations 2015

Appendix F