HYBRIDIZATION DYNAMICS OF INVASIVE CATTAIL (*TYPHACEAE*) STANDS AT PIERCE CEDAR CREEK INSTITUTE: A MOLECULAR ANALYSIS

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ABSTRACT

Three cattail taxa are recognized in Michigan USA: native *Typha latifolia* (broad-leaf cattail), the invasive *Typha angustifolia* (narrow-leaf cattail), and the hybrid of these two species *Typha* × *glauca*. *Typha angustifolia* and *T.* × *glauca* are of special interest because of their ability to aggressively spread and out-compete the native cattail *T. latifolia*. *Typha* × *glauca* has been shown to out-compete both its parental taxa and produce monospecific stands. We surveyed the Pierce Cedar Creek Institute (PCCI) property for cattails and located 25 distinct cattail marshes. We determined the total area of cattail marsh at PCCI to be roughly 10% of the 267 ha property. Cattail individuals were sampled from each of the 25 stands and random amplified polymorphic DNA markers were used to identify the individuals to species. We found that 20 of the 25 stands were monospecific for the native cattail, *T. latifolia*. Five of the stands were mixtures of the native *T. latifolia* and the introduced *T. angustifolia*, and *T.* × *glauca* was found in two of the mixed stands. We recommend removal of the invasive *T. angustifolia* and *T.* × *glauca* individuals and the establishment of a monitoring plan in order to maintain the long-term health of the cattail marshes at PCCI.

Keywords: *Typha* spp., RAPD markers, invasive species

INTRODUCTION

Species of *Typha* L. (Typhaceae), commonly known as cattails, are highly productive emergent plants that grow in a variety of wetland habitats throughout the world (McManus et al. 2002). In the northern USA and Canada three taxa of cattail have been recognized: *Typha latifolia* L. (broad-leaf cattail), *Typha angustifolia* L. (narrow-leaf cattail), and *Typha* × *glauca* Godr. (white cattail). *Typha latifolia* is a native plant species that is a keystone emergent in marsh communities throughout North America (Smith 2000). In the United States, broad-leaf cattail is native to all 50 states. In Canada, it occurs in all provinces and territories except Nunavut (USDA, NRCS 2012).

Typha angustifolia is thought to have been introduced to the eastern seaboard from Europe in the early 19th century (Stucky and Salamon 1987; Selbo and Snow 2004), although Shih and Finkelstein (2008) suggest it may be present in pollen cores dating back 1,000 years. Following its colonization of the Atlantic coast, *T. angustifolia* began to move inland slowly – but by the early 20th century it had begun a rapid westward expansion (Mills et al. 1993). Today, *T. angustifolia* is found in 42 of the 50 United States; it is absent from Florida, Georgia, Alabama, Mississippi, Texas, Utah, Arizona, Hawaii and Alaska. In Canada it is found in all provinces except Labrador and Newfoundland and is absent in the Yukon, Northwest, and Nunavut Territories (USDA, NRCS 2012). Because of its aggressive spread, *T. angustifolia* is considered an invasive species. It often out-competes native wetland species, including *T. latifolia*, to produce very dense monospecific stands (Grace and Harrison 1986).

Typha latifolia and *T. angustifolia* are obligate wetland species, meaning that they are always found in or near water. Both species generally grow in flooded areas; however, *T. latifolia* is typically found in waters that do not exceed 0.8 m while *T. angustifolia* prefers deeper

water, usually greater than 0.75 m. Where the two species are sympatric, they typically segregate by water depth (Travis et al. 2010).

According to Grace and Harrison (1986), both *T. latifolia* and *T. angustifolia* are self-compatible, wind pollinated, clonal species. The reproductive shoots of both species are monoecious, with staminate flowers occurring above pistillate flowers, and protogynous (pistillate flowers produced prior to staminate flowers). The pistillate flowers remain receptive to pollen for four weeks (Kuehn et al. 1999). While the protogynous condition would seem to facilitate out crossing (Smith 2000), Krattinger (1975) showed that cattails are largely self-fertilized and that vegetative reproduction occurs more frequently than sexual reproduction. In part, the two species can be distinguished by the presence or absence of a spike gap between the staminate and pistillate flowers (*T. latifolia* generally has no spike gap, whereas *T. angustifolia* has a spike gap ranging from 0.5 to 4 cm). *T. latifolia* often flowers later than *T. angustifolia* but overlap in flowering times can lead to hybridization between the species (Selbo and Snow 2004). In fact, *T. latifolia* and *T. angustifolia* appear to hybridize wherever the two occur sympatrically (Galatowitsch 1999; Kuehn et al. 1999; Olsen et al. 2009).

The third taxa, *Typha* × *glauca*, is the hybrid of *T. angustifolia* (maternal) and *T. latifolia* (paternal) (Grace and Harrison 1986; Kuehn et al. 1999); however, its hybrid status has long been disputed. *Typha* × *glauca* has been identified as a distinct species; a stabilized hybrid taxon; an introgressed taxon with *T.* × *glauca* representing a series of intermediates in a hybrid swarm; and also as a sterile F_1 hybrid (Kuehn et al. 1999 and references therein). Taxonomic descriptions of *T.* × *glauca* frequently identify the plant as a sterile F_1 hybrid; however, recent studies have revealed the presence of backcrossed and later generation hybrids indicating that *T.* × *glauca* has at least some degree of fertility (Snow et al. 2010; Kirk et al. 2011).

The spread of invasive taxa are of particular interest to evolutionary biologists and ecologists because of their ability to alter community structure and ecosystem function (Horvitz et al. 1998). *Typha* × *glauca* is considered to be a highly invasive species due to its aggressive range expansion and ability to dominate wetland habitats. According to Galatowitsch et al. (1999), several hypotheses have been advanced in an effort to explain how introduced plant species become invasive species. The Introgression/Hybrid Speciation hypothesis suggests that interspecific hybridization between an introduced taxon and a native taxon results in novel phenotypes with selective advantages. Therefore, hybridization between native and introduced species is considered to be one of the driving forces behind the evolution of invasiveness (Ellstrand and Schierenbeck 2000; Schierenbeck and Ellstrand 2009). Hybrids between native and introduced species have frequently been shown to have increased fitness with respect to their parental species as they possess greater genetic and phenotypic diversity than their parents (Kuehn et al. 1999; Ellstrand and Schierenbeck 2006; Kirk et al. 2011). This appears to be the case for *T.* × *glauca* which can colonize the entire range of water depths in which the parental species segregate (Travis et al. 2010). Several researchers (e.g., Zedler and Kercher 2004; Travis et al. 2010) have documented the competitive superiority of *T.* × *glauca* indicating its potential as a highly invasive taxon.

F_1 hybrids are generally expected to be morphologically intermediate to their parental taxa but this is not necessarily the case for the hybrid *T.* × *glauca*. The leaf width of the hybrid *T.* × *glauca* is believed to range from 6 mm to 16 mm; although leaf widths up to 21 mm have been reported. Kuehn et al. (1999) found considerable phenotypic variation in leaf width for each of the parental species. The leaf width of *T. angustifolia* ranged from 4.5 mm to 12 mm and the leaf width of *T. latifolia* ranged from 7.5 mm to 22 mm. Therefore, the leaf width of *T.* ×

glauca overlaps with the parental species making this trait unreliable for hybrid identification. Flowering in cattails is also an unreliable trait for taxonomic identification because cattails often exhibit poor flower production. Dickerman (1982) found that over a three-year period only three of 1,779 marked shoots flowered at Lawrence Lake in Barry County, Michigan. The degree of shading, including self-shading in dense stands (Grace and Wetzel 1982), and the depth of rhizome submergence (Grace 1989) affect flowering success. In addition to poor flowering and the overlap in morphological traits, backcrossed and advanced generation hybrids are phenotypically more similar to one of the parental taxa further complicating the identification of hybrid individuals through morphological traits (Kuehn et al. 1999; Selbo and Snow 2004; Snow et al. 2010). Because morphological traits can be highly variable, DNA markers are considered to be more reliable for the identification of cattail species and their hybrids (Kuehn et al. 1999; Selbo and Snow 2004).

This study examined 25 discrete marsh populations at Pierce Cedar Creek Institute in Hastings, Michigan, USA in order to gain an understanding of the distribution and abundance of *T. latifolia, T. angustifolia*, and *T.* × *glauca*. We used random amplified polymorphic DNA (RAPD) markers coupled with intensive field sampling of the 25 populations to identify individual cattails to species. Kuehn et al. (1999) developed species-specific RAPD markers for *T. latifolia* and *T. angustifolia.* RAPD analysis of genomic DNA is expected to produce species-specific RAPD banding patterns while the hybrid, *T.* × *glauca*, is expected to display the banding patterns of each parent. Because of the potential for *T. angustifolia* and *T.* × *glauca* to invade the marsh communities at PCCI and out-compete the native *T. latifolia*, baseline data on the presence and distribution of *Typha* spp. is imperative to the development of an effective wetland management plan for the Institute.

METHODS

Study Site

This research took place at Pierce Cedar Creek Institute (PCCI) near Hastings, Michigan, USA. PCCI is an environmental field station and nature center whose property consists of forest, field, open water, and a variety of wetland habitats. Of the 267 ha site, a total of 103 ha is considered wetland. Brewster Lake and approximately 1.9 km of Cedar Creek are contained on the property and many of the wetlands are associated with these bodies of water. Most of the property was previously farmed and many of the wetland habitats along Cedar Creek appear to be recovering from disturbance. In addition, a tributary to the creek has also been dammed by beavers below Brewster Lake. This has led to flooding and the creation of shallow water habitat which is ideal for cattail growth. The cattail composition at PCCI has not been previously studied, but a 2003 vascular plant inventory of the property indicated that only *T. latifolia* was present (Slaughter and Skean 2003).

Mapping of Cattail Stands

In April of 2012, we visually surveyed the property by traveling on foot along the trails, and also by canoeing Cedar Creek. After visual determination of the locations of cattail stands, each stand was mapped using a hand-held GPS unit (Garmin *e*-Trex 30). Mapping consisted of traveling the perimeter of each cattail stand with the GPS unit creating an electronic track file. This data was entered into a geographical information system (ArcGIS 10) to create a digital map depicting the location of each cattail stand on PCCI property (Fig. 1). Each track on the GPS, with the exception of the Brewster Lake track, was created by walking the perimeter of the cattail stand. The Brewster Lake track was created by rowing around the perimeter of the lake in a boat.

Taxon Sampling

Typha samples were collected from each identified cattail stand. Within each stand, we began collecting at the edge and traveled straight-line transects, by foot, through the stand, stopping at least every 10 m to collect leaf tissue from the nearest ramet (an individual shoot of a clonal organism). In larger stands, the distance between sampled ramets often exceeded 10 m. For stands that were too small to establish transects, or in cases where traveling in a straight line was impractical, sampled ramets were separated by a distance of no less than 10 m. Having a minimum distance of 10 m between samples has been shown to decrease the likelihood of sampling clone mates (Kuehn et al. 1999; Olsen et al. 2009; Snow et al. 2010). No *a priori* species assignments were made at the time of collection because *T. angustifolia, T. latifolia* and *T.* × *glauca* display significant overlap in morphological characteristics (Kuehn et al. 1999, Olsen et al. 2009), however we did sample from a representative range of morphological variants within each stand. We sampled by clipping approximately 7 cm of leaf tissue from the youngest leaf. The tissue was immediately placed into a plastic bag containing silica gel desiccant. Each sampled ramet was flagged and numbered along with the corresponding tissue sample.

Identification of Typha Species and Hybrids

Total genomic DNA was extracted from approximately 3 cm^2 of dried leaf tissue following a variation of the 2 x CTAB (cetyl trimethylammonium bromide) extraction method for high polysaccharide plants described by Nickrent (2006). DNA extractions were stored at -20 °C for use in genetic identification of species and hybrids.

We used RAPD molecular markers to identify *T. latifolia, T. angustifolia,* and *T.* × *glauca.* Total genomic DNA was amplified with PCR using RAPD primers A2 (TGCCGAGCTG) and A8 (GTGACGTAGG) (Eurofins mwg/Operon Huntsville, AL, USA).

Each 25 µL reaction mixture contained 19.7 µL ultra-sterile H_2O, 2.5 µL 1 × PCR buffer (200 mM Tris-HCl [pH 8.4], 500 mM KCl), 1 µL 50 mM $MgCl_2$, 1 µL of either RAPD - A2 or RAPD - A8 primer, 0.25 µL 10 mM PCR Nucleotide Mix (USB Corp. Cleveland, OH, USA), 0.05 µL Platinum® Taq DNA Polymerase (Invitrogen, Carlsbad, CA, USA), and 0.5 µL total genomic DNA.

The 25 µL reaction mixtures were incubated in an iCycler Thermal Cycler (Bio-Rad Laboratories, Inc., Hercules, CA, USA) programmed for 2 min at 94 ºC (initial denaturation); 25 cycles of 1 min at 94 ºC, 1 min 30 sec at 34.5 ºC, and 2 min 20 sec at 72 ºC followed by 20 cycles of 1 min at 94 ºC, 1 min 30 sec at 34.5 ºC, and 2 min 20 sec at 72 ºC with a 10 sec increase after each cycle.

RAPD products were separated by gel electrophoresis on 1.2% agarose gels for 1 h at 90 V. Gels were stained in an ethidium bromide bath for 15 min and photographed under a UV light source. Molecular weights of amplification products were estimated using a 1 kb plus DNA ladder (Invitrogen, Carlsbad, CA, USA). Primer A2 yields 5 species-specific bands with bands for *T. latifolia* at 1.5 kb, 1.0 kb, and 0.6 kb and bands for *T. angustifolia* at 1.2 kb and 0.8 kb; primer A8 produces 3 species-specific bands with *T. latifolia* having a band at 1.0 kb and *T. angustifolia* having bands at 2.0 kb and 1.8 kb (Kuehn 1999). F_1 *T.* × *glauca* individuals are expected to show all parental bands.

RESULTS

Twenty-five wetland communities containing *Typha* spp. were identified on PCCI property (Fig. 1). These ranged in individual area from 8 m^2 to 97,613 m^2 (Table 1). The largest stands were associated with the perimeter of Brewster Lake and the wetland complexes adjoining Cedar Creek. The smallest stand was a drainage ditch adjacent to the main road into the PCCI property. This road was constructed between 1999 and 2001 (Brown, Pers. Comm.). The cumulative area of all cattail stands totaled 259,878 m^2 (26.0 ha) which is approximately 9.72% of the PCCI property.

We collected tissue samples from a total of 370 individuals from across the 25 cattail stands. Of these, DNA was extracted from 252 randomly chosen individuals, representing all 25 stands. Photos of two representative electrophoresis gels of RAPD DNA fragments produced by PCR amplification of cattail individuals using primer A8 are shown in Figure 2. The species-specific bands and species identification are indicated on the photos. We identified most individuals to species using primer A8. For approximately 1% of the individuals we confirmed the primer A8 species identification using Primer A2. No difference in species identification was observed between the two primers. RAPD analysis allowed us to identify 212 of these individuals to species; 200 (94.34%) were *T. latifolia*, 9 (4.25%) *T. angustifolia*, and 3 (1.42%) were the hybrid *T.* × *glauca*.

Of the 25 cattail stands at PCCI, 20 were monospecific, consisting of only the native cattail, *T. latifolia*, four were mixed for *T. latifolia* and *T. angustifolia*, and two stands consisted of a mixture of both parental species and their hybrid *T.* × *glauca* (Table 1).

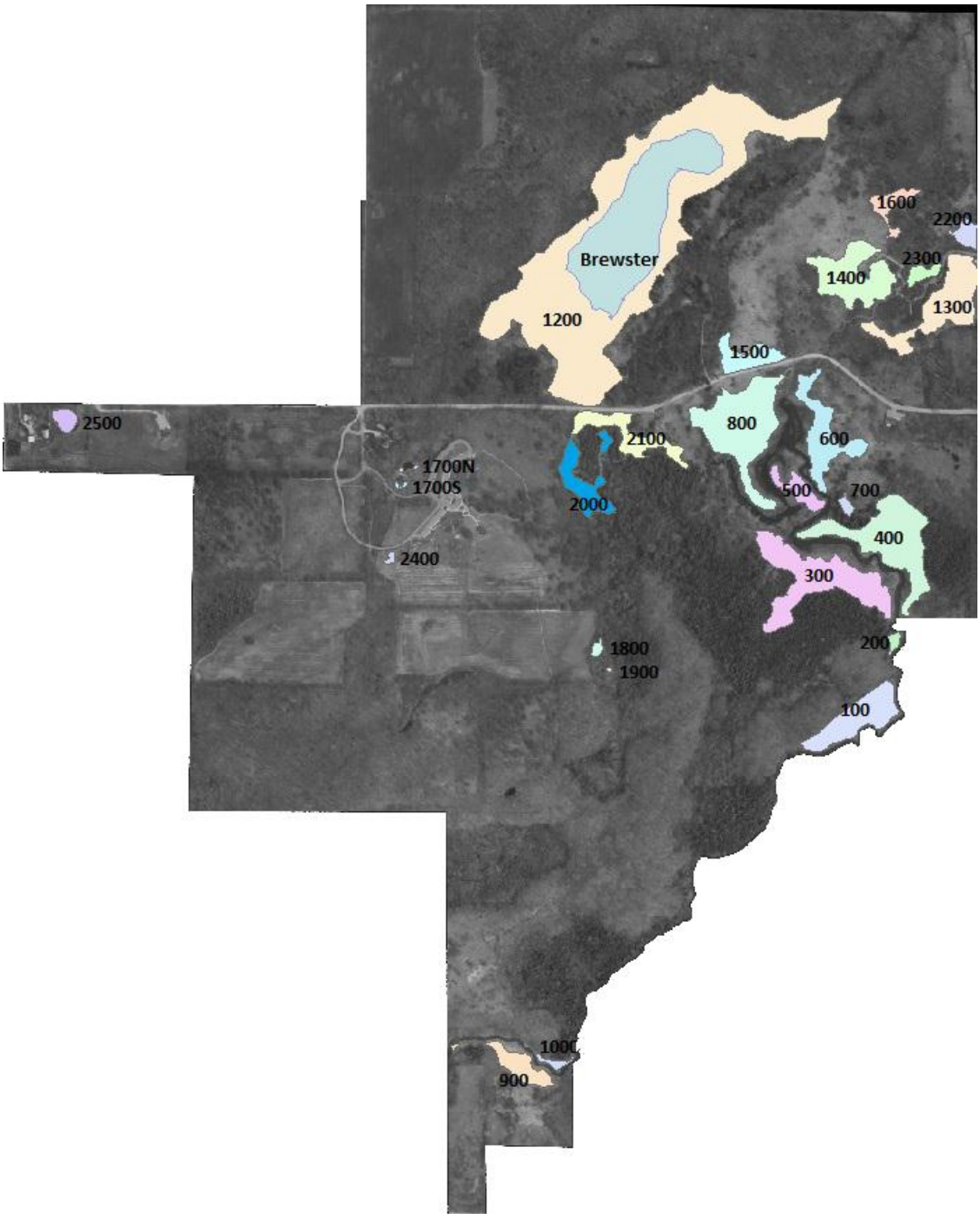
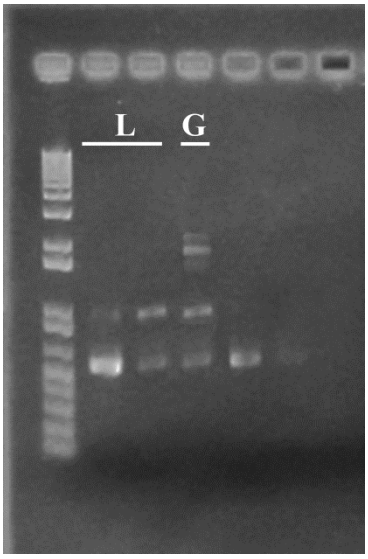


Figure 1. Cattail stands identified at Pierce Cedar Creek Institute near Hastings, Michigan. Numbers correspond with the coding assigned to sampled individuals. (See Table 1 for the species composition of each stand.)

Table 1. Area and species composition of the 25 cattail stands identified at Pierce Cedar Creek Institute. Area was calculated in Garmin *e-Trex* 30. The species composition of each stand (L - *Typha latifolia*, A - *Typha angustifolia*, and G - *Typha* × *glauca*) is presented as a percentage of each species per the total number of identified individuals from the stand.

Stand	Area (m^2)	% Species Composition
100	12181.00	L (100%)
200	717.82	L (100%)
300	27583.00	L (100%)
400	20048.00	L (100%)
500	3507.20	L (100%)
600	10751.00	L (75%), A (25%)
700	752.56	L (50%), A (50%)
800	22149.00	L (100%)
900	4607.00	L (100%)
1000	882.16	L (100%)
1200	97613.00	L (100%)
1300	14698.00	L (100%)
1400	14028.00	L (100%)
1500	5260.00	L (100%)
1600	3215.8.	L (100%)
1700S	207.03	L (33%), A (33%), G (33%)
1700N	177.02	L (100%)
1800	586.29	L (100%)
1900	95.06	L (100%)
2000	7133.76	L (100%)
2100	7695.90	L (77%), A (23%)
2200	2102.80	L (40%), A (20%), G (40%)
2300	1781.10	L (100%)
2400	371.96	L (66%), A (33%)
2500	1725.80	L (100%)
2600	7.63	L (100%)



B.

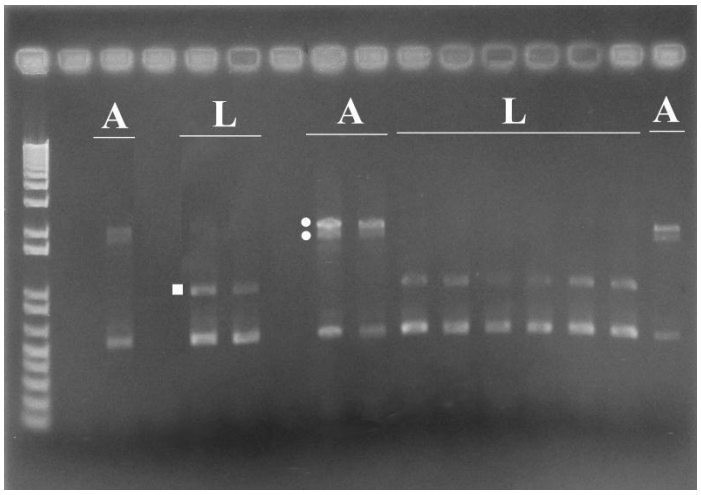


Figure 2. Random amplified polymorphic DNA (RAPD) phenotypes for cattail individuals identified using primer A8. The left lane contains a 1-kb plus DNA ladder size standard. A species-specific band for *Typha latifolia* (•) occurs at 1kb and two species-specific bands for *T. angustifolia* (▪) occur at 2.0kb and 1.8kb. A. One *T.* × *glauca* (G) and two *T. latifolia* (L) individuals identified by RAPD analysis. B. Eight *T. latifolia* (L) and four *T. angustifolia* (A) individuals identified by RAPD analysis.

DISCUSSION

This study documents the extent to which the native cattail *Typha latifolia*, the introduced and widespread cattail *T. angustifolia*, and the hybrid of these two species, *T. x glauca* occur at Pierce Cedar Creek Institute. Approximately 40% of the PCCI property is characterized as wetland habitat based on its soil type and hydrology (Brown, pers. comm.). However, many of these wetlands are visibly dry throughout much of the year. Because cattails prefer standing water, we were surprised to find that nearly 10% of the property consisted of cattail marshes. The density and species composition within each sampled wetland varied from what appeared to be a monospecific stand of cattails with one ramet immediately adjacent to another, to stands in which individual cattail ramets were separated by distances of approximately 10 m. Diversity appeared to be much higher in these stands with a variety of sedges, forbs, and shrubs occurring in between the *Typha* ramets.

Research has indicated that there is significant morphological overlap between cattail species and their hybrid (Kuehn et al. 1999). Our results demonstrate that the native cattail, *T. latifolia*, is the dominant cattail species at PCCI; 20 of 25 cattail stands were monospecific for *T. latifolia*. We did, however, find evidence of the widespread, introduced species, *T. angustifolia*, in five of the 25 stands and in two of these mixed stands the hybrid *T.* × *glauca* was found to be present. The homogenous *T. latifolia* stands were primarily associated with the wetlands surrounding Brewster Lake and Cedar Creek. It is likely that these stands experience lesser disturbance from changes in hydrology and water depth. *Typha latifolia* is reported to have superior growth and competitive ability over *T. angustifolia* in shallow, undisturbed, water habitats (Grace and Wetzel (1989). By contrast, *T. angustifolia* was shown to be competitively superior to *T. latifolia* in shallow water habitats when they were highly eutrophic (Weisner

1993). We found that mixed parental stands were located in areas that are likely experiencing greater disturbance because of their proximity to the PCCI trail system, roads, and other anthropogenic influences such as culverts which may be acting to alter hydrology and increase sedimentation. Stand 1700S was of particular interest because it is a small retention pond that was constructed on the PCCI property sometime between 1999 and 2001 (Brown, Pers. Comm.). The very nature of a retention pond suggests fluctuating water levels and sedimentation. Since the hybrid, *T. x glauca* can exist throughout the range of water depths occupied by its parental species it is not surprising that we identified a hybrid individual from this site.

It is well documented that when the native *T. latifolia* occurs sympatrically with the introduced *T. angustifolia* they can produce the hybrid *T.* × *glauca* (Galatowitsch 1999; Kuehn et al. 1999; Olsen et al. 2009). *Typha angustifolia* itself can out-compete the native cattail, *T. latifolia*, and the hybrid *T.* × *glauca* can out-compete both parental species (Grace and Harrison 1986). Because the 2003 survey of vascular plants at PCCI (Slaughter and Skean 2003) did not identify the occurrence of *T. angustifolia*, we believe it has moved onto the property within the last ten years. Since a large patch of *T. angustifolia* is directly adjacent to the trail system it is unlikely that the species was overlooked in the 2003 survey. Therefore, the presence of *T. angustifolia* is cause for concern regarding the long-term health of the native *T. latifolia* cattail marshes at PCCI. We recommend the removal of all *Typha angustifolia* individuals and hybrids from the PCCI property in order to ensure the long-term health of the *T. latifolia* cattail marshes. At the very least, we recommend that PCCI undertake a monitoring program to record the health of, and/or changes to, the cattail marshes on their property.

A cattail marsh monitoring program should keep track of fluctuations in hydrology and water levels, as well as, the numbers and locations of the invasive cattail species. *Typha*

angustifolia has been shown to prefer deeper water than *T. latifolia* (Travis et al. 2010). Therefore, if water levels increase around Brewster Lake or Cedar Creek, such as might be caused by beaver dams, the disturbance creates *T. angustifolia* appropriate habitat. Additionally, if a cattail marsh regularly receives additional water and nutrients from runoff, such as through the existing culvert systems, they are likely to experience increased growth of the invasive cattail species. An appropriate control technique would be to manipulate water levels so that the preferred *T. angustifolia* habitat is not available for colonization.

Optimally, all non-native cattail species would be removed from the PCCI property. According to the USDA NRCS Plant Guide (USDA, NRCS 2012) cattails can be removed by mowing the plants after the flowering heads are well-formed but before they have reached sexual maturity. This mowing treatment should be followed by a second mowing after 0.5 m – 1 m (2 - 3 feet) of new growth has occurred (typically 1 month). This treatment opens up habitat for other emergent vegetation and has been shown to be about 75% effective in eliminating undesirable cattail stands. Additionally, herbicides can be applied to the rooted portion of the cut ramets which should kill the submerged rhizome and prevent regrowth. We predict that without intervention, *T. angustifolia* and *T.* × *glauca* will spread through the marsh complexes at PCCI altering their species composition and the foraging behavior of many of the animal species dependent on them.

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