

***Report: Genetic Integrity of Long's bulrush at
Lac de l'École on the Tusket River***

Nova Scotia Species at Risk Conservation Fund project
number: NSSARCF09_05

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31 May 2011

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Introduction

Long's bulrush (*Scirpus longii* Fernald, Cyperaceae) is a wetland species of interest to botanists and conservationists. Members of the genus are rhizomatous perennials growing in dense mats and/or tussocks. Most species produce flowering culms on an annual basis (Whittemore and Schuyler 2003). *S. longii* is unique, directing more of its resources to the growth and extension of its rhizomes, producing large and long-lived clones (Hill and Johansson 1992), and flowering only when disturbed (as by fire, see Schuyler and Stasz 1985). As Long's bulrush allocates more of its resources to vegetative growth and inhabits continuously wet and peaty soils, it has been classified (Hill and Johansson 1992) as a *stress-tolerant* plant (Grime 1977) adapted to low concentrations of oxygen and other nutrients. However, the induction of flowering by disturbance indicates a facultative *ruderal* or weedy character (Grime 1977). Thus, Long's bulrush is adapted to an intermediate place in oligotrophic wetland succession, doing well once a peaty substratum is established, contributing to the development of a richer and drier soil, re-establishing itself (from seed and rhizome) quickly after drought and/or fire, and being excluded, eventually, by shrubs and trees.

The sister species of Long's bulrush, the woolly bulrush (*Scirpus cyperinus*), has a definite ruderal character – its rhizomes are less substantial than those of Long's bulrush (diameter: 1-1.5 cm vs. 1.5-3 cm, respectively), its leaves and culms are less robust, and its tussocks flower annually and do not spread aggressively (Whittemore and Schuyler 2003, Hill and Johansson 1992). According to Grime (1977), the woolly bulrush would be constitutively *ruderal*, *i.e.* completely adapted to disturbance. Woolly bulrush is common and widespread in eastern North America (Whittemore and Schuyler 2003) and Nova Scotia (Roland and Zinck 1998), occurring on the margins of wetlands and open still and running water, in forested swamp, and in ditches and other sites of anthropogenic disturbance, where soils are frequently but not necessarily continuously wet (personal observations).

Long's bulrush is an endangered species of the Atlantic Coastal Plain Flora (Wisheu *et al.* 1994). Its known range is from southwestern Nova Scotia to the pine barrens of southern New Jersey (Hill and Johansson 1992). Nova Scotian populations are of scientific interest – Has *S. longii* adapted to Nova Scotian conditions? What is the history of post-glacial migration of the species? – and of considerable concern in conservation. Long's bulrush occurs in Canada only in southwestern Nova Scotia and may be genetically distinct from populations in the USA. Long's bulrush is listed as *vulnerable* (Wisheu *et al.* 1994) under the Nova

Scotia Endangered Species Act (2006), of *special concern* under Canada's Species at Risk Act (2006), and is globally *imperiled* (NatureServe 2010). Long's bulrush is a *High Priority* species according to the *Recovery Strategy and Management Plan for Multiple Species of Atlantic Coastal Plain Flora in Canada* (Environment Canada and Parks Canada Agency 2010). This plan (p. 4) points out that, as ACPF populations in Nova Scotia are at the northern ends of their ranges and disjunct from populations in the USA, work is needed to determine the genetic distinctiveness and conservation-value of these populations.

In 2010, MacKay *et al.* reported on the genetics of several populations of Long's bulrush in the lower watershed of the Medway River in Queen's County, Nova Scotia. One of these populations is unusual in that it includes patches (< 1% of the area covered by *Scirpus*) that flower annually (Hill 1994) and which show some morphological traits of the wooly bulrush. This suggested that Long's bulrush and the wooly bulrush had hybridized to produce some plants that express the annual flowering-trait of the wooly bulrush putative parent species. MacKay *et al.* (2010) were able to show that these patches were indeed the product of hybridization. Unexpectedly, hybridization was also evident in the non-flowering part (> 99% of the area covered by *Scirpus*) of this population. Furthermore, hybridization was evident in one of two neighbouring (presumed "pure") Long's bulrush populations. Apparently, Long's bulrush is vulnerable to genetic invasion by its sister species – *S. longii* and *S. cyperinus* are not yet reproductively incompatible despite being quite distinct in morphology, ecology and reproductive biology. Induction of flowering of Long's bulrush (naturally or by human activity) puts this species at risk of loss by hybridization and introgression. Anthropogenic disturbance in the vicinity of Long's bulrush populations may be especially threatening as disturbance induces flowering of the species and provides opportunity for the always-flowering wooly bulrush (MacKay *et al.* 2010). If reproductively compatible, only differences in flowering seasons (early summer for Long's bulrush; late summer for the wooly bulrush; Roland and Zinck 1998) would inhibit the exchange of pollen between the species.

The primary objective of this project is to determine whether an important population of Long's bulrush in Nova Scotia has been compromised by hybridization with the wooly bulrush. A large population of Long's bulrush is found in a wetland at the northern end of Lac de l'École in the main course of the Tuskent River, Yarmouth County NS (Hill and Johansson 1992). The Tuskent River watershed is a *High Priority* watershed in the *Recovery Strategy and Management Plan for Multiple Species of Atlantic Coastal Plain Flora in Canada* (Environment Canada and Parks Canada Agency 2010). Unusually, flowering occurs annually in the Lac de l'École population (Hill and Johansson 1992; personal observations in 2009 and 2010), suggesting (MacKay *et al.* 2010) that

(1) induction of facultative flowering of Long's bulrush is occurring due to some regularly occurring environmental factors(s), and/or (2) at least some of the *Scirpus* in this population is the product of hybridization between Long's bulrush and the wooly bulrush and annual flowering is caused by genetic input from the latter parent species. As the population is in an isolated area, it is unlikely that the annual flowering of Long's bulrush at Lac de l'École, whether by (1) or (2), is due human activities. Thus, this population may be an example of natural hybridization – a “hybrid zone”. Demonstration of such hybridization would suggest Long's bulrush has had a long history of exposure to genetic invasion by its sister species – a conclusion that would inform the conservation of Long's bulrush, particularly efforts to isolate Long's bulrush from its wooly relative.

The technique developed for the investigation of the population genetics of Long's bulrush in the Medway River watershed (MacKay *et al.* 2010) will be used to look for genetic markers of the wooly bulrush in flowering and non-flowering individuals of the Lac de l'École Long's bulrush population. This simple technique involves isolation of small amounts of DNA from leaf tissue and characterization of that DNA by the RAPD (Random Amplified Polymorphic DNA) technique (MacKay *et al.* 2010). The work completed on the Medway River populations defined sets of species-specific genetic markers. This information will be combined with the Lac de l'École genetic information to determine whether some individuals at Lac de l'École are the products of hybridization.

This project is divided into three practical parts: (a) surveying and sampling the Lac de l'École bulrush population, and sampling wooly bulrush in the vicinity of this population; (b) conducting genetic and morphological analyses; and (c) reporting the conclusions of these analyses to the bodies responsible for conservation of Long's bulrush in Nova Scotia (*The Atlantic Coastal Plain Flora Recovery Team* and *The Nova Scotia Nature Trust*) and to the broader scientific community. These are described in full in NSSARCF09_05 (copy attached), with *Project Objectives 1, 2* and *3* corresponding to (a) above, *Project Objectives 4* and *5* corresponding to (b), and *Project Objective 6* corresponding to (c).

NSSARCF09_05 proposed a two-year project, with part (a) to be completed in summer 2010, part (b) to be completed by the end of 2011, and part (c) to be initiated in winter 2012. NSSARCF09_05 proposed that funding (\$16,041.40) for this project would be divided 60% (\$9,624.84) and 40% (\$6,416.56) between the NSSARCF and Mount Saint Vincent University's *Internal Standard Research Grant* program. The applicant was awarded \$8,000 by the NSSARCF. At this time (May 2011), the recipient of NSSARCF09_05 has not applied for a related

MSVU internal grant, as his duties as departmental chair have restricted research activities in the past two years. The monies provided by the NSSARCF have been used to complete part (a) only. Only NSSARCF monies have been used for part (a). This report describes the work done to complete part (a). Plans for completing parts (b) and (c) are discussed in Summary and Prospects.

Logistics and Methods

As proposed in NSSARCF09_05, three field trips were made to Lac de l'École in the summer of 2010. Lac de l'École is a small lake in the main course of the Tusket River, in Yarmouth County NS, immediately downstream from Gillifillan Lake and upstream from Wilson's Lake. See W2 on the *Tusket* page of *The Nova Scotia Atlas* (1:150,000). The lake is elongate (maximum length: 1.85 km; maximum width: 0.45 km) and constricted in its middle. Lac de l'École lies on a SE-to-NW axis, as do many of the lakes in the region. The shores of the lake not bounded by the peaty wetland at the northern end are predominantly rocky with some small shallow sandy coves (personal observation). The wetland harbouring the Long's bulrush population occurs at the northern end of Lac de l'École. See N 43° 56.3' W 65° 49.3' on the *20 P/30 Tusket Canadian Topographic Map* (1:50,000). As the run from Gillifillan Lake (*Indian Falls*) enters Lac de l'École 0.7 km from the northern end of the lake and the lake drains through its southwest corner (*Peter's Rapids*), the part of the lake adjacent to the northern wetland is not subject to the strong currents of the Tusket River flow. Two creeks drain through the northern wetland and southward into the lake: a larger creek running the length of the wetland along its western margin and a smaller creek in the southeast of the wetland. See NS aerial photograph no. 00315-102 (1:10,000) attached.

The dates of the three field trips (inclusive of travel days) were 7-11 June 2010, 2-5 July 2010, and 9-12 August 2010. On each trip, the applicant was accompanied by research assistant Maggie English, a senior Mount Saint Vincent University student. Travel between Halifax and Yarmouth County was by RM's personal vehicle. Accommodation was at the Vaughn Lake Bed and Breakfast in Gavelton, Yarmouth County (Dale Duncanson, proprietor). Access to the northern wetland at Lac de l'École was by a canoe provided at Lac de l'École by Larry Muise of Springhaven, Yarmouth County. The canoe was launched from where Rte. 308 passes the southern end of Lac de l'École.

The purpose of the field trip of 7-11 June 2011 was to survey the Long's bulrush population on Lac de l'École, in the northern wetland and along the eastern and western shores. This survey was initiated on 8 June and completed (except for the eastern shore) on 10 June. A GPS device was used to record positions (as named waypoints) of *Scirpus* plants (putative *S. longii* or other *Scirpus*), and to record our trail over the wetland and along the shores. Comparison of this trail to aerial photography ensured that all regions of the wetland were examined. This area is all of the unforested wetland south of N 43° 56.8' or where the western

creek is no longer visible in NS aerial photograph no. 00315-102. Notes were made on the boundaries of the population, the nature of the plant(s) observed (definitely or likely *S. longii*, *Scirpus* sp., no *Scirpus*, possible hybrid), occurrence of flowering culms, whether plants were lone, multiple, or in solid stands, whether plants occurred in tussocks or circular clones, and signs of disturbance (herbivory, ATV-tracks, erosion of wetland turf at the margins of the lake and creeks).

Note that NSSARCF09_05 proposed that specimens of *Scirpus* at Lac de l'École and in the vicinity of this lake would be obtained during the July and August field-trips, respectively. The following describes a modification of that plan, insofar as sampling at Lac de l'École extended into the August field-trip.

The primary purpose of the field-trip of 2-5 July 2010 was to collect specimens of putative Long's bulrush at Lac de l'École. NSSARCF09_05 proposed the acquisition of ~100 specimens (putative *S. longii*, other *Scirpus*, possible hybrids) at Lac de l'École and ~50 specimens (*Scirpus*) in the vicinity of the lake. The applicant received a scientific permit (copy attached) from NS Department of Natural Resources for harvest of 100 Long's bulrush plants. (MacKay *et al.* (2010) used 104 *Scirpus* specimens to effectively study genetic diversity and hybridization in three populations of Long's bulrush and surrounding woolly bulrush in the lower Medway River watershed.) Collection of putative Long's bulrush (flowering and non-flowering) in the northern wetland was initiated on 3 July and completed on 4 July. A survey of *Scirpus* on the eastern shore was completed on 4 July, as was collection of *Scirpus* specimens from that shore. A GPS device was used to record the origins of all specimens. Notes were made as above. Plants were removed by cutting the rhizome at least 5 cm from the base of the leaves. Specimens were folded, tied with flagging tape labelled with the name of the associated GPS waypoint, and stored in plastic bags. Upon return to shore, bags of specimens were immediately stored on ice in coolers. We returned to Halifax on 5 July. Specimens were processed 5-6 July in the Biology department at Mount Saint Vincent University. Each specimen was washed thoroughly in cool running tap-water and blotted. One or two leaves in best condition (fewest lesions from herbivory, mechanical damage, microbial infection) were removed from each specimen, washed and blotted. The remaining plant was placed in a standard botanical press. Once filled, the press was dried in a ventilated oven at 50 °C. Small (approx. 5 mm x 5 mm) pieces were cut from deep green regions near the bases of selected leaves, again avoiding lesions. Scissors and forceps were washed thoroughly between specimens. For each specimen, sets of leaf pieces (~12 pieces per set) were

placed in pairs of clean (unused) 12 mL-polypropylene tubes. Several grams of silica gel desiccant were placed in one of each pair of tubes and mixed with the leaf pieces. All tubes were closed with air-tight caps. Tubes with only leaf pieces are stored at -20 °C. Tubes with leaf pieces and desiccant are stored at room temperature. Whole plant specimens (dried for ~3 days with 2-3 changes of blotting materials) are stored at room temperature in (*Ziploc*) plastic bags with ~20 g of desiccant.

The objectives of the field-trip of 9-12 August were (i) to collect specimens of potential hybrids of Long's bulrush and other *Scirpus* in the northern wetland of Lac de l'École and the western shore of the lake, and (ii) to collect specimens of *Scirpus* (likely, the woolly bulrush *S. cyperinus*) in the vicinity of the Long's bulrush population at Lac de l'École. Objective (i) was completed on 11 August. Objective (ii) was completed on 12 August. For the latter, *Scirpus* was taken from fields, ditches and small wetlands from Gavelton to Canaan to Kegeshook Lake (~11 km east, ~5 km northeast and ~4 km northwest of Lac de l'École, respectively) and places in between. These sites are all in the lower Tusket River watershed. Specimens were obtained and notes and locations were recorded as described above. On 13 August, specimens were processed as above.

In the weeks after the July and August field-trips and lab-work described above, MSVU student Heather Mackie was employed on an intermittent basis to complete the drying and storage of plants specimens.

Preliminary Results

Two creek systems drain into Lac de l'École from the wetland at the northern end of the lake: a larger creek running the length of the wetland along its western margin and a smaller creek in the broad southeast of the wetland. See NS aerial photograph no. 00315-102 (1:10,000). The banks of the creeks are heavy, at least at the surface, with darkly-coloured mud and silt. At the shores of the lake and in the beds of the creeks, large blocks of muddy wetland turf have separated and/or are separating from the surrounding wetland, forming crevasses, peninsulas and islands. Peaty meadows of grasses, sedges, mosses and insectivorous plants start within ~5 m of the edges of the creeks. Thick beds of shrubs are found between the peaty meadows and the forest on the boundaries of the open wetland. The peaty meadows are broader near the margin of the lake. A broad (maximum width: ~200 m) irregularly-shaped meadow surrounds the eastern creek system. An elongate (maximum width: ~100 m) meadow surrounds the western creek system. North of N 43° 56.6', the shrubs come to the edge of the western creek, excluding the meadows.

Distribution of bulrushes at Lac de l'École

In the June field-trip, a survey was made of the northern wetland and the western shore of Lac de l'École. The presence/absence of putative Long's bulrush and/or other *Scirpus* and additional information was noted at 394 sites. As this information did not include positive identification of Long's bulrush, it cannot be used by itself to describe the distribution of this species at Lac de l'École.

In the July field-trip, putative Long's bulrush specimens were collected from the northern wetland, and *Scirpus* specimens were obtained from the eastern shore of the lake. In the August field-trip, specimens were collected of potential hybrids of Long's bulrush and of other *Scirpus* in the northern wetland of Lac de l'École and along the western shore of the lake. In July and August, *Scirpus* was noted at 199 sites at Lac de l'École and 195 *Scirpus* specimens were obtained from 180 of these sites. The determination of the identity of these specimens awaits morphological and genetic analyses.

Also in the August field-trip, 24 specimens of putative woolly bulrush (*S. cyperinus*) or other *Scirpus* were obtained from as many roadside sites in the vicinity of Lac de l'École.

Two replicate sets of leaf samples were prepared from all 219 *Scirpus* specimens. One set is stored at -20 °C. The other set is stored (desiccated) at room temperature. These samples will be used for the preparation of small quantities of DNA for DNA-fingerprinting and subsequent population genetic analysis by the RAPD technique (MacKay *et al.* 2010).

A precise description of the distribution of Long's bulrush and other *Scirpus* at Lac de l'École requires the completion of morphological and genetic analyses. This description will be anchored by the identification of the 219 collected specimens and their geographical co-ordinates. However, the survey and collecting work completed to date is sufficient to allow a general description of *Scirpus* at Lac de l'École. There are at least three distinct types of *Scirpus* at Lac de l'École: flowering putative *Scirpus longii* (fp*S*), non-flowering putative *Scirpus longii* (nfp*S*), and flowering *Scirpus* of one or more other species or flowering *Scirpus* produced by hybridization of these other species (f*S*).

In the field, fp*S* was identified by the height of its culms (~2 m), by the blackness of the bases of its involucre bracts, and by the fact that these plants had well-developed inflorescences in June whereas other species of NS *Scirpus* flower in late summer and fall (Roland and Zinck 1998). In the wetland at the northern end of Lac de l'École, fp*S* occurs along the shores of the lake, along the muddy banks of the two creek systems and sometimes partially submerged near the banks (never in the grass-and-sedge meadows). The heaviest densities of fp*S* occur on the banks where the creeks broaden to enter the lake. As one moves upstream along the creeks, the density of fp*S* decreases until only scattered lone plants are found. In either creek bed, no fp*S* was found north of N 43° 56.6'. Several hundred to a thousand fp*S* culms were observed in the northern wetland of Lac de l'École.

fp*S* is also found scattered along the shores of Lac de l'École adjoining the northern wetland. On the western shore, fp*S* occurs as far south as N 43° 56.2' (*i.e.*, to approx. one quarter the length of the lake). On the eastern shore, fp*S* occurs as far south as N 43° 55.9' (*i.e.*, to approx. three fifths the length of the lake). No fp*S* was found south of these positions.

In the field, nfp*S* was identified by the nature of its leaves (broader and longer than other grasses and sedges in the peaty meadows of this wetland), the robust nature of its rhizomes and its tendency to grow in clonal arcs and circles (Roland and Zinck 1998, Hill and Johansson 1992). nfp*S* occurs in the peaty grass and sedge meadows behind the creek and lake margins. Nearer these margins,

nfp*S*/ occurs in dense stands in which it is difficult to distinguish one clone from another. Beyond these stands (*i.e.*, towards the shrubs and then the woods), nfp*S*/ occurs as complete circular clones, arcs, small unorganized groups of plants and scattered lone plants. nfp*S*/ becomes rare as one approaches the shrubs and trees. An occasional nfp*S*/ was found isolated among the stands of shrubs. No nfp*S*/ was found along the shores of Lac de l'École.

The fp*S*/ growing along the muddy lake and creek margins is generally markedly taller (in its vegetative leaves) and darker green, than the nfp*S*/ growing in the peaty grass and sedge meadows. In fact, some of the nfp*S*/ is unusually small for vegetative *S. longii* (personal observations) and is obviously not thriving. Also, fp*S*/ occurs entirely in tussocks, whereas nfp*S*/ occurs as isolated plants, groups of plants, and as clonal arcs and circles.

f*S* (flowering *Scirpus*, presumed not *S. longii*) was characterized by its shorter culms (1-1.5 m), by the olive-green or green (not black as for fp*S*/) bases of its involucre bracts, by the immature (generally unopened) state of its inflorescence in June, and by the narrowness of its leaves (relative to fp*S*/ and nfp*S*/). In the northern wetland, f*S* occurs only along the muddy margins of the creeks and the lake and in the creek beds, mingled with the fp*S*/ (flowering putative *S. longii*). f*S* is outnumbered by fp*S*/ in the southern parts of the creek systems, but becomes more frequent as one moves upstream. Eventually (above N 43° 56.6') only f*S* occurs in the creek systems. As one proceeds to the north in the western creek system, shrubbery approaches and then overhangs the creek bed as f*S* is restricted to the creek bed and then disappears (north of N 43° 56.8').

f*S* is also found scattered along both shores of Lac de l'École from the north to the south ends of the lake, more frequently in the sandy shallow coves and with small communities of other wetland species than along the rocky parts of the shoreline.

Summary and Prospects

This report is a preliminary description of a survey of the Long's bulrush population at Lac de l'École in the watershed of the Tusket River, Yarmouth County NS, and an account of the collection of *Scirpus* at this lake and in its vicinity. This work contributes to the project *Genetic Integrity of Long's bulrush at Lac de l'École on the Tusket River* (NSSARCF09_05). The overall objectives of this project are to characterize genetic diversity and identity in Long's bulrush at Lac de l'École and determine whether this population has hybridized with other *Scirpus* species. In previous work, the investigator and his associates (MacKay *et al.* 2010) have shown that hybridization has occurred in Long's bulrush populations in the watershed of the lower Medway River, Queens County NS. As Long's bulrush at Lac de l'École is an important population of this endangered species in NS (Hill and Johansson 1992), it is critical, for conservation, to know whether the Lac de l'École population is genetically distinct in NS and whether this population is vulnerable to genetic invasion by its sister species, the wooly bulrush.

The purpose of the survey (NSSARCF09_05 *Project objective 1*) was to determine the distribution of all *Scirpus* at Lac de l'École. We were able to define at least three types of *Scirpus* at the lake: flowering putative *S. longii* (fp*S*), non-flowering putative *S. longii* (nfp*S*) and flowering *Scirpus* that was not *S. longii* (f*S*). At least several hundred culms of fp*S* were observed growing from tussocks on or near the muddy banks of the two creek systems that drain the wetland at the northern end of Lac de l'École and along the lakeshore of this wetland. Also, fp*S* is scattered along the lakeshores south of the northern wetland, as far south as approximately one-quarter and three-fifths of the western and eastern shores, respectively. nfp*S* occurs in the peaty grass-and-sedge meadows between the banks of the creek systems and the dense zones of shrubs that separate the meadows from the forest at the boundary of the wetland.

At Lac de l'École, fp*S* differs from nfp*S* not only in the presence of culms (flowering stems) in the former and the absence of culms in the latter, but also colour of the leaf blades (dark versus light green), robustness of whole vegetative plants (larger in fp*S*), and habit (tussocks in fp*S* *versus* single plants, groups and solid stands of plants, and clonal arcs and circles in nfp*S*). It is tempting to speculate that recurring disturbance and/or the nutrient-rich habitat at the margins of the creek systems and lake induces the facultative ruderal character (Grime 1977) of Long's bulrush. Thus physiological induction may be the

explanation, not hybridization with a constitutive ruderal species (*i.e.*, the wooly bulrush), for the regular, apparently annual, flowering of Long's bulrush at Lac de l'École. Certainly, the appearance of the fpSI in the field was typical for Long's bulrush – these plants did not show the mixture of *S. longii*- and *S. cyperinus*-traits observed for genetically-confirmed hybrid plants in the watershed of the lower Medway River (MacKay *et al.* 2010).

At Lac de l'École, other flowering *Scirpus* (fS) was found in the northern wetland and all along the western and eastern shores of the lake. In the northern wetland, it is entirely restricted to the muddy banks of the creek systems and the lakeshore of the wetland. fS is scattered among the (more frequent) fpSI at the lake margin and in the immediately adjacent regions of the creek systems. Moving northward (upstream) in the creek systems, fS becomes more frequent. Only fS occurs in the northern part (*i.e.*, above N 43° 56.6') of the western creek system and fS is eventually entirely excluded by shrubbery (north of N 43° 56.8'). Whether any of fS is the product of hybridization between Long's bulrush and the wooly bulrush remains to be seen. fS may be entirely *S. cyperinus* and/or some other *Scirpus* species.

The intermingling of fS and Long's bulrush in the northern wetland and along the shores of Lac de l'École, indicates that the genomes of at least two *Scirpus* species are present at this lake. Given the tendency of both species (presumably, *S. longii* and another *Scirpus* species) to flower naturally at Lac de l'École and the demonstrated capacity of Long's bulrush for hybridization (MacKay *et al.* 2010; Whittemore and Schuyler 2003), it will be important for the conservation of Long's bulrush to determine whether any *Scirpus* at this lake is the product of hybridization. Presumably, over the ages, there has been many opportunities for exchange of pollen between these species.

The collection of *Scirpus* at Lac de l'École (*Project objective 2*) was guided by the survey described above. The purpose of this collection was to provide material for morphological analysis and identification and for DNA-preparation and molecular population genetic analysis. These analyses (combined with similar analyses of *Scirpus* collected in the vicinity of Lac de l'École – see below) should allow resolution of the identities of fpSI and nfpSI, and, combined with the geographical co-ordinates of the origins of the collected specimens, the precise mapping of the distribution of *Scirpus* species, the different *S. longii* phenotypes (flowering tussocks, entirely vegetative plants and clones) and hybrids (if any) at Lac de l'École. In July and August 2011, 199 whole plant specimens of *Scirpus* were obtained from 180 sites at Lac de l'École. Of these specimens, 120 are

putative Long's bulrush (flowering and non-flowering plants) and 79 are putative other *Scirpus* species and/or the products of hybridization of these species. *Project 2* proposed that approximately 100 specimens would be obtained at Lac de l'École.

The purpose of collection in the vicinity of Lac de l'École (*Project objective 3*) was to provide material representing the local woolly bulrush (*S. cyperinus*) or other *Scirpus* for morphological and genetic analyses. When examining potential situations of hybridization and introgression, it's necessary to include representative samples of the two putative parent species. Genetic analyses depend on the identification of species-specific DNA-markers or species-distinctive frequencies of DNA-markers in populations. These are used as references for testing hypotheses of hybridization – the products of hybridization should show mixtures of the DNA-markers present in the putative parent species. As noted above, many of the plants obtained at Lac de l'École appear to be typical ("pure") Long's bulrush. In this project, the genetic data from these plants and the data obtained from *S. longii* populations in the Medway River (MacKay *et al.* 2010) will likely serve as representative of the Long's bulrush putative parent species. Regarding the other putative parent species, *Project objective 3* proposed that approximately 50 specimens of *Scirpus* (presumably, the common *S. cyperinus* or woolly bulrush) would be obtained from the vicinity of Lac de l'École. In August 2011, only 24 specimens of *Scirpus* were obtained from as many roadside sites in the vicinity of Lac de l'École. As the genetic data obtained from this project will be combined with that already obtained for populations in Medway River watershed (MacKay *et al.* 2010), including data for 30 *S. cyperinus* individuals, the deficiency in number of putative *S. cyperinus* individuals obtained at Lac de l'École should not present a difficulty. If the latter individuals seem genetically distinct from *S. cyperinus* in the Medway River watershed, then additional sampling of *Scirpus* in the vicinity of Lac de l'École may be required.

As a product of NSSARCF09_05, 219 whole plant specimens and duplicate sets of leaf samples from each of these plants reside in the Biology department of Mount Saint Vincent University. This collection represents Long's bulrush, other *Scirpus* species and, possibly, the products of hybridization of *Scirpus* species at Lac de l'École in the watershed of the Tusket River, Yarmouth County NS. Determination of the identity of these plants, the geographic distribution of the species involved and genetic relationships awaits analyses of the morphology of the plants and DNA (MacKay *et al.* 2010) extracted from the leaf samples. The recipient of NSSARCF09_05 will be on sabbatical leave from 1 July 2011 to

30 June 2012, during which time, he will be resident for ten months in Connecticut USA. During this time, the recipient's activities will include morphological analysis of the 219 whole plant specimens, and the writing of an application for an MSVU *Internal Standard Research Grant* to support the genetic analyses of the plants (*Project objectives 4 and 5* of NSSARCF09_05). It is possible that the morphological analysis will be sufficient to clearly indicate the disposition of Long's bulrush, other *Scirpus* species and any hybrids at Lac de l'École. This would allow communication of the status of Long's bulrush at Lac de l'École to the bodies responsible for conservation of the species (Atlantic Coastal Plain Recovery Team, Nova Scotia Nature Trust) in 2012. Conclusive evidence for or against hybridization at Lac de l'École depends on completion of the molecular population genetic analyses. Presuming receipt of financial support, the latter work would be conducted in 2012-2013 and results and conclusions would be available for communication to the bodies mentioned above and to the wider conservation biology communities (*Project objective 6*) in 2013.

Acknowledgements

The recipient of NSSARCF09_05 gratefully acknowledges the guidance and advice of Nicholas M. Hill, and the permission given by the NS Department of Natural Resources to collect Long's bulrush.

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