Visual-based monitoring (2016 to 2020) of direct impacts to wetland fishes from aerial and ground application of herbicide to control invasive European common reed (*Phragmites australis subsp. australis*).

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Visual-based monitoring (2016 to 2020) of direct impacts to wetland fishes from aerial and ground application of herbicide to control invasive European common reed (*Phragmites australis subsp. australis*).

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Reid, S.M., LeBaron, A., Soetemans, J., Bershatsky, J., Braun, H., and MacDonald, F. 2023. Visual-based monitoring (2016 to 2020) of direct impacts to wetland fishes from aerial and ground application of herbicide to control invasive European common reed (*Phragmites australis subsp. australis*). Can. Data Rep. Fish. Aquat. Sci. 1362: vi + 18 p.

European common reed (*Phragmites australis subsp. australis*) is an invasive perennial grass that has degraded Great Lakes coastal wetlands. Along the north shore of Lake Erie, an integrated pest management strategy (herbicide application, mechanical removal, and/or prescribed burns) has been implemented. Due to potential direct impacts of aerial and ground herbicide (active ingredient - glyphosate) application to wetland fishes, monitoring was done at Long Point Bay and Rondeau Provincial Park from 2016 to 2020. The monitoring design included visual surveys for distressed or dead fishes before and after herbicide application, and at control and treatment sites. At Crown Marsh (Long Point), surveys over three years detected 34 dead fishes from control and treatment ponds. While 85% of dead fishes were Bullhead *Ameiurus spp.*, observations also included Warmouth *Lepomis gulosus* (SARA: Special Concern) and recreationally important Northern Pike *Esox lucius*. Most dead fish were detected during 2016 surveys in the treatment area, or during 2016 and 2017 post-application surveys. In 2016, abnormal behaviour by juvenile Largemouth Bass *Micropterus salmoides* was observed on a limited scale before, and 2-3 days after herbicide application. No evidence of abnormal behavior or greater mortality risk was detected during surveys at Big Creek National Wildlife Area (Long Point) and Rondeau Provincial Park.

RÉSUMÉ

Reid, S.M., LeBaron, A., Soetemans, J., Bershatsky, J., Braun, H., and MacDonald, F. 2023. Visual-based monitoring (2016 to 2020) of direct impacts to wetland fishes from aerial and ground application of herbicide to control invasive European common reed (*Phragmites australis subsp. australis*). Can. Data Rep. Fish. Aquat. Sci. 1362: vi + 18 p.

Le roseau commun européen (*Phragmites australis* de la sous-espèce *australis*) est une herbe vivace envahissante qui a dégradé les zones humides côtières des Grands Lacs. Le long de la rive nord du lac Érié, une stratégie de lutte intégrée (application d'herbicides, enlèvement mécanique et/ou brûlage dirigé) a été mise en œuvre. En raison des répercussions directes éventuelles de l'application aérienne et terrestre d'herbicides (matière active – glyphosate) sur les poissons des zones humides, des travaux de surveillance ont été menés à Long Point Bay et au parc provincial Rondeau, de 2016 à 2020. Le plan de surveillance comprenait des relevés visuels de poissons en détresse ou morts avant et après l'application d'herbicide sur les sites de référence et de traitement. Au marais Crown (Long Point), des relevés menés sur trois ans ont permis de détecter 34 poissons morts dans les étangs de référence et de traitement. Tandis que 85 % des poissons morts étaient des barbottes (*Ameiurus* spp.), on a également observé des crapets sac-à-lait (*Lepomis gulosus*), espèce préoccupante selon la LEP et des grands brochets (*Esox lucius*), une espèce d'importance récréative. La plupart des poissons morts ont été détectés dans le cadre des relevés de 2016 visant de la zone de traitement, ou dans le cadre des relevés après traitement de 2016 et de 2017. En 2016, un comportement anormal d'achigans à grande bouche (*Micropterus salmoides*) juvéniles a été observé à une échelle limitée avant, et 2 à 3 jours après l'application d'herbicide. Aucun signe de comportement anormal ou de risque de mortalité plus élevé n'a été détecté dans le cadre des relevés effectués dans la réserve nationale de faune de Big Creek (Long Point) et dans le parc provincial Rondeau.

INTRODUCTION

European common reed (*Phragmites australis subsp. australis*) is an invasive perennial grass that was transported from Eurasia and has caused severe degradation to coastal wetlands and other nearshore areas in North America. Lake Erie coastal zones are highly vulnerable to invasion by common reed (Carlson Mazur et al. 2014). Wetlands along the north shore of Lake Erie provide important habitats for both recreationally and commercially important fishes and for fish species at risk. These species use wetlands as nursery habitats, spawning grounds, and to feed (Jude and Pappas 1992). At Long Point and Rondeau Bay, wetland ecosystems have recently been transformed by the spread of this invasive plant. A reduction in plant diversity has occurred and the spread of common reed has converted wetlands into more terrestrial environments, as it promotes the accumulation of sediment and organic matter (Wilcox et al. 2003; Schummer et al. 2012). Consequently, the spread of common reed results in many negative impacts to the ecosystem including loss of native wetland vegetation, reduction in habitat quality for wildlife, and a decrease in nitrogen and phosphorus availability (Findlay et al. 2002; Wilcox et al. 2003).

In response to wetland habitat loss following the common reed invasion, federal, provincial, and municipal governments and non-government conservation groups began implementing an integrated pest management strategy to control the spread of common reed and improve habitat conditions. The strategy includes herbicide application between August and October, mechanical removal of herbicide-treated common reed, and/or prescribed burning if conditions allow during winter months. Prior to 2016, efforts were largely unsuccessful in controlling spread due to the lack of registered herbicides for use in aquatic habitats in Canada. To address the growth of common reed in Rondeau Provincial Park (PP) and the Long Point region (includes Crown Marsh, Long Point and Turkey Point Provincial Parks, Long Point and Big Creek National Wildlife Areas, and private lands in the region and lower Big Creek Watershed), Health Canada's Pest Management Regulatory Agency approved the emergency registration of an herbicide (active ingredient glyphosate) in wet areas. The basis for the emergency registration was the serious threat common reed represented to species at risk and other valued species in these wetland complexes.

Generally, herbicide application methods have focused on aerial application (helicopter) for primary treatment of large dense stands of Phragmites growing in inaccessible or difficult terrain. Ground application methods (e.g. backpack, hydraulic sprayer operated from amphibious vehicles or boats) were utilized either to address sites unsuitable for aerial application including lower density Phragmites stands or for secondary follow up treatments to address regrowth as needed. Herbicide application generally occurred from mid-August to early October. The rate of application ranged between 4.4L/ha and 8.77L/ha, depending on conditions and application method.

In 2016, 2017 and 2018, aerial (helicopter) herbicide application was conducted at Long Point Crown Marsh (covering ~87.5ha, 31.15ha and 2.02ha respectively) and at Rondeau PP in 2016 (~100ha) to address large monoculture stands of Phragmites. Concurrently, for this time period, ground based application methods to address retreatment and satellite populations were conducted at Crown Marsh (2016:~10ha, 2017: ~67ha, 2018: 51.9Ha) and Rondeau PP (2017: ~17ha, 2018: 3ha). Additional ground treatments occurred at these sites in 2019 and 2020. At this time, the focus of primary control shifted to the Big Creek and Long Point National Wildlife areas. In 2019, ~9ha at Big Creek and Long Point National Wildlife areas were treated by ground application. In 2020, aerial and ground application occurred at Big Creek NWA (~176ha) and the Thoroughfare Unit of the Long Point NWA (~85ha).

A significant body of literature indicates that the overall risk to aquatic organisms from glyphosate use in wetlands and overwater situations is low (Solomon and Thompson 2003). In terms of impacts to fish, exposure to environmentally realistic exposure levels could result in sub-lethal effects such as reduced feeding activity (Giaquinto et al. 2017). Ecotoxicological studies have identified more harmful effects to fish can occur at much higher concentrations of glyphosate, and that fish may be more sensitive to the surfactant in the herbicide formulation than glyphosate (Pérez et al. 2011, Annett et al. 2014). Considering the unique nature of the project in Canada and expected sensitivities of wetland fishes at risk, there is great uncertainty regarding potential impacts. Therefore, monitoring the effectiveness of approaches that mitigate harm during glyphosate application as well as evaluating the benefits of common reed removal are important scientific activities to inform management.

DFO data reports are published to support the Fish and Fish Habitat Protection and Species at Risk programs by providing a description of field activities and to provide a medium for archiving data associated with sampling and monitoring of fishes and their habitat. In this study, visual-based surveys were done before and after glyphosate application at Long Point and Rondeau Provincial Park from 2016 to 2020. Monitoring indicators included visual observations of distressed (e.g. abnormal swimming behaviour) and/or dead fish. These activities were undertaken to meet monitoring requirements under the following Species at Risk Act Permits: 16-HCAA-00903, 17- HCAA-00862, 18-HCAA-01027, 19-HCAA-0700 and 19-HCAA-01966.

METHODS

The following sections describe the surveys done to assess the risk of direct harm to fish as a result of herbicide application in the Long Point region and Rondeau Provincial Park (Figure 1). Survey design was developed in consultation with DFO biologists. The design included surveys before and after herbicide application, and at control (reference) and treatment sites. Locations of monitoring sites are presented in Figures 2 – 5.

LONG POINT

Crown Marsh

From 2016 to 2018, a Before-After Control Impact (BACI) monitoring program was undertaken to assess the risk of fish mortality as a result of herbicide application. Each year, 5 ponds were surveyed: 3 ponds inside the herbicide application area, and 2 control (i.e. reference) ponds outside the application area (Table 1). Each of the ponds inside and outside the treated area were not directly connected to each other.

Single-day surveys were done prior to herbicide application, and at 2 time-periods after herbicide application. In 2016, surveys were done 14 days before aerial and ground herbicide application, and 3 days and 32 days after application. In 2017, surveys were done 23 to 26 days before aerial and ground herbicide application, 1 to 3 days after, and 30 days after herbicide application. In 2018, surveys were done 6 to 14 days before aerial and ground herbicide application, 1 to 9 days and 43 days after herbicide application. Timing of post-application surveys was affected by several factors including: health and safety concerns related to herbicide exposure, the waterfowl hunting schedule in Crown Marsh, weather, and availability of field staff. Therefore, timing of post-application surveys was variable across years, and across ponds.

Monitoring at each pond consisted of a visual survey of 20 randomly placed transects. The 10-metre long transects originated from the shoreline and were orientated towards the

centre of the pond. Along each transect, a 2 m wide swath of habitat was surveyed. Field staff wearing polarized sunglasses walked slowly along each transect, documenting the presence of dead fish or observations of abnormal fish swimming behaviour (distressed fish). The mean (± 1 standard deviation) search time along transects was 64 (± 15.8) seconds in 2016, 51 (± 11.6) seconds in 2017 and 58 (± 11.1) seconds in 2018.

Digital images of dead fishes and voucher specimens (bagged and stored frozen) were taken to assist with species identification. In 2016 and 2017, identification support was provided by staff from the Great Lakes Laboratory for Fisheries and Aquatic Sciences (Fisheries and Oceans Canada, Burlington, Ontario).

Within each year, among-pond and among-survey differences were assessed using the mean number (and 95% confidence intervals) of dead fishes per transect detected from each pond at each survey period. Confidence intervals were calculated using boot-strap methods (9999 resampling events).

Big Creek and Long Point National Wildlife Areas

Initially for 2019, a BACI design was planned for use in Big Creek National Wildlife Area. However, extremely dense stands of Phragmites and high-water levels prevented access by canoe or foot to proposed control (reference) sites. As a result, monitoring was modified to focus on the herbicide treatment area within Brown's Marsh at Big Creek National Wildlife Area. Herbicide application at Brown's Marsh occurred on September 3rd, 2019. Single day surveys occurred prior to application (August 26) and one day (September 4), 6 days (September 9) and 30 days (October 7) post herbicide application. Two open channel areas in the Brown's Marsh treatment area were surveyed by canoe. The first post-herbicide treatment survey was delayed by weather conditions that prevented safe canoe operation.

In 2020, a BACI monitoring program was planned for Big Creek Unit (BCNWA) and Thoroughfare Unit (LPNWA). In each NWA, surveys were conducted at 3 paired treated and untreated sites. Monitoring was planned to assess aerial and ground herbicide treatments. Surveys occurred prior to herbicide application, and 1 day post, 2 to 3 days post and 1 month post application. Unforeseen complications in procuring herbicide resulted in a delay in aerial herbicide application, which meant that herbicide was applied at two different time stamps within each unit. Ground application took place between August 18 and September 25. Aerial application at Thoroughfare Unit was on October 3 and 5, and at Big Creek Unit on October 5. As a result, only certain sites were surveyed alongside aerial herbicide application, whereas others were surveyed alongside ground application. Surveys at one site in the Big Creek Unit, and all sites in the Thoroughfare Unit were coordinated with aerial herbicide application, and occurred roughly at the planned timing of surveys. Surveys at two sites in the Big Creek Unit were coordinated with ground herbicide application, and occurred at the planned timing of surveys.

Monitoring of each treatment and control site consisted of a visual survey of 20 randomly placed transects, originating from one stationary location along the edge of a Phragmites stand and stretching 2 m wide, and 10 m long. Field staff wearing polarized sunglasses paddled slowly along each transect, documenting the presence of distressed or dead fish. In 2019, a voucher was taken to assist with species identification; preserved by fixation in 10% buffered formalin followed by storage in 70% ethanol. Identification support was provided by Fisheries and Oceans Canada.

RONDEAU PROVINCIAL PARK

In 2017 and 2018, monitoring at Rondeau Provincial Park was conducted on foot or by canoe (or other similar vessel as conditions allowed). During each year, a single 200 m long transect was surveyed at each of the 2 control sites, and the 2 treatment sites (Figure 5). Pairs of control and treatment sites were located on the Lake Erie side of the park, and on protected bay side of the park. Field staff wearing polarized sunglasses walked or paddled slowly along each transect, documenting the presence of dead fish or observations of abnormal fish swimming behaviour.

In 2017, a pre-treatment survey was done on August 31 and post-treatment surveys were done 1 day (October 4) and 3 days (October 7) after herbicide application. In 2018, a pre-treatment survey was done on September 3. Post-treatment surveys were done 1 day (Lake Erie side: September 13, bay side: September 19), 2-3 days (Lake Erie side: September 15, bay side: September 20), and 30 days (Lake Erie side: October 12, bay side: October 18) after herbicide application.

RESULTS

LONG POINT

Crown Marsh

Over the three-year period, visual surveys detected 36 dead or distressed fishes from control and treatment ponds (Table 2). Eighty-five percent of dead fishes were Bullhead (*Ameiurus sp*.). Other species were: Bowfin *Amia calva* (n=1), Northern Pike *Esox lucius* (n = 1), Tadpole Madtom *Noturus gyrinus* (n = 1), and Warmouth *Lepomis gulosus* (n = 2). Across all 3 years, only 2 dead fishes were detected from control ponds, and only 5 dead fishes were detected prior to herbicide application. Most dead fishes were detected during: (i) 2016 pre- and post-application surveys of ponds in the herbicide application area (n = 27), or (ii) post-herbicide application surveys in 2016 and 2017 (n = 26).

In 2016, 27 dead fishes were detected from the 3 ponds in the herbicide application area during pre- and post-application surveys. Only 1 dead fish was detected from control ponds. Typically, 2 to 3 days after herbicide application, more dead fishes were detected in ponds in the herbicide application area than outside. Compared to the pre-application survey, there was only a significant increase in the mean number of dead fish (per transect) at Pond 42 (Table 3). During post-application surveys, the number of dead fish detected in Pond 42 was 7 – 8 times higher than other ponds in the herbicide application area.

While most dead fishes were Bullhead (Brown Bullhead and unidentified *Ameiurus sp.*), observations of dead or distressed fish also included Warmouth (SARA: Special Concern) and Largemouth Bass and Northern Pike (two recreationally important species). A single dead Warmouth (total length = 80 mm) was collected from Pond 42 on September 19, 2016, and another dead Warmouth (total length = 113 mm) was collected from Pond 1 on October 18, 2016.

Observations of distress (abnormal swimming behaviour) by wetland fishes were rare. Observations of abnormal swimming behaviour by juvenile Largemouth Bass were reported at Pond 1 before, and 2-3 days after herbicide application. Abnormal behaviour consisted of swimming in circles at the surface, and the loss of equilibrium. Before herbicide application, normally swimming fishes were observed at 20% of surveyed transects. Shortly after herbicide application, normally swimming fishes were noted at 8% of transects surveyed in the treatment

area and 3% outside. Observations of normal swimming behaviour was reported 1 month after herbicide application at Pond 5 (1 transect) and at 10% of control pond transects.

In 2017, 6 dead fishes were detected from Ponds 3 and 5, located in the ground herbicide application area. Dead fishes were identified as Brown Bullhead (n=3), Black Bullhead (*Ameiurus melas*) (n=1), unidentified *Ictalurid sp.* (n=1), and Tadpole Madtom (*Noturus gyrinus*) (n=1). A single dead Brown Bullhead was detected from Pond 3 prior to herbicide application. After herbicide application, dead fish were only detected from Pond 5 (2 Brown Bullhead 24 hours after application, and 1 Black Bullhead, 1 unidentified *Ictalurid* sp., 1 Tadpole Madtom 30 days after application). No dead fishes were detected outside the herbicide application area. No distressed fish were observed at any pond. Observations of normal swimming fishes were only noted before herbicide application, at 17% of pond transects.

In 2018, only a single dead fish was detected during post-application visual surveys. On September 28th, a single dead Bowfin was detected from Pond 43 (located outside the herbicide application area). No other distressed or dead fishes were detected. Water samples collected by the University of Waterloo in 2018 within 24 hours, and one-month post-treatment at Long Point were below detection limits for glyphosate (0.001 ppm) and for alcohol ethoxylate (surfactant) (0.03 ppm).

Big Creek and Long Point National Wildlife Areas

In 2019, no dead or distressed fish were observed prior to herbicide application, one day after, or 6 days after herbicide application. On the final day of post-application surveys (October 7), one dead Black Bullhead was detected in the Big Creek NWA treatment area.

In 2020, no dead or distressed fish were found during any of the pre- and post-herbicide application surveys. Large schools of fish were observed in areas that had been treated with herbicide only a few days prior.

RONDEAU PROVINCIAL PARK

In 2017 and 2018, no dead or distressed fish were found during any of the pre- and post-herbicide application surveys.

DISCUSSION

Results from three years of monitoring at Crown Marsh (Long Point) indicate that aerial herbicide application adjacent to open-water habitats could increase the risk of fish mortality. However, this interpretation is largely based on differences in the number of dead Bullhead among ponds and monitoring periods, and therefore less applicable to other wetland fishes. The number and diversity of dead fishes detected during visual surveys was very small compared to recent seine-based samples of these ponds (Rook et al. 2016). No evidence of elevated mortality risk from aerial herbicide application was detected during surveys at Big Creek National Wildlife Area (Long Point) and Rondeau Provincial Park. We acknowledge that the numbers of dead fishes detected in this study were likely underestimated. Some of the expected bias results from the removal of carcasses by scavengers including crayfish and turtles (Ryon et al. 2000). Additionally, visual surveys tend to underestimate mortalities for small-bodied individuals and less abundant species (Labay and Buzan 1999; Kennedy et al. 2017).

At Crown Marsh treatment areas, the level of fish mortality varied among ponds and among treatment years. In 2016, visual-based surveys indicate increased fish mortality at Pond 42 within 2 to 3 days of, and 1 month after herbicide application. Compared to other ponds affected by herbicide application, Pond 42 is much smaller and more isolated from other open-

water habitats. The pond's physical characteristics may have contributed to the increase in fish mortality, reflecting a greater exposure to the herbicide and/or other stressors such as lower water level and higher water temperatures. In 2017, a much smaller number of dead fish were counted after herbicide application, and in 2018, no dead fish were detected in the treatment area. Lower mortality coincides with the extent of herbicide application, as most was ground-based spot treatments vs. larger scale aerial application.

Three variations of the visual survey design were used to monitor wetlands treated with herbicide: (1) BACI with multiple transects surveyed at each monitoring site – Crown Marsh (2016 – 2018) and BCNWA and LPNWA (2020); (2) BACI with a single transect surveyed at paired monitoring sites – Rondeau Bay; and (3) before and after surveys at only impact site – Brown's Marsh (2019). Some of the variation in surveys was the result of adapting to practical challenges to implementing the original survey design (#1). The complete BACI design used at Crown Marsh permitted a statistical-based interpretation of the impact of herbicide application. Such an assessment would not have been possible without control ponds, or without sufficient sample replication during surveys. Fortunately, a lack of dead fish detections at Rondeau Bay and Brown's Marsh meant such comparisons were unnecessary. During 2016, freezing was the voucher preservation method – the poor state of vouchers made confirmatory species identifications in the laboratory difficult. As implemented afterwards, formalin-fixation is the recommended voucher preservation method.

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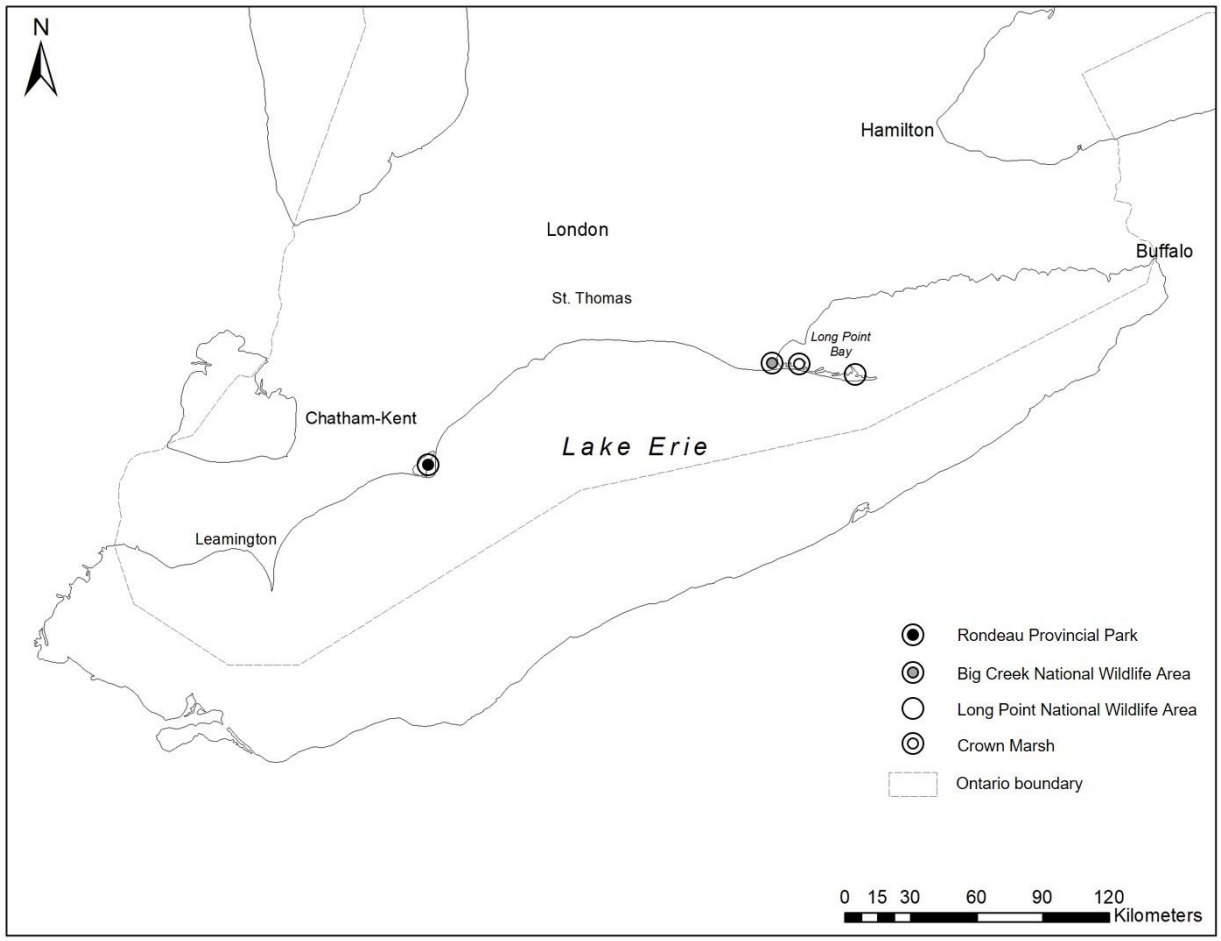

Figure 1. Locations of Lake Erie coastal wetlands where herbicide treatment of European common reed occurred from 2016 to 2020.

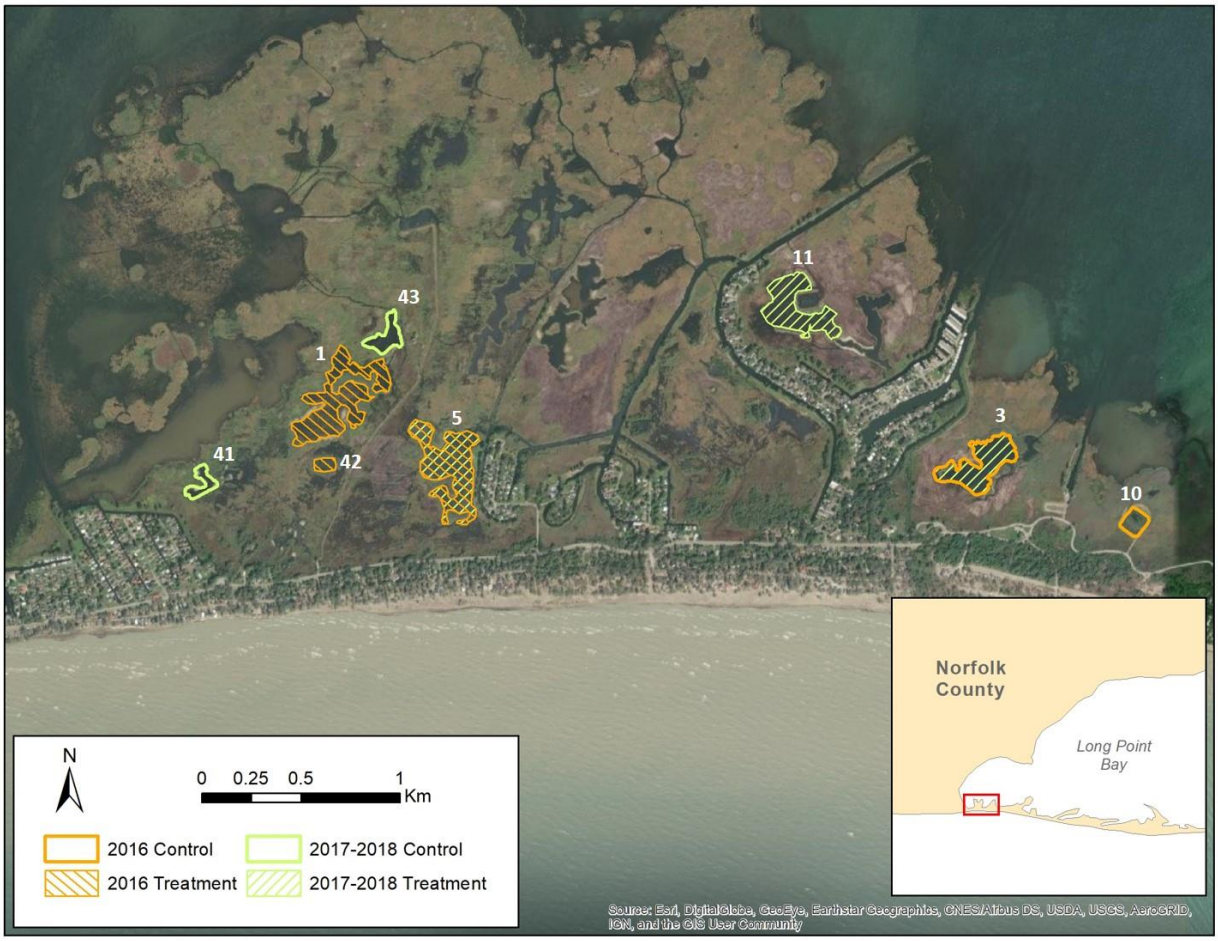

Figure 2. Locations of Crown Marsh ponds monitored with visual surveys in 2016, 2017 and 2018.

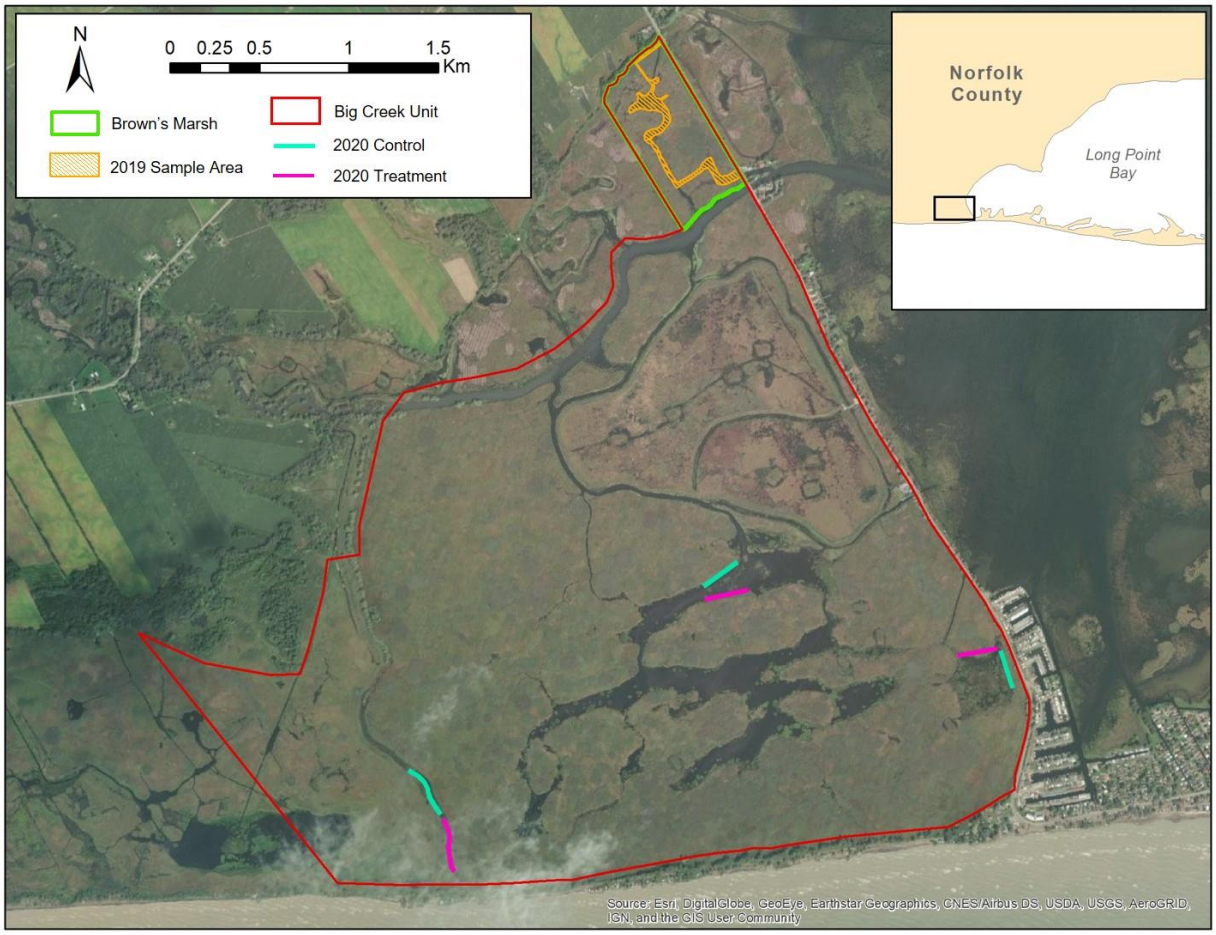

Figure 3. Locations of visual monitoring surveys at Big Creek NWA in 2019 and 2020.

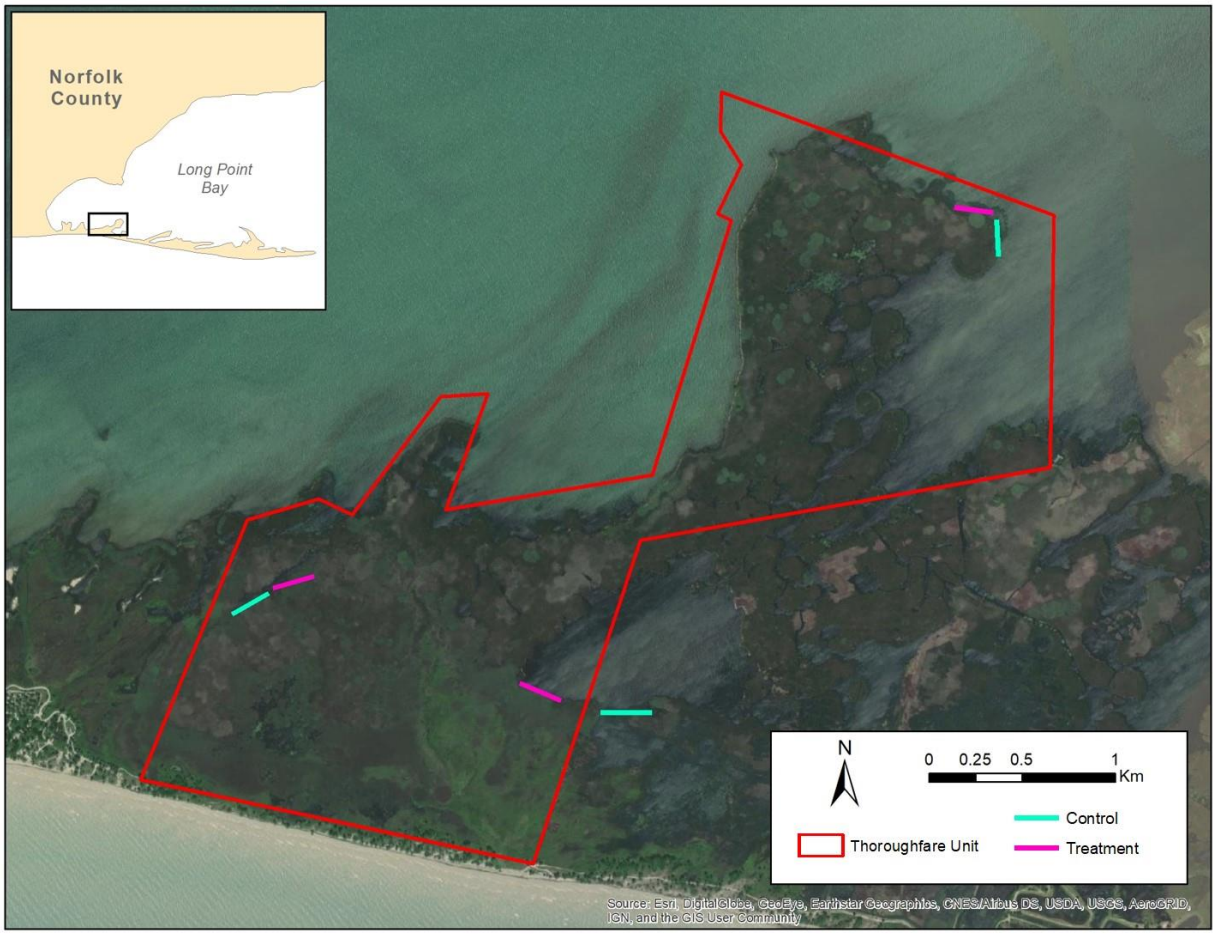

Figure 4. Locations of visual monitoring surveys at Thoroughfare Unit, Long Point NWA in 2020.

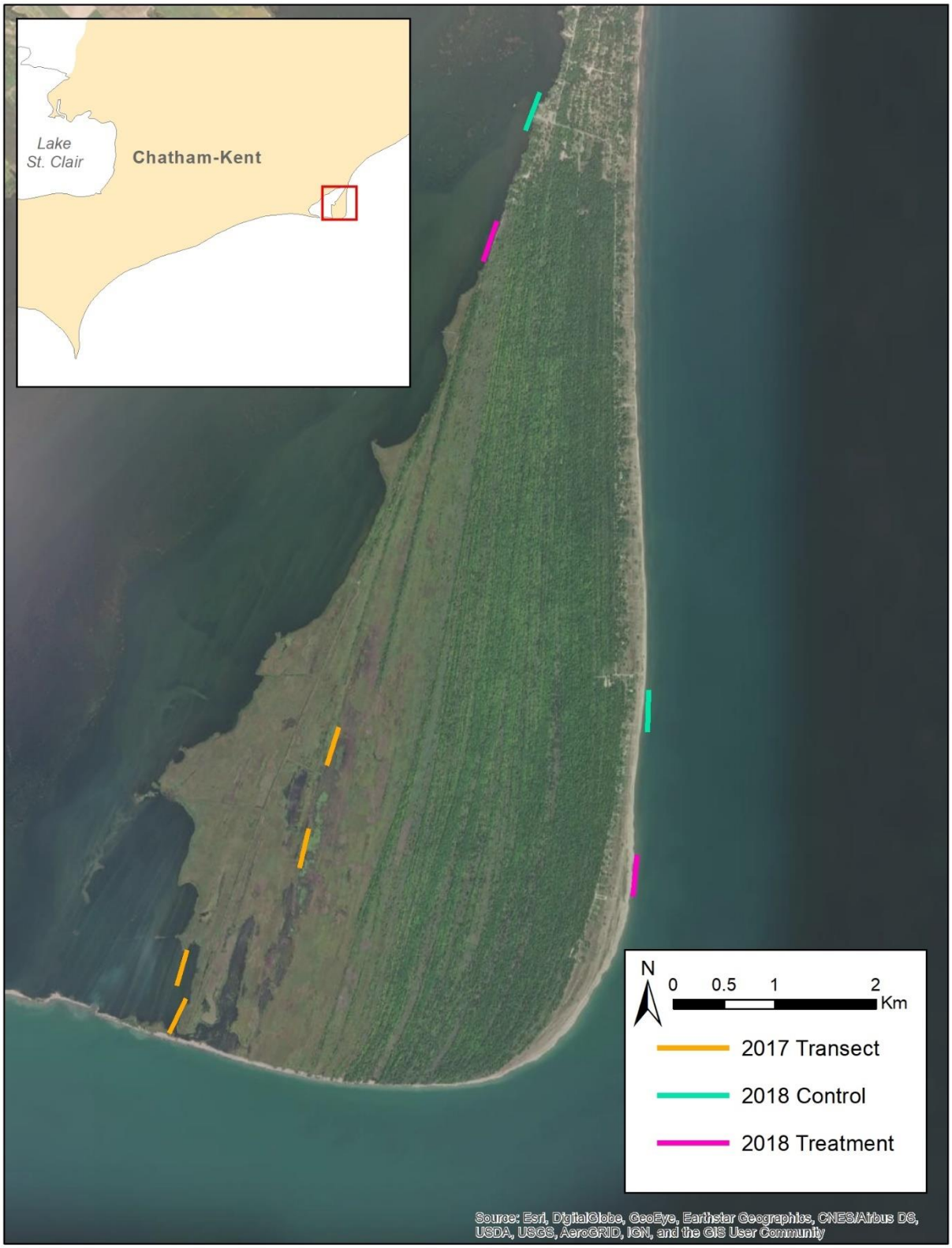

Figure 5. Locations of Rondeau Provincial Park visual monitoring surveys, 2017 and 2018.

Figure 6. Images of dead fishes detected during 2016 and 2017 visual surveys at Crown Marsh. a) Pond 42, transect 7, 18-Oct-2016 Bullhead sp. b) Pond 1, transect 14, 18-Oct-2016 Warmouth c) Pond 1, transect 4, 18-Oct-2016 Bullhead sp. d) Pond 5, transect 7, 11-Oct-2017 Ictalurid sp. e) Pond 3, transect 6, 18-Oct-2016 Bullhead sp. f) Pond 5, transect 14, 13-Sep-2017 Bullhead sp. g) Pond 5, transect 14, 13-Sept-2017 Bullhead sp.

Table 1. Locality and sampling timing for Crown Marsh visual monitoring surveys, 2016 to 2018.

Pond	Treatment/Control	Latitude	Longitude	Date	Before/After	Effort (seconds)
3	Control	42.5836	-80.3928	2-Sep-16	Before	49-101
10	Control	42.5820	-80.3857	2-Sep-16	Before	30-80
1	Treatment	42.5861	-80.4218	2-Sep-16	Before	45-90
5	Treatment	42.5839	-80.4164	2-Sep-16	Before	48-88
42	Treatment	42.5839	-80.4229	2-Sep-16	Before	38-91
3	Control	42.5836	-80.3928	19-Sep-16	After	47-95
10	Control	42.5820	-80.3857	19-Sep-16	After	45-82
1	Treatment	42.5861	-80.4218	19-Sep-16	After	47-142
5	Treatment	42.5839	-80.4164	19-Sep-16	After	57-82
42	Treatment	42.5839	-80.4229	19-Sep-16	After	50-97
3	Control	42.5836	-80.3928	18-Oct-16	After	45-82
10	Control	42.5820	-80.3857	18-Oct-16	After	61-97
1	Treatment	42.5861	-80.4218	18-Oct-16	After	42-57
5	Treatment	42.5839	-80.4164	18-Oct-16	After	42-120
42	Treatment	42.5839	-80.4229	18-Oct-16	After	50-80
41	Control	42.5830	-80.4277	16-Aug-17	Before	24-64
43	Control	42.5881	-80.4196	16-Aug-17	Before	31-75
3	Treatment	42.5836	-80.3928	16-Aug-17	Before	46-67
5	Treatment	42.5839	-80.4164	16-Aug-17	Before	42-59
11	Treatment	42.5885	-80.3994	16-Aug-17	Before	29-46
41	Control	42.5830	-80.4277	12-Sep-17	After	30-65
43	Control	42.5881	-80.4196	11-Sep-17	After	45-63
3	Treatment	42.5836	-80.3928	11-Sep-17	After	45-62
5	Treatment	42.5839	-80.4164	13-Sep-17	After	53-70
11	Treatment	42.5885	-80.3994	12-Sep-17	After	39-59
41	Control	42.5830	-80.4277	14-Sep-17	After	35-70
43	Control	42.5881	-80.4196	14-Sep-17	After	33-56
3	Treatment	42.5836	-80.3928	14-Sep-17	After	44-65
5	Treatment	42.5839	-80.4164	14-Sep-17	After	47-62
11	Treatment	42.5885	-80.3994	14-Sep-17	After	39-53
41	Control	42.5830	-80.4277	11-Oct-17	After	41-53
43	Control	42.5881	-80.4196	11-Oct-17	After	36-64
3	Treatment	42.5836	-80.3928	11-Oct-17	After	40-135
5	Treatment	42.5839	-80.4164	11-Oct-17	After	35-58
11	Treatment	42.5885	-80.3994	11-Oct-17	After	43-66
41	Control	42.5830	-80.4277	17-Sep-18	Before	36-78
43	Control	42.5881	-80.4196	13-Sep-18	Before	48-59

Table 1 (con't).

Pond	Treatment/Control	Latitude	Longitude	Date	Before/After	Effort (seconds)
3	Treatment	42.5836	-80.3928	17-Sep-18	Before	59-74
5	Treatment	42.5839	-80.4164	17-Sep-18	Before	34-60
11	Treatment	42.5885	-80.3994	13-Sep-18	Before	50-62
41	Control	42.5830	-80.4277	28-Sep-18	After	18-83
43	Control	42.5881	-80.4196	28-Sep-18	After	51-70
3	Treatment	42.5836	-80.3928	28-Sep-18	After	49-71
5	Treatment	42.5839	-80.4164	28-Sep-18	After	42-71
11	Treatment	42.5885	-80.3994	28-Sep-18	After	38-98
41	Control	42.5830	-80.4277	2-Oct-18	After	41-69
43	Control	42.5881	-80.4196	1-Oct-18	After	51-71
3	Treatment	42.5836	-80.3928	1-Oct-18	After	50-105
5	Treatment	42.5839	-80.4164	1-Oct-18	After	44-81
11	Treatment	42.5885	-80.3994	1-Oct-18	After	32-72
41	Control	42.5830	-80.4277	8-Nov-18	After	39-76
43	Control	42.5881	-80.4196	8-Nov-18	After	47-57
3	Treatment	42.5836	-80.3928	8-Nov-18	After	54-73
5	Treatment	42.5839	-80.4164	8-Nov-18	After	45-69
11	Treatment	42.5885	-80.3994	8-Nov-18	After	31-56

Table 2. Summary of species detections during Crown Marsh visual surveys, 2016 to 2018. Distressed (i.e. abnormal swimming behaviour) fishes are identified with an asterisk.

Pond	Treatment/Control	Date	Before/After	Transect Number	Species detected
1	Treatment	2-Sep-16	Before	14	*Micropterus salmoides**
5	Treatment	2-Sep-16	Before	9	*Ameiurus sp. (n=2)*
5	Treatment	2-Sep-16	Before	12	*Esox lucius*
42	Treatment	2-Sep-16	Before	18	*Unknown*
1	Treatment	19-Sep-16	After	16	*Ameiurus nebulosus*
1	Treatment	19-Sep-16	After	20	*Micropterus salmoides**
5	Treatment	19-Sep-16	After	8	*Ameiurus sp.*
5	Treatment	19-Sep-16	After	12	*Ameiurus sp.*
42	Treatment	19-Sep-16	After	2	*Ameiurus sp.*
42	Treatment	19-Sep-16	After	3	*Ameiurus nebulosus*
42	Treatment	19-Sep-16	After	4	*Ameiurus sp.*
42	Treatment	19-Sep-16	After	8	*Ameiurus nebulosus*
42	Treatment	19-Sep-16	After	10	*Ameiurus sp.*
42	Treatment	19-Sep-16	After	11	*Ameiurus sp. (n =2)*
42	Treatment	19-Sep-16	After	N/A	*Lepomis gulosus*
3	Control	18-Oct-16	After	6	*Ameiurus sp.*
1	Treatment	18-Oct-16	After	4	*Ameiurus sp.*
1	Treatment	18-Oct-16	After	6	*Ameiurus sp.*
1	Treatment	18-Oct-16	After	7	*Ameiurus sp.*
1	Treatment	18-Oct-16	After	14	*Lepomis gulosus*
5	Treatment	18-Oct-16	After	19	*Ameiurus sp.*
42	Treatment	18-Oct-16	After	3	*Ameiurus sp.*
42	Treatment	18-Oct-16	After	4	*Ameiurus sp.*
42	Treatment	18-Oct-16	After	5	*Ameiurus sp.*
42	Treatment	18-Oct-16	After	7	*Ameiurus sp.*
42	Treatment	18-Oct-16	After	14	*Ameiurus sp.*
42	Treatment	18-Oct-16	After	19	*Ameiurus sp.*
42	Treatment	18-Oct-16	After	20	*Ameiurus sp.*

Table 2 (con't).

Pond	Treatment/Control	Date	Before/After	Transect Number	Species detected
3	Treatment	16-Aug-17	Before	11	*Ameiurus nebulosus*
5	Treatment	13-Sep-17	After	3	*Ameiurus nebulosus*
5	Treatment	13-Sep-17	After	14	*Ameiurus nebulosus*
5	Treatment	11-Oct-17	After	1	*Ameiurus melas*
5	Treatment	11-Oct-17	After	1	*Noturus gyrinus*
5	Treatment	11-Oct-17	After	7	*Ictalurid sp.*
43	Control	28-Sep-18	After	1	*Amia calva*

Table 3. Comparison of mean (95% confidence interval range) number of dead fishes detected per transect during Crown Marsh visual surveys 2016 to 2018.

		Pre-application	Post-application	
		2016		
	Control/Treatment	**September 2**	**September 19**	**October 18**
Pond 3	Control	0	0	0.05 (0-0.1)
Pond 10	Control	0	0	0
Pond 1	Treatment	0	0.05 (0-0.1)	0.2 (0-0.35)
Pond 5	Treatment	0.15 (0-0.3)	0.1 (0-0.2)	0.05 (0-0.1)
Pond 42	Treatment	0.05 (0-0.1)	0.35 (0.10-0.35)	0.40 (0.15-0.65)
		2017		
		August 16	**September 12 to 14**	**October 11**
Pond 41	Control	0	0	0
Pond 43	Control	0	0	0
Pond 3	Treatment	0.05 (0-0.15)	0	0
Pond 5	Treatment	0	0.1 (0-0.24)	0.15 (0-0.38)
Pond 11	Treatment	0	0	0
		2018		
		September 13 & 17	**September 28 to October 2**	**November 8**
Pond 41	Control	0	0	0
Pond 43	Control	0	0.05 (0-0.15)	0
Pond 3	Treatment	0	0	0
Pond 5	Treatment	0	0	0
Pond 11	Treatment	0	0	0