Translocations and monitoring of Northern Leopard Frogs, *Lithobates pipiens*, at Brisco, BC.

by

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for

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EXECUTIVE SUMMARY

Ongoing monitoring of reintroduced leopard frogs at Brisco was carried out in 2018, and included the use of Songmeters, Nocturnal Calling Surveys and Visual Encounter Surveys. No leopard frogs were detected during the calling period.

A total of 1,685 northern leopard frog tadpoles and 110 metamorphs were translocated to the Brisco Release Pond (BRP). Of these, 1600 hatchling tadpoles from the Vancouver Aquarium were released after water changes on June 6, and 80 tadpoles were released the following week from eggs procured from the wild and hatched at the Calgary zoo. On August 15, 110 metamorphs and 5 tadpoles from the Vancouver Aquarium were also released. Metamorphosis occurred in mid-July.

No adult or juvenile leopard frogs were observed during 101 hours of survey time. Between July 31 and October 17, 263 Young of Year (YOY) were seen. Of these, 244 observations were in the BRP, 12 were on the riverbank to the south and 7 were across the river on Larry's Island. The mean snout-vent-length (SVL) of YOY was 50.7 mm (SD = 8.2, n = 161), mean shank length was 28.5 mm (SD = 5.2, n = 161) and average weight was 13.9 g (SD = 5.5, n = 161). The largest individual was 63.7 mm SVL and the heaviest weighed 28 g. Nuptial pads were first noticed on Sept 4. The smallest male with these pads had a SVL of 49.8 mm and weighed 12 g. Growth rates were highly variable, with SVL growing at 0.16 mm / day (SD = 0.15, n = 19) and Shank length at 0.09 mm / day (SD = 0.07, n = 22).

A subsample of YOY remained small throughout the survey period. It is likely they were individuals from the metamorph release of August 15, as a similar group that stayed consistently small through the season has never been detected in previous years.

Two malformations were observed. One YOY had shanks that differed by 5.4 mm. The other was a large tadpole brought from the Vancouver Aquarium on Aug 15 that could not swim upright and seemed to have a spinal curvature.

Chytridiomycosis was first observed on Sept 11 and a skin swab subsequently confirmed this. Thirty one of 88 YOY (35%) captured on or after September 11 showed gross evidence of chytrid fungus. Six YOY captured on September 10 and recaptured on Sept 25 had lost weight.

Overall body condition was poorer than 2014 and 2015, likely due to chytrid, which was not detected in those years. Body condition in 2018 was better than in 2016, a year which also had a chytrid outbreak.

Columbia spotted frogs were observed regularly, with all age classes apparent.

An important consideration going forward is the poor availability of tadpoles/egg masses from the wild and in captive rearing situations. The desired number of 8,000 or more tadpoles per year has only been obtained in one year of the previous six. Furthermore, the presence of chyrid fungus at the release site becomes more significant in light of the small number of individuals translocated.

Acknowledgements

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INTRODUCTION

The northern leopard frog, *Lithobates pipiens*, became extinct throughout most of its range in southeastern British Columbia in the 1970s and 1980s. The species persisted, however, in the Creston Valley Wildlife Management Area (CVWMA), and this became the source population for reintroductions back into historic range. The re-establishment of the leopard frog in the Columbia marshes became a Recovery Action identified by the Northern Leopard Frog Recovery Team (NLFRT) and included in the Recovery Strategy (Environment and Climate Change Canada 2017). Suitable habitat was identified in work carried out by MSc candidate Chris Carli, supplemented by input from the author (Ohanjanian and Carli 2010).

This document reports on the sixth year of reintroduction efforts to a pond (the Brisco Release Pond, or BRP, Figure 1) in the Columbia wetlands. Prior efforts had varied in their effectiveness due in large part to a reduced number of individuals available for translocation in all but one year (Ohanjanian et al. 2013, Ohanjanian 2014, 2015, 2016, 2017). The target of 8,000 translocated tadpoles was only achieved in 2016. In 2017, one individual was observed in spring, indicating that the species had over-wintered. No others were seen. A single male call was recorded calling on a Songmeter (Ohanjanian 2017). In 2018, ongoing monitoring, including Nocturnal Calling Male Surveys (NCS), Remote Sensing (Songmeter deployment) and Visual Encounter Surveys (VES), was undertaken in addition to translocations.

Figure 1. Northern leopard frog reintroduction sites at Brisco Release Pond in 2018.

METHODS

On April 26, 2018, five Songmeters (Wildlife Acoustics SM2+) were deployed at the BRP (two, one at both north and south ends), on Larry's Island (one) and on Muskrat Island (two) (Figure 1). These were set to record from 11 pm until 2 am every night until they were removed in late-June. The UTM coordinates of these can be found in Appendix A. After retrieval the sound files were scanned, using Songscope software, for the visual signature of the leopard frog's "snore" call

On-site Nocturnal Calling Surveys (NCS) (n = 18) were carried out weekly, beginning on April 28 and continuing until May 30 at three permanent stations at the north, middle and southern ends of the BRP. Two observers listened passively for 9 minutes at each station, and environmental conditions (water temperature, pH, conductivity, air temperature, wind speed and cloud cover) were recorded.

The numbers and age class of Columbia spotted frogs (*Rana luteiventris*) observed incidentally were recorded during all NCS and subsequent VES.

Hatchling tadpoles were transferred following the same protocol as in previous years, using translocation guidelines outlined in Kendell and Prescott (2007). Three translocations of tadpoles and metamorphs took place in 2018. Tadpoles from the Vancouver Aquarium arrived by air at Cranbrook Airport in plastic bags in a styrofoam container, and were driven to Brisco. Additional tadpoles were brought from the Calgary zoo. Water from the release pond was gradually added to acclimatize tadpoles to the different conditions. When pH and temperature were equalized, tadpoles were released into the pond. A third release of captive reared metamorphs from the Vancouver Aquarium also took place in August.

Amphibian hygiene protocols were followed to reduce likelihood of disease transmission (British Columbia 2008; Adama and Davidson 2007). Dedicated sets of waders and nets were used at Brisco, and amphibians were handled with Ziploc bags and nitrile gloves specific to each individual. As in previous years, weights (to nearest 0.5 g) and both snout-vent lengths (SVL) and shank lengths (to nearest 0.1 mm) were recorded using a Pesola scale and calipers. Overall health was noted by visual inspection and assessment of righting reflex. When chytrid symptoms were observed on September 11 (i.e. sloughing skin) no further captures were made that day to reduce transmission of the disease. A sample of skin from one individual was sent to the Animal Health Centre in Abbotsford BC for PCR testing and diagnosis. Subsequently, an enhanced protocol which involved disinfecting multiple nets with Virkon between each individual, was followed. Photos of dorsal spot patterns were taken for inclusion in the Brisco frog archive for potential Mark-Recapture population analysis in future and to determine growth rates and movements. All leopard frogs were geo-referenced using hand-held GPS units, and released within 5 minutes at their capture site.

RESULTS

Remote Sensing and Nocturnal Calling Surveys

No leopard frogs were detected on Songmeters. No leopard frogs were heard calling at the BRP in spite of ideal survey conditions on May 15 and May 22 when night time water temperatures were over 20°C and air temperatures ranged from 11.9 to 14.5°C. On May 30, the Columbia River was overflowing the banks of the BRP, reducing the water temperature to 14°C.

Translocations

On June 6, 1600 hatchling tadpoles were flown to Cranbrook from the Vancouver Aquarium. These tadpoles, from frogs that were captive reared, were transported as shipped in plastic bags to the BRP. They were kept in bags during three water changes prior to release at 16:30 into the Brisco Release Pond (Figure 1). Water conditions for the acclimation process during the release are shown in Table 1.

Table 1. Water temperature, pH and conductivity during tadpole reintroduction, June 6, 2018

		Aquarium water	BRP water
On arrival:	Water temp	16.0	19.1
	pH	9.7	8.2
	Conductivity	55.3	282
1st change	Water temp	17.1	
	pH	8.5	
	Conductivity	84.0	
2nd change	Water temp	17.6	
	pH	8.3	
	Conductivity	105.0	
3rd change	Water temp	18.8	
	pH	8.3	
	Conductivity	141.0	

Water depths at the release site were approximately 80 – 100 cm due to the rise and inflow of the Columbia River the previous week (Figure 2). Tadpoles were released in a stand of bulrush to provide good cover.

Figure 2. Tadpole release at Brisco Release Pond, June 6, 2018

An additional 80 tadpoles were brought from the Calgary zoo and released into the BRP in early June. Those came from eggs collected earlier in spring from the CVWMA (L. Randall, pers. comm.).

On August 15 a third translocation of 111 metamorphs and 6 tadpoles from the Vancouver Aquarium took place. These froglets appeared to be in good health, although they were small, and there was no mortality in transit from the aquarium. The size of a sub-sample of these frogs (n = 8) is shown in Table 2.

Table 2. Sizes of a subsample of captive-reared L. pipiens metamorphs released on Aug 15, 2019

Froglet ID	SVL (mm)	Wgt (g)
ID No. 1	23.6	--
ID No. 2	31.5	--
ID No. 3	33.8	3.4
ID No. 4	30.3	5.5[1]
ID No. 5	31	3.0
ID No. 6	33.3	3.0
ID No. 7	31.9	3.0
ID No. 8	34	3.5
Mean	31.2	
SD	3.3	

[1] Weight of this individual is likely a measuring error

Front legs of the tadpoles were not yet emerged. One was malformed and unable to swim upright. It was not released and is now in the BC Provincial Museum collection (Figure 3).

Figure 3. Malformed L. pipiens tadpole, Aug 15, 2018

Visual Encounter Surveys

No juvenile or adult leopard frogs from previous releases were observed during 4 hours of survey time in May, 2019 nor in the subsequent summer/fall surveys.

Between July 31 and October 17, a total of 263 Young-of-Year (YOY) were seen during 96:58 hours of VES. Of these, 244 observations were in the Brisco Release Pond itself, 12 were on the riverbank to the south, and 7 were on Larry's Island, across the Columbia River (Figure 1).

The first YOY was seen on July 31 near the release area. Its size (10 g) suggested that it had metamorphosed by mid-July. Some YOY had moved out of the BRP by Aug 14; two individuals were observed on the riverbank south of the BRP. On September 6, three YOY were first observed across the river on Larry's Island (Figure 1).

Morphometric data were obtained on 161 captures (Table 3). Twenty-eight YOY were re-captured one or more times[2]. The mean snout-vent length (SVL) of YOY in 2018 was 50.7 mm, the mean shank length was

[2] To allow comparisons with previous years, data from recaptures were included in calculations of the overall average of SVL, shank and weight.

28.6 and the average weight was 13.9 grams. The largest individual was 63.7 mm SVL on September 25, and the heaviest individual weighed 28 g.

Table 3. Sizes of L. pipiens YOY captured at Brisco, July 31 to Oct 17, 2018

	SVL (mm)	SHANK (mm)	WGT (g)
Mean	50.7	28.5	13.9
SD	8.2	5.2	5.5
Range	28 – 63.7	14.0 – 35.0	2 - 28
n	161	161	161

There was a subsample of YOY that remained small throughout the survey period. On September 18 and 25, nine[3] small individuals were captured (Table 4). Their ability to disperse did not seem impeded, however; very small individuals (only 3 g, and likely from the metamorph release of August 15) were foraging outside the BRP on the riverbank to the south on September 4.

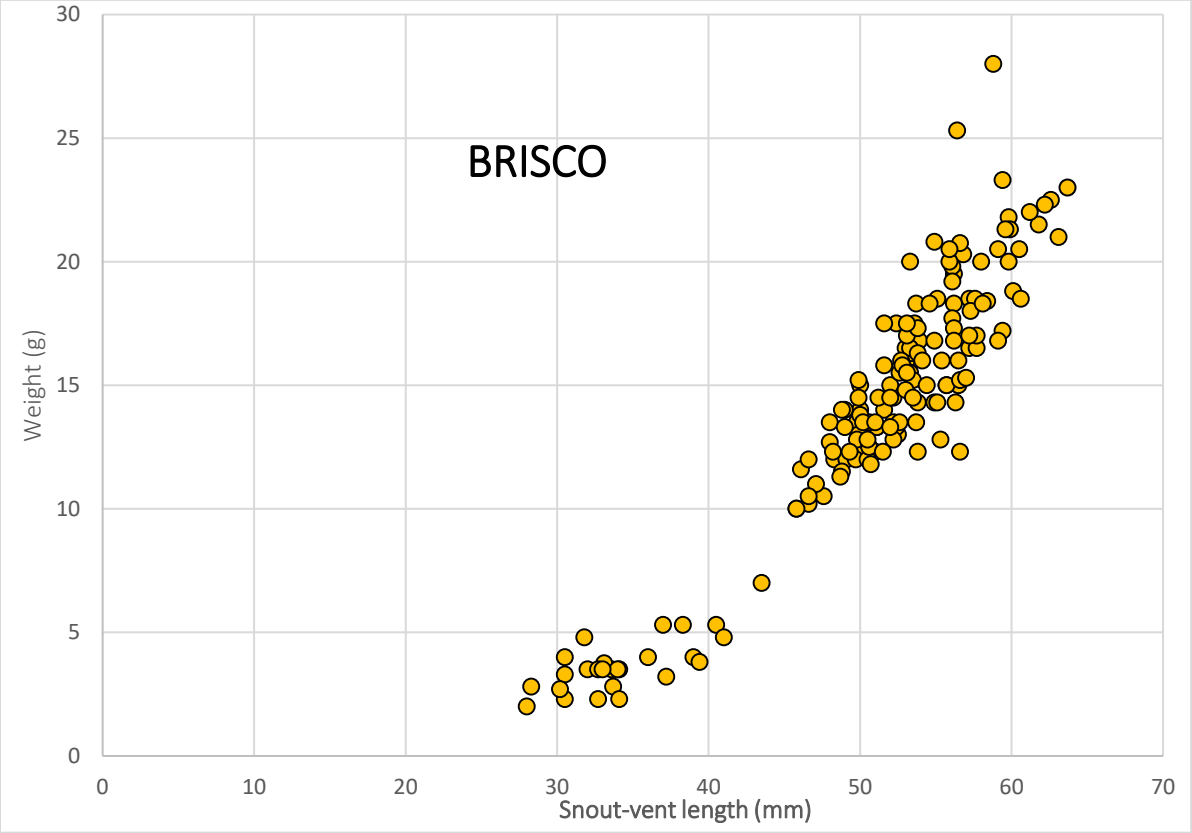
Table 4. Sizes of small L. pipiens YOY captured on September 18 and 25.

	SVL (mm)	Shank (mm)	Wgt (g)
	28.3	16	2.8
	33	16.2	3.5
	34.1	16	2.3
	38.3	21	5.3
	30.5	16.6	4
	37	19.7	5.3
	32.7	17.2	2.3
	31.8	20.4	4.8
	28	15.1	2
Mean	32.6	17.6	3.6
SD	3.5	2.2	1.3

This size disparity is also clear in the graph showing body condition in Figure 4. SVLs were clearly bimodal, showing a group of very small individuals distinct from the majority. These are likely individuals from the August 15 release of smaller, aquarium-reared metamorphs (see below Discussion).

[3] A tenth YOY was captured, however it is not included here as this individual had markedly different leg sizes (see below, Health). Nevertheless this individual was also extremely small (4.8 g).

Figure 4. Weight as a function of SVL for YOY at Brisco, 2018



Nuptial pads were first noticed on September 4 on four individuals. The smallest male with nuptial pads had a SVL of 49.8 mm and he weighed 12 g (n = 21 males).

Recaptures

Calculated growth rates of recaptured animals using both SVL and shank measurements were too variable to provide any comparisons with previous years (Table 5). Decreases in weight were detected for individuals that showed signs of chytridiomycosis when captured (sloughing skin). Six YOY captured on September 18 and recaptured one week later on September 25 lost 2g, 0.8g, 2.2g, 0.7g, 0.5g, and 0.2 g.

Table 5. Average daily growth rates of recaptured L. pipiens YOY using SVL and shank lengths, 2018

	Mean growth /day (mm)	SD	Range	n
SVL	0.16	0.15	0 – 0.56	19
Shank	0.09	0.07	0 – 0.31	22

Health

Chytridiomycosis was confirmed by the Animal Health Centre, Abbotsford, BC on an animal captured on September 11. This frog showed symptoms of early chytrid infection (sloughing skin) (Figure 5). Thirty one of 88 YOY (35%) captured on or after September 11 showed gross evidence of chytrid fungus, i.e. sloughing skin. The relative proportion of captured frogs apparently afflicted with chytrid increased over the following two months. On September 18, 9 of 33 (27%) of individuals captured had chytrid signs and this proportion was almost double on October 17, when 4 of 8 captured animals (50%) showed symptoms.

Figure 5. Field evidence of chytridiomycosis (later confirmed) Sept 11, Brisco Release Pond

No major deformities were encountered in 2018. However one individual on Sept 11 had different sized shanks; the left was 5.4 mm longer and bulkier than the right (Figure 6). It weighed 16 g and appeared healthy otherwise.

Figure 6. Young-of-Year L. pipiens with different shank sizes.

Other species

Although it was expected that the Pacific chorus frog would be heard in the spring, only one other frog species, the Columbia spotted frog, was detected at the BRP. This highly aquatic frog was seen during every VES of the BRP. All age classes were represented, and maximum of 9 adults and 43 YOY were seen on September 25.

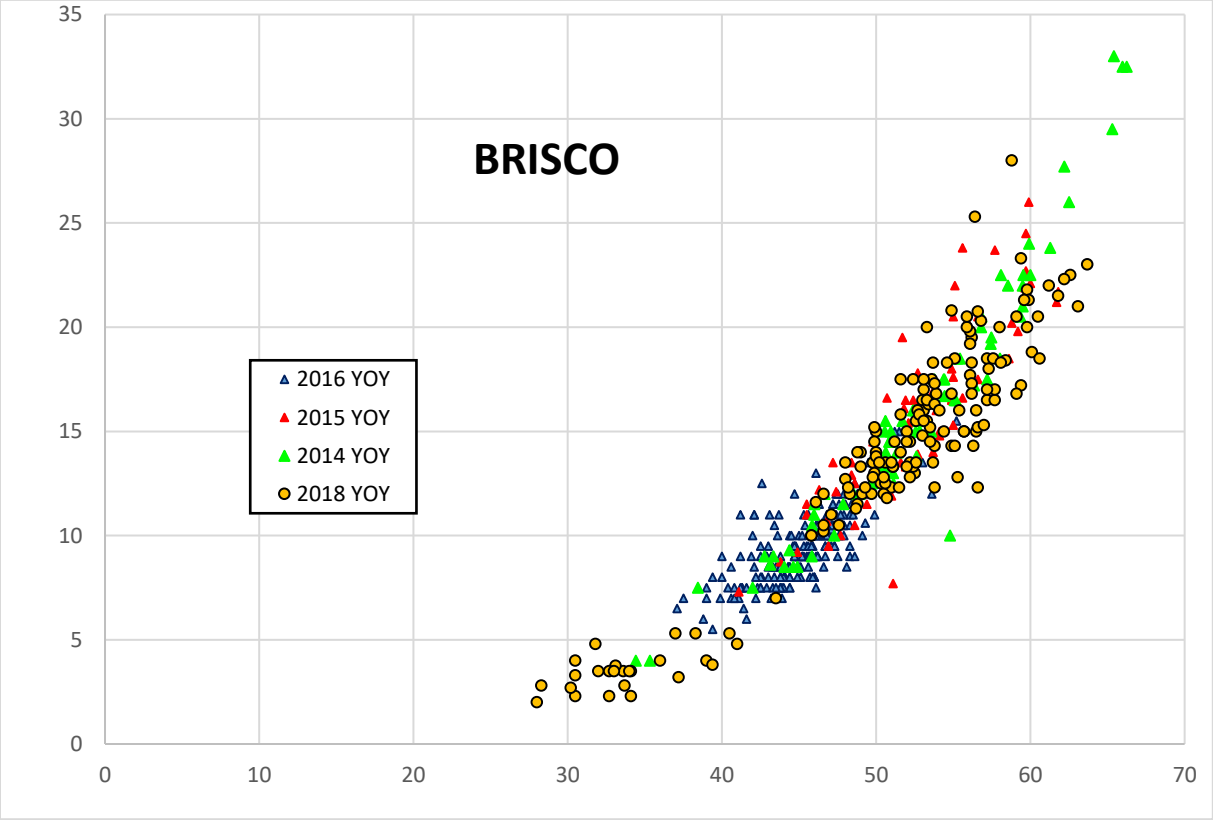
Common garter snakes (*Thamnophis sirtalis*) and sunfish were also observed in the BRP. The former were also present on Larry's Island.

DISCUSSION

As in previous years, tadpoles appear to have survived in good numbers to metamorphose into froglets. Almost 1800 individuals were introduced, and we would expect a survivorship of only 5-10% of tadpoles (i.e. 90 to 180 metamorphs). The number encountered shows that this expected tadpole survivorship was exceeded in 2018 at the BRP. When probability of detection is factored in, the total number of detections (263) plus the confirmed identification of at least 125 individuals suggest that well over 10% of the tadpoles reached metamorphosis. Therefore, despite the presence of predatory fish brought in from the river during high water, the habitat again provided good cover and adequate food for developing tadpoles.

In 2018, body condition of YOY leopard frogs appeared to be poorer overall than in 2014 and 2015, but better than in 2016 (Figure 7), especially in the larger size categories. This may reflect lower thriftiness due to chytridiomycosis – a disease that was not observed in 2014 or 2015.

Figure 7. Body condition of YOY at Brisco over multiple years.



Similarly, body condition and overall size of YOY in 2016 (when chytrid was first confirmed) was poorer than those previous two years. The organism responsible for this disease, *Batrachochytrium dendrobatidis* (Bd) is a pathogenic fungus that causes higher amphibian mortality at lower water temperatures. Chytridiomycosis outbreaks, therefore, are more typically observed in fall than in summer.

A larger number of captured YOY showed signs of chytrid on October 17 (50% of captures, n = 8) than on September 18 (27% of captures, n = 33), suggesting that the condition was increasing in its prevalence in the population. Even though ease of capture of sick individuals would bias any measure of the prevalence in the total population, it cannot be assumed that the many individuals that escaped over the survey period were *not* affected by this disease. Animals with sloughing skin in the earlier stages of chytridiomycosis appeared healthy and active and able to avoid capture.

It is likely that the presence of very small YOY throughout the sampling period reflects a size disparity between YOY that were released as tadpoles in early June vs those released as metamorphs on August 15. To support this, there is indirect evidence from previous years, when no metamorphs were released. By mid-September no YOY were smaller than 11 g (2014), 10.7 g (2015), and 7.5 g (2016). The body condition graph also clearly indicates the bimodal population size structure in 2018, not present in other years. This size disparity may be a reflection of underlying poorer health and thriftiness of the Vancouver Aquarium metamorphs or simply the fact that they metamorphosed one month later than the tadpoles. A third possibility is that the translocated metamorphs' growth was stunted or delayed by their need to adapt to the new environment. The smaller size going into winter would be disadvantageous and Altwegg and Reyer (2003) found that late metamorphosing individuals of other ranid species had a lower survival rate as well as slower growth. This is an area of research that could be undertaken with marked metamorphs, using, for example VIE, and could inform future translocations.

In 2017 a leopard frog call was heard on one night in spring and a single juvenile was observed once. Other than these detections, no other overwintering success has been confirmed at Brisco. Germano and Bishop (2008) report that the number of animals released during amphibian translocations significantly affects success, and Semlitsch (2002) suggests that the release of 10,000–50,000 eggs over several years is required to reach an adult population of 100 individuals. Furthermore, population viability analysis suggests that future work should focus on reducing the extinction risk (and therefore increase likelihood of population establishment) by reintroducing several populations with good connectivity between them (Tischendorf 2007). During the current project, the objective of translocating 8,000+ tadpoles per year was only achieved once, in 2016, due to a short supply of captive-bred individuals and fewer egg masses in the wild at Creston. Without adequate numbers of individuals to translocate, the reintroduction of leopard frogs into the Columbia marshes cannot be achieved.

An additional consideration going forward is the fact that much remains unknown about the effects of Bd, which was confirmed in leopard frogs in the BRP in 2016 and 2018. There is evidence that some leopard frogs at Creston developed resistance to Bd (Vourdouw et al. 2010). It is not known if any of the transplanted individuals avoided contagion, nor if any survived it. The small number of frogs available for transplant further complicates this, as it reduces the probability that individuals with resistance are included.

Further translocations to the Columbia marshes and elsewhere should be considered in this light.

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APPENDIX A. UTM coordinates of Songmeters deployed April 26, 2018

	Easting	Northing
Brisco Release Pond north	0550650	5628662
Brisco Release Pond south	0550894	5628421
Larry's Island	0551121	5628168
Muskrat Island south	0550622	5628795
Muskrat Island north	0550490	5629156