Identifying Factors Contributing to the Successful Spawning and Early Rearing of Cowichan Lake Lamprey (*Entosphenus macrostomus*)

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IDENTIFYING FACTORS CONTRIBUTING TO THE SUCCESSFUL SPAWNING AND EARLY REARING OF COWICHAN LAKE LAMPREY (*ENTOSPHENUS MACROSTOMUS*)

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ABSTRACT

Wade, J., Dealy, L., Hodes, V., and P. Grant. 2018. Identifying Factors Contributing to the Successful Spawning and Early Rearing of Cowichan Lake Lamprey (*Entosphenus macrostomus*). Can Manuscr. Rep. Fish. Aquat. Sci. 3169 v + 13 p.

An experiment was conducted in the summer of 2018 to add to the growing knowledge of the spawning and rearing requirements of Cowichan Lake Lamprey (*Entosphenus macrostomus*). In 2017 a preliminary study was conducted to identify spawning areas (Wade et al., 2018) and confirmed for the first time the use of alluvial fans for spawning and nest building in this species.

The goals of the 2018 experiment were to determine the spawning period as well as extent of habitat usage for spawning and early rearing of Cowichan Lake Lamprey; to further define the features of nests; to begin collecting environmental data during spawning and early rearing and; to refine field sampling methods for the use of eDNA in determining presence or absence of lamprey. As the majority of fish (29 of 31) were trapped at the Robertson River location in 2017, the study was conducted in this one tributary in 2018. Eight trap lines consisting of six traps each were installed from April 20 to June 29 when they were removed due to low water. No lamprey were captured in any traps, no lamprey were seen building nests.

It is likely that several factors contributed to the differences in habitat between the end of June 2017 and the same time in 2018 at the mouth of Robertson River. It is probable that winter and spring weather events over the past year were sufficiently forceful to both change the extent of the alluvial fan (wider) and deposit more sediment. How these attributes and others including water temperature, depth, or current, may or may not affect spawning or choice of spawning locations is unknown.

Comparing the catch rates and habitat features of the same spawning area in two consecutive years illustrates the importance of both understanding what physical conditions are necessary for spawning as well as recognizing that in a stochastic environment, it is necessary to protect all potential spawning and rearing habitat, not just that in which spawning has been observed as habitat conditions may change from year to year.

RÉSUMÉ

Wade, J., Dealy, L., Hodes, V. et Grant., P. 2018. Détermination des facteurs contribuant au succès du frai et de l'élevage précoce de la lamproie du lac Cowichan (*Entosphenus macrostomus*). Rapport manuscrit canadien des sciences halieutiques et aquatiques 3169 v + 13 p.

Au cours de l'été 2018, une expérience a été réalisée afin d'accroître les connaissances sur les besoins de la lamproie du lac Cowichan (*Entosphenus macrostomus*) en matière de frai et d'élevage. En 2017, une étude préliminaire a été menée pour relever les frayères (Wade *et al.*, 2018); elle a confirmé pour la première fois, chez cette lamproie, l'utilisation de cônes alluviaux pour le frai et la nidification.

Les objectifs de l'expérience de 2018 étaient les suivants : déterminer la période de frai ainsi que l'étendue de l'utilisation de l'habitat pour le frai et l'élevage précoce de la lamproie du lac Cowichan; mieux définir les caractéristiques des nids; commencer à recueillir des données environnementales pendant le frai et l'élevage précoce; améliorer les méthodes d'échantillonnage sur le terrain pour déterminer la présence ou non de lamproie au moyen d'ADNe. Comme la majorité des poissons (29 sur 31) ont été capturés à l'emplacement de la rivière Robertson en 2017, l'étude de 2018 a été menée dans ce tributaire. Du 20 avril au 29 juin, huit lignes de casiers composées de six pièges chacune ont été installées, puis enlevées en raison du faible niveau d'eau. Aucune lamproie n'a été capturée dans les pièges; aucune lamproie n'a construit de nids.

Il est probable que plusieurs facteurs aient contribué aux différences observées dans les habitats entre la fin juin 2017 et la même période en 2018 à l'embouchure de la rivière Robertson. Il est probable que des événements météorologiques s'étant produits au cours de l'hiver et du printemps de la dernière année aient été suffisamment violents pour modifier l'étendue du cône alluvial (plus large) et déposer plus de sédiments. On ne sait pas comment ces attributs et d'autres, y compris la température de l'eau, la profondeur ou le courant, peuvent ou non influer sur le frai ou le choix du lieu de frai.

La comparaison des taux de prises et des composantes de l'habitat d'une même frayère au cours de deux années consécutives illustre l'importance de comprendre les conditions physiques nécessaires au frai et de reconnaître qu'il est nécessaire, dans un environnement stochastique, de protéger tous les habitats potentiels de frai et d'élevage, pas seulement ceux dans lesquels le frai a été observé, car les conditions d'habitat peuvent varier d'année en année.

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INTRODUCTION

Cowichan Lake Lamprey (*Entosphenus macrostomus*) is a freshwater parasitic lamprey likely derived from Pacific Lamprey (*E. tridentatus*) within the last 10,000 years (Beamish & Wade 2018). It is an extreme endemic, present only in the Cowichan Valley watershed located on Vancouver Island, British Columbia. This includes Mesachie Lake, Bear Lake and Cowichan Lake as well as tributaries emptying into these water bodies. As Bear Lake is entirely contiguous with Cowichan Lake with no impediment to the movement of water or aquatic species it is considered to be Cowichan Lake for the purposes of this paper (Figure 1).

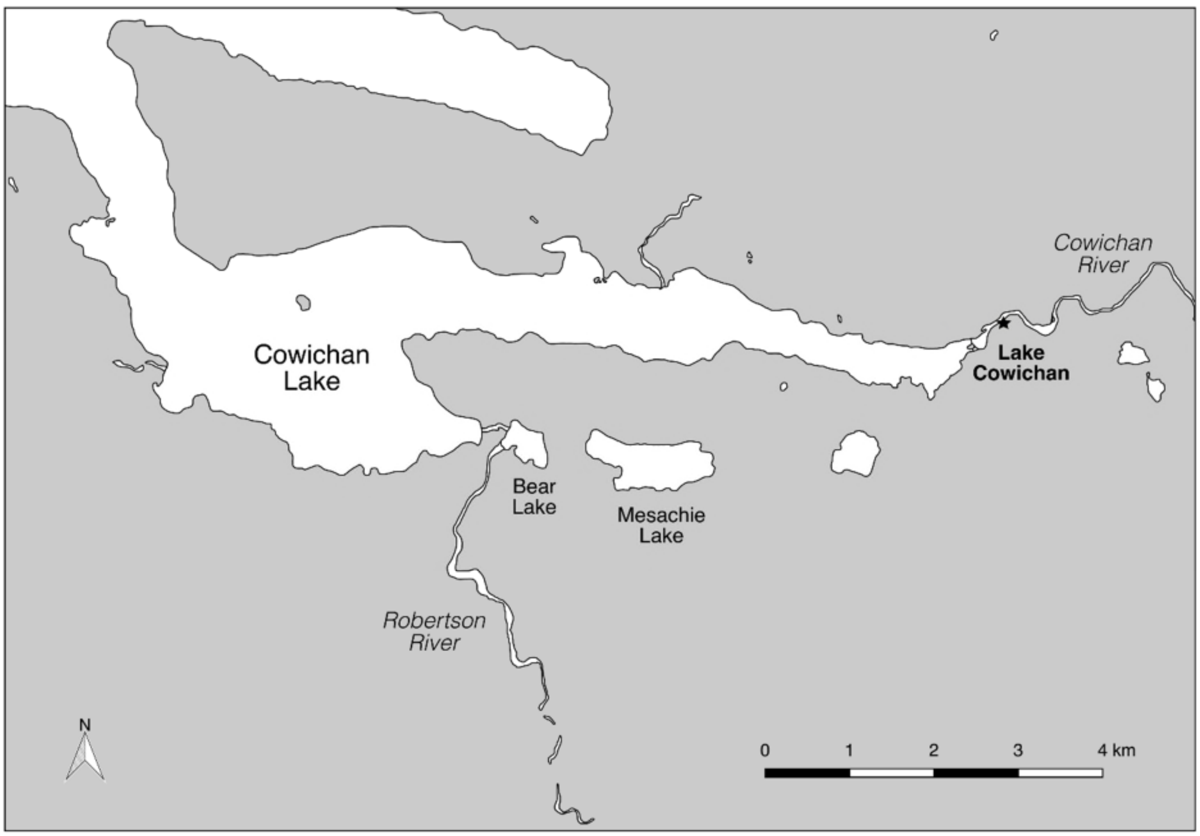

Figure 1. Map of 2018 study area showing location of Robertson River within the Cowichan Valley watershed, Vancouver Island, British Columbia.

Cowichan Lake Lamprey was first described in 1982 (Beamish 1982). For much of the 36 years since, little work has been conducted that contributes to the understanding of the ecology of the species. In recent years efforts to understand various aspects of the reproductive biology have been attempted to aid in the management of the species.

The spawning period has been estimated to occur from May to the end of July with peak spawning in June (Beamish & Wade, 2008). Mature lamprey have been observed at the confluence of Halfway Creek and Mesachie Lake (Beamish 1982; Beamish & Wade 2008) and in 2017, at the confluence of Robertson River and Cottonwood River with Bear Lake and Cowichan Lake respectively (Wade et al. 2018). The study conducted in 2017 (Wade et al. 2018) informed several outstanding reproductive questions including: the first observations of spawning and nest building in the species; the first report of sexual dimorphism in adults in spawning condition; habitat utilization (including pebble size) for nest building, spawning and early rearing. From the 2017 study it was not possible to determine the beginning and end of the spawning period and the extent of the tributaries used by lamprey to spawn.

The goals of the 2018 experiment were to determine the spawning period as well as extent of habitat usage for spawning and early rearing of Cowichan Lake Lamprey; to further define the features of nests; to begin collecting environmental data during spawning and early rearing and; to refine field sampling methods for the use of eDNA in determining presence or absence of lamprey. As the majority of fish (29 of 31) were trapped at the Robertson River location in 2017, it was considered to have the greatest potential for detecting species, therefore this the study was conducted in this one tributary in 2018.

CONTEXT

This study is an important contribution of information towards the continued survival of the species by seeking to identify spawning and rearing requirements to inform habitat protection goals. It addresses several items in the Recovery Strategy (DFO, 2007), recovery objectives in the Draft Action Plan and knowledge gaps in the identification of critical habitat (MacConnachie & Wade 2016).

Section 2.1 of the Recovery Strategy (DFO, 2007), *Biological Needs, Ecological Role and Limiting Factors*, states that as a limiting factor "all species require sufficient rearing and spawning habitat and a healthy food base." Knowledge to date does not allow the determination of "sufficient" spawning habitat of *E. macrostomus*. Potential spawning habitat in Cowichan Lake has been proposed (MacConnachie & Wade 2016) and can be further amended based on Wade et al. (2018). The extent to which tributaries are utilized or their characteristics such as temperature remains unknown. The intent of this project is to inform the extent of the spawning period and if the lamprey use the rivers in which to spawn.

Section 3 of the Recovery Strategy (DFO, 2007), *Threats*, lists *Water Use* as a threat to the species. There is a need to identify spawning areas in order to inform decision-making regarding water extraction from Cowichan Lake during typical and abnormal precipitation years. This has become an extremely important issue for local businesses and residents of the Cowichan Valley in recent years. The potential impacts of water extraction on lamprey spawning and early rearing are being considered in the proposed Cowichan Water Use Plan (WUP) options currently under consultation (https://cowichanwup.ca/about/, accessed August 2018).

Land Use is another potential threat identified in the Recovery Strategy. Since the Recovery Strategy was written in 2007, there has been further land development around Cowichan Lake near the shoreline. In order to inform developers and residents, there is a need to identify spawning and rearing areas before it is impacted. In addition, Section 6.2 of the Recovery Strategy, *Schedule of Studies*, specifically cites "observations on spawning locations" as being useful for identifying characteristics of suitable spawning habitat.

In summary, this project will inform the following three recovery objectives in the Draft Action Plan namely:

- Maintain a self-sustaining population of Cowichan Lake Lamprey (Vancouver Lamprey) within Cowichan and Mesachie lakes that is resilient to short-term habitat perturbations.
- Maintain, and where possible enhance, the ecological integrity of habitat for Cowichan Lake Lamprey (Vancouver Lamprey).
- Increase scientific understanding of Cowichan Lake Lamprey (Vancouver Lamprey) through additional investigation of its taxonomic status, natural history, critical habitat, and threats to the species' persistence.

This work will also inform the following two items identified in the draft Action Plan which were to be undertaken by DFO:

- Develop a robust monitoring plan to provide for a clear indication of the progress achieved towards securing the species' long term viability.
- Continue to investigate use of tributary habitats throughout the Cowichan Valley watershed.

This study also informs three knowledge gaps identified in the proposed critical habitat paper for Cowichan Lake Lamprey (MacConnachie & Wade, 2016):

- To develop a better understand of habitat use of different life stages of the species.
- To increase the current state of knowledge about the species in general and habitat use through trapping studies in Cowichan Lake.
- To identify spawning locations and habitat in Cowichan Lake.

METHODS

In order to determine the spawning window, traps were deployed at the end of April 2018 with the intention of removing them mid September 2018. This was not possible due to low water conditions and the study was terminated at the end of June 2018. As water levels were becoming low, and it became obvious that the study would be terminated early, snorkel surveys were added to the methods to determine if lamprey were using the alluvial fans for spawning or nest building. The alluvial fans were located further toward Bear Lake and in deeper water than the traps. Further decreases in water level also eventually resulted in termination of snorkel surveys.

TRAPPING

Eight trap lines were installed in Robertson River from the confluence with Bear Lake (trap line 1) upstream approximately 1000 m (trap line 8) (Figure 2). As this map was created using QGIS Version 2.18 and shapefiles available in the BC Data Catalogue (https://catalogue.data.gov.bc.ca/), the wetted area (blue) depicted in Figure 2 is greater than that observed during the 2018 field work.

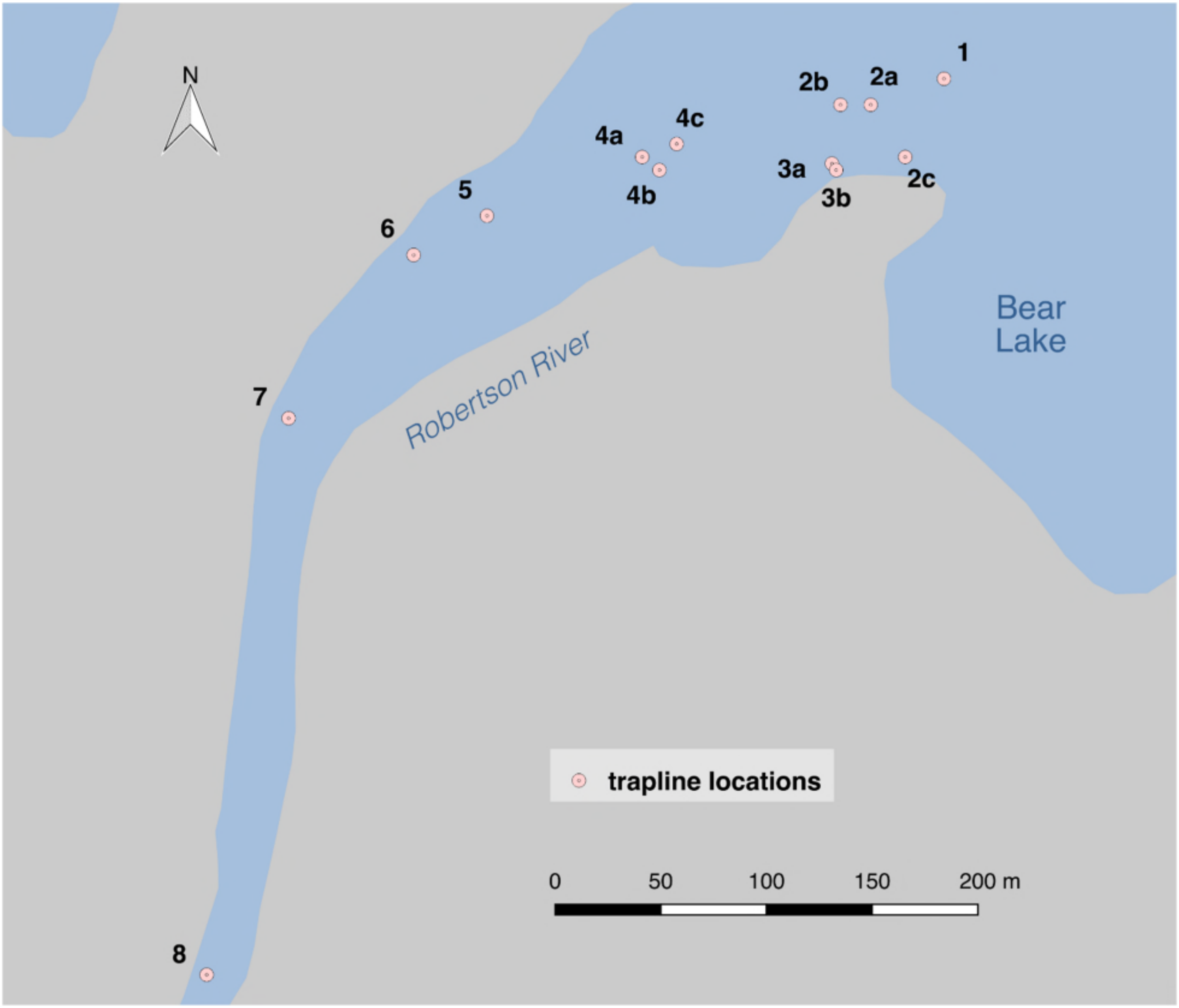

Figure 2. Map depicting trap line locations from the alluvial fan in Bear Lake upstream in Robertson River (1,2a/b/c, 3a/b, 4a/b/c, 5,6,7 and 8) for 2018 spawning survey. As trap lines needed to be moved due to low water they were numbered a,b,c in sequence with each subsequent move.

The trap line configuration was the same as described in Beamish and Wade (2008) and Wade et al. (2018). A trap line consists of a “weir” made of Vexar® plastic (9 mm hole diameter) held in place with rebar and cable ties, with six minnow traps (three per side) placed parallel to the weir in the substrate and tied in place to the rebar (Figure 3).

Figure 3. Photograph of trap line depicting Vexar® fence held in place with rebar, minnow traps visible at the base.

Trap lines were placed in line with the direction of water movement out of Robertson River. All sampling was performed as described in Wade et al. (2018).

SNORKELING

A total of seven snorkeling surveys were conducted in the alluvial fan of Robertson River along the transects depicted in Figure 4. Transects were snorkeled as described in Wade et al. (2018). Snorkeling was conducted continuously from location 1 to location 13 (Figure 4) and the total number of spawners recorded across all transects. These transects were selected to cover as much of the alluvial fan as possible.

Figure 4. Snorkel transects in the alluvial fan of Robertson River, 2018.

ENVIRONMENTAL OBSERVATIONS

Water depth was measured at the upper, middle and lower ends of each trap line once per week. A temperature data logger (TidbiT® v2 Temp UTBI-001) was affixed to some of the trap lines. Trap lines 4 and 7 did not have TidbiT®s; trap lines 2,3,5 and 8 had TidbiT®s throughout the experiment. The TidbiT® on trap line 6 was moved to trap line 1 on May 8, 2018.

Temperatures were automatically recorded at 10 minute intervals. For each trap line, the minimum, maximum, and average daily temperatures were determined when a full 24 hour period of temperature data was available.

eDNA SAMPLING

Environmental DNA (eDNA) based detection of finfish starts with collecting and filtering a water sample to obtain free-floating DNA from organisms living in that environment. DNA is extracted from the filter and used for DNA metabarcoding. This method uses PCR and high-throughput Next

Generation Sequencing to generate millions of DNA sequence reads from all target species in the sample simultaneously and with high sensitivity.

These sequence reads are designed to be species-specific, such that they can be matched to existing reference data for species identification. Metabarcoding can be used for detection of cryptic and rare taxa, and holds promise for overcoming current reliance on traditional monitoring methods.

The main purpose of this work is to perform optimization and field-validation of an existing freshwater finfish DNA metabarcoding method for use as a biosurveillance tool to aid in decision-making and management of Cowichan Lake Lamprey. For 2018, the goal is to create a reference library and refine sampling methodologies for Cowichan Lake Lamprey. Finclips collected in 2017 form the basis of the reference library. As Cowichan Lake Lamprey and Pacific Lamprey are closely genetically related, finclips from Pacific Lamprey from the Strait of Georgia were also collected to further refine the reference library. Field testing of the eDNA sampling methods is essential to verify that eDNA can readily be detected when the species are present. This work was undertaken in 2018 at five different sampling locations in Robertson River, throughout the trapping area; controls were taken on site at the beginning and end of sampling (Table 1).

Samples were taken on May 8, May 22, June 5 and June 19, 2018 before water levels became too low and the experiment was terminated. All samples were taken in accordance with methods developed by Dr. C. Abbott and her laboratory and based on Lacoursière-Roussel et al. (2018) and Majaneva et al. (2018). Results of the development of the reference library and sampling methodologies for Cowichan Lake Lamprey will not be presented here. Controls were taken before the first sample and after the last sample

Table 1. eDNA sampling locations in Robertson River, 2018.

Location	Latitude (ºN)	Longitude (ºW)
Upstream of trap line 8	48º 48′ 41.4″	124º 08′ 05.3″
Upstream of trap line 7	48º 48′ 49.4″	124º 08′ 03.3″
Between trap lines 5 and 4a	48º 48′ 53.6″	124º 07′ 57.0″
Above trap line 3a	48º 48′ 54.8″	124º 07′ 51.1″
In the lake, left facing downstream	48º 48′ 55.5″	124º 07′ 48.7″

RESULTS

TRAPPING

Due to low water, trap lines 2 and 4 had to be relocated twice and trap line 3 had to be relocated once. As they were reinstalled within approximately 3 m from the initial location, the total number of catch days is reported for the trap line as a whole not with each relocation. Traps were wet for a total of 523 days (Table 2).

Table 2. Summary of trap lines installed in Robertson River, 2018. Catch days= number of days traps were installed and functioned properly.

Trap Line	Date Installed (2018)	Date Removed (2018)	Total Number Catch Days	Latitude (ºN)	Longitude (ºW)
1	8 May	29 June	51	48º 48′ 55.3″	124º 07′ 48.1″
2a	20 April	7 June	69	48º 48′ 54.9″	124º 07′ 49.8″
2b	7 June	17 June		48º 48′ 54.9″	124º 07′ 50.5″
2c	17 June	29 June		48º 48′ 54.1″	124º 07′ 49.0″
3a	20 April	7 June	69	48º 48′ 54.0″	124º 07′ 50.7″
3b	7 June	29 June		48º 48′ 53.9″	124º 07′ 50.6″
4a	20 April	2 June	69	48º 48′ 54.1″	124º 07′ 55.1″
4b	2 June	17 June		48º 48′ 53.9″	124º 07′ 54.7″
4c	17 June	29 June		48º 48′ 54.3″	124º 07′ 54.3″
5	21 April	29 June	68	48º 48′ 53.2″	124º 07′ 58.7″
6	20 April	29 June	69	48º 48′ 52.6″	124º 08′ 00.4″
7	30 April	29 June	59	48º 48′ 50.1″	124º 08′ 03.3″
8	20 April	29 June	69	48º 48′ 41.6″	124º 08′ 05.2″
Total			**523**		

No Cowichan Lake Lamprey were caught throughout the experiment; 441 sculpin (sp.), 892 salmonids (sp.), 577 Three-spined Stickleback (*Gasterosteus aculeatus*), 22 Signal Crayfish (*Pacifastacus leniusculus*) and 28 Rough-skinned Newt (*Taricha granulosa*) were caught (Table 3).

Table 3. Catches from trap lines 1-8, Robertson River 2018.

Trap Line	Sculpin (sp.)	Salmonid (sp.)	Three-spined Stickleback	Signal Crayfish	Rough-skinned Newt
1	36	316	335	12	21
2a,b,c	44	94	215	1	6
3a,b	48	70	20	2	0
4a.b,c	59	165	6	2	0
5	69	73	1	1	1
6	47	34	0	1	0
7	73	60	0	2	0
8	65	80	0	1	0
Total	**441**	**892**	**577**	**22**	**28**

SNORKELING

A total of seven snorkel surveys were conducted in the alluvial fan along the transects identified in Figure 4. Surveys were conducted during the day on June 15, 17, 18, 21, 23, 25 and 27, 2018. No lamprey were observed. Visibility was 100% during each survey. Water levels were decreasing rapidly throughout the month making surveys difficult toward the end.

ENVIRONMENTAL OBSERVATIONS

Water depth measured at all trap lines decreased throughout the experiment, as is expected with little rainfall during the summer in this region (Figure 5). Trap lines 2,3 and 4 required relocation several times due to low water (Table 2). Comparisons between upstream and downstream reaches of Robertson River are not meaningful as trap locations were chosen based on appropriate substrate and water flow. Within the same general area water may be shallower or deeper due to sediment deposition and water flow. Near the end of the experiment, water depth and flow had become sufficiently low to create sandbars in the middle of Robertson River and isolate some trap lines entirely from river flow. By June 29th, the experiment was terminated because water depth and flow were insufficient to allow upstream movement of lamprey past trap line 3b (Figure 2).

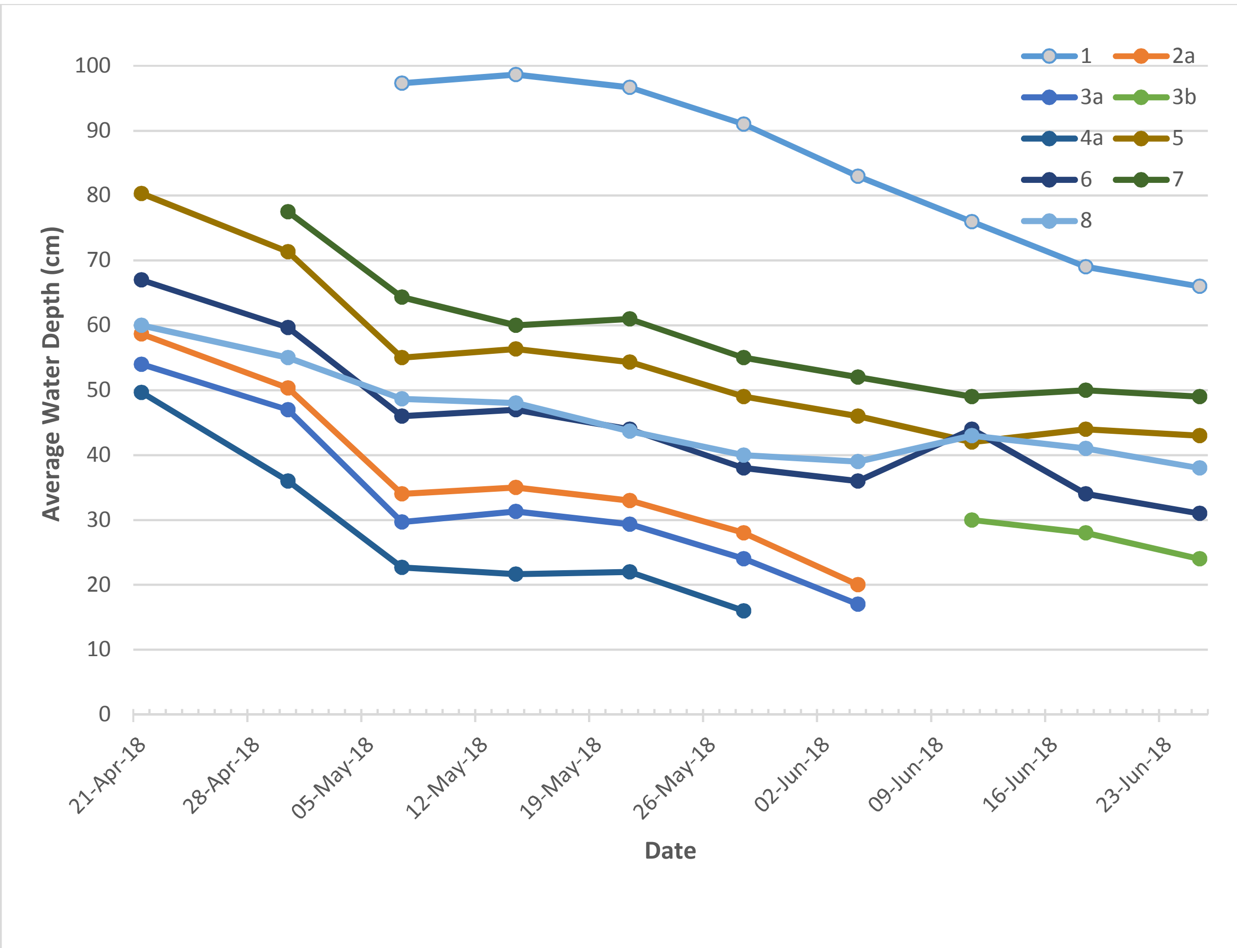

Figure 5. Average water depth from trap line (1-8) install to removal, Robertson River, 2018. Location of trap lines illustrated in Figure 2. Trap line locations where two or fewer depths were recorded were not included.

With a decrease in water depth over time, an increase in temperature was measured at all trap lines (Figure 6). Because some traps were moved due to low water it is difficult to compare temperature between trap lines. The lowest average daily water temperature recorded throughout the experiment was 5.18 °C (trap lines 3a and 5 on April 22, 2018); the highest average daily water temperature was 24.03 °C (trap line 1 on June 20, 2018) (Table 4).

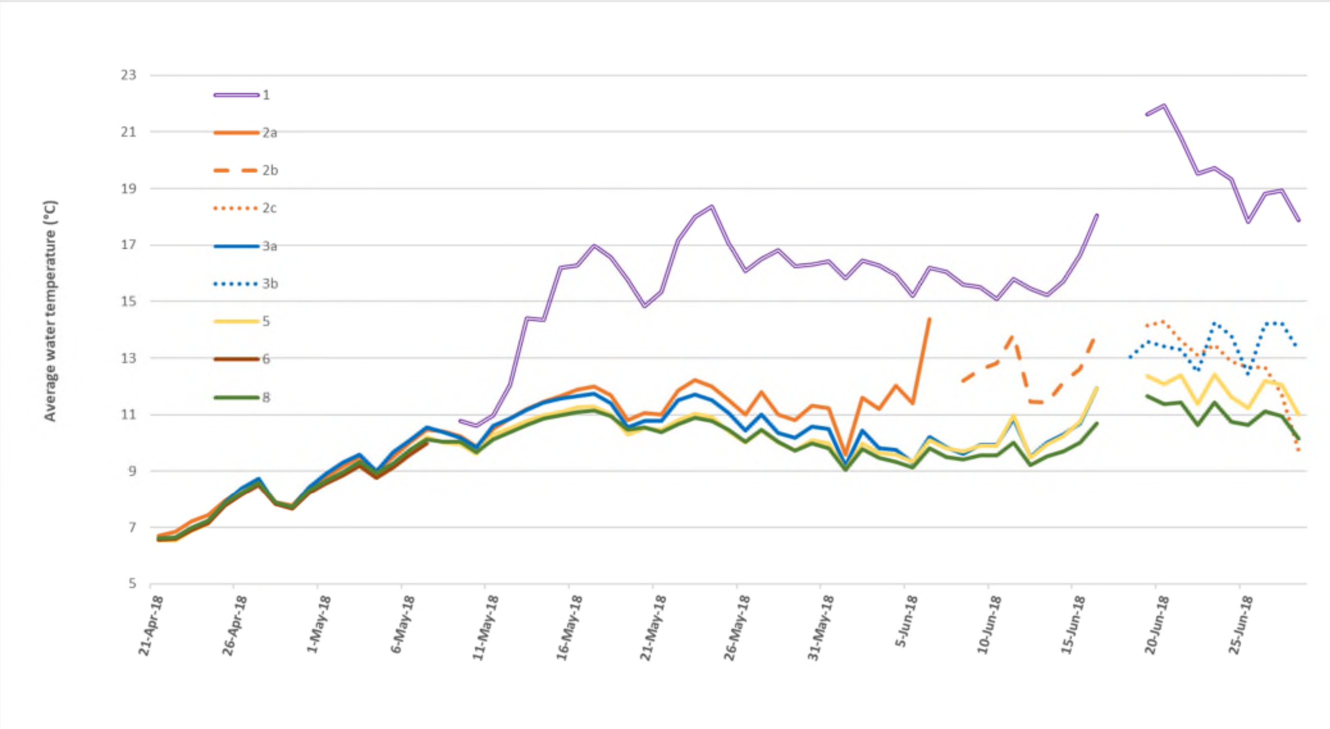

Figure 6. Average daily water temperature (^{0}C) at trap lines (20 April-28 June 2018) in Robertson River.

Table 4. Summary of TidbiT® derived temperature data from trap lines installed in Robertson River, 2018.

Trap Line	**Data Series (2018)**		**Max. Temp.**		**Min. Temp.**		**Average Mean Daily Temp. (ºC)**	**Mean Daily Temp.**		
	Start	**Stop**	**(ºC)**	**Date (2018)**	**(ºC)**	**Date (2018)**		**High (ºC)**	**Low (ºC)**	**SD**
1	9 May	28 June	24.03	20 June	8.74	11 May	16.43	21.93	10.60	2.36
2a	21 April	6 June	19.44	6 June	5.23	22 April	10.28	14.37	6.69	1.68
2b	8 June	16 June	20.01	11 June	10.00	13 June	12.55	13.91	11.42	0.89
2c	19 June	28 June	16.63	20 June	8.67	28 June	12.83	14.30	9.74	1.33
3a	21 April	16 June	15.32	23 May	5.18	22 April	9.94	11.95	6.56	1.33
3b	18 June	28 June	18.49	26 June	10.22	18 June	13.45	14.26	12.41	0.64
5	21 April	28 June	16.44	23 June	5.18	22 April	10.02	12.42	6.54	1.38
6	21 April	7 May	12.65	7 May	5.21	22 April	8.21	9.98	6.56	1.02
8	21 April	28 June	13.88	21 June	5.23	22 April	9.78	11.65	6.61	1.16

DISCUSSION

Although no lamprey were caught or were observed spawning and therefore we could not answer the questions we set out to, this study was none-the-less valuable in re-enforcing the importance of understanding the drivers for spawning and early rearing and ensuring habitat resilience long-term. This importance is illustrated by comparing a relatively successful year such as 2017 where 29 lamprey were caught and three spawning events were observed to 2018, where effort was increased substantially, and no lamprey were caught or observed spawning.

The features of alluvial fans such as substrate size, water depth and wetted area are going to be affected by stochastic events including snow melt, annual rainfall amounts and rainfall events. This is demonstrated in the differences in the alluvial habitat observed at the same times in 2017 and 2018 (Figure and Figure respectively). Figure (June 25, 2017) shows that the entire alluvial fan is wetted as compared to Figure (June 27, 2018) where large sandbars were created at the mouth of the river. Other visible changes include the movement of the cluster of large woody debris (A) from near the sandbar in 2017 to mid-stream in 2018.

Figure 7. Aerial photograph of the alluvial fan for Robertson River, June 25, 2017.

Figure 8. Aerial photograph of the alluvial fan for Robertson River, June 27, 2018.

What cannot be depicted in these figures is water depth. However, because of the success in catching lamprey in 2017, some traps were placed in similar locations and these can be compared. A trap line was placed at the tip of the sandbar in 2017 and 2018 (B in Figure and Figure respectively) and mid-stream outflow (C in Figure and Figure respectively). The average water depth at location B was 77 cm on June 30, 2017 (Wade et al., 2018), at location B on June 25, 2018 it was 66 cm. Average water depth at location C on June 30, 2017 was 56 cm (Wade et al., 2018) and 24 cm on June 25, 2018.

In general, the overall depth of the alluvial fan at the end of June 2018 was less compared to the same time in 2017. This observation is supported by water depth measurements at nests reported in 2017 (Wade et al. 2018) compared with the average water depth at trap lines during similar time periods in 2018. Four spawning lamprey were observed at the end of the alluvial fan on June 27th, 2017 in water 65 cm deep (Wade et al., 2018). During snorkel surveys at the end of June 2018 water depth was a maximum of approximately 55 cm. On June 23rd, 2017, two lamprey were observed spawning upstream of the large woody debris (A in Figure) near the sandbar, in water 42 cm deep (Wade et al., 2018); the same location on June 27th, 2018 (Figure) was superficially wetted. The third spawning site in 2017 was adjacent to the trap line indicated by C (Figure); depth of water at the spawning site was 58 cm on June 29th, 2017. Average water depth at approximately the same location on June 25th, 2018 was 24 cm. By the end of June, 2018 much of the area between the various clumps of woody debris (Figure and, transects 4-5, 6-7 and 7-8 in Figure 4) were too shallow to snorkel and had to be walked. These same areas were not measured in 2017, however, anecdotally, this area was not at risk of drying up by the end of June 2017. The freshwater discharge from the river was determined to be substantially less by the end of June 2018 as compared to the same time in 2017 as almost all flow from the river was subsurface; by the end of June 2017, the water continued to flow freely from the river. The effect on habitat coverage is depicted in Figure and Figure .

Such differences between years were not found when comparing average daily water temperatures. The same TidbiT® data logger as used in 2018 recorded temperatures at the lake-most trap line (B in Figure). Between June 21 and June 29, 2017, the average daily temperature at this trap line was 18.2 °C (Wade, 2017). The average daily water temperature between June 21 and June 28, 2018 at the same trap line (B in Figure) was 19.1°C. Unfortunately, in 2017 there was only one TidbiT® deployed so any conclusions as to the role of temperature as a cue to spawn or the potential combination of depth, water flow and temperature as a cue to spawn cannot be determined at this time.

It is likely that several factors contributed to the differences in habitat between the end of June 2017 and the same time in 2018 at the mouth of Robertson River. It is probable that winter and spring weather events over the past year were sufficiently forceful to both change the extent of the alluvial fan (wider) and deposit more sediment. This is reasonable given the movement of the large woody debris as depicted in Figure and Figure . Water depth in the alluvial fan was less in 2018 than 2017. This could be attributed to an overall decrease in water levels in Bear and Cowichan lakes and/or an increase in sediment deposition in the alluvial fan (i.e. the substrate was closer to the surface of the water because there was more sediment).

Comparing the catch rates and habitat features of the same spawning area in two consecutive years illustrates the importance of both understanding what physical conditions are necessary for spawning as well as recognizing that in a stochastic environment, it is necessary to protect all potential spawning and rearing habitat, not just that in which spawning has been observed as habitat conditions may change from year to year.

REFERENCES

Beamish, R. J., and Wade, J. 2008. Critical habitat and the conservation ecology of the freshwater parasitic lamprey, *Lampetra macrostoma*. Can. Field Nat. 122(4): 327-337.

Beamish, R. J. 1982. *Lampetra macrostoma*, a new species of freshwater parasitic lamprey from the west coast of Canada. Can. J. Fish. Aquat. Sci. 37: 736-747.

DFO. 2007. Recovery Strategy for the Vancouver Lamprey (*Lampetra macrostoma*) in Canada. Species at Risk Act Recovery Strategy Series, Fisheries and Oceans Canada, Ottawa, ix + 21 pp.

Lacoursière-Roussel, A., Howland, K., Normandeau, E., Grey, E.K., Archambault, P., Deiner, K., Lodge, D.M., Hernandez, C., Leduc, N., and Bernatchez, L. 2018. eDNA metabarcoding as a new surveillance approach for coastal Arctic biodiversity. Ecol. Evol. 1-15. DOI: 10.1002/ece3.4213.

MacConnachie, S.E.M., and Wade, J. 2016. Information in support of the identification of critical habitat for the Cowichan (Vancouver) Lamprey (*Entosphenus macrostomus*). DFO Can. Sci. Adv. Secretariat Res. Doc. 2016/109. vi + 17p.

Majaneva, M., Diserud, O.H., Eagle, S.H.C., Bostrom, E., Hajibabaei, M., and Ekrem, T. 2018. Environmental DNA filtration techniques affect recovered biodiversity. Sci. Rep. DOI:10.1038/s41598-018-23052-8.

Wade, J. 2017. Field Report: Trapping of Cowichan Lamprey (*Entosphenus macrostomus*) in Cowichan and Bear Lakes 2017. Prepared for the British Columbia Conservation Foundation 22p.

Wade, J., Dealy, L., and MacConnachie, S. 2018. First record of nest building, spawning and sexual dimorphism in the threatened Cowichan Lake lamprey *Entosphenus macrostomus*. End. Sp. Res. 35:39-45.

Wade, J., and MacConnachie, S. 2016. Cowichan Lake Lamprey (*Entosphenus macrostomus)* ammocoete habitat survey. 2012. Can. Manuscr. Rep. Fish. Aquat. Sci. 3088: iv + 15p.