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Water Monitoring for Neonicotinoid Pesticides in Lower Mainland and Okanagan Streams

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EXECUTIVE SUMMARY

Water quality monitoring was conducted by the British Columbia Government to contribute to the (pan-Canadian) Environmental Monitoring Working Group (EMWG) investigation into the presence of neonicotinoid (neonic) insecticides in streams across Canada. The sampling occurred bi-weekly during the 2017 and 2018 growing seasons in agricultural areas in the Lower Mainland and the Okanagan Valley.

Findings of the water quality data analyses:

Lower Mainland

- The highest maximum concentration of any neonicotinoid parameter measured in Lower Mainland sites was imidacloprid (9.6 x the *acute* Pest Management Regulatory Agency [PMRA] endpoint), followed by thiamethoxam (7.2 x the chronic PMRA endpoint), and clothianidin (4.2 x the chronic PMRA endpoint).
- The greatest mean concentration measured was imidacloprid (4.6 x the chronic PMRA endpoint) at the upstream Nicomekl River site, followed by clothianidin (below PMRA endpoints), and then thiamethoxam (below PMRA endpoints).
- The highest maximum and mean concentrations of imidacloprid and thiamethoxam were measured at the Nicomekl upstream site in 2017. The highest maximum concentration of clothianidin was measured at the Nicomekl downstream site in 2017.
- Concentrations of all neonics measured increased from 2017 to 2018 in the Sumas Lake Canal and the Cohilukthan Slough. By contrast, the concentrations of neonics in the Nicomekl River decreased from 2017 to 2018.
- *Potential Risk to Aquatic Invertebrates:*
    - The duration and concentration of neonic exposure (over multiple bi-weekly sampling events), combined with the cumulative nature of toxic effects, indicates that potential impacts to aquatic macroinvertebrates communities are likely in the Nicomekl River.
    - A higher percentage of non-detects and lower concentrations observed in the Sumas Canal and Cohilukthan Slough suggests a lower risk of adverse effects on aquatic organisms in the sampled areas.
- Examination of Nicomekl tributaries (located further upstream than the original upstream sampling site) confirmed the presence of all three neonics at higher maximum concentrations and greater within-site variability upstream of the original Nicomekl River sites. The highly elevated and pulsing nature of the releases suggests that the source may be effluent from a greenhouse or similar operation rather than drift from agricultural fields. Based on the magnitude of the acute endpoint exceedances measured in each of the sample sites across the three tributaries, the potential for impacts on aquatic insects is likely in these areas.

Okanagan

- For the primary neonics tested (thiamethoxam, clothianidin, and imidacloprid) in the Okanagan sites, concentrations above the reported detection limit (RDL) were detected in 2% of the samples analyzed in 2017. In 2018, all the samples measured were below the RDL. No PMRA endpoints were exceeded in the samples analyzed.
- *Potential Risk to Aquatic Invertebrates:*

- Based on the data reviewed, the high percentage of non-detects and the low concentrations observed (within 0.003 µg/L of the RDL) indicates a low risk of adverse effects on aquatic organisms in sampled areas.

- The supplementary benthic invertebrate analysis conducted (Appendix E) in Trout, Naramata, and Mission creeks supported the conclusion above as impacts specific to neonics were not identified. However, the study design and methods used limited the findings of the benthic invertebrate assessment.

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1. INTRODUCTION

In 2016, Health Canada's Pest Management Regulatory Agency (PMRA) released a Proposed Re-evaluation Decision (PRVD 2016-20) that proposed phasing out the use of imidacloprid, a neonicotinoid insecticide (neonic), for agricultural and outdoor use by 2019 to 2021. The PVRD simultaneously announced a review of the potential risks of two other neonics, thiamethoxam and clothianidin, on aquatic invertebrates (EMWG 2017).

In response to the PRVDs, the Environmental Monitoring Working Group (EWMG) was formed and tasked with developing and implementing an environmental monitoring strategy to investigate the presence of neonics in streams and to assess the associated impacts on aquatic invertebrates. As part of the EMWG, the B.C. Ministry of Agriculture (AGRI) and the Ministry of Environment and Climate Change Strategy (ENV) collaboratively conducted water quality sampling in British Columbia's Lower Mainland and Okanagan Valley, two regions with intensive agriculture.

Neonics have an unusual and highly varied effect on non-target aquatic organisms (Raby et al. 2018). Neonics were developed to inhibit the central nervous system of insects while having a very low toxicity to vertebrates and higher order animals (Jeschke et al. 2010; Tomizawa and Casida 2003). Toxicity tests with standard aquatic species, including *Daphnia magna*, rainbow trout (*Oncorhynchus mykiss*) and zebrafish (*Danio rerio*) demonstrate low acute toxicity (high LC50s), whereas neonics have been found to be highly toxic to aquatic macroinvertebrates, particularly insects (Raby et al. 2018). Toxicity studies on invertebrates have shown the binding of neonics to receptors (the physiological mechanism of toxicity) to be long-lasting, nearly irreversible, and cumulative over multiple exposure events, often prolonging exposure and causing delayed lethal effects (Morrissey et al. 2015; Sanchez-Bayo et al. 2016). For sensitive species (such as Ephemeroptera, Trichoptera, and Coleoptera taxa), short-term lethal effects have been observed at concentrations <1µg/L in water (Morrissey et al. 2015; Raby et al. 2018).

To assess the drift of neonics into the aquatic environment, water sampling was conducted upstream (for reference) and downstream from crops onto which neonics are generally applied. To supplement the water quality data, a benthic invertebrate community analysis was also conducted in the Okanagan to provide an additional line of evidence. The fine substrates in the Lower Mainland streams were not appropriate for invertebrate sampling using ENV's preferred methods. The following review provides a summary of the methods, and results, and provides an assessment of the potential risk to aquatic invertebrates in these freshwater systems.

2. METHODS

2.1 Lower Mainland Sites

Water samples were collected in three watersheds across the Lower Mainland in 2017 and 2018. Sumas Lake Canal, Nicomekl River, Cohilukthan Slough, and a reference site (in a non-agricultural area) were sampled bi-weekly between June 7th and September 11th in 2017 and May 8th to September 26th in 2018. An overview map of the sampling areas is provided in Appendix A, Figure A1. In Sumas Lake Canal and Nicomekl River, an upstream and downstream site were selected to isolate areas of neonic application (Sapsford et al. 2017). The sampling at Cohilukthan Slough was only conducted in a downstream location as there was no suitable upstream/control site.

In 2018, additional samples were collected in various tributaries of the Nicomekl River (upstream of the previous sampling sites). There were three main areas/tributaries sampled. The frequency and location of these samples varied due to conditions (i.e. some tributaries dried up in the summer months).

Overall, there were two sites in Tributary 1, two sites in Tributary 2, and three sites in Tributary 3 that were sampled at varying frequencies from May to September.

Okanagan Sites

Water samples were collected in five rivers/creeks in the Okanagan Valley in 2017 and 2018. The Okanagan River and Mission, Trout, Naramata, and Middle Vernon creeks were sampled bi-weekly from June 8th to September 12th in 2017, and from June 4th to August 30th in 2018. Each creek/river was sampled at two locations, one upstream and one downstream of agriculture, to isolate areas of neonic application. An overview map of the sampling areas is provided in Appendix A, Figure A2.

2.2 Water Sample Collection

The sampling was conducted by Triton Environmental in 2017 and by AGRI and ENV staff in 2018. The method of sample collection varied between sites. Grab samples were collected with gloved hands or using a bridge or pole sampler, depending on access to the water. Field data including temperature, pH, and specific conductivity were collected simultaneously using a YSI 600 XL meter.

In 2017 and 2018, water samples were shipped to ALS Labs in Burnaby, B.C. and analyzed according to the *Protocol for Surface Water Monitoring for Neonicotinoids* distributed by the EMWG (2016). The samples were tested for the presence of thiamethoxam, clothianidin, and imidacloprid in 2017. In 2018, additional neonics were analysed including acetamiprid, nitenpyram, and thiacloprid, as well as an emerging substitute for neonics, sulfoxaflor (in the sulfoximine class of insecticides). ALS has a reported detection limit (RDL) of 0.005 µg/L for neonics (and sulfoxaflor), except for thiamethoxam, which has a RDL of 0.004 µg/L.

To provide quality control for lab analyses, one duplicate sample was taken on each sampling date. In addition, one field blank on each sampling day was exposed to the ambient air conditions and included in the analysis to test for contamination due to site conditions or travel.

2.3 Analysis

2.3.1 Summary Statistics

Summary statistics including minimum, maximum and mean concentrations were calculated for each neonic parameter and site across the Lower Mainland and the Okanagan, where applicable. There was a high percentage of censored data (values below the reported detection limit). Mean concentrations were not calculated for sites with greater than 50% censored data as per the ENV guidance document (Huston & Juarez-Colunga 2009). For sites and parameters with less than 50% censored data, means were calculated using the Kaplan-Meier method (Helsel 2011). The Kaplan-Meier method is one of the non-parametric methods recommended for data sets with smaller sample sizes (n < 50 observations). The parameter means for each site were calculated using the NADA package (Lee 2017) in R Studio (version 3.5.3).

2.3.2 Comparison to PMRA Endpoints

In accordance with the EMWG, the concentrations of neonics were compared against the PMRA-assigned chronic and acute endpoints. PMRA endpoints exist for imidacloprid, clothianidin, and thiamethoxam (Table 1). To date, endpoints for acetamiprid, nitenpyram, thiacloprid, and sulfoxaflor have not been set. For comparison, the Canadian Council of Ministers of the Environment (CCME) has established an interim chronic guideline for aquatic life for imidacloprid of 0.23 µg/L. CCME guidelines have not been developed for thiamethoxam or clothianidin.

Table 1: PMRA-assigned chronic and acute endpoints for neonic concentrations in freshwater.

	Imidacloprid (µg/L)	Clothianidin (µg/L)	Thiamethoxam (µg/L)
Chronic Endpoint	0.041	0.020	0.026
Acute Endpoint	0.360	1.50	9.0

2.4 Benthic Invertebrate Sampling

To provide additional insight into potential effects on aquatic life, benthic invertebrate communities were sampled at three of the sites in the Okanagan Valley. Additional information on sample collection, analysis, and findings is provided separately in Appendix E.

3. RESULTS & DISCUSSION

3.1 Lower Mainland Results

The findings for imidacloprid, clothianidin, and thiamethoxam are further discussed below (full results are included in Appendix B in Table B1 and B2). Of the four insecticides added to the analysis in 2018 - acetamiprid, nitenpyram, thiacloprid, and sulfoxaflor – only acetamiprid was detected (twice) and at low concentrations of 0.008 and 0.0066 µg/L. As the supplementary insecticides included in the 2018 analysis were largely undetected (>99%), they are not discussed further in this report.

Imidacloprid

The concentration of imidacloprid ranged across the three sampled rivers in the Lower Mainland. The highest maximum and mean (±SD) concentrations were measured in the upstream site of the Nicomekl River in 2017 at 0.74 µg/L and 0.19 µg/L (± 0.23), respectively (Table 2). By comparison, a maximum concentration of 0.21 µg/L and a mean of 0.079 µg/L (± 0.068) were measured at the downstream Nicomekl River site in 2017. At the Sumas Lake Canal sites in 2017, concentrations above the RDL were not detected at the upstream sample site, while the Sumas downstream site had a maximum concentration of 0.013 ug/L (Table 2). At the Cohilukthan site, only one of eight samples analyzed in 2017 was above the reported detection limit (RDL), with a concentration of 0.0085 µg/L.

In 2018, the Nicomekl upstream site continued to have the highest concentrations of imidacloprid of all sites, but both the maximum and mean were lower than 2017 values. For every other site assessed, the maximum and mean concentrations (where applicable) were higher in 2018 than in 2017. The site with the highest percent increase in maximum imidacloprid concentration was the Nicomekl River downstream site (90.48% increase from 2017) (Table 3).

Table 2: Summary of the imidacloprid concentrations measured across the Lower Mainland sampling sites.

Stream	Sampling Position	Sample size (n)	Detections above RDL	Percentage of Censored Data	Minimum (µg/L)	Maximum (µg/L)	Mean* (µg/L)	Standard Deviation (µg/L)
2017								
Cohilukthan	Downstream	8	1	*87.5*	<RDL	0.0085	--	--
Nicomekl	Upstream	8	8	0	0.034	0.74	0.19	0.23
Nicomekl	Downstream	8	8	0	0.025	0.21	0.079	0.068
Sumas	Upstream	8	0	*100*	<RDL	<RDL	--	--
Sumas	Downstream	8	3	*62.5*	<RDL	0.013	--	--
Total		40	20	50	--			
Reference		8	0	*100*	<RDL	<RDL	--	--
2018								
Cohilukthan	Downstream	10	3	*70*	<RDL	0.012	--	--
Nicomekl	Upstream	10	10	0	0.014	0.57	0.11	0.18
Nicomekl	Downstream	10	10	0	0.0086	0.40	0.1	0.13
Sumas	Upstream	10	1	*90*	<RDL	0.0057	--	--
Sumas	Downstream	10	4	*60*	<RDL	0.023	--	--
Total		50	28	44	--			
Reference		10	0	*100*	<RDL	<RDL	--	--

Notes: * Means were calculated using Kaplan-Meier methods to account for uncensored data. Means were only calculated when the percentage of uncensored data was under 50%.

- Indicates N/A.

Table 3: Percent change in maximum and mean imidacloprid concentrations from 2017 to 2018.

Stream	Sampling Position	Percent change in maximum imidacloprid concentration from 2017 to 2018 (%)	Percent change in mean imidacloprid concentration from 2017 to 2018 (%)
Cohilukthan	Downstream	+ 41.2	--
Nicomekl	Upstream	- 23.0	- 10.8
Nicomekl	Downstream	+ 90.5	+ 10
Sumas	Upstream	--	--
Sumas	Downstream	+ 76.9	--

Notes: * Percent change was only calculated if values for 2017 and 2018 existed for each site and parameter.

\- Indicates N/A

Clothianidin

The highest maximum and mean concentrations of clothianidin were observed at the Nicomekl River downstream site at 0.16 µg/L and 0.028 µg/L, respectively, in 2017 (Table 4). Clothianidin was observed at the Sumas Lake Canal downstream site in 2017 and 2018 with a maximum concentration of 0.085 µg/L in 2018. The presence of clothianidin was also detected at Cohilukthan Slough in 2018, but was not detected in 2017. The presence of clothianidin at concentrations above the RDL was only measured at the three aforementioned sites, in five samples of 40 in 2017 and in eight of 50 samples in 2018 (Table 4).

Where clothianidin concentrations were above the RDL, the concentrations increased in 2018 (compared to 2017) in the Cohilukthan Slough and the Sumas Lake Canal downstream sites. The greatest change in the maximum concentration observed was an 877% increase at the Sumas Lake Canal downstream site (Table 5). In contrast, concentrations of clothianidin decreased in 2018 at the Nicomekl River downstream site. There was an 80.63% decrease in the maximum concentration observed in 2018. Note: calculating the percent change for many sites was not possible due to the high percentage of censored data.

Table 4: Summary of the clothianidin concentrations measured across the Lower Mainland sampling sites.

Stream	Sampling Position	Sample size (n)	Detections above RDL	Percentage of Censored Data	Minimum (µg/L)	Maximum (µg/L)	Mean* (µg/L)	Standard Deviation (µg/L)
				2017				
Cohilukthan	Downstream	8	0	*100*	<RDL	<RDL	--	--
Nicomekl	Upstream	8	0	*100*	<RDL	<RDL	--	--
Nicomekl	Downstream	8	4	50	<RDL	0.16	0.028	0.059
Sumas	Upstream	8	0	*100*	<RDL	<RDL	--	--
Sumas	Downstream	8	1	*87.5*	<RDL	0.0087	--	--
Total		40	5	87.5	--			
Control		8	0	*100*	<RDL	<RDL	--	--
				2018				
Cohilukthan	Downstream	10	1	*90*	<RDL	0.044	--	--
Nicomekl	Upstream	10	0	*100*	<RDL	<RDL	--	--
Nicomekl	Downstream	10	4	*60*	<RDL	0.031	--	--
Sumas	Upstream	10	0	*100*	<RDL	<RDL	--	--
Sumas	Downstream	10	3	*70*	<RDL	0.085	--	--
Total		50	8	84	--			
Control		10	0	*100*	<RDL	<RDL	--	--

Notes: * Means were calculated using Kaplan-Meier methods to account for uncensored data. Means were only calculated when the percentage of uncensored data was under 50%.

\- Indicates N/A.

Table 5: Percent change in maximum and mean clothianidin concentrations from 2017 to 2018.

Stream	Sampling Position	Percent change in maximum clothianidin concentration from 2017 to 2018 (%) *	Percent change in mean clothianidin concentration from 2017 to 2018 (%)*
Cohilukthan	Downstream	--	--
Nicomekl	Upstream	--	--
Nicomekl	Downstream	- 80.6	--
Sumas	Upstream	--	--
Sumas	Downstream	+ 877	--

Notes: * Percent change was only calculated if values for 2017 and 2018 existed for each site and parameter.
- Indicates N/A.

Thiamethoxam

The highest maximum concentration of thiamethoxam at 0.19 µg/L was observed at the Nicomekl River upstream site in 2017. The highest mean concentration measured was 0.0078 µg/L (± 0.0053) for five samples taken in the Sumas Lake Canal upstream site in 2018. However, as seen in Table 6, due to the low number of detections (two of 40 samples in 2017 and 13 of 50 in 2018), many averages were not calculated.

Thiamethoxam was detected more frequently and at more sample sites in 2018, compared to 2017. Thiamethoxam was not detected above the 0.004 ug/L RDL in the Sumas Lake Canal sites in 2017, but was measured at mean concentrations of 0.0078 ug/L and 0.0053 ug/L in 2018. In contrast, at the Nicomekl River sites, the mean concentration of thiamethoxam decreased in 2018 by 96.6% and 57.7% at the upstream and downstream sites, respectively (Table 7)

Table 6: Summary of the thiamethoxam concentrations measured across the Lower Mainland sampling sites.

Stream	Sampling Position	Sample size (n)	Detections above RDL	Percentage of Censored Data	Minimum (µg/L)	Maximum (µg/L)	Mean* (µg/L)	Standard Deviation (µg/L)
2017								
Cohilukthan	Downstream	8	0	*100*	<RDL	<RDL	--	--
Nicomekl	Upstream	8	1	*87.5*	<RDL	0.19	--	--
Nicomekl	Downstream	8	1	*87.5*	<RDL	0.0097	--	--
Sumas	Upstream	8	0	*100*	<RDL	<RDL	--	--
Sumas	Downstream	8	0	*100*	<RDL	<RDL	--	--
Total		40	2	*95*	--	--	--	--
Control		8	0	100	--	--	--	--
2018								
Cohilukthan	Downstream	10	0	100	<RDL	<RDL	--	--
Nicomekl	Upstream	10	3	*70*	<RDL	0.0064	--	--
Nicomekl	Downstream	10	1	90	<RDL	0.0041	--	--
Sumas	Upstream	10	5	50	<RDL	0.019	0.0078	0.0053
Sumas	Downstream	10	4	*60*	<RDL	0.014	--	--
Total		50	13	74	--	--	--	--
Control		10	0	100	--	--	--	--

Notes: * Means were calculated using Kaplan-Meier methods to account for uncensored data. Means were only calculated when the percentage of uncensored data was under 50%.

- Indicates N/A.

Table 7 : Percent change in maximum and mean thiamethoxam concentrations from 2017 to 2018.

Stream	Sampling Position	Percent change in maximum thiamethoxam concentration from 2017 to 2018*	Percent change in mean thiamethoxam concentration from 2017 to 2018**
Cohilukthan	Downstream	--	--
Nicomekl	Upstream	- 96.58	--
Nicomekl	Downstream	- 57.73	--
Sumas	Upstream	--	--
Sumas	Downstream	--	--

Notes: * Percent change was only calculated if values for 2017 and 2018 existed for each site and parameter.

- Indicates N/A

3.1.1 PMRA Endpoint Exceedances

By location: In 2017, the only PMRA endpoint exceedances observed were in the Nicomekl River at the upstream and the downstream sample sites (Table 8). A similar frequency of exceedances was observed in the Nicomekl River in 2018 at upstream and downstream sites (Table 9). In 2018, there were additional endpoint exceedances at the Cohilukthan Slough (clothianidin) and at the Sumas Lake Canal (clothianidin at the downstream site) (Table 9). The magnitude and duration of exceedances are provided in Table 8 and 9.

By type: Of the neonics analyzed, the greatest number of endpoint exceedances were imidacloprid (77%), followed by clothianidin (19%), and thiamethoxam (4%) (Table 8/9). There were three exceedances of the acute endpoint (0.36 µg/L) for imidacloprid and 17 exceedances of the chronic endpoints (0.041 µg/L) (Figure 1).

By date: The timing of peak neonic concentrations generally occurred during May/June, followed by another peak of lower magnitude in September/October. For imidacloprid, the highest concentrations detected were on the first sampling dates of 2017 and 2018 (Figure 1). It is not known whether imidacloprid was present in these systems prior to the first sampling event.

Table 8: Comparison of 2017 neonic data from the Lower Mainland to chronic and acute PMRA endpoints.

Stream	**Position**	**Neonic Parameter of Concern**	**Comparison to Chronic Endpoint**				**Comparison to Acute Endpoint**			
			Frequency of Exceedances (# detected / # sampling events)	**Magnitude of Exceedances** (Range of Exceedances of Chronic Endpoint [CE])	**Longest Duration of Exceedance** (# of consecutive events the endpoint was exceeded)	**Does the site mean exceed the chronic endpoint?**	**Frequency of Exceedances** (# detected / # sampling events)	**Magnitude of Exceedances** (Range of Exceedance of Acute Endpoint [AE])	**Longest Duration of Exceedance** (# of consecutive events the endpoint was exceeded)	**Does the site mean exceed the acute endpoint?**
Cohilukthan	Downstream only	No exceedances measured.								
Nicomekl	Upstream	Imidacloprid	5 / 8	1.1 – 4.98 x CE	3 events – August	Yes (4.6 x greater)	1 / 8	2.06 x AE	1 event – June	No
		Thiamethoxam	1 / 8	7.19 x CE	1 event - September	N/A	No acute exceedances measured.			
	Downstream	Imidacloprid	5 / 8	1.07 – 5.2 x CE	3 events – June	Yes (1.9 x greater)	No acute exceedances measured.			
Sumas	Upstream	No exceedances measured.								
	Downstream									

Table 9: Comparison of 2018 neonic data from the Lower Mainland to chronic and acute PMRA endpoints.

Stream	Position	Neonic Parameter of Concern	Comparison to Chronic Endpoint				Comparison to Acute Endpoint			
			Frequency of Exceedances (# detected / # sampling events)	**Magnitude of Exceedances** (Min – Max Exceedance of Chronic Endpoint)	**Longest Duration of Exceedance** (# of consecutive events the endpoint was exceeded)	**Does the site mean exceed the endpoint?**	**Frequency of Exceedances** (# detected / # sampling events)	**Magnitude of Exceedances** (Min – Max Exceedance of Acute Endpoint)	**Longest Duration of Exceedance** (# of consecutive events the endpoint was exceeded)	**Does the site mean exceed the endpoint?**
Cohilukthan	Downstream only	Clothianidin	1 / 10	2.21	1 week – September	N/A	No acute exceedances measured.			
Nicomekl	Upstream	Imidacloprid	3 / 10	1.68 – 6.05	2 events – September	Yes (2.7 x greater)	1 / 10	1.59	1 event – May	
	Downstream	Clothianidin	1 / 10	1.56	1 event – June	N/A	No acute exceedances measured.			
		Imidacloprid	4 / 10	1.04 – 6.9	3 events - May/June (includes acute exceedance)	Yes (2.4 x greater)	1 / 10	9.63	1 event – May (adjacent to chronic exceedances)	
Sumas	Upstream	No exceedances measured.								
	Downstream	Clothianidin	1 / 10	4.24	1 event – June	N/A	No acute exceedances measured.			

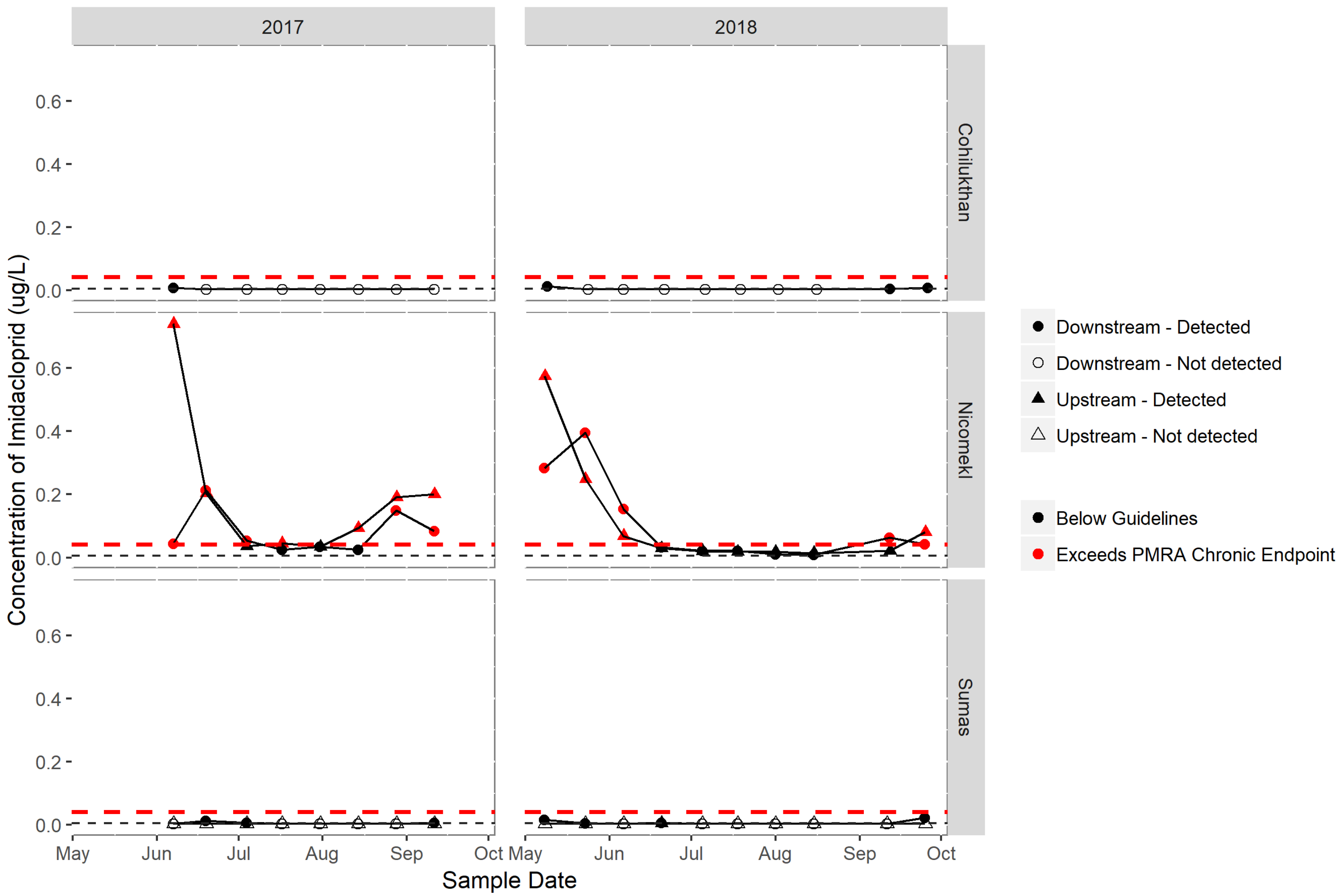

Figure 1: The concentration of imidacloprid detected at each sampling event at Cohilukthan, Nicomekl, and Sumas sites (from top to bottom) in 2017 and 2018 (left to right). The red dashed line represents the PMRA chronic endpoint and the black dashed line is the reported detection limit (RDL). Similar figures for clothianidin and thiamethoxam concentrations in each river are provided in Appendix C.

3.1.2 Summary of Lower Mainland Findings:

- The highest maximum concentration of any neonic parameter measured was imidacloprid followed by thiamethoxam and clothianidin. Imidacloprid, then clothianidin, and thiamethoxam had the greatest mean concentrations, respectively.
- The highest maximum and mean concentrations of imidacloprid and thiamethoxam were measured at the Nicomekl River upstream site in 2017. The highest maximum concentration of clothianidin was measured at the Nicomekl downstream site in 2017.
- Concentrations of all neonics increased in 2018 (compared to 2017) in the Sumas Lake Canal and Cohilukthan Slough sites. By contrast, the concentrations in the Nicomekl River sites decreased from 2017 to 2018 for all neonic parameters.

3.1.3 Examination of Nicomekl Tributaries

In response to the high concentrations of neonics observed at the upstream sampling site of the Nicomekl River (originally intended to be a no-neonic control site) in 2017, additional sampling was conducted in the upstream tributaries in 2018. The objective was to pinpoint the source tributary of the neonics and to find a more appropriate control site.

The analysis showed concentrations of neonics above PMRA endpoints in all tributary sites (Table 10, Figure 2; Appendix B, Table B3). Of the samples collected in the various tributaries, 37 of 42 results exceeded the chronic endpoints for imidacloprid and 10 of 42 exceeded the acute endpoints for imidacloprid. The maximum concentration of imidacloprid, found in Tributary 2, was 3.4 µg/L (9.4 x greater than the acute endpoint). Thiamethoxam concentrations exceeded chronic endpoints in 11 of 42 samples, at sampling sites 2 (6), 3 (1), and 3C (4). Clothianidin concentrations exceeded chronic endpoints in one of 42 samples (site 2). High concentrations (at least one result over the acute endpoint) of imidacloprid were observed at each sampling site.

The highest concentration of imidacloprid, measured in Tributary 2, exceeded the chronic endpoint by 82.9 x (Table 10, Figure 2). It is possible that each tributary contributes to elevated downstream concentrations of imidacloprid observed in the Nicomekl River. Interestingly, the timing of peak concentrations differed between sampling sites. The underlying cause of the differences in timing is not known but may reflect point source releases such as greenhouse effluent entering the waterways.

Table 10: Summary of the neonic endpoint exceedances observed at each Nicomekl tributary. A complete list of detections is provided in Appendix B, Table B3.

Tributary	Sampling Site	Neonic Parameter of Concern	Magnitude of Exceedances (Min – Max Exceedance of Chronic Endpoint)	Frequency of Exceedances (# detected / # sampling events, Endpoint)	Potential Sources of Insecticide*
1	1	Imidacloprid	1.7 – 22	3 / 5 Chronic 1 / 5 Acute	Agriculture: cranberries, blueberries Greenhouses, nurseries
	1A	Imidacloprid	1.1 – 14.8	4 / 5 Chronic 1 / 5 Acute	
2	2	Imidacloprid	1.3 – 82.9	10 / 11 Chronic 2 / 11 Acute	Agriculture: blueberries Greenhouses, nurseries
		Thiamethoxam	1.1 – 1.3	6 / 11 Chronic 0 / 11 Acute	
		Clothianidin	1.7	1 / 11 Chronic 0 / 11 Acute	
	2A	Imidacloprid	36	1 / 1 Chronic 1 / 1 Acute	Agriculture: blueberries Greenhouses, nurseries
3	3	Imidacloprid	1.4 – 42.9	11 / 12 Chronic 2 / 12 Acute	
		Thiamethoxam	1.0	1 / 12 Chronic 0 / 12 Acute	
	3C	Imidacloprid	1.28 – 35	4 / 4 Chronic 1 / 4 Acute	Stagnant water
		Thiamethoxam	3.5 – 5.5	4 / 4 Chronic 0 / 4 Acute	
	3D	Imidacloprid	5.7 – 69.5	4 / 4 Chronic 2 / 4 Acute	Greenhouses, nurseries

Note: * - as per Sapsford 2018.

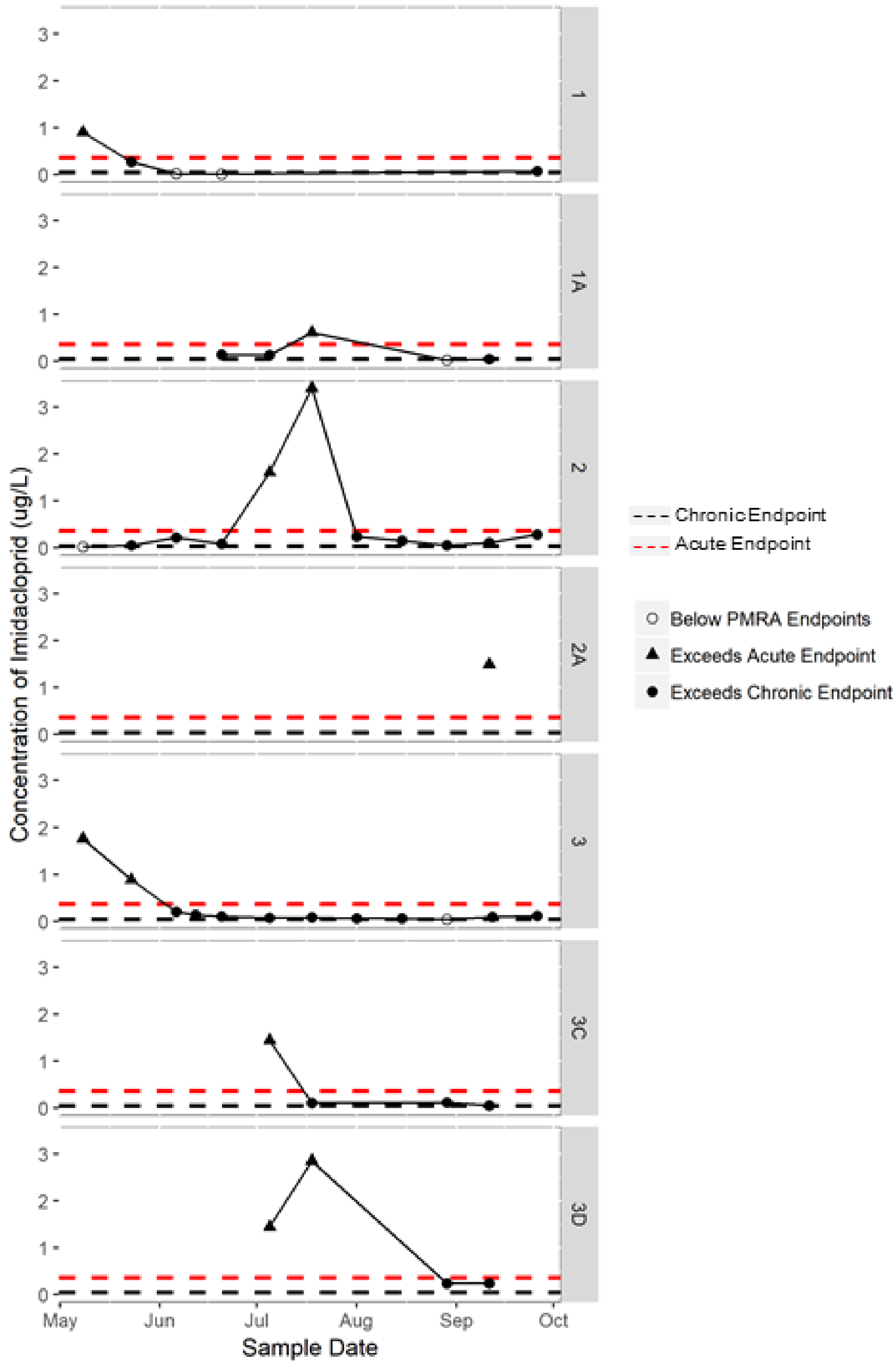

Figure 2: The concentration of imidacloprid detected at each tributary of the Nicomekl River in 2018. The black dashed line represents the PMRA chronic endpoint at 0.041 µg/L. The red dashed line represents the PMRA acute endpoint at 0.36 ug/L. Similar figures for clothianidin and thiamethoxam concentrations in each river are provided in Appendix D.

3.1.4 Pesticide Storage Inspections in Nicomekl watershed

Following the sampling for neonics in the Nicomekl River and tributaries in 2017 and 2018, ENV Integrated Pest Management (IPM) officers inspected ten pesticide storage areas in the Nicomekl River watershed on June 20 & 26, 2019. The intent of the inspections was to identify products containing neonics, assess whether products were being stored properly, and to provide information about the safe use of products and the importance of maintaining a buffer between fields and aquatic systems. Seven sites often had at least one neonic product stored. Several compliance actions were taken related to improper storage of pesticides. There was no risk of entry into aquatic waterways observed during the site visits.

3.2 Okanagan Sites

For the primary neonics tested (thiamethoxam, clothianidin, and imidacloprid), concentrations above the RDL were detected in two percent (five out of 240 [three parameters x 80 sampling events]) of the samples analyzed in 2017. In 2018, all the samples measured were below the RDL for the parameters analysed, including the four insecticides added to the analysis: acetamiprid, nitenpyram, thiacloprid, and sulfoxaflor.

The neonics measured above the RDL in 2017 included clothianidin (two samples), thiamethoxam (two samples), and imidacloprid (1 sample). All three neonics were detected at the upstream sampling site in Middle Vernon Creek (four samples). The additional detection of thiamethoxam was at the downstream site of Mission Creek. All the samples containing neonics were collected on either August 19, 2017 or September 12, 2017. The detections are provided in Appendix B, Table B4. For the samples analyzed, no PMRA acute or chronic endpoints were exceeded.

3.3 Benthic Invertebrate Biomonitoring Results

The results of the analysis are discussed in Appendix E.

4. CONCLUSION - POTENTIAL RISK TO AQUATIC INVERTEBRATES

4.1 Lower Mainland Sites

The water quality sampling conducted in the Lower Mainland confirms the presence of a neonic exposure pathway to the aquatic environment. In the Nicomekl River, concentrations of imidacloprid exceeding chronic and acute exposure endpoints were detected (often consecutively) through May to June, and intermittently in July, August, and September in 2017 and 2018. The duration of the exposure (over multiple bi-weekly sampling events), combined with the cumulative nature of toxic effects, indicated that impacts to aquatic macroinvertebrates communities are likely in the Nicomekl River. As the toxic effects of neonics on aquatic invertebrates are cumulative (and irreversible), it is important to determine if the concentration of imidacloprid remains elevated continuously during May/June, or if the exposure occurs in pulses (e.g. following rain events or pesticide application). To further understand the level of risk associated with neonics in the Nicomekl River, additional water quality sampling is recommended from April through June.

A higher percentage of non-detects and lower concentrations observed in the Sumas Canal and Cohilukthan Slough indicate a lower risk of adverse effects on aquatic organisms in the sampled areas of these watersheds.

4.1.1 Nicomekl River Tributaries

The additional samples collected in upstream tributaries of the Nicomekl River confirmed the presence of all three neonics at higher maximum concentrations and greater within-site variability compared to the original Nicomekl River sites. The highly elevated and pulsing nature of the releases suggests that the source may be effluent from a greenhouse or similar operation instead of drift from agricultural fields.

Based on the magnitude of the acute endpoint exceedances in each of the sample sites across the three tributaries, the potential for impacts to aquatic insects is likely in these areas.

4.2 Okanagan Sites

The high percentage of non-detects and the low concentrations observed (within 0.003 µg/L of the RDL) indicates a low risk to aquatic organisms in the areas that were sampled. The supplementary benthic invertebrate analysis conducted (see Appendix E) in three of the five creeks seemed to support this conclusion as effects specific to neonics could not be determined.

5. RECOMMENDATIONS

Based upon the results of the 2017/2018 sampling, recommendations for future sampling and monitoring are provided below.

Lower Mainland

- Sampling programs should begin earlier in the year to confirm the timing and magnitude of maximum concentrations (particularly in the Nicomekl River). Increased sampling (weekly) is recommended during peak application periods to understand the duration/variability of exposure. As neonics are highly water soluble, sampling immediately following rain events is recommended to determine maximum concentrations.
- As the concentrations of neonics in the tributary areas of the Nicomekl River were greater than the original upstream and downstream sample sites, any further monitoring efforts should be extended to these areas.

Okanagan

- As two of the three samples with detected neonic concentrations in the Okanagan Valley were located at the upstream sample site for Middle Vernon Creek, a different upstream /control site should be identified if sampling is to continue in this area. Further investigation into the upstream source of neonics should be considered with a focus on greenhouses or other potential point source inputs.
- If additional sampling is undertaken in the Okanagan, sampling should occur immediately following rain events. Investigation of creeks in the area that receive effluent from greenhouse operations should also occur.

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APPENDIX A: SITE OVERVIEW MAPS

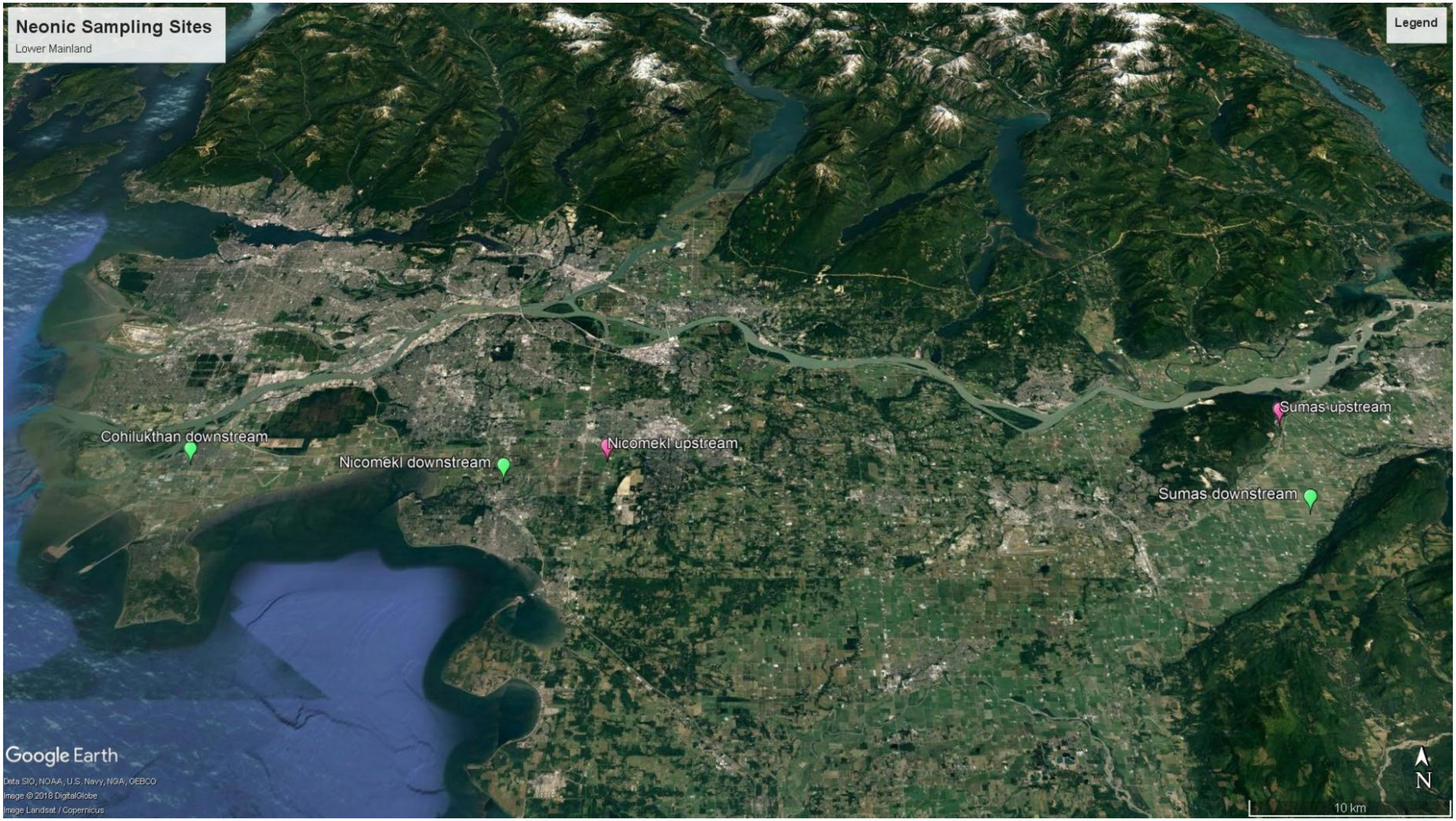

Figure A1: The upstream (pink) and downstream (green) sampling sites located in the Lower Mainland during the 2017/2018 sampling.

Figure A2: The upstream (blue) and downstream (purple) sampling sites located in the Okanagan Valley during the 2017/2018 sampling.

APPENDIX B: SUMMARY OF NEONIC DETECTIONS AND EXCEEDANCES FOR 2017 AND 2018

Table B1: A list of detection and exceedances for 2017 sampling in the Lower Mainland. Exceedances of the PMRA chronic endpoints are bolded, and concentrations above the PMRA acute endpoints are bolded and italicized.

Stream	Position	Date	Thiamethoxam	Clothianidin	Imidacloprid
Sumas	Upstream	7-Jun-17	< RDL	< RDL	< RDL
		19-Jun-17	< RDL	< RDL	< RDL
		4-Jul-17	< RDL	< RDL	< RDL
		17-Jul-17	< RDL	< RDL	< RDL
		31-Jul-17	< RDL	< RDL	< RDL
		14-Aug-17	< RDL	< RDL	< RDL
		28-Aug-17	< RDL	< RDL	< RDL
		11-Sep-17	< RDL	< RDL	< RDL
Sumas	Downstream	7-Jun-17	< RDL	< RDL	< RDL
		19-Jun-17	< RDL	0.0087	0.0133
		4-Jul-17	< RDL	< RDL	0.006
		17-Jul-17	< RDL	< RDL	< RDL
		31-Jul-17	< RDL	< RDL	< RDL
		14-Aug-17	< RDL	< RDL	< RDL
		28-Aug-17	< RDL	< RDL	< RDL
		11-Sep-17	< RDL	< RDL	0.006
Nicomekl	Upstream	7-Jun-17	< RDL	< RDL	***0.740***
		19-Jun-17	< RDL	< RDL	**0.204**
		4-Jul-17	< RDL	< RDL	0.0361
		17-Jul-17	< RDL	< RDL	**0.045**
		31-Jul-17	< RDL	< RDL	0.0339
		14-Aug-17	< RDL	< RDL	**0.0935**
		28-Aug-17	< RDL	< RDL	**0.191**
		11-Sep-17	**0.187**	< RDL	**0.201**
Nicomekl	Downstream	7-Jun-17	< RDL	**0.022**	**0.044**
		19-Jun-17	< RDL	**0.163**	0.213
		4-Jul-17	< RDL	0.0112	**0.0545**
		17-Jul-17	< RDL	< RDL	0.025
		31-Jul-17	< RDL	0.0056	0.0348
		14-Aug-17	< RDL	< RDL	0.0253

		28-Aug-17	< RDL	< RDL	**0.15**
		11-Sep-17	0.0097	< RDL	**0.0837**
Cohilukthan	Downstream	7-Jun-17	< RDL	< RDL	0.0085
		19-Jun-17	< RDL	< RDL	< RDL
		4-Jul-17	< RDL	< RDL	< RDL
		17-Jul-17	< RDL	< RDL	< RDL
		31-Jul-17	< RDL	< RDL	< RDL
		14-Aug-17	< RDL	< RDL	< RDL
		28-Aug-17	< RDL	< RDL	< RDL
		11-Sep-17	< RDL	< RDL	< RDL

Table B2: A list of detection and exceedances for 2018 sampling in the Lower Mainland. Exceedances of the PMRA chronic endpoints are bolded, and concentrations above the PMRA acute endpoints are bolded and italicized.

Stream	Position	Date	Thiamethoxam	Clothianidin	Imidacloprid	Acetamiprid
Sumas	Upstream	8-May-18	< RDL	< RDL	< RDL	< RDL
		23-May-18	< RDL	< RDL	< RDL	< RDL
		6-Jun-18	< RDL	< RDL	< RDL	< RDL
		20-Jun-18	0.014	< RDL	0.0057	< RDL
		5-Jul-18	0.0193	< RDL	< RDL	< RDL
		18-Jul-18	0.0095	< RDL	< RDL	< RDL
		1-Aug-18	0.0052	< RDL	< RDL	< RDL
		15-Aug-18	0.005	< RDL	< RDL	< RDL
		12-Sep-18	< RDL	< RDL	< RDL	< RDL
		25-Sep-18	< RDL	< RDL	< RDL	< RDL
Sumas	Downstream	8-May-18	< RDL	0.0116	0.017	< RDL
		23-May-18	< RDL	0.006	0.0058	< RDL
		6-Jun-18	0.0054	**0.085**	< RDL	< RDL
		20-Jun-18	0.0141	< RDL	0.0071	< RDL
		5-Jul-18	0.0078	< RDL	< RDL	< RDL
		18-Jul-18	0.0058	< RDL	< RDL	< RDL
		1-Aug-18	< RDL	< RDL	< RDL	< RDL
		15-Aug-18	< RDL	< RDL	< RDL	< RDL
		12-Sep-18	< RDL	< RDL	< RDL	< RDL
		25-Sep-18	< RDL	< RDL	0.0232	< RDL
Nicomekl	Upstream	8-May-18	0.0049	< RDL	***0.574***	< RDL
		23-May-18	0.0052	< RDL	**0.248**	< RDL
		6-Jun-18	0.0064	< RDL	**0.0688**	< RDL
		20-Jun-18	< RDL	< RDL	0.0302	< RDL
		5-Jul-18	< RDL	< RDL	0.0209	< RDL
		18-Jul-18	< RDL	< RDL	0.0183	< RDL
		1-Aug-18	< RDL	< RDL	0.0191	< RDL
		15-Aug-18	< RDL	< RDL	0.0144	< RDL

		12-Sep-18	< RDL	< RDL	0.0214	< RDL
		25-Sep-18	< RDL	< RDL	**0.0805**	< RDL
Nicomekl	Downstream	8-May-18	< RDL	0.0054	**0.283**	0.0066
		23-May-18	< RDL	0.0117	***0.395***	< RDL
		6-Jun-18	< RDL	0.0062	**0.154**	< RDL
		20-Jun-18	< RDL	0.0312	0.0329	< RDL
		5-Jul-18	< RDL	< RDL	0.0214	0.0053
		18-Jul-18	< RDL	< RDL	0.0225	< RDL
		1-Aug-18	< RDL	< RDL	0.0103	< RDL
		15-Aug-18	< RDL	< RDL	0.0086	< RDL
		12-Sep-18	< RDL	< RDL	**0.0635**	< RDL
		25-Sep-18	< RDL	< RDL	**0.0427**	< RDL
Cohilukthan	Downstream	9-May-18	< RDL	< RDL	0.0123	< RDL
		23-May-18	< RDL	< RDL	< RDL	< RDL
		6-Jun-18	< RDL	< RDL	< RDL	< RDL
		20-Jun-18	< RDL	< RDL	< RDL	< RDL
		5-Jul-18	< RDL	< RDL	< RDL	< RDL
		18-Jul-18	< RDL	< RDL	< RDL	< RDL
		1-Aug-18	< RDL	< RDL	< RDL	< RDL
		15-Aug-18	< RDL	< RDL	< RDL	< RDL
		12-Sep-18	< RDL	< RDL	0.0051	< RDL
		26-Sep-18	< RDL	0.0442	0.0076	< RDL

Table B3: A list of detection and exceedances for 2018 sampling in the tributaries of the Nicomekl River. Exceedances of the PMRA chronic endpoints are bolded, and concentrations above the PMRA acute endpoints are bolded and italicized. This is a complete list of samples collected in the tributaries.

Tributary	Sampling Site	Date	Imidacloprid	Thiamethoxam	Clothianidin
1	1	8-May-18	***0.904***	< RDL	< RDL
		23-May-18	**0.271**	< RDL	< RDL
		6-Jun-18	0.0191	< RDL	< RDL
		20-Jun-18	0.0094	< RDL	< RDL
		26-Sep-18	**0.0735**	0.0041	< RDL
	1A	20-Jun-18	**0.144**	< RDL	< RDL
		5-Jul-18	**0.131**	< RDL	< RDL
		18-Jul-18	***0.605***	< RDL	< RDL
		29-Aug-18	0.0212	< RDL	< RDL
		11-Sep-18	**0.0456**	< RDL	< RDL
2	2	8-May-18	0.0229	0.008	< RDL
		23-May-18	**0.059**	**0.0295**	< RDL
		6-Jun-18	**0.217**	**0.0276**	< RDL
		20-Jun-18	**0.094**	0.0212	< RDL
		5-Jul-18	***1.61***	**0.0328**	< RDL
		18-Jul-18	***3.4***	**0.0323**	< RDL
		1-Aug-18	**0.239**	0.0058	0.034
		15-Aug-18	**0.159**	**0.031**	0.007
		29-Aug-18	**0.0535**	**0.0275**	0.0074
		11-Sep-18	**0.112**	0.0235	0.006
		26-Sep-18	**0.289**	0.0052	< RDL
	2A	11-Sep-18	***1.49***	< RDL	< RDL

3	3	8-May-18	***1.76***	**0.0268**	< RDL
		23-May-18	***0.885***	0.0206	< RDL
		6-Jun-18	**0.204**	0.021	< RDL
		12-Jun-18	**0.133**	0.0129	< RDL
		20-Jun-18	**0.105**	0.0069	< RDL
		5-Jul-18	**0.0716**	0.0049	< RDL
		18-Jul-18	**0.0825**	0.0097	< RDL
		1-Aug-18	**0.0625**	0.0161	< RDL
		15-Aug-18	**0.0575**	0.0081	< RDL
		29-Aug-18	0.0344	< RDL	< RDL
		12-Sep-18	**0.0885**	0.0149	< RDL
		26-Sep-18	**0.114**	0.0046	< RDL
	3C	5-Jul-18	***1.44***	**0.144**	< RDL
		18-Jul-18	**0.109**	**0.146**	< RDL
		29-Aug-18	**0.114**	**0.121**	< RDL
		11-Sep-18	**0.0525**	**0.227**	< RDL
	3D	5-Jul-18	***1.44***	< RDL	< RDL
		18-Jul-18	***2.85***	0.0068	< RDL
		29-Aug-18	**0.234**	< RDL	< RDL
		11-Sep-18	**0.241**	< RDL	< RDL

Table B4: A list of detection and exceedances for sampling in the Okanagan Valley. Exceedances of the PMRA chronic endpoints are bolded, and concentrations above the PMRA acute endpoints are bolded and italicized. Samples with results below the RDL for each neonic are not included.

Stream	Position	Date	Thiamethoxam	Clothianidin	Imidacloprid
Middle Vernon Creek	Upstream	29-Aug-17	0.0058	0.0067	< RDL
		12-Sep-17	0.008	< RDL	0.0044
Mission Creek	Downstream	29-Aug-17	< RDL	< RDL	0.0041

APPENDIX C: CONCENTRATIONS OF NEONICS OVER TIME IN LOWER MAINLAND SAMPLING SITES

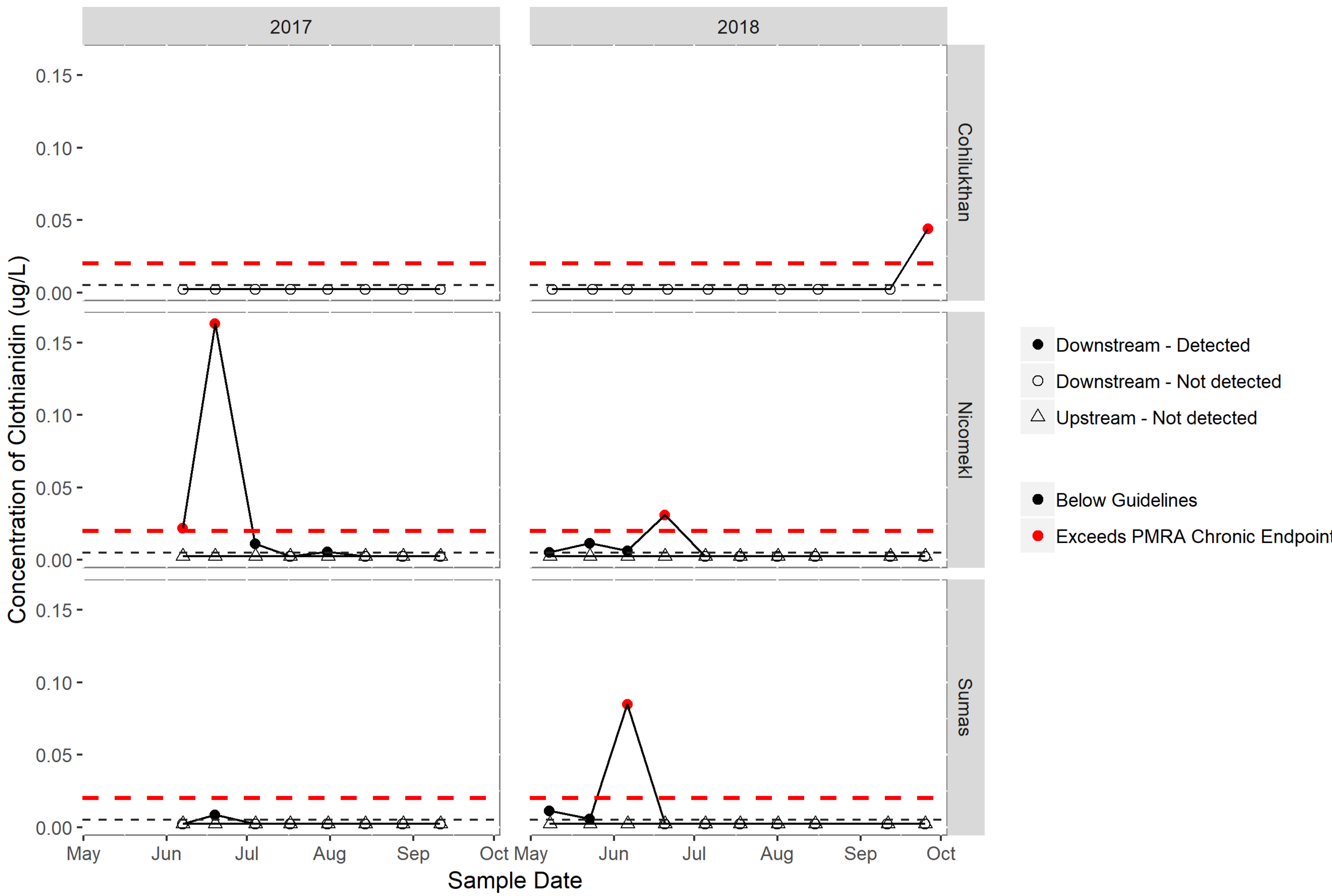

Figure C1: The concentration of clothianidin detected at Cohilukthan, Nicomekl, and Sumas sites (top to bottom) from 2017 and 2018 (left to right). The black dotted line represents the Reported Detection Limit (RDL) at 0.05 µg/L. The red line represents the PMRA standard endpoint at 0.020 µg/L

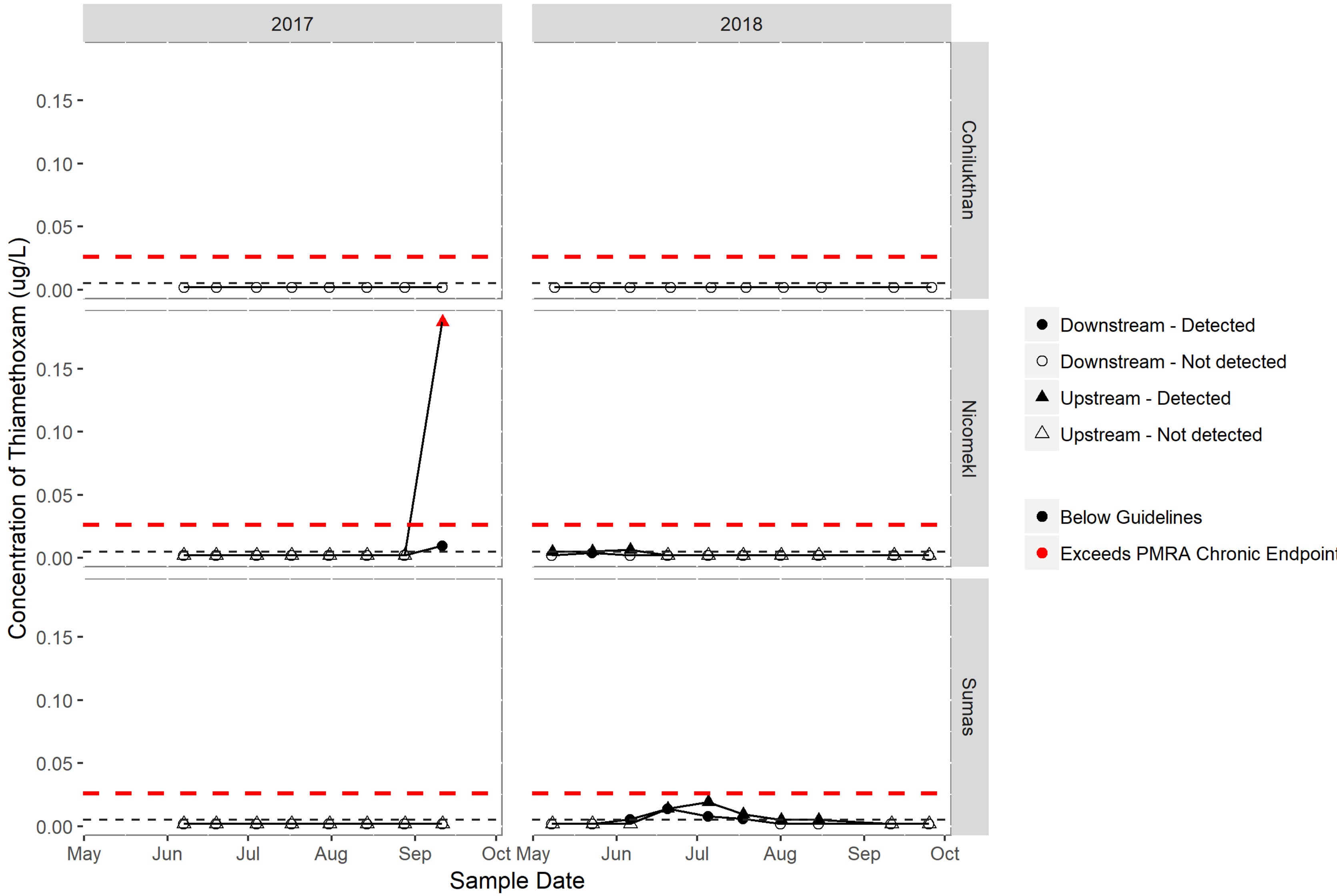

Figure C2: *The concentration of thiamethoxam detected at Cohilukthan, Nicomekl, and Sumas sites (top to bottom) from 2017 and 2018 (left to right). The black dotted line represents the Reported Detection Limit (RDL). The red line represents the PMRA standard chronic endpoint at 0.041 µg/L.*

APPENDIX D: CONCENTRATIONS OF NEONICS OVER TIME AT NICOMEKL TRIBUTARY SAMPLING SITES

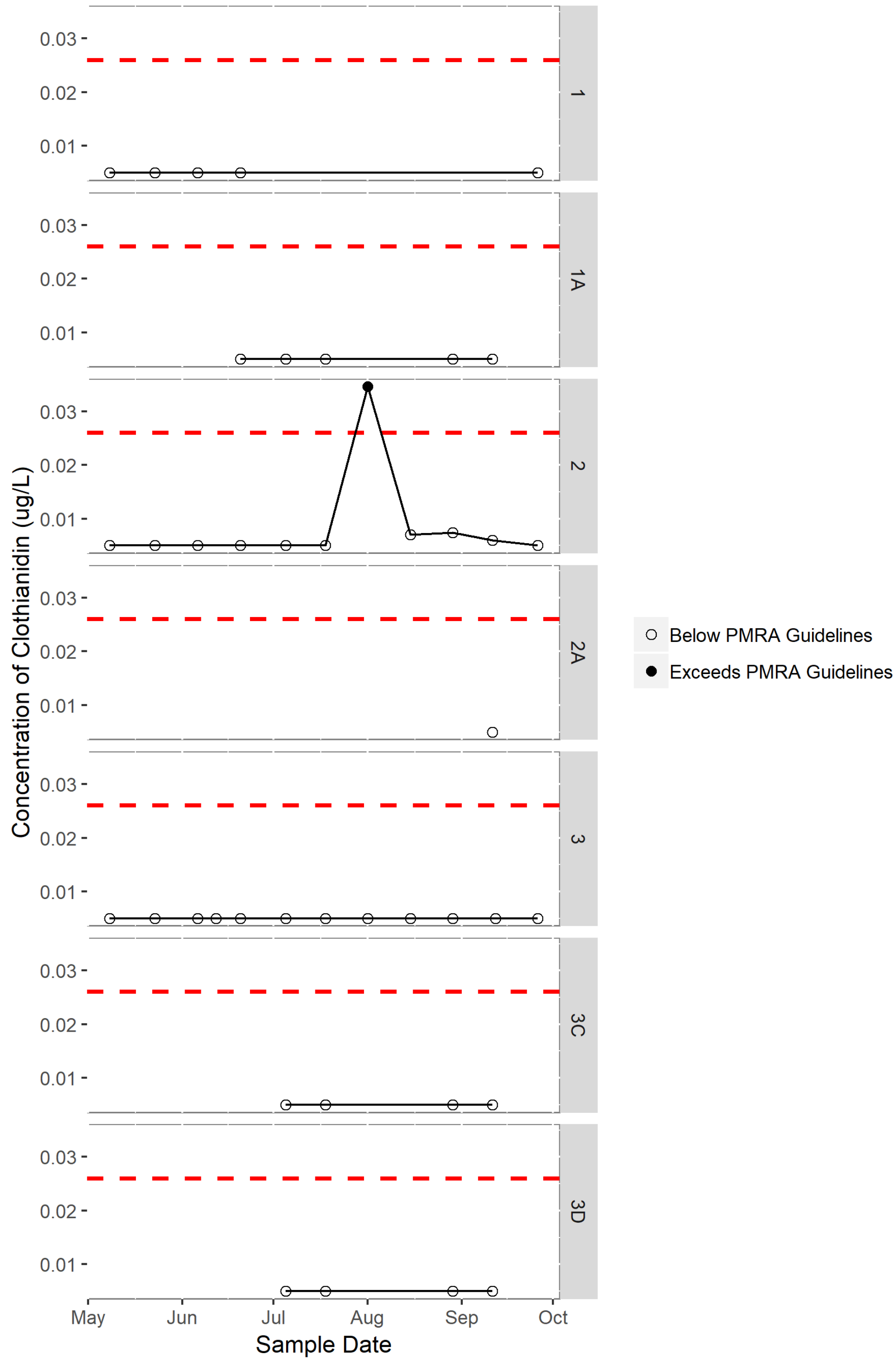

Figure D1: The concentration of clothianidin detected at each sampled tributary of the Nicomekl River in 2018. The red line represents the PMRA chronic endpoint at 0.020 µg/L.

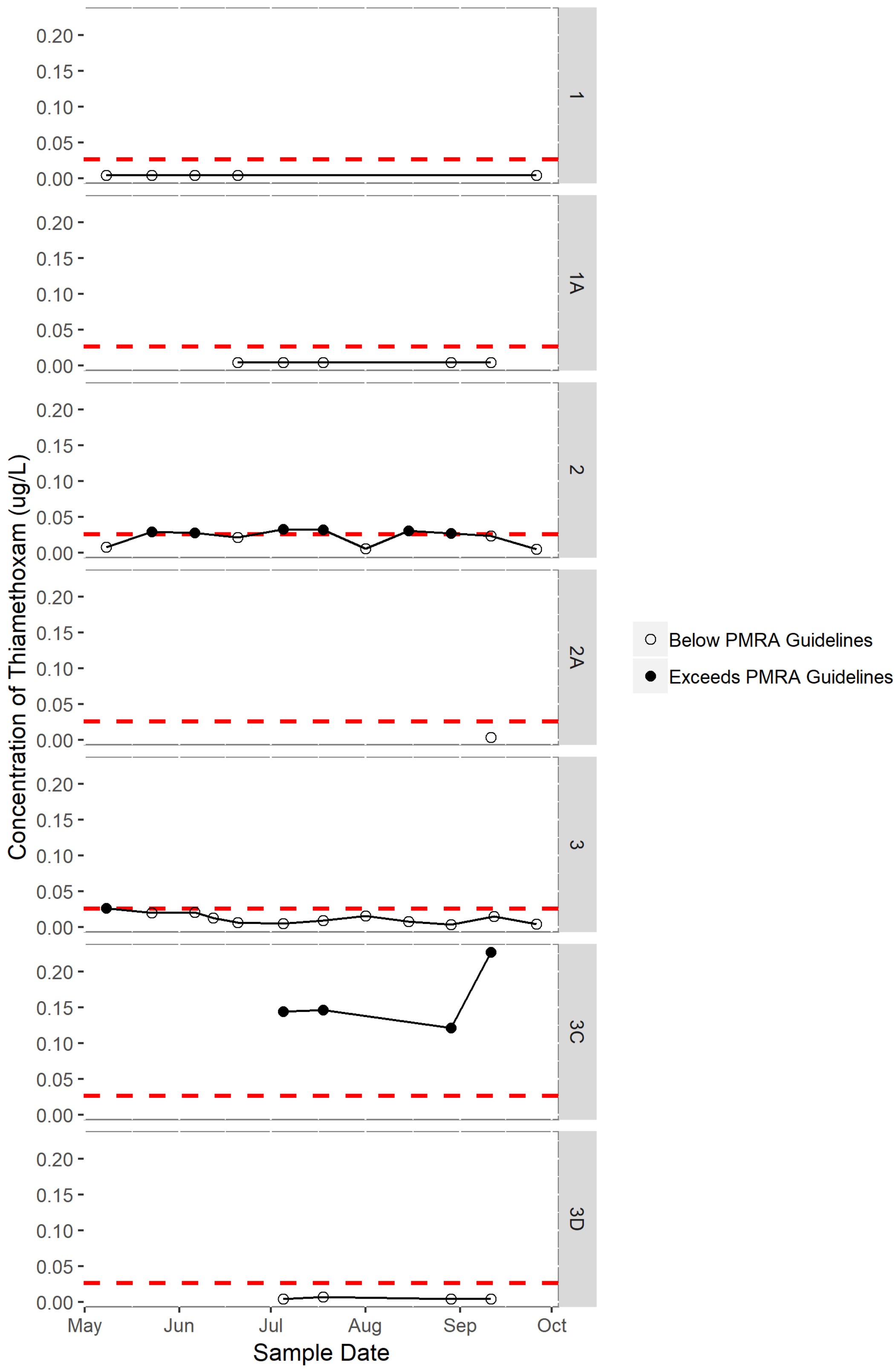

Figure D2: The concentration of thiamethoxam detected at each sampled tributary of the Nicomekl River in 2018. The red line represents the PMRA chronic endpoint at 0.026 µg/L.

APPENDIX E: BENTHIC INVERTEBRATE ANALYSIS OF OKANAGAN CREEKS

Background

Biomonitoring using freshwater benthic macroinvertebrates (herein referred to as BI) is used to complement water quality data and provide information on ecosystem condition (Canadian Aquatic Biomonitoring Network [CABIN] 2019). The addition of BI biomonitoring to an aquatic assessment provides a locally relevant effects measurement (i.e., the effect of a stressor on relevant biota compared to relying solely on the presence/magnitude of a stressor) (Bailey et al. 2004). BI are effective for biomonitoring because they are sedentary, site-specific, and live long enough to provide an indicator of preceding conditions in a waterbody for the weeks or months prior to sampling (CABIN 2019).

As mentioned in Section 1.0, neonics have an unusual and highly varied effect on non-target aquatic organisms (particularly insects) (Pestana et al. 2009; Raby et al. 2018). A comparison of acute and chronic toxicity test results by Raby et al. (2018) found that insects from the orders Ephemeroptera, Trichoptera, Coleoptera, and Diptera (particularly Chironomidae) are the most sensitive to neonics (findings also supported by Benton et al. 2017; Miles et al. 2017). Conversely, Cladocerans (*Daphnia magna*, *Ceriodaphnia dubia*; both commonly used for toxicity testing) were the least sensitive invertebrates by a magnitude of 100,000 times (Morrissey et al. 2015). Overall, the most sensitive aquatic invertebrates are insects, followed by crustaceans (e.g. amphipods), ostracods, decapods (shrimp), tubicifid worms, and mussels (Sanchez-Bayo et al. 2016).

Due to the unique toxic effects of neonics on certain orders of aquatic macroinvertebrates, BI monitoring was added to the 2018 monitoring to test if the impacts of neonics could be pinpointed. The objectives of the study were to 1) assess which assessment tools and metrics available within CABIN were useful in detecting neonic impacts; 2) identify any neonic-specific impacts, if possible; and 3) provide an overall comparison of BI communities at upstream and downstream sites. To provide insight into the potential biological effects of neonics in Okanagan Valley streams, BI sampling was conducted in Trout, Naramata and Mission creeks. BI sampling did not occur in the Lower Mainland as the sites did not meet the habitat requirements for CABIN sampling.

The following is a brief description of the study and the findings related to neonics.

Sampling Locations

BI community samples were collected by ENV staff at six locations, including the upstream and downstream sites of Trout, Naramata, and Mission creeks. The samples were collected using CABIN protocols on September 17th – 19th, 2018 (CABIN 2019). In the study design, upstream sites were intended to be reference sites, with no drainage from agricultural areas and the exposure sites were in areas of, or downstream of, high-density agriculture.

For each of the Okanagan sites, the primary land use adjacent to the creeks between the upstream and downstream sites was a mix of intensive agriculture and urban or semi-urban. Further upstream from the reference site (identified throughout the report and in Figure E1 below as the upstream site) the land use was primarily forested with areas of urban and semi-urban with limited amounts of agriculture. The type and distribution of land uses are similar across Okanagan sites.

Figure E1. Google Earth satellite imagery from the Mission Creek sampling sites. The upstream site is identified by a blue pin on the east side of the image. The downstream site is identified by a purple pin on the west side of the image. The land use distribution between sites is primarily agricultural on the south side of the creek and urban along the north side. A riparian zone of trees is present at varying widths along most of the stretch.

CABIN Assessment Methods

CABIN uses a reference condition approach (RCA) study design. RCA compares a test (exposure or potentially impacted) site to a group of physically and ecologically similar reference sites, instead of depending on a single upstream site for comparison (which almost always violates statistical assumptions of independence). The goals of RCA are to 1) establish a range of natural variability in BI communities within similar reference sites (grouped based upon environmental and habitat variables) and then 2) to determine if the BI community at a test site fits within the natural variability or falls outside (divergence) (CABIN 2019). The CABIN program provides several options to model, assess, and interpret environmental data to determine potential impacts to BI communities. The approaches I considered include 1) Benthic Assessment of Sediment (BEAST) analysis, 2) community metrics, and 3) a modified version of the River Invertebrate Prediction and Classification System (RIVPACS) analysis. Each approach is used to answer different questions about community-level biological change (Figure E2). Using a combination of tools increases confidence in the assessment and allows for more informed characterization of risk. Specific to this study, I used the 2017 Okanagan CABIN reference model (Strachan 2018).

Ability to detect neonic impacts

RIVPACs Analysis

RIVPACS uses presence/absence data to assess which taxa you would expect to find at a site (based upon matched reference sites) compared to the taxa observed. The output of RIVPACS is an Observed: Expected (O:E) ratio. If the O:E ratio is high (approaching 1), the BI communities are similar. The lower the O:E ratio, the more taxa are missing from a site, suggesting stressors to the BI community may be present. To determine if neonic-sensitive species were missing, I modified the traditional RIVPACS output to separate benthic invertebrate orders that are sensitive to neonics from less-sensitive orders. I then calculated O:E ratios for taxa with a greater than or equal to a probability of occurrence on 0.5 in reference sites. If the O:E ratio for neonic-sensitive species is greater upstream compared to downstream sites, it may be indicative of neonic-related stress to aquatic insects.

BEAST Analysis

The BEAST model uses classification and ordination statistical methods to group reference sites based upon habitat variables and then to identify deviance of the BI community observed from the expected reference community (Strachan & Reynoldson 2014). Ordination results provide a general comparison of the BI community at a test site to a group of reference sites. While results indicate if the community at the test site is divergent from reference condition, they do not indicate cause. Due to the study design and the multitude of potential anthropogenic stressors in the Okanagan streams, the BEAST analysis cannot be used to pinpoint impacts related to neonics.

Community Metrics

The community metrics in the CABIN program are used to determine the diversity and abundance of taxa (at the order level) and to assess how many pollution-sensitive organisms were observed. Similar to the findings of the BEAST analysis, community metrics provide limited insight into potential effects that could be attributed to neonics. Although pollution sensitive organisms such as Ephemeroptera and Trichoptera (and Plecoptera to a lesser extent) have been found to be sensitive to neonics, these organisms are sensitive to a range of pollutants. With the lack of control sites, any effects seen in the benthic invertebrate community cannot be attributed to neonic use.

Standard CABIN methods are designed to provide an overall assessment of the level of degradation of the BI community and do not easily assess specific types of stressors. However, a modified version of the RIVPACs analysis can potentially provide insight into the presence of neonic-specific impacts. This possibility is explored further below.

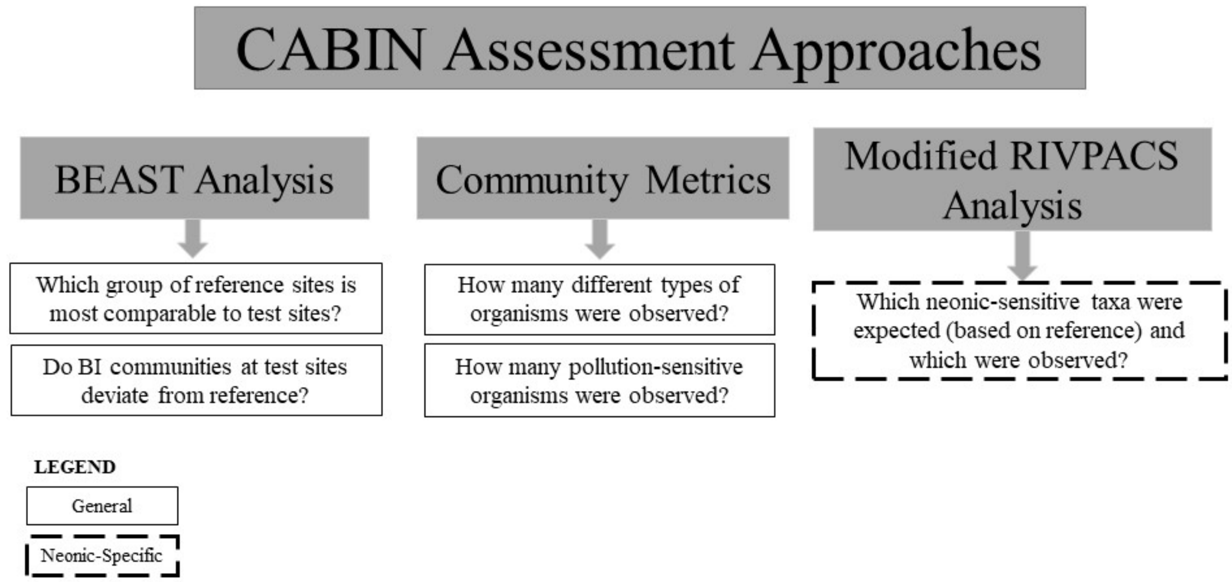

Figure E2: An overview of CABIN approaches and the questions posed by each (modified from CABIN 2019).

Assessment of Neonic-Specific Impacts

The results of the modified RIVPACS analysis on Trout Creek found that the O:E ratio for neonic-sensitive BI taxa was 0.63 at both the upstream and downstream sites (Table E1). As such, there does not appear to be neonic-specific impacts occurring disproportionately at the downstream site. The O:E ratio for all expected orders was also equal at upstream and downstream sites at 0.58.

Table E1: The results of the modified RIVPACS analysis for Trout Creek. All present taxa were denoted regardless of the probability of occurrence, but only the taxa with greater than or equal to 0.5 probability of occurrence were included in the calculation of the O:E ratios.

Order	Family	Probability of Occurrence	Present at upstream site	Present at downstream site
Coleoptera	Elmidae	0.82	Y	Y
Diptera	Ceratopogonidae	0.5	Y	Y
	Chironomidae	0.95	Y	Y
	Empididae	0.5	-	Y
	Psychodidae	0.68	Y	-
	Simuliidae	0.68	-	-
	Tipulidae	0.59	-	Y

Ephemeroptera	Baetidae	1	Y	Y
	Ephemerellidae	1	Y	Y
	Heptageniidae	1	Y	Y
	Leptophlebiidae	0.77	-	-
Trichoptera	Brachycentridae	0.86	Y	-
	Glossosomatidae	0.68	-	-
	Hydropsychidae	0.95	Y	Y
	Hydroptilidae	0.09*	-	Y
	Lepidostomatidae	0.55	Y	Y
	Rhyacophilidae	0.95	-	-
Sensitive Taxa - O:E (p≥0.50)			0.63 (10:2)	0.63 (10:2)
Haplotaxdis	Naididae	0.09*	Y	Y
Plecoptera	Capniidae	0.55	-	-
	Chloroperlidae	0.86	-	Y
	Nemouridae	1	-	-
	Perlidae	0.95	-	-
	Perlodidae	0.68	Y	Y
Pteronarcyidae	Pteronarcyidae	0.36*	Y	
Trombidiformes	Hydryphantidae	0.36*	Y	Y
	Hygrobatidae	0.32*	-	Y
	Lebertiidae	0.64	Y	-
	Sperchontidae	0.82	Y	Y
	Torrenticolidae	0.82	Y	Y
All Orders – O:E (p≥0.50)			0.58 (14:2)	0.58 (14:2)

At Naramata Creek, the results of the modified RIVPACS analysis showed that the O:E ratio for neonic-sensitive orders was 0.55 at upstream sites and 0.73 at downstream sites (Table E2). Based on the O:E ratios, downstream BI distribution more closely matched the reference conditions than upstream, suggesting no neonic-related effects between the upstream and downstream sites. The O:E ratio for all orders was 0.67 at upstream sites and 0.71 at downstream sites.

Table E2: The results of the modified RIVPACS analysis for Naramata Creek. All present taxa were denoted regardless of the probability of occurrence but only the taxa with greater than or equal to 0.5 probability of occurrence were included in the calculation of the O:E ratios.

Order	Family	Probability	Present at upstream site	Present at downstream site
Coleoptera	Dytiscidae	0.04*	Y	-
	Elmidae	0.84	-	Y
Diptera	Ceratopogonidae	0.48*	Y	Y
	Chironomidae	0.96	Y	Y
	Empididae	0.5	-	-
	Psychodidae	0.66	-	-
	Simuliidae	0.56	Y	Y
	Tipulidae	0.59	-	Y
Ephemeroptera	Ameletidae	0.5	-	-
	Baetidae	1	Y	Y
	Ephemerellidae	1	Y	Y
	Heptageniidae	1	Y	Y
	Leptophlebiidae	0.82	Y	Y
Sensitive Orders - O:E (p≥0.50)			6:1 (0.6)	8:1 (0.7)
Trichoptera	Brachycentridae	0.86	-	-
	Glossosomatidae	0.71	Y	Y
	Hydropsychidae	0.95	Y	Y
	Lepidostomatidae	0.57	Y	Y
	Limnephilidae	0.24*	Y	Y
	Psychomyiidae	0.03*	Y	
	Rhyacophilidae	0.95	Y	Y
Plecoptera	Capniidae	0.58	Y	-
	Chloroperlidae	0.88	Y	-
	Nemouridae	1	Y	Y
	Perlidae	0.94	Y	Y
	Perlodidae	0.71	-	Y
Pteronarcyidae	Pteronarcyidae	0.28*	Y	
Trombidiformes	Hygrobatidae	0.35*		Y

	Lebertiidae	0.64	-	Y
	Sperchontidae	0.74	Y	-
	Torrenticolidae	0.8	Y	Y
All Orders – O:E (p≥0.50)			16:2 (0.7)	17:2 (0.7)

The modified RIVPACS assessment at Mission Creek showed O:E ratios for neonic-sensitive orders as 0.56 at the upstream site and 0.69 at the downstream site (Table E3). Similar to the Naramata Creek observation, there appears to be more neonic-sensitive species observed at downstream sites indicating there is a low likelihood of neonic-specific effects between the upstream and downstream site. The O:E ratios for all expected species were 0.63 at upstream sites and 0.54 at downstream sites.

Table E3: The results of the modified RIVPACS analysis for Mission Creek. All present taxa were denoted regardless of the probability of occurrence but only the taxa with greater than or equal to 0.5 probability of occurrence were included in the calculation of the O:E ratios.

Order	Family	Probability	Present at upstream site	Present at downstream site
Coleoptera	Elmidae	0.82	Y	Y
Diptera	Ceratopogonidae	0.5	-	-
	Chironomidae	0.96	Y	Y
	Empididae	0.5	Y	Y
	Psychodidae	0.69	-	-
	Simuliidae	0.67	-	-
	Tipulidae	0.59	-	Y
Ephemeroptera	Baetidae	1	Y	Y
	Ephemerellidae	1	Y	Y
	Heptageniidae	1	-	Y
	Leptophlebiidae	0.77	Y	Y
Trichoptera	Brachycentridae	0.86	-	Y
	Glossosomatidae	0.68	-	-
	Hydropsychidae	0.95	Y	Y
	Lepidostomatidae	0.54	Y	Y
	Leptoceridae	0	Y	
	Rhyacophilidae	0.96	-	-
Sensitive Orders - O:E (p≥0.50)			9:16 (0.56)	11:16 (0.69)

Plecoptera	Capniidae	0.56	-	-
	Chloroperlidae	0.87	-	-
	Nemouridae	1	-	-
	Perlidae	0.95	Y	-
	Perlodidae	0.69	Y	-
Haplotaxdis	Naididae	0.09	Y	-
Trombidiformes	Hygrobatidae	0.31	-	Y
	Lebertiidae	0.64	Y	-
	Sperchontidae	0.81	Y	Y
	Torrenticolidae	0.82	Y	-
All Orders – O:E (p≥0.50)			15:24 (0.63)	13:24 (0.54)

Based upon the RIVPACS analysis, there was no evidence of impacts on neonic-sensitive orders at any of the downstream Okanagan sites in comparison to upstream sites. However, this assessment was limited as it used presence/absence data only, and it assumed that all species in an order have a similar sensitivity to neonics. Overall, this observation fits my expectations as the Okanagan sites had very low or no presence of neonics in the water-quality sampling.

General Assessment of Okanagan Sites

BEAST Model

The BEAST model output uses hybrid multidimensional scaling (HMDS) to plot test sites relative to reference sites. Confidence ellipses are placed around the reference communities and the test sites (in this study, both the upstream and downstream sites at each waterbody) are plotted in relation to their level of similarity with BI communities from similar reference sites in the wider geographic area (CABIN 2019). The confidence ellipses represent the following stream conditions:

- within 90% = similar to reference
- between 90% and 99% = mildly divergent from reference -10% of reference sites could also be here (Type I Error)
- between 99% and 99.9% = divergent from reference
- outside of the 99.9% = highly divergent from reference (CABIN 2019)

In the three Okanagan watercourses, the upstream sites were classified as similar to reference (Naramata Creek) to mildly divergent (Trout and Mission creeks) (Table E4). The downstream sites ranged from mildly divergent (Naramata and Mission creeks) to divergent (Trout Creek). For Trout Creek and Naramata Creek, the upstream site was more similar to reference conditions than the downstream indicating potential effects of stressors on BI communities between the two sites. At Mission Creek, the upstream and downstream sites had the same level of divergence (“Mildly Divergent”). ***Overall, BEAST analysis indicated that the BI community at the downstream sites of Trout and Naramata Creek were more impacted than the upstream sites, while the same level of potential effect was observed at***

Mission Creek upstream and downstream sites. Mission Creek was the only site of the three to have trace concentrations of neonics present in the 2017 water quality sampling.

Table E4: BEAST model results at the upstream and downstream sites. Yellow boxes indicate that sites are "Mildly Divergent", yellow indicates "Divergent", and boxes without color are similar to reference sites.

Watercourse	Location	Probability of Group Membership	BEAST Model Assessment
Trout Creek	Upstream	Group 2 - 100%	Mildly Divergent
	Downstream	Group 2 - 100%	Divergent
Naramata Creek	Upstream	Group 2 – 62.2%	Similar to Reference
	Downstream	Group 2 – 70.5%	Mildly Divergent
Mission Creek	Upstream	Group 2 – 99.8%	Mildly Divergent
	Downstream	Group 2 – 99.8%	Mildly Divergent

Community Metrics

Community metrics for measuring BI abundance and the number of pollution-sensitive individuals were calculated for each upstream and downstream site and compared to the reference group conditions.

Abundance

The number of taxa observed at each of the six locations ranged from 14 (Mission Creek downstream) to 21 (Naramata Creek upstream) compared to a mean of 24 (± 3.54 SD) taxa in the reference group (Table E5). The number of taxa observed at the upstream and downstream sites of all three creeks was lower than expected based on sites in the reference group. The number of taxa was higher at upstream sites for each creek but the differences between downstream sites were only one to two taxa.

Certain macroinvertebrate orders are sensitive to pollutants in streams. The most common pollution-sensitive taxa used for impact assessment are the Ephemeroptera, Plecoptera, and Trichoptera orders (referred to collectively as EPT). In streams with fewer EPT taxa than expected (compared to reference groups), stressors are more likely to be present. The total number of EPT taxa observed at the three sites ranged from 8to 14 compared to a reference mean value of 14.92 (± 2.30 SD) taxa (Table E2).

Based on the EPT taxa present in the three creeks, Naramata Creek is expected to have better water quality and less degraded conditions at the upstream (within 1 SD) compared to the downstream (between 1-2 SD) site. However, both Naramata Creek sites have a higher number of EPT taxa compared to Trout and Mission creeks. In Trout and Mission creeks, the number of EPT taxa was considerably lower (> 2 SD) than the reference group at upstream and downstream sites. Based on abundance metrics, it seems likely that similar stressors were present across upstream and downstream sites.

Number of Individuals

Related to the abundance of taxa observed at each site is the composition or number of individuals that fall within each taxa or taxa group (i.e. EPT). Therefore, to add context to the abundance metrics, the % EPT and % 2 dominant taxa were assessed at each upstream and downstream site (for all three creeks) and compared to the CABIN reference group. The % EPT metric indicates the proportion of BI assessed that are pollution-sensitive. The % 2 dominant taxa provides information about the uniformity of the biota of the site. Typically, degraded site conditions will have higher percentages of fewer taxa whereas higher quality sites will be more diverse.

The % EPT varied greatly across sites from 24.2% (Trout Creek downstream) to 88.7% (Naramata Creek upstream) compared to a reference group mean of 75.7% (± 10.1 SD) (Table E5). The low percentage of EPT individuals at Trout Creek matched the low number of EPT taxa previously described. Interestingly, the Mission Creek downstream site had a high percentage of EPT individuals despite only having eight taxa present (Table E2). These results suggest that despite having a lower number than expected taxa, select EPT taxa were able to thrive in Mission Creek.

The % 2 dominant taxa metric varied to a lesser extent across sites from 39.1% (Trout Creek downstream) to 67.9% (Mission Creek upstream) compared to a reference mean value of 46.5% (± 10.6 SD) (Table E5). Interestingly, Trout Creek was the least uniform site (based solely on the % 2 dominant metric) but also had the lowest EPT taxa and individuals.

The composition of the BI community largely matched the findings of the abundance assessment for the metrics assessed. ***The results of the metrics were similar at upstream and downstream sites within each creek indicating that similar stressors were present throughout the sampling area.*** The results further supported the higher water quality and overall conditions at Naramata Creek compared to Trout and Mission creeks. The metrics also indicated that Trout Creek had the most degraded BI community of the three assessed.

Table E5: Results of community metrics at Trout, Naramata, and Mission creeks. White boxes indicate the results were within 1 SD of reference. Yellow boxes indicate sites were between 1-2 SD from reference. Orange boxes refer to sites greater than 2 SDs from reference.

Watercourse	**Location**	**Abundance Metrics**		**Number of Individuals**	
		Total Number of Taxa	Number of EPT Taxa	% EPT	% 2 dominant taxa
Trout Creek	Upstream	19	8	44.3	39.1
	Downstream	18	8	24.2	56.9
Naramata Creek	Upstream	21	14	88.7*	57.6
	Downstream	20	12	83.6	56.7
Mission Creek	Upstream	16	8	69.3	45.6
	Downstream	14	8	86.8*	67.9
Predicted Group Reference – Mean ± SD		24 ± 3.54	14.92 ± 2.30	75.7 ± 10.1	46.5 ± 10.6

Note: * Indicates the value is outside of the standard deviation of the reference site in a positive direction.

Study Limitations

There were several key limitations in this study. Most importantly, the study design did not have an effective reference site for neonic use. Due to the multiple stressors present between upstream and downstream sites, including agriculture and urban, any impacts detected at downstream sites are likely due to a combination of stressors. Ideally, the study design would have included "no-neonic" reference sites with similar upstream land-use. Another important limiting factor is that neonics were not detected (at concentrations above the RDL) in two of the three sites sampled were detected at low concentrations in the other site (Mission Creek). Furthermore, the only neonic detections in Mission Creek occurred in 2017 and not in 2018 when the BI sampling was conducted. The absence of neonic detections during water sampling indicates that even if it were possible to isolate the impacts of neonics using CABIN methods, I would not expect to be able to detect the effects in the Okanagan study sites.

Conclusions

The objectives of the study were met as follows:

1). Is it possible to detect impacts due to neonic exposure using the CABIN methodology?

Overall, the standard CABIN methods are designed to provide an overall assessment of the level of degradation of the BI community and do not easily assess specific types of stressors. However, a modified version of the RIVPACs analysis can potentially provide insight into the presence of neonic-specific impacts.

2). Were any impacts related to neonic exposure identified?

Based upon the limited findings of the RIVPACS analysis, there was no evidence of impacts on neonic-sensitive orders at any of the downstream Okanagan sites in comparison to upstream sites.

3). What was the overall CABIN assessment of Okanagan creeks?

Overall, BEAST analysis indicated that the BI community at the downstream sites of Trout and Naramata creeks were more impacted than the upstream sites, while the same level of impact was observed at Mission Creek upstream and downstream sites. It is also worth noting that Mission Creek was the only site of the three to have trace concentrations of neonics present in the 2017 water quality sampling.

Overall, the results of the community metrics were similar at upstream and downstream sites within each creek indicating that similar stressors were present throughout the sampling area. Metrics indicated that Trout Creek had the most degraded BI community across upstream and downstream sites, compared to Naramata and Mission creeks.

A summary of the findings from the benthic invertebrate assessment in the three Okanagan Creeks is provided below.

Neonic-specific Impacts to BI Communities

Modified RIVPACS	Findings
Trout Creek	No neonic-specific impacts were detected. Upstream and downstream sites had similar presence of neonic-sensitive species.
Naramata Creek	No neonic-specific impacts were detected. Downstream site had a higher presence of neonic-sensitive species than upstream.
Mission Creek	No neonic-specific impacts were detected. Downstream site had a higher presence of neonic-sensitive species than upstream.

General Impacts to BI Communities

Beast Analysis	**Findings**
Trout Creek	The downstream site appeared to be more impacted than the upstream (upstream - Mildly Divergent, downstream - Divergent).
Naramata Creek	The downstream site appeared to be more impacted than upstream (upstream - Similar to Reference, downstream -Mildly Divergent).
Mission Creek	Similar levels of impact were assessed at upstream and downstream sites (Mildly Divergent) indicating potential stressors at both sites.
Community Metrics	**Findings**
Trout Creek	Upstream and downstream sites had fewer EPT taxa and % EPT than reference (both site values were > 2 standard deviations from reference) suggesting both sites showed anthropogenic impacts
Naramata Creek	Upstream and downstream sites had similar EPT taxa and % EPT as reference sites, suggesting little anthropogenic impact.
Mission Creek	Upstream and downstream sites had fewer EPT taxa (both sites were > 2 standard deviations from reference) suggesting both sites showed anthropogenic impacts. Downstream sites had higher % EPT taxa than upstream.

Recommendations

As the presence of neonics and potential impacts to BI communities were largely undetected in this study, further work in this area is not warranted at this time. Based upon the findings of this analysis, considerations for general neonic biomonitoring programs are provided below.

- The study design should be modified to add control and neonic-exposure streams (with confirmed neonic presence) with similar land-uses and stressors instead of using the upstream/downstream model.
- Although some limited aspects of the CABIN model were used in this assessment, a biomonitoring program targeted at identifying neonic-related impacts should be adopted in future monitoring.