Information Request Package 8 from the Review Panel for the Roberts Bank Terminal 2 Project Environmental Assessment: Responses

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IR8-01 Marine Vegetation – Biomass Estimates, Salinity: Additional Information

Information Source(s)

EIS Volume 2: Appendix 9.5-A, Figure 22

EIS Volume 3: Section 11, Table 11-14, Table 11-15, Table 11-16, Table 11-18

Context

The methodology adopted by the Proponent for its prediction of the biomass changes of native eelgrass, *Ulva*, biomat and intertidal marsh, as a function of salinity for existing and future conditions with the proposed Project during freshet, was not explicitly described in Section 11 of the EIS.

In Tables 11-14, 11-15, 11-16 and 11-18 of the EIS, several vegetation biomass estimates were reported that appeared to be anomalous when compared with the expectation that biomass as a function of salinity should be monomodal. For example:

- Table 11-14 reported zero (with Project) native eelgrass biomass for a salinity of 26-28 practical salinity units (PSU). In comparison, salinities of 24-26 PSU and 28-30 PSU had predicted biomass values of 11.0 and 264.4 tonnes respectively;
- Table 11-15 reported *Ulva* biomass (without Project) of 307.0 tonnes for salinity of 8-10 PSU in comparison with 722.4 tonnes for salinity of 6-8 PSU and 650.5 tonnes at 12-14 PSU;
- Table 11-18 reported intertidal marsh (with Project) biomass of 164.6 tonnes under 4-6 PSU in comparison with 276.9 and 269.4 tonnes under salinities of 2-4 and 6-8 PSU respectively.

It is unclear whether the outlier values within the tables represented anomalous maxima or minima or whether they reflected statistical variability or errors in the vegetation biomass calculations as a function of salinity.

Tables 11-14, 11-15, 11-16 and 11-18 reported biomass estimates to one tenth of a decimal point, implying a high degree of precision. Additional information is required to substantiate the reported precision presented in these tables.

Further, these tables reported biomass estimates as a function of salinity specifically for freshet conditions. The Fraser River hydrograph (Figure 22 of Appendix 9.5-A) indicated that the freshet is approximately 3 months in duration, while non-freshet conditions occur over the remaining 9 months of the year. An explanation is required as to whether the biomass values in Tables 11-14, 11-15, 11-16 and 11-18 were annual values scaled up from freshet conditions. Additional information is required to allow the prediction of changes in marine vegetation biomass as a function of salinity during non-freshet conditions.

Tables 11-14, 11-15, 11-16 and 11-18 of the EIS did not provide an indication of the relative importance of biomass differences to the total biomass in the local assessment area.

Information Request

Provide graphs of the data contained in Tables 11-14, 11-15, 11-16 and 11-18 of the EIS.

Provide an expanded description of the methods used to generate biomass estimates for native eelgrass, *Ulva*, biomat and intertidal marsh, as a function of salinity, for existing and future conditions with the proposed Project.

Provide an explanation for the vegetation biomass outliers as noted in the context for this information request. If the values referenced in the context are statistical artifacts or errors, recalculate the predicted biomass differences in Tables 11-14, 11-15, 11-16 and 11-18 of the EIS.

Provide justification for the reported level of precision, down to one tenth of a decimal point, for the vegetation biomass differences with the Project reported in Tables 11-14, 11-15, 11-16 and 11-18 of the EIS.

Provide the biomass estimates as a function of salinity for existing and future conditions with the Project for native eelgrass, *Ulva*, biomat and intertidal marsh for one year, including freshet and non-freshet conditions. Present the results in the same format as Tables 11-14, 11-15, 11-16 and 11-18 of the EIS, as well as graphs. Based on the revised estimates, provide an updated discussion for the evaluation of potential differences in marine vegetation biomass as a function of salinity for existing and future conditions with the Project and their implications for the assessment of effects on marine vegetation.

VFPA Response

Clarification

The VFPA would like to clarify that the numbers presented in EIS Tables 11-14, 11-15, 11-16, and 11-18 do not represent a comparison of existing biomass and predicted future biomass as a result of the Project. Rather, these represent the *proportion of existing vegetation biomass in tonnes* that coincides with the prevailing water column salinity regime, as expressed by salinity contours depicting the 50th percentile for both existing and future salinity conditions. The tables are not intended to represent a relationship between biomass and salinity for native eelgrass, *Ulva*, biomat, and intertidal marsh, nor are they intended to be predictive (i.e., no correlative relationship between salinity and marine vegetation productivity was established and used to estimate future biomass).

This analysis was used in the effects assessment as a line of evidence to determine Project-related effects to marine vegetation due to predicted changes in salinity (EIS Section 11.6). Specifically, this analysis quantified how much marine vegetation biomass, with the existing spatial distribution and productivity, coincides with the freshet and non-freshet salinity regimes for existing and future conditions with the Project.

The VFPA would also like to clarify that for the purposes of the EIS, the Fraser River non-freshet period is represented by a three-month period (October, November, and December), not nine months as was assumed in the context to this information request[1]. The core three-month freshet period (May, June, and July) is characterised by high Fraser River flows and a very similar tidal pattern to the core non-freshet period (October, November, and December); therefore, the differences in salinity conditions that occur at Roberts Bank during these two core periods are almost entirely related to differences in Fraser River discharge, making comparison between them more useful and appropriate than comparing two six-month periods.

Changes to productivity, expressed as biomass, that are expected to occur as a result of future changes in salinity were accounted for within the Roberts Bank ecosystem model but are not presented in EIS Tables 11-14, 11-15, 11-16, and 11-18. The link between physical variables (i.e., salinity, depth, wave height, and bottom current) and marine vegetation productivity was established by defining environmental preference functions, which describe the response of each sub-component to changes in these variables (see Section 2.12 of EIS Appendix 10-C for more information).

Part 1 – Provide graphs of the data contained in Tables 11-14, 11-15, 11-16 and 11-18 of the EIS.

Graphs of the data contained in EIS Tables 11-14, 11-15, 11-16, and 11-18 are provided in **Figures IR8-01-1** to **IR8-01-4** below.

[1] As per Figure 22 of EIS Appendix 9.5-A, the freshet, on average, starts in April and ends in September, and lasts six months with a core three-month period from May to July. The non-freshet period extends from October through March, with a core three-month period occurring from October to December.

Figure IR8-01-1 Proportion of Existing Native Eelgrass Biomass coinciding with 50th Percentile Water Column Salinity Increments for both Existing Conditions and Future Conditions with the Project during Freshet

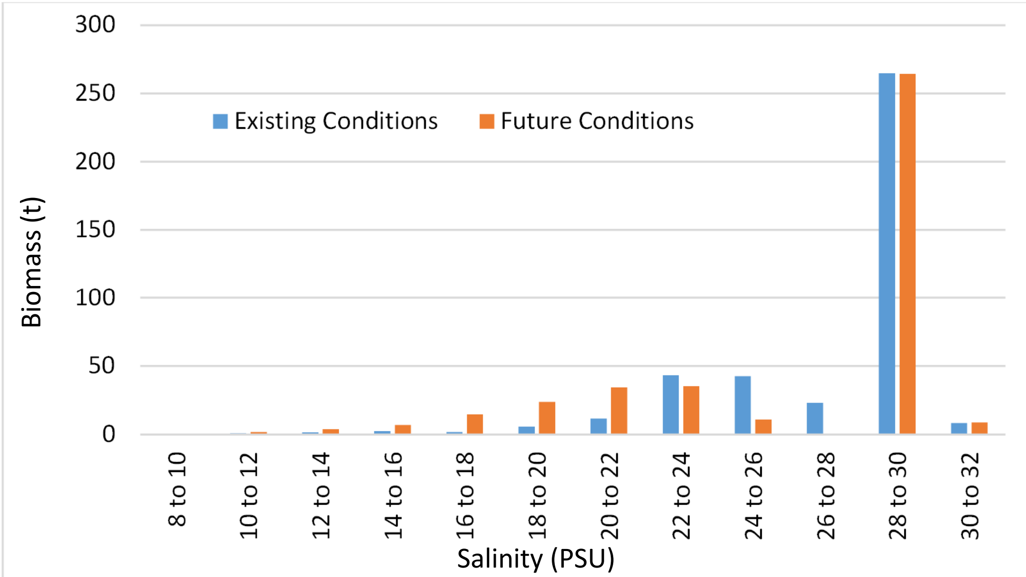


Figure IR8-01-2 Proportion of Existing Intertidal Marsh Biomass coinciding with 50th Percentile Water Column Salinity Increments for both Existing Conditions and Future Conditions with the Project during Freshet

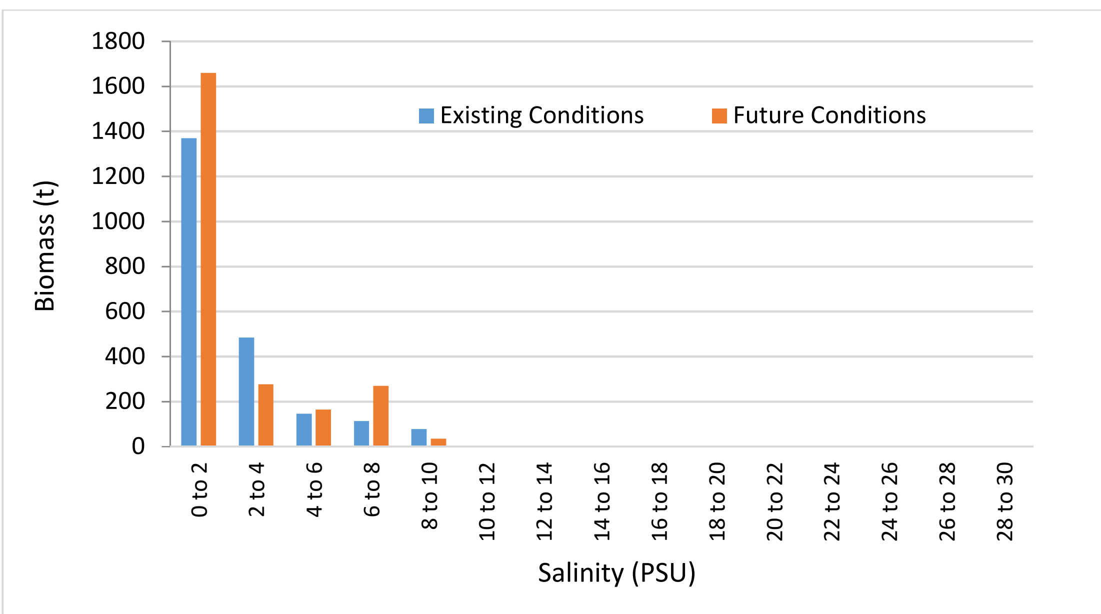


Figure IR8-01-3 Proportion of Existing *Ulva* Biomass coinciding with 50th Percentile Water Column Salinity Increments for both Existing Conditions and Future Conditions with the Project during Freshet

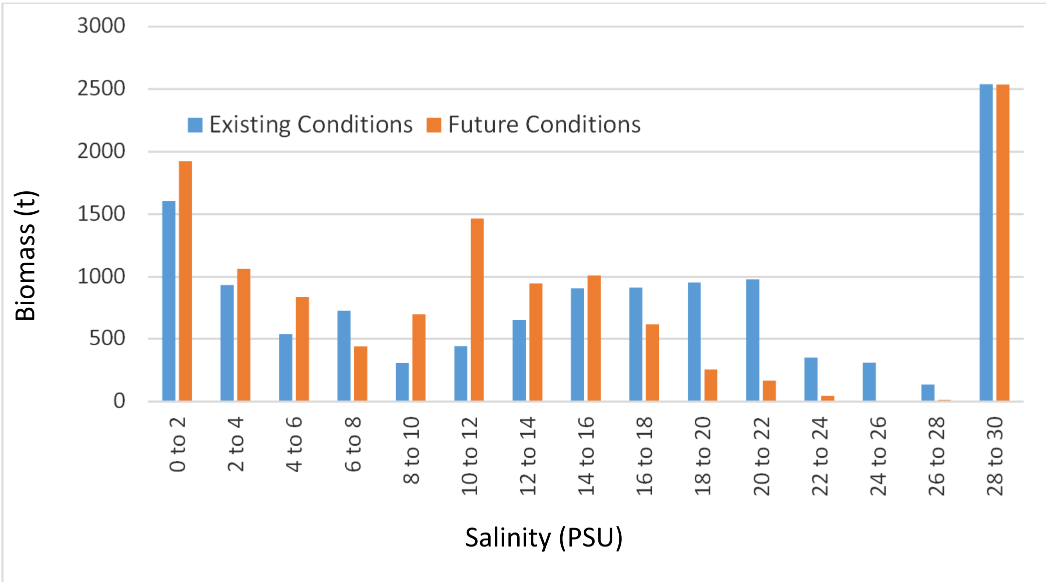
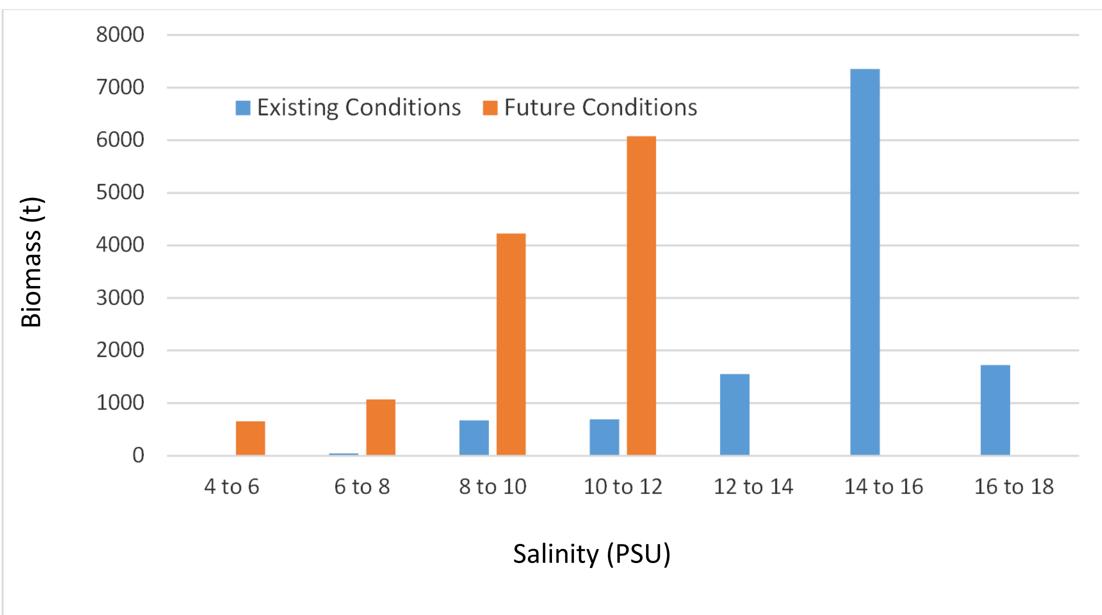


Figure IR8-01-4 Proportion of Existing Biomat Biomass coinciding with 50th Percentile Water Column Salinity Increments for both Existing Conditions and Future Conditions with the Project during Freshet



Part 2 – Provide an expanded description of the methods used to generate biomass estimates for native eelgrass, Ulva, biomat and intertidal marsh, as a function of salinity, for existing and future conditions with the proposed Project.

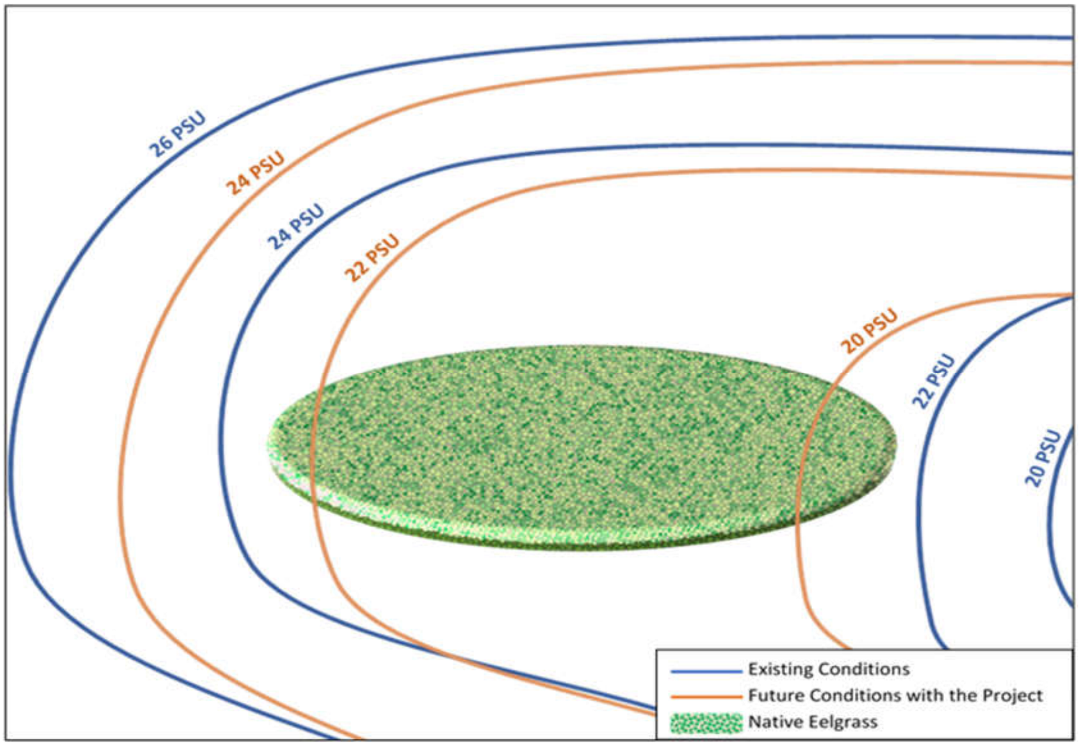
The VFPA would like to clarify that productivity changes in relation to Project-induced salinity changes were accounted for within the Roberts Bank ecosystem model along with other abiotic factors (i.e., depth, wave height, and bottom current velocity); however, the relative contribution of salinity, or any other individual abiotic factor, cannot be isolated. Therefore, to examine potential effects associated specifically with salinity, EIS Tables 11-14, 11-15, 11-16, and 11-18 were generated and qualitatively analysed. The data presented in EIS Tables 11-14, 11-15, 11-16, and 11-18, and **Figures IR8-01-1** to **IR8-01-4** above, present how the spatial distribution of the existing marine vegetation biomass coincides with the salinity regime, as expressed by the 50th percentile salinity value (using two practical salinity unit (PSU) increments) for existing and future conditions with the Project.

For example, **Figure IR8-01-5** shows a hypothetical polygon of native eelgrass that is located within a zone of variable water column salinity, which is represented by contours depicting 50th percentile salinity for existing and future with the Project conditions. Under existing conditions, 0 tonnes of the eelgrass is located within the zone with median salinity between 20 PSU and 22 PSU (i.e., none of the green circle falls within the blue 20-22 PSU contours in **Figure IR8-01-5**); however, under future conditions with the Project, 'X' tonnes will be exposed to median salinity between 20 PSU and 22 PSU (represented by the portion of the green circle within the orange 20-22 PSU salinity contours in **Figure IR8-01-5**). Note that existing biomass is used in both cases because this analysis is not predictive (i.e., increases or decreases in biomass as a function of salinity change were not calculated); instead, it is simply a GIS (geographic information system) mapping analysis where different salinity data layers (i.e., layers for both existing and future conditions) are overlain on the existing native eelgrass polygon, allowing for qualitative comparison of, for instance, whether more or less biomass will be exposed to its optimal salinity range.

The purpose of presenting these data is to show that, with the Project, the spatial distribution of salinity is expected to change, which has the potential to produce concomitant changes to the amount of productivity existing within each salinity contour (**Figure IR8-01-5**). For example, the 20 to 22 PSU salinity increment may occur over a relatively small area under existing conditions, and that area may change in size or distribution under future conditions that, by extension, would change the amount of vegetative biomass that exists within the 20 to 22 PSU bin (i.e., based on changing salinity contours only, as the biomass is spatially static for this analysis, as depicted in **Figure IR8-01-5** above). Essentially, these tables attempt to capture the amounts of existing vegetative biomass (in two PSU increments) that will be exposed to different salinities due to Project placement.

The methods used to generate existing biomass estimates for marine vegetation sub-components are similar to those reported for the Roberts Bank ecosystem model (see EIS Appendix 10-B and IR8-03 (CEAR Document #1199[2]) for more detailed descriptions).

Figure IR8-01-5 Intertidal Marsh Biomass and Associated 50th Percentile Water Column Salinity for Existing Conditions and Future Conditions with the Project during Freshet from EIS Table 11-18



The methods used to generate these biomass estimates for each marine vegetation sub-components are described below:

Native Eelgrass

1. Measure density (i.e., shoots per square metre)[3] empirically, and multiply by 10,000 to convert to hectares (ha).

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[2] CEAR Document #1199 From the Vancouver Fraser Port Authority to the Review Panel re: Response to Information Requests IR8-03 and IR8-09 (See Reference Document #1071).

[3] During Roberts Bank field surveys, native eelgrass shoots in a 0.25 m^2 quadrat were counted and extrapolated to one square metre by multiplying by 4. Field data also showed that one square metre of native eelgrass that had 100% ground cover had approximately 139 shoots of eelgrass, on average (TDR MVB-1 in Appendix AIR10-C of CEAR Document #388).

2. Estimate the biomass of one square metre using a combination of field data (density) and scientific literature (shoot weight)[4]. Calculate tonnes (t) per hectare by multiplying shoots per hectare (Step 1) by the average mass of an individual shoot (grams (g)) and converting to tonnes.
3. Overlay existing and future median salinity distributions on the Roberts Bank habitat map (EIS Figure 11-2)[5] in GIS software and bin eelgrass distribution in 2 PSU salinity increments (0-2 PSU, 2-4 PSU, etc.) based on its spatial overlap. Multiply eelgrass biomass (tonnes per hectare) by the sub-polygon area of the particular median salinity bin with which it overlaps and the percent cover within that sub-polygon.
4. Sum sub-polygon biomasses for each salinity bin.

The calculation below illustrates the above steps for eelgrass:

Step	Methodology
1	$\frac{Eelgrass\ shoots}{m^2} x \frac{10{,}000\ m^2}{ha} = \frac{Eelgrass\ shoots}{ha}$
2	$\frac{Eelgrass\ shoots}{ha} x \frac{g}{Eelgrass\ shoot} x \frac{1\ tonne}{1{,}000{,}000\ g} = \frac{Eelgrass\ tonnes}{ha}$
3	$\frac{Eelgrass\ tonnes}{ha} x\ Subpolygon\ area\ (ha)\ of\ a\ given\ salinity\ bin\ x\ Eelgrass\ \%\ cover = Eelgrass\ tonnes$
4	$Sum\ Eelgrass\ tonnes\ across\ all\ subpolygons\ of\ a\ particular\ salinity\ bin = Total\ Eelgrass\ biomass\ in\ that\ salinity\ bin$

Intertidal Marsh

Calculations of intertidal marsh biomass within salinity increments involved a similar method to that presented for eelgrass above, including field sampling of densities (using quadrats)

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[4] A sub-sample of native eelgrass shoots was collected and weighed to estimate an average shoot weight. The shoot weight was verified by reviewing the scientific literature (e.g., Moody 1978, Harrison 1982, Beal et al. 2004).

[5] The Roberts Bank habitat map uses complex polygons to visually represent the distribution of various vegetation sub-components in the local assessment area (LAA). The habitat map polygons are comprised of many smaller polygons (i.e., sub-polygons), each with underlying data, including percent cover.

and combining these empirical values with weight values found in the literature to generate biomass estimates.

There are differences in productivity between brackish marsh and salt marsh which have been well documented in the scientific literature (e.g., Węski et al. 2010). Due to these differences, biomasses for each marsh type were calculated separately for a particular salinity bin and then summed together at the end to generate one value for the overall intertidal marsh sub-component. This was done because estimating average intertidal marsh productivity (tonnes per hectare) would overestimate the intertidal marsh biomass affected by changes in salinity along the northwest side of the causeway, for example, as this is relatively low productivity salt marsh (i.e., low biomass). Estimating the biomass affected solely as salt marsh was a more accurate estimate of the biomass affected by the change in salinity in this area. This was also done so it was more directly comparable to the Roberts Bank ecosystem model.

Ulva

1. *Ulva* biomass was estimated to be 5 kilograms (kg) per square metre (m^2) based on a thorough review of the scientific literature (Vadas et al. 2004, Schaadt 2005, Zhang et al. 2014). This estimate was converted to tonnes (t) per hectare (ha).
2. Overlay existing and future salinity distributions on the Roberts Bank habitat map (EIS Figure 11-2) in GIS software and bin *Ulva* distribution in 2 PSU salinity increments (0-2 PSU, 2-4 PSU, etc.) based on its spatial overlap.
3. Multiply *Ulva* biomass (tonnes per hectare) by the sub-polygon area of the median salinity bin with which it overlaps and the percent cover within that sub-polygon.
4. All sub-polygon biomasses were then summed.

The calculation below illustrates the above steps for *Ulva*:

Step	Methodology
1	$\frac{Ulva\ kg}{m^2} x \frac{10{,}000\ m^2}{ha} x \frac{1\ tonne}{1{,}000\ kg} = \frac{Ulva\ tonnes}{ha}$
2	$\frac{Ulva\ tonnes}{ha} x\ Subpolygon\ area\ (ha)\ of\ a\ given\ salinity\ bin = Ulva\ tonnes\ of\ a\ given\ salinity\ bin$
3	$Ulva\ tonnes\ of\ a\ given\ salinity\ bin\ x\ Ulva\ \%\ cover = Ulva\ tonnes\ of\ a\ given\ salinity\ bin\ corrected\ for\ \%\ cover$
4	$Sum\ Ulva\ tonnes\ across\ all\ subpolygons\ of\ a\ particular\ salinity\ bin = Total\ Ulva\ biomass\ in\ that\ salinity\ bin$

Biomat

In the literature, biomat is often described as a microbial mat, which can take different forms and be composed of a variety of single and multi-cellular organisms (Porada and Bouougri 2007, Franks and Stolz 2009). For the purposes of the assessment, biomat was defined as cyanobacteria (blue-green algae) and associated diatoms, which are similar components to biofilm (EIS Sections 11.5.4 and 11.5.5). Therefore, the existing biomass estimate for biomat was based on a similar methodology to biofilm.

1. The total mass (tonnes) of chlorophyll ɑ for the study area[6] was calculated by multiplying the average mass (mg) of chlorophyll ɑ per square meter (m^2), as determined by the hyperspectral survey (WorleyParsons 2014), by the biofilm study area.
2. This was converted to tonnes of carbon of microphytobenthos (MPB) using a factor of 40 (de Jonge 1980, WorleyParsons 2014). A factor of 40 was used as it was the mean carbon content to chlorophyll ɑ ratio of estuarine benthic diatoms observed by de Jonge (1980).
3. This is estimated to be approximately 7% of carbon content of biofilm; therefore, tonnes of MPB was multiplied by 100/7 resulting in the total carbon content of biofilm.
4. To convert to dry mass, multiplied total carbon content by 50, as C accounts for approximately 2% of mass. Water content of biofilm is estimated to be approximately 48% at Roberts Bank (Kuwae et al. 2008, 2012).
5. Dry mass was multiplied by 2 to obtain the total wet biomass of biofilm.
6. The wet biomass of biofilm was divided by the biofilm study area to determine the average tonnes of biofilm per hectare. The average production of biomat at Roberts Bank has been recorded as approximately 8 mm for a one-month period during peak growing conditions observed in July and August (NHC 2013). Biofilm was estimated to be an average of 4 mm thick; therefore, biofilm tonnes per hectare was multiplied by 2 so that the thickness was the same as biomat (8 mm).
7. Overlay existing and future salinity contours on the Roberts Bank habitat map (EIS Figure 11-2) in GIS software and bin biomat distribution in 2 PSU salinity increments (0-2 PSU, 2-4 PSU, etc.) based on its spatial overlap. Multiply biomat biomass (tonnes per hectare) by the sub-polygon area of the particular salinity bin with which it overlaps and the percent cover within that sub-polygon.
8. All sub-polygon biomasses were then summed.

[6] The total mass of chlorophyll ɑ within the study area was estimated from the hyperspectral survey data (WorleyParsons 2014).

The following formula illustrates and summarises the method outlined above:

Step	Methodology
1	$\frac{mg\ Chl\ a}{m^2} x \frac{1{,}000{,}000\ m^2}{km^2}\ x\ \frac{1\ tonne}{1{,}000{,}000\ \mathrm{mg}}\ x\ Biofilm\ study\ area\ (km^2)$ $= \text{Tonnes}\ of\ Chl\ a\ in\ study\ area$
2	$Tonnes\ of\ Chl\ a\ x\ 40\ =\ Tonnes\ of\ MPB\ carbon$
3	$Tonnes\ of\ MPB\ carbon\ x\ \frac{100}{7} = Total\ carbon\ content\ of\ biofilm$
4	$Total\ carbon\ content\ of\ biofilm\ x\ 50\ = Total\ biofilm\ dry\ weight\ (t)$
5	$Total\ dry\ weight\ x\ 2\ =\ Total\ wet\ biofilm\ weight\ (t)$
6	$\frac{Total\ wet\ weight\ (t)}{Biofilm\ study\ area\ (ha)}\ x\ 2\ =\ Biomat\ wet\ weight\ (\frac{t}{ha})$
7	$\frac{Biomat\ tonnes}{ha}\ x\ Subpolygon\ area\ (ha) of\ a\ given\ salinity\ bin\ x\ biomat\ \%\ cover$ $= Biomat\ tonnes\ in\ a\ particular\ subpolygon$
8	$Sum\ biomat\ tonnes\ across\ all\ subpolygons\ of\ a\ particular\ salinity\ bin$ $=\ Total\ biomat\ biomass\ in\ that\ salinity\ bin$

Part 3 – Provide an explanation for the vegetation biomass outliers as noted in the context for this information request. If the values referenced in the context are statistical artifacts or errors, recalculate the predicted biomass differences in Tables 11-14, 11-15, 11-16 and 11-18 of the EIS.

As explained in Parts 1 and 2 above, the data presented in EIS Tables 11-14, 11-15, 11-16, and 11-18 are representations of *proportions of existing* vegetation biomass that will be exposed to the future salinity condition and, as such, there are no outliers. For this reason, it is not appropriate or possible to compare the values with an expected statistical monomodal curve. Changes in marine vegetation biomass as a function of salinity change, along with other abiotic variables, are provided in EIS Appendices 10-B, 10-C, and 10-D. When the data in the tables are considered as intended, there are no outliers, statistical artifacts, or errors in the values presented.

Part 4 – Provide justification for the reported level of precision, down to one tenth of a decimal point, for the vegetation biomass differences with the Project reported in Tables 11-14, 11-15, 11-16 and 11-18 of the EIS.

As described above, the values in EIS Tables 11-14, 11-15, 11-16, and 11-18 are not statistically derived. The 'difference' column in these tables does not reflect a statistical prediction between existing and future conditions and the values presented therefore do not represent the level of precision associated with a prediction. The 'difference' column in these tables reflects the exposure of various parts of the marine vegetation polygon to median salinity, which is expected to change in the future with the Project. There is no uncertainty around future biomass because no prediction is made, only the coincidence with the existing biomass with future salinity conditions is reflected. The precision down to one tenth of a decimal point is considered appropriate because this reflects the level of precision in the mapping.

Part 5 – Provide the biomass estimates as a function of salinity for existing and future conditions with the Project for native eelgrass,* Ulva*, biomat and intertidal marsh for one year, including freshet and non-freshet conditions. Present the results in the same format as Tables 11-14, 11-15, 11-16 and 11-18 of the EIS, as well as graphs. Based on the revised estimates, provide an updated discussion for the evaluation of potential differences in marine vegetation biomass as a function of salinity for existing and future conditions with the Project and their implications for the assessment of effects on marine vegetation.

Biomass estimates as a function of salinity are not provided, as explained in Parts 1 and 2 of this response. However, the *proportion* of biomass associated with 2 PSU salinity increments are provided for native eelgrass, *Ulva*, biomat, and intertidal marsh for both existing and future conditions in both tabular and graphic form. Tabular data for freshet conditions are presented in EIS Tables 11-14, 11-15, 11-16, and 11-18, while graphic data for freshet conditions are presented in the response to Part 1 of this response above. Both tabular and graphic data for non-freshet conditions are presented below in **Tables IR8-01-1** to **IR8-01-4** and **Figures IR8-01-6** to **IR8-01-9**.

Overall, when considering biomass changes due to salinity over the course of a year (i.e., under both freshet and non-freshet conditions), there are no implications for the assessment of effects on marine vegetation. In the EIS, results were presented for freshet conditions only, as opposed to averaged over a year, because this was the most conservative approach. Salinity changes are most pronounced during freshet because melting of the snowpack leads to maximum discharges of freshwater from the Fraser River. Freshet also overlaps temporally with when marine vegetation productivity is at, or is approaching, its peak (i.e., spring and summer), as more sunlight facilitates more photosynthesis. Therefore, the approach outlined in the EIS illustrates the maximum biomass that might be affected by the maximum changes in salinity and is thus considered conservative. Presenting the results averaged over a year would dilute predicted effects on marine vegetation, as freshwater input would be lower (relative to freshet) such that salinity changes would be less pronounced and there would be less standing stock biomass over the winter, when days are shorter.

Differences in biomass distribution across salinity bins due to the Project are considered negligible for non-freshet conditions, as changes in salinity are minor during this time of year and will remain within the range of what marine vegetation sub-components experience under existing conditions (EIS Section 9.7.8; **Tables IR8-01-1** to **IR8-01-4**, **Figures IR8-01-6** to **IR8-01-9**).

Slight differences in total biomass between existing conditions and future conditions with the Project, presented in the last row of **Tables IR8-01-1** to **IR8-01-4**, are attributed to 1) direct loss and mortality of marine vegetation due to the Project footprint (i.e., modelled future salinity conditions include the Project footprint, and marine vegetation cannot occur within these infilled areas (see EIS Section 11.6.1.1, EIS Table 11-12)), and 2) rounding of biomass values.

Native Eelgrass

As outlined in the EIS, salinity-driven changes in native eelgrass productivity during the freshet period under future conditions with the Project are anticipated to be negligible (EIS Section 11.6.2.1, EIS Table 11-14; **Figure IR8-01-1**). Similarly, salinity-driven changes in native eelgrass productivity outside of freshet are also expected to be negligible (**Table IR8-01-1**; **Figure IR8-01-6**). Native eelgrass occurs within a salinity range of freshwater to marine (i.e., 0 to 42 PSU); however, 10 to 30 PSU is optimum for productivity (Phillips et al. 1983, Phillips 1984, Thom et al. 2012). During the non-freshet period, under both existing and future conditions, all native eelgrass biomass remains within its optimum salinity range. Therefore, changes in productivity due to changes in salinity during the non-freshet period are considered negligible for native eelgrass.

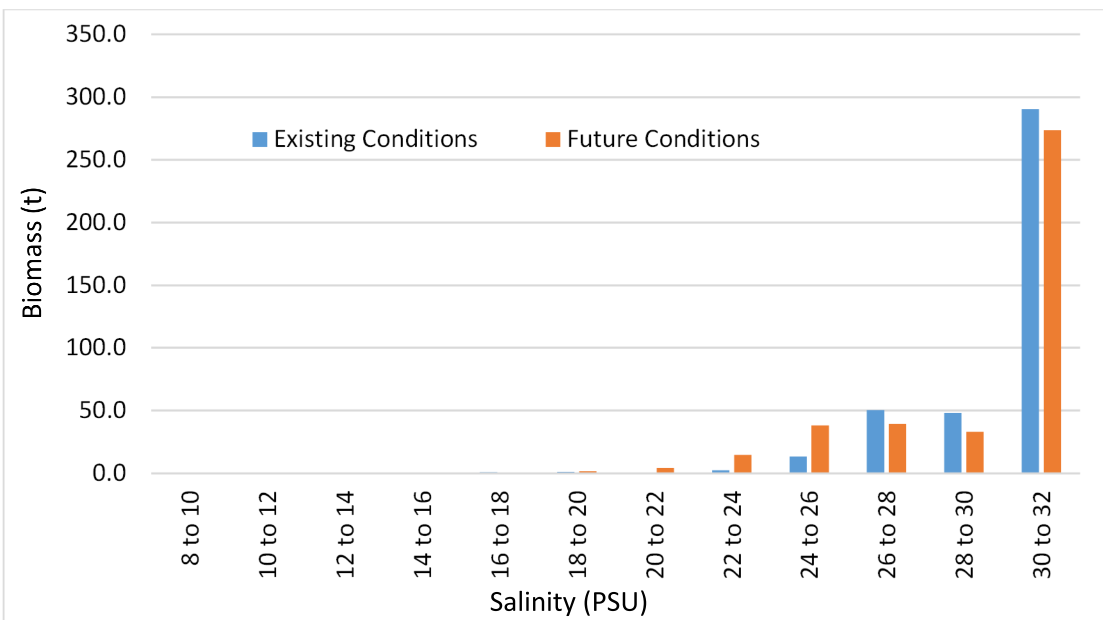
Table IR8-01-1 Proportion of Existing Native Eelgrass Biomass coinciding with 50th Percentile Water Column Salinity Increments for both Existing Conditions and Future Conditions with the Project during Non-freshet

Native Eelgrass Salinity Range (PSU)	Biomass (t)		
	Existing Salinity Conditions	Future Salinity Conditions with Project	Difference
0 to 2	0	0	0
2 to 4	0	0	0
4 to 6	0	0	0
6 to 8	0	0	0
8 to 10	0	0	0
10 to 12	0	0	0
12 to 14	0	0	0
14 to 16	0	0	0

Native Eelgrass Salinity Range (PSU)	Biomass (t)		
	Existing Salinity Conditions	Future Salinity Conditions with Project	Difference
16 to 18	1	0	1
18 to 20	1	2	1
20 to 22	0	4	4
22 to 24	2	15	12
24 to 26	13	38	25
26 to 28	50	39	-11
28 to 30	48	33	-15
30 to 32	291	274	-17
TOTAL	406	405	-1*

Note: *The difference between existing conditions biomass and future conditions with Project biomass is due to direct loss and mortality due to the Project footprint (EIS Section 11.6.1.1, EIS Table 11-12) and the rounding of biomass values.

Figure IR8-01-6 Proportion of Existing Native Eelgrass Biomass coinciding with 50th Percentile Water Column Salinity Increments for both Existing Conditions and Future Conditions with the Project during Non-freshet



Intertidal Marsh

Salinity-driven changes in intertidal marsh productivity during the freshet period under future conditions with the Project were characterised in the EIS as positive (EIS Section 11.6.3.2, EIS Table 11-18; **Figure IR8-01-3**); this is based on literature, which reported brackish and freshwater conditions favour intertidal marsh productivity (Crain et al. 2004, Więski et al.

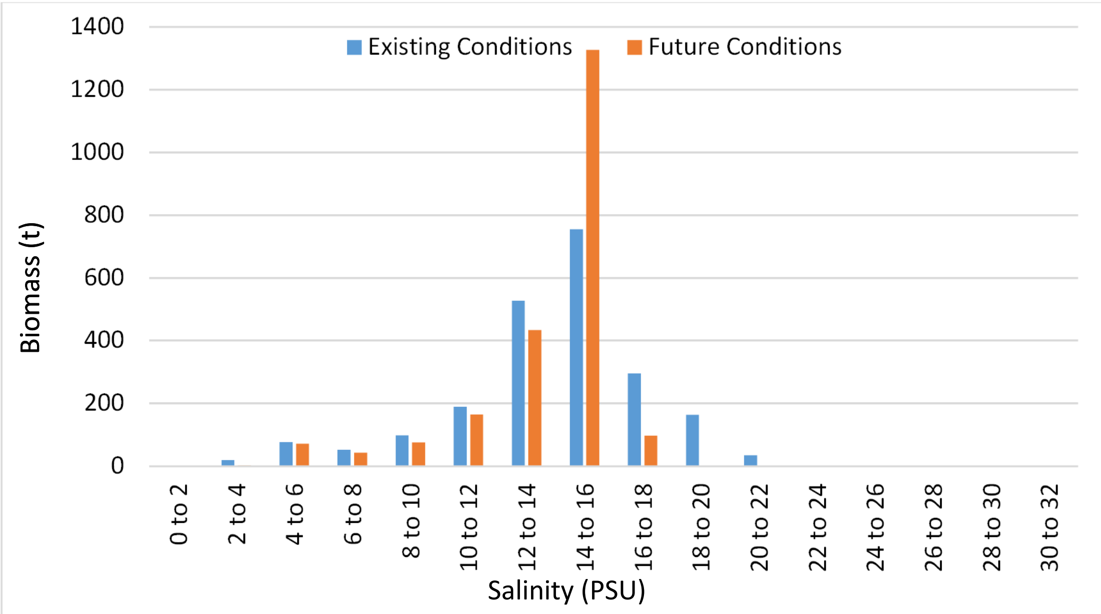
2010, Woo and Takekawa 2012) and corroborated by the Roberts Bank ecosystem model, which predicted that 335 tonnes of intertidal marsh biomass will be gained under future conditions. Salinity changes were identified as a major driver of predicted gains, specifically the increased spatial overlap with less saline waters particularly during freshet. As depicted in **Table IR8-01-2** and **Figure IR8-01-7** below, the Project will change the distribution of saline water within the study area such that, during non-freshet periods, most intertidal marsh biomass will fall within the 14 to 16 median salinity bin. As this lies within the brackish range (approximately 2 to 20 PSU), changes in salinity due to the Project are considered to have a positive effect on intertidal marsh productivity during non-freshet periods.

Table IR8-01-2 Proportion of Existing Intertidal Marsh Biomass coinciding with 50th Percentile Water Column Salinity Increments for both Existing Conditions and Future Conditions with the Project during Non-freshet

Intertidal Marsh Salinity Range (PSU)	Biomass (t)		
	Existing Salinity Conditions	Future Salinity Conditions with Project	Difference
0 to 2	0	0	0
2 to 4	20	2	-18
4 to 6	77	72	-5
6 to 8	52	43	-9
8 to 10	99	75	-23
10 to 12	189	165	-24
12 to 14	528	433	-95
14 to 16	756	1,327	571
16 to 18	296	98	-198
18 to 20	164	0	-163
20 to 22	35	0	-35
22 to 24	1	0	-1
24 to 26	1	0	-1
26 to 28	1	0	-1
28 to 30	0	0	0
30 to 32	1	1	0
TOTAL	2,220	2,216	-4*

Note: *The difference between existing conditions biomass and future conditions with Project biomass is due to direct loss and mortality due to the Project footprint (EIS Section 11.6.1.1, EIS Table 11-12) and the rounding of biomass values.

Figure IR8-01-7 Proportion of Existing Intertidal Marsh Biomass coinciding with 50th Percentile Water Column Salinity Increments for both Existing Conditions and Future Conditions with the Project during Non-freshet



Ulva

As outlined in the EIS, salinity-driven changes in *Ulva* productivity during the freshet period under future conditions with the Project are anticipated to be negligible (EIS Section 11.6.2.1, EIS Table 11-15; **Figure IR8-01-2**); this is attributed to the species' wide salinity tolerance (0 to 51 PSU; Reed and Russell 1979, Young et al. 1987), it naturally occurring in both freshwater and marine environments (Martins et al. 1999, Messyasz and Rybak 2010), and the Roberts Bank ecosystem model indicating that salinity is not a key driver for *Ulva* productivity (as detailed in EIS Appendix 10-D). During non-freshet conditions, the change in *Ulva* biomass across salinity increments remains largely unchanged relative to existing conditions (**Table IR8-01-3**; **Figure IR8-01-8**). Under future non-freshet conditions, *Ulva* biomass will still be distributed within its natural range and where optimal growth has been observed to occur (15 and 20 PSU; Martins et al. 1999). Therefore, changes in productivity due to changes in salinity during the non-freshet period are considered negligible for *Ulva*.

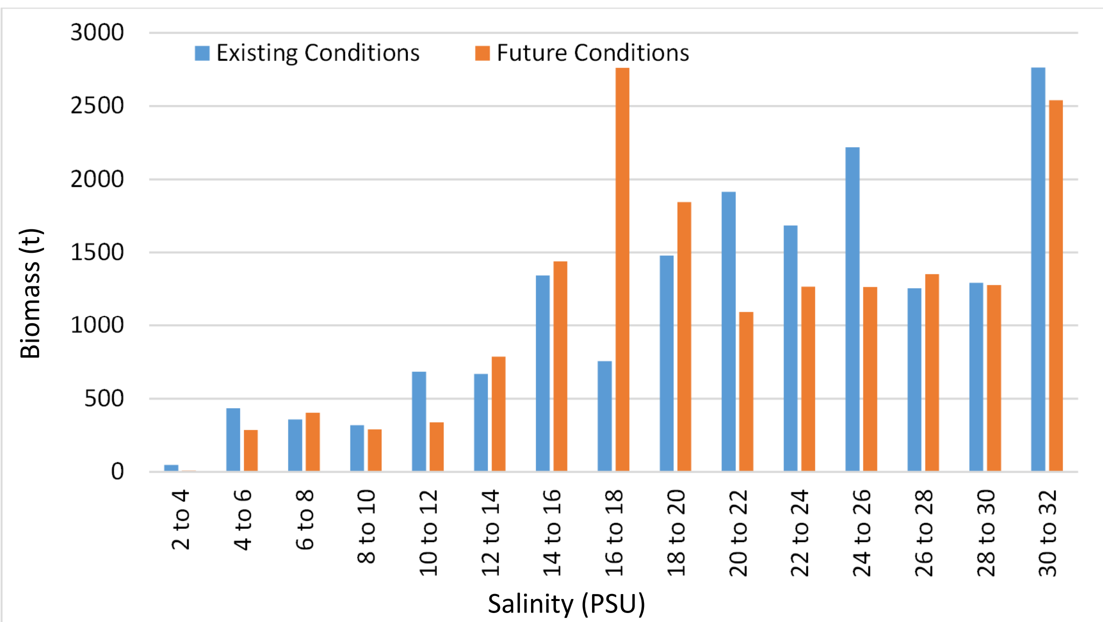
Table IR8-01-3 Proportion of Existing *Ulva* Biomass coinciding with 50th Percentile Water Column Salinity Increments for both Existing Conditions and Future Conditions with the Project during Non-freshet

***Ulva* Salinity Range (PSU)**	**Biomass (t)**		
	Existing Salinity Conditions	**Future Salinity Conditions with Project**	**Difference**
0 to 2	0	0	0.0
2 to 4	47	7	-41

Ulva Salinity Range (PSU)	Biomass (t)		
	Existing Salinity Conditions	Future Salinity Conditions with Project	Difference
4 to 6	435	285	-150
6 to 8	357	403	47
8 to 10	318	289	-29
10 to 12	682	338	-344
12 to 14	667	787	120
14 to 16	1,344	1,441	97
16 to 18	755	2,761	2,005
18 to 20	1,479	1,845	366
20 to 22	1,915	1,093	-821
22 to 24	1,684	1,267	-418
24 to 26	2,218	1,264	-954
26 to 28	1,256	1,352	96
28 to 30	1,293	1,279	-15
30 to 32	2,762	2,539	-223
TOTAL	17,212	16,950	-264*

Note: *The difference between existing conditions biomass and future conditions with Project biomass is due to direct loss and mortality due to the Project footprint (EIS Section 11.6.1.1, EIS Table 11-12) and the rounding of biomass values.

Figure IR8-01-8 Proportion of Existing *Ulva* Biomass coinciding with 50th Percentile Water Column Salinity Increments for both Existing Conditions and Future Conditions with the Project during Non-freshet



Biomat

Salinity-driven changes in biomat productivity during the freshet period under future conditions with the Project were characterised in the EIS as negligible (EIS Section 11.6.2.1, EIS Table 11-16; **Figure IR8-01-3**); this is based on literature, which reported low salinities do not appear to affect cyanobacteria productivity (Moisander et al. 2002), and corroborated by sensitivity analyses run for the Roberts Bank ecosystem model, which indicated that salinity was not a driving abiotic factor for biomat productivity (as described in Section 3.2 EIS Appendix 10-D).

As outlined above, the Project will change the distribution of saline water within the study area such that under future conditions, biomat will be exposed to lower median salinities on average; under existing conditions, the majority of biomat biomass occurs from 18 to 22 PSU, while under future conditions during this same period, the majority of biomat biomass will occur within the 14 to 18 PSU contours (**Table IR8-01-4**; **Figure IR8-01-9**). Despite the shift, the future scenario reflects the range most biomat biomass is distributed within under existing conditions during freshet (i.e., 12 to 18 PSU; **Figure IR1-01-3**). Since future biomass distribution across salinity bins overlaps with existing patterns, Project-related changes in salinity are expected to negligibly affect biomat productivity.

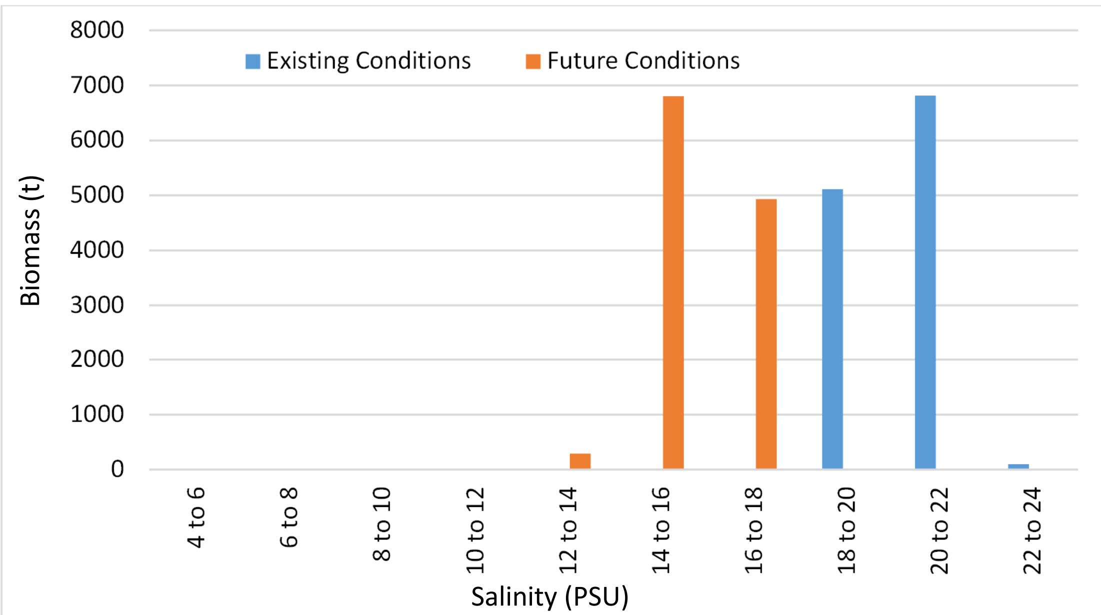
Table IR8-01-4 Proportion of Existing Biomat Biomass coinciding with 50th Percentile Water Column Salinity Increments for both Existing Conditions and Future Conditions with the Project during Non-freshet

Biomat Salinity Range (PSU)	Biomass (t)		
	Existing Salinity Conditions	**Future Salinity Conditions with Project**	**Difference**
0 to 2	0	0	0
2 to 4	0	0	0
4 to 6	0	0	0
6 to 8	0	0	0
8 to 10	0	0	0
10 to 12	0	0	0
12 to 14	0	289	289
14 to 16	0	6,801	6,801
16 to 18	0	4,930	4,930
18 to 20	5,111	0	-5,111
20 to 22	6,815	0	-6,815
22 to 24	94	0	-94
24 to 26	0	0	0
26 to 28	0	0	0
28 to 30	0	0	0

Biomat Salinity Range (PSU)	Biomass (t)		
	Existing Salinity Conditions	Future Salinity Conditions with Project	Difference
30 to 32	0	0	0
TOTAL	12,020	12,020	0*

Note: *There is no difference between existing conditions biomass and future conditions with Project biomass due to no direct loss and mortality due to the Project footprint (EIS Section 11.6.1.1, EIS Table 11-12).

Figure IR8-01-9 Proportion of Existing Biomat Biomass coinciding with 50th Percentile Water Column Salinity Increments for both Existing Conditions and Future Conditions with the Project during Non-freshet



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IR8-02 Marine Vegetation – Biomass Estimates, Salinity: Statistics

Information Source(s)

EIS Volume 3: Section 11, Table 11-19, Figure 11-11, Figure 11-12, Figure 11-13

Context

In Table 11-19 of the EIS, the Proponent reported predictions of mean water column salinity changes of biofilm area during freshet and non-freshet flows that would be attributed to the proposed Project. Estimates were provided for the 5 biofilm zones (upper intertidal, mid-intertidal, lower intertidal, Canoe Passage and inter-causeway).

Differences between the mean salinity values for existing conditions and conditions with the Project were utilized to evaluate the potential for reduced productivity for the marine-influenced biofilm assemblage during freshet.

Salinity estimates were reported as means with associated standard deviations. Changes in salinity estimates were based on mean differences, but the standard deviations of the differences were not reported.

Figures 11-11, 11-12, and 11-13 of the EIS depicted representations of the salinity values used to generate Table 11-19. These figures did not reflect the statistical variability of the differences in salinity. To provide a robust statistical comparison, it is necessary to report and analyze the statistical variability of the differences.

Information Request

Update Table 11-19 to reflect the statistical confidence predicted for changes in salinity of the biofilm area corresponding to existing conditions and conditions with the proposed Project for freshet and non-freshet periods, including:

- the standard deviations for the two columns reporting differences in salinity in Table 11-19; and
- standard deviations for the differences between the means in salinity predictions.

Provide a discussion of the implications of incorporating statistical variability in the evaluation of the potential for reduced productivity for marine and freshwater biofilm assemblages as a function of salinity differences during freshet and non-freshet periods.

VFPA Response

Clarification

To clarify the data requested by the Panel, as stated in the context to this information request, salinity estimates were reported as means for five biofilm zones for the freshet (May to June) and non-freshet (October to December) periods and reported in EIS Table 11-19. Changes in salinity estimates were based on differences between the means (i.e., by subtracting the existing conditions (baseline) means and with proposed project means). This method of determining the difference between mean salinities does not allow for an estimate of variability to be calculated. Therefore, to answer the information request and provide an estimate of variability, the VFPA calculated salinity differences for each (spatial) point in the model under existing conditions and conditions with the proposed project in place for each period. From this dataset, the mean salinity change (hereafter, referred to as 'mean difference') and associated variability with the proposed project in place could be calculated for each zone and temporal period. In some instances, this procedure resulted in slight changes in the difference values presented in EIS Table 11-19 compared to those reported in this response.

Also, in reviewing EIS Table 11-19, it was determined that the salinity difference for Canoe Passage during the non-freshet period with the proposed project in place was erroneously reported as -1.7 practical salinity units (PSU) and should have been reported as 1.7 PSU. This error has been corrected in the revised presentation of the data.

Update Table 11-19 to reflect the statistical confidence predicted for changes in salinity of the biofilm area corresponding to existing conditions and conditions with the proposed Project for freshet and non-freshet periods, including: the standard deviations for the two columns reporting differences in salinity in Table 11-19; and standard deviations for the differences between the means in salinity predictions.

Standard Deviations for the Two Columns Reporting Differences in Salinity in Table 11-19

Differences between mean salinity predictions for the five biofilm zones were reported under existing conditions and conditions with the proposed project in place for the freshet and non-freshet periods in EIS Table 11-19 (EIS Section 11.6.3.5). The means and standard deviations of the two columns reporting differences in salinity in Table 11-19 are -2.8 PSU ± 2.7 (standard deviation (SD)) and -2.0 PSU ± 2.7 (SD) for the freshet and non-freshet periods, respectively.

Standard Deviations for the Differences between the Means in Salinity Predictions

As stated in the Clarification section above, to assess the potential variability in salinity changes within biofilm zones for freshet and non-freshet periods, spatial differences in salinity between existing conditions and conditions with the proposed project were determined and the mean differences and associated standard deviations were calculated (**Table IR8-02-1**). Similar to information reported in EIS Table 11-19, differences in salinity levels between existing conditions and conditions with the potential project varied between <1 PSU for the Canoe Passage zone close to the Fraser River to approximately -5.5 PSU for the Upper Intertidal zone during the spring freshet (May to July) (**Table IR8-02-1**). Variability in the mean difference estimates ranged from ±0.1 PSU (SD) in the Inter-causeway zone to ±3.4 PSU (SD) in the Mid-intertidal zone. During the freshet, variability in mean difference estimates for the Canoe Passage and Upper-intertidal zones were ±1.0 PSU (SD) and ±1.9 PSU (SD), respectively.

During the non-freshet period, mean salinity differences between existing conditions and with project conditions changed little compared to values reported in EIS Table 11-19 (**Table IR8-02-1**). Similar to information reported in the EIS, the greatest difference in salinity between conditions is predicted to occur in the Lower intertidal zone, experiencing a mean salinity approximately -3 PSU lower than existing conditions. Overall, mean differences in salinity between existing conditions and conditions with the proposed project ranged from -3.0 PSU to 1.7 PSU. Similar to results reported in the EIS, the salinity levels in the Inter-causeway are not predicted to change (i.e., difference between conditions <1%) (**Table IR8-02-1**). Variability in the mean difference estimates ranged from ±0.1 PSU (SD) in the Inter-causeway zone to ±3.5 PSU (SD) in the Lower-intertidal zone.

Table IR8-02-1 Mean Water Column Salinity Changes of Biofilm Area, as Defined by Hyperspectral Imaging, during Freshet and Non-freshet Flows Attributed to RBT2 (originally presented as EIS Table 11-19 in EIS Section 11.6.3.5)

Predominant Influence	Biofilm Zone	Freshet (May to July) Mean Salinity (PSU)			Non-Freshet (October to December) Mean Salinity (PSU)		
		Existing Conditions	With Project	Mean Difference	Existing Conditions	With Project	Mean Difference
Marine	*Upper intertidal*	9.3 ± 4.4	5.8 ± 2.7	-3.5 ± 1.9	17.9 ± 1.3	14.9 ± 0.8	~~-3.0~~ -2.9 ± 1.5
Marine	*Mid-intertidal*	16.0 ± 2.5	10.4 ± 1.4	~~-5.6~~ -5.5 ± 1.4	21.8 ± 1.4	16.6 ± 1.1	-5.2 ± 1.3
Marine	*Lower intertidal*	21.5 ± 2.6	16.6 ± 2.0	~~-4.9~~ -4.6 ± 3.4	26.7 ± 2.0	23.5 ± 2.2	~~-3.2~~ -3.0 ± 3.5
Freshwater	*Canoe Passage*	5.5 ± 3.5	5.5 ± 4.1	< 1 ± 1.0	14.4 ± 3.4	16.1 ± 3.9	~~-1.7~~ 1.7 ± 1.0
Undefined	Inter-causeway	28.8 ± 0.2	28.9 ± 0.2	< 1 ± 0.1	31.4 ± 0.1	31.2 ± 0.2	< 1 ± 0.1

Notes:

- Variance presented as standard deviation.
- Changes to EIS Table 11-19, reported in EIS Section 11.6.3.5, are shown in red.
- Crossed-out numbers represent salinity differences (reported in EIS Table 11-19) derived from subtracting mean Existing Conditions and With Project salinities that differ slightly from mean differences calculated from the population of difference values used to assess the statistical variability of potential salinity changes.

Provide a discussion of the implications of incorporating statistical variability in the evaluation of the potential for reduced productivity for marine and freshwater biofilm assemblages as a function of salinity differences during freshet and non-freshet periods

Statistical variability was incorporated into the evaluation of the potential for reduced productivity for biofilm assemblages as a function of salinity for both the freshet and non-freshet periods as explained in EIS Section 11.6.3.4, EIS Appendix 15-B, and EIS Appendices 10-B, 10-C, and 10-D.

Since submission of the EIS, additional information on salinity has been collected (see response to IR8-04 of CEAR Document #1110[1]) at seven monitoring stations distributed across the local assessment area (LAA) salinity gradient. This new information shows that under existing conditions there is large variation in salinity across Roberts Bank due to variable Fraser River flow and tidal mixing. The Review Panel, in IR12-09 (CEAR Document #1206[2], has requested further information on statistical variability in this dataset, which will be provided and discussed in that response.

New information presented in IR8-04 (CEAR Document #1110), and additional studies conducted during the 2017 spring freshet (see *Biofilm Dynamics during 2017 Northern Migration (Technical Data Report)*, submitted under separate cover), have increased the understanding of biofilm ecology at Roberts Bank and potential effects resulting from varying salinity levels on biofilm productivity. Key findings from this work pertinent to informing the assessment of potential effects from the proposed project on biofilm productivity are as follows:

- Biofilm is better adapted to different salinity conditions in an estuarine environment than previously understood;
- Comparable biofilm fatty acid productivity levels have been documented during the spring freshet in freshwater and marine locations under three freshets of differing size, indicating an adaptation to variable salinity conditions;
- Effects from changing salinity on biofilm productivity have been potentially over estimated, as salinity has been found to largely play a secondary role to other abiotic factors (e.g., the amount of time mudflats are not inundated allowing diatoms to photosynthesize) in promoting biofilm productivity;
- Biofilm fatty acid levels are primarily driven by the amount of time photosynthetic diatoms are exposed to the sun (i.e., not inundated), which is driven by the tidal/lunar cycle, and will not be affected by the Project. It is therefore likely that a consistent supply of biofilm-associated fatty acids are annually produced during the freshet;

[1] CEAR Document #1110 From the Vancouver Fraser Port Authority to the Review Panel re: Response to Information Request IR8-04 (See Reference Document #1071).

[2] CEAR Document #1206 From the Review Panel to the Vancouver Fraser Port authority re: Information Request Package 12.

- The assertion that there are two biofilm assemblages (i.e., fresh and marine) is not supported by two years of additional field study (2016 and 2017), as no difference in community composition has been found among sites sampled across the salinity gradient. The biofilm community within the LAA is more accurately described as estuarine in nature; and
- The changes in salinity due to the Project likely fall within the natural range of variability experienced during freshet; therefore, changes to biofilm with the proposed project in place are anticipated to be minor and within levels currently experienced naturally. More information on this topic will be presented as part of the VFPA's response to IR12-09.

Therefore, in light of the new information collected since submission of the EIS, and due to the small statistical variability in mean difference estimates reported in **Table IR8-02-1** compared to salinity levels (and associated estimates of variability) predicted with the project in place, potential changes to biofilm productivity described in the EIS are considered conservative and are not affected by the mean salinity difference requested as part of this information request.

IR8-03 Marine Vegetation – Biomass Estimates, Existing Conditions

Information Source(s)

EIS Volume 3: Section 11.5, Figure 11-2

Context

In Section 11.5 of the EIS, the Proponent provided biomass estimates under existing conditions for each marine vegetation sub-component within the local assessment area during the summer. The biomass values reported were:

- Native eelgrass - 407 tonnes
- Non-native eelgrass - 17 tonnes
- Intertidal marsh - 2,220 tonnes
- *Ulva* - 17,268 tonnes
- Kelp - 373 tonnes
- Rockweed - 300 tonnes
- Biomat - 12,019 tonnes
- Biofilm - 19,486 tonnes

It is unclear what the source of these estimates is, and how this information contributed to the assessment of the effects of the proposed Project on marine vegetation**.**

Information Request

Provide the source of the biomass estimates under existing conditions for each marine vegetation sub-component and an explanation of the method used to calculate these estimates.

Provide an explanation of how the biomass values for existing conditions contribute to the assessment of effects on marine vegetation.

VFPA Response

Provide the source of the biomass estimates under existing conditions for each marine vegetation sub-component and an explanation of the method used to calculate these estimates.

The biomass value estimates under existing conditions were estimated using a combination of Roberts Bank field data and scientific literature. The data and/or scientific literature used, as well as the method, are described below in **Table IR8-03-1** for each marine vegetation sub-component.

Table IR8-03-1 Sources and Methodology of Calculating Biomass Estimates for Marine Vegetation Sub-components

<table>
<tr><th>Sub-component</th><th>Sources of Data</th><th>Method</th></tr>
<tr><td colspan="3">Eelgrass</td></tr>
<tr><td>Native Eelgrass</td><td>• RBT2 field studies (TDR MVB-1 in Appendix AIR10-C of CEAR Document #388[1])
• Moody 1978a, Harrison 1982a, Beal et al. 2004</td><td>The estimate is based on calculations using data from site-specific field surveys and scientific literature.
1. Field data (shoot counts, percent cover, eelgrass shoot weight) were used to estimate the biomass of one square metre (m^2) of native eelgrass. Roberts Bank field surveys included counting native eelgrass shoots in 0.25 m^2 quadrats (TDR MVB-1 in Appendix AIR10-C of CEAR Document #388). Counts within each quadrat were multiplied by 4 to get the number of shoots per square metre. Field data also showed that on average one square metre of native eelgrass that had 100% ground cover had approximately 139 shoots of eelgrass. This density was then converted to shoots per square kilometre (km^2).
2. A sub-sample of native eelgrass shoots was collected and weighed to estimate an average shoot weight. The shoot weight was verified by reviewing the scientific literature (e.g., Moody 1978a, Harrison 1982a, Beal et al. 2004).
3. Shoots per square kilometre (Step 1) was then multiplied by the average mass (grams (g)) of an individual shoot (Step 2) and converted to tonnes (t) of native eelgrass per square kilometre (km^2).
4. The productivity (tonnes per km^2) of native eelgrass was then multiplied by the area (km^2) of each native eelgrass sub-polygon and its associated percent cover.
5. All sub-polygon biomasses were then summed.
The following formula illustrates and summarises the method outlined above:
(1) $\frac{Eelgrass\ shoots}{m^2} x \frac{1{,}000{,}000\ m^2}{km^2} x \frac{mass\ (g)}{Eelgrass\ shoot} x \frac{1\ tonne}{1{,}000{,}000\ g} = \frac{Eelgrass\ tonnes}{km^2}$
(2) $\frac{Eelgrass\ tonnes}{km^2}\ x\ Subpolygon\ area\ (km^2)\ x\ \text{Subpolygon}\ eelgrass\ \%\ cover\ = Eelgrass\ tonnes$
(3) $Sum\ eelgrass\ biomass\ across\ all\ subpolygons = Total\ existing\ eelgrass\ biomass$</td></tr>
<tr><td>Non-native Eelgrass</td><td>• RBT2 field studies (TDR MVB-1 in Appendix AIR10-C of CEAR Document #388)
• Harrison 1982b</td><td>The estimate is based on calculations using data from site-specific field surveys and scientific literature.
1. Field data (shoot counts, percent cover, eelgrass shoot weight) were used to estimate the biomass of one square metre (m^2) of non-native eelgrass. Roberts Bank field surveys included counting non-native eelgrass shoots from a sub-sample of 0.0625 m^2 quadrats (TDR MVB-1 in Appendix AIR10-C of CEAR Document #388). Counts within each quadrat were multiplied by 16 to get the number of shoots per square metre. Field data also showed that on average one square metre of non-native eelgrass that</td></tr>
</table>

[1] CEAR Document #388 From Port Metro Vancouver to the Canadian Environmental Assessment Agency re: Completeness Review - Responses to Additional Information Requirements Follow-Up (See Reference Document # 345) including 22 Technical Data Reports.

Sub-component	Sources of Data	Method
		had 100% ground cover had approximately 1,000 shoots of eelgrass. This density was then converted to shoots per square kilometre (km^2). 2. A sub-sample of non-native eelgrass shoots was collected and weighed to estimate an average shoot weight. The shoot weight was verified by reviewing the scientific literature (e.g., Harrison 1982b). 3. Shoots per square kilometre (Step 1) was then multiplied by the average mass (grams (g)) of an individual shoot (Step 2) and converted to tonnes (t) of non-native eelgrass per square kilometre (km^2). 4. The productivity (tonnes per km^2) of non-native eelgrass was then multiplied by the area (km^2) of each non-native eelgrass sub-polygon and its associated percent cover. 5. All sub-polygon biomasses were then summed. The following formula illustrates and summarises the method outlined above: (1) $\frac{Eelgrass\ shoots}{m^2}\ x\ \frac{1{,}000{,}000\ m^2}{km^2}\ x\ \frac{mass\ (g)}{Eelgrass\ shoot}\ x\ \frac{1\ tonne}{1{,}000{,}000\ g} = \frac{Eelgrass\ tonnes}{km^2}$ (2) $\frac{Eelgrass\ tonnes}{km^2}\ x\ Subpolygon\ area\ (km^2)\ x\ \text{Subpolygon}\ eelgrass\ \%\ cover\ = Eelgrass\ tonnes$ (3) $Sum\ eelgrass\ biomass\ across\ all\ subpolygons = Total\ existing\ eelgrass\ biomass$
Intertidal Marsh		
Intertidal Marsh	• RBT2 field studies (TDR MVB-1 in Appendix AIR10-C of CEAR Document #388) • Moody 1978b, Karagitzides 1987, Karagitzides & Hutchinson 1991	The estimate is based on calculations using data from site-specific field surveys and scientific literature. 1. Percent cover data from Roberts Bank were collected using a 0.25 m^2 quadrat at marsh survey locations throughout the local assessment area (LAA) (TDR MVB-1 in Appendix AIR10-C of CEAR Document #388). 2. Productivity (mass per unit area) estimates from the scientific literature were used to calculate the biomass of intertidal marsh under existing conditions. Site-specific estimates were available from Moody (1978b), Karagitzides (1987), and Karagitzides and Hutchinson (1991). 3. Intertidal marsh community composition varies with salinity. Intertidal marsh communities were distinguishable across the LAA. Brunswick Point has a brackish species composition, the inter-causeway has a salt marsh community, and north of Roberts Bank causeway has a mix of the two communities (TDR MVB-1 in Appendix AIR10-C of CEAR Document #388; Figure 2-1). 4. To incorporate the marsh community composition within the three areas described above, and the varying productivity of the species present, the various species biomasses contributed proportionally to their percent cover. 5. The productivity (tonnes per km^2) of intertidal marsh was then multiplied by the area (km^2) of each sub-polygon containing intertidal marsh and its associated percent cover. 6. All sub-polygon biomasses were then summed. The following formula illustrates and summarises the method outlined above. An example for brackish marsh is provided.

Sub-component	Sources of Data	Method
		(1) $\frac{American\ bulrush\ mass\ (g)}{m^2}\ x\ Average\ \%\ cover = American\ bulrush\ proportional\ biomass\ (\frac{g}{m^2})$ $\frac{Lyngbye's\ sedge\ mass\ (g)}{m^2}\ x\ Avg\ \%\ cover = Lyngbye's\ sedge\ proportional\ biomass\ (\frac{g}{m^2})$ $\frac{seacoast\ bulrush\ mass\ (g)}{m^2}\ x\ Avg\ \%\ cover = seacoast\ bulrush\ proportional\ biomass\ (\frac{g}{m^2})$ $\frac{Common\ cattail\ mass\ (g)}{m^2}\ x\ Avg\ \%\ cover = Common\ cattail\ proportional\ biomass\ (\frac{g}{m^2})$ (2) Sum proportional $biomasses = Avg\ of\ 1\ m^2\ of\ brackish\ marsh\ biomass\ \left(\frac{g}{m^2}\right)$ (3) $Average\ biomass\ \left(\frac{g}{m^2}\right)\ x\ \frac{1{,}000{,}000\ m^2}{km^2}\ x\ \frac{1\ tonne}{1{,}000{,}000\ g}\ =$ Average brackish marsh biomass $\left(\frac{tonnes}{km^2}\right)$ (4) $\frac{Marsh\ tonnes}{km^2}\ x\ Subpolygon\ area\ (km^2)\ x$ Subpolygon intertidal marsh % $cover\ =\ Intertidal\ marsh\ tonnes$ (5) $Sum\ intertidal\ marsh\ biomass\ across\ all\ subpolygons =\ Total\ existing\ intertidal\ marsh\ biomass$ (6) The same calculation for the salt marsh and the mixed brackish and salt marsh communities (7) *Sum all three community biomass estimates = Total intertidal marsh existing biomass*
Macroalgae		
Ulva	• RBT2 field studies (TDR MVB-1 in Appendix AIR10-C of CEAR Document #388) • Vadas et al 2004, Schaadt 2005, Zhang et al. 2014	The estimate is based on calculations using data from site-specific field surveys and scientific literature. Biomass samples of *Ulva* were not collected as part of RBT2 field studies. 1. An estimate of 5 kilogram (kg) per square metre (m^2) was used after a thorough review of the scientific literature (Vadas et al. 2004, Schaadt 2005, Zhang et al. 2014). 2. This estimate was converted to tonnes (t) per square kilometre (km^2). 3. The productivity (tonnes per km^2) of *Ulva* was then multiplied by the area (km^2) of each sub-polygon containing *Ulva* and its associated percent cover. Percent cover data was collected during field studies at Roberts Bank. Percent cover was estimated by sampling 0.25 m^2 quadrats on the mudflats within the LAA.

Sub-component	Sources of Data	Method
		4. All sub-polygon biomasses were then summed. The following formula illustrates and summarises the method outlined above: (1) $\frac{Ulva\ kg}{m^2} x \frac{1{,}000{,}000\ m^2}{km^2} x \frac{1\ tonne}{1{,}000\ \text{kg}} = \frac{Ulva\ tonnes}{km^2}$ (2) $\frac{Ulva\ tonnes}{km^2}\ x\ Subpolygon\ area\ (km^2)\ x\ Subpolygon\ Ulva\ \%\ cover\ =\ Ulva\ tonnes$ (3) $Sum\ Ulva\ biomass\ across\ all\ subpolygons = Total\ existing\ Ulva\ biomass$
Rockweed	• RBT2 field studies (TDR MVB-1 in Appendix AIR10-C of CEAR Document #388) • Guillaumont et al. 1993	The estimate is based on calculations using data from site-specific field surveys and scientific literature. Biomass samples were not collected as part of RBT2 field studies. 1. An estimate of 5.75 kilogram (kg) per square metre (m^2) was used after a thorough review of the scientific literature (Guillaumont et al. 1993). 2. This estimate was converted to tonnes (t) per square kilometre (km^2). 3. The productivity (tonnes per km^2) of rockweed was then multiplied by the area (km^2) of each sub-polygon containing rockweed and an average percent cover of 25% was used. Percent cover data was collected during field studies at Roberts Bank. Percent cover was estimated by sampling 0.25 m^2 quadrats along the rocky foreshore of the existing terminal. 4. All sub-polygon biomasses were then summed. The following formula illustrates and summarises the method outlined above: (1) $\frac{Rockweed\ kg}{m^2} x \frac{1{,}000{,}000\ m^2}{km^2} x \frac{1\ tonne}{1{,}000\ \text{kg}} = \frac{\text{Rockweed}\ tonnes}{km^2}$ (2) $\frac{\text{Rockweed}\ tonnes}{km^2}\ x\ Subpolygon\ area\ (km^2)\ x\ \text{Subpolygon rockweed}\ \%\ cover\ =\ Rockweed\ tonnes$ (3) $Sum\ \text{rockweed}\ biomass\ across\ all\ subpolygons = Total\ existing\ \text{rockweed}\ biomass$
Kelp	• RBT2 field studies (TDR MVB-1 in Appendix AIR10-C of CEAR Document #388) • Wort 1955, Druehl and Hsiao 1977, Anderson et al. 2007, Fehr et al. 2010 and 2012	Bull kelp (*Nereocystis luetkeana*) and sugar wrack kelp (*Saccharina latissima*) are the two dominant species of kelp present within the LAA (Fehr et al. 2010 and 2012). The estimate is based on calculations using data from site-specific field surveys and scientific literature. Biomass samples were not collected as part of RBT2 field studies. 1. An estimate of 12.9 kilogram (kg) per square metre (m^2) was used as a combined productivity estimate for both kelp species after a thorough review of the scientific literature (Wort 1955, Druehl & Hsiao 1977, Anderson et al. 2007, Fehr et al. 2010 and 2012). 2. This estimate was converted to tonnes (t) per square kilometre (km^2). 3. The productivity (tonnes per km^2) of kelp was then multiplied by the area (km^2) of each sub-polygon containing kelp and its associated percent cover. Percent cover data was collected during field studies at Roberts Bank. Percent cover was estimated by sampling 0.25 m^2 quadrats along the rocky foreshore of the existing terminal. 4. All sub-polygon biomasses were then summed.

Sub-component	Sources of Data	Method
		The following formula illustrates and summarises the method outlined above: (1) $\frac{Kelp\ kg}{m^2}\ x\ \frac{1{,}000{,}000\ m^2}{km^2}\ x\ \frac{1\ tonne}{1{,}000\ \text{kg}} = \frac{Kelp\ tonnes}{km^2}$ (2) $\frac{Kelp\ tonnes}{km^2}\ x\ Subpolygon\ area\ (km^2)\ x\ \text{Subpolygon}\ kelp\ \%\ cover\ = Kelp\ tonnes$ (3) $Sum\ kelp\ biomass\ across\ all\ subpolygons = Total\ existing\ kelp\ biomass$
Biomat		
Biomat	• RBT2 field studies (TDR MVB-1 in Appendix AIR10-C of CEAR Document #388) • WorleyParsons 2014a • NHC 2013 • de Jonge 1980, Porada and Bouougri 2007, Kuwae et al. 2008, Franks and Stolz 2009, Kuwae et al. 2012	The estimate is based on calculations using data from site-specific field surveys and scientific literature. Biomass samples were not collected as part of RBT2 field studies. In the literature, biomat is often described as microbial mat, which can take different forms and be composed of a variety of single and multi-cellular organisms (Porada and Bouougri 2007, Franks and Stolz 2009). For the purposes of the assessment, biomat was defined as cyanobacteria (blue-green algae) and associated diatoms, which are similar components to biofilm (EIS Sections 11.5.4 and 11.5.5). Therefore, the existing biomass estimate for biomat was based on a similar methodology to biofilm. 1. The total mass of chlorophyll a for the study area was converted to carbon content of microphytobenthos (MPB) using a factor of 40 (de Jonge 1980, WorleyParsons 2014a). 2. This is estimated to be approximately 7% of carbon content of biofilm; therefore, tonnes of MPB was multiplied by 100/7 resulting in the total carbon content of biofilm. 3. To convert to dry mass, multiplied total carbon content by 50, as C accounts for approximately 2% of mass. Water content of biofilm is estimated to be approximately 48% at Roberts Bank (Kuwae et al. 2008, 2012), so dry mass was multiplied by 2 to obtain the total wet biomass of biofilm. 4. Average production of biomat at Roberts Bank has been recorded as approximately 8 mm for a one month period during peak growing conditions observed in July and August (NHC 2013). 5. Biofilm was estimated to be an average of 4 mm thick; therefore, biomat biomass was multiplied by 2 so that the thickness was the same as biomat (8 mm). The following formula illustrates and summarises the method outlined above: (1) $\frac{mg\ Chl\ a}{m^2}\ x\ \frac{1{,}000{,}000\ m^2}{km^2}\ x\ \frac{1\ tonne}{1{,}000{,}000\ \text{mg}}\ x\ Biofilm\ study\ area\ (km^2) = \text{T}onnes\ of\ Chl\ a$ (2) $Tonnes\ of\ Chl\ a\ x\ 40\ = Tonnes\ of\ MPB$ (3) $Tonnes\ of\ MPB\ x\ \frac{100}{7} = Total\ carbon\ content$ (4) $Total\ carbon\ content\ x\ 50\ = Total\ biofilm\ dry\ weight\ (t)$ (5) $Total\ weight\ (dry\ mass)\ x\ 2\ = Total\ wet\ biofilm\ weight\ (t)$ (6) $\frac{Total\ wet\ weight\ (t)}{Biofilm\ study\ area\ (km^2)}\ x\ 2\ = Biomat\ wet\ weight\ (\frac{t}{km^2})$

Sub-component	Sources of Data	Method
		(7) $Biomat\ wet\ weight\ \left(\frac{t}{km^2}\right)\ x\ Biomat\ study\ area\ (km^2)\ =\ Biomat\ weight\ weight\ (t)$
Biofilm		
Biofilm	• WorleyParsons 2014a • WorleyParsons 2014b. • de Jonge 1980, Kuwae et al. 2008, Kuwae et al. 2012	Biomass of all biofilm at Roberts Bank was estimated using abundance of chlorophyll ɑ (Chl ɑ) data identified by the hyperspectral imagery and converted to tonnes (t) based on the scientific literature. 1. The total mass of chlorophyll ɑ for the study area was converted to carbon content of microphytobenthos (MPB) using a factor of 40 (de Jonge 1980, WorleyParsons 2014a). 2. This is estimated to be approximately 7% of carbon content of biofilm; therefore, tonnes of MPB was multiplied by 100/7 resulting in the total carbon content of biofilm. 3. To convert to dry mass, multiplied total carbon content by 50, as C accounts for approximately 2% of mass. Water content of biofilm is estimated to be approximately 48% at Roberts Bank (Kuwae et al. 2008, 2012), so dry mass was multiplied by 2 to obtain the total wet biomass of biofilm. The following formula illustrates and summarises the method outlined above: (1) $\frac{mg\ Chl\ a}{m^2}\ x\ \frac{1{,}000{,}000\ m^2}{km^2}\ x\ \frac{1\ tonne}{1{,}000{,}000\ mg}\ x\ Chl\ \alpha\ distribution^2\ (km^2) = Tonnes\ of\ Chl$ (2) $Tonnes\ of\ Chl\ a\ x\ 40\ =\ Tonnes\ of\ MPB$ (3) $Tonnes\ of\ MPB\ x\ \frac{100}{7} = Total\ carbon\ content$ (4) $Total\ carbon\ content\ x\ 50\ = Total\ biofilm\ dry\ weight\ (\ t)$ (5) $Total\ biofilm\ dry\ weight\ (t)\ x\ 2\ =\ Total\ biofilm\ wet\ weight\ (t)$

[2] Chl ɑ distribution was based on the hyperspectral survey conducted within the LAA (WorleyParsons 2014a).

Provide an explanation of how the biomass values for existing conditions contribute to the assessment of effects on marine vegetation.

The biomass values for existing conditions were used in the lines of evidence for the assessment of effects on marine vegetation. The biomass values represent empirical estimates of existing biomass within the LAA and were used as reference points to quantitatively assess changes in productivity due to the Project; for example, they were used to quantify direct mortality due to the Project footprint. The biomass values were also used as inputs to the Roberts Bank ecosystem model reflecting estimates of existing biomass under current conditions (EIS Appendices 10-B and 10-C). A sensitivity analysis of the ecosystem model biomass input values was performed to see what effect variability or uncertainty in these input values had on the results of the ecosystem model (EIS Appendix 10-D, CEAR Document #984[3]); results showed that the model was robust to uncertainty in marine vegetation biomass input values.

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IR8-04 Marine Vegetation – Biofilm

Information Source(s)

EIS Volume 3: Section 11

ECCC response to Information Request ECCC IR-02 (CEAR Doc#960)

Context

In its response to Review Panel information request ECCC IR-02, Environment and Climate Change Canada stated that a multi-year baseline sampling program could provide the spatial and temporal variability in biofilm composition and abundance at Brunswick Point. This sampling could be used to characterize biofilm taxonomy; essential fatty acid profiles; and to correlate to salinity, sediment grain size, currents and wave action. It was the view of Environment and Climate Change Canada that these information gaps identified could be addressed through new studies conducted over a minimum of three years.

Although the Department advised that this information could not be generated within the timeline of the environmental assessment of the proposed Project, information is required to determine whether there are additional samples that are being or could be utilized for further analyses on biofilm**.**

Information Request

Confirm whether the Proponent has collected samples that were not reported in the EIS that could be available for analysis to provide information regarding the spatial and temporal variability in biofilm composition, biofilm taxonomy; essential fatty acid profiles or other factors.

Indicate whether further analyses of these samples are underway or contemplated by the Proponent.

VFPA Response

The VFPA confirms that additional biofilm sampling and analysis have been completed since the submission of the EIS. Sampling was undertaken in both 2016 and 2017. The results and analysis of the 2016 program are presented in this response. The results from the 2017 sampling program are currently being analysed and will be submitted to the Panel registry once reporting is complete.

This response provides the background and rationale for the additional studies as well as commentary on how the 2016 results compare with the findings and conclusions presented in the EIS. Two standalone reports are attached to this response as **Appendices IR8-04-A** and **IR8-04-B**.

The results of the 2016 additional studies complement the findings of the EIS and adds to the overall understanding of biofilm science. In summary, the results align with the following conclusions in the EIS:

- Impacts to western sandpiper (WESA) have largely been avoided through the placement of the marine terminal almost six kilometres away from their main feeding areas on Roberts Bank;
- The availability of food at Roberts Bank with the Project in place will not be limiting for migrating WESA;
- Ongoing salinity monitoring continues to show that under existing conditions there is large variation in salinity across Roberts Bank due to variable Fraser River flow and tidal mixing;
- Biofilm is abundant across the existing salinity gradient, found in both freshwater- and marine-influenced locations; and
- The Project will not affect the availability or quality of food available to migrating WESA.

In addition, the results of the literature review undertaken as part of the 2016 study show that shorebirds migrate long distances successfully with and without polyunsaturated fatty acids (PUFA). Rather, it is the availability of foods that can be converted to monosaturated fatty acid (MUFA) and saturated fatty acid (SFA) that seem to play a more significant role in migratory flight. Regardless, given that PUFA, MUFA, and SFA are found in abundances across the salinity gradient and the fact that the availability of MFA and SFA are primarily driven by mudflat exposure time, the Project will not affect the availability or quality of food available to migrating WESA.

Background to the Additional Biofilm Studies

EIS Studies

As reported in the EIS, biofilm sampling at Roberts Bank was undertaken between 2012 and 2013. The results of the analysis are presented in Section 11.0 of the EIS. Further information is also provided in WorleyParsons (2015*a*, 2015*b*, 2015*c*, 2015*d*, 2015*e*) and TDR MVB-1 in Appendix AIR10-C of Additional Information Request #10 (AIR-12.04.15-10 of CEAR Document #388[1]). The survey design and sample analysis were informed by a number of meetings with Environment Canada (EC) as well as a Shorebirds and Biofilm Technical Advisory Group (TAG). The first meeting with EC was held in December 2011, with the study program presented to EC in the spring of 2012 to solicit feedback prior to initiation of fieldwork. The TAG met on four occasions in the fall of 2012 through the winter of 2013 and included world renowned shorebird, biofilm, and remote sensing experts from Canada, Japan, and the U.S.A. representing government, university, and industry. Information gained through the TAG process informed the 2013 study program.

[1] CEAR Document #388 From Port Metro Vancouver to the Canadian Environmental Assessment Agency re: Completeness Review - Responses to Additional Information Requirements Follow-Up (See Reference Document # 345) including 22 Technical Data Reports.

Based on this feedback from consultation, a suite of studies was initiated on shorebirds, biofilm, and invertebrates to better understand the existing supply and availability of these food sources to shorebirds, in particular WESA. The suite of studies is described in Tables 11-4, 12-4, and 15-5 of the EIS.

The assessment on biofilm was structured around changes to biomass and diversity (see Table 11-2 of the EIS). The density of the photopigment Chlorophyll *a* was used as an indicator to detect changes to biomass whilst biofilm assemblage composition was used as an indicator to detect changes to diversity. In addition, information on biofilm distribution, fat, and carbohydrates was also collected. In respect to fat, information on total lipids was sampled in the 2013 biofilm sampling program with the results presented in WorleyParsons (2015*a*). As reported in Section 4.1 and in Figure 4.1-4 of WorleyParsons (2015*a*), total lipids were found in spring, summer, and winter with spring and summer possessing the highest and comparable lipid levels. Spatially, there were no statistical differences in total lipid concentrations at Roberts Bank between freshwater-influenced and marine water-influenced locations in areas used by WESA north of the Roberts Bank causeway.

Information on fatty acids was also collected as part of a study on August 6, 2013 with the results presented in TDR MVB-1 in Appendix AIR10-C (AIR-12.04.15-10 of CEAR Document #388). The purpose of the study was to determine, using fatty acid signatures, whether historic salt marshes had been present at Roberts Bank as a precursor to the biomat habitat currently present at Roberts Bank. Whilst the study was not specifically undertaken on biofilm, the results provide useful context regarding the presence of fatty acids in other primary producing habitats at Roberts Bank. The results confirmed that high levels of PUFAs (also referred to as essential fatty acids) comprised 24% to 45% of the total fatty acid concentrations in areas in the upper intertidal and in the inter-causeway area.

The results of these studies, conducted in 2013, indicate that total lipids and fatty acids are present across seasons and Roberts Bank in a range of habitats.

Environment and Climate Change Canada Consultation Post EIS Submission

Since submission of the EIS in March 2015, the VFPA has held a number of meetings with Environment Canada / Environment and Climate Change Canada (ECCC) on the results of the coastal bird and biofilm assessment. EC staff raised the issue of lipid rich diatoms and their potential importance to WESA at a meeting held on August 14, 2015.

In a subsequent meeting held on November 23, 2015, the VFPA team met with ECCC and other federal agencies to discuss the Roberts Bank ecosystem model. At this meeting, ECCC provided an overview of the potential importance of highly unsaturated fatty acids (HUFA) and PUFA properties of diatoms and their importance to WESA. ECCC stated that they would be conducting fieldwork in spring 2016 to better understand fatty acids in diatoms. At this meeting, the VFPA agreed to meet with ECCC to better understand this issue. In a meeting held on February 18, 2016, ECCC presented to the VFPA a suite of studies they and others planned to undertake in the forthcoming northward migration period.

Following this meeting, in light of the feedback received from ECCC, the VFPA made the decision to undertake further biofilm sampling in the forthcoming northern migration period in April and May 2016. Recognising that both the VFPA and ECCC would be sampling at Roberts Bank at the same time, a data sharing agreement was entered into by both parties. The data sharing agreement set out the terms on which both parties would share their respective 2016 data. The agreement was finalised on May 26, 2016 and is valid through to December 16, 2017. VFPA data for the 2016 program was shared with ECCC on November 23, 2017.

VFPA 2016 Study Program Aims and Objectives

In line with ECCC's submissions to the Panel (CEAR Documents #960[2], #574[3], and #581[4]), and information collected as part of the EIS, the VFPA scoped the two studies to obtain information to answer the following questions:

- Question 1: What are the key diatoms that WESA require to meet their nutritional needs during spring migration?

  This question was posed based on comments raised by ECCC as articulated in CEAR Documents #574 and #581, such as:

  - "*Biofilm is made up of a variety of diatom assemblages, which vary spatially and temporally in response to fluctuating environmental conditions*" (CEAR Document #581);
  - "*predicted changes in water salinity…*" due to the Project *"could result in the elimination of specific marine influenced diatom species in the area where foraging activity of the Western Sandpiper is most intense (where marine-influenced biofilm is predicted to decrease)*." (CEAR Document #574); and
  - "*Preliminary data suggest that certain energy- and lipid-rich diatom species are key components of the marine-influenced biofilm of the upper intertidal zone. The EIS does not identify the diatom species that are believed necessary for successful Western Sandpiper migration*" (CEAR Document #581).

- Question 2: Are there differences in WESA usage across Roberts Bank?

  This question was posed based on comments raised by ECCC as articulated in CEAR Documents #574 and #581, such as:

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[2] CEAR Document #960 From Environment and Climate Change Canada to the Review Panel re: Response to Information Requests issued by the Review Panel on April 5, 2017 (See Reference Document 950).

[3] CEAR Document #574 From Environment and Climate Change Canada to the Review Panel re: Comments on the information relating to the environmental assessment of the Roberts Bank Terminal 2 Project.

[4] CEAR Document #581 From Environment and Climate Change Canada to the Review Panel re: Comments on the information relating to the environmental assessment of the Roberts Bank Terminal 2 Project.

- “*Western Sandpipers forage intensively on biofilm on the upper intertidal areas of Roberts Bank (0-350m from shore)*” (CEAR Document #574); and
- “*Data from the Roberts Bank stopover site suggest that Western Sandpipers depend upon lipid-rich diatom patches blooming in upper intertidal biofilm*.” (CEAR Document #581).

- Question 3: What are the spatial and temporal fatty acid profiles in biofilm during northward migration?

  This question was posed based on comments raised by ECCC as articulated in CEAR Documents #574 and #581, such as:

  - The biofilm at Roberts Bank *“occurs in a narrow temporal and spatial window and is heavily influenced by salinity, as well as temperature*.” (CEAR Document #574); and
  - *“access to lipids, especially omega-3 fatty acids known as Highly Unsaturated Fatty Acids (HUFA) and Polyunsaturated Fatty Acids (PUFA), produced by diatoms in biofilm, may be critical to long-distance migrating shorebirds”* (CEAR Document #581).

- Question 4: What are the key, limiting abiotic and biotic factors influencing the occurrence, distribution, and productivity of the biofilm community?

  This question was posed based on comments raised by ECCC as articulated in CEAR Document #581, such as:

  - “*The key* diatom *species on Roberts Bank may be estuarine in nature; however, the precise salinity range within which the implicated species can survive and bloom has yet to be determined. The physical and/or chemical factors that drive these diatoms to bloom at Roberts Bank are also unknown. Salinity appears to play a primary role, and nutrients, temperature, and light, are also likely important factors influencing the timing and extent of lipid production*.”

- Question 5: Would the key abiotic and biotic factors influencing biofilm be affected by the Project? If so, how and to what extent?

  These questions were posed based on comments raised by ECCC as articulated in CEAR Document #581, such as:

  - “*The key diatom species on Roberts Bank may be estuarine in nature; however, the precise salinity range within which the implicated species can survive and bloom has yet to be determined. The physical and/or chemical factors that drive these diatoms to bloom at Roberts Bank are also unknown. Salinity appears to play a primary role, and nutrients, temperature, and light, are also likely important factors influencing the timing and extent of lipid production*.”

- Question 6: What are the predicted population/species level consequences if the Project causes these key diatom species to become depleted or to disappear altogether?

  This question was posed based on comments raised by ECCC as articulated in CEAR Documents #574 and #581, such as:

  - "research *studies at Roberts Bank suggest that omega-3 fatty acids produced by diatoms (within the biofilm) may be critical for shorebirds to undertake long-distance migrations."* (CEAR Document #574); and
  - "*Without additional information, there is a reasonable possibility that the Project could adversely affect the entire species of Western Sandpiper*." (CEAR Document #581).

The above questions also speak to ECCC's most recent submission to the Panel (CEAR Document #1091[5]) in relation *to "Predicted changes in salinity and associated potential effects to lipid-rich biofilm and shorebirds".*

The first study, 'Shorebird and Biofilm Dynamics during Northward Migration' (**Appendix IR8-04-A**; hereafter 'the Biofilm Dynamics Study'), investigated factors affecting biofilm abundance and community composition at seven sites across Roberts Bank, from freshwater-influenced sites close to Canoe Passage to more marine-influenced sites close to the Roberts Bank causeway. Sampling was initiated prior to WESA arriving at Roberts Bank, continued through migration, and was terminated with the cessation of migration. The study was undertaken from April 18 to May 12, 2016. Information on WESA usage across Roberts Bank was also collected with the aim of comparing the results with previous information collected on usage in 2012 and 2013 as part of the RBT2 environmental assessment. For consistency, samples of biofilm were submitted to the same laboratory at Ryerson University used by ECCC for fatty acid analysis.

The second study, 'Investigation of Selective Feeding of Biofilm Communities by Shorebirds during Northern Migration' (**Appendix IR8-04-B**; hereafter 'the Selective Feeding Study'), investigated whether WESA selectively feed on specific diatoms. This study involved the collection of fecal droppings from captured WESA on five days between April 21 and April 26, 2016 at Roberts Bank in freshwater- and marine-influenced areas. Droppings were preserved, and their contents subsequently sent for taxonomic analyses.

Results from the VFPA's 2016 biofilm studies and information from existing RBT2 studies and peer-reviewed literature pertinent to addressing the questions outlined above are presented below.

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[5] CEAR Document #1091 From Environment and Climate Change Canada to the Review Panel re: Response to Information Requests issued by the Review Panel on September 27, 2017 (See Reference Document #1063).

Question 1: What are the key diatoms that WESA require to meet their nutritional needs during spring migration?

Similar to previous studies (WorleyParsons 2015*a*, *b*, *c*), the Biofilm Dynamics Study documented a diversity of diatom genera (n = 55) within the biofilm community at Roberts Bank during WESA migration. Also, similar to previous studies, diatoms of the genera *Nitzschia*, *Navicula*, and *Achnanthidium* comprised the dominant genera at all sites (i.e., freshwater- and marine-influenced sites). Statistical analyses found no difference in community composition between sites, and no difference between the composition of freshwater-influenced sites close to Canoe Passage and marine-influenced sites in the upper intertidal zone west of the Roberts Bank causeway in the 2016 samples.

Similar to the Biofilm Dynamics Study, the Selective Feeding Study documented a diversity of diatom genera (n = 28) in WESA fecal droppings, with *Nitzschia, Navicula,* and *Achnanthidium* dominating WESA diets, comprising 92% of the diatoms consumed at both freshwater- and marine-influenced sites. These three genera were found in every dropping sample besides one, which was comprised of 100% *Navicula*. All other genera were documented in less than two-thirds of droppings in low abundances. Results from the Selective Feeding Study indicated diets of WESA at Canoe Passage and Upper Intertidal sites were not statistiscally different, indicating WESA fed on the same abundant diatoms in freshwater- and marine-influenced locations.

Results also indicated the potential for selective feeding by WESA on diatoms in the common diatom genera *Navicula* and *Achnanthidium. Navicula* comprised the largest portion of diatoms in WESA droppings at both Canoe Passage and Upper Intertidal sites (45% and 43%, respectively, from April 23 to 26). These porportions are approximately 10% to 15% higher than those documented in biofilm sediment samples (i.e., 36% and 26%, respectively) collected during the same periods within the same sites. This phenomina also occurred for the genus *Achnanthidium*, possibly indicating preferential foraging for *Navicula* or *Achnanthidium*. Conversely, proportions of *Nitzschia* found in WESA droppings were below those documented in sediment samples, possibly indicating avoidance of *Nitzschia*. Although these results were not statistically significant, the similarity of findings from two distinct locations provides an indication of potential selective foraging on these three genera of diatoms, which have been documented to be abundant across Roberts Bank during northward migration (WorleyParsons 2015*a*, **Appendix IR8-04-A**).

While potential evidence for selective feeding by WESA was observed, the phenomena was documented in both freshwater- and marine-influenced habitats on common, abundant genera that are available across the salinity gradient. Also, as fatty acids abundance was similar in both freshwater- and marine-influenced habitats (see below), results from the 2016 studies, and previously documented WESA distribution and abundance patterns (Hemmera 2014), provide evidence that marine-influenced habitats are not unique or preferred.

Question 2: Are there differences in WESA usage across Roberts Bank?

Consistent with previous research (TDR CB-1 in Appendix AIR10-C of Additional Information Request #10 (AIR-12.04.15-10 of CEAR Document #388, EIS Appendix 15-B)), the Biofilm

Dynamics Study showed the highest shorebird usage during northward migration occurring in habitats close to Canoe Passage and in the Upper Intertidal zone close to Brunswick Dyke. Furthermore, during the 2012 and 2013 WESA northward migration, sandpiper use of freshwater-influenced locations close to Canoe Passage was consistently as high or higher than more marine-influenced areas close to the Brunswick Dyke / Roberts Bank Causeway (TDR CB-1) indicating a potential preference for foraging in more freshwater-influenced habitats. Shorebirds feeding in saline environments must expend energy to osmoregulate salts consumed when foraging, which can be energetically expensive (Gutiérrez et al. 2011). Research has shown that foraging in more saline environments can increase a shorebird's basil metabolic rate and daily energy consumption by 17% and 20%, respectively (Gutiérrez et al. 2011) making more saline habitats energetically more expensive. As a primary goal of WESA during northward migration is to reach the breeding grounds as quickly as possibly to secure breeding territories, feeding in habitats that meet nutritional needs while minimising physiological demands is likely a preferred strategy. These physiological benefits coupled with the availability of the same community of diatoms in freshwater- and marine-influenced habitats, abundant fatty acids close to Canoe Passage (see below), and the open mudflats of Canoe Passage being a potentially safer foraging location from hunting falcons compared to the more enclosed Upper Intertidal zone may make freshwater-influenced areas within Roberts Bank preferred habitats and also explain shorebird distribution patterns documented in 2012 and 2013.

Question 3: What are the spatial and temporal fatty acid profiles in biofilm during northward migration?

Spatially, the Biofilm Dynamics Study documented a large number of fatty acids (28) in biofilm across all sites. These fatty acids were documented at all sites on nearly all surveys, indicating the suite of fatty acids was produced across the salinity gradient from freshwater- to marine-influenced sites. Additionally, fatty acid levels at sites close to Canoe Passage were often similar to sites in the marine upper intertidal zone. This distribution of high fatty acids abundance in freshwater- and marine-influenced areas aligns with the previously documented high Chlorophyll *a* and lipid abundances (WorleyParsons 2015*b*) and WESA usage (TDR CB-1 in Appendix AIR10-C of CEAR Document #388) in the Canoe Passage and Upper Intertidal areas during northward migration. In addition, total PUFA levels were found in similar abundance between sites located in freshwater- and marine-influenced areas indicating they were available to foraging WESA using both habitat types.

Temporally, the Biofilm Dynamics Study findings indicate that in 2016, overall biofilm Chlorophyll *a* and fatty acid abundances did not differ during the duration of the study (April 18 to May 12). The high abundance of fatty acids at Roberts Bank has been documented to be sustained through the summer, as prior studies conducted in support of the RBT2 Project found biofilm-associated lipid and fatty acid levels to be present during the summer period coinciding with WESA southward migration (Hemmera 2013, WorleyParsons 2015*a*). Furthermore, results from the 2013 study found lipid levels in spring and summer did not differ at Roberts Bank, but were statistically much lower in winter (WorleyParsons 2015*a*).

The regime of high fatty acid levels in spring and summer and lower levels in winter more accurately support a paradigm similar to deciduous trees that leaf out in the spring as conditions improve, maintain their productivity through summer, and eventually drop their leaves in fall becoming dormant when conditions become less favourable in winter. Based on the existing data, it is likely that fatty acid levels at Roberts Bank increase in spring prior to WESA arriving on site, potentially coinciding with the annual late-March phytoplankton bloom in the water of the Strait of Georgia (Allen and Wolfe 2013), and are maintained through summer.

Question 4: What are the key, limiting abiotic and biotic factors influencing the occurrence, distribution, and productivity of the biofilm community?

A primary objective of the Biofilm Dynamics Study was to investigate factors affecting Chlorophyll *a* and fatty acid abundance during northward migration at Roberts Bank. The key factors positively influencing biofilm were increases in the amount of time mudflats were exposed (i.e., not inundated) and increases in the 5th percentile water column salinity (PSU). Increases in mudflat exposure time were the primary factor driving total fatty acid, MUFA, and SFA abundance. For total MUFA and most SFA modelled, there was little evidence of other abiotic factors influencing abundance levels. For factors affecting total fatty acid abundance, modelling indicated a weak to moderate influence from increases in 5th percentile water column salinity. In contrast, most PUFA levels were positively and more strongly influenced by increases in 5th percentile water column salinity and were weakly to moderately influenced by increases in mudflat exposure time. Evidence that water column temperature affected biofilm abundance were limited.

Question 5: Would the key abiotic and biotic factors influencing biofilm be affected by the Project? If so, how and to what extent?

Results from the Biofilm Dynamics Study indicate that the Project is unlikely to affect the availability of fatty acids (i.e., MUFA, SFA, and PUFA) to northward migrating WESA. As MUFA and SFA levels are primarily associated with mudflat exposure time and not changes in salinity, results from the 2016 Biofilm Dynamics Study suggest that a consistent supply of MUFA and SFA are available annually, regardless of the salinity regime. In addition, the study found numerically lower, but not statistically different, levels of PUFA under very ‘fresh’ conditions close to the Fraser River outflow compared to more marine locations close to the Roberts Bank causeway. This indicates that despite the effects of salinity, diatoms produce PUFA in both freshwater- and marine-influenced habitats in roughly similar abundances at Roberts Bank. It is therefore likely that regardless of the salinity change brought about by the Project, PUFA will be found across Roberts Bank during WESA northward migration.

Results from this study also inform biofilm and shorebird ecology within the Fraser River estuary that go beyond potential salinity changes brought on by the Project. The size and timing of the annual Fraser River spring freshet[6] have been documented to fluctuate greatly, ranging from peak discharges of 535 m^3/second to 8,500 m^3/second during WESA northward migration since 1912. This range in discharges likely causes the salinity regime at Roberts Bank to vary greatly and likely overwhelms potential salinity changes brought about by the

[6] Freshet: the seasonal flood of freshwater from a river from melted snow into the sea.

Project. Regardless of the size of Fraser River discharge and resulting salinity regime, results from the Biofilm Dynamics Study suggest there is an existing abundance of MUFA, SFA, and PUFA that occur in biofilms across the salinity spectrum annually during spring migration that will be unaltered with the Project in place. Therefore, it stands to reason that the potential seasonal changes to salinity levels at Roberts Bank with the Project in place will have little effect on overall fatty acid availability to foraging WESA or their ability to successfully migrate.

Question 6: What are the predicted population/species level consequences if the Project causes these key diatom species to become depleted or to disappear altogether?

Results from the Biofilm Dynamics Study provides evidence that the Project is unlikely to affect the availability of fatty acids (including essential fatty acids) during WESA northward migration. Therefore, population consequences resulting from the Project are also considered unlikely. This is further reinforced in the scientific literature where there is some debate concerning whether PUFA consumption is required to facilitate long-distance flight (Maillet and Weber 2006, 2007, Nagahuedi et al. 2009, Guglielmo 2010).

For example, research shows that shorebirds migrate long distances successfully with and without consuming PUFA, placing the critical nature of PUFA in question (Guglielmo 2010). However, what is critical to fueling WESA migratory flight appears to be the availability of foods that can be converted to MUFA and SFA that are stored by WESA in adipose tissue during migration (Egeler and Williams 2000). Previous research has shown that 80% to 90% of all fatty acids stored by WESA to fuel migratory flight are C_{16} and C_{18} fatty acids (i.e., palmitate, 16:0; palmitoleate, 16:1; stearate, 18:0; oleate, 18:1) (Egeler and Williams 2000). In contrast, PUFA comprise approximately 5% of stored fats, with long-chained omega-3 fatty acids comprising less than 1% of stored fats (Egeler and Williams 2000). Therefore, PUFA and omega-3 fatty acids are not considered a primary factor in directly fueling migration. The Biofilm Dynamics Study documented MUFA and SFA comprising approximately 74% of the total fatty acids available at Roberts Bank. Regardless of whether PUFA are essential to sustaining WESA migration, results from the Biofilm Dynamics Study indicate omega-3 and omega-6 PUFA are produced by biofilm across the existing study area from freshwater- to marine-influenced sites.

One of the primary results from the Biofilm Dynamics Study was the finding of strong positive correlations among biofilm productivity parameters (i.e., Chlorophyll *a*, fatty acids, and carbohydrate levels) indicating effects to biofilm are expressed similarly across the suite of biofilm parameters. This finding indicates that Chlorophyll *a* is potentially a good proxy for fatty acid levels in biofilm. The VFPA initiated a second expanded biofilm dynamics study during spring 2017, in which this finding will be further investigated. Data from the 2017 study are currently being analysed and will be available in early 2018.

Update on 2017 Biofilm Sampling Program

A second field season of the Biofilm Dynamics Study was conducted in the spring of 2017. Chlorophyll *a*, fatty acid, carbohydrate, and taxonomy data have recently been received from the labs and analyses of the data is currently underway.

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Appendices

Appendix IR8-04-A	Shorebird and Biofilm Dynamics during Northward Migration
Appendix IR8-04-B	Investigation of Selective Feeding of Biofilm Communities by Shorebirds during Northern Migration

APPENDIX IR8-04-A

SHOREBIRD AND BIOFILM DYNAMICS DURING NORTHWARD MIGRATION

Shorebird and Biofilm Dynamics during Northward Migration

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EXECUTIVE SUMMARY

The western sandpiper (*Calidris mauri*, WESA) is a small migratory shorebird whose distribution is restricted to North and South America. During northward migration, the intertidal region of Roberts Bank supports approximately half of the migrating WESA population using the Fraser River estuary (FRE). Recent research has shown that WESA at Roberts Bank derive between 38% to 68% of their total daily energy requirements from biofilm, a thin (~2 mm), dense layer of predominantly organic material found on, and just below, the surface of freshwater, marine, and estuarine sediments.

The biofilm at Roberts Bank is believed to be derived from microphytobenthos (diatoms) found in marine ocean waters, freshwater outflow from the Fraser River (Canoe Passage), and diatoms inhabiting the FRE. Each species of diatom is adapted to a particular set of environmental conditions under which they photosynthesise, grow, and produce Chlorophyll *a*, fatty acids, carbohydrates, and protein (amino acids) that provide energy and nutrients to foraging birds, marine invertebrates and fish. Certain types of fatty acids, such as polyunsaturated fatty acids (PUFA, e.g., eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)) and monounsaturated fatty acids (MUFA, e.g., oleic acid and palmitoleic acid) produced by biofilm-associated diatoms are theorised to be nutritionally and/or physiologically important to long distance migrants, such as WESA. To examine the relationship between abiotic factors, biofilm parameters (fatty acid, Chlorophyll *a*, and total carbohydrate abundance), and microphytobenthos community composition a study was conducted during the 2016 WESA northward migration at Roberts Bank.

Twenty-eight fatty acids were identified and quantified in the biofilm community during the study. Most fatty acids (>99%), Chlorophyll *a*, and Total Carbohydrates were found at all stations, during every sampling event. The fatty acids found in greatest abundance were the saturated fatty acid (SFA) palmitic acid (16:0), the MUFA palmitoleic acid (16:1n-7), the PUFAs EPA (20:5n-3), and DHA (22:6n-3). Palmitic acid and palmitoleic acid are known to be important contributors to WESA fattening during northward migration, and EPA and DHA are believed to be potentially important factors in facilitating long distance migratory flight.

Statistically significant positive correlations among biofilm parameters (i.e., Chlorophyll *a*, fatty acids, and total carbohydrate levels) were documented during the study indicating effects to biofilm are expressed similarly across the suite of productivity parameters. As Chlorophyll *a* is one of the easiest and most cost-effective biofilm parameters to assess, it is potentially good proxy for assessing fatty acid abundances at Roberts Banks.

During spring migration, stations A, C, and I, having the longest mudflat exposure time, regularly possessed greater abundance of many biofilm parameters than other stations. Station I, located close to Canoe Passage, typically possessed levels similar to stations A and C, located close to the Roberts Bank causeway, indicating the potential presence of elevated biofilm fatty acid and Chlorophyll *a* abundance across the salinity gradient. Station X was located at a lower tidal elevation than most sites, resulting in shorter exposure periods, and regularly possessed the lowest levels of the suite of biofilm parameters analysed. Total Chlorophyll a, fatty acids, PUFA, MUFA, and SFA abundance levels at Roberts Bank did

not change over the duration of the 2016 northward migration; although levels varied at stations within the study area.

Six of the 28 fatty acids, plus 6 summary fatty acids measures (Total Fatty Acids, Total PUFA, Total MUFA, Total SFA, Total Omega 3, and Total Omega 6 fatty acids) and Chlorophyll *a* and Total Carbohydrates were analysed to determine factors influencing their abundance (i.e., mg biofilm parameter/m^2). The amount of time mudflats were exposed (i.e., not inundated) and the 5^{th} percentile water column salinity (PSU) were positively correlated with the abundance of numerous biofilm parameters. Increases in mudflats exposure time were the primary factor driving Total Fatty Acid, MUFA, and SFA abundance. For Total MUFA (including 16:1n-7), and most SFA modelled, there was little evidence of other abiotic factors influencing abundance levels. For factors affecting Total Fatty Acids, modelling indicated a weak to moderate influence from increases in 5^{th} percentile water column salinity. In contrast, most PUFA levels were positively and more strongly influenced by increases in 5^{th} percentile water column salinity and were weakly to moderately influenced by increases in mudflat exposure time. An exception to this was EPA abundance, which was weakly to moderately influenced by mudflat exposure time and $5^{th}/95^{th}$ percentile salinity levels. Evidence for changes to water column temperature to influence biofilm abundance levels was limited.

MUFA and SFA comprised 74% and PUFA comprised 26% of Total Fatty Acid levels. As MUFA and SFA levels are primarily associated with mudflat exposure time and not changes in salinity, results from this study suggest that MUFA and SFA abundance should be less influenced by the Fraser River freshet, which annually inputs large quantities of freshwater over Roberts Bank mudflats during WESA migration. As PUFA abundance appear to be primarily influenced by salinity levels it is likely that PUFA levels across Roberts Bank fluctuate annually in association with the size and timing of the Fraser River freshet whose maximum discharge has been documented to fluctuate 15-fold, between 535 m^3/second and 8,500 m^3/second, during WESA northward migration since 1912. However, this study documented the biofilm community producing PUFA across the salinity gradient from freshwater dominated sites close to the Fraser River to more marine stations close to the Roberts Bank causeway. It is therefore likely that regardless of the size of the spring freshet PUFA are found across Roberts Bank during WESA northward migration.

Similar to previous studies, diatoms of the genera *Nitzschia, Navicula,* and *Achnanthidium* were the dominant genera at all sites. No differences in microphytobenthic community composition among stations across the duration of the study was documented. However, a shift in the overall microphytobenthic community was found between April 25 and 29 after the spring tide. This change was accompanied by decreases in the dominant taxa and the introduction of previously absent taxa (e.g., *Planothidium* spp., *Tryblionella* spp., *Fragilaria* spp., and *Encyonema* spp.).

Key findings of this research are:

1. A large number of fatty acids (28) were documented in the biofilm community during the study. All fatty acids were documented across Roberts Bank from freshwater- to marine-influenced intertidal sites.
2. Fatty Acids in greatest abundance were the SFA palmitic acid (16:0), the MUFA palmitoleic acid (16:1n-7), and the PUFAs EPA (20:5n-3), and DHA (22:6n-3). MUFA, PUFA, and SFA abundances were found at similar levels at freshwater and marine-influenced sites. MUFA and SFA comprised 74% and PUFA comprised 26% of total fatty acid levels. MUFA and SFA are the primary fatty acids used by WESA to fuel migration (comprising ~95% of stored fats) and are therefore available to foraging WESA across the salinity gradient. The role of PUFA, specifically omega 3 and 6 fatty acids, to facilitate long-distance flight is a topic of debate within the scientific literature. Regardless, omega 3 and 6 PUFA were available to foraging WESA across the salinity gradient at Roberts Bank during northward migration.
3. No statistical change in fatty acid abundance was documented over the duration of the study, except in two fatty acids that increased in abundance. This increase appeared to be driven by elevated levels across stations on the final day of sampling (May 12).
4. The biofilm parameter Chlorophyll *a*, which has been used in prior research to assess biofilm biomass, was statistically and positively correlated with the abundance of numerous fatty acids. This finding indicates the potential use of Chlorophyll *a* abundance as a proxy for assessing fatty acid abundances.
5. MUFA and SFA levels were documented to be primarily driven by increases in mudflat exposure time, with some evidence they were affected by changes in water column salinity levels. Therefore, results suggest that MUFA and SFA abundance should be less influenced by the annual Fraser River freshet, which inputs large quantities of freshwater over the Roberts Bank mudflats, and should be annually abundant at freshwater- and marine-influenced sites during WESA northward migration.
6. PUFA abundance was documented to be primarily influenced by salinity levels. Therefore, it is likely that PUFA levels across Roberts Bank fluctuate annually in association with the size and timing of the Fraser River freshet. Although PUFA levels were positively associated with increases in salinity, the affect from salinity did not translate in large differences in total PUFA abundance between freshwater- and-marine influenced sites (i.e., abundances were not statistically different between sites). This study also documented the biofilm community producing PUFA across the salinity gradient. It is therefore likely that regardless of the size of the spring freshet, and associated salinity change, PUFA are found across Roberts Bank during WESA northward migration.
7. A large number of diatom genera (55) comprise the biofilm community during WESA northward migration. However, three genera (*Nitzschia*, *Navicula*., and *Achnanthidium*) dominate the community. During the 2016 northward migration, the dominant genera were distributed across Roberts Bank, with no difference in community composition documented. A parallel study investigating the composition of WESA diet found *Nitzschia*, *Navicula*., and *Achnanthidium* comprised 92% of the diatoms consumed at both freshwater- and marine-influenced sites. These genera have been documented to be abundant during previous northward migrations and potentially form an important part of WESA foraging ecology.
8. The similarity in diatom community composition and fatty acid abundances between freshwater- and marine-influenced sites may explain the previously documented high use of the Canoe Passage and Upper Intertidal areas by WESA during northward migration. As less saline sites have been documented to be physiologically less energy demanding to foraging shorebirds compared to more saline sites, it is possible that the freshwater-influenced Canoe Passage site represents higher quality habitat compared to the Upper Intertidal area close to Brunswick Dyke.

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1.0 INTRODUCTION

1.1 BACKGROUND

The western sandpiper (*Calidris mauri*, WESA) is a small migratory shorebird whose distribution is restricted to North and South America. During northward migration, the intertidal region of Roberts Bank, between the Roberts Bank causeway northwest to Canoe Passage (adjacent to Brunswick Marsh) has been shown to support approximately half of the migrating WESA population using the Fraser River estuary (FRE) during migration (Hemmera 2014). Northward migration occurs between mid-April and mid-May (Butler et al. 1987), with the peak of migration occurring between April 24 and May 3 (Drever et al. 2014). It is estimated that an average of 600,000 sandpipers (~17% of the entire population) use the area between Canoe Passage and the Roberts Bank causeway annually during northward migration (Drever et al. 2014).

Until the mid-2000s, small marine invertebrates (i.e., meio- and macrofauna such as polychaetes and bivalve molluscs) were believed to comprise WESA diet while at the FRE. In 2005, biofilm was first theorised to be consumed by WESA, with the first paper confirming biofilm in WESA diet published in 2008 (Kuwae et al. 2008). Recent research has shown that WESA at Roberts Bank derive between 38% and 68% of their total daily energy requirements from biofilm (Kuwae et al. 2008, 2012, Jardine et al. 2012).

Biofilm is a thin (~2 mm), dense layer of inorganic and organic substances (e.g., microphytobenthos[1], microbes, and organic detritus) found on the surface of shallow freshwater, marine, and estuarine sediments. Biofilm productivity is driven by photosynthetic eukaryotic organisms, primarily diatoms, which excrete extracellular polymeric substances that form a mucilaginous matrix creating a microenvironment that binds the community and promotes attachment to sediment. As the microphytobenthic community is photosynthetic, it is constrained by the depth of maximum light penetration to approximately the top 2 mm of sediments (De Brouwer and Stal 2001, Herlory et al. 2004).

The biofilm at Roberts Bank is believed to derive from diatoms found in marine ocean waters, freshwater outflow from the Fraser River (Canoe Passage), and diatoms inhabiting the FRE. Each species of diatom is adapted to a particular set of environmental conditions. Under optimal conditions diatoms can produce large amounts of biomass (e.g., Chlorophyll *a*, carbohydrates, and protein), while sub-optimal conditions (e.g., changes in salinity, temperature, light concentration or nutrients) that “stress” diatoms can inhibit photosynthesis, but promote fatty acid synthesis (Chu et al. 1996, Tzovenis et al. 1997, Pasquet et al. 2014, Schnurr and Allen 2015). Certain types of fatty acids, such as polyunsaturated fatty acids (PUFA) (e.g., eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)) and monounsaturated fatty acids (MUFA) (e.g., oleic acid and palmitoleic acid) and saturated fatty acids (SFA) produced by biofilm-associated

[1] Microphytobenthos: microscopic, photosynthetic eukaryotic algae (diatoms) and cyanobacteria that live on sediment surfaces

diatoms are theorised to be nutritionally and/or physiologically important to long distance migrants, such as WESA (Guglielmo et al. 1998, Egeler and Williams 2000, Guglielmo 2010).

The Environmental Assessment (EA) conducted on the proposed expansion of the existing container port at Roberts Bank (referred to as the Roberts Bank Terminal 2 Project [RBT2]) stated that the expansion of the terminal may alter the existing water column salinity in areas of Roberts Bank where biofilm currently exists (EIS, v3, s11.6.3.5 (PMV 2015a)). Environment and Climate Change Canada (ECCC) expressed concern that potential salinity changes may affect biofilm-associated fatty acid production at Roberts Bank. To examine the relationship between abiotic factors, such as salinity, and biofilm biomass/fatty acid production in the microphytobenthic community, a study was conducted during the 2016 WESA northward migration period at Roberts Bank.

1.2 Study Objectives

To further understand biofilm ecology, biofilm dynamics during northward migration were studied to:

1) Obtain data on biofilm biomass, fatty acid, and microphytobenthic community composition in areas of known WESA usage across Roberts Bank;
2) Identify and quantify the fatty acids produced by biofilm at Roberts Bank;
3) Investigate spatial and temporal differences in selected biofilm biomass parameters (Chlorophyll *a* and carbohydrate levels) and fatty acid levels at Roberts Bank during the WESA northward migration period;
4) Investigate potential relationships between biofilm biomass parameters and fatty acid levels;
5) Investigate potential correlations between fatty acid abundance and abiotic factors, including water column salinity levels;
6) Identify the microphytobenthic community composition (to diatom genera) found in biofilm at Roberts Bank during northward migration;
7) Investigate temporal changes to the microphytobenthic community during the northward migration period; and
8) Investigate linkages between biofilm parameter[2] abundances and WESA foraging through the collection of shorebird fecal droppings densities (i.e., # WESA droppings/15-m^2) at biofilm sampling locations.

[2] Biofilm parameters: components of biofilm (i.e., Chlorophyll *a*, carbohydrate, and fatty acids) known to be potentially useful to WESA to fuel northward migration.

2.0 METHODS

2.1 STUDY AREA

Biofilm sampling and shorebird droppings surveys were conducted at seven locations on Roberts Bank (**Figure 2-1**); each sampling station incorporated a conductivity/depth/temperature logger to record water column salinity. Stations were distributed across Roberts Bank to cover a range of predicted salinity conditions (NHC 2014). Sampling stations included five of nine long-term salinity monitoring stations deployed in March through the remainder of 2016 (stations A, C, J, I, H), and two temporary stations deployed specifically for this study (stations Y and X) between mid-April and June 2016.

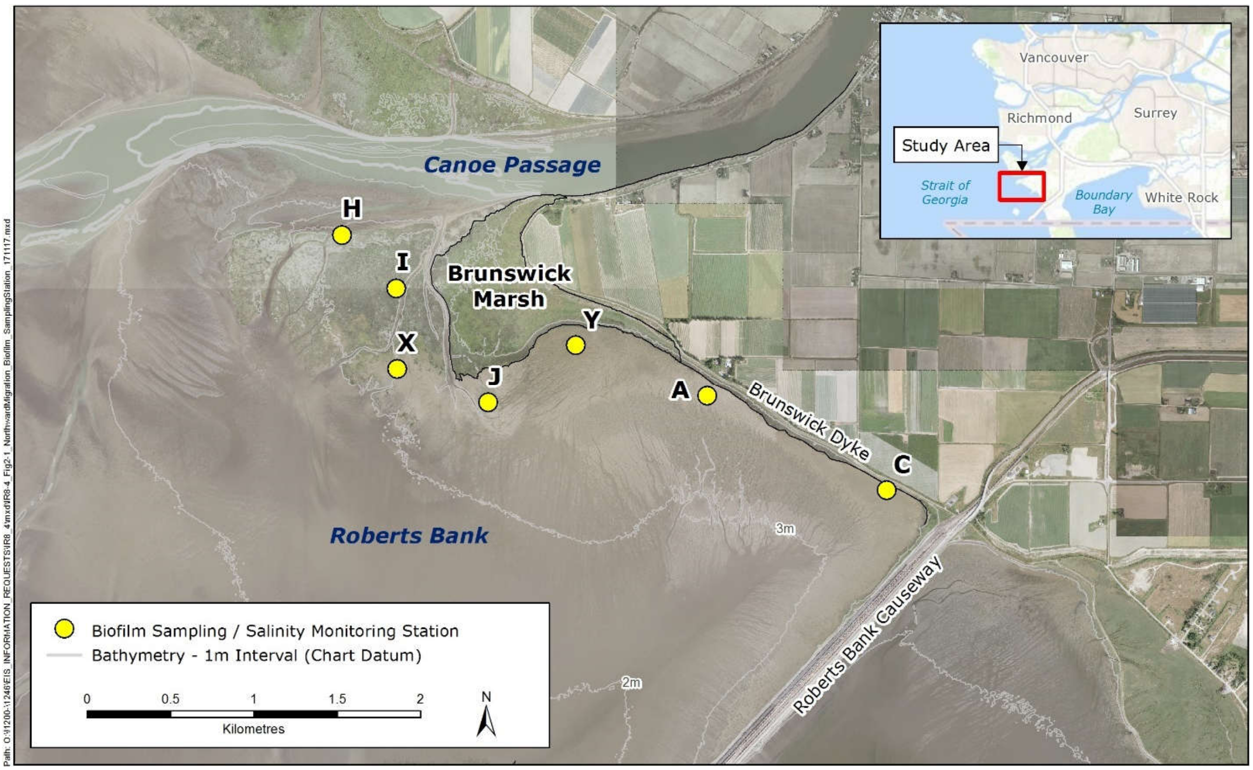

Figure 2-1 Biofilm, Shorebird, and Salinity Monitoring Stations within the Roberts Bank Study Area

2.2 TEMPORAL SCOPE

Biofilm sampling and shorebird droppings surveys were conducted during six sampling events over the course of the northward shorebird migration (**Table 2-1**). Two sampling events were scheduled to occur prior to (April 18 & 23), during (April 25 & 29), and after (May 6 & 12) the typical peak of WESA migration. As biofilm productivity and microphytobenthic community composition can vary with the tidal cycle (WorleyParsons 2015*a*), surveys were also timed to occur across the Spring and Neap tidal cycle to capture predicted highs and lows of the biofilm community during the WESA northward migration period.

Table 2-1 Sampling Event Dates and Tide States

Date of Sampling Event	Tide State
April 18, 2016	Neap-Spring Tide Transition
April 23, 2016	Spring Tide
April 25, 2016	Spring-Neap Tide Transition
April 29, 2016	Neap Tide
May 6, 2016	Spring Tide
May 12, 2016	Neap Tide

2.3 FIELD STUDY METHODS

Details of the methods employed to collect data on biofilm, shorebird usage, and salinity are outlined below. For all data collection, surveyors navigated to the sampling/monitoring stations using handheld GPS units. Biofilm and shorebird droppings samples were only collected on days with no precipitation.

In addition to data collected on biofilm, shorebird droppings and salinity, photographs of the habitat (i.e., substrate and vegetation) were taken during the initial sampling event at each station. Photographs were also taken during each sampling event around stations located in areas on emergent ephemeral marsh (Stations G, H, and I). Photos captured habitat in all cardinal directions from the salinity monitoring station (**Figure 2-2**) and a representative 1 m^2 quadrat at the droppings sampling location (**Figure 2-3**). Surveyors also noted time of day for each station visit, and visually assessed the substrate type (i.e., mud, muddy-sand, sandy-mud, sand), and dominant vegetation (e.g., none, emergent sedges, eelgrass (*Zostera japonica*)) at each station.

Figure 2-2 Example of Habitat Documentation of Survey Stations: Photographs of Habitat in Four Cardinal Directions at Station H

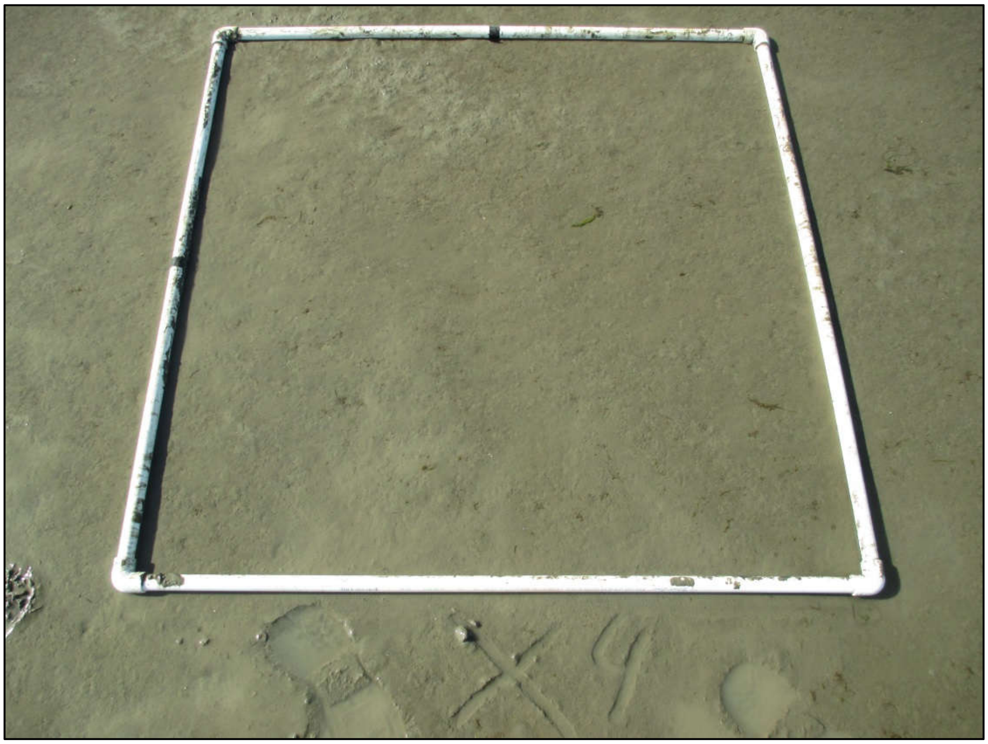

Figure 2-3 Representative Habitat Photo with Quadrat used for Shorebird Droppings Surveys

2.3.1 Biofilm Sample Collection and Storage

Biofilm samples were collected using a 41 mm wide plastic putty knife (hereafter “scraper”) (**Figure 2-4**) to scrape the top 2 mm of biofilm/sediment sample off the sediment surface along a 20 cm length (i.e., 82 cm^2 surface area). The depth of the scrape was controlled with the angled edge of the scraper, which was measured to be 2 mm. This was done by in-field estimation and reflects an approximate sampling depth. The scraper was rinsed with de-ionised water between each sample. Samples from successive surveys were taken adjacent to one another resulting in a total of six side-by-side scrapes at each monitoring station.

During each survey, one sample was collected from each station for Chlorophyll *a*/Total Carbohydrate, and Fatty Acid analysis (see **Section 2.5** for a description of taxonomic classification sample collection and storage methods). The sample was placed in a 60 mL black centrifuge tube, which was labelled and sealed in an individual amber bag with a second label. The samples were stored in a handheld cooler with dry ice until they could be transferred to a -80ºC freezer at the end of the day. The samples were held in the -80ºC freezer until they could be transferred to the laboratory for analysis. All samples were shipped on dry ice and remained below -30ºC during transport.

Figure 2-4 Plastic Putty Knife (with dimensions) Used for Scrape Samples

2.3.2 Biofilm Sample Processing

Forty-five samples (42 biofilm samples plus 3 duplicate samples) were collected in the field during April and May 2016. Chlorophyll *a* and carbohydrate samples were transported to Aquatech Enviroscience Laboratories (AEL) (Victoria, B.C.) for analyses. Per ECCC recommendations, fatty acid samples were transported to Ryerson University (Toronto, ON) and analysed by Dr. Michael Arts and Dr. Peter Schnurr. Samples were placed in Styrofoam coolers with dry ice and transited for less than 24 hours. Upon receiving the samples, laboratory personnel verified the temperature and confirmed the samples were accounted for in the Chain of Custody (COC). A unique laboratory ID number was issued to each sample vial. The laboratory ID number was associated with the field number provided on the centrifuge tube and inside the sealed amber bag to assure tracking of each sample.

The laboratory IDs were logged and a backup data file was created. Each wet sample was weighed, freeze-dried at -60°C, and then re-weighed to determine dry weight. Once dried, the samples were homogenised using a pestle and mortar, grinding the sample until all obvious clumps had been broken and the sample was a fine powder. Separate subsamples from the homogenised sample were used for Chlorophyll *a* (100 to 200 mg subsample) and Total Carbohydrate (100 mg subsample) analyses. Specific methods are described in **Sections 2.4.1** and **2.4.2**.

Three separate biofilm biomass metrics were measured:

- **Chlorophyll *a*** – Chlorophyll *a* is the primary photosynthetic pigment of all oxygen-producing photosynthetic organisms and is present in all algae, cyanobacteria, and other photosynthetic organisms. It is a measure of density and primary productivity potential and used as a measure of biofilm biomass;
- **Total Carbohydrate** – the energy absorbed by Chlorophyll *a* (as well as accessory pigments such as Fucoxanthin) is transformed into glucose, which is a measure of Total Carbohydrate; and
- **Fatty Acids and Fatty Acid Methyl Esters (FAME)** – Fatty acids are the major component of lipids with fatty acid methyl esters being chemically unique types of fatty acids. Fatty acids are considered a measure of caloric and nutrient value for predators, with the fatty acids EPA (20:5n-3), DHA (22:6n-3), palmitic acid (16:0), palmitoleic acid (16:1n-7) and stearic acid (18:0) (amongst others) noted as potentially important fatty acids to long distance migrants such as WESA (Egeler and Williams 2000, Guglielmo 2010). For a list of fatty acids see **Appendix A**.

2.4 BIOFILM PARAMETERS

2.4.1 Chlorophyll *a*

The assessment of Chlorophyll *a* is a common method of estimating biofilm biomass and the potential productivity of photosynthetic organisms in the marine environment (Underwood and Smith 1998, Kuwae et al. 2008, Boyer et al. 2009, Jardine et al. 2012, Macdonald et al. 2012, MacDonald et al. 2012). For this study, the concentration of Chlorophyll *a* in each sample was measured by fluorimetry. Fluorimetry measures the fluorescence emitted by compounds when exposed to ultraviolet or other intense radiant energy. Chlorophyll *a* produces fluorescence of a characteristic colour and wavelength, allowing identification and quantification of several significant compounds in biological specimens.

Protocol

The Chlorophyll *a* content of sediment samples was determined using an extraction procedure utilising a mixture of High Performance Liquid Chromatography (HPLC) grade Acetone:Methanol at a 90:10 ratio. The measurements were performed using a Fluorescent Spectrometer from TURNER (model 7200-000, using Trilogy Module CHL-NA, Model #46, Turner Designs, Sunnyvale, CA, USA).

Prior to extraction, samples were re-lyophilised for two hours to remove any residual water, frost or ice. Chlorophyll *a* was extracted by mixing 50 to 100 mg of dry sediment in 5 mL of degassed mixture of Acetone:Methanol (90:10 by volume) in 15 mL glass tube, then vortexed. The sample tubes were flushed with N2, wrapped in aluminium foil to protect from the light, then stored in the dark at -10 to -20°C for 12 hours, before being vortexed again. The tubes were then centrifuged for five (5) minutes to achieve a better separation of the solvent extract and sediment. An aliquot of the extract (1 to 2 mL) was transferred into a clean 12 x 75 mm disposable glass test tube, where Raw Fluorescence Units (RFU) were read on the fluorimeter.

2.4.2 Total Carbohydrate

Carbohydrates are an important component of the extracellular polymeric substances (EPS) secreted by microphytobenthos found in biofilm. Carbohydrates are found in two different phases: liquid (colloidal) or solid (bound). Carbohydrates that are easily extracted with water from sediments are termed "colloidal carbohydrates" (Underwood et al. 1995) and include EPS and simple sugars (Taylor et al. 1999). The carbohydrates that remain in the sediment after colloidal carbohydrate extraction are termed “bound carbohydrate”. The protocol used to measure the Total Carbohydrate concentration of a sediment sample is designed for samples that have not been subjected to any extraction procedure; therefore, all carbohydrates, including intracellular, extracellular, and particle-bound material, were hydrolysed and measured. Total Carbohydrate levels were determined based on the amount of glucose.

Protocol

The analytical protocol used for Total Carbohydrate in sediments followed the methodologies described by Underwood et al. (1995) and Dubois et al. (1956). The analytical system included a Pharmacia Biotech model Ultraspec 2000 and a UV-Vis Spectrometer.

Total Carbohydrate concentrations were measured by creating a phenol-sulphuric acid assay. Two mL of distilled H2O was added to 100 mg of freeze-dried homogenised sediment in a 15 mL conical plastic tube. Then, 1 mL of 5 % phenol aqueous solution and 5 mL of concentrated H_2SO_4 was added to the mixture. Once the production of gas ceased, the mixture was shaken in a vortex and put in a water bath for one hour at 30ºC. The mixture was then centrifuged at 3,000 rotations per minute (RPM) for 15 minutes. The tubes were allowed to stand for 10 minutes, then shaken and placed for 10 to 20 minutes in a water bath at 30°C. The absorbance of the supernatant was measured using a spectrometer at 485 nm.

2.4.3 Fatty Acids

Fatty acids are a form of lipid used to store energy in organisms and play a important role in the construction of biological structures, such as cell membranes. Marine microalgae including diatoms are the primary producers of certain fatty acids, with certain types of fatty acids known to be important to the health of higher vertebrate organisms (e.g., omega-3 fatty acids such as EPA and DHA) (Guglielmo 2010).

Protocol

Biofilm samples were stored at -80°C. Samples were lyophilised using a Labconco Freezone 2.5 Freeze Drier. Dry biofilm samples were then homogenised with a mortar and pestle. A portion (~3 g) of the homogenised biofilm was used to determine ash free dry weight (AFDW) to normalise the fatty acid data to organic content. It was also used to determine the inorganic content of the biofilm sample (i.e., sediment).

A second portion of the homogenised biofilm was used to determine FAME content. Since 20 mg of organic content was the desired amount of sample to extract from, and the inorganic fraction of the biofilm was high (~92 to 99% inorganic), a calculated mass of sample (organic + inorganic) was determined for each sample. Therefore, depending on the organic content of the respective samples, between ~230 and 1,700 mg of sample was used for initial extractions.

Once samples were prepared for analysis, FAME were extracted using the Folch Method (Folch et al. 1957). During extraction 10.2 µg of tricosylic acid (23:0) was added to each sample as an internal standard. Tricosylic acid was used because it is rarely produced in nature. The aqueous phase of the extractant was discarded, so that only neutral lipid species were considered for analysis. Chloroform was evaporated under a nitrogen blanket prior to re-dissolving extracted lipids in 1 mL of hexane.

Fatty acids were then methylated to produce fatty acid methyl esters (FAME) with a 1% solution of H_2SO_4 in anhydrous methanol and 1 mL of hexane at 90°C for 90 minutes. A VWR Digital 2 Block Heater was used for maintaining the temperature of the tubes during methylation. Fatty acids were extracted twice with 4 mL hexane aliquots and re-dissolved in a known volume of hexane after evaporation under a nitrogen blanket. Aliquots of 50 or 100 µl were used to gravimetrically determine the total FAME content extracted from the biofilms. The remaining fatty acid solution was used to identify and quantify fatty acids within the biofilm sample using gas chromatography (GC) and flame ionisation detection (FID).

All FAME were analysed with an Shimadzu GC-2010PLUS. The column used was a Supelco SP-2560 (100 m x 0.25 mm x 0.2 µm, Agilent). The GC was run on splitless with an initial column temperature of 60°C. The column temperature was then increased to 180°C at a rate of 15°C per minute, followed by a ramping rate of 2°C per minute to 240°C, and a hold time of 5 minutes to elute all remaining fatty acids. The carrier gas was helium flowing at a rate of 1.2 mL/min, while the column flow rate was 1.2 mL/min. Injector and detector temperatures were both 250°C. Peaks were identified by matching retention times of fatty acid standards. Standard fatty acids methyl ester mix GLC68F (Nu-Chek Prep.) was used for the calibration curve and identification of peaks. Additionally, GLC436 (Nu-Chek Prep.) was used for peak identification.

2.4.4 Data Treatment and Statistical Analysis

Biofilm parameters (Chlorophyll *a*, carbohydrates, and fatty acids) were expressed as an estimate of abundance per square meter (e.g., mg fatty acids/m^2) of intertidal mudflats. During field sampling, care was used to remove only the top 2 mm of sediment when collecting biofilm samples. However, slight variations in sampling depth taken over the length of the 20 cm scrape, variation in sediment grain size, and differing levels of water content within sediments led to differing amounts of sediment collected ranging from ~ 6 to 19 g (dry mass). As biofilm is restricted to the top 2 mm of sediment, the variable collection depth can act to “dilute” biofilm concentrations when more non-biofilm containing material, associated with larger sample weights, is collected in a sample (**Figure 2-5a**). Expressing biofilm abundance on a per square meter basis (**Figure 2-5b**), instead of as a concentration per unit weight of sediment, largely corrects for the dilution and variable sediment water content issues and was the rationale for expressing biofilm availability on a surface area basis.

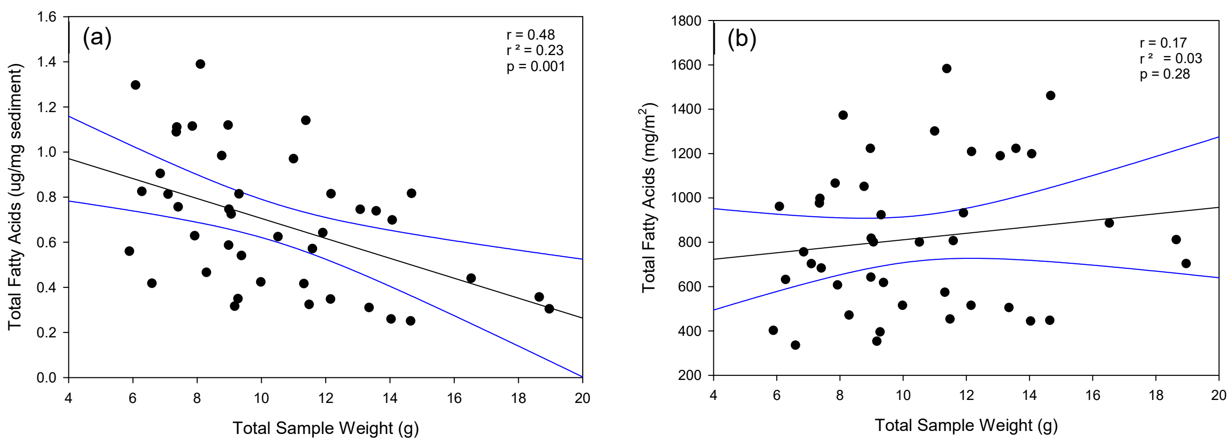

Figure 2-5 Relationship between Total Fatty Acid Levels and the Amount of Sediment Collected during Field Sampling when Expressed as (a) the Concentration of a Biofilm Parameter per Unit Mass of Sediment vs. (b) Abundance per Unit Area of Mudflats

All analyses were completed using SYSTAT 13.1 (Chicago, USA). Prior to analyses, biofilm biomass indicators (Chlorophyll *a*, Total Carbohydrate) and fatty acid were tested for normality using the Shapiro-Wilk test. If parameters were found to possess non-normal distributions, attempts were made to normalise the data using accepted transformations. Transformed parameters are presented in (**Table 2-2**).

Table 2-2 Normality Test Results and Transformations of Biofilm Biomass and Fatty Acid Parameters

Biofilm Parameter	Shapiro-Wilk Statistic (p-value)		Transformation
	Untransformed Data	Transformed Data	
14:0	0.95 (<0.05)	0.95 (0.08)	Square root
14:1	0.91 (<0.01)	0.95 (0.09)	Square root
15:1	0.90 (<0.01)	0.95 (0.08)	Square root
16:1n-7	0.93 (<0.01)	0.95 (0.05)	Square root
17:0	0.92 (<0.01)	0.96 (0.16)	Square root
18:1n-7	0.85 (<0.01)	0.96 (0.12)	Square root
18:1n-9	0.92 (<0.05)	0.96 (0.15)	Square root
18:2n-6	0.93 (<0.05)	0.96 (0.20)	Square root
18:3n-3	0.91 (<0.01)	0.96 (0.12)	Square root
18:3n-6	0.90 (<0.01)	0.97 (0.22)	ln+1
20:0	0.92 (<0.05)	0.96 (0.16)	Square root
20:1n-9	0.92 (<0.05)	0.96 (0.13)	Square root
20:4n-6	0.88 (<0.01)	0.97 (0.44)	ln+1
21:0	0.91 (<0.01)	0.96 (0.18)	ln+1
22:1n-9	0.93 (<0.05)	0.96 (0.15)	Square root
22:2	0.95 (<0.05)	0.97 (0.28)	Square root
22:6n-3	0.94 (<0.05)	0.97 (0.27)	Square root
24:1n-9	0.93 (<0.05)	0.96 (0.21)	Square root
Total Omega 3	0.94 (<0.05)	0.96 (0.21)	Square root
Total Omega 3+6	0.94 (<0.05)	0.97 (0.24)	Square root
Total Omega 6	0.91 (<0.01)	0.96 (0.10)	Square root
Total MUFA	0.95 (<0.05)	0.96 (0.22)	Square root
Total PUFA	0.94 (<0.05)	0.97 (0.24)	Square root
Total Carbohydrates	0.95 (<0.05)	0.96 (0.16)	Square root
Total Sample Weight: Chlorophyll *a*/Carbohydrates	0.92 (0.004)	0.98 (0.82)	ln+1
Total Sample Weight: Fatty Acids	0.94 (0.021)	0.98 (0.53)	ln+1

Summary statistics were calculated for the 28 fatty acids documented in the biofilm community during the study. Eight of the 28 fatty acids known to be potentially important to migrating WESA, plus Chlorophyll *a*, Total Carbohydrates, and 7 summary fatty acids metrics (i.e., Total Fatty Acids, Total MUFA, Total SFA, Total Omega 3, Total Omega 6, Total PUFA, and Total Lipids) were investigated for correlations between parameters. Eight fatty acids plus six fatty acid summary metrics and Chlorophyll *a* and Total Carbohydrates were investigated for spatial and temporal differences and abiotic factors potentially affecting biofilm parameters.

Transformed data were used to test for statistical significance using parametric analyses (e.g., Analysis of Covariance (ANCOVA), Pearson Correlations, General Linear Models (GLM)); however, graphed relationships are presented using untransformed data when necessary to aid interpretation. Despite expressing biofilm abundance on a per square meter basis, the total amount of sediment collected in the field (total sample weight) was found to explain additional variability for some biofilm parameters and was included as a covariate in statistical analyses.

To test for relationships among measured parameters (e.g., Chlorophyll *a*, Total Carbohydrates, fatty acid levels), Pearson correlation analyses were conducted using a Bonferroni adjusted p-value to account for increased likelihood of Type I error (falsely rejecting a null hypothesis) resulting from multiple comparisons.

The effects of temporal and spatial variation (i.e., sample date and station) were tested for significance against the following biofilm parameters:

- Chlorophyll *a* abundance (mg/m^2);
- Total Carbohydrate abundance (mg/m^2); and
- Total and individual fatty acid abundance (mg/m^2).

To analyse the data, repeated one-way ANCOVA tests were used to determine if the measured parameters differed among stations while controlling for total sample weight. Repeated GLMs were used to investigate differences in parameters by sampling dates.

The results obtained with a one-way ANCOVA serve only to indicate whether the means of the dependent variable differ significantly when considering the independent variable. In order to determine specific differences within each variable (i.e., differences among individual stations/dates), a Bonferroni *post-hoc* test was used. The Bonferroni p-value is adjusted to account for multiple tests, allowing for a consistent alpha (α) to control for Type I error. Temporal and spatial interactions of parameters were assessed by reviewing time series trends.

Water column salinity (expressed as practical salinity units [PSU]), water column temperature (C°), and the amount of time mudflats were exposed within a tidal cycle were recorded every five minutes using CTD-Diver dataloggers (Van Essen Instruments 2016) deployed at stations A, C, H, I and J. Fifth, 50th (median), and 95th percentile salinity and temperature estimates were calculated for each station for the previous 24-hour, 48-hour, 72-hour, and 96-hour periods prior to sampling to inform the conditions potentially affecting biofilm parameters. The percentage of time mudflats were exposed (i.e., not covered by water) was calculated for the same periods at each station by determining when the CTD-Diver dataloggers, placed at the mudflat-water interface, at each station were "dry" and not covered by water. This created 28 potential variables (3 percentiles x 4 periods x 2 parameters [salinity and temperature] + 4 mudflat exposure periods) that could be used to inform variables affecting biofilm abundance.

To reduce the number of variables available for modelling and address issues of collinearity, a Pearson Correlation analyses was conducted for each abiotic parameter (e.g., 5th percentile water column temperature) to investigate the strength of correlation between temporal periods (i.e., 5th percentile water column temperature over the 24-hours vs. 48-hours vs. 72-hours vs. 96-hours prior to biofilm sampling). The correlation coefficients for each time period were averaged and the period with the largest mean coefficient was included in model development. All time periods within an abiotic parameter were highly correlated (**Table 2-3**). When coefficients between time periods did not vary (e.g., 48-hour, 72-hour, and 96-hour time periods for 5th percentile water column salinity = 0.99) the 72-hour time period was selected as it was highly correlated within all abiotic parameters across time periods.

Table 2-3 Mean Pearson Correlation Coefficients Calculated across 24-Hour, 48-Hour, 72-Hour, and 96-Hour Periods at Monitoring Stations at Roberts Bank (April 18 to May 12, 2016).

Variable	Duration of Time Prior to Biofilm Sampling			
	24-Hour	48-Hour	72-Hour	96-Hour
5th Percentile Water Column Salinity	0.97	0.99	**0.99**	0.99
50th Percentile Water Column Salinity	0.96	0.97	**0.98**	0.95
95th Percentile Water Column Salinity	0.95	0.96	**0.96**	0.93
5th Percentile Water Column Temperature	0.87	0.88	**0.91**	0.81
50th Percentile Water Column Temperature	0.93	0.96	**0.97**	0.94
95th Percentile Water Column Temperature	0.86	0.93	**0.93**	0.92
Percent Time Mudflat Exposure	0.97	0.98	**0.99**	0.97

To further refine the variables available during Akaike's Information Criterion (AIC) model selection, the distribution of temperature and salinity data within 5th, 50th, and 95th percentile variables were visually inspected and a Pearson's Correlation analyses between variables was conducted. In general, the 50th percentile water column temperature and salinity variables were found to be highly correlated with 5th and 95th percentile measures. During backward stepwise regression modelling (see below), 5th and 95th percentile temperature and water column salinity variables showed the strongest and most consistent correlation with abundance measurements compared to 50th percentile values, with 50th percentile variables not being included in the final model for any biofilm parameter. Therefore, 5th and 95th percentile values were judged to be the most informative, and 50th percentile temperature and water column salinity variables were excluded from AIC modelling. Variables used to model abiotic factors potentially affecting biofilm as part of the AIC modelling are shown in **Table 2-4**.

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Table 2-4 Variables used in AIC Modelling to Model Abiotic Influence on Biofilm Parameters

Variable	Definition
5th Percentile Water Column Salinity	5th percentile salinity for the 72-hour period preceding sample collection
95th Percentile Water Column Salinity	95th percentile salinity for the 72-hour period preceding sample collection
5th Percentile Water Column Temperature	5th percentile water temperature (Celsius) for the 72-hour period preceding the sampling day
95th Percentile Water Column Temperature	95th percentile water temperature (Celsius) for the 72-hour period preceding the sampling day
Percent Time Mudflat Exposure	The percentage of time the sampling station was exposed (i.e., not inundated) during the 72-hour period prior to sampling
Total Sample Weight (g)	The total dry mass of biofilm sediment sample

Backwards stepwise regression and Akaike's Information Criterion (AIC) analyses were used to assess the importance of abiotic factors on biofilm parameters. AIC, an information-theoretic approach that does not restrict variable inclusion into a model based on a variable's significance level, was used to evaluate models (Burnham and Anderson 2002). Models were evaluated using Akaike's Second-Order Information Criterion for small sample size (AIC_c). Models with $\Delta AIC_c < 2$ were considered to have substantial empirical support, $4 \leq \Delta AICc \leq 7$ had considerably less support, and models with $\Delta AICc > 10$ possessed virtually no support (Burnham and Anderson 2002). To understand the importance of each abiotic variable and its contribution to influencing biofilm parameters a balanced variable set of models were evaluated and summed Akiake model weights were calculated (Burnham and Anderson 2002, Arnold 2010). Under this approach, a set of models were constructed that included all combinations of the abiotic variables in **Table 2-4**. This created a model set of 31 models, with each variable represented in 16 models (**Table 2-5**). The summed weights computed using this approach therefore represent a relative weighting of each variable's importance and its ability to influence biofilm parameters such as fatty acids, carbohydrates, and chlorophyll *a*. The variable Total Sample Weight was added as a covariate to each model.

During backward stepwise regression modelling, all candidate variables listed in **Table 2-4** plus median (50^{th} percentile) water column salinity and temperature variables were entered into the model. At each step, the least significant variable with a p-value > 0.05 was removed from the model and previously removed variables were allowed to re-enter the model if they explained more variability than retained variables. This process continued until only statistically significant variables ($p > 0.05$) remained in the model.

Table 2-5 Complete Balanced Model Set used in AIC Analyses

Model ID	Model	# of Parameters (K)
1	5th Percentile Salinity + Total Sample Weight	4
2	95th Percentile Salinity + Total Sample Weight	4
3	5th Percentile Temperature + Total Sample Weight	4
4	95th Percentile Temperature + Total Sample Weight	4
5	Percent Time Mudflats Exposed + Total Sample Weight	4
6	5th Percentile Salinity + 95th Percentile Salinity + Total Sample Weight	5
7	5th Percentile Salinity + 5th Percentile Temperature + Total Sample Weight	5
8	5th Percentile Salinity + 95th Percentile Temperature + Total Sample Weight	5
9	5th Percentile Salinity + Percent Time Mudflats Exposed + Total Sample Weight	5
10	95th Percentile Salinity + 5th Percentile Temperature + Total Sample Weight	5
11	95th Percentile Salinity + 95th Percentile Temperature + Total Sample Weight	5
12	95th Percentile Salinity + Percent Time Mudflats Exposed + Total Sample Weight	5
13	5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	5
14	5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	5
15	95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	5
16	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + Total Sample Weight	6
17	5th Percentile Salinity + 95th Percentile Salinity + 95th Percentile Temperature + Total Sample Weight	6
18	5th Percentile Salinity + 95th Percentile Salinity + Percent Time Mudflats Exposed + Total Sample Weight	6
19	5th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	6
20	5th Percentile Salinity + 5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	6
21	5th Percentile Salinity + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	6
22	95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	6
23	95th Percentile Salinity + 5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	6
24	95th Percentile Salinity + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	6

Model ID	Model	# of Parameters (K)
25	5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	6
26	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	7
27	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	7
28	5th Percentile Salinity + 95th Percentile Salinity + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	7
29	5th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	7
30	95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	7
31	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	8

2.4.5 Field Duplicates

Field duplicates were collected to assess the variability due to the data collection method and noted high spatial variability. Three field duplicates were collected per field sampling equalling ~ 7% of the total samples collected. The laboratory duplicate was run on a randomly assigned sample to measure the precision of the sample analysis and methodology of each analysis. Field duplicates were collected to measure the precision of sampling effectiveness (e.g., is the sample an appropriate representation of the site). The field duplicates were collected from the same sampling station, though a cleaned core and scraper were used to collect the sample. A comparison of the field duplicate to the sample was conducted to establish the percent variability in data due to sample collection protocols.

2.5 TAXONOMY

2.5.1 Sample Collection and Storage

One sample was collected at each site during each survey event for taxonomic identification. The sample was placed in a 60 mL black centrifuge tube, filled with 5% Lugol's solution in filtered seawater, and gently shaken to mix the sediment and the preservative solution. The tubes were labeled and placed in an individual sealable amber bag with a second label. The samples were stored in a backpack and transported to a light-proof cooler at the end of each day. The samples were held in a dark cooler at room temperatures until they were transferred to EcoAnalysts Inc. (Moscow, Idaho) for taxonomic identification to genus level.

2.5.2 Sample Processing

EcoAnalysts used the Palmer Maloney counting chamber method for counting and identifying microphytobenthos to genus level. To homogenise and evenly disperse the cells, samples were gently inverted a minimum of 30 times, then 0.1 mL of the sample was randomly collected and placed in a Palmer Maloney counting chamber using a micropipette. The counting chamber contains the sample within a defined area on a glass microscope slide.

A Leica DMIL Light Emitting Diode (LED) compound light microscope with 20X, 40X, and 63X objective magnifications were used to visually identify microphytobenthos. This microscope was integrated with a Leica DFC450 digital camera (5 megapixel resolution). The combination of microscope and camera allowed single cells greater than ~ 4-5 µm to be identified. Identification was focused on nanoplankton species. Once in the counting chamber, the samples were assessed for concentration.

Optimal efficiency and accuracy of identification/enumeration occurs at ~ 15-20 units per field of view. If the sample concentration was too high or low, the subsample was adjusted by adding or removing small volumes of sample and filtered seawater. If the volume was changed, the new volume and concentration were recorded on the datasheet.

Individual cells or natural units were identified using the latest taxonomic references (Tomas 1997, Sheath and Wehr 2003, Siver et al. 2005, John et al. 2011). The term natural units is used to distinguish between a single cell and colonial algae. Colonial algae can form aggregations of multiple cells. In this instance, the aggregation is defined as one natural unit. For each multi-cellular natural unit, the total number of cells were counted.

Microphytobenthos were identified and counted to the genus level until 300 natural units were encountered. Counts were conducted using the transect method, where taxonomists follow a straight line across the entire counting chamber, enumerating and identifying all organisms within the field of view. The identification and enumeration process takes approximately an hour to complete per sample; however, instances did occur where samples were in extremely low densities, thus making it difficult to achieve 300 natural units within a reasonable amount of time. In order to process these samples in an efficient manner, samples with low densities were enumerated for a maximum period of two hours. This level of effort was deemed sufficient to provide comparable taxonomic densities.

2.5.3 Data Analysis

Calculating Cell Density

To estimate microphytobenthos cell density in each sample, the length, width, and depth of each counting chamber were recorded and multiplied to calculate the volume. If applicable, the counted volume was multiplied by the dilution or concentration factor. This density was then multiplied by the total sample volume (mL) to provide a total number of cells per sample. The number of cells per sample was extrapolated to the entire 1 m^2 quadrat. The total number of cells per sample was divided by the surface area and multiplied by 10,000 cm^2/m^2.

Community Structure Indices

Measures of community structure, including total and individual taxa density were graphed to illustrate spatial and temporal patterns.

2.5.4 Community Analysis

Taxonomic density data were imported into PRIMER-E (Plymouth, UK) and log+1 transformed to increase normality. The Bray-Curtis coefficients were calculated for all pairwise combinations of samples and plotted using a non-Parametric Multidimensional Scaling Analysis (nMDS). The nMDS is a dimensionless ordination plot where axes are unitless and the distance between points is relative to their similarity. Communities plotted close to each other are considered to be similar while communities plotted farther away are considered to be dissimilar. A Cluster Analysis using group average linkages was conducted to provide visual comparisons of the different levels of similarity.

Analysis of Similarity

To test for spatial and temporal difference in taxonomic composition, community similarity coefficients were assessed using the Analysis of Similarity (ANOSIM) test in PRIMER-E. The ANOSIM analysis uses the Bray-Curtis coefficients to rank the order of dissimilarity among defined groups. An R-statistic is calculated based on the difference of the mean rank similarity between and within groups as a factor of the total number of samples. The R-statistic is a scaled ratio between -1 and 1 with values close to zero indicating high similarity. A positive value means most similar samples are within a defined group; negative values means most similar samples are outside of a defined group. To test for significance of the R-statistic, random permutations are run with samples being randomly assigned to groups. The R-statistic from the random permutations is then compared against the observed R-statistics to determine if it is significantly different compared to random groupings. If the R-value is significant, it concludes that the samples within groups are more similar (to the degree of the R-value) than would be expected by random chance.

Similarity Percentage

A Similarity Percentage (SIMPER) analysis was undertaken in PRIMER-E to identify individual taxa and their influence in different temporal and spatial samples. The SIMPER analysis (Clarke 1993) uses the average Bray-Curtis coefficients among samples within each assigned group, and compares these means among groups. As the Bray-Curtis coefficient is based on the contribution of each taxon, the average dissimilarity between pairwise category comparisons can be expressed in terms of the average percent dissimilarity contributed per taxa. For each pairwise comparison, individual taxa are listed based on the average dissimilarity. The SIMPER results breakdown the community comparison and identify which taxa are dominant within a given habitat and which taxa have the greatest dissimilarity between groups.

2.6 SALINITY MONITORING

Hydrodynamic modelling conducted by Northwest Hydraulic Consultants (NHC) has predicted that the RBT2 Project may result in temporary changes in water column salinity at Roberts Bank (EIS, v2, s9.7.8 (PMV 2015b)).

Data were recorded on abiotic intertidal water parameters at Roberts Bank from March 2016 throughout the freshet and WESA northward migration period. Automated datalogging sensors (models: Schlumberger CTD-Diver and Solinst LTC Levelogger Junior) were used to collect water temperature, water pressure, and conductivity data at five-minute intervals at five stations starting in March and an additional two stations in April through May. The data raw were entered into the Aquarius Time-Series database (Aquarius Informatics Inc. 2017) and salinity values (PSU) were calculated from the conductivity and temperature data. Water parameter sensors were deployed at the sediment-water interface at all stations to assess salinity where biofilm occurs, and at a height of 0.3 m at three stations to assess vertical mixing of fresh and saltwater (**Figure 2-6, Figure 2-7**). Data were downloaded from sensors during field visits on a monthly basis and sent to NHC for processing with AQUARIUS software designed to manage water quality data.

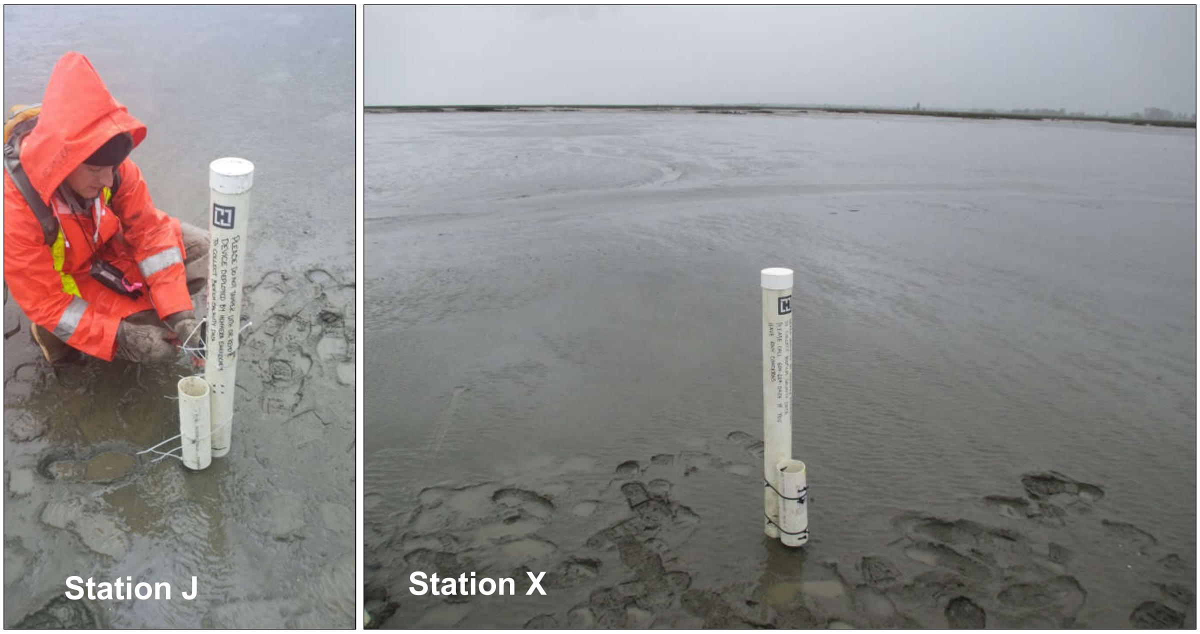


Figure 2-6 Station Housings Showing Installation of Sensor at a Height of 30 cm at Station J and Housing for Sensor at the Sediment-Water Interface at Station J and X

Figure 2-7 View of Sensor within Housing at the Sediment-Water Interface

2.7 Shorebird Foraging Use

Shorebird foraging use was assessed using methods developed by the Centre for Wildlife Ecology (CWE) of Simon Fraser University (Pomeroy 2005, Hemmera 2014), which involves counting droppings within a defined area (i.e., droppings density). Dropping density provides a sensitive and convenient measure of the intensity of spatial use because they are produced frequently (i.e., 0.48 droppings/minute for WESA) by foraging shorebirds and are washed away during high tide periods (Pomeroy 2005). Typically, droppings are washed off the mudflats twice daily with the high tide. However, during lower high tides droppings in the upper intertidal zone may not be completely cleared. Tide charts were examined and dropping counts at locations that were not completely flooded prior to sampling were removed from the analyses.

Shorebird droppings can be distinguished from those of gulls, ducks, and other larger bird species as they are typically smaller than the size of a Canadian quarter (<2.5 cm diameter). In addition, dunlin droppings can be smaller or larger than the size of a Canadian dime, while WESA droppings are generally dime-sized or smaller (Pomeroy 2005) and black bellied plover droppings are typically larger than a dime. While overlap in droppings size across shorebirds prevents definitive assignment to species, for qualitative assessments of differences in size class distributions, droppings were recorded in two categories, dime-sized or smaller, and dime to quarter size. To allow shorebirds time to feed and deposit droppings on the mudflats, sampling began at least 30 minutes after the tide had receded from each station. Dropping surveys were not conducted after periods of rainfall that reduced dropping presence.

At each station, droppings survey locations were determined with random bearings and within ≤ 25 m from the biofilm/salinity station. Bearings were varied by adding 60° to a randomly selected bearing for the initial survey to provide evenly distributed sampling around the salinity monitoring station over the six sampling events (6 x 60° = 360°). Surveyors recorded the number of droppings at each location within 15, 1-m^2 quadrats (**Figure 2-8**) (5 perpendicular to the survey bearing on either side and 5 along the bearing). To minimise null data, which provides misleading inference of droppings densities and complicates statistical analyses, additional 15-m^2 surveys (maximum 3) were conducted immediately in front of the first survey when no droppings were observed. Droppings densities were calculated by dividing the number of droppings counted within the number of 1-m^2 quadrats sampled.

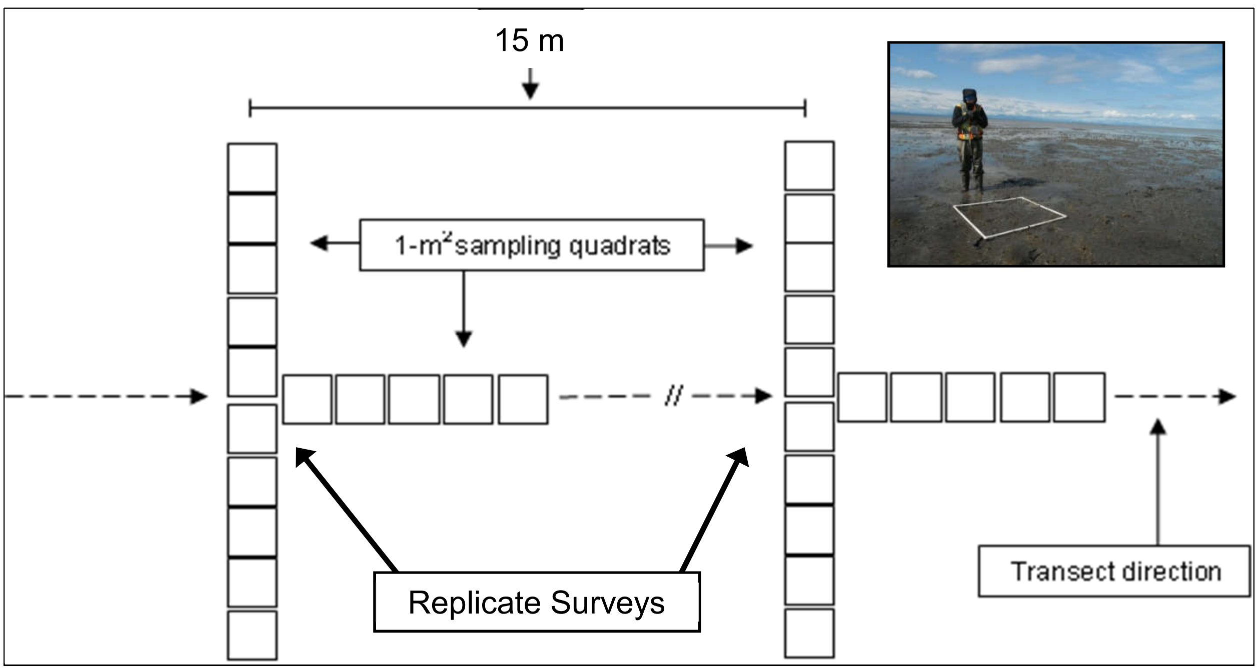


Figure 2-8 Design of Droppings Surveys to Assess Shorebird use of Intertidal Mudflats. Inset photograph shows a biologist inspecting a 1-m^2 quadrat for shorebird droppings. Replicate surveys were only conducted if no droppings were detected in the first survey.

2.7.1 Statistical Analysis

All analyses were completed using SYSTAT 13.1 (Chicago, USA). Prior to analyses shorebird usage indicators (dropping density < dime and < quarter in size) were tested for normality using the Shapiro-Wilks test. Both parameters were found to possess non-normal distributions due to a large number of samples (~40%) containing no or few droppings. Attempts made to normalise the data using transformations were unsuccessful. Therefore, non-parametric statistics were used.

Kruskal-Wallis non-parametric one-way ANOVA was used to assess differences among stations in droppings densities. The results obtained with a Kruskal-Wallis test serve only to indicate whether the parameters differ significantly when considering the independent variable. If a significant result was found, specific differences among stations were investigated using the Conover-Inman Test for All Pairwise Comparisons. Temporal and spatial interactions of parameters were assessed by reviewing time series trends.

To test for relationships between dropping densities and biofilm biomass (Chlorophyll *a*, Total Carbohydrates) and fatty acid parameters a non-parametric Spearman correlational analysis was used.

It should be noted that spatial and temporal autocorrelation were not able to be accounted for due to lack of replication in the sampling design and therefore data from sampling points collected at the same station on subsequent days are not independent.

3.0 RESULTS

3.1 BIOFILM BIOMASS AND FATTY ACID ABUNDANCE

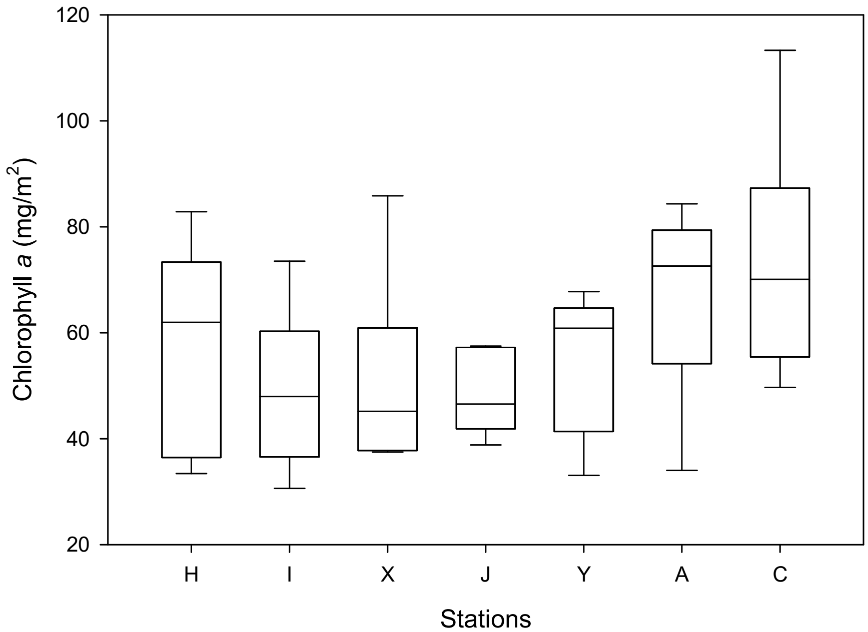
Except in one instance (18:1n-7 at station H on April 18), all 28 fatty acids analysed plus Chlorophyll *a* and carbohydrates were found at every station, on every sampling event, indicating all parameters were produced by biofilms across the entire spatiotemporal range and across the suite of abiotic conditions experienced within the study area.

3.1.1 Chlorophyll *a*

Mean biofilm Chlorophyll *a* levels varied among stations (**Table 3-1**). Stations A and C, located in the upper intertidal zone close to the Roberts Bank Causeway, possessed the highest Chlorophyll *a* levels, followed by the Station H located close to Canoe Passage. Stations J and X, located between the Upper Intertidal Zone and Canoe Passage, and Station I, located adjacent to Canoe Passage, contained the lowest Chlorophyll *a* levels. A one-way ANCOVA found statistically significant differences in Chlorophyll *a* abundance among stations ($F_{6,34} = 5.2$, $p = 0.001$) (**Figure 3-1**). Pairwise comparisons found significant differences between Stations A, C and Stations J, X, and Y.

Table 3-1 Biofilm Chlorophyll *a* Summary Statistics per m² of Intertidal Mudflats for Stations Sampled at Roberts Bank (April 18 to May 12, 2016)

Station	n	Mean (mg/m²)	Standard Deviation	Minimum	Maximum
A	6	67	18	34	84
C	6	73	23	50	113
H	6	58	19	33	83
I	6	49	15	31	74
J	6	48	8	39	57
X	6	51	18	37	86
Y	6	55	14	33	68



Bonferroni Pairwise Comparison Test Results		
Station	**Station**	**p-Value**
A	J	0.04
A	X	0.05
C	J	< 0.01
C	X	< 0.01
C	Y	0.04

Figure 3-1 Chlorophyll *a* Abundance per m² of Intertidal Mudflats at Roberts Bank Sampling Stations (April 18 to May 12, 2016) and Table Showing Statistically Significant Differences among Stations based on ANCOVA Results Controlling for Sample Collection Weight.

Overall Chlorophyll *a* abundance during the course of the study varied between a mean of 48 and 71 mg/m² (**Table 3-3, Figure 3-2**). A GLM determined changes in Chlorophyll *a* abundance were not statistically different across the duration of the study ($F_{1,40} = 0.76$, $p = 0.39$) or among sampling dates ($F_{5,36} = 1.4$, $p = 0.25$).

Table 3-2 Biofilm Chlorophyll *a* Summary Statistics per m² of Intertidal Mudflats by Sampling Date at Roberts Bank (April 18 to May 12, 2016).

Sampling Date	n	Mean (mg/m²)	Standard Deviation	Minimum	Maximum
18-Apr	7	61	28	33	113
23-Apr	7	54	18	31	83
25-Apr	7	58	17	37	79
29-Apr	7	48	11	33	64
06-May	7	52	16	37	84
12-May	7	71	10	57	86

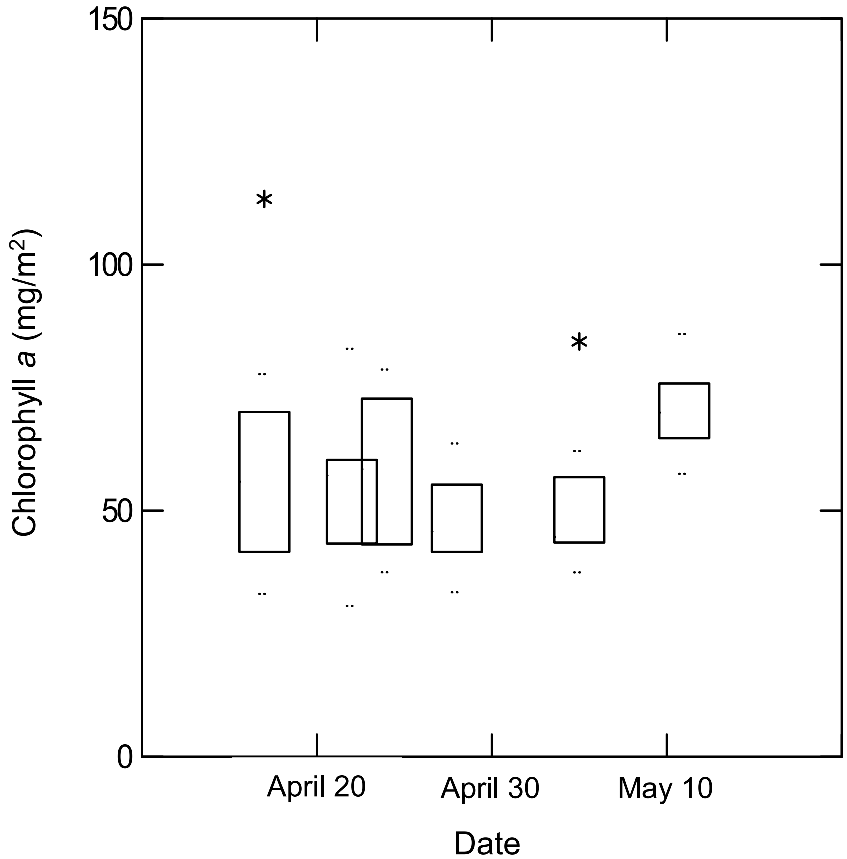


Figure 3-2 Temporal Variation in Biofilm (Chlorophyll *a*) Abundance per m² of Intertidal Mudflats at Roberts Bank (April 18 to May 12, 2016).

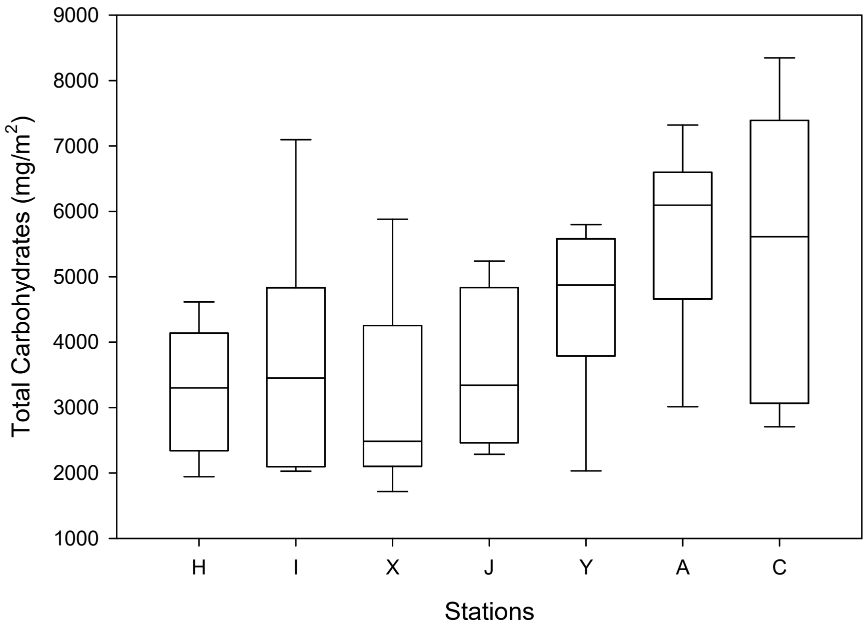
3.1.2 Carbohydrates

The mean spatial abundance of carbohydrates among stations varied between ~3.1 g/m² to 5.7 g/m² (**Table 3-3**). Similar to Chlorophyll *a* (**Table 3-1** and **Figure 3-1**), carbohydrate abundance was highest at stations A and C (**Table 3-3** and **Figure 3-3**), with statistically significant differences documented among Stations A / C and H, J, X, and Stations Y and X ($F_{6,35} = 13.0$, $p < 0.001$) (**Figure 3-3**). Station I was not statistically different from any other station.

Table 3-3 Biofilm Total Carbohydrate Summary Statistics per m² of Intertidal Mudflats for Stations Sampled at Roberts Bank (April 18 to May 12, 2016).

Station	n	Mean (g/m²)	Standard Deviation	Minimum	Maximum
A	6	5.7	1.5	3.0	7.3
C	6	5.4	2.2	2.7	8.3
H	6	3.3	1.0	1.9	4.6
I	6	3.7	1.9	2.0	7.1

Station	n	Mean (g/m²)	Standard Deviation	Minimum	Maximum
J	6	3.6	1.2	2.3	5.2
X	6	3.1	1.5	1.7	5.9
Y	6	4.6	1.4	2.0	5.8



Bonferroni Pairwise Comparison Test Results		
Station	Station	p-Value
A	H	< 0.001
A	J	< 0.001
A	X	< 0.001
C	H	0.01
C	J	0.01
C	X	< 0.001
X	Y	0.05

Figure 3-3 Carbohydrate Abundance per m² of Intertidal Mudflats at Roberts Bank Sampling Stations (April 18 to May 12, 2016) and Table Showing Statistically Significant Differences among Stations based on ANCOVA Results Controlling for Sample Collection Weight

Overall total carbohydrate abundance differed by ~42% over the course of the study, ranging from 3.3 to 5.6 g/m² (**Table 3-4**). The lowest mean carbohydrate abundance was documented on April 23, with the highest mean abundance documented 19 days later on May 12. Differences in carbohydrate abundance across the duration of the study ($F_{1,40} = 3.4$, $p = 0.07$) and among sampling dates ($F_{5,36} = 2.2$, $p = 0.08$) were not statistically significant.

Table 3-4 Biofilm Total Carbohydrate Summary Statistics per m² of Intertidal Mudflats by Sampling Date at Roberts Bank (April 18 to May 12, 2016)

Sampling Date	n	Mean (g/m²)	Standard Deviation	Minimum	Maximum
18-Apr	7	4.2	2.3	2.0	8.3
23-Apr	7	3.3	1.0	2.1	4.9
25-Apr	7	4.2	2.2	1.7	7.3
29-Apr	7	4.0	1.3	2.5	5.4
06-May	7	3.5	1.3	1.9	5.9
12-May	7	5.9	1.0	4.6	7.1

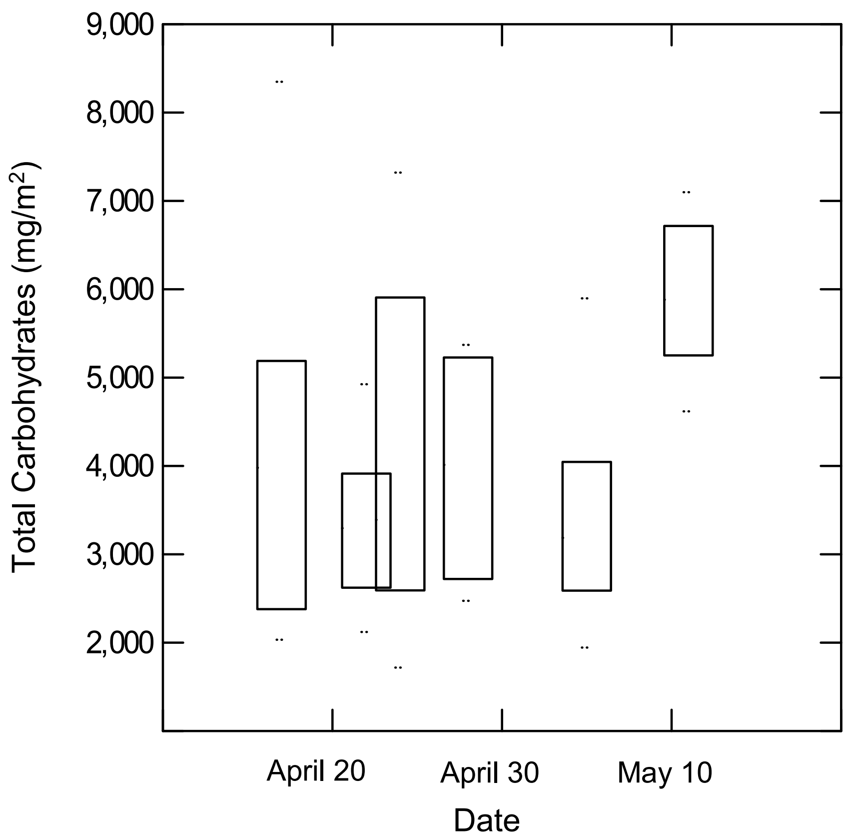


Figure 3-4 Temporal Variation of Total Carbohydrate Abundance per m² of Intertidal Mudflats at Roberts Bank (April 18 to May 12, 2016)

3.1.3 Fatty Acids

The SFAs myristic acid (14:0), palmitic Acid (16:0), and stearic acid (18:0), the MUFA palmitoleic acid (16:1n-7), and the PUFAs EPA (20:5n-3), and DHA (22:6n-3) were the most abundant fatty acids documented, ranging from means of 32 mg/m^2 to 182 mg/m^2 (Error! Reference source not found.**, Appendix B**). Mean abundances of all fatty acids ranged from 3 mg/m^2 (20:3n-3) to 182 mg/m^2 (16:1n-7). Twenty-two of the 28 fatty acids analysed were documented possessing mean abundances of < 30 mg/m^2 and 13 were found at abundances < 10 mg/m^2. Fatty acids with the lowest abundances were the SFA 15:1 and the PUFAs 20:3n-3, 20:3n-6, 22:1n-9, and 22:2. Mean MUFA (291 mg/m^2) and SFA abundances (315 mg/m^2) were comparable and were ~50% higher than PUFA levels (208 mg/m^2) (**Appendix B**).Error! Reference source not found.On average, Total Fatty Acid levels were comprised of 39% SFA, 36% MUFA, and 25% PUFA. Mean levels of Omega 6 fatty acids (46 mg/m^2), a type of PUFA, were the lowest of all fatty acid types (**Figure 3-5**). Fatty acid summary statistics and summary statistics by stations are presented in **Appendix B** and **C**, respectively.

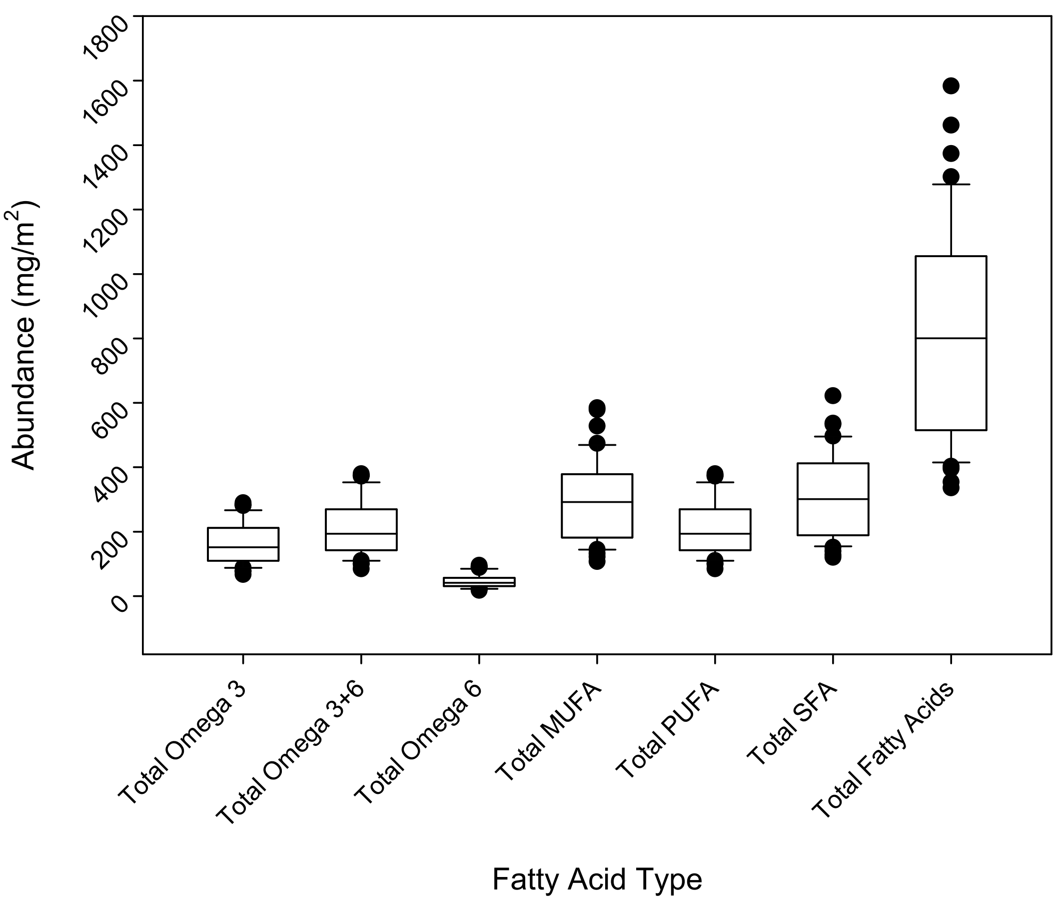


Figure 3-5 Abundance of Omega 3, Omega 6, Monounsaturated (MUFA), Polyunsaturated (PUFA), and Saturated Fatty (SFA) Acids per m^2 of Intertidal Mudflats in Biofilm at Roberts Bank (April 18 to May 12, 2016) across all Stations and Sampling Periods. Black dots indicate outliers.

3.1.3.1 Spatial Distribution

Mean Total Fatty Acid levels varied among stations (**Figure 3-6**), but followed a similar pattern as Chlorophyll *a* (**Figure 3-1**) and Total Carbohydrates (**Figure 3-3**). Stations A (1.1 g/m^2) and C (1.1 g/m^2) possessed the highest mean Total Fatty Acid levels, followed by station I (0.9 g/m^2), Y (0.9 g/m^2), and H (0.7 g/m^2). Stations J (0.5 g/m^2) and X (0.5 g/m^2) possessed the lowest levels and were ~55% lower than A and C.

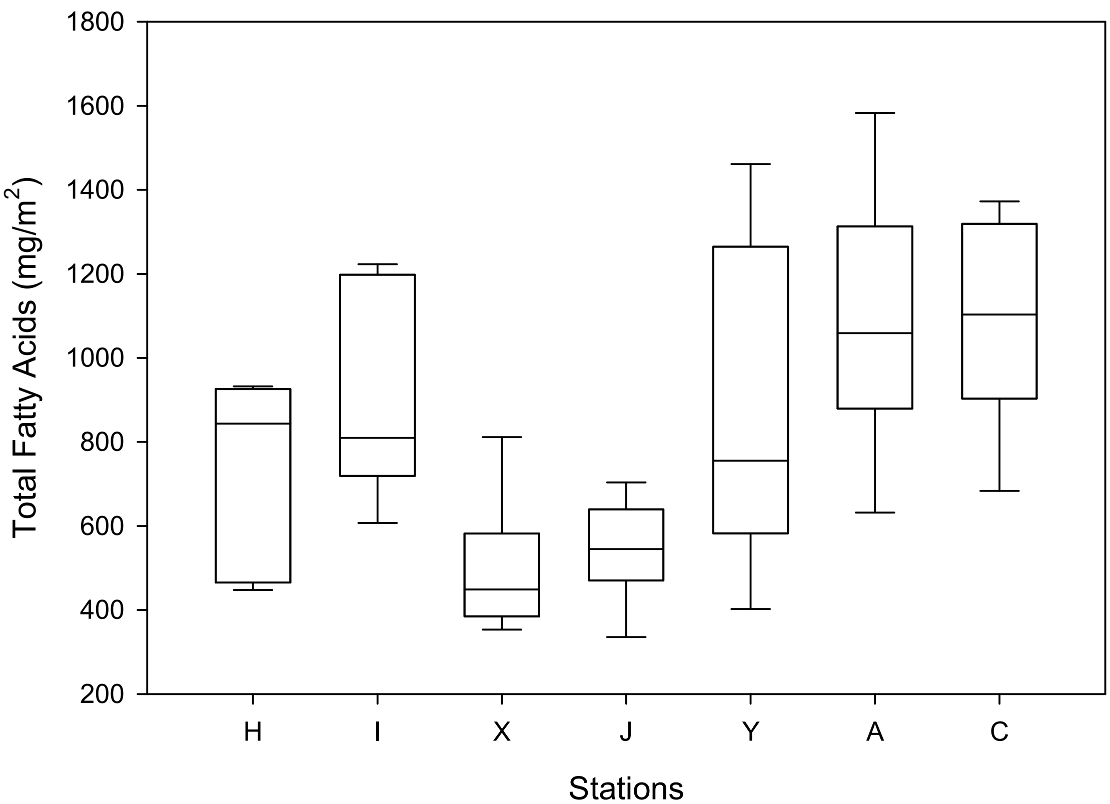


Figure 3-6 Total Fatty Acid Abundance per m^2 of Intertidal Mudflats by Sampling Station at Roberts Banks (April 18 to May 12, 2016). See Table 3-5 for Bonferroni pairwise comparison test results.

A one-way ANCOVA, conducted to determine differences in biofilm Total Fatty Acid abundance among stations while controlling for Sample Weight found statistically significant differences ($F_{6,34} = 13.5$, $p < 0.001$) (**Table 3-5**). Pairwise comparisons found significant differences among Stations A/C and Stations H/J/X, and Station I/Y and Stations J/X. The general pattern of fatty acid levels at Stations A/C > Stations H/J/X/Y was repeated for many of the other fatty acids and types of fatty acids tested (**Table 3-5**). For example, for PUFA, with the exception of EPA, at Stations A/C were higher than Stations H/J/X. This pattern also held true for Total SFA, 16:0, and 18:0. For EPA, Stations A/C were higher than J/X, but levels were not statistically different from the remaining stations. MUFA, particularly 16:1n-7, were another exception. For 16:1n-7, the mean abundance at Y was statistically larger than levels at Station X, but not different among other stations. Stations X consistently possessed the lowest levels of all stations regardless of the biofilm parameter.

Table 3-5 Results from ANCOVA Tests Showing Differences in Fatty Acid Biofilm Parameter Abundance among Stations at Roberts Banks (April 18 to May 12, 2016) Controlling for Sample Collection Weight

Parameter	$F_{6,34}$	p-Value	Bonferroni Pairwise Comparison Test
Total Fatty Acids	13.5	<0.001	H,J,X<A,C ; J,X<I,Y
Polyunsaturated Fatty Acids (PUFA)			
Total PUFA	9.7	<0.001	H,J,X<A,C ; Y<A ; X<I
20:5n-3 (EPA)	5.1	0.001	J,X<A,C
22:6n-3 (DHA)	14.6	<0.001	H,I,J,X,Y<A,C ; X<I,Y
Omega 3	8.2	<0.001	H,J,X,Y<A ; J,X<C
Omega 6	17.4	<0.001	H,I,J,X,Y<A,C ; X<I,Y
Monounsaturated Fatty Acids (MUFA)			
Total MUFA	10.5	<0.001	H,J,X<A,C ; J,X<I,Y
16:1n-7	3.9	0.004	X<Y
18:1n-7	4.3	0.003	J,X < A,I
18:1n-9	15.9	<0.001	H,I,J,X,Y < A,C
Saturated Fatty Acids (SFA)			
Total SFA	18.3	<0.001	H,J,X<A,C,I ; J,X<Y
14:0	16.2	<0.001	J,X<A,C,H,I ; Y<I, X<Y
16:0	14.9	<0.001	H,J,X< A,C ; H,J,X<I ; J,X<Y
18:0	24.6	<0.001	H,J,X,Y<A,C ; X<H ; J,X,Y<I

Notes: All analyses were performed on transformed variables where appropriate (see **Table 2-2**).

3.1.3.2 Temporal Distribution

For most fatty acids no statistical change in abundance was documented over the duration of the study (**Table 3-6**). The two fatty acid parameters demonstrating significant increases in abundance were 18:1n-9 and Total Omega 6 fatty acids. For each parameter, the increase appeared to be driven by elevated abundance levels across stations on the final day of sampling (May 12) (**Figure 3-7**). Differences in biofilm parameters documented within stations are reported in **Section 3.1.3.3**.

Table 3-6 Results from GLM Analyses Showing Differences in Fatty Acid Biofilm Parameters among Sampling Dates at Roberts Banks (April 18 to May 12, 2016) Controlling for Sample Collection Weight

Parameter	P-value
14:0	0.26
16:0	0.11
16:1n-7	0.14

Parameter	P-value
18:0	0.19
18:1n-7	0.52
18:1n-9	0.02
20:5n-3 (EPA)	0.17
22:6n-3 (DHA)	0.06
Omega 3	0.14
Omega 6	0.03
Total MUFA	0.10
Total PUFA	0.10
Total SFA	0.07
Total Fatty Acids	0.07

Notes: All analyses were performed on transformed variables where appropriate (see **Table 2-2**).

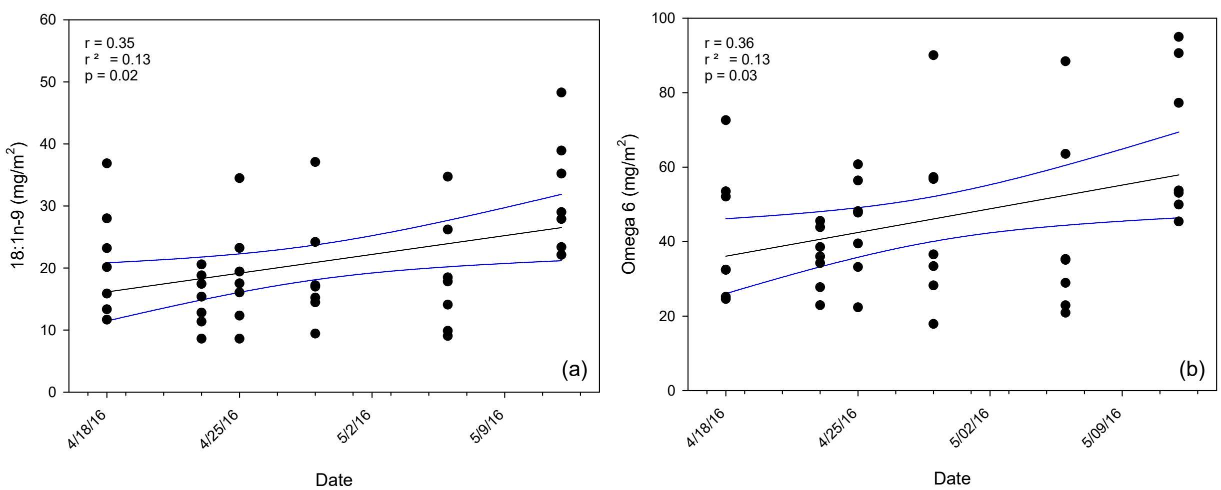


Figure 3-7 Temporal Variation of 18:1n-9 (a) and Omega 6 Fatty Acid (b) Abundance at Roberts Bank (April 18 to May 12, 2016). Blue lines indicate 95% confidence interval.

3.1.3.3 Spatial-Temporal Trends

Although productivity parameter levels across the study area did not differ among sampling dates, changes were documented within individual stations (**Figure 3-8** to **Figure 3-10**). These changes often differed between stations; however, most stations followed similar trajectories. For example, Chlorophyll a, Total Fatty Acid, Total PUFA, Total MUFA, and Total SFA followed the same overall temporal trends within stations (**Figure 3-8**).

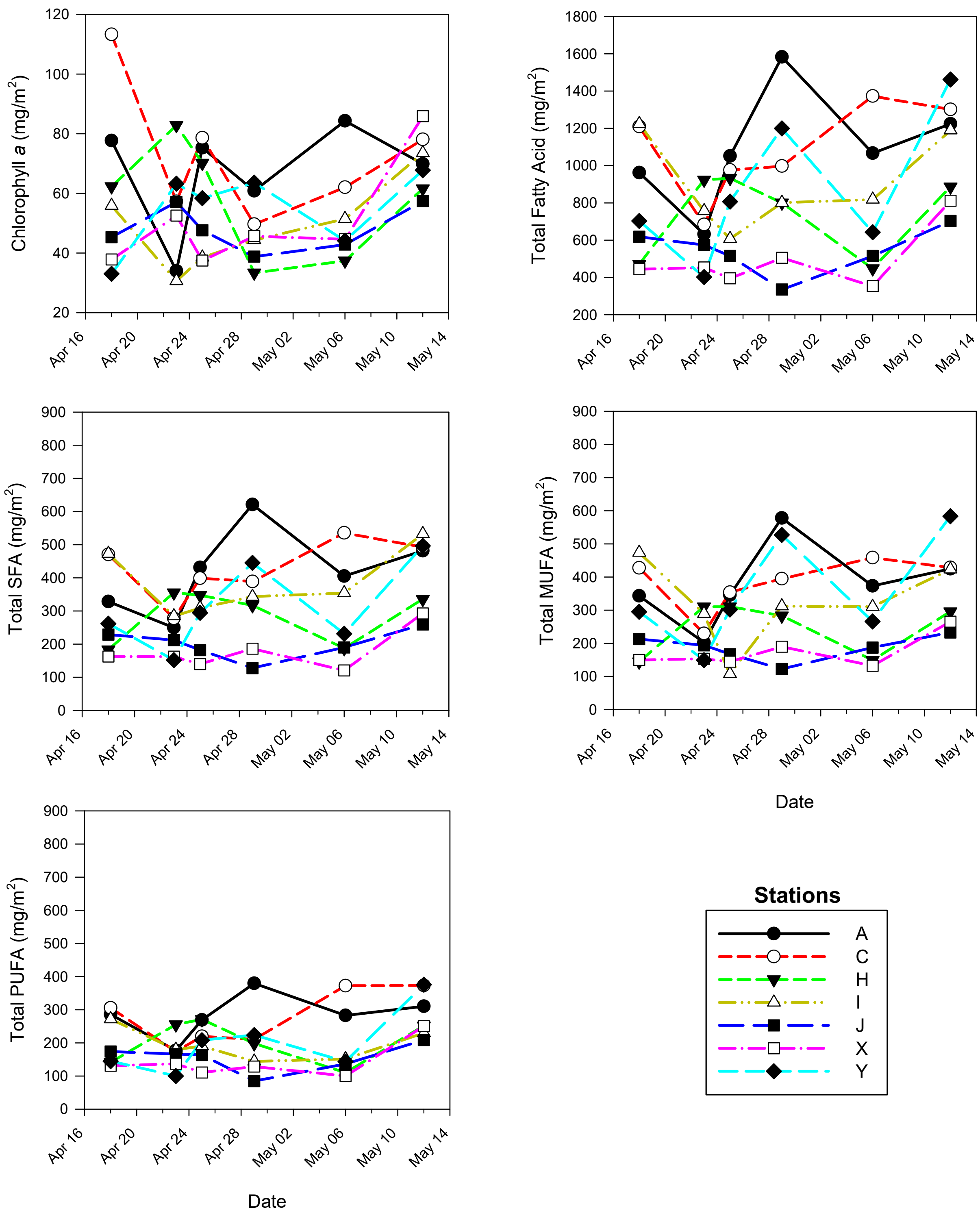


Figure 3-8 Temporal Trends for Chlorophyll a, Total Fatty Acid, SFA, MUFA, and PUFA for Individual Stations across all Sampling Events at Roberts Bank. Note: differences in y-axis scale between inset figures are intentional to allow differences in levels over time to be observed.

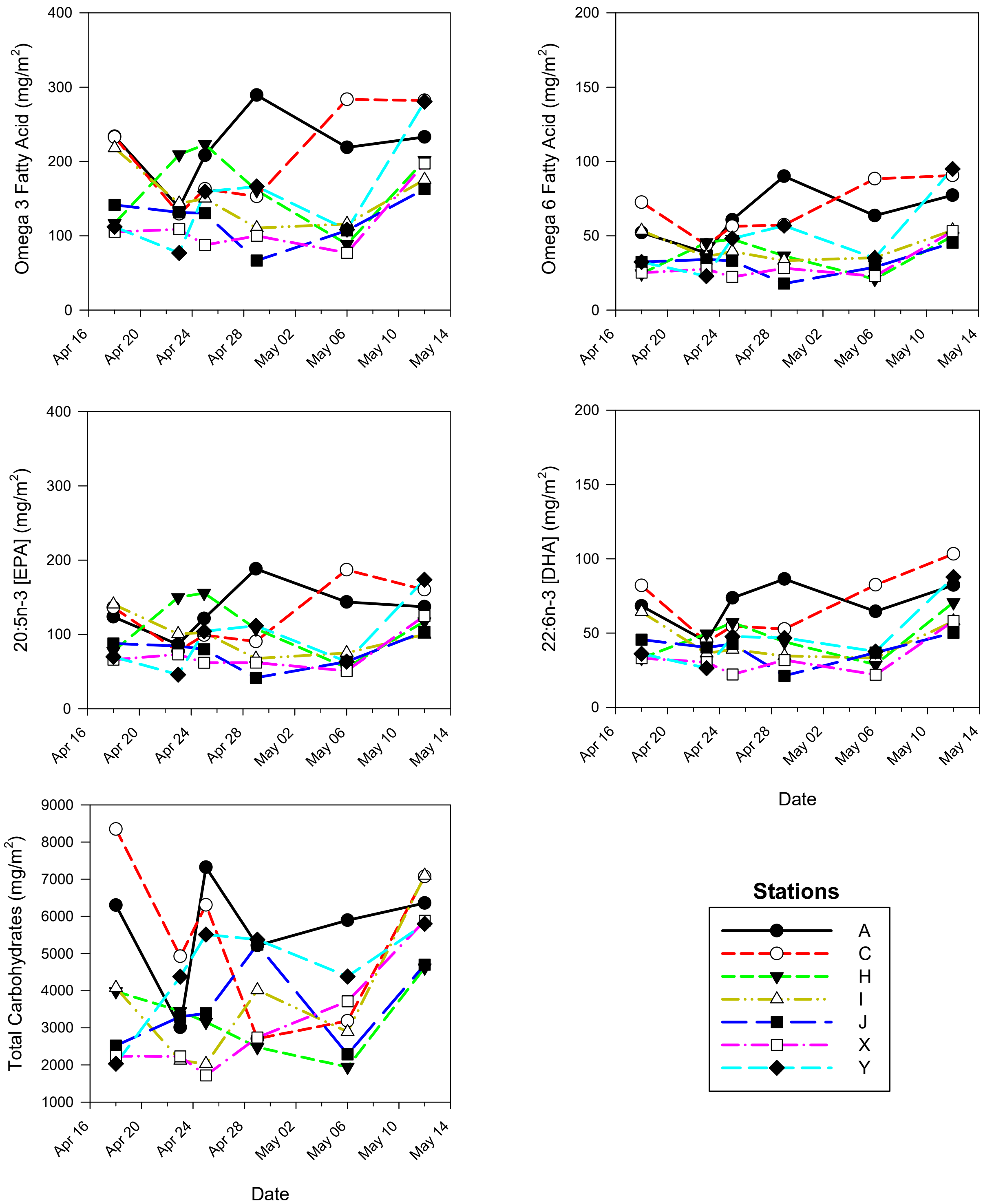


Figure 3-9 Temporal Trends for Total Omega 3, Omega 6, EPA, DHA Fatty Acids, and Total Carbohydrates for Individual Stations across all Sampling Events at Roberts Bank. Note: differences in y-axis scale between inset figures are intentional to allow differences in levels over time to be observed.

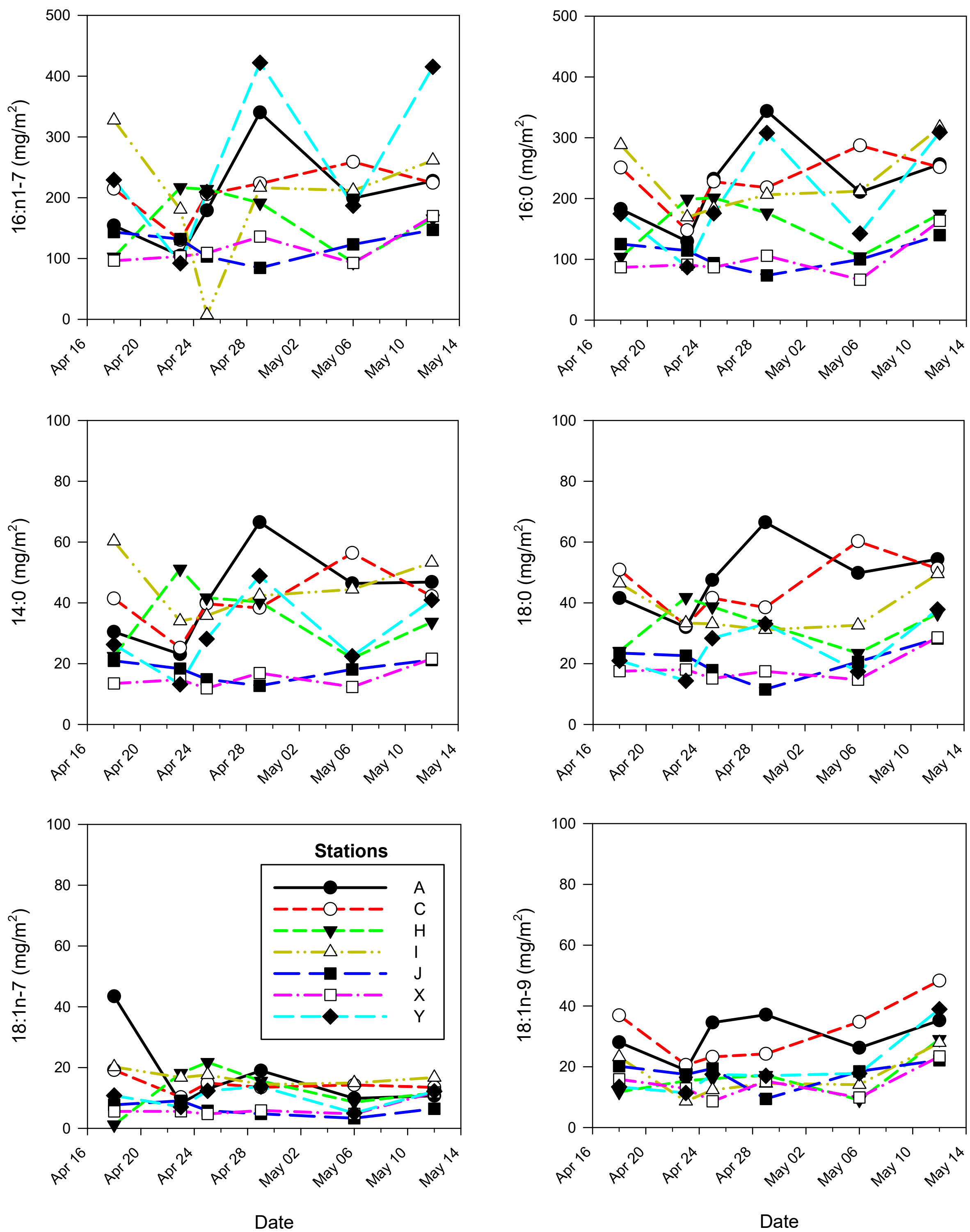


Figure 3-10 Temporal Trends for 14:0, 16:0, 18:0, 16:1n-7, 18:1n-7, and 18:1n-9 Fatty Acids for Individual Stations across all Sampling Events at Roberts Bank. Note: differences in y-axis scale between inset figures are intentional to allow differences in levels over time to be observed.

3.1.4 Correlations Among Biofilm Parameters

Seventy-seven percent of individual focal fatty acids and fatty acid types showed strong statistically significant positive correlations ($r > 0.70$) with each other, and 98% of focal fatty acid parameters were positively correlated ($p<0.05$) (**Table 3-7**). Positive correlations also occurred between biofilm biomass parameters Chlorophyll *a* and Total Fatty Acid ($r = 0.59$, $p < 0.001$), Chlorophyll *a* and Total Carbohydrates ($r = 0.79$, $p < 0.001$), and Total Carbohydrates and Total Fatty Acid ($r = 0.58$, $p < 0.001$) (**Figure 3-11**). Chlorophyll *a* showed significant positive correlations with Total Fatty Acid, PUFA, MUFA, and SFA, with correlation coefficients ranged from 0.53 (MUFA) to 0.66 (PUFA). Chlorophyll *a* was also positively correlated with the omega 3 fatty acids EPA ($r = 0.60$) and DHA ($r = 0.67$). Overall, chlorophyll *a* was positively correlated with 70% of all biofilm parameters analysed ($n = 37$, listed in **Appendix B**).

Correlation coefficients among fatty acid types were also highly correlated and ranged from 0.93 between MUFA and SFA ($p<0.001$) to 0.84 between MUFA and PUFA ($p<0.001$) (**Table 3-7**). Eighty-seven percent of all biofilm parameters ($n = 37$) were statistically correlated with each other, and no parameters demonstrated negative correlations.

Table 3-7 Pearson Correlation Coefficients and Significance Among Biofilm Parameter Abundance

Parameters	Chlorophyll *a*	14:0	16:0	16:1n-7	18:0	18:1n-7	18:1n-9	20:5n-3	22:6n-3	Total Omega 3	Total Omega 6	Total PUFA	Total MUFA	Total SFA	Total Fatty Acids	Total Lipids
14:0	0.45															
16:0	0.50	*0.94*														
16:1n-7	0.40	*0.71*	*0.80*													
18:0	**0.59**	*0.90*	*0.87*	**0.59**												
18:1n-7	0.41	*0.69*	*0.65*	0.42	*0.66*											
18:1n-9	**0.64**	**0.58**	*0.70*	**0.55**	*0.78*	0.41										
20:5n-3	**0.60**	*0.78*	*0.79*	**0.62**	*0.87*	**0.60**	*0.76*									
22:6n-3	*0.67*	*0.72*	*0.79*	**0.60**	*0.87*	**0.56**	*0.95*	*0.90*								
Total Omega 3	*0.65*	*0.78*	*0.81*	**0.62**	*0.89*	**0.64**	*0.85*	*0.98*	*0.96*							
Total Omega 6	**0.64**	*0.77*	*0.87*	*0.68*	*0.89*	**0.56**	*0.90*	*0.90*	*0.95*	*0.94*						
Total PUFA	*0.66*	*0.79*	*0.83*	**0.64**	*0.90*	**0.63**	*0.87*	*0.97*	*0.97*	*1.00*	*0.97*					
Total MUFA	**0.53**	*0.86*	*0.94*	*0.92*	*0.82*	**0.61**	*0.75*	*0.79*	*0.81*	*0.82*	*0.88*	*0.84*				
Total SFA	**0.56**	*0.94*	*0.98*	*0.75*	*0.94*	*0.65*	*0.79*	*0.84*	*0.86*	*0.87*	*0.92*	*0.89*	*0.93*			
Total Fatty Acids	**0.59**	*0.90*	*0.97*	*0.81*	*0.92*	**0.64**	*0.83*	*0.89*	*0.90*	*0.91*	*0.95*	*0.93*	*0.96*	*0.98*		
Total Lipids	0.52	*0.79*	*0.83*	**0.55**	*0.85*	**0.64**	*0.70*	*0.72*	*0.78*	*0.77*	*0.80*	*0.79*	*0.76*	*0.86*	*0.83*	
Total Carbohydrates	*0.79*	0.39	0.52	0.43	**0.53**	0.32	*0.68*	0.44	**0.64**	**0.54**	**0.62**	**0.57**	**0.56**	**0.56**	**0.58**	0.51

Notes: Statistical tests were conducted on the abundance of each parameters per square meter (i.e., mg/m^2).
A Bonferroni correction was used to adjust p-values to account for multiple comparison tests.
Significant p-values are represented with the following formatting: **p < 0.001**, *p < 0.05.*

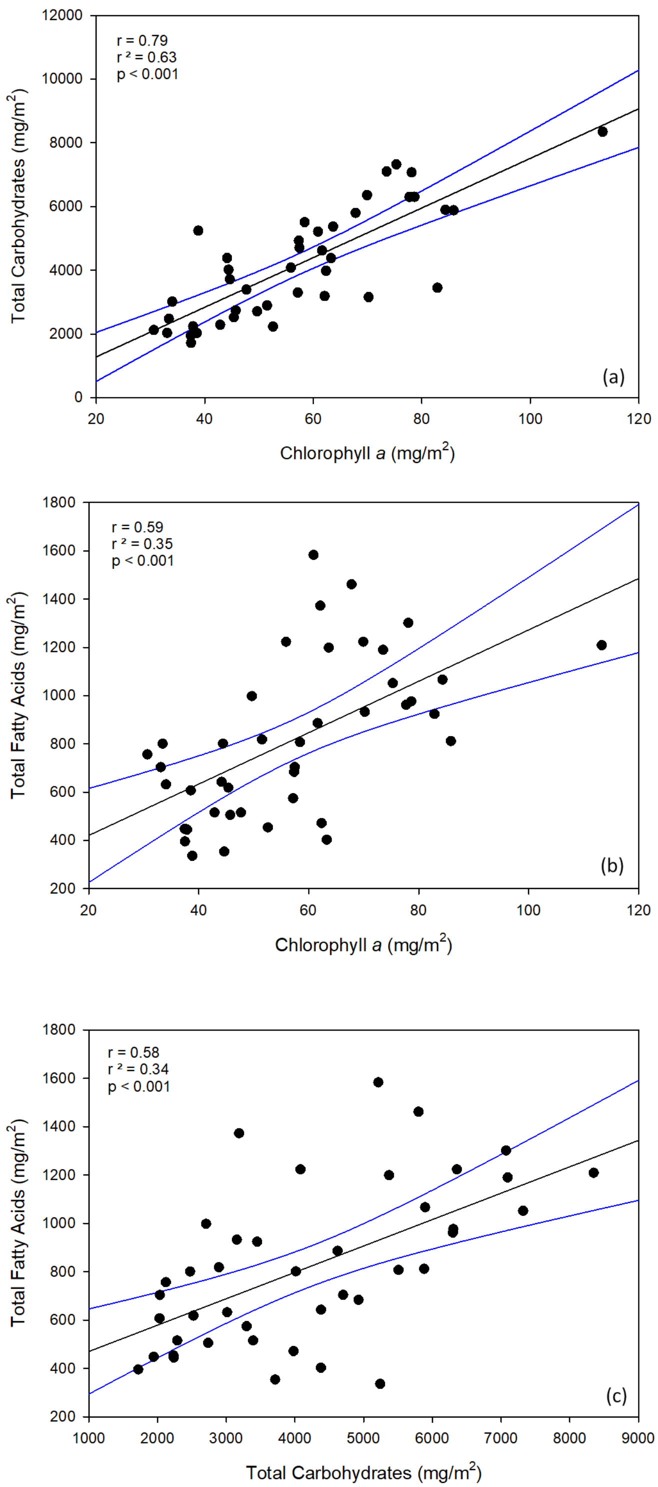


Figure 3-11 Relationships between Chlorophyll a and Carbohydrates (A), Chlorophyll *a* and Total Fatty Acids (B), and Carbohydrates and Total Fatty Acids (C). Blue lines indicate 95% confidence interval.

3.1.5 Abiotic Effects on Biofilm

Abiotic factors with the potential to affect biofilm abundance were graphed by date and station to aid in interpretation of results (**Figure 3-12**). Mudflat exposure, 5^{th} and 95^{th} percentile water column salinity, and 5^{th} and 95^{th} percentile water column temperature varied spatially and temporally over the duration of the study. All stations followed a similar pattern for the amount of time mudflats were exposed following a receding tide (**Figure 3-12A**). On April 18 mudflat exposure time was greatest and ranged from ~80% of the day (~19 hours) for Stations A, C, and I to 53% (~12.6 hour) for Station H. Mudflat exposure time decreased through April 23-25 and slowly increased again toward the end of April. During the duration of the study, Stations A, C, and I tracked closely having similar exposure periods. Station H was exposed for ~38% less time per day (6.1 hours/day ± 1.4 SD) compared to A, C and I. Station J was exposed 13.4 hours/day (± 1.7 SD) and was intermediate to A/C/I and H.

Stations closest to Canoe Passage had the lowest 5^{th} and 95^{th} percentile salinities (**Figure 3-12B** and **C**). The 5th percentile salinities at Stations H, I, and J over the duration of the study averaged 0.24 PSU (± 0.38 SD). Fifth percentile salinities were generally higher and more variable at Stations A and C (9.8 PSU ± 6.1 SD). A similar trend was documented at stations A and C for 95^{th} percentile water column salinity. Ninety-fifth percentile salinities at Stations H and I varied more compared to 5^{th} percentile levels and ranged from 11.4 PSU to 0.1 PSU ($\bar{x}$ = 3.1 PSU ± 3.4 SD) over the duration of the study.

Fifth percentile water column temperatures for all five monitoring stations were less variable than exposure and salinity estimate (**Figure 3-12D**). Temperatures varied from a low of 8.8°C at station H to a high of 12.9°C at station A during the duration of the study. The highest mean temperatures were found at stations A (11.1°C ± 1.3 SD) and C (11.5°C ± 1.1 SD), which were approximately one degree higher than the mean temperatures documented at stations H (10.5°C ± 1.0 SD), I (10.3°C ± 1.1 SD), and J (10.8°C ± 1.3 SD). Variation in 95^{th} percentile water column temperatures was greater than the 5^{th} percentile (**Figure 3-12E**). Mean 95^{th} percentile temperatures at stations A (19.1°C ± 4.4 SD) and C (19.6°C ± 4.7 SD) were the highest over the duration of the study and differed from each other by less than one degree. Intermediate temperatures were documented at station J (17.6°C ± 3.4 SD), while the lowest mean temperatures were documented at stations H (12.9°C ± 1.4 SD) and I (14.2°C ± 3.1 SD). Ninety-fifth percentile water column temperatures were more variable over the duration of the study compared to 5^{th} percentile temperatures (**Figure 3-12E**).

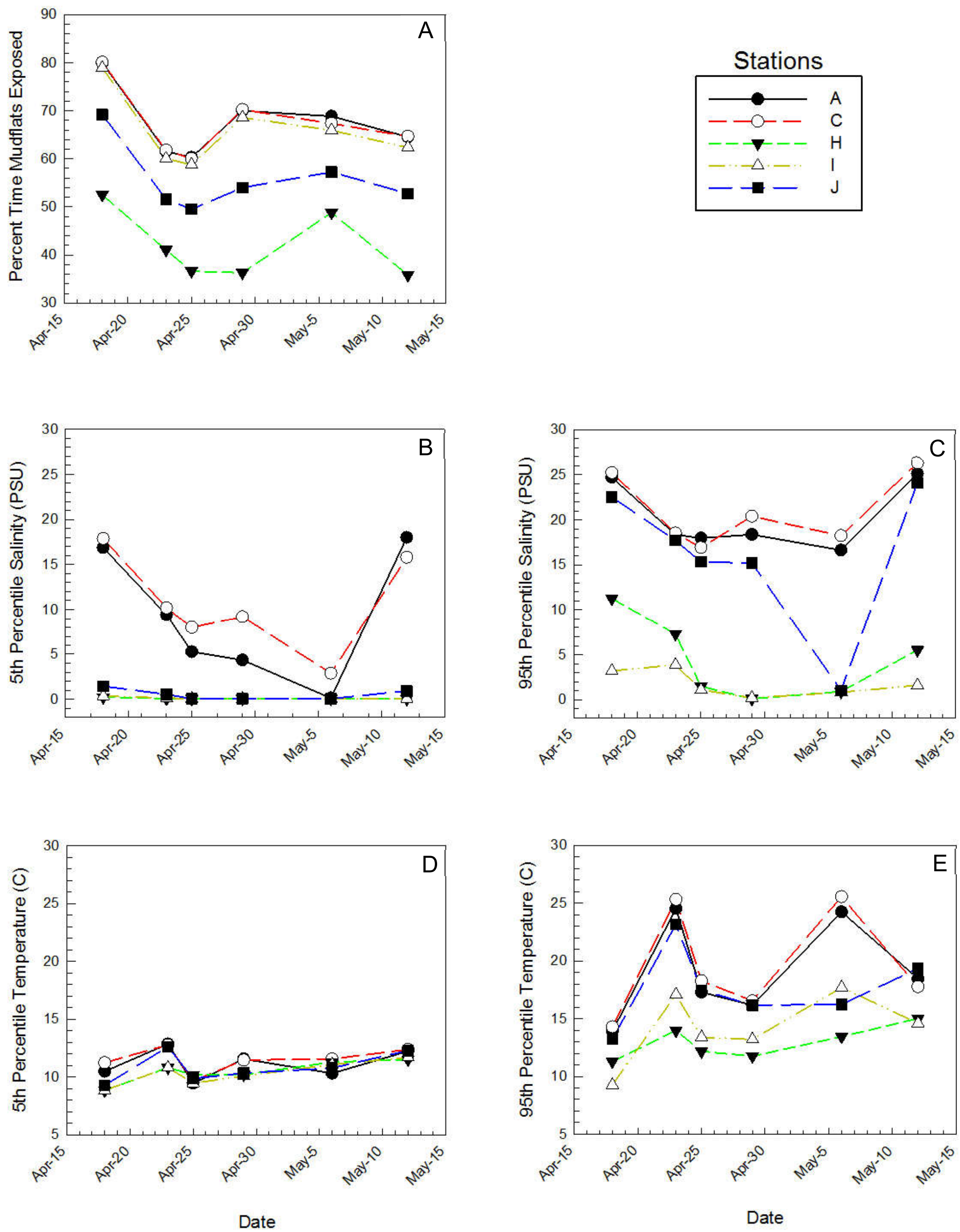


Figure 3-12 Temporal Changes in Abiotic Factors Potentially Affecting Biofilm Abundance at Monitoring Stations, Roberts Bank (April 18 to May 12, 2016). (A) = percent of time mudflats were exposed over the 72 hours prior to sampling; (B) and (C) = 5th and 95th percentile water column salinity over the 72 hours prior to sampling, respectively; (D) and (E) = 5th and 95th percentile water column temperature over the 72 hours prior to sampling, respectively.

AIC_c modelling results are presented in **Sections 3.1.5.1** to **Section 3.1.5.5** for summary biofilm parameters. Model results for individual fatty acids are summarized in **Section 3.1.5.6**. As discussed previously (see **Section 2.4.4**), the amount of sediment collected for analyses (i.e., Total Sample Weight) varied among samples and was included as a covariate in modelling. Therefore, Total Sample Weight is not discussed or presented as factor influencing biofilm abundance, but is included in models to explain added variability associated with the sampling technique used to collect biofilm in the field.

3.1.5.1 Chlorophyll a

AIC_c results indicated models with the most support of factors influencing biofilm Chlorophyll *a* levels contained the variables: 5th percentile salinity and 5th percentile water temperature (**Table 3-8**). However, summed Akaike model weights across the entire model-set (**Appendix D**) indicate 5th percentile salinity ($\sum w_i = 0.93$) was a more important factor affecting biofilm abundance than water temperature, or any other variable, as its summed weights were two to four times as large as the summed weights of the other factors in the analyses (**Table 3-9**).

Results from the backward stepwise regression indicated the model containing 5th percentile salinity and total sample weight was the most parsimonious model, explaining the most variability of all potential models ($r = 0.60$, $F_{1,28} = 7.7$, $P = 0.002$).

Table 3-8 Variables Influencing Chlorophyll *a* (square root transformed) Abundance in Biofilm based on Akaike's Second-Order Information Criterion (AICc) for the Best Models Tested. Only models with substantial empirical support (ΔAIC_c < 2.0, Burnham and Anderson 2002) are presented.

Model	Model ID	# of Parameters (K)	AIC_c	ΔAIC_c	Akaike Weight (w_i)
5th Percentile Salinity + Total Sample Weight	1	4	256.54	0.00	0.27
5th Percentile Salinity + 5th Percentile Temperature + Total Sample Weight	7	5	256.99	0.45	0.21

Notes: For the full set of models evaluated using AIC (i.e., 31 models) see **Appendix D**.

The variables 5th percentile salinity and the amount of time the mudflats were exposed (mudflat exposure time) were positively associated with Chlorophyll *a* abundance in all models (**Table 3-9, Figure 3-13**). The effects from 5^{th} percentile temperature and 95^{th} percentile salinity and temperature varied among models.

Chlorophyll *a* levels showed a wide range of abundance values, particularly at the lower end of the salinity spectrum, ranging from ~30 mg/m^2 to 84 mg/m^2 (**Figure 3-13**). Chlorophyll *a* levels at the higher end of the 5th percentile salinity spectrum ranged from ~57 mg/m^2 to 113 mg/m^2.

Table 3-9 Relative Importance of Variables Potentially Influencing Chlorophyll *a* Levels in Biofilm based of Summed Akaike Weights and the Direction of Change

Variables	Sum of Weights (w_i)	Direction of Change (+ / -)
5th Percentile Salinity	0.93	+
5th Percentile Temperature	0.42	+ / -
95th Percentile Salinity	0.22	+ / -
Percent Time Mudflats Exposed	0.21	+
95th Percentile Temperature	0.21	+ / -

Notes: Direction of Change: + = increases in variable positively affect the biofilm parameter considered (e.g., Chlorophyll *a*, fatty acids, etc.), - = increases in variable negatively affect the biofilm parameter considered.

Chlorophyll *a* was produced by biofilms sampled across the salinity gradient (**Figure 3-13**). Under lower 5th salinity conditions (5th percentile PSU ≤ 5, n = 21) Chlorophyll *a* levels averaged 54 mg/m^2 ± 15 SD. Under moderate 5th percentile salinities (5 < PSU ≤ 10, n = 4) Chlorophyll *a* levels averaged 59 mg/m^2 ± 21 SD. Under high 5th percentile salinities levels (PSU > 10, n = 5) Chlorophyll *a* levels averaged 79 mg/m^2 ± 21 SD. Differences in mean Chlorophyll *a* abundance between the low and high salinity conditions were statistically significant after correcting for multiple comparisons ($F_{2,27}$ = 4.4, p = 0.02).

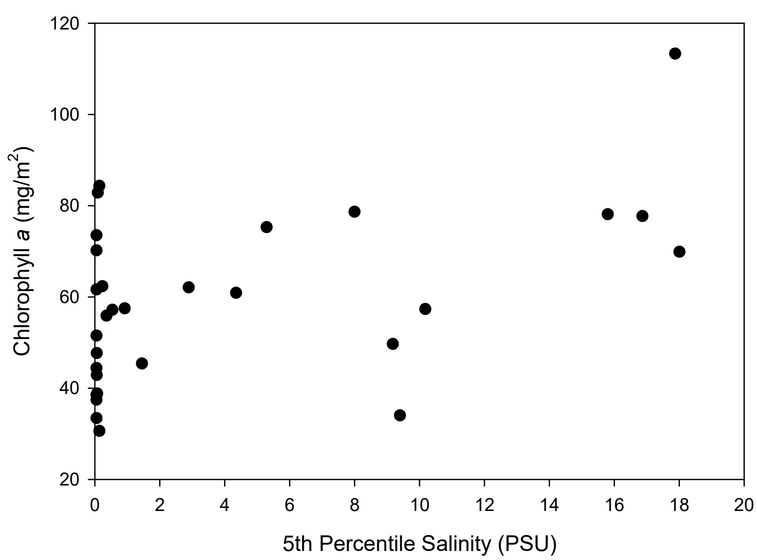


Figure 3-13 Association between Fifth Percentile Salinity as measured in Practical Salinity Units (PSU) and Chlorophyll *a* Levels

3.1.5.2 Total Fatty Acid Abundance

AIC_c results indicated models with the most support to influence biofilm Total Fatty Acid levels contained the variables representing the amount of time the mudflats were exposed over the previous 72-hour period and the 5th percentile water column salinity (**Table 3-10**). However, summed Akaike model weights across the entire model-set (**Appendix D**) indicated mudflat exposure time ($\sum w_i = 0.88$) was a more important factor affecting biofilm abundance compared to salinity ($\sum w_i = 0.41$) (**Table 3-11, Figure 3-14**). Both variables were positively correlated with increased fatty acid levels (**Figure 3-14**). Water temperature (5th and 95th percentile) and 95th percentile water column salinity were not found to be significant predictors of total fatty acid levels.

Results from the backward stepwise regression indicated the model containing only the percentage of time mudflats were exposed to be the most parsimonious model, explaining the most variability of all potential models ($r = 0.44$, $F_{1,28} = 6.7$, $P < 0.01$).

Table 3-10 Variables Influencing Total Fatty Acid Abundance in Biofilm based on Akaike's Second-Order Information Criterion (AICc) for the Best Models Tested. Only models with substantial empirical support (∆AICc < 2.0, Burnham and Anderson 2002) are presented.

Model	Model ID	# of Parameters (K)	AIC_c	ΔAIC_c	Akaike Weight (w_i)
Percent Time Mudflats Exposed + Total Sample Weight	5	4	427.31	0.00	0.29
5th Percentile Salinity + Percent Mudflat Emersion + Total Sample Weight	9	5	428.39	1.07	0.17

Notes: For the full set of models evaluated using AIC (i.e., 31 models) see **Appendix D**.

Table 3-11 Relative Importance of Variables Potentially Influencing Total Fatty Acid Abundance in Biofilm based of Summed Akaike Weights and the Direction of Change.

Variables	Sum of Weights (w_i)	Direction of Change (+ / -)
Percent Time Mudflats Exposed	0.88	+
5th Percentile Salinity	0.41	+
95th Percentile Temperature	0.20	+ / -
95th Percentile Salinity	0.20	+ / -
5th Percentile Temperature	0.18	+ / -

Notes: Direction of Change: + = increases in variable positively affect the biofilm parameter considered (e.g., Chlorophyll *a*, fatty acids, etc.), - = increases in variable negatively affect the biofilm parameter considered.

Fatty acids were produced by biofilms sampled across the salinity gradient (**Figure 3-14**). Under lower salinity conditions (5th percentile PSU ≤ 5, n = 21) Total Fatty Acid levels averaged 0.8 g/m^2 ± 0.3 SD. Under moderate 5th percentile salinities (5 < PSU ≤ 10, n = 4) fatty acid levels averaged 0.9 g/m^2 ± 0.2 SD. Under high 5th percentile salinities levels (PSU > 10, n = 5) fatty acid levels averaged 1.1 g/m^2 ± 0.3 SD. Differences in mean total fatty acid abundance among salinity conditions were not statistically significant ($F_{2,27}$ = 1.5, p = 0.24).

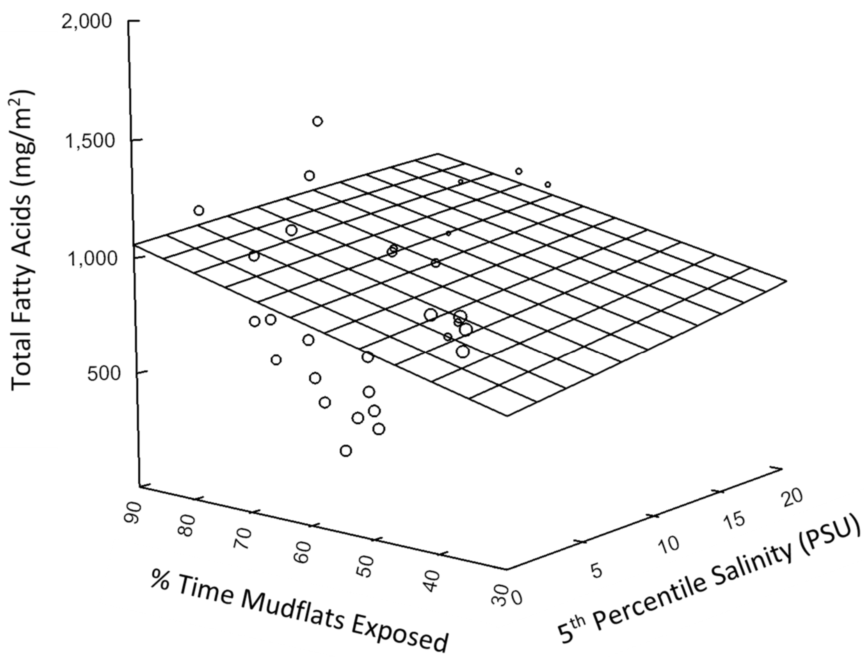


Figure 3-14 Relationship between Total Fatty Acid Abundance, the Percentage of Time Mudflats are Exposed, and the Fifth Percentile Salinity

3.1.5.3 Total PUFA

AIC_c results indicated models with the most support to influence biofilm Total PUFA levels contained the variables representing the amount of time the mudflats were exposed over the previous 72-hour period and the 5th percentile water column salinity (**Table 3-12**). However, unlike Total Fatty Acids, summed Akaike model weights across the entire model-set (**Appendix D**) indicated 5th percentile water salinity to be more important in affecting biofilm abundance ($\sum w_i = 0.76$) compared to mudflats exposure time ($\sum w_i = 0.34$) (**Table 3-13**, **Figure 3-15**). Both variables were positively correlated with increased PUFA levels (**Table 3-13, Figure 3-15**). Summed Akaike weights for 95th percentile water column salinity were similar to that for mudflat exposure time indicating some potential influence of PUFA abundance. Fifteen of 16 models in the model-set demonstrated a positive relationship between 95th percentile water column salinity and PUFA abundance. Water temperature (5th and 95th percentile) were not found to be significant predictors of total PUFA levels.

Results from the backward stepwise regression indicated the model containing the 5th percentile salinity (Model ID 1) to be the most parsimonious model, explaining the most variability of all potential models (r = 0.48, $F_{1,28} = 8.3$, $P < 0.007$).

Table 3-12 Variables Influencing Total Polyunsaturated Fatty Acid (PUFA) Abundance in Biofilm based on Akaike's Second-Order Information Criterion (AICc) for the Best Models Tested. Only models with substantial empirical support (∆AICc < 2.0, Burnham and Anderson 2002) are presented.

Model	Model ID	# of Parameters (K)	AIC_c	ΔAIC_c	Akaike Weight (w_i)
5th Percentile Salinity + Total Sample Weight	1	4	141.64	0.00	0.27
5th Percentile Salinity + Percent Time Mudflats Exposed + Total Sample Weight	9	5	143.38	1.74	0.11

Notes: For the full set of models evaluated using AIC (i.e., 31 models) see **Appendix D**.

Table 3-13 Relative Importance of Variables Potentially Influencing Total PUFA Levels in Biofilm based of Summed Akaike Weights and the Direction of Change

Variables	Sum of Weights (w_i)	Direction of Change (+ / -)
5th Percentile Salinity	0.76	+
Percent Time Mudflats Exposed	0.34	+
95th Percentile Salinity	0.31	+/-
95th Percentile Temperature	0.21	+/-
5th Percentile Temperature	0.21	+/-

PUFA were produced by biofilms sampled across the salinity gradient (**Figure 3-15**). Under lower salinity conditions (5th percentile PSU ≤ 5, n = 21) PUFA abundance averaged 0.21 g/m^2 ± 0.08 SD. Under moderate 5th percentile salinities (5 < PSU ≤ 10, n = 4) PUFA levels averaged 0.22 g/m^2 ± 0.04 SD. Under high 5th percentile salinities levels (PSU > 10, n = 5) PUFA levels averaged 0.29 g/m^2 ± 0.07 SD. Differences in mean PUFA abundance among salinity conditions were not statistically significant ($F_{2,27} = 2.4$, $p = 0.11$).

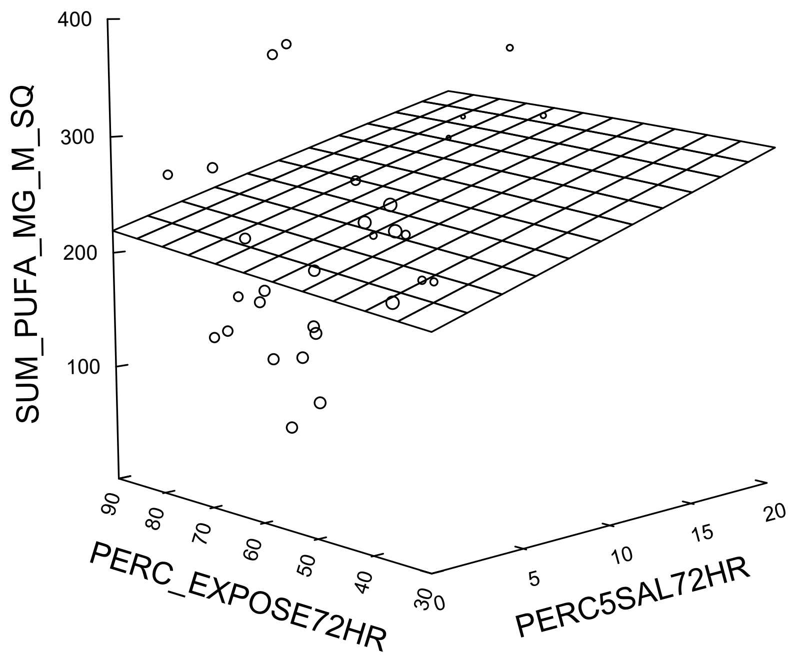


Figure 3-15 Relationship between Polyunsaturated Fatty Acid (PUFA) Levels, the Percentage of Time Mudflats are Exposed, and the Fifth Percentile Salinity

3.1.5.4 Total MUFA

AIC_c results indicated models with the most support to influence biofilm Total MUFA levels contained the variable percent of time mudflats were exposed (Model ID 5), and percent of time mudflats were exposed plus the 5th percentile water column salinity over the previous 72-hour period Model ID 9) (**Table 3-14**). Summed Akaike model weights across the entire model-set (**Appendix D**) indicated mudflat exposure time ($\sum w_i = 0.92$) was the variable with the most influence on biofilm MUFA levels, with all other variables possessing $\sum w_i \leq 0.34$ (**Table 3-15**). In all models, mudflat exposure time and 5^{th} percentile water column salinity were positively associated with increases in biofilm MUFA abundance. The direction of influences for the remaining variables (5th percentile temperature, 95th percentile salinity, 95th percentile temperature) varied depending on the other variables included in the model.

Results from the backward stepwise regression indicated the model containing the percentage of time mudflats were exposed (Model ID 5) to be the most parsimonious model, explaining the most variability of all potential models ($r = 0.47$, $F_{1,28} = 7.7$, $P < 0.01$).

Table 3-14 Variables Influencing Total Monounsaturated Fatty Acid (MUFA) Abundance in Biofilm based on Akaike's Second-Order Information Criterion (AICc) for the Best Models Tested. Only models with substantial empirical support (∆AICc < 2.0, Burnham and Anderson 2002) are presented.

Model	Model ID	# of Parameters (K)	AIC_c	ΔAIC_c	Akaike Weight (w_i)
Percent Time Mudflats Exposed + Total Sample Weight	5	4	158.05	0.00	0.33
Percent Time Mudflats Exposed + 5th Percentile Salinity + Total Sample Weight	9	5	159.69	1.64	0.15

Notes: For the full set of models evaluated using AIC (i.e., 31 models) see **Appendix D**.

Table 3-15 Relative Importance of Variables Potentially Influencing Total MUFA Abundance in Biofilm based of Summed Akaike Weights and the Direction of Change

Variables	Sum of Weights (w_i)	Direction of Change (+ / -)
Percent Time Mudflats Exposed	0.92	+
5th Percentile Salinity	0.34	+
5th Percentile Temperature	0.20	+/-
95th Percentile Salinity	0.20	+/-
95th Percentile Temperature	0.18	+/-

MUFA were produced by biofilms sampled across the salinity gradient (**Figure 3-16**). Under lower salinity conditions (5th percentile PSU ≤ 5, n = 21) MUFA levels averaged 0.28 g/m^2 ± 0.13 SD. Under moderate 5th percentile salinities (5 < PSU ≤ 10, n = 4) MUFA levels averaged 0.32 g/m^2 ± 0.08 SD. While under high 5th percentile salinities levels (PSU > 10, n = 5) MUFA levels remained unchanged averaging 0.37 g/m^2 sediment ± 0.09 SD. Differences in mean MUFA abundance among salinity conditions were not statistically significant ($F_{2,27}$ = 1.4, p = 0.27).

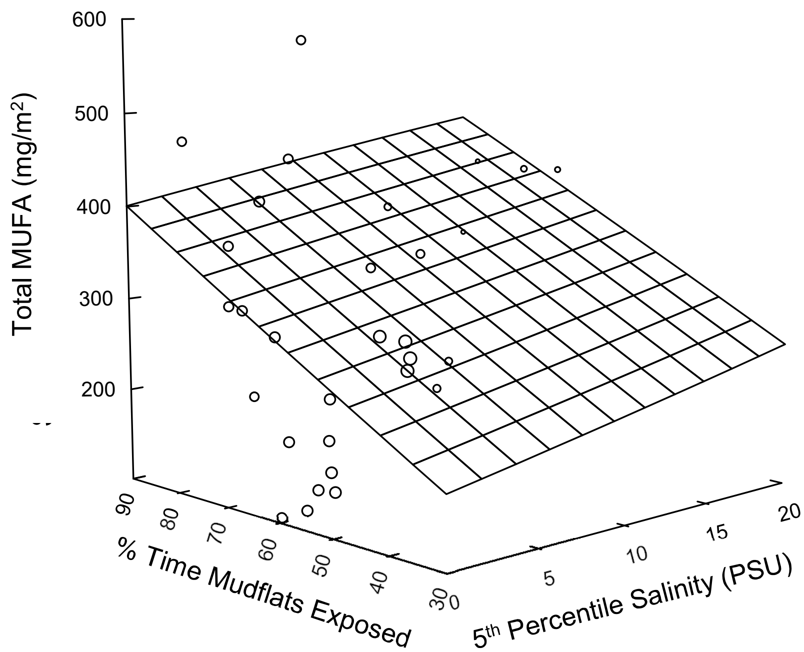


Figure 3-16 Relationship between Monounsaturated Fatty Acid (MUFA) Levels, the Percentage of Time Mudflats are Exposed, and the Fifth Percentile Salinity

3.1.5.5 Total SFA

AIC_c results for SFA were very similar to those for MUFA. Analyses indicated models with the most support to influence biofilm Total SFA levels contained the variable representing the amount of time the mudflats were exposed over the previous 72-hour period (**Table 3-16**). Model ID 9, comprised by the variables the percentage of time mudflats were exposed and the 5th percentile water column salinity possessed an ΔAIC_c score of 2.02 indicating some evidence for salinities influence on Total SFA levels (**Appendix D**). However, summed Akaike model weights across the entire model-set (**Appendix D**) indicated mudflat exposure time ($\sum w_i = 0.92$) was the variable with the most influence on biofilm SFA levels, as all other variables possessed $\sum w_i \leq 0.32$ (**Notes:** For the full set of models evaluated using AIC (i.e., 31 models) see **Appendix D**.

Table 3-17). All variables in the model set were positively associated with biofilm SFA abundance, although evidence for their influence on SFA abundance is not strong.

Results from the backward stepwise regression indicated the model containing the percentage of time mudflats were exposed (Model ID 5) to be the most parsimonious model, explaining the most variability of all potential models ($r = 0.69$, $P < 0.001$).

Table 3-16 Variables Influencing Total Saturated Fatty Acid (SFA) Levels in Biofilm based on Akaike's Second-Order Information Criterion (AICc) for the Best Models Tested. Only models with substantial empirical support (ΔAICc < 2.0, Burnham and Anderson 2002) are presented

Model	Model ID	# of Parameters (K)	AIC_c	ΔAIC_c	Akaike Weight (w_i)
Percent Time Mudflats Exposed + Total Sample Weight	5	4	372.73	0.00	0.35

Notes: For the full set of models evaluated using AIC (i.e., 31 models) see **Appendix D**.

Table 3-17 Relative Importance of Variables Potentially Influencing Total SFA Levels in Biofilm based of Summed Akaike Weights and the Direction of Change

Variables	Sum of Weights (w_i)	Direction of Change (+ / -)
Percent Time Mudflats Exposed	0.92	+
5th Percentile Salinity	0.32	+
95th Percentile Temperature	0.22	+
95th Percentile Salinity	0.19	+
5th Percentile Temperature	0.19	+

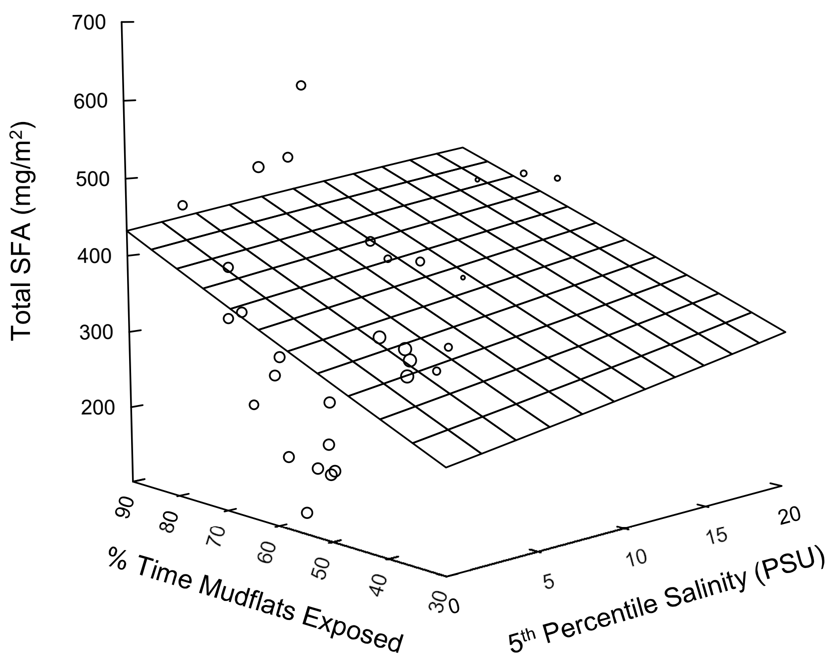


Figure 3-17 Relationship between Saturated Fatty Acid (SFA) Levels, the Percentage of Time Mudflats are Exposed, and the Fifth Percentile Salinity

SFA were produced by biofilms sampled across the salinity gradient (**Figure 3-17**). Under lower salinity conditions (5th percentile PSU ≤ 5, n = 21) SFA levels averaged 0.26 ug/mg sediment ± 0.12 SD. Under moderate 5th percentile salinities (5 < PSU ≤ 10, n = 4) SFA levels averaged 0.28 ug/mg sediment ± 0.12 SD. Under high 5th percentile salinities levels (PSU > 10, n = 5) SFA levels averaged 0.27 ug/mg sediment ± 0.12 SD. Differences in mean SFA abundance among salinity conditions were not statistically significant ($F_{2,27}$ = 1.1, p = 0.35).

3.1.5.6 Summary of Biofilm Parameters

AIC_c modelling and summed Akaike model weights indicated increases in mudflat exposure time and 5th percentile water column salinity levels were the primary factors positively influencing biofilm parameter abundance (e.g., Chlorophyll *a* and fatty acid levels) (**Table 3-18**). Each of these parameters had strong to moderate influence on 12 of the 16 biofilm parameters tested. Ninety-fifth percentile water column salinity levels, and 5th and 95th percentile water column temperature levels were found not to have a strong influence on any parameter and moderate influence over 2-4 of the 16 biofilm parameters evaluated (**Table 3-18**).

Chlorophyll *a* and total carbohydrate abundances were primarily influenced by changes in 5th percentile water column salinity, while Total Fatty Acid levels were primarily influenced by the length of time the mudflats were exposed and secondarily by 5th percentile water column salinity levels (**Table 3-18**).

Total MUFA abundance, including levels of 16:1n-7, were positively influenced by increases in mudflats exposure time with little evidence that production was affected by water column salinity or temperature. In contrast, 18:1n-7 and 18:1n-9 were more strongly influenced by 5th percentile water column salinity and to a lesser extant a combination of 95th percentile water column salinity, 95th percentile water column temperature, and mudflat exposure time. 18:1n-7 and 18:1n-9 comprised ~4-7% of total MUFA abundance (see **Appendix B**) and had little influence on the overall factors influencing MUFA abundance.

Factors influencing SFA abundance were similar to those of MUFA showing a strong positive influence by increases in the percentage of time mudflats were exposed. However, summed Akaike weights indicated potential moderate positive effects from increases in 5th and 95th percentile water column salinity levels for the fatty acids 18:0 and 14:0, respectively.

Total PUFA, omega 3, omega 6, and individual fatty acids 20:5n-3 (EPA) and 22:6n-3 (DHA) abundances were primarily influenced by increases in 5th percentile salinity levels, with increases in mudflat exposure time, 95th percentile salinity, and 95th percentile water column temperature (for total omega 6 fatty acids) having moderate to low positive influence on abundance.

Table 3-18 Summary of the Relative Importance of Abiotic Factors Potentially Influencing Biofilm Parameter Abundance based on the Sum of Akaike Weights ($\sum w_i$) across the Entire Model Set (n = 31 models)

Biofilm Parameter	Abiotic Factors				
	Percent Time Mudflats Exposed	5th Percentile Salinity	95th Percentile Salinity	5th Percentile Temperature	95th Percentile Temperature
Chlorophyll *a*	0.21	0.93	0.22	0.42	0.21
Total Carbohydrates	0.32	0.96	0.23	0.35	0.21
Fatty Acids					
Total Fatty Acid	0.88	0.41	0.20	0.20	0.18
Polyunsaturated Fatty Acids (PUFA)					
Total PUFA	0.34	0.76	0.31	0.21	0.21
Omega 3 Fatty Acids	0.31	0.72	0.32	0.23	0.20
Omega 6 Fatty Acids	0.53	0.81	0.28	0.22	0.34
20:5n-3 (EPA)	0.32	0.45	0.39	0.23	0.23
22:6n-3 (DHA)	0.34	0.93	0.28	0.24	0.24
Monounsaturated Fatty Acids (MUFA)					
Total MUFA	0.92	0.34	0.20	0.20	0.18
16:1n-7	0.78	0.24	0.22	0.22	0.21
18:1n-7	0.34	0.73	0.52	0.26	0.43
18:1n-9	0.58	0.84	0.46	0.26	0.36
Saturated Fatty Acids (SFA)					
Total SFA	0.92	0.32	0.22	0.19	0.19
14:0	0.75	0.31	0.51	0.21	0.23
16:0	0.96	0.25	0.32	0.19	0.19
18:0	0.72	0.54	0.21	0.20	0.21

Notes:

1. The maximum possible sum of Akaike Weights ($\sum w_i$) = 1.0. Variables with $\sum w_i$ approaching 1.0 indicates strong support for their ability to influence biofilm abundance parameters.
2. Cell coloration: Black: $\sum w_i > 0.66$; dark gray: $0.66 \geq \sum w_i \geq 0.33$; white: $\sum w_i < 0.33$
3. For the full set of models evaluated using AIC (i.e., 31 models) see **Appendix D**

3.2 MICROPHYTOBENTHIC COMMUNITY COMPOSITION

The microphytobenthic community during the 2016 northward migration was dominated by *Nitzschia* spp. and *Navicula* spp., representing 34.4% and 30.3% of the total observed community, respectively (**Figure 3-18**). The genus *Achnanthidium* spp. accounted for 8.9% of the total observed microphytobenthos community.

Achnanthidium spp. accounted for a larger proportion of the community at Stations J, X, and Y (**Figure 3-18**). Across all stations, *Nitzschia* and *Navicula* spp. were relatively consistent, accounting for ~2/3rds of the total observed community (**Figure 3-18**; **Appendix E**). Total microphytobenthos abundance, along with the key taxa *Nitzschia* spp., *Navicula* spp., and *Achnanthidium* spp., were observed to decrease at all sites after April 25 (**Figure 3-19**).

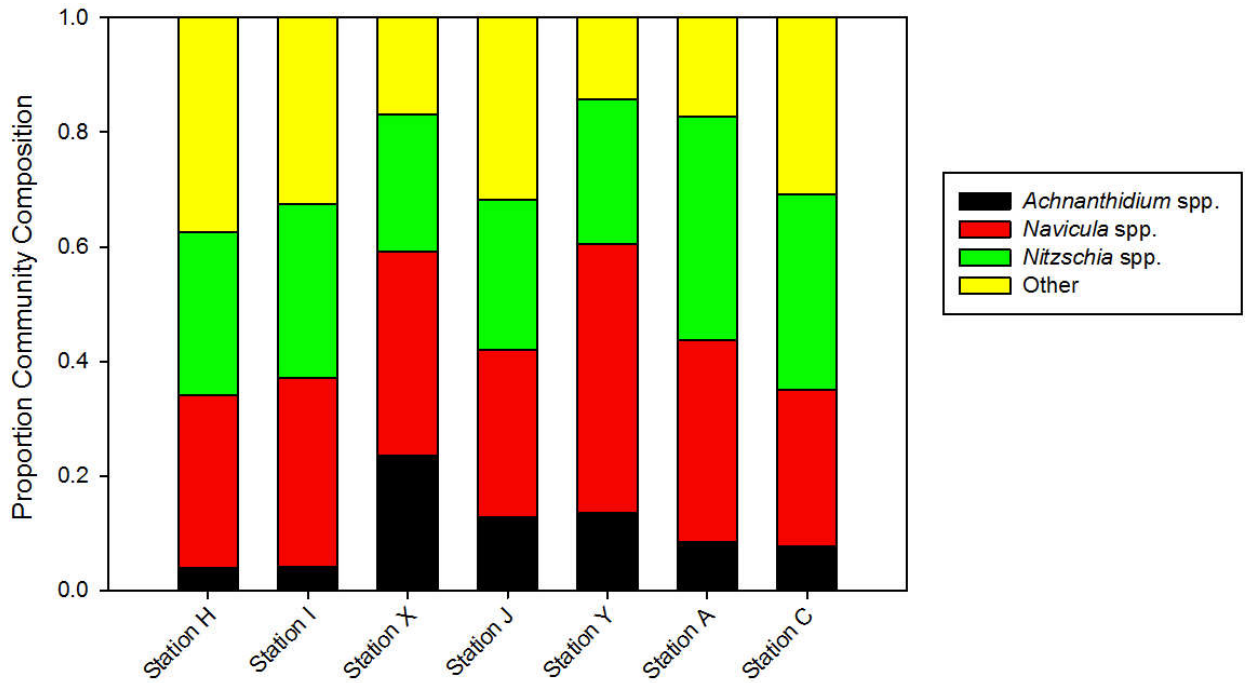


Figure 3-18 Spatial Microphytobenthos Community Composition during the 2016 Northward Migration Study

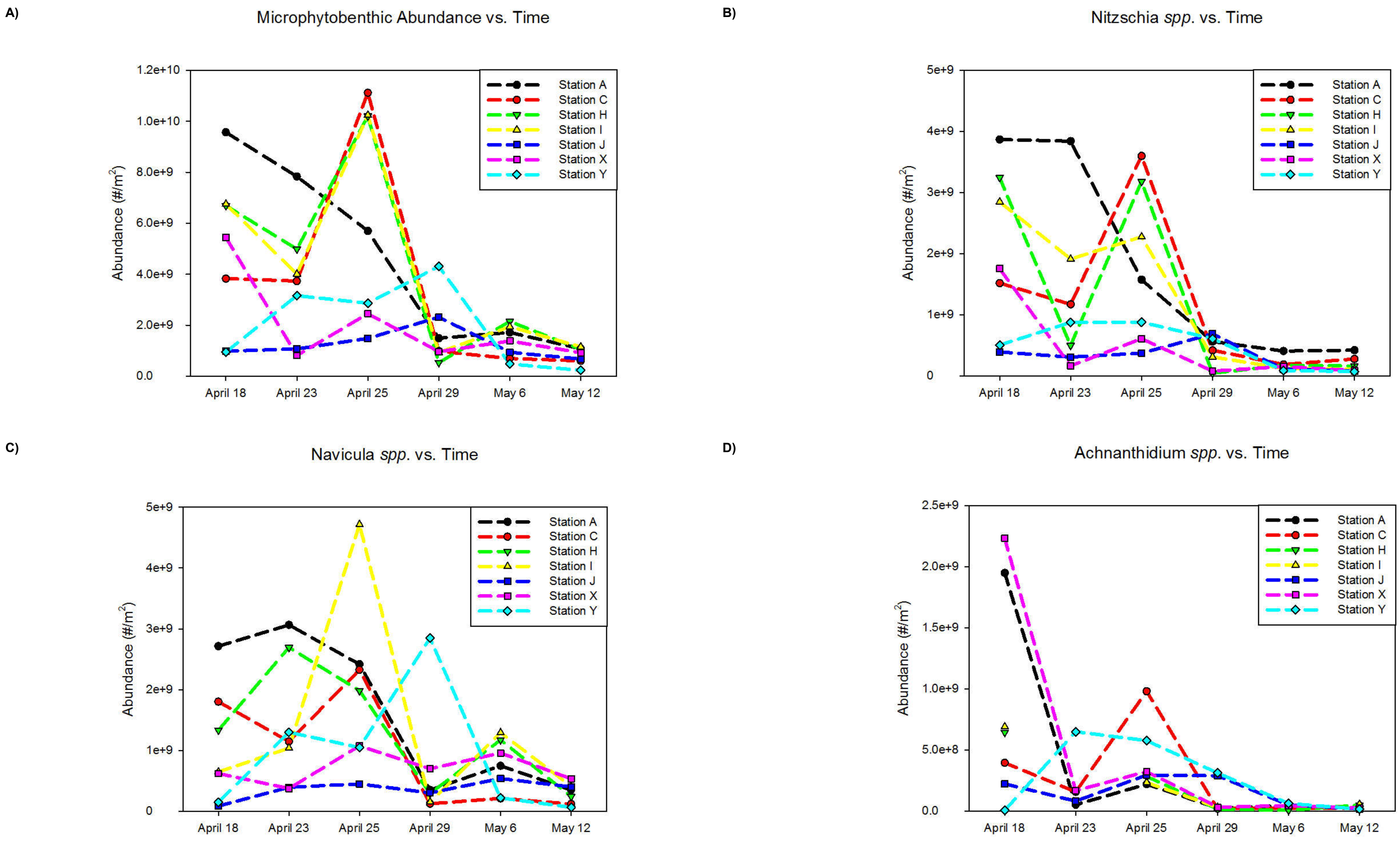


Figure 3-19 Spatial and Temporal Trends in Total Microphytobenthos (A), *Nitzschia* spp. (B), *Navicula* spp. (C), and *Achnanthidium* spp. (D)

3.2.1 Community Composition Patterns

No significant difference in the microphytobenthic community among stations was documented (R = 0.07, p = 0.13); however, a significant difference was observed temporally (R = 0.36, p = 0.001). This temporal difference is evident in the nMDS as the April 18 – April 25 microphytobenthic community strongly separated from the April 29 – May 12 community (**Figure 3-20**). Using all stations across Roberts Bank nested within each sampling date, a stronger temporal difference was observed (R = 0.50, p = 0.001). The associated Single Linkage Cluster Analysis is shown in **Figure 3-21** and indicates a statistically significant change in the microphytobenthic community across Roberts Bank between April 25 and 29.

The results of a Similarity Profile analysis (SIMPROF) confirmed that each cluster is unique from the other (Pi = 3.49, p < 0.001). A summary of temporal differences is provided in **Table 3-19**.

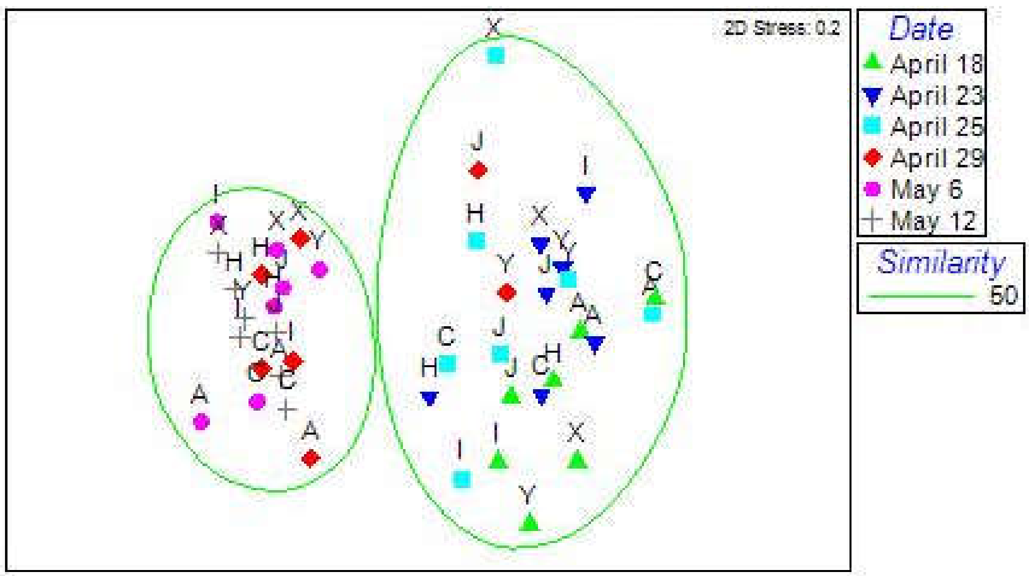


Figure 3-20 Non-Parametric Multi-Dimensional Scaling Analysis (nMDS) of Microphytobenthos Community at Roberts Bank during 2016 Northward Migration

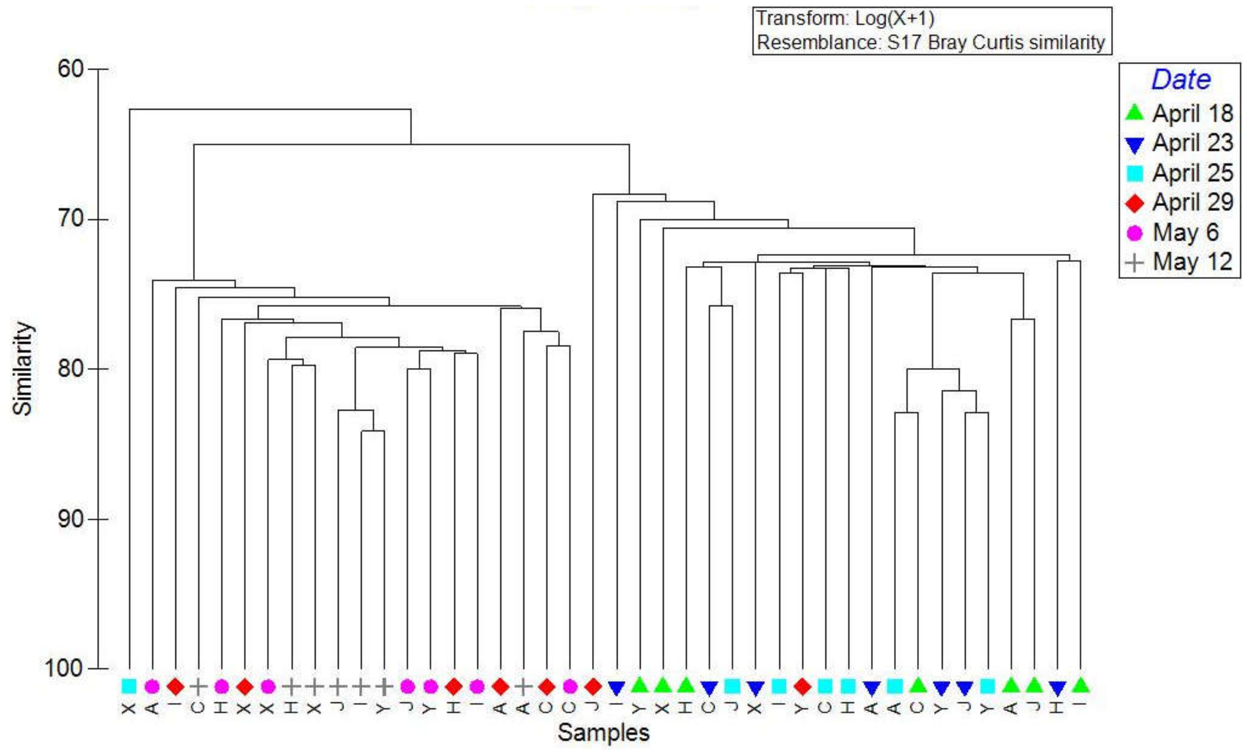


Figure 3-21 Single Linkage Cluster Analysis of Microphytobenthos Community at Roberts Bank during 2016 Northward Migration

Table 3-19 Analysis of Similarities (ANOSIM) of Biofilm Sampling Dates. Bolded values indicate a significance at $p < 0.05$

Dates	18-Apr	23-Apr	25-Apr	29-Apr	6-May
23-Apr	0.19				
25-Apr	0.13	0.06			
29-Apr	**0.59**	**0.54**	**0.38**		
6-May	**0.96**	**0.94**	**0.75**	0.00	
12-May	**0.95**	**0.96**	**0.82**	0.15	0.01

3.2.2 Temporal Community Patterns

Based on the cluster analysis, community data were separated into the three significantly different clusters:

- Cluster 1 – All Stations on April 18-25 data, Station J and Y on April 29
- Cluster 2 – All Stations on April 29-May 12 (except Station J and Y on April 29)
- Cluster 3 – Station X on April 25 (considered an outlier).

Comparisons between Cluster 1 (April 18-25) and Cluster 2 (April 29 - May12) showed the community differences were primarily driven by changes in sub-dominant diatom taxa as opposed to changes in dominant taxa (i.e., *Nitzschia* spp, *Navicula* spp., and *Achnanthidium* spp.) (see the "Dissimilarity" and "Contributed %" column in **Table 3-20**).

Several taxa not document from April 18 to 25 were observed between April 29 and May 12 including *Planothidium* spp., *Tryblionella* spp., and *Encyonema* spp. At the same time, relatively minor decreases were observed in the dominant *Nitzschia* spp., *Navicula* spp., and *Achnanthidium* spp., which contributed minimally to the SIMPER results. Despite this, the presence of new species and/or increased abundances of minor species occurring after April 25 changed the overall community composition within the study area.

Table 3-20 Similarity Percentage (SIMPER) Results Between April 18-25 and April 29-May 12, 2016 Sampling at Roberts Bank

Species	Cluster 1 April 18-25 Average Abundance	Cluster 2 April 29-May 12 Average Abundance	Dissimilarity	Contributed %	Cumulative %
Unknown pennales spp.	-	17.04	8.30	5.38	5.38
Planothidium spp.	-	17.00	7.47	5.37	10.75
Tryblionella spp.	-	14.27	2.20	4.44	15.19
Fragilaria spp.	2.52	14.42	2.02	4.15	19.33
Encyonema spp.	-	11.40	1.62	3.65	22.99
Amphora spp.	3.39	11.32	1.48	3.37	26.36
Melosira spp.	5.81	12.69	1.38	3.31	29.67
Hippodonta spp.	3.33	10.62	1.20	3.19	32.87
Aulacoseira spp.	7.08	12.83	1.33	2.98	35.85
Diatoma spp.	8.05	10.91	1.15	2.89	38.74
Cylindrotheca spp.	-	8.96	1.02	2.84	41.58
Eunotia spp.	-	8.80	1.15	2.73	44.30
Thalassiosira spp.	8.18	3.25	0.98	2.62	46.92
Bacillaria spp.	7.18	6.48	1.04	2.57	49.49
Ulnaria spp.	-	8.10	1.04	2.50	52.00
Unknown centrales spp.	15.04	12.27	1.00	2.35	54.34
Cymbella spp.	4.76	5.69	0.91	2.28	56.62
Epithemia spp.	4.86	5.67	0.92	2.25	58.87
Tabularia spp.	-	7.24	0.94	2.18	61.05
Skeletonema spp.	5.59	3.11	0.83	2.08	63.13
Diploneis spp.	0.76	6.33	0.86	2.06	65.18
Eucocconeis spp.	-	6.38	0.84	1.98	67.16

Species	Cluster 1	Cluster 2	Dissimilarity	Contributed %	Cumulative %
	April 18-25 Average Abundance	April 29-May 12 Average Abundance			
Surirella spp.	14.34	15.88	0.95	1.87	69.04
Gomphonema spp.	15.13	15.37	0.85	1.72	70.75
Achnanthidium spp.	17.71	16.23	0.82	1.42	-
Nitzschia spp.	20.86	18.88	1.76	0.64	-
Navicula spp.	20.74	19.67	1.53	0.44	-

3.2.3 Salinity Influence of Microphytobenthic Composition

A correlation between water column salinity measurements and nMDS values was conducted to determine salinity's influence on microphytobenthic community composition. A moderately negative relationship occurred between MDS1 and 24 hour (r_s = -0.38), 48 hour (r_s = -0.42), 72 hour (r_s = -0.47), and 96 hour salinity (r_s = -0.47) (**Figure 3-22**), indicating that 38 to 47% of the variation in the microphytobenthic community composition can be explained by water column salinity. Also, when viewing the nMDS plot, the community clusters (April 18-25 and April 29-May 12) aligned along nMDS1, indicating that the change in community composition was related to relative changes in water column salinity.

Based on visual interpretation of bubble plots, combined with correlations of individual taxa, the influence of salinity over several different taxa can be inferred. **Figure 3-23** presents the nMDS plot of salinity stations (similar to **Figure 3-22**), with icons scaled to the 24 hr. salinity values. Overall, *Nitzschia* spp. and *Achnanthidium* spp. tended to increase with increasing salinity in contrast to *Cylindrotheca* spp., *Encyonema* spp., *Fragilaria* spp., and *Planothidium* spp. which tended to decrease (**Figure 3-23**).

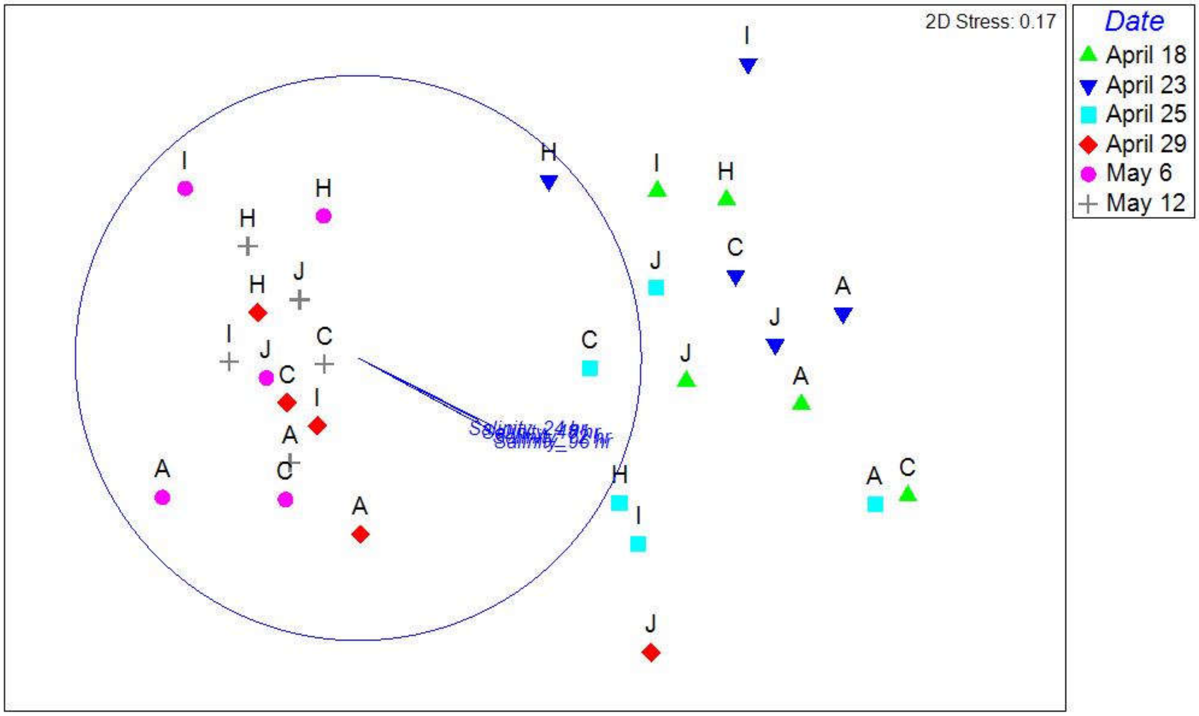


Figure 3-22 nMDS Plot of Salinity Monitoring Stations with Spearman Rank Correlation Overlaid

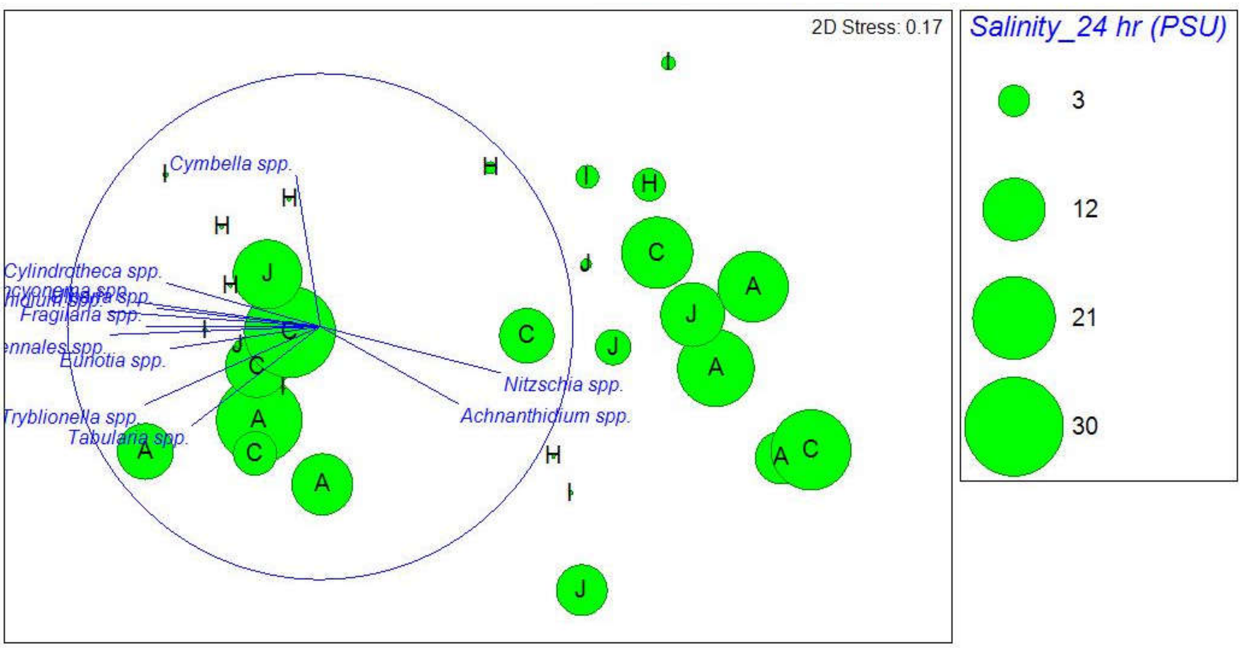


Figure 3-23 nMDS Plot Showing the Relationship between Biofilm Taxonomy with 24 hr Salinity Values Bubble Plots

3.3 SHOREBIRD FORAGING USE

3.3.1 Spatial and Temporal Analyses

Shorebird dropping densities were recorded at the seven sampling stations during the six sampling events (n = 42). However, dropping estimates for stations A, C, and Y (in the upper intertidal zone) on April 29 and May 12 were excluded from analyses as high tides were too low to inundate the mudflats around the stations and dropping densities reflected multiple periods of usage. These sampling events had very high droppings densities and it is likely the low-high tide concentrated shorebirds in the upper intertidal zone when the remainder of the mudflat was flooded and unavailable for foraging. Therefore, the sample size used in the analyses was reduced to 36 (**Table 3-21**).

A Pearson correlation analysis showed the two dropping density estimates based on droppings size (i.e., # droppings less than a dime in size and # of droppings less than a Canadian quarter in size) to be highly correlated (r = 0.99). Therefore, one metric, droppings less than a dime, was selected for analyses as this size category has been documented to be indicative of WESA droppings (Pomeroy 2005).

Mean dropping densities at stations averaged across the migratory period ranged from 0.7 droppings/15 m^2 at Station J to 20.9 droppings/15 m^2 at Station Y (**Table 3-21**, **Figure 3-24**). There was high variability in dropping densities across space and time, with ~25% of sampling events at stations detecting no droppings. Locations consistently supporting shorebird droppings were Stations X, Y, and I. Locations with the fewest occurrences of droppings were Stations H and J. The highest single day dropping density (70) was documented at Station I close to Canoe Passage. A Kruskal-Wallis non-parametric ANOVA found no difference in dropping densities between stations (H = 10.1, p = 0.12).

Table 3-21 Mean Dropping Densities, Standard Deviations, and Sample Sizes of Shorebird Droppings Documented at Sampling Stations during Northward Migration

Station	N	Mean # Droppings / 15 m^2	SD	Minimum Droppings / 15 m^2	Maximum Droppings / 15 m^2	% Surveys with Droppings
A	4	7.5	5.2	0.0	12	75%
C	4	3.8	3.5	0.0	8	75%
H	6	3.8	6.4	0.0	16	50%
I	6	12.5	28.2	0.0	70	83%
J	6	0.7	1.0	0.0	2	50%
X	6	1.0	0.6	0.3	2	100%
Y	4	20.9	19.8	2.5	38	100%

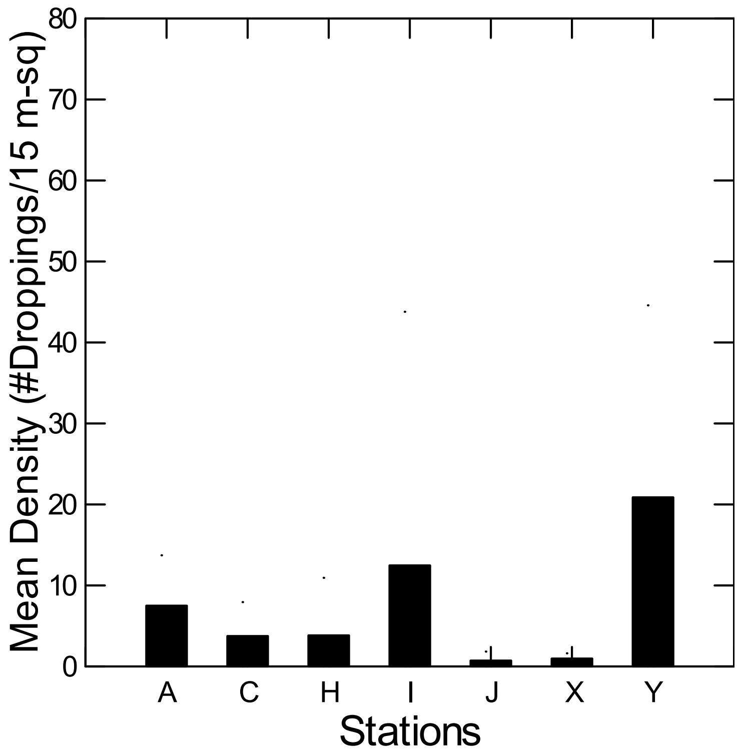


Figure 3-24 Mean Shorebird Dropping Density (Bars) and Standard Deviation (Whiskers) during WESA Northward Migration at Robert Banks

The temporal pattern associated with dropping densities varied by station (**Figure 3-25)**. Densities at Station I were low (≤ 2 droppings/15 m^2) during the beginning to the migratory period, increased during the peak of WESA migration to 70 droppings/15 m^2 toward the end of April, and returned to low levels through mid-May. Other locations, such as Stations J and X, consistently possessed low usage with dropping densities remaining ≤ 2 droppings/15 m^2 throughout the migratory period. Station Y experienced high densities early in the season and also on April 29 and May 12 when the station remained exposed during the low-high tide. This pattern also occurred at Stations A and C.

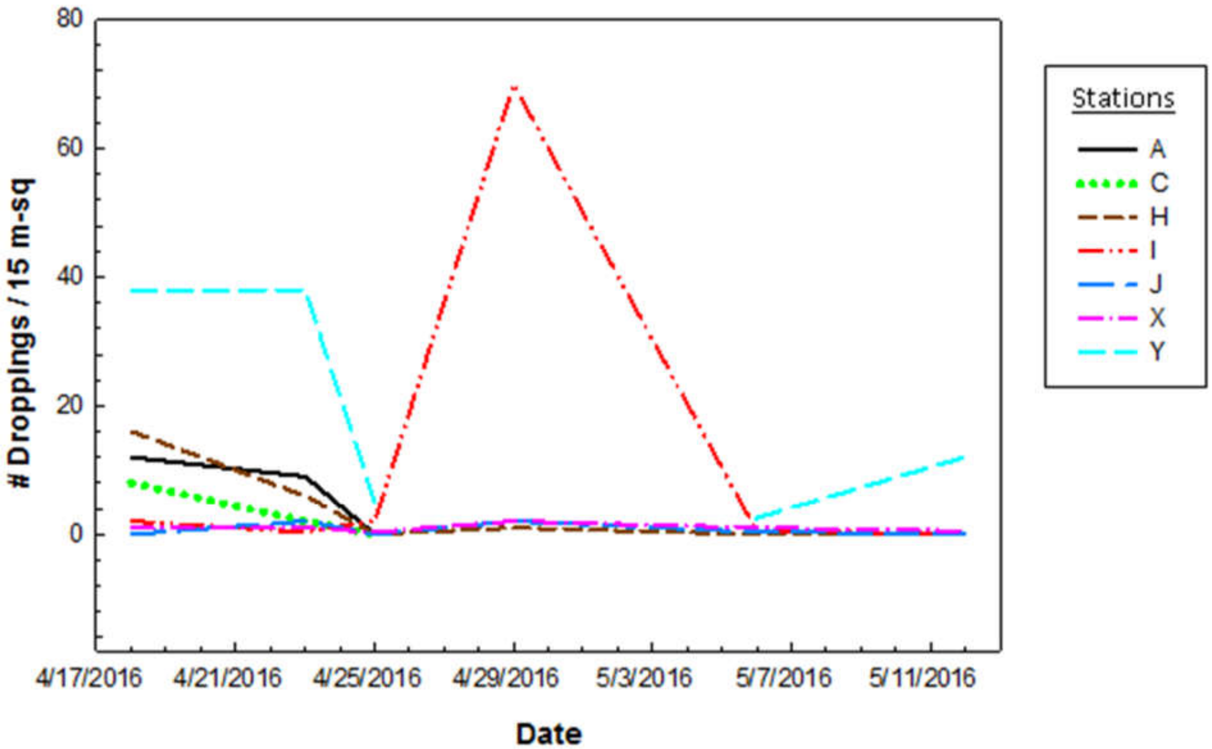


Figure 3-25 Temporal Patterns in Shorebird Dropping Densities at Stations during WESA Northward Migration at Roberts Banks. Note: Densities for Stations A, C, and Y on April 29 and May 12 are excluded

3.3.2 Correlation Analysis

No correlation between dropping densities and biofilm biomass parameters (Chlorophyll *a* and Total Carbohydrates) or fatty acid were documented (**Table 3-22**). Spearman correlation coefficients were low to moderate for all parameters ranging from -0.13 to 0.27.

Table 3-22 Spearman Correlation Coefficients between WESA Dropping Densities (#/15 m^2) and Focal Biofilm Parameter Abundances and Fatty Acid Concentrations

Biofilm parameter	Correlation Coefficient (P)
Chlorophyll *a*	0.11
Total Carbohydrates	0.12
Fatty Acids	
Total Fatty Acid	0.08
Polyunsaturated Fatty Acids (PUFA)	
Total PUFA	0.08
20:5n-3 (EPA)	0.07
22:6n-3 (DHA)	-0.06
Omega 3 Fatty Acids	0.07
Omega 6 Fatty Acids	0.04
Monounsaturated Fatty Acids (MUFA)	
Total MUFA	0.09
16:1n-7	0.07
18:1n-7	0.12
18:1n-9	-0.07
Saturated Fatty Acids (SFA)	
Total SFA	0.07
14:0	0.22
16:0	0.07
18:0	0.14

4.0 DISCUSSION

4.1 BIOFILM BIOMASS AND FATTY ACID ABUNDANCE

4.1.1 Relationship Among Biofilm Parameters

Eighty-seven percent of the biofilm parameters tested (n = 37, **Appendix B**) and 98% of focal fatty acid parameters (n = 15, **Table 3-7**) showed positive statistically significant correlations with each other. Positive relationships included those among Chlorophyll *a* and 11 of 15 biofilm parameters (**Table 3-7**). These relationships between parameters indicate that effects to biofilm are generally expressed similarly across the suite of parameters (i.e., Chlorophyll *a*, fatty acids, and carbohydrates).

Abundance was expressed similarly across biofilm parameters among stations. For example, when stations were ranked according to the abundance levels of each biofilm parameter, Stations A and C ranked as the first or second highest abundance 75% to 88% of the time. Station I consistently possessed the next highest abundance estimates, including possessing the highest (i.e., ranked 1st) abundance estimates ~30% of the time. Stations H and Y ranked in the middle (i.e., 3rd, 4th, or 5th highest ranking) 88% to 94% of the time, respectively. Station J was ranked 6th 75% of the time, and station X had the lowest biofilm abundance levels 88% of the time.

These results suggest that knowledge of one biofilm parameter could facilitate the estimation of the remaining suite of parameters and the positive or negative effects documented in a single parameter are likely indicative of effects to other biofilm parameters. Previous biofilm studies conducted at Roberts Bank used Chlorophyll *a* abundance as a measure of biofilm biomass and to assess how abiotic factors affect biofilm (LGL and Hemmera 2014, WorleyParsons 2014, 2015*b*). Based on results from this study, it is consistent that results from those studies can inform potential effects to biofilm-associated fatty acid and carbohydrate abundance.

Results also suggest that analyses for Chlorophyll *a* in intertidal mudflat sediments is potentially a cost effect and reliable way to measure and monitor biofilm abundance at Roberts Bank compared to analyses of other parameters. The current method used to measure Chlorophyll *a* is through laboratory analysis of biofilm sediment samples. While accurate and one of the cheaper known methods of assessing biofilm, the procedure typically takes 30 to 60 days to obtain results and if multiple samples are taken can become costly. Newly emerging methods, such as the use of a BenthoTorch (BBE 2017), may prove more cost effective as they measure and report Chlorophyll *a* abundance of biofilm-associated diatoms in the field within seconds, allowing for real-time results, trend monitoring, and the ability to sample a large area quickly and efficiently.

4.1.2 Distribution and Abundance of Biofilm Parameters

A consistent pattern in the levels of biofilm parameters was documented between stations, with the stations having the longest mudflats exposure time (i.e., Stations A, C, and I) regularly possessing the highest biofilm parameter abundances. Despite documented differences in parameter levels, almost all fatty acids (>99%), plus Chlorophyll *a* and Total Carbohydrates, were documented at every station on every sampling event, indicating all productivity parameters were produced by biofilms across the entire spatiotemporal range and across the suite of abiotic conditions experienced within the study area. This included the documentation of high levels of MUFA, SFA, and PUFA under both freshwater dominated conditions (e.g., Station I, 5th percentile salinity < 5 PSU) and more marine water dominated environments (e.g., Stations A and C, 5th percentile salinity > 10 PSU).

Approximately 90% of the energy for migratory flight in birds comes from fat deposits with the remaining 10% comprised largely of protein (Guglielmo 2010). In this study, the fatty acids in greatest abundance were the SFA palmitic acid (16:0), the MUFA palmitoleic acid (16:1n-7), and the PUFA EPA (20:5n-3), and DHA (22:6n-3), all of which are potentially important to migrating WESA. Previous research has shown that 80 to 90% of all fatty acids stored by WESA are C_{16} and C_{18} fatty acids (i.e., the SFA palmitate [16:0] and stearate [18:0], and the MUFA palmitoleate [16:1] and oleate [18:1]) (Egeler and Williams 2000) similar to fatty acids documented in this study. During spring migration, the percentage of MUFA (e.g., 16:1 and 18:1) stored by WESA has been shown to increase, possibly because MUFA have a lower melting point and are easier to metabolize (Egeler and Williams 2000, Guglielmo 2010) than SFA (e.g., 16:0, 18:0). Despite this shift, the same four fatty acids (i.e., 16:0, 16:1, 18:0, 18:1) accounted for ~86% of fatty acids deposited in male and female WESA during northward migration (speaking to their potential importance (Egeler and Williams 2000)). Within this study, 16:0, 16:1 (including 16:1n-7), 18:0, 18:1 fatty acids comprised 52% of the total fatty acid abundance documented. The importance of these fatty acids to migrating WESA is further supported by research indicating that WESA are capable of physiologically manipulating fatty acids prior to storing fats to achieve the necessary profile to fuel migratory flight (e.g., converting SFA to MUFA) (Egeler and Williams 2000, Egeler et al. 2003).

Compared to MUFA and SFA, which can originate from dietary sources and physiological manipulation, PUFA, such as EPA and DHA, and other essential fatty acids cannot be manufactured by birds and must be obtained through dietary sources alone (Egeler et al. 2003, Guglielmo 2010). Levels of PUFA in adipose tissue of migrating WESA have been documented in small quantities (~5%), with long-chained omega 3 and omega 6 fatty acids comprising less than 1% of stored fats (Egeler and Williams 2000). Therefore, PUFA, omega 3, and omega 6 fatty acids likely do not play a primary role in directly fueling migratory flight. However, it has been hypothesised that omega 3 PUFAs, such as EPA, increase migratory performance by enhancing the functional capacity of membranes and thereby increase the aerobic capacity of flight muscles (Maillet and Weber 2006, 2007). This theory has not been proven, nor has it been proven that incorporation of omega 3 PUFAs in adipose tissues and cell membranes improve exercise performance or are necessary for endurance flight (Guglielmo 2010).

Regardless of the essential nature of PUFA, as mentioned previously, omega 3 and 6 PUFA (including EPA and DHA) were produced by the biofilm community across the spatiotemporal range and abiotic gradient documented during spring 2016. AIC model results indicated PUFA abundance was positively correlated with 5th percentile water column salinity levels. Despite this, an ANCOVA did not document a difference in total PUFA or EPA abundance between stations located within areas of higher salinity close to the Roberts Bank causeway (i.e., Stations A and C) and Station I, located close to the freshwater outflow of the Fraser River, indicating the potential for similar PUFA/EPA abundance to occur across the known salinity gradient of the study area. Similarly, total PUFA abundance was documented not to differ statistically between samples collected in high 5th percentile water column salinity locations vs. low 5th percentile salinity locations. The lack of statistical difference in PUFA abundance between high and low salinity sites could be the results of the limited sample sizes available for analyses; however, both areas (i.e., close to the Fraser River outflow vs. Roberts Bank Causeway/Brunswick Dyke) are heavily used by foraging WESA during northward migration (**Figure 4-1a**) indicating both areas likely satisfy WESA nutritional needs.

During the 2012 and 2013 WESA northward migration, sandpiper use of the less saline locations close to Canoe Passage was consistently equal to or greater than in more saline areas close to the Brunswick Dyke/Roberts Bank Causeway (Hemmera 2014). Notable in the 2012 and 2013 **Figure 4-1** shorebird distributions are the shift in usage closer to Canoe Passage into likely more freshwater conditions in 2012 (**Figure 4-1a**). In 2012, the annual freshet was a large event approaching a 20-year recurrence flood resulting in elevated freshwater discharge from the Fraser River (**Figure 4-1b**). The large freshet likely decreased salinity levels across a larger area of mudflats than in typical years and may have benefited shorebirds, as the high usage of areas dominated by freshwater input likely has physiological benefits. Shorebirds feeding in saline environments must expend energy to osmoregulate salts consumed when foraging, which can be energetically expensive. Gutiérrez et al. (2011) documented a 17 and 20% increase in basal metabolic rate and daily energy consumption, respectively, in shorebirds using saltwater compared to freshwater environments indicating the environment was energetically more expensive. Additionally, the body mass of shorebirds feeding within saltwater habitats were 9-16% lower than birds using freshwater habitats. As a primary goal of WESA during northward migration is to reach the breeding grounds as quickly as possibly to secure breeding territories, feeding in habitats that meet nutritional needs while minimizing physiological demands is likely a good strategy and may make freshwater dominated areas within estuaries preferential habitats.

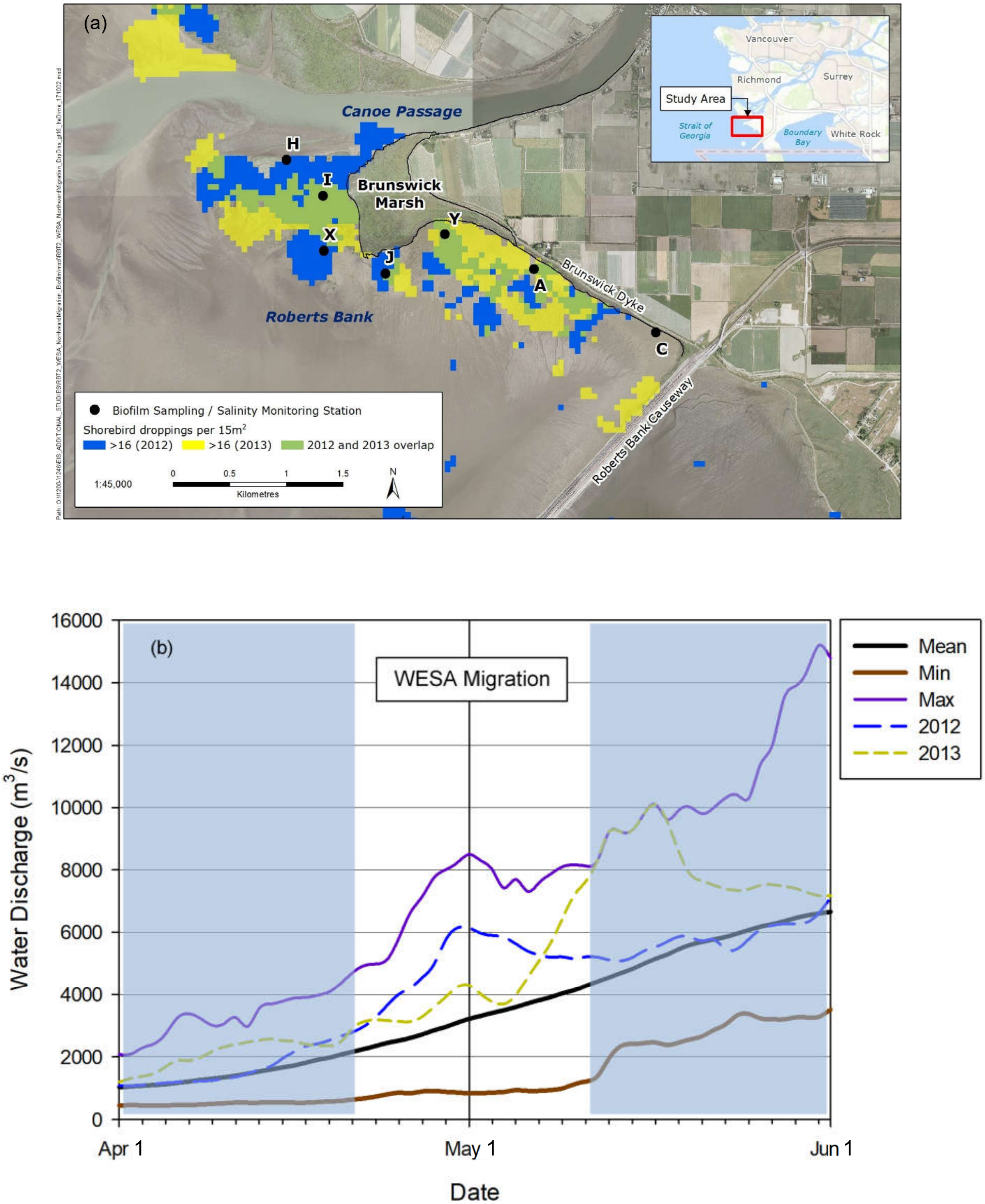


Figure 4-1 Areas of High Western Sandpiper Usage of the Study Area during Northward Migration in 2012 and 2013 Based on Sandpiper Droppings Densities less than a Canadian Dime in Size (a) and Historical (1912 to 2016) Fraser River Freshet Discharge Rates (b). Figure (a) is modified from data presented in Hemmera (2014) to present areas of greatest usage by WESA. Water data are derived from the Government of Canada (2017) Water Office for Fraser River Station at Hope, BC (08MF005).

4.1.3 Factors Affecting Biofilm Parameters

Increases in the percentage of time the intertidal mudflats were exposed (i.e., not inundated) and increases in 5th percentile water column salinity were found to be the primary factors correlated with biofilm abundance levels. Increases in mudflat exposure time were strongly associated with increases in total fatty acid, total MUFA (including 16:1n-7), and total SFA (including 14:0, 16:0, 18:0) abundance. For total MUFA and total SFA there was little evidence that changes in salinity affected abundance levels. These results were supported by the regression and AIC analyses, and by examining mean fatty acid levels under differing salinity conditions (**Section 3.1.5**). An exception to this was the MUFA 18:1n-7 and 18:1n-9, which was influenced more by a suite of factors including changes 5th and 95th percentile water column salinity levels, mudflats exposure time, and potentially 95th percentile water column temperature. 18:1n-7 and 18:1n-9 comprised ~1% and 3% of the total fatty acid abundance, respectively (**Appendix B**). Total fatty acid levels were primarily driven by increases in mudflat exposure time, with some evidence indicating a smaller influence from changes in 5th percentile water column salinity (**Table 3-18**, **Figure 3-14**).

In contrast, positive increases in most PUFA abundance were primarily associated with increases in 5th percentile water column salinity and secondarily influenced by increases in mudflat exposure time (**Table 3-18**). This relationship was true for levels of Total PUFA, 22:6n-3 (DHA), and omega 6 fatty acids. In contrast, 20:5n-3 (EPA) abundance was influenced roughly equally by 5th and 95th percentile water column salinity. There was little evidence that water column temperature was a factor driving PUFA abundance levels.

The result that Chlorophyll *a* levels in biofilm were positively correlated with water column salinity aligns with previous research (LGL and Hemmera 2014, WorleyParsons 2014). Similar to most other biofilm parameters, there was little evidence that Chlorophyll *a* levels were influenced by changes in water column temperature.

Collectively, these results indicate the majority of fatty acid production at Roberts Bank during the 2016 WESA northward migration was driven by mudflat exposure, as increases in exposure time drove MUFA and SFA production, which accounted for 74% of Total Fatty Acid abundance. This finding is substantiated by AIC results indicating mudflats exposure time as the primary driver of Total Fatty Acid production, with salinity having a secondary influence (**Table 3-18**). Mudflat exposure is largely driven by the spring-neap tidal cycle associated with the 29.5-day lunar cycle. As the lunar cycle is a regular phenomenon that does not fluctuate, it is logical that there's a dependable standing stock supply of fatty acid at Roberts Bank available to migrating WESA and other organisms at this time of year.

As PUFA abundance appear to be primarily influenced by salinity levels it is likely that PUFA levels across Roberts Bank fluctuate annually in association with the size and timing of the Fraser River freshet whose maximum discharge has been documented to fluctuate between 535 m^3/second and 8,500 m^3/second during WESA northward migration since 1912 (**Figure 4-1b**) (Government of Canada 2017). However, as noted above, the biofilm community produces PUFA across the salinity gradient from freshwater dominated sites close to Canoe Passage to more marine stations in the upper intertidal zone close to the Roberts Bank causeway. It is therefore likely that regardless of the size of the spring freshet PUFA such as EPA and DHA are found across Roberts Bank during WESA northward migration.

The explanatory power of abiotic factors potentially affecting biofilm abundance (e.g., 5th, 50th, 95th percentiles of abiotic variables) appear to be influenced by the timeframe over which they're summarised. For example, this study repeatedly sampled biofilm over a period of days and found the 5th and 95th percentile water column salinity values to be most informative in explaining biofilm biomass estimates. This is in contrast to previous research that investigated biofilm biomass over an approximately four week period and found that 50th percentile salinity values showed the strongest and most consistent correlations (LGL and Hemmera 2014). It is possible that over longer time frames, variation in an abiotic parameter resulting from variation in the daily tidal cycle and the 29.5-day spring-neap tide cycle cause the extreme values (e.g., 5th and 95th percentiles) to be less informative than the median condition at a location (**Figure 4-2**). Regardless, results for these analyses align with previous studies showing an increase in biofilm abundance with increasing salinity.

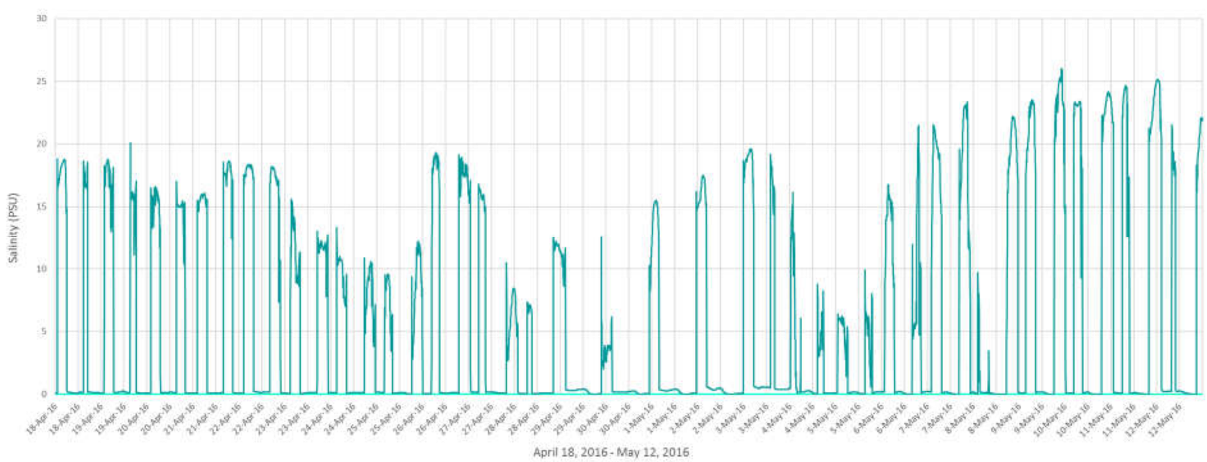


Figure 4-2 Variation in Salinity Levels at Station A from April 18 to May 12, 2016. Values of zero are indicative of when water did not cover the salinity sensor and the mudflats were exposed.

4.2 MICROPHYTOBENTHIC COMMUNITY COMPOSITION

Similar to previous studies (WorleyParsons 2015*b*), diatoms of the genera *Nitzschia, Navicula,* and *Achnanthidium* were distributed across the salinity gradient from Canoe Passage to the Roberts Bank causeway and were the dominant genera at all sites during WESA spring migration. Based on 2013 data (WorleyParsons 2015*b*), it appears that these genera also dominate the intertidal zones of Roberts Bank through summer, with *Nitzschia* and *Navicula* remaining dominant in winter and *Achnanthidium* decreasing in abundance. The presence of these genera in high numbers at Roberts Bank over multiple years potentially indicates they are a fixture, and potentially an important component, of the Roberts Bank diatom community.

The statistically significant temporal difference in the microphytobenthic community composition documented in this study followed two noticeable patterns. First, after the spring tide (which ended on April 25), total microphytobenthos density was observed to decrease (**Figure 3-19a**). This appears to have been caused by changes in sub-dominant diatom taxa as opposed to changes in dominant taxa (i.e., *Nitzschia* spp, *Navicula* spp., and *Achnanthidium* spp.). Two of these taxa, *Nitzschia* spp. and *Achnanthidium* spp., were inferred to have positive relationships with salinity (**Figure 3-23**). At this point, several previously absent taxa came into the community including *Planothidium* spp., *Tryblionella* spp., *Fragilaria* spp., and *Encyonema* spp. (**Table 3-20**).

The Spring 2013 community possessed only three dominant taxa, with defined habitat preferences: *Nitzschia* spp. (predominantly marine), *Navicula* spp. (mostly marine with some freshwater species) and *Achnanthidium* spp. (freshwater) (WorleyParsons 2014, 2015*b*). This dominance allowed for general conclusions to be made regarding freshwater influence based on the presence/dominance of *Achnanthidium* spp. However, in the 2016 Northward Migration study, *Achnanthidium* spp. was sub-dominant. The reason for these differences are not known at this time, but may be related to inter-annual differences in freshet conditions and potential subsequent influences on coastal hydrology patterns.

The results from this study regarding biofilm community composition largely align with results from a study of shorebird fecal droppings conducted to investigate species of diatoms consumed by foraging WESA during northward migration and the potential for selective feeding (Hemmera 2017). Similar to this study, a diversity of diatom genera (n = 28) were documented in droppings with *Navicula* spp., *Achnanthidium* spp., and *Nitzschia* spp. as dominant taxa, comprising 92% of the diatoms in WESA diets. Also, no statistical difference in the diatom community composition among stations was documented in this study. Similarly, the composition of diets of birds caught close to Canoe Passage did not differ from those close to the Roberts Bank Causeway in the upper intertidal zone (Hemmera 2017). The alignment of these studies seems to indicate that the diatom community at Roberts Bank is diverse, but dominated by a few abundant taxa. These dominant taxa were distributed across the Roberts Bank in 2016 and dominated WESA diets.

4.3 SHOREBIRD FORAGING USE

Consistent with previous research (Hemmera 2014, LGL and Hemmera 2014), this study documented the highest shorebird usage occurring in habitats close to Canoe Passage and in the Upper Intertidal zone close to Brunswick Dyke. Although dropping densities were numerically greater (**Table 3-21**) at these locations, they were not found to be statistically different from other stations sampled in 2016. It is suspected that limitations resulting from a low sample size (n=36 for droppings densities plots) and lack of replicate sampling at stations may have hampered the ability to detect spatial and temporal differences in shorebird usage and explain this difference. Data used in the SFOM consisted of numerous dropping transect surveys covering hundreds of plots over the course of the migratory period (LGL and Hemmera 2014). Droppings surveys for this study were restricted to 15 m^2 around each station, which may not have been sufficient to quantify WESA usage around the area adjacent to sampling stations.

5.0 CONCLUSIONS

This study successfully enhanced and informed the understanding of biofilm ecology, community composition, distribution, and factors affecting biofilm parameter abundance. Key findings of this study include:

1. A large number of fatty acids (28) were documented in the biofilm community during the study. All fatty acids were documented across Roberts Bank from freshwater- to marine-influenced intertidal sites.
2. Fatty acids in greatest abundance were the SFA palmitic acid (16:0), the MUFA palmitoleic acid (16:1n-7), and the PUFAs EPA (20:5n-3), and DHA (22:6n-3). MUFA, PUFA, and SFA abundances were found at similar levels at freshwater and marine-influenced sites. MUFA and SFA comprised 74% and PUFA comprised 26% of total fatty acid levels. MUFA and SFA are the primary fatty acids used by WESA to fuel migration (comprising >95% of stored fats) and are available to foraging WESA across the salinity gradient. PUFA are believed to potentially facilitate long-distance flight and are also available across the salinity gradient.
3. No statistical change in fatty acid abundance was documented over the duration of the study, except in two fatty acids (i.e., 18:1n-9 and total omega 6 fatty acids) that increased in abundance. This increase appeared to be driven by elevated levels across stations on the final day of sampling (May 12).
4. The biofilm parameter Chlorophyll *a*, which has been used in prior research to assess biofilm biomass, was statistically and positively correlated with the abundance of numerous fatty acids. This finding indicates the potential use of Chlorophyll *a* abundance as a proxy for assessing fatty acid abundances.
5. MUFA and SFA levels were documented to be primarily driven by increases in mudflat exposure time, with some evidence they were affected by changes in water column salinity levels. Therefore, results suggest that MUFA and SFA abundance should be less influenced by the annual Fraser River freshet, which inputs large quantities of freshwater over Roberts Bank mudflats, and should be annually abundant at freshwater- and marine-influenced sites during WESA northward migration.
6. PUFA abundance was documented to be primarily influenced by salinity levels. Therefore, it is likely that PUFA levels across Roberts Bank fluctuate annually in association with the size and timing of the Fraser River freshet. However, although PUFA levels were positively associated with increases in salinity the affect from salinity did not translate in large differences in total PUFA abundance between freshwater- and-marine influenced sites (i.e., abundances were not statistically different between sites). This study also documented the biofilm community producing PUFA across the salinity gradient. It is therefore likely that regardless of the size of the spring freshet, and associated salinity change, PUFA are found across Roberts Bank during WESA northward migration.
7. A large number of diatom genera (25) comprise the biofilm community during WESA northward migration. However, three genera (*Nitzschia*, *Navicula*., and *Achnanthidium*) dominate the community. During the 2016 northward migration, the dominant genera were distributed across

Roberts Bank, with no difference in community composition documented. A parallel study investigating the composition of WESA diet found *Nitzschia*, *Navicula*., and *Achnanthidium* comprised 92% of the diatoms consumed at both freshwater- and marine-influenced sites. These genera have been documented to be abundant during previous northward migrations and potentially form an important part of WESA foraging ecology.

8. The similarity in diatom community composition and fatty acid abundances between freshwater- and marine-influenced sites may explain the previously documented high use of the Canoe Passage and Upper Intertidal areas by WESA during northward migration. As less saline sites have been documented to be physiologically less energy demanding to foraging shorebirds compared to more saline sites, it is possible that the freshwater-influenced Canoe Passage site represents higher quality habitat compared to the Upper Intertidal area close to Brunswick Dyke.

6.0 STUDY LIMITATIONS

The sample size of this study was relatively small (i.e., n = 30 to 42 depending on the analyses) and lacked replicate sampling at stations, preventing a more detailed analysis of factors influencing biofilm abundance within stations through the migratory period and assessment of biofilm variability. Despite this limitation, statistically significant results informing factors influencing biofilm abundance, and the spatial and temporal distribution of biofilm parameters across the study area were achieved. Future work conducted under a replicate design, to better inform spatial and temporal variability in abundance levels and factors affecting abundance, would increase the robustness of conclusions.

7.0 CLOSURE

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8.0 STATEMENT OF LIMITATIONS

This Work was performed in accordance with the contract for Environmental Services for the Panel Phase of the Roberts Bank Terminal 2 Project between Hemmera Envirochem Inc. ("Hemmera") and Vancouver Fraser Port Authority ("Client"), dated January 1, 2016 ("Contract"). This Report has been prepared by Hemmera and Advisian, based on fieldwork conducted by Hemmera and Advisian, for sole benefit and use by Vancouver Fraser Port Authority. In performing this Work, Hemmera and Advisia have relied in good faith on information provided by others, and has assumed that the information provided by those individuals is both complete and accurate. This Work was performed to current industry standard practice for similar environmental work, within the relevant jurisdiction and same locale. The findings presented herein should be considered within the context of the scope of work and project terms of reference; further, the findings are time sensitive and are considered valid only at the time the Report was produced. The conclusions and recommendations contained in this Report are based upon the applicable guidelines, regulations, and legislation existing at the time the Report was produced; any changes in the regulatory regime may alter the conclusions and/or recommendations.

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APPENDIX A
Fatty Acid Methyl Ester Key

Appendix A List of Fatty Acids

Common Name	Chemical Name	Isomer
Myristic acid	Tetradecanoic acid	14:0
Myristoleic acid	cis-9-tetradecanoic acid	14:1
Pentadecanoic acid	Pentadecanoic acid	15:0
-	cis-10-pentadecenoic acid	15:1
Palmitic acid (PAM)	Hexadecanoic acid	16:0
Palmitoleic acid	9-hexadecenoic acid	16:1n-7
Margaric acid	Heptadecanoic acid	17:0
-	cis-10-heptadecanoic acid	17:1
Stearic acid (STA)	Octadecanoic acid	18:0
Vaccenic acid	11-octadecenoic acid	18:1n-7
Oleic acid (OLA)	cis-9-octadenoic acid	18:1n-9
Linoleic acid (LNA)	cis-9,12-octadecadienoic acid	18:2n-6
α-Linoleic acid (ALA)	9,12,15-octadecatrienoic acid	18:3n-3
γ-Linoleic acid (GLA)	9,12,15-octadecatrienoic acid	18:3n-6
Arachidic acid	Eicosanoic acid	20:0
Gondoic acid	cis-11-eicosenoic acid	20:1n-9
Eicosadienoic acid	cit-11,14-eicosadienoic acid	20:2n-6
Eicosatrienoic acid (ETE)	cis-11,14,17-eicosatrienoic acid	20:3n-3
Dihomo-γ-linolenic acid (DGLA)	cis-8,11,14-eicosatrienoic acid	20:3n-6
Arachidonic acid (ARA)	5,8,11,14-eicosatetraenoic acid	20:4n-6
Eicosapentaenoic acid (EPA)	5,8,11,14,17-eicosapentaenoic acid	20:5n-3
Heneicosylic acid	Heneicosanoic acid	21:0
Behenic acid	Docosanoic acid	22:0
Erucic acid	13-docosenoic acid	22:1n-9
Docosadienoic acid	cis-13,16-docosadienoic acid	22:2
Docosahexaenoic acid (DHA)	4,7,10,13,16,19-docosahexaenoic acid	22:6n-3
Lignoceric acid	Tetracosanoic acid	24:0
Nervonic acid	15-tetracosanoic acid	24:1n-9

APPENDIX B

Biofilm Parameter Summary Statistics

Appendix B Summary Abundance Statistics of All Biofilm Parameters Sampled at Roberts Bank (April 18 to May 12, 2016). Units are mg/m^2.

Biofilm Parameter	N	Mean (mg/m^2)	Standard Deviation	Minimum	Maximum	Percent of Total Fatty Acids
14:0	42	32	15	12	67	4%
14:1	42	28	21	4	81	3%
15:0	42	9	4	4	21	1%
15:1	42	4	3	1	11	1%
16:0	42	179	75	67	344	22%
16:1n-7	42	182	86	7	422	22%
17:0	42	27	15	7	62	3%
17:1	42	19	12	5	48	2%
18:0	42	33	14	11	67	4%
18:1n-7	42	12	7	1	43	1%
18:1n-9	42	21	10	9	48	3%
18:2n-6	42	11	5	4	22	1%
18:3n-3	42	8	3	3	16	1%
18:3n-6	42	7	3	3	14	1%
20:0	42	9	4	4	18	1%
20:1n-9	42	16	7	6	35	2%
20:2n-6	42	8	3	3	15	1%
20:3n-3	42	3	5	1	33	< 1%
20:3n-6	42	3	2	1	7	< 1%
20:4n-6	42	17	8	7	42	2%
20:5n-3	42	102	39	42	188	12%
21:0	42	6	3	2	12	1%
22:0	42	7	3	2	12	1%
22:1n-9	42	3	1	1	6	< 1%
22:2	42	4	2	1	7	< 1%
22:6n-3	42	50	20	21	103	6%
24:0	42	13	6	3	26	2%
24:1n-9	42	6	3	2	13	1%
Total Omega 3	42	162	62	67	289	20%
Total Omega 6	42	46	20	18	95	6%
Total MUFA	42	291	128	107	584	36%
Total PUFA	42	208	81	85	379	25%
Total SFA	42	315	130	120	621	39%
Total Fatty Acids	42	818	328	336	1583	100%
Chlorophyll a	42	57	18	31	113	-
Total Lipids	42	1399	560	494	2845	-
Total Carbohydrates	42	4188	1751	1717	8347	-

APPENDIX C

Biofilm Parameter Summary Statistics by Station

Appendix C Summary Statistics of All Biofilm Parameters at Roberts Bank by Station (April 18 to May 12, 2016).

Station: A					
Biofilm Parameter	**n**	**Mean (mg/m^2)**	**Standard Deviation**	**Minimum**	**Maximum**
14:0	6	42	15	23	67
14:1	6	56	17	34	81
15:0	6	13	3	8	15
15:1	6	6	4	2	10
16:0	6	226	72	130	344
16:1n-7	6	201	80	105	340
17:0	6	42	14	26	62
17:1	6	32	14	12	48
18:0	6	49	12	32	67
18:1n-7	6	17	13	8	43
18:1n-9	6	30	7	19	37
18:2n-6	6	16	4	10	22
18:3n-3	6	9	2	6	11
18:3n-6	6	10	3	6	14
20:0	6	12	4	7	18
20:1n-9	6	5	1	3	6
20:2n-6	6	10	3	6	14
20:3n-3	6	8	13	2	33
20:3n-6	6	4	1	3	6
20:4n-6	6	23	8	13	34
20:5n-3	6	133	33	86	188
21:0	6	9	2	5	11
22:0	6	8	3	3	12
22:2	6	5	1	4	6
22:6n-3	6	70	15	44	86
24:0	6	19	5	12	26
24:1n-9	6	8	2	5	10
Total Fatty Acids	6	1086	313	632	1583
Total PUFA	6	284	66	177	379
Total MUFA	6	378	123	202	578
Total SFA	6	420	128	249	621
Total Omega 3	6	220	49	138	289
Total Omega 6	6	64	18	39	90
Chlorophyll a	6	67	18	34	84
Total Lipids	6	2010	611	1168	2845
Total Carbohydrates	6	5683	1477	3012	7320

Appendix C (cont.) **Summary Statistics of All Biofilm Parameters at Roberts Bank by Station (April 18 to May 12, 2016).**

Station: C					
Biofilm Parameter	**n**	**Mean (mg/m^2)**	**Standard Deviation**	**Minimum**	**Maximum**
14:0	6	41	10	25	56
14:1	6	51	19	23	75
15:0	6	13	5	9	21
15:1	6	7	3	2	11
16:0	6	231	47	148	287
16:1n-7	6	210	43	130	259
17:0	6	45	12	29	60
17:1	6	32	8	19	40
18:0	6	46	10	33	60
18:1n-7	6	14	3	10	19
18:1n-9	6	31	11	21	48
18:2n-6	6	17	4	11	22
18:3n-3	6	10	3	6	15
18:3n-6	6	10	3	6	14
20:0	6	13	4	9	18
20:1n-9	6	5	1	3	6
20:2n-6	6	12	3	8	15
20:3n-3	6	3	1	2	3
20:3n-6	6	5	1	3	6
20:4n-6	6	25	8	16	35
20:5n-3	6	125	43	77	187
21:0	6	9	3	5	12
22:0	6	9	2	7	11
22:2	6	5	1	4	7
22:6n-3	6	70	23	44	103
24:0	6	20	5	14	25
24:1n-9	6	9	3	6	13
Total Fatty Acids	6	1090	255	684	1373
Total PUFA	6	276	87	173	373
Total MUFA	6	382	83	229	458
Total SFA	6	427	92	277	536
Total Omega 3	6	207	68	129	284
Total Omega 6	6	68	19	44	91
Chlorophyll a	6	73	23	50	113
Total Lipids	6	1861	355	1332	2381
Total Carbohydrates	6	5423	2222	2705	8347

Appendix C (cont.) Summary Statistics of All Biofilm Parameters at Roberts Bank by Station (April 18 to May 12, 2016).

Station: H					
Biofilm Parameter	n	Mean (mg/m^2)	Standard Deviation	Minimum	Maximum
14:0	6	35	12	22	51
14:1	6	19	7	8	26
15:0	6	7	3	4	13
15:1	6	3	1	1	5
16:0	6	160	45	104	201
16:1n-7	6	164	54	94	217
17:0	6	23	9	11	35
17:1	6	13	6	6	24
18:0	6	33	8	23	42
18:1n-7	6	13	7	1	22
18:1n-9	6	16	7	9	29
18:2n-6	6	9	3	4	12
18:3n-3	6	7	2	4	11
18:3n-6	6	5	2	3	8
20:0	6	8	2	6	11
20:1n-9	6	3	1	2	4
20:2n-6	6	9	3	5	13
20:3n-3	6	2	1	1	3
20:3n-6	6	2	1	1	3
20:4n-6	6	12	4	7	17
20:5n-3	6	110	40	55	156
21:0	6	4	2	2	7
22:0	6	7	2	4	9
22:2	6	3	1	2	5
22:6n-3	6	47	16	29	71
24:0	6	10	4	3	15
24:1n-9	6	5	2	3	9
Total Fatty Acids	6	744	225	447	933
Total PUFA	6	204	67	110	271
Total MUFA	6	249	80	144	310
Total SFA	6	287	80	183	355
Total Omega 3	6	167	54	89	223
Total Omega 6	6	38	12	21	50
Chlorophyll a	6	58	19	33	83
Total Lipids	6	1220	345	795	1564
Total Carbohydrates	6	3269	975	1944	4618

Appendix C (cont.) Summary Statistics of All Biofilm Parameters at Roberts Bank by Station (April 18 to May 12, 2016).

Station: I					
Biofilm Parameter	**n**	**Mean (mg/m^2)**	**Standard Deviation**	**Minimum**	**Maximum**
14:0	6	45	10	34	60
14:1	6	36	6	30	45
15:0	6	7	3	4	12
15:1	6	6	1	4	7
16:0	6	230	59	169	317
16:1n-7	6	201	108	7	327
17:0	6	28	9	20	47
17:1	6	21	7	10	30
18:0	6	38	8	31	50
18:1n-7	6	17	2	14	20
18:1n-9	6	17	7	9	28
18:2n-6	6	11	3	9	15
18:3n-3	6	9	4	5	15
18:3n-6	6	6	2	4	8
20:0	6	9	3	6	14
20:1n-9	6	3	1	2	6
20:2n-6	6	8	2	6	12
20:3n-3	6	2	1	1	3
20:3n-6	6	2	1	2	3
20:4n-6	6	14	3	10	18
20:5n-3	6	98	26	67	141
21:0	6	5	2	2	7
22:0	6	8	3	6	12
22:2	6	3	1	1	5
22:6n-3	6	44	13	33	64
24:0	6	13	5	9	21
24:1n-9	6	5	2	3	8
Total Fatty Acids	6	899	250	607	1223
Total PUFA	6	194	49	144	272
Total MUFA	6	319	127	107	474
Total SFA	6	383	98	284	533
Total Omega 3	6	152	40	110	218
Total Omega 6	6	42	9	33	54
Chlorophyll a	6	49	15	31	74
Total Lipids	6	1588	354	1082	2021
Total Carbohydrates	6	3705	1883	2028	7097

Appendix C (cont.) **Summary Statistics of All Biofilm Parameters at Roberts Bank by Station (April 18 to May 12, 2016).**

Station: J					
Biofilm Parameter	**n**	**Mean (mg/m^2)**	**Standard Deviation**	**Minimum**	**Maximum**
14:0	6	18	3	13	21
14:1	6	7	4	4	14
15:0	6	9	3	4	12
15:1	6	2	0	1	2
16:0	6	108	24	74	140
16:1n-7	6	122	24	85	147
17:0	6	17	5	9	25
17:1	6	10	3	5	14
18:0	6	21	6	11	28
18:1n-7	6	6	2	3	9
18:1n-9	6	18	4	9	22
18:2n-6	6	7	2	4	8
18:3n-3	6	6	1	3	7
18:3n-6	6	5	1	3	6
20:0	6	7	2	4	8
20:1n-9	6	3	1	2	4
20:2n-6	6	6	2	3	8
20:3n-3	6	1	0	1	2
20:3n-6	6	2	1	1	3
20:4n-6	6	12	5	7	20
20:5n-3	6	77	22	42	103
21:0	6	5	1	3	7
22:0	6	5	1	4	7
22:2	6	3	1	1	3
22:6n-3	6	39	10	21	50
24:0	6	10	2	7	13
24:1n-9	6	5	1	3	6
Total Fatty Acids	6	544	124	336	703
Total PUFA	6	155	42	85	208
Total MUFA	6	186	38	122	233
Total SFA	6	200	45	128	259
Total Omega 3	6	123	33	67	163
Total Omega 6	6	32	9	18	45
Chlorophyll a	6	48	8	39	57
Total Lipids	6	984	333	528	1430
Total Carbohydrates	6	3572	1177	2286	5241

Appendix C (cont.) Summary Statistics of All Biofilm Parameters at Roberts Bank by Station (April 18 to May 12, 2016).

Station: X					
Biofilm Parameter	**n**	**Mean (mg/m^2)**	**Standard Deviation**	**Minimum**	**Maximum**
14:0	6	15	4	12	22
14:1	6	7	4	4	14
15:0	6	7	3	4	11
15:1	6	1	1	1	2
16:0	6	100	33	67	163
16:1n-7	6	118	30	93	170
17:0	6	14	7	7	27
17:1	6	8	4	5	16
18:0	6	19	5	15	29
18:1n-7	6	6	3	5	12
18:1n-9	6	14	5	9	23
18:2n-6	6	6	2	4	10
18:3n-3	6	5	2	3	9
18:3n-6	6	4	1	3	7
20:0	6	5	2	4	10
20:1n-9	6	2	1	1	4
20:2n-6	6	5	2	4	8
20:3n-3	6	2	2	1	6
20:3n-6	6	2	1	1	3
20:4n-6	6	12	6	9	24
20:5n-3	6	73	26	51	125
21:0	6	4	2	2	7
22:0	6	5	3	2	9
22:2	6	2	1	1	4
22:6n-3	6	33	13	22	58
24:0	6	9	4	5	16
24:1n-9	6	4	2	2	7
Total Fatty Acids	6	494	164	354	811
Total PUFA	6	143	55	100	250
Total MUFA	6	172	49	132	265
Total SFA	6	177	61	120	292
Total Omega 3	6	113	43	77	197
Total Omega 6	6	30	12	22	53
Chlorophyll a	6	51	18	37	86
Total Lipids	6	800	241	494	1105
Total Carbohydrates	6	3085	1527	1717	5881

Appendix C (cont.) **Summary Statistics of All Biofilm Parameters at Roberts Bank by Station (April 18 to May 12, 2016).**

Station: Y					
Biofilm Parameter	**n**	**Mean (mg/m^2)**	**Standard Deviation**	**Minimum**	**Maximum**
14:0	6	30	13	13	49
14:1	6	22	10	12	35
15:0	6	8	4	5	17
15:1	6	4	1	2	5
16:0	6	200	90	87	309
16:1n-7	6	259	132	92	422
17:0	6	20	9	12	36
17:1	6	17	9	10	35
18:0	6	25	9	14	38
18:1n-7	6	10	3	5	14
18:1n-9	6	19	10	11	39
18:2n-6	6	11	5	5	19
18:3n-3	6	7	4	4	16
18:3n-6	6	7	4	3	14
20:0	6	8	3	4	14
20:1n-9	6	3	1	2	4
20:2n-6	6	7	3	4	13
20:3n-3	6	2	1	1	3
20:3n-6	6	3	2	2	7
20:4n-6	6	20	12	9	42
20:5n-3	6	95	46	46	174
21:0	6	5	3	3	12
22:0	6	6	2	3	9
22:2	6	3	1	2	6
22:6n-3	6	47	21	26	88
24:0	6	12	5	7	21
24:1n-9	6	5	3	3	11
Total Fatty Acids	6	869	390	402	1462
Total PUFA	6	199	98	100	376
Total MUFA	6	354	167	149	584
Total SFA	6	313	132	151	496
Total Omega 3	6	151	72	77	281
Total Omega 6	6	48	26	23	95
Chlorophyll a	6	55	14	33	68
Total Lipids	6	1327	478	791	1812
Total Carbohydrates	6	4578	1382	2032	5800

APPENDIX D

AIC_c Model Results

Appendix D AIC$_c$ Model Results for Chlorophyll *a* at Roberts Bank (April 18 to May 12, 2016).

Model #	Model	AIC$_c$	ΔAICc	w_i
1	5th Percentile Salinity + Total Sample Weight	256.54	0.00	0.27
2	95th Percentile Salinity + Total Sample Weight	261.52	4.98	0.02
3	5th Percentile Temperature + Total Sample Weight	267.71	11.17	0.00
4	95th Percentile Temperature + Total Sample Weight	267.62	11.08	0.00
5	Percent Time Mudflats Exposed + Total Sample Weight	263.38	6.84	0.01
6	5th Percentile Salinity + 95th Percentile Salinity + Total Sample Weight	259.39	2.85	0.06
7	5th Percentile Salinity + 5th Percentile Temperature + Total Sample Weight	256.99	0.45	0.21
8	5th Percentile Salinity + 95th Percentile Temperature + Total Sample Weight	259.32	2.77	0.07
9	5th Percentile Salinity + Percent Time Mudflats Exposed + Total Sample Weight	258.98	2.44	0.08
10	95th Percentile Salinity + 5th Percentile Temperature + Total Sample Weight	263.73	7.18	0.01
11	95th Percentile Salinity + 95th Percentile Temperature + Total Sample Weight	263.43	6.89	0.01
12	95th Percentile Salinity + Percent Time Mudflats Exposed + Total Sample Weight	263.14	6.60	0.01
13	5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	270.50	13.96	0.00
14	5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	266.28	9.74	0.00
15	95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	266.27	9.73	0.00
16	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + Total Sample Weight	260.03	3.49	0.05
17	5th Percentile Salinity + 95th Percentile Salinity + 95th Percentile Temperature + Total Sample Weight	262.25	5.71	0.02
18	5th Percentile Salinity + 95th Percentile Salinity + Percent Time Mudflats Exposed + Total Sample Weight	262.12	5.58	0.02
19	5th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	259.45	2.91	0.06
20	5th Percentile Salinity + 5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	260.02	3.48	0.05
21	5th Percentile Salinity + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	262.00	5.45	0.02
22	95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	266.48	9.94	0.00
23	95th Percentile Salinity + 5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	265.84	9.30	0.00
24	95th Percentile Salinity + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	265.55	9.01	0.00
25	5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	269.42	12.88	0.00
26	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	262.86	6.32	0.01
27	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	263.38	6.84	0.01
28	5th Percentile Salinity + 95th Percentile Salinity + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	265.32	8.78	0.00
29	5th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	262.86	6.32	0.01
30	95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	268.94	12.40	0.00
31	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	266.59	10.05	0.00

Note: The set of 31 models is balanced, containing all combinations of abiotic variables, with each abiotic variable represented in 16 models.

Appendix D (cont.) AIC_c Model Results for Total Carbohydrates at Roberts Bank (April 18 to May 12, 2016).

Model #	Model	AIC_c	ΔAICc	w_i
1	5th Percentile Salinity + Total Sample Weight	231.20	0.00	0.25
2	95th Percentile Salinity + Total Sample Weight	237.41	6.21	0.01
3	5th Percentile Temperature + Total Sample Weight	247.33	16.13	0.00
4	95th Percentile Temperature + Total Sample Weight	247.05	15.86	0.00
5	Percent Time Mudflats Exposed + Total Sample Weight	239.62	8.43	0.00
6	5th Percentile Salinity + 95th Percentile Salinity + Total Sample Weight	233.66	2.47	0.07
7	5th Percentile Salinity + 5th Percentile Temperature + Total Sample Weight	232.27	1.07	0.15
8	5th Percentile Salinity + 95th Percentile Temperature + Total Sample Weight	234.09	2.90	0.06
9	5th Percentile Salinity + Percent Time Mudflats Exposed + Total Sample Weight	232.27	1.08	0.15
10	95th Percentile Salinity + 5th Percentile Temperature + Total Sample Weight	239.98	8.78	0.00
11	95th Percentile Salinity + 95th Percentile Temperature + Total Sample Weight	239.58	8.39	0.00
12	95th Percentile Salinity + Percent Time Mudflats Exposed + Total Sample Weight	237.30	6.10	0.01
13	5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	249.95	18.75	0.00
14	5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	242.23	11.04	0.00
15	95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	242.25	11.05	0.00
16	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + Total Sample Weight	234.84	3.65	0.04
17	5th Percentile Salinity + 95th Percentile Salinity + 95th Percentile Temperature + Total Sample Weight	236.72	5.52	0.02
18	5th Percentile Salinity + 95th Percentile Salinity + Percent Time Mudflats Exposed + Total Sample Weight	235.24	4.04	0.03
19	5th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	233.98	2.79	0.06
20	5th Percentile Salinity + 5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	234.22	3.02	0.06
21	5th Percentile Salinity + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	235.42	4.23	0.03
22	95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	242.73	11.53	0.00
23	95th Percentile Salinity + 5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	240.35	9.15	0.00
24	95th Percentile Salinity + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	240.04	8.84	0.00
25	5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	245.33	14.14	0.00
26	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	237.41	6.22	0.01
27	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	237.34	6.14	0.01
28	5th Percentile Salinity + 95th Percentile Salinity + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	238.63	7.44	0.01
29	5th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	236.64	5.44	0.02
30	95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	243.47	12.28	0.00
31	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	240.40	9.21	0.00

Note: The set of 31 models is balanced, containing all combinations of abiotic variables, with each abiotic variable represented in 16 models.

Appendix D (cont.) AIC_c Model Results for Total Fatty Acids at Roberts Bank (April 18 to May 12, 2016).

Model #	Model	AIC_c	ΔAICc	w_i
1	5th Percentile Salinity + Total Sample Weight	430.68	3.37	0.05
2	95th Percentile Salinity + Total Sample Weight	434.73	7.41	0.01
3	5th Percentile Temperature + Total Sample Weight	436.67	9.36	0.00
4	95th Percentile Temperature + Total Sample Weight	437.11	9.80	0.00
5	Percent Time Mudflats Exposed + Total Sample Weight	427.31	0.00	0.29
6	5th Percentile Salinity + 95th Percentile Salinity + Total Sample Weight	433.52	6.21	0.01
7	5th Percentile Salinity + 5th Percentile Temperature + Total Sample Weight	433.46	6.15	0.01
8	5th Percentile Salinity + 95th Percentile Temperature + Total Sample Weight	433.58	6.27	0.01
9	5th Percentile Salinity + Percent Time Mudflats Exposed + Total Sample Weight	428.39	1.07	0.17
10	95th Percentile Salinity + 5th Percentile Temperature + Total Sample Weight	437.58	10.26	0.00
11	95th Percentile Salinity + 95th Percentile Temperature + Total Sample Weight	437.52	10.21	0.00
12	95th Percentile Salinity + Percent Time Mudflats Exposed + Total Sample Weight	430.06	2.75	0.07
13	5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	439.53	12.22	0.00
14	5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	429.86	2.54	0.08
15	95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	430.10	2.78	0.07
16	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + Total Sample Weight	436.57	9.26	0.00
17	5th Percentile Salinity + 95th Percentile Salinity + 95th Percentile Temperature + Total Sample Weight	436.66	9.34	0.00
18	5th Percentile Salinity + 95th Percentile Salinity + Percent Time Mudflats Exposed + Total Sample Weight	431.18	3.87	0.04
19	5th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	436.49	9.18	0.00
20	5th Percentile Salinity + 5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	431.54	4.22	0.04
21	5th Percentile Salinity + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	431.53	4.21	0.04
22	95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	440.32	13.01	0.00
23	95th Percentile Salinity + 5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	432.98	5.66	0.02
24	95th Percentile Salinity + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	433.18	5.87	0.02
25	5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	432.99	5.68	0.02
26	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	439.67	12.36	0.00
27	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	434.61	7.30	0.01
28	5th Percentile Salinity + 95th Percentile Salinity + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	434.42	7.10	0.01
29	5th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	434.95	7.64	0.01
30	95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	436.37	9.06	0.00
31	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	438.00	10.69	0.00

Note: The set of 31 models is balanced, containing all combinations of abiotic variables, with each abiotic variable represented in 16 models.

Appendix D (cont.) AIC_c Model Results for Total Polyunsaturated Fatty Acids (PUFA) at Roberts Bank (April 18 to May 12, 2016).

Model #	Model	AIC_c	ΔAICc	w_i
1	5th Percentile Salinity + Total Sample Weight	141.64	0.00	0.27
2	95th Percentile Salinity + Total Sample Weight	144.52	2.88	0.06
3	5th Percentile Temperature + Total Sample Weight	149.96	8.32	0.00
4	95th Percentile Temperature + Total Sample Weight	149.93	8.29	0.00
5	Percent Time Mudflats Exposed + Total Sample Weight	145.37	3.73	0.04
6	5th Percentile Salinity + 95th Percentile Salinity + Total Sample Weight	144.16	2.52	0.08
7	5th Percentile Salinity + 5th Percentile Temperature + Total Sample Weight	144.23	2.59	0.07
8	5th Percentile Salinity + 95th Percentile Temperature + Total Sample Weight	144.41	2.77	0.07
9	5th Percentile Salinity + Percent Time Mudflats Exposed + Total Sample Weight	143.38	1.74	0.11
10	95th Percentile Salinity + 5th Percentile Temperature + Total Sample Weight	147.41	5.77	0.02
11	95th Percentile Salinity + 95th Percentile Temperature + Total Sample Weight	147.32	5.68	0.02
12	95th Percentile Salinity + Percent Time Mudflats Exposed + Total Sample Weight	145.29	3.65	0.04
13	5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	152.73	11.09	0.00
14	5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	147.86	6.22	0.01
15	95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	147.67	6.03	0.01
16	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + Total Sample Weight	146.92	5.28	0.02
17	5th Percentile Salinity + 95th Percentile Salinity + 95th Percentile Temperature + Total Sample Weight	147.31	5.67	0.02
18	5th Percentile Salinity + 95th Percentile Salinity + Percent Time Mudflats Exposed + Total Sample Weight	146.30	4.66	0.03
19	5th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	146.13	4.49	0.03
20	5th Percentile Salinity + 5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	146.37	4.73	0.03
21	5th Percentile Salinity + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	146.36	4.72	0.03
22	95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	150.45	8.81	0.00
23	95th Percentile Salinity + 5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	148.44	6.80	0.01
24	95th Percentile Salinity + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	148.44	6.80	0.01
25	5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	150.81	9.17	0.00
26	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	149.57	7.93	0.01
27	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	149.52	7.88	0.01
28	5th Percentile Salinity + 95th Percentile Salinity + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	149.69	8.05	0.00
29	5th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	148.72	7.08	0.01
30	95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	151.88	10.24	0.00
31	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	152.48	10.84	0.00

Note: The set of 31 models is balanced, containing all combinations of abiotic variables, with each abiotic variable represented in 16 models.

Appendix D (cont.) AIC_c Model Results for Eicosapentaenoic Acid (EPA, 20:5n-3) at Roberts Bank (April 18 to May 12, 2016).

Model #	Model	AIC_c	ΔAICc	w_i
1	5th Percentile Salinity + Total Sample Weight	307.49	0.00	0.18
2	95th Percentile Salinity + Total Sample Weight	307.85	0.36	0.15
3	5th Percentile Temperature + Total Sample Weight	310.38	2.89	0.04
4	95th Percentile Temperature + Total Sample Weight	310.22	2.73	0.05
5	Percent Time Mudflats Exposed + Total Sample Weight	308.56	1.07	0.10
6	5th Percentile Salinity + 95th Percentile Salinity + Total Sample Weight	310.05	2.56	0.05
7	5th Percentile Salinity + 5th Percentile Temperature + Total Sample Weight	310.16	2.67	0.05
8	5th Percentile Salinity + 95th Percentile Temperature + Total Sample Weight	310.34	2.85	0.04
9	5th Percentile Salinity + Percent Time Mudflats Exposed + Total Sample Weight	309.99	2.50	0.05
10	95th Percentile Salinity + 5th Percentile Temperature + Total Sample Weight	310.66	3.17	0.04
11	95th Percentile Salinity + 95th Percentile Temperature + Total Sample Weight	310.69	3.20	0.04
12	95th Percentile Salinity + Percent Time Mudflats Exposed + Total Sample Weight	310.13	2.64	0.05
13	5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	313.11	5.62	0.01
14	5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	311.42	3.93	0.02
15	95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	311.24	3.75	0.03
16	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + Total Sample Weight	312.90	5.41	0.01
17	5th Percentile Salinity + 95th Percentile Salinity + 95th Percentile Temperature + Total Sample Weight	313.20	5.71	0.01
18	5th Percentile Salinity + 95th Percentile Salinity + Percent Time Mudflats Exposed + Total Sample Weight	312.88	5.39	0.01
19	5th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	312.61	5.12	0.01
20	5th Percentile Salinity + 5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	312.99	5.50	0.01
21	5th Percentile Salinity + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	313.08	5.58	0.01
22	95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	313.81	6.32	0.01
23	95th Percentile Salinity + 5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	313.23	5.74	0.01
24	95th Percentile Salinity + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	313.28	5.79	0.01
25	5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	314.36	6.87	0.01
26	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	316.01	8.52	0.00
27	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	316.11	8.62	0.00
28	5th Percentile Salinity + 95th Percentile Salinity + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	316.32	8.83	0.00
29	5th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	315.80	8.31	0.00
30	95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	316.66	9.17	0.00
31	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	319.55	12.06	0.00

Note: The set of 31 models is balanced, containing all combinations of abiotic variables, with each abiotic variable represented in 16 models.

Appendix D (cont.) AIC$_c$ Model Results for Docosahexaenoic Acid (DHA, 22:6n-3) at Roberts Bank (April 18 to May 12, 2016).

Model #	Model	AIC$_c$	ΔAICc	w_i
1	5th Percentile Salinity + Total Sample Weight	93.94	0.00	0.26
2	95th Percentile Salinity + Total Sample Weight	99.15	5.21	0.02
3	5th Percentile Temperature + Total Sample Weight	108.49	14.55	0.00
4	95th Percentile Temperature + Total Sample Weight	108.47	14.53	0.00
5	Percent Time Mudflats Exposed + Total Sample Weight	101.74	7.80	0.01
6	5th Percentile Salinity + 95th Percentile Salinity + Total Sample Weight	96.03	2.08	0.09
7	5th Percentile Salinity + 5th Percentile Temperature + Total Sample Weight	96.26	2.32	0.08
8	5th Percentile Salinity + 95th Percentile Temperature + Total Sample Weight	96.60	2.66	0.07
9	5th Percentile Salinity + Percent Time Mudflats Exposed + Total Sample Weight	95.10	1.16	0.15
10	95th Percentile Salinity + 5th Percentile Temperature + Total Sample Weight	102.03	8.09	0.00
11	95th Percentile Salinity + 95th Percentile Temperature + Total Sample Weight	101.85	7.91	0.01
12	95th Percentile Salinity + Percent Time Mudflats Exposed + Total Sample Weight	98.84	4.90	0.02
13	5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	111.21	17.26	0.00
14	5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	103.91	9.97	0.00
15	95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	103.62	9.68	0.00
16	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + Total Sample Weight	98.41	4.47	0.03
17	5th Percentile Salinity + 95th Percentile Salinity + 95th Percentile Temperature + Total Sample Weight	99.17	5.23	0.02
18	5th Percentile Salinity + 95th Percentile Salinity + Percent Time Mudflats Exposed + Total Sample Weight	97.68	3.74	0.04
19	5th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	96.99	3.05	0.06
20	5th Percentile Salinity + 5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	97.91	3.97	0.04
21	5th Percentile Salinity + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	97.93	3.99	0.04
22	95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	104.96	11.02	0.00
23	95th Percentile Salinity + 5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	101.99	8.05	0.00
24	95th Percentile Salinity + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	101.97	8.03	0.00
25	5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	106.74	12.80	0.00
26	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	100.41	6.47	0.01
27	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	100.64	6.70	0.01
28	5th Percentile Salinity + 95th Percentile Salinity + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	101.07	7.12	0.01
29	5th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	99.16	5.22	0.02
30	95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	105.40	11.46	0.00
31	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	102.93	8.99	0.00

Note: The set of 31 models is balanced, containing all combinations of abiotic variables, with each abiotic variable represented in 16 models.

Appendix D (cont.) AIC_c Model Results for Omega 3 Fatty Acids at Roberts Bank (April 18 to May 12, 2016).

Model #	Model	AIC_c	ΔAICc	w_i
1	5th Percentile Salinity + Total Sample Weight	134.25	0.00	0.27
2	95th Percentile Salinity + Total Sample Weight	136.69	2.44	0.08
3	5th Percentile Temperature + Total Sample Weight	141.50	7.25	0.01
4	95th Percentile Temperature + Total Sample Weight	141.49	7.25	0.01
5	Percent Time Mudflats Exposed + Total Sample Weight	137.65	3.40	0.05
6	5th Percentile Salinity + 95th Percentile Salinity + Total Sample Weight	136.86	2.61	0.07
7	5th Percentile Salinity + 5th Percentile Temperature + Total Sample Weight	136.60	2.35	0.08
8	5th Percentile Salinity + 95th Percentile Temperature + Total Sample Weight	137.14	2.89	0.06
9	5th Percentile Salinity + Percent Time Mudflats Exposed + Total Sample Weight	136.36	2.11	0.09
10	95th Percentile Salinity + 5th Percentile Temperature + Total Sample Weight	139.49	5.24	0.02
11	95th Percentile Salinity + 95th Percentile Temperature + Total Sample Weight	139.31	5.06	0.02
12	95th Percentile Salinity + Percent Time Mudflats Exposed + Total Sample Weight	138.02	3.77	0.04
13	5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	144.35	10.10	0.00
14	5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	140.40	6.16	0.01
15	95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	140.31	6.06	0.01
16	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + Total Sample Weight	139.35	5.11	0.02
17	5th Percentile Salinity + 95th Percentile Salinity + 95th Percentile Temperature + Total Sample Weight	139.97	5.73	0.02
18	5th Percentile Salinity + 95th Percentile Salinity + Percent Time Mudflats Exposed + Total Sample Weight	139.33	5.08	0.02
19	5th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	138.89	4.64	0.03
20	5th Percentile Salinity + 5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	139.13	4.88	0.02
21	5th Percentile Salinity + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	139.49	5.24	0.02
22	95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	142.46	8.21	0.00
23	95th Percentile Salinity + 5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	141.12	6.87	0.01
24	95th Percentile Salinity + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	141.07	6.82	0.01
25	5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	143.46	9.21	0.00
26	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	142.31	8.06	0.00
27	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	142.30	8.05	0.00
28	5th Percentile Salinity + 95th Percentile Salinity + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	142.76	8.51	0.00
29	5th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	141.82	7.57	0.01
30	95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	144.51	10.26	0.00
31	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	145.58	11.33	0.00

Note: The set of 31 models is balanced, containing all combinations of abiotic variables, with each abiotic variable represented in 16 models.

Appendix D (cont.) AIC$_c$ Model Results for Omega 6 Fatty Acids at Roberts Bank (April 18 to May 12, 2016).

Model #	Model	AIC$_c$	ΔAICc	w_i
1	5th Percentile Salinity + Total Sample Weight	100.61	0.00	0.17
2	95th Percentile Salinity + Total Sample Weight	104.58	3.97	0.02
3	5th Percentile Temperature + Total Sample Weight	110.91	10.30	0.00
4	95th Percentile Temperature + Total Sample Weight	110.63	10.02	0.00
5	Percent Time Mudflats Exposed + Total Sample Weight	104.52	3.91	0.02
6	5th Percentile Salinity + 95th Percentile Salinity + Total Sample Weight	102.83	2.22	0.06
7	5th Percentile Salinity + 5th Percentile Temperature + Total Sample Weight	103.49	2.88	0.04
8	5th Percentile Salinity + 95th Percentile Temperature + Total Sample Weight	101.91	1.30	0.09
9	5th Percentile Salinity + Percent Time Mudflats Exposed + Total Sample Weight	100.73	0.12	0.16
10	95th Percentile Salinity + 5th Percentile Temperature + Total Sample Weight	107.13	6.52	0.01
11	95th Percentile Salinity + 95th Percentile Temperature + Total Sample Weight	107.29	6.68	0.01
12	95th Percentile Salinity + Percent Time Mudflats Exposed + Total Sample Weight	103.21	2.60	0.05
13	5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	113.23	12.62	0.00
14	5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	105.31	4.70	0.02
15	95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	104.48	3.87	0.02
16	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + Total Sample Weight	105.99	5.38	0.01
17	5th Percentile Salinity + 95th Percentile Salinity + 95th Percentile Temperature + Total Sample Weight	104.99	4.38	0.02
18	5th Percentile Salinity + 95th Percentile Salinity + Percent Time Mudflats Exposed + Total Sample Weight	103.48	2.87	0.04
19	5th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	103.69	3.08	0.04
20	5th Percentile Salinity + 5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	103.72	3.11	0.04
21	5th Percentile Salinity + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	101.88	1.27	0.09
22	95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	110.28	9.67	0.00
23	95th Percentile Salinity + 5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	105.72	5.11	0.01
24	95th Percentile Salinity + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	105.59	4.97	0.01
25	5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	107.52	6.91	0.01
26	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	107.08	6.47	0.01
27	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	106.81	6.20	0.01
28	5th Percentile Salinity + 95th Percentile Salinity + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	105.32	4.71	0.02
29	5th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	104.47	3.86	0.02
30	95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	108.94	8.33	0.00
31	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	108.06	7.45	0.00

Note: The set of 31 models is balanced, containing all combinations of abiotic variables, with each abiotic variable represented in 16 models.

Appendix D (cont.) AIC_c Model Results for Total Monounsaturated Fatty Acids (MUFA) at Roberts Bank (April 18 to May 12, 2016).

Model #	Model	AIC_c	ΔAICc	w_i
1	5th Percentile Salinity + Total Sample Weight	162.67	4.62	0.03
2	95th Percentile Salinity + Total Sample Weight	166.52	8.47	0.00
3	5th Percentile Temperature + Total Sample Weight	167.76	9.72	0.00
4	95th Percentile Temperature + Total Sample Weight	168.37	10.33	0.00
5	Percent Time Mudflats Exposed + Total Sample Weight	158.05	0.00	0.33
6	5th Percentile Salinity + 95th Percentile Salinity + Total Sample Weight	165.41	7.37	0.01
7	5th Percentile Salinity + 5th Percentile Temperature + Total Sample Weight	165.52	7.48	0.01
8	5th Percentile Salinity + 95th Percentile Temperature + Total Sample Weight	165.55	7.51	0.01
9	5th Percentile Salinity + Percent Time Mudflats Exposed + Total Sample Weight	159.69	1.64	0.15
10	95th Percentile Salinity + 5th Percentile Temperature + Total Sample Weight	169.29	11.24	0.00
11	95th Percentile Salinity + 95th Percentile Temperature + Total Sample Weight	169.28	11.24	0.00
12	95th Percentile Salinity + Percent Time Mudflats Exposed + Total Sample Weight	160.94	2.89	0.08
13	5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	170.48	12.44	0.00
14	5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	160.53	2.48	0.10
15	95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	160.91	2.87	0.08
16	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + Total Sample Weight	168.53	10.49	0.00
17	5th Percentile Salinity + 95th Percentile Salinity + 95th Percentile Temperature + Total Sample Weight	168.56	10.51	0.00
18	5th Percentile Salinity + 95th Percentile Salinity + Percent Time Mudflats Exposed + Total Sample Weight	162.21	4.17	0.04
19	5th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	168.68	10.63	0.00
20	5th Percentile Salinity + 5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	162.81	4.77	0.03
21	5th Percentile Salinity + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	162.84	4.79	0.03
22	95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	171.78	13.74	0.00
23	95th Percentile Salinity + 5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	163.66	5.61	0.02
24	95th Percentile Salinity + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	164.06	6.02	0.02
25	5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	163.52	5.47	0.02
26	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	171.87	13.83	0.00
27	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	165.58	7.54	0.01
28	5th Percentile Salinity + 95th Percentile Salinity + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	165.48	7.43	0.01
29	5th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	166.21	8.17	0.01
30	95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	166.96	8.91	0.00
31	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	169.24	11.19	0.00

Note: The set of 31 models is balanced, containing all combinations of abiotic variables, with each abiotic variable represented in 16 models.

Appendix D (cont.) AIC$_c$ Model Results for Palmitoleic Acid (16:1n-7) at Roberts Bank (April 18 to May 12, 2016).

Model #	Model	AIC$_c$	ΔAICc	w_i
1	5th Percentile Salinity + Total Sample Weight	158.76	3.64	0.05
2	95th Percentile Salinity + Total Sample Weight	159.64	4.52	0.03
3	5th Percentile Temperature + Total Sample Weight	159.83	4.71	0.03
4	95th Percentile Temperature + Total Sample Weight	160.11	4.99	0.03
5	Percent Time Mudflats Exposed + Total Sample Weight	155.12	0.00	0.33
6	5th Percentile Salinity + 95th Percentile Salinity + Total Sample Weight	161.62	6.50	0.01
7	5th Percentile Salinity + 5th Percentile Temperature + Total Sample Weight	161.66	6.54	0.01
8	5th Percentile Salinity + 95th Percentile Temperature + Total Sample Weight	161.62	6.51	0.01
9	5th Percentile Salinity + Percent Time Mudflats Exposed + Total Sample Weight	158.01	2.89	0.08
10	95th Percentile Salinity + 5th Percentile Temperature + Total Sample Weight	162.45	7.34	0.01
11	95th Percentile Salinity + 95th Percentile Temperature + Total Sample Weight	162.44	7.32	0.01
12	95th Percentile Salinity + Percent Time Mudflats Exposed + Total Sample Weight	157.92	2.80	0.08
13	5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	162.47	7.35	0.01
14	5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	157.90	2.78	0.08
15	95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	158.01	2.89	0.08
16	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + Total Sample Weight	164.77	9.65	0.00
17	5th Percentile Salinity + 95th Percentile Salinity + 95th Percentile Temperature + Total Sample Weight	164.76	9.64	0.00
18	5th Percentile Salinity + 95th Percentile Salinity + Percent Time Mudflats Exposed + Total Sample Weight	160.93	5.82	0.02
19	5th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	164.72	9.60	0.00
20	5th Percentile Salinity + 5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	161.05	5.93	0.02
21	5th Percentile Salinity + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	161.15	6.03	0.02
22	95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	165.13	10.01	0.00
23	95th Percentile Salinity + 5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	160.83	5.71	0.02
24	95th Percentile Salinity + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	161.07	5.95	0.02
25	5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	160.82	5.70	0.02
26	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	168.16	13.04	0.00
27	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	164.21	9.10	0.00
28	5th Percentile Salinity + 95th Percentile Salinity + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	164.35	9.24	0.00
29	5th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	164.22	9.11	0.00
30	95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	164.15	9.03	0.00
31	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	167.91	12.79	0.00

Note: The set of 31 models is balanced, containing all combinations of abiotic variables, with each abiotic variable represented in 16 models.

Appendix D (cont.) AIC_c Model Results for Vaccenic acid (18:1n-7) at Roberts Bank (April 18 to May 12, 2016).

Model #	Model	AIC_c	ΔAICc	w_i
1	5th Percentile Salinity + Total Sample Weight	90.75	2.13	0.07
2	95th Percentile Salinity + Total Sample Weight	94.02	5.40	0.01
3	5th Percentile Temperature + Total Sample Weight	94.03	5.41	0.01
4	95th Percentile Temperature + Total Sample Weight	92.11	3.49	0.03
5	Percent Time Mudflats Exposed + Total Sample Weight	91.56	2.95	0.05
6	5th Percentile Salinity + 95th Percentile Salinity + Total Sample Weight	88.62	0.00	0.20
7	5th Percentile Salinity + 5th Percentile Temperature + Total Sample Weight	93.10	4.49	0.02
8	5th Percentile Salinity + 95th Percentile Temperature + Total Sample Weight	90.11	1.49	0.09
9	5th Percentile Salinity + Percent Time Mudflats Exposed + Total Sample Weight	93.00	4.38	0.02
10	95th Percentile Salinity + 5th Percentile Temperature + Total Sample Weight	96.86	8.25	0.00
11	95th Percentile Salinity + 95th Percentile Temperature + Total Sample Weight	94.74	6.12	0.01
12	95th Percentile Salinity + Percent Time Mudflats Exposed + Total Sample Weight	93.46	4.84	0.02
13	5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	91.95	3.34	0.04
14	5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	94.46	5.85	0.01
15	95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	92.07	3.45	0.04
16	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + Total Sample Weight	91.46	2.84	0.05
17	5th Percentile Salinity + 95th Percentile Salinity + 95th Percentile Temperature + Total Sample Weight	90.74	2.12	0.07
18	5th Percentile Salinity + 95th Percentile Salinity + Percent Time Mudflats Exposed + Total Sample Weight	90.30	1.68	0.09
19	5th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	92.42	3.81	0.03
20	5th Percentile Salinity + 5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	95.75	7.14	0.01
21	5th Percentile Salinity + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	92.67	4.05	0.03
22	95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	94.94	6.32	0.01
23	95th Percentile Salinity + 5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	96.45	7.83	0.00
24	95th Percentile Salinity + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	95.12	6.50	0.01
25	5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	92.42	3.80	0.03
26	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	94.06	5.44	0.01
27	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	93.60	4.98	0.02
28	5th Percentile Salinity + 95th Percentile Salinity + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	92.95	4.33	0.02
29	5th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	94.95	6.33	0.01
30	95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	95.70	7.08	0.01
31	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	96.48	7.86	0.00

Note: The set of 31 models is balanced, containing all combinations of abiotic variables, with each abiotic variable represented in 16 models.

Appendix D (cont.) AIC$_c$ Model Results for Oleic Acid (OLA, 18:1n-9) at Roberts Bank (April 18 to May 12, 2016).

Model #	Model	AIC$_c$	ΔAICc	w_i
1	5th Percentile Salinity + Total Sample Weight	74.38	0.81	0.09
2	95th Percentile Salinity + Total Sample Weight	77.14	3.57	0.02
3	5th Percentile Temperature + Total Sample Weight	90.88	17.31	0.00
4	95th Percentile Temperature + Total Sample Weight	90.35	16.78	0.00
5	Percent Time Mudflats Exposed + Total Sample Weight	81.88	8.32	0.00
6	5th Percentile Salinity + 95th Percentile Salinity + Total Sample Weight	74.30	0.73	0.10
7	5th Percentile Salinity + 5th Percentile Temperature + Total Sample Weight	77.19	3.63	0.02
8	5th Percentile Salinity + 95th Percentile Temperature + Total Sample Weight	75.59	2.02	0.05
9	5th Percentile Salinity + Percent Time Mudflats Exposed + Total Sample Weight	73.57	0.00	0.14
10	95th Percentile Salinity + 5th Percentile Temperature + Total Sample Weight	80.01	6.44	0.01
11	95th Percentile Salinity + 95th Percentile Temperature + Total Sample Weight	80.04	6.47	0.01
12	95th Percentile Salinity + Percent Time Mudflats Exposed + Total Sample Weight	74.77	1.20	0.08
13	5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	93.06	19.49	0.00
14	5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	82.80	9.23	0.00
15	95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	81.55	7.98	0.00
16	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + Total Sample Weight	77.21	3.64	0.02
17	5th Percentile Salinity + 95th Percentile Salinity + 95th Percentile Temperature + Total Sample Weight	77.11	3.54	0.02
18	5th Percentile Salinity + 95th Percentile Salinity + Percent Time Mudflats Exposed + Total Sample Weight	74.25	0.69	0.10
19	5th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	75.27	1.70	0.06
20	5th Percentile Salinity + 5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	76.72	3.15	0.03
21	5th Percentile Salinity + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	74.51	0.95	0.09
22	95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	83.14	9.57	0.00
23	95th Percentile Salinity + 5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	77.78	4.21	0.02
24	95th Percentile Salinity + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	77.71	4.14	0.02
25	5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	84.67	11.10	0.00
26	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	78.29	4.72	0.01
27	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	77.65	4.08	0.02
28	5th Percentile Salinity + 95th Percentile Salinity + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	76.95	3.39	0.03
29	5th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	75.29	1.72	0.06
30	95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	81.14	7.57	0.00
31	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	78.85	5.28	0.01

Note: The set of 31 models is balanced, containing all combinations of abiotic variables, with each abiotic variable represented in 16 models.

Appendix D (cont.) AIC$_c$ Model Results for Total Saturated Fatty Acids (SFA) at Roberts Bank (April 18 to May 12, 2016).

Model #	Model	AIC$_c$	ΔAICc	w_i
1	5th Percentile Salinity + Total Sample Weight	377.53	4.80	0.03
2	95th Percentile Salinity + Total Sample Weight	381.34	8.61	0.00
3	5th Percentile Temperature + Total Sample Weight	381.97	9.24	0.00
4	95th Percentile Temperature + Total Sample Weight	382.34	9.61	0.00
5	Percent Time Mudflats Exposed + Total Sample Weight	372.73	0.00	0.35
6	5th Percentile Salinity + 95th Percentile Salinity + Total Sample Weight	379.91	7.18	0.01
7	5th Percentile Salinity + 5th Percentile Temperature + Total Sample Weight	380.34	7.61	0.01
8	5th Percentile Salinity + 95th Percentile Temperature + Total Sample Weight	380.42	7.69	0.01
9	5th Percentile Salinity + Percent Time Mudflats Exposed + Total Sample Weight	374.75	2.02	0.13
10	95th Percentile Salinity + 5th Percentile Temperature + Total Sample Weight	384.13	11.40	0.00
11	95th Percentile Salinity + 95th Percentile Temperature + Total Sample Weight	384.21	11.48	0.00
12	95th Percentile Salinity + Percent Time Mudflats Exposed + Total Sample Weight	375.55	2.82	0.08
13	5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	384.82	12.08	0.00
14	5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	375.40	2.67	0.09
15	95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	375.58	2.85	0.08
16	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + Total Sample Weight	383.01	10.28	0.00
17	5th Percentile Salinity + 95th Percentile Salinity + 95th Percentile Temperature + Total Sample Weight	382.96	10.23	0.00
18	5th Percentile Salinity + 95th Percentile Salinity + Percent Time Mudflats Exposed + Total Sample Weight	376.59	3.86	0.05
19	5th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	383.44	10.71	0.00
20	5th Percentile Salinity + 5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	377.90	5.17	0.03
21	5th Percentile Salinity + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	377.90	5.17	0.03
22	95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	387.02	14.29	0.00
23	95th Percentile Salinity + 5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	378.31	5.58	0.02
24	95th Percentile Salinity + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	378.54	5.81	0.02
25	5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	378.52	5.79	0.02
26	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	385.86	13.13	0.00
27	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	379.98	7.25	0.01
28	5th Percentile Salinity + 95th Percentile Salinity + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	379.54	6.81	0.01
29	5th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	381.34	8.61	0.00
30	95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	381.75	9.02	0.00
31	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	383.06	10.33	0.00

Note: The set of 31 models is balanced, containing all combinations of abiotic variables, with each abiotic variable represented in 16 models.

Appendix D (cont.) AIC$_c$ Model Results for Myristic Acid (14:0) at Roberts Bank (April 18 to May 12, 2016).

Model #	Model	AIC$_c$	ΔAICc	w_i
1	5th Percentile Salinity + Total Sample Weight	104.50	3.19	0.04
2	95th Percentile Salinity + Total Sample Weight	104.75	3.44	0.03
3	5th Percentile Temperature + Total Sample Weight	105.11	3.80	0.03
4	95th Percentile Temperature + Total Sample Weight	104.72	3.41	0.03
5	Percent Time Mudflats Exposed + Total Sample Weight	101.47	0.16	0.17
6	5th Percentile Salinity + 95th Percentile Salinity + Total Sample Weight	104.30	2.99	0.04
7	5th Percentile Salinity + 5th Percentile Temperature + Total Sample Weight	107.01	5.70	0.01
8	5th Percentile Salinity + 95th Percentile Temperature + Total Sample Weight	106.71	5.41	0.01
9	5th Percentile Salinity + Percent Time Mudflats Exposed + Total Sample Weight	104.34	3.04	0.04
10	95th Percentile Salinity + 5th Percentile Temperature + Total Sample Weight	107.65	6.34	0.01
11	95th Percentile Salinity + 95th Percentile Temperature + Total Sample Weight	107.48	6.17	0.01
12	95th Percentile Salinity + Percent Time Mudflats Exposed + Total Sample Weight	101.31	0.00	0.19
13	5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	107.47	6.16	0.01
14	5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	104.20	2.89	0.04
15	95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	103.73	2.42	0.06
16	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + Total Sample Weight	107.23	5.92	0.01
17	5th Percentile Salinity + 95th Percentile Salinity + 95th Percentile Temperature + Total Sample Weight	107.45	6.14	0.01
18	5th Percentile Salinity + 95th Percentile Salinity + Percent Time Mudflats Exposed + Total Sample Weight	102.90	1.59	0.09
19	5th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	109.86	8.56	0.00
20	5th Percentile Salinity + 5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	107.35	6.04	0.01
21	5th Percentile Salinity + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	106.88	5.57	0.01
22	95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	110.45	9.14	0.00
23	95th Percentile Salinity + 5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	104.40	3.09	0.04
24	95th Percentile Salinity + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	104.46	3.15	0.04
25	5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	106.83	5.52	0.01
26	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	110.37	9.06	0.00
27	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	106.32	5.01	0.02
28	5th Percentile Salinity + 95th Percentile Salinity + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	106.27	4.96	0.02
29	5th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	110.25	8.94	0.00
30	95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	107.81	6.50	0.01
31	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	109.77	8.46	0.00

Note: The set of 31 models is balanced, containing all combinations of abiotic variables, with each abiotic variable represented in 16 models.

Appendix D (cont.) AIC$_c$ Model Results for Palmitic Acid (PAM, 16:0) at Roberts Bank (April 18 to May 12, 2016).

Model #	Model	AIC$_c$	ΔAICc	w_i
1	5th Percentile Salinity + Total Sample Weight	345.15	6.49	0.01
2	95th Percentile Salinity + Total Sample Weight	347.91	9.24	0.00
3	5th Percentile Temperature + Total Sample Weight	348.00	9.33	0.00
4	95th Percentile Temperature + Total Sample Weight	348.08	9.41	0.00
5	Percent Time Mudflats Exposed + Total Sample Weight	338.67	0.00	0.34
6	5th Percentile Salinity + 95th Percentile Salinity + Total Sample Weight	346.81	8.14	0.01
7	5th Percentile Salinity + 5th Percentile Temperature + Total Sample Weight	347.83	9.16	0.00
8	5th Percentile Salinity + 95th Percentile Temperature + Total Sample Weight	347.80	9.13	0.00
9	5th Percentile Salinity + Percent Time Mudflats Exposed + Total Sample Weight	341.46	2.79	0.09
10	95th Percentile Salinity + 5th Percentile Temperature + Total Sample Weight	350.78	12.11	0.00
11	95th Percentile Salinity + 95th Percentile Temperature + Total Sample Weight	350.63	11.97	0.00
12	95th Percentile Salinity + Percent Time Mudflats Exposed + Total Sample Weight	340.47	1.80	0.14
13	5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	350.56	11.89	0.00
14	5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	341.57	2.90	0.08
15	95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	341.44	2.77	0.09
16	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + Total Sample Weight	349.82	11.15	0.00
17	5th Percentile Salinity + 95th Percentile Salinity + 95th Percentile Temperature + Total Sample Weight	349.96	11.29	0.00
18	5th Percentile Salinity + 95th Percentile Salinity + Percent Time Mudflats Exposed + Total Sample Weight	341.89	3.22	0.07
19	5th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	350.92	12.26	0.00
20	5th Percentile Salinity + 5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	344.60	5.93	0.02
21	5th Percentile Salinity + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	344.42	5.75	0.02
22	95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	353.42	14.75	0.00
23	95th Percentile Salinity + 5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	343.39	4.72	0.03
24	95th Percentile Salinity + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	343.60	4.93	0.03
25	5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	344.38	5.71	0.02
26	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	353.04	14.37	0.00
27	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	345.32	6.65	0.01
28	5th Percentile Salinity + 95th Percentile Salinity + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	345.16	6.49	0.01
29	5th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	347.77	9.10	0.00
30	95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	346.78	8.11	0.01
31	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	348.82	10.15	0.00

Note: The set of 31 models is balanced, containing all combinations of abiotic variables, with each abiotic variable represented in 16 models.

Appendix D (cont.) AIC$_c$ Model Results for Stearic Acid (STA, 18:0) at Roberts Bank (April 18 to May 12, 2016).

Model #	Model	AIC$_c$	ΔAICc	w_i
1	5th Percentile Salinity + Total Sample Weight	241.06	0.94	0.13
2	95th Percentile Salinity + Total Sample Weight	245.55	5.42	0.01
3	5th Percentile Temperature + Total Sample Weight	246.95	6.82	0.01
4	95th Percentile Temperature + Total Sample Weight	247.30	7.17	0.01
5	Percent Time Mudflats Exposed + Total Sample Weight	240.13	0.00	0.20
6	5th Percentile Salinity + 95th Percentile Salinity + Total Sample Weight	243.80	3.67	0.03
7	5th Percentile Salinity + 5th Percentile Temperature + Total Sample Weight	243.94	3.81	0.03
8	5th Percentile Salinity + 95th Percentile Temperature + Total Sample Weight	243.84	3.71	0.03
9	5th Percentile Salinity + Percent Time Mudflats Exposed + Total Sample Weight	240.61	0.48	0.16
10	95th Percentile Salinity + 5th Percentile Temperature + Total Sample Weight	248.25	8.12	0.00
11	95th Percentile Salinity + 95th Percentile Temperature + Total Sample Weight	248.44	8.31	0.00
12	95th Percentile Salinity + Percent Time Mudflats Exposed + Total Sample Weight	242.83	2.70	0.05
13	5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	249.84	9.71	0.00
14	5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	242.39	2.26	0.07
15	95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	242.52	2.39	0.06
16	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + Total Sample Weight	246.94	6.81	0.01
17	5th Percentile Salinity + 95th Percentile Salinity + 95th Percentile Temperature + Total Sample Weight	246.58	6.45	0.01
18	5th Percentile Salinity + 95th Percentile Salinity + Percent Time Mudflats Exposed + Total Sample Weight	243.29	3.17	0.04
19	5th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	246.64	6.51	0.01
20	5th Percentile Salinity + 5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	243.75	3.62	0.03
21	5th Percentile Salinity + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	243.55	3.43	0.04
22	95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	251.33	11.20	0.00
23	95th Percentile Salinity + 5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	245.51	5.38	0.01
24	95th Percentile Salinity + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	245.65	5.53	0.01
25	5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	245.50	5.37	0.01
26	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Total Sample Weight	249.15	9.02	0.00
27	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	246.69	6.56	0.01
28	5th Percentile Salinity + 95th Percentile Salinity + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	245.90	5.77	0.01
29	5th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	246.90	6.77	0.01
30	95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	248.92	8.80	0.00
31	5th Percentile Salinity + 95th Percentile Salinity + 5th Percentile Temperature + 95th Percentile Temperature + Percent Time Mudflats Exposed + Total Sample Weight	249.10	8.97	0.00

Note: The set of 31 models is balanced, containing all combinations of abiotic variables, with each abiotic variable represented in 16 models.

APPENDIX E

Taxonomic Composition of Microphytobenthic Community

Appendix E - Taxonomic Composition of Microphytobenthic Community

	Station A						Station C						Station H						Station I						Station J						Station X						Station Y					
Row Labels	18-Apr	23-Apr	25-Apr	29-Apr	06-May	12-May	18-Apr	23-Apr	25-Apr	29-Apr	06-May	12-May	18-Apr	23-Apr	25-Apr	29-Apr	06-May	12-May	18-Apr	23-Apr	25-Apr	29-Apr	06-May	12-May	18-Apr	23-Apr	25-Apr	29-Apr	06-May	12-May	18-Apr	23-Apr	25-Apr	29-Apr	06-May	12-May	18-Apr	23-Apr	25-Apr	29-Apr	06-May	12-May
Achnanthes spp.										0.70%	1.00%													0.30%																		
Achnanthidium spp.	20.40%	0.70%	3.90%	1.60%	2.60%	2.30%	10.30%	4.20%	8.80%	2.00%	3.60%	3.60%	9.60%		2.80%	3.00%	0.30%	4.70%	10.20%		2.30%	3.30%		4.90%	22.80%	7.60%	19.90%	12.50%	5.30%	2.60%	41.00%	20.60%	13.20%	3.30%	3.30%	2.60%	0.70%	20.60%	20.10%	7.20%	13.00%	7.00%
Amphora spp.						1.00%			1.60%	0.70%	1.30%	1.00%			1.20%	0.30%	0.30%	0.30%			2.00%		0.30%	0.30%				2.20%	0.70%						0.70%	0.30%					1.00%	0.30%
Anabaena spp.																																										
Asterionella formosa																			4.80%	1.60%																						
Aulacoseira spp.						0.30%				0.30%	0.30%			0.30%		0.30%	0.30%	0.30%		0.60%		0.30%	0.30%	0.30%		1.00%		2.90%	1.00%	1.70%	1.60%	1.90%		1.30%	0.70%	0.70%		1.60%	1.30%	1.30%	0.30%	0.30%
Bacillaria spp.				0.30%		0.30%		0.60%	1.00%	0.30%	0.70%		3.00%	1.30%			0.30%		1.60%		2.90%	1.00%		0.30%	0.30%		1.30%			0.70%							0.70%					
Biddulphia spp.						0.30%																															1.00%		1.00%			
Campylodiscus spp.								1.60%		0.70%	0.30%	0.30%																														
Chroococcus spp.																					1.00%																					
Cladophora glomerata																										0.30%					0.30%											
Cocconeis spp.	0.90%	2.60%	6.40%	0.30%	1.70%	1.00%	0.30%	1.60%	6.90%	1.00%	3.00%	2.30%		0.30%	6.50%	0.30%			1.00%		2.90%	0.30%	0.70%	1.00%	5.60%	4.30%	1.00%	4.20%	1.30%	0.70%	3.60%		3.40%	1.30%	1.00%	0.70%	1.00%	1.20%	1.70%	1.30%	2.00%	0.70%
Coelastrum spp.															5.60%													2.90%					1.80%									
Coscinodiscus spp.											0.30%						0.30%																									
Cyclotella spp.	0.30%											0.30%					0.30%		5.40%						8.30%					0.30%	1.60%						6.30%					
Cylindrotheca spp.											0.70%					0.30%		15.00%				20.40%	11.70%	23.00%						2.30%				0.30%		8.60%						0.70%
Cymbella spp.												0.30%	5.60%	0.70%			0.70%	0.70%	1.00%					0.60%			0.70%			0.30%	1.00%	0.90%		0.30%	0.70%							
Diatoma spp.			2.30%		0.30%			0.60%	1.30%	1.00%		0.70%	1.70%	0.70%		2.60%	0.70%	2.00%	0.30%	1.30%	1.60%		0.70%	1.00%	0.70%					0.30%				2.00%	0.70%	1.70%				1.00%		0.70%
Didymosphenia spp.				0.70%																				0.30%	1.00%			0.30%									0.30%					
Diploneis spp.										1.00%		0.30%				0.30%	0.30%						0.30%	0.00%			1.30%		0.30%	0.30%											0.30%	
Encyonema spp.					0.30%					0.30%						1.30%		0.70%				2.00%	0.30%	1.00%					1.00%	0.30%				0.70%	0.30%	0.30%					1.70%	0.30%
Entomoneis spp.													1.30%	0.70%				0.30%																		0.30%				1.30%		
Epithemia spp.				1.00%						0.30%	1.00%	0.30%		4.00%				0.70%	1.00%		1.60%	0.70%			1.00%			4.20%		0.30%										0.30%		
Eucocconeis spp.				1.30%	0.30%	0.30%				1.00%	0.30%	0.30%				0.30%																									0.30%	
Eunotia spp.				0.30%	0.30%	0.30%				0.30%						2.00%	0.30%							0.30%					0.30%	0.30%				1.00%								0.30%
Fallacia spp.					0.30%																		0.70%	0.30%																		0.30%
Fragilaria spp.				1.60%	1.70%	1.70%			1.60%	1.00%		1.30%		0.70%	2.20%	1.60%	0.30%	1.30%				0.30%	1.30%	1.30%					1.00%	1.30%			4.60%		1.30%	2.00%					1.00%	0.30%
Gomphoneis spp.					0.30%											0.30%						0.30%	0.30%																			
Gomphonema spp.	1.20%	1.00%		1.30%		0.30%			1.60%	0.30%	0.30%	0.70%	3.70%	4.00%	9.00%	3.90%	2.00%	1.70%	4.20%	1.60%	2.90%	3.30%	0.70%	1.60%	3.00%	7.60%	5.20%	4.80%	2.30%	2.00%	2.30%	2.20%		2.00%	1.60%	1.00%		1.60%	2.00%	1.60%	2.00%	1.30%
Gyrosigma spp.	0.30%	1.60%	8.70%	5.90%	5.00%	2.70%	0.30%	9.40%	10.50%	6.60%	4.30%	2.30%	4.70%	3.30%	5.20%	3.00%	1.30%	0.30%	7.30%	6.40%	9.50%	5.30%	1.00%	1.30%	3.00%	1.30%	3.60%	5.40%	1.60%	0.70%	0.30%	0.30%		1.00%	0.70%		5.60%	2.20%		0.70%	0.30%	3.00%
Hannaea spp.		1.00%						2.90%						3.00%	1.50%			0.30%		3.50%													2.80%									
Hippodonta spp.								1.60%			0.30%	0.30%		3.70%		11.50%	21.50%	22.60%					2.30%	3.90%			3.30%	4.80%	1.00%	2.00%			2.10%		1.00%	5.90%						0.30%
Leptolyngbya spp.									0.70%								0.30%							0.60%				0.30%	0.70%	0.30%			1.50%								0.30%	
Melosira spp.				2.30%	1.30%	0.70%			3.30%	1.30%	2.60%	1.30%		1.00%	1.50%		0.30%	1.00%	1.30%		1.00%	1.60%		1.30%					3.60%	0.30%	1.60%					0.30%	1.70%				0.70%	1.70%
Meridion spp.											0.30%											0.70%							0.30%													
Navicula spp.	28.40%	39.10%	42.40%	23.50%	43.60%	32.70%	47.10%	30.70%	20.90%	12.50%	30.70%	19.70%	19.90%	54.00%	19.40%	54.80%	54.70%	22.90%	9.60%	26.00%	46.10%	16.40%	66.40%	35.60%	8.90%	36.90%	30.30%	13.10%	57.20%	59.70%	11.40%	46.20%	43.90%	72.60%	69.40%	58.10%	15.60%	41.10%	36.60%	66.10%	45.50%	28.90%
Nitzschia spp.	40.40%	49.00%	27.70%	37.80%	23.80%	39.00%	39.70%	31.40%	32.40%	42.60%	27.10%	46.10%	48.50%	10.00%	31.20%	8.20%	8.10%	15.30%	42.20%	47.80%	22.20%	33.60%	6.50%	9.70%	40.10%	28.90%	25.10%	29.70%	11.80%	11.60%	32.20%	20.30%	24.80%	8.30%	11.10%	9.60%	53.30%	27.70%	30.70%	14.10%	18.30%	32.20%
Oedogonium spp.																										0.70%																
Oscillatoria sp.	1.90%																																				1.70%					
Pinnularia spp.						0.70%					0.30%						0.30%	0.30%																								
Planothidium spp.				1.60%	1.70%	1.30%				3.00%	3.00%	3.30%				1.30%	2.60%	2.70%				0.30%	3.60%	2.30%					6.30%	5.00%				2.00%	2.90%	3.30%					9.30%	5.60%
Pleurosigma spp.				0.30%	0.30%																																					
Pseudanabaena spp.										0.30%							0.30%	0.30%																0.30%		0.70%					0.30%	
Rhoicosphenia spp.					0.30%											0.30%																		0.30%	0.70%	0.30%						
Rhopalodia spp.					0.70%							0.30%												0.30%						0.30%							1.70%					0.30%
Skeletonema spp.				0.30%								0.30%		0.70%	1.90%				2.90%	1.30%		0.30%				1.00%												0.30%	1.30%		0.30%	
Stauroneis spp.				0.30%	0.30%																								0.30%													
Staurosira spp.																																			0.30%							
Surirella spp.	4.00%	3.60%		2.30%	1.00%	0.30%		7.40%	3.90%	2.30%	2.30%	0.70%	1.00%	9.30%	7.10%	1.00%	0.70%	1.70%	6.40%	5.10%	2.00%	3.00%	0.30%	2.30%	3.60%	2.30%	2.60%		0.70%	0.70%		2.20%	1.80%	0.70%	0.30%	0.30%	1.00%		1.70%	1.60%	0.30%	0.70%
Synedra spp.			1.30%																		0.30%							4.20%												0.30%		
Tabellaria spp.						0.30%						0.30%			1.50%						1.60%			0.30%								0.60%								1.60%		
Tabularia spp.				0.30%	0.30%	0.30%				1.00%	0.70%	0.70%										0.30%		0.30%																		1.30%
Thalassiosira spp.		1.30%		0.70%	0.30%			2.30%	3.60%			0.70%		1.00%					1.00%							1.00%	2.90%				2.90%			0.30%			7.00%		1.00%			
Tryblionella spp.				12.70%	8.30%	8.70%				14.90%	8.90%	8.60%				0.30%		1.70%				4.30%		1.60%					1.30%	0.30%				0.70%	0.70%	1.00%						7.00%
Ulnaria spp.					0.70%	0.30%						0.30%						0.70%				0.70%	0.30%	0.60%						1.00%					0.30%							0.70%
Unknown centrales sp.	2.20%																								1.70%														2.60%			
Unknown centrales spp.			7.40%	0.70%	1.00%	1.00%	2.30%	5.50%	2.00%	0.70%	1.00%	0.30%	1.00%	1.30%	3.40%	0.70%	0.70%	0.30%		4.80%		0.70%		1.00%		7.00%	2.90%	8.60%	0.30%	1.00%		4.70%					2.60%	3.70%		1.30%	0.30%	1.30%
Unknown pennales spp.				2.60%	3.60%	4.00%				4.00%	5.60%	3.30%				2.30%	2.90%	2.30%				1.00%	2.30%	2.30%					1.60%	3.60%				1.70%	2.60%	2.30%					2.70%	4.70%

APPENDIX IR8-04-B

INVESTIGATION OF SELECTIVE FEEDING OF BIOFILM COMMUNITIES BY SHOREBIRDS DURING NORTHERN MIGRATION

Investigation of Selective Feeding of Biofilm Communities by Shorebirds during Northward Migration

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July 2017

HEMMERA

EXECUTIVE SUMMARY

Western sandpiper (*Calidris mauri*, WESA) and dunlin (*Calidris alpina*) were captured from freshwater- (i.e., Canoe Passage) and marine-influenced (Upper Intertidal) sites at Roberts Bank, and fecal samples (droppings) were collected to provide a direct measure of the types and proportions of diatoms consumed as a step in determining whether WESA selectively feed on specific genera, and to investigate differences in WESA diets across Roberts Bank. Droppings from 14 WESA from the Canoe Passage site, all WESA droppings (n=5) collected from the Upper Intertidal site, and 1 dunlin dropping sample from the Canoe Passage location were sent for taxonomic analyses.

A total of 28 diatom genera were observed in droppings samples. Droppings samples from Canoe Passage had a higher number of genera (26) and genera contributing at least 1% of diatom biomass (15) as compared to Upper Intertidal samples (15 and 10, respectively). Diatoms of the genus *Navicula* comprised the largest portion of biomass in shorebird droppings from the Canoe Passage and the Upper Intertidal locations averaging approximately 44% of total diatom biomass in samples. *Achnanthes/Achnanthidium* and *Nitzschia* were the other most consistently observed diatom genera with average biomass comprising approximately 9% and 6% of the total, respectively. *Navicula* was observed in all WESA droppings samples analysed while *Achnanthes/Achnanthidium* and *Nitzschia* were observed in all but one sample from Canoe Passage in which 100% of diatoms were *Navicula*. The other genera that made up more than 5% of diatom biomass at either site were *Amphora, Surirella, Melosira*, and *Trachyneis*, but were found in lower proportions of droppings at each site ranging from 20% to 64%. All other genera comprised <1% of the total biomass of diatoms at sites. The proportional composition of diatoms in WESA droppings derived from abundance (count) data (units/ml) differed from estimates based on biomass (µg/ml) for some genera. However, *Navicula*, *Achnanthes*/*Achnanthidium*, and *Nitzschia* dominated abundance estimates comprising just over 90% of all diatoms counted at both sites. The dunlin droppings sample from Canoe Passage was comprised of the diatom genera: *Amphora*: 43%, *Navicula*: 29%, *Achnanthes/Achnanthidium*: 17%, and *Trybloniella*: 10%.

Results indicated diets of WESA at Canoe Passage and Upper Intertidal sites, and diets across sampling dates, were similar and not statistiscally different (ANOSIM = 0.17; perMANOVA: p = 0.13). These results imply that diets (based on diatom composition of droppings) of WESA caught at one site were as similar or more similar to the diets of birds caught outside their site compared to birds caught within the same site. This phenomenon also applied to bird capture dates, and aligns with results from a parallel study that found no significant differences in biofilm diatom community composition within intertidal sediments at sampling locations across Roberts Bank. These results indicate a diverse diatom community with diatom genera of many species well dispersed across Roberts Bank.

Navicula comprised the largest portion of diatoms in WESA droppings at both Canoe Passage and Upper Intertidal sites (45% and 43%, respectively, from April 23 to 26). These porportions are approximately 10 to 15% higher than those documented in biofilm sediment samples (i.e., 36% and 26%, respectively) collected during the same periods within the same sites. Higher proportions of diatoms in droppings relative to biofilm sediment samples were also observed for the genus *Achnanthidium* at both capture locations, possibly indicating preferential foraging for *Navicula* and *Achnanthidium*. Conversely, proportions of *Nitzschia* found in WESA droppings were below those documented in sediment samples at both sites, possibly indicating avoidance of *Nitzschia*. Despite the small sizes and limited temporal scope of this study, the similarity of findings from two distinct locations provides an indication of potential selective foraging on these genera of diatoms, which are abundant across Roberts Bank during northward migration. Given similarity of diet composition at both capture locations, diet composition reported here likely applies across Roberts Bank.

Invertebrate parts comprised 77% to 86% of dropping biomass compared to diatoms at both the Canoe Passage and Upper Intertidal capture sites, respectively. This result appears to emphasise the importance of invertebrates as a food source for migrating WESA. However, previous studies provide evidence that invertebrate parts are over-represented in fecal droppings and results from this study align with prior research.

Key findings of this study include:

1. Diets of WESA at fresh-water influenced locations (Canoe Passage) and the marine-influenced locations (Upper Intertidal) were similar and not statistiscally different.
2. The diatom genera *Navicula*, *Achnanthes*/*Achnanthidium*, and *Nitzschia* were abundant in biofilm sediments and found to be the dominant genera in WESA diets, being found in 95% of droppings and comprising 92% of diatoms in droppings.
3. Based on the prevalence and abundance of diatom genera in biofilm sediments and WESA droppings, and similarities of findings from two distinct locations at Roberts Bank, this study provides an indication of potential selective foraging by WESA on the genera *Navicula* and Achnanthes/Achnanthidium.
4. Given the similarity of diet composition at distinct capture sites, located ~ 3 km apart, the diet composition reported in this study likely applies across Roberts Bank.

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1.0 INTRODUCTION

Microphytobenthos[1] (hereafter referred to as biofilm) is an important component of western sandpiper (*Calidris mauri* [WESA]) diet. Recent studies suggest that biofilm contributes 50% to 68% of daily energetic requirements for WESA foraging at Roberts Bank during the northward migration with the remainder satisfied by marine invertebrates (Kuwae et al. 2008). A primary component of biofilm is comprised of photosynthetic diatoms. To understand whether WESA rely heavily on specific diatom communities or genera of diatoms found in biofilm, a field program was conducted during the 2016 northern spring migration to collect and examine contents of feces (i.e. droppings) deposited by foraging WESA. As diatom bodies (termed frustules) are composed largely of hard silica, diatoms consumed by WESA pass through the digestive tract and are present in droppings and available for identification.

1.1 STUDY OBJECTIVES

The objectives of this study were:

1) To examine WESA droppings in order to provide a direct measure of the types and proportions of diatoms consumed as a step in determining whether WESA selectively feed on specific genera; and
2) To investigate differences in WESA diets based on the diatom community composition documented in droppings collected at two sites at Roberts Bank (i.e., Canoe Passage and the Upper Intertidal zone).

This report has focused on the documentation of field, laboratory and analytical methodologies, and the presentation of data and analytical results. A limited degree of high-level interpretation is provided in **Section 4**.

[1] Microphytobenthos: microscopic, photosynthetic eukaryotic algae (diatoms) and cyanobacteria that live on sediment surfaces

2.0 METHODS

2.1 STUDY AREA

The study area consists of two intertidal locations at Roberts Bank: Capture location 1, adjacent to Canoe Passage; and capture location 2 in the upper intertidal zone south of Brunswick Dyke (**Figure 2-1**).

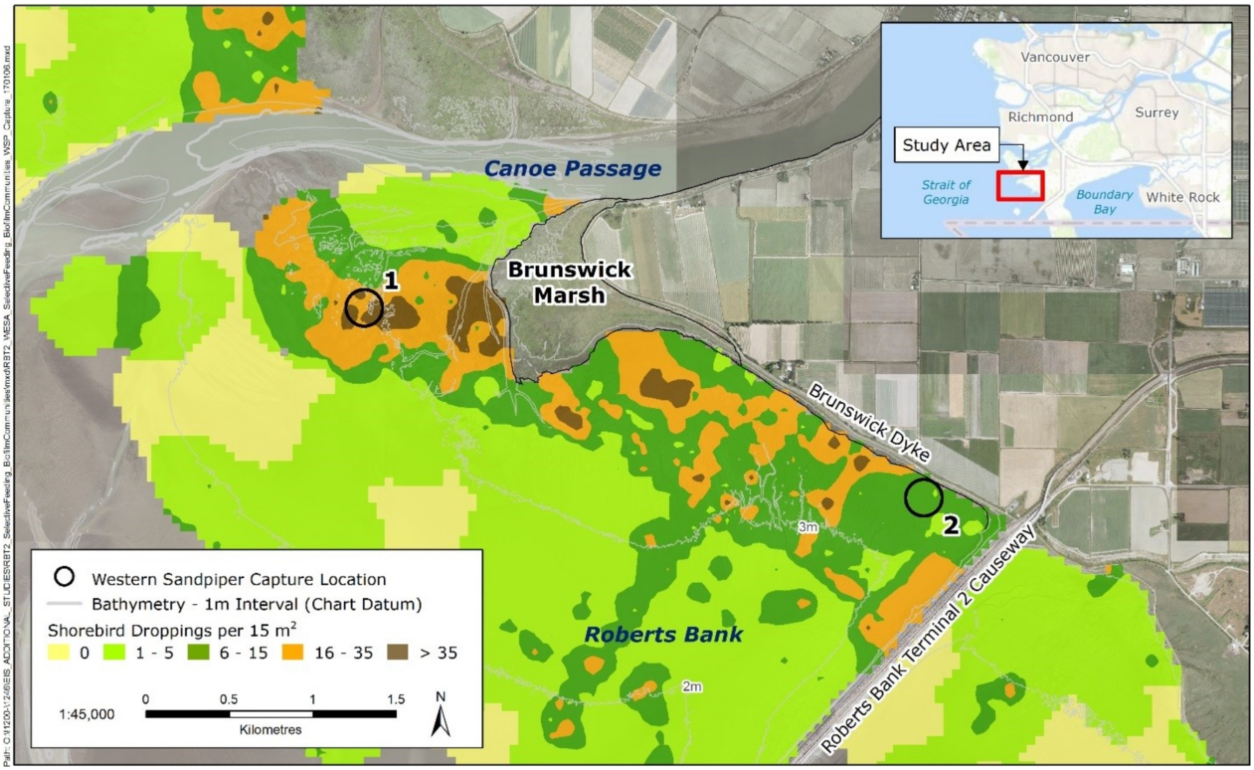

Figure 2-1 Capture Locations for Western Sandpiper at Canoe Passage (1) and the Upper Intertidal (2), April 22 to April 26, 2016 (dropping densities based on field data collected during the 2012 and 2013 northward migrations; see Hemmera 2014).

Capture location 1, hereafter 'Canoe Passage', represents mudflat habitat with relatively high inputs of freshwater from the Fraser River which flows through the passage. Capture location 2, hereafter 'Upper Intertidal', represents more saline mudflat habitat which receives less freshwater flow from the Fraser River. Samples from Canoe Passage were collected at sites with high shorebird dropping densities recorded during field studies conducted in 2012 and 2013 (**Figure 2-1**) (Hemmera 2014). Habitat at Canoe Passage was characterised by emergent sedge vegetation, uneven terrain with small pools and channels, and relatively solid substrate containing a mix of sand mud and clay (**Figure 2-2A**). Samples from the Upper Intertidal were collected from sites where concentrated groups of WESA were observed during the 2016 northward migration. Habitat within the Upper Intertidal location had no vegetation, even terrain without pools or channels, and soft sediments comprised of mud (**Figure 2-2B**). The concentrated densities of WESA at these locations provided the best opportunity to capture the number of WESA required to meet sampling objectives (see **Section 2.3**). Birds captured at both sites were observed to be actively feeding.

Figure 2-2 Photograph of Mist Nets and Habitat at the Canoe Passage (A) and Upper Intertidal (B) Capture Locations

2.2 SAMPLE COLLECTION AND LEVEL OF EFFORT

Shorebirds are abundant in the Fraser River estuary (FRE) throughout most of the year, but especially during migration when Roberts Bank hosts the highest densities of shorebirds throughout the FRE (Butler and Vermeer 1994, Sutherland et al. 2000, Butler et al. 2002). Northward migration of WESA occurs between mid-April and mid-May (Butler et al. 1987). Trapping was conducted on three days between April 22 and April 26, 2016 at Canoe Passage and on four days within the Upper Intertidal (**Table 2-1**). Sampling at Canoe Passage was concluded on April 25 after sampling objectives were met. Droppings samples were only obtained at the Upper Intertidal location on April 26. All captures were obtained within the biofilm sediment sampling window implemented to investigate biofilm community composition at Roberts Bank (see Hemmera and Advisian 2017).

Table 2-1 Sample Schedule and Locations and Samples Obtained and Analysed

Sample Date	Sampling Location	Captured Birds	Droppings Samples	Samples Analysed
April 22, 2016	Canoe Passage	6	5	1
April 23, 2016	Canoe Passage	20	19	7
April 25, 2016	Canoe Passage	18	17	7
April 26, 2016	Upper Intertidal	5	5	5
	Total	49	46	20

2.3 FIELD STUDY METHODS

Droppings can be collected directly off intertidal mudflats (Kuwae et al. 2008, 2012, Jardine et al. 2012); however, contamination of droppings by diatoms on surficial sediments makes this option less than ideal. Therefore, droppings were collected from captured birds rather than from the mudflat surface to ensure that samples were representative of prey consumed by WESA and were not influenced by the biofilm community occurring at the site.

A meeting between Hemmera and Environment and Climate Change Canada (ECCC) biologists was held prior to the first day of sampling to standardised methodologies between shorebird capture teams. A crew of a minimum of four biologists experienced in avian capture and handling techniques used mist nets to capture WESA and obtain droppings samples. Sampling within the Canoe Passage location was led by Hemmera personnel, while sampling within the Upper Intertidal location was led by ECCC personnel. ECCC personnel accompanied Hemmera biologists to the Canoe Passage site on the April 25 sampling event to assist with bird captures and sample processing. Within the sampling locations, mist nets were set in areas where birds were observed foraging and transiting in the highest concentrations (**Figure 2-2**). Appropriate federal scientific collection permits and animal care approvals were obtained prior to capturing shorebirds.

Droppings were obtained from birds immediately following capture and up to 30 minutes after capture. At the Canoe Passage sampling location, captures were conducted on falling tides at least three hours after high tide. The tide-specific sampling schedule was designed to minimise the potential for droppings to reflect prey consumed in terrestrial habitats which are occasionally used as roosting sites by WESA during high tides. At the Upper Intertidal site, attempts were made to capture WESA on the rising tide, at least five hours after high tide; however, WESA flocks avoided nets during this period and captures were only successful on the falling tide.

2.3.1 Fecal Sample Collection

Upon capture, individual birds were placed in plastic holding containers with cloth covers (**Figure 2-3A**). Birds were held in containers until droppings were observed (typically within 5 to 10 minutes) (**Figure 2-3B**). Droppings were then rinsed into plastic centrifuge tubes (50 mL) using deionised water (**Figure 2-3C**). Following transfer to centrifuge tubes, droppings samples were diluted with Lugols iodine solution to a standard tea colour (yellow-brown) (**Figure 2-3D**) and temporarily stored in a dark cooler to prevent sample degradation. Following dropping collections, birds were processed for standard morphological measurements and plumage characteristics (i.e., bill length for sex assignment, plumage coloration for age determination; Prater et al. 1977). Containers were rinsed with deionised water and dried with a cloth between uses. Following field work, samples were refrigerated until they were sent for analysis. Samples were monitored and additional Lugols solution was added if the sample turned clear or light yellow.

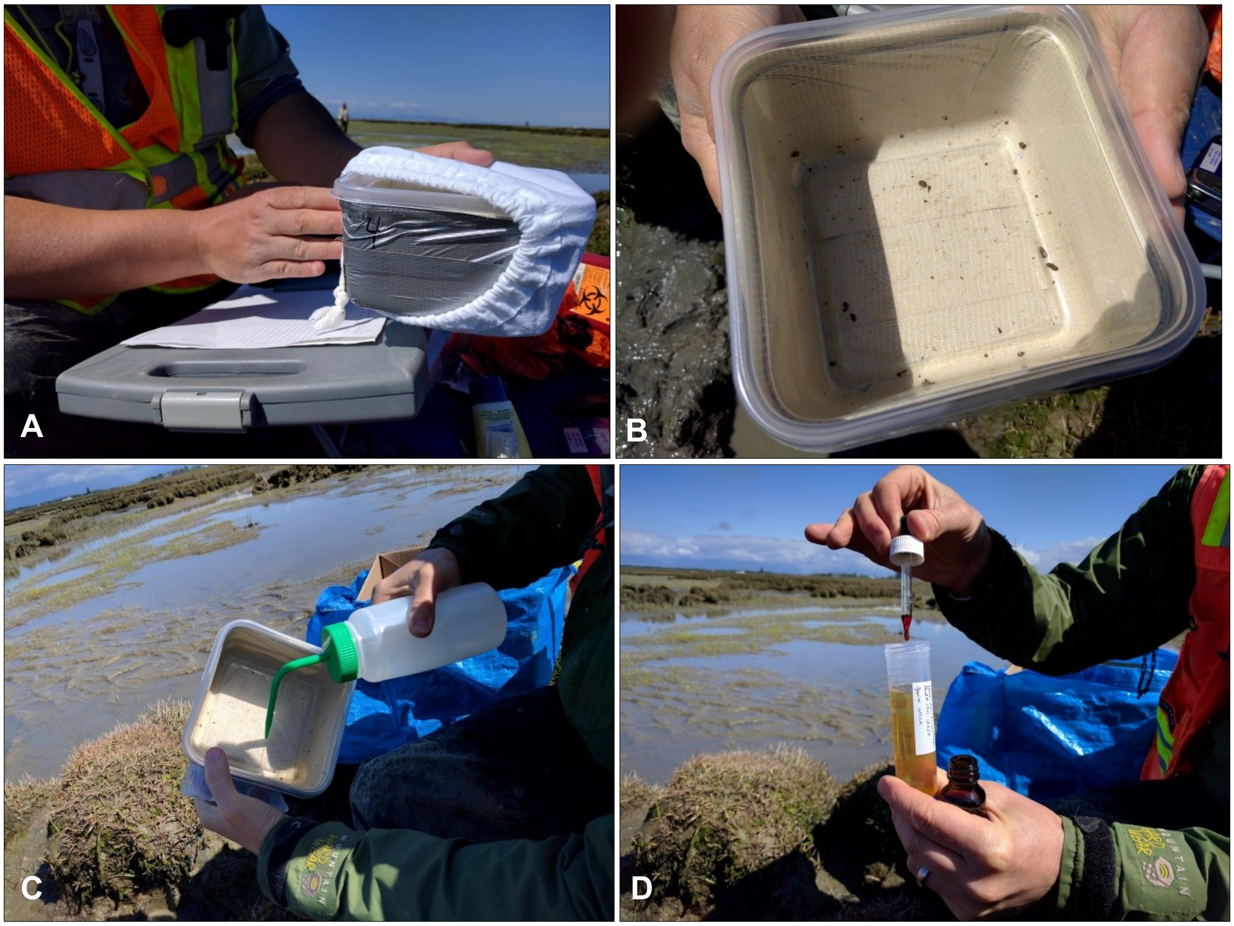

Figure 2-3 Photographs of Droppings Sample Collection Methods: A – Plastic holding container with cloth lid; B – Holding container with droppings sample; C – Rinse of droppings sample and centrifuge tube; D – Sample in centrifuge tube showing color following addition of Lugols solution

The sampling objective for the study was to obtain droppings from 20 individuals with approximately equal representation from both capture locations. Where more samples were available than required, samples were selected based on volume (i.e., lowest volume samples excluded from consideration) and a random selection from the remaining samples was conducted by assigning a random number to each sample and selecting samples with the highest numbers. Sample volume was measured as the height of solid sample mass visible within centrifuge tubes. The samples with heights <4 mm were excluded to ensure sufficient material for accurate determination of composition. This final sample selection was reviewed to ensure representation from all capture dates and inclusion of all age and sex classes.

2.3.2 Taxonomic Identification of Prey

WESA droppings samples preserved in Lugols iodine solution were shipped to Algal Taxonomy and Ecology Inc., Stony Mountain, MB. Analyses were completed by algal taxonomy specialist Hedy Kling M.Sc. in the summer and fall of 2016 on the recommendation of ECCC technical advisors.

Droppings composition analyses followed a standard inverted microscope method for phytoplankton analysis outlined by Findlay and Kling (1998) and for benthic algal and algal sediment analysis according to Kling (1998) and Findlay et al. (1998). Each droppings sample (consisting of shorebird droppings, rinse water, and Lugols solution) was mixed to homogenise the droppings within the sample solution and sub-sampled into a 2 ml settling chamber using either 1 or 2 ml of the sample solution depending on the density of sample contents (e.g., diet items and sediments). The sub-samples were allowed to settle for 12 hours and examined using a Leica Diavert inverted microscope. If the organisms and sediment formed a single layer on the glass cover slip the sample was analysed directly; however, if the silt clay detritus layer was thick enough to obscure diatoms or other organisms, it was further diluted and re-settled until a single layer could be clearly analysed. Where feasible (i.e., for relatively large diet items) diet items on half of the settling chamber bottom were counted. Smaller diet items too numerous to count across the entire chamber were counted along a transect across the chamber bottom to provide a representative sample that could be scaled up to provide an estimate of count for the sample. Estimates of volume and biomass were calculated for each diet item and for each size class of diet items when there were multiple. Biomass calculations were based on these standard methods using the geometric shape of the diet item body, calculated from length and width measurements with the 3rd dimension approximated according to the references above. Flotation appendages or gelatinous mucilage associated with algae were not included in these calculations. A specific gravity of one was used to convert biovolume to biomass (i.e., 1 cm^3 = 1 g). Dilutions required to obtain an appropriate sample layer were recorded and included in the final calculations of counts and biomass together with the initial volume of sample taken and the total sample volume. In addition to the algae and diatoms, remains of other organisms found in the sample (e.g., spine and bristles of polychaete and oligochaete worms, other invertebrate parts) were also recorded to provide an indication of other diet items and their contribution to the diet relative to diatoms. Sediments were not recorded as they provide no energetic value to sandpipers and are ingested in association with a variety of diet items. Taxonomic analyses yielded an estimate of total count for diatoms and other items observed in the sample as well as an estimate of biomass based on the volume calculations described above. Taxonomic classification for diatoms and other algae was provided for the most part to genus level and in some cases where possible, to tentative species assignments or was left as indeterminate (i.e., No ID diatom). Invertebrates could not be classified to genus because most of these organisms' bodies are digested and the parts that remain are fragmented. Consequently, invertebrate parts were typically not classified beyond class (e.g., polychaeta, oligochaeta, bivalvia).

Additionally, diatoms were identified as having passed through a shorebird gut or not, based on the presence/absence of soft parts. Diet items with soft parts that would have been digested had they passed through a digestive tract were likely transferred from the mudflat to the sample on the feet of WESA. Items that were not likely to have passed through a shorebird gut were recorded, but noted as such and excluded from subsequent data analyses.

2.4 DATA ANALYSES

2.4.1 Summarisation of Taxonomic Data

Droppings samples contained varying amounts of sediment, a common finding in shorebirds that ingest intertidal biofilm and invertebrates. In addition to variable sediment content, droppings samples contained varying amounts of total biomass. These sources of variability were controlled for by summarising the droppings composition data as the fraction of total biomass comprised by each diet item. These proportions provided a measure of diet contribution that is comparable across samples, regardless of total sample biomass or sediment content.

Two approaches to compare the relative contribution of different diatoms to a shorebird's diet across samples and groups (e.g., capture location) were conducted. In both cases diatoms were categorised by genus. The first approach summed the biomass (µg/ml) of all diatoms within a genus and calculated the proportional biomass by genera in each bird's diet as described above. This metric was believed to be most pertinent to foraging shorebirds as it calculates a proportional mass of diatoms consumed by shorebirds. The second approach used a count of the number of diatom cells (individual diatoms) or units (diatom aggregates) (units/ml) to calculate a proportional contribution of diatoms to shorebird's diet. As the size of diatoms can vary greatly, results from this metric can differ from the first measure using biomass and are provided for comparative purposes.

These metrics of diet composition were summarised by capture location and for all samples by calculating the mean proportion and the standard deviation around that mean for each genus. The proportion of droppings samples that contained each diatom genus was also determined for each capture location and for all samples.

A similar approach was taken to assess the biomass contribution of diatoms to the diet relative to invertebrates, the other major diet component observed in droppings samples. The proportion of total diatom biomass and invertebrate biomass were determined for each individual and means and standard deviations for the two diet categories were determined for each capture location.

2.4.2 Community Analysis of Diatom Proportion Data

Diatom composition data were analysed with PRIMER statistical software (v.6.1.2, Primer-E Ltd.) to identify key indicator diatoms that drive the differences and similarities in composition across samples, and to assess the significance of differences in diatom proportions between groups (e.g., capture location, sample date, sex, age class).

A similarity percentage analysis (SIMPER) was used to identify key indicator diatom genera (i.e., diatoms that have the most influence on diatom community similarity) by comparing the proportions of diatom genera biomass in droppings by capture location. SIMPER identifies the amount each taxon-genus pair contributes to the Bray-Curtis similarity within a group, and dissimilarity (δ) between groups. In order to gain a sense of how consistently each genus contributes, the dissimilarity value was divided by the standard deviation (SD) across samples. Similarity/SD values greater than 1.4 indicate a strong indicator species (Clarke and Warwick 2001). Dissimilarity was also presented as a percent of total dissimilarity to show relative contribution of each species. The SIMPER results breakdown the community comparison and identify which taxa are dominant within a given habitat and which taxa have the greatest dissimilarity between groups.

To test whether similarities in diatom composition between groups were statistically significant, a multivariate analysis of similarities (ANOSIM) was employed. ANOSIM is analogous to the univariate one-way analysis of variance tests (ANOVA). Essentially, it tests the null hypothesis that there are no differences between groups of samples (e.g., there is no difference between the diatom community in droppings at Canoe Passage and the Upper Intertidal) (Clarke and Gorley 2006). The significance statistic is a percentage with values less than 5% (equating to a p-value of 0.05) indicating significant differences. To provide further support for analysis of differences between groups, a permutational MANOVA (perMANOVA) was conducted using the software package PERMANOVA (Anderson 2001, Andersen et al. 2008). PerMANOVA allows for partitioning of variation and multivariate factorial experiments (e.g., combined analysis of differences among sample dates and capture locations) by calculating the sum of squared interpoint distances to determine within group variation, which addresses many common violations of analysing ecological data. Significance ($P<0.05$) is determined by permutation tests used to generate a distribution of F under the null hypothesis of no relationship, termed pseudo-F.

To illustrate how samples relate based upon diatom genera composition, non-metric multidimensional scaling (nMDS) was used to explore relationships between the species composition of the different sites. nMDS is commonly used in ecological studies because it avoids many unrealistic assumptions not met by ecological data of other methods such as principal components analysis (Clarke and Gorley 2006). nMDS creates a map of the samples in an ordination diagram based upon the degree of dissimilarity between samples due to their species abundance. When viewing an ordination, objects which are “more similar” to each other will occur closer together; while objects that are dissimilar will occur further apart.

3.0 RESULTS

3.1.1 Shorebird Captures and Samples Sent for Analysis

A total of 49 WESA were captured during field efforts, but only 5 were captured in the Upper Intertidal site. Consequently, more samples were analysed from the Canoe Passage site (n=14) as compared to the Upper Intertidal (n=5). The 20th droppings sample analysed was from a dunlin (*Calidris alpina*) captured at Canoe Passage for comparative purposes.

3.1.2 Fecal Composition

Invertebrate parts comprised the majority of dropping biomass at both the Canoe Passage and Upper Intertidal capture sites (**Table 3-1**, **Figure 3-1**). The dunlin droppings sample from Canoe Passage had similar proportions as Upper Intertidal WESA (0.85 invertebrates, 0.15 diatoms).

Table 3-1 Proportions of Diatom Biomass Relative to Other Digested Components of Western Sandpiper Droppings Samples

	Canoe Passage		Upper Intertidal	
	Mean	Standard Deviation	Mean	Standard Deviation
Diatoms	0.229	0.272	0.141	0.161
Sponge	0.003	0.010	0	-
Invertebrates	0.769	0.270	0.859	0.161

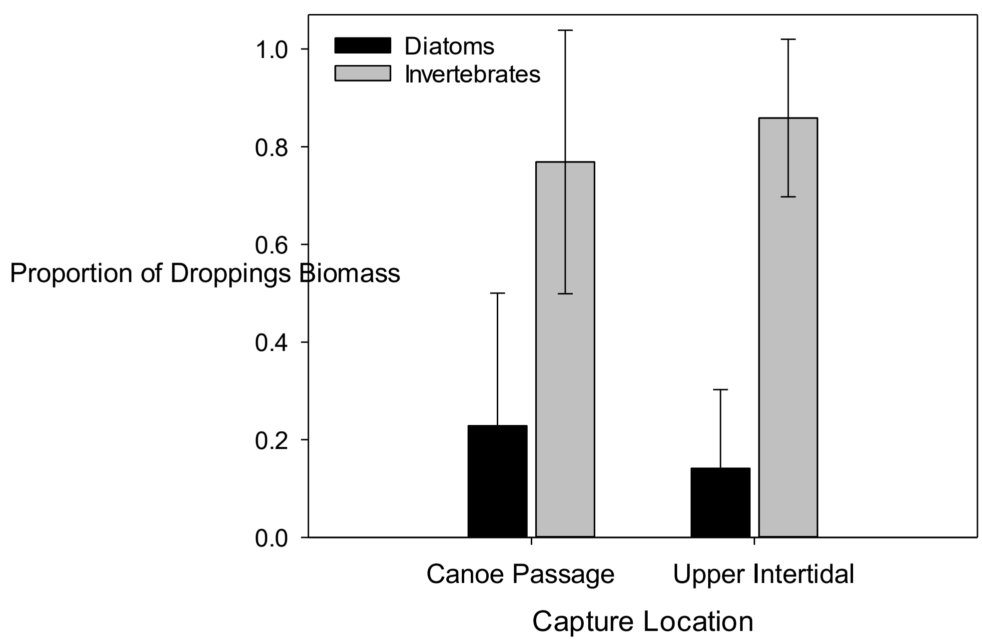

Figure 3-1 Mean (Bars) and Standard Deviation (Whiskers) of Diatoms and Invertebrates Biomass in Western Sandpiper Droppings Samples at Canoe Passage (n = 14) and Upper Intertidal (n = 5) Sites

A total of 28 diatom genera were observed in droppings samples, of which 17 comprised at least 1% of biomass (µg/ml) at either Canoe Passage or the Upper Intertidal capture locations. Less than 1% of diatoms in any sample were unidentified and categorised as "No ID diatoms" (**Table 3-2**). The majority (≥60%) of biomass was comprised of four genera which, on average, occurred in similar proportions across the two sites. In order from greatest to smallest proportion of droppings samples, these genera were *Navicula, Achnanthidium, Amphora,* and *Nitzschia*. Diatoms of the genus *Navicula* comprised the largest portion of biomass in shorebird droppings from Canoe Passage (**Figure 3-2A**) and the Upper Intertidal (**Figure 3-2B**) averaging approximately 44% of all diatom biomass in samples (**Table 3-2**). *Achnanthes/Achnanthidium, Amphora* and *Nitzschia* were the other most consistently observed diatom genera with average biomass comprising approximately 9%, 6% and 6% of the total, respectively (**Table 3-2**). *Navicula* was observed in all WESA droppings samples analysed while *Achnanthes/Achnanthidium* and *Nitzschia* were observed in all but one sample from Canoe Passage in which 100% of diatoms were *Navicula*. Genera that made up more than 5% of diatom biomass at just one site were *Surirella, Melosira*, and *Trachyneis*. These genera were less consistent in their presence and proportions in samples (**Table 3-2**). While mean proportions of *Amphora* were similar at both capture sites, the genus was only observed in 21% of samples in Canoe Passage as compared to 60% in the Upper Intertidal, and the overall standard deviation (0.134) was more than double the mean (0.060) indicating high variability in the amount of *Amphora* occurring within samples at Canoe Passage (**Table 3-2**). *Surirella* was observed in 64% of samples from Canoe Passage, but not in any samples from the Upper Intertidal. *Melosira* was observed in three samples from both capture locations in low proportions of biomass (<12%) in all but one sample from the Upper Intertidal in which *Melosira* comprised 74.9% of diatoms (**Table 3-2**). *Trachyneis* was also observed in three samples from each capture location, but was consistently more abundant in Upper Intertidal samples (**Table 3-2**). Droppings samples from Canoe Passage had a higher number of genera (26) and genera contributing at least 1% of diatom biomass (15) as compared to Upper Intertidal samples (15 and 10, respectively). The dunlin droppings sample from Canoe Passage was comprised of the diatom genera in order of decreasing proportion: *Amphora*: 0.43; *Navicula*: 0.29; *Achnanthes/Achnanthidium*: 0.17; *Trybloniella*: 0.10.

A complete list of diatom genera observed in droppings and their biomass proportions in samples is presented in **Table 1** of **Appendix A**. A complete list of diet items and biomass recorded within each sample is presented in **Table 1** of **Appendix B**.

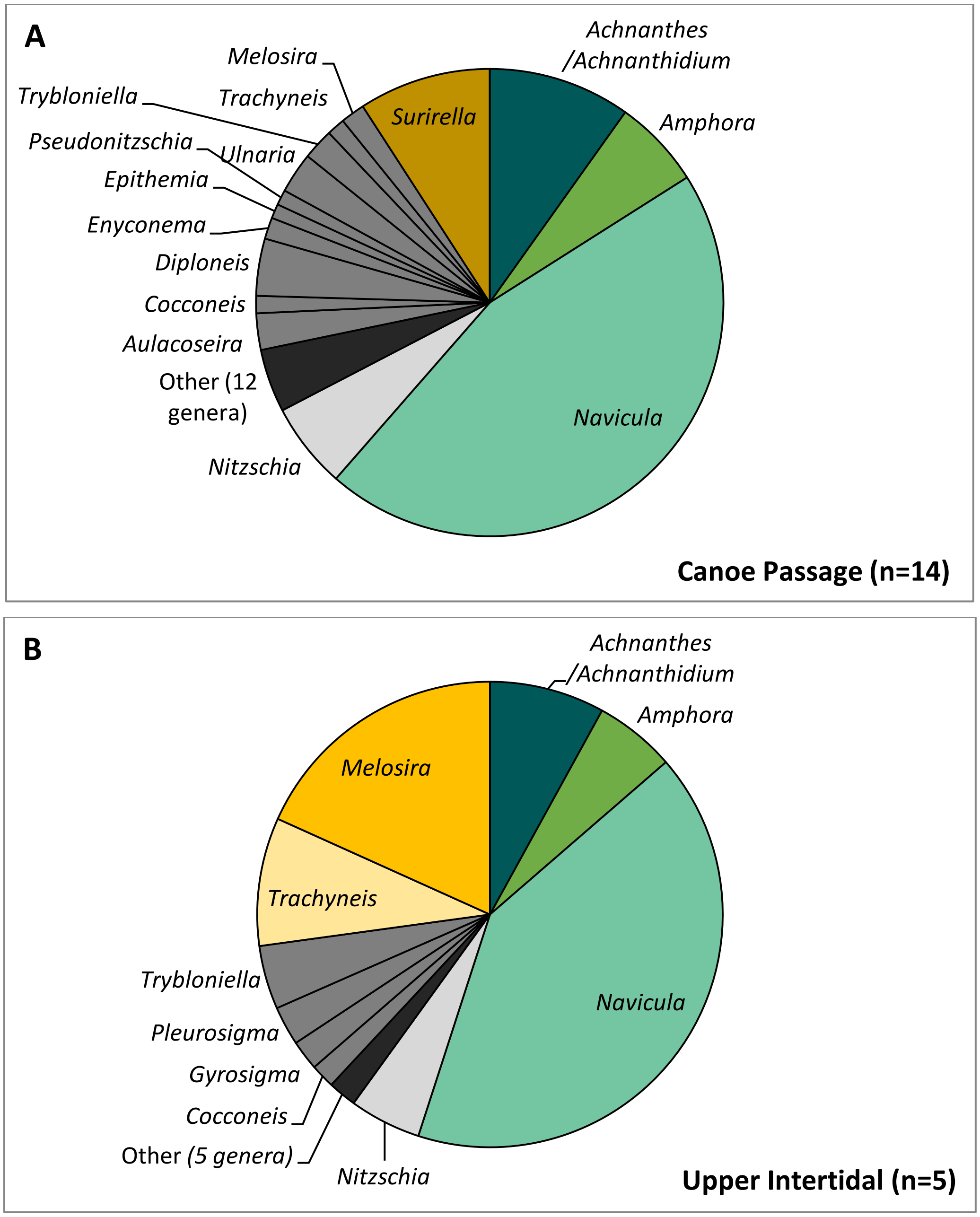

Figure 3-2 Mean Proportion of Diatom Genera Biomass (µg/ml) in Western Sandpiper Droppings from Canoe Passage (A) and Upper Intertidal (B) Capture Locations

Note: Diatom genera comprising <1% of total diatoms are grouped within the category "Other". Diatoms comprising >1% to <5% of Canoe Passage and Upper Intertidal samples are shown in dark grey. Sample sizes are shown in the lower right-hand corner in parentheses beside capture location name. Proportional biomass data is presented in **Table 3-2**.

Table 3-2 Mean Biomass (µg/ml) Proportions, Standard Deviation (SD), and % Presence of Diatoms in samples of Western Sandpiper Droppings by Capture Location and Genus

	Total (n = 19)			Canoe Passage (n = 14)			Upper Intertidal (n = 5)		
Diatom Genera	**Mean (µg/ml)**	**SD**	**% Presence**	**Mean (µg/ml)**	**SD**	**% Present**	**Mean (µg/ml)**	**SD**	**% Presence**
Navicula	0.443	0.222	100	0.454	0.213	100	0.413	0.271	100
Achnanthes /Achnanthidium	0.094	0.076	95	0.099	0.085	93	0.080	0.045	100
Surirella	0.067	0.102	47	0.091	0.110	64	0	-	0
Amphora	0.060	0.134	32	0.062	0.155	21	0.056	0.058	60
Melosira	0.060	0.172	32	0.017	0.040	21	0.183	0.320	60
Nitzschia	0.057	0.084	95	0.059	0.093	93	0.050	0.057	100
Trachyneis	0.033	0.070	32	0.013	0.025	21	0.089	0.119	60
Diploneis	0.029	0.127	5	0.040	0.148	7	0	-	0
Trybloniella	0.027	0.049	42	0.022	0.040	43	0.044	0.072	40
Ulnaria	0.021	0.058	37	0.028	0.066	50	0	-	0
Aulacoseira	0.019	0.056	11	0.025	0.065	14	0	-	0
Cocconeis	0.013	0.024	32	0.012	0.026	21	0.017	0.020	60
Enyconema	0.011	0.028	16	0.015	0.032	21	0	-	0
Gyrosigma	0.010	0.030	16	0.007	0.025	7	0.021	0.041	40
Pseudonitzschia	0.008	0.034	5	0.011	0.040	7	0	-	0
Epithemia	0.007	0.024	11	0.010	0.027	14	0	-	0
Pleurosigma	0.007	0.031	5	0	-	0	0.027	0.061	20
Thalassiosira	0.007	0.030	5	0.009	0.035	7	0	-	0
Cylindrotheca	0.005	0.014	21	0.005	0.016	14	0.005	0.010	40
Rhicosphenia	0.005	0.020	5	0.006	0.023	7	0	-	0
Tabellaria	0.004	0.017	5	0.005	0.020	7	0	-	0
Fragilaria	0.003	0.006	26	0.004	0.007	29	0	-	0
Diatoma	0.002	0.006	11	0.001	0.004	7	0.005	0.010	20
Gomphonema	0.002	0.007	11	0.003	0.008	14	0	-	0
Skeletonema	0.002	0.010	5	0	-	0	0.009	0.020	20
Cyclotella	0.001	0.003	11	0.001	0.004	14	0	-	0
Eunotia	0.001	0.003	11	0.001	<0.001	7	0.001	0.001	20
Diatom (No ID)	< 0.001	0.002	5	0.001	0.002	7	0	-	0
Reimeri	< 0.001	0.001	5	<0.001	0.001	7	0	-	0
Genus Count			**26**			**15**			**28**

The proportional composition of diatoms in droppings derived from abundance (count) data (units/ml) differed from estimates based on biomass (µg/ml) for some genera. *Navicula*, *Achnanthes* /*Achnanthidium*, and *Nitzschia* dominated abundance estimates comprising just over 90% of all diatoms counted at both sites (**Table 3-3**, **Figure 3-3**). Similar to results based on biomass data, *Navicula* averaged 43% to 44% of diatoms at the Upper Intertidal and Canoe Passage locations, respectively. *Achnanthes*/*Achnanthidium* and *Nitzschia* were also found at very similar abundaces between sites, differing by 5% to 7% (**Table 3-3**). Abundances of all other genera individually averaged ≤ 5% of fecal samples.

Differences between biomass and abundance estimates are likely due to large size differences between some genera. For example, diatoms of the genera *Achnanthes*/*Achnanthidium* tended to be small in size (mean dimensions = 11 x 4 µm), while others, such as the genera *Trachyneis*, were much larger (mean dimensions = 62 x 17 µm). While *Achnanthes*/*Achnanthidium* is much more abundant than *Trachyneis* (38% vs. 1% of diet at the Upper Intertidal site, **Table 3-3**, **Figure 3-3**) they were present in comparable biomass (8% vs. 9%,**Table 3-2**, **Figure 3-2**) as many fewer *Trachyneis* are required to equal the same biomass as *Achnanthes*/*Achnanthidium*.

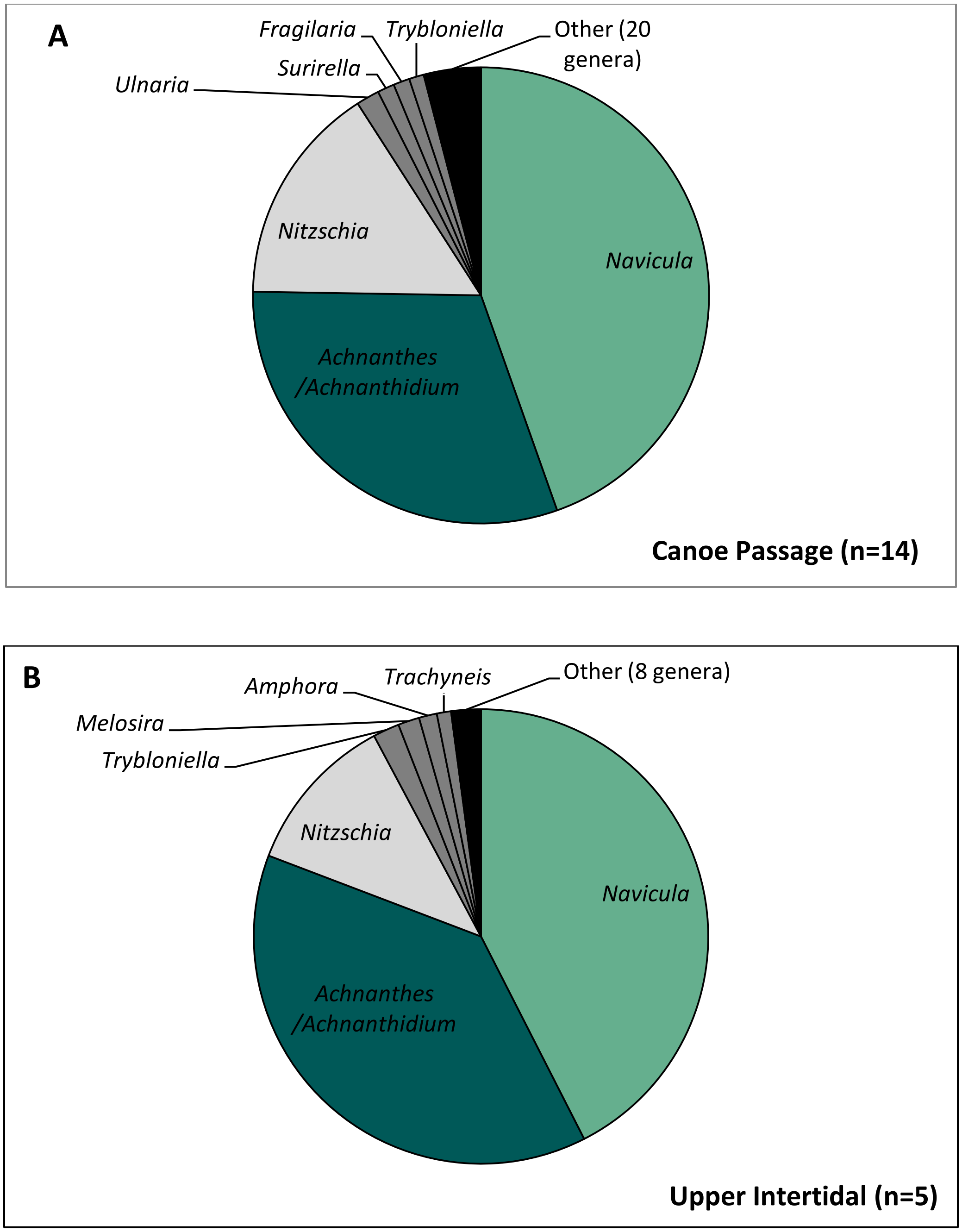

Figure 3-3 Mean Proportion (Cell Units/ml) of Diatom Genera in WESA Droppings from Canoe Passage (A) and Upper Intertidal (B) Capture Locations

Note: Diatom genera comprising <1% of total diatoms are grouped within the category "Other". Diatoms comprising <5% of Canoe Passage and Upper Intertidal samples are shown in grey. Sample sizes are shown in the lower right hand corner in parentheses beside capture location name. Proportional abundance data is presented in **Table 3-3**.

Table 3-3 Mean Proportions (Cell Units/ml) and Standard Deviation (SD) of Diatom in samples of WESA Droppings by Capture Location and Genus

Diatom Genera	Total (n = 19)		Canoe Passage (n = 14)		Upper Intertidal (n = 5)	
	Mean (units/ml)	SD	Mean (units/ml)	SD	Mean (units/ml)	SD
Navicula	0.440	0.186	0.446	0.214	0.425	0.083
Achnanthes /Achnanthidium	0.327	0.156	0.307	0.172	0.383	0.087
Nitzschia	0.145	0.131	0.157	0.148	0.114	0.069
Ulnaria	0.016	0.048	0.016	0.048	0	-
Trybloniella	0.013	0.020	0.010	0.017	0.019	0.026
Surirella	0.012	0.013	0.012	0.013	0	-
Fragilaria	0.009	0.021	0.012	0.024	<0.001	<0.001
Amphora	0.008	0.014	0.006	0.013	0.013	0.016
Gomphonema	0.007	0.022	0.007	0.022	0	-
Melosira	0.005	0.015	0.001	0.003	0.015	0.029
Skeletonema	0.005	0.011	0	-	0.005	0.011
Cylindrotheca	0.004	0.008	0.003	0.007	0.007	0.009
Enyconema	0.004	0.008	0.004	0.008	0	-
Trachyneis	0.004	0.011	0.001	0.003	0.010	0.02
Aulacoseira	0.003	0.009	0.003	0.009	0	-
Cocconeis	0.003	0.010	0.004	0.012	0.002	0.003
Diatom (No ID)	0.003	0.012	0.003	0.012	0	-
Diatoma	0.002	0.005	0.001	0.002	0.004	0.009
Cyclotella	0.001	0.003	0.001	0.003	0	-
Diploneis	0.001	0.005	0.001	0.005	0	-
Epithemia	0.001	0.003	0.001	0.003	0	-
Eunotia	0.001	0.003	<0.001	<0.001	0.003	0.006
Pseudonitzschia	0.001	0.004	0.001	0.004	0	-
Gyrosigma	<0.001	0.001	<0.001	0.002	0.001	0.001
Pleurosigma	<0.001	0.001	0	-	<0.001	0.001
Reimeri	<0.001	0.001	<0.001	0.001	0	-
Rhicosphenia	<0.001	0.001	<0.001	0.001	0	-
Tabellaria	<0.001	0.001	<0.001	0.001	0	-
Thalassiosira	<0.001	0.002	<0.001	0.002	0	-
Genus Count		**26**		**15**		**28**

Note: For the percent occurrence of diatom genera across fecal samples see the % Presence column in **Table 3-2**.

3.1.3 Community Analyses

3.1.3.1 Individual Western Sandpipers

Results of ANOSIM and perMANOVA tests revealed no significant differences in community compostion between the date fecal samples were collected or capture location (i.e., Canoe Passage vs. Upper

Intertidal) (ANOSIM: 16.5% (equivalent p = 0.165); perMANOVA: p = 0.129). All but one outlying sample from the Upper Intertidal shared 35% similarity in diatom genera composition. Also, samples sharing 50% similarity were not grouped by sample date or capture location (**Figure 3-4**) indicating some droppings were more similar to droppings from other dates or locations than to samples collected on the same date or within the same location.

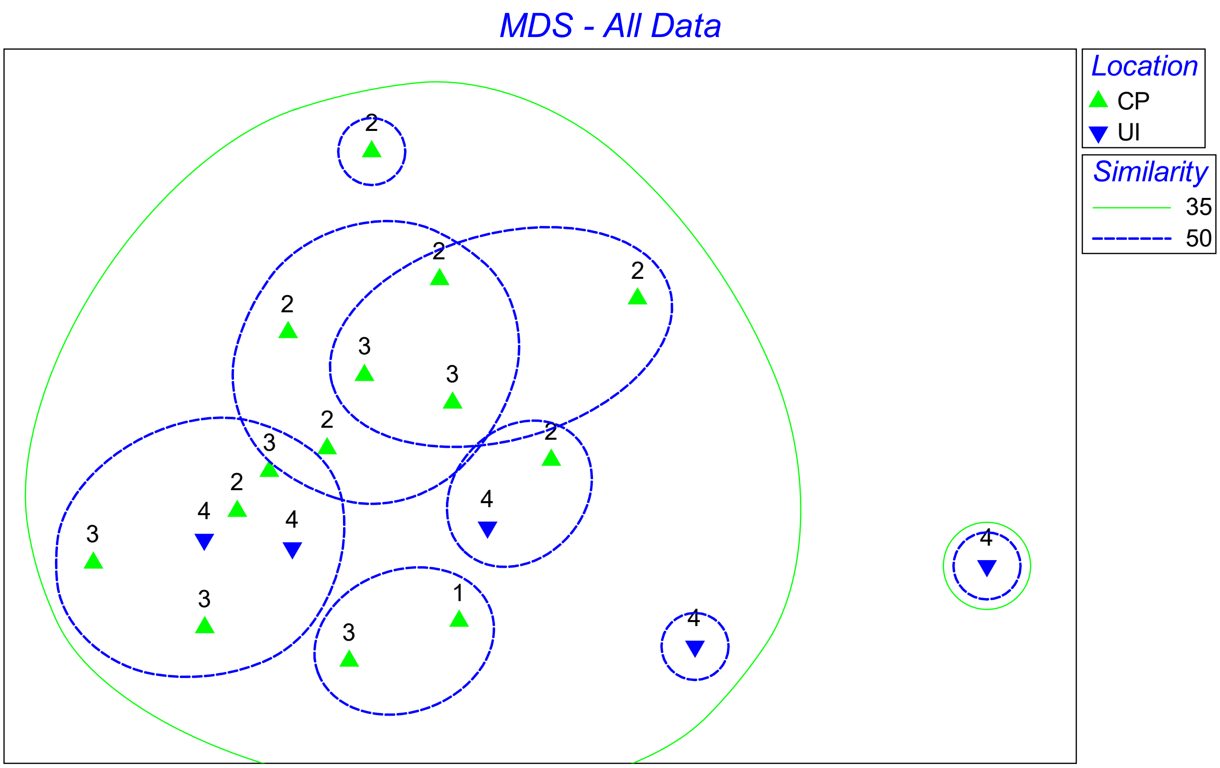

Figure 3-4 Ordination Diagram Illustrating the Degree of Diatom Composition Dissimilarity as Distance between Sample Symbols based on the Biomass (µg/ml) of Genera

Note: Samples from Canoe Passage (CP) and Upper Intertidal (UI) capture locations are labelled with green and blue symbols, respectively. Labels 1, 2, 3, and 4 indicate the date of sample collection and correspond to sample dates April 22, 23, 25, and 26, respectively. MDS = multidimensional scaling.

Results of SIMPER analyses showed that diatom composition was more similar amongst samples from Canoe Passage (average similarity: 45.91) than amongst samples from the Upper Intertidal (average similarity: 38.99); however, similarity values at both capture locations reflect substantial dissimilarity across samples (i.e., average similarity <50). The lack of similarity of samples within groups likely contributes to the nonsignificant differences in diatom community composition between groups. SIMPER analyses found *Navicula* contibuted 73% and 63% to the difference in community composition within samples from Canoe Passage and the Upper Intertidal, respectively (**Table 3-4**). *Navicula* was the only genus with a Similarity/SD ratio over 1.4 at both capture locations, and *Achnanthes/Achnanthidium* was the only other genus with such a high ratio at the Upper Intertidal capture location. Thus, these two genera are considered to drive variability among fecal droppings in diatom composition in diet. *Achnanthes/Achnanthidium* contributed an additional 11% and 13% to the difference in diatom composition for the two sites with the majority of remaining contributions made up by *Surirella*, *Nitzschia*, *Amphora*, *Melosira* and *Trachyneis* genera. These seven diatom genera accounted for over 95% of the differences in composition at both sites (**Table 3-4**).

Table 3-4 SIMPER Analysis Results for Diatom Genera in Western Sandpiper Droppings Samples from Canoe Passage and Upper Intertidal Capture Locations

Diatom Genera	Average Similarity	Similarity/SD	Percent Contribution	Cumulative Percent Contribution
Canoe Passage				
Navicula	33.63	2.88	73.26	73.26
Achnanthes /Achnanthidium	5.04	1.06	10.97	84.24
Surirella	3.22	0.60	7.02	91.26
Nitzschia	1.99	0.91	4.33	95.59
Amphora	0.45	0.11	0.98	96.57
Trybloniella	0.38	0.36	0.83	97.40
Ulnaria	0.26	0.20	0.56	97.96
Trachyneis	0.17	0.18	0.38	98.34
Aulacoseira	0.17	0.10	0.38	98.71
Enyconema	0.16	0.18	0.34	99.05
Melosira	0.15	0.13	0.33	99.38
Cocconeis	0.13	0.17	0.29	99.67
Fragilaria	0.06	0.25	0.14	99.81
Epithemia	0.05	0.10	0.10	99.91
Gomphonema	0.02	0.10	0.05	99.96
Cylindrotheca	0.02	0.10	0.03	99.99
Cyclotella	0.00	0.10	0.01	100.00
Upper Intertidal				
Navicula	24.55	1.60	62.98	62.98
Achnanthes /Achnanthidium	5.15	1.68	13.21	76.20
Melosira	2.11	0.55	5.42	81.62
Amphora	2.06	0.58	5.28	86.90
Trachyneis	2.01	0.37	5.16	92.05
Nitzschia	1.91	1.64	4.89	96.94
Trybloniella	0.55	0.32	1.42	98.36
Cocconeis	0.49	0.45	1.25	99.61
Gyrosigma	0.10	0.32	0.26	99.87
Cylindrotheca	0.05	0.32	0.13	100.00

4.0 DISCUSSION

4.1 DIET COMPOSITION

Results indicated diets of WESA at Canoe Passage and Upper Intertidal, and diets across sampling dates, were similar and not statistiscally different (ANOSIM = 0.17; perMANOVA: p = 0.13). For example, the diets (based on diatom composition of droppings) of birds caught at one site were as similar or more similar to the diets of birds caught outside their site compared to those caught within their capture location. Likewise, droppings collected on different dates were also similar, indicating diets did not differ over the course of the study.

Twenty-eight genera of diatoms were found in WESA droppings, with the number of genera fed on by individual birds varying from 1 to 13. Similar to previous studies of the biofilm community (WorleyParsons 2015*a*), WESA droppings from Canoe Passage possessed higher taxonomic richness compared to the Upper Intertidal site. Despite this diversity and variability, three genera were found in 95% of droppings, *Navicula*, *Achnanthes*/*Achnanthidium*, and *Nitzschia* across sites. Research has shown these genera to be abundant at Robert Bank in other years (WorleyParsons 2014, 2015*a*, *b*) and based on their high prevalence and abundance in droppings may influence WESA foraging. A parallel study found *Achnanthes* to comprise a very small proportion (0.15%) of the *Achnanthes*/*Achnanthidium* complex (Hemmera and Advisian 2017), and therefore this complex can be considered almost entirely represented by diatoms of the genus *Achnanthidium*. Other genera making up proportional biomasses >5% were *Amphora*, *Surirella*, *Melosira*, and *Trachyneis*; however, these genera occurred within only 32% to 47% of birds in variable abundances across individuals and capture locations. Thus, diatoms belonging to these other genera seem less likely to be a driver of foraging preferences. These results indicate a variable, diverse diatom community, with diatom genera of many species well dispersed across Roberts Bank.

4.2 SELECTIVE FEEDING

Navicula comprised the largest portion of diatoms in WESA droppings at both Canoe Passage and Upper Intertidal sites (45% and 43%, respectively, from April 23 to 26, **Table 4-1**). These porportions are approximately 10 to 15% higher than those documented in biofilm sediment samples (i.e., 36% and 26%, respectively) collected during the same periods within the same sites. Higher proportions of cells in droppings relative to biofilm sediment samples were also observed for the genus *Achnanthidium* at both capture locations (**Table 4-1**), possibly indicating preferential foraging for *Navicula* and *Achnanthidium*. Conversely, proportions of *Nitzschia* found in WESA droppings were below those documented in sediment samples (Hemmera and Advisian 2017) at both sites, possibly indicating avoidance of *Nitzschia*. Despite the limited sample sizes and temporal scope of this study, the similarity of findings from two distinct locations indicates potential selective foraging on three genera of diatoms that are abundant across Roberts Bank during northward migration (WorleyParsons 2015*a*, Hemmera and Advisian 2017).

Table 4-1 Comparison of the Percent of Diatoms of Dominant Genera in WESA Droppings to those Documented in Biofilm Sediment

Diatom	Canoe Passage		Upper Intertidal	
Genera	Droppings	Biofilm Sediment	Droppings	Biofilm Sediment
Navicula	45% ± 21 SD	36% ± 16 SD	43% ± 8 SD	26% ± 7 SD
Nitzschia	16% ± 15 SD	28% ± 16 SD	11% ± 7 SD	32% ± 1 SD
Achnanthidium	31% ± 17 SD	1% ± 17 SD	38% ± 9 SD	7% ± 5 SD

Notes:
1. Droppings data summarised here were from samples collected on April 23, 25 (Canoe Passage; n = 14) and April 26 (Upper Intertidal; n = 5).
2. Biofilm sediment data summarised here were from samples collected on April 23 and 25 at two sites in Canoe Passage (n = 4) and one site in the Upper Intertidal (n = 2). For biofilm sediment sampling methods and results see (Hemmera and Advisian 2017).

It also should be noted that on the basis of prey biomass, benthic invertebrates (e.g., polycheates, bivalve mollusks) dominated WESA droppings, as invertebrate parts were four times more abundant compared to diatom frustules (**Figure 3-1**). A substantial portion of invertebrate biomass is soft tissue that is absorbed during digestion, so the biomass of invertebrates ingested by WESA is likely substantially larger than that observed in droppings. In contrast to the large proportion of invertebrate parts observed in droppings reported here, studies using stable isotope analyses of stomach contents suggest biofilm contributes 50% to 68% of daily energetic requirements for WESA foraging at Roberts Bank during the northward migration (Kuwae et al. 2008). Kuwae et al. (2008) analysed stomach contents and fecal droppings and provided evidence that invertebrate parts are over-represented in droppings. In an analyses of stomach contents from 97 WESA, invertebrates fragments comprised a mean of 8.6% by volume, whereas biofilm-associated diatoms comprised 76.1% of total volume. Additionally, stable isotope signatures of stomach contents were very similar to those of biofilm and microphytobenthos indicating that the majority of the organic material in stomachs was derived from biofilm (Kuwae et al. 2008). In contrast, stable isotope signatures of WESA droppings were more similar to invertebrates indicating invertebrates were over represented in droppings (Kuwae et al. 2008). Thus, the results of this study correspond with previous research.

As WESA are highly mobile, it should also be recognised that droppings collected from the Canoe Passage and the Upper Intertidal capture locations do not necessarilly reflect biofilm consumed at those sites. If a bird was caught shortly after arriving at a capture location the diet reflected in droppings would be reflective of the area(s) it previously foraged. Such movements potentially contribute to the variability in diatom composition of droppings within capture locations. Thus, this study does not offer conclusive evidence that prey consumed at the specific capture locations are similar; however, it does suggest that composition of diatoms in the diet are similar across different regions of the mudflat. Due to the distance between the two capture locations (~ 3 km), and abundance of foraging habitat in the study area, it is considered unlikely that the similarity between sites documented in this study is due to birds feeding at one capture site and being caught at the other. Despite this uncertainty, this study provides evidence that the primary diatom genera consumed at Roberts Bank is *Navicula* with the genera *Achnanthes/Achnanthidium* being second most consumed. Given the movement of shorebirds described above and the similarity of diet composition at both capture locations, diet composition reported here likely applies across Roberts Bank.

5.0 CONCLUSIONS

Key findings of this study include:

1. Diets of WESA at fresh-water influenced locations (Canoe Passage) and the marine-influenced locations (Upper Intertidal) were similar and not statistiscally different.
2. The diatom genera *Navicula*, *Achnanthes*/*Achnanthidium*, and *Nitzschia* were abundant in biofilm sediments and found to be the dominant genera in WESA diets, being found in 95% of droppings and comprising 92% of diatoms in droppings.
3. Based on the prevalence and abundance of diatom genera in biofilm sediments and WESA droppings, and similarities of findings from two distinct locations at Roberts Bank, this study provides an indication of potential selective foraging by WESA on the genera *Navicula* and *Achnanthes*/ *Achnanthidium*.
4. Given the similarity of diet composition at distinct capture sites, located ~ 3 km apart, the diet composition reported in this study likely applies across Roberts Bank.

6.0 CLOSURE

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APPENDIX A

Proportions of Diatom Genera in Fecal Samples by Biomass

Table 1 Proportions of Diatom Genera Biomass (µg/ml of droppings sample) Reported in Western Sandpiper Droppings Samples from Two Capture Locations at Roberts Bank during the 2016 Northward Migration

Canoe Passage (samples 37–74)

Band Number: 1701-126*XX*

Upper Intertidal (samples 25–31)

Band Number: 2661-710*XX*

Diatom Genus Category	37	40	41	42	43	45	48	55	58	59	61	66	71	74	25	26	27	30	31
Achnanthes /Achnanthidium	0.051	0.160	0.073	0.283	0.077	0.125	0.042	0.122	0.037	0.143	0.018	0.013	0.235	-	0.105	0.137	0.086	0.023	0.048
Amphora	0.384	0.013	-	-	-	-	-	-	0.464	-	-	-	-	-	0.098	0.054	-	-	0.130
Aulacoseira	-	-	-	-	-	0.196	-	-	-	-	0.157	-	-	-	-	-	-	-	-
Cocconeis	-	-	0.073	-	-	-	0.027	-	-	-	-	0.068	-	-	-	0.042	-	0.035	0.007
Cyclotella	0.003	0.014	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cylindrotheca	-	-	-	0.059	0.014	-	-	-	-	-	-	-	-	-	-	0.022	-	-	0.005
Diatom (No ID)	-	-	-	-	-	-	-	-	-	-	-	0.009	-	-	-	-	-	-	-
Diatoma	-	-	-	-	-	-	-	-	-	0.014	-	-	-	-	-	0.023	-	-	-
Diploneis	-	-	-	-	0.554	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Enyconema	0.062	-	-	-	-	-	-	-	-	0.040	0.106	-	-	-	-	-	-	-	-
Epithemia	-	-	-	-	-	-	0.097	-	-	0.043	-	-	-	-	-	-	-	-	-
Eunotia	-	-	0.015	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.003
Fragilaria	-	-	0.022	0.016	-	0.010	-	0.007	-	-	-	-	-	-	-	-	-	-	<0.001
Gomphonema	-	-	-	-	-	0.020	-	-	-	-	0.023	-	-	-	-	-	-	-	-
Gyrosigma	-	-	-	-	-	-	-	-	-	-	-	0.094	-	-	-	-	-	0.010	0.094
Melosira	-	-	0.015	-	-	0.112	-	-	-	0.109	-	-	-	-	-	0.048	-	0.749	0.116
Navicula	0.358	0.275	0.517	0.632	0.251	0.166	0.388	0.315	0.463	0.354	0.417	0.568	0.656	1.000	0.615	0.353	0.768	0.141	0.191
Nitzschia	0.039	0.030	0.057	0.010	0.025	0.039	0.005	0.355	0.036	0.060	0.152	0.002	0.024	-	0.015	0.149	0.011	0.027	0.047
Pleurosigma	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.136	-	-
Pseudonitzschia	-	-	-	-	-	-	-	0.148	-	-	-	-	-	-	-	-	-	-	-
Reimeri	-	-	0.002	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rhicosphenia	-	-	-	-	-	-	-	-	-	0.087	-	-	-	-	-	-	-	-	-
Skeletonema	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.044
Surirella	-	0.361	0.145	-	0.063	0.133	0.252	-	-	0.150	0.086	0.004	0.085	-	-	-	-	-	-
Tabellaria	-	-	0.074	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Thalassiosira	-	-	-	-	-	-	0.129	-	-	-	-	-	-	-	-	-	-	-	-
Trachyneis	0.070	-	-	-	-	0.052	-	0.053	-	-	-	-	-	-	-	0.172	-	0.015	0.259
Tryblionella	0.033	0.145	-	-	0.016	0.039	0.059	-	-	-	-	0.009	-	-	0.166	-	-	-	0.055
Ulnaria	-	0.003	0.007	-	-	0.109	0.001	-	-	0.001	0.041	0.233	-	-	-	-	-	-	-

APPENDIX B

Biomass of All Diet Items

Table 1 Biomass of all diet items (µg/ml of droppings sample) Reported in Western Sandpiper Droppings Samples from Two Capture Locations at Roberts Bank during the 2016 Northward Migration

	Canoe Passage Band Number: 1701-126XX														**Upper Intertidal Band Number: 2661-710XX**					
Diet Item Category	**37**	**40**	**41**	**42**	**43**	**45**	**48**	**55**	**58**	**59**	**61**	**66**	**71**	**74**	**25**	**26**	**27**	**30**	**31**	**Grand Total**
Diatoms																				
Achnanthes /Achnanthidium	0.057	0.096	0.076	0.127	0.117	0.150	0.125	0.017	0.008	0.309	0.008	0.032	0.050		0.088	0.029	0.016	0.020	0.242	**1.567**
Amphora	0.431	0.008							0.102						0.082	0.012			0.650	**1.284**
Aulacoseira						0.235					0.074									**0.309**
Cocconeis			0.075				0.080					0.163				0.009		0.030	0.035	**0.392**
Cyclotella	0.003	0.008																		**0.011**
Cylindrotheca				0.027	0.022											0.005			0.025	**0.078**
Diatom (No ID)												0.021								**0.021**
Diatoma										0.031						0.005				**0.036**
Diploneis					0.845															**0.845**
Enyconema	0.069									0.086	0.050									**0.205**
Epithemia							0.288			0.092										**0.380**
Eunotia			0.015																0.017	**0.032**
Fragilaria			0.023	0.007		0.012		0.001											0.001	**0.044**
Gomphonema						0.024					0.011									**0.034**
Gyrosigma												0.226						0.009	0.469	**0.704**
Melosira			0.015			0.134				0.235						0.010		0.647	0.579	**1.621**
Navicula	0.402	0.165	0.534	0.284	0.382	0.199	1.152	0.044	0.102	0.766	0.195	1.364	0.140	0.049	0.513	0.075	0.142	0.122	0.954	**7.582**
Nitzschia	0.044	0.018	0.059	0.005	0.038	0.047	0.015	0.050	0.008	0.130	0.071	0.004	0.005		0.013	0.032	0.002	0.024	0.234	**0.796**
Pleurosigma																	0.025			**0.025**
Pseudonitzschia								0.021												**0.021**
Reimeri			0.002																	**0.002**
Rhicosphenia										0.188										**0.188**
Skeletonema																			0.222	**0.222**
Surirella		0.216	0.150		0.096	0.160	0.750			0.325	0.040	0.010	0.018							**1.765**
Tabellaria			0.077																	**0.077**
Thalassiosira							0.384													**0.384**
Trachyneis	0.078					0.063		0.008								0.037		0.013	1.294	**1.492**
Trybloniella	0.037	0.087			0.025	0.047	0.174					0.021			0.138				0.276	**0.806**
Ulnaria		0.002	0.007			0.131	0.003			0.003	0.019	0.560								**0.724**
Diatom Subtotal	*1.122*	*0.599*	*1.033*	*0.450*	*1.526*	*1.200*	*2.971*	*0.141*	*0.219*	*2.164*	*0.467*	*2.400*	*0.213*	*0.049*	*0.834*	*0.214*	*0.185*	*0.864*	*4.998*	*21.646*

Canoe Passage — **Band Number: 1701-126XX**

Upper Intertidal — **Band Number: 2661-710XX**

Diet Item Category	37	40	41	42	43	45	48	55	58	59	61	66	71	74	25	26	27	30	31	Grand Total
Invertebrates																				
Arthropod	0.131	0.213	25.245	0.509	0.373	76.895	9.425		10.570	7.996	0.033	262.628	8.508		1.962	0.524	24.035	1.089	60.430	**490.567**
Invert (no ID)	1.039	0.964		5.181	0.945	0.344		0.010	2.562		0.128	3.393	0.106	1.367		22.233	0.480	0.050		**38.800**
Mollusk												68.635								**68.635**
Nematode		0.236																		**0.236**
Oligochaete							0.019		0.307				0.033		0.553	0.090		0.058	0.998	**2.057**
Polychaete		0.256	0.456		1.527	0.423	1.658			0.578	0.230	0.254	0.225				0.564	0.277	4.057	**10.504**
Invertebrate Subtotal	*1.170*	*1.669*	*25.701*	*5.690*	*2.844*	*77.663*	*11.101*	*0.010*	*13.438*	*8.574*	*0.390*	*334.910*	*8.872*	*1.367*	*2.515*	*22.847*	*25.078*	*1.474*	*65.485*	*610.799*
Sponges																				
Sponge														0.055						**0.055**
Grand Total	**2.291**	**2.268**	**26.734**	**6.140**	**4.370**	**78.863**	**14.072**	**0.151**	**13.658**	**10.737**	**0.857**	**337.310**	**9.085**	**1.471**	**3.349**	**23.060**	**25.264**	**2.338**	**70.483**	**632.500**

IR8-05 Marine Vegetation – Project Interactions

Information Source(s)

EIS Volume 2: Section 9.5, Figure 9.5-27, Appendix D of Appendix 9.5-A

EIS Volume 3: Appendix 10-B, Section 11, Table 11-10, Table 11-12

Context

Appendix D of Appendix 9.5-A provided an investigation of the potential for erosion of the tidal flats and formation of channels associated with widening the causeway, based on prior occurrence of such effects during construction of the Deltaport Third Berth Terminal.

Figure 9.5-27 in Section 9.5 of the EIS showed a prediction of the extent of temporary drainage channel formation during the construction phase associated with the causeway. Approximately 20% of the area within the zone delineated in Figure 9.5-27 was assumed to be occupied by channels and deposited sediment. The Proponent also stated that the channels adjacent to Deltaport Third Berth Terminal have persisted since their formation because waves and currents in that area are too small to redistribute sediments. Due to more energetic conditions, the Proponent anticipated that the channels on the north side of the widened causeway for Roberts Bank Terminal 2 were expected to infill once the area between the dyke and the causeway is filled and water no longer drains through the dyke. The Proponent proposed to mitigate potential effects through Project design measures.

Section 11 of the EIS did not explicitly discuss the effects from habitat loss on marine vegetation from the channels north of the expanded causeway. It is unclear whether the area of the proposed Project footprint in Table 11-12 of the EIS included the channels that were expected to be formed during the construction of the expanded causeway.

Information Request

Provide a description of how the channel formation from seepage north of the widened causeway was considered in the effects assessment for marine vegetation.

Confirm whether the channels were included in the Project footprint in Table 11-12 of the EIS. If the channels were not included, provide an assessment of the effects on marine vegetation should the channels persist throughout the life of the proposed Project.

VFPA Response

Provide a description of how the channel formation from seepage north of the widened causeway was considered in the effects assessment for marine vegetation.

The formation of small drainage channels from seepage north of the widened causeway during causeway dyke infilling was not considered in the effects assessment for marine vegetation, as potential changes resulting from seepage were assessed in the coastal geomorphology assessment as being both temporary and reversible (EIS Section 9.5.8.1).

The VFPA has committed to reducing potential adverse effects of temporary channel formation related to tidal waters drainage during causeway dyke construction through detail design (EIS Table 35-2).

Confirm whether the channels were included in the Project footprint in Table 11-12 of the EIS. If the channels were not included, provide an assessment of the effects on marine vegetation should the channels persist throughout the life of the proposed Project.

The area of potential drainage channel formation was not included in the estimate of habitat loss due to the Project footprint (provided in EIS Table 11-12), as the channels are not components of the terminal, causeway, or tug basin Project areas[1]. Although the channels were not included in the Project footprint, an assessment of the effects on marine vegetation is not provided as channels are not expected to persist throughout the life of the proposed Project. This is because, as outlined in EIS Section 9.5.8.1, energetic conditions on the north side of the Roberts Bank causeway will promote the redistribution of sediments and infilling of channels that potentially form (if unmitigated). Hence, it is expected that channel formation will be temporary and reversible (i.e., will not persist long term). In addition, the VFPA has committed to ensuring the study and management of potential channel formation that is predicted to occur while the causeway containment dykes are filled with sand, and will incorporate detail design changes as required (EIS Section 9.5.8.1). Therefore, potential channel formation will be mitigated through design (i.e., avoidance mitigation).

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[1] The Project area is outlined in a yellow boundary in EIS figures. Refer to Table 2-1 in the Project Construction Update (CEAR Document #1210) for the components included in the Project area (Updated EIS Table 4-1 in CEAR Document #1210).

IR8-06 Marine Vegetation – Physical Setting, Crest Protection Feature

Information Source(s)

EIS Volume 2: Section 9.5, Figure 9.5-34

EIS Volume 3: Section 11, Figure 11-2

Context

In Figure 9.5-34 of the EIS, the Proponent identified the existing crest protection structure near the existing tug basin. However, Figure 11-2 of the EIS did not indicate a habitat type as would be expected for marine vegetation over a hard substrate feature in that area, and the potential influence of the existing crest protection on marine vegetation is not further discussed in Section 11.

It is unclear whether the existing crest protection structure has been considered in the analysis of the potential effects of the proposed Project on marine vegetation**.**

Information Request

Explain whether the existing crest protection feature near the existing tug basin was considered as hard substrate in the lines of evidence for the description of existing conditions as well as the assessment of effects of the proposed Project on marine vegetation.

Discuss the potential implications on vegetation productivity estimates of including the existing crest protection structure as habitat for marine vegetation that prefer hard substrate (e.g. rockweed).

VFPA Response

Explain whether the existing crest protection feature near the existing tug basin was considered as hard substrate in the lines of evidence for the description of existing conditions as well as the assessment of effects of the proposed Project on marine vegetation.

The existing crest protection structure near the existing tug basin was considered as hard substrate in both empirical and Roberts Bank ecosystem model lines of evidence for the description of existing conditions as well as the assessment of effects of the proposed Project on marine vegetation.

For the empirical line of evidence, the area of the crest protection structure was used in calculations of existing macroalgae biomass. Further, the vegetation associated with the crest protection structure was considered in the effects assessment via the mechanism of direct mortality, where field data was used to empirically calculate the biomass and area lost due to the Project footprint (EIS Section 11.6.1, EIS Table 11-12). Specifically, EIS Table 11-12 shows that it is estimated that approximately 7 tonnes of rockweed biomass will be lost due to tug basin expansion, which includes crest protection removal. However, it is important to note that a replacement crest protection feature is proposed that is marginally larger than the existing one (approximately 5,880 m^2 versus 4,540 m^2), such that there will be a net increase (1,340 m^2) in hard substrate for macroalgal attachment; this increase in hard substrate was not factored into calculations because specific measurements of the new crest protection feature were not available at the time of EIS submission.

For the Roberts Bank ecosystem model line of evidence, the area of the existing crest protection feature was considered as hard substrate within the model. As outlined in EIS Appendix 10-C, each grid cell in the model (1 hectare (ha)) was assigned a percentage to reflect the proportion of hard substrate found within it; thus, the grid cell that includes the crest protection feature does account for its presence. For example, assuming the crest protection structure is contained entirely within one grid cell (as opposed to being split across two or three), if the size of the crest protection is 0.45 ha, and each grid cell is 1 ha, the cell would be classified as 45% hard substrate.

While the replacement crest protection feature will be slightly larger than the existing one, this was not included in the model because, as outlined above, specific measurements of the new crest protection feature were not available at the time of EIS submission. Therefore, the model used the same percentages of hard substrate in the cells associated with the tug basin for both existing and future conditions, such that no change in productivity associated with the crest protection was captured. Even if the measurements were available earlier, because both existing and proposed crest protection features exist on a sub-pixel spatial scale (i.e., less than 1 ha in size), the 'future with Project' scenario would not be able to account for the difference in productivity specifically associated with this structure alone. However, this is predicted to have a negligible effect on the productivity estimate for future conditions because of the small size difference between the two features (gain of 1,340 m^2). Qualitatively, it is anticipated that, with the new crest protection feature, there would be a slight increase in macroalgae productivity due to a slight increase in hard substrate area; therefore, the approach described in the EIS likely led to an underestimation of future macroalgae biomass and can thus be considered a conservative prediction of project effects on marine vegetation.

Discuss the potential implications on vegetation productivity estimates of including the existing crest protection structure as habitat for marine vegetation that prefer hard substrate (e.g. rockweed).

As outlined above, implications of including or excluding the crest protection feature in existing productivity calculations are negligible because the existing structure occupies a small area, and supports a minimal amount of productivity, relative to the overall amount of hard substrate habitat and associated macroalgal productivity in the local assessment area of Roberts Bank. Similarly, implications of including or excluding the replacement crest protection feature in future productivity calculations (i.e., in calculations of productivity change) are negligible because the net difference in hard substrate habitat associated with the structure under existing and future conditions is also small.

IR8-07 Marine Vegetation – Effects Assessment, Eelgrass

Information Source(s)

EIS Volume 3: Section 11.6.3.1

Context

In Section 11.6.3.1 of the EIS, the Proponent stated that a decrease of 12 tonnes of native eelgrass is the worst-case scenario as an effect from changes in sedimentation and coastal processes as a result of the proposed Project. The source of this value, however, was not provided in Section 11.

Information Request

Describe the method used to quantify the worst-case scenario of an effect to native eelgrass biomass due to changes in sedimentation and coastal processes.

VFPA Response

The method used to quantify the worst-case scenario of an effect to native eelgrass biomass (i.e., 12 tonne loss) due to changes in sedimentation and coastal processes involves spatially overlaying site-specific empirical eelgrass data with results from the coastal geomorphology study, and is reflective of the multiple lines of evidence approach used to assess Project effects (see the response to IR5-29 of CEAR Document #1185[1], specifically Appendix IR5-29-1, for more details). This worst-case approach assumes that the entire area affected by increased sedimentation (EIS Section 9.5.8.2, EIS Table 9.5-6, EIS Figure 9.5-35) will no longer support *any* native eelgrass productivity, which is highly unlikely (rationale provided in text below); thus, these calculations are considered conservative by reflecting the worst-case scenario.

The estimate is based on calculations using data from site-specific field surveys sampling native eelgrass productivity.

[1] CEAR Document #1185 From the Vancouver Fraser Port Authority to the Review Panel re: Responses to Information Requests IR5-28 and IR5-29 (See Reference Document #975).

The method used to quantify the worst-case scenario is outlined in the steps below:

1. During field surveys, measure eelgrass density (e.g., shoots per square metre)[2] empirically, and multiply by 10,000 to convert to hectares.
2. Estimate the average biomass (grams (g)) of an individual shoot of eelgrass using a combination of field data and scientific literature[3].
3. Calculate tonnes (t) per hectare by multiplying shoots per hectare (Step 1) by the average mass of an individual shoot (Step 2; grams (g)) and converting to tonnes (t).
4. Overlay the area of increased sedimentation (EIS Figure 9.5-35, Zone 4) on the Roberts Bank (marine vegetation local assessment area) habitat map (EIS Figure 11-2)[4] in GIS (geographic information system) software in order to quantify the area of each native eelgrass sub-polygon that overlaps with the area of sedimentation (Zone 4).
5. Multiply the productivity (tonnes per hectare) of native eelgrass by the area of the native eelgrass sub-polygon that overlaps with the area of sedimentation (Zone 4).
6. Multiply the tonnes of eelgrass within Zone 4 (calculated in Step 5) by the percent cover of native eelgrass within the overlapping area of sedimentation.
7. Sum all sub-polygon biomasses.

The following formula illustrates and summarises the method outlined above:

$$\frac{Eelgrass\ shoots}{m^2}\ x\ \frac{10{,}000\ m^2}{ha}\ x\ \frac{g}{Eelgrass\ shoot}\ x\ \frac{1\ tonne}{1{,}000{,}000\ g} = \frac{Eelgrass\ tonnes}{ha}$$

$$\frac{Eelgrass\ tonnes}{ha}\ x\ Subpolygon\ area\ of\ overlap\ (ha)\ x\ Eelgrass\ \%\ cover\ = Eelgrass\ tonnes$$

$$Sum\ Eelgrass\ biomass\ across\ all\ subpolygons = \ Total\ Eelgrass\ biomass\ \text{affected}$$

While increased sedimentation (EIS Figure 9.5-35, Zone 4) is expected from deposition of fine sediment that is currently carried in suspension (mainly silt), future sedimentation rates are expected to be low given that there is a limited annual supply (EIS Section 9.5.6.1; EIS Appendix 9.5-A, Section 4.2); this assumption is corroborated by 40 years of elevation data (from bathymetric surveys), which shows relatively little change in bed levels over that time period (see IR2-07 of CEAR Document #961[5]). Additionally, although the average wave height experienced in Zone 4 is expected to be smaller, the area will continue to be exposed to waves

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[2] During Roberts Bank field surveys, native eelgrass shoots in a 0.25 m^2 quadrat were counted, and extrapolated to one square metre by multiplying by 4. Field data also showed that one square metre of native eelgrass that had 100% ground cover had approximately 139 shoots of eelgrass, on average (Roberts Bank Terminal 2 Technical Data Report Marine Vegetation).

[3] A sub-sample of native eelgrass shoots was collected and weighed to estimate an average shoot weight. The shoot weight was verified by reviewing the scientific literature (e.g., Moody 1978, Harrison 1982, Beale et al. 2004).

[4] The Roberts Bank habitat map uses complex polygons to visually represent the distribution of various vegetation sub-components in the local assessment area. The habitat map polygons are comprised of many smaller polygons (i.e., sub-polygons) each with underlying data, including percent cover.

[5] CEAR Document #961 From the Vancouver Fraser Port Authority to the Review Panel re: Revised Responses to Information Request Package 2 (See Reference Documents #946 and #908).

from the northwest in a similar way as under Existing Conditions as well as experience regular tidal exchange, such that a large increase in fine sediment retention (which may lead to deposition) is not expected. Thus, for these reasons, the worst-case scenario is unlikely to manifest. See IR5-21 of CEAR Document #1140[6] for a similar discussion related to Zone 7, where increased turbidity is predicted to occur.

This method was used because it is not possible to reliably predict future sedimentation rates associated with fine suspended sediment and any associated potential productivity losses with existing modelling tools, including via the Roberts Bank ecosystem model. The coastal geomorphology hydrodynamic model (EIS Appendix 9.5-A) estimated sedimentation due to bedload sediment (e.g., coarser silt and sand) and not sedimentation due to suspended sediment (e.g., fine silts and clays). It is generally accepted within the engineering community that predictions of bed level changes arising from suspended sediment transport are not reliable. Therefore, empirical calculations in conjunction with scientific literature, experience from past environmental assessments (e.g., Deltaport Third Berth), and other models (e.g., the coastal geomorphology hydrodynamic model and its predicted change in tidal current velocity) were used. This is reflective of the multiple lines of evidence approach used to assess Project-related effects on marine vegetation.

References

Beal, B. F., R. L. Vadas Sr., W. A. Wright, S. Nickl, and N. W. Lermond. 2004. Annual Aboveground Biomass and Productivity Estimates for Intertidal Eelgrass (*Zostera marina L.*) in Cobscook Bay, Maine. Northwestern Naturalist 11:197–224.

Harrison, P. G. 1982. Spatial and Temporal Patterns in Abundance of Two Intertidal Seagrasses, *Zostera americana* Den Hartog and *Zostera marina* L. Aquatic Botany 12:305–320.

Moody, R. 1978. Habitat, Population and Leaf Characteristics of *Zostera marina L.* on Roberts Bank, British Columbia. Master's Thesis, University of British Columbia.

[6] CEAR Document #1140 From the Vancouver Fraser Port Authority to the Review Panel re: Responses to Information Requests IR5-03, IR5-21, IR6-06, IR6-07, IR6-17, and IR6-36 (See Reference Documents #975 and #991).

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IR8-08 Marine Vegetation – Effects Assessment

Information Source(s)

EIS Volume 2: Section 8

EIS Volume 3: Section 11, Table 11-23, Section 17

CEAR Doc#547

Context

In Section 8 of the EIS, the Proponent described the effects assessment methodology based on the systematic identification of effects as adverse, significant, and likely; coupled with the identification of mitigation measures. However, the presentation of information in Section 11 did not clearly correspond to a systematic assessment of effects as outlined in Section 8.

The Proponent's analysis of effects for the marine vegetation sub-components in Section 11 was presented in various ways. The information was organized by:

- effects conclusion (negligible vs. predicted);
- pathway of effect (direct loss and mortality, changes in water quality, changes to sedimentation and coastal processes, and biotic interactions);
- effect type (productivity vs. biofilm assemblage); and
- sub-component (eelgrass, intertidal marsh, macroalgae, biomat, and biofilm).

Further, mitigation measures proposed in Section 11 and 17 of the EIS were not explicitly associated with the potential effects on marine vegetation to which they could apply. For example, Table 11-23 of the EIS referenced several plans, but did not state the specific measures that were intended to mitigate adverse effects, nor did it explain how the measures would be commensurate with predicted effects.

In addition, the Proponent stated that the Roberts Bank Ecosystem Model (EwE model) was intended to provide one line of evidence to assess the potential effects of the proposed Project on the Roberts Bank ecosystem, and that this would then be used with other lines of evidence to make the final assessment (CEAR Doc#547). Although Section 11 provided an assessment based on multiple lines of evidence for biofilm, it is unclear how the effects assessment uses lines of evidence other than ecosystem modelling for other sub-components.

The Proponent also stated that the objective of the EwE model was not to provide an assessment of Project effects for each functional group at a fine temporal scale, but to estimate changes in productive potential, with and without the Project, at the ecosystem level (CEAR Doc#547). However, ecosystem modelling results were used in the effects assessment for marine vegetation by functional group. In the case of intertidal marsh, adverse effects are predicted due to direct habitat loss and changes in biotic interactions, but specific mitigation measures and residual effects were not described further based on predicted gains in productivity. It is unclear how adverse effects from the Project occurring from the start of construction, and during the life of the Project, would be mitigated by considering predicted gains in productivity for future conditions.

Information Request

Provide, in tabular format, the effects assessment for each marine vegetation sub-component. For each sub-component, organize the information systematically to demonstrate:

- the potential adverse effects associated with each pathway of effects;
- the specific mitigation measures comprised in environmental management plans, Project design, and offsetting plans directly applicable to each potential adverse effect;
- the description of residual effects in terms of their magnitude, geographic extent, frequency, duration and reversibility and social and ecological context, as applicable;
- the rationale for whether the residual effects are considered significant; and,
- the rationale for whether the significant effects are considered likely.

In the table, identify the source for the information presented such as the EIS section number, information request response number, CEAR document number or other references. Clearly indicate where information is provided that is not currently on the registry.

In the table, provide lines of evidence other than ecosystem modelling (e.g. literature, empirical studies, professional judgement, Indigenous knowledge) in cases where the conclusions relied exclusively on ecosystem modelling. Describe how the different lines of evidence were utilized in the rationale to reach the effects assessment conclusions.

In the table, changes in productivity predicted through ecosystem modelling should not be used as the sole basis for conclusions regarding effects assessment. For example, a predicted gain in productivity through ecosystem modelling would not justify that mitigation measures are not required. Changes in productivity predicted through ecosystem modelling may however serve to inform the determination of significance for residual effects and their likelihood, where applicable.

VFPA Response

This response provides full technical information and table as requested by re-organising information presented in the EIS (Table IR8-08-A1 of **Appendix IR8-08-A**); no new information is presented. However, as recognised in the context to this information request, information for biofilm has not been repeated here as information on the multiple lines of evidence approach was already presented in the EIS.

Clarification

The VFPA would like to clarify that, contrary to the context to this information request, the presentation of information in EIS Section 11.0 does correspond to the systematic assessment of effects outlined in EIS Section 8.0, as shown in **Table IR8-08-1** below.

Table IR8-08-1 Systematic Assessment Methodology used in EIS Section 11.0

EIS Section 8.0 Methodology	Corresponding EIS Section 11.0 Methodology
Establishment of Assessment Boundaries (8.1.3)	Assessment Boundaries (11.3)
Description of Existing Conditions (8.1.4)	Existing Conditions (11.5), organised by sub-component
Identification of Interactions and Project Effects (8.1.5)	Future Conditions with the Project (11.6)[a]
Mitigation measures (8.1.6)	Mitigation Measures (11.7)
Characterisation of Residual Effects and Context (8.1.7)	Characterisation of Residual Effects and Context (11.8)
Determination of Significance of Residual Adverse Effects (8.1.8)	Determination of Significance of Residual Adverse Effects (11.9)
Cumulative Effects Assessment (8.1.9)	Cumulative Effects Assessment (11.10)
Monitoring and Follow-up Programs (8.1.10)	Monitoring and Follow-up Programs (11.12)

Notes: a. As is the case for all valued component (VC) chapters, EIS Section 11.6 starts with identifying Project-VC interactions (presented in EIS Tables 11-10 and 11-11); mechanisms affecting productivity are identified in EIS Section 11.6.1; negligible effects are discussed in EIS Section 11.6.2, organised by mechanism and for each sub-component as relevant; potential effects are assessed in EIS Sections 11.6.3 (productivity) and 11.6.4 (assemblage composition), and organised by sub-component.

The VFPA would also like to clarify that the Roberts Bank ecosystem model (RB model) provides useful indications of the relative impacts on each functional group, using biomass ratios as the primary indicator of both direct and indirect impacts. These model outputs can be aggregated to higher levels of resolution (e.g., at the ecosystem level; see response to IR3-24 of CEAR Document #984[1]), but the outputs are also valid at the resolution of functional groups, particularly given the extensive sensitivity analyses completed in the EIS and in subsequent submissions to the Panel (e.g., CEAR Document #547[2], question 2.7; responses to IR3-09, IR3-23 of CEAR Document #984).

Additionally, the context to this information request states that it is unclear how the effects assessment uses lines of evidence other than ecosystem modelling for sub-components other than for biofilm. The VFPA would like to clarify that a multiple lines of evidence approach was used for all sub-components, as explained throughout the response below.

For increased clarity, a discussion, by marine vegetation sub-component, is provided below on how the multiple lines of evidence for each marine vegetation sub-component were used

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[1] CEAR Document #984 From the Vancouver Fraser Port Authority to the Review Panel re: Responses to Information Request Package 3 (See Reference Document # 928).

[2] CEAR Document #547 From the Vancouver Fraser Port Authority to the Review Panel re: Answers to preliminary technical questions submitted during the completeness phase from Fisheries and Oceans Canada, Natural Resources Canada, and Environment and Climate Change Canada, concerning the ecosystem modelling to support the Roberts Bank Terminal 2 Project environmental review (NOTE: Updated September 28th, 2016).

to inform the productivity change conclusion. See IR5-29 of CEAR Document #1185[3] for a complete discussion on how multiple lines of evidence were incorporated into marine VC effects assessments.

Mechanisms (i.e., effects pathways) that have the potential to affect the productivity of marine vegetation are described in EIS Section 11.6.1 and Project-environment interactions that were not expected to contribute to productivity change (i.e., negligible effect) are described in EIS Section 11.6.2.

Assessment of Native Eelgrass Productivity Change

1. Model Evidence

Roberts Bank Ecosystem Model Key Run

For native eelgrass, the key run output predicted that, under future conditions with the Project, productivity would increase by 11 tonnes (t) or 4% relative to 'Future without the Project' (EIS Appendix 10-C). Independent of any other lines of evidence, this result is considered 'negligible' as per the common classification system categories of negligible, minor, moderate, and high[4].

Roberts Bank Ecosystem Model Sensitivity Analyses

Sensitivity analyses were conducted to evaluate the robustness of key run values to uncertainty in input variables and indicate whether the productivity predictions for each sub-component may be over- or underestimated (EIS Appendix 10-D). In particular, to test for sensitivity in input assumptions, a Monte Carlo approach was used (refer to the *Preamble in Support of Responses to IR3-01 to IR3-24* (CEAR Document #984) for more details); this analysis yielded the exact same result as the key run: a 4% increase in native eelgrass productivity.

Further, visual validation and goodness of fit metrics—including percent correct classification (PCC), sensitivity and specificity evaluations, and Cohen's Kappa—provide additional confidence of model predictions[5]. As discussed in IR3-21 (CEAR Document #984), accuracy of the RB model prediction for native eelgrass was high, with a PCC of 90% and showed substantial agreement based on the Kappa statistic (0.68). The model performed well in forecasting both native eelgrass presence (sensitivity = 86%) and absence (specificity = 91%). The high degree of agreement between the model and the habitat map likely reflect

[3] CEAR Document #1185 From the Vancouver Fraser Port Authority to the Review Panel re: Responses to Information Requests IR5-28 and IR5-29 (See Reference Document #975).

[4] In CEAR Document #314 From Port Metro Vancouver to the Canadian Environmental Assessment Agency re: Completeness Review - Responses to Additional Information Requirements (See reference document # 271) for the Environmental Impact Statement, productivity change was characterised according to the following categories: Negligible = 0% to 5% change; Minor = 6% to 30% change; Moderate = 31% to 60% change; High = 61% to 100% change.

[5] See CEAR Documents #314 and #547 for a description of PCC, sensitivity, and specificity validation methods; see IR3-21 (CEAR Document #984) for a description of Cohen's Kappa methods.

the within-year stability of eelgrass distribution resulting in a close alignment of potential productivity and species distribution.

Overall, results from the RB model, sensitivity analyses, and validation exercises together indicate that the direction of change for native eelgrass is likely positive, but that increases are small relative to existing productivity such that they will be difficult to measure (i.e., 'negligible').

2. Empirical Estimates of Changes in Productivity

Direct Mortality: Native eelgrass distribution partially overlaps with the footprints of Project components; as a result, some direct mortality cannot be avoided and is expected to lead to corresponding decreases in productivity. Productivity loss from terminal and causeway footprints was accounted for in the RB model; however, the net positive result reflects indirect gains in productivity from geomorphic changes due to Project placement, which improved native eelgrass growing conditions.

Empirical calculations (see EIS Section 11.6.3.1) exclude indirect gains and suggest that native eelgrass biomass can be expected to decrease by approximately 3 t, or 1%, due to the terminal and causeway footprints. Additionally, mortality associated with tug basin expansion was not factored into the RB model; empirical calculations for this area indicate an additional 2 t may be lost, bringing the total value of footprint loss (i.e., terminal, causeway, and tug basin) to 5 t.

Sedimentation: Native eelgrass comprises 17 hectares (ha) of the 40 ha predicted to experience increased deposition (see EIS Section 11.6.3.1). Long-term accumulation of fine-grained sediments is anticipated to increase the elevation of this area which, in a worst-case scenario, was conservatively assumed to make the area unsuitable for native eelgrass. The anticipated effect is a decrease of 12 t of native eelgrass that currently exists in this area (worst-case scenario; see response to IR8-07 of CEAR Document #1193[6] for more details relating to this prediction).

Wave Shadow: Native eelgrass exists in approximately 4 ha (representing a biomass of 2 t) of the 70 ha area where wave energy is also expected to decrease (EIS Section 9.5). Wave energy decreases may increase native eelgrass productivity in this area as wave energy has been shown to be a limiting factor in the distribution of native eelgrass (Stevens and Lacy 2011). It is estimated that native eelgrass productivity could possibly increase in this area by 4 t to 8 t.

Summing the empirical calculations outlined above (i.e., -5 t - 12 t + 6 t[7]), an 11 t (4%) decrease in productivity can conservatively be estimated. This falls within the negligible

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[6] CEAR Document #1193 From the Vancouver Fraser Port Authority to the Review Panel re: Response to Information Requests IR8-06 and IR8-07 (See Reference Document #1071).
[7] 6 t was used as the average of the empirical range of 4-8 t presented in the 'wave shadow' section above.

change range of 0% to 5% and, if merged with model evidence (which accounts for direct/footprint loss as well as indirect effects), no change is indicated (pre-mitigation).

3. Literature Evidence

As described in IR5-29 (CEAR Document #1185), both scientific literature and government or industry reports were used as a line of evidence in the assessment to inform and/or support predictions for all marine biophysical VCs, including marine vegetation. Literature was incorporated into both the existing conditions section as well as predictions of future conditions with the Project, specifically to support the characterisation of potential effects. For example, as outlined in Step 2 above, empirical calculations indicated that, over time, sedimentation may lead to a 12 t loss of native eelgrass productivity. This was corroborated by literature, which suggested that increased sedimentation may potentially lead to mortality due to smothering (Short and Wyllie-Echeverria 1996, Mills and Fonseca 2003). When sedimentation is more extreme, it has been documented that burial of 25% of the leaf length can cause up to 50% mortality of native eelgrass shoots (Mills and Fonseca 2003).

Native eelgrass, however, is also capable of rapidly growing longer internodes to raise leaves above sediment in areas where above-normal sedimentation occurs (Hemminga and Duarte 2000, Cabaço and Santos 2007). As outlined in EIS Section 9.5, it is not possible to accurately predict future rates of deposition of the fine sediment fraction that is carried in suspension, but Zone 4 on the north side of the causeway is expected to experience a slight increase in sedimentation rates with the Project, above the existing slow rates of accumulation. Because sedimentation rates are expected to be slight, literature suggests that eelgrass will be able to cope with such changes; thus the literature evidence supports a negligible ranking for change in native eelgrass productivity.

4. Evidence from Previous Environmental Assessments and other IC/VC Conclusions

Another line of evidence that contributed to the evaluation of native eelgrass productivity change included information gathered and lessons learned from previous environmental assessments at Roberts Bank, specifically the Deltaport Third Berth Project (DP3). The ability to draw from past project experience provides site-specific evidence as to how the Roberts Bank ecosystem responds to development and, in some cases, establishes a robust time-series that strengthens predictions.

For instance, the characterisation of effects to native eelgrass as ‘negligible’ is also supported by an understanding of effects of nearby projects and scientific literature (e.g., Stevens and Lacy 2011). The area colonised by native eelgrass increased dramatically between 1967 and 2003 in the inter-causeway area (Durance 2004), which may have been due to a number of factors, including changes in tidal flow, deflection of the silty Fraser River plume (increasing light availability) due to the construction of the Deltaport Terminal, and the BC Ferries Terminal causeway protecting the eelgrass bed from southeasterly winter storms. Similarly, the native eelgrass bed area north of the Roberts Bank causeway has increased over 15-fold since 1994 (Tarbotton and Harrison 1996, Triton 2004), possibly due to a wave shadow created by the Deltaport Terminal. To date, it appears that development at Roberts Bank has had a positive influence on native eelgrass (EIS Section 11.5.1.5).

Additionally, as described in IR5-29 (CEAR Document #1185), the assessment of potential effects of the Project on VCs considered data generated and conclusions drawn within a particular intermediate component (IC) or VC section to inform subsequent VC sections, as appropriate. The marine vegetation assessment relied on data from the coastal geomorphology and marine water quality assessments, as described below.

Conclusions of the coastal geomorphology assessment were incorporated into the native eelgrass effects assessment as a mechanism of potential productivity changes. In addition to potential effects on productivity as a result of sedimentation, described in Step 2 above, during construction and operation phases of the proposed Project, changes to current velocity are predicted to occur (EIS Section 9.5.8.2). Marine sediments that are highly mobile due to tidal currents, wave exposure, or sediment composition can cause successive burial and erosion as well eelgrass mortality (Hemminga and Duarte 2000). Current velocity of 50 centimetres per second (cm/s) inhibits production, although 120 cm/s to 180 cm/s is the upper tolerance range (Fonseca and Kenworthy 1987, Fonseca and Bell 1998, Koch 2001). Existing current velocities over Roberts Bank tidal flat are generally below 50 cm/s (EIS Section 9.5.6). Further, areas of increased current velocity (EIS Section 9.5.8.2) are not expected to spatially overlap with existing native eelgrass biomass and therefore will not contribute to productivity changes.

Conclusions of the marine water quality assessment were also incorporated into the native eelgrass assessment as a pathway of potential productivity change (see EIS Sections 11.6.2.1 and 11.6.3.1). Dispersion modelling results were used to show that, on the north side of the causeway, spatial overlap of the total suspended solids (TSS) plume within the intertidal and shallow subtidal (0 m chart datum (CD) to -10 m CD) zone occupied by eelgrass will be minimal, and that the levels predicted are within the range of TSS levels that eelgrass currently experiences. In the inter-causeway area, TSS generated by dredging activities at the tug basin are expected to cause an increase in sedimentation in the lower intertidal and the shallow subtidal zones, in areas where native eelgrass currently exists. Sediment re-suspension due to dredging at the tug basin was modelled (EIS Section 9.6.8), and TSS concentrations greater than 5 milligrams per litre (mg/L) are predicted to occur less than 30% of the time in the low intertidal zone. Overall, dredging the tug basin is anticipated to have a minor negative influence on the productivity of eelgrass during the construction phase, but does not alter the category ranking for eelgrass productivity change due to the temporary and localised nature of the disturbance.

5. Proposed Mitigation

Because Project-environment interactions for native eelgrass are expected to be negligible over the long-term, specific mitigation measures for this sub-component are not proposed. However, native eelgrass will benefit from some of the generic mitigation measures described below and in EIS Sections 17.0 and 33.0.

Consistent with standard practice in environmental assessment, specific mitigation plans will be developed during the detailed design and permitting processes, in consultation with relevant regulators and with input by Indigenous groups. Specific measures will be determined at that time. General measures are described below.

Avoidance

- The placement of the terminal in the subtidal zone will avoid and reduce direct mortality to native eelgrass.

Reduction

- Implementation of the Construction Environmental Management Plan (CEMP) will help mitigate against construction-related mechanisms of productivity change. For example, erosion and run-off controls (e.g., silt fences) may be utilised during land-based construction and operation activities to minimise the likelihood of increased TSS or sedimentation in the nearshore environment at Roberts Bank.

Offsetting

- Proposed onsite offsetting includes 3 ha of native eelgrass, not to mitigate effects to eelgrass itself because the potential effect is categorised as negligible, but to mitigate effects to other marine biophysical VCs, including Dungeness crabs, bivalve shellfish, and Pacific salmon. There is precedent for conducting eelgrass transplants at Roberts Bank and there is high confidence in the success and effectiveness of this measure (the VFPA's forthcoming response to IR7-29 will provide more detail).

Summary of Potential Project-related Effects to Native Eelgrass Productivity

The RB model predicted an 11 t (4%) increase in native eelgrass productivity and results of sensitivity analyses confirmed this estimate was robust to uncertainty in input parameters. Empirical calculations of direct mortality due to the terminal and causeway footprint (-3 t), tug basin (-2 t), sedimentation (-12 t), and wave shadow (+6 t) effects sums to an 11 t (4%) decrease. Both modelling and empirical estimates individually fall within the negligible change range of 0% to 5% of the existing biomass and, if merged, indicate zero change (without mitigation). The overall productivity of eelgrass within the local assessment area (LAA) is not expected to be compromised over the long-term, given that (i) direct biomass loss represents a small proportion of overall native eelgrass biomass (1%); (ii) habitat suitability and growing conditions are expected to improve as a result of Project placement; (iii) general mitigation measures, though not proposed to mitigate adverse effects to eelgrass, will nonetheless benefit eelgrass; in particular, Project placement in the subtidal zone is considered to be effective in minimising overlap with the native eelgrass bed; and (iv) onsite eelgrass transplants are planned to mitigate potential effects for other VCs, such as marine invertebrates, marine fish, and coastal birds, but will also benefit eelgrass in the LAA and further counter construction-associated mortality.

Considering this evidence in light of information from past environmental assessments and IC studies, such as the fact that development at Roberts Bank has facilitated eelgrass increase and expansion over time, the potential effect of the proposed Project on native eelgrass productivity is anticipated to be, overall, negligible even without mitigation. Therefore, no specific mitigation is warranted for native eelgrass, though it will benefit from general mitigation measures proposed for other reasons, and no measurable residual adverse effects on native eelgrass are predicted.

Assessment of Non-Native Eelgrass Productivity Change

1. Model Evidence

Roberts Bank Ecosystem Model Key Run

The key run forecasted that, under future conditions with the Project, non-native eelgrass production will remain essentially unchanged, with a 0.05 t (1%) decrease in productivity relative to 'Future without the Project' conditions (EIS Appendix 10-C). Independent of any other lines of evidence, this result is considered 'negligible' as per the common classification system categories of negligible, minor, moderate, and high.

Roberts Bank Ecosystem Model Sensitivity Analyses

Sensitivity analyses showed that the key run estimate was robust to uncertainty in input parameters (EIS Appendix 10-D). The Monte Carlo analysis yielded the exact same result as the key run: a 1% decrease in non-native eelgrass productivity.

Further, as discussed in IR3-21 (CEAR Document #984), accuracy of the RB model prediction for non-native eelgrass was moderate. Overall, PCC was 80% and the Kappa value indicated moderate agreement (0.42). The model performed slightly better at forecasting presence of non-native eelgrass (sensitivity = 88%) than forecasting its absence (specificity = 79%).

Overall, results from the RB model, sensitivity analyses, and goodness of fit validation exercises together indicate that the direction of change for non-native eelgrass is likely slightly negative, but that decreases are so small relative to existing productivity such that they will be difficult to measure (i.e., 'negligible').

2. Empirical Estimates of Changes in Productivity

Direct Mortality: Direct mortality due to the causeway footprint was empirically estimated to cause a 0.3 t (<2%) decrease in non-native eelgrass productivity. This aligns well with results from model evidence (see above) and supports a negligible productivity change category rating.

Sedimentation: As outlined in EIS Section 11.5.1.1, in the Fraser river estuary, non-native eelgrass occurs at higher elevations (i.e., between +2 m CD and +3 m CD) and in relatively muddier areas compared to native eelgrass. Thus, an increase in non-native eelgrass productivity is anticipated to occur over the long-term due to predicted increases in tidal flat elevation and the accumulation of finer sediments as a result of Project placement; however, the productivity increase is difficult to quantify.

Wave Shadow: The predicted 70 ha wave shadow is not expected to influence the productivity of non-native eelgrass. This is because wave energy does not appear to limit non-native eelgrass distribution; in fact, relative to native eelgrass, *Zostera japonica* is more breakage-resistant and structurally flexible (Shafer et al. 2007) and thus capable of withstanding areas of greater wave energy (Fong 1998).

Overall, empirical evidence suggests a less than 2% decrease in non-native eelgrass productivity, based on direct footprint loss. This falls within the negligible change range of 0% to 5%.

3. Literature Evidence

In the case of non-native eelgrass, literature was used to characterise existing conditions as well as contextualise predicted effects. For example, Harrison and Bigley (1982) report that non-native eelgrass is primarily an annual species that recruits from seed each year, enabling it to be opportunistic and re-colonise quickly after disturbance. Additionally, literature suggests that salinity is generally not a strong controlling factor on the distribution and productivity of non-native eelgrass in North America (Kaldy 2006, Shafer 2007, Shafer et al. 2011), such that predicted Project-related salinity changes are not anticipated to affect non-native eelgrass during the freshet period. This evidence provides further support for a negligible rating for productivity change.

4. Evidence from Previous Environmental Assessments and other IC/VC Conclusions

As discussed above, the marine vegetation assessment relied on data from the coastal geomorphology and marine water quality assessments.

Conclusions of the coastal geomorphology assessment were incorporated into the non-native eelgrass effects assessment as a mechanism of potential productivity changes. For example, during construction and operation, increased sedimentation due to increased turbidity, a back-eddy, and lower current velocities is predicted to occur over an area of 40 ha on the north side of where the proposed terminal and existing terminal will meet (see EIS Section 9.5.8). These predicted IC effects will contribute to improved growing conditions for non-native eelgrass, which prefers quiescent areas of higher elevation and finer sediments, such that non-native eelgrass is expected to increase over the long-term.

Conclusions of the marine water quality assessment were also incorporated into the non-native eelgrass assessment as a pathway of potential productivity change (see EIS Sections 11.6.2.1 and 11.6.3.1). Dispersion modelling results showed that while Project-generated TSS plumes are predicted to overlap with areas of non-native eelgrass, predicted concentrations are within the range that non-native eelgrass currently experiences[8]; further,

[8] In the EIS, a scenario of 85% retention of fill material during land development was used to assess effects of total suspended solids on marine VCs. However, as outlined in Sections 3.1.5 and 3.1.6 in the Project Construction Update (PCU, CEAR Document #1210), the amount of fine material discharged is

interaction will only take place during high tides, and daytime low tides will allow enough light for photosynthesis (EIS Section 11.6.2.1). Therefore, increased turbidity levels due to terminal placement are not expected to affect non-native eelgrass, and the productivity change category rating remains negligible.

Additionally, TSS generated by dredging activities at the tug basin are expected to cause an increase in sedimentation in the lower intertidal and the shallow subtidal zones of the inter-causeway—this is not expected to spatially overlap with non-native eelgrass distribution.

5. Proposed Mitigation

Because Project-environment interactions for non-native eelgrass are predicted to be negligible over the long-term, specific mitigation measures for this sub-component are not proposed. However, non-native eelgrass will benefit from some of the generic mitigation measures described below and in EIS Sections 17.0 and 33.0.

Avoidance

- The placement of the terminal in the subtidal zone will avoid and reduce direct mortality to non-native eelgrass.

Reduction

- Implementation of the CEMP will help mitigate against construction-related mechanisms of productivity change. For example, erosion and run-off controls (e.g., silt fences) may be utilised during land-based construction and operation activities to minimise the likelihood of increased TSS or sedimentation in the nearshore environment at Roberts Bank.

Summary of Potential Project-related Effects to Non-native Eelgrass Productivity

Ecosystem modelling, accounting for both direct and indirect effects, determined that non-native eelgrass biomass would decrease by 0.05 t due to the Project. This is in alignment with the small proportion (<0.5 t, 2%) that will experience direct mortality due to the Project footprint. Changes in coastal geomorphic processes (i.e., sedimentation) is expected to improve environmental conditions and positively influence non-native eelgrass productivity, though quantification is not possible. Empirical and literature evidence suggest that such longer-term gains may at least partially, if not fully, offset short-term construction phase losses (i.e., direct mortality), as indicated by the RB model. Overall, predicted productivity changes in non-native eelgrass are slight and unlikely to be measurable against natural variation, even without mitigation. Therefore, no specific mitigation is warranted for non-native eelgrass, though it will benefit from general mitigation measures proposed for other reasons, and no measurable residual adverse effects on non-native eelgrass are predicted.

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now assumed to be 3% from all sources (i.e., a 97% retention rate). Refer to the PCU (CEAR Document #1210) for more details.

Assessment of Intertidal Marsh Productivity Change

1. Model Evidence

Roberts Bank Ecosystem Model Key Run

The key run forecasted that intertidal marsh production will increase by 335 t (25%) under 'Future with the Project' conditions relative to 'Future without the Project' (EIS Appendix 10-C). Independent of any other lines of evidence, this result is considered 'minor' (i.e., minor increase) as per the common classification system categories of negligible, minor, moderate, and high.

The key abiotic factor driving intertidal marsh productivity was salinity, due to the brackish and salt marsh communities that occur within the LAA. The increase in productivity is considered to be due to improved growing conditions due to changes in salinity and not due to an increase in areal distribution (refer to EIS Appendix 10-D). Areas that will experience lower salinity year-round may shift from a less productive salt marsh plant community to a more productive brackish marsh community.

Roberts Bank Ecosystem Model Sensitivity Analyses

Sensitivity analyses showed that the key run estimate was robust to uncertainty in input parameters (EIS Appendix 10-D). The Monte Carlo analysis yielded the exact same result as the key run: a 25% increase in intertidal marsh productivity.

Further, as discussed in IR3-21 (CEAR Document #984), validation exercises indicated that the RB model forecasted the distribution of tidal marsh with an adequate level of accuracy. PCC was 91% and Kappa agreement was fair (0.3). The model performs moderately well in forecasting intertidal marsh presence (sensitivity = 63%) while it performs very well at forecasting absences (specificity = 92%).

Overall, results from the RB model, sensitivity analyses, and goodness of fit validation exercises together indicate that the direction of change for intertidal marsh is likely positive, and that increases are substantial enough to be considered minor.

2. Empirical Estimates of Changes in Productivity

Direct Mortality: Intertidal marsh distribution partially overlaps with the footprint of the widened causeway; as a result, some direct mortality cannot be avoided and is expected to lead to corresponding decreases in productivity. Productivity loss from the causeway footprint was accounted for in the RB model; however, the net positive result reflects indirect gains in productivity from geomorphic changes due to Project placement, which improved intertidal marsh growing conditions. Empirical calculations exclude indirect gains and suggest that intertidal marsh biomass can be expected to decrease by approximately 1 t (<0.1%).

3. Literature Evidence

Literature was incorporated into both the existing conditions section as well as predictions of future conditions with the Project, specifically to support the characterisation of potential effects. For example, as outlined in Step 1 above, the RB model predicted an increase in intertidal marsh productivity largely driven by changes in salinity. Literature corroborates this prediction, as lower salinities generally increase productivity of intertidal marsh plants (Crain et al. 2004, Woo and Takekawa 2012).

4. Evidence from Previous Environmental Assessments and other IC/VC Conclusions

Another line of evidence that contributed to the evaluation of intertidal marsh productivity change included information gathered and lessons learned from previous anthropogenic development and environmental work at Roberts Bank. For instance, the characterisation of effects to intertidal marsh as a 'minor increase' is believed to be a reasonable assumption based on historical trends. As outlined in EIS Section 11.5.2 and Appendix AIR13-A of CEAR Document #388[9], from approximately 1880 until present, small- to large-scale human activities in the Fraser River estuary have altered natural sedimentation rates (Hales 2000). In particular, installation of river-training structures and sea dykes has led to increases in marsh area over time at Roberts Bank; specifically, Hales (2000) estimated a 123% increase in intertidal marsh area at Brunswick Point from 1930 to 1994.

Conclusions of the coastal geomorphology assessment were incorporated into the intertidal marsh effects assessment as a mechanism of potential productivity changes. For example, results of the coastal geomorphology study were used to support the prediction that Project-related changes in sediment deposition will also contribute to increased intertidal marsh productivity (see EIS Section 11.6.3.2). Intertidal marsh habitat in the LAA is characterised by emergent vascular plants in the elevation range of +3.2 m CD to +4.8 m CD, which is regularly covered during high tides (Mason and Booth 2004, G. L. Williams and NHC 2009). As outlined for native eelgrass above, as well as in the response to IR8-07 (CEAR Document #1193), increased sedimentation is expected from deposition of fine sediment that is currently carried in suspension (mainly silt; EIS Figure 9.5-35, Zone 4). Over time, it was assumed that increased sedimentation could lead to increased bed elevation that, in turn, could provide habitat for intertidal marsh (similar to the existing causeway shoreline, which supports fringing intertidal marsh). However, as outlined in the response to IR8-07 (CEAR Document #1193), this scenario should be interpreted with caution because (i) there is a limited annual supply of fine sediment; (ii) 40 years of elevation data (from bathymetric surveys) show relatively little change in bed levels over that time; and (iii) it is not possible to reliably predict future sedimentation rates associated with fine suspended sediment. However, changes to sediment deposition from Project placement are anticipated to have a positive (but unquantifiable) effect on intertidal marsh productivity over the long term.

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[9] CEAR Document #388 From Port Metro Vancouver to the Canadian Environmental Assessment Agency re: Completeness Review - Responses to Additional Information Requirements Follow-Up (See Reference Document # 345) including 22 Technical Data Reports.

Conclusions of the marine water quality assessment were also incorporated into the intertidal marsh assessment as a pathway of potential productivity change (see EIS Sections 11.6.2.1 and 11.6.3.1). Dispersion modelling results showed that Project-generated TSS plumes will not encroach into mid to high intertidal elevations occupied by intertidal marsh; thus, elevated TSS levels from Project construction activities are predicted to have a negligible influence on intertidal marsh productivity.

5. Proposed Mitigation

Because Project-environment interactions for intertidal marsh are predicted to be positive over the long-term, specific mitigation measures for this sub-component are not proposed. However, intertidal marsh will benefit from some of the generic mitigation measures described below and in EIS Sections 17.0 and 33.0.

Avoidance

- The placement of the terminal in the subtidal zone will avoid and reduce direct mortality to intertidal marsh.

Reduction

- Implementation of the CEMP will help mitigate against construction-related mechanisms of productivity change. For example, erosion and run-off controls (e.g., silt fences) may be utilised during land-based construction and operation activities to minimise the likelihood of increased TSS or sedimentation in the nearshore environment at Roberts Bank.

Offsetting

- Proposed onsite offsetting includes 15 ha of intertidal marsh, not to mitigate effects to marsh itself because the potential effect is categorised as net positive, but to mitigate effects to other marine biophysical VCs, including bivalve shellfish and Pacific salmon.

Summary of Potential Project-related Effects to Intertidal Marsh Productivity

The RB model predicted a 335 t (25%) increase in intertidal marsh productivity and results of sensitivity analyses confirmed this estimate was robust to uncertainty in input parameters. Adjusting this estimate to include empirical calculations of direct mortality (1 t; <0.1%) has negligible influence. This evidence in light of information from past environmental assessments and IC studies, such as substantial marsh expansion over time due to river training structures and sea dykes, and positive effects of salinity and sedimentation, points towards a minor increase in intertidal marsh productivity with the Project. As such, no specific mitigation is proposed for intertidal marsh and no residual adverse effects are predicted.

Assessment of* Macroalgae *(Ulva) Productivity Change

1. Model Evidence

Roberts Bank Ecosystem Model Key Run

The key run forecasted that *Ulva* productivity will decrease by 583 t (8%) under 'Future with the Project' conditions relative to 'Future without the Project' (EIS Appendix 10-C). Independent of any other lines of evidence, this result is considered 'minor' as per the common classification system categories of negligible, minor, moderate, and high.

Roberts Bank Ecosystem Model Sensitivity Analyses

Sensitivity analyses showed that the key run estimate was robust to uncertainty in input parameters (EIS Appendix 10-D). The Monte Carlo analysis yielded the exact same result as the key run: an 8% decrease in *Ulva* productivity.

Further, as discussed in IR3-21 (CEAR Document #984), the RB model forecasted the distribution of green algae with average accuracy, compared to visual observations. Overall, PCC was 59% and Kappa values indicated slight agreement (0.15). The model performance for presence and absence forecasts was similar (sensitivity = 52% and specificity = 63%). These results are considered reasonable given *Ulva*'s ephemeral nature, life history, and environmental drivers of distribution.

Overall, results from the RB model, sensitivity analyses, and goodness of fit validation exercises together indicate that the direction of change for *Ulva* is likely negative, and that decreases are considered minor.

2. Empirical Estimates of Changes in Productivity

Direct Mortality: Empirical calculations indicate that construction of the Project, primarily widening of the causeway, will cause direct loss of approximately 259 t of *Ulva* (EIS Table 11-12), which represents 2% of its estimated total biomass within the LAA. The magnitude of loss calculated empirically is considerably less (i.e., by 294 t) than that predicted by the RB model for reasons explained in the 'Literature Evidence' sub-section below (i.e., oversensitivity to salinity). Furthermore, although the *Ulva* biomass within the LAA largely occurs on the mudflats, there is some biomass that deposits on hard substrate (rip-rap) surrounding the existing terminal as well; since the proposed Project will increase the amount of hard substrate, more habitat will be made available for *Ulva* deposition within the LAA. This evidence supports a productivity change category rating of 'negligible' (i.e., 2% loss).

3. Literature Evidence

As is the case for other marine vegetation sub-components, literature was incorporated into both the existing conditions section as well as predictions of future conditions with the Project, specifically to support the characterisation of potential effects. For example, while the direct loss of *Ulva* due to the Project footprint is unavoidable (see Step 2 above), it is considered an opportunistic alga that is capable of swift re-establishment through both spore dispersal

(Amsler and Searles 1980, Zechman and Mathieson 1985) and drift movement by tidal action (Schaadt 2005). *Ulva* abundance and distribution varies widely intra- and inter-annually in time, and is ephemeral and patchy in space (see EIS Section 11.5.3), making it difficult to model with accuracy.

Because *Ulva*'s ephemeral nature makes its deposition and productivity difficult to predict, literature was used to override RB model outputs. One example is in terms of how changes in salinity will affect *Ulva* productivity. In the RB model, *Ulva* was given a strong environmental preference for higher salinities (>20 practical salinity units (PSU)) (EIS Appendix 10-C, Figure 2-23), based on an empirical snapshot of its current distribution at Roberts Bank (i.e., sampling abiotic data layers under the habitat map); however, because (i) *Ulva* is ephemeral and drifts with the tides and (ii) because the habitat map shows only dominant vegetation types in an area (i.e., *Ulva* deposition occurs over a wider area in the LAA, but is not dominant everywhere), model inputs are not necessarily representative of the range of salinity preference/tolerance for *Ulva* in general (Reed and Russell 1979, Martins et al. 1999, Kamer and Fong 2000). This may have overemphasised *Ulva*'s sensitivity to salinity, which appeared to contribute to the predicted productivity decrease. Literature suggests that *Ulva* is actually quite salinity tolerant and occurs over a wide salinity range (Reed and Russell 1979, Kamer and Fong 2000), but that the optimum range lies between 15 PSU to 20 PSU (Martins et al. 1999). Salinity over the tidal flat varies from near 0 PSU to 33 PSU during a rising or falling tide (approximately 6 hours) (EIS Section 9.7.6), and existing *Ulva* biomass occurs across this wide salinity range. For example, while *Ulva intestinalis* growth rate was found to be depressed by extremely low salinities (i.e., ≤ 5 PSU), increased nutrient levels have been shown to decrease the negative effects of reduced salinity on *U. intestinalis* (Kamer and Fong 2001), enabling the alga to inhabit estuaries where freshwater input reduces salinity but is nutrient rich.

Taken together, literature evidence suggests that *Ulva* productivity loss was likely overestimated by the RB model and that productivity is unlikely to be affected by the Project over the long term and thus supports a productivity change category rating of 'negligible'.

4. Evidence from Previous Environmental Assessments and other IC/VC Conclusions

As outlined in IR5-29 (CEAR Document #1185) and for other sub-components above, another line of evidence that contributed to the evaluation of productivity change of sub-components includes information gathered and lessons learned from previous environmental assessments at Roberts Bank, specifically DP3. The adaptive management strategy (AMS) was an eight-year DP3 follow-up study (2007 to 2014) designed to provide an early warning system, so that steps could be taken to mitigate risks well before valued ecosystem components are affected. While not part of the core AMS program, juvenile crab habitat monitoring was completed to fulfill the VFPA's commitments under the Owner's Table of Commitments and Assurance for the DP3 Project to determine whether *Ulva* nursery habitat re-establishes itself along the newly created foreshore (Hemmera 2015).

A juvenile crab habitat monitoring survey was conducted over a nine-week period from July 7 to September 5, 2014. In addition to quantifying juvenile Dungeness crab density and size, the density and distribution of *Ulva* mat and hummocks, as important juvenile habitat, was also quantified. Study results demonstrate that juvenile Dungeness crabs and *Ulva* re-established on the mudflat adjacent to the DP3 footprint (Hemmera 2015). This study provides empirical evidence of natural recovery of juvenile crab nursery habitat, which not only informed predictions for the RBT2 assessment, but also helped increase confidence in the productivity change category rating of 'negligible' by providing empirical evidence of natural recovery occurring at Roberts Bank post-DP3.

Conclusions of the coastal geomorphology assessment were incorporated into the *Ulva* effects assessment as a mechanism of potential productivity changes. The area of increased turbidity is predicted to interact with approximately 950 t of *Ulva* biomass (EIS Table 11-13). Effects due to an increase in turbidity levels are anticipated to be negligible, as the increase is expected to be within the range of natural variation and increases in turbidity levels are only predicted for short durations during a flood tide in the intertidal zone. As a result, changes in productivity of *Ulva* due to changes in turbidity levels were considered negligible and the productivity change category rating of 'negligible' remains.

Additionally, during construction and operation, increased sedimentation due to increased turbidity, a back-eddy, and lower current velocities is predicted to occur. This increase is expected to affect an area of 40 ha shoreward of the terminal (EIS Section 9.5.2; EIS Figure 9.5-35, Zone 4). Approximately 57 t of *Ulva* biomass within an area of 2.5 ha may be affected by increased deposition. As *Ulva* occurs in a wide range of elevations (Park and Hwang 2011) and disperses by spores (Amsler and Searles 1980, Zechman and Mathieson 1985), distribution and abundance is not anticipated to be affected by changes in sediment deposition. *Ulva* is tolerant to sedimentation (Eriksson and Johansson 2005), and will re-colonise areas where sediment accumulation has occurred (Pomeroy and Stockner 1976, Lotze et al. 1999). Changes in productivity of *Ulva* due to sedimentation are therefore not anticipated and the rating of 'negligible' productivity change remains.

5. Proposed Mitigation

No long-term adverse effects are anticipated for *Ulva*; therefore, specific mitigation measures for this representative species are not proposed.

However, *Ulva* will benefit from some of the generic mitigation measures described below and in EIS Sections 17.0 and 33.0:

Avoidance

- The terminal will be placed predominantly in the subtidal zone to avoid and minimise direct mortality to *Ulva*.
- Rocky shoreline will also be included in portions of the causeway perimeter, which is substrate for *Ulva* attachment and facilitate *Ulva* productivity.

Reduction

- Implementation of the CEMP will help mitigate against construction-related mechanisms of productivity change. For example, erosion and run-off controls (e.g., silt fences) may be utilised during land-based construction and operation activities to minimise the likelihood of increased TSS or sedimentation in the nearshore environment at Roberts Bank.

Summary of Potential Project-related Effects to *Ulva* Productivity

The ecosystem model predicted a 583 t (8%) decrease in *Ulva* productivity and results of sensitivity analyses confirmed this estimate was robust to uncertainty in input parameters. Other lines of evidence, including empirical calculations, literature, the DP3 AMS program, and other IC sections collectively suggest that the RB model prediction is likely overestimated and that productivity change would be more accurately characterised by a 'negligible' rating. While a decrease in productivity resulting from the direct loss of *Ulva* due to the Project footprint and predicted changes in sedimentation is unavoidable, *Ulva* is an opportunistic alga that is capable of swift re-establishment through both spore dispersal and drift movement by tidal action, and will quickly re-colonise habitat within the LAA. Therefore, the potential effect of the Project on *Ulva* productivity is expected to be negligible, even without mitigation. Therefore, no specific mitigation is warranted for *Ulva*, though it will benefit from general mitigation measures proposed for other reasons, and no measurable residual adverse effects on Ulva are predicted.

Assessment of Macroalgae (Brown Algae) Productivity Change

1. Model Evidence

Roberts Bank Ecosystem Model Key Run

The RB model modelled rockweed and kelp together as a functional group called brown algae, such that changes in biomass for these two species were not individually estimated. The model estimated that brown algae would decrease in biomass by 53 t (12%) (EIS Appendix 10-C). Independent of any other lines of evidence, this result is considered 'minor' as per the common classification system categories of negligible, minor, moderate, and high.

Roberts Bank Ecosystem Model Sensitivity Analyses

Sensitivity analyses showed that the key run estimate was robust to uncertainty in input parameters (EIS Appendix 10-D). The Monte Carlo analysis yielded the exact same result as the key run: a 12% decrease in brown algae productivity.

Further, as discussed in IR3-21 (CEAR Document #984), accuracy of the RB model prediction for brown algae was high, with a PCC of 95% and a moderate agreement based on the Kappa statistic (0.41). Model accuracy was high in forecasting both brown algae presence (sensitivity = 96%) and absence (specificity = 95%).

Overall, results from the RB model, sensitivity analyses, and goodness of fit validation exercises together indicate that the direction of change for macroalgae is likely negative, and that decreases are considered minor.

2. Empirical Estimates of Changes in Productivity

Direct Mortality: Direct loss of rockweed due to overlap with the Project footprint is estimated to be 83 t, or 28%. This result is higher magnitude than that predicted by the RB model, because it does not reflect longer-term indirect gains in productivity from increased hard substrate habitat for rockweed attachment. Therefore, this value is considered to capture temporary loss, limited to the construction phase. The rating of productivity change remains 'minor', as it is between a 6% and 30% change.

It is estimated that 2 t of kelp will be directly lost within the terminal footprint, where it overlaps the subtidal reefs, which represents approximately 1% of the total kelp biomass within the LAA. This is considered negligible due to the small proportion of the existing biomass being affected.

Overall, empirical evidence suggests a productivity change rating category of minor for macroalgae.

3. Literature Evidence

Similar to other marine vegetation sub-components, literature was incorporated into both the existing conditions section as well as predictions of future conditions with the Project, specifically to support the characterisation of potential effects. For example, direct loss of both rockweed and kelp productivity is expected to be short-term due to their ability to quickly re-colonise (Lubchenco 1980, 1983); this is attributed to inherent biological characteristics, which include reproduction and dispersal via spores, such that supply from nearby unaffected areas will likely help counter direct loss.

4. Evidence from Previous Environmental Assessments and other IC/VC Conclusions

Conclusions of the coastal geomorphology assessment were incorporated into the macroalgae effects assessment as a mechanism of potential productivity changes. Effects due to predicted increases in turbidity levels are anticipated to be negligible for both rockweed and kelp, as the increase is expected to be within the range of natural variation and increases in turbidity levels are only predicted for short durations during a flood tide in the intertidal zone. As a result, changes in macroalgae productivity due to changes in turbidity levels are considered negligible.

During construction and operation, increased sedimentation due to increased turbidity, a back-eddy, and lower current velocities is predicted to occur; this increase is expected to affect an area of 40 ha shoreward of the terminal (EIS Section 9.5.2; EIS Figure 9.5-35, Zone 4). Predicted changes in sedimentation do not spatially overlap with rockweed nor kelp distribution and are therefore considered negligible.

Conclusions of the marine water quality assessment were also incorporated into the macroalgae effects assessment as a mechanism of potential productivity changes, salinity in particular. Rockweed occurs in the upper intertidal zone (i.e., high elevation) and will not be submerged in lower salinity water for long periods of time. No published information was found on salinity effects on rockweed photosynthesis; however, similar species (*Fucus serratus* and *F. vesiculosus*) experienced a slight decrease in photosynthesis at low (6 PSU) salinity (Munda and Kremer 1977). Re-colonising rockweed will not experience average salinity during freshet as low as 6 PSU; therefore, it is anticipated that changes in salinity due to RBT2 will not measurably influence rockweed productivity. Further, at Roberts Bank, kelp is found entirely in subtidal waters. Given that changes in salinity are not predicted for subtidal areas, no interaction is expected and, therefore, kelp productivity will not be affected.

Overall, before mitigation, a potential minor adverse effect is predicted for kelp, a representative species of the macroalgae sub-component.

5. Proposed Mitigation

The following measures are proposed to mitigate potential adverse effects to macroalgae due to the Project (EIS Sections 17.0 and 33.0):

Avoidance

- The Project will increase the amount of hard substrate habitat in the LAA suitable for brown algae attachment. The terminal caisson structures, along with the rip-rap/rocky shoreline in portions of the causeway perimeter, are expected to provide additional attachment habitat, thereby substantially increasing macroalgae productive potential.

Reduction

- Implementation of the CEMP will help mitigate against construction-related mechanisms of productivity change. For example, erosion and run-off controls (e.g., silt fences) may be utilised during land-based construction and operation activities to minimise the likelihood of increased TSS or sedimentation in the nearshore environment at Roberts Bank.

Offsetting

- Proposed onsite offsetting includes up to 2 ha of subtidal rocky reefs, at depths suitable for macroalgal attachment; this is expected to further increase brown algae productivity by approximately 258 t, which far outweighs the predicted loss.
- Proposed onsite offsetting also includes a sandy gravel beach that will promote rockweed attachment, which will further enhance productivity.

Summary of Potential Project-related Effects to Brown Algae Productivity

The RB model and sensitivity analyses predicted a 53 t (12%) decrease in macroalgae productivity. The direction of change is in alignment with empirical estimates. Direct mortality will decrease rockweed productivity by 83 t (28%) in the short-term and is considered a minor decrease. In contrast, an estimated 2 t of kelp will be directly lost within the terminal footprint, where it overlaps the subtidal reefs, which is 1% of the kelp biomass within the LAA. Empirical calculations are of higher magnitude because, unlike the RB model, they do not account for indirect gains in productivity resulting from Project placement; these gains are substantial, as Project infrastructure (i.e., caissons, rip-rap, pilings) is expected to add a considerable amount of hard substrate habitat, suitable for brown algae colonisation. The amount of hard substrate added exceeds the amount lost during construction; thus, once colonised, the LAA is predicted to support more brown algae productivity than under existing conditions. Additionally, offsetting in the form of subtidal rocky reefs and sandy gravel beach habitat is expected to further increase availability of hard substrate attachment habitat by over 2 ha, further enhancing brown algal productive potential within the LAA. Literature suggests both rockweed and kelp possess biological attributes (i.e., spore dispersal) that facilitate quick recolonisation of rocky habitat that will increase in area due to the Project.

Changes in salinity, TSS, and sedimentation will have a negligible effect on rockweed productivity either due to future salinity contours remaining within rockweed's range of tolerance or not overlapping spatially; for kelp, predicted changes do not overlap spatially with its subtidal distribution.

While the Project is expected to result in a temporary (i.e., construction phase only) minor adverse effect on rockweed productivity, the implementation of mitigation measures including Project placement, design (i.e., increase in rocky substrate), and offsetting (i.e., subtidal rocky reef creation) ensures that net changes to macroalgae productivity will be negligible over the long-term and no measurable residual adverse effects are predicted.

Assessment of Biomat Productivity Change

1. Model Evidence

Roberts Bank Ecosystem Model Key Run

For biomat, the key run output predicted that productivity would decrease by 356 t or 29% relative to 'Future without the Project' conditions (EIS Appendix 10-C). Independent of any other lines of evidence, this result is considered 'minor' as per the common classification system categories of negligible, minor, moderate, and high. The extent of this change does not fully align with other lines of evidence . Differences are attributed to the inability of the model to capture the extent to which biomat can modify its surrounding environment and the overestimated effects of predation in the food web, discussed in further detail below.

Roberts Bank Ecosystem Model Sensitivity Analyses

Sensitivity analyses showed that the key run estimate was robust to uncertainty in input parameters (EIS Appendix 10-D). The Monte Carlo analysis yielded the exact same result as the key run: a 29% decrease in biomat productivity.

Further, as discussed in IR3-21 (CEAR Document #984), accuracy of the RB model prediction for biomat was high. Overall, PCC was 97% and the Kappa value indicated moderate agreement (0.45). The model was highly accurate in forecasting both presence (sensitivity = 100%) and absence (specificity = 97%).

Overall, results from the RB model, sensitivity analyses, and goodness of fit validation exercises together indicate that the direction of change for biomat is likely negative, and that decreases are considered minor.

2. Empirical Estimates of Changes in Productivity

Direct mortality to biomat will not occur because the Project footprint and biomat do no spatially overlap.

3. Literature Evidence

Similar to other marine vegetation sub-components, a literature review was conducted and the resulting analysis informed both the existing conditions section as well as predictions of future conditions with the Project, specifically to support the characterisation of potential effects. For example, biomat establishes, expands, and self-propagates by modifying environmental characteristics, such as water velocity, of its surroundings. Biomat slows water current velocities, which increases sedimentation and the vertical and horizontal growth of more biomat (Stal 2010). Abiotic parameters included in the RB model were unable to capture the unique way in which biomat interacts with and modifies its physical environment and the productivity increases that result from that modification and, as such, the predicted decline in biomat productivity is considered overestimated.

Further, food web-related influences are likely driving the negative model prediction for biomat due to increases in macrofauna (27% or 788 t; EIS Table 12-8) and waterfowl (8% or 1 t; EIS Table 15-10). However, the magnitude of change in macrofauna is considered an overestimation by at least 5% (EIS Section 12.6.3), such that predicted losses of biomat productivity due to macrofaunal grazing are likely overestimated. A review of existing literature did not indicate biomat comprises any portion of waterfowl diet and waterfowl were not observed consuming biomat during baseline studies for RBT2 (TDR MVB-1 in Appendix AIR10-C of CEAR Document #388). This would suggest that the magnitude of predicted biomat loss would be less than predicted by the model.

Consistent with the lines of evidence approach taken for this assessment (see IR5-29 of CEAR Document #1185) taking into account this scientific understanding of biomat's characteristics as well as a more realistic estimate of grazing pressure, the modelling evidence conclusion of 'minor' was therefore adjusted to a productivity change category rating of 'negligible'.

4. Evidence from Previous Environmental Assessments and other IC/VC Conclusions

Conclusions of the coastal geomorphology assessment were incorporated into the biomat effects assessment as a mechanism of potential productivity changes. Geomorphic studies show that biomat within the LAA is accreting and increasing in the LAA (EIS Appendix 9.5-A). Further, predicted changes in turbidity levels during the construction and operation phases, as well as predicted areas of sedimentation, are not expected to overlap spatially with biomat (EIS Table 11-13). Taken together, this supports a negligible productivity rating.

Conclusions of the marine water quality assessment, particularly changes in salinity, were also incorporated into the biomat effects assessment as a mechanism of potential productivity change. Changes in salinity are predicted to occur within biomat areas: as shown in EIS Table 11-16, more biomat biomass will experience salinities between 4 PSU and 12 PSU in the future with the Project compared to existing conditions. Salinity was not determined by the RB model to be a driving abiotic factor for the decrease (as described in EIS Appendix 10-D); this is supported by Moisander et al. (2002) who reported that low salinities do not affect cyanobacteria productivity. As a result, changes in salinity from the Project are not anticipated to affect biomat, further supporting a productivity category rating of negligible.

5. Proposed Mitigation

Because Project-environment interactions for biomat are predicted to be negligible, specific mitigation measures for this sub-component are not proposed. However, biomat will benefit from some of the generic mitigation measures described below and in EIS Sections 17.0 and 33.0.

Avoidance

- The placement of the terminal in the subtidal zone will completely avoid direct mortality to biomat.

Reduction

- Implementation of the CEMP will help mitigate against construction-related mechanisms of productivity change. For example, erosion and run-off controls (e.g., silt fences) may be utilised during land-based construction and operation activities to minimise the likelihood of increased TSS or sedimentation in the nearshore environment at Roberts Bank.

Summary of Potential Project-related Effects to Biomat Productivity

The RB model and sensitivity analyses suggest biomat productivity will decrease by 29%, or 356 t, due to indirect effects of the Project; however, as stated above, the RB model overestimates such loss. As discussed in EIS Section 11.6.3.4, the extent of this change does not fully align with other lines of evidence; differences are attributed to the inability of the model to capture the extent to which biomat is capable of modifying its surrounding environment and the overestimated effects of macrofauna and waterfowl grazing in the food web. Conclusions pertaining to biomat predominantly rest on two key points: 1) There will be no direct mortality due to the Project and there is no spatial overlap between predicted changes in TSS and sedimentation with biomat; and, 2) while biomat will, on average, experience reduced salinity during freshet with the Project relative to existing conditions, the literature indicates salinity is not a key factor for biomat productivity. Considering all of these lines of evidence, changes in biomat productivity due to the Project are characterised as negligible even without mitigation. No residual adverse effects are predicted for this sub-component.

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Appendices

Appendix IR8-08-A Marine Vegetation Sub-component Summary of Effects

APPENDIX IR8-08-A

MARINE VEGETATION SUB-COMPONENT SUMMARY OF EFFECTS

Table IR8-08-A1 Marine Vegetation Sub-component Summary of Effects Based on Lines of Evidence, Mitigation, Productivity Change Conclusion, and Description of Residual Effects

Sub-component	Effect Mechanism	Potential Adverse Effects	Lines of Evidence: Empirical Estimate (tonnes (t) & % of existing biomass)	Lines of Evidence: Ecosystem Model Productivity Change (%; t) Gain	Lines of Evidence: Ecosystem Model Productivity Change (%; t) Loss	Lines of Evidence: Other Evidence (literature, past Projects, IC sections etc.)	Mitigation	Productivity Change Conclusion	Residual Effects Characterisation	Significance Rationale/ Likelihood
Eelgrass										
Native	• Direct Mortality • Changes in Water Quality: TSS during construction • Changes to Sedimentation and Coastal Processes: Sedimentation & wave shadow (EIS Section 11.6.3.1)	Loss of productivity	Direct mortality: 5 t loss (4%) Increased sedimentation:12 t loss (worst-case scenario; 3%) Wave shadow will increase productivity by 4 t to 8 t (1 to 2%) Net: 11 t loss	4% 11 t	-	• Native eelgrass can withstand relatively extreme sedimentation (Hemminga and Duarte 2000, Mills and Fonseca 2003, Cabaço and Santos 2007). • Wave shadows facilitate eelgrass colonisation and productivity (Stevens and Lacy 2011). • Native eelgrass area at Roberts Bank has been increasing since the early 1970s (Tarbotton and Harrison 1996) due to construction of the Roberts Bank causeway, which reduced turbidity (and increased light availability) in the inter-causeway area, and created the wave shadow (Tarbotton and Harrison 1996).	1. Avoidance (EIS Section 11.7.1) • Placing the marine terminal predominantly within the subtidal zone. 2. Construction Environmental Management Plans (EIS Section 33.3) • Construction Compliance Monitoring Plan • Dredging and Sediment Discharge Plan • Sediment and Erosion Control Plan 3. Onsite Offsetting (EIS Section 17.3.2.20) • Transplanting approximately 3 ha of native eelgrass • Site restoration will occur as soon as possible following construction activities	Negligible	None	n/a
Non-native	• Direct Mortality • Changes to Sedimentation & Coastal Processes: Sedimentation (EIS Section 11.6.3.1)	Loss of productivity	Direct mortality: 0.3 t loss (2 %)	-	1% 0.05 t	• Quick to colonise available habitat (Harrison and Bigley 1982). • Accumulation of finer sediments over time may increase bed elevation, making growing conditions more suitable (Harrison and Bigley 1982), but the productivity increase is difficult to quantify.	1. Avoidance (EIS Section 11.7.1) • Placing the marine terminal predominantly within the subtidal zone. 2. Construction Environmental Management Plans (EIS Section 33.3) • Construction Compliance Monitoring Plan • Dredging and Sediment Discharge Plan • Sediment and Erosion Control Plan	Negligible	None	n/a

Sub-component	Effect Mechanism	Potential Adverse Effects	Lines of Evidence				Mitigation	Productivity Change Conclusion	Residual Effects Characterisation	Significance Rationale/ Likelihood
			Empirical Estimate (tonnes (t) & % of existing biomass)	Ecosystem Model Productivity Change (%; t)		Other Evidence (literature, past Projects, IC sections etc.)				
				Gain	Loss					
Intertidal Marsh										
Intertidal Marsh	• Direct Mortality • Changes in Water Quality: Salinity • Changes to Sedimentation & Coastal Processes: Sedimentation • Biotic Interactions (EIS Section 11.6.3.2)	Loss of productivity	Direct mortality: 1 t loss (<0.1 %)	25% 335 t	-	• Marsh plants are quick to re-colonise (Erfanzadeh et al. 2010). • Lower salinities generally increase productivity of intertidal marsh plants (Crain et al. 2004, Woo and Takekawa 2012). • Marsh has been increasing over time in LAA due to river training and dyking (Hales 2000)	1. Avoidance (EIS Section 11.7.1) • Placing the marine terminal predominantly within the subtidal zone. 2. Construction Environmental Management Plans (EIS Section 33.3) • Construction Compliance Monitoring Plan • Dredging and Sediment Discharge Plan • Sediment and Erosion Control Plan 3. Onsite Offsetting (EIS Section 17.3.2.20) • Construction of ~15 ha of intertidal salt marsh • Site restoration will occur as soon as possible following construction activities	Minor increase	None	n/a
Macroalgae										
Ulva	• Direct Mortality • Biotic Interactions (EIS Section 11.6.3.3)	Loss of productivity	Direct mortality: 259 t (2%)	-	8% 583 t	• *Ulva* is ephemeral and drifts in and out of the LAA based on tidal currents; thus, quick re-colonisation will occur (Amsler and Searles 1980, Zechman and Mathieson 1985). • Amount of hard substrate is increasing due to the Project, which is also used by *Ulva*. • *Ulva*'s salinity preference input into the RB model is based on a snapshot of its distribution; it is able to drift and deposit across the entire LAA and exhibits broad salinity tolerance (Reed and Russell 1979, Martins et al. 1999, Kamer and Fong 2000). This led to *Ulva* being sensitive to predicted changes in salinity, which contributed to the predicted decrease in future *Ulva* productivity.	1. Avoidance (EIS Section 11.7.1) • Placing the marine terminal predominantly within the subtidal zone • Incorporation of rocky shoreline in portions of the terminal causeway perimeters 2. Construction Environmental Management Plans (EIS Section 33.3) • Construction Compliance Monitoring Plan • Dredging and Sediment Discharge Plan • Sediment and Erosion Control Plan	Negligible	None	n/a

Sub-component	Effect Mechanism	Potential Adverse Effects	Lines of Evidence: Empirical Estimate (tonnes (t) & % of existing biomass)	Lines of Evidence: Ecosystem Model Productivity Change (%; t) Gain	Lines of Evidence: Ecosystem Model Productivity Change (%; t) Loss	Lines of Evidence: Other Evidence (literature, past Projects, IC sections etc.)	Mitigation	Productivity Change Conclusion	Residual Effects Characterisation	Significance Rationale/ Likelihood
Brown Algae (Rockweed and Kelp)	• Direct Mortality • Biotic Interactions (EIS Section 11.6.3.3)	Loss of productivity	Direct mortality (rockweed): 83 t (28%) Direct mortality (kelp): 2 t (1%)	-	12% 53 t	• Swift re-colonisation will occur after construction is complete (temporal loss) (Lubchenco 1980, 1983). • Amount of hard substrate is increasing due to the Project.	1. Avoidance (EIS Section 11.7.1) • Placing the marine terminal predominantly within the subtidal zone • Incorporation of rocky shoreline in portions of the terminal causeway perimeters 2. Construction Environmental Management Plans (EIS Section 33.3) • Construction Compliance Monitoring Plan • Dredging and Sediment Discharge Plan • Sediment and Erosion Control Plan 3. Onsite Offsetting (EIS Section 17.3.2.20) • Construction of sandy gravel beach • Construction of subtidal reefs for kelp habitat	Negligible	None	n/a
Biomat										
Biomat	• Biotic Interactions (EIS Section 11.6.3.4)	Loss of productivity	-	-	29% 356 t	• The RB model is unable to characterise the way biomat establishes and propagates (EIS Section 9.5.6); therefore, anticipated future accretion and productivity are not captured by the model. • Food web-related influences are predicted to have a negative effect on biomat due to increases in macrofaunal grazers (27% or 788 t). The increase in macrofaunal grazers was determined to be an overestimation (EIS Section 12.6.3). An increase in other waterfowl (8% or 1.3 t) also negatively effects biomat. A literature review did not find any information whether waterfowl consume biomat and it was not observed during RBT2 baseline studies (TDR MVB-1 in Appendix AIR10-C of CEAR Document #388).	None	Negligible	None	n/a

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IR8-09 Marine Vegetation – Invasive Species

Information Source(s)

EIS Volume 3: Section 11

Proponent Response to Additional Information Requirements of July 31, 2017 (CEAR Doc#314) IR3

Proponent Response to Follow-up Additional Information Requirements of Dec 4, 2015 (CEAR #388) IR3

Context

The Proponent excluded the invasive intertidal marsh plant, English cordgrass (Spartina anglica), from the marine vegetation assessment and stated that ongoing eradication work by the British Columbia Spartina Working Group may result in the elimination of this invasive plant from Roberts Bank (CEAR Doc#314).

In its response to follow-up information requirement #3 from the Canadian Environmental Assessment Agency (CEAR Doc#388), the Proponent indicated that, despite effects by the Province of British Columbia to manage Spartina, it may never be fully eradicated at Roberts Bank. The Proponent further stated that, in the hypothetical and unlikely situation that Spartina is not eradicated, it would typically have negative consequences for native species and habitats since it is an invasive species.

Information is required regarding the management program and the status of ongoing eradication efforts of Spartina at Roberts Bank and the current and past distribution and biomass of Spartina in the local and regional assessment areas.

Information Request

Provide a description of the ongoing management of Spartina anglica at Roberts Bank by the Province of British Columbia. Summarize the status of the eradication efforts to eliminate Spartina in the local and regional assessment areas. Include a map that shows the past and current distribution of Spartina in these areas.

VFPA Response

The VFPA would like to clarify that *Spartina anglica* management and eradication is the responsibility of the Province of British Columbia. The information provided in this response is based on publicly available reports from the B.C. Spartina Working Group (BCSWG) and was not collected by the VFPA firsthand.

Provide a description of the ongoing management of Spartina anglica at Roberts Bank by the Province of British Columbia.

Since the 1980s, *Spartina* plants have expanded northward from California, Oregon, and Washington into B.C. English cordgrass (*Spartina anglica*) was first detected in the Fraser River delta in 2003. In response to *Spartina*'s increasing presence in B.C., the Province joined the Pacific Coast Collaborative West Coast plan to eradicate *Spartina* from its waters by 2018 (Pacific Coast Collaborative 2008). These cross border partnerships with Washington, Oregon, and California are aimed at reducing the ecological and economic impacts of invasive species and promoting ocean health.

In 2004, the BCSWG was formed, with a focus on employing early detection and rapid response methods to eradicate *Spartina*. This group has mapped infestations, removed plants by machine and by hand, conducted evaluations on effectiveness, and undertaken public education and training. BCSWG is a multi-agency partnership and consists of representatives from the following organisations:

- City of Delta;
- City of Port Moody;
- City of Surrey;
- Coastal Invasive Species Committee;
- Community Mapping Network;
- Invasive Species Council of Metro Vancouver;
- Ducks Unlimited Canada (DUC);
- Environment and Climate Change Canada – Canadian Wildlife Service;
- Fisheries and Oceans Canada;
- Friends of Semiahmoo Bay Society;
- Ladner Rotary Club;
- Metro Vancouver;
- Ministry of Environment (MOE);
- Ministry of Forests, Lands, & Natural Resource Operations (FLNRO);
- Vancouver Fraser Port Authority; and
- Vancouver Island Conservation Lands Management Program.

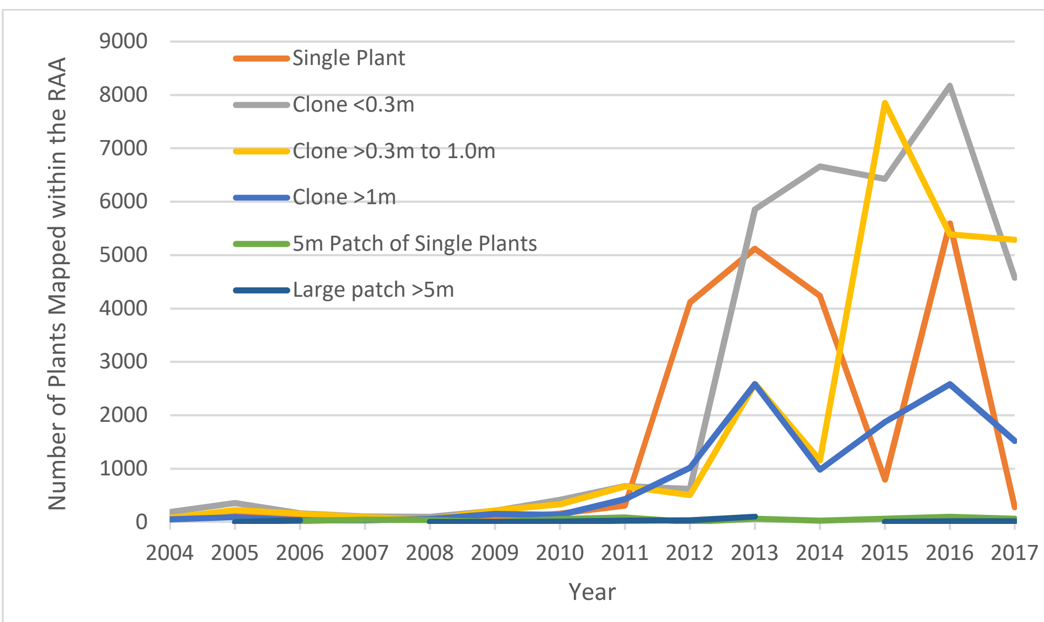
Summarize the status of the eradication efforts to eliminate Spartina in the local and regional assessment areas.

Spartina infestations are currently limited to a total of less than 22 hectares in B.C. (BCSWG 2016) (**Figure IR8-09-1**). Preventing and controlling the spread at the early stages of expansion is the ongoing management priority because allowing this species to spread uncontrolled will result in widespread alteration and loss of important intertidal habitats, which would be detrimental to a multitude of species, and would require considerably greater resources to control in the future. This is particularly important in B.C., where known infestations of the species are within the relatively early stages of growth, existing as pioneer infestations characterised by small clones or individual plants and not yet exhibiting vast monocultures (Dresen et al. 2010).

The BCSWG undertakes a variety of activities on an annual basis, aimed at identifying *Spartina* infestations and removing them. *Spartina* control efforts run year round from April 1 to March 31. Intertidal flats are surveyed between May and October by summer crews and partnership agencies, where plants are mapped using GPS (global position system) and smart devices to track population trends and spatial locations*.* The program achieves complete mapping of the intertidal foreshore at Roberts Bank and in Boundary Bay from Point Roberts to Peace Arch Crossing / Drayton Harbor. From 2003 to 2013, removal techniques focused on manual removal through hand digging and mechanical removal through excavator burial. To date, manual and mechanical removal methods were met with limited success for *S. anglica* (BCSWG 2016; see *Spartina* distribution figures presented in **Appendix IR8-09-A**).

Consequently, herbicide treatment was incorporated in late summer of 2013 as part of an integrated pest management plan to control *Spartina* infestations in the Lower Mainland. Herbicide application has been focused on *S. anglica* in the geographic areas of Roberts Bank and Boundary Bay in the Lower Mainland; the herbicide used is 'Habitat' (active ingredient: Imazapyr) with a surfactant (Ag Surf II). Five consecutive years of complete herbicide treatment on *S. anglica* at Roberts Bank have been conducted and three non-consecutive years of complete treatment have been done at Boundary Bay. In general, *S. anglica* populations in the Lower Mainland are recently trending downward, with some sites demonstrating more success than others*.* Overall, there has been a greater than 30% reduction in the number of *S. anglica* plants in the local assessment area and some sites have seen greater than 50% reduction (BCSWG 2016).

Figure IR8-09-1 Number of *Spartina* Plants Mapped in the Regional Assessment Area from 2004 to 2017



Note: RAA = regional assessment area
Source: Data provided by Ducks Unlimited Canada.

Include a map that shows the past and current distribution of Spartina in these areas.

Figures IR8-09-A1 to IR8-09-A28 in **Appendix IR8-09-A** show the distribution of *Spartina* mapped within the local and regional assessment areas from 2004 to 2017 by *Spartina* size class. It should be noted that annual mapping reflects variation in the level of detection effort and resources from year to year and thus may not wholly accurately depict trends in actual abundance/distribution over time.

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Appendices

Appendix IR8-09-A *Spartina* Distribution Maps

APPENDIX IR8-09-A
SPARTINA DISTRIBUTION MAPS

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Legend

- BOUNDARY OF PROJECT AREA
- MARINE VEGETATION LAA
- CANADA-U.S.A. BORDER

SPARTINA PLANTS
- SINGLE PLANT
- < 0.3 m DIAMETER
- >1.0 m DIAMETER

0 1 2
Kilometres
1:40,000
NAD 1983 UTM Zone 10N

N

Note:
Spartina Data provided by Ducks Unlimited Canada

PORT of vancouver

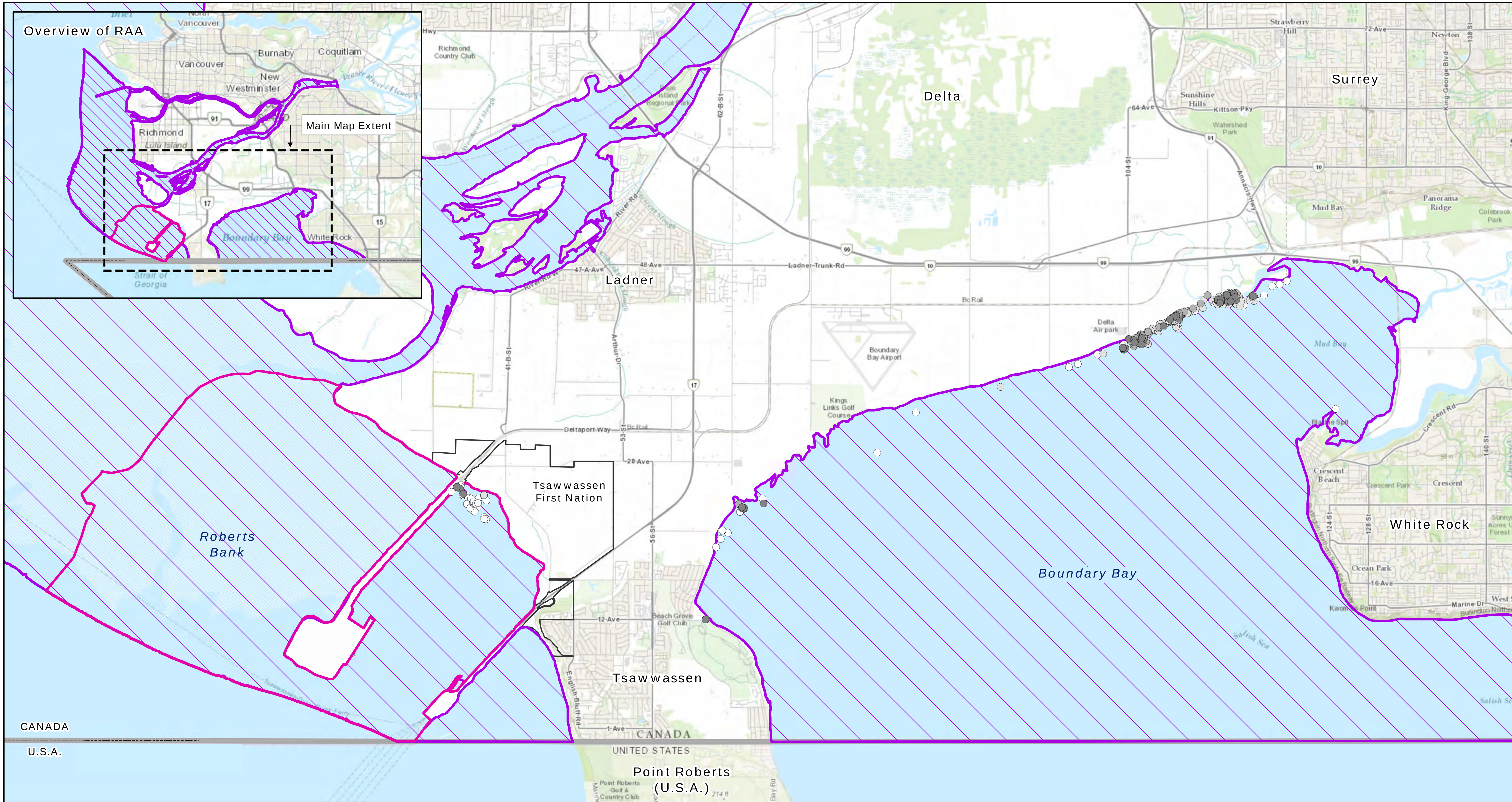
ROBERTS BANK TERMINAL 2

SPARTINA DISTRIBUTION WITHIN THE MARINE VEGETATION LAA 2004

DATE: 05/14/2018

FIG No. IR8-09-A1

Path: O:\1200-1\1246\EIS_INFORMATION_REQUESTS\IR8_09\mxd\IR8_09_Ax-even-pages_SpartinaDistribution_RAA_180514.mxd



Legend

- BOUNDARY OF PROJECT AREA
- MARINE VEGETATION LAA
- MARINE VEGETATION RAA
- CANADA-U.S.A. BORDER

SPARTINA PLANTS

- SINGLE PLANT
- < 0.3 m DIAMETER
- 0.3 - 1.0 m DIAMETER
- >1.0 m DIAMETER

0 2 4
Kilometres
1:75,000
NAD 1983 UTM Zone 10N

Note:
Spartina Data provided by Ducks Unlimited Canada

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ROBERTS BANK TERMINAL 2

SPARTINA DISTRIBUTION WITHIN THE MARINE VEGETATION RAA 2004

DATE:	FIG No.
05/14/2018	IR8-09-A2

Basemap Sources: Esri, HERE, DeLorme, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, MapmyIndia, © OpenStreetMap contributors, and the GIS User Community; Imagery Source: BING Maps Aerial

Path: O:\1200-1246\EIS_INFORMATION_REQUESTS\IR8_09\mxd\IR8_09_Ax-odd-pages_SpartinaDistribution_LAA_180514.mxd

Legend

BOUNDARY OF PROJECT AREA

MARINE VEGETATION LAA

CANADA-U.S.A. BORDER

SPARTINA PLANTS

- SINGLE PLANT
- < 0.3 m DIAMETER
- 0.3 - 1.0 m DIAMETER
- >1.0 m DIAMETER
- >5 m LARGE PATCH OF PLANTS

0 1 2 Kilometres

1:40,000

NAD 1983 UTM Zone 10N

N

Note:
Spartina Data provided by Ducks Unlimited Canada

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ROBERTS BANK TERMINAL 2

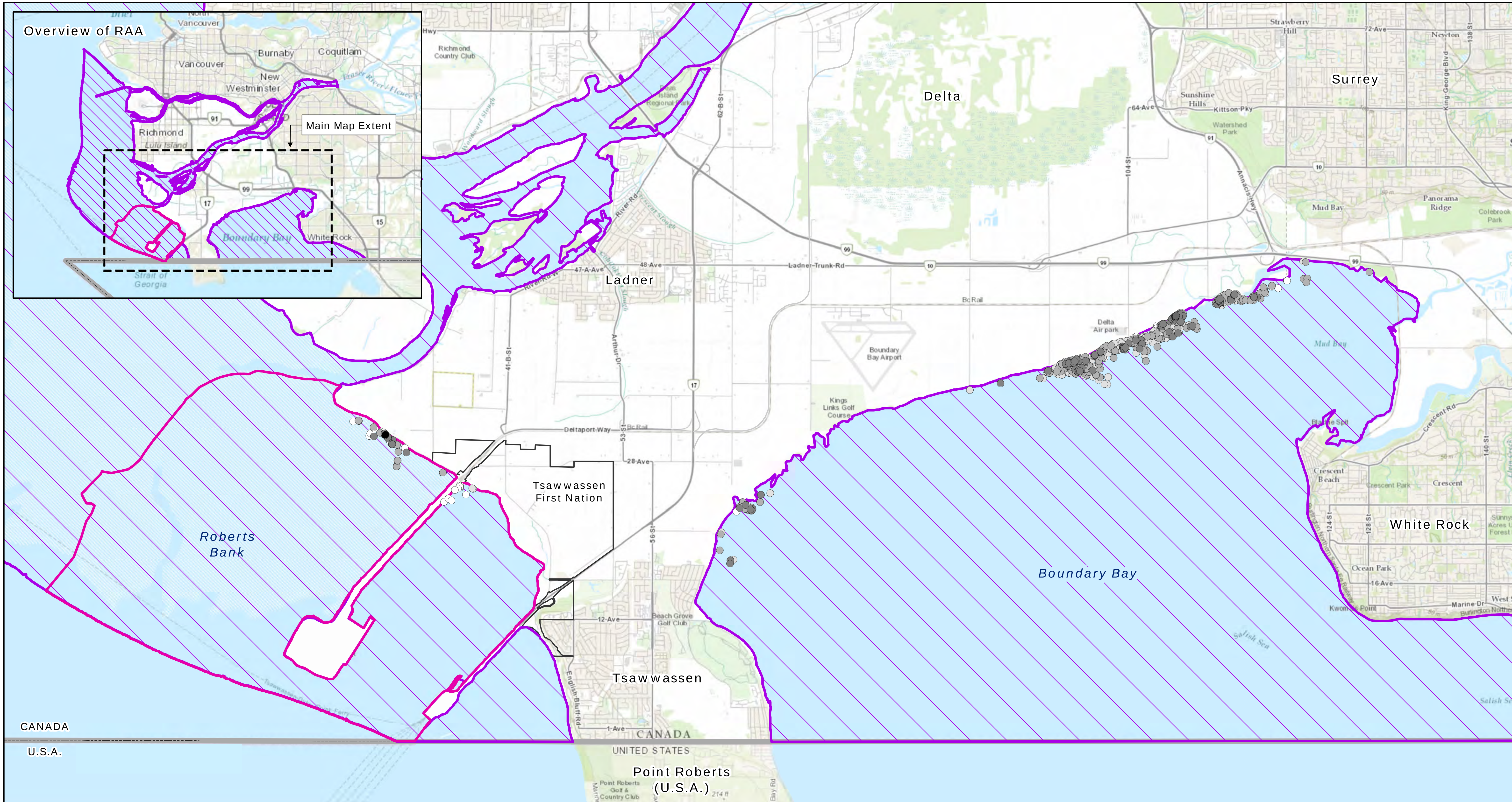
SPARTINA DISTRIBUTION WITHIN THE MARINE VEGETATION LAA 2005

DATE: 05/14/2018

FIG No. IR8-09-A3

Basemap Sources: Esri, HERE, DeLorme, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, MapmyIndia, © OpenStreetMap contributors, and the GIS User Community; Imagery Source: BING Maps Aerial

Path: O:\11200-11246\EIS_INFORMATION_REQUESTS\IR8_09\mxd\IR8_09_Ax-even-pages_SpartinaDistribution_RAA_180514.mxd



Legend

BOUNDARY OF PROJECT AREA
MARINE VEGETATION LAA
MARINE VEGETATION RAA
CANADA-U.S.A. BORDER

SPARTINA PLANTS
- SINGLE PLANT
- < 0.3 m DIAMETER
- 0.3 - 1.0 m DIAMETER
- >1.0 m DIAMETER
- ~5 m PATCH OF PLANTS
- >5 m LARGE PATCH OF PLANTS

0 2 4
Kilometres
1:75,000
NAD 1983 UTM Zone 10N

Note:
Spartina Data provided by Ducks Unlimited Canada

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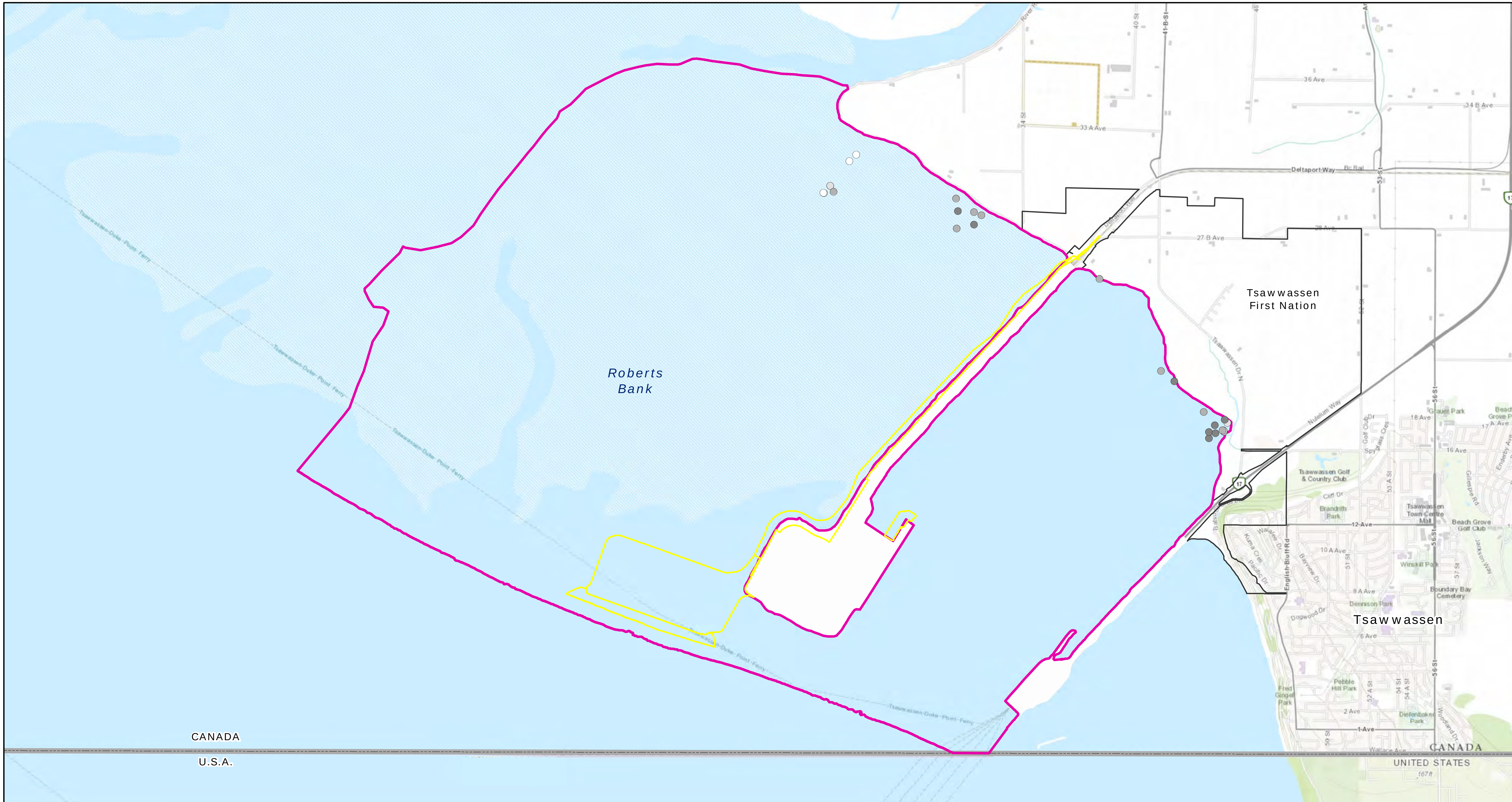
ROBERTS BANK TERMINAL 2

SPARTINA DISTRIBUTION WITHIN THE MARINE VEGETATION RAA 2005

DATE:	FIG No.
05/14/2018	IR8-09-A4

Basemap Sources: Esri, HERE, DeLorme, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, MapmyIndia, © OpenStreetMap contributors, and the GIS User Community; Imagery Source: BING Maps Aerial

Path: O:\1200-1246\EIS_INFORMATION_REQUESTS\IR8_09\mxd\IR8_09_Ax-odd-pages_SpartinaDistribution_LAA_180514.mxd



Legend

BOUNDARY OF PROJECT AREA	
MARINE VEGETATION LAA	
CANADA-U.S.A. BORDER	

SPARTINA PLANTS
- SINGLE PLANT
- < 0.3 m DIAMETER
- 0.3 - 1.0 m DIAMETER
- >1.0 m DIAMETER

0 1 2
Kilometres
1:40,000
NAD 1983 UTM Zone 10N

N

Note:
Spartina Data provided by Ducks Unlimited Canada

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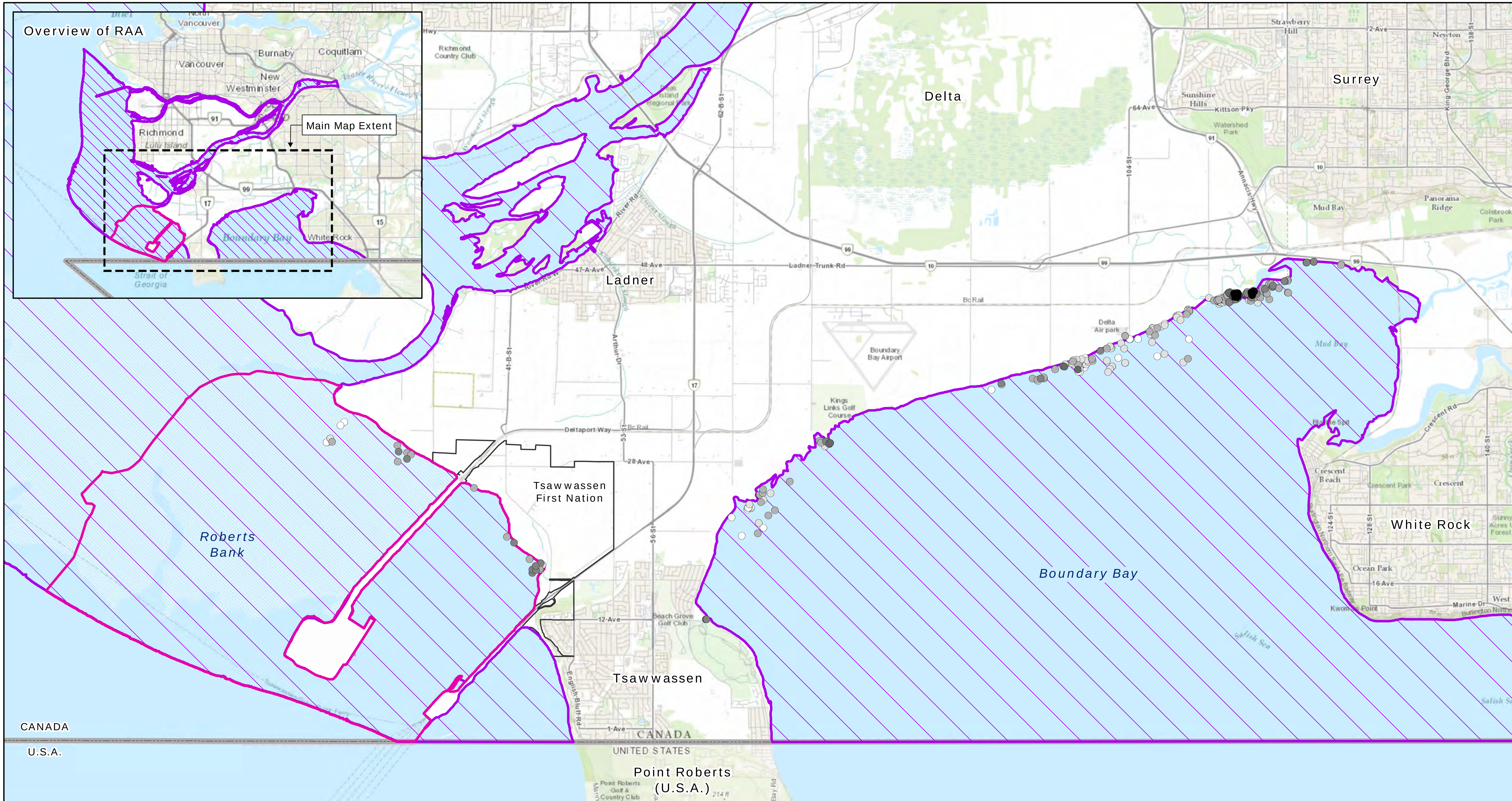
ROBERTS BANK TERMINAL 2

SPARTINA DISTRIBUTION WITHIN THE MARINE VEGETATION LAA 2006

DATE:	FIG No.
05/14/2018	IR8-09-A5

Basemap Sources: Esri, HERE, DeLorme, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, MapmyIndia, © OpenStreetMap contributors, and the GIS User Community; Imagery Source: BING Maps Aerial

Path: O:\11200-\1246\EIS_INFORMATION_REQUESTS\IR8_09\mxd\IR8_09_Ax-even-pages_SpartinaDistribution_RAA_180514.mxd



Legend

BOUNDARY OF PROJECT AREA	
MARINE VEGETATION LAA	
MARINE VEGETATION RAA	
CANADA-U.S.A. BORDER	

SPARTINA PLANTS

- SINGLE PLANT
- < 0.3 m DIAMETER
- 0.3 - 1.0 m DIAMETER
- >1.0 m DIAMETER
- ~5 m PATCH OF PLANTS
- >5 m LARGE PATCH OF PLANTS

0 2 4
Kilometres
1:75,000
NAD 1983 UTM Zone 10N

N

Note:
Spartina Data provided by Ducks Unlimited Canada

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ROBERTS BANK TERMINAL 2

SPARTINA DISTRIBUTION WITHIN THE MARINE VEGETATION RAA 2006

DATE:	FIG No.
05/14/2018	IR8-09-A6

Basemap Sources: Esri, HERE, DeLorme, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, MapmyIndia, © OpenStreetMap contributors, and the GIS User Community; Imagery Source: BING Maps Aerial

Path: O:\1200-1246\EIS_INFORMATION_REQUESTS\IR8_09\mxd\IR8_09_Ax-odd-pages_SpartinaDistribution_LAA_180514.mxd

Legend

- BOUNDARY OF PROJECT AREA
- MARINE VEGETATION LAA
- CANADA-U.S.A. BORDER

SPARTINA PLANTS

- SINGLE PLANT
- < 0.3 m DIAMETER
- 0.3 - 1.0 m DIAMETER
- >1.0 m DIAMETER
- ~5 m PATCH OF PLANTS

0 1 2
Kilometres
1:40,000
NAD 1983 UTM Zone 10N

N

Note:
Spartina Data provided by Ducks Unlimited Canada

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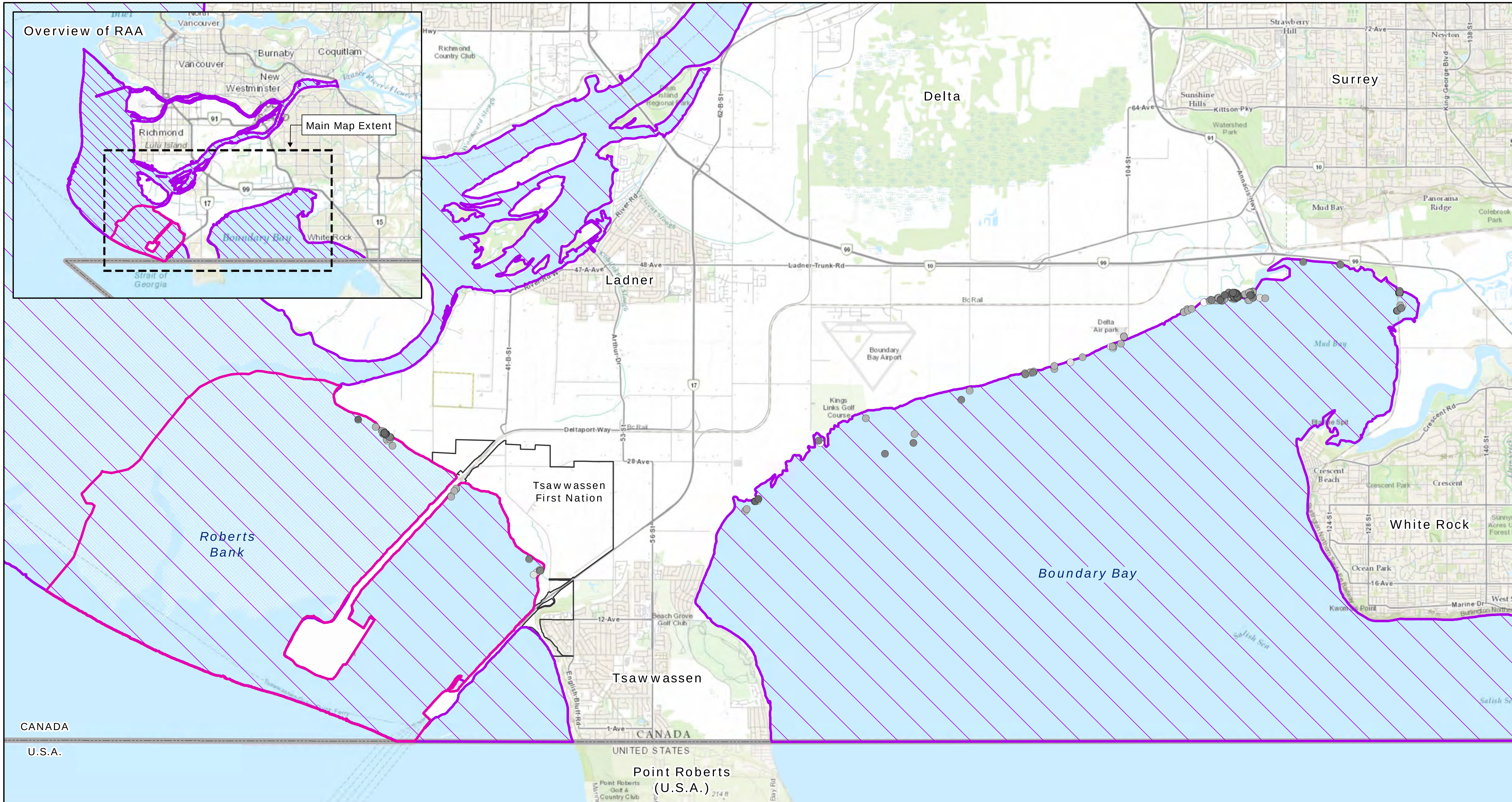
ROBERTS BANK TERMINAL 2

SPARTINA DISTRIBUTION WITHIN THE MARINE VEGETATION LAA 2007

DATE:	FIG No.
05/14/2018	IR8-09-A7



Path: O:\11200-\1246\EIS_INFORMATION_REQUESTS\IR8_09\mxd\IR8_09_Ax-even-pages_SpartinaDistribution_RAA_180514.mxd

Legend

- BOUNDARY OF PROJECT AREA
- MARINE VEGETATION LAA
- MARINE VEGETATION RAA
- CANADA-U.S.A. BORDER

SPARTINA PLANTS

- SINGLE PLANT
- < 0.3 m DIAMETER
- 0.3 - 1.0 m DIAMETER
- >1.0 m DIAMETER
- ~5 m PATCH OF PLANTS

0 2 4
Kilometres
1:75,000
NAD 1983 UTM Zone 10N

N

Note:
Spartina Data provided by Ducks Unlimited Canada

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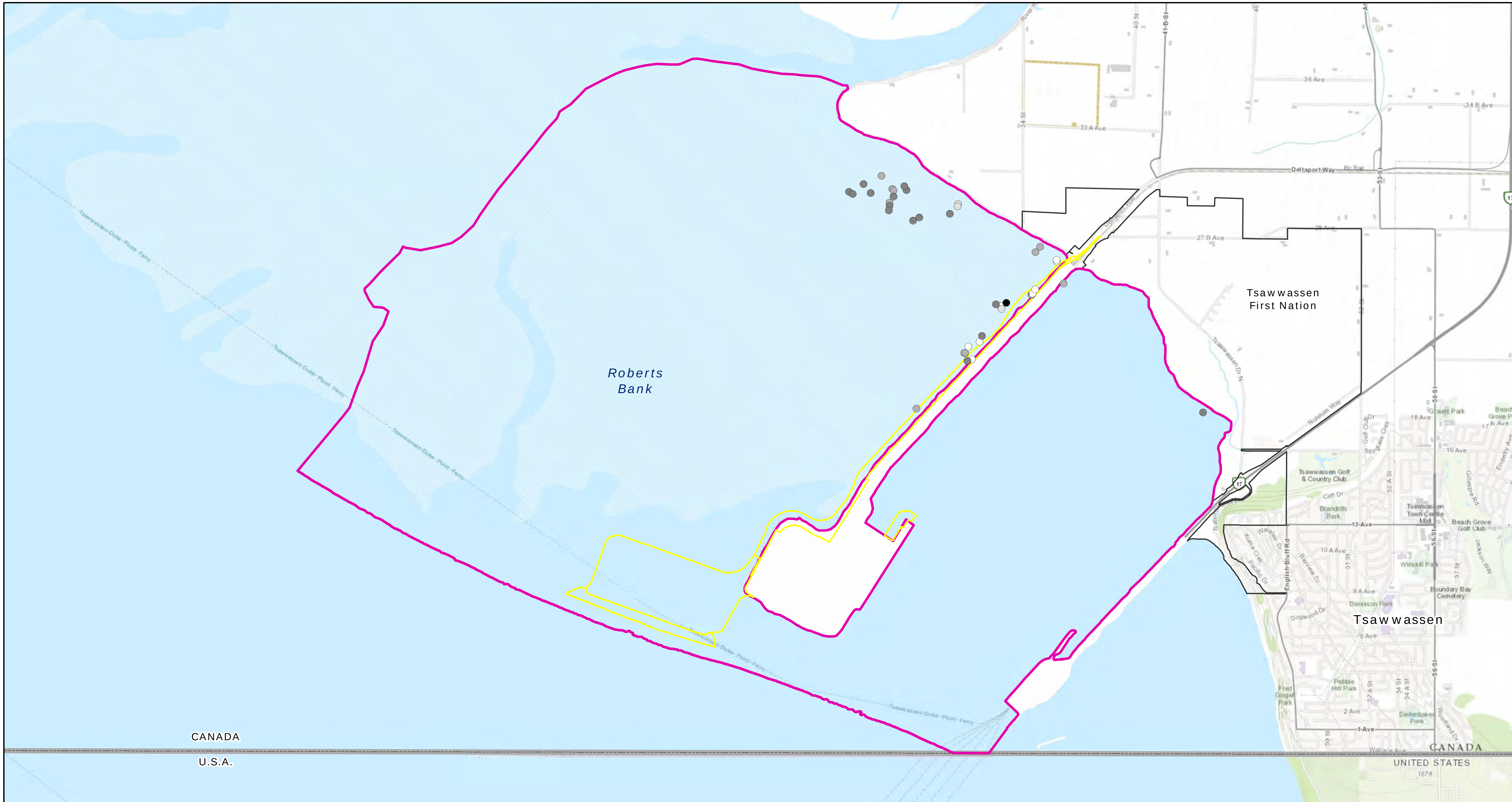
ROBERTS BANK TERMINAL 2

SPARTINA DISTRIBUTION WITHIN THE MARINE VEGETATION RAA 2007

DATE:	FIG No.
05/14/2018	IR8-09-A8

Basemap Sources: Esri, HERE, DeLorme, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, MapmyIndia, © OpenStreetMap contributors, and the GIS User Community; Imagery Source: BING Maps Aerial

Path: O:\1200-1246\EIS_INFORMATION_REQUESTS\IR8_09\mxd\IR8_09_Ax-odd-pages_SpartinaDistribution_LAA_180514.mxd



Legend

- BOUNDARY OF PROJECT AREA
- MARINE VEGETATION LAA
- CANADA-U.S.A. BORDER

SPARTINA PLANTS

- SINGLE PLANT
- < 0.3 m DIAMETER
- 0.3 - 1.0 m DIAMETER
- >1.0 m DIAMETER
- >5 m LARGE PATCH OF PLANTS

0 1 2
Kilometres
1:40,000
NAD 1983 UTM Zone 10N

N

Note:
Spartina Data provided by Ducks Unlimited Canada

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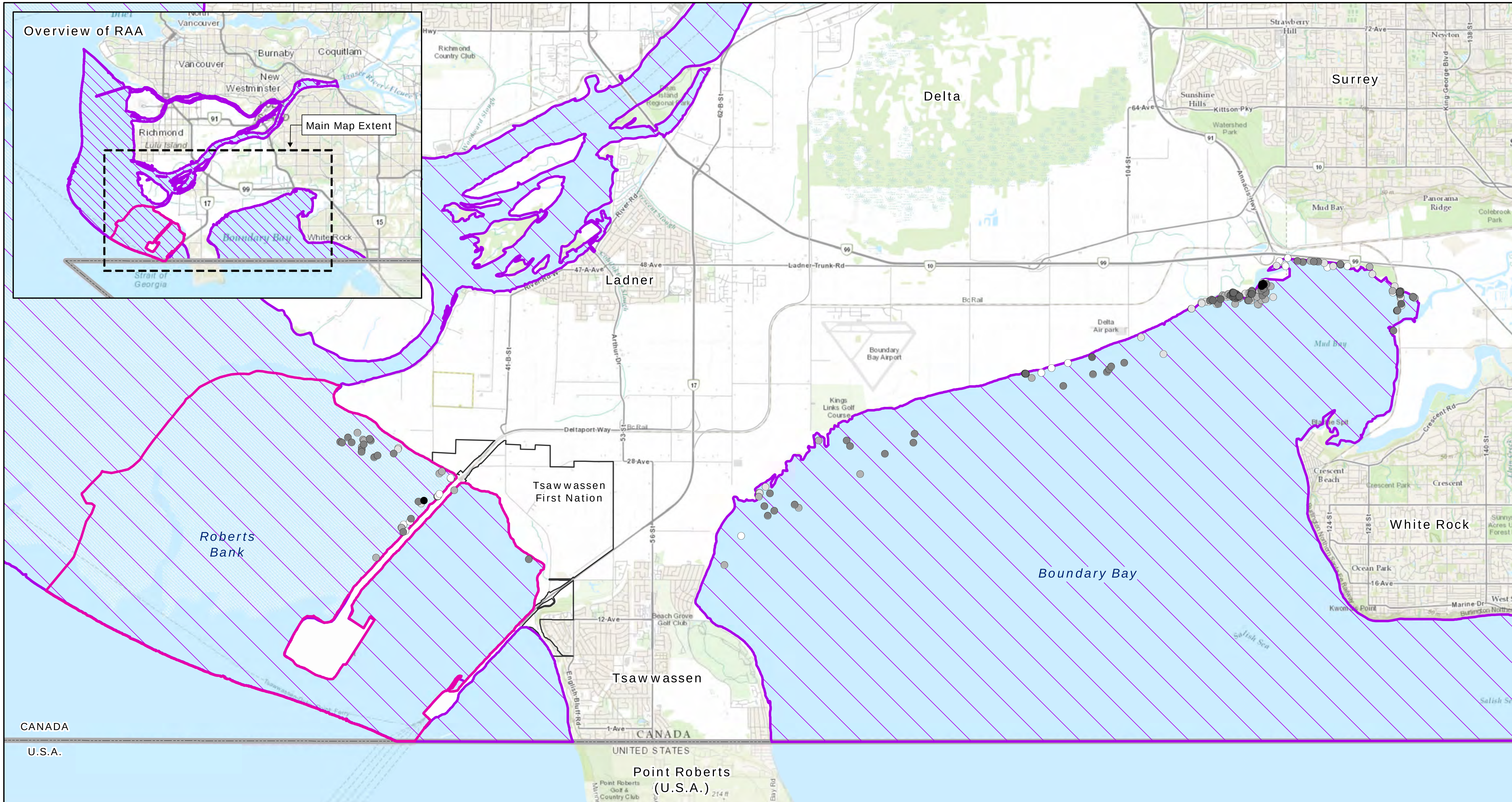
ROBERTS BANK TERMINAL 2

SPARTINA DISTRIBUTION WITHIN THE MARINE VEGETATION LAA 2008

DATE:	FIG No.
05/14/2018	IR8-09-A9

Basemap Sources: Esri, HERE, DeLorme, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, MapmyIndia, © OpenStreetMap contributors, and the GIS User Community; Imagery Source: BING Maps Aerial



Path: O:\11200-1246\EIS_INFORMATION_REQUESTS\IR8_09\mxd\IR8_09_Ax-even-pages_SpartinaDistribution_RAA_180514.mxd

Legend

- BOUNDARY OF PROJECT AREA
- MARINE VEGETATION LAA
- MARINE VEGETATION RAA
- CANADA-U.S.A. BORDER

SPARTINA PLANTS

- SINGLE PLANT
- < 0.3 m DIAMETER
- 0.3 - 1.0 m DIAMETER
- >1.0 m DIAMETER
- ~5 m PATCH OF PLANTS
- >5 m LARGE PATCH OF PLANTS

0 2 4
Kilometres
1:75,000
NAD 1983 UTM Zone 10N

Note:
Spartina Data provided by Ducks Unlimited Canada

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ROBERTS BANK TERMINAL 2

SPARTINA DISTRIBUTION WITHIN THE MARINE VEGETATION RAA 2008

DATE: 05/14/2018

FIG No. IR8-09-A10

Basemap Sources: Esri, HERE, DeLorme, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, MapmyIndia, © OpenStreetMap contributors, and the GIS User Community; Imagery Source: BING Maps Aerial

Legend

BOUNDARY OF PROJECT AREA	
MARINE VEGETATION LAA	
CANADA-U.S.A. BORDER	

SPARTINA PLANTS
- SINGLE PLANT
- < 0.3 m DIAMETER
- 0.3 - 1.0 m DIAMETER
- >1.0 m DIAMETER
- ~5 m PATCH OF PLANTS

0 1 2
Kilometres
1:40,000
NAD 1983 UTM Zone 10N

N

Note:
Spartina Data provided by Ducks Unlimited Canada

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ROBERTS BANK TERMINAL 2

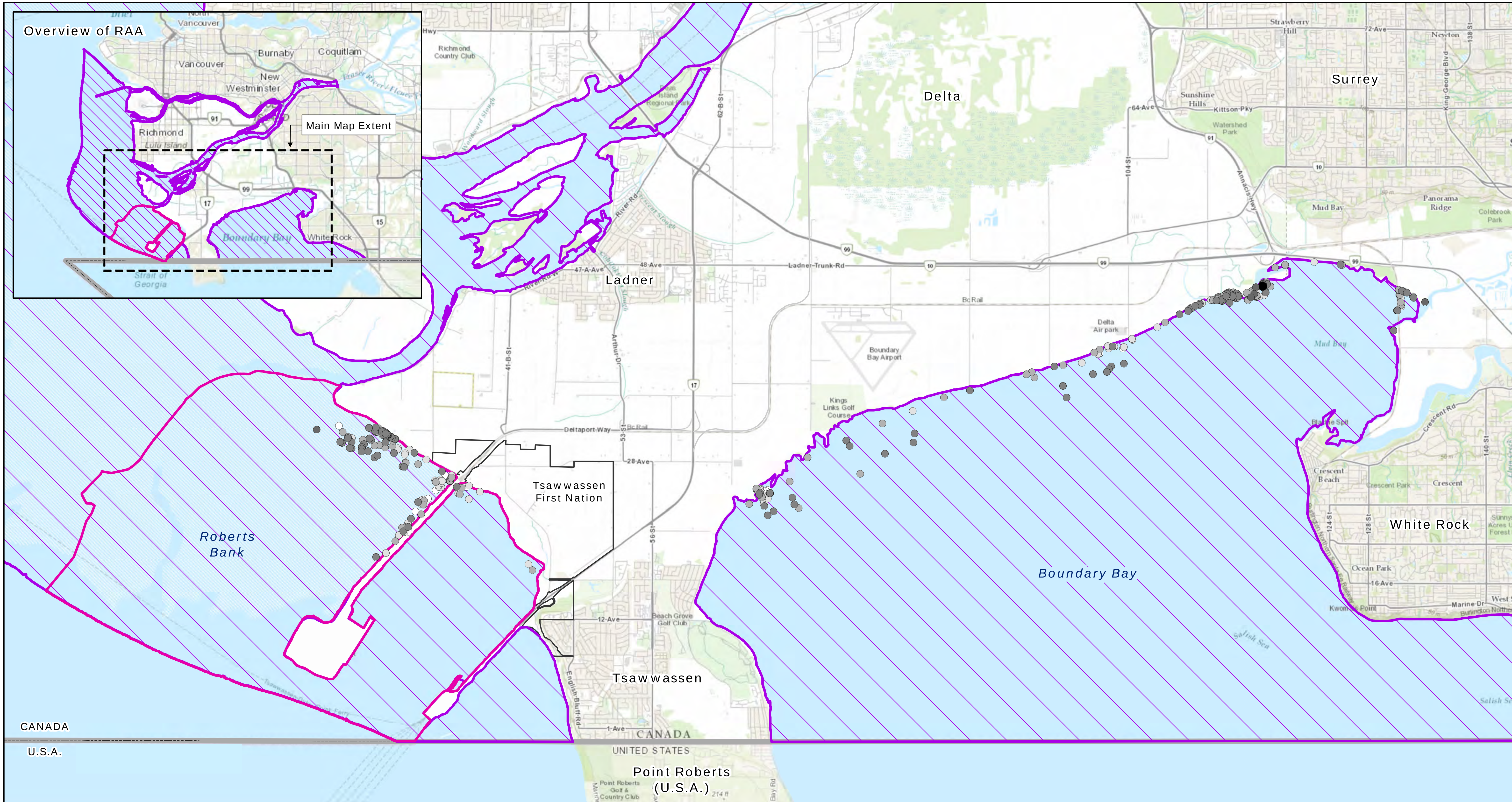
SPARTINA DISTRIBUTION
WITHIN THE MARINE VEGETATION LAA
2009

DATE: 05/14/2018

FIG No. IR8-09-A11

Basemap Sources: Esri, HERE, DeLorme, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, MapmyIndia, © OpenStreetMap contributors, and the GIS User Community; Imagery Source: BING Maps Aerial

Path: O:\!1200-1246\EIS_INFORMATION_REQUESTS\IR8_09\mxd\IR8_09_Ax-odd-pages_SpartinaDistribution_LAA_180514.mxd



Legend

- BOUNDARY OF PROJECT AREA
- MARINE VEGETATION LAA
- MARINE VEGETATION RAA
- CANADA-U.S.A. BORDER

SPARTINA PLANTS

- SINGLE PLANT
- < 0.3 m DIAMETER
- 0.3 - 1.0 m DIAMETER
- >1.0 m DIAMETER
- ~5 m PATCH OF PLANTS
- >5 m LARGE PATCH OF PLANTS

Note:
Spartina Data provided by Ducks Unlimited Canada

0 2 4
Kilometres
1:75,000
NAD 1983 UTM Zone 10N

N

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ROBERTS BANK TERMINAL 2

SPARTINA DISTRIBUTION WITHIN THE MARINE VEGETATION RAA 2009

DATE: 05/14/2018

FIG No. IR8-09-A12

Path: O:\1200-1246\EIS_INFORMATION_REQUESTS\IR8_09\mxd\IR8_09_Ax-odd-pages_SpartinaDistribution_LAA_180514.mxd

Legend

Yellow line	BOUNDARY OF PROJECT AREA
Magenta line	MARINE VEGETATION LAA
Grey line	CANADA-U.S.A. BORDER

SPARTINA PLANTS
- SINGLE PLANT
- < 0.3 m DIAMETER
- 0.3 - 1.0 m DIAMETER
- >1.0 m DIAMETER
- ~5 m PATCH OF PLANTS
- >5 m LARGE PATCH OF PLANTS

0 1 2
Kilometres
1:40,000
NAD 1983 UTM Zone 10N

N

Note:
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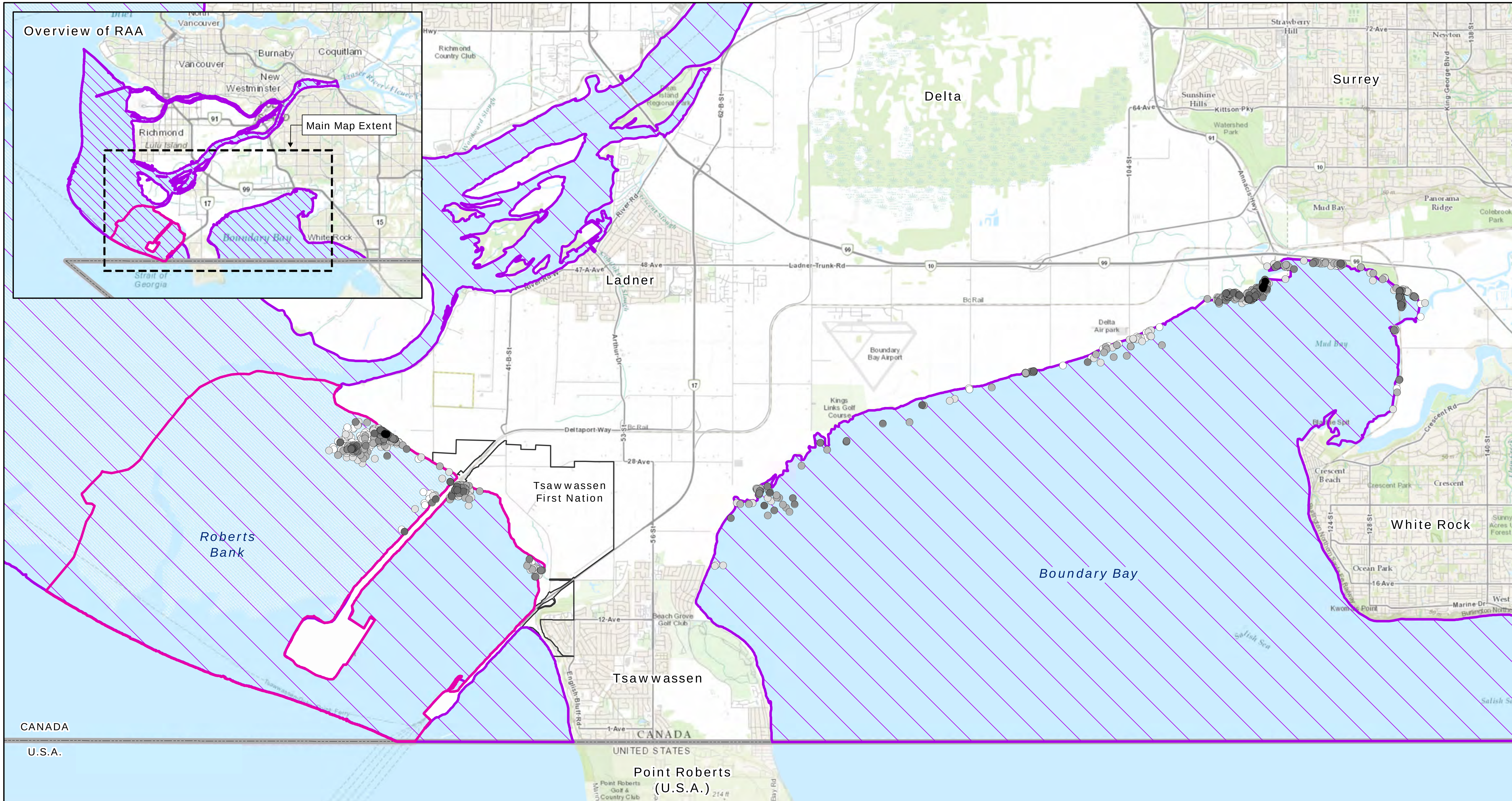
ROBERTS BANK TERMINAL 2

SPARTINA DISTRIBUTION WITHIN THE MARINE VEGETATION LAA 2010

DATE:	FIG No.
05/14/2018	IR8-09-A13

Basemap Sources: Esri, HERE, DeLorme, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, MapmyIndia, © OpenStreetMap contributors, and the GIS User Community; Imagery Source: BING Maps Aerial



Path: O:\1200-1246\EIS_INFORMATION_REQUESTS\IR8_09\mxd\IR8_09_Ax-even-pages_SpartinaDistribution_RAA_180514.mxd

Legend

- BOUNDARY OF PROJECT AREA
- MARINE VEGETATION LAA
- MARINE VEGETATION RAA
- CANADA-U.S.A. BORDER

SPARTINA PLANTS

- SINGLE PLANT
- < 0.3 m DIAMETER
- 0.3 - 1.0 m DIAMETER
- >1.0 m DIAMETER
- ~5 m PATCH OF PLANTS
- >5 m LARGE PATCH OF PLANTS

0 2 4
Kilometres
1:75,000
NAD 1983 UTM Zone 10N

N

Note:
Spartina Data provided by Ducks Unlimited Canada

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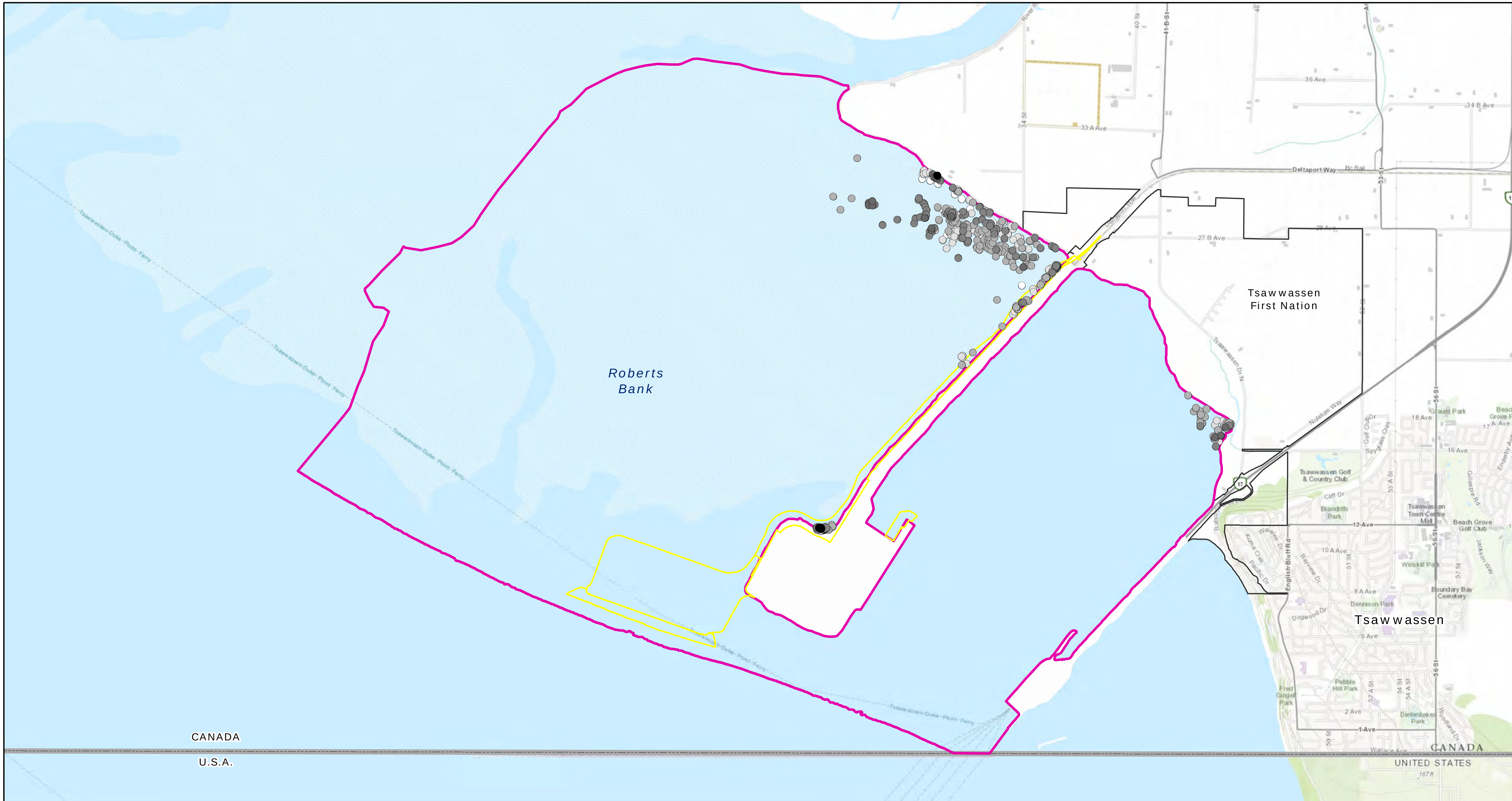
ROBERTS BANK TERMINAL 2

SPARTINA DISTRIBUTION WITHIN THE MARINE VEGETATION RAA 2010

DATE:	FIG No.
05/14/2018	IR8-09-A14

Basemap Sources: Esri, HERE, DeLorme, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, MapmyIndia, © OpenStreetMap contributors, and the GIS User Community; Imagery Source: BING Maps Aerial



Legend

BOUNDARY OF PROJECT AREA	
MARINE VEGETATION LAA	
CANADA-U.S.A. BORDER	

SPARTINA PLANTS
- SINGLE PLANT
- < 0.3 m DIAMETER
- 0.3 - 1.0 m DIAMETER
- >1.0 m DIAMETER
- ~5 m PATCH OF PLANTS
- >5 m LARGE PATCH OF PLANTS

0 1 2
Kilometres
1:40,000
NAD 1983 UTM Zone 10N

N

Note:
Spartina Data provided by Ducks Unlimited Canada

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ROBERTS BANK TERMINAL 2

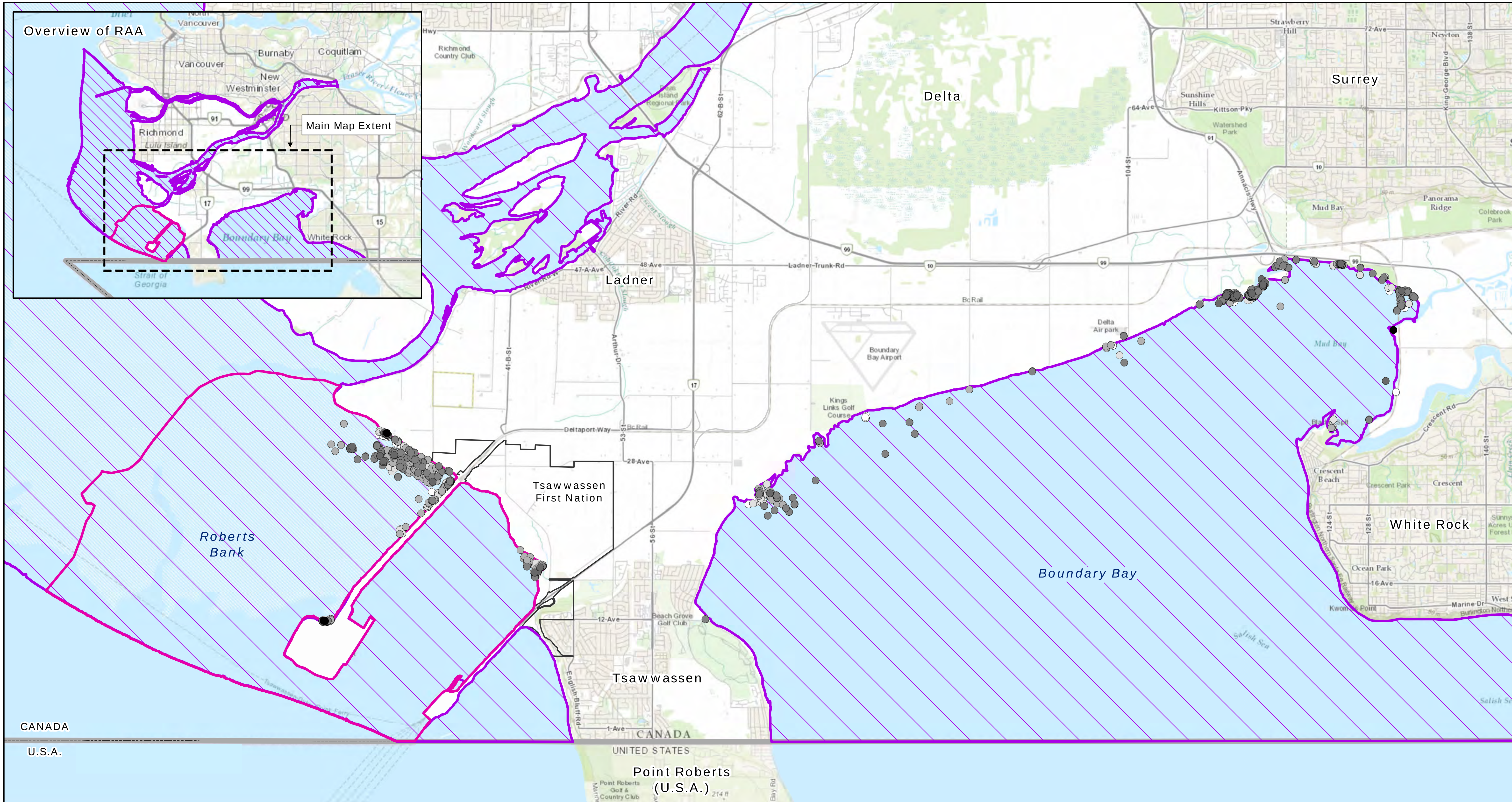
SPARTINA DISTRIBUTION
WITHIN THE MARINE VEGETATION LAA
2011

DATE:	FIG No.
05/14/2018	IR8-09-A15

Basemap Sources: Esri, HERE, DeLorme, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, MapmyIndia, © OpenStreetMap contributors, and the GIS User Community; Imagery Source: BING Maps Aerial

Path: O:\1200-1246\EIS_INFORMATION_REQUESTS\IR8_09\mxd\IR8_09_Ax-odd-pages_SpartinaDistribution_LAA_180514.mxd

Path: O:\11200-\1246\EIS_INFORMATION_REQUESTS\IR8_09\mxd\IR8_09_Ax-even-pages_SpartinaDistribution_RAA_180514.mxd



Legend

- BOUNDARY OF PROJECT AREA
- MARINE VEGETATION LAA
- MARINE VEGETATION RAA
- CANADA-U.S.A. BORDER

SPARTINA PLANTS

- SINGLE PLANT
- < 0.3 m DIAMETER
- 0.3 - 1.0 m DIAMETER
- >1.0 m DIAMETER
- ~5 m PATCH OF PLANTS
- >5 m LARGE PATCH OF PLANTS

Note:
Spartina Data provided by Ducks Unlimited Canada

0 2 4
Kilometres
1:75,000
NAD 1983 UTM Zone 10N

N

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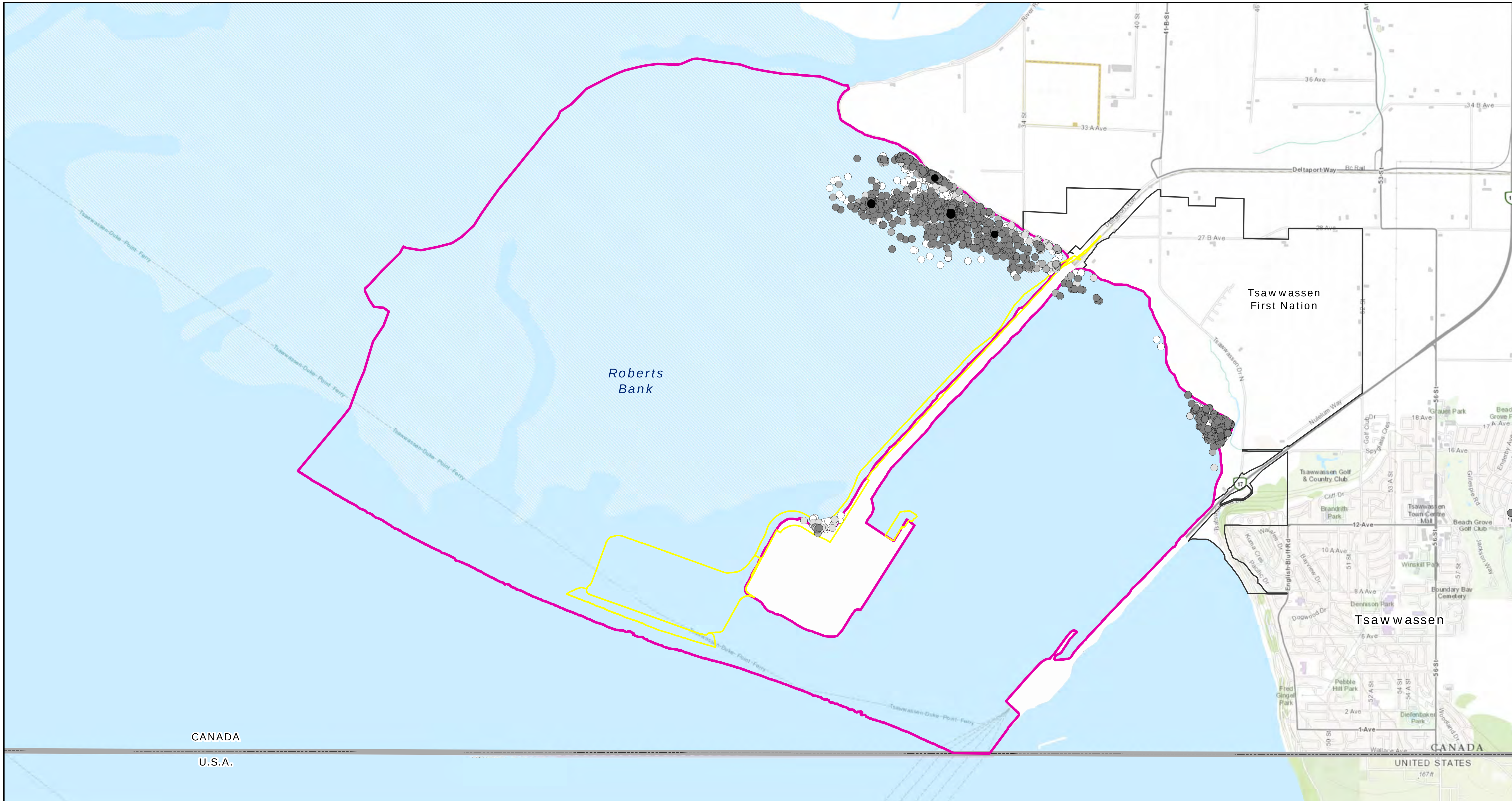
ROBERTS BANK TERMINAL 2

SPARTINA DISTRIBUTION WITHIN THE MARINE VEGETATION RAA 2011

DATE:	FIG No.
05/14/2018	IR8-09-A16

Basemap Sources: Esri, HERE, DeLorme, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, MapmyIndia, © OpenStreetMap contributors, and the GIS User Community; Imagery Source: BING Maps Aerial

Path: O:\1200-1246\EIS_INFORMATION_REQUESTS\IR8_09\mxd\IR8_09_Ax-odd-pages_SpartinaDistribution_LAA_180514.mxd



Legend

BOUNDARY OF PROJECT AREA

MARINE VEGETATION LAA

CANADA-U.S.A. BORDER

SPARTINA PLANTS

- SINGLE PLANT
- < 0.3 m DIAMETER
- 0.3 - 1.0 m DIAMETER
- >1.0 m DIAMETER
- ~5 m PATCH OF PLANTS
- >5 m LARGE PATCH OF PLANTS

0 1 2
Kilometres
1:40,000
NAD 1983 UTM Zone 10N

Note:
Spartina Data provided by Ducks Unlimited Canada

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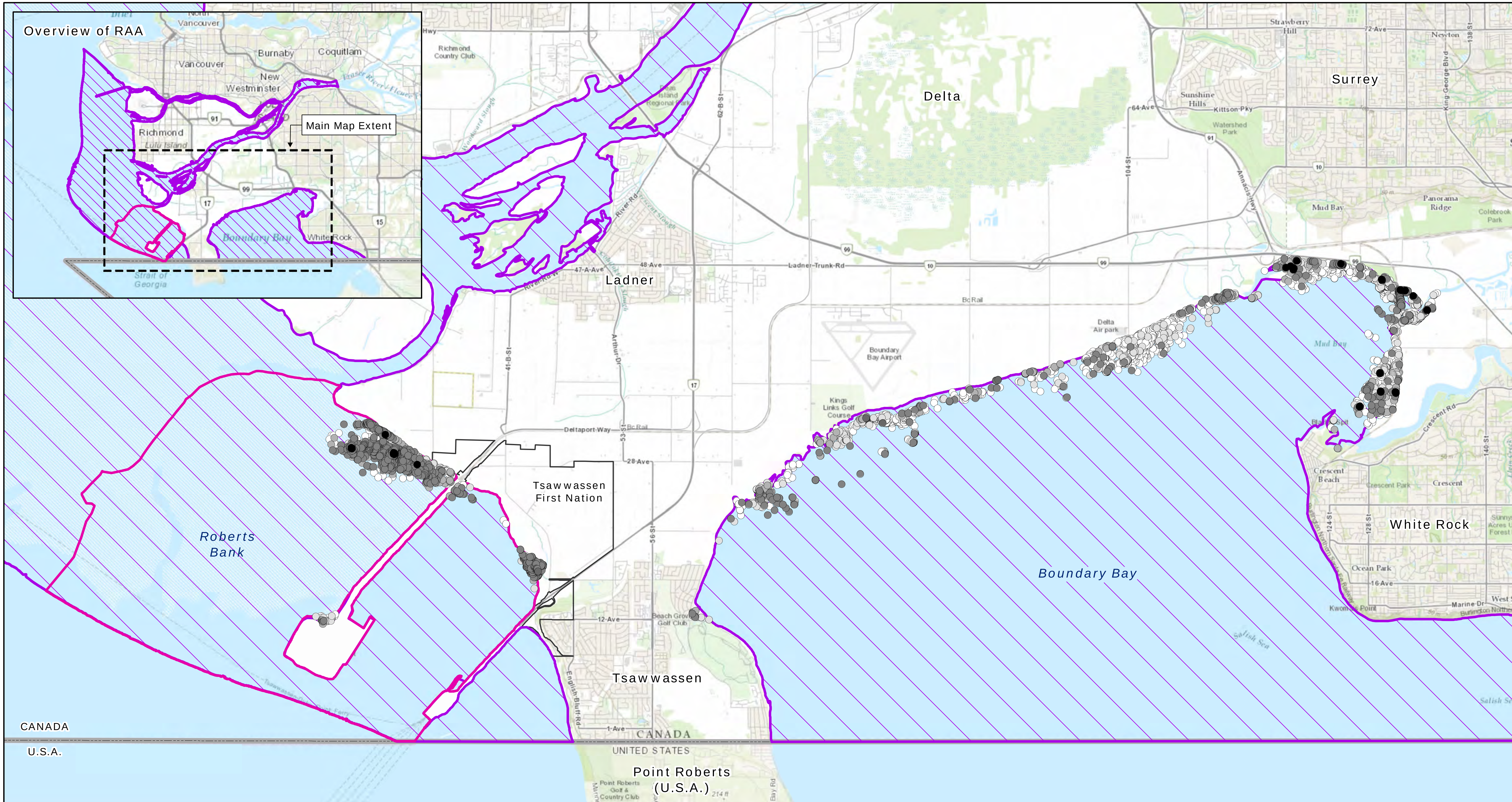
ROBERTS BANK TERMINAL 2

SPARTINA DISTRIBUTION WITHIN THE MARINE VEGETATION LAA 2012

DATE:	FIG No.
05/14/2018	IR8-09-A17

Basemap Sources: Esri, HERE, DeLorme, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, MapmyIndia, © OpenStreetMap contributors, and the GIS User Community; Imagery Source: BING Maps Aerial

Path: O:\11200-\1246\EIS_INFORMATION_REQUESTS\IR8_09\mxd\IR8_09_Ax-even-pages_SpartinaDistribution_RAA_180514.mxd



Legend

- BOUNDARY OF PROJECT AREA
- MARINE VEGETATION LAA
- MARINE VEGETATION RAA
- CANADA-U.S.A. BORDER

SPARTINA PLANTS

- SINGLE PLANT
- < 0.3 m DIAMETER
- 0.3 - 1.0 m DIAMETER
- >1.0 m DIAMETER
- ~5 m PATCH OF PLANTS
- >5 m LARGE PATCH OF PLANTS

0 2 4
Kilometres
1:75,000
NAD 1983 UTM Zone 10N

Note:
Spartina Data provided by Ducks Unlimited Canada

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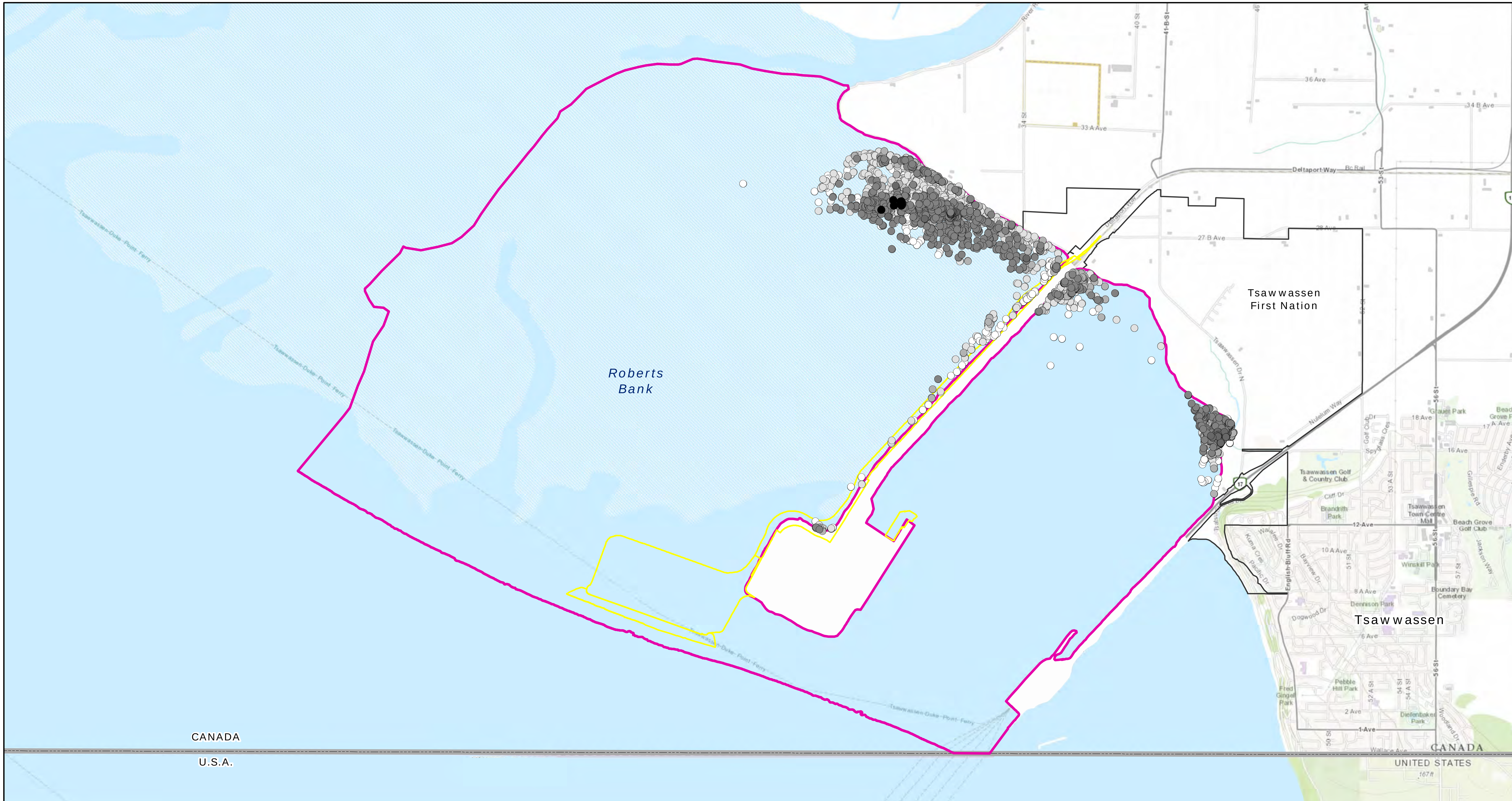
ROBERTS BANK TERMINAL 2

SPARTINA DISTRIBUTION WITHIN THE MARINE VEGETATION RAA 2012

DATE:	FIG No.
05/14/2018	IR8-09-A18

Basemap Sources: Esri, HERE, DeLorme, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, MapmyIndia, © OpenStreetMap contributors, and the GIS User Community; Imagery Source: BING Maps Aerial



Legend

- BOUNDARY OF PROJECT AREA
- MARINE VEGETATION LAA
- CANADA-U.S.A. BORDER

SPARTINA PLANTS

- SINGLE PLANT
- < 0.3 m DIAMETER
- 0.3 - 1.0 m DIAMETER
- >1.0 m DIAMETER
- ~5 m PATCH OF PLANTS
- >5 m LARGE PATCH OF PLANTS

0 1 2
Kilometres
1:40,000
NAD 1983 UTM Zone 10N

N

Note:
Spartina Data provided by Ducks Unlimited Canada

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ROBERTS BANK TERMINAL 2

SPARTINA DISTRIBUTION WITHIN THE MARINE VEGETATION LAA 2013

DATE:	FIG No.
05/14/2018	IR8-09-A19

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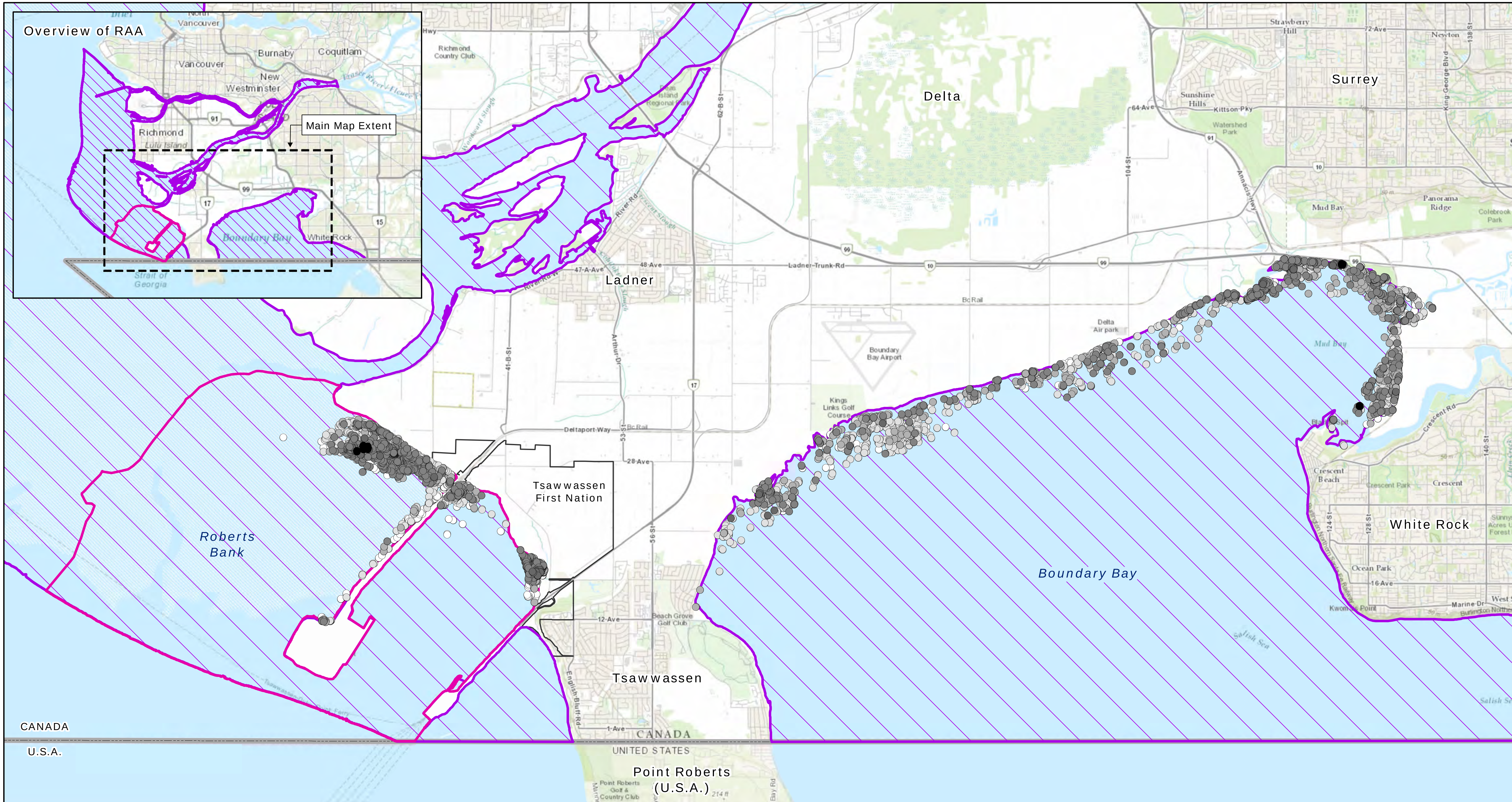

Legend

- BOUNDARY OF PROJECT AREA
- MARINE VEGETATION LAA
- MARINE VEGETATION RAA
- CANADA-U.S.A. BORDER

SPARTINA PLANTS

- SINGLE PLANT
- < 0.3 m DIAMETER
- 0.3 - 1.0 m DIAMETER
- >1.0 m DIAMETER
- ~5 m PATCH OF PLANTS
- >5 m LARGE PATCH OF PLANTS

Note:
Spartina Data provided by Ducks Unlimited Canada

0 2 4
Kilometres
1:75,000
NAD 1983 UTM Zone 10N

N

PORT of vancouver

ROBERTS BANK TERMINAL 2

SPARTINA DISTRIBUTION WITHIN THE MARINE VEGETATION RAA 2013

DATE:	FIG No.
05/14/2018	IR8-09-A20

Basemap Sources: Esri, HERE, DeLorme, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, MapmyIndia, © OpenStreetMap contributors, and the GIS User Community; Imagery Source: BING Maps Aerial

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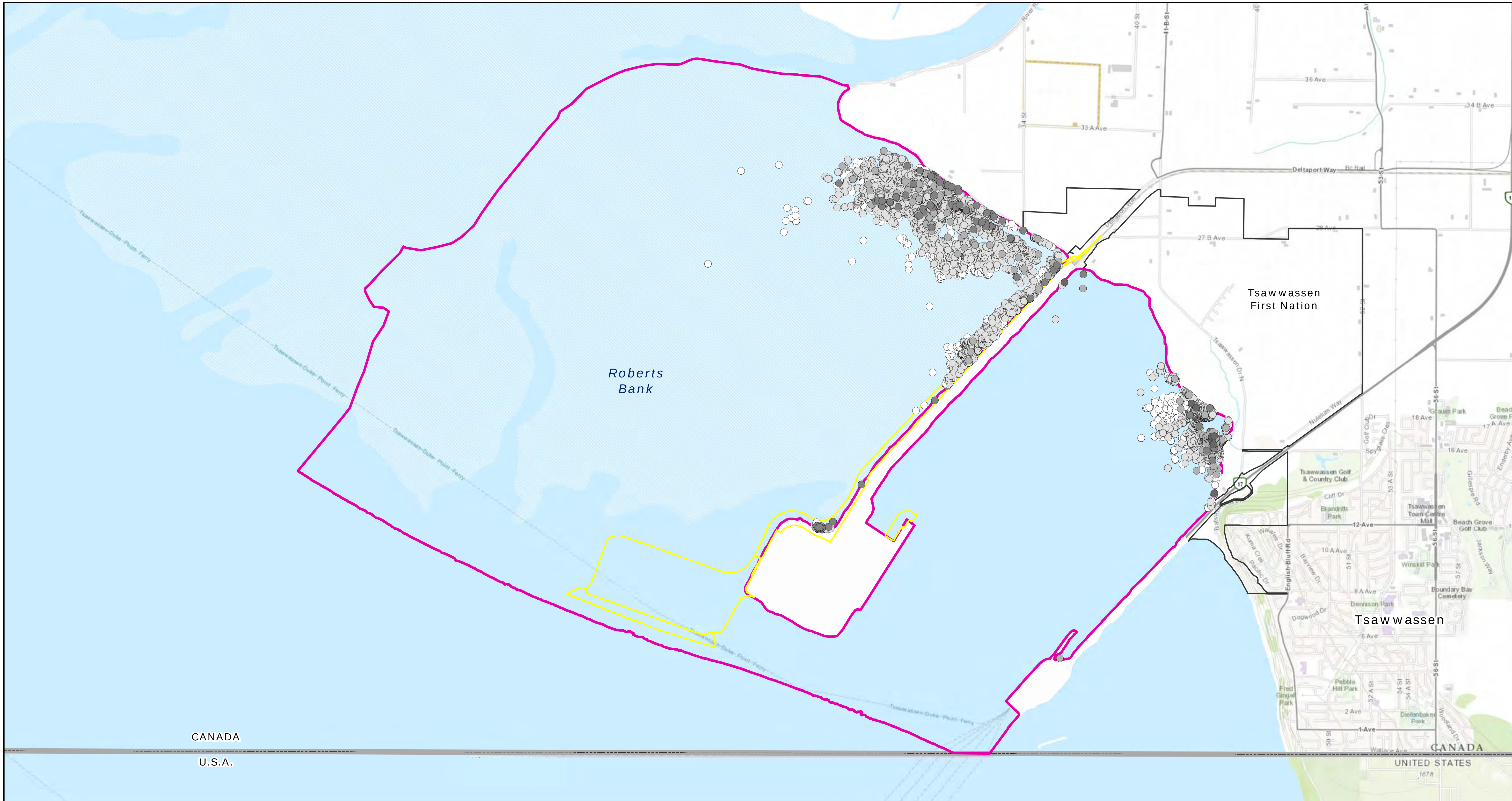

Legend

- BOUNDARY OF PROJECT AREA
- MARINE VEGETATION LAA
- CANADA-U.S.A. BORDER

SPARTINA PLANTS

- SINGLE PLANT
- < 0.3 m DIAMETER
- 0.3 - 1.0 m DIAMETER
- >1.0 m DIAMETER
- ~5 m PATCH OF PLANTS

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Kilometres

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NAD 1983 UTM Zone 10N

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Note:
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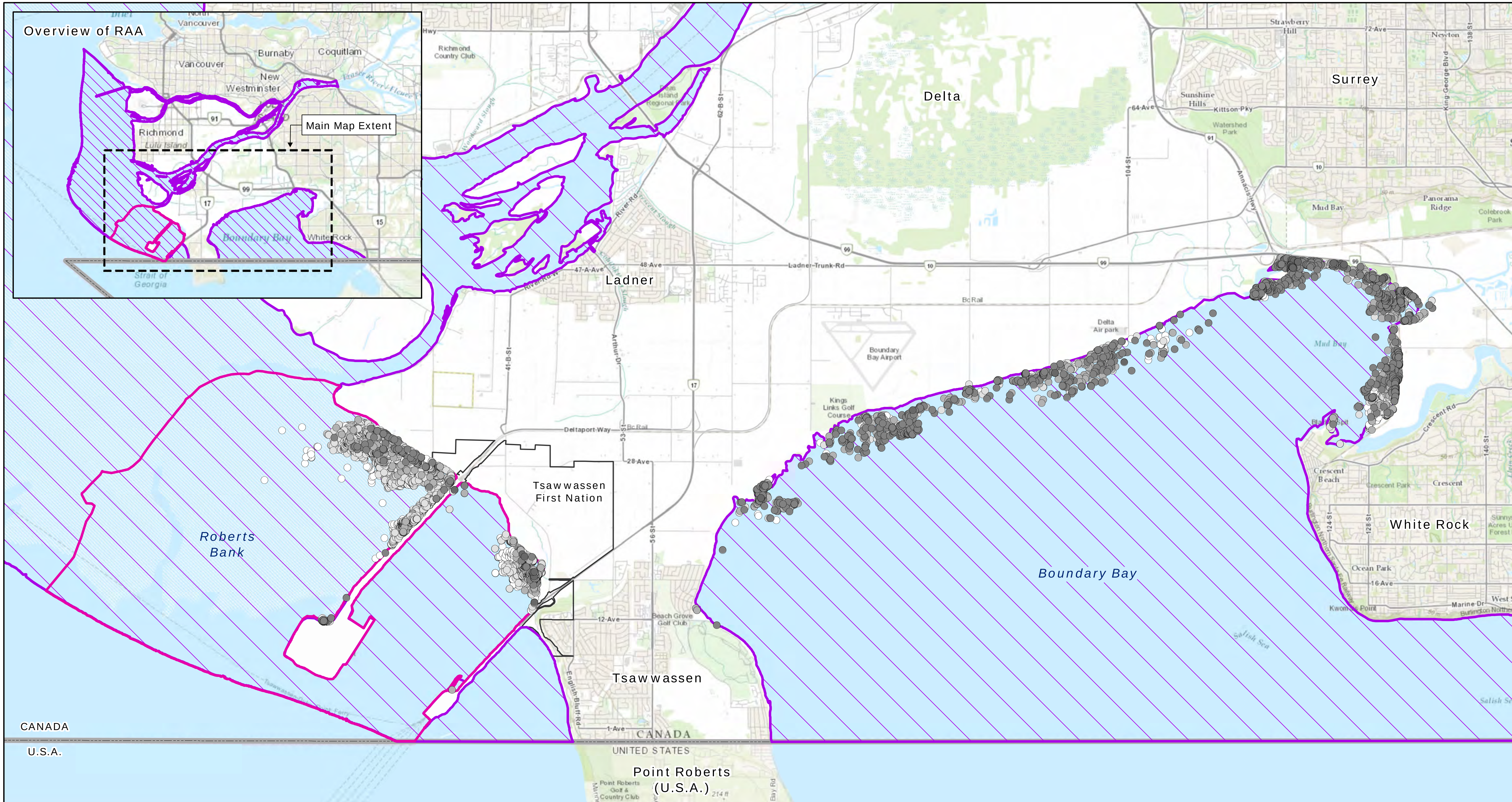
ROBERTS BANK TERMINAL 2

SPARTINA DISTRIBUTION WITHIN THE MARINE VEGETATION LAA 2014

DATE:	FIG No.
05/14/2018	IR8-09-A21

Basemap Sources: Esri, HERE, DeLorme, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, MapmyIndia, © OpenStreetMap contributors, and the GIS User Community; Imagery Source: BING Maps Aerial

Path: O:\11200-\1246\EIS_INFORMATION_REQUESTS\IR8_09\mxd\IR8_09_Ax-even-pages_SpartinaDistribution_RAA_180514.mxd



Legend

- BOUNDARY OF PROJECT AREA
- MARINE VEGETATION LAA
- MARINE VEGETATION RAA
- CANADA-U.S.A. BORDER

SPARTINA PLANTS
- SINGLE PLANT
- < 0.3 m DIAMETER
- 0.3 - 1.0 m DIAMETER
- >1.0 m DIAMETER
- ~5 m PATCH OF PLANTS

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Kilometres
1:75,000
NAD 1983 UTM Zone 10N

Note:
Spartina Data provided by Ducks Unlimited Canada

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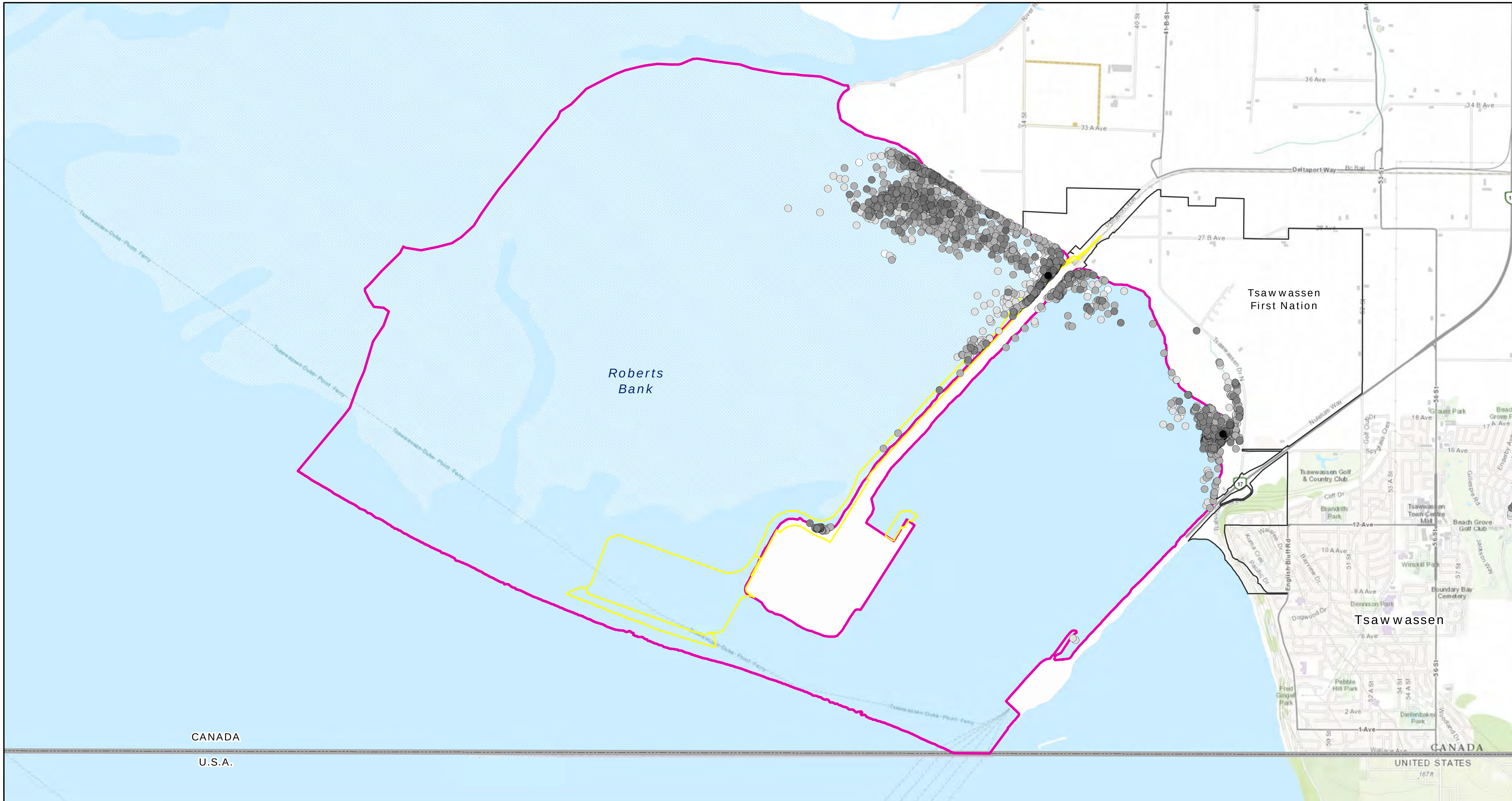
ROBERTS BANK TERMINAL 2

SPARTINA DISTRIBUTION WITHIN THE MARINE VEGETATION RAA 2014

DATE:	FIG No.
05/14/2018	IR8-09-A22

Basemap Sources: Esri, HERE, DeLorme, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, MapmyIndia, © OpenStreetMap contributors, and the GIS User Community; Imagery Source: BING Maps Aerial

Path: O:\1200-1246\EIS_INFORMATION_REQUESTS\IR8_09\mxd\IR8_09_Ax-odd-pages_SpartinaDistribution_LAA_180514.mxd



Legend

- BOUNDARY OF PROJECT AREA
- MARINE VEGETATION LAA
- CANADA-U.S.A. BORDER

SPARTINA PLANTS

- SINGLE PLANT
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- 0.3 - 1.0 m DIAMETER
- >1.0 m DIAMETER
- ~5 m PATCH OF PLANTS
- >5 m LARGE PATCH OF PLANTS

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Kilometres
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NAD 1983 UTM Zone 10N

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Note:
Spartina Data provided by Ducks Unlimited Canada

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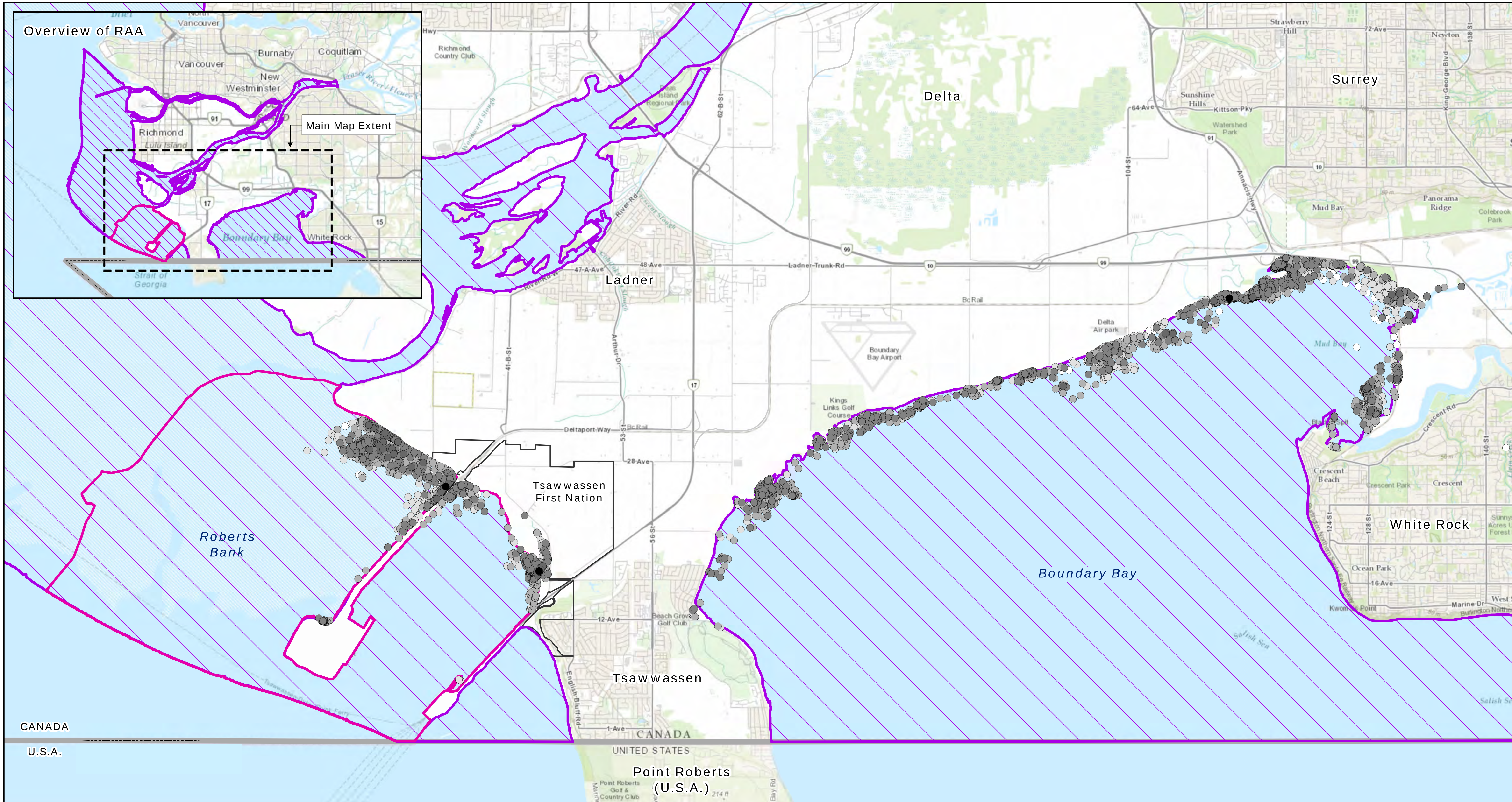
ROBERTS BANK TERMINAL 2

SPARTINA DISTRIBUTION WITHIN THE MARINE VEGETATION LAA 2015

DATE:	FIG No.
05/14/2018	IR8-09-A23

Basemap Sources: Esri, HERE, DeLorme, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, MapmyIndia, © OpenStreetMap contributors, and the GIS User Community; Imagery Source: BING Maps Aerial

Path: O:\1200-\1246\EIS_INFORMATION_REQUESTS\IR8_09\mxd\IR8_09_Ax-even-pages_SpartinaDistribution_RAA_180514.mxd



Legend

- BOUNDARY OF PROJECT AREA
- MARINE VEGETATION LAA
- MARINE VEGETATION RAA
- CANADA-U.S.A. BORDER

SPARTINA PLANTS
- SINGLE PLANT
- < 0.3 m DIAMETER
- 0.3 - 1.0 m DIAMETER
- >1.0 m DIAMETER
- ~5 m PATCH OF PLANTS
- >5 m LARGE PATCH OF PLANTS

0 2 4
Kilometres
1:75,000
NAD 1983 UTM Zone 10N

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Note:
Spartina Data provided by Ducks Unlimited Canada

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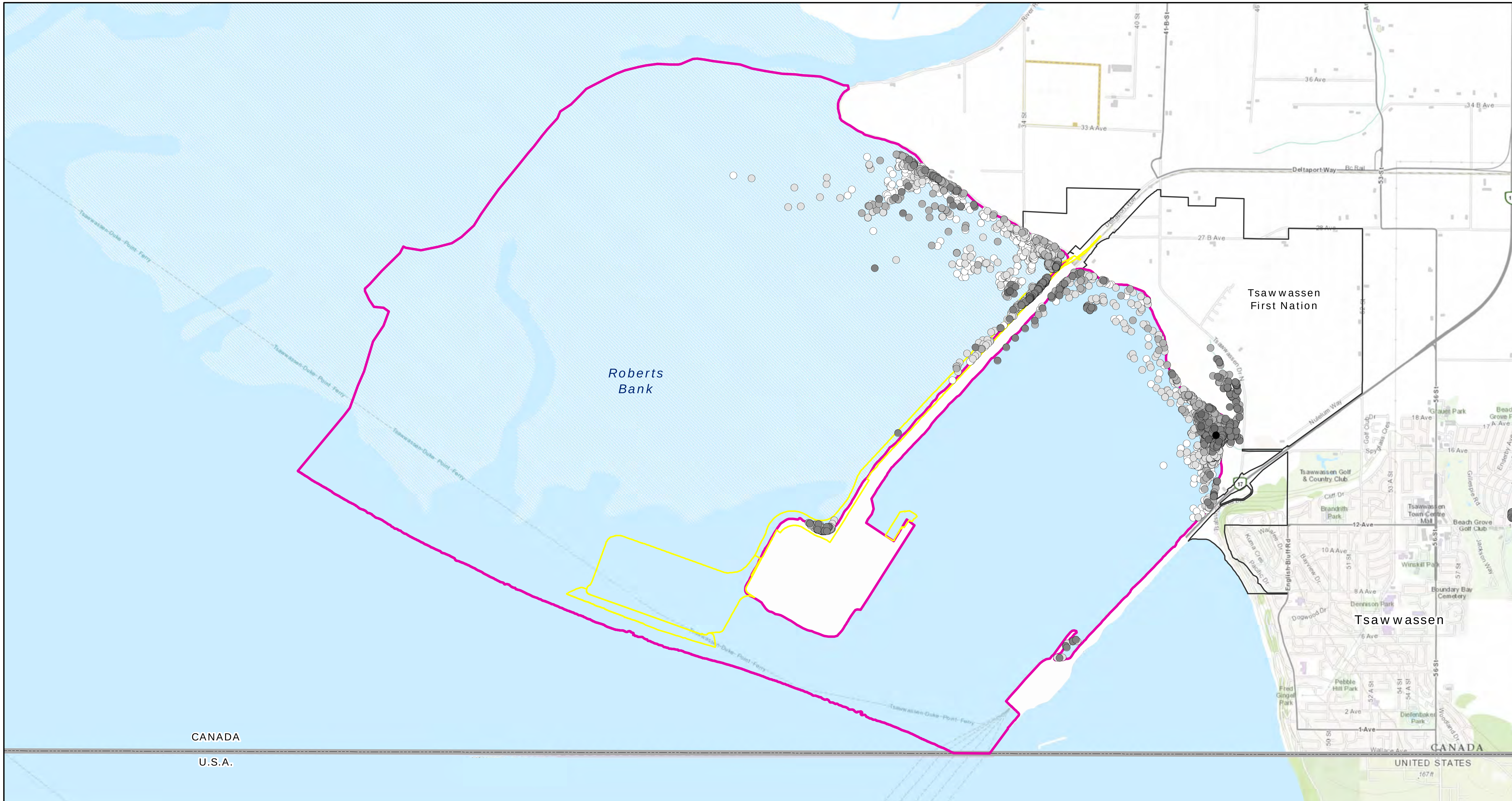
ROBERTS BANK TERMINAL 2

SPARTINA DISTRIBUTION WITHIN THE MARINE VEGETATION RAA 2015

DATE:	FIG No.
05/14/2018	IR8-09-A24

Basemap Sources: Esri, HERE, DeLorme, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, MapmyIndia, © OpenStreetMap contributors, and the GIS User Community; Imagery Source: BING Maps Aerial

Path: O:\1200-1246\EIS_INFORMATION_REQUESTS\IR8_09\mxd\IR8_09_Ax-odd-pages_SpartinaDistribution_LAA_180514.mxd



Legend

- BOUNDARY OF PROJECT AREA
- MARINE VEGETATION LAA
- CANADA-U.S.A. BORDER

SPARTINA PLANTS
- SINGLE PLANT
- < 0.3 m DIAMETER
- 0.3 - 1.0 m DIAMETER
- >1.0 m DIAMETER
- ~5 m PATCH OF PLANTS
- >5 m LARGE PATCH OF PLANTS

0 1 2
Kilometres
1:40,000
NAD 1983 UTM Zone 10N

N

Note:
Spartina Data provided by Ducks Unlimited Canada

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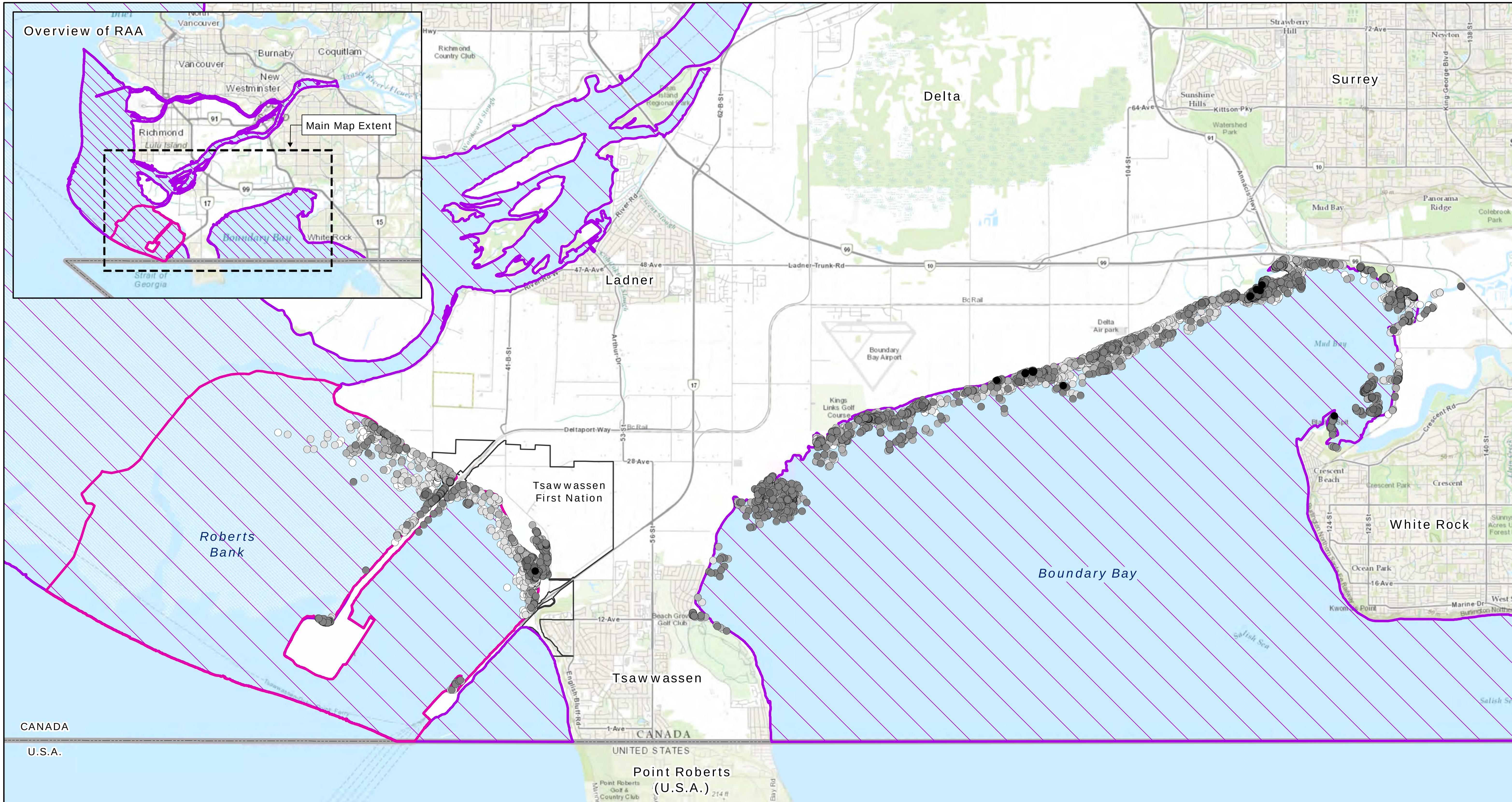
ROBERTS BANK TERMINAL 2

SPARTINA DISTRIBUTION WITHIN THE MARINE VEGETATION LAA 2016

DATE:	FIG No.
05/14/2018	IR8-09-A25

Basemap Sources: Esri, HERE, DeLorme, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, MapmyIndia, © OpenStreetMap contributors, and the GIS User Community; Imagery Source: BING Maps Aerial



Legend

BOUNDARY OF PROJECT AREA

MARINE VEGETATION LAA

MARINE VEGETATION RAA

CANADA-U.S.A. BORDER

SPARTINA PLANTS

- SINGLE PLANT
- < 0.3 m DIAMETER
- 0.3 - 1.0 m DIAMETER
- >1.0 m DIAMETER
- ~5 m PATCH OF PLANTS
- >5 m LARGE PATCH OF PLANTS

Note:
Spartina Data provided by Ducks Unlimited Canada

0 2 4
Kilometres
1:75,000
NAD 1983 UTM Zone 10N

N

PORT of vancouver

ROBERTS BANK TERMINAL 2

SPARTINA DISTRIBUTION WITHIN THE MARINE VEGETATION RAA 2016

DATE:	FIG No.
05/14/2018	IR8-09-A26

Path: O:\1200-1246\EIS_INFORMATION_REQUESTS\IR8_09\mxd\IR8_09_Ax-odd-pages_SpartinaDistribution_LAA_180514.mxd

Legend

Yellow outline	BOUNDARY OF PROJECT AREA
Magenta outline	MARINE VEGETATION LAA
Grey line	CANADA-U.S.A. BORDER

SPARTINA PLANTS

- SINGLE PLANT
- < 0.3 m DIAMETER
- 0.3 - 1.0 m DIAMETER
- >1.0 m DIAMETER
- ~5 m PATCH OF PLANTS
- >5 m LARGE PATCH OF PLANTS

0 1 2
Kilometres
1:40,000
NAD 1983 UTM Zone 10N

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Note:
Spartina Data provided by Ducks Unlimited Canada

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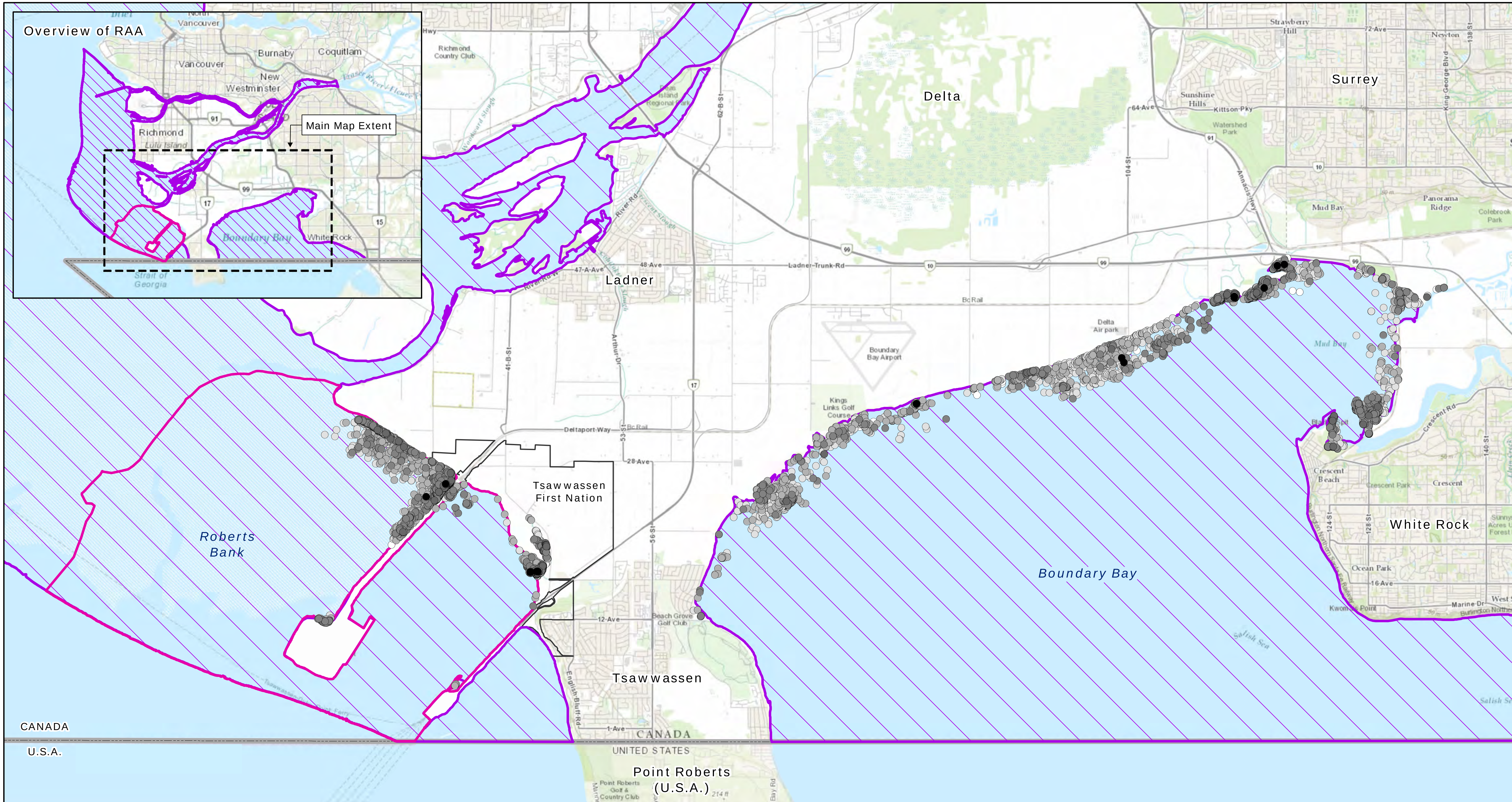
ROBERTS BANK TERMINAL 2

SPARTINA DISTRIBUTION WITHIN THE MARINE VEGETATION LAA 2017

DATE:	FIG No.
05/14/2018	IR8-09-A27

Basemap Sources: Esri, HERE, DeLorme, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, MapmyIndia, © OpenStreetMap contributors, and the GIS User Community; Imagery Source: BING Maps Aerial

Path: O:\11200-11246\EIS_INFORMATION_REQUESTS\IR8_09\mxd\IR8_09_Ax-even-pages_SpartinaDistribution_RAA_180514.mxd



Legend

- BOUNDARY OF PROJECT AREA
- MARINE VEGETATION LAA
- MARINE VEGETATION RAA
- CANADA-U.S.A. BORDER

SPARTINA PLANTS

- SINGLE PLANT
- < 0.3 m DIAMETER
- 0.3 - 1.0 m DIAMETER
- >1.0 m DIAMETER
- ~5 m PATCH OF PLANTS
- >5 m LARGE PATCH OF PLANTS

0 2 4
Kilometres
1:75,000
NAD 1983 UTM Zone 10N

Note:
Spartina Data provided by Ducks Unlimited Canada

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ROBERTS BANK TERMINAL 2

SPARTINA DISTRIBUTION WITHIN THE MARINE VEGETATION RAA 2017

DATE: 05/14/2018

FIG No. IR8-09-A28

Basemap Sources: Esri, HERE, DeLorme, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, MapmyIndia, © OpenStreetMap contributors, and the GIS User Community; Imagery Source: BING Maps Aerial

IR8-10 Outdoor Recreation – Changes to Environmental Setting

Information Source(s)

EIS Volume 2: Section 8.1.5, Section 9.3.11

EIS Volume 4: Sections 24.6.1 to 24.6.4, Table 24-1, Table 24-5, Table 24-6, Section 25.6.1

Context

In Table 24-5 and 24-6 of the EIS, the Proponent described potential interactions of the proposed Project with outdoor recreation for the construction and operation phases, respectively. The Proponent determined that for all outdoor recreation sub-components identified in Table 24-1 of the EIS, predicted changes in noise and visual resources would not alter the character of the environmental setting at locations where activities would take place.

Further, the Proponent indicated that the change to the quality of experience for all outdoor recreation sub-components would be negligible, and therefore did not carry these interactions forward to an assessment of potential effects. In the subsequent sections (Section 24.6.1 to 24.6.4), the Proponent provided a rationale for why the effect was considered to be negligible. For example, regarding recreational boating in Section 24.6.1, the Proponent indicated that although perceptible changes to visual and noise conditions are anticipated during Project construction and operation, future conditions with the Project would be consistent with the industrialized character of the existing environmental setting at Roberts Bank. Similar explanations were provided for the other outdoor recreation sub-components.

However, as stated by the Proponent in Section 8.1.5 of the EIS, all interactions between the Project and valued components that have the potential to result in measurable adverse effects prior to mitigation would be identified and an effects assessment would be carried out. In Section 25.6.1 of the EIS, the Proponent indicated that there would be a potential change to daytime visual resources and carried this effect forward to an effects assessment. As stated by the Proponent in Section 9.3.11, the Project is expected to increase noise levels within the local study area to varying degrees during construction and operation. Since noise and visual resources are key parameters that contribute to the environmental setting indicator used to assess effects on outdoor recreation, it is unclear why changes to the environmental setting were considered to be negligible for all outdoor recreation sub-components if there were measurable changes to both noise and daytime visual resources predicted. An effects assessment should be carried out for all outdoor recreation sub-components that may experience a measurable change in environmental setting.

Information Request

Carry out an effects assessment for all outdoor recreation sub-components that may experience a measurable change in environmental setting (i.e. noise and visual resources) as a result of the proposed Project which includes:

- a description of mitigation measures that would be applied to mitigate the effects of the Project on outdoor recreation sub-components;
- an assessment of the residual effects after the implementation of the above mitigation measures; and
- if there are residual effects predicted, provide a characterization of the effect and a prediction of the significance of those effects.

VFPA Response

An effects assessment on all outdoor recreation sub-components of the environmental setting, including from a change in visual resources and noise, has been carried out as described in EIS Section 24.0. This response clarifies why Project effects on the environmental setting for outdoor recreation were appropriately identified as negligible, even though measurable residual effects on visual resources and changes in noise levels (the key parameters that contribute to the environmental setting indicator for outdoor recreation) were identified.

Rationale for Negligible Effects Determination on Outdoor Recreation Environmental Setting

A residual measurable Project effect on visual resources (as described in EIS Sections 25.8.1 and 25.8.2) and changes in noise conditions (as outlined in EIS Section 9.3.9) do not directly translate to a measurable effect on outdoor recreation through changes in quality of environmental setting. The indicators for the assessment of effects on outdoor recreation, via the quality of environmental setting effect pathway, were different from the assessment indicators for visual resources (valued component) and noise (intermediate component).

For the visual resources pathway, the qualitative assessment of effects on the quality of environmental setting for outdoor recreation was based on anticipated residual effects on the *existing landscape character and quality of daytime and nighttime visual resources*. In contrast, the visual resources assessment was based on changes to indicators of visual quality class (daytime), and changes to light trespass and sky glow classifications (nighttime). Effects on visual resources as described in EIS Sections 25.8.1 and 25.8.2 were characterised based on visual alterations that would occur in the local assessment area (LAA) as a result of changes in these indicators. Similarly, for the noise pathway, the assessment of effects on the environmental setting for outdoor recreation was based on a qualitative assessment of Project-related changes to the overall characteristic of the noise environment. In contrast, the noise assessment was based on changes to quantitative indicators of various noise parameters[1].

Considering the assessment indicator framework described above, the qualitative assessment of Project effects on the quality of environmental setting for outdoor recreation for all sub-components, and specifically the identification of a negligible Project effect for this pathway, was based on the following:

---

[1] The parameters assessed in the noise assessment include continuous noise (measured by day-night average level (L_{dn}), daytime equivalent level (L_d), and nighttime equivalent level (L_n)) as well as transient, impulsive, and low-frequency noise and vibration (EIS Section 9.3).

- The existing characteristics, of visual quality and the noise environment, of the LAA that contribute to the existing environmental setting for outdoor recreation;
- Changes to the visual landscape characteristics and noise environment as a result of the Project; and
- The anticipated expectations and values of outdoor recreational users with respect to the visual quality and noise landscape character in the LAA, both existing and with the Project.

Changes to Visual Resources and Effects on Outdoor Recreation Environmental Setting

The existing environmental setting within the outdoor recreation LAA is characteristic of an altered visual environment from existing port facilities, as well as alterations to the natural landscape consisting of agricultural lands interspersed with residential and commercial areas, a network of roads, and a rail corridor (EIS Sections 25.8.1, 25.8.2, and 25.8.3, and AIR-12.04.15-13, Schedule 13-7 (CEAR Document #388[2])). Specifically, from the visual points of reception (PORs) in the LAA (which are representative of areas where land and marine-based outdoor recreation activities occur and where changes in landscape character and effects on the environmental setting could be experienced), the Deltaport and Westshore terminals are dominant in the existing viewscapes, including cranes, coal piles, and ships at existing port terminals. The existing nighttime visual conditions consist predominantly of lighting from existing port facilities at Roberts Bank terminals, the BC Ferries terminal, and causeways, as well as greenhouses, and commercial and residential and road lighting. The visual environment in the outdoor recreation LAA is further represented by the existing visual quality class which describes level and character of visible alterations for these sites, as described in EIS Section 25.5.3. From representative water-based outdoor recreation receptor sites (i.e., BC Ferries Duke Point route and BC Ferries Swartz Bay routes), the existing visual class is rated as 'maximum modification' (highest rating), where alterations are easy to see, large in scale, and a dominant element in the landscape. The existing visual quality class for land-based outdoor recreation receptor sites (i.e., BC Ferries causeway, Tsawwassen First Nation Outer Dyke, which are representative of the nearby shore-based walking trails and hunting areas in the outdoor recreation LAA, including Brunswick Point Trail) are also rated as 'maximum modification'. The existing visual quality class for the more distant Alaksen National Wildlife Area recreation site (i.e., where bird watching occurs) is rated as 'modification', where visible alterations are easy to see and either large in scale and of natural appearance or small to medium in scale with angular characteristics.

With proposed mitigation (as described in EIS Section 25.7), Project residual effects on visual resources include the following:

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[2] CEAR Document #388 From Port Metro Vancouver to the Canadian Environmental Assessment Agency re: Completeness Review - Responses to Additional Information Requirements Follow-Up (See Reference Document # 345) including 22 Technical Data Reports.

- Visible alterations to daytime visual resources in the LAA that are considered to be low magnitude, which is defined as 'minor, not resulting in an overall change in visual quality classifications' compared to existing conditions;
    - The identified marine-based and land-based recreation sites located within the outdoor recreation LAA are expected to retain their maximum modification visual class rating with the Project (EIS Section 25.6.1). At the Alaksen National Wildlife Area site, the visual class rating is defined as 'maximum modification', where new facilities will be seen as a continuation of the existing terminal facilities extending the massing of cranes and lower height visual features along the horizon (EIS Section 25.6.1); and
- Visible alterations to nighttime visual resources in the LAA that are considered to be low magnitude, with the change in light trespass CIE (Commission Internationale de L'Éclairage) classification (one level) predicted to occur only at one point of reception (N-POR11 immediately south of the Project area near the existing Westshore Terminals at Roberts Bank) and change in sky glow CIE classification at one POR (N-POR1 on Galiano Island).

While Project construction and operation will add new visible industrial features in the Roberts Bank area that will appear more prominent at closer distances (i.e., where marine-based activities occur), with mitigation, Project-related alterations will result in only a minor increase in visibility of anthropogenic features (EIS Sections 24.6.2.2 and 25.9.2). The characteristics of Project features will be consistent with, and will not alter, the current landscape character for daytime and nighttime visual resources. As a result, the potential effect to the outdoor recreation sub-components through the environmental setting pathway related to change in visual conditions is considered negligible.

Changes to Noise Levels and Effects on Outdoor Recreation Environmental Setting

The existing environmental setting in the LAA with respect to noise levels is characteristic of a dynamic noise environment, with noise generated from a number of diverse sources, including the existing Roberts Bank terminals, causeway and traffic, rail activity on the Roberts Bank causeway, BC Ferries, various road traffic, residential construction, farming activities, aircraft, and noise from marine vessels in transit (EIS Section 9.3.7.1).

During Project construction and operations, noise increases at locations where land-based outdoor recreation occurs are predicted to be below 3 dBA (EIS Appendix 9.3-A, Appendix H, and EIS Section 24.5.4), which is below the perception level for the average person (EIS Section 24.6.1)[3]. Daytime noise increases where recreational boating is concentrated (i.e., east of the BC Ferries causeway) and where windsport activities are concentrated (i.e., between BC Ferries causeway and Point Roberts) area also projected to be less than 3 dBA (EIS Section 24.5.1).

Recreational fish and seafood harvesting are outdoor recreation activities occurring in the LAA, which could experience noticeable changes in noise levels (i.e., greater than 3 dBA) (EIS

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[3] The average person cannot perceive sound increases of smaller than 3 dBA (EIS Section 24.6.1).

Section 24.6.2.2). Marine areas where recreational fish and/or seafood harvesting occurs in the LAA include 1) north and west of the existing Roberts Bank terminals (and the proposed RBT2 terminal); 2) in the inter-causeway area and near the BC Ferries Terminal, on both the west and east side of BC Ferries causeway, and 3) at the mouth of the Fraser River.

Harvesting activities to the north and west of the Project area occur within 1 km of the proposed RBT2 terminal where daytime noise levels are already influenced by existing port activity, and are expected to be above 50 dBA in the future without the Project. Changes in daytime noise levels in the marine areas to the north and west, closest to the Project (i.e., within 1 km) are predicted to be greatest and most noticeable during construction (7.4 to 12.9 dBA) and operation (7.9 to 13 dBA) phases of the Project. Changes in daytime noise levels in marine areas to the north and west, and further away than 1 km of the proposed RBT2 terminal during construction and operation phases are predicted to be lower (EIS Appendix 9.3-A, Appendix H).

Harvesting activities in the inter-causeway area take place between 3 to 4 km to the east of the Project (the centre of the RBT2 terminal), where daytime noise levels are already above 55 to 60 dBA[4] in existing conditions, and are predicted to remain similar in expected conditions (future without the Project) (EIS Section 24.6.2.2). Changes in daytime noise levels in the inter-causeway area 3 to 4 km to the east of the Project (as well as in areas to the east of the BC Ferries Terminal) are expected to be below 3 dBA in both construction and operations (EIS Appendix 9.3-A, Appendix H).

Harvesting activities at the mouth of the Fraser River within the LAA take place approximately 6 to 7 km north of the Project. Changes in daytime noise levels during Project construction are not predicted to be noticeable at these distances (i.e., the increases are predicted to be 1.3 to 1.4 dBA compared to expected conditions). The increases in noise levels during Project operation may be noticeable at this distance (approximately 3.5 to 3.6 dBA); however, future noise levels with Project operation are predicted to remain below 55 dBA in these areas (44.6 to 46.7 dBA, as shown in EIS Appendix 9.3-A, Appendix H).

Noise mitigation proposed within the Construction and Operation Noise Management Plans (EIS Section 33.3.6 and 33.4.4) will reduce noise levels in marine environments.[5] It is anticipated that the sounds of recreational fish and crab harvester vessel engines and the water and wind environment would further limit the perceptibility of noise changes from the Project, with the level of impact dependent on factors such as wind direction and weather conditions. Based on these factors, changes to noise conditions in areas used for marine-based outdoor recreation, and specifically for recreational crab and salmon fishing (EIS Sections 9.3.9.2, 9.3.9.3, and 24.6) are predicted to not incrementally alter (and would be consistent with) the existing overall noise landscape character at Roberts Bank and the marine recreational environmental setting.

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[4] 55 dBA is the Health Canada threshold for speech comprehension, as defined in the human health assessment (EIS Section 27.5.3.1).

[5] All noise level changes noted in this response and in EIS Sections 9.3 and 24.0 are predicted levels before implementation of noise mitigation.

While it is noted above that measurable changes to visual and noise conditions are anticipated during Project construction and operations, recreational users' expectations and values pertaining to the existing visual landscape character and noise environment in the LAA are expected to reflect those of a semi-urban and industrial environment. It is expected that the incremental change in visible built features, light, and noise from the Project that would be experienced by recreational users in the LAA, would not be viewed by these users as uncharacteristic of the environmental setting for outdoor recreation, within which they recreate. It is expected that recreational marine users will experience a small non-measurable (negligible) alteration to existing visual landscape character and noise environment and, in turn, to the environmental setting for outdoor recreation in the LAA.

Mitigation Measures to Mitigate Effects of the Project on Outdoor Recreation Environmental Setting and Residual Effects Characterisation

As described above, the potential effect on outdoor recreation via the quality of environmental setting pathway is determined to be negligible, and therefore no mitigation specific to outdoor recreation has been proposed. Consistent with EIS methodology (EIS Section 8.1.5), characterisation of residual effects or determination of the significance of residual effects is not required for negligible effects. However, the implementation of mitigation measures to address Project effects on daytime and nighttime visual resources (as described in EIS Section 25.7) and Project effects on noise levels (as described in EIS Sections 33.3.6 and 33.4.4) committed to in the EIS would contribute to reducing effects to the visual and noise landscape character and the environmental setting for outdoor recreation. These mitigation measures include the following:

- Measures to avoid or reduce potential effects on visual resources:
    - Crane color optimisation; and
    - Lighting design and operation – light management plans;
- Measures to avoid or reduce potential effects on noise:
    - A construction noise management plan with the following measures:
        - Scheduling higher noise-generating activities during weekdays and during daytime;
        - Shutting down equipment and vehicles when not in use;
        - Utilising equipment that produces less noise; and
        - Awareness and training for construction crew;
    - An operations noise management plan with the following measures:
        - Optimising tonality for equipment alarms to limit audibility on shore while meeting safety requirements;
        - Operator awareness and training; and
        - Regularly maintaining equipment (e.g., lubricating pulleys and other moving parts, replacing deteriorated exhaust mufflers, maintaining engine efficiency through servicing).

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IR8-11 Visual Resources – Technical Boundaries

Information Source(s)

EIS Volume 2: Section 8.1.3.4, Section 9.3

EIS Volume 4: Section 24.3.4, Section 25

Proponent Response to Follow-Up Additional Information Requirements of Dec 4, 2015 (CEAR Doc#412): IR13, Schedule 13-9

Context

In Section 8.1.3.4 of the EIS, the Proponent indicated that technical boundaries, such as unavoidable data gaps or model limitations, can constrain the identification or assessment of potential effects of the proposed Project on a valued component. In Section 24.3.4 of the EIS, the Proponent indicated that the technical boundaries for assessing the quality of environmental setting for outdoor recreation were dependent on those identified in both Section 9.3 Noise and Vibration and Section 25.0 Visual Resources. Although there was some information provided in Section 9.3 of the EIS regarding uncertainty, sensitivity, and reliability of the field work performed and regarding the noise propagation modelling and mapping, there were no technical boundaries described in Section 25 of the EIS.

In Schedule 13-9 of its response to follow-up information requirement #13 from the Canadian Environmental Assessment Agency, the Proponent indicated that data availability for use in the characterization of conditions prior to and following the influence of past and future projects and activities were technical boundaries to the assessment of cumulative effects for visual resources. However, it is unclear whether there are additional technical boundaries that may have limited the assessment of the potential effects of the Project on visual resources.

Information Request

Provide a description of the technical boundaries to the assessment of effects of the proposed Project on visual resources, as referenced in Section 24.3.4 but not presented in Section 25 of the EIS.

VFPA Response

No technical boundaries were identified in EIS Section 25.0 for visual resources, as sufficient information was available to understand the existing character of visual resources within the study area and to assess the potential effects of the Project. The approach followed guidance from established assessment frameworks (i.e., B.C. Visual Resource Management, Commission Internationale de l'Éclairage) and used current photos from key representative viewpoints (EIS Appendix 25-B) and detailed Project 3-D models and design information (EIS Appendix 25-B).

The statement in EIS Section 24.3.4 referenced in the context to this information request expresses that the outdoor recreation assessment relies on the conclusions of other assessments (including visual resources and noise and vibration), and therefore incorporates any technical boundaries that are identified for those assessments. The statement was intended to refer generally to this dependency. While there are technical boundaries for the noise and vibration assessment, none were identified in EIS Section 25.0 for the visual resources assessment, in accordance with established visual assessment guidelines, and application of appropriate data and analysis.

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IR8-12 Visual Resources – Night-time Spatial Boundaries

Information Source(s)

EIS Volume 4: Section 25.3.1, Figure 25-1

EIS Volume 2: Section 9.4, Section 9.4.5.2, Appendix 9.4-A

Context

In Figure 25-1 of the EIS, the Proponent presented the spatial boundaries used in the visual resources effects assessment. The night-time visual resources local assessment area was established as a 60 kilometre-radius surrounding the proposed Project. The assessment of effects of the Project on night-time visual resources was based on the technical studies of light described in Section 9.4 and Appendix 9.4-A of the EIS.

As stated by the Proponent in Section 9.4.5.2 of the EIS, points of reception for the light effects assessment were located 0.6 to 37 km away from the Project. However, although the Proponent indicated in Section 25.3.1 of the EIS that lighting associated with the Project was not anticipated to be visible at distances greater than 60 km from the Project area, there were no points of reception located between 37 and 60 km from the Project to support this conclusion.

Additional information is required to determine whether representative points of reception between 37 and 60 km away from the Project could experience changes in sky glow classification and night-time visual resources.

Information Request

Describe the potential for representative points of reception between 37 and 60 km away from the proposed Project to experience a change in sky glow classification and night-time visual resources as a result of the Project.

VFPA Response

EIS Section 25.3.1 identifies a nighttime visual resources local assessment area (LAA) that was conservatively based on preliminary desktop mapping that identified 60 km as the maximum extent within which the Project is expected to potentially affect visual resources during the night. Through subsequent field measurement of existing light conditions and modelling of changes in light trespass and sky glow from the Project (as presented in EIS Tables 9.4-9 and 9.4-10), it was determined that at N-POR12 (Valdez Island), located at 37 km distance from the Project, there is no predicted change in illuminance from the existing level of 0.004 lux. The location of N-POR12 is shown in EIS Figure 25-2. In addition, measurements and subsequent modelling predicted changes in sky glow from 144% to 146% brightness above a natural dark sky would not result in a change in Sky Glow CIE Zone

Classification at N-POR12. As a result, predicted changes in light levels would not appear as a noticeable change to nighttime visual resources at N-POR12.

As indicated in EIS Section 9.4.5.3, attenuation due to distance between the light sources and points of reception (PORs) decreases the potential for the Project to result in noticeable changes to light levels as the distance of the POR from the Project increases. Based on light modelling results, measurable changes in light trespass and sky glow with the Project are not anticipated at N-PORs located beyond 37 km from the Project. Consequently, measurable Project effects on nighttime visual resources beyond 37 km from the Project were not anticipated, and the potential for representative PORs in the spatial area between 37 km and 60 km within the nighttime visual resources LAA were therefore not included.

IR8-13 Visual Resources – Expectations for Visual Quality

Information Source(s)

Proponent Response to Follow-Up Additional Information Requirements of July 31, 2015 (CEAR Doc#412): IR13, Schedule 13-9

EIS Volume 4: Section 25.2.1, Figure 25-2

Context

In Schedule 13-9 of its response to follow-up information requirement #13 from the Canadian Environmental Assessment Agency, the Proponent indicated that expectations for visual quality were likely to reflect the cultural values of persons residing, working and recreating in a semi-urban and industrial environment that is part of a dynamic, growing metropolitan region, and that built features such as the proposed Project are not uncharacteristic of such an environment. Further, the Proponent stated that by taking the social context and expectations of viewers for visual quality within the semi-urban setting that surrounds the Project site and the industrial character of the Roberts Bank area into consideration, the effects of the Project in combination with the effects of other projects and activities that have been (and are being) carried out were therefore expected to be not significant for the visual resources valued component.

However, in Section 25.2.1 of the EIS, the Proponent also stated that daytime viewscapes in the vicinity of the Project area have recognized scenic value and support tourism and recreational values by providing setting for activities such as whale watching, fishing, and harvesting of seafood and waterfowl.

Additional information is required to determine the expectations of viewers for visual quality at daytime points of reception located within the visual resources local assessment area as presented in Figure 25-2 of the EIS.

Information Request

Provide information regarding the expectations of viewers for visual quality in the local assessment area for visual resources for each point of reception as presented in Figure 25-2 of the EIS, and indicate the source of this information.

VFPA Response

The source of information used in the EIS to understand expectations of viewers for daytime points of reception, termed as D-PORs, located within the visual resources daytime local assessment area (LAA) included the following:

- An understanding of the type of outdoor land and marine use activities, and level of use carried out within the LAA;

- Existing viewing conditions at each D-POR as described in EIS Appendix 25-A;
- Reference to existing visual and lighting impact assessment studies for other projects within the RBT2 Project area (i.e., Deltport Third Berth and Deltaport Terminal Road and Rail Improvement Project) that provided additional context about activities and conditions at viewing locations (EIS Section 25.4); and
- Guidance from the British Columbia Visual Landscape Inventory Procedures and Standards Manual (B.C. MoF 1997) on defining dimensions of viewing conditions that include viewer type, viewing distance, and existing conditions for each D-POR (EIS Table 25-9).

Established frameworks for visual impact assessment (VIA) commonly draw on knowledge from studies on visual perception of landscapes to understand the interaction between the viewer and the landscape (BC MoF 1997, LI/IEMA 2002, USDI BLM 1986, US DoT 2015). They emphasize that context affects the interaction as human receptors bring different expectations to different types of places and situations. Within the LAA, it is predicted that viewers' expectations would reflect an awareness of the existing visually evident land uses located around the Project and the Project's general setting within the Metro Vancouver region (EIS Section 25.5.2).

VIA frameworks commonly employ viewer characterisations as a practical tool to predict the relationship between viewers and the landscape in order to describe the relative expectations or level of concern for visual change. Based on identified activities, level of use, and existing viewing conditions at D-PORs described in the EIS, **Table IR8-13-1** provides further information related to viewers and their expectations for visual quality using definitions in the British Columbia Visual Landscape Inventory Procedures and Standards Manual.

Table IR8-13-1 Point of Reception Viewer Characterisation

Point of Reception	Point of Reception Name	Viewer Type	Distance to Existing Terminals (km)	Viewer Conditions and Expectations and for Visual Quality
D-POR1	BC Ferries Duke Point Route	Travel corridor	2.0	A majority of viewers would have moderate expectations/concerns for visual quality based on the following: • Large numbers of viewers; • Moderate viewing duration within mid-ground viewing distance; and • Secondary importance to the activity or experience pursued (i.e., passenger ferry).

Point of Reception	Point of Reception Name	Viewer Type	Distance to Existing Terminals (km)	Viewer Conditions and Expectations and for Visual Quality
D-POR2	BC Ferries Causeway	Travel corridor	2.6	A majority of viewers would have moderate expectations/concerns for visual quality based on the following: • Large numbers of viewers; • Short viewing duration within mid-ground viewing distance; and • Secondary importance to the activity or experience pursued (i.e., motorist).
D-POR3	Tsawwassen First Nation, Outer Dyke	Cultural importance	4.7	A majority of viewers would have high expectations/concerns for visual quality based on the following: • Moderate numbers of viewers; • Long viewing duration within mid-ground viewing distance; and • Scenic quality is typically of primary importance to the activity or experience pursued (i.e., residents).
D-POR4	Point Roberts, U.S.A. Mid-way Western Shore	Residential	6.4	A majority of viewers would have high expectations/concerns for visual quality based on the following: • Moderate numbers of viewers; • Long viewing duration within middle-ground viewing distance; and • Scenic quality is typically of primary importance to the activity or experience pursued (i.e., residents).
D-POR5	BC Ferries Swartz Bay Route	Travel corridor	12.0	A majority of viewers would have moderate expectations/concerns for visual quality based on the following: • Large numbers of viewers; • Moderate viewing duration within background viewing distance; and • Secondary importance to the activity or experience pursued (i.e., passenger ferry).
D-POR6	Richmond, Garry Point Park	Recreation	11.5	A majority of viewers would have high expectations/concerns for visual quality based on the following: • Moderate numbers of viewers; • Short viewing duration within background viewing distance; and • Scenic quality is typically of primary importance to the activity or experience pursued (i.e., recreation and tourism).

Point of Reception	Point of Reception Name	Viewer Type	Distance to Existing Terminals (km)	Viewer Conditions and Expectations and for Visual Quality
D-POR7	Mayne Island, Bennett Bay Ecological Reserve	Recreation and tourism	19.3	A majority of viewers would have high expectations/concerns for visual quality based on the following: • Moderate numbers of viewers; • Short to moderate viewing duration within background viewing distance; and • Scenic quality is typically of primary importance to the activity or experience pursued (i.e., recreation and tourism).
D-POR8	Saturna Island, Mid-Island	Residential	24.4	A majority of viewers would have high expectations/concerns for visual quality based on the following: • Low numbers of viewers; • Long viewing duration within background viewing distance; and • Scenic quality is typically of primary importance to the activity or experience pursued (i.e., rural residents).
D-POR9	Galiano Island, Dionisio Point Provincial Park	Recreation and tourism	31.3	A majority of viewers would have high expectations/concerns for visual quality based on the following: • Low numbers of viewers; • Short to moderate viewing duration within background viewing distance; and • Scenic quality is typically of primary importance to the activity or experience pursued (i.e., recreation and tourism).
D-POR10	Alaksen National Wildlife Area (federal lands)	Recreation and tourism	9.3	A majority of viewers would have moderate expectations/concerns for visual quality based on the following: • High numbers of viewers; • Short to moderate viewing duration within background viewing distance; and • Scenic quality is typically of secondary importance to the activity or experience pursued (i.e., bird watching).

Notes: km = kilometres; mid-ground = 1 to 8 km viewing distance; background = greater than 8 km; moderate viewing duration = temporary static viewpoint (e.g., highway rest stops) or slow moving water; short viewing duration = limited to glimpses; long viewing duration = sustained opportunities (e.g., communities, campgrounds, etc.)

References

British Columbia Ministry of Forests (B.C. MoF). 1997. Visual Landscape Inventory Procedures and Standards Manual. Forest Practices Branch for the Culture Task Force, Resources Inventory Committee. ISBN 0-7726-3359-2. pp. 71

Landscape Institute and the Institute of Environmental Management and Assessment (LI/IEMA). 2002. Guidelines for Landscape and Visual Impact Assessment (First Edition). Spon Press, New York.

United States Department of the Interior (USDI). 1986. Visual Resource Inventory. Bureau of Land Management Manual Handbook H-8410-1, Rel. 8-28. Washington, DC.

United States Department of Transportation (U.S. DoT). 2015. Federal Highway Administration - Guidelines for the Visual Impact Assessment of Highway Projects. Available at https://www.environment.fhwa.dot.gov/guidebook/documents/VIA_Guidelines_for_Highway_Projects.asp. Accessed November 2017.

IR8-14 Visual Resources – Influence of Agricultural Land on Landscape Character

Information Source(s)

Proponent Response to Follow-Up Additional Information Requirements of July 31, 2015 (CEAR Doc#412): IR13, Schedule 13-9

EIS Volume 4: Section 25.8.3, Section 26.5.3, Figure 26-5, Figure 26-6

Context

In Schedule 13-9 of its response to follow-up information requirement #13 from the Canadian Environmental Assessment Agency, the Proponent referred to the Roberts Bank area as having a semi-urban setting with industrial character. Furthermore, when describing the context for the effects of the proposed Project on visual resources in Section 25.8.3 of the EIS, the Proponent stated that the prominent visual features adjacent to the Project area include: cranes, coal piles and ships at the port terminals; the Roberts Bank causeway and overpasses; ferries, the ferry terminal and causeway; residential and commercial neighbourhoods; greenhouses; and train and road corridors within a larger regional context.

In Schedule 13-9, the Proponent also indicated that the incremental change that would be introduced by the Project would contribute a minor increase in the visibility of anthropogenic features and artificial lighting and the characteristics of the Project features would be consistent with the existing landscape character.

However, in Section 26.5.3 of the EIS, the Proponent highlighted that:

- the main Metro Vancouver Regional Growth Strategy designations in the land use local assessment area outside of federal lands are industrial and agricultural;
- Tsawwassen First Nation Lands within the land use local assessment area are designated under the Tsawwassen First Nation Land Use Plan for agricultural, industrial, and infrastructure uses; and
- approximately 105 ha of land within the local assessment area is part of the Agricultural Land Reserve, where farming is recognized as the priority use.

Although the Proponent repeatedly referred to the industrial character of the surrounding environment in its assessment of effects to visual resources, it did not highlight the presence of agricultural land in the daytime visual resources local assessment area and its influence on the character of the landscape in the local assessment area.

Information is required to determine the influence of agricultural land on landscape character when describing the context for effects of the Project on visual resources.

Information Request

Indicate how the consideration of agricultural land in the description of landscape character and context for residual effects may change the determination of significance presented in Section 25.9.2 of the EIS**.**

VFPA Response

Clarification

It is acknowledged in Section 25.5.2 of the EIS, where existing conditions of the Project setting are described, that agricultural lands (including greenhouses that use night lighting) are an evident land use and are interspersed with residential development, commercial areas, and road and railway corridors. The assessment of visual effects of the Project focuses on the discernable visual setting as evident from the points of reception (PORs).

As illustrated in EIS Appendix 25-B, Figures 25B-B1 to 25-B30, perspective views of the Project area from PORs used for the assessment do not demonstrate agricultural land features and characteristics as a dominant visual feature in the viewscapes. As the presence of agricultural land has been considered in the description of landscape character and as context for residual visual effects, there is no change to the determination of significance presented in Section 25.9.2 of the EIS.