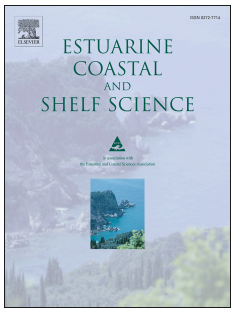
Accepted Manuscript



Seasonal changes in fatty acid composition of estuarine intertidal biofilm: Implications for western sandpiper migration

Peter J. Schnurr, Mark C. Drever, Hedy J. Kling, Robert W. Elner, Michael T. Arts

PII: S0272-7714(19)30066-6

DOI: https://doi.org/10.1016/j.ecss.2019.04.047

Reference: YECSS 6213

To appear in: *Estuarine, Coastal and Shelf Science*

Received Date: 11 February 2019

Revised Date: 23 April 2019

Accepted Date: 28 April 2019

Please cite this article as: Schnurr, P.J., Drever, M.C., Kling, H.J., Elner, R.W., Arts, M.T., Seasonal changes in fatty acid composition of estuarine intertidal biofilm: Implications for western sandpiper migration, *Estuarine, Coastal and Shelf Science* (2019), doi: https://doi.org/10.1016/j.ecss.2019.04.047.

SEASONAL CHANGES IN FATTY ACID COMPOSITION OF ESTUARINE INTERTIDAL BIOFILM: IMPLICATIONS FOR WESTERN SANDPIPER MIGRATION

Peter J. Schnurr[1*], Mark C. Drever[2], Hedy J. Kling[3], Robert W. Elner[2], Michael T. Arts[1]

[1]Department of Chemistry and Biology, Ryerson University, 350 Victoria Street, Toronto, Ontario, M5B 2K3, Canada

[2]Pacific Wildlife Research Centre, Environment and Climate Change Canada, 5421 Robertson Road, Delta, British Columbia, V4K 3N2, Canada

[3]Algal Taxonomy & Ecology Incorporated. PO Box 761 Stony Mtn, PO 8050e PR 321N, Stony Mountain, Manitoba, R0C 3A0, Canada

Corresponding Author:
*Peter J. Schnurr
Department of Chemistry and Biology
Ryerson University
350 Victoria Street
Toronto, Canada
M5B 2K3
peter.schnurr@mail.utoronto.ca
647-967-9403

Declarations of interest: none

ABSTRACT

For shorebirds, long distance migration is an energy-demanding activity, and lipids (largely comprised of fatty acids) with their high energy density are an ideal fuel. Diatoms in intertidal biofilms provide a rich source of fatty acids for fuel and for critical physiological functions. We compared the composition of intertidal biofilm on mudflats at Roberts Bank, a major stopover site for shorebirds in the Fraser River estuary, between two seasons: spring, during the northward breeding migration of Western Sandpipers (*Calidris mauri*), and winter, when no migrating shorebirds are present. Mass fractions of fatty acids in biofilm (µg fatty acids/g sample in the upper 2 mm of biofilm-containing sediment) in April were 3-7× higher than in winter (January and February). This difference included total saturated, monounsaturated, polyunsaturated, omega-3 (n-3), and omega-6 (n-6) fatty acids, as well as individual fatty acids such as palmitoleic acid (16:1n-7), eicosapentaenoic acid (EPA; 20:5n-3), and docosahexaenoic acid (DHA; 22:6n-3). In addition, organic content was ~25% higher in spring compared to winter. The microphytobenthos in spring biofilm was dominated by marine-influenced diatoms (primarily from the genera *Nitzschia* and *Navicula*) which made up >50% (µg/ml) of total biofilm biomass. Higher fatty acid and organic content in biofilm during spring provide shorebirds with both energy and physiologically important fatty acids to support their migration. These findings are consistent with the 'green wave' hypothesis, whereby bird migration broadly coincides temporally with the availability of energy and essential nutrients. The role of diatoms as purveyors of important fatty acids to shorebirds underscores the need for new conservation policies that protect the abundance of organic and fatty acid content of intertidal biofilm at estuarine stopover sites.

Keywords: biofilm; diatoms; fatty acids; estuaries; Western Sandpiper; green wave hypothesis

Abbreviations:
Omega-3 (n-3)
Omega-6 (n-6)
Eicosapentaenoic acid (EPA)
Docosahexaenoic acid (DHA)
Extracellular polymeric substances (EPS)
Omega-3 long chain polyunsaturated fatty acids (n-3 LC-PUFA)
Saturated fatty acids (SFA)
Monounsaturated fatty acids (MUFA)
Polyunsaturated fatty acids (PUFA)
Photon flux densities (PFD)
Ash free dry weight (AFDW)
Gas chromatography (GC)
Flame ionization detection (FID)
Fatty acid methyl esters (FAME)

1 Introduction

Biofilm, consisting primarily of microphytobenthos (mostly diatoms) and bacteria, serves various functions on intertidal sediments (Paterson et al. 2003), including nutrient recycling (Decho 2000), and physical stabilization (Bellinger et al. 2005). Biofilms can exhibit high rates of primary productivity (Underwood and Kromkamp 1999), and along with associated extracellular polymeric substances (EPS), are consumed by a range of taxa in estuarine food webs, including invertebrates (Herman et al. 2000), fish (Carpentier et al. 2014) and shorebirds (Kuwae et al. 2008; Mathot et al. 2010). Shorebirds can derive 50-68% of their daily energetic requirements during migration from biofilm (Elner et al. 2005; Kuwae et al. 2008). These discoveries have prompted calls for conservation efforts designed to preserve the processes underlying the nutrient dynamics of intertidal sediments and for better understanding of the role of biofilm as a food resource for migratory birds (Jiménez et al. 2015; Mathot et al. 2018).

Shorebirds that migrate long distances depend on stopover sites with specific characteristics (Albanese and Davis 2015). Paramount among these is safe access to abundant, predictable, and high-quality food resources, allowing the replenishment of fuel and essential

nutrient stores (Warnock 2010). Diatoms within biofilm communities are a rich source of fatty acids (Huggins et al. 2004; Passarelli et al. 2015). Shorebirds assimilate fatty acids through either predation on invertebrates that have fed on diatoms and other algal taxa (Quinn et al. 2017), or grazing directly on surficial biofilm (Elner et al. 2005; Kuwae at al. 2008). Although fatty acids are known to be a primary fuel for high endurance migration in birds (McWilliams et al. 2004), the relative importance of individual n-3 and n-6 PUFA or the importance of having specific ratios of SFA, MUFA, and PUFA (i.e. degree of saturation) have not been unequivocally resolved (Guglielmo 2018; Price 2010).

Dietary fatty acid composition affects the energetic performance of birds (Guglielmo 2010) such that migrating birds could benefit by selecting food containing specific fatty acids (McWilliams et al. 2004). For example, consumption of biofilm by Semipalmated Sandpipers (*Calidris pusilla*) in the Bay of Fundy was higher on intertidal mudflats where biofilm had greater proportions of n–3 PUFA (Quinn et al. 2017). Compared to more (non-polar) saturated fatty acids, more (polar) PUFA may have higher solubility in water ('like dissolves like'), hence may be more easily mobilized in cellular cytosol leading to the speculation that they may be a preferred source of energy (Price 2010). However, more research is needed, as mobilization from stored triacylglycerol involves many steps, any of which could be rate-limiting. In addition, n-3 LC-PUFA, such as eicosapentaenoic acid [EPA; 20:5n-3] and docosahexaenoic acid [DHA; 22:6n-3] cannot be efficiently synthesized by many vertebrates and must be obtained "pre-formed" in the diet (Arts et al. 2001). Although not exhaustively substantiated, n-3 LC-PUFA may provide physiological benefits such as increased cell membrane fluidity (i.e. more appropriately stated as decreases in membrane lipid order), permeability, and protein activity (Guglielmo 2018; Maillet and Weber 2007; Price 2010; Weber 2009). These fatty acids are

found in high levels in mitochondria-rich and high-contraction-frequency muscles, such as in the pectorals of migrating birds (Infante et al. 2001) and may enhance recovery from inflammation associated with long distance migration (Price 2010). Finally, diatoms are the major source of n-3 LC-PUFA, including EPA and DHA, in marine food webs (Hixson et al. 2015), and their potential utility to migratory shorebirds makes them particularly valuable compared to other food sources. In sum, improved understanding of the factors that affect the availability and composition of fatty acids in biofilm in general is a prerequisite to elucidating the value of this resource for shorebirds.

Within a migratory flyway, the onset of photosynthetic activity during spring in the northern hemisphere has a northward progression, known as the 'green wave' (Schwartz 1998), a phenomenon driven by environmental factors, especially temperature and photon flux density (PFD). Birds arriving at stopover sites coincident with the 'green wave' can maximize their access to food during migration to their breeding grounds (Marra et al. 2005). The trophic level of migrating sandpipers declines as they move northward (Beninger et al. 2011), indicating an increasing consumption of primary producers. At estuarine stopovers, diatom blooms are a component of the 'green wave,' and provide migrating shorebirds with an abundance of fatty acids (Mathot et al. 2018). Currently, the ecological processes underpinning the availability of intertidal diatoms grazed by shorebirds at stopover sites are not well understood, and as such, appropriate conservation strategies cannot be adequately developed (Mathot et al. 2018).

The present study characterized temporal patterns in the organic and fatty acid content mass fraction within intertidal mudflat biofilm on Roberts Bank in the Fraser River Estuary, British Columbia (Sutherland et al. 2013). The estuary and delta form Canada's most important stopover area for shorebirds along the Pacific Flyway (Butler 1994), with the Roberts Bank area

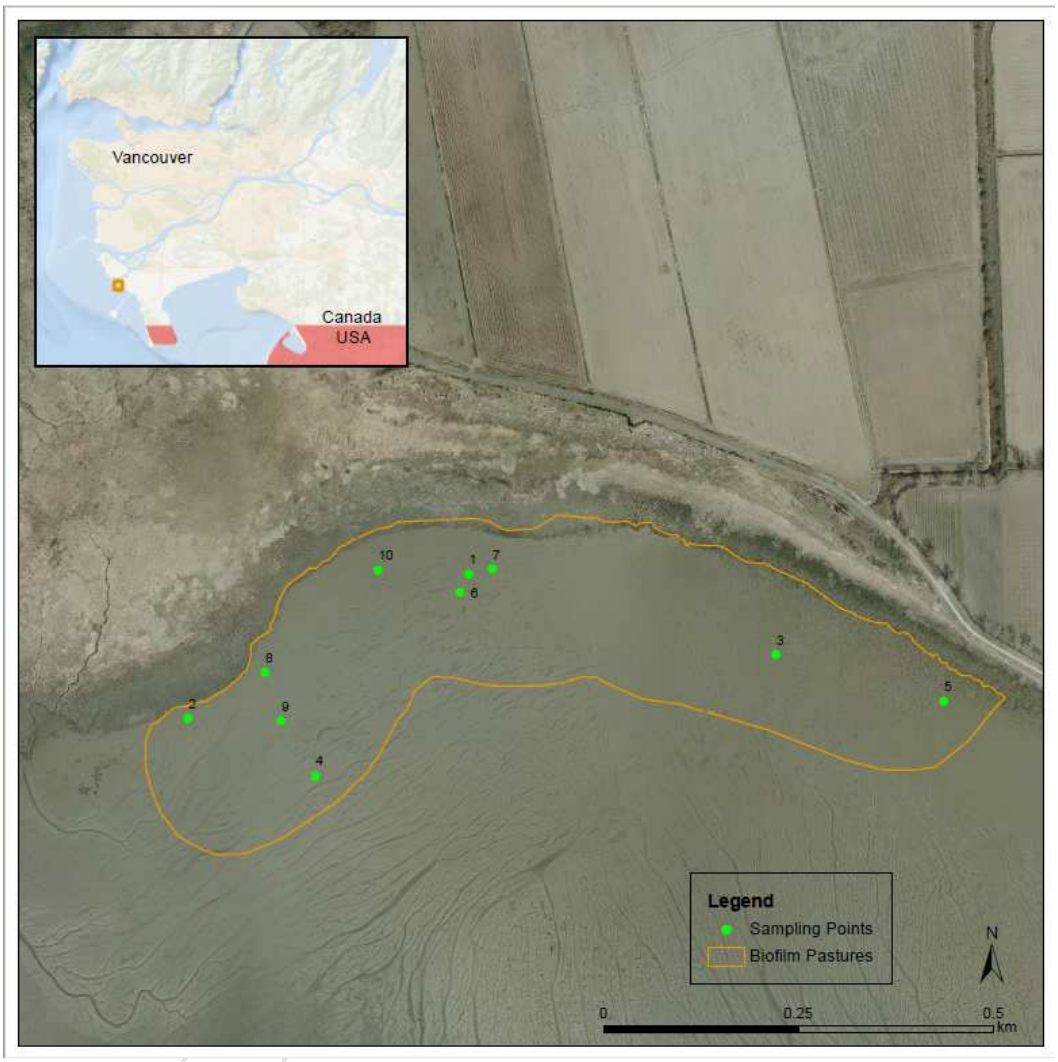
having the highest usage (Jardine et al. 2015). Approximately 1.2 million shorebirds use the estuary and delta annually (Butler and Campbell 1987), including hundreds of thousands of Western Sandpipers (*Calidris mauri*) and Dunlin (*Calidris alpina*) that migrate through the estuary during April and May en route to Arctic breeding grounds (Drever et al. 2014). Both species forage on epifaunal and infaunal portions of marine ecosystems (Mathot et al. 2010; Sutherland et al. 2000) and are demonstrated biofilm grazers (Beninger et al. 2011; Kuwae et al. 2008). We determined biofilm organic and fatty acid content on Roberts Bank between two seasons (i.e. spring and winter) to compare the ecosystem during Western Sandpiper migratory periods and non-migratory periods. These mudflat biofilms are characterized mostly as transient epibenthic biofilm, but sometimes epibenthic microbial mats (Beninger 2018), depending on season and location in which samples were taken. Throughout this paper they will simply be referred to as 'biofilm.' Also, we investigated specific diatom communities and taxa within these biofilms that could be sources of fatty acids for migrating shorebirds during spring (April).

2 Materials and Methods

2.1 Biofilm Sampling

Single biofilm samples were collected from ten mudflat locations in the upper intertidal zone of Roberts Bank on two occasions in spring 2016 (April 23 and 25, 2016), while triplicate samples were taken at five of the ten locations in winter 2017 (January 26 and February 28, 2017). A boundary was determined around an area known to be highly utilized by Western Sandpipers during their stopovers (Drever et al. 2014) and an ArcGIS random site generator used to select the 10 locations within the boundary (Fig. 1). The spring sampling dates were chosen to coincide with expected peak northward migration (Drever et al. 2014), and the winter dates were selected to compare biofilm organic and fatty acid content during the non-migratory period.

123 Ambient temperature and PFD data for a location <20 km from the study site was provided by
124 the Burns Bog Flux Tower project team (full measurement details are in Lee et al. 2017).



125
126 **Fig. 1** Biofilm sampling locations on Roberts Bank, British Columbia, Canada. Bounding box in
127 upper left inset indicates location of study site relative to the city of Vancouver.
128
129 Biofilm was collected by using a spatula to scrape the top ~2 mm layer from the intertidal
130 sediment sample. Visual inspection (with the unaided human eye) of both spring and winter
131 samples revealed that macro-invertebrates (>1 mm) did not contribute significantly to the overall
132 biofilm biomass, but when present, were removed from samples with tweezers before organic
133 and fatty acid content analysis. Microinvertebrates (<1 mm) within the samples may have

contributed small amounts of biomass and fatty acids compared to the biofilm biomass. The area per sample was standardized to 10 x 10 cm by using a plastic template. Each sample was placed into a 50 ml Falcon Tube and stored at -80°C to stabilize the biochemical integrity of the lipids. Samples were collected for taxonomic analysis by taking a small (5 ml) sub-volume of the above samples and placing them in separate 15 ml Falcon™ tubes. Samples for taxonomy analysis were collected during the spring sampling campaign, but not winter. The samples were preserved in seawater containing Lugol's iodine solution (mixed at a concentration to make a yellow-brown colored solution) before being stored at -6°C and transported to the Algal Taxonomy and Ecology Inc. laboratory (Stony Mountain, Manitoba, Canada).

2.2 Biofilm Homogenization and Ash Free Dry Weight

Frozen samples were lyophilized using a Labconco Freezone 2.5 Freeze Drier. Dry biofilm samples were then homogenized with a mortar and pestle. Each sample was analyzed for ash free dry weight (AFDW) composition because: 1) it could be used as an indicator for overall biofilm organic content (i.e. organic material within the sediment) (g/g); 2) biofilm samples were ~92-98% (g/g) inorganic sediment (clay, silt, and sand), so it was useful to normalize analytes to AFDW before calculating total amount of fatty acids in the sample. Ash free dry weight was determined by taking a portion (~3 g) of the homogenized biofilm sample, ashing in a 550°C oven for 24 h to volatize all organic material, and re-weighing to measure the difference in weight before and after combustion.

2.3 Lipid Extraction and Fatty Acid Methyl Ester Derivatization

A second portion of the homogenized biofilm was used to determine the types and quantities of fatty acids (i.e. fatty acid content) of biofilm. Since 20 mg of organic content was

156 the desired amount of sample to extract, but organic fraction of the biofilms were variable (~2-
157 8% (g/g) organic), a calculated amount of sample (organic + inorganic) was determined for each
158 sample. Therefore, depending on the organic content of the respective samples, between ~300
159 and 700 mg of sample was used for initial extractions.

160 Once samples were prepared for analysis, lipids were extracted using the Folch Method
161 (Folch et al. 1957). During extraction 10.2 µg of tricosylic acid (23:0) was added to each sample
162 as an internal standard. Tricosylic acid was used because it is rarely produced in nature. The
163 aqueous phase was discarded, and the chloroform:methanol layer was evaporated under a
164 nitrogen blanket prior to re-dissolving extracted lipids in 1 ml of hexane.

165 Lipids were then methylated with a 1% (v/v) solution of H_2SO_4 in anhydrous methanol
166 and 1 ml of hexane at 90°C for 90 min. A VWR Digital 2-Block Heater was used to maintain the
167 temperature during methylation. Fatty acids were extracted twice with 4 ml hexane aliquots and
168 re-dissolved in a known volume of hexane after evaporation under a nitrogen blanket. Aliquots
169 of 100 µl were used to gravimetrically determine the total fatty acid content extracted from the
170 biofilms. The remaining fatty acid solution was used to identify and quantify fatty acids within
171 the biofilms using gas chromatography (GC) and flame ionization detection (FID).

172 Fatty acid methyl esters (FAME) were analyzed with a Shimadzu GC2010 Plus and an
173 AOC-20i autosampler. The column used was a Supelco SP-2560, 100 m length and 0.25 mm
174 inner diameter. The GC was run on splitless mode with an initial column temperature of 60°C.
175 The column temperature was then increased to 180°C at a rate of 15°C per min, followed by a
176 ramping rate of 2°C per min to 240°C, and a hold time of 5 min to elute all remaining fatty acids.
177 Helium was used as the carrier gas at a flow rate of 1.2 ml/min. Injector and detector

178 temperatures were both 250°C. Peaks were identified by matching retention times to those in a
179 fatty acid standard (37-component FAME mix, Supelco, catalog No. 47885-U).

180 2.4 Fatty Acid Content

181 To determine the mass fraction of fatty acids in a sample (i.e. upper 2 mm of biofilm), the
182 following calculation was developed to account for the amount of biomass and the amount of
183 fatty acids within that biomass, where organics equals biofilm AFDW:

$$\mu g\ \text{fatty acid/g sample} = \mu g\ \text{fatty acid/g organics} \times \text{g organics/g sample}$$

184 2.5 Chlorophyll-a Determination

185 A portion of each homogenized sample was used to determine chlorophyll-*a* content of
186 the biofilm. The procedure was an adaptation from Arar (1997). As was the case with lipid/fatty
187 acid determination, 20 mg of organic content was targeted, so the amount used for extraction was
188 calculated from the organic mass fraction of each sample. Chlorophyll was extracted by grinding
189 the sample in a grinding tube with 10 ml of 9:1 acetone:water for 2 min at 500 g. The sample and
190 biomass were transferred from the grinding tube to a centrifugation tube and steeped in a dark
191 refrigerator (4°C) for 20-23 h. Samples were then centrifuged at 675 *g* for 15 min before
192 transferring the supernatant to a quartz cuvette (1 cm^3) for analysis by spectrophotometry
193 (Agilent Cary 60 UV-Vis Spectrophotometer). The instrument was zeroed with a 9:1
194 acetone:water blank. Absorbance (abs) values were measured at 750 nm, 664 nm, 647 nm, and
195 630 nm and applied to an equation developed by Arar (1997) (chlorophyll-*a* (mg/L) = 11.85 x
196 abs 664 nm – 1.54 x abs 647 nm – 0.08 x abs 630 nm); abs750 was subtracted from each
197 absorbance measurement at the 3 wavelengths for chlorophyll-*a* because abs750 accounted for
198 suspended materials affecting absorbance readings for chlorophyll content. Chlorophyll-*a* mass

(mg) within each sample was determined by multiplying chlorophyll-*a* concentration (mg/L) by volume of acetone:water used (10 ml). Chlorophyll-*a* mass fraction of each biofilm sample was determined by dividing the extracted chlorophyll-*a* mass by mass of sample extracted (i.e. mg Chlor-*a*/g biofilm). The amount of phaeophytin contribution from each sample was not assessed because differentiating chlorophyll contributions from living versus dead biomass was not of interest, but rather, our focus was on determining chlorophyll content of standing stock biomass.

2.6 Biofilm Measures and Statistical Analyses

Ash free dry weight, or organic content expressed as the organic fraction of the sample weight (g/g), was used as the indicator for overall organic content of biofilm. The lipid/fatty acid content was normalized to AFDW as well as per gram of sample. Total lipids, chlorophyll-*a* and fatty acids were normalized to gram of sample in the upper 2 mm to account for the differences in organic content between spring and winter. We summed the fatty acid mass fractions into major groups based on their saturation levels, including total values for SFA, MUFA, and PUFA, including n-3, and n-6 fatty acids. Also, we considered a suite of individual fatty acids, among them 16:0, 16:1n-7, 18:0, 18:1n-9, EPA and DHA - C16 and C18 fatty acids comprised 80 to 90% of all fatty acids found in depot fat of migrating sandpipers (Egeler and Williams 2000). We used the sum total of palmitoleic acid (16:1n-7), EPA, and DHA as a biomarker for diatoms (Shin et al. 2008).

We tested for seasonal differences in each biofilm measure using a General Linear Mixed Model using the *lme4* package in R (Bates et al. 2015). We $\log_e$-transformed response variables (percent organic content, total lipid, chlorophyll-a, and amounts of fatty acids, expressed on a per gram of sediment), and modelled them as a function of Season (Spring 2016 or Winter 2017) as fixed effect in a model that included location identifier as a random term. The inclusion of

location as a random term allowed us to account for both consistent spatial differences and the uneven spatial sampling effort between the two seasons. Due to the log-e transformation, zero values were replaced with the minimum non-zero value for each response variable divided by 10. The approach may have resulted in an underestimation of seasonal differences but allowed us to meet the distributional assumptions of mixed effects models. We tested for significance of the Season term with a t-test using the Satterthwaite's method (package *lmerTest* in R [Kuznetsova et al. 2017]). For each model, we checked the residuals for normalcy and heteroscedasticity, and calculated pseudo-R^2 values (Nakagawa et al. 2017) using package *piecewiseSEM* (Lefcheck 2016) in R. Two pseudo-R^2 values were derived for each model: the marginal R^2 considers only the variance explained by the fixed effects, and the conditional R^2 by both the fixed and random effects.

2.7 Composition Analyses

Community composition of recently living microphytobenthos and invertebrates in biofilm samples was determined via microscopic study as per Findlay and Kling (1998) and Kling (1998). Our field studies did not include winter taxonomy sampling, so only spring taxonomy analysis was conducted. A subsample of each field collection was diluted and subsampled into a 2 ml settling chamber, allowed to settle for a 12 h period and examined using a Leica Diavert inverted microscope at 144 to 960x magnification. Only cells containing chloroplasts or having an indication that they were recently living were included in the analysis. Large organisms were enumerated at the low power and the smaller, more numerous cells were enumerated at the higher power. Measures of length and width were used to obtain estimates of cell volume for each taxa. These volumes are obtained by routine measurements of 30-50 cells of an individual taxa and application of the geometric formula best fitted to the shape of the

245 protoplast, excluding floatation appendages or mucilage (Rott 1981). Estimates of taxon-specific
246 volume and total counts of each taxon were used to calculate total volume, which was converted
247 to biomass assuming a specific gravity of 1 (1 cm^3 = 1 g). Taxonomic classification was done to
248 genus, and species when possible.

249 3 Results

250 3.1 Temporal Changes in Biofilm Organic and Fatty Acid Content

251 All measures of fatty acids varied strongly between spring 2016 and winter 2017, although
252 patterns varied by each measure (Table 1). Linear mixed effects models indicated a significant
253 difference between seasons for all variables except Chlorophyll-a (Table 1). Models had mixed
254 explanatory performance, based on conditional pseudo-R^2 values, and the variance explained
255 tended to be higher for individual fatty measures (range of R^2: 0.28-0.96) than for summed
256 biofilm measures (range of R^2: 0.16-0.49). The marginal R^2 values (variance explained by fixed
257 effects) were in general large fractions of the conditional R^2 values (total variance explained),
258 indicating that seasonal differences were larger than the spatial differences accounted by the
259 random effect of station ID (Table 1).

260 **Table 1. Parameter estimates of mixed effects models depicting biofilm measures as a function of season**. Beta refers to
261 parameter values for fixed effects with standard errors (SE) that denote the average difference in values observed in Winter 2017
262 relative to Spring 2016. Marginal R^2 considers only the variance explained by the fixed effects, and the conditional R^2 by both the
263 fixed and random effects (the total variance explained by the model).

Measure	Beta	SE	DF	t-value	P-value	Marginal R^2	Conditional R^2
Organic Content	-0.210	0.076	47	-2.77	0.008	0.13	0.30
Total lipid	-0.543	0.146	48	-3.72	0.001	0.20	0.46
Chlorophyll-*a*	-0.133	0.093	47	-1.43	0.158	0.04	0.16
SFA	-1.343	0.238	46	-5.65	<0.001	0.40	0.46
MUFA	-1.980	0.337	46	-5.87	<0.001	0.42	0.49
PUFA	-0.954	0.195	46	-4.89	<0.001	0.33	0.41
Total n-3	-1.587	0.348	46	-4.56	<0.001	0.30	0.31
Total n-6	-2.004	0.586	47	-3.42	0.001	0.20	0.20
16:0	-2.882	0.505	47	-5.70	<0.001	0.39	0.50
Palmitoleic acid (16:1n-7)	-0.933	0.189	47	-4.93	<0.001	0.31	0.49
18:0	-4.240	0.822	47	-5.16	<0.001	0.36	0.36
18:1n-9	-5.197	0.165	46	-31.41	<0.001	0.95	0.96
EPA (20:5n-3)	-1.693	0.447	47	-3.79	<0.001	0.22	0.35
DHA (22:6n-3)	-4.123	0.288	47	-14.32	<0.001	0.80	0.84
Sum of palmitoleic acid, EPA, DHA	-1.109	0.220	47	-5.035	<0.001	0.33	0.48

All biofilm measures were higher in spring 2016 relative to winter 2017. Based on fitted means from mixed effects models, organic content in spring averaged 5.0% compared to ~4.1% (g/g) in winter, a relative difference of ~23% (Fig. 2). In contrast, Chlorophyll-*a* content in spring averaged 0.065 mg/g sample, and was not significantly different from the 0.056 mg/g sample observed in winter (Fig. 2). Total lipid was substantially higher in spring (1.45 mg/g sample) than in winter (0.84 mg/g sample), an increase of 1.7× relative to winter (Fig. 2). The higher springtime total lipid compared to winter was mirrored by differences in all summed fatty acid measures, such that the average springtime mass fractions were 3-7× the values observed in winter (Fig. 2). Total fatty acids in spring averaged 653.2 µg/g sample, relative to 173.8 µg/g sample in winter. Saturated fatty acids in spring (243.8 µg/g sample) were ~7× values from winter (33.5 µg/g sample). MUFA in spring (238.1 µg/g sample) were ~3× values from winter (91.2 µg/g sample). Similarly, PUFA in spring (164.6 µg/g sample) was ~5× values from winter (33.3 µg/g sample). Total n-3 fatty acids in spring (124.5 µg/g sample) were ~ 7× values from winter (16.6 µg/g sample), and total n-6 fatty acids in spring (34.5 µg/g sample) were ~6× values observed in winter (5.8 µg/g sample).

The most abundant fatty acids in both seasons were 16:0, 16:1n-7, and EPA (Fig. 3; Table 2; Appendix 1). Mass fractions of individual fatty acids considered were higher in spring relative to values observed in winter (Fig. 3; Table 2). Based on predicted values, mass fractions of palmitic acid (16:0) averaged 177.7 µg/g sample in spring, nearly 18× the values observed in winter (10.0 µg/g sample). Palmitoleic acid (16:1n-7) averaged 223.2 µg/g sample in spring, 2.5× the value from winter (87.5 µg/g sample). EPA averaged 109.2 µg/g sample in spring, 5.4× the value from winter (20.2 µg/g sample). Some of the individually considered fatty acids had near zero values in winter (e.g., 18:1n-9 and DHA), and showed strong differences to values

observed in the spring (Fig. 3). The sum of palmitoleic, EPA, and DHA, which served as a marker for diatom abundance, during spring was 3.0× the value observed during winter. Also, each response variable was determined on a per gram of organic content (AFDW) basis, and showed similar seasonal trends for all response variables (Table 2; Appendix 1; Appendix 3).

Table 2. Summary of fatty acids profiles. Means and standard deviations are expressed as Mass Fraction of Fatty Acid Methyl Esters (µg FAME/g of biofilm sample; obtained by multiplying by proportion of organic content in samples – see Methods). N for spring 2016 = 19; N = winter 2017 = 30.

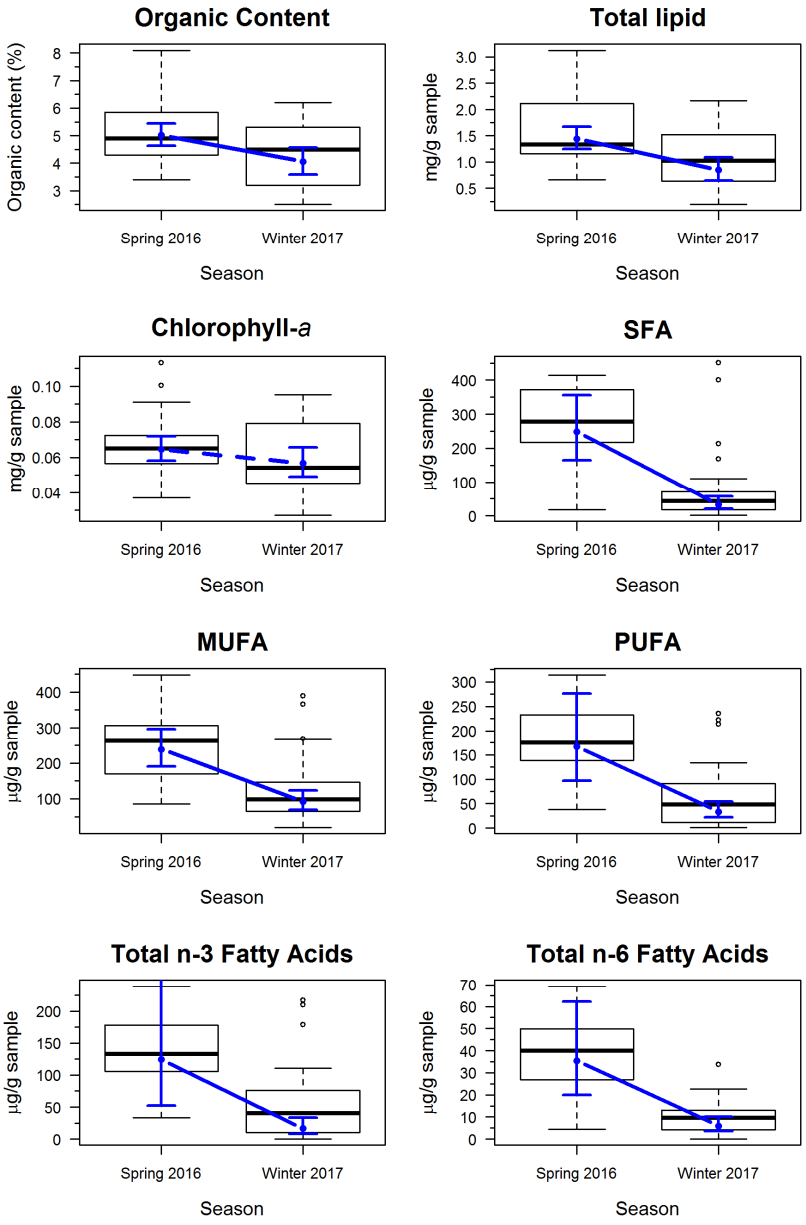
Peak	Systematic Nomenclature	Trivial Nomenclature	Molecular Formula	Spring 2016 Mean	Spring 2016 SD	Winter 2017 Mean	Winter 2017 SD
1	Tetradecanoic acid	Myristic acid	14:0	33.69	10.43	4.12	7.21
2	cis-9-tetradecanoic acid	Myristoleic acid	14:1	0.86	0.95	0.00	0.00
3	Pentadecanoic acid	Pentadecanoic acid	15:0	31.36	31.88	3.01	4.39
4	cis-10-pentadecenoic acid	-	15:1	5.50	5.83	0.26	0.97
5	Hexadecanoic acid	Palmitic acid	16:0	187.41	56.53	48.71	69.96
6	9-hexadecenoic acid	Palmitoleic acid	16:1n-7	237.71	86.18	118.03	86.82
7	Heptadecanoic acid	Margaric acid	17:0	1.27	2.95	0.00	0.00
8	cis-10-heptadecanoic acid	-	17:1	14.17	20.19	2.42	5.61
9	Octadecanoic acid	Stearic acid	18:0	32.08	9.07	10.11	20.70
10	trans-9-octadenoic acid	Elaidic acid	18:1n-9t	0.00	0.00	0.00	0.00
11	cis-9-octadenoic acid	Oleic acid	18:1n-9c	10.32	5.08	0.02	0.10
12	trans-9,12-octadecadienoic acid	Linolelaidic acid	18:2n-6t	0.51	0.20	0.14	0.77
13	cis-9,12-octadecadienoic acid	Linoleic acid (LNA)	18:2n-6c	8.44	3.62	0.14	0.46
14	Eicosanoic acid	Arachidic acid	20:0	1.81	0.87	0.00	0.00
15	9,12,15-octadecatrienoic acid	γ-Linoleic acid (GLA)	18:3n-6	5.97	2.30	0.02	0.06
16	cis-11-eicosenoic acid	Gondoic acid	20:1n-9	1.59	0.86	0.00	0.00
17	9,12,15-octadecatrienoic acid	α-Linolenic acid (ALA)	18:3n-3	4.12	1.20	1.76	1.98
18	cis-11,14-Eicosadienoic acid	Eicosadienoic acid	20:2n-6	5.45	2.77	4.74	4.46
19	Docosanoic acid	Behenic acid	22:0	5.78	2.12	1.79	1.41
20	cis-8,11,14-eicosatrienoic acid	Dihomo-γ-linolenic acid	20:3n-6	1.42	0.88	0.00	0.00
21	13-docosenoic acid	Erucic acid	22:1n-9	0.56	0.55	0.02	0.09
22	cis-11, 14, 17 - eicosatrienoic acid	Eicosatrienoic acid	20:3n-3	0.08	0.14	0.32	1.73
23	5,8,11,14-eicosatetraenoic acid	Arachidonic acid (ARA)	20:4n-6	20.15	7.98	4.86	3.16
24	cis-13,16-docosadienoic acid	Docosadienoic acid	22:2	4.19	1.43	0.13	0.39
25	Tetracosanoic acid	Lignoceric acid	24:0	7.50	2.51	3.27	2.19

26	5,8,11,14,17-eicosapentaenoic acid	Eicosapentaenoic acid (EPA)	20:5n-3	116.41	38.00	53.78	52.96
27	15-tetracosanoic acid	Nervonic acid	24:1n-9	0.002	0.003	0.017	0.095
28	4,7,10,13,16,19-docosahexaenoic acid	Docosahexaenoic acid (DHA)	22:6n-3	0.466	0.215	0.026	0.083

294

295

296



297

298 **Fig. 2 Organic content, total lipid, chlorophyll-a and amounts of major groups of fatty**
299 **acids at Roberts Bank, British Columbia, Canada, during spring 2016 and winter 2017**.
300 Box plots represent the distribution of observed values, where midline is the median, with the
301 upper and lower limits of the box being 75th and 25th percentiles. Whiskers extend up to 1.5× the
302 interquartile range, and outliers are depicted as points. Blue circles indicate predicted means
303 from linear mixed effects models, and bounds are 95% predictions intervals from fixed effects.
304 Dashed lines indicate no significant differences between seasons.

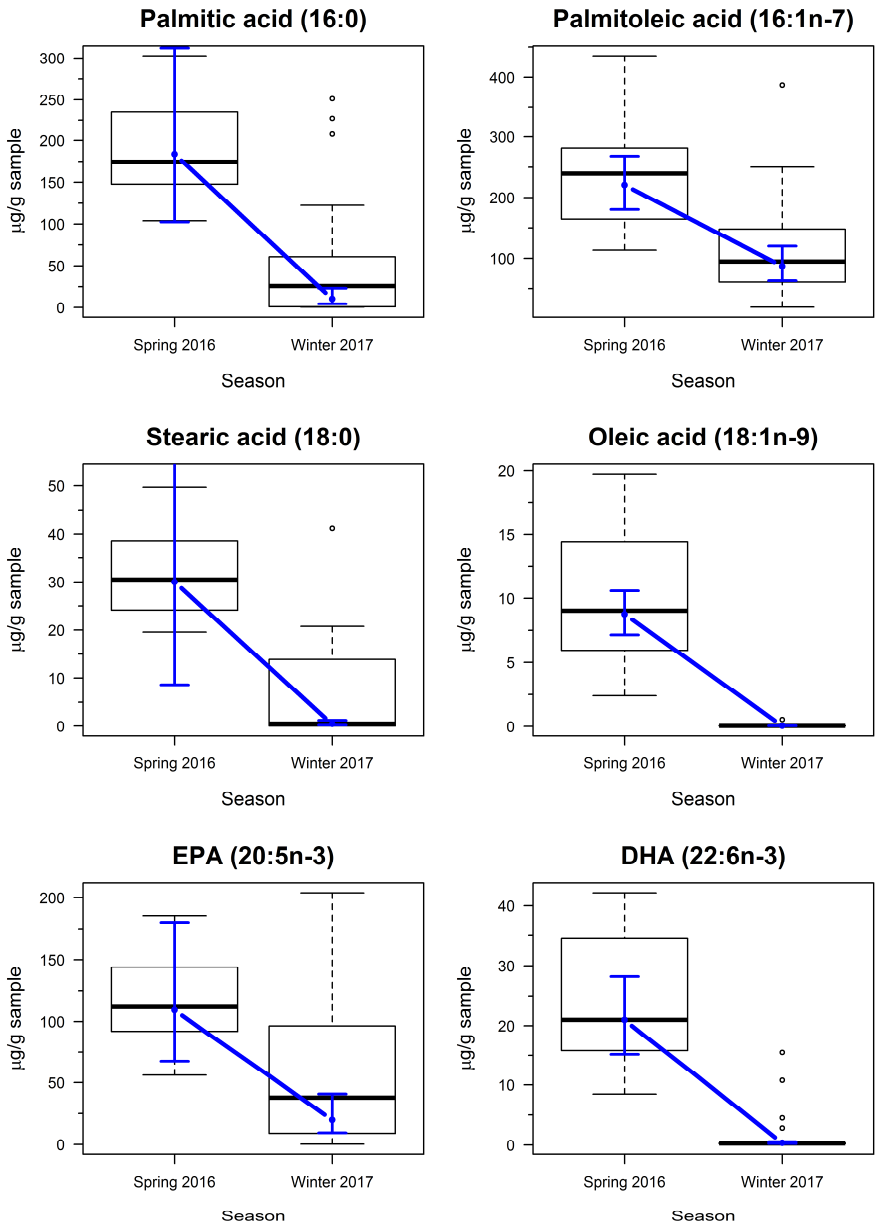
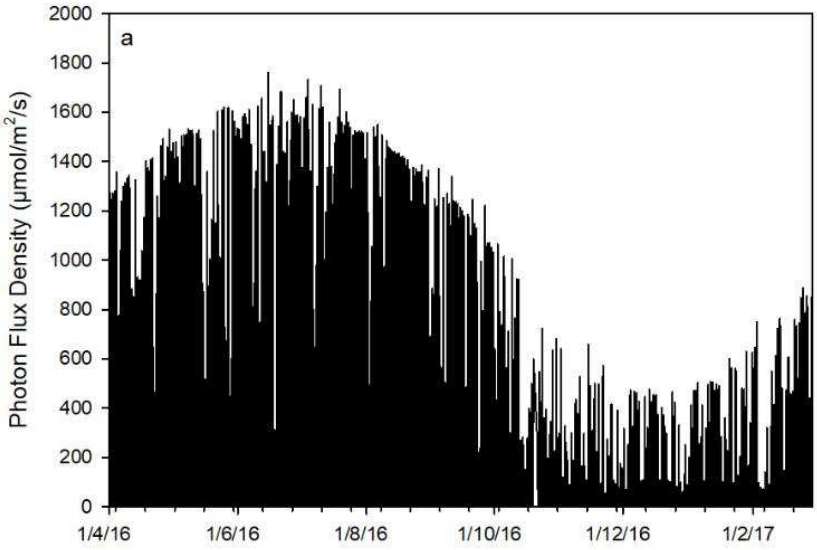


Fig. 3 Amounts of individual fatty acids at Roberts Bank, British Columbia, Canada, during spring 2016 and winter 2017. Box plots represent the distribution of observed values, where midline is the median, with the upper and lower limits of the box being 75th and 25th percentiles. Whiskers extend up to 1.5× the interquartile range, and outliers are depicted as points. Blue circles indicate predicted means from linear mixed effects models, and bounds are 95% predictions intervals from fixed effects. Dashed lines indicate no significant differences between seasons.

3.2 Photon Flux Density and Temperatures across Seasons

The PFD was low during the winter months compared to spring. Specifically, average daytime PFD ranged between 800 and 1400 µmol/m^2/s in April 2016, contrasting with values during January and February 2017 that ranged between 200 and 400 µmol/m^2/s (Fig. 4a). A similar trend occurred with the average ambient temperatures observed during the two time periods (Fig. 4b).



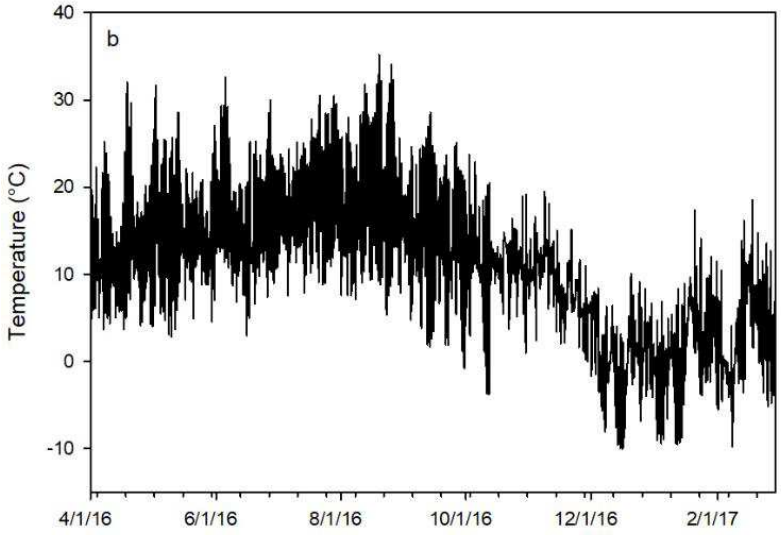


Fig. 4 Photon flux density (a) ambient temperature (b) in the study area from 1 January 2016 to 31 March 2017. Photon flux density (µmol/m^2/s) and temperature (ºC) measurements were taken at Burns Bog, Richmond, British Columbia

3.3 Composition Analyses

The community composition of April biofilm samples encompassed several major taxonomic groups (Table 3), with >50% (µg/ml) of the total biomass being composed of Bacillariophyta (diatoms). The most common diatom genera were the *Nitzschia* and *Naviculoid* complexes, which occurred in all samples, followed by *Gyrosigma*, the *Achanthes* and *Achnanthidium* complex, *Cylindrotheca*, and *Tryblionella* (Appendix 2). Cyanophyta were the second most common taxon in the microphytobenthos, and occurred in 75% of samples but made up small fractions of each sample (<1% [µg/ml]). The most common Cyanophyta genera were *Phormidium*, *Leptolyngbya*, and *Pseudanabaena*. The remaining algal taxa included Chlorophyta, Euglenophyta, and Pyrrophyta, which occurred in few or only one sample, and in small proportions (Table 3). In addition to algae, several groups of invertebrates were present (Table 3), of which the most common were nematodes, which occurred in all samples and made

up 17% (μg/ml) of the total biomass (Appendix 2). Zooplankton, including nematodes, copepods and other zooplankton remains, made up ~35% (μg/ml) of biomass in the samples. Protists, flagellates, and sponge spicules were found in small quantities in a few samples (Appendix 2). Plastic fibers were identified in 20% of all samples collected.

Table 3. **Percent biomass (μg/ml) of major taxonomic groups in 20 samples of biofilm on Roberts Bank, British Columbia, Canada, collected on 21 & 23 April 2016.** Groups are ranked by order of percent contribution to overall biomass. Biomass (%) is expressed as the percent of the total biomass across all samples (20172 μg/ml).

Group	Group Biomass (%)
Bacillariophyta	54.68
Zooplankton	35.10
Protists	6.47
Plastic	1.59
Cyanophyta	0.94
Chlorophyta	0.43
Sponges	0.29
Euglenophyta	0.22
Flagellates	0.16
Pyrrophyta	0.07
Rotifera	0.04
Chrysophyta	0.002

4 Discussion

Although previous studies have investigated diatom-containing biofilm grazed by shorebirds (Jardine et al. 2015; Kuwae et al. 2012; Mathot et al. 2010; Quinn et al. 2017), our study is the first to simultaneously examine biofilm community composition and seasonal availability of a wide range of important fatty acids from an intertidal habitat related to shorebird presence. Our findings highlight the role of estuarine biofilm as a purveyor of important essential nutrients on Roberts Bank for hundreds of thousands of shorebirds during their breeding

354 migration. Of all sites in the Fraser River estuary and delta, shorebirds are found in the highest
355 densities on Roberts Bank during spring migration (from mid-April to mid-May each year)
356 (Butler 1994; Drever et al. 2014; Jardine et al. 2015). The availability of total and specific fatty
357 acids in microphytobenthos, particularly n-3 LC-PUFA (including EPA and DHA) that are not
358 found in primary producers in terrestrial habitats (Hixon *et al.* 2015), may help explain why
359 muddy intertidal estuaries are favored by shorebirds, not only on Roberts Bank but throughout
360 the world (Butler et al. 2001; Mathot et al. 2018).

361 The significantly higher fatty acid mass fractions in spring compared to winter may be a
362 result of three (non-mutually exclusive) processes: i) seasonal species turnover within the biofilm
363 community, including increases in invertebrate populations (i.e. succession), ii) a wintertime
364 decrease in the proportion of biofilm diatom biomass compared to detritus and other organic
365 matter, and iii) physiological changes within extant species induced by seasonal changes in
366 environmental conditions (i.e. resulting in an increase in per cell individual fatty acid synthesis
367 rates).

368 In terms of species turnover, the spring 2016 samples were dominated by *Nitzschia* and
369 *Navicululoid* genera, two common complexes in epipelic microphytobenthos (Underwood 2001)
370 known for having high lipid content (Shifrin & Chisholm 1981). The presence of these diatoms
371 mirrored taxa identified in biofilm and stomach contents of Western Sandpipers collected on
372 Roberts Bank in April 2004 (Beninger et al. 2011). While there were no taxonomic data from the
373 2017 winter samples, a study in the same area found *Nitzschia* and *Navicululoid* genera were also
374 abundant during the winter of 2014 (Worley Parsons 2015). The same study found
375 *Achnanthidium*, predominantly a freshwater diatom genus, was abundant in the spring of 2013
376 (Worley Parsons 2015). In contrast, this taxon made up a small fraction (<1%) of our spring

2016 samples (Appendix 2), and its abundance was thus unlikely to have resulted in the differences in fatty acid contents we observed between winter and spring. Nonetheless, community composition of algal communities can have strong effects on fatty acid profiles (Galloway and Winder 2015), and the effect of seasonal species turnover on fatty acid contents of intertidal biofilm needs to be explored further.

The invertebrate fraction (35%) in spring samples could have contributed to the higher lipid and fatty acid contents within the biofilm matrices. Although we did not conduct taxonomic analysis of biofilms sampled in winter, studies suggest there are large increases in invertebrate populations in spring (Sahan *et al.* 2007). This population increase is associated with increases in access to high quality food (i.e. fatty acid rich algae biomass) (Ahlgren *et al.* 1997; Goedkoop *et al.* 2000; Sahan *et al.* 2007) and rising temperatures positively affecting metabolic rates (Sahan *et al.* 2007). Lipid accumulation in invertebrates is likely the result of grazing on lipid/fatty acid-rich diatoms (Goulden and Place 1990). Microinvertebrates are entrenched in the biofilm matrices, so they contribute to the food of migrating shorebirds but, regardless of the organism being consumed, ultimately the majority of lipids/fatty acids within estuarine mudflats are derived principally from algae, particularly diatoms.

Fatty acid mass fraction may be higher in spring compared to winter due to higher proportions of diatom-contributed biomass within the organic component of the biofilm. These biomass dynamics are influenced by major seasonal changes in PFD, temperature, and tidal patterns and which are characteristic features of temperate estuarine systems. Photon flux densities (Jensen and Revsbech 1989; Schnurr and Allen 2015; Schnurr *et al.* 2016) and temperature (Blanchard et al. 1997; Jiang and Gao 2004; Kudo et al. 2000; Scholz and Liebezeit 2013) are higher in spring compared to winter (Fig. 4a & b), creating conditions conducive to

enhanced rates of photosynthesis and growth in microphytobenthos. Concomitantly, the lowest tides in the study area occur nocturnally in winter and change to diurnal tides in summer; a switch that occurs near the spring equinox in March (Thomson 1981). This switch results in extended exposure times of the intertidal zone (i.e. less attenuation of light from the overlying water column) during daylight hours in spring relative to winter, and stimulates photosynthetic activity. Since diatoms are the main primary producers in intertidal biofilm, increases in photosynthetic activity likely cause increases in diatom biomass in the organic fraction of the sediment. Such a mechanism is supported by the tripling of the sum of palmitoleic acid, EPA, and DHA, the diatom-associated fatty acids, during spring relative to winter.

Mass fractions of fatty acids may also be higher in spring compared to winter if changes in environmental conditions induce physiological changes within diatom biomass/cells. Increases in PFD are known to cause a fatty acid accumulation response in algae (Piepho et al. 2012; Wainman et al. 1999; Wang et al. 2013), which likely contributes to the significantly greater individual and aggregate biofilm fatty acid contents in spring compared to winter. Increased PFDs are known to upregulate the fatty acid synthesizing enzyme acetyl CoA carboxylase, and increase NADPH, which is used to synthesize fatty acids (Wainman et al. 1999). Although high temperatures (within a certain range) can also cause a fatty acid accumulation response (Scholz and Liebezeit 2013; Thompson and Guo 1992; Wainman et al. 1999), mass fractions of many PUFA, particularly EPA and DHA, are significantly reduced if temperatures become too high (Jiang and Gao 2004; Scholz and Liebezeit 2013). Spring temperatures during our study period were considered within temperature ranges suitable for fatty acid accumulation, including PUFA.

Average daily discharges of the Fraser River increase rapidly with the annual spring snow melt (the freshet) during April and May, from 1000 m^3/s during winter months to 7000

423 m^3/s in June (Foreman et al. 2001). This freshet is accompanied by rapid changes in salinity and
424 water chemistry, which may have also contributed to the observed fatty acid accumulation
425 response in spring. Changes in salinity can affect metabolism of silicon and enhance lipid
426 production in oleaginous marine diatoms (Adams and Bugbee 2014). Additionally, nutritional
427 stress (especially nitrogen or silicon) induced by lower nutrient inputs into the estuary from
428 freshet conditions (Harrison et al. 1991; Rodolfi et al. 2009; Yin et al. 1995) can cause algae,
429 including diatoms, to reallocate carbon from growth to storage, resulting in the accumulation of
430 fatty acids (Chelf 1990; Grosse et al. 2018; Mus et al. 2013; Schnurr et al. 2013; Shifrin and
431 Chisholm 1981). The increase in both organic and fatty acid content are collectively considered a
432 'bloom,' which generally happens annually when environmental conditions become more
433 favorable.

434 Environmental conditions may also explain why the mass fraction of chlorophyll-*a* in
435 spring was not significantly different than winter, as might be expected to accompany the greater
436 organic content observed in spring. Algae regulate their photoreceptors (i.e. chlorophyll)
437 according to the needs of their photosystems and in response to their environmental conditions
438 (Melis et al. 1999). In this case, the high PFD conditions in springtime may cause down-
439 regulation in the amount of chlorophyll photoreceptors to prevent photo oxidation as microalgae
440 photosystems become saturated at ~ 400 micromoles/m^2/s (Melis 2009). However, the lower
441 PFD conditions in winter (Fig. 4a) likely caused up-regulation of chlorophyll content to
442 maximize the capture of limited photons during this time. As such, the unchanged levels of
443 chlorophyll-*a* are logical despite the significant increase in diatom-related biomass. Thus, in this
444 context, chlorophyll-*a* content is a poor proxy for algae biomass abundance in intertidal mudflat
445 biofilms.

The elevated PUFA levels (particularly EPA and DHA) in spring biofilm compared with winter biofilm coincided with the springtime arrival of Western Sandpiper on northward migration (Drever et al. 2014), and could be a factor in their migration success (Price 2010). Consumption of PUFA has various beneficial effects, including enhancement of unsaturation levels of muscle membranes (i.e. and which decreases average membrane lipid order) in migratory birds (Maillet and Weber 2006; Weber 2009), which increases overall permeability, transmembrane lipid transport and protein activity (Maillet and Weber 2007; Weber 2009). Also, Semipalmated Sandpipers (*Calidris pusilla*) fed high EPA and DHA diets upregulated enzymes involved in oxidative capacity in cellular mitochondria (Maillet and Weber 2007). In Ruby-throated Hummingbirds *(Archilochus colubris),* pectoral muscles showed an association between high mitochondrial density and DHA content, suggesting that DHA affects the high contraction rate of their wings (Infante et al. 2001). Further, EPA (which is abundant in diatoms) may facilitate muscle recovery after strenuous migrations, given that this fatty acid is a precursor for anti-inflammatory eicosanoids (Price 2010). Since migratory shorebirds cannot produce long-chain PUFA *de novo* (Viegas *et al.* 2017), and likely only make EPA and DHA from shorter-chain PUFA with limited efficiency, it is arguably advantageous for them to ingest PUFA pre-formed in their diet through the consumption of diatoms and invertebrates that have consumed diatoms.

Once shorebirds reach their breeding grounds, n-3 LC-PUFA, particularly EPA and DHA, may serve essential roles in bird development, growth and reproduction. For example, Tree Swallows (*Tachycineta bicolor*) fed a EPA-rich diet demonstrated significant increases in brain DHA, resulting in increased reproductive success, measured as number of fledglings, hatch and fledge rate, and egg and chick number (Twining et al. 2018). While the physiological

469 pathways of biofilm-derived fatty acids during shorebird migration and onto the breeding
470 grounds remain uncertain, the known roles of DHA (and EPA) in brain development and
471 reproductive success in other vertebrates are indications that negative carryover effects could
472 result if the abundance of organic and fatty acid content of biofilm at migratory stopover habitats
473 is compromised.

474 Our findings highlight the dynamic nature of intertidal biofilm as a food resource for
475 shorebirds, and associated seasonal changes in organic and fatty acid content of biofilm
476 coinciding with shorebird migration. Biofilm biomass and fatty acid content levels observed in
477 the spring are unlikely to be maintained throughout the summer due to the combined adverse
478 effects of high temperatures (Blanchard et al. 1997; Jiang and Gao 2004; Kudo et al. 2000,
479 Scholz and Liebezeit 2013) and the photo-inhibitory effects of high PFDs (Chen et al. 2011;
480 Benemann 2013; Melis 2009) on microphytobenthos. The high mass fractions of fatty acids in
481 spring, when large flocks of shorebirds are moving through the area, is consistent with the 'green
482 wave' hypothesis (Marra et al. 2005; Schwartz 1998), whereby bird migration coincides with the
483 availability of key nutrients at stopover sites. Conversely, if elevated levels of fatty acids are
484 available only during a limited temporal window, there may be a 'match -mismatch' scenario,
485 whereby if the resources required to successfully migrate are compromised when the birds arrive,
486 population-level consequences may ensue (Cushing 1990; Koeller et al. 2009; Jones and
487 Creswell 2010). Such a scenario would be exacerbated by the large variance in the seasonal
488 influx of fatty acids against the inflexibility of the migrating Western Sandpipers to adjust their
489 schedule (Clark and Butler 1999).

490 High production and cycling of nutrients are key features of intertidal sediments that
491 support migratory shorebirds (Saint-Béat et al. 2013), yet there is little understanding of how

492 grazing by shorebirds affects biofilm productivity. The complexity and sensitivity of intertidal
493 biofilm systems are further underscored by the possibility of feedback mechanisms between
494 biofilm and shorebirds, where shorebird droppings add dissolved nutrients and affect
495 microphytobenthic diatom growth and biochemical composition (Jauffrais et al. 2015). Thus,
496 studies which couple research on fatty acid profiling, biofilm ecology, and avian physiology are
497 required to better understand the contribution of biofilms to shorebird migration. Finally, there
498 are broader ecosystem implications surrounding the conservation of intertidal biofilm
499 communities, given that essential fatty acids, produced by the microphytobenthos in biofilms,
500 move up trophic levels through invertebrates (Middelburg et al. 2000; Richoux and Froneman
501 2008), fish, waterbirds, and mammals (Colombo et al. 2016). In summary, our findings
502 underscore the need for the conservation of intertidal biofilm habitats in estuarine systems,
503 especially in the context of maintaining their core functionality in providing nutritionally
504 important fatty acids to shorebirds (and other consumers) in coastal food webs.

505 **Acknowledgements**

506 This work was supported by a Natural Science and Engineering Research Council of Canada
507 Discovery Grant (#04537-2014) to MTA and Environment and Climate Change Canada funding
508 to RWE and MCD. Special thanks to the Burns Bog Flux Tower project team for temperature
509 and photon flux density data. We thank our volunteers who collected biofilm, including Sean
510 Boyd, Amos Chow, Sarah Cheng, Andrea McLeod, Catherine Jardine, Britney Niedzielski, Elsie
511 Krebs, Holly Middleton, Andrew Huang, and Karen Devitt. Francisca Olaya Nieto and Jeffrey
512 Thomas prepared Fig 1.

513

5 References

1) Adams, C. and Bugbee, B. 2014. Enhancing lipid production of the marine diatom *Chaetoceros gracilis*: synergistic interactions of sodium chloride and silicon. Journal of Applied Phycology. 26:1351-1357.
2) Ahlgren, G., Goedkoop, W., Markenstein, H., Sonesten, L., Boberg, M. 1997. Seasonal variations in food quality for pelagic and benthic invertebrates in Lake Erken – the role of fatty acids. Freshwater Biology. 38:555-570.
3) Albanese, G. and Davis, C.A. 2015. Characteristics within and around stopover wetlands used by migratory shorebirds: Is the neighborhood important? Condor. 117:328-340.
4) Arar, E.J. 1997. In Vitro Determination of Chlorophylls a, b, c1 + c2 and Pheopigments in Marine and Freshwater Algae by Visible Spectrometry (Method 446.0). National Exposure Research Laboratory Office of Research and Development U.S. Environmental Protection Agency Cincinnati, Ohio.
5) Arts, M.T., Ackman, R.G., Holub, B.J. 2001. "Essential fatty acids" in aquatic ecosystems: a crucial link between diet and human health and evolution. Canadian Journal of Fisheries and Aquatic Sciences. 58:122-137.
6) Bates, D., Maechler, M., Bolker, B., Walker, S. 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software. 67:1-48.
7) Bellinger, B.J., Abdullahi, A.S., Gretz, M.R., Underwood, G.J.C. 2005. Biofilm polymers: relationship between carbohydrate biopolymers from estuarine mudflats and unialgal cultures of benthic diatoms. Aquatic Microbial Ecology. 38:169-180.
8) Benemann, J. 2013. Microalgae for Biofuels and Animal Feeds. Energies. 6:5869-5886.

9) Beninger, P.G., Elner, R.W., Morancais, M., Decottignies, P. 2011. Downward trophic shift during breeding migration in the shorebird *Calidris mauri* (Western Sandpiper). Marine Ecology Progress Series. 428:259-269.

10) Beninger, Peter. G. 2018. Introduction: Mudflat Basics. In, *Mudflat Ecology*. Springer International Publishing AG. 201. Online. pp 1-9.

11) Blanchard, G.F., Guarini, J.M., Gros, P., Richard, P. 1997. Seasonal effect on the relationship between the photosynthetic capacity of intertidal microphytobenthos and temperature. Journal of Phycology. 33:723-728.

12) Butler, R.W. and Campbell, R.W. 1987. The birds of the Fraser River delta: populations, ecology and international significance. Occasional Paper. Ottawa, Canada: Canadian Wildlife Service. 73 pages. Available at: http://publications.gc.ca/collections/collection_2018/eccc/CW69-1-65-eng.pdf

13) Butler, R.W. 1994. Distribution and abundance of Western Sandpipers, Dunlins, and Black-bellied Plovers in the Fraser River estuary. Occasional Paper. Ottawa, Canada: Canadian Wildlife Service. 78 pages. Available at: http://publications.gc.ca/collections/collection_2018/eccc/CW69-1-83-eng.pdf

14) Butler, R.W., Davidson, N.C., Morrison, R.G. 2001. Global-scale shorebird distribution in relation to productivity of near-shore ocean waters. Waterbirds. 24:224-232.

15) Carpentier, A., Como, S., Dupuy, C., Lefrançois, C., Feunteun, E. 2014. Feeding ecology of *Liza* spp. in a tidal flat: Evidence of the importance of primary production (biofilm) and associated meiofauna. Journal of Sea Research. 92:86-91.

16) Chelf, P. 1990. Environmental control of lipid and biomass production in two diatom species. Journal of Applied Phycology. 2:121-129.

17) Chen, X., Goh, Q.Y., Tan, W., Hossain, I., Chen, W.N., Lau, R. 2011. Lumostatic strategy for microalgae cultivation utilizing image analysis and chlorophyll a content as design parameters. Bioresource Technology. 102:6005-6012.

18) Clark, C. and Butler R.W. 1999. Fitness components of avian migration: a dynamic model of Western Sandpiper migration. Evolutionary Ecology Research. 1:443-447.

19) Colombo, S.M., Wacker, A, Parrish, C.C., Kainz, M.J., Arts, M.T. 2017. A fundamental dichotomy in long-chain polyunsaturated fatty acid abundance between and within marine and terrestrial ecosystems. Environmental Reviews. 25:163-174.

20) Cushing, D. H. 1990. Plankton production and year-class strength in fish populations: an update of the match/mismatch hypothesis. Advances in Marine Biology. 26:249-293.

21) Decho, A.W. 2000. Microbial biofilms in intertidal systems: an overview. Continental Shelf Research. 20:1257-1273.

22) Drever, M.C., Lemon, M.J., Butler, R.W., Millikin, R.L. 2014. Monitoring populations of Western Sandpipers and Pacific Dunlins during northward migration on the Fraser River Delta, British Columbia, 1991–2013. Journal of Field Ornithology. 85:10-22.

23) Egeler, O. and Williams, T.D. 2000. Seasonal, age, and sex-related variation in fatty-acid composition of depot fat in relation to migration in Western Sandpipers. The Auk. 117:110-119.

24) Elner, R.W., Beninger, P.G., Jackson, D.L., Potter, T.M. 2005. Evidence of a new feeding mode in western sandpiper (*Calidris mauri*) and dunlin (*Calidris alpina*) based on bill and tongue morphology and ultrastructure. Marine Biology. 146:1223-1234.

25) Findlay, D.L. and Kling, H.J. 1998. Protocols for Measuring Biodiversity: Phytoplankton in Freshwater. Winnipeg, Manitoba: Department of Fisheries and Oceans.

583 26) Folch, J., Lees, M., Sloane Stanley, G.H. 1957. A simple method for the isolation and
584 purification of total lipids from animal tissues. Journal of Biological Chemistry. 226:497-
585 509.
586 27) Foreman, M.G.G., Lee, D.K., Morrison, J., Macdonald, S., Barnes, D., Williams, I.V.
587 2001. Simulations and retrospective analyses of Fraser watershed flows and temperatures.
588 Atmosphere-Ocean. 39:89-105.
589 28) Galloway, A.W.E. and Winder, M. 2015. Partitioning the relative importance of
590 phylogeny and environmental conditions on phytoplankton fatty acids. PLoS ONE. 10:
591 e0130053.
592 29) Goedkoop, W., Sonesten, L., Ahlgren, G., Boberg, M. 2000. Fatty acids in profundal
593 benthic invertebrates and their major food resources in Lake Erken, Sweden: seasonal
594 variation and trophic indications. Canadian Journal of Fisheries and Aquatic. 57:2267-
595 2279.
596 30) Goulden, C.W. and Place, A.R. 1990. Fatty acid synthesis and accumulation rates in
597 daphniids. Journal of Experimental Zoology. 256:168-178.
598 31) Grosse, J., Brussard, C.P.D., Boschker, H.T.S. 2018. Nutrient limitation driven dynamics
599 of amino acids and fatty acids in coastal phytoplankton. Limnology and Oceanography.
600 64:302-316.
601 32) Guglielmo, C.G. 2010. Move that fatty acid: fuel selection and transport in migratory
602 birds and bats. Integrative and Comparative Biology. 50:336–345.
603 33) Guglielmo, C.G. 2018. Obese super athletes: fat-fueled migration in birds and bats.
604 Journal of Experimental Biology. 221:jeb165753. doi:10.1242/jeb.165753

605 34) Harrison, P.J., Clifford, P.J., Cochlan, W.P., Yin, K., St. John, M.A., Thompson, P.A.,
606 Sibbald, M.J., Albright, L.J. 1991. Nutrient and plankton dynamics in the Fraser River
607 plume, Strait of Georgia, British Columbia. Marine Ecology Progress Series. 70:291-304.
608 35) Herman P.M.J., Middelburg, J.J., Widdows, J., Lucas, C.H., Heip, C.H.R. 2000. Stable
609 isotopes as trophic tracers: combining field sampling and manipulative labelling of food
610 resources for macrobenthos. Marine Ecology Progress Series. 204:79-92.
611 36) Hixson, S.M., Sharma, B., Kainz, M.J., Wacker, A. Arts, M.T. 2015. Production,
612 distribution, and abundance of long-chain omega-3 polyunsaturated fatty acids: a
613 fundamental dichotomy between freshwater and terrestrial ecosystems. Environmental
614 Reviews. 23:414-424.
615 37) Huggins K., Frenette J.J., Arts, M.T. 2004. Nutritional quality of biofilms with respect to
616 light regime in Lake Saint-Pierre (Québec, Canada). Freshwater Biology. 49:945-959.
617 38) Infante, J.P., Kirwan, R.C., Brenna, J.T. 2001. High levels of docosahexaenoic acid
618 (22:6n-3)-containing phospholipids in high-frequency contraction muscles of
619 hummingbirds and rattlesnakes. Comparative Biochemistry and Physiology Part B:
620 Biochemistry and Molecular Biology. 130:291-298.
621 39) Jardine, C.B., Bond, A.L., Davidson, P.J., Butler, R.W., Kuwae, T. 2015. Biofilm
622 consumption and variable diet composition of Western Sandpipers (*Calidris mauri*)
623 during migratory stopover. PloS One. 10:e0124164.
624 40) Jauffrais, T., Drouet, S., Turpin, V., Méléder, V., Jesus, B., Cognie, B., Raimbault, P.,
625 Cosson, R.P., Decottignies, P., Martin-Jézéquel, V. 2015. Growth and biochemical
626 composition of a microphytobenthic diatom (*Entomoneis paludosa*) exposed to shorebird

(*Calidris alpina*) droppings. Journal of Experimental Marine Biology and Ecology. 469:83-92.

41) Jensen, J. and Revsbech, N.P. 1989. Photosynthesis and respiration of a diatom biofilm cultured in a new gradient growth chamber. FEMS Microbiology Ecology. 62:29-38.

42) Jiang, H. and Gao, K. 2004. Effects of lowering temperature during culture on the production of polyunsaturated fatty acids in the marine diatom *Phaeodactylum tricornutum* (Bacillariophyceae). Journal of Phycology 40:651-654.

43) Jiménez, A., Elner, R.W., Favaro, C., Rickards K., Ydenberg, R.C. 2015. Intertidal biofilm distribution underpins differential tide-following behavior of two sandpiper species (*Calidris mauri* and *Calidris alpina*) during northward migration. Estuarine, Coastal and Shelf Science. 155:8-16.

44) Jones, T. and Creswell, W. 2010. The phenology mismatch hypothesis: are declines of migrant birds linked to uneven global climate change? Journal of Animal Ecology. 79:98-108.

45) Kling, H.J. 1998. A summary of past and recent plankton of Lake Winnipeg, Canada using algal fossil remains. Journal of Paleolimnology. 19:297-307.

46) Koeller, P., Fuentes-Yaco, C., Platt, T., Sathyendranath, S., Richards, A., Ouellet, P., Orr, D., Skulladdir, U., Wieland, K., Savard, L., Aschan, M. 2009. Basin-scale coherence in phenology of shrimps and phytoplankton in the North Atlantic Ocean. Science. 324:791-793.

47) Kudo, I., Miyamoto, M., Noiri, Y., Maita, Y. 2000. Combined effects of temperature and iron on the growth and physiology of the marine diatom *Phaeodactylum tricornutum* (Bacillariophyceae). Journal of Phycology. 36:1096-1102.

650 48) Kuwae, T., Beninger, P.G., Decottignies, P., Mathot, K.J., Lund, D.R., Elner, R.W. 2008.
651 Biofilm grazing in a higher vertebrate: the western sandpiper, *Calidris mauri*. Ecology.
652 89:599-606.
653 49) Kuwae, T., Miyoshi, E., Hosokawa, S., Ichimi, K., Hosoya, J., Amano, T., Moriya, T.,
654 Kondoh, M., Ydenberg, R.C. and Elner, R.W. 2012. Variable and complex food web
655 structures revealed by exploring missing trophic links between birds and biofilm.
656 Ecology Letters 15:347-356.
657 50) Kuznetsova, A., Brockhoff, P.B. Christensen, R.H.B., 2017. lmerTest package: tests in
658 linear mixed effects models. Journal of Statistical Software. 82(13).
659 51) Lee, S-C., Christen, A., Black, A.T., Johnson, M.S., Jassal, R.S., Ketler, R., Nesic, Z.,
660 Merkens, M. 2017. Annual greenhouse gas budget for a bog ecosystem undergoing
661 restoration by wetting. Biogeosciences. 14:2799-2814.
662 52) Lefcheck, J.S., 2016. piecewiseSEM: Piecewise structural equation modelling in R for
663 ecology, evolution, and systematics. Methods in Ecology and Evolution. 7:573-579.
664 53) Maillet, D. and Weber, J. M. 2006. Performance-enhancing role of dietary fatty acids in a
665 long-distance migrant shorebird: the semipalmated sandpiper. Journal of Experimental
666 Biology. 209:2686-2695.
667 54) Maillet, D. and Weber, J. M. 2007. Relationship between n-3 PUFA content and energy
668 metabolism in the flight muscles of a migrating shorebird: evidence for natural doping.
669 Journal of Experimental Biology. 210:413-420.
670 55) Marra, P. P., Francis, C. M., Mulvihill, R. S., & Moore, F. R. 2005. The influence of
671 climate on the timing and rate of spring bird migration. Oecologia. 14:307-315.

672 56) Mathot, K.J., Lund, D.R., Elner, R.W. 2010. Sediment in stomach contents of western
673 sandpipers and Dunlin provide evidence of biofilm feeding. Waterbirds. 33:300-306.
674 57) Mathot, K.J., Piersma, T., and Elner, R.W. 2018. Shorebirds as integrators and indicators of
675 mudflat ecology. In: *Mudflat Ecology*, P.G. Beninger (Editor) Mudflat Ecology. Switzerland:
676 Springer Nature. pp 309-338.
677 58) McWilliams S.R., Guglielmo C., Pierce B., Klaassen M. 2004. Flying, fasting, and
678 feeding in birds during migration: a nutritional and physiological ecology perspective.
679 Journal of Avian Biology. 35:377-393.
680 59) Melis, A., Neidhardt, J., Benemann, J.R. 1999. *Dunaliella salina* (Chlorophyta) with
681 small chlorophyll antenna sizes exhibit higher photosynthetic productivities and photon
682 use efficiencies than normally pigmented cells. Journal of Applied Phycology. 10:515-
683 525.
684 60) Melis, A. 2009. Solar energy conversion efficiencies in photosynthesis: Minimizing the
685 chlorophyll antennae to maximize efficiency. Plant Science. 177:272-280.
686 61) Middelburg, J.J., Barranguet, C., Boschker, H.T., Herman, P.M., Moens, T., Heip, C.H.
687 2000. The fate of intertidal microphytobenthos carbon: An in situ ^{13}C labeling study.
688 Limnology and Oceanography 45:1224-1234.
689 62) Mus, F., Toussaint, J.P., Cooksey, K.E., Fields, M.W., Gerlach, R., Peyton, B.M.,
690 Carlson, R.P. 2013. Physiological and molecular analysis of carbon source
691 supplementation and pH stress-induced lipid accumulation in the marine diatom
692 *Phaeodactylum tricornutum*. Applied Microbiology and Biotechnology. 97:3625-3642.

63) Nakagawa, S., Johnson, P.C. and Schielzeth, H., 2017. The coefficient of determination R^2 and intra-class correlation coefficient from generalized linear mixed-effects models revisited and expanded. Journal of the Royal Society Interface. 14:20170213.

64) Passarelli, C., Meziane, T., Thiney, N., Boeuf, D., Jesus, B., Ruivo, M., Jeanthon, C., Hubas, C. 2015. Seasonal variations of the composition of microbial biofilms in sandy tidal flats: Focus of fatty acids, pigments and exopolymers. Estuarine, Coastal and Shelf Science. 153:29-37.

65) Paterson, D.M., Perkins, R., Consalvey, M., & Underwood, G.J. 2003. Ecosystem function, cell micro-cycling and the structure of transient biofilms. In, *Fossil and Recent Biofilms*. Springer, Dordrecht. pp 47-63.

66) Piepho, M., Arts, M.T., Wacker, A. 2012. Species-specific variation in fatty acid concentrations of four phytoplankton species: does phosphorus supply influence the effect of light intensity or temperature. Journal of Phycology. 48:64-73.

67) Price, E.R. 2010. Dietary lipid composition and avian migratory flight performance: Development of a theoretical framework for avian fat storage. Comparative Biochemistry and Physiology, Part A. 157:297-309.

68) Quinn, J.T., Hamilton, D.J., Hebert, C.E. 2017. Fatty acid composition and concentration of alternative food of Semipalmated Sandpipers (*Calidris pusilla*) in the upper Bay of Fundy, Canada. Canadian Journal of Zoology. 95:565-573.

69) Richoux, N.B. and Froneman, P.W. 2008. Trophic ecology of dominant zooplankton and macrofauna in a temperate, oligotrophic South African estuary: a fatty acid approach. Marine Ecology Progress Series. 357:121-137.

715 70) Rodolfi, L., Zittelli, G.C., Bassi, N., Padovani, G., Biondi, N., Bonini, G., Tredici, M.R.
716 2009. Microalgae for oil: strain selection, induction of lipid synthesis and outdoor mass
717 cultivation in a low-cost photobioreactor. Biotechnology and Bioengineering. 102:100-
718 112.
719 71) Rott, E. 1981. Some results from phytoplankton counting intercalibrations.
720 Schweizerische Zeitschrift für Hydrologie. 43:34-62.
721 72) Sahan, E., Sabbe, K., Creach, V., Hernandez-Raquet, G., Vyverman, W., Stal, L.J.,
722 Muyzer, G. 2007. Community structure and seasonal dynamics of diatom biofilms and
723 associated grazers in intertidal mudflats. Aquatic Microbial Ecology. 47:253-266.
724 73) Saint-Béat, B., Dupuy, C., Bocher, P., Chalumeau, J., De Crignis, M., Fontaine, C.,
725 Guizien, K., Lavaud, J., Lefebvre, S., Montanié, H. and Mouget, J.L. 2013. Key features
726 of intertidal food webs that support migratory shorebirds. Plos One. 8(10):p.e76739.
727 74) Schnurr, P.J., Espie, G.S., Allen, D.G. 2013. Algae biofilm growth and the potential to
728 stimulate lipid accumulation through nutrient starvation. Bioresource Technology.
729 136:337-344.
730 75) Schnurr, P.J. and Allen, D.G. 2015. Factors affecting algae biofilm growth and lipid
731 production: A review. Renewable and Sustainable Energy Reviews. 52:418-429.
732 76) Schnurr, P.J., Molenda, O., Edwards, E., Espie, G.S., Allen, D.G. 2016. Improved
733 biomass productivity in algal biofilms through synergistic interactions between photon
734 flux density and carbon dioxide concentration. Bioresource Technology. 219:72-79.
735 77) Scholz, B. and Liebezeit, G. 2013. Biochemical characterization and fatty acid profiles of
736 25 benthic marine diatoms isolated from the Solthörn tidal flat (southern North Sea).
737 Journal of Applied Phycology. 25:453-465.

738 78) Schwartz MD. 1998. Green-wave phenology. Nature. 394:839–840.
739 79) Shifrin, N.S. and Chisholm, S.W. 1981. Phytoplankton lipids: Interspecific differences
740 and effects of nitrate, silicate and light-dark cycles. Journal of Phycology. 17:374-384.
741 80) Shin, P.K., Yip, K.M., Xu, W.Z., Wong, W.H., Cheung, S.G. 2008. Fatty acid as markers
742 to demonstrating trophic relationships among diatoms, rotifers and green-lipped mussels.
743 Journal of Experimental Marine Biology and Ecology. 357:75-84.
744 81) Sutherland, T.F., Shepherd, P.C.F., Elner, R.W. 2000. Predation on meiofaunal and
745 macrofaunal invertebrates by western sandpipers (*Calidris mauri*): evidence for dual
746 foraging modes. Marine Biology. 137:983-993.
747 82) Sutherland, T.F., Elner, R.W., O'Neill, J.D. 2013. Roberts Bank: Ecological Crucible of
748 the Fraser River Estuary. Progress in Oceanography. 115:171–180.
749 83) Thomson, R. E. 1981. Oceanography of the British Columbia coast. Canadian Special
750 Publication of Fisheries and Aquatic Sciences. 56. 291 pages. Available at: https://waves-
751 vagues.dfo-mpo.gc.ca/Library/487.pdf
752 84) Thompson, P.A. and Guo, M. 1992. Effects of variation in temperature. I. on the
753 biochemical composition of eight species of marine phytoplankton. Journal of Phycology.
754 28:481-488.
755 85) Twining, C.W., Shipley, J.R., Winkler, D.W. 2018. Aquatic insects rich in omega-3 fatty
756 acids drive breeding success in a widespread bird. Ecology Letters. 21:1812-1820.
757 86) Underwood G.J.C. and Kromkamp J. 1999. Primary production by phytoplankton and
758 microphytobenthos in estuaries. Advances in Ecology Research. 29:93-153.
759 87) Underwood, G.J.C. 2001. Microphytobenthos. Encyclopedia of Ocean Sciences. 3:1770–
760 1777.

761 88) Viegas I., Araújo P.M., Rocha A.D., Villegas A, Jones J.G, Ramos J.A, Masero J.A.,
762 Alves J.A. 2017. Metabolic plasticity for subcutaneous fat accumulation in a long-
763 distance migratory bird traced by 2H2O. Journal of Experimental Biology. 220:1072-
764 1078.
765 89) Wainman, B.C., Smith, R.E.H., Rai, H., Furgal, J.A. 1999. Irradiance and lipid
766 production in natural algal populations. In, *Lipids in Freshwater Ecosystems*. Springer,
767 New York. pp 45-70.
768 90) Wang, L., Li, Y., Sommerfeld, M., Hu, Q. 2013. A flexible culture process for production
769 of the green microalga *Scenedesmus dimorphus* rich in protein, carbohydrate or lipid.
770 Bioresource Technology. 129:289-295.
771 91) Warnock, N. 2010. Stopping vs. staging: the difference between a hop and a jump.
772 Journal of Avian Biology. 41:621-626.
773 92) Weber, J.M. 2009. The physiology of long-distance migration: extending the limits of
774 endurance metabolism. The Journal of Experimental Biology. 212:593-597.
775 93) WorleyParsons. 2015. Roberts Bank Terminal 2 Technical Data Report Biofilm Annual
776 Variability Study. Technical Data Report, Prepared by WorleyParsons Canada, Prepared
777 for Port Metro Vancouver, Burnaby, B.C. 102 pages. Available at:
778 http://www.robertsbankterminal2.com/wp-content/uploads/RBT2-Biofilm-Annual-
779 Variability-TDR-Main-Text1.pdf
780 94) Yin, K., Harrison, P. J., Pond, S., Beamish, R. J. 1995. Entrainment of nitrate in the
781 Fraser River estuary and its biological implications. I. Effects of the salt wedge.
782 Estuarine, Coastal and Shelf Science. 40:505–528.

783

6 Appendices

Appendix 1. Summary of fatty acids profiles. Means and standard deviations are expressed as Mass Fraction of Fatty Acid Methyl Esters (µg FAME/mg ash free dry weight (AFDW) of biofilm sample). N for spring 2016 = 19; N = winter 2017 = 30.

				Spring 2016		**Winter 2017**	
Peak	Systematic Nomenclature	Trivial Nomenclature	Molecular Formula	Mean	SD	Mean	SD
1	Tetradecanoic acid	Myristic acid	14:0	0.644	0.127	0.089	0.153
2	cis-9-tetradecanoic acid	Myristoleic acid	14:1	0.015	0.015	0.000	0.000
3	Pentadecanoic acid	Pentadecanoic acid	15:0	0.531	0.440	0.065	0.089
4	cis-10-pentadecenoic acid	-	15:1	0.093	0.085	0.008	0.035
5	Hexadecanoic acid	Palmitic acid	16:0	3.675	1.083	1.050	1.604
6	9-hexadecenoic acid	Palmitoleic acid	16:1n-7	4.734	1.870	2.548	1.728
7	Heptadecanoic acid	Margaric acid	17:0	0.020	0.047	0.000	0.000
8	cis-10-heptadecanoic acid	-	17:1	0.225	0.293	0.048	0.105
9	Octadecanoic acid	Stearic acid	18:0	0.619	0.117	0.192	0.378
10	trans-9-octadenoic acid	Elaidic acid	18:1n-9t	0.000	0.000	0.000	0.000
11	cis-9-octadenoic acid	Oleic acid	18:1n-9c	0.196	0.083	0.000	0.002
12	trans-9,12-octadecadienoic acid	Linolelaidic acid	18:2n-6t	0.010	0.003	0.003	0.016
13	cis-9,12-octadecadienoic acid	Linoleic acid (LNA)	18:2n-6c	0.162	0.059	0.003	0.009
14	Eicosanoic acid	Arachidic acid	20:0	0.034	0.012	0.000	0.000
15	9,12,15-octadecatrienoic acid	γ-Linoleic acid (GLA)	18:3n-6	0.119	0.047	0.000	0.001
16	cis-11-eicosenoic acid	Gondoic acid	20:1n-9	0.032	0.018	0.000	0.000
17	9,12,15-octadecatrienoic acid	α-Linolenic acid (ALA)	18:3n-3	0.080	0.019	0.037	0.044
18	cis-11,14-Eicosadienoic acid	Eicosadienoic acid	20:2n-6	0.104	0.043	0.094	0.081
19	Docosanoic acid	Behenic acid	22:0	0.109	0.026	0.036	0.026
20	cis-8,11,14-eicosatrienoic acid	Dihomo-γ-linolenic acid	20:3n-6	0.028	0.017	0.000	0.000
21	13-docosenoic acid	Erucic acid	22:1n-9	0.010	0.009	0.000	0.002
22	cis-11, 14, 17 - eicosatrienoic acid	Eicosatrienoic acid	20:3n-3	0.001	0.003	0.008	0.043
23	5,8,11,14-eicosatetraenoic acid	Arachidonic acid (ARA)	20:4n-6	0.390	0.133	0.107	0.055
24	cis-13,16-docosadienoic acid	Docosadienoic acid	22:2	0.080	0.015	0.003	0.010

25	Tetracosanoic acid	Lignoceric acid	24:0	0.143	0.028	0.074	0.044
26	5,8,11,14,17-eicosapentaenoic acid	Eicosapentaenoic acid (EPA)	20:5n-3	2.286	0.716	1.103	1.075
27	15-tetracosanoic acid	Nervonic acid	24:1n-9	0.002	0.003	0.017	0.095
28	4,7,10,13,16,19-docosahexaenoic acid	Docosahexaenoic acid (DHA)	22:6n-3	0.466	0.215	0.026	0.083

788

789

790 **Appendix 2**. **Occurrence and percent biomass (µg/ml) of major taxonomic groups in 20**
791 **samples of biofilm on Roberts Bank, British Columbia, Canada, collected on 21 April 2016.**
792 Taxa are ranked by order of occurrence (number of samples). Biomass (%) is expressed as the
793 percent of the total biomass across all samples (20172 µg/ml). Taxa are identified to genera or
794 species where possible.

Group	Taxon	Occurrence	Biomass (%)	Group Biomass (%)
Bacillariophyta	Naviculoidbiraphid (large, cftrachyneis/plagiotropis)	20	11.95	54.68
	Nitzschia	20	2.06	
	Navicula	20	1.30	
	Nitzschia (small)	18	2.80	
	Navicula (large)	16	8.80	
	Navicula (small)	16	0.45	
	Naviculagregaria	15	1.05	
	Gyrosigma	15	0.92	
	Achanthes/Achnanthidium	15	0.42	
	Cylindrothecagracilis	14	3.02	
	Tryblionella	14	2.31	
	Tryblionellaconstricta	11	0.53	
	Achnanthes/Achnanthidium (small)	11	0.38	
	Gyrosigmabalticum	8	2.11	
	Nitzschia sigma	8	0.39	
	Amphora	7	0.62	
	Gyrosigmaacuminatum	6	0.23	
	Gyrosigmaattenuatum	5	0.61	
	Cylindrothecaclosterium	5	0.12	
	Campylodiscus	4	7.16	
	Cocconeis	4	0.47	
	Diploneis	4	0.32	
	Skeletonemacostatum	4	0.16	
	Small unidentified centric diatoms	4	0.14	
	Ulnaria ulna	4	0.02	
	Gyrosigmafasciola	4	0.01	
	Thalassiosira	3	0.85	
	Entomoneis	3	0.47	
	Nitzschiatryblionella	3	0.08	
	Melosiranumuloides	3	0.05	
	Unidentified benthic diatoms	3	0.04	

Group	Taxon	Occurrence	Biomass (%)	Group Biomass (%)
	Campylodiscushibernicus	2	0.25	
	Tryblionellalevadensis	2	0.09	
	Paraliasulcata	2	0.03	
	Aulacoseirasubarctica	2	0.02	
	Podsira	1	2.18	
	Auxospore	1	0.62	
	Fallacia	1	0.32	
	Surirella	1	0.28	
	Biddulphia	1	0.26	
	Nitzschialinearis	1	0.21	
	Luticola	1	0.13	
	Melosira	1	0.10	
	Skeletonema	1	0.07	
	Navicula cf. phylepta	1	0.06	
	Placoneis	1	0.06	
	Gyrosigmadistortum	1	0.03	
	Naviculaprotracta	1	0.03	
	Cyclotellastriata	1	0.02	
	Cyclotellameninghiniana	1	0.02	
	Frustulia	1	0.02	
	Aulacoseira	1	0.01	
	Gyrosigmascalproides	1	0.01	
	Detonella	1	0.01	
	Naviculacryptocephela	1	0.01	
	Gyrosigmalittorale	1	0.01	
	Nitzschiapalaceae	1	0.005	
	Fragilariacapucina	1	0.004	
	Gyrosigmaprolongatum	1	0.003	
	Tabellariafenestrata	1	0.002	
	Naviculadscusis	1	0.001	
	Fragilaria	1	0.0001	
Zooplankton	Nematode	20	17.27	35.10
	Copepod (unidentified)	4	9.65	
	Crustacean nauplii	4	2.74	
	Zooplankton remains	2	5.35	
	Zooplankton	1	0.10	
Protists	Ciliate	3	1.21	6.47
	Difflugia	2	4.66	

Group	Taxon	Occurrence	Biomass (%)	Group Biomass (%)
	Tintinnids/ Tintinnopsis	2	0.40	
	Thecate amoeba	2	0.15	
	Strobilidium	1	0.05	
	Strombidium	1	0.003	
Plastic	Plastic fiber	4	1.59	1.59
Cyanophyta	Phormidium	12	0.36	0.94
	Leptolyngbya	9	0.37	
	Pseudanabaena	8	0.07	
	Chroococcus	2	0.10	
	Oscillatoria	1	0.04	
	Spirulina	1	0.004	
	Aphanothecebachmanii	1	0.000	
	Unidentified filamentous blue-green algae	5	0.10	
Chlorophyta	Filamentous green algae	1	0.31	0.43
	Planktonemalauterborni	1	0.01	
	Monoraphidium	1	0.002	
	Closterium	1	0.001	
Sponges	Sponge spicule	2	0.29	0.29
Euglenophyta	Euglenid	3	0.18	0.22
	Trachelomonas	1	0.05	
Flagellates	Silicoflagellate	1	0.16	0.16
	Colorless flagellate	1	0.005	
Pyrrophyta	Gymnodinium	1	0.07	0.07
Rotifera	Filinina	1	0.04	0.04
Lipids	Lipids	15	0.01	0.01
Chrysophyta	Ochromonas	1	0.002	0.002

795

796

Appendix 3. Organic content, total lipids, chlorophyll and amounts of major groups of fatty acids at Roberts Bank, British Columbia, Canada, during spring 2016 and winter 2017, expressed as the fraction of Ash Free Dry Weight (AFDW). Box plots represent the distribution of observed values, where midline is the median, with the upper and lower limits of the box being 75th and 25th percentiles. Whiskers extend up to 1.5X the interquartile range, and outliers are depicted as points. Blue circles indicate predicted means from linear mixed effects models, and bounds are 95% prediction intervals from fixed effects. Dashed lines indicate no significant differences between seasons.