Slime, Safety and Shorebirds: Biofilm Production and Grazing by Migrating Western Sandpipers *(Calidris mauri)*

by

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Ethics Statement

Abstract

The quality of stopover sites for migrant shorebirds is thought to be determined by food availability and safety from predators. This thesis investigates this interaction on an estuarine mudflat in British Columbia, where migrant western sandpipers graze biofilm. I measured biofilm concentration and grazing intensity on transects across the mudflat. I found that the concentration of biofilm rose 4.1 mg m^{-2} hr^{-1} during tidal emersion periods, with total accumulation matching that removed by sandpipers during grazing visits. During the higher-intensity (10 – 100 fold, based on daily sandpiper counts) northward migration, biofilm concentration increased and grazing decreased with proximity to the shoreline. In contrast, during southward migration biofilm was uniformly high. A danger manipulation experiment supported a trade-off with biofilm concentration: grazing declines with danger, but less so where biofilm is higher. Together the results indicate that dynamic trophic interactions between danger, sandpipers and biofilm create spatial patterns in biofilm concentration.

Keywords: western sandpiper; biofilm; spring migration; grazing; predation-danger

I dedicate this work to my family.

To my parents for supporting me and believing that I can do anything I set my mind to, and to my sisters for their unconditional love and friendship. I am truly grateful.

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Table of Contents

List of Tables

List of Figures

Chapter 1.

Introduction

1.1. Study Species

1.1.1. Western Sandpiper

Western sandpipers (*Calidris mauri*) are small (25-50g) migratory shorebirds. Differential migrants, western sandpipers overwinter between northern Oregon, USA, and Peru, with males located north of females and juveniles found on the northern and southern extremes of the non-breeding region (Nebel et al. 2002). In spring, western sandpipers migrate north along the Pacific Flyway to breeding grounds in Alaska and eastern Siberia. Northward migration is rapid to ensure breeding ground arrival corresponds with Arctic snowmelt date (Liebezeit et al. 2014). Timely northward migration is motivated by reproduction and biological fitness. Adults that arrive at breeding grounds later nest later (Bêty et al. 2003; Hupp et al. 2006), and offspring that hatch following peak resource abundance have low survival rates (Meijer et al. 1990; Sedinger et al. 1995). It takes between 1-2 weeks for western sandpipers to complete their northward migration. Some birds fly as far as 5,000 km in this short timespan (Weber et al. 1998). For western sandpipers both migration and reproduction are energetically costly (Piersma et al. 2003; Cresswell et al. 2004). On average, western sandpipers can store 8-10% of their weight as reserve fuel (Butler et al. 1987). However, stored energy alone cannot sustain the long-distance flights of these small shorebirds. Western sandpipers must forage intensely while on migration to replenish fuel stores depleted during flight. While migrating, sandpipers feed and rest at several “stopover sites”, coastal mud and sandflats located along the Pacific Flyway. Stopover sites are rich in food resources and provide the fuel sandpipers require during migration (Iverson et al. 1996; Warnock and Bishop 1998).

Previously, shorebird diets were believed to consist wholly of epibenthic, and infaunal, macro- and meiofaunal invertebrates. Foraging techniques included surface feeding (pecking), deep probing, and surface tension transport of small prey suspended in water (Barbosa and Moreno 1999; Estrella et al. 2007). Recently, through examination of bill and tongue physiology (Elner et al. 2005), high definition foraging footage, and stomach isotope analysis (Kuwae et al. 2008; Kuwae et al. 2012), biofilm was found to constitute 45-59% of total western sandpiper diet. Telephoto-video recordings show western sandpipers graze biofilm in a manner distinct from pecking, probing, or surface-tension transport (Kuwae et al. 2008). Coarse denticles on the upper interior bill, and dense tongue-spines, collect biofilm from the sediment surface, while the bill is opened and closed to draw biofilm toward the throat (Kuwae et al. 2008). The entire sequence occurs in under 0.3 seconds for rapid ingestion of biofilm.

1.1.2. Biofilm

Intertidal biofilm is found on the surface of tidal flats in the top 2-mm of sediment. Biofilm is a community of microalgae and bacteria, their mucous secretions, and sediment particles (Decho 2000). Intertidal biofilm communities, also known as microphytobenthos (MPB), are composed predominantly of diatoms and cyanobacteria (Cibic et al. 2007; Underwood 2010). Carbon, fixed during photosynthesis, is diverted into exopolymeric substances (EPS), a mucus-like secretion that binds biofilm communities to inorganic substrate (Stal and de Brouwer 2003). EPS is rich in carbohydrates (70-95% polysaccharides and remainder lipoproteins), which are easily digested, and burned for immediate energy, or stored as fat (Yallop et al. 1994; De Winder et al. 1999; Hoskins et al. 2003). Biofilm is also rich in fatty-acids, particularly linoleic or polyunsaturated fatty-acids (PUFAs) (Quinn et al. 2017). Fat is energy dense and provides much of the fuel shorebirds use during migration (Lindström 1991; Gannes 2001; Mcwilliams et al. 2004). In many species, including shorebirds, these short-chained PUFAs are more easily mobilized than other fatty-acids, and are speculated to improve flight performance in long-distance migrants (Raclot 2003; Maillet 2006; Price et al. 2008). Energy dense and quick-burning, biofilm is a high-quality food source for shorebirds on migration.

However, benthic biofilm found on mudflats is difficult to study. In intertidal systems, Chl-*a* and EPS in the top 2 mm of sediment are positively correlated (Underwood and Smith 1998; De Winder et al. 1999; De Brouwer and Stal 2002). Chl-*a* concentration (mg m^{-2}) or content (mg) are common biofilm biomass approximation measures (Elner et al. 2005; Kuwae et al. 2008; Jardine et al. 2015). Fluorescence is the most common biofilm-estimation technique, however there are several challenges associated with this procedure. Traditional fluorescence biofilm-estimation methods are expensive, labour-intensive, and destructive to sensitive clay-rich habitats. As a result, biofilm research is often restricted by sample size limitations over geographic space and repeated measurements over time.

1.2. Study System

During northward migration, western sandpipers use coastal stopover sites along the Pacific Flyway from southern wintering-grounds to northern breeding-grounds in Alaska (Wilson 1994). Between mid-April to mid-May, migrating western sandpipers pass through the Georgia Strait, British Columbia, stopping at mud and sandflats to rest and refuel (Buchanan 1988; Iverson et al. 1996). The Fraser River Estuary, in which 95% of the western sandpiper population stage annually, is a designated Important Bird Area, a Western Hemisphere Shorebird Reserve Network (WHSRN) site, and is a wetland of international significance under the Ramsar Convention on Wetlands. Roberts Bank is a large mudflat, 27 km^2 at lowest tide, located south of the Fraser River Estuary in Delta, British Columbia (49° 03' 25.20" N, 123° 10' 23.40" W). A 20-50 m wide strip of saltmarsh and a 2-m high dike borders Roberts Bank. Tides at Roberts Bank are semidiurnal with the time of maximum tide shifting ~ 45 minutes later each day.

In spring, hundreds of thousands of migrating western sandpipers stop at Roberts Bank to feed on benthic invertebrates and biofilm. Western sandpipers begin feeding on the mudflat as the tide falls, and continue to forage within 600 m of the shoreline throughout the tidal-exposure period (Jiménez et al. 2015). During high tide western sandpipers roost in nearby farm fields. The dike at Roberts Bank provides cover for merlins (*Falco columbarius*) and peregrine falcons (*Falco peregrinus*) that hunt shorebirds. Falcons are most successful when hunting close to shore where tall saltmarsh grasses conceal their attacks.

Studies find that while some shorebird populations are stable or rising, other species are declining (Bart et al. 2007; Morrison et al. 2012; Wetlands International 2012; Studds et al. 2017). Stopover sites, critical refuelling habitats for migrating shorebirds (Studds et al. 2017), are threatened by shoreline degradation from sea-level rise and urban, and industrial, coastal development (Murray et al. 2014; Murray et al. 2018). Much of the shoreline surrounding the Fraser River Estuary is industrialized. At

Roberts Bank a large coal and container port is located on the eastern edge of the mudflat. In 2013, the Vancouver Fraser Port Authority proposed construction of an additional marine container terminal to be located alongside the existing port infrastructure at Roberts Bank. The proposed terminal-construction could alter tidal regimes and sedimentation patterns (Sutherland et al. 2013), which may affect biofilm production and shorebird habitat quality. My research will help to characterize the role of biofilm in intertidal ecosystems and their significance to the diet, and population viability, of western sandpipers. This thesis will provide valuable information for decision-makers regarding the inclusion of biofilm in environmental assessments and consideration of critical shorebird habitat for pendant and future coastal development.

1.3. Thesis Outline

This study assesses the spatio-temporal dynamics of intertidal-biofilm production, and western sandpiper foraging behaviour, during northward migration. Chapter 2 focuses on diurnal and seasonal variation in biofilm density and the factors influencing biofilm production and standing-crop density. Using a novel biofilm measurement technique, this chapter aimed to determine how biofilm density at a stopover site; 1) changes over an intertidal period, 2) varies between seasons and years, and 3) responds to grazing by migrating western sandpipers across the mudflat. Chapter 3 experimentally tests the hypothesis that western sandpipers trade-off safety and biofilm at a stopover site under increased predation danger. This chapter examined how sandpipers adjust foraging behaviour in response to increased danger over a tidal-emersion period. In the final chapter I summarize the findings of this study, discuss its limitations, and suggest areas for further research.

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Chapter 2.

Fine-scale biofilm measurement on a temperate mudflat with the Chlorophyll Content Meter-300

2.1. Introduction

For migratory shorebirds, food supply is an essential component of habitat quality. At coastal estuary “stopover sites” migrating shorebirds feed intensively, depositing fat into reserve fuel stores (Lindstrom and Piersma 1993). High-quality stopover sites supply the energy shorebirds require for long-distance flight and future fecundity (Myers et al. 1987; O’Reilly and Wingfield 1995; Bishop and Warnock 1998; Clark and Butler 1999; Mehlman et al. 2005). Despite the importance of pre-nesting activities to reproductive success, stopover sites are one of the least studied aspects of shorebird migration (Arzel et al. 2006; Drent et al. 2007; Legagneux et al. 2012; Lyons et al. 2018). One of the greatest threats to stopover sites worldwide is coastal development (Murray et al. 2014). In order to assess habitat quality, and the potential risks to shorebirds from coastal development, food availability at stopover sites must be quantified.

Each spring, western sandpipers (*Calidris mauri*) migrate 5,000 - 10,000 km, over the span of a few weeks, to reach their summer breeding grounds in Alaska (Wilson 1994). Along their journey, sandpipers stop at large coastal estuaries to rest and refuel. Estuaries are some of the most productive ecosystems on earth (Nixon et al. 1986; MacIntyre et al. 1996). Where freshwater and marine environments intersect, estuaries ferry nutrients and organic matter between rivers and the ocean. These inputs support estuarine community assemblages, often characterized by high biomass (Pinckney and Zingmark 1993). Most estuarine mudflat primary production comes from microphytobenthos (MPB), communities of diatoms, cyanobacteria and sediment, also known as biofilm. Biofilm plays a large ecological role in providing food for a wide array of species across multiple trophic levels, including: polychaetes (Decho and Lopez 1993), snails (Whitlatch and Obrebski 1980), copepods (Decho and Moriarty 1990), and fish (Almeida 2003). Previously, western sandpiper diets were thought to primarily consist of macroinvertebrate and meiofaunal prey, such as molluscs, crustaceans, polychaetes and insects (Zwarts et al. 1990, Skagen and Oman 1996). However, through functional morphology, video recordings and stomach contents, and stable isotope analysis, recent studies determined that western sandpipers also graze biofilm (Kuwae et al. 2008; Mathot et al. 2010). It is estimated that biofilm provides up to 50% of western sandpiper daily energy requirements during diurnal foraging and 68% including nocturnal activities (Kuwae et al. 2008). Coastal development, and ensuing hydrodynamic shifts, may pose direct and indirect threats to biofilm and, subsequently, the shorebirds that consume it. Maintaining the integrity of physical and biological processes governing the intertidal system, which includes biofilm production, is a conservation concern (Emmerson et al. 2001; Lundkvist et al. 2007). For healthy populations of shorebirds like the western sandpiper to persist, sufficient high-quality food at stopover sites is integral.

Biofilm can account for a large portion of western sandpiper diet (Kuwae et al. 2008). However, biofilm has proven difficult to study given that measurement typically requires labour intensive, and often expensive, extraction methods (Murphy and Underwood 2006; Murphy et al. 2008; Coelho et al. 2011). As a result, the fine-scale spatio-temporal dynamics between western sandpipers and biofilm is poorly understood (Elner et al. 2005; Kuwae et al. 2008; Mathot et al. 2010; Kuwae et al. 2012). Intertidal biofilm may be influenced by bottom-up as well as top-down factors, which often vary through time and space. Bottom-up processes influence primary production, while grazers exert top-down pressure on biofilm standing-crop density. Nutrient availability (Kocum et al. 2002), temperature (Salleh and McMinn 2011), and light (Smith and Underwood 2000; Savelli et al. 2018), enhance primary production and biofilm growth, but also fluctuate seasonally in temperate climates. In comparison, little is known about the effect that grazing has upon intertidal biofilm growth and regeneration.

In many producer-consumer relationships grazing reduces biomass, but the rate at which reduction occurs varies across ecosystems and time-scales. Pulses of migratory animals graze intensively over short periods, which may result in immediate and significant reduction in primary-producer biomass (Bauer and Hoye 2014). Alternatively, repeated grazing may promote accelerated biofilm regeneration. For example, barnacle geese (*Branta leucopsis*) in the Dutch Waddenzee repeatedly grazed red fescue grass to levels that sustained regrowth of protein-rich plant tissue (Ydenberg and Prins 1981). Similarly, but over a longer time-period, rocky-shore biofilm biomass increased in snail-grazed enclosures several months after grazing (Skov et al. 2010; Savelli et al. 2018). How biofilm production on a temperate mudflat varies over short and long time-periods, as influenced by top-down and bottom-up processes, has yet to be quantified.

This chapter considers the interaction between biofilm and western sandpipers at Roberts Bank, a large coastal mudflat in southern British Columbia, Canada (49° 03' 25.20" N, 123° 10' 23.40" W). Here, I introduce a low-cost, rapid, and non-destructive alternative method to estimate primary productivity (chlorophyll-*a*) on a mudflat to quantify biofilm density over fine spatial and temporal scales. The procedures described in this chapter aim to develop convenient and repeatable field methods to measure biofilm abundance and western sandpiper foraging activity. Through the novel application of this technology I explore variation in temporal biofilm density, both diurnal and seasonal, as well as the effect of biofilm grazing by western sandpipers across space. This chapter asks how biofilm density at a stopover site: 1) changes over a tidal-exposure period, 2) varies between seasons and years, and 3) responds to grazing by migrating western sandpipers across the upper intertidal. I predict that: 1) biofilm will increase over a tidal exposure period, 2) biofilm density will be higher in summer in response to higher average temperature and insolation, and 3) biofilm growth will increase due to enhanced regeneration following grazing by western sandpipers.

2.2. Methods

2.2.1. Study Site

Field work was conducted at Roberts Bank, British Columbia, Canada (49° 03' 25.20" N, 123° 10' 23.40" W), from 23-29 April, 2016 and 18 April - 8 Sept, 2017. Roberts Bank is a 27 km^2 intertidal mudflat located east and south of Brunswick Point on the mouth of the Fraser River Estuary. A designated Important Bird Area, Roberts Bank provides a critical resting and refuelling site for migratory shorebirds during northward migration (April – May). The area of the upper intertidal studied covered 48,000 m^2 (400 m x 120 m) (Figure 2.1).

2.2.2. Data Collection

Biofilm

Diatoms compose most of the photosynthetically active portion of MPB biomass; therefore concentrations of chlorophyll-*a* (a pigment essential for photosynthesis) provide an indirect measure of biofilm (Underwood and Smith 1998; De Brouwer and Stal 2002; Pomeroy and Butler 2005; Beninger et al. 2011). Conventional biofilm estimation methods involve sediment sampling in the field and chlorophyll-*a* (Chl-*a*) extraction in a lab. I measured Chl-*a* with an Opti-Sciences Chlorophyll Content Meter (CCM-300) (https://www.optisci.com/ccm-300.html). The CCM-300 uses a fiber-optic cable with a 3 mm diameter probe that emits an excitation wavelength (700 nm – 710 nm). The light emitted is absorbed by Chl-*a* in the sample under the probe, and re-emitted as fluorescence at a longer wavelength (730 nm – 740 nm). The CCM-300 has two solid-state, high sensitivity detectors, which provide simultaneous, dual-detection of wavelengths from 700 nm - 710 nm and 730 nm – 740 nm. The chlorophyll fluorescence ratio F735/F700 (termed CFR) is linearly proportional to Chl-*a* content in the range of 41 to 675 mg m^{-2} (r^2 >0.95) (Gitelson et al. 1999). The 3 mm probe allows the CCM-300 to measure Chl-*a* on curved or uneven surfaces and samples that are too small for traditional absorption techniques.

I mounted the CCM-300 on a stand composed of a hollow arm extending 60 cm perpendicular from a square base with a 4 mm hole drilled through its center, in which the probe rested (Figure 2.2). The CCM-300 unit faced skyward when resting on a flat surface and the attached fiber-optic cable ran through the hollow arm of the stand. This allowed the sensor to rest 1 mm from the bottom of the base. When placed on the surface of the mudflat the stand enabled rapid and repeatable measurement of the top biofilm layer. Between each measurement I rinsed the surface of the probe with filtered water to prevent carry over. To increase measurement accuracy, five measurements from the same location were averaged into one measurement using the CCM-300's built-in function.

I calibrated CCM-300 CFR measurements with lab-analyzed Chl-*a* from mud samples collected at Roberts Bank in 2017. Biofilm was measured in paired 1 m^2 plots at each of seven sites using the CCM-300 and established sampling methods (Hemmera Envirochem Inc. 2018). Mud samples were extracted concurrently with CCM-300 measurements from separate sampling plots located 1 m apart. A 7.6 cm wide putty knife and a ruler were used to scrape off the top 2 mm of mudflat in a 10 cm long strip (Figure 2.3). Immediately after collection, the mud samples were placed into a 60 mL centrifuge tube, which was sealed in an individual amber bag and stored in a portable cooler with dry ice. Once collected, all mud samples were transferred to a -80°C freezer prior to lab analysis. Samples were shipped in styrofoam coolers with dry ice, and remained below -30°C. Mud samples were analyzed for Chl-*a* at Aquatech Enviroscience Laboratories (AEL, Victoria, B.C.) using a Fluorescent Spectrometer from TURNER (model 7200-000, using Trilogy Module CHL-NA, Model #46, Turner Designs, Sunnyvale, CA, USA). In the lab, each mud sample was weighed, freeze-dried at -60°C, and re-weighed to calculate dry weight. The dried samples were then homogenized into a fine powder using a mortar and pestle and a subsample (40 to 200 mg) removed from each. Chl-*a* content of sediment samples was extracted with a mixture of High Performance Liquid Chromatography (HLPC) grade Acetone: Ethanol at a 90:10 ratio.

Ecological Variables

Several environmental variables have been shown to influence biofilm production including temperature, precipitation, solar radiation, and nutrient concentration. Mean daily temperature (C°) and mean daily precipitation (mm) for Vancouver International Airport (49°11'41.000" N, 123°11'02.000" W), were retrieved from Environment and Climate Change Canada’s historical database. Solar radiation (W m^{-2}) was recorded at the University of British Columbia Totem Field climate station (49.2562° N, 123.2494° W), retrieved from http://ibis.geog.ubc.ca/~achristn/data.html. Discharge is correlated with nutrient input and, because nutrients were not directly measured, serve as a proxy measure. To quantify nutrient input from the Fraser River freshet, discharge (m^3 s^{-1}) from the Fraser River at Hope hydrometric station (Station: 08MF005; 49°23'09" N, 121°27'15" W) was retrieved from Environment and Climate Change Canada’s historical database. Environment Canada notes that 2017 hydrometric data are preliminary and subject to revision. I extracted these data for the 2016 and 2017 sampling periods, and for the migration and summer (June-August) periods for a 27-year climate normal (1991-2018), excluding solar radiation for which long-term data was unavailable.

Western Sandpipers

To estimate mudflat usage by western sandpipers I measured number of droppings m^{-2} and proportion of footprints m^{-2} (Pomeroy 2006). Western sandpipers leave droppings that are white and circular (approximately 1.5 cm diameter), and their footprints comprise three branching lines (approximately 2.5 cm in diameter). Both droppings and footprints are well preserved on the mudflat and easily identified (Figure 2.4 a). At Roberts Bank the mudflat is inundated with water during the rising tide, which effectively removes any trace of droppings and footprints with the receding tide (Sutherland et al. 2000). I estimated footprints as the proportion of western sandpiper footprints left in the mud of a 1 m^2 plot in 5% bins (Kautsky 2013; Figure 2.4 b).

2.2.3. Study Design

Field procedures were carried out during the western sandpiper northward migration period (~20 April – 5 May) in 2016 and 2017. A pilot study in spring 2016 developed the basic field protocols. Biofilm and dropping counts were measured from 23 - 29 April, on two parallel transects spaced 15 m apart. In spring 2017 I expanded the sampling protocol to include six 400-m-long transects, spaced 40 m apart, oriented perpendicular to shore (Figure 2.5). I collected biofilm measurements from twenty marked plots spaced evenly, 20 m apart, along each transect (120 plots total). I measured droppings and footprints in five 1-m^2 subplots perpendicular to each of the 120 biofilm-sampling plots. Counts and droppings in subplots were averaged for each plot before analysis. In 2017, I took bi-weekly measurements from June to September for a total of 10 days.

I installed western sandpiper exclosures at nine plots along six transects, spaced evenly across the study area (Figure 2.5). Exclosure devices are commonly used to assess avian predation (Schneider and Harrington 1981, Sewell 1996, Sutherland et al. 2000). However, exclosures often have inadvertent effects or “artefacts” including alteration of: water flow, sediment deposition, and biological and chemical aspects. I chose a basic exclosure design that would deter birds from entering exclosure plots while reducing inadvertent effects on the surrounding environment. Exclosures consisted of four PVC pipes (50 cm x 3 cm) rising 30 cm above the mud surface to form a 1 m^2 plot. I tied flagging tape around the perimeter, and across the interior, of the exclosure, and added 15 cm streamers to deter birds from entering the plot (Figure 2.6). At each exclosure plot, I measured biofilm inside the exclosure and in a 1 m^2 control plot adjacent to the exclosure. Order of plot measurement was randomly assigned. I removed all exclosures on 10 May 2017.

2.2.4. Statistical Analysis

To calibrate CCM-300 biofilm measurements I fit a linear regression between CCM-300 CFRs and lab-extracted Chl-*a* concentrations from mud samples. Before analysis, replicate measurements from the same site were averaged for each measurement method.

I investigated Chl-*a* concentration over mudflat exposure time using a linear mixed effects model, with sampling day and transect as random effects. I calculated exposure time as the length of time between a receding tidal height of 3.2 m (sea level height at which mudflat exposure occurs at Roberts Bank) and the time of measurement. None of the assumptions of equal variance, normal residuals, influential points, or linearity were violated. The Variance Inflation Factor (VIF) of both predictors (dropping density and time since high tide) are tolerable and the degree of multicolinearity is low.

To explore variation in biofilm density across season and year I performed a one-way analysis of variance (ANOVA) test to compare mean Chl-*a* densities observed during spring migration in 2016 and 2017, and summer 2017. A multiple pairwise-comparison Tukey Honest Significant Difference (HSD) tested for differences in daily mean Chl-*a*, temperature, precipitation, PAR, discharge from the Fraser River, between the three measurement periods. The assumptions of an independent, random sample with normal distributions and comparable standard deviations and standard errors from the mean (variance) were met.

I also explored the effect of western sandpiper grazing on biofilm density in 2016 using a Welch two-sample t-test with droppings as evidence of western sandpiper grazing (refer to Appendix A for a comparison between sandpiper-presence indicators; droppings and footprints). I tested for a difference in Chl-*a* between grazed plots (droppings count > 0) and ungrazed plots (dropping count = 0). Assumptions of independence, equal variance and a normal distribution were all met, and a one-tailed t-test run to test if Chl-*a* levels were higher in areas with an absence of grazing evidence. A paired t-test compared mean Chl-*a* density in control and treatment (exclosure) plots, and a one-tailed t-test determined if Chl-*a* was greater in exclosure plots. A Tukey's Honest Significant Difference (HSD) multiple comparisons tested for differences between plots with and without evidence of grazing across 2016 and 2017 sampling periods.

I conducted an Analysis of Covariance (ANCOVA) to test for an effect of distance from shore (covariate) on Chl-*a* density in spring and summer of 2017. Separate linear mixed effects models were fit for 2017 spring and summer, with day and transect as random effects. In the spring migration model I included dropping density along with distance as a Chl-*a* predictor. None of the assumptions of equal variance, normal residuals, influential points, or linearity were violated. VIF of both predictors (dropping density and distance from shore) were tolerable and the degree of multicolinearity low. I calculated western sandpiper grazing intensity as the ratio of dropping density (m^{-2}) to Chl-*a* density (mg m^{-2}) over distance from shore during 2017 spring migration. To test for a difference in mean grazing intensity (droppings:Chl-*a*) across distance from shore I conducted a Kruskal-Wallis rank sum test and post-hoc Dunn multiple comparison test. All assumptions of the non-parametric Kruskal-Wallis test were satisfied.

The CCM-300 was unable to generate a CFR on 28% of attempted measures. These missing values or "non-detects" were treated as NA for all analyses. Missing values were missing completely at random and only plots with both pairs of measurements were included in analyses. With a sample size of 54 paired measurements, the assumptions of a paired t-test were satisfied. All confidence intervals (CI) are based on 95% probability and all analyses were carried out using the software program *R* version 3.1.4. (R Development Core Team, 2015).

2.3. Results

2.3.1. Calibration

There was a significant positive relationship between CFR measurements and chemically extracted Chl-*a* (mg m^{-2}). The remainder of this thesis describes biofilm in units of Chl-*a* density (mg m^{-2}) derived from the relationship described in Figure 2.7.

2.3.2. Seasonal and Annual Biofilm Variation

Mean Chl-*a* density differed between sampling periods (F = 210, $p < 0.001$; Figure 2.8; Table 2.1). Mean Chl-*a* was 7.7 mg m^{-2} greater during spring migration in 2016 than in 2017 ($p < 0.001$), and 9.3 mg m^{-2} greater in summer than in spring 2017 ($p < 0.001$). Mean Chl-*a* was similar in summer 2017 and spring 2016 (p = 0.114). Table 2.2 summarizes daily means of environmental variables during the 2016 and 2017 sampling periods and the 27-year average from 1991-2017. During migration in 2016, mean solar radiation and discharge from the Fraser River were higher than in 2017, and mean temperature and precipitation similar (Figure 2.9). Mean temperature, solar radiation, and discharge from the Fraser River were higher, and precipitation lower, in summer 2017 than spring 2017 (Figure 2.9).

2.3.3. Biofilm Growth and Grazing

Chl-*a* content was positively correlated with tidal exposure duration (F = 55; $p < 0.001$; Figure 2.10): after the mudflat was exposed, Chl-*a* increased 4.1 (0.6 SE) mg m^{-2} every hour.

Mean Chl-*a* density was 11.9 (1.2 SE) mg m^{-2} lower in plots grazed by western sandpipers than in ungrazed plots (t = 7.92, p-value <0.001, Table 2.5; Figure 2.11). Ungrazed plots contained 0 droppings m^{-2} and grazed plots contained an average of 4 (SE 0.2) droppings m^{-2}. Mean Chl-*a* outside of bird exclosures was 5.3 (2.0 SE) mg m^{-2} lower than Chl-*a* levels inside (t = 3, p-value = 0.05; Figure 2.11). Average dropping density at control plots was 0.3 (SE 0.09) droppings m^{-2}. While statistically different, mean Chl-*a* density at grazed plots was more similar to control plots, with a difference of 5.6 mg Chl-*a* m^{-2} (t = 3.33, p = 0.006), than ungrazed plots to exclosure plots, which differed by 13.5 mg Chl-*a* m^{-2} (t = 8.64, $p < 0.001$).

2.3.4. Spatial Patterns of Grazing-Intensity

In 2017, Chl-*a* density across distance from shore differed between spring and summer at Roberts Bank (Figure 2.12). In spring, Chl-*a* decreased with increasing distance from shore while in summer Chl-*a* did not differ across distance (F = 0.4; p = 0.531). Spring Chl-*a* decreased 0.03 (SE 0.002) mg m^{-2} with every 1 m increase in distance, holding dropping density constant (F = 181; $p < 0.001$), and 0.6 (SE 0.2) mg m^{-2} with each additional dropping (F = 9, p = 0.003). Overall biofilm density at any distance was greater in summer than spring.

In the first 300 m from shore, mean dropping density and mean Chl-*a* density across distance were inversely related (Figure 2.13). Biofilm density was greatest 20 m from shore and decreased with distance. In contrast, dropping density was lowest 20 m from shore, increased with distance to ~ 150 m, and decreased slightly around 350 m. Mean grazing intensity (dropping:Chl-*a* density) differed over distance from shore (Figure 2.15). The lowest grazing intensity occurred in the first 20-60 m from shore, which was distinctly different from grazing intensity further from shore ($p<0.001$).

2.4. Discussion

2.4.1. Calibration

The CCM-300 effectively generates rapid, reliable, and non-destructive Chl-*a* measurements in the field, based on data in this study. The relationship between chemically extracted Chl-*a* from mud samples and CFR measurements was logical and consistent with the CCM-300 low detection limit of 41 mg Chl-*a* m^{-2} (CFR =0.31) reported by Gitelson et al. (1999). This detection limit corresponds closely with the lowest measurements obtained at Roberts Bank. Conventional biofilm estimation methods involve labour intensive and costly lab-extraction of Chl-*a* from field samples. In addition to financial and logistic limitations, these methods also damage the sampling area, which prevents repeated measurements over time. Future biofilm studies interested in increasing sample size and reducing research expenses should consider the CCM-300 to measure Chl-*a*. I recognize that my ground-truthed sample size is small and recommend the collection of more paired measurements to improve the instrument's calibration accuracy.

The CCM-300 generated some missing (NA) CFR measurements. These NA values were independent of space or time, as I found no spatial or temporal patterns in NA distribution. To explain the missing values, I first considered a low detection threshold hypothesis (Helsel and Lee 2006). The random distribution of NAs over time and space rendered this explanation unlikely, as I expected more non-detects (lower-limit CFR values) immediately following tidal emersion or where sandpiper grazing had occurred. Alternatively, I believe the NAs arose from non-ideal sampling substrate conditions. The mud, on which biofilm was measured, is a heterogeneous surface covered in an extensive system of ephemeral dendritic channels, and ridges, created by tidal movement. At Roberts Bank, channel surface water depths range from ~1 cm to 20 cm, and pore-water content ranges from high, within channels, to low, on ridges. Following an intense rain event the CCM-300 did not yield CFRs at plots where measurement attempts prior to precipitation had been successful. This could indicate that surface water, from drainage channels or precipitation ponding on the mudflat, interfered with Chl-*a* detection. The CCM-300 may be unable to detect re-emitted fluorescence from diatoms at extreme high and low pore-water contents and surface water levels, resulting in random non-detection.

2.4.2. Seasonal and Annual Biofilm Variation

While top-down and bottom-up processes influence primary production, biofilm research has focused primarily on abiotic controls over biofilm growth. Previous studies found many environmental factors influence biofilm density, including light (Smith and Underwood 2000; Perkins et al. 2001; Salleh and McMinn 2011), temperature (Salleh and McMinn 2011), rain (Tolhurst et al. 2006), sediment grain-size (Watermann et al. 1999), salinity (Underwood and Provot 2000; Orvain et al. 2014), tidal processes (Tolhurst et al. 2003; Mitbavkar and Anil 2004), and nutrient availability (Underwood 2002). Evidence suggests that warm, sunny, and dry springs generate high biofilm productivity on intertidal mudflats, therefore annual variation in local patterns of insolation, temperature, and precipitation may affect biofilm density between years. The size and timing of the spring freshet could also influence year-to-year biofilm production. Salinity and nutrient supply at Roberts Bank is highly dependent upon considerable, and often rapid, discharge of spring melt from the Fraser River. Timing of the freshet is determined by the rate and quantity of snowmelt in the Fraser River drainage basin, compounded by spring precipitation and temperature. An influx of freshwater, delivered by the freshet, may lower salinity levels creating conditions favourable to some diatom species, while increased nutrient deposition may increase primary production on the mudflat. However, recent studies at Roberts Bank found conflicting evidence of how abiotic factors affect biofilm density (Hemmera Envirochem Inc. 2018). These mixed results convey some uncertainty in the foundation upon which to base environmental correlate hypotheses.

Biofilm at Roberts Bank varied seasonally and between migration periods in 2016 and 2017. Mean Chl-*a* was lower in spring 2017 than spring 2016, and higher in summer compared to spring 2017. It is possible some of the observed variation could be the result of abiotic effects, as outlined above. On average, the 2016 migration period received more solar radiation and discharge from the Fraser River than the 2017 migration period. High discharge and solar radiation rates in 2016 may have enhanced primary production, and thus increased biofilm density, on the mudflat. Likewise, high biofilm density in summer could be the result of higher temperatures, insolation and discharge, compared to spring. However, there is disagreement over abiotic effects on biofilm production (Hemmera Envirochem Inc. 2018). Furthermore, these environmental variables were measured at meteorological towers 20-30 km from the sampling area; a scale too coarse to inspire confidence in observed correlations. To better determine biofilm response to abiotic factors, climate variables should be measured on a local scale to reflect mudflat microclimate variation.

For biofilm growth and continuity, top-down constraints may be just as important as bottom-up processes (Russell et al. 2013; Cheverie et al. 2014; Savelli et al. 2018). In addition to abiotic factors, biofilm growth also responds to grazing pressure (Pinckney et al. 2003; Hicks et al. 2011; Orvain et al. 2014). Western sandpiper northward migration is often rapid due to biological constraints on reproductive success (Drent et al. 2007). Accordingly, western sandpipers stopping at Roberts Bank in spring feed in high concentrations over a few days (Butler et al. 1987). In contrast, southward migration is segregated over time by age and sex, and western sandpiper densities at Roberts Bank are much lower in summer than spring (Butler et al. 1987; Mathot et al. 2007). Intense spring grazing, absent in summer when far fewer sandpipers stopover, may have added top-down pressure to biofilm growth at Roberts Bank. I hypothesize that western sandpiper grazing during northward migration led to low spring biofilm levels. Western sandpipers rapidly remove large quantities of invertebrate biomass (Quammen 1981; Mathot and Elner 2004), directly and indirectly affecting biophysical aspects of intertidal communities (Erwin 1996), therefore it is likely that biofilm is similarly influenced by grazing. I will explore this hypothesis in greater detail below.

2.4.3. Biofilm Growth and Variation

Following mudflat exposure at Roberts Bank, Chl-*a* density increased with tidal exposure duration. This result is consistent with observations and experimental evidence of benthic diatom diurnal rhythmic migration (Herlory et al. 2004, Mitbavkar and Anil 2004). As the tide recedes, and the mudflat is exposed, epipelic diatoms migrate vertically to the top 2 mm of the sediment surface where photosynthesis takes place (Herlory et al. 2004). These diatoms continue to grow and aggregate at the surface, increasing biomass over time until tidal inundation signals a reverse vertical-migration below the surface. To understand foraging behaviour it is important to consider the temporal variation in biofilm over an intertidal period. Grazers adopt many strategies to maximize energy intake. For example, barnacle geese wintering in the Dutch Waddenzee repeatedly grazed red fescue grass to levels that promoted regeneration of protein-rich tissue (Ydenberg and Prins 1981). To maximize energy intake, western sandpipers may delay grazing to allow biofilm density to build up over time. Foragers might also graze biofilm in waves to enable primary production, and regeneration, to occur between grazing events. Food web and foraging behaviour models of intertidal ecosystems should account for biofilm production over a tidal emersion period, as behaviour may be linked to biofilm density over time.

I found evidence to suggest that sandpiper grazing reduced biofilm density at Roberts Bank during spring migration. Mean Chl-*a* density decreased 12 mg m^{-2} in grazed areas in 2016, and 5.3 mg m^{-2} outside exclosures in 2017. This equates to an 18% decrease in available biofilm (measured at ungrazed plots) in 2016, and a 10% decrease (measured at exclosures) in 2017. One study estimated a single western sandpiper, while stopping at Roberts Bank, might consume up to 190 g (190 000 mg) of biofilm per day (Table 2.4). However, this estimate seems high given western sandpipers weigh, on average, 25 g during migration. Kuwae et al.'s (2008) estimates are based on the assumption that western sandpipers spend 80% of a 12.6-hour tidal exposure period foraging. Presumably, western sandpipers also spend time roosting and flying between foraging patches in addition to grazing at stopovers. Reducing the proportion of time spent foraging, from 80% to a conservative 50%, yields a slightly more reasonable daily biofilm ingestion estimate of 120 g sandpiper $^{-1}$ (Table 2.3). Thus, a flock of 100,000 sandpipers may consume 120,000 kg (1.2×10^7 mg) of biofilm per day (Table 2.4). While the mudflat is 27 km^2 at maximum tidal exposure, sandpiper spatial usage is not equal across the mudflat. Most sandpipers forage in the upper intertidal region, which covers an area ~ 2 km^2 (Jiménez et al. 2015). Assuming an average biofilm density of 60 mg m^{-2} upper intertidal biofilm density is ~ 1.2×10^8 mg m^{-2} (Table 2.4). At a rate of 120 g day^{-1}, 100,000 western sandpipers would consume ~ 10% of available biofilm, which reflects levels of Chl-*a* grazing observed in this study (10-18%). However, biofilm production, as described above, increases 4.1 mg m^{-2} with every additional hour of exposure. Biofilm regeneration over a tidal-exposure period may have masked a larger grazing effect, depending on the time between grazing and measurement. Therefore, western sandpipers likely removed more biofilm than was measured, as production would replace that which had been grazed, thus dampening the observed grazing effect. It is clear western sandpipers do not graze 100% of available biofilm at Roberts Bank. Whether biofilm is left behind after grazing because sandpiper energy requirements were satisfied, or low concentrations of biofilm are difficult and energetically costly to forage, remains unknown.

Results from the exclosure experiment support my hypothesis, generated from 2016 observations, that western sandpipers graze down daily biofilm standing-crop during spring migration. However, the effect observed in 2016 (12 mg m^{-2}) was twice as large as the experimental effect found in 2017 (5.3 mg m^{-2}). Although the results are consistent and support my hypothesis, I believe the exclosure experiment suffered from scale issues. Such complications are common in animal behaviour studies. The 1 m^2 plots (originally chosen for logistic reasons) encompass a very small fraction of the mudflat available to foragers. Sandpipers may have avoided exclosure and, subsequently, adjacent control plots in favour of undisturbed areas nearby. I believe this experiment could be improved, and a larger effect observed, by increasing the size of exclosures, and spacing control plots farther apart to reduce unintended control-plot avoidance. To correct for avoidance issues I conducted a large-scale field experiment on spatial-usage, described in Chapter 3.

2.4.4. Spatial Patterns of Grazing-Intensity

During western sandpiper spring migration at Roberts Bank, biofilm density decreased with increasing distance from shore. In contrast, summer biofilm density was greater than spring, and remained constant across distance. Abiotic factors, such as solar radiation and temperature, could have increased biofilm density between seasons, however the effect should have been uniform across space. Instead, the spatial biofilm gradient observed in spring was absent in summer. Seasonal climate variations should affect the mudflat evenly across space, varying the intercept of the biofilm gradient without affecting the slope. Changes in biofilm density across distance from shore could arise from sediment grain-size gradation on the mudflat, or earlier tidal-exposure close to shore. Macro-invertebrate density in tidal flats decreases with distance from shore in association with sediment distribution along a shallow bed-slope (Kennish 1990; Pomeroy 2006). Perhaps, similar to macroinvertebrates, biofilm growth is enhanced by fine-grained sediments that settle close to shore (Kennish 1986). However, if sediment size influences biofilm production the same gradient should be observed in both summer and spring. Alternatively, earlier exposure to sunlight, and thus earlier biofilm production, could lead to higher biofilm biomass close to shore. As water recedes from a tidal flat, the seafloor with highest elevation, closest to shore, is exposed first. The slope at Roberts Bank is very shallow and the entire mudflat is exposed in ~ 30 minutes. According to biofilm growth estimates, this 30 minute ‘head start’ would only confer a 2 mg m^{-2} advantage to the closest plot (20 m from shore) over the furthest plot (400 m from shore), far less than the ~10 mg m^{-2} difference observed. Regardless, sediment size distribution and tidal exposure at Roberts Bank do not undergo seasonal changes. The seasonal difference in biofilm density over distance from shore indicates that the factor, which affected biofilm during spring, was not effective in summer. Since biofilm density decreased in regions of western sandpiper grazing, I hypothesize the biofilm gradient was grazed down by western sandpipers during spring migration according to optimal foraging theory.

At Roberts Bank, spring biofilm density decreased over distance from shore while dropping density increased, providing further evidence of a grazing effect. Lowest sandpiper grazing intensity (dropping:biofilm density ratio) occurred 20-60 m from shore. In summer, biofilm density was uniform across distance. Assuming the processes governing biofilm production are constant between spring and summer, dropping measurements and experimental evidence, suggest that a pulse of western sandpipers grazed biofilm in an onshore-offshore gradient during spring migration. The observed pattern of decreasing biofilm with increasing distance from shore is similar to a giving up density (GUD), a measure of how much food foragers leave behind in a resource patch (Brown 1988). Many migrating species, such as wildebeest, locusts (Bauer and Hoye

2014), and barnacle geese (Ydenberg and Prins 1981), impact resident community processes and ecosystem functions through intense pulses of herbivory. At Roberts Bank, western sandpipers appear to have altered the short-term standing biofilm crop across the mudflat; grazing less intensely in areas close to shore while removing a greater extent from areas further from shore. Danger across a mudflat increases with proximity to the shoreline, where visibility is obstructed and predation-risk is high. The biofilm GUD gradient at Roberts Bank could signify that western sandpipers trade-off biofilm and safety at Roberts Bank, as observed with macroinvertebrates at Boundary Bay, BC (Pomeroy 2006). In Chapter 3, I experimentally test the hypothesis that western sandpipers trade-off food and safety at Roberts Bank, resulting in a biofilm gradient across the upper intertidal.

2.5. Conclusion

In this chapter, I described methods for simple, reproducible, biofilm estimation on an intertidal mudflat. I observed biofilm density variation between measurement years and seasons, and suggested several possible explanations. I found biofilm increased over time during diurnal emersion, while western sandpipers reduced biofilm through intense grazing during spring migration. There is agreement that migrating shorebirds consume substantial quantities of biofilm at Roberts Bank, and I have shown that production is important to maintain biofilm densities. The level to which shorebirds graze biofilm could indicate satiation, or low biofilm concentrations that confer an energetic cost. The biofilm concentrations at which shorebirds are physiologically able, and energetically motivated, to forage is an important question for future research.

2.6. Figures

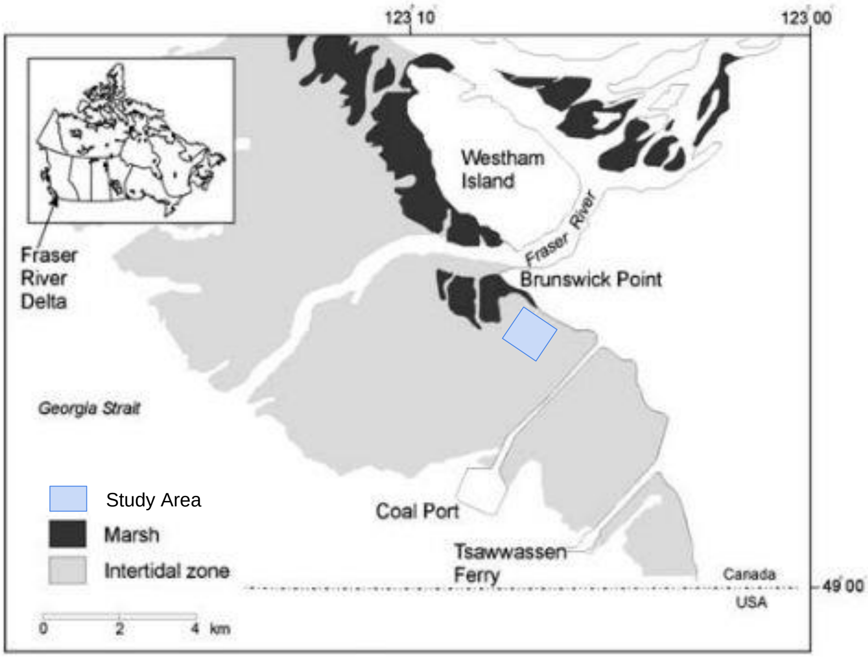

Figure 2.1. Roberts Bank, Fraser River delta, British Columbia, Canada. Retrieved from Mathot et al. 2010.

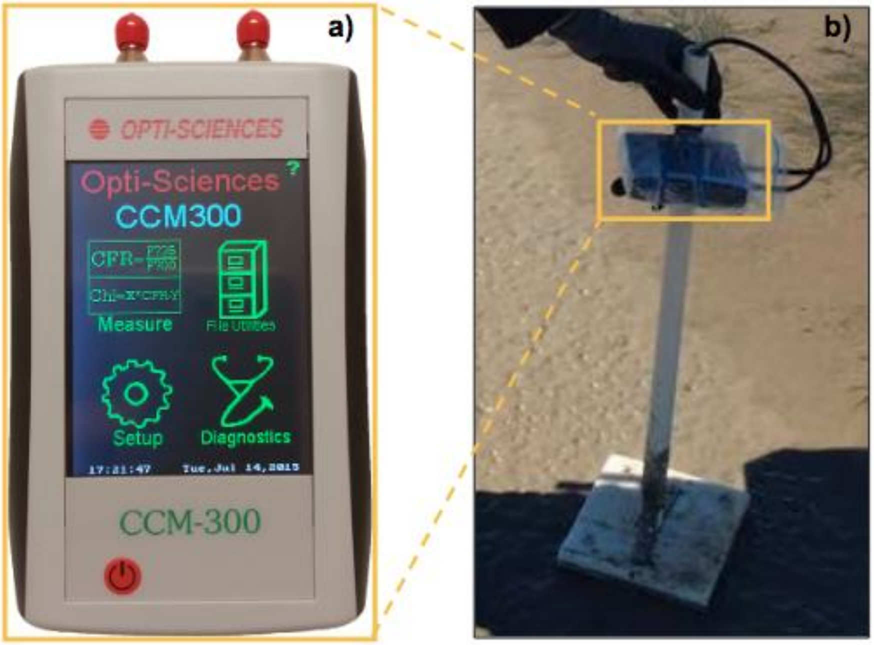

Figure 2.2. **The CCM-300 (12 cm x 9 cm x 3 cm) (a) was secured to a stand composed of an arm (60 cm length) and a square base (17 cm x 17 cm) with a 4 mm hole drilled through its center (b).**

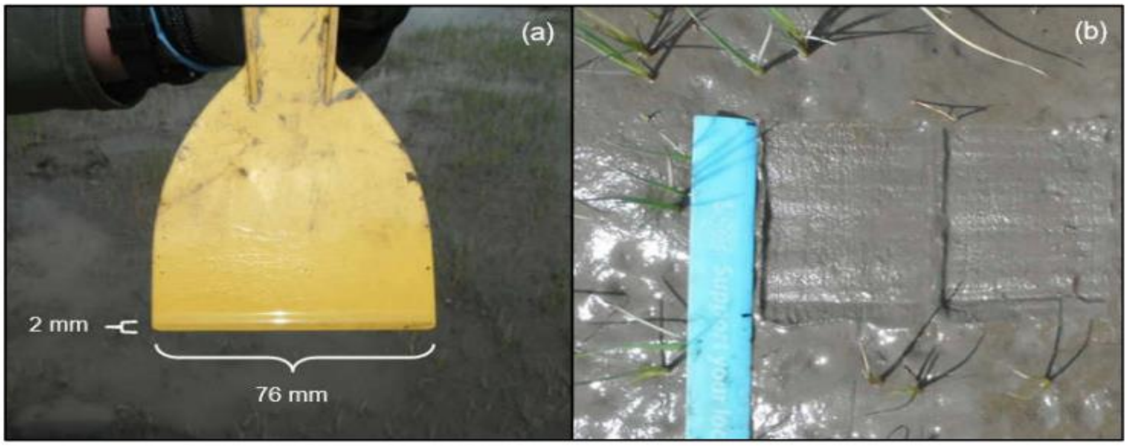

Figure 2.3. **Plastic putty knife (with dimensions) used to scrape biofilm samples (a) and mudflat after biofilm was removed, with ruler indicating the 10 cm distance that was scraped (b). Photos retrieved from Hemmera Envirochem Inc. (2018).**

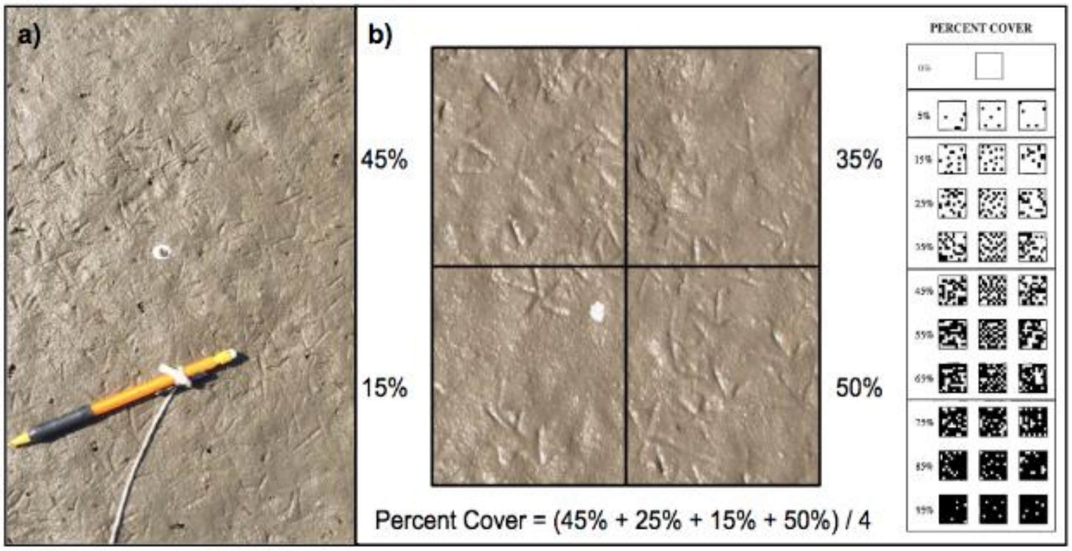

Figure 2.4. Western sandpiper footprints and dropping, pencil included for scale (a) and example calculation of percent cover to estimate proportion of footprints (b).

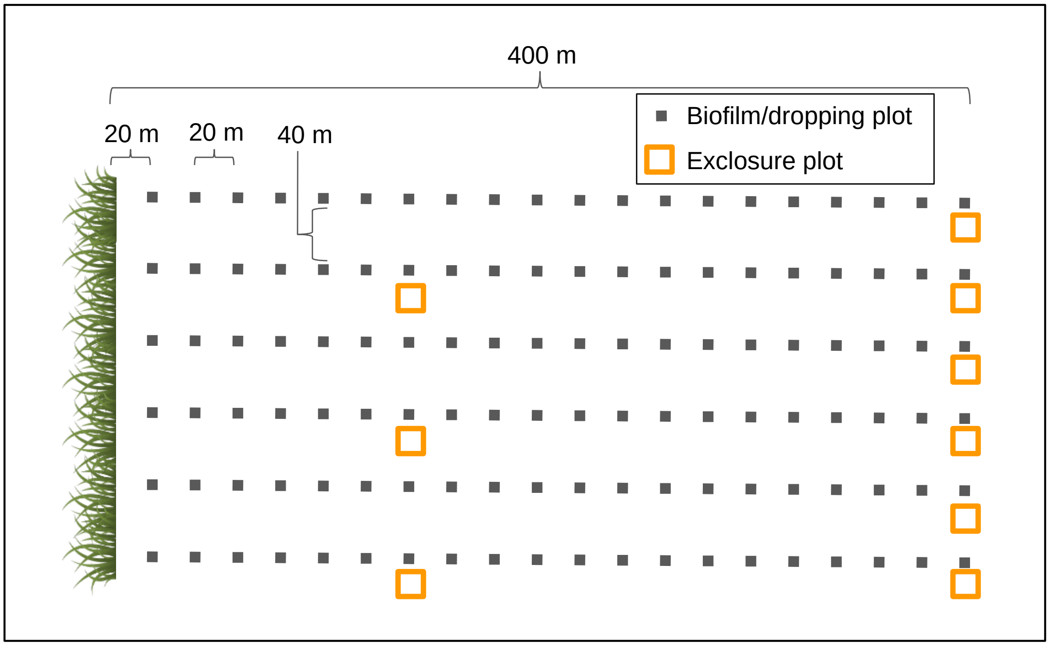

Figure 2.5. 2017 transect sampling design indicating location of transects with respect to the shoreline and placement of exclosure plots adjacent to biofilm and dropping sampling plots.

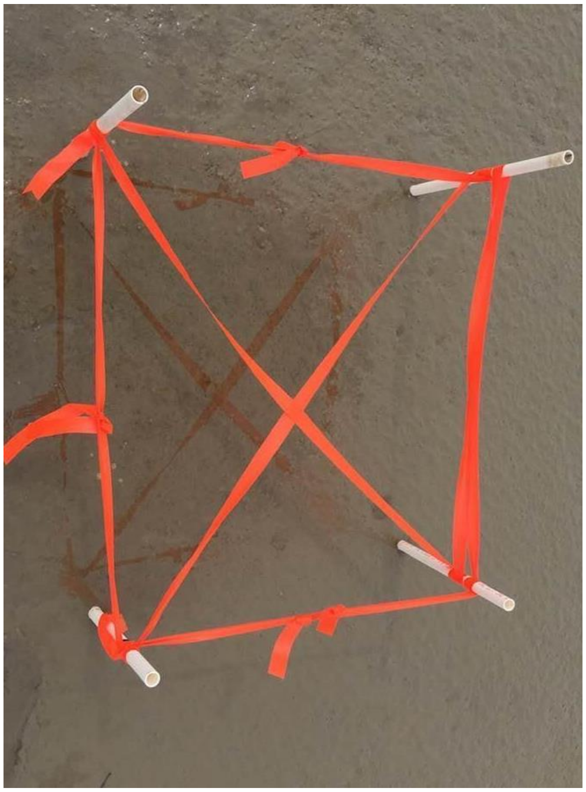

Figure 2.6. Western sandpiper exclosure plot consisting of 4 pieces of PVC, extending 15 cm out of the mud in a 1 m x 1 m square, encircled by flagging tape.

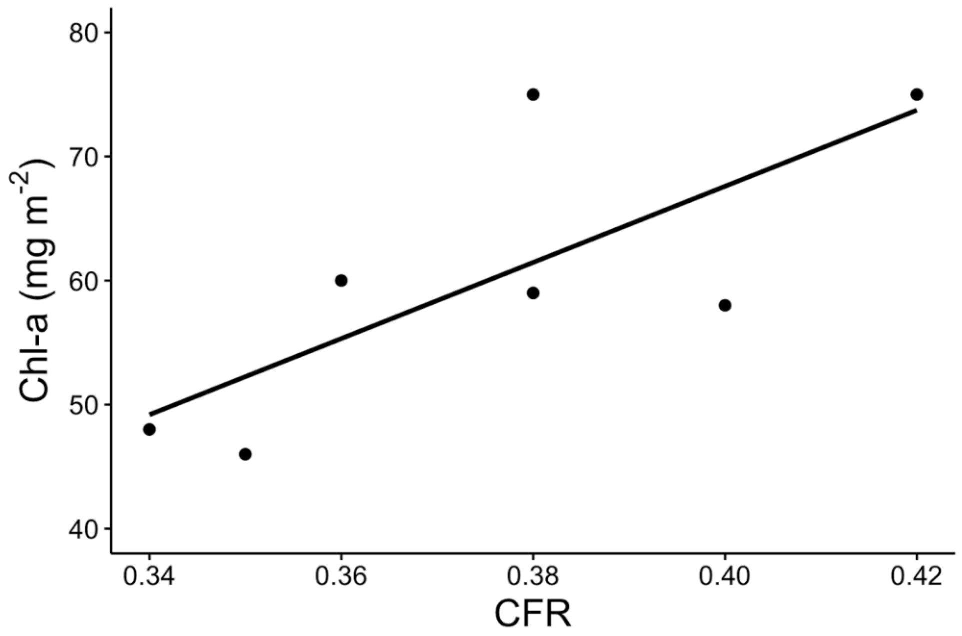

Figure 2.7. **Chlorophyll-*a* concentration (mg m^{-2}) derived from a linear model of chemically-extracted Chl-*a* from mud samples and concurrent CCM-300 CFR measurements. The calibration equation is: Chl-*a* = -55.16 + 306.89*CFR ($p=0.05$, $R^2 = 0.56$, adjusted $R^2 = 0.48$). Gittelson equation is: Chl-*a* = - 391 + 634*CFR ($R^2 = 0.96$).**

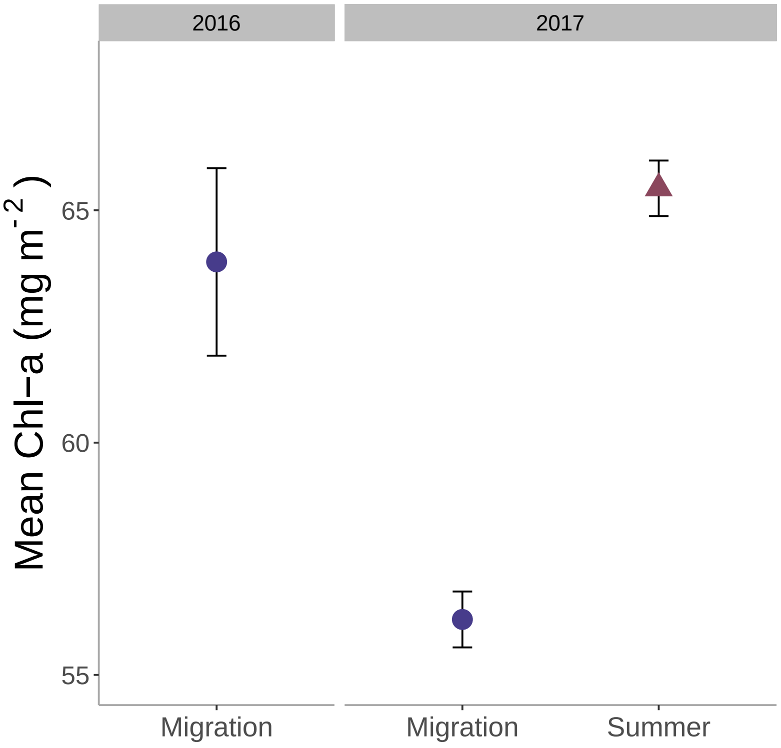

Figure 2.8. **Mean Chl-a (mg m^{-2}) measured during spring migration of 2016 and 2017, and summer 2017, with 95% confidence intervals.**

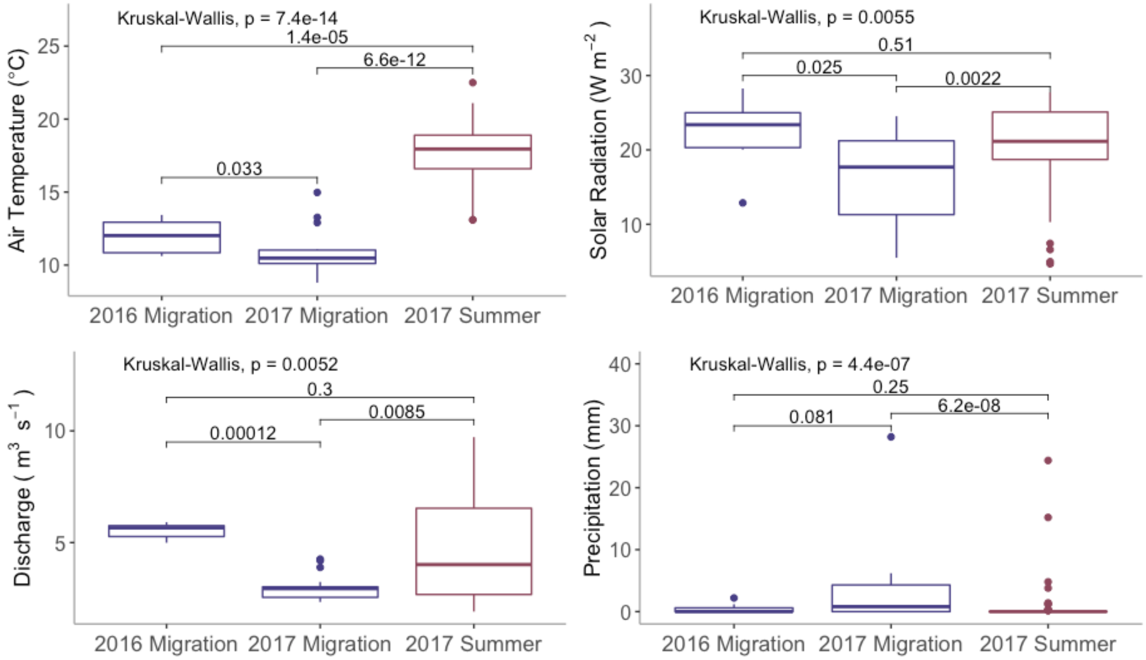

Figure 2.9. **Mean temperature (°C), precipitation (mm), solar irradiance (W m^{-2}), and discharge from the Fraser River (m^3 s^{-1}) during the 2016 and 2017 migration and 2017 summer biofilm sampling periods. Labels indicate p-values of pairwise comparisons between sampling periods for each variable. For plotting purposes, discharge (m^3 s^{-1}) and solar radiation (W m^{-2}) were scaled (x 10^3 and x 10^2, respectively).**

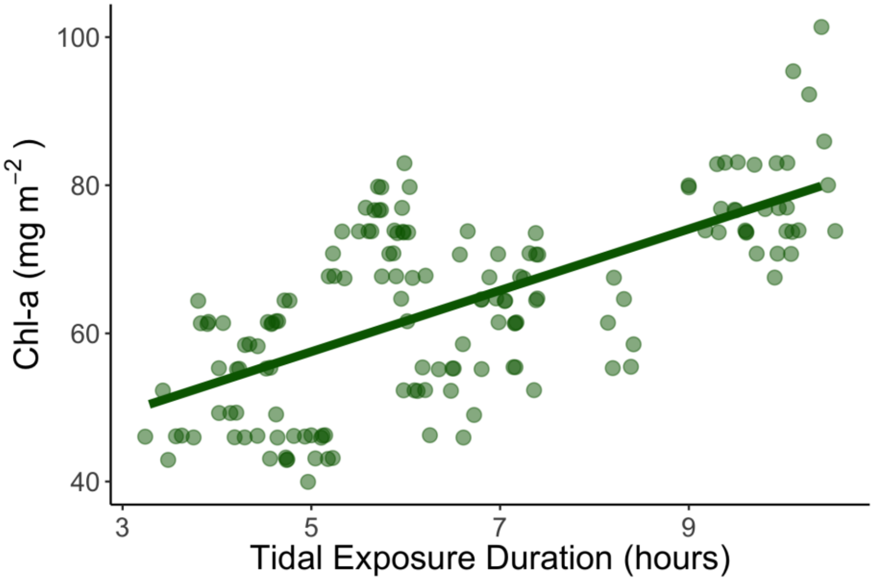

Figure 2.10. **Linear model describing the increase in Chl-*a* density (mg m^{-2}) with duration of mudflat exposure (hours).**

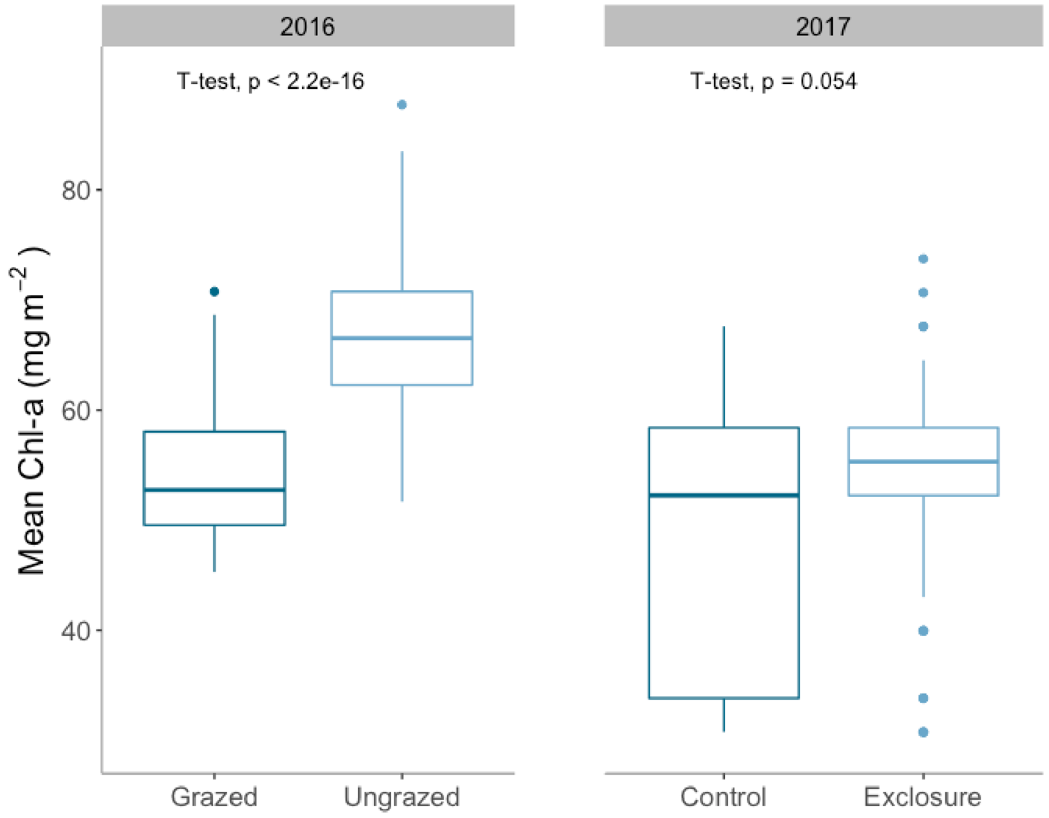

Figure 2.11. **Mean Chl-*a* density (mg m^{-2}) in grazed and ungrazed plots (2016) and in exclosure (ungrazed) and control (grazed) plots (2017). Error bars are 95% confidence intervals. Grazed plots contained an average of 4 droppings m^{-2}.**

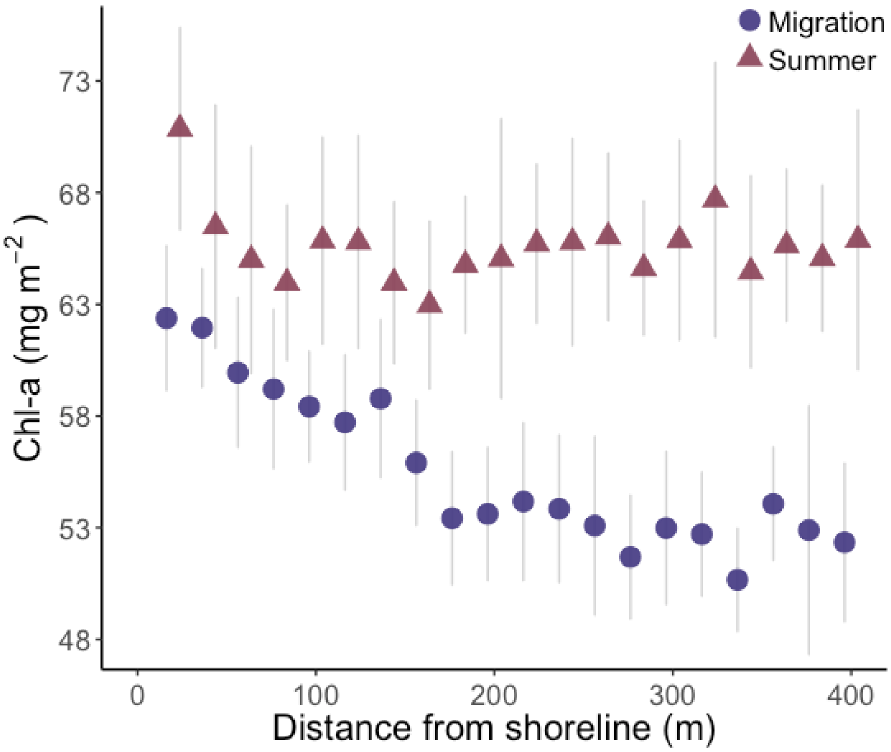

Figure 2.12. **Mean Chl-*a* (mg m^{-2}) across distance from shore during spring migration and late summer in 2017.**

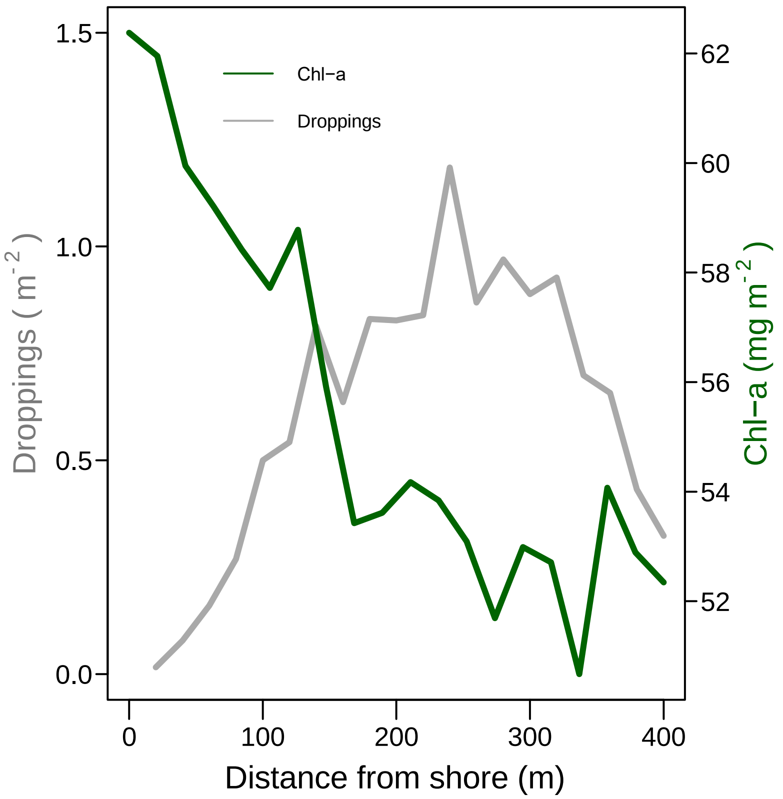

Figure 2.13. **Mean dropping (m^{-2}) and Chl-*a* (mg m^{-2}) density over distance from shore (m) during 2017 migration.**

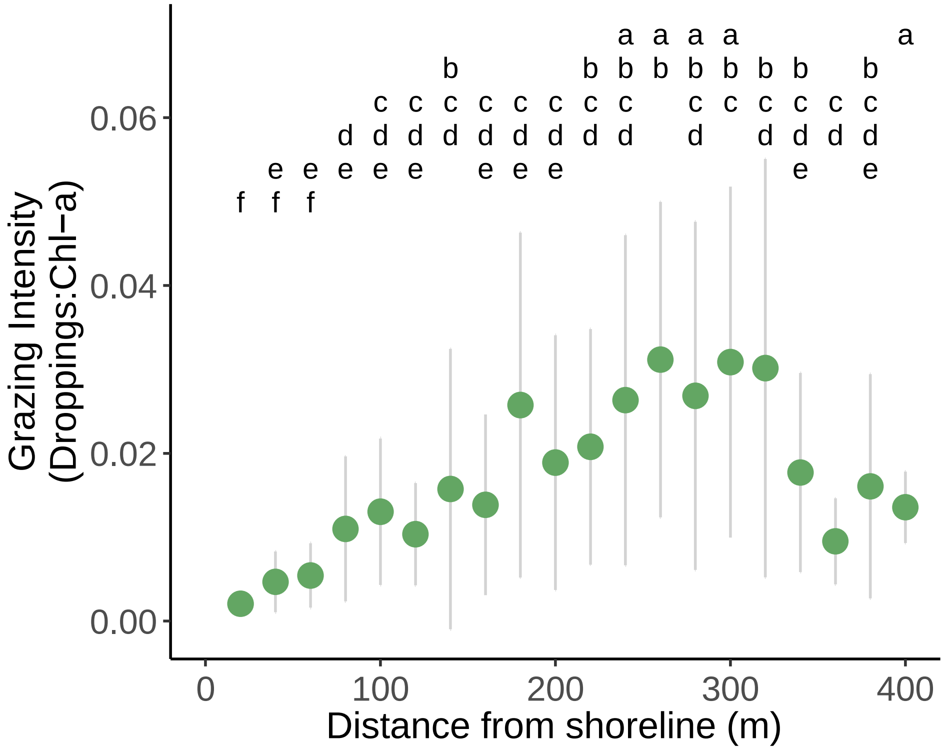

Figure 2.14. **Mean grazing intensity (ratio of dropping (m^{-2}) to Chl-*a* (mg m^{-2}) density) across distance from shore during 2017 migration, with 95% confidence limits around the ratio of paired dropping to Chl-a density measurements and Dunn multiple comparison composite letter display. The Dunn test found grazing intensity across distances fell into six groups (a-f). Distances that are not significantly different belong to the same group, and are expressed colour (distances that share a colour in the vertical columns are similar). Note that 20, 40, and 60 m from shore belong to the same group (f), which is dissimilar from all other distances.**

2.7. Tables

Table 2.1. Mean Chl-*a* density (mg m^{-2}) measured during spring migration of 2016 and 2017, and summer 2017, with standard error of the mean and 95% confidence intervals (LCL=lower confidence limit, UCL=upper confidence limit).

Time Period	n	Mean	SE	LCL	UCL
Migration-2016	6	63.9	1.0	61.9	65.9
Migration-2017	13	56.2	0.3	55.8	56.8
Summer-2017	8	65.5	0.3	64.9	66.1

Table 2.2. Mean temperature (°C), precipitation (mm), solar irradiance (W m^{-2}), discharge from the Fraser River (m^3 s^{-1}) and droppings (m^{-2}), in 2016, 2017 and averages from 1991-2018 during migration and summer (June-August).

	Year	Temp (°C)	Precipitation (mm)	Solar Irradiance (W m^{-2})	Discharge (m^{3} s^{-})	Droppings (m^{-2})
Migration	2016	11.9	0.5	221.6	5516	1.5
	2017	10.8	3.3	157.7	2977	0.6
	1991-2018	11.2	2.2	NA	3630	NA
Summer	2017	17.6	0.6	207.9	4793	NA
	1991-2018	17.4	1.3	NA	4565	NA

Table 2.3 Mean Chl-*a* density (mg m^{-2}) in grazed and ungrazed plots (2016), and mean Chl-*a* density in exclosure and control plots (2017). LCL = 95% lower confidence limit, UCL = 95% upper confidence limit. Grazed plots contained an average of 4 droppings m^{-2}.

Year	Group	n	Mean	SE	LCL	UCL
2016	Ungrazed	86	66.6	0.9	65.1	68.0
	Grazed	58	54.6	0.7	52.8	56.5
2017	Exclosure	54	54.4	1.7	50.9	57.8
	Control	54	49.1	1.6	45.8	52.3

Table 2.4. Estimated biofilm ingestion rate of western sandpipers and biofilm density at Roberts Bank (adapted from Kuwae et al. 2008).

Measurements	Value	Reference
Foraging action rate (actions min^{-1}); A	121	Kuwae et al. (2008)
Scraping biofilm mass (mg wet mass action^{-1}); B	2.6	Elner et al. (2005)
Available foraging time (h d^{-1}); C	12.6	Kuwae et al. (2008)
Foraging time in G (%); D	50	Adapted from Kuwae et al. (2008)
Biofilm mass ingesting rate (g wet mass d^{-1}); $E = A \times B \times C \times D$	120	Adapted from Kuwae et al. (2008)
Average biofilm density (mg m^{-2}); F	60	This study
Sandpiper foraging area (2 km^2); G	2	Jimenez et al. (2015)
Biofilm density in sandpiper foraging area (mg m^{-2}); $H = F \times G$	1.2×10^8	

2.8. References

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Chapter 3.

Western sandpiper (*Calidris mauri*) stopover site usage: trade-offs between biofilm and predation-danger

3.1. Introduction

Food availability, and the risks associated with food acquisition, influence foraging behaviour (Lima and Dill 1990). Foragers must balance the benefit of energy intake with the mortality cost of utilizing dangerous environments. Such “trade-offs”, between food and danger, directly affect foraging decisions. Migratory shorebirds stopping at coastal estuaries must feed intensively to gain the energy necessary for long-distance flight and fecundity (Myers et al. 1987; O’Reilly and Wingfield 1995; Warnock and Bishop 1998; Clark and Butler 1999; Mehlman et al. 2005). Intense foraging lowers vigilance while increasing weight; and inattentive, heavy shorebirds are less likely to evade predator attacks (Dierschke 2003). Predation risk also influences shorebird stopover-site selection and length of stay. Falcons pose the greatest risk to migratory shorebirds foraging at a stopover site**.** Foragers select and remain for longer periods at large (safe) sites if food abundance is equal to small (dangerous) sites (Lank et al. 2003; Ydenberg et al. 2004; Pomeroy et al. 2006). Trade-off decisions can also reveal information about site quality. Danger may affect how foragers assess the value of food within a site, thus predation risk and food quality, and availability, determine site quality (Quinn and Cresswell 2004; Butler et al. 2005). Understanding these trade-off decisions, the mechanisms that drive them, and how they influence stopover site-selection, is important for effective migratory shorebird conservation.

An increasing number of studies stress the ecological role of danger to migratory species (Diershke 1998, Lank et al. 2003; Ydenberg et al. 2004; Bauer et al. 2010; Dekker et al. 2011; Hope et al. 2011). Shorebirds respond to danger through increased vigilance, flock aggregation, and relocation to safer resource patches (Lima and Dill 1990, Brown and Kotler 2004, Pomeroy et al. 2006). Behaviours that reduce the risk of predation do so at the cost of time spent foraging and the amount of food foraged.

Foragers often allocate more time to resource patches in which food availability compensates for safety, and vice versa (Kotler et al. 2010). For example, semipalmated sandpipers (*Calidris pusilla*) preferentially selected safe sites when adequate food supply was present (Sprague et al. 2008). Dierschke (2003) also found evidence of a trade-off when lightweight passerines foraged more intensely, and in higher-risk habitats, than heavy birds.

Despite increased recognition of top-down effects on foraging decisions, few experiments have tested migrant response to predation. While trade-offs have been documented in numerous species, under varying conditions, most experiments are conducted in controlled environments. Results inferred from controlled settings are imperfect because not all aspects of complex natural systems can be replicated. Field experiments are invaluable to ecological research because factors of interest can be manipulated without altering the system. However, large-scale field experiments are difficult to implement, particularly when studying dynamic relationships. In 2003, western sandpiper (*Calidris mauri*) spatial-usage was measured along an artificial danger gradient at Boundary Bay, BC, to test the influence of predation-danger on macroinvertebrate foraging behaviour (Pomeroy et al. 2006). Recently, biofilm has been shown to account for 45-59% of western sandpiper diet while on migration. It is unknown how predation influences biofilm foraging decisions, and whether western sandpipers trade-off biofilm and safety.

Chapter 2 revealed that biofilm concentration fluctuates over a tidal-emersion period. Biofilm increases with tidal-exposure time, due to increased primary production, and grazing lowers the biofilm standing-crop in grazed patches. Therefore, trade-off decisions depend on food availability and risk of predation over an intertidal period. Foraging behaviour may vary throughout an intertidal cycle in response to biofilm fluctuation. Many species trade-off food and danger (Lindstrom 1990), including the western sandpiper (Pomeroy et al. 2006), but field experiments have not considered how trade-off decisions might change in response to biofilm fluctuation over a tidal-exposure period.

This chapter tests the hypothesis that migrating western sandpipers base foraging decisions on biofilm availability, and predation risk, over a tidal-emersion period. During annual northward migration, on average over 600,000 western sandpipers stop to forage biofilm on the 27 km^2 mudflat at Roberts Bank (49° 03' 25.20" N, 123° 10' 23.40" W). There, peregrine falcons (*Falco peregrinus*) and merlins (*Falco columbarius*) launch stealth attacks, which pose the greatest threat to sandpiper safety (Ydenberg et al. 2002; Lank et al. 2003). Western sandpipers are most successfully predated close to shore where tall grasses provide cover for falcon strikes (Cresswell 1994; Dekker and Ydenberg 2004; Cresswell and Quinn 2010). To observe how western sandpipers trade-off food and safety at Roberts Bank I manipulated danger on the mudflat with visual obstructions. Using time-lapse photography, I compared western sandpiper spatial usage between visually obstructed treatment-transects, and unobstructed control-transects, over a tidal-emersion period.

In this chapter I explore how western sandpipers forage at a stopover-site, across space and over time. I predict, 1) that fewer western sandpipers will forage on obstructed transects where predation danger is high, as observed by Pomeroy et al. (2006). This effect should, 2) be greatest close to the obstruction, as predator detection decreases with proximity to cover. Spatial usage should also be influenced by biofilm availability, which is variable throughout a tidal-emersion period. Therefore I hypothesize, 3) that western sandpipers will take greater risks to acquire biofilm accumulated over an intertidal period, following initial foraging and subsequent biofilm reduction in safe areas.

3.2. Methods

3.2.1. Study site

This study took place at Roberts Bank, British Columbia, Canada (49° 03' 25.20" N, 123° 10' 23.40" W), from 18 April - 7 May 2017, and 19 April - 3 May 2018. Roberts Bank is a 27 km^2 intertidal mudflat located east of Brunswick Point on the mouth of the Fraser River estuary (Figure 3.1). At the edge of the mudflat is a 20-40 m wide saltmarsh rimmed by a 2-m high dike. A designated Important Bird Area (IBA), Roberts Bank provides a critical resting and refuelling site for migratory shorebirds during northward migration (April – May). Western sandpipers are small (~25 g) migratory shorebirds that stopover at Roberts Bank in large number each spring to feed on biofilm. The greatest threats to sandpipers at Roberts Bank are peregrine falcons and merlins (Ydenberg et al. 2002, Lank et al. 2003). I studied the upper intertidal region of Roberts Bank along six transects, which covered 48,000 m^2 (400 m x 120 m), and two experimental sites, each 8,000 m^2 (30 m x 200 m).

3.2.2. Study Design

To observe how western sandpipers forage for biofilm in response to danger at Roberts Bank I repeated a food-danger trade-off experiment originally conducted at Boundary Bay, BC, in 2003 (Pomeroy et al. 2006). The tidal-flat at Boundary Bay is composed primarily of sand while silt and clay predominate at Roberts Bank (Sewell 1996). Sand at Boundary Bay is redistributed with each rising tide, and human disturbances (from walking, kneeling, etc.) are smoothed over. In contrast, disturbances to the Roberts Bank mudflat leave impressions in the mud that remain for several weeks. I modified the methods outlined by Pomeroy et al. (2006) to better suit the clay-rich mud at Roberts Bank. Instead of counting droppings at the end of each experimental period, I counted western sandpipers throughout the experiment using time-lapse cameras.

I placed paired (control and treatment) transects at two sites on the Roberts Bank upper intertidal, ~ 500 m apart (Figure 3.2). Transects began 20 m from shore and extended 220 m perpendicular to shore. Control and treatment (obstruction) transects were spaced 20 m apart. Each transect was marked with a 10 m x 10 m grid of white PVC stakes that extended 15 cm above the mud surface (Figure 3.2; Figure 3.3).

Food abundance on large tidal flats decreases with distance from shore (Pomeroy 2006; Figure 3.4a). Biofilm abundance during northward migration at Roberts Bank followed the same general pattern of macroinvertebrate density found at Boundary Bay, where food abundance decreases with distance from shore (Pomeroy 2006; Figure 3.4). This biofilm density gradient enabled comparison between usage on the shoreward (high food) and oceanward (low food) sides of the obstruction. Falcons are stealth predators that rely on speed and cover to ambush unsuspecting prey. Most sandpiper fatalities occur close to shore where skyview is obstructed by a sloping shoreline or tall vegetation (Cresswell 1994, Decker and Ydenberg 2004, Cresswell and Quinn 2010).

Predation danger was increased on experimental transects with a visual obstruction placed 120 m from shore (Figure 3.2; Figure 3.3). At the midway point of each transect (120 m from shore), I installed four rebar posts, which extended 1 m above the mud surface in a line oriented parallel to shore at 5 m intervals. To these posts I attached the obstruction: a 1 m x 20 m, opaque, black tarp reinforced with four 1-m long, PVC pipes spaced 5 m apart. Sliding the pipes over the rebar posts enabled efficient tarp deployment. Control transects contained only rebar posts without an obstruction tarp. I alternated the obstruction transect at both sites to control for site effects, and rotated the obstruction-tarps between the two sites every third day to control for tarp effects.

At each transect, six cameras (Browning Strike Force Elite HD, model BT-5HDE) captured 1 photo min^{-1} (24 cameras in total) over a 5-hour tidal-exposure period. I attached cameras to PVC poles 1.5 m above the mud surface. Two cameras faced the shore (North) at 120 m and 235 m from shore, while another two cameras faced the ocean (South) at 15 m and 120 m from shore (Figure 3.3). To cover the blind spot closest to the barrier, I set up two cameras at 115 m and 125 m from shore, and 15 m east of the transect. The obstructions were deployed at treatment transects just as the falling tide revealed the mudflat (~ 3.4 m above sea level). The experiment began once all 12 cameras were recording and the obstruction tarp was in place. I ran the experiment on 14 days at site 1, and 15 days at site 2. High-tide height post-sunrise on April 23, 24, and 25 did not reach 3.2 m, thus transects were not submerged during high tide. On these days the experiment took place during periods of both rising and falling tide. After 5 hours, I concluded the experiment, removed the obstructions and turned off the cameras to prolong battery-life.

Time-lapse photos were processed in ®Paint. Every 5th photo (photo 5 min^{-1}) was analyzed. The furthest plot analyzed was 30 m from the camera. The number of western sandpipers in 20 (10 m x 10 m) plots, and 2 (5 x 10 m) plots, were counted for each transect (44 plots per site and 88 plots total) in 5-minute intervals.

For a description of study design and field methods for data collected in 2017 please refer to Chapter 2.

3.3. Statistical Analysis

At food-danger trade-off transects, I calculated western sandpiper spatial usage as: (total sandpipers m^{-2}) x (5 min photo^{-1}). I trimmed daily treatment-control transect pair measurements so experiment duration was equal at all plots. On Day of Year (DOY) 114 the experiment could not be deployed due to logistical issues. To maintain experimental consistency between replicates I removed DOY 114. Results with and without DOY 114 were similar.

I compared mean spatial usage (western sandpipers m^{-2} min^{-1}) on entire control and obstruction transects with a paired Wilcoxon Signed Rank Test. Before analysis I averaged across site and day for each level of danger (distance from shore and obstruction). Assumptions of dependence and a non-parametric distribution were met.

I calculated the difference between spatial-usage of high and low danger areas by subtracting western sandpiper usage (m^{-2} min^{-1}) at treatment plots from control plots (control-treatment). A positive difference indicated that western sandpiper use of control plots was greater. I compared the difference in spatial usage (control-treatment) between ocean and shore sides of the obstruction with a paired t-test. With linear mixed effects models, specifying day and site as random effects, I described the difference in spatial usage (control-treatment) over distance from the obstruction on shore and ocean sides of the obstruction. Both models satisfied the assumptions of linearity, however, spread of the ocean model residuals increased with distance from the obstruction. Despite the unequal variance, the linear model was chosen to describe the ocean side data to simplify interpretation between models.

I grouped transect plots into three bins; 20-90 m from shore, 90-150 m from shore, and 150-220 m from shore. The 20-90 m bin represents distances close to shore where tall grasses act as natural obstruction for both control and treatment transects. The 90-150 m bin encompasses the area closest to the obstruction on treatment transects, and the 150-220 m bin includes plots furthest from both the shore and the obstruction. The experiment was divided into Time 1, counts from the first half of the experiment (hours 0-2.5), and Time 2, counts from the second half of the experiment (hours 2.5-5). A standard zero-inflated negative binomial generalized linear mixed model (GLMM), with DOY as a random effect, was used to test for an interaction between time, distance, and transect on sandpiper usage. A negative binomial distribution was chosen over poisson because the data appear over-dispersed due to among-individual heterogeneity. Results of the poisson and negative binomial model were similar and the negative binomial selected after comparison of Akaike's Information Criterion (AIC) weights.

All confidence intervals (CI) are based on 95% probability and all analyses were carried out using *R* version 3.1.4. (R Core Team, 2015).

3.4. Results

3.4.1. Food-danger Trade-off

At each of the two experimental sites, 16 cameras recorded 60 time-lapse photos per day. The experiment ran on 15 days at Site 1 and 14 days at Site 2 for a total of 15 replicates (Table 3.1). In total, 1,452 western sandpipers were counted at Site 1, and 265 at Site 2 (Table 3.1). Mean sandpiper usage on treatment transects was 1.69 m^{-2} min^{-1} compared to 2.05 m^{-2} min^{-1} on control transects (Table 3.2). Western sandpiper spatial usage differed between treatment and control transects (Figure 3.5). On average, western sandpiper usage on treatment transects was lower by 0.36 (SE 0.1) western sandpipers (m^{-2} min^{-1}) than on control transects ($Z = 0.24$, $p < 0.001$; Table 3.2). Overall usage was slightly higher on the oceanward side of the obstruction, with the difference between control and treatment transects significantly different (0.66 (SE 0.16) m^{-2} min^{-1}, $Z = 0.34$, $p < 0.001$; Table 3.2). No evidence of a difference was found between usage of control and treatment transects on the shoreward side of the obstruction (0.04 (SE 0.14) m^{-2} min^{-1}, $Z =0.16$, $p = 0.773$; Figure 3.5).

Mean dropping density pattern, across distance from shore, in 2018 was similar to 2017 (Figure 3.6). Mean dropping density in 2018 was 2.0 droppings m^{-2}, which was greater than mean dropping density in 2017 (0.6 droppings m^{-2}).

On either side of the obstruction (ocean and shore), the difference in western sandpiper usage (m^{-2} min^{-1}) between control and treatment transects differed. The difference in spatial usage between control and treatment plots on the ocean side of the obstruction was 0.59 (SE 0.2) sandpipers m^{-2} min^{-1} greater than the shore side of the obstruction ($t = 3$, $p = 0.004$; Figure 3.7). The difference in western sandpipers (m^{-2} min^{-1}) between control and treatment transects was greatest near the obstruction and decreased with distance on both shore and ocean sides (Figure 3.6). The mean difference (control-treatment) decreased by 0.015 (SE 0.005) western sandpipers (m^{-2}

min^{-1}) on the ocean side of the obstruction (F = 9, p = 0.002; Figure 3.6) and by 0.014 (SE 0.003) western sandpipers (m^{-2} min^{-1}) on the shore side of the obstruction (F = 15, p < 0.001). While the rates of decrease with distance from the obstruction are similar, the difference in western sandpipers (m^{-2} min^{-1}) (control-treatment) was greater on the ocean side of the obstruction (Figure 3.7).

3.4.2. Trade-offs over a Tidal-exposure Period

A main effect of time on western sandpiper usage was found, in which sandpiper usage increased 2.8 (SE 1.3) western sandpipers m^{-2} min^{-1} between Time 1 and Time 2 (Z = 3.62, p-value < 0.001). The main effect of transect was also significant, with 1.71 (SE 1.28) fewer sandpipers m^{-2} min^{-1} on treatment transects compared to control (Z=2.14, p-value = 0.03). On treatment transects, the interaction between distance from shore and time during the experiment on sandpiper usage m^{-2} min^{-1} was significant (Z = 2.59, p-value = 0.009; Figure 3.8). Sandpiper usage increased between Time 1 and Time 2 on treatment transects in plots 20-90 m from shore by 4.9 (SE 1.8) sandpipers m^{-2} min^{-1}. Sandpiper usage did not change between Time 1 and Time 2 on control transects, nor at plots 90-150 m, and 150-220 m from shore, on treatment transects (Z = 0.11, p-value = 0.910).

3.5. Discussion

3.5.1. Food-danger Trade-off

During northward migration at Roberts Bank, western sandpipers used high-risk, visually obstructed transects less than low-risk, unobstructed transects. Western sandpipers spent less time grazing close to the obstruction and on the shoreward side of transects; areas of low visibility and thus high predation-danger. These results agree with observations from a similar experiment conducted at Boundary Bay (Pomeroy et al. 2006). However, the treatment effect (control-treatment spatial usage) at Boundary Bay was over three times greater than at Roberts Bank, with 1.5 and 0.3 droppings m^{-2} respectively (sandpiper and dropping calibration is described in Appendix A). Spatial usage is dependent on food resource availability and predation danger, but in natural ecosystems these factors are rarely fixed. I propose western sandpipers make trade-off decisions depending on current food resource abundance and predation-risk, which may vary between sites and years. The difference in sandpiper usage of safe and dangerous areas observed at Roberts Bank and Boundary Bay (Pomeroy et al. 2006) could be due to spatial and temporal variation in food quality and quantity, landscape features, predator presence, and individual condition.

Sandpiper food resources, both quality and quantity, may differ between sites. Biofilm forms on silt and clay-rich mudflats while invertebrates predominate in more porous soil-environments, such as the sandy tidal-flats of Boundary Bay (Kennish 1990; Yates et al. 1993; Stal and de Brouwer 2003). Biofilm is fatty-acid rich and may be considered a high-quality food item when compared to invertebrates, which are partly indigestible, have a high search and handling time, and yield less energy (Sutherland et al. 2000; Kuwae et al. 2008). If sandpipers feed primarily on low-quality invertebrates at Boundary Bay, spatial usage of dangerous areas should be low because the potential food energy would not outweigh the risk of predation. In contrast, high-quality biofilm at Roberts Bank might offset predation risk resulting in greater time spent foraging in dangerous areas. The smaller difference in spatial usage between dangerous and safe areas at Roberts Bank, as compared to Boundary Bay, could indicate that western sandpipers are willing to risk more to acquire high-quality biofilm than low-quality invertebrates. Other species also consume biofilm on intertidal mudflats, such as dunlin (*Calidris alpina*) (Drouet et al. 2015), snails (Whitlatch and Obrebski 1980), polychaetes (Decho and Lopez 1993) and copepods (Hamilton et al. 2006). Presence and abundance of these species, over time and space, may correspond to variation in biofilm availability in different years and sites. The sandy and porous intertidal zone at Boundary Bay may provide more suitable habitat for biofilm-feeding polychaetes and copepods than the clay-rich and low-porosity sediment at Roberts Bank. At Boundary Bay, invertebrates may deplete biofilm availability close to shore; thereby reducing the benefits accrued from foraging in dangerous areas. Prior to migration north, dunlin forage in the Fraser River Delta in spring, overlapping with western sandpiper stopover. Dunlin abundance can vary between sites in the same year, and at the same site over time (Drever et al. 2014). Large flocks of dunlin might compete for biofilm with western sandpipers at Roberts Bank, altering food abundance. High competition with biofilm-feeders in a particular site or year may decrease biofilm availability and influence western sandpiper trade-off decisions.

Shoreline features provide the cover that falcons use to hunt sandpipers on a mudflat. The type, shape, and extent of cover at a site will affect the degree of danger (Dekker and Ydenberg 2004). The shape and extent of saltmarsh cover at Boundary Bay and Roberts Bank vary. Boundary Bay is crescent shaped, 60 km^2 at low-tide, and is bordered by a 10-200 m wide saltmarsh. In comparison, Roberts Bank is 27 km^2 at low tide, bordered by a 20-40 m wide saltmarsh. While both sites are large and relatively low danger, the proportion of habitat within 150 m from cover is greater at Boundary Bay than Roberts Bank (Hope 2018). Cover determines the danger gradient from shore, which may influence trade-off decisions at a stopover site. The wide saltmarsh border at Boundary Bay could provide considerable cover for falcons, resulting in higher spatial usage of safe areas. Predator presence also influences western sandpiper foraging behaviour, which varies over time and space (Hope et al. 2014). In theory, when falcons are present sandpiper usage of safe areas would be high, and difference in spatial usage (control-treatment) would be large, like that observed at Boundary Bay. Competition and predation were not controlled for at Boundary Bay nor at Roberts Bank, thus either, or both, factors could have influenced the experimental results (Pomeroy et al. 2006).

Individual condition could also influence western sandpiper trade-off decisions at a stopover site. Upon arrival on a mudflat, lightweight birds will take greater and more frequent risks than heavy birds that require less energy (Olsson et al. 2002; Dierschke 2003; Pomeroy et al. 2008). Information on individual condition and associated foraging behaviour would provide valuable insight into food-predation trade-off decisions, however such data are difficult to acquire. A high proportion of lightweight sandpipers foraging in a specific year or site may be pressured to forage in more dangerous areas to meet energy requirements, and vice versa.

3.5.2. Trade-offs over a Tidal-exposure Period

Where food abundance is low, on the ocean side of the obstruction, the difference in sandpiper usage between control and treatment transects was greatest. Western sandpipers spent more time in safe but low-biofilm density areas, following trade-off theory predictions and results from Boundary Bay (Pomeroy et al. 2006). Despite predation risk, spatial usage of control and treatment transects overall was similar on the shore side of the obstruction. However, spatial usage of control and treatment plots close to shore (20-90 m) was greater than the natural background-pattern. I hypothesize that this difference is due to changes in spatial-usage over time, in response to dynamic processes of biofilm growth and grazing. The dynamic spatio-temporal relationship between biofilm and sandpipers throughout tidal emersion could explain the overall similar usage of safe and dangerous areas close to shore, despite low visibility and increased predation-danger.

In the first half of the experiment, sandpipers traded food for safety through greater usage of safe areas (control transects). In the second half of the experiment, usage of dangerous areas (obstruction transects 20-90 m from shore) increased. Since predation-danger was experimentally manipulated, this result suggests that trade-off decisions may change over an intertidal period in response to changes in food resource abundance and availability. Initial grazing took place in safe areas (during the first half of the experiment), thus reducing biofilm in safe areas for subsequent grazing. Ungrazed biofilm in dangerous areas would therefore be high, relative to safe grazed-areas, in the second half of the experiment following initial foraging. I speculate that over time, sandpiper usage switched from safe to dangerous areas in response to greater biofilm availability in ungrazed, dangerous-areas following initial grazing. Additionally, rates of biofilm production in ungrazed areas may be higher than grazed areas due to longer, undisturbed sunlight-exposure. The change in biofilm availability, from relatively equal upon emersion to reduced levels in safe areas over an intertidal cycle, may explain why more western sandpipers used obstruction transects close to shore at Roberts Bank than Boundary Bay. These results suggest, when biofilm is uniform across distance following tidal exposure, western sandpipers graze safe (control) areas first according to trade-off theory. After initial grazing, the incentive to forage in dangerous areas increases due to high food availability relative to previously grazed safe-areas. These results suggest biofilm availability, which varies over a tidal-exposure period in response to production and grazing, influences western sandpiper trade-off decisions between food and safety.

The pattern and extent of western sandpiper spatial usage of trade-off transects was similar to natural transects, indicating normal mudflat usage during the experiment. However, control plots 90-130 m form shore (adjacent to the obstruction) had lower densities of western sandpipers than the natural pattern. The lower than expected usage could be due to unintended tarp-effects on control plots near the obstruction. In spring

2018, several studies were conducted concurrently at Roberts Bank, thus space on the mudflat was limited. Although 20 m of empty mudflat separated control and treatment transects, some predation-risk might have been conferred to control plots adjacent the obstruction.

Despite increased danger from the obstruction-tarp, I was unable to control for raptor presence. It is possible that some of the behaviours observed in this experiment were enhanced when falcons were present.

3.6. Conclusion

During spring migration, western sandpipers graze biofilm at Roberts Bank in a giving up density gradient following food-predation trade-off theory. Sandpiper trade-off decisions, between food and safety, appear to vary over space and time. After tidal exposure most foraging occurs in safe areas. Over a tidal-emersion period, the incentive to graze in dangerous areas where food is ungrazed increases. High-quality biofilm appears to provide a strong incentive for western sandpipers to forage in more dangerous areas where biofilm is more abundant.

3.7. Figures

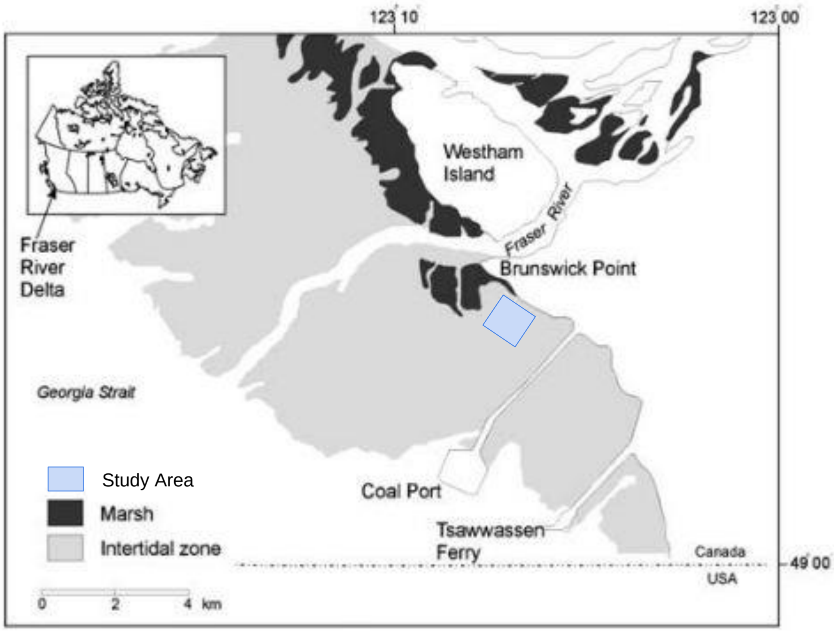

Figure 3.1. **Roberts Bank, Fraser River delta, British Columbia, Canada. Retrieved from Mathot et al. 2010.**

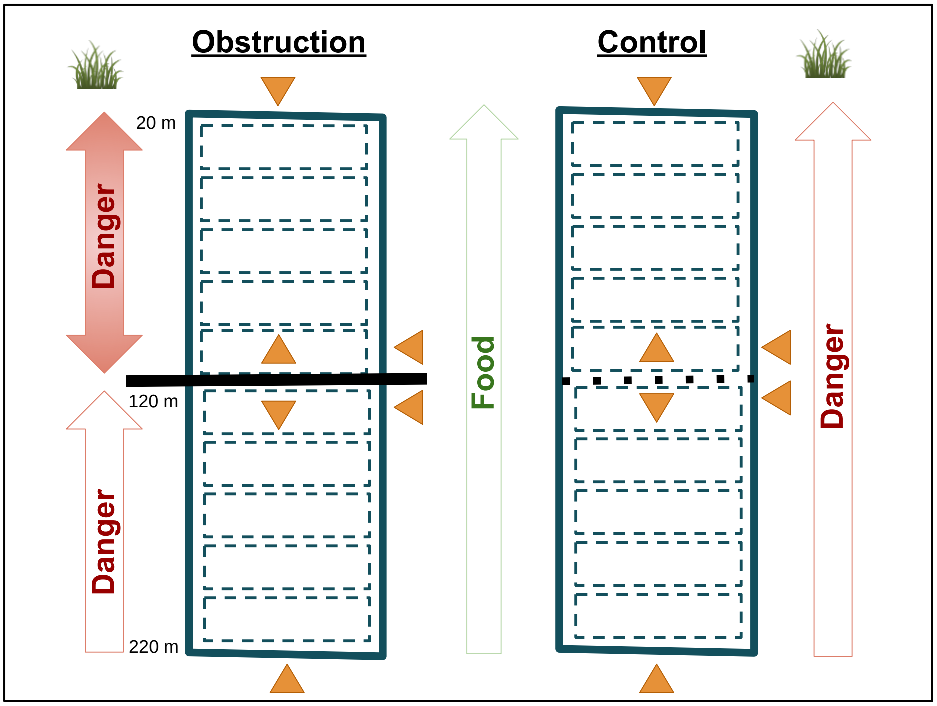

Figure 3.2. Food-danger experiment design. Paired control and treatment (obstruction) transects are outlined in blue with dashed lines showing 10 m² plots. The black line represents the obstruction-tarp located 120 m from, and perpendicular to, shore. Orange triangles indicate camera location and orientation. Arrows indicate the direction of increasing danger (red) and food abundance (green).

Figure 3.3. Treatment transect with the obstruction tarp installed 120 m from shore. White, 15 cm tall, stakes mark the corners of each 10 m x 10 m plot.

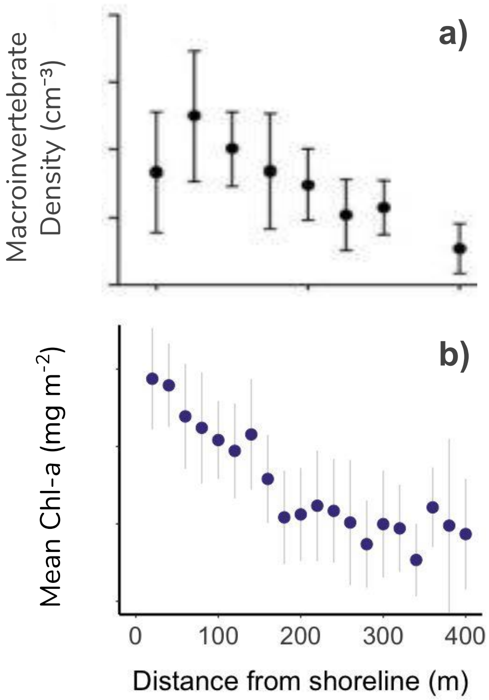

Figure 3.4. **Distribution of macroinvertebrates at Boundary Bay, BC, adapted from Pomeroy (2006), sampled in 2002 (a); and mean Chl-*a* (mg m^{-2}) across distance from shore during spring migration and late summer in 2017 at Roberts Bank, BC, (b).**

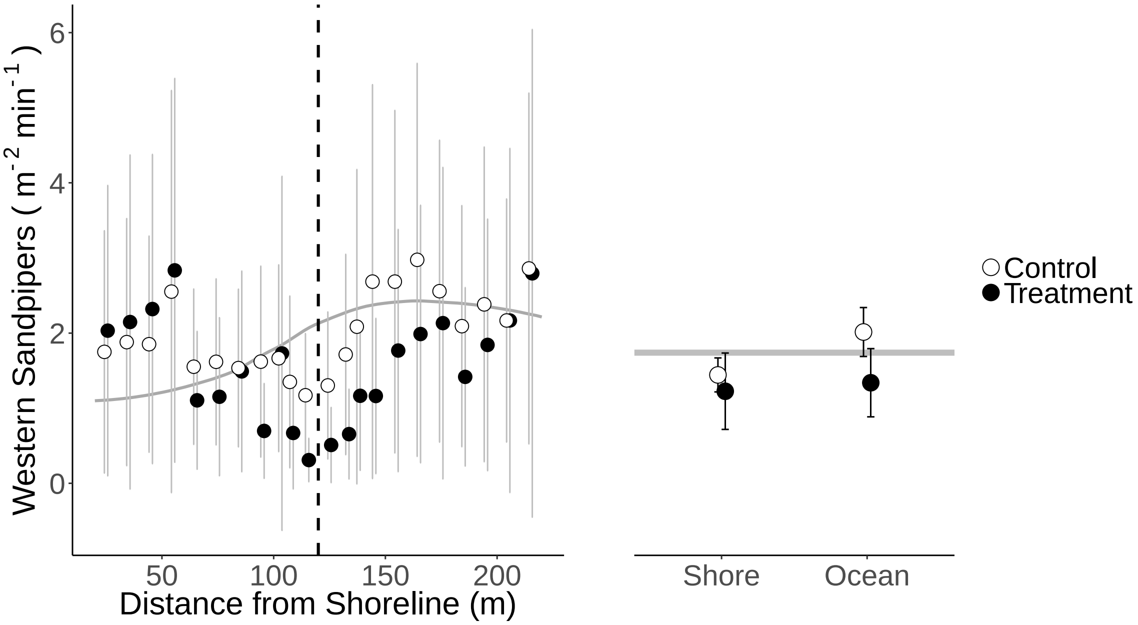

Figure 3.5. **The effect of an experimental obstruction (indicated by dashed vertical line, 120 m from shore) on western sandpiper spatial usage at a stopover site during northward migration (left panel). Mean western sandpiper usage (m^{-2} min^{-1}) on control transects is shown with open circles, and closed circles on treatment transects. Error bars are 95% confidence intervals. The solid gray line is the natural pattern of western sandpipers (m^{-2} min^{-1}) observed on six transects at Roberts Bank during 2018 spring migration. Refer to appendix 1 for natural pattern.**

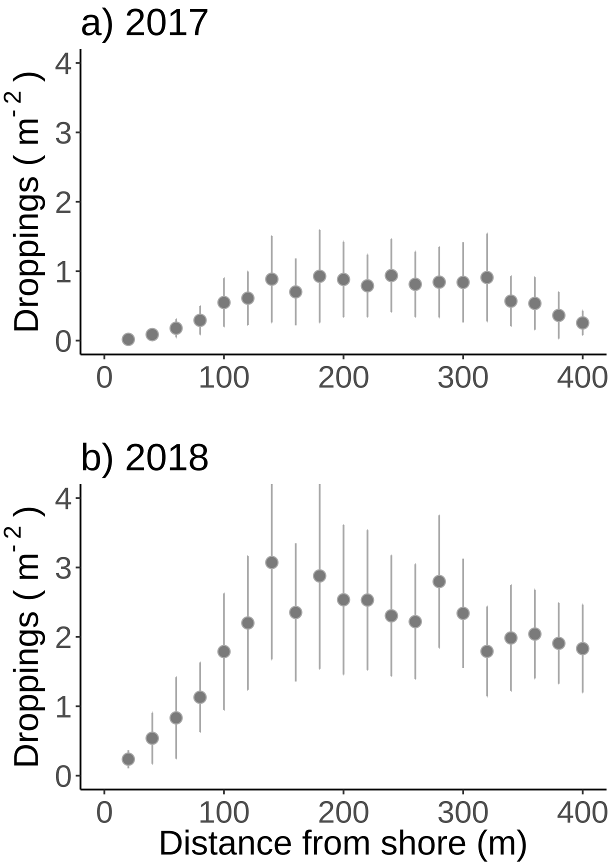

Figure 3.6. **Mean dropping density (m^{-2}) across distance from shore during 2017 spring migration a), and 2018 spring migration b). Error bars are 95% confidence intervals. The dropping density pattern is similar between years, with a steep increase between 20 m and 140 m from shore and a plateau ~ 150 m from shore. However, overall dropping density magnitude was greater in 2018.**

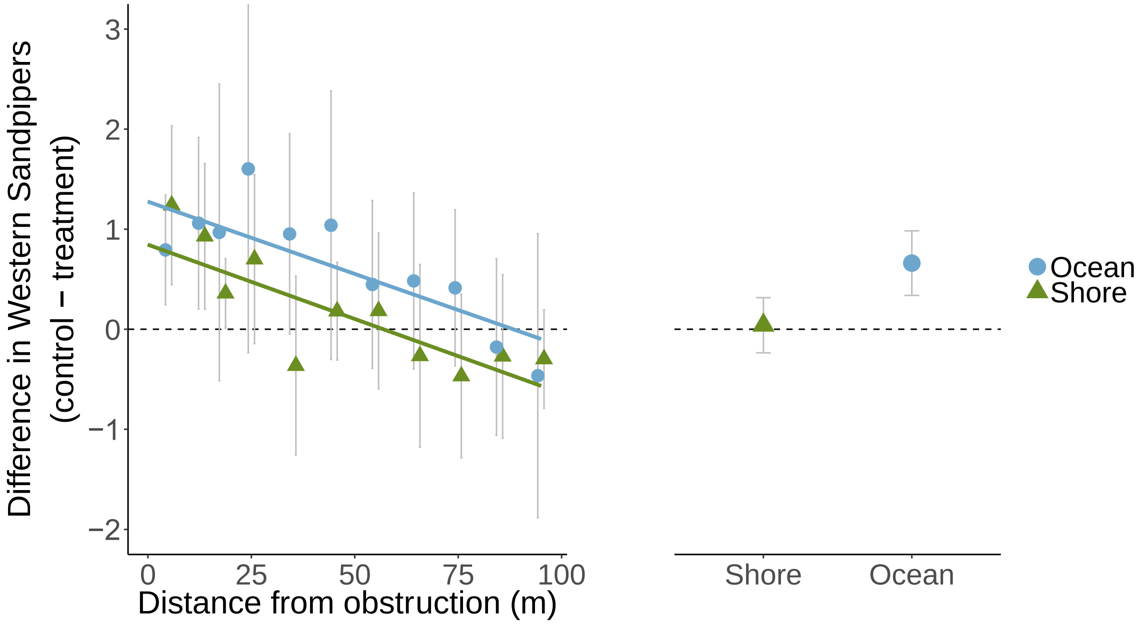

Figure 3.7. **Difference between mean western sandpipers (m^{-2} min^{-1}) on control and treatment transects on ocean and shore sides of the obstruction (left panel). Mean difference between mean western sandpipers (m^{-2} min^{-1}) on control and treatment transects on ocean and shore sides of the obstruction, with 95% confidence intervals (right panel).**

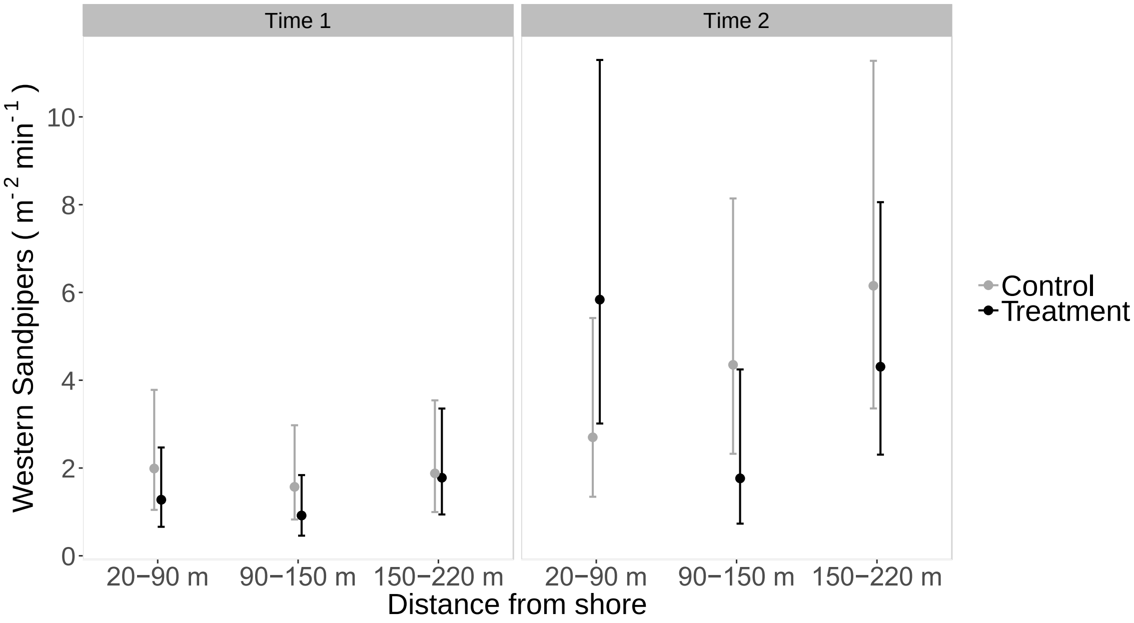

Figure 3.8. Western sandpipers (m^{-2} min^{-1}) on control and treatment (obstruction) transects in Time 1 (the first 2.5 hours of the experiment) and Time 2 (the second 2.5 hours of the experiment) at distances 20-90 m from shore, 90-150 m from shore, and 150-220 m from shore. Marginal effects from a generalized linear mixed model of the interaction between time, distance from shore, and transect (control and treatment).

3.8. Tables

Table 3.1. Summary of total cameras, photos, days the experiment was run, and total western sandpipers counted from photos at each experimental site. (An experimental site contains a control and a treatment transect).

	Site 1	Site 2
Cameras	16	16
Photos camera^{-1} day^{-1}	60	60
Photos day^{-1}	960	960
Days	15	14
Total western sandpipers counted	1452	265

Table 3.2. Test of difference in mean western sandpipers (m^{2}min^{-2}) between paired treatment and control transects with standard error of the difference (SE) and p-value for t-tests between whole transects and ocean and control sides of the obstruction.

	Transect	n	Mean	Difference	SE	P-value
Whole Transects	Treatment	401	1.69	0.36	0.10	<0.001
	Control		2.05			
Ocean	Treatment	207	1.69	0.66	0.16	<0.001
	Control		2.35			
Shore	Treatment	194	1.69	0.04	0.14	0.773
	Control		1.73			

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Chapter 4.

Conclusions

4.1. Summary

Previous intertidal biofilm research has been limited by traditional estimation methods, which require high inputs of time, effort, and capital. As a result, current estimates of biofilm density on intertidal mudflats are crude. In Chapter 2, I used the CCM-300, a novel Chl-*a* measurement technique, to estimate intertidal biofilm density at fine spatial-resolution across annual, seasonal, and diurnal time-scales. I have shown that the CCM-300 generates reliable, repeatable, and instantaneous Chl-*a* measurements in the field, and provides a solution to some of the current challenges in intertidal biofilm research. The methods described in this study present a suitable alternative to traditional biofilm measurement techniques.

At Roberts Bank, biofilm increased over an intertidal period, was lower in areas of western sandpiper grazing, and decreased with increasing distance from shore during spring. Lowest biofilm densities in grazed areas (Figure 4.1b) corresponded to densities 300-400 m from shore during spring migration (Figure 4.1c) and densities in the first hours following tidal emersion (Figure 4.1a). Highest biofilm densities measured at the end of a tidal emersion period (Figure 4.1a) align with values measured in ungrazed areas (Figure 4.1b) and in summer (Figure 4.1c). The offshore-onshore biofilm gradient in spring suggests that western sandpipers, stopping over in large number to feed on biofilm, grazed down the biofilm standing-crop according to optimal foraging theory. Western sandpiper droppings were inversely related to biofilm density across a danger gradient (distance from shore). A predation-danger experiment found that western sandpipers trade-off food and safety at Roberts Bank. This result suggests that the spring biofilm-density gradient was produced by migrants grazing down the biofilm standing-crop with decreasing frequency in dangerous areas closest to shore.

Chapter 3 experimentally tested the hypothesis that migrating western sandpipers trade-off biofilm availability and safety at Roberts Bank, BC, Canada. Migrant shorebirds used dangerous areas (on obstructed transects, and the area closest to the obstruction) less than relatively safe areas (control transects, and the areas furthest from the obstruction). The difference in control and treatment transect usage was greatest on the oceanward side of the obstruction, where safety was high but food was low. These results reflect the findings of a similar experiment conducted in 2003 at Boundary Bay, BC, Canada (Pomeroy et al. 2006), and provide evidence that migrant sandpipers trade-off food and safety by adjusting mudflat spatial-usage. However, I also observed changes in spatial usage over time, suggesting that trade-offs depend on biofilm resource availability over a tidal-emersion period. As biofilm density increases with sunlight-exposure, and decreases from grazing pressures, western sandpipers may switch usage from safe to dangerous areas over an intertidal period.

4.2. Limitations

This study is limited by paired Chl-*a* estimates, derived from lab-analysed mud samples and CCM-300 CFR measurements. Due to logistic and financial constraints only seven mud samples were analyzed for Chl-*a* density. To generate a more robust calibration, between traditional lab-analysed Chl-*a* estimates and CCM-300 field measurements, more paired samples are required.

Another limitation was the CCM-300's sensitivity to standing water and prolonged exposure to saline environments. Chl-*a* detection decreased after heavy rain-events, which saturated the mudflat and left pools of standing water. Vancouver, BC, receives a high annual volume of precipitation; therefore measuring biofilm in spring was a challenge. However, Chl-*a* estimation is rapid, which enabled collection of an unprecedented number of measurements despite restriction during, and immediately following, rain events. Another concern was damage to the instrument from corrosion brought on by extended contact with ocean sediment and saltwater. As a precaution to future users, I recommend rinsing the CCM-300 Chl-*a* sensor of mud and saltwater, thoroughly drying the instrument, and storing it in a safe, dry environment.

This study is also limited by a lack of biofilm data for spring 2018, in which the food-safety trade-off experiment was conducted. Technical difficulties prohibited the use of the CCM-300 in 2018, which prevented Chl-*a* measurement. Although there is a precedent to assume food availability is similar between years (Pomeroy et al. 2006), biofilm measurements throughout the experiment would further support the hypothesis that sandpipers adjust subsequent foraging in response to lower biofilm levels in previously grazed areas.

4.3. Future Research

This study was the first to estimate in situ biofilm density through Chl-*a* measurement using the CCM-300. Future biofilm research should consider investing in the CCM-300 as a practical tool to measure benthic biofilm in intertidal systems.

While I observed changes in biofilm in response to tidal exposure and grazing by shorebirds, it remains unclear how disruption of existing ecological and hydrological processes will affect biofilm production. I have identified several factors that may influence biofilm production and standing-crop density over various timescales. Variation in biofilm appears to be linked to sunlight, temperature, and nutrient concentrations, however the mechanisms are uncertain. Furthermore, some studies have reported nocturnal biofilm foraging (Hebert 2011; Quinn 2011; Kuwae et al. 2008), which questions some of the assumptions about diatom response to sunlight during tidal emersion. It is important to understand the abiotic systems that either enhance, or constrain, biofilm production so that we may accurately assess the impacts of ecosystem change on biofilm abundance. Biofilm is an important component of marine food webs and therefore a critical area of study. Future biofilm research should take into account the temporal variation in biofilm density on tidal flats, and experimentally test how growth is influenced by environmental factors such as sunlight, temperature, and nutrients.

I proposed several theories as to how the current state of food and danger at stopover sites might influence trade-off decisions in migrating western sandpipers over time and space. These hypotheses are unsupported by food abundance measurements, providing an area for further research. Data on individual bird condition was beyond the scope of this study but could explain individual foraging behaviour, and risk assessment, by migrating shorebirds.

4.4. Figures

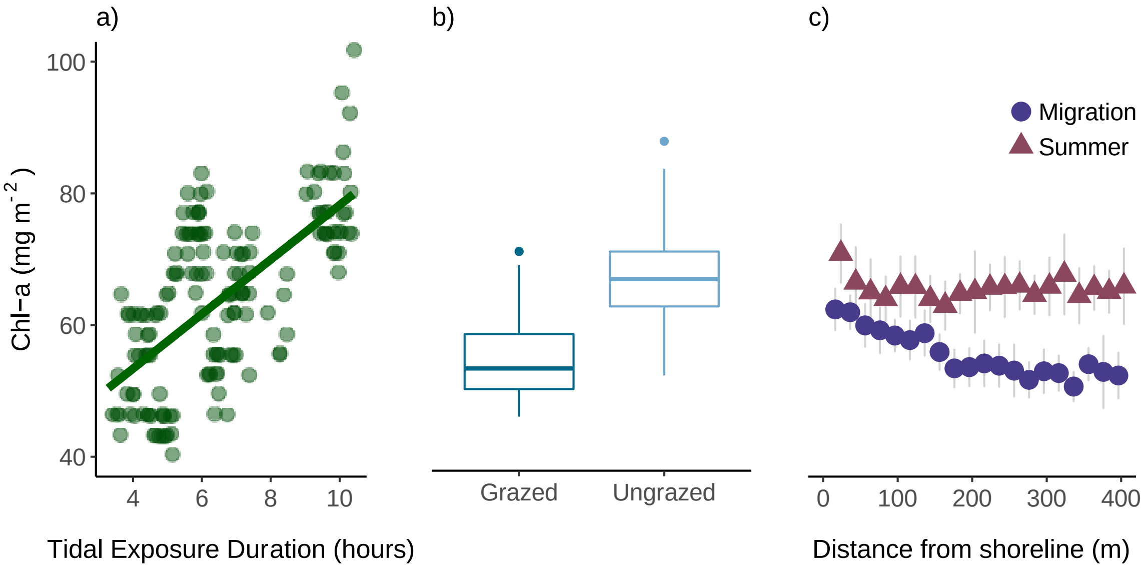

Figure 4.1. **Biofilm density a) over a tidal exposure period, b) in areas with (grazed) and without sandpiper grazing (ungrazed), and c) with distance from shore during migration and summer. Biofilm is measured as Chl-*a* (mg m^{-2}).**

4.5. References

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Appendix.

Sandpiper-proxy Correlations

While foraging on exposed tidal mudflats, shorebirds leave behind droppings and footprints. These proxy measurements are often used as indicators of shorebird presence and foraging intensity. Many shorebird studies report dropping-rates from observations of individual birds (Pomeroy 2006; Kuwae et al. 2008). Such measurements are time-consuming and only describe dropping rates for a few individuals, despite frequent (and perhaps erroneous) generalization to an entire flock. While common in the literature, the methods and data used to generate these estimates are often vague, and the estimates themselves mere footnotes within larger, more complex, studies. Dropping estimates are often used to explain or validate significant results and conclusions about shorebird foraging behaviour, thus it is important that these estimates are representative. Footprints, while not as often cited, also provide a measure of shorebird presence, and, arguably, a measure of time or energy spent foraging. Surveys are another common method to estimate shorebird habitat use and flock size. However, survey counts are often criticized as a poor indicator of presence (Dickie et al. 2014). Furthermore, presence does not simply denote foraging intensity because shorebirds also invest time roosting and evading predators while at stopovers.

Shorebird research is logistically challenged by shorebird habitats, vast and often isolated tidal flats. During migration, shorebirds forage and rest on tidal mudflats, coastal stopover-sites rich in food resources. Coastal mudflats are submerged during high tide and inaccessible for long periods, yet are difficult to traverse when exposed and very sensitive to disturbance. During tidal-immersion periods, shorebird droppings and tracks are removed by the gently ebbing tide (Sutherland et al. 2000). Human footprints, however, are not so easily erased. At Roberts Bank, BC, impressions from bootprints remain visible for weeks, and even months in particularly clay-rich areas. Despite the potential for unintended effects upon repeated measurements over short time-scales like shorebird migrations (often 2-3 weeks), research-related disturbances to mudflats is still a challenge. One solution is to conduct shorebird surveys (using a telescope) from a distance without accessing the mudflat being surveyed. However, long-distance observations lack the ability to track fine-scale movement of large flocks. Time-lapse cameras offer an alternative solution. Common in animal behaviour studies trail-cameras are unobtrusive, low-disturbance once installed, and capture large amounts of information for relatively little effort.

Field designs aim to maximize a study's confidence in its results while minimizing time, effort, and fiscal expenditures. Here I describe some common methods to estimate shorebird presence, and foraging intensity, and a less common method for measurement in sensitive habitats. This appendix asks how dropping density and proportion of tracks compare to counts of western sandpipers and describes methods for counting shorebirds while minimizing habitat disturbance using time-lapse cameras.

Methods

I measured the number of droppings m^{-2} and proportion of footprints m^{-2} in plots at Roberts Bank to estimate mudflat usage by western sandpipers. Foraging western sandpipers leave white, circular (approximately 1.5 cm diameter) droppings, and footprints comprised of three branching lines (approximately 2.5 cm in diameter). Both droppings and footprints are easily discernible on mudflats during low tide (Figure A1).

Western sandpiper droppings and footprints were measured on 11 days between 19 April, 2018 and 3 May, 2018. Trail cameras (Browning Strike Force Elite HD, model BT-5HDE) were placed at 10 plots to count western sandpipers m^{-2} min^{-1}. Cameras were fixed 1.5 m above the mud surface, atop PVC pipes, 15 m south of each plot, and oriented north to reduce glare. Four, white, PVC stakes marked the corners of each camera plot (Figure A2). Once the plot became visible (following tidal emersion), I counted western sandpipers in 1-minute intervals (1 photo = 1 minute) until the time when droppings and footprints were recorded at each plot (Figure A3).

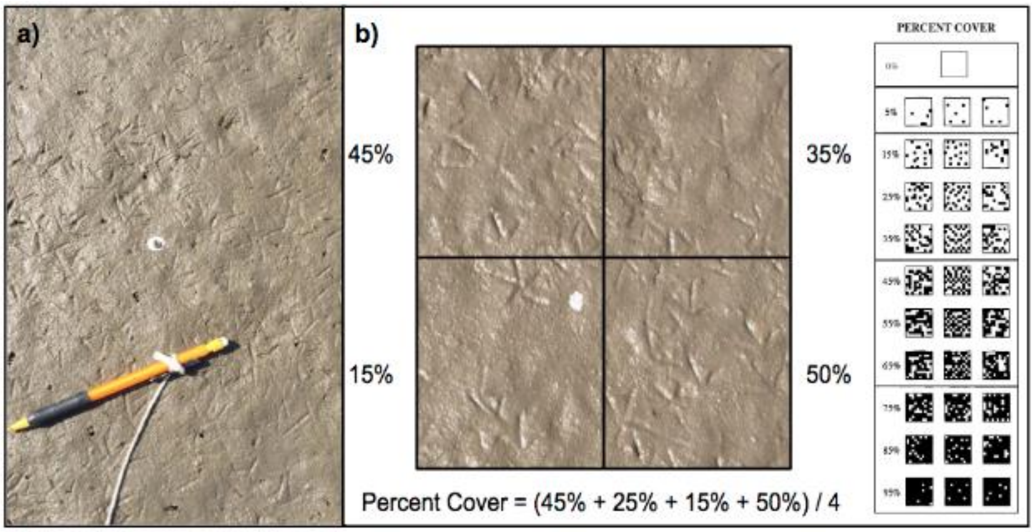

Figure A1. Western sandpiper footprints and dropping, pencil included for scale (a) and example calculation of percent cover to estimate proportion of footprints (b). Footprints were estimated as the proportion of western sandpiper tracks left in the mud of a 1 m^2 plot in 5% bins (McIntyre and Eleftheriou 2005).

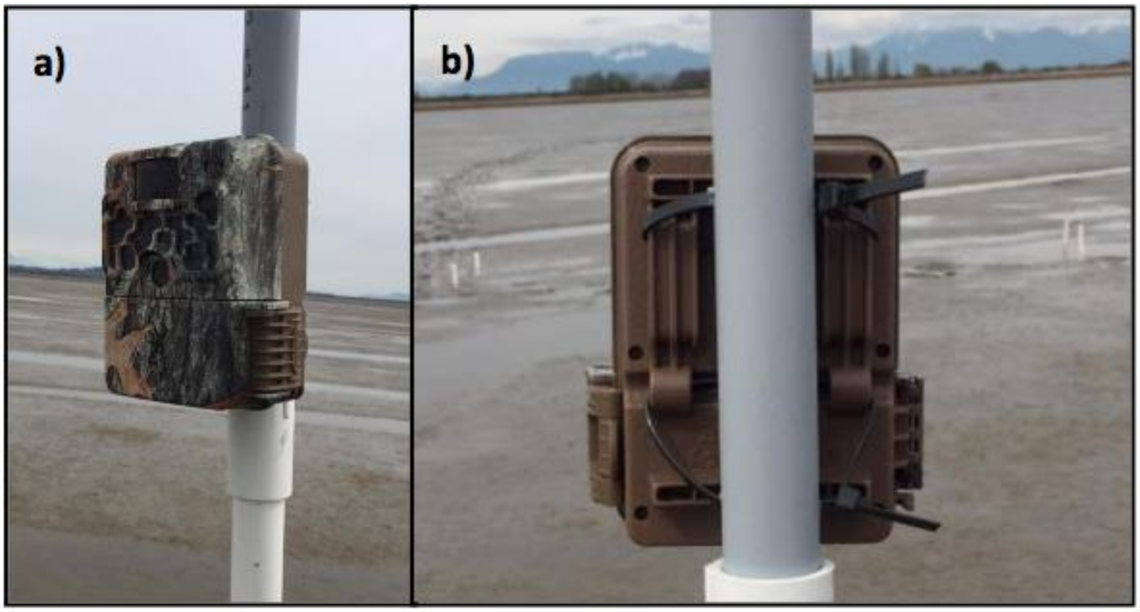

Figure A2. Cameras (Browning Strike Force Elite HD, model BT-5HDE) attached to PVC poles 1.5 m above the mud surface so as to remain in place throughout the study period and stay clear of the high tide (a). Cameras faced plots marked with 15 cm white stakes in which I counted droppings and tracks, and western sandpipers from photos (b).

Figure A3. **Time-lapse photo of western sandpipers foraging in a camera plot marked with 15-cm, white stakes. Two western sandpipers are within the sampling plot in this photo.**

Statistical Analysis

To estimate droppings western sandpiper^{-1} m^{-2}, and compare dropping rates per bird at Roberts Bank to those found in other studies, I fit a linear mixed effects model of dropping density (m^{-2}) and western sandpipers (m^{-2} min^{-1}), with plot and day as random effects. Model residuals satisfied all assumptions. To predict western sandpiper counts from dropping density, I used a simple linear regression. Model assumptions were satisfied by taking the log of western sandpiper count (+1) and the daily mean per plot. I used this model to convert background droppings, measured on 2018 'natural' transects, for comparison with western sandpiper measurements on experimental and control transects.

Results

I found a strong linear relationship between dropping density and western sandpipers ($p < 0.001$; Figure A4): which found that western sandpipers produced 0.4 (SE 0.01) droppings m^{-2} min^{-1} (Figure A4; $p<0.001$).

I found a moderate nonlinear relationship between western sandpiper footprint (m^{-2}) and dropping (m^{-2}) densities (Figure A6). As dropping density increased the variance in footprint density increased until saturation at ~ 10 droppings (m^{-2}).

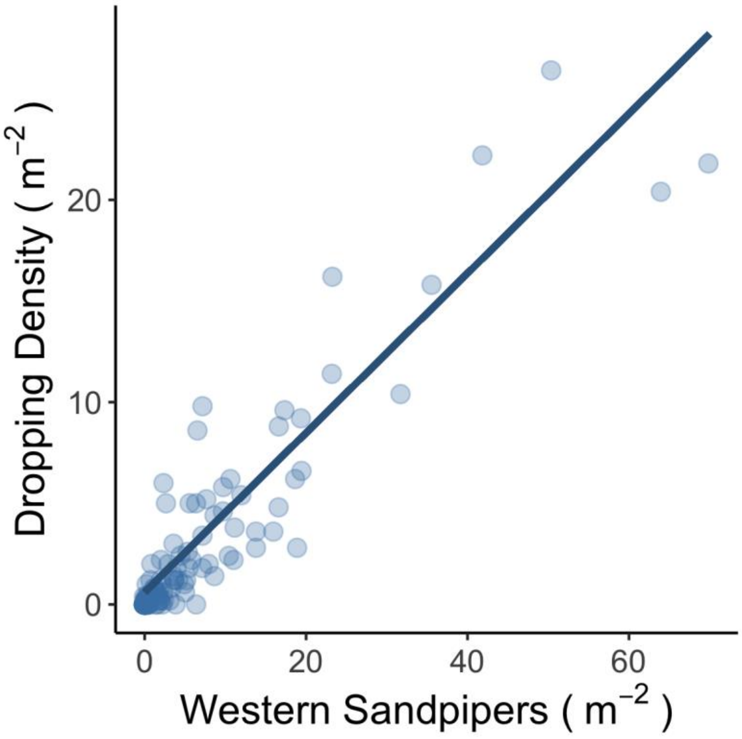

Figure A4. **Relationship between droppings m^{-2} and number of western sandpipers m^{-2} observed within camera plots at Roberts Bank.**

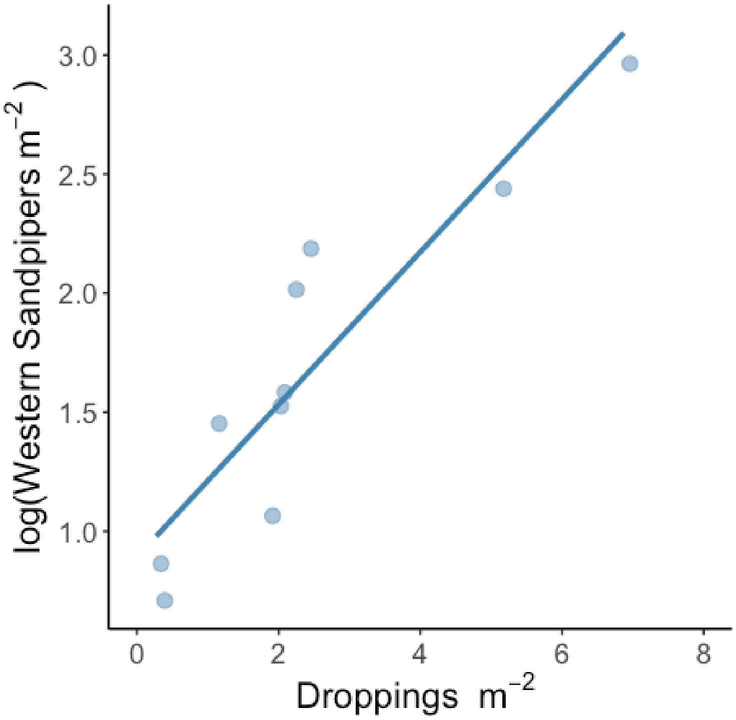

Figure A5. **Relationship between droppings m^{-2} and number of log(western sandpipers m^{-2}) observed within camera plots at Roberts Bank.**

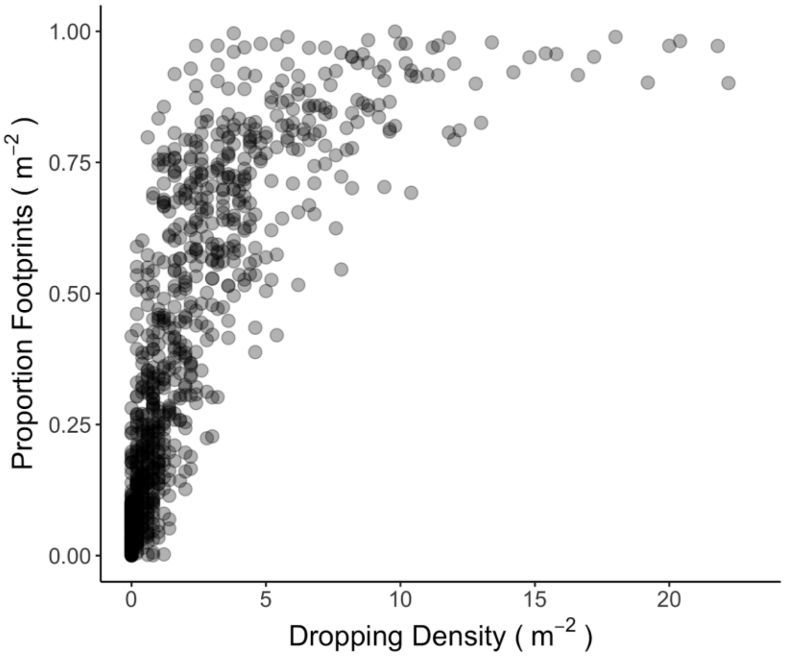

Figure A6. **Relationship between dropping density (m^{-2}) and proportion of footprints (m^{-2}) observed at Roberts Bank during spring migration.**

Discussion

Droppings provide a record of time spent grazing, while footprints record steps taken. I measured droppings, footprints, and western sandpipers to determine which indicator of presence best represented western sandpiper foraging. I observed a strong positive relationship between droppings and western sandpipers and a non-linear relationship between tracks and droppings. At low dropping density, more footprints were produced per dropping, which suggests that, at times, sandpipers are walking faster or spent more time searching for food. In contrast droppings appear to be strong indicators of western sandpiper presence and grazing intensity. Shorebird studies commonly approximate foraging behaviour with dropping density and the results of this study justify such assumptions. Thus, dropping density (m^{-2}) was chosen as a proxy to evaluate western sandpiper grazing intensity in this thesis. While not discussed here, footprint density offers additional insight into foraging behaviour, such as search effort.

Pomeroy (2006) and Kuwae et al. (2008) both estimated that western sandpipers produced droppings at a steady rate of ~ 0.5 droppings min^{-1}. These estimates are similar to the ~0.4 droppings min^{-1} that I observed at Roberts Bank. However, these studies estimate dropping rate through observation of individual birds over time. These results support the assumption that large flocks of western sandpipers rapidly metabolize food while foraging on a stopover site.

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