The “Secret Garden”: Microphytobenthic Biofilms and the Foraging Ecology of Calidridine Sandpipers

by

Ariam Jiménez Reyes

M.Sc. (Zoology and Ecology), University of Havana, 2004
B.Sc., University of Havana, 2000

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Name:	**Ariam Jiménez Reyes**
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Examining Committee:	**Chair: Dr. J. Mattsson**, Associate Professor

Dr. R.C. Ydenberg,
Senior Supervisor
Professor

Dr. R.W. Elner
Supervisor
Emeritus Scientist
Environment Canada

Dr. D.J. Green
Supervisor
Associate Professor

Dr. W.J. Palen
Internal Examiner
Assistant Professor

Dr. D.J. Hamilton
External Examiner
Associate Professor,
Department of Biology
Mount Allison University

Date Defended/Approved: 17 January 2013

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Abstract

Food availability is well-known to have a strong influence on where and how shorebirds forage. A recent discovery is that microphytobenthic (MPB) biofilms, previously unknown to be used as food by any avian species, are an important component of the diet of small calidridine sandpipers. This dissertation investigates the as yet uninvestigated relations between the behavior and distribution of foraging calidridine sandpipers and MPB biofilm availability. I studied three calidridine species - western sandpiper (*Calidris mauri*), dunlin (*Calidris alpina*), least sandpiper (*Calidris minutilla*) - at three estuarine sites: Roberts Bank, an intertidal stopover site in Canada; and at Río Máximo and Tunas de Zaza, coastal stop-over sites in Cuba. Of these species, western sandpipers show the greatest modification of tongue and bill morphology for biofilm feeding. Infrared photography from ground and air and sediment sampling were used to assess the abundance of biofilm and the small benthic invertebrates fed on by sandpipers. Counts of shorebirds and measures of dropping density were conducted to assess bird distribution and habitat use.

At Roberts Bank, the highest MPB biomass was found in the upper intertidal (0-750 m from shore, tide height range: 3.0-3.5 m), consisting of a diatomaceous biofilm, one the two major classes of MPB biofilms. Foraging dunlins closely followed the ebbing tide, exploiting the upper intertidal only briefly. In contrast, western sandpipers exploited the entire exposed tidalflat surface, spending much more time in the upper intertidal, matching MPB biomass more closely than the distribution of benthic invertebrates. Both Cuban sites showed a seasonal change in the type of MPB biofilm, shifting from cyanobacterial mats during southbound stopovers to diatomaceous biofilms during winter. In contrast to the foraging activity of western sandpipers at Roberts Bank, least sandpipers used foraging habitats in accordance with benthic invertebrate density rather than MPB biomass. I review the available evidence and conclude that the reason for this difference is that cyanobacterial mats provide poorer nutrition than diatomaceous biofilms.

Keywords: calidridine sandpipers, microphytobenthos, biofilm, biofilm feeding, food, habitat use

Dedication

I dedicate this dissertation to my family, especially …

to my Mom for her eternal optimism and perseverance;

to my Dad for teaching me the importance of imagination and dreams;

to Laura for her patience, understanding, encouragement, and love;

to my son, Darío, for refreshing my dreams

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1. General introduction

Shorebirds (Aves: Scolopacidae) devote time and energy to seasonal migration between breeding and wintering areas (Morrison and Myers 1987). The “fuel” that supplies such demands comes from food obtained in a wide range of climatic regions and habitats. Accordingly, there have been many studies on the diet and feeding ecology of migratory shorebirds (Goss-Custard *et al.* 1991, Yates *et al.* 1993, Mathot *et al.* 2007). Understanding the ecological processes supporting the food webs of shorebirds, as well as the foraging ecology patterns of the birds themselves is critical to effective conservation (West *et al.* 2005, Taft and Haig 2006).

Recent investigations have unveiled evidence of new feeding mechanisms and unexpected food sources for shorebirds (Sutherland *et al.* 2000, Kuwae *et al.* 2008, Kuwae *et al.* 2012). Previously, shorebird diet had been described as encompassing a variety of suspended, epibenthic and infaunal macroinvertebrate taxa (Goss-Custard *et al.* 1991, Tsipoura and Burger 1999). These are obtained through a combination of three main foraging modes: surface feeding (pecking) which targets epifauna, deeper foraging (probing) to capture infaunal prey (Zweers and Gerristen 1997) and surface tension transport to feed on small prey suspended in the water column (Rubega 1997, Estrella *et al.* 2007). In addition to these feeding mechanisms and prey types utilized by shorebirds, subsequent experimental studies have revealed that the birds can utilize smaller prey within the meiofaunal size fraction (0.063 - 0.5 mm) (Sutherland *et al.* 2000). Further, examination of the bill and tongue ultra-structure of western sandpiper and dunlin (Elner *et al.* 2005), combined with detailed video examination on foraging and isotope analysis (Kuwae *et al.* 2008, Kuwae *et al.* 2012), revealed that biofilm constitutes a new and important food source for shorebirds. The findings open fresh perspectives on shorebird biology and a need to re-evaluate the foraging ecology of shorebirds in intertidal systems through the ecological lens of biofilm. The main goal of my study was to examine the feeding ecology shorebirds, with special emphasis on biofilm as a novel food source.

1.1. Biofilm: a microscopic landscape on intertidal estuaries

Biofilm is a generic term used to define matrix-enclosed communities of microorganisms attached to surfaces (Branda *et al.* 2005). Such communities are complex and dynamic, resembling microbial landscapes where ecological process and functions take place within and throughout the slimy matrix produced by the microorganisms (Battin *et al.* 2007).

Biofilms are considered the dominant mode of microbial life in most aquatic ecosystems (Branda *et al.* 2005, Battin *et al.* 2007). Particularly in estuaries and shallow coastal systems, the heterogeneous mix of microalgae and bacteria associated with sediment surface secrete a matrix of mucilaginous extracellular polymers (EPS) to form a microbial biofilm (Decho 2000). Thus, biofilm is a community of microbial cells (Underwood *et al.* 2005) whose activities may be structured or enhanced by the EPS matrix sourrounding the cells (Underwood and Paterson 2003).

1.2. Biofilm forming microorganisms in intertidal systems

A complex arrangement of benthic microalgae, autotrophic and heterotrophic bacteria inhabits intertidal biofilm communities (Cammen and Walker 1986, Carman 1990). Although estuarine biofilms are comprised of a diverse mix of microorganisms, the microphytobenthic fraction is the main agent responsible for the formation of highly dense biofilms on the surface of intertidal sediments (Yallop *et al.* 2000, Stal 2003). Microphytobenthos (MPB) is an artificial grouping of algae and photosynthetic bacteria, with diatoms and cyanobacteria being the dominant constituents (Cibic *et al.* 2007, Underwood 2010). These photoautotrophic microorganisms are important constituents of biofilm communities, constructing and reorganizing the biofilm matrix during every emersion period (Tolhurst *et al.* 2003).

Two distinctive types of MPB can be distinguished in intertidal biofilms, based on their photoautotrophic microorganisms dominance: cyanobacterial mats and diatomaceous biofilms (Stal 2003). In marine fine sandy sediments, microbial mats are composed of an upper layer of cyanobacteria that fuel underlying layers of associated bacterial groups (chemoorganotrophic bacteria, sulfate-reducing bacteria, anoxygenic bacteria, green sulfur bacteria) through the products of oxygenic photosynthesis (Stal 2003). Diatomaceous biofilms are usually found on silt-clay rich sediments forming a layer up to 3-4 mm on top of the sediment surface, with epipelic diatoms being the most abundant group (Underwood 2010). Although bacterial activity is present (Cammen and Walker 1986, Yallop *et al.* 2000), these biofilms do not give rise to laminated communities (Stal 2003). Disregarding which MPB group prevails (cyanobacteria or diatoms), both systems of phototrophic microalgae share the attribute of producing copious amounts of extracellular polymeric substances (EPS; Yallop *et al.* 1994, de Winder *et al.* 1999).

1.3. Mucilaginous matrix

The EPS is the product of metabolism of the microorganisms constituting biofilm and forms the matrix that cements the organisms together and to the substratum (Decho 2000, Underwood 2010). The EPS produced by the microorganisms exist as tightly bound (capsular), loosely adhered (slime type) or as free dissolved matter (component of dissolved organic matter, DOM) (Underwood and Paterson 2003). The EPS is composed largely of polysaccharides (70% - 95%) with the balance made by lipoproteins (Taylor *et al.* 1999, de Brouwer and Stal 2001, Hoskins *et al.* 2003).

Microphytobenthic organisms divert up to 80 % of the carbon they fix during the daylight emersion period into EPS (Stal 2003) . Such adaptation has been proposed as a convenient mechanism to divert excess absorbed energy, which would otherwise cause oxidative damage (Underwood *et al.* 2005). Nevertheless, EPS is not merely a expensive loss of energy but rather fulfills a variety of functions beneficial to the microorganisms and the landscape they form (Underwood and Paterson 2003, Underwood 2010). At the microscopic landscape level, the highly hydrated nature of the EPS enhances water storage and protects the microorganisms from desiccation during low tide periods (Decho 2000). The EPS matrix confers shelter to MPB making them less accessible to grazers (Plante 2000). Moreover, EPS offers a stable and optimum environment to microorganisms for growth, to bind them to the sediment surface, or to facilitate cell motility (Battin *et al.* 2007, Underwood 2010). At the intertidal landscape-level, both EPS and MPB strongly influence sediment stability at intertidal systems (Paterson 1989, Yallop *et al.* 2000, Tolhurst *et al.* 2003) and represent an important labile organic carbon pool in these habitats (Decho 2000). Specifically, carbohydrates and microorganisms encompassing biofilm are proven an important food source for polychaetes (Decho and Lopez 1993), brittlestar (Hoskins *et al.* 2003), snails (Whitlatch and Obrebski 1980), copepods (Decho and Moriarty 1990), shrimps (Abreu *et al.* 2007), fishes (Almeida 2003), and most recently, shorebirds (Kuwae *et al.* 2008, Kuwae *et al.* 2012).

1.4. Biofilm estimation in intertidal systems

Biofilm may be a predictable resource in intertidal systems, but as for any living community, this biogenic surface exhibits natural rhythm of variability (Herlory *et al.* 2004, Underwood *et al.* 2005). Studies have shown that biofilm (both MPB biomass and its metabolic product, the EPS) often varies over short to long term temporal and spatial scales in relation to environmental factors such as light (Smith and Underwood 2000, Perkins *et al.* 2001), sediment (Watermann *et al.* 1999), temperature (Defew *et al.* 2004), salinity (Underwood and Provot 2000), tides and exposure time (Tolhurst *et al.* 2003, Mitbavkar and Anil 2004), rain (Tolhurst *et al.* 2006), nutrient availability (Underwood 2002) and grazing (Pinckney *et al.* 2003, Hicks *et al.* 2011).

Understanding biofilm in intertidal systems involves the challenge of detecting the variations in the “invisible” layer of microorganisms on the sediment surface. The assessment of microalgal biomass through the analysis of its spectral properties is a technique used in estuarine biofilm studies (Serôdio *et al.* 1997, Kazemipour *et al.* 2012). Detection of small quantities of chlorophyll-*a* (Chl-*a*) in coastal environments (water or sediment) has been employed since the publishing of Lorenzen’s equations for determination of chlorophyll and pheo-pigments (Lorenzen 1967). Because photosynthetic MPB are the main contributors of estuarine biofilms communities (Cibic *et al.* 2007, Underwood 2010), the quantification of Chl-*a* concentration (Chl-*a* per unit area of sediment) or Chl-*a* content (Chl-*a* per unit mass of sediment) have been used as a proxy for MPB biomass and productivity in estuarine systems (Serôdio *et al.* 1997, Degré *et al.* 2006, Hicks *et al.* 2011). In this thesis, I assume that the determination of Chl-*a* can be further used as a proxy for biofilm abundance (MPB biomass and EPS content) in intertidal systems. Such an assumption is supported by the positive correlations between Chl-*a* and soluble glucose-rich EPS found in the upper most sediment layers (0-2 mm) of intertidal systems where diatoms are the main intertidal producer (Underwood and Smith 1998, de Winder *et al.* 1999, de Brouwer and Stal 2001).

1.5. Thesis outline

The overall objective of my study was to assess the extent to which aspects of shorebird ecology (such as spatial distribution and behavioral patterns) coincides with biofilm presence and availability. The thesis consists of six independent chapters which address topics related to biofilm and the foraging ecology of migrating calidridine sandpipers. Specifically, the primary objectives addressed were to: 1) use remote sensing methods to assess and map the MPB biomass spatial variation at an important stop-over habitat for shorebird (Chapter 2), 2) examine the type of MPB biofilms and biomass variation at tropical sites in order to assess biofilm presence and availability for calidridine sandpipers during southward migration and winter (Chapter 4), and 3) examine how calidridine sandpiper behavioral patterns and habitat use at foraging habitats coincides with both biofilm and traditional prey availability (Chapter 3 and 5). A common factor linking each chapter is the inclusion and analysis of biofilm as a potential variation source underlying sandpiper feeding ecology at estuarine systems.

The challenge to assess shorebird prey availability increases many fold when a new and “invisible” food source is taken into account (biofilm). Remote sensing has been described as the science and art of obtaining information about an object, area, or phenomenon through the analysis of data acquired by a device that is not in contact with the investigation subject (Lillesand and Kiefer 2000). In Chapter 2, I explore the use of remote sensing as a tool to assess MPB biomass as a proxy for biofilm availability at Roberts Bank. Sediment sampling, ground-based digital color infrared images (CIR) and aerial CIR imagery acquisitions were conducted over the intertidal mudflat. I examined the efficacy of two broad wavelength bands (R, 600-700 nm, and NIR, 750-1350 nm) and four derived vegetation index (DVI, RVI, NDVI, and SAVI) to assess Chl-*a* content as a measure of MPB biomass. Also, ground-truth sampling (Chl-*a* content and digital CIR measurements) and aerial CIR imagery taken on Apr. 25th 2008 were used to map MPB biomass variation on the whole study site.

Daily averages of more than 100,000 sandpipers concentrate at Roberts Bank during the northward migration (23 Apr. - 3 May.), with a ratio of approximately 80 % western sandpiper to 20 % dunlin (Jardine *et al.* 2012). Competitive interactions may arise between such congeners with some degree of overlap in their invertebrate diet (Senner *et al.* 1989). With the emergence of biofilm as a potential food source for shorebirds' examining how these species segregate in time and space during the onset of the ebbing tide could add new understanding to their competitive interactions. Thus, in Chapter 3, I examine the feeding distribution of western sandpiper and dunlin during the ebbing tide at the upper intertidal of Roberts Bank. The basic attributes indicative of biofilm grazing have been described for both species (Elner *et al.* 2005). However, western sandpipers appear to be more adapted for this feeding mode than dunlin (Elner *et al.* 2005, Mathot *et al.* 2010, Kuwae *et al.* 2012). Taking into account such differences, I explore several predictions on the distribution of both shorebird species during the ebbing tide in relation to biofilm availability. In general, I expected that, under a competition scenario, western sandpiper would use habitats that favour biofilm abundance more frequently and for longer than dunlin.

Understanding the role of biofilms in the sandpiper foraging ecology requires the understanding of biofilm significance at tropical sites used as stop-over sites and/or wintering areas. Little is known about spatial and temporal variation in MPB biofilm availability at tropical sites. Chapter 4 reports on the type of MPB biofilm and biomass at two Cuban estuarine sites (Río Máximo and Tunas de Zaza) exhibiting different type of flood-emersion regime ("wind tide" and tidal, respectively). The study covered calidridine southbound migration (October 2009) and wintering residency (January 2009).

Food availability is a major factor determining shorebird distribution (Goss-Custard *et al.* 1991, Yates *et al.* 1993). Biofilm has recently been added to the items that calidridine sandpipers can incorporate into their diet (Kuwae *et al.* 2008, Kuwae *et al.* 2012, Quinn and Hamilton 2012). However, in contrast to benthic invertebrates, little is known about the set of conditions that makes biofilm available to shorebirds. In Chapter 5, I test the hypothesis stating that the type of MPB biofilm (cyanobacterial mats and diatomaceous biofilm) mediates the relationship between biofilm availability and foraging spatial use by migrant small-bodied sandpipers. The hypothesis was based on the poor nutritional value of cyanobacterial mats to biofilm grazing organisms (Chapter 4). Thus, positive relationships between biofilm availability and sandpiper spatial use are predicted only for diatomaceous biofilm sites. I assessed the extent to which MPB biomass, macro- and meiofaunal invertebrates influence on patterns of sandpiper foraging habitat use at stop-over sites exhibiting different classes of biofilm (cyanobacterial mats: Río Máximo and Tunas de Zaza, Cuba; diatomaceous biofilms: Roberts Bank, Canada).

Finally, in Chapter 6, I summarize all the findings and discuss the implications for research and conservation, with special emphasis on the role of biofilm in shorebird ecology.

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2. Ground and aerial based remote sensing using color infrared images for assessing microphytobenthic biomass at Roberts Bank, Fraser River Estuary, British Columbia, Canada

2.1. Abstract

Here I tested the efficacy of color-infrared photography (CIR) to assess microphytobenthic (MPB) biomass at Roberts Bank. I conducted two separate spatial scale assessments of MPB biomass using two broad wavelengths: Red (R, 600-700 nm) and Near-infrared (NIR, 750-1350 nm). First, I explored the use of a modified digital camera to assess MPB biomass from a ground-based study involving most of the intertidal used by shorebirds. Second, I used aerial CIR imagery to map MPB distribution over the emerged mudflat at Roberts Bank. The ground-based assessment revealed that only the NIR reflectance was positively associated with Chl-*a* content. Such a relationship was further improved by considering the percentage of water content in the sediment sample. Aerial CIR imagery revealed a heterogeneous distribution of MPB biomass with highest values converging to the upper intertidal (0-750 m from shore). The results indicate that CIR imagery is a practical method for rapid qualitative assessment of MPB biomass.

Keywords: remote sensing, color-infrared photography, biofilm distribution, microphytobenthos biomass, Roberts Bank,

2.2. Introduction

Food availability is a critical variable associated with shorebird abundance and distribution in non-breeding sites (Goss-Custard *et al.* 1991, Cohen *et al.* 2010).

Recently, estuarine microphytobenthic biofilms have been demonstrated to be a major food source for small sandpipers (Kuwae *et al.* 2012). Accordingly, there is a need to quantify the biofilm availability in estuarine habitats in order to accurately assess habitat quality in shorebird non-breeding sites.

Assessment of shorebird food requirements in intertidal systems necessarily involve some of the most laborious and tedious tasks in shorebird foraging ecology (A. Jiménez pers. obs.). The challenge increases many-fold when a new and "invisible" food source, such as surficial biofilm, is considered. Estuarine biofilms are comprised in great extent by mycrophytobenthos (MPB) which is an artificial grouping of benthic microalgae such as diatoms and photosynthetic cyanobacteria (Admiraal *et al.* 1984, Underwood and Kromkamp 1999). Also, the benthic MPB is the main determinant of the extracellular polymeric substances (EPS) forming the carbohydrate rich biofilm matrix (Stal 2003). Thus, MPB biomass over the exposed intertidal sediment can be used as an index of biofilm abundance (Grinham *et al.* 2007, Mathot *et al.* 2007, Kuwae *et al.* 2008).

Conventionally, MPB biomass have been determined indirectly by estimating the amount of chlorophyll-*a* (Chl-*a*) extracted from sediment cores (Yallop *et al.* 1994, Herlory *et al.* 2004, Grinham *et al.* 2007). Such a labour-intensive and sometimes expensive method has the additional inconvenience of "destroying" the sampling area; an important limitation in studies seeking to provide contiguous measurements of Chl-*a* over time (Murphy and Underwood 2006, Murphy *et al.* 2008, Coelho *et al.* 2009). The use of remote sensing tools offers an alternative means to obtain contiguous quantitative measurements of benthic Chl-*a* in estuarine mudflats (Serôdio *et al.* 1997, Serôdio *et al.* 2001, Jesus *et al.* 2006, Murphy *et al.* 2006, Coelho *et al.* 2009).

Spectroradiometric techniques are among the most commonly used remote sensing tools (Paterson *et al.* 1998, Decho *et al.* 2003, Combe *et al.* 2005, Murphy *et al.* 2008). Such techniques rely on the reflectance spectra of plant and soil components as governed by their individual optical properties (Tucker 1979). High absorption of incident sunlight in the visible red (R, 600-700 nm) and strong reflectance in the near-infrared (NIR, 750-1350 nm) portions of the electromagnetic spectrum by photosynthetically active plant tissue is distinctive from the reflectance of soil or water (Tucker 1979, Lillesand and Kiefer 2000). The reflected spectral bands can be used in a quantitative way to estimate vegetation biomass indices (Jordan 1969, Tucker 1979, Huete 1988, Gilabert *et al.* 2002).

Most remote sensing work has been applied and tested with terrestrial vegetation (Woodcock and Strahler 1987, Wessman *et al.* 1988, Hall *et al.* 1991, Martin *et al.* 1998). The microalgae components of estuarine biofilm share the same spectral properties as plants because they contain Chl-*a* as one of the most important photosynthetic pigments. However, the smaller amount of Chl-*a* in MPB biofilms and the greater proportion of substratum contributing to the remotely sensed signal makes the quantification of Chl-*a* more difficult than, say, canopies of terrestrial vegetation (Combe *et al.* 2005, Murphy *et al.* 2005). Also, the finer-scale distribution of microalgae patches over the wet sediment increases the challenge of using remote sensing tools in intertidal habitats (Kelly *et al.* 2001). Available and free satellite imagery has too coarse resolution (e.g., 30-120m, Landsat TM) for accurately mapping MPB biofilms at estuarine systems. Therefore, research studies aiming to map MPB biofilm distribution need to rely on high resolution satellite imagery (SPOT), aerial color-infrared (CIR) photography or other hyperspectral imaging airborne system as a more appropriate source of data (Combe *et al.* 2005, Costa *et al.* 2007). Although the potential problems above should not be ignored (relatively low Chl-a concentration in MPB compared to terrestrial vegetation, and MPB fine scale spatial distribution), the use of spectroradiometric remote sensing techniques has yielded valuable information for intertidal habitats where MPB is the main photosynthetic primary producer (Murphy *et al.* 2004, Combe *et al.* 2005, Murphy *et al.* 2005, Pomeroy and Butler 2005, Murphy and Underwood 2006, Murphy *et al.* 2006)

The underlying hypothesis of this work is that benthic Chl-*a* in estuarine mudflats can be assessed by using CIR photography to quantifying its distinctive spectral characteristics in the visible R and the NIR. The aims of this study were to (1) investigate the utility of digital CIR photography to assess benthic Chl-*a* on emerged areas of the Roberts Bank mudflat, and (2) estimate and map the spatial distribution of MPB biomass across the emerged intertidal using airborne CIR imagery. Here, the efficacy of broad R and NIR spectral bands and three vegetation indices was investigated by comparing them to *in situ* measurements of Chl-*a* content of surface sediment (Chl-*a* normalized to the dry mass of the sediment in each sample). Previous studies using CIR in intertidal systems have used either infrared photographic films (Pomeroy and Butler 2005) or multispectral digital cameras (Murphy *et al.* 2004) which are difficult to operate in the field and/or are expensive in terms of equipment cost and image processing. The approach of this study was to use an affordable digital camera, within which the principal components had been modified to obtain the R and NIR spectral bands, thus greatly reducing cost and increasing flexibility in the field. Also, I examined the correspondence between the ground-truth CIR digital images, CIR airborne imagery and Chl-*a* content during an emersion period at Roberts Bank and mapped the spatial distribution of biofilm biomass over this shorebird habitat.

2.3. Methods

2.3.1. Study area

All fieldwork was carried out on Roberts Bank, in the southern arm of the Fraser River Estuary (49°03'N, 123°09'W), British Columbia, Canada. Roberts Bank is a mesotidal estuary with tidal range of 0.0-3.8 m (Zharikov *et al.* 2009). The immediate study area encompassed a large estuarine intertidal mudflat (approximately, 2.5 x 4.0 km) which extends from Brunswick Point towards a causeway supporting a port facility (Fig. 2.1). The mudflat is separated from agricultural fields by dikes and its upper reaches are colonized by brackish marsh. For the purpose of this study only the 'non-vegetated' intertidal was considered. The dominant taxonomic group of MPB in Robert Banks mudflats is diatoms (Kuwae *et al.* 2008, Beninger *et al.* 2011). The intertidal flats sediment grades landwards from sand to soft mud substrate (Williams and Hamilton 1995). The site represents an important habitat for resident and migratory birds, especially shorebirds which can reach flock sizes in the hundreds of thousands (Butler 1994, Butler *et al.* 2002). The Fraser River Estuary is officially included in three international conservation programs for its significance to birds: Globally Important Bird Area, "Hemispheric Important Area" by the Western Hemispheric Shorebird Reserve Network (WHSRN) and Ramsar wetland of International Importance (Hill *et al.* 2012).

2.3.2. Sediment sampling

Color-infrared digital camera data and *in situ* sediment samples for assessing Chl-*a* content data were obtained during low tide (N = 94). The paired set of samples was taken at randomly selected points across the intertidal. All samples were collected between 07:00 and 14:00 h in April/May 2008 within a range of 2.0 - 2.5 h after the sediment became exposed in a particular sampling site.

Digital camera data

Digital CIR imagery for the sediment surface was acquired using a modified digital camera Canon 30D of 8.2 million pixels fitted with a Canon EF 100mm f/2.8 macro lens (Astro Hutech). Image data are quantized to 8 bit (0 - 255 data range) resolution. Two major modifications were performed in the camera. First, the internal near-infrared filter was removed facilitating the acquisition of images on the NIR spectral band. Secondly, a 3D lens was modified in order to be attached on top of the lens while holding two 58 mm filters. The modification consisted in a rectangular aluminum platform (150 x70 mm, 4 mm thick), painted in black and glued on top of the 3D lens plastic case. Two holes were made on the platform just over the prisms to screw in each filter. The resulting light-weight system was fitted (screwed in) to the Canon lens. Such modifications allowed the simultaneous acquisition of two different broad spectral band images (Red and NIR).

The filter selection was made according to the Wratten numbers system. I used a Hoya 25A filter for acquisition of broad R band. This is a strong contrast red filter used for color separation and infrared photography in black and white film and blocks light below 580nm (50% of transmittance at 600 nm). The broad NIR spectral band was obtained by using a Tiffen #87 filter, which is often used as a standard infrared filter as it has a sharp cut-off at 730nm (50% of transmittance at 820 nm), and transmits freely throughout the infrared region.

The camera was configured to produce an image of 3504 x 2336 pixels, covering an area of sediment of approximately 75 x 50 mm. The approximate resolution of each pixel was 0.001 mm. The camera was mounted on a black tripod at 0.85 m above the sediment surface. A grey plastic caliper was placed above the sediment area to be sampled and included in every image as a reference coordinate system to couple both spectral images with the *in situ* Chl-*a* content data.

Digital CIR images were acquired under natural sunlight. In order to standardize images for variations in ambient light conditions, an 18% reflective humidity-insensitive grey card (Novoflex Controlcard) was used as a reflectance standard. The grey card was placed within the sampled area such that it was always included on the top left corner of every image. The integration (exposure) time for both spectral bands was optimized to ambient light conditions using camera link software tools. Care was taken to properly focus the area of interest. In order to do so, I previously experimented to understand and mark the correct amount of focus shift between images taken with the R and NIR filters. For each sampling point, a set of four to eight images was taken to provide a reasonable range of images with proper focus and exposure time in each spectral band. Images were recorded as RAW image format and saved onto a laptop PC until processing.

***In situ* Chl-*a* data.**

To validate the accuracy of the image-derived estimation of benthic Chl-*a*, *in situ* sediment samples were collected from within the area of interest imaged by the camera for laboratory analysis. A modified 60 cc syringe (apex removed and edges sharpened) with a 26 mm inner diameter was used as a core sampler to remove a standard sediment surface area. The modified corer was pushed into the sediment and the plunger lifted until approximately 10-15 mm of sediment was expressed in the barrel. Care was taken to prevent contact between the plunger and the sediment surface. The plunger was removed from the syringe, placed in the opposite end and pushed to extrude the first 2 mm of sediment. Biofilm samples were taken by slicing the top 2 mm off the sediment with a spatula. Samples were placed in plastic labeled zip-loc bags and stored in a cool box with ice, returned to the laboratory and stored in the dark at -20℃.

2.3.3. Airborne remotely sensed data

Airborne imagery

On 25 April 2008 between 15:00 and 16:35 h., airborne images were acquired using the Cessna 180g/185C turbo photo conversion aircraft from the Department of

Geography of the Simon Fraser University. Images were taken during low tide when the intertidal zone was completely exposed. At the time of acquisition, atmospheric conditions were clear and winds were calm. The airplane flew at an altitude of 2,900 m at approximately 167 km/h. Six tracks were flown, orientated approximately northeast-southwest, starting with the most eastern strip and working westwards to keep pace with low water conditions in the estuary.

Images were obtained using Nikon F-250 motor driven camera for aerial reconnaissance mounted in the front camera port (16'') of the aircraft that had been modified for aerial survey. The exposure was at 1/75 second at f:4.0 using a standard #15 (Bausch & Lomb) filter. The film was Kodak Infrared Aerochrome III 1443 which contains 3 emulsion layers sensitive to green (500-600 nm), red (600-700 nm) and near infrared (700-800 nm). A yellow (#15) filter was used to remove blue wavelength from exposure on all layers.

Ground-truth sampling

Ground-truth sampling was carried out on 25 April 2008 between 12:00-14:40 h. Fifteen sampling points (4x4 m) were positioned across the intertidal for ground reference survey. Reference points were distributed every 300 m covering a wide range of the intertidal (from 50 m to >1600 m from shore). Points were always set on completely emerged sites. Reference aerial targets, consisting in white plastic bags (2.5 m^2), were placed at the north corner of each sampling point to allow for posterior georegistration and accuracy validation of the airborne CIR imagery. Ground-truth digital CIR imagery and *in situ* benthic Chl-*a* samples were taken following the above methodology. In addition to the digital photograph and sediment samples, I took notes on the presence of macroalgae or any other physical feature (type of sediment, distance to poodles, ridges and runnels and channels) that could be associated later with the airborne imagery data.

2.3.4. Imagery processing

Ground digital CIR images saved as RAW format were transformed to TIFF format for further processing. Format transformation was done using the free image processing software DCRAW. I used the free ImageJ 1.39u software to extract the pixel values (Digital Number, DN) of the area of interest, which was a measure of raw reflectance data.

In order to compare images obtained under different light conditions and camera integration times, it was necessary to calibrate individual spectral bands to reflectance. Reflectance calibration was done by standardizing the brightness of pixels in each image to the grey card following Murphy *et al.* (2004). For both bands in each image, the raw DN values over the grey card were extracted and averaged (average number of image pixels was 180). The DN values in the area of interest of the image were converted to relative reflectance (ρ) using the following equation:

ρ (%) = DN (image) x RG / DN (grey card);

where,

DN (image) = DN at each image pixel,

RG = Reflectivity of the grey card (18%),

DN (grey card) = Average DN of the image values extracted over the grey card.

Methods to extract information on plant pigments from remotely sensed data are constrained in part by the number and width of spectral bands available. For this study, only two broad spectral bands (R and NIR) were sensed by the modified digital camera and this limited the way the data could be analyzed. I used the spectral reflectance of the two single bands sampled and four vegetation indices to correlate with benthic Chl-*a*. Vegetation indices used in this study were: 1) the Difference Vegetation Index (DVI = NIR – R, Tucker 1979), 2) the Ratio Vegetation Index (RVI = NIR / R, Jordan 1969), 3) the Normalized Difference Vegetation Index (NDVI = (NIR-R) / (NIR+R), Rouse *et al.* 1973), 4) and the Soil Adjusted Vegetation Index (SAVI = [(NIR-R) / (NIR+R+0.5)] x 1.5, Huete 1988). All these indices exclusively use both the NIR and R reflectance band and the SAVI uses additional constants to deal with potential interferences from the soil background.

Thirty-one CIR images were obtained from the aerial remote sensing survey. Wide overlapping between images was purposely achieved in order to construct a mosaic image of the Roberts Bank intertidal. To obtain false CIR slides, the Infrared film was processed using standard Kodak negative processing to specified gamma by HAS in Dayton, Ohio. Film was scanned at 10 microns with direct linear conversion using an EXON Expression 1640XL photogrammetrically calibrated flatbed scanner. Scanned slides had a ground resolution of 1x1 m. The digitized images are represented by 24-bit true color with three bands: 8-bit red (R), 8-bit green (G) and 8-bit blue (B). For each pixel in the image, the primary color value is represented by a DN within the range of 0 to 255 for each spectral band. All image registration was undertaken at SFU in the SFU Remote Sensing Laboratory using “ER Mapper” image processing software. At each ground reference point, nine pixels were randomly selected to extract the mean DN in each spectral band. The average pixel values were used as measure of reflectance data.

2.3.5. Laboratory analysis

Frozen sediment samples for Chl-*a* content determination were transferred to 20 ml scintillation vials, weighed to the nearest 0.01 g and thawed in the dark before analysis. Ten ml of 90 % acetone solution was added to the vials and samples were thoroughly shaken for 2 min. in a vortex and placed into a dark box at -4°C for 24 h for extraction. The amount of Chl-*a* in the supernatant was measured spectrophotometrically using a Genesys 10uv spectrophotomer. Absorbance at 665 and 750 nm were measured; samples were acidified with two drops of 0.1 N HCL, mixed, left for 1 min. and absorbance was measured again at the same wavelengths. Concentration of corrected Chl-*a* was calculated using Lorenzen’s spectrophometric equations (Lorenzen 1967). Then, samples were oven dried for 48 h at 70°C and weighted again to obtain the dry weight. Microphytobenthic Chl-*a* was expressed as $\mu g\ g^{-1}{}_{\text{dry sediment (DM)}}$ (weigth-normalized values). The sediment water content (WC, %) was calculated by (Murphy *et al.* 2004):

$$WC\ (\%) = (Wt_{wet} - Wt_{dry}) / Wt_{wet} \times 100$$

where, Wt_{wet} and Wt_{dry} are the wet and dry weight of the sediment sample, respectively.

2.3.6. *Statistical analysis*

Data were analyzed using the software program R 2.10.0 (R Development Core Team 2009). Chlorophyll-*a* content was transformed using natural logarithms (ln (Chl-*a* +1)) to meet the normality of distribution and homogeneity of variance assumptions. All model results were visually inspected to confirm that they satisfied these assumptions. Values presented in the manuscript are means ± standard deviations (S.D.).

To investigate the utility of ground-based digital CIR images to assess Chl-*a* content, the reflectance data for R, NIR, and vegetation indices (DVI, RVI, NDVI and SAVI) were regressed against the *in situ* Chl-*a* content data using general linear models. To assess support for these six competing models, Akaike's Information Criterion adjusted for small sample size (AIC_c) was calculated for all models in the set (Burnham and Anderson 2002). Competitive models were defined as those within two AIC_c units (ΔAIC_c) from the best model (lowest AIC_c value); all others were considered as having definite evidence ($\Delta AIC_c = 2\text{-}4$), weak evidence ($\Delta AIC_c = 4\text{-}7$) or no evidence ($\Delta AIC_c > 7$) to be the best approximating model.

Because indices constructed from NIR reflectance can be affected by soil wetness (Huete 1988, Gilabert *et al.* 2002, Coelho *et al.* 2009), I examined the effect of sediment water content (WC) on the remote sensing assessment of Chl-*a* content. I did not hypothesize any WC effect *a priori* using the original set of models. Rather, I assessed the influence of WC in a secondary modeling exercise, using only the model(s) contained in the best subset ($\Delta AIC_c \leq 2$). I used multiple linear regressions to examine the influence of WC on the assessment of Chl-*a* content through digital CIR imagery. In this case, the set of models considered: 1) the main effect of a particular wavelength reflectance or vegetation index, 2) the main effect of WC, 3) the additive effect between these two predictor variables, 4) the interaction between predictors, and 5) the intercept only or Null model. The relative variable importance (RVI) was examined by summing Akaike's weight (ω_i) over all models containing the variable of interest. If there was no unambiguously best supported model in the set ($\omega_i > 0.9$), a model averaging approach was conducted over models having some level of evidence ($\Delta AIC_c \leq 7$). Therefore, I adjusted Akaike's weight over selected models and calculated model-averaged parameter estimates by multiplying explanatory variables coefficients with the model

Akaike's weight and summing these products over models. To assess reliability and precision, unconditional standard errors and 95% confidence intervals (95%CI) were generated from the model-averaged parameter estimates (Burnham and Anderson 2002). Also, I presented R^2 or adjusted R^2 values to describe overall model fit.

Digital ground-truth and airborne spectral reflectance data were correlated using the measurements taken over the 15 reference points. *In situ* Chl-*a* content data were plotted against both the ground-truth and airborne NIR reflectance band data. The strength of such relationships was determined by linear regression analyses.

2.4. Results

2.4.1. Predicting benthic Chl-a content through ground-based digital remote sensing

Chlorophyll-*a* content over Roberts Bank intertidal ranged from 6.0 to 33.2 $\mu g\ g^{-1}{}_{DM}$ with an average of 14.9 ± 6.3 $\mu g\ g^{-1}{}_{DM}$ for all samples analyzed (N = 94). Mean R and NIR reflectance was 15.8 ± 1.7 % (range: 12.3 – 19.9 %) and 15.2 ± 1.6 % (range: 11.4 – 18.2 %), respectively. The sediment water content averaged 41.3 ± 11.4 % (range: 25.8 – 63.3 %).

The initial modeling of Chl-*a* content as predicted by reflectance data and vegetation indices unambiguously revealed that NIR reflectance best described variations in benthic Chl-*a* content (Table 2.1). The NIR model had an Akaike weight of 0.99, whereas no other candidate model provided reasonable support to the data. In the best model, the natural logarithm transformed Chl-*a* content increased with increasing NIR reflectance (ln (Chl-*a* content +1) = 0.378 + 0.148 NIR). The model explained 34% of the total variation. Because the R spectral band and the four vegetation indices explained none or few of the *in situ* Chl-*a* content, they were not considered further.

The second modeling exercise showed the need of considering both the NIR and sediment WC to best describe benthic Chl-*a* content in Roberts Bank intertidal (Table 2.2). The best approximating model in the candidate set contained the additive effect of NIR reflectance and sediment water content. The Akaike weight of that model was 0.49.

However, the model featuring the interaction between NIR reflectance and WC followed the best model rather closely with ΔAIC_c of 0.18 and an Akaike weight of 0.44 (Table 2.2). The model considering only the main effect of water content on Chl-*a* content acquired some support ($\Delta AIC_c = 3.97$, $\omega_2 = 0.07$). The models that did not acquire support within this subset where those not including sediment WC effects, suggesting that sediment wetness has an important role in variation of benthic Chl-*a* content. Also, the inclusion of sediment wetness improved the model performance ($R^2_{adj} = 0.55 – 0.58$).

The examination of the relative importance of each predictor variable showed that both NIR reflectance and sediment WC were most important with a total weight of 1.00, compared to 0.48 for the interaction term. Model averaged parameter estimates and their unconditional standards error were calculated using models 2, 3 and 4 as they had most of the support within the set ($\Delta AIC_{c\ 2,3\ \text{and}\ 4} = 0.99$). Although both the NIR reflectance and water content typically bounded zero, effects sizes and their confidence intervals strongly favored positive effects (Table 2.2). Such results are indicative of strong but moderately imprecise and uncertain effects. The effect of the interaction between NIR reflectance and sediment WC was negative and small ($\beta_{NIR*WC} = - 0.001$), and its 95% confidence interval widely bounded zero (Table 2.2).

Figure 2.2 represents the relationship between benthic Chl-*a* content and NIR for samples with WC above or below the median (median WC = 38%, N = 94). Samples with WC above 38% had higher benthic Chl-*a* content and a more pronounced slope in the Chl-*a* content and NIR reflectance relationship than those samples with WC below the median value. The relationships showed considerable amount of scatter around the line of best fit, especially for wetter sediment with larger amounts of Chl-*a* content. For example, at sites with WC > 38% the relative NIR reflectance at values close to 17% (N= 6, range: 16.9 - 17.1 %) had corresponding amounts of Chl-*a* content ranging from 9.8 - 27.5 $\mu g\ g^{-1}{}_{DM}$.

2.4.2. Airborne imagery

Near-infrared and R spectral bands obtained from digital ground-truth images and airborne CIR imagery were highly correlated ($r_{Red} = 0.61$, $P = 0.001$; $r_{NIR} = 0.83$, $P < 0.001$, respectively). There was a positive and significant relationship between benthic

Chl-*a* content and the ground-truth NIR reflectance band ($R^2_{14} = 0.43$, $P = 0.008$). Similarly, there was a positive and significant relationship between benthic Chl-*a* content and the airborne NIR reflectance band ($\ln(\text{Chl-}a\ \text{content}+1) = 1.31 + 0.02 \cdot \text{NIR}_{\text{airborne}}$, $R^2_{14} = 0.47$, $P = 0.005$). I further examined the relationship between benthic Chl-*a* content and the NDVI index obtained using the airborne-based R and NIR reflectance data. The relationship between Chl-*a* content and the NDVI index was marginally significant and explained 49 % of the total variation ($\ln(\text{Chl-}a\ \text{content}+1) = 0.97 + 0.03 \cdot \text{NDVI}$, $R^2_{14} = 0.49$, $P = 0.042$).

Maps of the benthic Chl-*a* content were constructed by applying the two regression equations based on airborne reflectance data to the matching CIR imagery (Fig. 2.3A and 2.3B, NIR reflectance and NDVI index, respectively). Since both the NIR reflectance and NDVI index had only moderate relationship with benthic Chl-*a* content ($R^2 < 0.50$), I conducted a qualitative visual interpretation of the maps using relative estimates of Chl-*a* contents. Intertidal Chl-*a* content distribution was described as patchy with high, medium or low Chl-*a* content. Patches with high Chl-*a* content appeared as red and orange tones whereas yellow and blue areas were indicative of sites with medium and low Chl-a content, respectively. In the maps, red and orange tones use to correspond with agricultural fields and marsh vegetation which are expected to exhibit more Chl-*a* content than intertidal primary producers (MPB and macroalgae).

The NIR reflectance for benthic Chl-*a* content in this study varied from bright orange to blue (Fig. 2.3A). Data derived from aerial NIR reflectance showed the highest Chl-*a* contents in the upper section of the intertidal, specifically between 0 and 750m from shore. Within this intertidal section there was a heterogeneous distribution of benthic Chl-*a* content (from light yellow to orange) with two large patches located in the northern and central part of this intertidal region. There was a sharp contrast in Chl-*a* content between the initial section of the intertidal and the subsequent intertidal region. Such contrast was more notable in the region around Brunswick Point where large areas seemed to have had low or no benthic Chl-*a*. Also, a large channel running parallel to the jetty causeway showed a low benthic Chl-*a* content feature. An apparent continuous patch of benthic Chl-*a* content was identified along the intertidal section adjacent to the jetty causeway. Similarly, strip and oval chapped orange patches appeared in scattered fashion over the western and central part of the map. A closer examination to the original images suggested that both the large orange patch adjacent to the jetty causeway and the rest of the scattered patches were probably caused by the reflection of sunlight over the wet sediment.

The visual interpretation of the map based on the NDVI index also showed large benthic Chl-*a* content patches in the intertidal close to shore (Fig. 2.3B). However, the comparison between both maps revealed important features not detected by the exclusive use of the NIR reflectance data. The NDVI index is more sensitive to vegetation features than just the NIR reflectance; thus, agricultural fields and marsh are best detailed in this map. Consequently, benthic Chl-*a* contents in the NDVI image are predominantly represented by yellow tones. Considering the above, the NDVI map showed two areas within the intertidal exhibiting orange patches which may be indicative of Chl-*a* content readings from non-microphytobenthos organisms. The largest orange patch within the intertidal was located towards the northwestern region of Brunswick Point. Another orange patch was detected towards the end of the large channel that runs parallel to the jetty causeway. A further subtle difference of the NDVI map (Fig. 2.3B) in comparison to the NIR map is the presence of yellow areas both sides of the large channel. The feature is notable towards the end of the channel. The absence of the large orange patch adjacent to the jetty causeway as well as the scattered strip and oval orange patches is consistent with the “artificial” nature of the signal (sunlight reflection into the camera). The presence of three similar orange arcs within the western region is consistent with the observed effect of sunlight reflection over the wet sediment.

2.5. Discussion

2.5.1. Digital CIR photography to estimate Chl-a on exposed areas of the Roberts Bank mudflat

In this study, I have presented results illustrating that ground-based remote sensing can be used for rapidly assessing intertidal biofilm biomass governed by diatomaceous MPB in estuarine habitats. I found that among the two broad-band digital CIR data and the derived vegetation indices used, only the calibrated NIR band offered useful information for benthic Chl-*a* content assessment. The NIR reflectance readings explained 34 % of the total variation of Chl-*a* content. Further, the linear fit between the NIR reflectance band and the benthic Chl-*a* can be improved by considering the sediment water content in the sample (R^2 values up to 0.58).

The amount of explained variance in Chl-*a* content by using NIR reflectance data was consistent with the literature (Pomeroy and Butler 2005, van der Wal and Herman 2007). The results are, however, contrary to those reported by Murphy *et al.* (2004), who did not find relationship between the Chl-*a* and the NIR reflectance. The underlying rationale for the wide variation in the capability to predict benthic Chl-*a* (from non existing to moderately positive relationships) from indices constructed from NIR bands can be based on the spectral properties of the algal assemblages themselves (Murphy *et al.* 2005). Given that the reflectance at NIR bands is dominated by scattering properties of the plant tissues (Knipling 1970), differences in the structure of the microalgae (e.g. unicellular or filamentous) could influence on the measured values (DN) of NIR spectral reflectance (Méléder *et al.* 2003, Kazemipour *et al.* 2012). For example, Murphy *et al.* (2005) stated that the light scattering properties of diatoms and filamentous cyanobacteria are likely to be different given their differences in the arrangement of internal structures (fucoxantin/chl-*a* ratio), external morphology (unicellular and filamentous, respectively), and the way they are distributed over the sediment (biofilms and mats, respectively). Unfortunately, there have been no studies investigating the influence of the microalgae structure on the NIR reflectance readings (Murphy *et al.* 2005). However, the documented lack of relationship between the NIR reflectance and the benthic Chl-*a* (Murphy *et al.* 2004) was obtained in a mudflat covered by filamentous algae, whereas those studies showing a positive relationship between Chl-*a* and NIR reflectance (R^2 values ranging from 0.21-0.58) have been conducted in areas dominated by diatomaceous biofilms (Pomeroy and Butler 2005, van der Wal and Herman 2007, this study). Therefore, future remote sensing studies assessing values of benthic Chl-*a* at intertidal systems need to take into account the heterogeneous nature of the MPB assemblages as their different light scattering properties may preclude the use of indices constructed from NIR bands.

The digital camera worked well for acquiring images over the sediment surface under a range of light conditions. The use of this system has several advantages over the conventional infrared slide film or multispectral digital cameras. The most valuable aspect of the digital system used, aside from its affordability (~ C$ 1,500), is the ability to quickly save and store as many images as the researcher needs and to process them easily and free of charge, if using free data image software (e.g. ImageJ). Also, the platform that fixes the two filters to the camera proved useful because it allowed two spectral images of the same subject to be obtained simultaneously. The results showed here proved that the R filter did not provide useful information in MPB biomass assessment. Future studies should consider the coupled use of NIR and yellow filters to simultaneously obtain information on benthic Chl-*a* content and sediment water content. The above rationale comes from the consideration of the significant relationship between the specular reflection in the yellow region of the spectrum (500 - 530 nm) and the water content at the uppermost sediment layers (0 - 600 µm) (Coelho *et al.* 2009). Given the importance of the sediment water content measurements for estimating benthic Chl-*a* through NIR reflectance (Table 2.2), simultaneous remote sensing measurements of NIR and yellow reflectance on the sediment may notably improve the estimates of intertidal MPB biomass.

In summary, the results from the use of a modified digital CIR camera showed that the system is useful to make rapid assessments of benthic Chl-*a* content. If researchers are investigating small variations in MPB biomass over the intertidal they need to rely on more accurate but expensive portable field spectrometry devices (Murphy *et al.* 2008). However, the digital NIR images coupled with measurements of sediment water content represent a useful alternative for quickly delivering qualitative information to assess relative diatomaceous biofilm abundance at intertidal habitats. By using the camera system developed here, researchers would be able to rank sites in accordance to MPB biomass. The technique could be further improved by experimenting over gradients of Chl-*a* content and water content to precisely document the Chl-*a* threshold over which differences in NIR reflectance could be detected.

2.5.2. Spatial distribution of MPB biomass across the Roberts Bank's emerged intertidal

In contrast to the shorebird's invertebrate prey, the MPB has spectral characteristics that allow researchers to map MPB biofilm spatial distribution over the entire intertidal habitat by using remote sensing tools. Roberts Bank was the first site where biofilm feeding by western sandpiper was documented (Elner *et al.* 2005, Kuwae *et al.* 2008, Mathot *et al.* 2010, Beninger *et al.* 2011). However, up until to now, there was no detailed information about the MPB biofilm spatial distribution on Roberts Bank. A previous remote sensing study based on hyperspectral images mapped the intertidal vegetation communities on the variable exposed sediment at Roberts Bank, but did not consider MPB biofilm (Costa *et al.* 2007). The latter study was spatially restricted towards the northwestern part of Roberts Bank and focused on classifying marsh vegetation. Thus, the variations in the spectral Chl-*a* signature from the "non-vegetated" intertidal at Roberts Bank were overlooked.

Although the moderate relationship between the airborne NIR reflectance and the ground-truthed Chl-*a* content prevented predictions of MPB biomass in terms of $\mu g\ g^{-1}{}_{DM}$, both maps consistently showed the heterogeneous distribution of the benthic Chl-*a* within the intertidal. At the least, low, medium and high Chl-*a* contents can be distinguished. Such qualitative assessment is of practical value because it can reduce the amount of field work when looking for overall biofilm distribution. The visual interpretation of the maps illustrate that the rich MPB biofilm area comprises less than 30% of the whole intertidal at Roberts Bank, mostly restricted to the upper intertidal (0 - 750 m from shore, tide height range: 3.0 - 3.5 m). Taking into account the high values of MPB biomass and sediment water content at the upper intertidal, I speculate that this is the more likely scenario for biofilm feeding by shorebirds. Future detailed field studies to determine the effects of the distribution of MPB biofilm on sandpiper foraging ecology (behavior and distribution of foraging sandpipers) can be mainly focused on the upper intertidal.

The MPB distribution in the intertidal created a range of Chl-*a* content and NIR reflectances that were evident in both the ground-truth digital images and the aerial CIR photographs. There was high correspondence between ground-truth digital spectral bands and those taken by airborne CIR photographs. Further, the NIR reflectance obtained from ground and aerial survey showed almost the same moderate relationship with the ground-truth Chl-*a* content data. Therefore, the broad spatial variations in Chl-*a* content detected through walking and photographing random point samples in the intertidal were consistently documented in the maps of MPB biomass spatial distribution acquired through aerial CIR photography.

The main disadvantage of the information obtained from the aerial CIR was the lack of necessary resolution to discriminate between macroalgae and MPB Chl-*a* content. Previous studies (Méléder *et al.* 2003, Combe *et al.* 2005, Murphy *et al.* 2008) have experimented, combining laboratory reflectance spectra-biomass measurements with field work conducted with more sophisticated remote sensing tools (i.e., Digital Airborne Imaging Spectrometer, Compact Airborne Spectrographic Imager). Such approaches have allowed the developing of more specific indices capable of discriminating between intertidal producers (Combe *et al.* 2005, Kazemipour *et al.* 2012). Data collected in this study were limited by the broad spectral sensitivity of the film used and the spectral transmission of the filter. Hence, the usefulness and application of the aerial CIR imagery used in this study is dependent on ground reconnaissance surveys and the acquisition of appropriate ground-truth data. Although this limitation necessarily involves some laborious ground sampling, the further application of airborne CIR remote sensing has the potential to provide detailed and comprehensive qualitative estimates of MPB biomass distribution over the whole intertidal area. A useful alternative explored in the present study was to draw inferences based on the combination of maps derived from NIR reflectance and NDVI index. Images on the NIR could be affected by sediment wetness (Huete 1988) while the NDVI is more sensitive to changes in the green vegetation (Tucker 1979). Thus, the contrast between both maps may help in detecting discrepancies in estimates of benthic Chl-*a* contents possibly caused by the presence of macroalgae or eelgrass. The case is supported by the detection only in the NDVI map of a large orange patch in the northwestern section of Brunswick Point. The Chl-*a* signature detected in the NDVI map is consistent with the observation of Cyperaceae patches (*Carex* spp., sedge) within this region as recorded during ground reconnaissance (this study) and documented previously in a remote sensing study (Costa *et al.* 2007). Also, the contrast between both maps helped identify the “noise” caused by the specular reflection of sunlight into the camera on some areas of the intertidal.

Currently, understanding of biofilm occurrence throughout the intertidal banks of the Fraser River delta system is lacking. Qualitative assessment of benthic MPB biomass distribution by using remote sensing on a regional scale has potential application in this internationally important wetland. Exploring the potential isolation of regionally important estuarine biofilm areas within the Fraser River delta system is of primary concern for local management plans because it could reveal the vulnerability of these sites to threats such as coastal development or high-impact erosion events (e.g. extreme storms) (Hill *et al.* 2012). Other issue not yet explored, concerns the dynamic nature of estuarine biofilms. The option to include a temporal component does exist if multiple sets of images are acquired over time. The time frame for airborne CIR imagery acquisition will depend on the temporal scale of the specific research question (seasonally or annually). Any of the two approaches (spatial and temporal) can allow for monitoring changes in MPB biomass and its potential influence on shorebird distribution. Such assessment is particularly important considering the biogeographic significance of the Fraser River delta system in the conservation of western sandpiper (Butler *et al.* 1987, Butler 1994, Butler *et al.* 2002).

2.6. Acknowledgements

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2.8. Tables

Table 2.1. ***Rankings of six linear models assessing benthic Chl-a content (ln+1 transformed [µg $g^{-1}{}_{DM}$]) through ground-based digital remote sensing at Roberts Bank's intertidal, British Columbia in April and May, 2008. Variables tested included Red relative reflectance (R), Near-infrared relative reflectance (NIR), Difference Vegetation Index (DVI), Ratio Vegetation Index (RVI), Normalized Difference Vegetation Index (NDVI), and Soil Adjusted Vegetation Index (SAVI). Models were ranked by Akaike's information criterion corrected for small sample size (AIC_c for the best model was 58.92), where K is the number of parameters in the model, ΔAIC_c is the difference in AIC_c between the top-ranked model and the model in question, ω_i is the Akaike weight, which reflects the relative likelihood of the model, and R^2 is a measure of overall model fit.***

Model term	K	ΔAIC_c	ω_i	R^2
NIR	3	0.00	0.99	0.34
DVI	4	23.04	1×10^{-5}	0.18
RVI	4	23.93	1×10^{-5}	0.17
SAVI	4	24.24	1×10^{-5}	0.17
NDVI	4	24.27	1×10^{-5}	0.17
R	3	39.13	3×10^{-9}	<0.01

Table 2.2. Model selection results from the general linear model assessment of variation in Chl-a content by considering Near-infrared reflectance (NIR) and sediment water content (WC). Models are ranked by Akaike's information criterion corrected for small sample size (AIC_c for the best model was 5.50), where K is the number of parameters in the model, ΔAIC_c is the difference in AIC_c between the top-ranked model and the model in question, ω_i is the Akaike weight, which reflects the relative likelihood of the model, and adjusted R^2 as a measure of overall model fit. Model-averaged parameters (β) and their 95% confidence intervals (95% CI) were calculated across models with some level of evidence (models 2-4).

	Model terms							
Model ID	**Intercept**	**NIR**	**WC**	**NIR*WC**	***K***	**ΔAIC_c**	**ω_i**	**R^2_{adj}**
3	x	x	x		4	0.00	0.49	0.57
4	x	x	x	x	5	0.18	0.44	0.58
2	x		x		3	3.97	0.07	0.55
1	x	x			3	40.49	$8x10^{-9}$	0.34
5	x				2	77.82	$6x10^{-18}$	0.00
RVI[a]	1.00	1.00	1.00	0.48				
β	0.341	0.098	0.040	-0.001				
95%CI inf.	-2.080	-0.046	-0.017	-0.003				
95%CI sup.	2.761	0.242	0.097	0.001				

[a] Relative variable importance

2.9. Figures

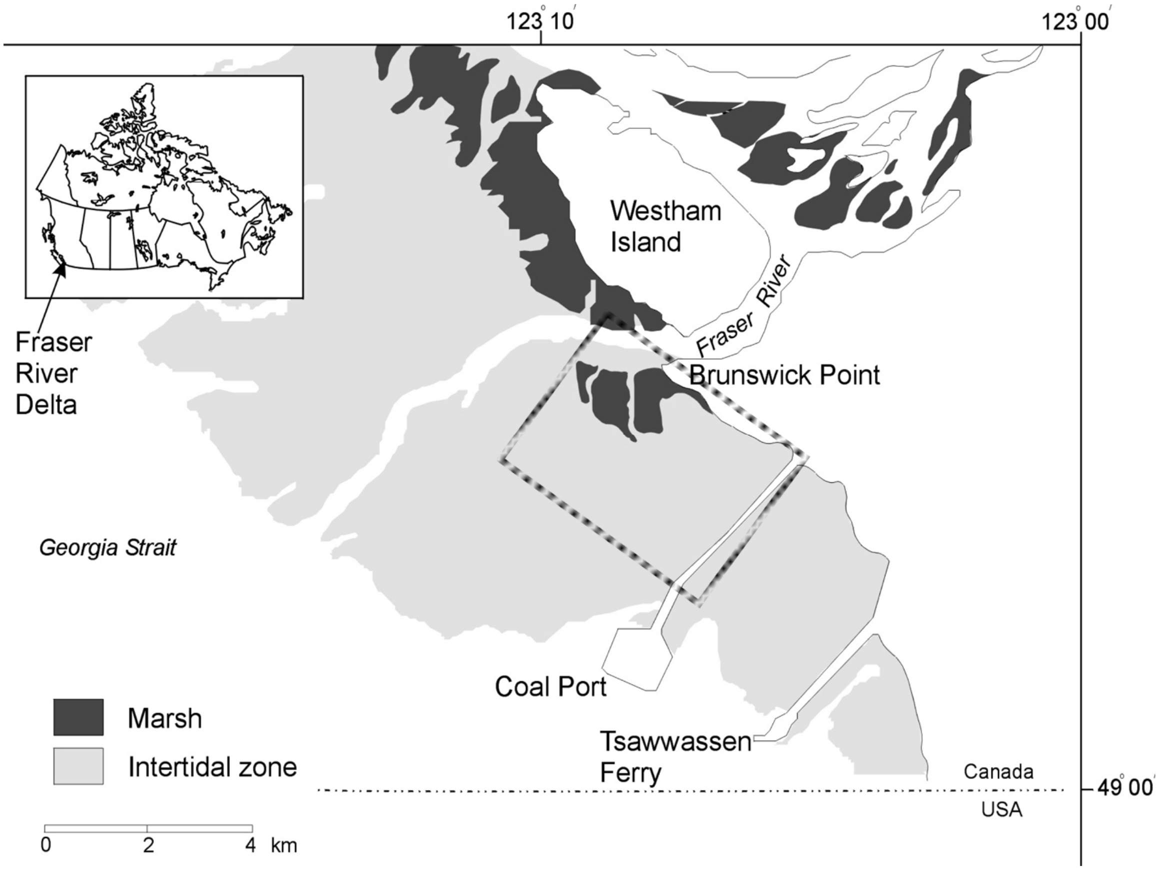


Figure 2.1. Map of Roberts Bank, British Columbia, showing the study site (rectangle) where ground-truth sampling took place.

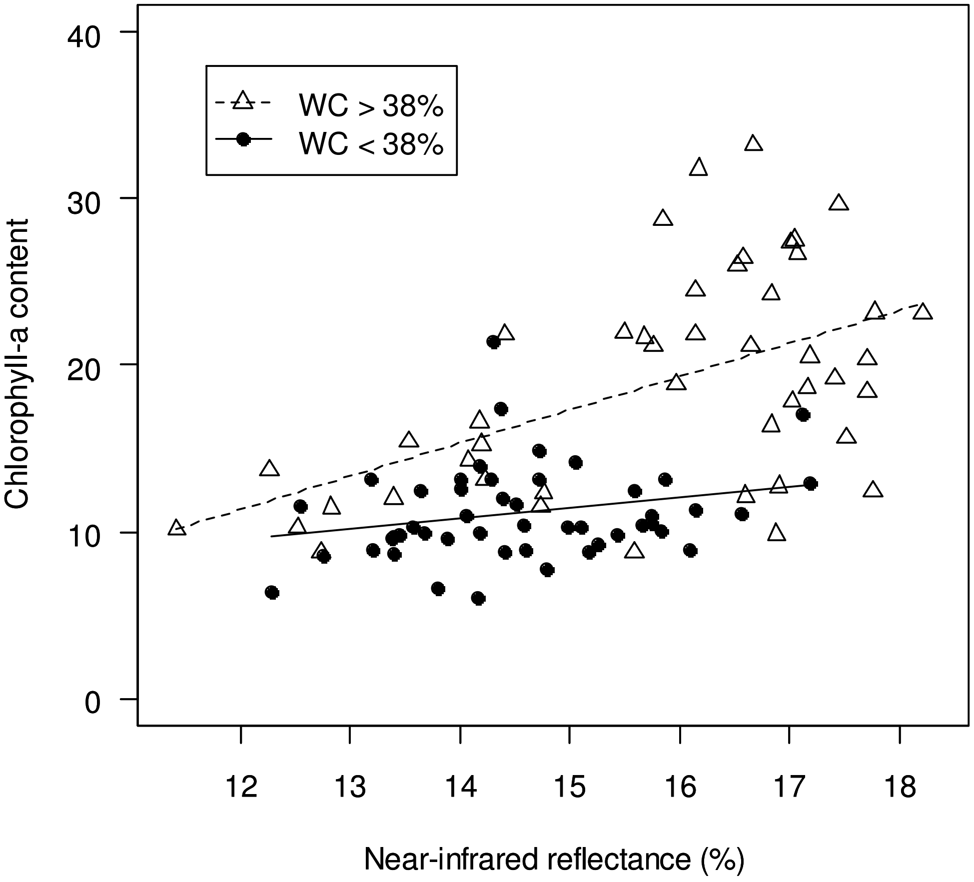
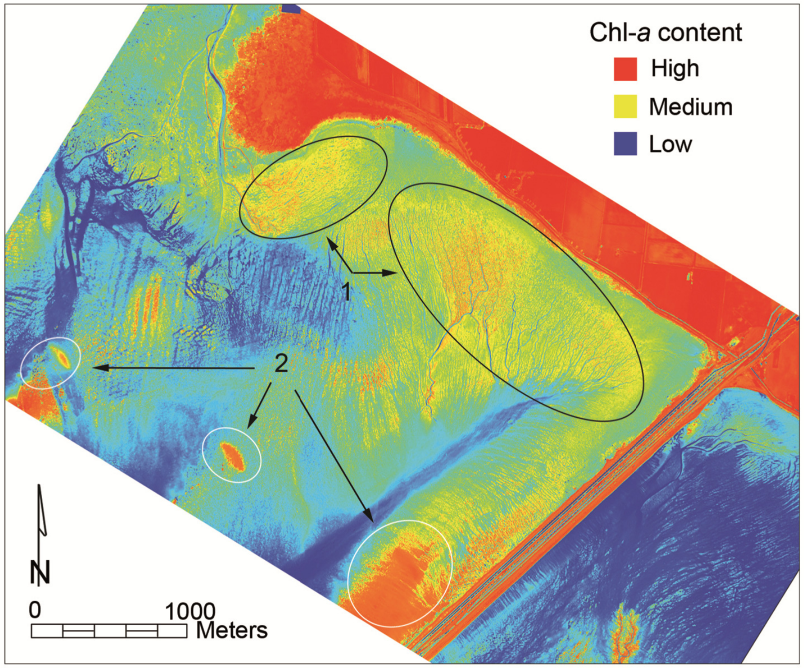


Figure 2.2. ***Benthic chlorophyll-a content (µg g^{-1} $_{DM}$) in relation to Near-infrared reflectance (%) of sediment samples with water content above (open triangles and dashed line) or below (solid circles and line) the median value (WC_{median} = 38%). Sediment samples were taken during the western sandpiper (Calidris mauri) spring migration at Roberts Bank, British Columbia, Canada.***

A)

$\ln(\text{Chl-}a\text{ content} + 1) = 1.31 + 0.02 \cdot \text{NIR}, R^2 = 0.47, P = 0.005$



B)

$\ln(\text{Chl-}a\text{ content} + 1) = 0.97 + 0.03 \cdot \text{NDVI}, R^2 = 0.49, P = 0.042$

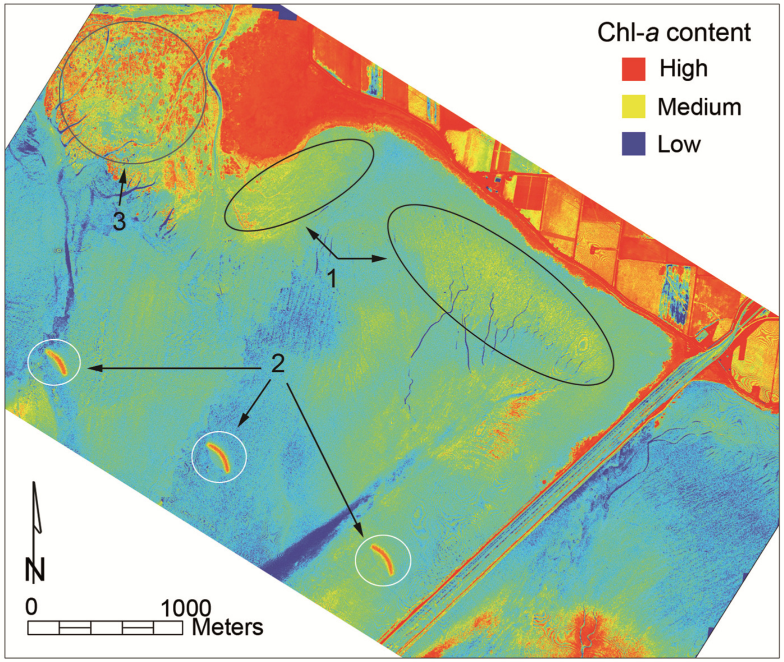


Figure 2.3. **Relative benthic micorphytobenthic (MPB) biomass assessment at Roberts Bank using aerial colour infrared images to estimate chlorophyll-a (Chl-a) content. Images were obtained on April 25th, 2008. Regression equations were constructed using ground-truth sampling data for Chl-a content (µg $g^{-1}{}_{DM}$) and the aerial reflectance readings at these points (N = 15). (A) Estimation of Chl-a content from near-infrared (NIR) reflectance. (B) Estimation of Chl-a content from normalized difference vegetation index (NDVI). Ellipses 1: largest patches of benthic MPB biomass at the upper intertidal. Ellipses 2: high reflectance patches caused by specular reflection of sunlight over the wet sediment. Ellipse 3: patches with high Chl-a content probably caused by the mix of MPB and Cyperaceae patches (Carex spp., sedge).**

3. Following the tide: Can biofilm distribution explain habitat segregation of two sympatric sandpipers (*Calidris mauri* and *Calidris alpina*) during the ebb tide?

3.1. Abstract

Inter-specific competition may be expected between western sandpiper (*Calidris mauri*) and dunlin (*Calidris alpina*) converging simultaneously in the upper intertidal at Roberts Bank during the onset of the ebbing tide. Of these species, western sandpipers show the greatest modification of tongue and bill morphology for biofilm feeding. I hypothesized that these species will segregate foraging niches at the upper intertidal based on their different aptitudes for biofilm grazing. I determined western sandpiper and dunlin "tide-following" behaviour and distribution at the upper intertidal during the ebb tide. In addition, I assessed sediment grain size distribution and food availability (microphytobenthic (MPB) biomass and invertebrate abundance) at the initial and final section of the upper intertidal (150-350 and 450-650 m from shore, respectively). Foraging dunlins closely followed the ebbing tide, exploiting the upper intertidal only briefly. In contrast, western sandpipers did not follow the tide, spending much more time in the initial section of upper intertidal. Microphytobenthic biomass and water content were highest in the initial section of the upper intertidal, indicating greater biofilm availability for shorebirds in the first 350 m from shore. Invertebrate density did not differ between sections of the upper intertidal. Overall, western sandpiper behaviour and distribution matched more closely the MPB biofilm availability than invertebrate availability. The findings suggest that western sandpipers minimize potential inter-specific competition for food based on their greatest propensity for biofilm feeding.

Keywords: biofilm feeding, food availability, segregation, western sandpiper, dunlin, tide following

3.2. Introduction

During migration, huge flocks of many shorebird species converge simultaneously on a wide variety of wetlands (Hicklin 1987, Engilis *et al.* 1998, Andrei *et al.* 2006, Ge *et al.* 2006, Rodrigues 2009). Under such circumstances, competitive processes may be expected to emerge in a group where diet, foraging methods and feeding habitats usually overlap on non-breeding sites (Tsipoura and Burger 1999, Andrei *et al.* 2009). Competitive processes on non-breeding habitats have been proposed to have a pivotal role in shorebird population regulation, survival and conservation (Baker and Baker 1973). Therefore, understanding the processes underlying patterns of niche partitioning with respect to food, feeding methods, timing and spacing can contribute to conservation in shorebirds (Goss-Custard 1980, Lifjeld 1984, Mathot *et al.* 2007).

Tides have been described as one of the most important factors affecting shorebird distribution while foraging (Connors *et al.* 1981, Granadeiro *et al.* 2006). During high tide, the intertidal flats are inundated and shorebirds are restricted to roosting sites or inland areas. As the tide recedes intertidal habitats become increasingly available to foraging shorebirds. The intensity of competition between shorebirds is necessarily regulated by the tidal cycle. Highest foraging interference between species occurs at the onset of the receding tide when available feeding space is limited to the uppermost intertidal areas (Recher 1966). At this time, niche partitioning strategies in similar ecomorphological species are expected to be most pronounced. Partitioning strategies between species may manifest through changes in patterns of habitat use in a way that allow individuals to maximize their intake rate under an interference competition scenario (Senner *et al.* 1989, Rosa *et al.* 2007).

Every year, for approximately 15 days, over two million sandpipers coexist in Roberts Bank, British Columbia, during their northbound migration (Butler 1994). The mixed-species sandpiper foraging flocks at this internationally important estuarine mudflat are mostly western sandpiper (*Calidris mauri*) and dunlin (*Calidris alpina*) (Butler and Vermeer 1994). Both species are capable of tactile and visual feeding techniques (probing and pecking, respectively) and have been reported foraging close to the tide line during ebbing and rising tides (Brennan *et al.* 1985, Colwell and Landrum 1993, Butler *et al.* 2002). The species exhibit moderate to high degree overlap on invertebrate consumption (both prey composition and size) when they inhabit the same wintering or stop-over sites (Couch 1966, Senner *et al.* 1989). Despite many similarities in their foraging behaviour, western sandpiper and dunlin exhibit a differential capacity to feed on biofilm (Elner *et al.* 2005, Mathot *et al.* 2010, Kuwae *et al.* 2012).

Biofilm represents an important food source for intertidal organisms (Decho 1990, Decho and Moriarty 1993, Hoskins *et al.* 2003, Abreu *et al.* 2007), including small-bodied sandpipers such as western sandpiper and dunlin (Kuwae *et al.* 2012). The intertidal biofilm is a matrix-enclosed community of microphytobenthos (MPB), bacteria, and organic detritus (Kuwae 2002) glued together and cemented to the sediment surface by copious amounts of extracellular polymeric substances (EPS) secreted by the microorganisms living within the biofilm (Stal 2003, Underwood and Paterson 2003). In intertidal habitats, biofilm abundance as measured by MPB biomass increases with the proportion of mud in the sediment (Underwood and Kromkamp 1999, Watermann *et al.* 1999). On a diel pattern, biofilm abundance (both MPB biomass and secreted EPS) builds up in the sediment surface as exposure time increases (Herlory *et al.* 2004, Hanlon *et al.* 2006) as result of vertical migration of motile diatoms positioning themselves in the photic zone (Mitbavkar and Anil 2004, Underwood *et al.* 2005).

A differential capacity for biofilm feeding between western sandpiper and dunlin was initially suggested by differences observed in their feeding apparatus (Elner *et al.* 2005). The higher density and length of keratinized lateral spines found on the western sandpiper’s tongue as compared to dunlin appeared indicative of a greater reliance of western sandpiper on biofilm (Elner *et al.* 2005). Investigation of the diets of both species through stomach content analysis (Mathot *et al.* 2010) and stable isotope assessments (Kuwae *et al.* 2012) confirmed that western sandpiper extensively use biofilm as food source whereas dunlin using the same habitat make comparatively less use of biofilm.

The differential biofilm grazing capability of western sandpiper and dunlin (Elner *et al.* 2005, Mathot *et al.* 2010, Kuwae *et al.* 2012) has opened fresh perspectives on their biology and may be relevant in exploring segregation patterns in two species which share foraging habitat and diet (Couch 1966, Senner *et al.* 1989). The way biofilm and benthic invertebrates distribute within the upper section of the intertidal is critical to understand how western sandpiper and dunlin coexist during the initial phase of the ebbing tide. In this paper, I examined niche partitioning strategies between western sandpiper and dunlin during the time the ebbing tide exposed the upper intertidal habitat at Roberts Bank (0-750 m from shore). I hypothesized that these species will segregate their foraging niches during the onset of the ebbing tide based on their different aptitudes for biofilm grazing (Elner *et al.* 2005, Kuwae *et al.* 2012). I predicted that if biofilm feeding is more prevalent in western sandpiper than in dunlin then western sandpiper will (*i*) feed farther from the tide line than dunlin as biofilm abundance and accessibility increases in exposed sediments, (*ii*) preferentially feed in the areas with highest fine sediment and water content as such conditions promotes biofilm availability (both MPB biomass and biofilm handling), and (*iii*) spend more time than dunlin feeding in the upper intertidal as this region holds the highest biofilm abundance in Roberts Bank's mudflat as measured by MPB biomass estimates (Chapter 2). In order to test the hypothesis and predictions, I determined 1) the "tide following" behaviour and distribution of western sandpiper and dunlin in the upper intertidal zone at Robert Banks during the time it took the area to become exposed. In addition, I assessed 2) the physical characteristics (sediment grain size and water content), and 3) food availability (both MPB biofilm and invertebrates) within the upper intertidal zone at Robert Banks.

3.3. Methods

3.3.1. Study site

Roberts Bank is an extensive estuarine habitat (27 km^2) contributing to the intertidal zone of the Fraser River Delta (49˚03' N, 123˚09' W; Fig. 3.1). Roberts Bank intertidal flats represent an important stop-over habitat for migratory shorebirds during the northward migration (from April 20th through May 10th), hosting approximately two-million shorebirds with major contributions from western sandpiper and dunlin (Butler 1994). The tides are semi-diurnal during spring (tidal range 0.0 - 3.8 m) and sediment is primarily fine sand with the proportion of sand increasing towards the low water mark (Eisma 1998, Zharikov *et al.* 2009). An extensive dendritic system of sinuous channels occupies approximately 40% of the upper intertidal and extends down to the mid-intertidal. The tidal flats at Roberts Bank also host the Deltaport (coal and container terminals) and the Tsawwassen ferry terminals (Fig. 3.1). Both sites located at the south edge of the bank are connected to the mainland by causeways. The study site was located at Brunswick Point, a marsh promontory on the western edge of Roberts Bank. Specifically, the study took place in the upper intertidal, the area comprising the first 750 m from shore and extending from the marsh shore at Brunswick Point to the northern zone of the dendritic channel system.

3.3.2. Data collection

Study design

Two spatial designs were used for this study (Fig. 3.1). First, I used a 500 m transect perpendicular to the shore to determine western sandpiper and dunlin “tide following” behaviour and distribution along the upper intertidal during the ebbing tide. Also, the transect was used to assess sediment grain size variations within the upper intertidal. The transect started 150 m from shore to ensure birds were feeding by the time such area became uncovered and avoid counting aggregations of roosting birds waiting for the tide to recede. The transect was marked every 50 m with plastic poles (length: 150 cm, diameter: 2.5 cm) pushed into the sediment leaving approximately 1 m above the surface. Poles were wrapped up with orange tape to enhance visibility from the shore. Another two smaller poles (50 cm above surface) were located at 5 m of the primary pole (East and West). The small poles were used as a visual aid to delineate a 5 m radius circular area where shorebird counts were conducted. The second spatial design used in this study was envisioned to assess food availability (MPB biofilm and benthic invertebrates) and shorebird habitat use after the exposure period. The upper intertidal at Brunswick Point was divided into two spatial sectors parallel to the shore. These sections were the initial section of the upper intertidal, which comprised the area between 150 and 350 m from shore, and the final section of the upper intertidal involving the area between 450 and 650 m from shore. A gap of 100 m was established between the two sections to preclude overlapping effects when examining a rather continuous habitat.

Sandpiper counts

Fifteen bird counts were conducted during two northward migration periods: April 24 - May 6 2008 (N = 10), and April 26 - April 30 2009 (N = 5). Counts were carried out using a 40x spotting scope from a vantage point at Brunswick Point marsh, approximately 250 meters from and perpendicular to the centre of the transect. Counts were done during the morning receding tide beginning between 05:26 and 09:09 h., depending on the daily tide conditions and subject to sufficient light to allow shorebird identification. Three of these counts were excluded from the analysis because either the count started too late (April 26 2008, count started when tide line was at 300 m from shore) or too few birds were present in the transect (average number of 2.3 and 2.4 birds per count in May 6 2008 and Apr. 26 2009, respectively). The resulting sample size was 12 counts with a minimum average of 25 birds per count.

Each sampling day a count sequence started at the moment when the tide edge passed the marker nearest to shore (150 m from shore) and lasted 5 min. after it passed the last marker in the upper intertidal (650 m from shore). Three components were recorded for each count sequence (N = 183): (1) time at the beginning of the count sequence, (2) location of the tide edge with respect to the markers (time was noted once a marker was uncovered), and (3) estimated species abundance at every uncovered marker. Birds were counted if they were within a 5 m radius of the primary pole and estimated abundances are expressed as bird density (number of shorebirds counted in a 5 m radius circumference). Shorebirds roosting on the transect were not included in the counts, although their presence on the transect occurred at a low frequency (16 of 183 total count sequences). Short time intervals counts were chosen to allow a high resolution “picture” of the shorebirds using the area in relation to the time involving the exposure of the upper intertidal. On average, the number of shorebirds at each uncovered marker was recorded once every three minutes (range: 1 to 6 min.). To standardize daily counts, observations were pooled every 6 min. time interval. I used average shorebird number when more than a count sequence was performed in the 6 min. interval.

Once the upper intertidal was fully exposed we carried out bird counts in four 100 m x 100 m plots (two plots per upper intertidal section) to assess total habitat use for each sandpiper species. Such counts were conducted from Apr. 27 through Apr. 30, 2009 (N = 4). Birds were counted at 15 min intervals during a 105 min counting session each day. Birds resting or sleeping were excluded from counts.

Sediment sampling

A 26 mm internal diameter syringe previously modified (apex removed and edges sharpened) was used to sample sediment for assessments of grain size, water content, and food availability. The sampling core was identical to that used in previous research on western sandpiper on this and other studied sites (Sutherland *et al.* 2000, Pomeroy and Butler 2005, Pomeroy 2006, Mathot *et al.* 2007).

Sediment samples for grain size analysis were collected along the 500 m transect at the end of the 2009 migration period (May 8th). Three sediment cores (30 mm deep) were taken at random points at less than 1 m from each marker (N = 46). Sampling for food assessment was conducted in the two spatial sectors (initial and final upper intertidal), but protocols for biofilm and invertebrates differed in sampling day, time and sediment depth.

Eight random points were sampled on April 28 and 29 2008 at four separate times over the tidal emersion period (08:00 to 17:00 h.) for biofilm availability. Biofilm availability here was estimated through the determination of MPB biomass (biofilm abundance) and sediment water content (biofilm handling). Chlorophyll-*a* (Chl-*a*) is a known estimator for MPB biomass in intertidal sediments (Underwood and Smith 1998, Pomeroy and Butler 2005a, Hanlon *et al.* 2006). Therefore, I used Chl-*a* content as an index of biofilm availability. Samples were taken immediately (08:00 h.), 1 hour (09:00 h.) and 3 hours (11:00 h.) after the sediment became exposed. These sampling times represent the full time range western sandpiper can devoted to feed in the upper intertidal at Brunswick Point during the low tide period (A. Jiménez pers. obs.). The last sample was taken before immersion (17:00 h.). The modified core was pushed into the sediment and the plunger lifted until approximately 30 mm of sediment was expressed in the barrel. Care was taken to prevent contact between the plunger and the sediment surface. The plunger was removed from the syringe, placed in the opposite end and pushed to extrude the sediment. Biofilm samples were taken by slicing the top 2 mm of the mud surface extracted with the core. Samples were placed in properly labelled plastic Ziploc, stored in a cooler with ice and frozen at -20℃ within 2 h. after collecting.

Sediment samples for benthic invertebrate assessment were collected between April 30 and May 2, 2008. Eleven replicate samples were taken in each spatial sector once the shorebird counting had concluded (approximately 1.5 h. after sediment exposure). Samples for prey assessment were obtained using the same protocol as for biofilm, but in this case the samples were obtained by slicing the first 10 mm of the mud surface. I collected only the first 10 mm of sediment for invertebrate assessment for two reasons. First, focal observations on western sandpiper feeding at Robert Banks have revealed that epifaunal foraging encompass most of the feeding repertoire of males and females during the northward migration (96 % and 84 %, respectively, Mathot and Elner 2004). Secondly, invertebrates within this sediment layer make the highest contribution in number and biomass to the total invertebrate density at northern western sandpiper non-breeding sites (Mathot *et al.* 2007). As with biofilm samples, invertebrates samples were placed in labelled Ziploc bags and stored in a cooler until transportation to the laboratory. All samples were frozen at -20℃ within 2 h. after collecting.

3.3.3. Laboratory analysis

Sediment samples for grain size analysis were weighed wet and dry (oven dried for 48 h at 70℃) to the nearest 0.01 g. Samples were soaked for 24 hours in a solution of Sodium hexametaphosphate (6.2 g/l) to dissolve agglomerated particles. Sediment grain size was analyzed using a wet sieving technique. Grain size was divided into five categories, including coarse sand (≥ 0.500 mm), medium sand (0.250 - 0.500 mm), fine sand (0.125 – 0.250 mm), very find sand (0.063 – 0.125 mm), and mud (≤ 0.063 mm). The samples were rinsed with tap water for 10 minutes through every sieve, starting with the largest mesh width and preceding to the smallest one. The resulting four size sediment fractions were dried again and masses were recorded. The mud fraction for each sample was determined as the difference between the initial (before sieving) and final (after sieving) sediment dry weight. Sediment grain size composition represented the contribution in biomass of each sediment fraction to the total sample biomass and was expressed as a percentage.

Frozen samples for Chl-*a* and water content assessment were weighed to the nearest 0.01 g, thawed in the dark, transferred to scintillation vials with 10 ml of 90 % acetone solution, thoroughly shaken for 2 min in a vortex and placed into a dark box at -4°C for 24 h for extraction. The amount of Chl-*a* in the supernatant was measured spectrophotometrically using a Genesys 10uv spectrophotomer. Absorbance at 665 and 750 nm were measured; samples were acidified with two drops of 0.1 N HCL, mixed, left for 1 min and absorbance was measured again at the same wavelengths. Concentration of corrected Chl-*a* was calculated using Lorenzen's method (Lorenzen 1967). Then, samples were oven dried for 48 h at 70°C and weighted again to obtain the dry weight. Sediment water content was calculated as the difference between wet and dry weight and expressed as percentage. Chlorophyll-*a* content was expressed as weight-normalized values ($\mu g\ g^{-1}{}_{\text{dry sediment (DM)}}$).

Benthic samples for macro-faunal invertebrates were thawed and then sieved through 0.5 mm mesh using filtered water. The retained material was preserved in labelled vials with 85 % ethanol until they were sorted and counted. Although both sandpipers may be using meiofaunal invertebrates that would have passed the 0.5 mm sieve (Sutherland *et al.* 2000), only macrofaunal prey were measured in this study. Using a stereomicroscope (Leica Wild M8) at 60x, I sorted, identified and counted all invertebrates of taxonomic groups documented in western sandpiper and dunlin diet (Wolf 2001, Andrei *et al.* 2009). Invertebrate density is offered as number of individuals per sampling core.

3.3.4. Data Analysis

Tide following index and shorebird distribution analyses

A tide following index (TFI) was calculated to compare the intensity of tide following foraging behavior between sandpiper species. The TFI was defined as the proportion of birds of a given species within 0 to 100 m of the ebbing tide edge. The TFI was calculated by dividing the number of birds within two markers of the tide edge by the total number of birds of that species present on the transect at each count interval. As a proportion, TFI values varied between 0 and 1 which indicates tide line avoidance or strict tide following, respectively.

Because the research was constrained to a narrow transect embedded into a 118 ha study site the measured shorebird response to the tide was also influenced by the amount of habitat available at a given time. The upper intertidal at Brunswick Point becomes exposed in a widening pattern, resembling a funnel shape. Most of the available habitat for shorebirds when the ebbing tide started was constrained to the vicinity of the transect (the narrow part of the funnel). As the ebbing tide progressed, wider sections of the habitat become exposed simultaneously. Decreasing bird densities and TFI values were expected to occur as consequence of the increasing mudflat availability. Therefore, decreasing numbers of sandpipers in the transect as the ebbing tide progressed was not necessarily the result of birds avoiding the tide edge but rather due to birds spreading out into the broader upper intertidal as more became exposed. To better understand the tide following behavior of the studied species, I assumed that TFI measured in the transect will proportionally decrease with the increase in the available foraging area during the ebbing tide. An expected TFI was devised for each day based on the amount of habitat available when each marker became exposed. The expected TFI represents the behaviour for an ideal strict tide follower which experience density declines proportionally to the amount of habitat available during the ebbing tide (Table 3.1). The expected TFI was created by determining the proportion of mudflat area being exposed when each marker became emerged (Table 3.1).

To estimate the proportion of mudflat exposed SOCET SET photogrammetry software was used to create customized contours on a digital aerial photograph map of Roberts Bank. Rectified aerial images (orthophotos) were overlaid and viewed in stereo display and polygons were traced that “contained” area exposed on mudflat as tide moved from an upper to a lower marker. Such an approach, which relied heavily on knowledge of tide patterns of the study area, provided a coarse but practical estimate of the area exposed as the tide line moved every 50 m down the transect. The method was chosen because the changes in elevation on Roberts Bank are so slight that an accurate contour map could not be created using an automated contour mapping program (Hill *et al.* 2012).

To examine the possible departures from an ideal tide follower, I calculated the difference between the expected and the observed TFI for a given species at a given time interval count (N = 183). The difference result was named TFI deviance and it offers a comparable magnitude on how shorebirds behave in relation to the water line during the ebbing tide. The TFI deviance varies between -1 and 1. Values close to zero indicate counts where birds behaved similarly to what was expected for a strict tide follower. Negative values are indicative of counts where birds were following the tide at higher proportion than expected, whereas positive values signal counts where a high proportion of birds were at more than 100 m from the tide edge.

Statistical analysis

Differences in TFI deviance between species at each marker were assessed using a paired Student t-test. Sediment grain size composition (only the mud fraction), Chl-*a* content, sediment water content, and invertebrate density were compared between the initial and final sections of the upper intertidal. Sediment grain size and invertebrate density variations between the upper intertidal sections were assessed using a paired Student t-test. Temporal and spatial variation in Chl-*a* and water content were tested using a two-way analysis of variance (ANOVA). *A posteriori* Tukey's pair-wise comparison test was also performed to elucidate possible differences between levels of a factor. When necessary, transformations were used to achieve the assumptions of homogeneity and normality (ln (Chl-*a* + 1)).

All computations and statistical tests were performed using the freely available statistical software R 2.10.0 (R Development Core Team 2009). If not otherwise stated, I present means with standard deviation (± SD). All tests were two-tailed and the level of significance was $P < 0.05$.

3.4. Results

3.4.1. *Tide following index and spatial distribution patterns of western sandpiper and dunlin*

The initial observed TFI values for both species started close to the expected TFI, but as tide receded neither of the two sandpiper species coincided exactly with an ideal tide follower (Fig. 3.2). On average, the TFI deviance in dunlin had lower magnitude than that detected in western sandpiper (0.25 ± 0.27 and 0.46 ± 0.27, respectively; Paired t-test: $t_{182} = 8.603$, $P < 0.001$), indicating that for most of the observations the proportion of dunlin close to the water line was higher than the proportion of western sandpiper at the same spatio-temporal point.

The relationship between the expected and observed TFI in dunlin was stable over the tide ebbing period, resembling a close to linear trend, as envisioned for an ideal tide follower whose numbers on the transect depends linearly on the amount of habitat being exposed (Fig. 3.2). Dunlin's major departures from the expected TFI occurred on the final section of the transect (from 450 to 600 m), reaching a maximum TFI deviance at 500 m from the shore (Table 3.2). In contrast, the observed trend in western sandpiper's TFI showed a substantial decline when the tide edge exposed the 250 m marker and reached the highest difference from the expected TFI at 450 m from shore (Table 3.2). The greatest difference in the TFI deviance between species emerged at the 400 m marker when western sandpiper showed a value 3.3 times higher than the one recorded for dunlin (Table 3.2).

The tide following behavior of western sandpiper and dunlin drove their distribution patterns during the ebbing tide on the upper intertidal. The general shorebird distribution pattern (Fig. 3.3) can be described as follows: 1) western sandpipers were more constrained to the initial section of the upper intertidal for most of the observation period and, in general, spent more time in the area than dunlin; 2) western sandpiper densities in the initial section of the upper intertidal were more evenly distributed in the sampled time and the highest abundance occurred distant from the tide line, such a pattern was notable once the tide line has passed the 350 m marker; 3) highest densities of dunlin occurred close to the tide line and birds ranged longer distances from shore than western sandpipers; and, 4) dunlin were scarce or absent from most of the initial section of the upper intertidal after the site had been exposed for more than 50 minutes, on average. Nine of the 12 sampled days (75 %) fall within the described distribution pattern. For the remaining sampled days (N = 3; Apr. 29 and May 5 2008, and Apr. 27 2009) western sandpiper distribution patterns resembled those described for dunlin. Dunlin never demonstrated the most common distribution pattern of its smaller congener.

Overall, western sandpiper density was higher than dunlin density after the ebbing tide fully exposed the upper intertidal (Fig. 3.4, western sandpiper: 104.7 ± 115.7 birds/ha, dunlin: 12.4 ± 34.2 birds/ha, Student t-test: $t_{126} = -5.80$, $P < 0.001$). Similar numbers of western sandpiper were detected at both sections of the upper intertidal (initial and final sections) once the tide line had passed the 650 m marker. However, temporal declines started immediately at the initial section of the upper intertidal. Western sandpiper density in the closest section to shore reached its minimum 60 min after the counts in the exposed upper intertidal had started. At this time, bird numbers started to decline in the final section of the upper intertidal. Few dunlins were present in the initial section of the upper intertidal after the site was completely exposed. Highest densities of dunlin were detected in the final section of the upper intertidal immediately after the area was exposed. The species completely abandoned the area 60 min after the counts in the exposed upper intertidal started.

3.4.2. Sediment grain size composition

The sediment in the upper intertidal at Brunswick Point was predominantly mud and very fine sand (Fig. 3.5). Samples along the intertidal transect showed an opposite gradient in the proportion of these sediment fractions. Mud content comprised most of the sample sediment weigh (62.9 to 86.1 %) and showed a steady decline with distance from shore. On average, the initial section of the upper intertidal contained 10 % more mud than the final section (80.2 ± 0.1 % and 70.1 ± 0.1 %, respectively, paired Student t-test: $t_{29} = 7.30$, $P < 0.001$). The opposite trend was observed in the smallest sand fraction, which tended to increase with distance from the shoreline. The remaining sand fractions contributed less than 7 % to the total dry sediment weight at each individual marker and showed stable values along the transect. Coarse sand contributed the least among all sediment fractions analyzed.

3.4.3. Spatial and temporal biofilm availability.

The initial section of the upper intertidal had greater Chl-*a* content in the top 2 mm of the sediment than the final section (Fig. 3.6; $F_{1,56} = 62.59$, $P < 0.001$). Chlorophyll-*a* content during the emersion period averaged 21.6 ± 5.8 $\mu g\ g^{-1}_{\text{dry sediment}}$ in the first 350 m from shore whereas an average of 12.7 ± 1.9 $\mu g\ g^{-1}_{\text{dry sediment}}$ was recorded in the final section of the upper intertidal. There was no significant temporal variation in Chl-*a* content ($F_{3,56}$ = 0.78, P = 0.508), although samples collected in the initial section of the upper intertidal showed a temporal trend toward a progressive increment in Chl-*a* content values which reached a maximum average of 24.1 ± 5.0 µg $g^{-1}_{dry\ sediment}$ three hours after emersion (11:00 h.). In both sections, Chl-*a* content values one hour before flooding were similar to those measured three hours after emersion. There were no intertidal section x sampling time interaction for Chl-*a* content ($F_{3,56}$ = 1.11, P = 0.351).

Mean water content in the top 2 mm varied with intertidal section and time ($F_{1,56}$ = 746.60, P < 0.001; $F_{3,56}$ = 13.48, P < 0.001, respectively). Approximately, the first 350 m of the upper intertidal contained 20 % more water than the final section (Fig. 3.6, initial = 60.0 ± 5.3 %, final = 40.0 ± 2.5 %). Overall, water content in the sediment decreased 6 % during the emersion time (08:00 h., 53.8 ± 13.4 %; 17:00 h., 47.9 ± 12.0 %). The interaction between the intertidal section and the emersion time proved significant for sediment water content ($F_{3,56}$ = 9.37, P < 0.001). *Post hoc* comparisons indicated differences for all combinations involving the two intertidal sections (Fig. 3.6). Pair-wise combination within the initial upper intertidal showed that the percentage of water content was significantly higher at the first sampling time (08:00 h., 7.1 to 10.4 %, P < 0.001 for all combinations). There were no differences in water content between the remaining sampling times. Within the final section of the upper intertidal differences were detected in samples taken at 17:00 h. and those obtained at 08:00 and 09:00 h. (P = 0.039 and 0.041, respectively). Samples taken before flooding had 4.7 % less water than those taken at 08:00 and 09:00 h.

3.4.4. Invertebrate availability.

Overall, seven invertebrate taxa were found at the upper intertidal (Table 3.3). All taxa occurred in both sections, except for ostracods which were only found in one sample in the first 350 m from shore. Macrofaunal invertebrates were numerically dominated by polychaetes and bivalve molluscs which provided together 87 % of overall invertebrate density (54 % and 33 %, respectively). Mean invertebrate density was similar for both upper intertidal sections (initial: 35.3 ± 13.9 indiv./core; final: 34.3 ± 13.5 indiv./core; Student t test, t_{10} = - 0.16, P = 0.876). Mean density for each individual taxon did not differ significantly among the upper intertidal sections (Paired t-test, t_{10} = -2.01 to 0.899, P = 0.07 to 0.985).

3.5. Discussion

3.5.1. Species differ in "tide following" behavior and distribution

Here I demonstrated that foraging western sandpiper and dunlin adopted different "tide following" behavior during the onset of the ebbing tide at Roberts Bank. Following the water edge is the initial response of both species when tide starts to recede in the upper intertidal at Brunswick Point. However, once the first 350 m of the upper intertidal is exposed, a high proportion of western sandpipers stop progressing down the mudflat whereas most of the dunlin continue moving with the tide line. Consequently, the distribution of western sandpiper and dunlin at the upper intertidal differs during the ebb tide (Fig. 3.3). Western sandpiper makes intensive use of the initial section of the upper intertidal (first 350 m from shore) and their density remains high and constant even when the sediments had been exposed for approximately one hour. Moreover, once the upper intertidal is completely exposed, western sandpipers continue foraging across the site for up to 2 hours. In contrast, dunlin highest densities continually change with the tide line position, exploiting briefly but equally the whole extent of the upper intertidal habitat.

Although some studies have reported similar differences in the "tide following" behaviour of western sandpiper and dunlin (Couch 1966, Senner *et al.* 1989), most of the literature have catalogued them as "tide followers" with strong preferences to forage near the tide edge (Colwell and Landrum 1993, Warnock and Takekawa 1995, Butler *et al.* 2002, Granadeiro *et al.* 2006). As the former was true for dunlin feeding at Brunswick Point, the results differed from those reported for the western sandpiper in this area (Butler *et al.* 2002). I suggest that differences between my results and those previously documented for western sandpiper at Roberts Bank (Butler *et al.* 2002) may be a consequence of the different spatial and temporal scales used. Western sandpiper may follow the tide down and up at Roberts Bank through a tidal cycle (Butler *et al.* 2002), but when restricting the observations to the onset of the ebbing tide they clearly exhibit a preference for feeding at more than 100 m away from the tide edge.

3.5.2. Can MPB biofilm availability explain habitat segregation of western sandpiper and dunlin during the ebbing tide?

I predicted that, given that biofilm feeding is more prevalent in western sandpiper than dunlin (Mathot *et al.* 2010, Kuwae *et al.* 2012), western sandpiper will behave and distribute according to biofilm availability. All the predictions were supported by the results. Western sandpiper behaved as a “non-tide follower”, preferentially used the section with more biofilm availability (150-350 m from shore), and spent more time than dunlin in the upper intertidal which correspond with the area with highest biofilm abundance at Roberts Bank. Hence, western sandpiper behaviour and distribution matches more closely the observed distribution of diatomaceous biofilm than the distribution recorded for benthic invertebrates.

The initial section of the upper intertidal exhibits three important features indicative of greater biofilm availability for shorebirds (Fig. 3.6): 1) higher MPB biomass, 2) exclusive occurrence of MPB biomass builds up trend during the emersion period, and 3) higher sediment water content. The higher MPB biomass detected in the first 350 m from shore is consistent with the positive relationship between MPB biomass and mud content (Yallop *et al.* 1994, Jesus *et al.* 2009). In general, the upper intertidal at Brunswick Point is comprised mostly of fine sediment (mud). However, the sediment at the initial section of the upper intertidal had 10% of more mud content than the final section. I speculate that such a difference was the main determinant for the differential distribution on MPB biomass and water content.

The higher water content at the initial section of the upper intertidal may be of relevant importance for biofilm use by western sandpiper. Food availability for shorebirds is not only related with the resource abundance (i.e., MPB biomass), but also to the accessibility and cost of feeding on such resource (Zwarts and Wanink 1993). The proposed mechanism of biofilm ingestion involves copious amounts of wet mud (Elner *et al.* 2005, Kuwae *et al.* 2008). Hence, the higher water content in the closest section from shore may be indicative of relative higher biofilm accessibility and less cost associated with its use compared to biofilm use in the sandier section of the upper intertidal.

One alternative explanation for the prolonged use of the upper intertidal by western sandpiper is that the birds were targeting invertebrates trapped within areas still covered by a layer of water (Rosa *et al.* 2007) . Another invertebrate oriented explanation for the prolonged use of the upper intertidal by western sandpiper is that by using the muddiest sediments at the flat the birds may take advantage of the area with highest sediment penetrability (Quammen 1982), allowing to the birds to spend more time probing for invertebrates. These two alternative explanations indicating invertebrate use by western sandpiper at the upper intertidal do not necessarily exclude the hypothesis of western sandpiper distribution according biofilm availability. Biofilm feeding would likely not exclude the birds from encountering and feeding on benthic invertebrates inhabiting the upper intertidal. Moreover, feeding on biofilm may help explain the use of meiofaunal resources, such as copepods (Sutherland *et al.* 2000), prey presumably difficult to detect by visual clues but consumed while embedded in the biofilm mucilaginous matrix (Elner *et al.* 2005).

Discerning how much biofilm or invertebrates contribute to western sandpiper behaviour and distribution at the upper intertidal will require comparative analyses not carried out in the present study (e.g. simultaneously measuring the relationship between the availability of different food sources - biofilm and invertebrates - and bird distribution). Nonetheless, grazing on biofilm hypothesis appears the most parsimonious once we take into account the accompanying considerations. First, previous studies have shown that western sandpiper at Roberts Bank relies extensively on biofilm to supply their daily energy requirements (Kuwae *et al.* 2008, Beninger *et al.* 2011, Kuwae *et al.* 2012). Given the sharp contrast in MPB biomass between the upper and the mid-lower intertidal sections (Chapter 2; high and low MPB biomass, respectively), is to be expected that most of the biofilm intake by western sandpiper occurs within the upper intertidal. The observed intensive use of the upper intertidal by western sandpiper is consistent with the high contribution of biofilm to the species diet (Kuwae *et al.* 2008, Beninger *et al.* 2011).

Second, in contrast to the mid and lower intertidal, the upper intertidal at Brunswick Point drains rapidly after exposure. Specifically, after 20 min. of emersion shallow pools and channels are scarce within the studied area. Further, neither shallow pools nor channels were present within the transect where bird counts were conducted during the ebb tide. Therefore, I consider that the activity of benthic invertebrates trapped in areas covered by water was not a major determinant for western sandpiper distribution at the upper intertidal. In addition, if western sandpiper were exclusively feeding on benthic invertebrates on drained sediments exposed for that long (i.e. more than 2 h.), I would expect to observe a high frequency of probing in the bird's feeding repertoire. However, previous studies in this site have shown that probing occurrence is considerably lower than pecking for both sexes of this species (Sutherland *et al.* 2000, Mathot and Elner 2004). Pecking is by far the main feeding mode used by western sandpiper at Roberts Bank (Mathot and Elner 2004). The mechanism for feeding on biofilm can be perceived by a human observer as multiple pecking at a high speed (Elner *et al.* 2005, Kuwae *et al.* 2008, Kuwae *et al.* 2012). Hence, the high pecking rates previously described at Roberts Bank may be related to western sandpipers making extensive use of biofilm (Kuwae *et al.* 2008).

In contrast to the foraging behaviour of western sandpiper, dunlins' tide following behaviour and distribution within the upper intertidal is consistent with a shorebird relying on visual clues for feeding on benthic invertebrates (Granadeiro *et al.* 2006, Santos *et al.* 2010). Even tactile foragers, such as dunlin, rely extensively on visual cues for feeding (Santos *et al.* 2009). Benthic invertebrates are more active, therefore visible, when there is water over the tidal flat (Vader 1964, Rosa *et al.* 2007, Santos *et al.* 2010). Thus, a foraging sandpiper positioning close to the moving tide line will favour its intake rate by enhancing the encounter rate with active invertebrate prey. The larger body size and tarsi of dunlin will benefit them over western sandpiper in maintaining a position ahead, within or close to the water line as tide recedes. By reaching deeper waters before western sandpipers, dunlin will take advantage of the presumably active annelids and molluscs. In contrast, the smaller size of western sandpiper would constraint their encounters with active prey close to the tide line. This is because by the time western sandpipers arrive in available shallow areas, invertebrate availability may have experienced a reduction due to dunlin exploitation and/or disturbance (Maurer 1984).

Rather than consuming and reducing the standing stock of invertebrates (exploitation competition), dunlin position ahead western sandpipers may elicit escape behaviour in prey (interference competition, Goss-Custard 1980). Consequently, prey availability for western sandpipers will be reduced and thus its intake rate of benthic invertebrates. By feeding on biofilm at the upper intertidal western sandpipers can avoid the decline in intake rate due to the interference competition from dunlin.

On three occasions western sandpiper behaved as dunlin and followed the tide through the upper intertidal. I could not find any satisfactory explanation for this change in behaviour. However, Senner *et al.* (1989) reported that western sandpiper changed its "non-tide following" behaviour towards "tide following" behaviour when dunlin were largely absent from the area. The authors suggested that the shift in western sandpiper feeding microhabitat in the absence of dunlin was indicative of competitive interactions between species. The interpretation provided by Senner *et al.* (1989) is in line with the competition scenario and further partitioning strategies between western sandpiper and dunlin at the upper intertidal of Brunswick Point.

As a general conclusion, I suggest that during the ebb tide western sandpiper behave and distribute at the upper intertidal of Brunswick Point in a way that minimize potential inter-specific competition for food. The spatial segregation pattern between western sandpiper and dunlin can be accounted for the greater propensity for biofilm feeding by western sandpiper and the different biofilm availability within the upper intertidal.

3.6. Acknowledgements

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3.7. References

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3.8. Tables

Table 3.1. Area measurements at Brunswick Point, Roberts Bank, British Columbia and values for the tide following index (TFI) expectation line.

Polygon	Area of polygon (m^2)	Cumulative area (m^2)	Proportion of total area	Expected decrease in TFI	TFI value expectation
Shore-150[a]	783364	783364	0.163	0.000	1.000
150-200	216073	999437	0.045	0.000	1.000
200-250	101460	1100897	0.021	0.021	0.979
250-300	173343	1274240	0.036	0.057	0.943
300-350	267011	1541251	0.055	0.112	0.888
350-400	144189	1685440	0.030	0.142	0.858
400-450	281685	1967125	0.058	0.201	0.799
450-500	235693	2202818	0.049	0.250	0.750
500-550	337063	2539854	0.070	0.320	0.680
550-600	465034	3004888	0.097	0.416	0.584
600-650	631360	3636248	0.131	0.547	0.453
650-low tide	1180560	4816808	0.245	N/A	N/A

[a] The first polygon comprises the area from the shore to our first marker (at 150 m from shore). Polygons were drawn to include elevations between those of neighboring markers. Expected TFI decrease is cumulative and does not begin until the 200 m marker is exposed because up to this point all birds have a TFI equals to 1 (according to our tide-following definition birds are within two markers of the tide edge).

Table 3.2. Tide following deviance of western sandpipers (**Calidris mauri*****) and dunlin (*****Calidris alpina*****) at different distances from shore at the upper intertidal of Brunswick Point, Roberts Bank, British Columbia. Tide following deviance represents the difference between an ideal strictly tide follower and the observed tide following index recorded for both shorebird species during the ebbing tide. The table offers the number of counts made at each marker (N) and the results of a paired t-test between the tide following deviance for both shorebirds.***

Distance from shore (m)		**Western Sandpiper**	**Dunlin**	**Paired t test[a]**
	N	**Mean ± SD**	**Mean ± SD**	
150	24	0.13 ± 0.34	0.08 ± 0.29	0.44^{ns}
200	19	0.34 ± 0.30	0.28 ± 0.30	0.56^{ns}
250	25	0.55 ± 0.30	0.29 ± 0.25	4.15***
300	20	0.64 ± 0.18	0.34 ± 0.36	6.13***
350	16	0.60 ± 0.32	0.25 ± 0.28	4.31***
400	23	0.57 ± 0.24	0.17 ± 0.24	7.95***
450	12	0.71 ± 0.07	0.42 ± 0.28	4.19***
500	4	0.65 ± 0.04	0.43 ± 0.14	2.60^{ns}
600	3	0.50 ± 0.14	0.41 ± 0.16	0.54^{ns}
650	37	0.34 ± 0.18	0.21 ± 0.30	3.18**

[a] Significant differences at $P < 0.05$ (*), <0.01 (**) and <0.001 (***).

Table 3.3. Density (mean number of individuals per core ± standard error) of benthic invertebrates occurring at the initial (150 – 350 m) and final (450 – 650 m) sections of the upper intertidal at Roberts Bank.

Invertebrate taxa	**Distance from shore (m)**			
	150 – 350 (N = 11)		**450 – 650 (N = 11)**	
	Mean ± SD	**Min - Max**	**Mean ± SD**	**Min – Max**
Nematodes	2.4 ± 2.9	0 -10	0.5 ± 0.8	0 - 2
Molluscs	10.2 ± 7.7	1 -23	13.00 ± 10.1	1 - 34
Polychaetes	18.9 ± 12.6	5 - 47	18.8 ± 7.8	2 - 27
Ostracods	4	0 - 4	-	-
Copepods	1.0 ± 1.5	0 - 4	5	0 - 5
Tanaids	0.5 ± 0.8	0 -2	0.7 ± 1.0	0 - 3
Cumaceans	1.9 ± 3.1	0 - 10	0.6 ± 0.9	0 -3

3.9. Figures

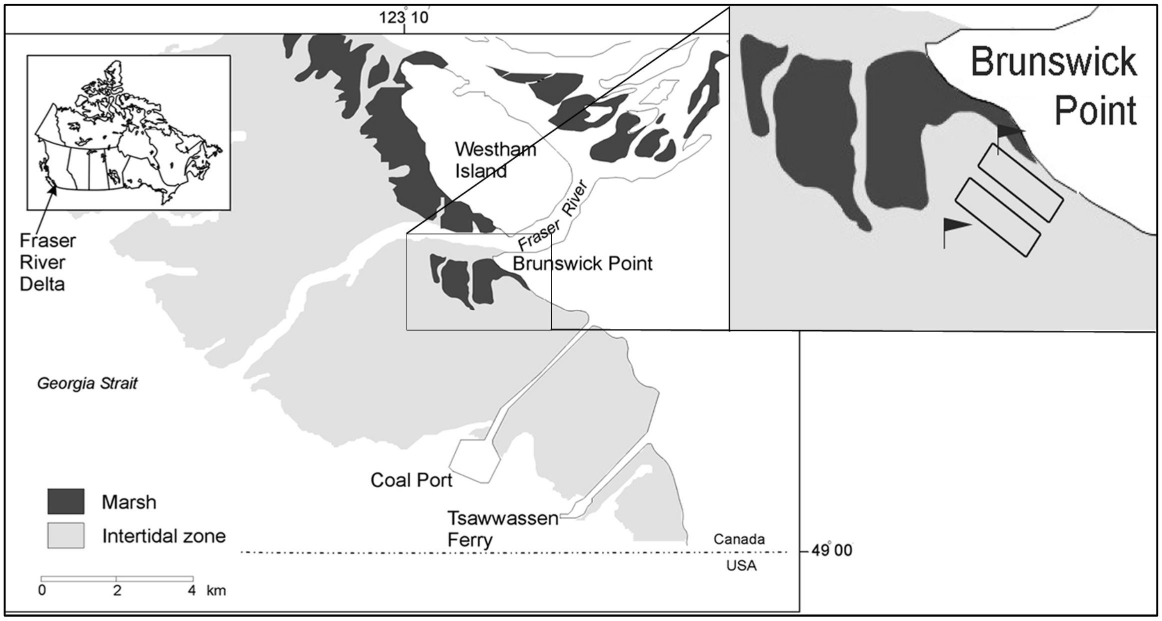

Figure 3.1. Map of Roberts Bank, British Columbia, showing the study site (Brunswick Point) and the two spatial sampling designs. Flags are indicating the origin and end of a 500 m transect used to assess sandpiper's tide following behavior and distribution. Rectangles indicate the initial and final sections (150-350 m and 450-650 m from shore, respectively) of the upper intertidal where MPB biofilm and invertebrate samples were obtained.

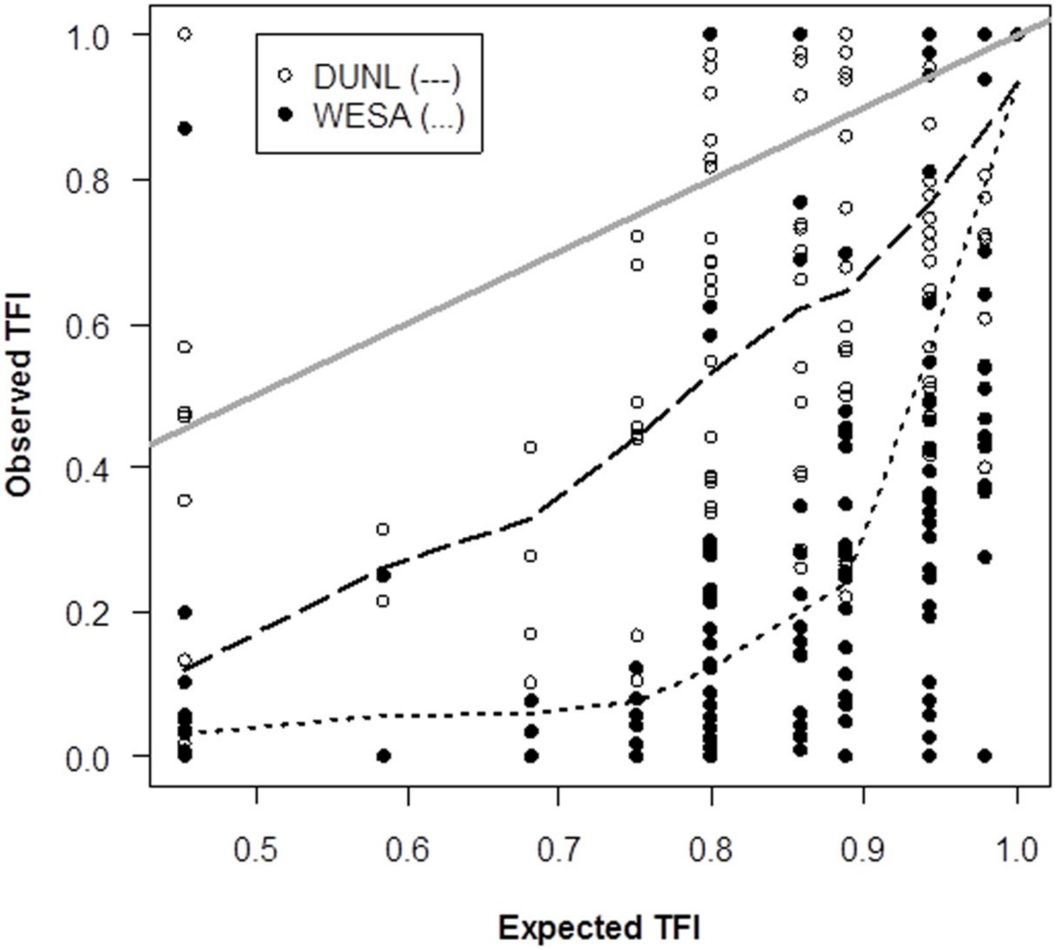

Figure 3.2. Relationship between the expected and observed tide following index (TFI, proportion of birds within 100 m from the water edge) of western sandpiper (WESA, Calidris mauri) and dunlin (DUNL, Calidris alpina) at Brunswick Point upper intertidal, Robert Banks, British Columbia. Trend lines show an ideal tide follower (thick grey line) and the tide following behavior of western sandpiper (closed circles and dotted line) and dunlin (open circles and dashed line). Lines for western sandpiper and dunlin represent lowess smoothing (f = 0.5).

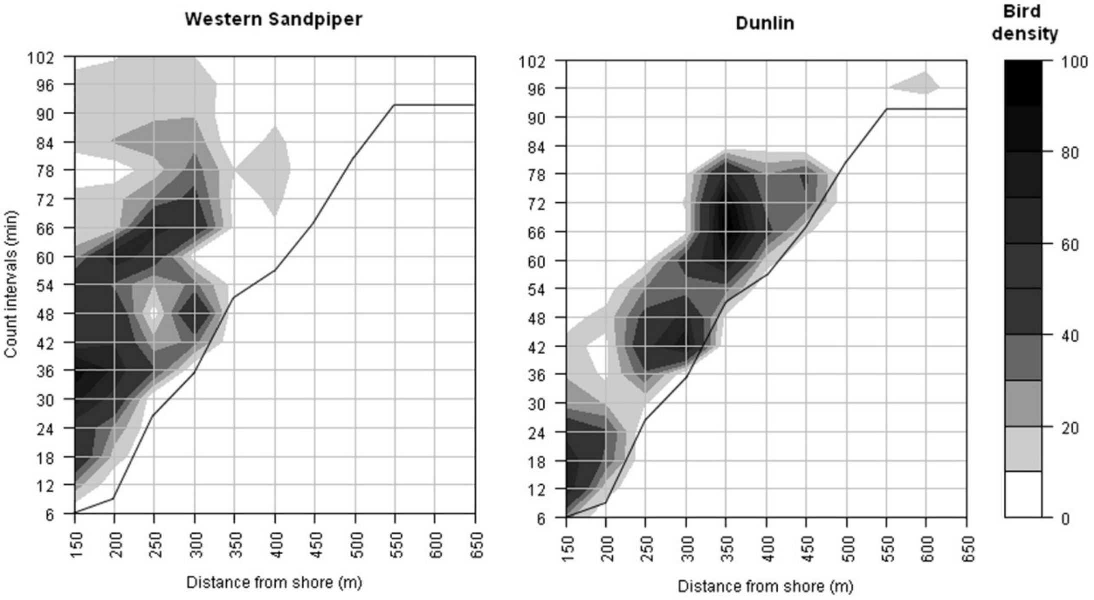

Figure 3.3. Typical spatial (distance from shore - m) and temporal (elapsed time after the first 150 m got exposed - min) variation in western sandpiper (Calidris mauri) and dunlin (Calidris alpina) density during the time it took to uncovered the first 650 m of the upper intertidal at Brunswick Point, Roberts Bank, British Columbia. The figure represents the shorebird distribution pattern recorded for April 30th, 2009. The line represents the tide line position at each count. For representation purpose the maximum bird density (number of birds in a 5 m^2 radius circumference) was fixed to a 100 bird density value. Only four counts on this day exceeded the number of 100 birds at a given time (150 m, 33 min: 104 WESA; 350 m, 57 min: 190 DUNL; 350 m, 60 min: 190 DUNL; and 350 m, 66 min: 114 DUNL).

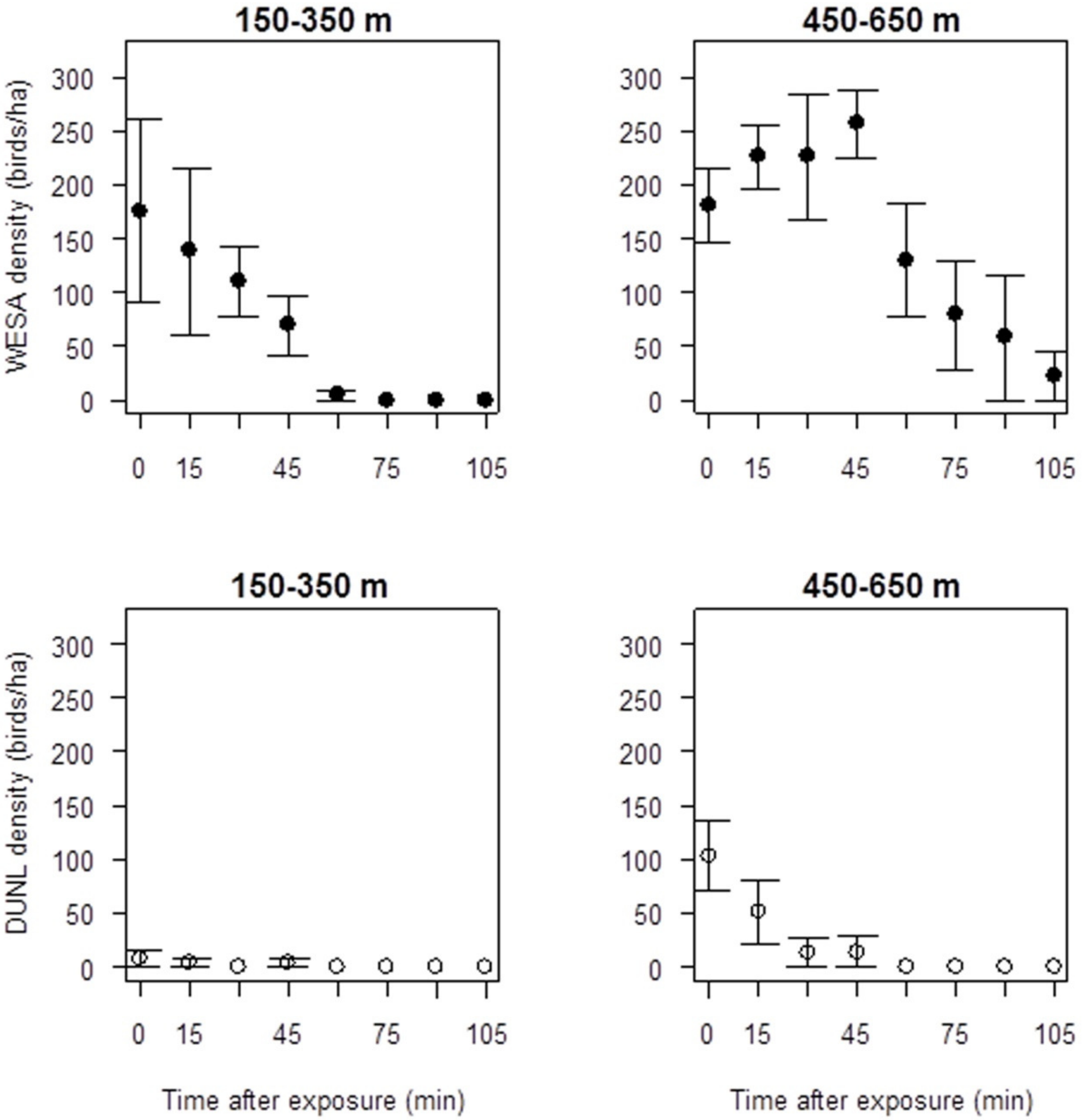

Figure 3.4. ***Western sandpiper (WESA, closed circles) and Dunlin (DUNL, open circles) density (birds/ha) at the initial (150-350 m from shore) and final (450-650 m from shore) section of the upper intertidal at Brunswick Point. Bird counts were conducted after the receding tide had exposed the first 650 m of the mudflat. Errors bars are ± SE.***

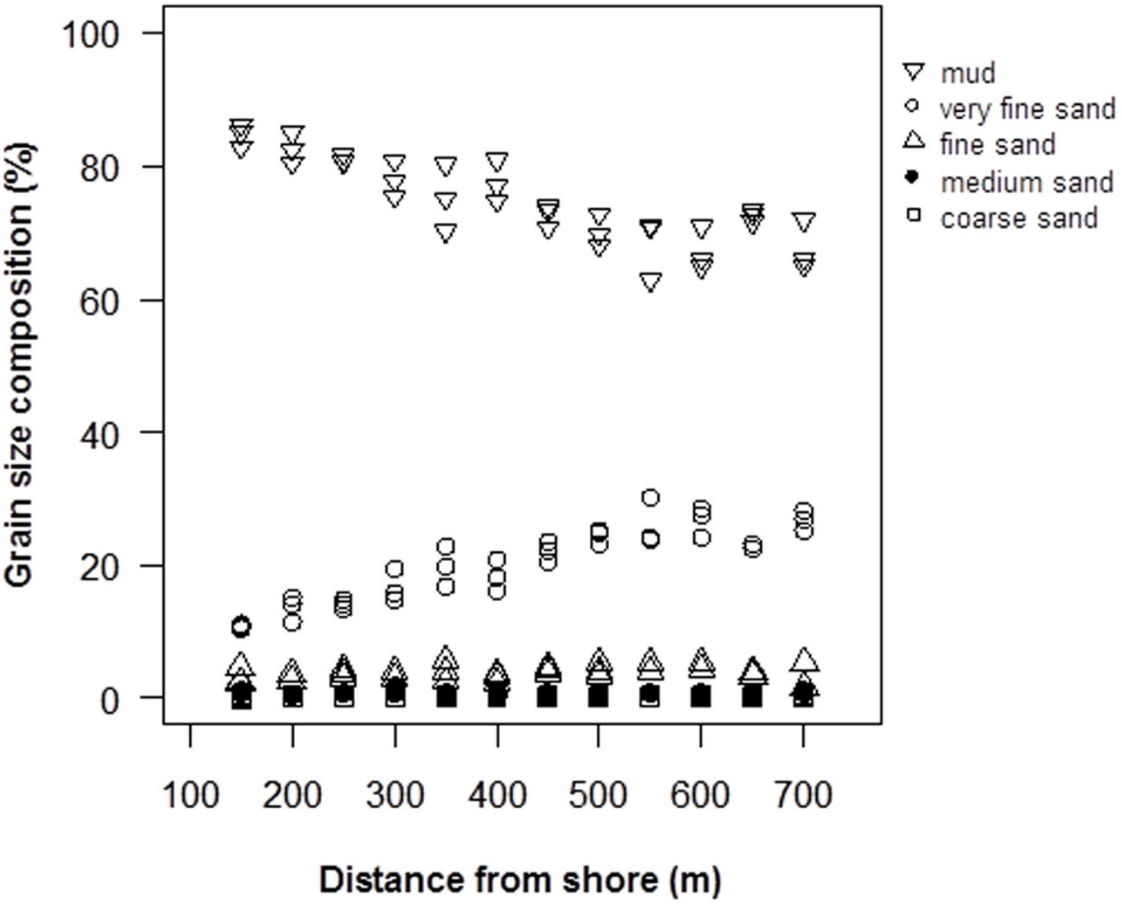

Figure 3.5. ***Sediment grain size composition along a 700 m long transect at the upper intertidal at Brunswick Point, Roberts Bank, British Columbia.***

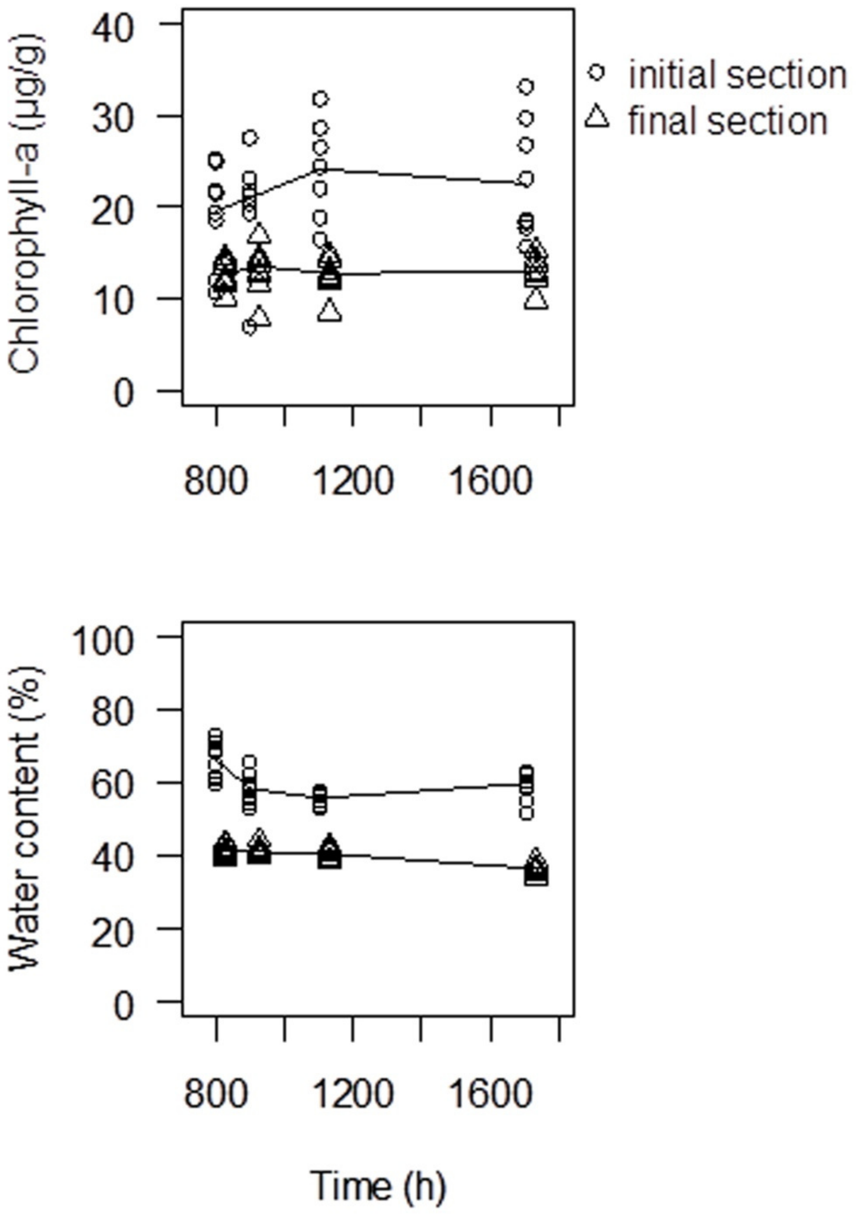

Figure 3.6. Spatial and temporal variation in Chlorophyll-a content (µg $g^{-1}{}_{dry\ sediment}$) and water content (%) in the top 2 mm of the sediment at the initial (open circles, 150 -350 m) and final (open triangles, 450 – 650 m) spatial sections of Roberts Bank's upper intertidal. Samples were collected at four time intervals during the emersion time: immediately after exposed (0800), 1 hour after emersion (0900), 3 hours after emersion (1100) and before immersion (1700). Data points for the initial and final upper intertidal spatial sections are offset from one another for clarity.

4. Microphytobenthic biofilms and biomass in two Cuban estuaries used by shorebirds

4.1. Abstract

Little is known about microphytobenthic (MPB) biofilms in tropical intertidal sites. Neither temporal variation in biofilm availability nor environmental factors that influence MPB biofilms have been investigated in tropical intertidal sites used by migrating and/or wintering sandpipers. Intertidal MPB biofilms were investigated in October 2008 and January 2009 in two Cuban estuarine flats (Río Máximo and Tunas de Zaza) exhibiting different types of flood-emersion regime. Cyanobacterial mats and diatomaceous biofilm were distinguished at both sites based on biofilm morphological features (color, texture, thickness and layering) judged by sight. Cyanobacterial mats were the dominat type of biofilm in Oct. 2008 at both sites, whereas diatomaceous biofilms were present in Jan. 2009. Microphytobenthic biomass varied between 0.5 and 724.9 µg g^{-1}_{DM}. The lowest MPB biomass averages were recorded in Jan. 2009. Measurements of sediment mud content, temperature and salinity suggested that temperature was the most important variable driving the temporal shift in MPB biofilms and biomass at the studied sites. Monthly censuses of small-bodied sandpipers ("peeps") at Río Máximo showed that the area was mainly used as a southward stop-over site (Sep.-Nov., 3212.3 ± 2557.4 peeps) and only as many as half of the sandpipers were observed in the wintering period (Dec.-Feb., 1493.5 ± 1470.4 peeps). The data suggest that sandpipers will encounter cyanobacterial mats while using Cuban estuaries as southward stop-over sites, whereas diatomaceous biofilms will be available for birds during the wintering period.

Keywords: cyanobacterial mats, diatomaceous biofilm, Cuba, stop-over site, calidridine sandpipers, migration phenology

4.2. Introduction

Microphytobenthic biofilms are complex and dynamic assemblages of microorganisms living within a matrix of mucilaginous extracellular polymers (EPS) on the sediment of intertidal ecosystems (Decho 2000, Barranguet *et al.* 2005). Diatoms and photosynthetic cyanobacteria are the main biotic constituents of the microphytobenthos (MPB) in intertidal systems (Underwood and Barnett 2006), and the major producers of the EPS that form the mucilaginous matrix surrounding the microbial community (Stal 2003, Underwood and Paterson 2003, Dijkman *et al.* 2010). The MPB biofilms play a crucial role at the base of the coastal trophic web (Decho 2000, Degré *et al.* 2006, Hicks *et al.* 2011). Specifically, the photosynthetic microorganisms along with the rich EPS matrix they secrete provide an important food resource for a wide diversity of grazers (e.g., polychaetes, Decho and Lopez 1993; crustaceans, Decho and Moriarty 1990, Abreu *et al.* 2007; molluscs, Whitlatch and Obrebski 1980; echinoderms, Hoskins *et al.* 2003; fishes, Almeida 2003, Lefrançois *et al.* 2011).

Recent studies have also demonstrated that MPB biofilms can be an important food source for shorebirds (Elner *et al.* 2005, Kuwae *et al.* 2008, Kuwae *et al.* 2012). The extent of biofilm usage by shorebirds varies depending on shorebirds traits (body mass, and tongue spine length and area) and environmental conditions (sediment grain size, and biofilm density) (Kuwae *et al.* 2012). Small-bodied sandpipers (< 30 g) are better adapted for feeding on biofilm than larger shorebirds (Elner *et al.* 2005, Kuwae *et al.* 2012). Spatio-temporal variations in environmental conditions can constraint the use of biofilm by sandpipers (Kuwae *et al.* 2012, Quinn and Hamilton 2012). For example, Kuwae *et al.* (2012) found intra-specific variability in biofilm use with higher biofilm contribution to western sandpiper's daily energy expenditure at muddy sites (Roberts Bank, 58%) than at sandy areas (Boundary Bay, 37%). However, much of the current understanding about MPB biofilms (Yallop *et al.* 1994, Defew *et al.* 2004, Underwood *et al.* 2005) and biofilm consumption by shorebirds (Kuwae *et al.* 2008, Kuwae *et al.* 2012, Quinn and Hamilton 2012) is based on studies conducted at northern temperate habitats.

The few MPB biofilm studies in the tropics have been focused on investigating MPB adaptations to tropical marine habitats (Underwood 2002), assessing spatial and temporal variations of epilithic cyanobacteria in rocky shores (Nagarkar and Williams 1999, Hutchinson and Williams 2003), or addressing the role of MPB biofilms in the food web structure of rocky shores (Williams *et al.* 2000, Nagarkar *et al.* 2004), streams (March and Pringle 2003) and rivers (Lefrançois *et al.* 2011). Little is known about MPB biofilms in tropical intertidal sites. Neither environmental factors that influence MPB biofilms nor temporal variation in biofilm availability have been investigated in tropical intertidal sites used by migrating and/or wintering sandpipers. Given that sandpipers spend 8 months of their annual cycle in subtropical and tropical sites (Morrison and Myers 1987) there is a need to understand the occurrence, availability and spatiotemporal variation in MPB biofilms at these habitats. The objectives of this study were to: 1) qualitatively describe the type of MPB biofilm observed at two tropical locations with two types of tidal cycle, 2) describe the seasonal variation in the type of MPB biofilm and biomass, 3) evaluate abiotic factors that may underlie the spatial and/or temporal variation of MPB biofilms in the studied sites, and 4) describe monthly variations of small-bodied sandpipers during the migratory period (Sep. – Apr.) in Cuba.

4.3. Methods

4.3.1. Study sites

Microphytobenthic biofilms were sampled from two estuarine habitats used by shorebirds as stop-over and wintering sites: 1) Río Máximo (21°44’N, 67°31’W), Camagüey, and 2) Tunas de Zaza (21°38’N, 79°32’W), Sancti-Spiritus, Cuba. Río Máximo and Tunas de Zaza are subtropical estuarine sites located in north and south central Cuba, respectively (Fig. 4.1). Both sites share general landscape commonalities such as large mudflat areas (Río Máximo: 2.6 x 1.2 km; Tunas de Zaza: 3.0 x 1.5 km) surrounded by shallow lagoons and bordered by red (*Rhizophora mangle*) and black (*Avicennia germinans*) mangrove forest. The sites differ in flood-emersion regime. Semidiurnal tides affect Río Máximo, while Tunas de Zaza is under the effect of mixed tides. Mean tidal height is 0.70 m and 0.25 m for Río Máximo and Tunas de Zaza, respectively. Besides the described inter-site differences in the tide type and height, the sites also experience different influences of wind during the tidal cycle. The mudflat at Río Máximo shows a flood-exposure pattern consistent with ‘wind flats’ in which onshore north winds push the water toward the coast. The effect of north winds with speeds over 15 km/h during high tide produce floods that cover the entire flat (A. Jiménez, unpubl. data). The area remains flooded as long as the winds are over 15 km/h. Only 25-50% of the flat at Río Máximo is covered during high tide when winds are lower than 10 km/h. The effects of north winds on the tidal cycle at Río Máximo are evident from October to December. At Tunas de Zaza the wind effect on mudflat flooding is negligible. The high tide regularly floods 75% of Tunas de Zaza flat and 50% of the area remains covered by water during low tide. Both sites are protected areas and considered Cuban Important Bird Areas due to is numbers of waterbirds (Aguilar 2009).

4.3.2. Data collection

Sampling was primarily conducted during the time the two estuarine flats were being used as southward stop-over habitats (October 10-20, 2008) and as wintering sites (January 20-27, 2009). Sampling occurred between 2.0 - 2.5 h after the sediment became exposed because of either tidal or wind effects. Samples were taken randomly from shorebird foraging habitats which were defined for this study as areas (~ 6 ha.) holding the highest congregations of foraging shorebirds.

Microphytobenthic biofilm classification

Biofilms were classified as either cyanobacterial mats or diatomaceous biofilms. Classification was based on morphological features that can be judged by sight. Detectable characteristics such as color, texture, thickness and layering of the sediment were the main attributes considered (Eisma 1998, Dijkman *et al.* 2010). Diatomaceous biofilms were determined when the sediment surface did not exhibit visible coloring or ranged from a faint green to a golden-brown coloration (caused by the presence of fucoxanthin pigments), and there were no observable filamentous texture or stratified layers on the sediment. Cyanobacterial mats were assumed to occur when biofilms formed a thick green mat of macroscopically filamentous structures elevated from the surrounding sediment. Cyanobacterial mat sediment cores exhibited noticeable colored banding patterns in the top 5 mm of sediment.

Microphytobenthic biomass sampling

To assess MPB biomass at each site and period (Table 4.1), I measured chlorophyll-*a* content (Chl-*a*, $\mu g\ g^{-1}{}_{\text{dry sediment}}$) in the upper 2 mm of the sediment. As MPB (diatoms and cynobacteria) are the major component of biofilms in intertidal systems (Yallop *et al.* 2000, Underwood and Barnett 2006), I used Chl-*a* content as a proxy to assess MPB biomass (Herlory *et al.* 2004, Pomeroy and Butler 2005). Sediment samples were collected with a 26 mm internal diameter syringe previously modified (60 cc syringe, apex removed and edges sharpened). The core was pushed into the sediment and the plunger lifted until approximately 15 mm of sediment was expressed into the barrel. Care was taken to prevent contact between the plunger and the sediment surface. The plunger was removed from the syringe, placed in the opposite end and pushed to extrude the first 2 mm of sediment. Biofilm samples were taken by slicing with a spatula the top 2 mm off the sediment. Samples were placed in labelled plastic zip-loc bags, stored in a cool box with ice, returned to the laboratory and stored in the dark at -20℃.

Abiotic features

Within the shorebird foraging habitats at Río Máximo and Tunas de Zaza, nine random sediment samples for mud content determination were collected in October 2008 (Table 4.1). Forty-mm deep sediment samples were collected with the modified syringe. Additionally, surface sediment temperature and pore-water salinity were measured for each site and period (Table 4.1) using a thermometer (VWR L37823; ± 0.1 ºC) and a hand-held portable refractometer (Vista; ± 1 ‰), respectively.

Bird counts

Because of logistic constraints, shorebird counts (from Sep. 2008 to Apr. 2009) were conducted only at Río Máximo. Morning shoreline counts (07:00-10:00 h.) were conducted when 30-50 % of the intertidal flat was exposed. Birds were counted while the observer walked eastward along the low tide mark, covering an area of 1.0 x 0.3 km (0.3 km^2). Birds were identified and counted using 10x50 binoculars and 20-60x spotting scope. In all, 112 censuses were made, each lasting 2-3 h. The number of censuses per month ranged from 8 to 22. I attempted to count individual birds, but large flocks (> 500 individuals) were counted in units of 10, 50 or 100 birds. For the objectives of this study, I only report the number of small-bodied sandpipers (*Calidris* sp., mostly least, semipalmated and western sandpipers) grouped under “peeps”.

4.3.3. Laboratory analyses

Frozen Chl-*a* content samples were transferred to 20 ml scintillation vials, weighed to the nearest 0.01 g and then thawed in the dark before analysis. Ten ml of 90 % acetone solution was added to the vials and samples were thoroughly shaken for 2 min in a vortex and placed into a dark box at -4℃ for 24 h for extraction. The amount of Chl-*a* in the supernatant was measured spectrophotometrically using a Milton Roy Spectronic 301 spectrophotomer. Absorbance at 665 and 750 nm were measured; samples were acidified with two drops of 0.1 N HCL, mixed, left for 1 min and absorbance was measured again at the same wavelengths. Concentration of corrected Chl-*a* was calculated using Lorenzen’s spectrophometric equations (Lorenzen 1967). Then, samples were oven dried for 48 h at 70℃ and weight again to obtain the dry weight.

Sediment samples for mud content (sediment grain size < 63 µm) determination were weighed wet and dry (oven dried for 48 h at 70℃) to the nearest 0.01 g. Samples were soaked for 24 hours in a solution of Sodium hexametaphosphate (6.2 g/l) to dissolve agglomerated particles of sediment. Each sample was passed through a 63 µm sieve by wet sieving technique. The sediment retained in the sieve (sandy fraction) was dried again and weighed. The difference of weight between the whole sample and the sandy fraction was considered as the mud fraction and expressed as a percentage of the total sediment weight.

4.3.4. Statistical analyses

Data were analyzed using R version 2.10.0 (R Development Core Team 2009). The assumptions of normality and homogeneity of variance were assessed visually for all models results. When violations were detected, they were managed through transformation (ln (Chl-*a* + 1); square root (“peeps” numbers)) or use of non-parametric techniques. Values presented in the manuscript are means ± standard deviations (S.D.), unless otherwise stated.

I used a two-way ANOVA to test for the effects of site (Río Máximo and Tunas de Zaza) and season (shorebird southward migration and wintering residency) on changes in Chl-*a* content at Cuban estuaries. Sediment mud content variation between sites was assessed by Mann-Whitney U test. A separate two-way ANOVA with site and season as factors was used to test for variations in temperature and salinity at Cuban estuaries. A one-way ANOVA was conducted to assess overall differences in "peeps" numbers during the migratory period (southward migration: Sep.-Nov., wintering residency: Dec.-Feb., and northward migration: Apr.-Mar.) at Río Máximo. I used the Tukey HSD test for pair-wise post-hoc comparisons. I considered statistical results to be significant at $P < 0.05$.

4.4. Results

4.4.1. Spatiotemporal variations in the type of MPB biofilms and biomass

Both sites showed the presence of cyanobacterial mats during October 2008 (Fig. 4.2A). The dark green mat extending for most of the shorebird foraging habitats exhibited a regularly spaced pattern of elevated hummocks alternating with water-filled hollows (Fig. 4.2A). At the hummocks, the layer was up to 6 mm thick whereas the water-filled hollows showed bare sediments with no visible coloring or a faint green coloring. In January 2009, none of the sites showed evidence of cyanobacterial mats. Instead, some areas of the sediment exhibited a thin (~1 mm) golden-brown 'skin' indicative of diatomaceous biofilms (Fig. 4.2B). The shift from cyabobacterial mats in October 2008 towards a diatomaceous biofilm in January 2009 occurred at both sites (Fig. 4.2).

The Chl-*a* content of the MPB biofilms in Cuban estuaries ranged from 0.5 to 724.9 $\mu g\ g^{-1}{}_{DM}$ (Fig. 4.3). Season ($F_{1,75} = 92.81$, $P < 0.001$) and site ($F_{1,75} = 4.13$, $P = 0.045$) had significant and marginally significant effects in Chl-*a* content, respectively. There was significant interaction between these main effects ($F_{1,75} = 15.59$, $P < 0.001$). The seasonal effect was more prominent at Tunas de Zaza, where the Chl-*a* content dropped from 206.1 ± 198.2 $\mu g\ g^{-1}{}_{DM}$ in October to 1.4 ± 0.9 $\mu g\ g^{-1}{}_{DM}$ in January (Fig.

4.3). Overall, Chl-*a* content tended to be higher at Tunas de Zaza than Río Máximo (89.8 ± 164.2 µg g^{-1} $_{DM}$ and 67.8 ± 153.3 µg g^{-1} $_{DM}$, respectively), but the site effect depended largely on season. Differences between sites were only noticeable in January, when Río Máximo exhibited ten times the amount of Chl-*a* content detected in Tunas de Zaza (14.7 ± 12.8 µg g^{-1} $_{DM}$, 1.4 ± 0.9 µg g^{-1} $_{DM}$, respectively).

4.4.2. Environmental parameters

Sediment mud content was similar in the two studied estuarine flats ($U = 30.0$, $P = 0.354$). Both sites showed high content of mud with average values of 72.1 ± 22.2 %, and 84.0 ± 8.3 % at Río Máximo and Tunas de Zaza, respectively.

Sediment temperature significantly decreased during the study period and was on average 4.5 °C higher in October than in January ($F_{1,71} = 71.45$, $P < 0.001$). Sediment temperature was significantly higher in Tunas de Zaza than in Río Máximo ($F_{1,71} = 13.40$, $P < 0.001$). The interaction between season and site resulted in significant differences in sediment temperature ($F_{1,71} = 16.93$, $P < 0.001$). Sediment temperature in Río Máximo and Tunas de Zaza was similar at both sites during October (Fig. 4.4A, 26.1 ± 2.3°C and 26.0 ± 2.3°C, respectively). However, in January, sediment temperature at Río Máximo decreased by approximately 6.8 °C, whereas at Tunas de Zaza temperature dropped 2.4 °C (Fig. 4.4A).

Salinity measurements in October 2008 were not significantly different from January 2009 (Fig. 4.4B, $F_{1,65} = 2.94$, $P = 0.091$). However, sites showed marked differences in salinity (Fig. 4.4B, $F_{1,65} = 63.47$, $P < 0.001$). Accordingly, Río Máximo and Tunas de Zaza can be considered as brackish (18.5 ± 12.9‰) and saline (48.5 ± 6.1‰) estuaries, respectively. There was no significant difference as result of the interaction between period and sites ($F_{1,65} = 0.01$, $P = 0.918$).

4.4.3. Migration phenology of small-bodied sandpipers at Río Máximo

The ANOVA indicated significant differences in the average number of “peeps” during the migration period ($F_{2,109} = 21.78$, $P < 0.001$). *Post-hoc* comparisons indicated significant differences between all periods ($P = < 0.001 – 0.02$). The sandpipers were more abundant during the southward migration (Sep.-Nov., 3212.3 ± 2557.4, range: 120 – 12,000), less abundant during the wintering residency (Dec.-Feb., 1493.5 ± 1470.4, range: 13 - 5000) and least abundant during the northward migration (Mar.-Apr., 552.8 ± 678.1, range: 15 – 3000) (Fig. 4.5).

4.5. Discussion

Few studies exist that document the variations in MPB biofilms at tropical intertidal areas used by Neartic sandpipers (Mathot *et al.* 2007). The present study indicates that shorebirds face notable temporal changes in the type of MPB biofilm and biomass while using tropical intertidal flats. Cyanobacterial mats were the dominant MPB biofilms during the southward migration and diatomaceous biofilms were present during the wintering period. The data suggest that temperature was the most important variable driving the seasonal MPB shift from cyanobacterial mats in October 2008 towards diatomaceous biofilms in January 2009.

4.5.1. The MPB biofilms in Cuban estuaries

During October 2008 the MPB biofilm at the Cuban estuaries showed the distinctive pattern of a dense cyanobacterial mat. The average MPB biomasses recorded in October 2008 were similar to those documented on senescent cyanobacterial mats sampled in temperate areas during the summer (Dijkman *et al.* 2010). Cyanobacterial mats such as those described here for Río Máximo and Tunas de Zaza, have been observed at other Cuban intertidal areas (Tiscornia, Havana; Sancti-Spiritus coastal lagoons; La Jaiba, Villa Clara) during the shorebird southward migration (August to October, A. Jiménez pers. obs.). Thus, it is to be expected that shorebirds migrating through Cuba during the southward migration will commonly encounter intertidal areas covered by cyanobacterial mats.

Diatomaceous biofilms were also present at the two Cuban estuaries, but they were only common during the shorebird wintering residency (January 2009). The shift in the type of MPB biofilm was notable both by *in situ* sight as by measurements of MPB biomass. Average MPB biomasses in January 2009 were 147 and 8 times lower in magnitude than the previously (October 2008) measured at Tunas de Zaza and Río Máximo, respectively. Of note, the average and variation range values of diatomaceous biomass at Río Máximo (14.1 µg g^{-1}_{DM}, and 1.4 - 46.5 µg g^{-1}_{DM}, respectively) were remarkably similar to those recorded for Roberts Bank, Canada during the northward migration of western sandpipers (Chapter 2; 25 Apr.- 4 May 2008). Hence, sandpipers wintering in tropical sites such as Río Máximo (brackish flats) may find biofilms consistent with the characteristics described in other areas where biofilm feeding by shorebirds occurs (Kuwae *et al.* 2008, Beninger *et al.* 2011).

4.5.2. Microphytobenthic biofilm seasonal shift

The most striking and consistent pattern at the two tropical estuaries was the marked seasonality in the MPB biofilm. Despite the inter-site differences in flooding regime (A. Jiménez pers. obs.), and salinity (brackish and marine), both areas showed the same temporal trend in the type of MPB biofilm. The seasonal pattern showed an alternation between cyanobacterial mats and diatomaceous biofilms, with cyanobacteria being dominant in October 2008 and diatoms in January 2009. Previous visits to Río Máximo indicate that the MPB transition is gradual. Cyanobacterial mats can be seen in Río Máximo from September to early November, whereas diatomaceous biofilm have been observed in the area in January and February.

The described seasonal shift in the type of MPB biofilm is in accord with results documented at temperate intertidal flats (Watermann *et al.* 1999, Scholz and Liebezeit 2012). The MPB biofilm transitions are postulated to be triggered by the effect of irradiance and ambient temperature (Admiraal *et al.* 1984, Barranguet *et al.* 2005). Cyanobacteria are more temperature tolerant MPB than diatoms (van der Grinten *et al.* 2005) and studies show that the intertidal biofilm is dominated by cyanobacteria when ambient temperatures are above 25 ℃ (Watermann *et al.* 1999, Defew *et al.* 2004). The seasonal MPB biofilm shift at Cuban estuaries from cyanobacterial mats in October (23-31 ℃) to diatomaceous biofilm in January (14-26 ℃) is in accord with superior cyanobacteria growth at higher temperatures (van der Grinten *et al.* 2005).

4.5.3. *Shorebird phenology*

The results show that the estuarine flat at Río Máximo is visited by the largest numbers of "peeps" during southward migration (Fig. 4.5). Similar patterns of shorebird migration phenology have been documented at other Cuban (Acosta *et al.* 1992, Pérez-Martínez 2011) and Caribbean sites (e.g. Puerto Rico, Wunderle *et al.* 1989, Collazo *et al.* 1995; Yucatán, Correa-Sandoval and Contreras-Balderas 2008). Therefore, the counts conducted at Río Máximo should reflect shorebird migration phenology in Cuba fairly accurately. The higher average in "peeps" numbers during southward migration indicate that Río Máximo (as well as most of the Caribbean shorebird habitats, Wunderle *et al.* 1989) is mostly used as a southward stop-over site by migrant small-bodied sandpipers.

4.5.4. *Implications for biofilm feeding by shorebirds*

Little is known about the role of the type of MPB biofilm (cyanobacterial mat or diatomaceous biofilm) on the shorebird biofilm feeding behavior (Quinn and Hamilton 2012). To date, the evidence for biofilm feeding by shorebirds comes from temperate sites dominated by diatomaceous biofilms (Kuwae *et al.* 2008, Kuwae *et al.* 2012, Quinn and Hamilton 2012). The use of cyanobacteria dominated biofilms by foraging shorebirds has not been explicitly documented for temperate or tropical sites. The dominance of cyanobacterial mats in Cuban estuarine areas during the shorebird southward migratory period plus to the fact that shorebirds are more numerous in Cuba during southward migration offer a great opportunity to examine the use of cyanobacterial mats by foraging shorebirds.

Understanding the role of cyanobacterial mats for biofilm feeding shorebirds is important given that cyanobacteria are generally considered as poor food for many grazing species (Lukesová and Frouz 2007). Besides the fact that cyanobacterial mats are less rich in carbohydrates (Yallop *et al.* 1994) and fatty acids (Dijkman *et al.* 2010) compared with diatomaceous biofilms, cyanobacteria also secrete capsular polymeric substances which act as a refractory barrier against the digestion of microbial cells (Decho and Lopez 1993). In contrast to cyanobacteria, diatoms inhabiting mudflats do not form structured sheaths of refractory exopolymers (Stal 2003). Thus, diatomaceous biofilm are presumably more nutritious and digestible than cyanobacterial mats. Further, cyanobacteria are also known to produce allelopathic compounds, which among other functions can offer protection against grazers (Leflaive and Ten-Hage 2007). Allelopatic compounds with toxic effects for invertebrate grazers have been found in freshwater benthic cyanobacteria (Becher and Jüttner 2005). Assuming different attractiveness (nutritional value) and digestibility (capsular exopolymers and allelopathic compunds) between both types of biofilm, I hypothesize that shorebirds will preferentially graze on diatomaceous biofilms rather than cyanobacterial mats. Such an hypothesis predicts that small-bodied sandpipers will rely on biofilm at tropical sites during the winter and northward migration. Also, the hypothesis predicts that the reliance on biofilm will be higher at temperate than tropical sites (Beninger *et al.* 2011), and at muddy than sandy habitats (Kuwae *et al.* 2012).

The outcomes emerging from these predictions are important in a future environmental scenario where climate change (i.e. rising temperature) goes hand in hand with changes in land use (e.g. causeway construction, port development, dredging). If sandpipers are not using cyanobacterial mats as food source, then the synergistic effect of higher temperatures and human enhanced sediment erosion and eutrophication processes at intertidal systems (Paerl and Huisman 2009) can lead to critical spatiotemporal shortage of diatomaceous biofilm availability for sandpipers.

4.6. Acknowledgements

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4.8. Tables

Table 4.1. ***Sample sizes of sediment mud content, temperature, pore-water salinity and chlorophyll-a content at two Cuban estuaries (Río Máximo and Tunas de Zaza) during the shorebird southward migration (Oct. 2008) and wintering residency (Jan. 2009).***

	Río Máximo		**Tunas de Zaza**	
Variables (units)	**Oct. 2008**	**Jan. 2009**	**Oct. 2008**	**Jan. 2009**
Mud content (%)	9	-	9	-
Temperature (°C)	20	17	20	18
Salinity (‰)	20	11	20	18
Chl-*a* content ($\mu g\ g^{-1}{}_{DM}$)	18	19	17	25

4.9. Figures

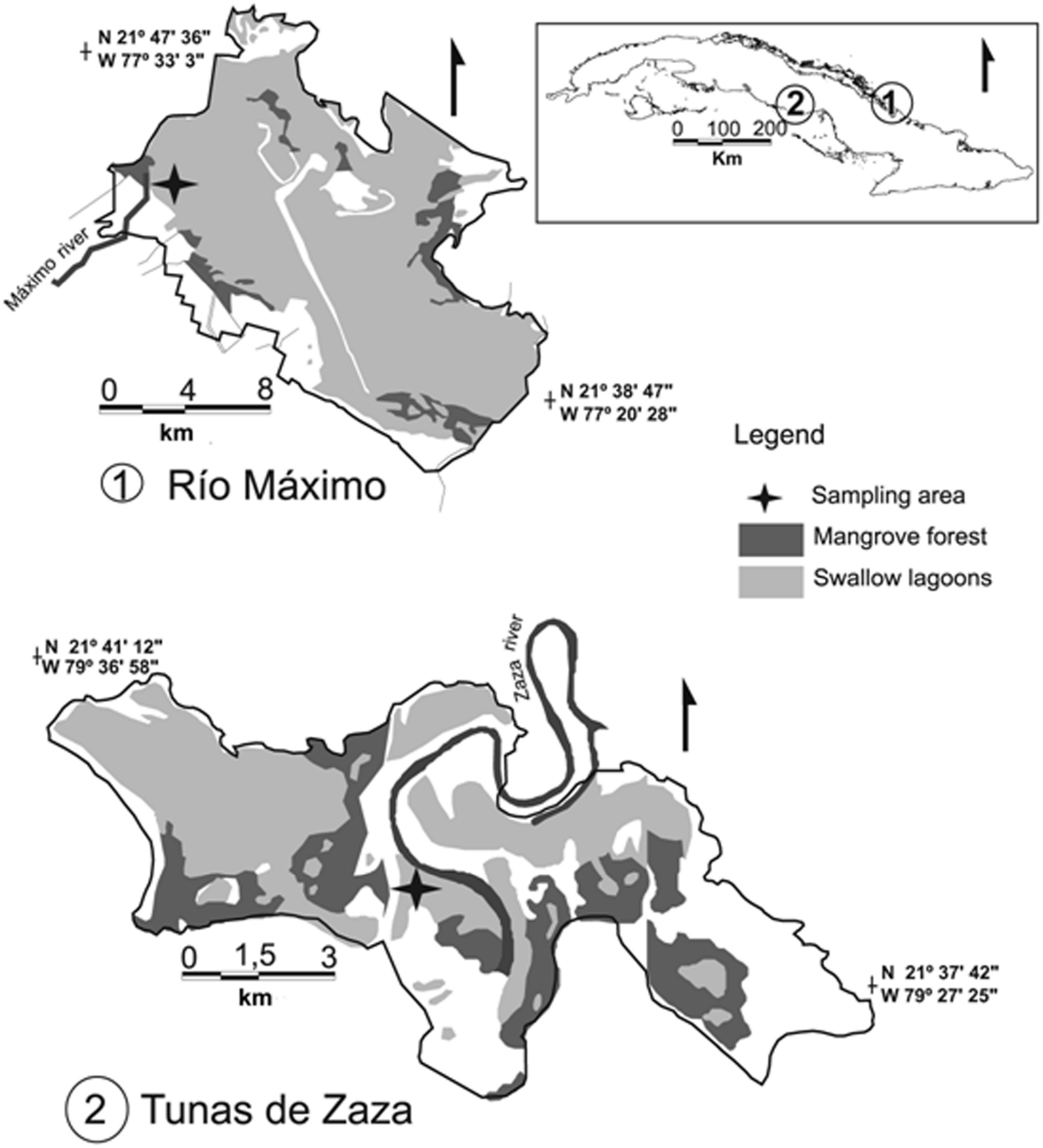

Figure 4.1. ***Map of the Cuban archipelago showing the two estuarine locations (1: Río Maximo, Camagüey; 2: Tunas de Zaza, Sancti Spitirus) where samples were collected***

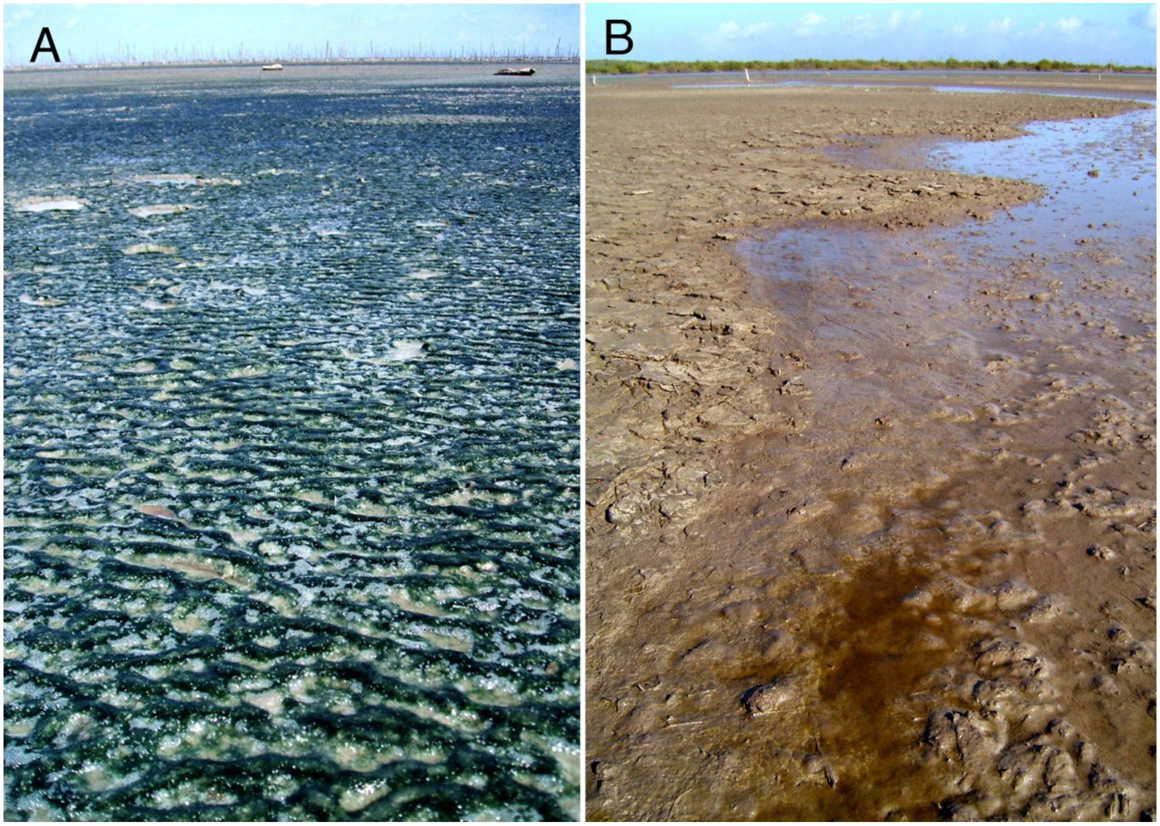

Figure 4.2. ***Seasonal shift of microphytobenthic biofilms at Río Máximo estuary (A: cyanobacterial mat in October 2008; B: diatomaceous biofilm in January 2009).***

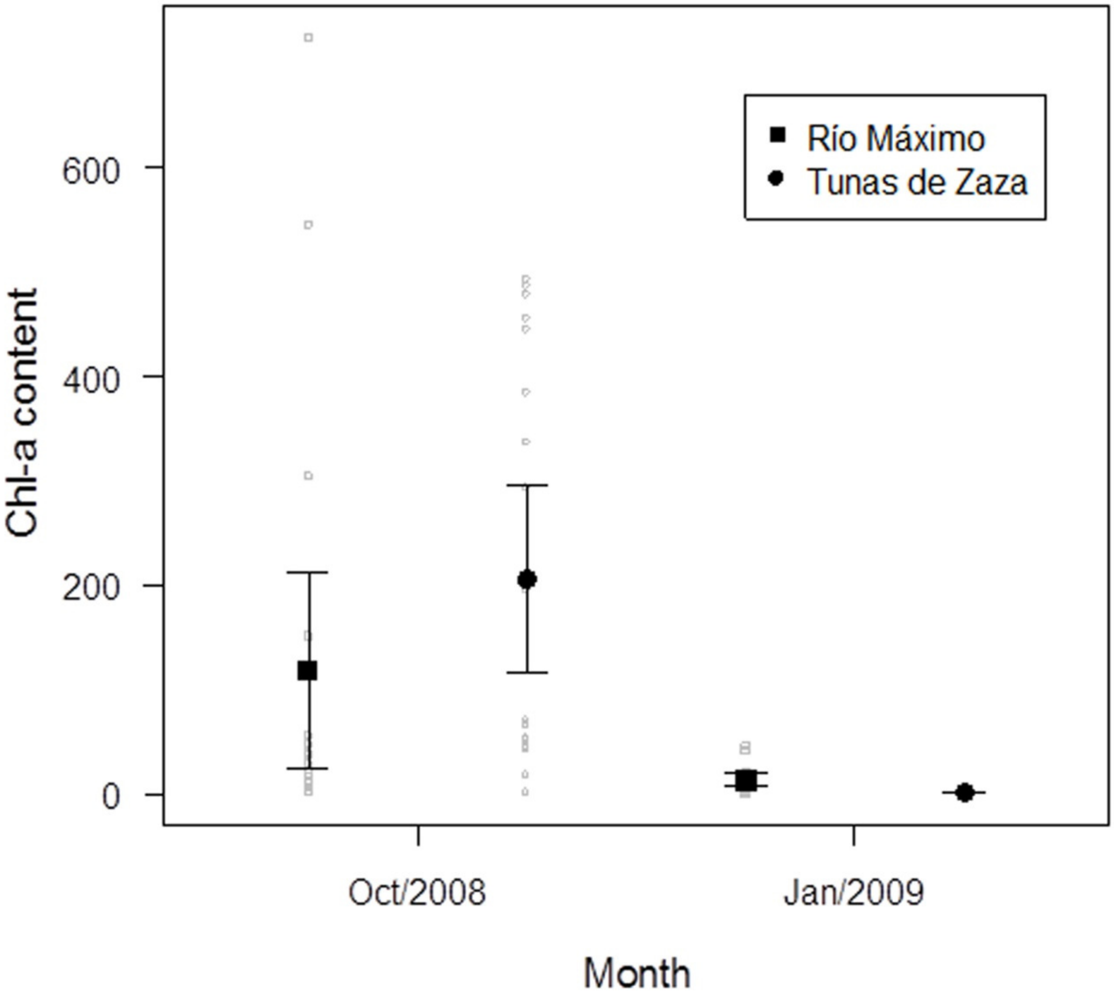

Figure 4.3. **Temporal variation in chlorophyll-a content (Chl-a, µg g^{-1}_{DM}) at Río Máximo and Tunas de Zaza estuaries during the shorebird southward migration (October 2008) and wintering residency (January 2009). Values are means ± 95% confidence intervals.**

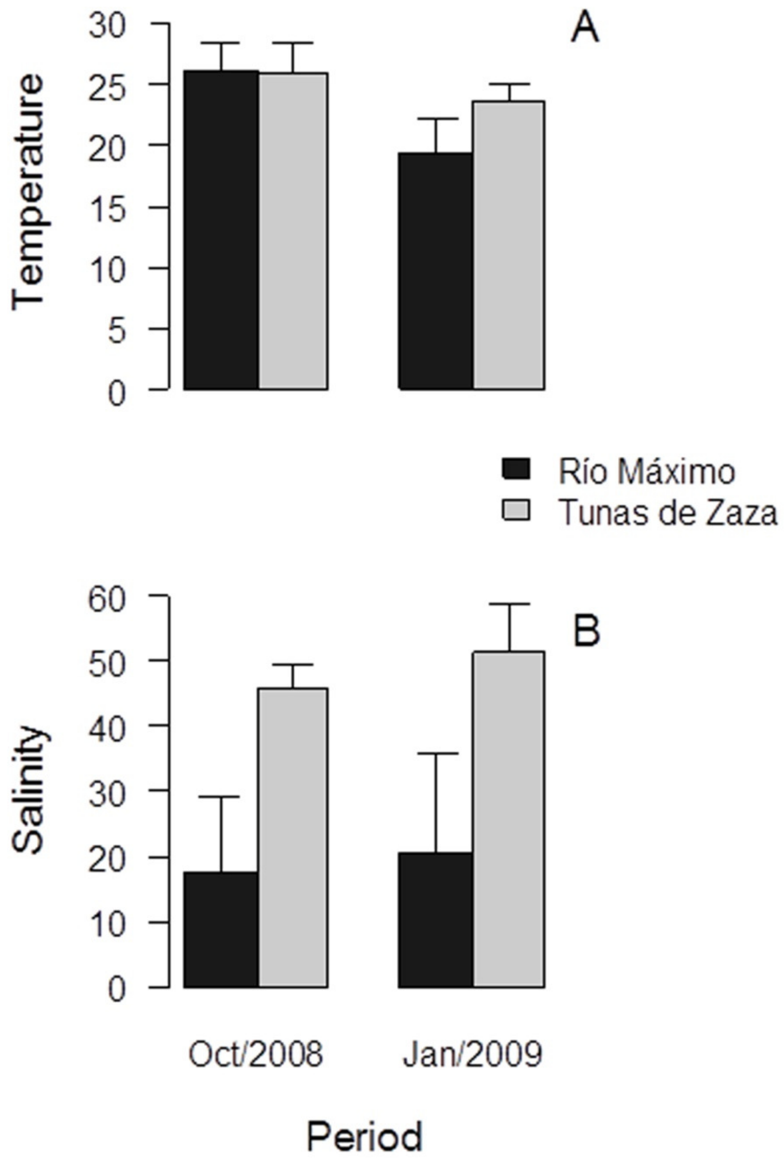

Figure 4.4. ***Sediment temperature (A, °C) and salinity (B, ‰) variation at Río Máximo (black bars) and Tunas de Zaza (grey bars) estuaries during the shorebird southward migration (October 2008) and wintering residency (January 2009). Values are means ± SD.***

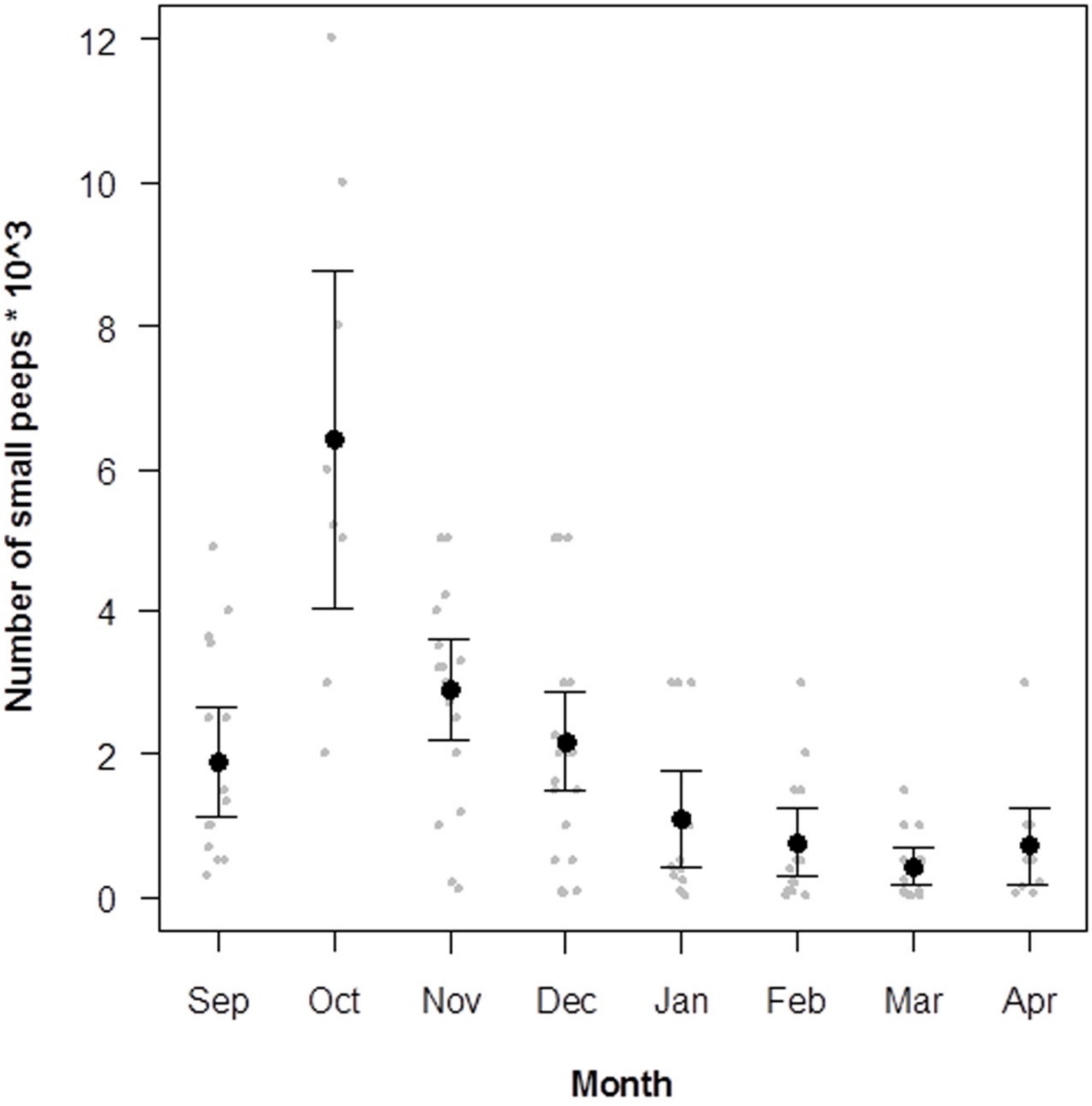

Figure 4.5. ***Monthly counts of small "peeps" (least, semipalmated and western sandpipers) at Río Máximo estuary, Cuba. Grey dots represents individual counts. Sample sizes are as follows: September, N=15; October, N=8; November, N=18; December, N=22; January, N=12; February, N=14; March, N=13; April, N=10. Values are means ± 95% confidence intervals.***

5. Does the type of microphytobenthic biofilm mediate shorebird foraging habitat use?: The role of biofilm and invertebrates on sandpiper distribution within the foraging habitat

5.1. Abstract

There is a lack of information about the role of biofilm availability on shorebird distribution. In theory, cyanobacterial mats should be less attractive to small-bodied biofilm grazing sandpipers, I hypothesize that the type of microphytobenthic biofilm (MPB, cyanobacterial mats and diatomaceous biofilm) mediates relationships between biofilm availability and spatial foraging patterns of the birds. I examined the extent to which MPB biomass, macro- and meiofaunal invertebrates influence sandpiper foraging habitat use at stop-over sites dominated by cyanobacteria (RM: Río Máximo, TZ: Tunas de Zaza) and diatomaceous biofilms (RB: Roberts Bank). Southward stop-over sites (RM and TZ) showed more MPB biomass and less invertebrate density than the northward stop-over site (RB). Sandpipers (mostly least sandpipers, *Calidris minutilla*) at RM and TZ used foraging habitats in accordance with macrofaunal invertebrate density. The positive relationship between birds and invertebrate density only operated for prey available at 0-10 mm sediment depth. Northward migrating western sandpipers (*Calidris mauri*) at RB upper intertidal sites concentrated their foraging efforts in patches with high MPB biomass. Overall, results appear to indicate that cyanobacteria-dominated biofilms are not used by shorebirds for biofilm feeding. I suggest that diatomaceous biofilm availability can be considered as an important limiting factor conditioning biofilm feeding and further sandpiper spatial distribution.

Keywords: biofilm availability, cyanobacterial mats, diatomaceous biofilm, calidridine sandpipers, habitat use, food availability

5.2. Introduction

Food availability at stop-over sites is pivotal for migrating shorebirds seeking to replenish their energy stores (Tsipoura and Burger 1999, Cohen *et al.* 2010). Accordingly, multiple studies have found a positive correlation between shorebird abundance and prey availability, but such relationships mostly apply across large spatial scales (e.g. when comparing between estuaries or distinctive habitats) (Wolff 1969, Goss-Custard *et al.* 1991, Yates *et al.* 1993, Taft and Haig 2006). Studies conducted at finer spatial scales (stations 10 – 100 m apart) (Wilson 1990, Wolff and Michaelis 2008, Zou *et al.* 2008) or exclosure experiments (Wilson 1991, Hamer *et al.* 2006, Kober and Bairlein 2006) have usually revealed either weak, no, or inverse relationships. Failure to "prove" fine scale positive relationships between shorebirds distribution and food may be caused, among other factors (e.g., predation danger, interference competition), by not considering the overall availability of food resources accessible to birds (Sutherland *et al.* 2000, Kuwae *et al.* 2012).

Migratory shorebirds have been described as opportunistic foragers by taking prey, hitherto benthic invertebrates, as they are available (Pienkowski 1983, Piersma *et al.* 1993, Santos *et al.* 2009). A flexible diet may assist shorebirds to deposit fat reserves as they migrate across thousands of kilometers (Davis and Smith 2001, Andrei *et al.* 2009, MacDonald *et al*. 2012). The wide spectrum of food sources that shorebirds may include in their diet is not limited to the benthic macro-invertebrates (invertebrates ≥ 0.5 mm, hereafter macrofaunal invertebrates) commonly considered in most habitat-use and resource-selection studies conducted on this group of birds (Zwarts and Wanink 1993, Pomeroy 2006, Santos *et al.* 2010). Zwarts *et al.* (1990) showed that approximately 40 % of the invertebrates eaten by shorebirds can pass through a 0.6 mm sieve. However, it took a further ten years, to demonstrate through exclosure experiments that western sandpipers (*Calidris mauri*) were able to remove meiofaunal (0.063 mm ≤ benthic invertebrates < 0.5 mm) as well as macrofaunal invertebrates (Sutherland *et al.* 2000). The ultimate capacity of shorebirds to use "unsuspected" food resources comes from the growing evidence for biofilm grazing by small-bodied sandpipers (< 30 g in body mass) (Elner *et al.* 2005, Kuwae *et al.* 2008, Mathot *et al.* 2010, Kuwae *et al.* 2012). Energy budget estimations have revealed that shorebird reliance on biofilm seems to be temporally- (Beninger *et al.* 2011) and spatially- (Kuwae *et al.* 2012, Quinn and Hamilton 2012) dependent, reaching a maximal contribution of about 70% of total diet in some localities.

Intertidal biofilm comprises a thin layer (< 3 mm) of a matrix-enclosed community of micro-organisms attached to the sediment surface (Decho 2000, Stal 2003). The matrix includes benthic bacteria and microphytobenthos (MPB) along with organic detritus and carbohydrate-rich exopolymeric substances mostly secreted by the MPB fraction (Underwood and Paterson 2003). Microphytobenthos is an artificial grouping of microalgae (diatoms) and photosynthetic bacteria (cyanobacteria) which are the main primary producers in most intertidal systems (Stal 2003, Debenay *et al.* 2007) and enhance benthic-pelagic coupling through the formation of biofilm (Leguerrier *et al.* 2003, Caramujo *et al.* 2005). Depending on the sediment grain-size and the ambient temperature, two different types of MPB biofilm may colonize the sediment surface. Cyanobacterial mats are favoured at high ambient temperatures and coarser sediments, whereas diatomaceous biofilms dominate at low temperatures and on mud (Watermann *et al.* 1999, Stal 2003). Cyanobacterial mats may also occur on mudflats but mostly during the summer and autumn months; seasonal shift in MPB dominance takes place as temperature decreases with diatom blooms occurring in the spring (van der Grinten *et al.* 2005).

In comparison to benthic invertebrate prey, little is known about the conditions that make biofilm available to shorebirds (Kuwae *et al.* 2008, Kuwae *et al.* 2012). In particular, there is a lack of information about the role of the two common types of MPB biofilm on sandpiper distribution while foraging. Given the poor nutritional quality (Yallop *et al.* 1994, de Winder *et al.* 1999, Huggins *et al.* 2004) and digestibility of cyanobacterial mats (Decho and Lopez 1993, Caramujo *et al.* 2005, Lukesová and Frouz 2007), these may be a less attractive class of biofilm for feeding shorebirds than diatomaceous biofilms (Chapter 4).

I hypothesized that the type of MPB biofilm mediates the relationship between biofilm availability and spatial use of foraging habitat by migrant small-bodied sandpipers. The hypothesis is based on the theoretical lower attractiveness of cyanobacterial mats compared to diatomaceous biofilm for grazing shorebirds. Thus, positive relationships between biofilm availability and sandpiper spatial use are more likely to emerge in habitats dominated by diatomaceous biofilm than in cyanobacteria-dominated sites. In the present study, I attempt to assess the extent to which MPB biomass, and macro- and meiofaunal invertebrates influence patterns of sandpiper foraging habitat use at stop-over sites with different MPB dominance. The hypothesis predicts that: (1) the foraging habitat use by sandpipers at stop-over sites dominated by cyanobacterial mats will not match biofilm availability and habitat use will be primarily influenced by invertebrate availability, and (2) the availability of biofilm will primarily influence foraging habitat use by sandpipers in stop-over sites dominated by diatomaceous biofilm.

5.3. Methods

5.3.1. Study sites

The study was carried out in three estuarine mudflats encompassing Cuban southward (Río Máximo, RM, 21°44'03.2"N, 67°31'17.9"W, and Tunas de Zaza, TZ, 21° 38' 34.4" N, 79°32'48.1"W) and Canadian northward shorebird stop-over sites (Roberts Bank, RB, 49°05'40"N, 123°12'41"W). Given that the method used for assessing sandpiper habitat use depends on the presence of high bird densities (see section 5.3.2 -Shorebird foraging habitat use-), the study at each particular habitat was restricted to periods of sandpipers peak abundances (RB: northward migration, April 26 – May 30, 2008, Butler 1994; RM and TZ: southward migration, October 10-20, 2008, Chapter 4).

Río Máximo and TZ are brackish (18.5 ± 12.9‰) and saline (48.5 ± 6.1‰) estuaries located in the north and south coast of the central part of Cuba, respectively. Both sites contain mudflats surrounded by mangrove forest and shallow coastal lagoons. Tides are semidiurnal in RM and mixed in TZ, and mean tidal heights are 0.70 m and 0.25 m, respectively. The upper 4 cm of sediment at both sites is characterized by mud composed of > 70% silt and clay (Chapter 4). Flooding regimen in RM sediments are highly dependent on north winds (i.e., wind-driven tides) driving water from the adjacent coastal lagoon to the mudflat (A. Jiménez unpubl. data). During the study, dense mats of filamentous cyanobacteria dominated the sediment surface (Chapter 4) and ambient temperature ranged between 23°C and 31°C.

Roberts Bank is a 6,000 ha. estuarine intertidal mudflat in the Fraser River delta, British Columbia. All work was conducted in the upper intertidal where the combination of high MPB biomass (Chapter 2) and fine sediments (>63% silt and clay, Chapter 3) may enhance shorebird biofilm feeding. The tidal height in this mesotidal estuary ranges from 0.0 - 3.8 m (Zharikov *et al.* 2009). Ambient temperature during sampling ranged from 11°C to 22°C. The dominant taxonomic group of MPB during shorebird northward migration at RB mudflats is diatoms (Kuwae *et al.* 2008, Beninger *et al.* 2011).

5.3.2. Study design

In this study, the inferences of sandpiper's spatial use are constrained to the foraging habitat within the studied estuaries. I defined foraging habitat as an area (~ 6 ha.) holding the highest congregations of foraging shorebirds. Sampling procedures followed the same basic design at each site. Sampling was conducted 1.5 to 2 h after emersion to guarantee shorebirds had enough time to use the area. Samples for food availability (biofilm, macro-, and meiofaunal invertebrates) and shorebird spatial use were taken along three transects located within the shorebird foraging habitat. Transects were 200 m long, spaced 100 m between each other and oriented perpendicular to shore. Each transect encompassed 40 units, each measuring 10 m x 50 m. A total of 20 units (6 or 7 random units per transect) were selected from each southward stop-over site and 30 units were sampled for the northward stop-over site (10 random units per transect).

Prey sampling

Two sediment samples were obtained within the randomly selected sampling units to examine for biofilm and benthic invertebrate availability. Samples were taken with a 26 mm internal diameter syringe (60 cc) which had been previously modified (apex removed and edges sharpened). Such a sampling core allows comparison with previous studies (Sutherland *et al.* 2000, Pomeroy and Butler 2005, Mathot *et al.* 2007).

Samples for biofilm were taken by slicing the top 2 mm of the mud surface extracted with the core. In Cuban estuaries, sediment samples for invertebrate assessment were taken up to 30 mm depth. Such a depth reflects the approximate range least, semipalmated, and western sandpipers could reach according to their bill length (15.4 - 29.2 mm) (Pyle 2008). Sediment samples were sliced into three 10 mm sections (0-10 mm, 11-20 mm, 21-30 mm). At RB, sediment samples for invertebrate assessment were taken only at 10 mm depth. Individual samples were placed in labelled plastic Ziploc bags and stored in a cooler, returned to the laboratory and stored at -20℃ until processing.

Shorebird foraging habitat use

In this manuscript I used the density of shorebird droppings as an index of cumulative shorebird foraging habitat use. The number of droppings in a 1 m^2 quadrat was highly correlated with the number of droppings in a 16 m^2 quadrat ($r_{68} = 0.91$, $P < 0.001$). Thus, sampling for shorebird dropping was conducted in a 16 m^2 quadrat (4 x 4 m) to minimize the amount of zero values in the sample. The area where prey samples were taken was used as the reference center for the sampling quadrat.

Dropping density has been used as evidence of spatial use of sandpipers in a given area during a tidal cycle (McCurdy *et al.* 1997, Pomeroy 2006, Zharikov *et al.* 2009). Dropping density can provide a measure of foraging shorebird activity during the tidal cycle because they are produced frequently while birds search for prey on the exposed intertidal sediments (e.g., 0.5 dropping $min.^{-1}$ at RB, Kuwae *et al.* 2008; 0.2 dropping $min.^{-1}$ at RM, A. Jiménez unpubl. data) and are washed away during high tide. Cumulative indexes, such as this, are preferable over instantaneous bird counts when the goal is to determine overall habitat use because the index considers both bird abundance and length of stay (Hamilton *et al.* 2003). Because the number of shorebirds at stopover sites varies on a daily basis (e.g. Fig. 4.5 in Chapter 4), dropping density at each quadrat was adjusted by the total number of droppings counted in all quadrats in the sampling day. The daily based proportion of droppings at each sampled unit helped to standardize for daily variation in shorebird numbers.

Inferences on habitat use drawn from dropping density are only useful in situations where a single species is numerically dominant or when the species involved have easily identifiable droppings. At RB, both conditions were met because western sandpiper are by far the most abundant shorebird during northbound migration (Butler 1994) and their droppings are smaller than dunlin (*Calidris alpina*), the only other common shorebird present at the estuary at the same time (Chapter 3). However, in Cuban estuaries assessing shorebird habitat use through dropping density was not as easy as on RB. Foraging sandpiper flocks in Cuban habitats are mostly comprised of least, semipalmated and western sandpipers (Mugica *et al.* 2006, Acosta *et al.* 2011), but there is no information about the proportions at which each species occurs. Further, the similar size of these species (Pyle 2008) suggests that there would be no clear dropping size differentiation. Thus, no attempt to distinguish between species droppings at Cuban estuaries was made in this study. However, shorebird counts within the foraging habitat were conducted at RM (N = 17) and TZ (N = 16) to account for species composition and contribution to the mixed sandpiper foraging flock. Hence, I assumed that inferences drawn for sandpiper foraging habitat use in Cuban estuaries are most likely for species contributing the most to mixed foraging flocks.

5.3.3. Laboratory analysis

Chlorophyll-*a* is a known estimator for MPB biomass in intertidal sediments (Underwood and Smith 1998, Pomeroy and Butler 2005, Hanlon *et al.* 2006). Therefore, I used Chl-*a* content as an index of biofilm availability. Frozen sediment samples for Chl-*a* content determination were transferred to 20 ml scintillation vials, weighed to the nearest 0.01 g and thawed in the dark before analysis. Ten ml of 90 % acetone solution was added to the vials and samples were thoroughly shaken for 2 min. in a vortex and placed into a dark box at -4 ℃ for 24 h. for extraction. The amount of Chl-*a* in the supernatant was measured spectrophotometrically using a Genesys 10uv spectrophotomer. Absorbances at 665 and 750 nm were measured; samples were acidified with two drops of 0.1 N HCL, mixed, left for 1 min. and absorbance was measured again at the same wavelengths. Concentration of corrected Chl-*a* was calculated using Lorenzen's spectrophometric equations (Lorenzen 1967). Chlorophyll-*a* content was expressed as $\mu g\ g^{-1}{}_{\text{dry sediment (DM)}}$ (weigth-normalized values).

Samples for benthic invertebrate assessment were filtered through two stacked sieves with mesh sizes of 0.5 and 0.063 mm. The material retained in the sieves was used to assess the macro- and meiofaunal density, respectively. For the meiofaunal fraction, I took into account only the density of the two most abundant organisms: nematodes and copepods (Sutherland *et al.* 2000, Pinckney *et al.* 2003). In order to separate copepods and nematodes from the sediments retained in the 0.063 mm sieve, I re-suspended the sample in 200 ml of sugar saturated solution (950 g of brown sugar in 1200 ml of water). The suspension was allowed to settle for 20 min. before the supernatant was decanted through the 0.063 mm sieve. The process was repeated three times. The method allowed a copepod and nematode extraction efficiency > 90 % (A. Gonzalez and A. Jiménez, in preparation). The macro- and meiofaunal invertebrates were transferred to labelled 20 ml vials containing 85 % ethanol. Using a stereomicroscope (Leica Wild M8) at 60x, I sorted, identified (Culter 1986), and counted all invertebrates of taxonomic groups documented in shorebird diets (Wolf 2001, Andrei *et al.* 2009). Invertebrate density is given as number of individuals per sampling core.

5.3.4. Data analysis

I performed statistical analyses with R version 2.10.1 (R Development Core Team 2009). Assumptions of normality and homogeneity of variance were assessed visually for all models results. When violations were detected, they were managed through transformation (natural logarithm + 1 transformation for Chl-*a* content and macrofauna density, and square root transformation for daily standardized dropping count).

Contingency tables were employed to test whether the vertical distribution of benthic invertebrates showed differences between the two sampled Cuban estuaries. Analysis of Variance (ANOVA) was conducted to assess overall differences in prey abundance (MPB biomass and benthic invertebrates) at the studied stop-over sites. To allow for comparability between study sites, only the upper most sediment section was compared (0-10 mm). When significant main effects were found, a post-hoc Tuckey's test was used to isolate differences between levels of factors. Pearson correlation coefficients were used to test relationships between food sources in each stop-over site. If not otherwise stated, here I present means with standard deviation (± SD). All tests were two-tailed and the level of significance was $P < 0.05$.

I used multiple linear regression models and an information-theoretic approach to explore the simultaneous effect of MPB biomass, macro- and meiofaunal invertebrate density on sandpiper habitat use. The response variable in my analysis was the daily standardized dropping proportion and the predictor variables included the MPB biomass (CHL) and the macro- (MACRO) and meiofaunal density (MEIO). The CHL was assessed through the measurement of sediment Chl-*a* content. All benthic invertebrates recovered in the macrofaunal fraction (except for nematodes) have been described in the shorebirds diet (Wolf 2001, Andrei *et al.* 2009). Therefore, macrofaunal invertebrate were grouped under the variable MACRO (overall number of macrofaunal invertebrates per core). The interaction between the copepod (COP) and nematode (NEM) density was used to address the variable MEIO. The use of the interaction between these two meiofaunal resources accounted for the presumed shorebird preference for copepods but no nematodes as revealed by: (1) the presence of copepods in shorebird stomach and dropping contents (Wolf 2001, Mathot *et al.* 2010), (2) the shorebird depletion effect on copepods and the lack of such effect on nematodes as measured by exclosure experiments (Sutherland *et al.* 2000), and (3) the lack of nematodes in shorebird stomach contents (Recher 1966, Senner *et al.* 1989, Davis and Smith 2001, Andrei *et al.* 2009) or feces (Wolf 2001, Kober and Bairlein 2006). Therefore, the use of the interaction between copepods and nematodes took into account both the overall meiofaunal density and the individual contribution of these small invertebrates in the foraging patch. The effect of location (LOC: RM and TZ) was also considered in models run for southward stop-over sites. Individual quadrats were treated as the sampling unit.

Because southward (Cuba) and northward (Canada) stop-over sites included different MPB and shorebird assemblages, migratory periods and predictor variables (LOC in Cuban models), I conducted separate analyses for each. A set of 15 *a priori* candidate models was tested for the southward stop-over sites, and a set of 8 *a priori* candidate models was tested for the northward stop-over site. Only additive effects between predictor variables were considered in order to reduce the number of possible models and minimize over-fitting the data (Anderson and Burnham 2002). There was an additional consideration for the analysis for Cuban southward stop-over sites. I conducted separated analyses of sandpiper habitat use under two different levels of prey availability for shorebirds in Cuban estuaries. My intention was to examine the effect of prey with different level of availability (“available” and “highly available”) for explaining sandpiper habitat use. Thus, I pooled the benthic invertebrates recovered in the three sectioned layers of sediment and classified this as “available” (0-30 mm). The same set of models was run when taking into account only the prey recovered from the first 10 mm of sediment and I designated this invertebrate fraction as “highly available” (0-10 mm). The Cuba “highly available” set of models is comparable with prey availability measured for RB.

I used Akaike’s information criterion adjusted for small sample sizes (AIC_c) to rank candidate models (Burnham and Anderson 2002). When the difference in AIC_c (ΔAIC_c) between the top-ranked model and the model in question was <2.0, the model was considered to have substantial empirical support in relation to all models examined (Burnham and Anderson 2002). Models with $\Delta AIC_c = 2\text{-}4$ were considered to have some evidence; all others were considered as having weak evidence ($\Delta AIC_c = 4\text{-}7$) or no evidence ($\Delta AIC_c > 7$) to be the best approximating model. Akaike weights (ω_i) were used to determine the relative likelihood of each model given the data. In the absence of an unambiguously best supported model ($\omega_i \geq 0.9$), I conducted the recommended modeling averaging approach over the set of models (Burnham and Anderson 2002). The inclusion of the null model in the best subset was interpreted as a lack of relevant predictors in the analysis. Using the resulting subset of models I calculated the relative variable importance weight (RVI), which is a measure of the importance for each variable, by summing model weights for all models containing that variable (Burnham and Anderson 2002). I used estimates of model averaged slope coefficients (β), unconditional standard errors and their 95 % confidence intervals to assess direction, magnitude and reliability of detected relationships (Burnham and Anderson 2002). I also reported R^2 as a measure of model fit (Symonds and Mousalli 2011).

5.4. Results

5.4.1. Benthic invertebrate vertical distribution at Cuban southward stop-over sites

Benthic invertebrate density for Cuba followed a decreasing gradient with sediment depth. Highest abundances of both invertebrate fractions were observed in the top 10 mm of the sediment (Fig. 5.1). The top layer of sediment held 59 % and 51 % of total macrofauna density in RM and TZ, respectively (Fig. 5.1A). There were differences between southward stop-over sites in the number of macrofaunal invertebrates per sediment layer (Chi-squared = 15.70, d.f. = 2, $P < 0.001$). The first 10 mm of sediment in RM had 35 % more macrofaunal invertebrates than the second layer (11-20 mm), whereas in TZ the difference between the first and the second layer was 14 %.

The meiofaunal fraction showed a steeper decreasing gradient in the sediment than the one detected for the macrofaunal fraction (Fig. 5.1B). The top 10 mm contained 92 % and 93 % of the total meiofaunal resources counted in the sediment cores obtained in RM and TZ, respectively. Differences in the meiofaunal density according to sediment depth were detected between sites (Chi-squared = 7.72, d.f. = 2, $P = 0.021$).

5.4.2. Prey availability at shorebird stop-over sites

Overall, the two southward stop-over sites were characterized by low densities of benthic invertebrates and high Chl-*a* content when compared to the northward stop-over site (Table 5.1). The ANOVAs indicated a significant difference in the average food source abundances among the stop-over sites (Chl-*a* content: $F_{2,67} = 32.15$, $P < 0.001$; macrofaunal density: $F_{2,67} = 21.06$, $P < 0.001$; meiofaunal density: ANOVA, $F_{2,67} = 7.48$, $P = 0.001$). *Post-hoc* comparisons indicated that average Chl-*a* content was significantly higher at southward stop-over sites than at the northward stop-over site ($P < 0.001$ for both pair-wise combination), while there were no significant differences in average Chl-*a* content between the two southward stop-over sites ($P = 0.170$). The average macrofaunal density was significantly higher in the northward stop-over site than at the two Cuban estuaries (RB-RM: $P < 0.001$, RB-TZ: $P < 0.001$). Average macrofaunal density was not significantly different between the two southward Cuban estuaries (P =

0.822). The northward stop-over site also showed higher average meiofaunal density than the southward stop-over sites (RB-RM: P = 0.006, RB-TZ: P = 0.004), while there was no significant differences between the Cuban sites (P = 0.990). Differences in overall meiofaunal density between study areas was entirely due to significant differences in nematode density at each stop-over site ($F_{2,67}$ = 139.51, $P < 0.001$). Copepod density was not significantly different between stop-over sites ($F_{2,67}$ = 2.35, P = 0.103).

5.4.3. Benthic invertebrate composition

Macrofaunal invertebrates in Cuban estuaries were composed of 10 taxa (Fig. 5.2). Diptera (pupae and larvae of Chironomidae and Ephydridae) and aquatic hemiptera (Corixidae) made up 90 % of the total density of macrofaunal invertebrates found in RM. In TZ the most numerically abundant macrofaunal resource was tanaids (53 %) followed by diptera (25 %). Ostracods made moderate contributions to the total macrofauna density at TZ (9 %). The remaining taxa (Foraminiferida, Nematoda, Sipunculidae, Polychaeta, Oligochaeta and Plecoptera) were present in small numbers and none accounted for > 3 % of the total.

Eight macrofaunal invertebrate taxa were found at RB. Polychaetes made the most important numerical contribution to the macrofaunal fraction (67 %), followed by nematodes (12 %) and bivalves (9 %). Crustaceans such as copepods, amphipods, ostracods, and tanaids made up the remaining macrofaunal invertebrate faction, but none of them contributed to more than 5 % to the total macrofauna density.

The two taxa considered for assessing the meiofaunal fraction of benthic invertebrates (nematodes and copepods) differed in numerical importance between subtropical and temperate stop-over sites (Fig. 5.3). Copepod density was higher than nematode density in the Cuban estuaries, whereas the opposite trend was found at RB. The copepod dominance was especially noticeable in RM where nematode density was extremely low.

5.4.4. Associations between food sources

There were significant correlations between some of the potential food sources in the TZ estuary, but none at RM (Table 5.2). At TZ, copepod density was negatively correlated with macrofaunal density and positively associated with Chl-*a* content. These associations were strongest when analyses were conducted taking into account only the "highly available" prey (0-10 mm depth). However, when analysing the whole sample depth (0-30 mm) a moderate negative correlation emerged between the macrofaunal density and Chl-*a* content.

Significant positive correlations occurred in RB between Chl-*a* content and the density of the two meiofaunal components analyzed (Chl-*a* *vs*. Copepod: $r_{28} = 0.49$, $P = 0.010$; Chl-*a* *vs*. Nematode: $r_{28} = 0.62$, $P < 0.001$). Also, there was a strong positive correlation between the two meiofaunal components ($r_{28} = 0.65$, $P < 0.001$). The macrofaunal density did not show any significant association with the rest of the measured potential food sources ($r_{28} = 0.22 – 0.36$, $P = 0.063 – 0.326$).

5.4.5. Shorebird assemblage in southward stop-over site and overall dropping density by site

Overall abundance of small-bodied sandpipers was greater at RM than TZ (RM: 7050.0 ± 3607.6; TZ: 1833.3 ± 683.1; $t_{10} = 3.48$, $P = 0.006$). Mixed flocks of small sandpipers were dominated by least sandpiper which accounted for 70% and 80% of individuals within flocks for RM and TZ, respectively (Fig. 5.4). There was a significant difference in flock composition between Cuban stop-over sites (Chi-square = 27.95, d.f. = 2, $P < 0.001$). The difference was caused by a higher proportion of semipalmated sandpipers in the mixed foraging flocks at RM compared to TZ.

Dropping density in the studied estuaries ranged from 0 to 128 droppings per 16 m^2. The ANOVA indicated a significant difference in the average dropping density among the stop-over sites ($F_{2,67} = 37.51$, $P < 0.001$). *Post-hoc* comparisons indicated that average dropping density was significantly higher at the northward stop-over site (RB: 55.9 ± 29.2, range: 23-128, N = 30) than the two southward stop-over sites (TZ: 18.3 ± 22.5, range: 0-77, N = 20; RM: 9.6 ± 8.6, range: 0-28, N = 20) ($P < 0.001$ for both pair-wise comparisons), while there was no significant difference in average dropping density between the two southward stop-over sites ($P = 0.463$).

5.4.6. Shorebird foraging habitat use in southward stop-over sites with cyanobactertial mats

When all "available" food sources were considered (0-30 mm sediment deep), habitat use was best explained by the null model (Table 5.3) suggesting that birds were randomly distributed within the foraging habitat. A plausible alternative ($\Delta AIC_c < 2$) was the model that featured macrofaunal invertebrates (MACRO). Another seven models acquired mentionable support from the data ($\Delta AIC_c < 4$). The inclusion of the null model as the top ranked model within the set ($\omega_{1,2,5,6,9,10,14,12,13} = 0.94$), and the relatively low Akaike weight and poor fit ($\omega_2 = 0.20$, $R^2 = 0.05$, respectively) of the second best competing model indicated that shorebird habitat use could not be reliably modeled only by the effect of the different food sources available. Therefore, results from the data set considering the "available" prey were excluded from any further analyses.

Unlike the analysis conducted with the "available" prey data, substantially lower uncertainty in model selection emerged when data was analyzed only for the "highly available" prey (0-10 mm of sediment, Table 5.4). Four models out of 15 received more than 90% of Akaike weight ($\omega_{2,6,9,13} = 0.93$) and the null model was not among them. The models featuring the effect of macrofaunal density and the additive effect between macrofaunal density and MPB biomass (CHL) had the strongest support and were equally parsimonious ($\Delta AIC_c < 2.0$). These two models represent 71 % of total Akaike weight and despite the relatively low power in the modelling effort ($R^2 = 0.20$), the top two models carried 16 and 12 times more support than the null model, respectively. Models including the meiofaunal invertebrate fraction were ranked below the null model and received either weak or no support from the data ($\Delta AIC_c = 7.56 – 12.97$).

Figure 5.5 shows the relationship between shorebird habitat use and prey availability as featured by the most parameterized best supported model. Sandpiper foraging habitat use increased as macrofaunal density increased in the foraging patches. Such a relationship seemed more important in MPB biomass rich patches (> 50 µg $g^{-1}{}_{DM}$, N = 17) than at those patches with low Chl-*a* content (< 50 µg $g^{-1}{}_{DM}$, N = 19).

The most important predictor over the subset of four models accounting for more than 90% Akaike weight (Table 5.5) was macrofaunal density. The relative variable importance (RVI) value of 1.0 clearly indicated that any model without this variable contributed little to the overall summed model weight. The effect of macrofaunal density over shorebird habitat use was positive and its confidence limit did not overlap zero, indicating consistent effect across models (Table 5.5). Chlorophyll-*a* content had a moderate RVI compared with the other analysed predictor (LOC), but both predictors showed confidence limits overlapping the zero value. Such results are indicative of a poor effect of Chl-*a* content and study location on shorebird foraging habitat use.

5.4.7. Shorebird foraging habitat use in a northward stop-over site with diatomaceous biofilm

Shorebird foraging habitat use was best explained by the model containing only the MPB biomass estimator (Table 5.6, ω_5 = 0.69, R^2 = 0.46). The evidence ratio between the best model and the null model indicated that shorebird habitat use explained by Chl-*a* content was about 2,851 times more likely than expected from random. Overall, models including Chl-*a* content ranked higher and had a better overall fit (R^2 = 0.43 - 0.46) than all other models not considering this variable (R^2 = 0.05 - 0.09). Models considering the single or additive effect of the two invertebrate fractions (macro- and meiofaunal invertebrates) were ranked below the null model and had no support from the data (Table 5.6, ΔAIC_c = 16.68 – 19.81).

On the basis of model averaging using the two best ranked models (Table 5.6, $\Delta AIC_c < 4$, $\omega_{5,6}$ = 0.91), shorebird foraging habitat use was best described by the positive effect of MPB biomass (Fig. 5.6; CHL, β = 0.121 ± 0.025) which had the highest possible RVI (1.0) and the 95% confidence interval did not include zero (0.071, 0.171). Contrary to MPB biomass, macrofaunal density had a negative effect on the response variable; it also had considerable smaller RVI (0.24), effect size (β = -0.003 ± 0.009) and its 95% confidence interval widely bounded zero (-0.021, 0.014).

5.5. Discussion

The findings suggest that the type of MPB biofilm can mediate the relationship between biofilm availability and biofilm feeding by small-bodied sandpipers. Overall results appear to indicate that cyanobacteria-dominated biofilms are less attractive for biofilm feeding shorebirds. Sandpiper foraging habitat use was invertebrate-oriented at tropical sites dominated by cyanobacterial mats, whereas a biofilm-oriented habitat use mode was noted at the northward stop-over site exhibiting diatomaceous biofilms.

5.5.1. Foraging habitat use at southward and northward stop-over sites

Flocks of small-bodied sandpipers (mostly least sandpipers) used the foraging habitat according to macrofaunal density at Cuban southward stop-over sites dominated by cyanobacterial mats. The result is consistent with previous published works (Colwell and Landrum 1993, Sánchez *et al.* 2006, Cohen *et al.* 2010), which found migratory sandpipers occurring in highest numbers at sites with proportionally greater invertebrate availability. However, such a food-based distribution pattern was only evident when considering the “highly available” prey (0-10 mm deep). Neither meiofaunal invertebrates nor MPB biomass explained the sandpiper distribution pattern within the foraging habitat. The relationship between sandpipers’ foraging habitat use and macrofaunal invertebrates was consistent between sites (RM and TZ), irrespective of differences in environmental variables (i.e. flooding-emersion regime, salinity), prey availability (Fig. 5.1) and composition (Fig. 5.2), and sandpiper numerical abundance (Fig. 5.4). Therefore, the prediction stating that foraging habitat use by small-bodied sandpipers in cyanobacterial dominated stop-over sites will be primarily influenced by invertebrate availability was supported by the Cuban southward migration data.

Also, the second prediction stating that availability of biofilm (MPB biomass) will primarily influence foraging habitat use by sandpipers at sites dominated by diatomaceous biofilm was supported by the data collected from the northward stop-over site (RB). The data obtained at the upper intertidal in RB showed unambiguously that western sandpiper foraging habitat use during northward migration was primarily influenced by MPB biomass. Western sandpipers feeding in the upper intertidal section at RB were less sensitive to benthic invertebrate availability than to biofilm availability. Such a finding is contrary to multiple studies demonstrating the overwhelming importance of invertebrate prey in determining the spatial distribution of small-bodied sandpipers (Goss-Custard *et al.* 1991, Yates *et al.* 1993, Sutherland *et al.* 2000). However, the positive association between MPB biomass and western sandpiper spatial use is consistent with the important role of biofilm in the species diet (Kuwae *et al.* 2008, Beninger *et al.* 2011, Kuwae *et al.* 2012). These latter studies demonstrate that benthic diatoms and biofilm are by far the most important food source of western sandpiper, contributing to 45 – 70 % of the species' total diet. Therefore, the present study lends additional support to the importance of intertidal biofilm for migrant western sandpipers using RB during the northward migration.

Moreover, the results at Roberts Bank provide strong support for the hypothesis that the western sandpiper is capable of using inter-specific niche partitioning strategies (Chapter 3) based on their greater capability to consume MPB biofilm (Elner *et al.* 2005). Contrary to dunlin, western sandpiper "non-tide following" behaviour and extensive use of the intertidal section closest to shore (< 350 m from shore) matched more closely with the distribution of diatomaceous biofilm than with invertebrate density within the upper intertidal (Chapter 3). However, this study (Chapter 3) did not simultaneously measure food availability and shorebird abundance at the same spatio-temporal point. Therefore, alternative explanations for the extensive use of the upper intertidal by western sandpipers also consider the less likely possibility of the species using invertebrates either trapped in shallow pools or burrowed in the sediment. The sampling design and the data obtained at Roberts Bank in the current study (Chapter 5) overcome the limitations of the previous study (Chapter 3). The results obtained here do not provide support for the invertebrate-oriented alternative hypotheses trying to explain the peculiar behaviour and distribution of western sandpiper at the upper intertidal. Rather, the data strongly agree with western sandpiper distribution according to the general pattern of MPB biofilm distribution in Roberts Bank's upper intertidal zone.

5.5.2. Food sources

The high MPB biomass values at southward stop-over sites are consistent with values recorded for cyanobacteria dominated biofilms (Dijkman *et al.* 2010). Also, the greatest relative abundance of “highly available” invertebrates at the northern stop-over site is in accord with previous studies (Mathot *et al.* 2007, Purwoko and Wolff 2008), showing that intertidal invertebrate density tends to be higher at northern than southern sites.

Interestingly, the patterns of habitat use exhibited by foraging sandpipers followed the opposite direction of food availability at each site. At sites with higher MPB biomass (RM and TZ), sandpipers used foraging habitat in accordance with invertebrate density, while at the site with higher invertebrate density (RB), foraging habitat use was related to MPB biomass. At least three mechanisms can be hypothesized to explain the contradictory result between food availability and sandpiper habitat use. The different food-based orientation in habitat use at stop-over sites can be caused either by the effect of *i)* inter-specific differences in sandpipers’ biofilm preferences (western sandpiper in RB, and a mix of three sandpipers dominated by least sandpiper in RM and TZ) (Kuwae *et al.* 2012), *ii)* temporal changes in birds dietary preferences as triggered by temporally different physiological demands (e.g. seasonal changes in digestive enzyme activities or metabolic requirements) (Beninger *et al.* 2011) or *iii*) spatio-temporal changes in MPB biofilm availability (Recher 1990) as measured by the type of MPB biofilm.

The data obtained in this study did not provide support for any of the proposed hypotheses; therefore, differentiating between them will require further comparative analyses. However, I speculate that the more likely hypothesis states that opposite directions between food availability and patterns of calidridine sandpiper habitat use are influenced by spatio-temporal changes in the type of MPB biofilm (*iii*). My speculation is based on the existing knowledge of sandpiper biofilm feeding ecology (Beninger *et al.* 2011, Kuwae *et al.* 2012, Quinn and Hamilton 2012) and the known differences in the nutritious quality of cyanobacteria and diatomaceous biofilms (Huggins *et al.* 2004, Dijkman *et al.* 2010, Lukesová and Frouz 2007).

First, the inter-specific differences in biofilm preference hypothesis (*i*) is challenged by results showing that biofilm feeding adaptations (length and total area of tongue spines) are negatively associated with shorebird body size (Kuwae *et al.* 2012). Thus, the small size of least and semipalmated sandpipers suggests that these species are as well adapted as western sandpipers for biofilm feeding. Further, plasma isotopic analysis of semipalmated sandpiper at Bay of Fundy supports biofilm consumption by the species (Quinn and Hamilton 2012). Also, a similar study on least sandpiper at Roberts Bank suggests biofilm use, especially by females (Gardiner 2012).

Second, biofilm feeding by sandpipers during early southward migration (8 July – 14 September, Gardiner 2012; August, Kuwae *et al.* 2012; 10 – 19 August, Quinn and Hamilton 2012) disagrees with a temporal change in birds' dietary preferences as triggered by temporally different physiological demands (*ii*). However, a study analysing different tissue compartments (muscle, liver, and stomach content) of western sandpipers captured at Roberts Bank did show a temporal change in biofilm consumption by western sandpipers (Beninger *et al.* 2011). The later study revealed a gradual downward shift in the mean trophic position of western sandpipers diet as northward migration progressed. Contrary to other stop-over sites on northward migration, the isotope signatures from birds at Roberts Bank indicated large contribution of diatoms (> 75 %) and particulate organic matter (20 %), and low contribution of suspension-feeding bivalves (< 5 %) (Beninger *et al.* 2011). The authors suggested that the progressive shift to lower trophic levels may be linked to a physiological transition associated with changes in digestive enzyme activities during northward migration (Stein *et al.* 2005).

The documented results regarding a temporal biofilm dietary shift by sandpipers (Beninger *et al.* 2011) certainly does not rule this out as a possible cause for differences in sandpiper habitat use at southward and northward stop-over sites in this study. Nonetheless, a seasonal change in sandpiper physiological requirements is not the only feasible explanation for a seasonal effect on sandpipers' MPB biofilm use. Temporal differences in MPB biofilm use by birds can also emerge as a result of seasonal shifts between diatomaceous and cyanobacterial biofilms (Chapter 4), hence, supporting the spatio-temporal differences in biofilm use as an effect of temporal changes in the type of MPB biofilm (*iii*). Given that cyanobacterial mats are dominant at higher temperatures (i.e. lower latitudes areas) and assuming sandpipers are less attracted by cyanobacteria biofilms (see below), it is possible to expect a temporal shift in sandpiper biofilm consumption such as that detected by Beninger *et al.* (2011). Higher biofilm use by sandpipers is expected to occur at northern sites during spring, when temperatures favour diatoms over cyanobacteria (Watermann *et al.* 1999).

Finally, further support for spatio-temporal differences in biofilm use by shorebirds due to different types of MPB biofilm comes from notable differences in the nutritious quality of cyanobacteria and diatoms (i.e. nutrient content, Huggins *et al.* 2004; digestibility, Caramujo *et al.* 2005, Lukesová and Frouz 2007). The basic energy blocks, carbohydrates and fatty acids, are present in both classes of MPB biofilms (Huggins *et al.* 2004). However, diatomaceous biofilms are richer in easily digestible carbohydrates (de Winder *et al.* 1999) and long-chain poly-unsaturated fatty acids (Huggins *et al.* 2004, Dijkman *et al.* 2010) than cyanobacterial mats. In particular, diatoms, but not cyanobacteria, contain eicosapentaneoic acid (20:5 n-3) and docosahexaneoic acid (22:6 n-3) (Dijkman *et al.* 2010), two fatty acids that have been shown to enhance the development of sandpiper flight muscles in preparation for long distance migration (Maillet and Weber 2006, 2007). Further, the diatom content of storage lipids (palmitic acid, 16:0; plamitoleic acid, 16:1), essential fatty acids (linoleic acid, 18:2 n-6), and lipids associated with better metabolic performance (20:5 n-3, 22:6 n-3) (Dijkman and Krompkan 2006, Dijkman *et al.* 2010) are similar to fatty acid content recorded in shorebird prey (e.g. *Corophium*, Napolitano and Ackman 1990).

On the other hand, diatomaceous biofilm contains much more colloidal than capsular carbohydrates, while the opposite trait is found in cyanobacterial mats (Yallop *et al.* 1994, de Winder *et al.* 1999). This difference between colloidal and capsular carbohydrates in biofilms has important ramifications for biofilm feeding, as capsular carbohydrates usually act as a defence against grazing in biofilm-forming cyanobacteria (Decho and Lopez 1993). Caramujo *et al.* (2005) experimentally showed that copepods are capable of digesting diatoms; however, more than half of all cyanobacterial cells remained intact after gut passage suggesting that ingestion rates may differ among MPB classes. The inability of grazers to digest cyanobacteria may increase in older and mature mats (Barranguet *et al.* 2005) such as those observed at Cuban sites. Also, the palatability of cyanobacteria for a grazer can be compromised by their ability to produce toxic compounds (Lukesová and Frouz 2007, O'Neil *et al.* 2012). For example, 6 (*Anabaena*, *Synechococcus*, *Calothrix*, *Lyngbya*, *Nodularia*, and *Nostoc*) out of 8 genera of cyanobacteria used by Dijkman *et al.* (2010) to characterize the fatty acid profile of intertidal microbial mats are known to produce hepatotoxins and neurotoxins (O'Neil *et al.* 2012). Cyanobacterial poisoning or intoxication have been reported in waterbirds (e.g. flamingos, coots, grebes; see Stewart *et al.* 2008). In shorebirds, avian vacuolar myelinopathy (AVM), a fatal neurological disease caused by a cyanobacterium toxin (Williams *et al.* 2007), has been confirmed in one killdeer (Fisher *et al.* 2006) and was the most likely cause of 54 mortality events of migrating calidridine sandpipers in Brazil (Buheler *et al.* 2010).

Therefore, although at first glance southward stop-over sites might seem to have higher biofilm availability (MPB biomass) than northward stop-over sites, this may not be the case. Given the differences between the two types of MPB biofilms, biofilm availability should not be assessed only based on MPB biomass. Rather, a clear understanding of the predominant MPB type (cyanobacterial or diatomaceous biofilm) should be the first step for assessing biofilm availability. Biofilm availability for sandpipers may be more complex than just discerning between the two major classes of MPB. Intrinsic (e.g. species composition, carbohydrate and fatty acid content, age, toxic compunds) and extrinsic (e.g. water content, grain size, salinity, biofilm-prey ratio) characteristics of MPB biofilms may be a determining factor in shorebird decisions on biofilm use (Quinn and Hamilton 2012). Further research is needed to untangle the complex patterns of biofilm consumption by shorebirds.

5.5.3. Relationship between cyanobacterial mats and invertebrates

Although cyanobacterial mats may be a poor food source for sandpipers, the results in the cyanobacteria rich habitats (RM and TZ) showed that patches with different MPB biomass (a variable related to mat thickness) were apparently not equal in value to migrant sandpipers (Fig. 5.5). The positive relationship between sandpiper habitat use and macrofaunal invertebrates was more notable in patches with thick mats (MPB biomass $> 50\ \mu g\ g^{-1}_{DM}$) than thin mats (MPB biomass $< 50\ \mu g\ g^{-1}_{DM}$). Such an effect was not caused by higher abundances of prey in thicker cyanobacterial mats as shown by the lack or negative association between MPB biomass and macrofaunal invertebrate density at RM and TZ, respectively.

I speculate that the thickness of the cyanobacterial mat could have an indirect effect on least sandpiper habitat use by increasing the availability of benthic invertebrate prey (Gonzalez 2011). The mechanism explaining a positive change in prey availability as mat thickness increases is based on the physical changes occurring on sediments covered by cyanobacterial mats. Cyanobacterial mats change sediment texture by retaining wetness and increasing sediment erosion and resistance (Stal 2003). If sediment resistance increases with mat thickness then invertebrate and birds will find higher resistance to burrowing or probing actions at thicker mats. Therefore, the steeper positive relationship between bird habitat use and macrofaunal invertebrates at patches with high MPB biomass (thicker mats) may be due to higher availability (vulnerability) of prey to pecking sandpipers.

5.5.4. Conclusions

As a general conclusion, I suggest that diatomaceous biofilm availability may be a limiting factor for biofilm feeding by sandpipers. Once occurring in the foraging habitat, diatomaceous biofilm biomass can be an important factor conditioning spatial and temporal distribution of foraging sandpipers. However, the data obtained during this study are only “snapshots” at defined times (southward or northward migration) and locations (foraging spots within tropical and temperate sites). Expanding our understanding of the role of MPB biofilm in the foraging ecology of small-bodied sandpipers requires additional study on the specific influence of cyanobacterial mats, especially with respect to nutritional and potential toxic effects on shorebirds and their invertebrate prey. I suggest that researchers consider a wider variety of food sources and availability levels that may be related with the use of foraging habitats by migrating shorebirds.

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5.8. Tables

Table 5.1. Overall prey abundance by food source (MPB biofilm, macro-, and meiofaunal invertebrates) at southward (Río Máximo and Tunas de Zaza, Cuba) and northward (Roberts Bank, Canada) shorebird stop-over sites during the 2008 migratory period.

		Río Máximo (N = 20)		Tunas de Zaza (N = 20)		Roberts Bank (N = 30)	
Predictor variable	**Variable ID/ unit**	**Mean (±SD)**	**Range**	**Mean (±SD)**	**Range**	**Mean (±SD)**	**Range**
Macrofauna density	MACRO/ individuals per core	16.5 (±8.6)	5-34	19.9 (±14.6)	2-54	45.4 (±23.7)	15-107
Meiofauna density	MEIO/ individuals per core	362.9 (±243.1)	26-1114	494.8 (±439.6)	38-1474	716.0 (±337.9)	150-1798
Copepod density	COP/ individuals per core	334.8 (±233.9)	23-1051	349.9 (±357.8)	1-1318	231.0 (±126.6)	33-557
Nematode density	NEM/ individuals per core	3.1 (±8.3)	0-37	95.2 (±123.5)	0-420	485.0 (±253.3)	113-1241
Chlorophyll-*a* content	CHL/ $\mu g\ g^{-1}{}_{DM}$	118.6 (±203.0)	2.6-724.9	206.1 (±198.2)	0.8-491.4	14.1 (±7.3)	5.2-41.6

Table 5.2. Pearson correlation coefficients for macrofaunal invertebrates (MACRO), copepod (COP) and nematode (NEM) density and chlorophyll-a content (CHL) at two Cuban estuaries at two different levels of availability for shorebirds ("highly available": 0-10 mm depth, and "available": 0-30 mm depth).

		Study site					
		Río Máximo			Tunas de Zaza		
Depth (mm)		**MACRO**	**COP**	**NEM**	**MACRO**	**COP**	**NEM**
0-10	COP	0.28			-0.71***		
	NEM	0.06	0.32		0.27	-0.10	
	CHL	-0.09	-0.32	0.07	-0.42	0.63**	0.03
0-30	COP	0.40			-0.53*		
	NEM	0.08	0.30		0.15	-0.05	
	CHL	-0.10	-0.35	0.04	-0.49*	0.62**	0.05

Table 5.3. ***Rankings of 15 linear models assessing least sandpiper (Calidris minutilla) habitat use (square root (daily standardized dropping count [droppings per 16 m^2]) at two Cuban southward stop-over sites dominated by cyanobacterial mats. Variables tested included macrofaunal invertebrate density (MACRO), meiofaunal invertebrate density (COP*NEM), microphytobenthic biomass (CHL), and location (LOC, Río Máximo and Tunas de Zaza). Invertebrate densities took into account "available" prey (0-30 mm sediment depth). Models were ranked by Akaike's information criterion corrected for small sample size (AIC_c for the best model was 0.35), where K is the number of parameters in the model, ΔAIC_c is the difference in AIC_c between the top-ranked model and the model in question, ω_i is the Akaike weight, which reflects the relative likelihood of the model, and R^2 is a measure of overall model fit.***

Mode ID	Model term	K	ΔAIC_c	ω_i	R^2
1	NULL	2	0.00	0.288	-
2	MACRO	3	0.71	0.202	0.05
5	CHL	3	2.27	0.084	0.01
6	CHL+MACRO	4	2.44	0.054	0.08
9	LOC+MACRO	4	2.45	0.041	0.01
10	LOC+COP*NEM	6	3.33	0.021	-0.02
14	LOC+CHL+COP*NEM	7	3.43	0.006	-0.05
12	LOC+CHL	4	3.88	0.010	0.09
13	LOC+CHL+MACRO	5	3.89	0.009	-0.02
3	COP*NEM	5	5.27	0.092	<0.01
11	LOC+MACRO+COP*NEM	7	6.44	0.012	0.04
15	LOC+MACRO+CHL+COP*NEM	8	6.69	0.003	-0.04
7	CHL+COP*NEM	6	6.95	0.052	0.12
4	MACRO+COP*NEM	6	7.87	0.085	0.01
8	MACRO+CHL+COP*NEM	7	9.46	0.041	-0.03

Table 5.4. Rankings of 15 linear models assessing least sandpiper (Calidris minutilla) habitat use (square root (daily standardized dropping count [droppings per 16 m^2]) at two Cuban southward stop-over sites (Río Máximo and Tunas de Zaza) dominated by cyanobacterial mats. Variables tested included macrofaunal invertebrate density (MACRO), meiofaunal invertebrate density (COP*NEM), microphytobenthic biomass (CHL), and location (LOC, Río Máximo and Tunas de Zaza). Invertebrate densities took into account "highly available" prey (0-10 mm sediment depth). Models were ranked by Akaike's information criterion corrected for small sample size (AIC$_c$ for the best model was -5.16), where K is the number of parameters in the model, ΔAIC$_c$ is the difference in AIC$_c$ between the top-ranked model and the model in question, ω_i is the Akaike weight, which reflects the relative likelihood of the model, and R^2 is a measure of overall model fit.

Model ID	Model	K	ΔAIC$_c$	ω_i	R^2
2	MACRO	3	0.00	0.395	0.20
6	CHL+MACRO	4	0.47	0.312	0.20
9	LOC+MACRO	4	2.34	0.123	0.15
13	LOC+CHL+MACRO	5	2.71	0.102	0.18
1	NULL	2	5.52	0.025	-
4	MACRO+COP*NEM	6	7.56	0.009	0.11
5	CHL	3	7.79	0.008	0.00
8	MACRO+CHL+COP*NEM	7	8.84	0.005	0.11
10	LOC+COP*NEM	6	8.89	0.005	0.07
11	LOC+MACRO+COP*NEM	7	8.96	0.004	0.12
14	LOC+CHL+COP*NEM	7	9.33	0.004	0.11
12	LOC+CHL	4	9.40	0.004	-0.03
15	LOC+MACRO+CHL+COP*NEM	8	9.42	0.004	0.16
3	COP*NEM	5	11.04	0.002	-0.03
7	CHL+COP*NEM	6	12.97	0.001	-0.04

Table 5.5. ***Summary of relative variable importance (RVI), weighted parameter estimates (β), unconditional standard errors (SE_u) and 95% confidence interval for variables occurring in the best subset of models ($\omega_{2,6,9,13} = 0.93$) explaining variation in shorebird foraging habitat use in Cuban estuaries.***

				95% CI	
Parameter	**RVI**	***β***	**SE_u**	**Lower**	**Upper**
Intercept	1.00	0.052	0.136	-0.215	0.319
MACRO[a]	1.00	0.110	0.038	0.036	0.184
CHL[b]	0.44	0.014	0.020	-0.026	0.054
$LOC_{\text{Tunas de Zaza}}$[c]	0.24	-0.009	0.026	-0.060	0.041

[a] Ln (Macrofauna density+1), macrofaunal invertebrates *per* sampling core

[b] Ln (Chlorophyll-*a* + 1), µg g^{-1}_{DM}

[c]Localities: Río Máximo and Tunas de Zaza

Table 5.6. ***Rankings of eight linear models assessing western sandpiper (Calidris mauri) habitat use (square root (daily standardized dropping proportion [droppings per 16 m^2]) at a Canadian northward stop-over site (Roberts Bank, British Columbia) dominated by diatomaceous biofilm. Variables tested included macrofaunal invertebrate density (MACRO), meiofaunal invertebrate density (COP*NEM), and microphytobenthic biomass (CHL). Invertebrate densities took into account "highly available" prey (0-10 mm sediment depth). Models were ranked by Akaike's information criterion corrected for small sample size (AIC_c for the best model was -80.24), where K is the number of parameters in the model, ΔAIC_c is the difference in AIC_c between the top-ranked model and the model in question, ω_i is the Akaike weight, which reflects the relative likelihood of the model, and R^2 is a measure of overall model fit.***

Model ID	Model term	K	ΔAIC_c	w_i	R^2
5	CHL	3	0.00	0.693	0.46
6	CHL+MACRO	4	2.72	0.215	0.43
7	CHL+COP*NEM	6	4.89	0.076	0.46
8	MACRO+CHL+COP*NEM	7	8.56	0.015	0.44
1	NULL	2	9.33	0.000	-
2	MACRO	3	10.96	0.000	0.05
3	COP*NEM	5	11.28	0.000	0.09
4	MACRO+COP*NEM	6	14.10	0.000	0.09

5.9. Figures

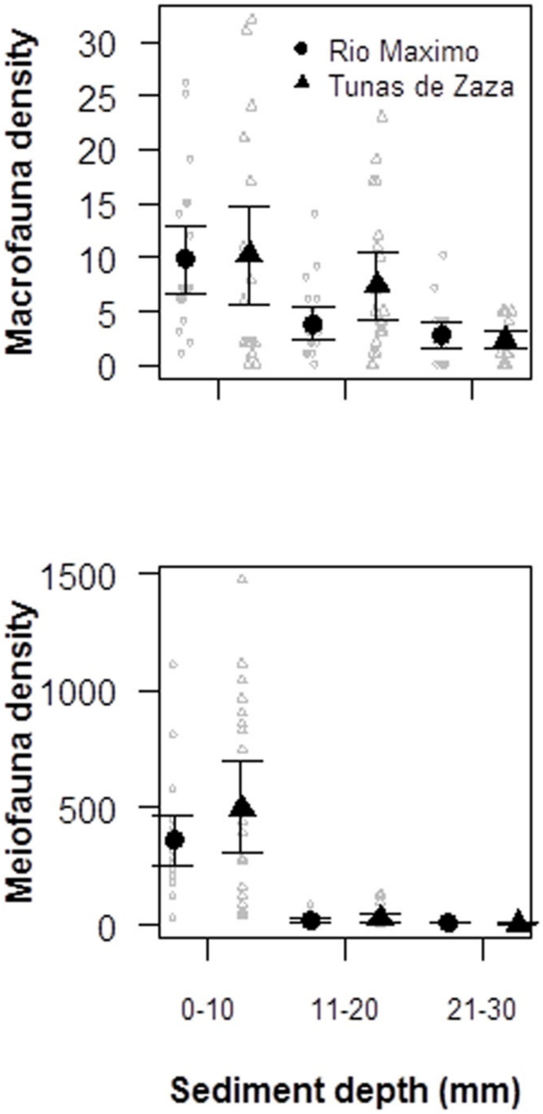

FIgure 5.1 ***Mean benthic invertebrate density (invertebrates per core) in three sediment depth ranges at the Cuban estuaries of Río Máximo and Tunas de Zaza. Values represent mean ± 95% CI.***

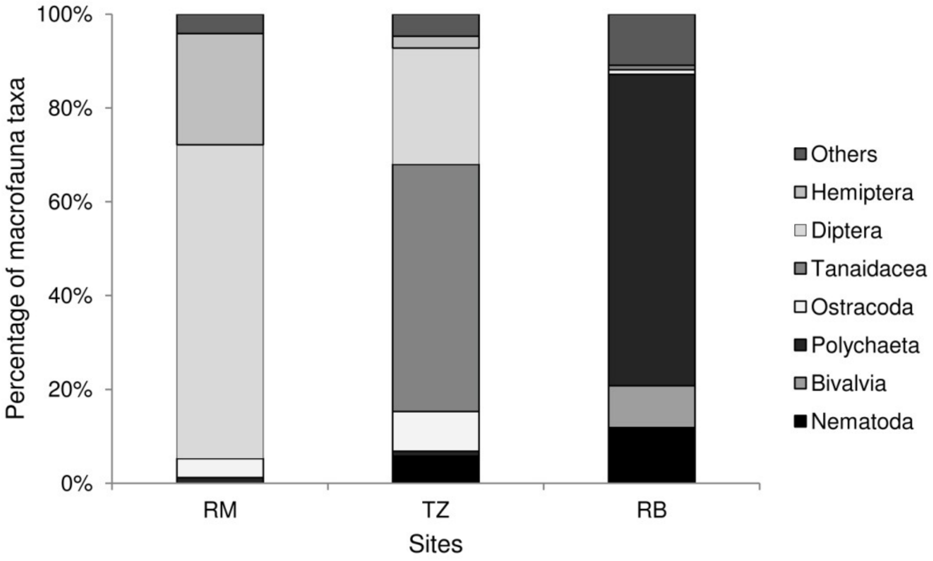

Figure 5.2. ***Percentage of macrofaunal invertebrate taxa at three shorebird stop-over sites (southward stop-over sites: Río Máximo, RM, and Tunas de Zaza, TZ; northward stop-over site: Roberts Bank, RB).***

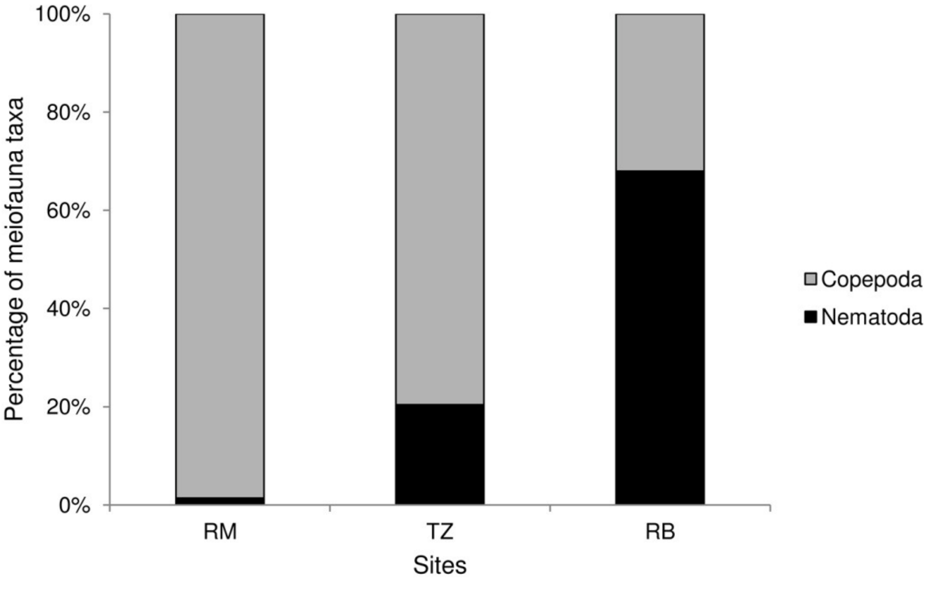

Figure 5.3. ***Percentage of two meiofaunal invertebrate taxa collected at three shorebird stop-over sites (southward stop-over sites: Río Máximo, RM, and Tunas de Zaza, TZ; northward stop-over site: Roberts Bank, RB).***

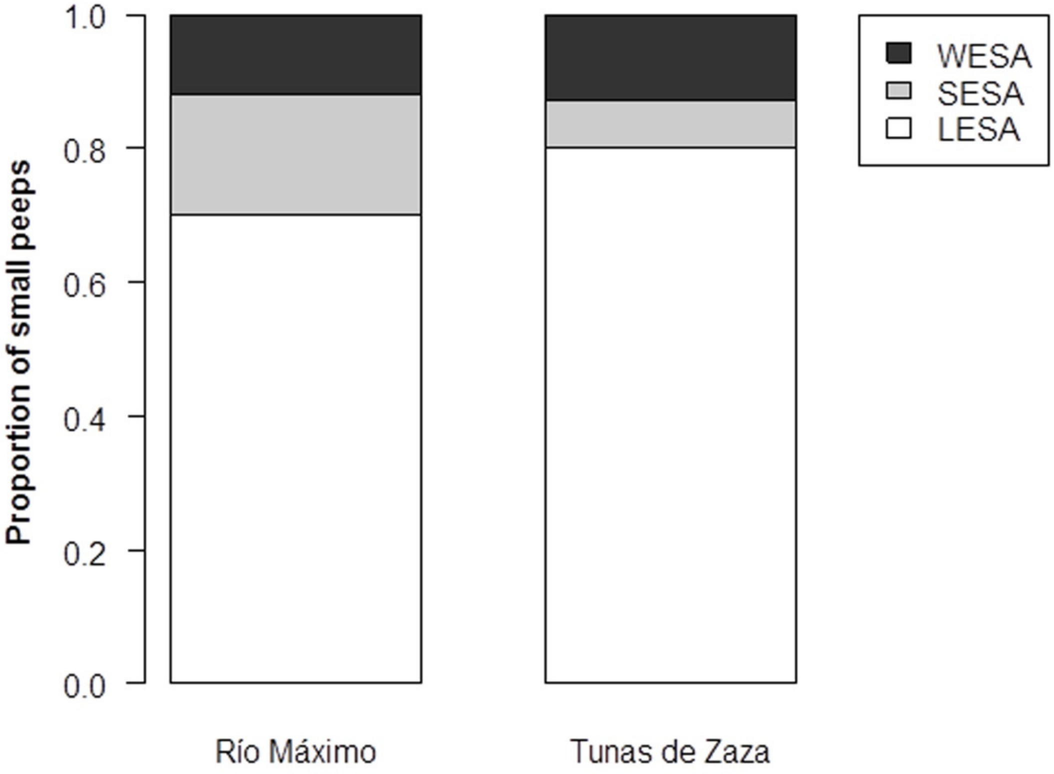

Figure 5.4. ***Small-bodied sandpiper proportion (LESA: least sandpiper, SESA: semipalmated sandpiper, WESA: western sandpiper) at two Cuban southward stop-over sites (Río Máximo, N=17; Tunas de Zaza, N=16).***

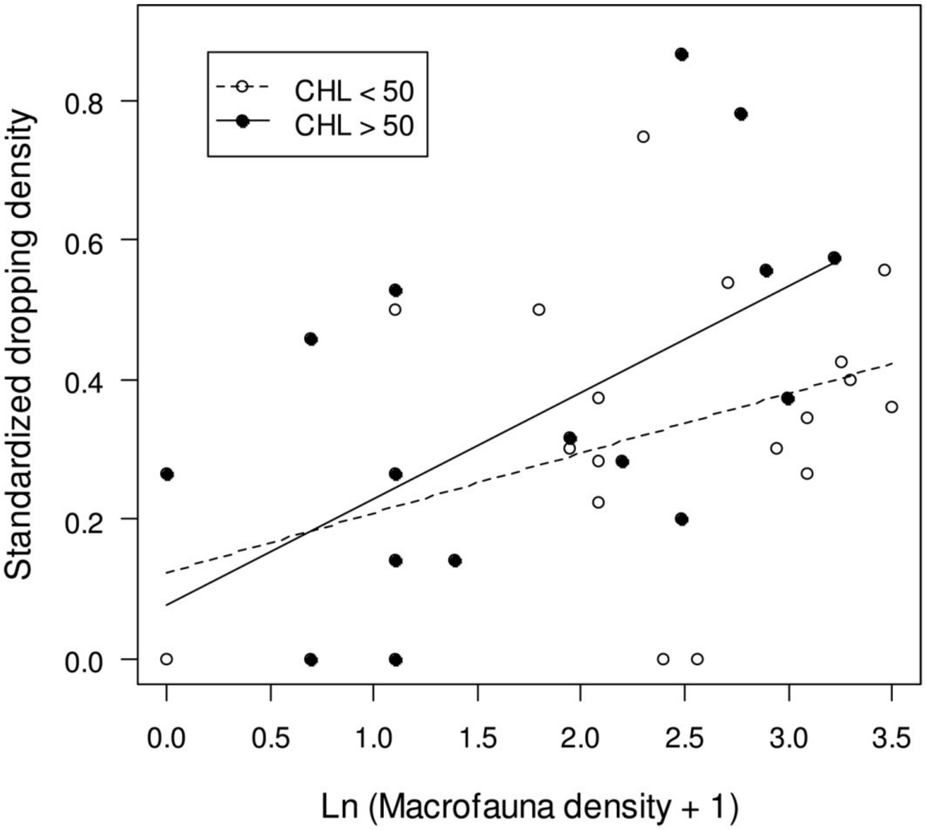

Figure 5.5. ***Relationship between macrofaunal invertebrate density (individuals per core) and the daily standardized dropping density as an estimator of sandpiper foraging habitat use at two Cuban southward stop-over sites (Río Máximo and Tunas de Zaza) dominated by cyanobacterial mats. The open and filled dots represent samples taken at poor (N = 19, range: 0.80 – 49.04 µg g^{-1} $_{DM}$) and rich (N = 17, range: 51.76 – 724.88 µg g^{-1} $_{DM}$) MPB biomass patches, respectively. Microphytobenthic biomass was assessed by sediment chlorophyll-a content (CHL).***

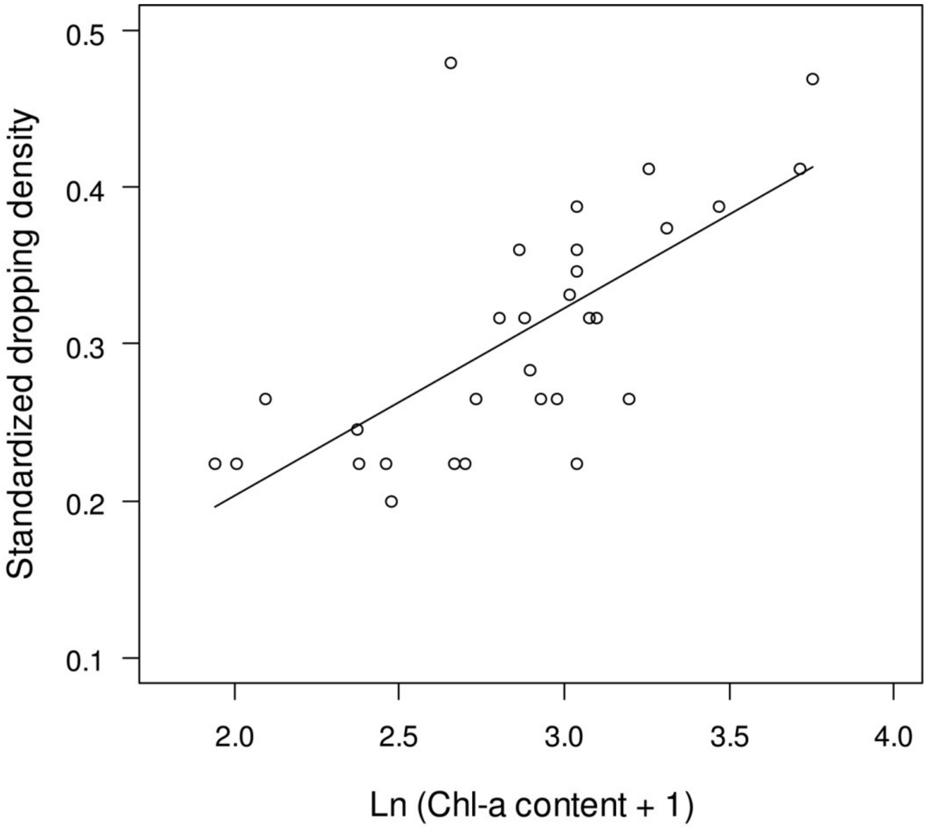

Figure 5.6. ***Relationship between chlorophyll-a content (Chl-a content, µg $g^{-1}{}_{DM}$) and the daily standardized dropping density as an estimator of sandpiper foraging habitat use at a Canadian northward stop-over site (Roberts Bank) dominated by diatomaceous biofilm. Chlorophlly-a content was used as an estimator of sediment MPB biomass.***

6. General synthesis

Microphytobenthos (MPB) on the surface of mudflats give rise to an efficient primary production system (Underwood and Kromkamp 1999) in which MPB forms a temporally dynamic biogenic structure rich in carbohydrates: biofilm (Decho 2000). Among many other ecological functions, this “unnoticed” garden growing in the intertidal has proved to be a crucial food supply in the diet of migrant small-bodied sandpipers (Kuwae *et al.* 2012). Therefore, the concept of intertidal biofilm has become central to the understanding of the foraging behaviour of migrant shorebirds.

In this chapter, I synthesize the findings and implications of my dissertation. The chapter is divided into four sections. In the first section, an overview of the thesis is presented, where the main results and conclusions of each chapter are summarised. In the second section, the implications of the main findings of this thesis are presented. The third and four sections discuss limitations of the study and possible future directions, respectively.

6.1. Summary

The general introduction presented in Chapter 1 provides the relevant background regarding intertidal MPB biofilm. In Chapter 2, the practical utility of both ground and aerial color-infrared photography in assessing diatomaceous biomass (MPB biomass as measured by Chl-*a* content) was tested at Roberts Bank. The use of ground-based digital near-infrared (NIR) images of the sediment surface coupled with measurements of sediment water content offered valuable rapid information of MPB biomass at fine scale sampling (7.5 x 5.0 cm). While the MPB biomass predictions emerging from the NIR images are not as accurate as other reflectance indices (Murphy *et al.* 2005), the affordability and rapid processing makes this method a tool capable of delivering qualitative information for estimating diatomaceous biomass availability. Also, the aerial CIR imagery proved useful to assess the MPB biomass distribution through the Roberts Bank intertidal, although the application is dependent on ground reconnaissance and ground-truth data. The visual comparison of NIR and NDVI reflectance images helped to differentiate between MPB and other intertidal vegetation. The aerial CIR imagery revealed the heterogeneous distribution of MPB biomass at Roberts Bank and highlighted the significance of the upper intertidal (0-750 m from shore, tide height from 3.0-3.5 m) as the zone with highest MPB biomass and sediment water content.

In Chapter 3, I explored whether the coexistence of western sandpiper and dunlin at the upper intertidal at Roberts Bank could be explained by their documented differential capacity to feed on biofilm (Elner *et al.* 2005, Mathot *et al.* 2010, Kuwae *et al.* 2012) and the food availability distribution within the area. Using bird counts and a tide following index (TFI), I showed that a higher than expected proportion of western sandpipers stopped following the tide when the initial section of the upper intertidal was completely exposed. Western sandpiper tended to concentrate and spent more time than dunlin at the first 350 m of the upper intertidal. Dunlin followed the tide line as the ebbing tide progressed and, in general, exploited briefly the upper intertidal. The initial section of the upper intertidal (150-350 m from shore) had more mud content, MPB biomass and water content than the final section (450-750 m). Results suggested that biofilm feeding is more advantageous in the initial section of the upper intertidal. No clear trend in benthic invertebrate density with upper intertidal sections was detected. The results are consistent with the hypothesis stating that the differential biofilm capability between the studied species underlies their spatial segregation within the upper intertidal during the ebbing tide.

In Chapter 4, I described the variations in the type of MPB biofilms and their biomass at two tropical sites used by shorebirds in Cuba (RM, Río Máximo; TZ, Tunas de Zaza). In addition, I evaluated abiotic factors that may underlie the MPB biofilm variations and described monthly variations in small calidridine sandpiper numbers. Despite abiotic differences between sites (flood-emersion regime and salinity), both Cuban estuaries showed a seasonal change in the type of MPB biofilms, shifting from cyanobacterial mats during southbound stopovers to diatomaceous biofilms during winter. Microphytobenthic biomass varied between 0.5 and 724.9 µg g^{-1}_{DM}. The lowest

MPB biomass averages were recorded in Jan. 2009 (14.7 and 1.4 µg $g^{-1}{}_{DM}$ at RM and TZ, respectively). Sandpiper average numbers were higher during southward migration (> 3,000 birds) than during winter (~1,500 birds) or northward migration (~500 birds). The data suggest that calidridine sandpipers will encounter cyanobacterial mats while using Cuban estuaries as southward stop-over sites, whereas diatomaceous biofilms will be available for birds during the winter. Given the poor quality (Yallop *et al.* 1994, Dijkman *et al.* 2010) and digestibility (Decho and Lopez 1993, Leflaive and Ten-Hage 2007) of cyanobacteria, I proposed that sandpipers will preferentially use diatomaceous biofilms rather than cyanobacterial mats. The hypothesis was further tested in the thesis (Chapter 5).

Food, hitherto benthic invertebrates, availability is known as a critical variable influencing the distribution of foraging sandpipers (Goss-Custard *et al.* 1991). However, the role of biofilm availability on sandpiper distribution has not been inferred. I hypothesized that the dominant type of MPB (cyanobacteria or diatom) influences foraging shorebird distribution in such a way that biofilm availability (MPB biomass) will be important at sites dominated by diatomaceous biofilms (Chapter 4). In Chapter 5, assessments of sandpiper habitat use in accordance to food availability (MPB biomass, benthic macro-, and meiofaunal invertebrates) were conducted at cyanobacterial and diatomaceous biofilm stop-over sites. The results strongly suggested that diatomaceous biofilm availability may be more important in determining sandpiper distribution in northward stop-over sites; whereas the importance of benthic invertebrates is predominantly in its role as a food source in cyanobacterial mat dominated southward stop-over sites. Given the available evidence showing cyanobacteria as a poor quality food source for diverse grazers and the findings at Cuban estuaries, I conclude that, for calidridine sandpipers, cyanobacterial mats are poorer in nutrition than diatomaceous biofilms. Therefore, diatomaceous biofilm availability could be a limiting factor for biofilm feeding sandpipers.

6.2. Implications of the results on sandpiper foraging ecology

Shorebird distribution (Wolff 1969, Colwell and Landrum 1993) and foraging behaviour (Estrella *et al.* 2007, Kuwae *et al.* 2010) is determined in large extent by the availability of their prey. Therefore, information on the fluctuations of biofilm, a novel and important food supplement for small-bodied sandpipers (Kuwae *et al.* 2008, Kuwae *et al.* 2012), becomes essential in any future study trying to elucidate the complex relationship between shorebirds and their food supply. There are only a handful studies on the role of intertidal biofilm in shorebird foraging ecology (Elner *et al.* 2005, Kuwae *et al.* 2008, Mathot *et al.* 2010, Beninger *et al.* 2011, Kuwae *et al.* 2012, MacDonald *et al.* 2012, Quinn and Hamilton 2012). Most of them have focused on gathering evidence of biofilm consumption by shorebirds (Elner *et al.* 2005, Kuwae *et al.* 2008, Beninger *et al.* 2011, MacDonald *et al.* 2012, Quinn and Hamilton 2012) and in examining the extent to which biofilm feeding occurs in shorebirds (Mathot *et al.* 2010, Kuwae *et al.* 2012). The current dissertation is the first step in examining the role of biofilm in the foraging behaviour and distribution of migratory shorebirds.

6.2.1. Conceptual model for biofilm feeding in shorebirds

Important spatial (Chapter 2) and temporal (Chapter 4) differences in the type of MPB biofilm and their biomass occur in shorebird stop-over sites. MPB is mainly responsible for biofilm formation in intertidal systems (Stal 2003) and is an important basal component of the biofilm consumed by shorebirds (Kuwae *et al.* 2008, Beninger *et al.* 2011, Kuwae *et al.* 2012). Thus, changes in MPB composition, biomass and water content could offer important insights into biofilm availability for grazing sandpipers.

To be considered as available for shorebirds, a food source needs to be detectable, accessible and digestable (Zwarts and Wanink 1993). The conditions and parameters influencing the availability of benthic invertebrates for shorebirds are well summarized and exemplified elsewhere (Piersma *et al.* 1993, Zwarts and Wanink 1993). However, because biofilm feeding is a new foraging mode for shorebirds, understanding the set of conditions that makes biofilm available is lacking. Here, I discuss the potential implications of the spatial and temporal variations in the type of MPB biofilms and their biomass on the 1) accessibility and 2) digestibility conditions for biofilm availability to shorebirds. The detectability of biofilms by shorebirds is presented in the third section of this chapter (Limitations).

Biofilm accessibility

Biofilm accessibility to shorebirds is temporally constrained by the 1) flood-emersion regime and 2) time of sediment emergence. Shorebirds generally use feeding grounds that are only temporally available because they are subject to emersion and inundation patterns. Thus, biofilm accessibility for shorebirds will primarily depend on the time the flats are emerged. Intertidal mudflats ruled by periodical tidal cycles (e.g. Roberts Bank or Tunas de Zaza) are available for birds on a predictable way. The sediment surface where biofilm develops will be available for shorebirds for approximately two periods of six hours over the course of 24-hours. The conditions for habitat availability are more irregular in other systems, such as those which depend on the prevailing wind direction and speed to expose the sediments (e.g., Río Máximo). In such systems, habitat availability, and consequently biofilm accessibility, will be more episodic or unpredictable because emersion and inundation patterns are induced by wind direction and strength.

Time of emergence of the foraging habitat not only controls the available foraging time for birds but also corresponds with a degree of dryness in the sediment, which can be crucial for biofilm grazing. Sediment pore water content rapidly decreases over an emersion period (Paterson 1989, Coelho *et al.* 2009). High water content in the sediment may facilitate the mucilaginous biofilm matrix adhering both to the shorebird distal spines and the mucus on the tongue (Elner *et al.* 2005). Also, water can have an important role on the transportation of the grazed biofilm through the bill through the formation of a dense but liquefied bolus containing sediment and biofilm (Kuwae *et al.* 2008, Kuwae *et al.* 2012). Thus, water content can represents an index for biofilm handling time. Biofilm handling time will be minimized in sediments containing high water content (muddy sediments). As sediment dries out, biofilm handling time will increase and biofilm profitability decrease until a point where shorebirds switch to a different prey or habitat (Chapter 3). Therefore, time of emergence of the sediment as well as other conditions affecting the water content (wind speed) in the sediment are crucial when considering biofilm accessibility for shorebirds.

Once the temporal conditions for biofilm accessibility are fulfilled, shorebirds will encounter a food source available on the sediment surface and presumably extending over many hundreds of square meters of intertidal habitat. However, given that biofilm is a discreet structure a few millimeters thick on the surface sediment (3-4 mm the most) (Underwood and Kromkamp 1999, de Brouwer and Stal 2001, Underwood and Paterson 2003), shorebirds would need to cover large areas of the foraging habitats to satisfy their daily energetic requirements. Thus, a shorebird can be expected to maximize its biofilm intake rate by visiting patches with high biofilm biomass. Among all the variables affecting the patchy distribution of biofilm, sediment grain-size constitutes an important influencing factor on biofilm type and biomass (Stal 2003). As shown by this (Chapter 3) and other studies (Underwood and Kromkamp 1999, Du *et al.* 2009), diatomaceous biofilm biomass positively correlates with fine sediments. Areas with higher proportion of fine sediment will also retain sediment wetness for longer than sand dominated areas (Chapter 3). Thus, the low energy areas of the intertidal where fine sediment tends to deposit hold the highest MPB biomass and sediment wetness. In estuarine areas, such habitats generally occur towards the shoreline (Eisma 1998). The value of the upper intertidal region is not only related to the MPB biomass, but also with the nutritional value of biofilms. Colloidal carbohydrates and glucose-rich EPS tend to be maximal in fine sediment sites (de Brouwer and Stal 2001, de Brouwer *et al.* 2003) where positive linear relationship between MPB biomass and carbohydrates only occurs (Underwood and Smith 1998, Blanchard *et al.* 2000, de Brouwer and Stal 2001). Therefore, shorebirds foraging extensively on biofilm can be presumed to primarily use sites with high proportion of fine sediment because of the advantages resulting from the combination of biofilm quality (biomass and nutritional value) and handling time (water content). There is recent evidence supporting the above prediction (Kuwae *et al.* 2012); the authors showed that shorebird reliance on biofilm was considerably higher at muddy than sandy locations.

Biofilm digestibility

Chlorophyll-*a* content has extensively been used as a proxy for MPB biomass assessment (Underwood and Smith 1998) and in proper environmental conditions (sediments with high silt content) is tightly related with the production of the mucilaginous biofilm matrix (de Brouwer and Stal 2001). However, to assess biofilm availability for shorebirds the estimation of Chl-*a* content needs to be coupled with the identification of the dominant type of MPB (Chapter 5). Such a need is especially important in light of the theoretically poor significance of cyanobacteria for intertidal grazers (Decho and Lopez 1993, Lukesová and Frouz 2007).

Understanding the factors that promote the formation of mature cyanobacterial mats may prove useful to predict no use of biofilm by shorebirds. Warm temperatures (> 25℃) and sandy sediments are abiotic features that favour cyanobacteria over diatoms (Watermann *et al.* 1999, Stal 2003, Jesus *et al.* 2009). Researches need to acknowledge the link between temperature, cyanobacterial mats and the poor nutritional quality of this type of MPB for biofilm feeding shorebirds. Such an analysis is especially relevant for studies sampling at latitudinal distant sites (Mathot *et al.* 2007). For example, coupling seasonal shifts of biofilm assemblages with a theoretical less attractiveness of cyanobacterial mats for birds may indicate a “migratory dietary shift strategy” in shorebirds (Beninger *et al.* 2011). Under such a scenario, biofilm grazing by shorebirds will be primarily occurring in temperate stop-over sites during the northward migration. Shorebird southward migration starts in late July and extends until October (Colwell 2010). During this period, warm temperatures at stop-over sites will favour the presence of cyanobacterial mats, especially at southern wintering locations where temperatures tend to be higher during the shorebird non-breeding period (Chapter 4). Shorebird wintering habitats may experience a decreasing trend in cyanobacterial mats dominance with a turnover at the end of the period (November-January) toward a diatom dominated biofilm. Diatomaceous biofilm will tend to dominate intertidal habitats for a brief period in subtropical and tropical habitats (January-February). Moreover, in northern temperate sites the lower temperatures favour high diatomaceous biofilm biomass from March to May (de Brouwer *et al.* 2000), which coincides with the time northward migrating shorebirds arrive at temperate stop-over sites such as Roberts Bank (Butler *et al.* 1987).

On the other hand, understanding the factors that enhance diatomaceous biofilm availability may help to further explain the variable reliance on biofilm exhibited by sandpipers (Kuwae *et al.* 2012, Quinn and Hamilton 2012). Birds using mudflats rely more in biofilm as food supply than those inhabiting sandflats (Kuwae *et al.* 2012). Predictions on inter- or intra-site biofilm availability and consumption by shorebirds can be inferred by considering the existing relationship between sediment grain-size, MPB biomass and sediment wetness. Specifically, in mesotidal estuaries the negative relationship between the tidal high and the proportion of mud content in the sediment (Eisma 1998) suggest that the highest consumption of biofilm by shorebirds will tend to occur at the higher parts of the flats (Chapter 3). Such prediction may have important ramifications in the estimation of biofilm contribution to daily energy expenditure (Kuwae *et al.* 2008, Kuwae *et al.* 2012) by restricting the assessment to the time shorebirds devote within the higher flats.

In summary, shorebirds grazing on unfiltered biofilm might be restricted to the more nutritional and digestible diatomaceous biofilms which is dominant in most of the estuarine systems when temperatures are under 20℃. Thus, on a seasonal scale, biofilm grazing may occur more frequently at the end of the winter residency and/or during the spring migration, making the northern stop-over sites the likely habitat scenario for biofilm feeding. On these sites, biofilm will be more available for shorebirds on fine sediment grain sections of the mudflat which is commonly the closest section to shore. On a daily pattern basis, biofilm availability will show a complex periodicity, controlled by timing of the emersion period, irradiance and wind speed, making the first 2 hours of daylight low tides the time window where biofilm biomass and wetness enhance biofilm feeding by shorebirds.

6.2.2. Implications for conservation and management of shorebird habitats

The dynamic nature and heterogeneous distribution of intertidal biofilms (de Brouwer and Stal 2001) have clear implications for shorebird management. The theoretical model for biofilm forming assumes that diatomaceous biofilm are highly favoured in sediments with high silt content and moderate shear stress (Underwood and Paterson 1993a, Blanchard *et al.* 2000). Also, high diatom densities on muddy sediments are correlated with higher nutrient content in silty-rich sediments (Scholz and Liebezeit 2012). The basic attributes for biofilm formation (high silt content, moderate shear stress and high nutrient content) are tightly interconnected and their interactions subsequently lead to a cascade of events (de Brouwer *et al.* 2003). The exudation of EPS by MPB increases the mud content, cohesiveness, erosion threshold (de Brouwer *et al.* 2000) and, consequently, stimulates increase in densities of diatoms (Weerman *et al.* 2010). Intertidal MPB biofilms are thought to be very resilient with estimates between 15 -23 days to reach 95 % of the original MPB biomass after a strong perturbation (Guarini *et al.* 2000). However, even under such short timeframe for recovery, the consequences for shorebirds can be vast if the perturbation coincides with their arrival.

Removal of benthic MPB by biocide usage (de Boer 1981, Underwood and Paterson 1993b), changes in hydrological processes (Blanchard *et al.* 2000) or notable top-down effects of herbivores (Daborn *et al.* 1993, Weerman *et al.* 2011) can lead to rapid synergistic effects of erosion events and biofilm disappearance. Significant reductions of diatom biomass and the ensuing decrease in mud content can reverse the cascade of biofilm forming processes resulting in sediment with low cohesiveness, low silt content and no diatomaceous biofilm (Weerman *et al.* 2010). Dramatic changes in the estuary hydrology (e.g. dredging, harbor or causeway construction) may also compromise biofilm feeding habitat by facilitating downshore spread of macroalgae (Lopes *et al.* 2006, García-Robledo *et al.* 2012), invasive intertidal vegetation (Buchanan 2003, Li *et al.* 2009) or invasive grazers (Hamilton *et al.* 2003).

The conservation implications are clear. The environmental quality of biofilm rich stop-over sites must be maintained so that biofilm availability for shorebirds remains adequate (West *et al.* 2005). Loss of biofilm rich habitat would likely lead to interference between feeding birds and thus a reduction in food intake by some. Competitive effects would not be restricted to intraspecific interactions (Chapter 5). Because the considerable resource overlap between different sandpiper species (Baker and Baker 1973, Andrei *et al.* 2009), the loss of a feeding supplement such as biofilm is thus likely to heighten interspecific competition and so affect different bird species to different extent (Evans and Pienkowski 1983). Therefore, a major conservation priority is safeguarding the habitat (the upper intertidal zones) which may be particularly important to provide sufficient biofilm availability. Industrial pollution and land reclamation for human activity should be prevented or minimized at sites where shorebirds depend on biofilm availability as a source of energy.

6.3. Limitations of the study

Several of the potential limiting factors associated with methods and results have been addressed in each chapter. For example, one of the major early issues was the followed strategy to measure biofilm availability. In this dissertation, I have used the estimation of MPB biomass as the methodological approach to assess biofilm availability. The rationale for such an approach was based on the general consensus of MPB being the main biotic determinant for biofilm formation at intertidal systems (Stal 2003, Underwood 2010). However, assessing biofilm by pigment analysis may not account for the whole complexity of biofilm (Decho 2000). Near-infrared images of the sediment surface have the advantage of covering large sections of the available habitat, but their low prediction capability for Chl-*a* content undermine its use for quantitative analyses such as the performed for shorebird habitat use (Chapter 3 and 5). Spectrophotometric laboratory assessments for sediment Chl-*a* content are more accurate in terms of quantitative estimates of MPB biomass. However, this laborious method can take more than one hour per sample (sum of time devoted to field sampling, Chl-*a* extraction and laboratory processing), which in turn limits the number of coupled samples (invertebrates and biofilm) needed to minimize variability at large sample plots. More importantly, both methods assume that shorebirds assess biofilm availability by visual means (biofilm detectability by birds). While this may be the case, shorebirds are equipped with an extensive battery of “sensory pits” (Nebel *et al.* 2005), taste buds (Elner *et al.* 2005) and are thought to have a well-developed sense of taste (Gerritsen *et al.* 1983, Van Heezik *et al.* 1983); all these characteristics may assist shorebirds in assessing biofilm “quality” in terms of biofilm nutrient content (carbohydrate or fatty acids).

Sediment carbohydrate content estimation is especially relevant because the dynamic relationship between MPB and EPS (Blanchard *et al.* 2000, Hanlon *et al.* 2006). An important fraction of the EPS produced (colloidal carbohydrates) is readily dissolved in the water during high tide (Hanlon *et al.* 2006). Colloidal carbohydrates can represent up to 30-60 % of the EPS produced by MPB during the emersion time (Smith and Underwood 2000). Colloidal carbohydrates have the potential to bind with sediment particles, organic matter and microorganisms forming stable aggregates or flocs (Kumar *et al.* 2004). The resulting flocculant particles are suspended in the water during high tide and are deposited on the sediment when the tide recedes (Dyer *et al.* 2000). Also, flocculant particles from marine diatom can contribute to the total carbohydrate pool deposited on the emerged sediment surface (Verney *et al.* 2009). Thus, as soon as the receding tide uncovers the sediment, shorebirds may find a ready to use carbohydrate food source which is not necessarily linked with the benthic Chl-*a* signature. There are also important changes in EPS productivity as MPB biomass reach its biotic capacity (Orvain *et al.* 2003). During the MPB stationary phase of growing, there is a change in MPB carbohydrate metabolism and most of the produced carbohydrates (~80 %) are of refractory nature (Orvain *et al.* 2003). Therefore, if shorebirds are relying on taste to assess biofilm patch quality they will likely avoid mature or senescent MPB biofilms patches. Given the existing dynamic nature between MPB biomass and EPS, there is a need to investigate whether shorebirds assess biofilm availability by visual or taste clues. The findings can overcome the existing difficulties on biofilm assessment by human observers (R. W. Elner com. pers.).

Other potentially limiting factor of data presented here are the inferences based on different species (Chapter 5). Although biofilm feeding is extended in small-bodied sandpipers (Kuwae *et al.* 2012) and recent evidence shows that semipalmated sandpiper (Quinn and Hamilton 2012) and least sandpiper (Gardiner 2012) may be using biofilm at northward stop-over sites, better approaches will be obtained by comparing spatial and temporal changes in biofilm consumption in a single species. In order to examine more thoroughly the temporal changes in biofilm use (MPB type hypothesis), studies in tropical sites with high densities of wintering shorebirds are desirable. Key wintering areas of western sandpiper such as the Gulf Coast of Mexico (Morrison *et al.* 2009) and the Upper Bay of Panama (Morrison *et al.* 1998) are the likely scenario to test that hypothesis.

6.4. Future directions

The present study offers some insight into the circumstances that may allow shorebirds to use biofilm. Further examination is required to test the proposed factors and hypothesis driving spatial (e.g. MPB type hypothesis; sediment grain size and biofilm feeding) and temporal (e.g. water content threshold for biofilm feeding) biofilm availability for shorebirds. Similarly, the effects of grazing competing invertebrates or predation risk on biofilm feeding shorebirds should be considered, as should the whole range of habitats shorebirds use during their annual cycle.

Other areas of future interest are very specific. For example, organisms feeding on microalgae and cyanobacteria need particular type of enzymes (cellulase, xylanase and pectinase) for the complete digestion of both cellulose cell walls and the gelatinous envelopes of microalgae (Lukesová and Frouz 2007). The presence of such enzymes has not been investigated thus far in grazing shorebirds. Morphological and physiological variations for biofilm feeding might be worthy to pursuing. Intra-specific variations in the density of tongue spines and enzymes may occur in individuals as an effect of season, habitat or individual specialization.

One of the most intriguing findings in shorebird biofilm feeding is the reported downward trophic shift during the northward migration of western sandpipers (Beninger *et al.* 2011). In this dissertation I have used the seasonal variation in the type of MPB biofilm (Chapter 4) and the theoretically less attractiveness of cyanobacterial mats (Chapter 5) in an attempt to explain the extensive use of biofilm at northern latitudes as migration to breeding sites progress. However, a dietary shift caused by an increase on the resource availability is not the only possible hypothesis capable to explain the progressive downward trophic shift experimented by migrating western sandpipers (Beninger *et al.* 2011). Changes in feeding behaviour and resource use can be caused by the differing demands or physiological requirements of birds as they proceed through their moult and reproductive cycles (Recher 1990). Such changes are shown by an increased specialization on particular resources (Recher 1990), as the high reliance on biofilm documented in western sandpiper's diet at Roberts Bank (Kuwae *et al.* 2008, Beninger *et al.* 2011). Biofilm is commonly perceived as an important energy source for shorebirds (Kuwae *et al.* 2008) because intertidal MPB is a rich source of nutritionally available lipid (Dijkman *et al.* 2010) and the mucilaginous matrix is loaded with digestible carbohydrates (glucose content in EPS is up to 85 %) (de Brouwer and Stal 2001). However, biofilm may also be an important source for carotenoids (Pennington *et al.* 1988, Cibic *et al.* 2007), a crucial nutrient with dual roles in reproductive processes: signalling and physiology (Svensson and Wong 2011).

Carotenoids are biologically active yellow, orange, and red pigments synthesized by plants and photosynthetic micro-organisms (Matsuno 2001). Animals must obtain carotenoids from their diets (Matsuno 2001). Carotenoids carry out many physiological functions in birds, including important roles as antioxidants, immunostimulants, and precursors to vitamin A (Svensson and Wong 2011). Also, in many birds, carotenoids play an important role in colourful sexual signals, where they are thought to honestly reflect individual quality (Blount *et al.* 2003, Giraudeau *et al.* 2011). Carotenoids concentration in feathers and/or eggs have been positively correlated with physical condition (Blount and Matheson 2006, Newbrey and Reed 2011), mating choice (Velando *et al.* 2006, Toomey and McGraw 2012), breeding performance (Hipfner *et al.* 2010, Safran *et al.* 2010), and nestling development (Biard *et al.* 2005, McGraw *et al.* 2005). There is not documented evidence that shows whether carotenoids are important for the common rusty-red alternate plumage feathers of breeding shorebirds, as it does in many other birds (Olson and Owens 2005). However, Piersma and Jukema (1993) stated that nutritional/resource factors seemed to play an important role on the breeding plumage molt of Bar-tailed Goodwit (*Limosa laponica*).

Crustaceans are considered the main source of carotenoids for Charadriiformes (Sánchez *et al.* 2009, Hipfner *et al.* 2010). However, the MPB inhabiting intertidal biofilms is a prime source of carotenoids for sandpipers (Beninger *et al.* 2011). If carotenoids are critical nutrients for overall individual fitness, one may expect that shorebirds take advantage on available biofilm as a main source of carotenoids. Such preference for biofilm carotenoids will be magnified at sites such as Roberts Bank where crustacean availability is low (Seaman 2003, Pomeroy and Butler 2005, this study). The critical importance of carotenoids for sandpipers is shown by their preference for red *Artemia* even when by doing so sandpipers increased their exposure to parasites (Sánchez *et al.* 2009). Curiously, such a cost is comparable with the findings of western sandpipers risking to predation by feeding during approximately two hours in the most dangerous area at Roberts Bank (Chapter 3, 150-350 m from shore). The use of the upper intertidal by western sandpipers may be explained if the benefit of feeding on biofilm is higher than the energetic cost resulting from being in the site with more predation danger. A benefit of such magnitude can be related to the acquisition of a highly energetic food supply and/or the ingestion of spatial-temporal limited nutrient (Houston *et al.* 2011).

Overall, biofilm feeding in shorebirds opens a new door for numerous interesting questions. Do shorebirds use biofilm as a source of energy, nutrients, or both? Is feeding for biofilm a safer way to use the most dangerous zone in the intertidal or is biofilm such a "rare" food supplement at some sites that the reward by using biofilm balances the cost of predation? Does the resources contained in the biofilm (energy/nutrients) and is use by shorebirds change as an effect of season or habitat? And if so, do shorebirds change their foraging behaviour and distribution accordingly? If biofilms are a major source of carotenoids and considering both that individual's circulating carotenoids profile is temporally dynamic (Safran *et al.* 2010) and shorebirds are "income breeders" (Jamieson 2009), do shorebirds forage for biofilm in the breeding areas? Hopefully the hypotheses and ideas stated in this thesis can lead to a broad set of testable predictions that should prompt further research in the field of shorebird foraging behaviour.

6.5. References

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