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Intertidal biofilm distribution underpins differential tide-following behavior of two sandpiper species (*Calidris mauri* and *Calidris alpina*) during northward migration

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ABSTRACT

The discovery that some shorebird species graze heavily on biofilm adds importance to elucidating coastal processes controlling biofilm, as well as impetus to better understand patterns of shorebird use of intertidal flats. Western sandpipers (*Calidris mauri*) and dunlin (*Calidris alpina*) stopover in the hundreds of thousands on the Fraser River estuary, British Columbia, Canada, during northward migration to breeding areas. Western sandpipers show greater modification of tongue and bill morphology for biofilm feeding than dunlin, and their diet includes more biofilm. Therefore, we hypothesized that these congeners differentially use the intertidal area. A tide following index (TFI) was used to describe their distributions in the upper intertidal during ebbing tides. Also, we assessed sediment grain size, biofilm (= microphytobenthic or MPB) biomass and invertebrate abundance. Foraging dunlin closely followed the ebbing tide line, exploiting the upper intertidal only as the tide retreated through this area. In contrast, western sandpipers were less prone to follow the tide, and spent more time in the upper intertidal. Microphytobenthic biomass and sediment water content were highest in 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 more closely matched MPB biofilm availability than invertebrate availability. Conservation of sandpipers should consider physical processes, such as tides and currents, which maintain the availability of biofilm, a critical food source during global migration.

1. Introduction

Physical processes over and within soft intertidal zones underlie complex spatial and temporal patterns in the distribution and abundance of biota (e.g. Sewell, 1996). For example, tidal cycles and wave action control intertidal flat hydrodynamics (Le Hir et al. 2000), which influence sediment grain size and penetrability (Jackson et al. 2005), thus structuring constituent epifaunal (above-sediment) and infaunal (within-sediment) invertebrate communities (Whitlatch, 1977). In turn, avian foraging on invertebrates is facilitated by the morphological adaptations of the particular bird species (Nebel et al. 2005). For long-billed shorebirds, the suitability of a foraging habitat is governed by the interaction between bill length and sediment penetrability; longer bills and softer sediments resulting in greater predation success on infaunal prey (Nebel et al. 2005). Conversely, for short-billed birds, foraging habitat is determined by the availability of epifaunal invertebrates (Nebel et al. 2005), which in turn is associated with sediment grain-size and high tidal exposure (Whitlatch, 1977). Thus, shorebird adaptations, morphological and behavioural, for feeding on invertebrates are linked to physical processes on mudflats.

Intertidal biofilm, a ubiquitous thin layer of adhering microorganisms, provides a further example of a biotic community driven by physical processes occurring on intertidal mudflats. Intertidal biofilm comprises a matrix-enclosed community of microphytobenthos (MPB), bacteria, and organic detritus (Kuwae, 2002)

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bound together and stuck to the sediment surface by copious amounts of extracellular polymeric substances (EPS) secreted by microorganisms living within the biofilm (Stal, 2003; Underwood and Paterson, 2003). As intertidal exposure time increases (Herlory et al. 2004; Hanlon et al. 2006), biofilm builds up as a result of vertical migration of motile diatoms positioning themselves in the photic zone (Mitbavkar and Anil, 2004; Underwood et al. 2005).

Western sandpipers (*Calidris mauri*) and dunlin (*Calidris alpina)* are able to graze on intertidal biofilm as well as peck and probe to exploit macro- and meio-faunal invertebrate prey (Elner et al. 2005; Mathot et al. 2010; Kuwae et al. 2012). Both shorebird species have been observed foraging close to the tide line during ebbing and rising tides (Brennan et al. 1985; Colwell and Landrum, 1993; Butler et al. 2002) and are reported to exhibit a moderate to high degree of overlap in invertebrate consumption (prey composition and size; Couch, 1966; Senner et al. 1989). However, despite similarities in foraging behaviour, their capacities differ due to differences in feeding morphology and bill length (Elner et al. 2005). In particular, the higher density and length of keratinized lateral spines on the western sandpiper's tongue as compared to dunlin are indicative of a greater reliance on biofilm (Elner et al. 2005).

Tides are one of the most important factors affecting the distribution of foraging shorebirds (Connors et al. 1981; Granadeiro et al. 2006). During high tide, intertidal flats are inundated and shorebirds are restricted to roosting sites or inland areas. However, as the tide recedes, intertidal habitats become increasingly available for foraging shorebirds, exposing both invertebrate prey and biofilm (Colwell and Landrum, 1993). Thus, the discovery that small-bodied sandpipers graze on biofilm (Kuwae et al. 2008) has opened a fresh dimension for understanding the physical and biotic factors responsible for shaping western sandpiper and dunlin foraging patterns.

Roberts Bank on the Fraser River Estuary, British Columbia, Canada, is an internationally important stopover and wintering area for shorebirds along the Pacific flyway, with over one million shorebirds estimated to use the area annually (Butler and Campbell, 1987). In particular, hundreds of thousands of western sandpipers arrive in late April to early May each year, en route from wintering areas along the coasts of Central and South America to their Arctic breeding grounds (Butler, 1994). Their numbers are supplemented by tens of thousands of dunlin that overwinter in the estuary as well as migrate through to their breeding grounds in Alaska (Butler and Campbell, 1987). We used the opportunity to examine how the distribution of western sandpipers and dunlin related to the availability of invertebrates and biofilm as the ebbing tide exposed the intertidal habitat. We hypothesized that the two species would segregate their foraging behaviours as the tide retreats based on their different aptitudes for biofilm grazing (Elner et al. 2005; Kuwae et al. 2012). We predicted that if biofilm feeding is more prevalent in western sandpipers, then as the tide ebbs, western sandpipers will (1) feed farther from the tide line than dunlin as biofilm abundance and accessibility increases in exposed sediments; (2) preferentially feed in the areas with highest fine sediment and water content as such conditions promote biofilm abundance, and (3) spend more time than dunlin feeding in the upper intertidal as this region has the highest biofilm abundance on Roberts Bank, as measured by MPB biomass. We measured the dispersion of western sandpipers and dunlin in the upper intertidal zone at Robert Banks, focusing on the behavior of individuals foraging on the intertidal area exposed by the ebbing tide. Also, we examined physical characteristics (sediment grain size and water content), and food abundance (MPB biofilm and invertebrates) within the upper intertidal zone at Robert Banks to determine their relation to shorebird foraging distribution.

2. Methods

2.1. Study site

Roberts Bank is an 8000 ha intertidal area forming part of the Fraser River estuary (49°03′ N, 123°09′ W; Fig. 1). The bank environment comprises a complex of riparian boundaries, intertidal marshes, mud and sand flats, eelgrass meadows, macroalgae and biofilms (Sutherland et al. 2013). Hundreds of thousands of mixed-species shorebirds, primarily western sandpipers and dunlin, use the estuary (Butler, 1994; Butler and Vermeer, 1994). Drever et al. (2014) assessed the population sizes and stopover times of western sandpiper and dunlin during their breeding migration through the estuary over the period 1991–2013.

Tides are semidiurnal with a range of 0.0–3.8 m (North American Datum). Sediments are clay and sand, with the proportion of sand increasing towards the low water mark (Eisma, 1998; Zharikov et al. 2009). An extensive dendritic channel system occupies the upper intertidal and extends down to the mid-intertidal. Also, Roberts Bank hosts a large coal and container port, as well as the Tsawwassen ferry terminal, both connected to the mainland by causeways. Our study site was located off Brunswick Point, a salt marsh promontory north of these causeways.

2.2. Shorebird distribution

We measured the dispersion of western sandpipers and dunlin across the intertidal area as the tide ebbed. A 500 m transect marked with 1 m poles at 50 m intervals was established, with two shorter (0.5 m) markers delineating a circle of 5 m radius around each pole, within which shorebirds were counted (Fig. 1). Flocks of sandpipers often roosted at high tide, but by the time the tide had fallen to the top of the transect 150 m from the high tide line all western sandpipers and dunlin were actively foraging.

Counts were conducted during the morning receding tide beginning between 0525 h and 0910 h, depending on the daily tide conditions and subject to sufficient light to allow shorebird identification. A count sequence began as the receding tide line reached the first marker at 150 m from the high tide line (tidal height 3.6 m), and terminated when it reached the final marker at 650 m from shore (tidal height 2.7 m). Counts were carried out using a 40× spotting scope from a vantage point on Brunswick Point, located 250 m from the centre of the transect. We made sweeps of the transect at 6 minute intervals, recording the location of the tide line with reference to the markers, and the numbers of western sandpipers and dunlin foraging within the 5 m radius circle of each marker. Only days with a minimum average of 25 birds per counts were considered. We completed count sequences on eight days during northward migration in 2008 (April 24 – May 6) and on four days in 2009 (April 26 – April 30). In all, 158 counts were made over the 12 days, for a mean of 13.2 counts per day.

We compared the intensity of tide line foraging for both western sandpipers and dunlin with a tide following index (TFI), defined as the proportion of birds of a given species foraging within 100 m of the ebbing tide edge. The TFI was calculated for each species (western sandpiper and dunlin) and count interval (n = 158) using the formula:

$$\mathrm{TFI_i} = \mathrm{Tide\ followers_i}/\mathrm{Total\ birds_i},$$

where ‘Tide followers’ denotes the number of individuals of the species foraging within 100 m of the tide edge, and ‘Total birds’ is

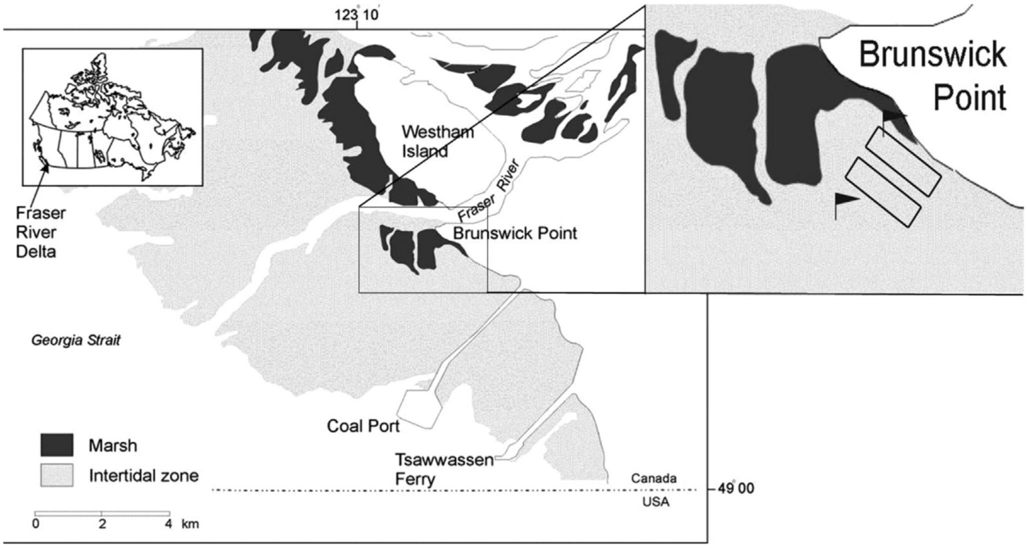

Fig. 1. Roberts Bank, British Columbia, showing the study site (Brunswick Point) and the two spatial sampling designs. Flags indicate the origin and end of a 500 m transect used to assess sandpiper's tide following behavior and distribution. Rectangles indicate the initial and final sections (150–350 m and 450–650 m from shore, respectively) of the upper intertidal where microphytobenthic biofilm and invertebrate samples were obtained.

the total number of individuals of that species recorded along the transect during the same count interval.

In principle, the value of TFI can vary between 0 (no birds foraging with 100 m of the tide line) and 1 (strict tide following). However, the upper intertidal at Brunswick Point is funnel-shaped, so that the area available along the tide edge increases more rapidly than the overall area as the tide ebbs, and thus biasing the TFI to underestimate tide-following intensity. We calculated the corrected-TFI value expected for a strict tide following species at Roberts Bank by estimating the proportion of the total intertidal area within 100 m of the tide line as each transect marker became emerged (Table 1).

For both western sandpipers and dunlin, we calculated the difference between the observed TFI and that expected for a strict tide follower. We termed the difference 'TFI deviance'. The TFI deviance can vary between 0 and 1. Values close to zero (i.e. expected = observed) indicates strict tide following, with all individuals foraging within 100 m of the receding tide edge. A positive difference (i.e. expected > observed) indicates avoidance of the tide line, with greater values indicating more avoidance. Occasional small negative differences (i.e. expected < observed) indicate measurement errors, for example due to underestimates of habitat availability.

Table 1
Area measurements at Brunswick Point, Roberts Bank, British Columbia and values for the tide following index (TFI) expectation line.

Polygon	Area of polygon (ha)	Cumulative area (ha)	Proportion of total area	Expected decrease in TFI	TFI value expectation
Shore-150[*]	78.3	78.3	0.163	0	1
150–200	21.6	99.9	0.045	0	1
200–250	10.1	110.0	0.021	0.021	0.979
250–300	17.3	127.4	0.036	0.057	0.943
300–350	26.7	154.1	0.055	0.112	0.888
350–400	14.4	168.5	0.03	0.142	0.858
400–450	28.1	196.7	0.058	0.201	0.799
450–500	23.5	220.2	0.049	0.25	0.75
500–550	33.7	253.9	0.07	0.32	0.68
550-600	46.5	300.4	0.097	0.416	0.584
600–650	63.1	363.6	0.131	0.547	0.453
650-low tide	118.0	481.6	0.245	N/A	N/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).

To determine how distribution and abundance changes in relation to food source, we also carried out counts in two 4 ha plots in the intertidal on four occasions (April 27–30, 2009; see Fig. 1). The upper of these was termed the 'initial section', and the lower the 'final section'. Once the TFI protocol was completed, we counted foraging sandpipers in these plots during eight successive 15 min intervals. Birds resting or sleeping were excluded from counts.

2.3. Invertebrate abundance

We measured macrofaunal abundance in the top 10 mm of sediment as these invertebrates make the greatest contribution in both numbers and biomass to total invertebrate density at western sandpiper stopover sites (Mathot et al. 2007). Eleven randomly placed samples were collected from each of two ('initial' and 'final') 4 ha plots established in the intertidal area (see Fig. 1) between April 30 and May 2, 2008. We used a 26 mm internal diameter syringe with apex removed and edges beveled (see Sutherland et al. 2000; Pomeroy and Butler, 2005; Pomeroy, 2006; Mathot et al. 2007) to collect invertebrates from sediments. The modified syringe was pushed into the sediment and the plunger pulled until 30 mm of sediment was drawn into the barrel. We took care to prevent contact between the plunger and the sediment surface. The plunger was removed from the syringe, inserted into the opposite end of the barrel and pushed to extrude the sediment. Samples were stored on ice until being stored in a freezer.

In the laboratory, samples were thawed and sieved through 0.5 mm mesh using filtered water. Although sandpiper species may consume meiofauna (which would have passed through the 0.5 mm sieve; Sutherland et al. 2000), we assessed only macrofaunal in this study. The retained material was preserved in labelled vials with 85% ethanol. Using a stereomicroscope (Leica Wild M8) at 60×, we identified, sorted and counted macroinvertebrates of major taxonomic groups, as documented in studies of western sandpiper and dunlin diet (Wolf, 2001; Andrei et al. 2009). Invertebrate density is expressed as the number of individuals per sample.

2.4. Biofilm abundance

We used a 26 mm internal diameter syringe with apex removed and edges bevelled (as described above) to draw samples to measure MPB biofilm abundance. Eight randomly placed cores were collected in each of two ‘initial’ and ‘final’ 4 ha plots established in the intertidal (Fig. 1). The cores were drawn immediately (0800 h), 1 h (0900 h), 3 h (1100 h) and 8 h (1700 h) after the sediment became exposed on April 28 and 29, 2008. Biofilm samples were taken by slicing the top 2 mm of the mud surface extracted with the core. The material was placed in labelled Ziploc bags, stored on ice in a cooler, and frozen at −20 °C within 2 h of collection.

We measured chlorophyll-*a* (Chl-*a*) content, a known estimator for MPB biomass in intertidal sediments (Underwood and Smith, 1998; Pomeroy and Butler, 2005; Hanlon et al. 2006). In the laboratory, the frozen samples 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 by absorbance at 665 and 750 nm using a Genesys 10uv spectrophotometer. 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). Next, 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 ($\mu g\ g^{-1}$ dry sediment (DM)).

2.5. Sediment grain size

A 26 mm internal diameter syringe with apex removed and edges beveled (as described above) was used to core sediment. Samples were collected along the 500 m transect at the end of the 2009 migration period (May 8), with three sediment cores (30 mm deep) taken at random points ~1 m from each marker ($n = 33$).

Samples were weighed both wet and dry (oven dried for 48 h at 70 °C) to the nearest 0.01 g. Samples were soaked for 24 h in a solution of sodium hexametaphosphate (6.2 g/l) to dissolve agglomerated particles. Grain size was assessed 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 through the successive sieves with tap water for 10 min. The resulting sand fractions were dried and weighed again. Sediment grain size composition is represented as the percentage contribution in mass of any particular fraction to the total.

2.6. Statistical analyses

To test whether differences in TFI deviance vary with distance from shore, we conducted an ANOVA with difference in the species TFI deviance (DUNL − WESA) for a given observation as the dependent variable and distance from shore as the independent variable. If a significant effect of distance from shore on the difference of species TFI deviance occurred, we conducted a paired t-test with Bonferroni corrections to examine at which distance from shore the species TFI deviance differed. Sediment grain size composition (mud fraction only) and overall invertebrate density were compared between the initial and final sections of the upper intertidal using a two-sample t-test. We examined spatial differences between macrofaunal benthic assemblages at the 22 sampled sites at two intertidal sections using Bray–Curtis similarity measures. Analysis of similarity (ANOSIM; Quinn and Keough, 2002) was performed on the similarity matrix of differences between intertidal sections based on the multivariate structure of the macrofaunal assemblages using 999 permutations in the test. We used ordination by non-metric multidimensional scaling (NMDS) based on the Bray–Curtis similarity matrix to visualize the similarity pattern of the macrofaunal assemblages at the studied intertidal sections.

Temporal and spatial variation in Chl-*a* and water content were compared between the initial and final sections of the upper intertidal and sampling intervals were tested using two-way analyses of variance (ANOVA), with *a posteriori* Tukey's HSD pairwise comparisons. When necessary, transformations were used to achieve the assumptions of homogeneity and normality (ln (Chl-$a + 1$)). Most computations and statistical tests were performed using the R statistical software version 2.10.0 (R Development Core Team, 2009). The computer package PRIMER (Clarke and Gorley, 2006) was used for the ANOSIM test and the NMDS representation. The final configuration of the presented NMDS was the best solution (i.e. exhibited the lowest ‘stress’ values) from a minimum of 100 random starts. Unless otherwise made explicit, we present means with standard deviation (±SD). All tests were two-tailed and the level of significance was $P < 0.05$.

3. Results

3.1. Shorebird distribution

During the high tide period, both western sandpipers and dunlin roosted along the marsh, in close proximity to the high tide line. The two species differed in their dispersion over the mudflat as the tide receded and foraging area became available (Fig. 2). The progression of TFI over the tidal cycle over all observation days is summarized in Fig. 3. Both species began close to the TFI value expected of a strict tide follower, but the observed TFI fell with the tide. Distance from shore had a significant effect on the species differences in TFI deviance (ANOVA, $F_{9,148} = 2.70$, $P = 0.006$). At all markers western sandpiper followed the tide less intensively than dunlin. Such a difference was significant in 4 of 10 cases after Bonferroni correction (Table 2).

The relationship between expected and observed TFIs over a count sequence more closely approximated a linear trend (Fig. 3) for dunlin than for western sandpipers. The major departure from expected TFI values for dunlin occurred in the lowest section of the transect (450–600 m), reaching its highest deviance around 500 m (Table 2). In contrast, the TFI for western sandpipers declined as the tide edge exposed the 250 m marker and reached its highest deviance at 450 m (Table 2). The greatest difference between species occurred at the 400 m marker, when TFI deviance for western sandpiper was 3.3 times greater than that recorded for dunlin (Table 2).

The general shorebird distribution pattern, shown in Fig. 2, can be described as follows: 1) western sandpipers were more constrained to the higher portion of the upper intertidal for most of the count sequence; 2) western sandpiper density in the initial section of the upper intertidal was evenly distributed, and the highest abundance occurred far from the tide line, notable especially once the tide line had passed the 350 m marker; 3) the highest densities of dunlin occurred close to the tide line, with dunlin ranging greater distances from shore than western sandpipers; 4) Dunlin were scarce or absent from most of the upper portion after the site had

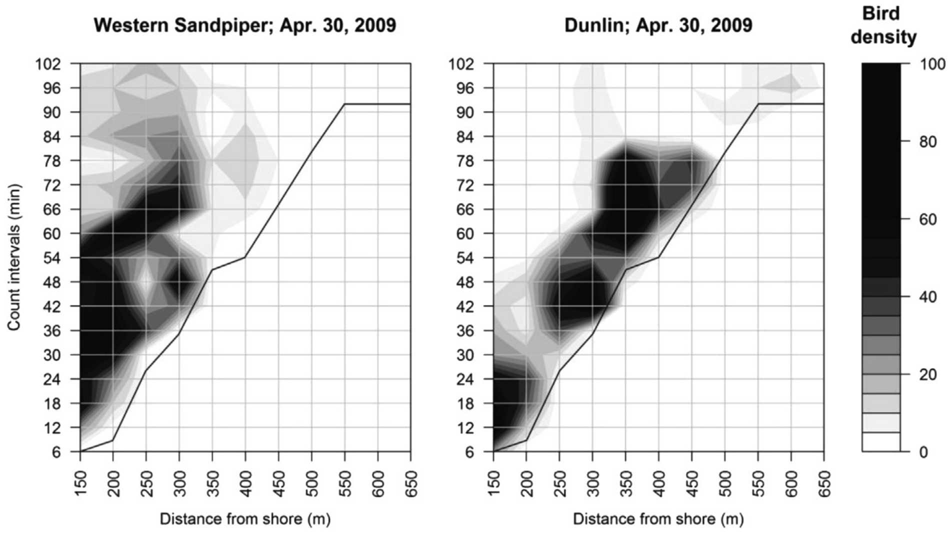

Fig. 2. Typical spatial (distance from shore – m) and temporal (elapsed time after the first 150 m got exposed – min) variation in western sandpiper (WESA, *Calidris mauri*) and dunlin (DUNL, *Calidris alpina*) density during the time it took to uncovered the first 650 m of the upper intertidal at Brunswick 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. Note that the maximum bird density represented is 100 birds per 5 m-radius circle. 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).

been exposed for more than approximately 50 min. Nine of the 12 sampled days (75%) generally followed this pattern. On the remaining three days (April 29, 2008; May 5, 2008; April 27, 2009) western sandpiper distribution patterns resembled those described for dunlin. Dunlin, in contrast, never demonstrated the distribution pattern of western sandpipers, their smaller congener.

Once the upper intertidal was fully exposed, the density of western sandpiper was higher than dunlin (Fig. 4, western sandpiper: 104.7 ± 115.7 birds/ha; dunlin: 12.4 ± 34.2 birds/ha, t-test: $t_{126} = -5.80$, $P < 0.001$). Similar numbers of western sandpipers were detected in both 1 ha plots once the tide line had passed the 650 m marker, but temporal declines started immediately at the initial section of the upper intertidal. Western sandpiper density in the section closest to shore reached its minimum 60 min after the counts began. At this time, all shorebird numbers started to decline in the final section of the upper intertidal. Few dunlin were present in the initial section of the upper intertidal after the site was completely exposed. The 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 (tidal height 2.7 m).

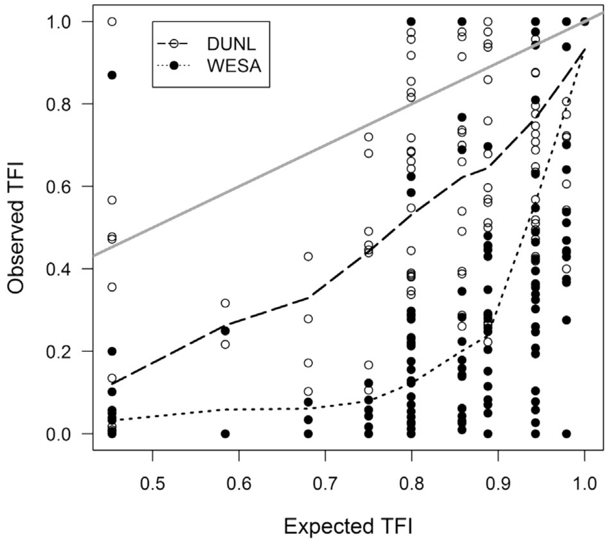

Fig. 3. Relationship between the expected and observed tide following index (TFI, proportion of birds within 100 m from the water edge) of western sandpiper (WESA, *Calidris mauri*) and dunlin (DUNL, *Calidris alpina*) at Brunswick Point upper intertidal, Robert Banks, British Columbia. Trend lines show 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).

3.2. Invertebrate abundance

Seven invertebrate taxa were found at the upper intertidal (nematodes, molluscs, polychaetes, ostracods, copepods, tanaids, cumaceans). All taxa occurred in both sections (Fig. 5), except for

Table 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.

Distance from shore (m)	N	Western sandpiper	Dunlin	P Value[*]
		Mean ± SD	Mean ± SD	
150	24	0.12 ± 0.34	0.08 ± 0.28	0.664
200	19	0.33 ± 0.31	0.28 ± 0.32	0.583
250	25	**0.55 ± 0.29**	**0.29 ± 0.24**	**<0.001**
300	20	**0.64 ± 0.19**	**0.33 ± 0.34**	**<0.001**
350	16	**0.60 ± 0.30**	**0.25 ± 0.26**	**<0.001**
400	23	**0.57 ± 0.23**	**0.17 ± 0.26**	**<0.001**
450	11	0.72 ± 0.04	0.43 ± 0.27	0.004
500	4	0.65 ± 0.04	0.43 ± 0.14	0.081
550	3	0.50 ± 0.14	0.41 ± 0.16	0.642
600	13	0.35 ± 0.24	0.22 ± 0.32	0.170

[*] Differences between the tide following deviance for western sandpipers and dunlin were analysed by paired t-test. Statistically significant differences are shown in bold based upon P < 0.001 after Bonferroni correction.

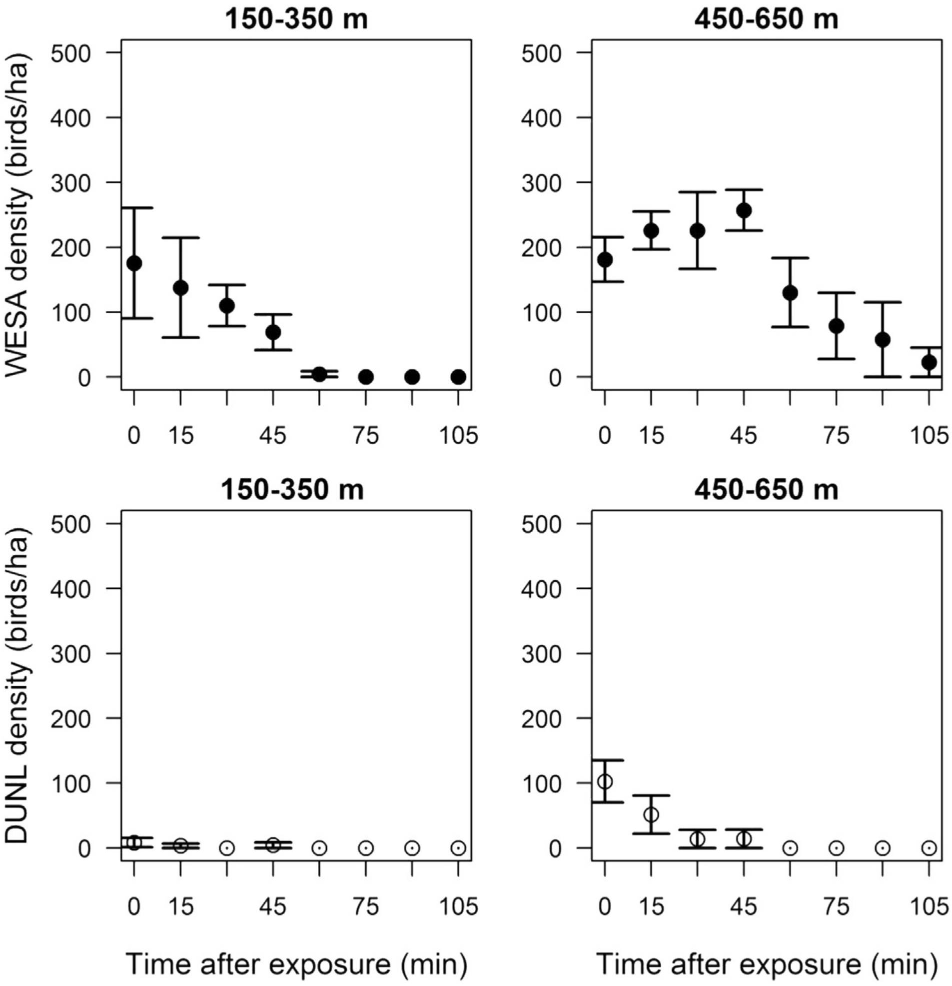

Fig. 4. Western sandpiper (WESA, closed circles) and dunlin (DUNL, open circles) density (birds/ha) at the initial (150–350 m from shore) and final (450–650 m from shore) section of the upper intertidal at Brunswick Point. Bird counts were conducted after the receding tide had exposed the first 650 m of the mudflat. Errors bars are ± SE.

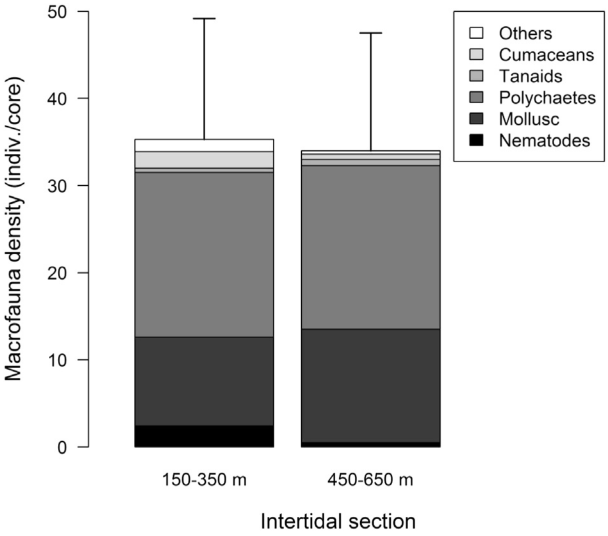

Fig. 5. Mean stacked density (±SD) of macrofaunal benthic invertebrates at two intertidal sections within the upper intertidal (150–350 m and 450–650 m from shore) in Roberts Bank, British Columbia, Canada. “Others” includes copepods and ostracods.

ostracods that were found in only one sample in the initial section. Macrofaunal invertebrates were dominated by polychaetes and bivalve molluscs, which together provided 87% of the overall invertebrate density (Fig. 5; 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; t-test, $t_{20} = -0.17$, $P = 0.866$).

The multivariate structure of the macrofaunal assemblage was not significantly different between the initial section and final section (ANOSIM, $R = -0.024$, $P = 0.605$). The NMDS ordination suggests that macroafaunal assemblages sampled at 150–350 m from shore were similar to those sampled within the final section of the upper intertidal (450–650 m; Fig. 6). Samples collected at 150–350 m from shore showed a higher dispersion in the plot suggesting a notable variability in the assemblage structure.

3.3. *Biofilm abundance*

The Chl-*a* content in the top 2 mm of the sediment from the initial section of the upper intertidal was greater than in the final section ($F_{1,56} = 62.59$, $P < 0.001$; see Fig. 7). Chl-*a* content during the emersion period averaged 21.6 ± 5.8 $\mu g\ g^{-1}$ dry sediment in the initial section, whereas an average of 12.7 ± 1.9 $\mu g\ g^{-1}$ dry sediment was recorded in the final section of the upper intertidal. There was no significant temporal variation in Chl-*a* content ($F_{3,56} = 0.78$,

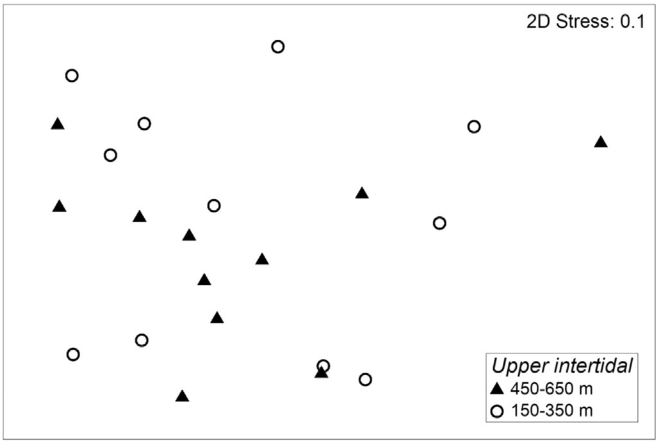

Fig. 6. Non-metric multidimensional scaling (NMDS) plot of benthic macrofaunal assemblages at 22 random points sampled in Brunswick Point, Roberts Bank. The spatial sections of the upper intertidal were superimposed on benthic community ordination: open circles = initial section of the upper intertidal (150–350 m from shore), filled triangles = final section of the upper intertidal (450–650 m from shore).

$P = 0.508$). The intertidal section × sampling time interaction for Chl-*a* content was not significant ($F_{3,56} = 1.11$, $P = 0.351$), suggesting that the difference in Chl-*a* among sections was consistent throughout the day. Overall, the initial section showed more intra-site variation for both Chl-*a* and water content than those located in the final section of the transect (Fig. 7).

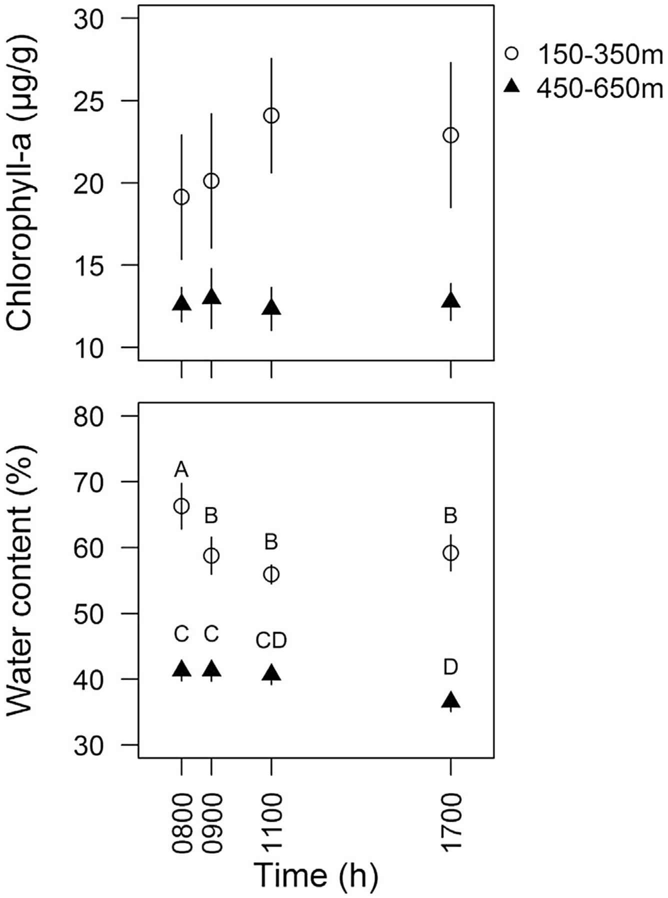

Fig. 7. Spatial and temporal variation in mean (95% CI) Chlorophyll-a content (μg $g^{-1}{}_{\text{dry sediment}}$) and mean (95% CI) water content (%) in the top 2 mm of the sediment at the initial (open circles, 150–350 m) and final (open triangles, 450–650 m) spatial sections of Roberts Bank's upper intertidal. Samples were collected at four time intervals during the emersion time: immediately after exposed (0800), 1 h after emersion (0900), 3 h after emersion (1100) and before immersion (1700).

The results showed that change in mean water content throughout the day depended on the intertidal section (two-way ANOVA: intertidal section × emersion time, $F_{3,56} = 9.37$, $P < 0.001$). *Post hoc* comparisons indicated that the sediment at the initial section had higher percentage of water content than the sediment at the final section of the intertidal, especially immediately after the sediment became exposed (Fig. 7).

3.4. Sediment grain size

Sediment in the upper intertidal off Brunswick Point was comprised of mud and sand (Fig. 8). Mud predominated (86.1–62.9% by weight) and showed a gradual decline with distance from shore. On average, the higher portion of the upper intertidal contained 10% more mud than the lower portion (80.3 ± 4.3% *vs.* 71.5 ± 4.2%, respectively; t-test: $t_{31} = 5.86$, $P < 0.001$). The fine sand fraction, in contrast, increased with distance from the shoreline. The remaining sand fractions contributed less than 7% to the total dry sediment weight, with coarse sand contributing the least of sediment fractions analyzed.

4. Discussion

The surficial homogeneity of intertidal flats belies the underlying spatial and temporal complexity of the biota (Underwood and Chapman, 1996). In the absence of confounding factors, such as predation risk (Pomeroy, 2006), the species, abundance and foraging modes of shorebirds over an intertidal flat can be expected to reflect the availability of food. Food availability is related not only with abundance (i.e., MPB biomass), but also with preference, accessibility and cost of feeding on the resource (e.g. Zwarts and Wanink, 1993). Here, we present results that suggest patterns in shorebird distribution are heavily influenced by the availability of biofilm. Our results show that western sandpipers and Pacific dunlin adopt different foraging behaviors during the period of ebbing tide at Roberts Bank, in accordance with our first hypothesis. Initially, both species follow the receding tide edge, but once the top 350 m of the upper intertidal was exposed, a high proportion of western sandpipers stop progressing down the mudflat.

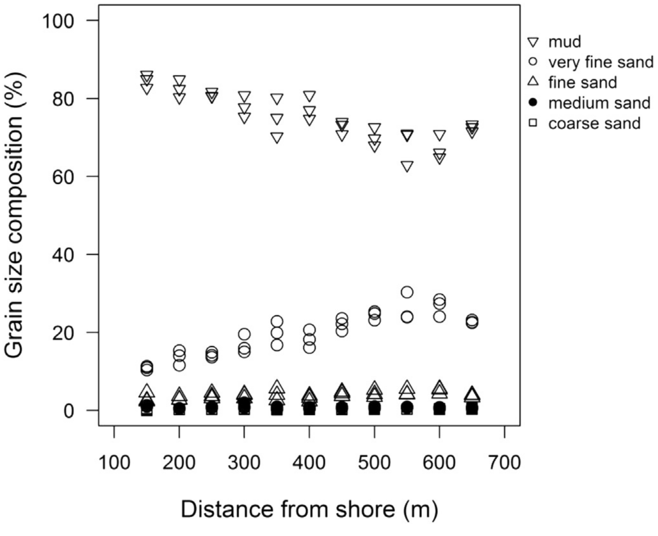

Fig. 8. Sediment grain size composition along a 700 m long transect at the upper intertidal at Brunswick Point, Roberts Bank, British Columbia.

In contrast, dunlin continually shifted with the tide line position, exploiting briefly the entire extent of the upper intertidal.

We found that western sandpipers fed in areas conducive to biofilm abundance, i.e. areas with high water content and fine sediment, in accordance with our second hypothesis. The higher MPB biomass detected in the first 350 m from shore is consistent with the documented 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), and the mud content increases with elevation of the intertidal area. Also, we found that western sandpipers spent more time than dunlin feeding in the upper intertidal, in accordance with our third hypothesis. Further, due to its profile, the upper intertidal sediment retains a high water content, which we surmise is important for western sandpipers as the mechanism proposed for biofilm ingestion involves copious amounts of wet mud (Elner et al. 2005; Kuwae et al. 2008).

There are various possible explanations for the prolonged use of the upper intertidal by western sandpipers. First, they might have been targeting invertebrates trapped in shallow pools (Rosa et al. 2007). However, after only 20 min of emersion, pools and channels were scarce over our study area, and we consider that predation on such invertebrates was not an important factor. A second possible invertebrate-oriented explanation is that by using the muddiest sediments, the shorebirds were taking advantage of feeding areas with highest sediment penetrability (Quammen, 1982), allowing them to probe for large invertebrates. If so, a high frequency of probing should have been observed, whereas previous studies on our site have shown that probing rates are markedly lower than pecking rates for both sexes (Sutherland et al. 2000; Mathot and Elner, 2004). Thirdly, there is the possibility that western sandpipers were targeting meiofaunal invertebrates bound into the biofilm. However, multiple lines of evidence, including food source mixing models, stomach content analyses and energy budgets, demonstrate that biofilm and microphytobenthos are the major food sources for western sandpipers on Roberts Bank (Kuwae et al. 2008, 2012).

Most previous literature has categorized western sandpipers as well as dunlin as ‘tide followers’, with strong preferences for foraging near the tide edge (Colwell and Landrum, 1993; Warnock and Takekawa, 1995; Butler et al. 2002; Granadeiro et al. 2006; but see; Couch, 1966; Senner et al. 1989). Although biofilm was not explicitly recognized by researchers in the aforesaid publications, the noted presence of fine sediments (which are conducive to biofilm formation), suggests that biofilm grazing may have been underlying tide following behaviours in the Mud River Estuary, San Francisco (Colwell and Landrum, 1993), Tagus Estuary, Portugal (Granadeiro et al. 2006) and Hartney Bay, Alaska (Senner et al. 1989). In sum, our results may not be unique to Roberts Bank and could be relevant across intertidal areas worldwide.

The underlying reason why on 3 of our 12 visits, western sandpipers behaved as dunlin and followed the tide through the upper intertidal remains unclear. Senner et al. (1989) reported that western sandpipers changed from ‘non-tide following’ to ‘tide following’ behaviour when dunlin were largely absent from the area, suggesting that the shift indicates competitive exclusion of western sandpipers by dunlin from preferred foraging options. Together, these observations indicate that non-tide following by western sandpipers is facultative and conditions such as, variously, relative food availability over the intertidal area (possibly due either to the presence of competitors and/or actual changes in the spatial and temporal dynamics of biofilm production), day-to-day changes in predation risk over the intertidal (cf. Pomeroy, 2006), and/or other unknown factors make either tide or non-tide following the better option. Whatever the reason(s), our results uphold our prediction that due to their greater propensity for biofilm grazing (Mathot et al. 2010; Kuwae et al. 2012), the foraging dispersion of western sandpipers, at least in the upper intertidal and in the period following high tide, more closely matches the observed distribution of biofilm than the distribution of macroinvertebrates.

Biofilm grazing as the most parsimonious explanation for our findings on differential tide following is further supported by other considerations. First, the intensive use of the upper intertidal by western sandpiper aligns with biofilm being the major component of their diet at Roberts Bank (Kuwae et al. 2008; Beninger et al. 2011; Kuwae et al. 2012). Secondly, rapid pecking is the predominant feeding mode used by western sandpipers on Roberts Bank (Mathot and Elner, 2004), and is also reflective of biofilm grazing and stomach contents (Elner et al. 2005; Kuwae et al. 2008, 2012). In comparison, the tide following of dunlin is consistent with a more tactile foraging strategy, relying extensively on visual cues for feeding on epifauna (Granadeiro et al. 2006; Santos et al. 2009, 2010). Benthic invertebrates are more active and therefore visible when immersed (Vader, 1964; Rosa et al. 2007; Santos et al. 2010). Thus, a sandpiper foraging in shallow water along the receding tide line will likely benefit from an enhanced encounter rate with epifaunal prey.

Intertidal and estuarine habitats, and the species that rely on them, are increasingly threatened as shorelines become modified by anthropogenic structures and sea-level rise (Bulleri and Chapman, 2010; Hill et al. 2013). In particular, coastal developments and dredging can alter tidal regimes and sedimentation patterns (Pratolongo et al. 2013), which may, in turn, affect biofilm production and shorebird habitat quality (Sutherland et al. 2013). Adding to these concerns, many shorebird species worldwide are experiencing population declines (Wetlands International, 2006). While the reason(s) for the declines are unclear (Thomas et al. 2006; Catry et al. 2011) shorebird conservation practices have, hitherto, not encompassed the important role that intertidal biofilm is now known to play in shorebird diets, in general, and fuelling breeding migration, in particular. Based on our study, we recommend that environmental assessments for coastal development and conservation strategies for shorebirds need to explicitly consider the physical and biotic processes that produce and replenish biofilm.

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