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BIOFILM GRAZING IN A HIGHER VERTEBRATE: THE WESTERN SANDPIPER, *CALIDRIS MAURI*

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Abstract. We show that a higher vertebrate can graze surficial intertidal biofilm, previously only considered a food source for rasping invertebrates and a few specialized fish. Using evidence from video recordings, stomach contents, and stable isotopes, we describe for the first time the grazing behavior of Western Sandpipers (*Calidris mauri*) and estimate that biofilm accounts for 45–59% of their total diet or 50% of their daily energy budget. Our finding of shorebirds as herbivores extends the trophic range of shorebirds to primary consumers and potential competitors with grazing invertebrates. Also, given individual grazing rates estimated at seven times body mass per day and flock sizes into the tens of thousands, biofilm-feeding shorebirds could have major impacts on sediment dynamics. We stress the importance of the physical and biological processes maintaining biofilm to shorebird and intertidal conservation.

Key words: *Calidris mauri*; foraging behavior; grazing behavior; intertidal ecosystems; natural diet; shorebirds; surficial intertidal biofilm; trophic ecology; Western Sandpiper.

INTRODUCTION

Worldwide declines in shorebird populations (Wetlands International 2006) underscore the need to better understand their biology, and in particular their feeding ecology. Shorebirds peck and probe for food from intertidal flats and wetlands. Although their natural diets are diverse, macroinvertebrates such as polychaetes, molluscs, crustaceans, and insects have been considered their principal prey (e.g., Zwarts et al. 1990, Skagen and Oman 1996). However, observations of feeding behavior, stomach contents, and field experiments show that macrofauna (prey >0.5 mm) alone cannot account for their complete diet (Sewell 1996), and the contribution of meiofaunal prey (<0.5 mm) is now recognized (Zwarts et al. 1990, Sutherland et al. 2000).

Recently, examination of bill and tongue morphology of two calidrid species presented a functional case for unfiltered grazing on surficial biofilm (Elner et al. 2005). However, whether biofilm was actively or only incidentally grazed and how much it contributed to diet was unknown. Biofilm consists of a thin (0.01–2 mm) yet dense layer of microbes, organic detritus, and sediment in a mucilaginous matrix of extracellular polymeric substances together with non-carbohydrate components secreted by microphytobenthos and benthic bacteria (Characklis and Marshall 1990). Rasping invertebrates and some highly specialized fish have long been considered as the sole consumers of biofilm (Decho 1990, Horn and Ojeda 1999).

In this first report of biofilm feeding for any higher vertebrate, we present evidence from video images of feeding behavior, stomach content analyses, stable isotope techniques, and energy budget models to identify the contribution of biofilm and associated organisms to the diet of Western Sandpipers (*Calidris mauri*; see Plate 1).

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MATERIALS AND METHODS

Video images

Foraging and defecation by Western Sandpipers were recorded (30 frames/s) using a digital camcorder (Canon, XL1S) with telephoto lens (Canon, EF 400 mm f/2.8L IS USM) through a lens adaptor (Canon, EF Adaptor XL). Video images with a focal length of 2880 mm (82.3× optical magnification) were acquired by this system. Video images were replayed and analyzed frame-by-frame to document the feeding actions of individual sandpipers (Kuwae 2007).

Sampling

Western Sandpipers were collected (Permit #59-03-0398 and amendment #1 from Environment Canada) from Roberts Bank (49°05' N, 123°12' W) on the Fraser River estuary, British Columbia, Canada, during northward migration (April/May 2003 and 2004). Their stomachs were fixed with 80% ethanol and contents identified to the lowest possible taxonomic level. The frequency of occurrence and estimated percentage of total stomach volume were recorded for individual taxa (Swynnerton and Worthington 1940), and sediment subsamples of each stomach were examined for unbroken microphytobenthos. Nine stomach samples were frozen, freeze-dried, and powdered for isotopic analyses. A further nine samples were frozen for individual photopigment analysis.

Western Sandpiper droppings (<30 min after defecation) were collected from the intertidal of Roberts Bank ($n = 20$, 3 May 2005; $n = 89$, 5 May 2005). To test if biofilm formed on mud sediment surfaces contributes to the food sources for the sandpipers, surface sediments (up to ~1 mm depth) were collected from the site using a toothbrush ($n = 20$, 10 May 2005). No macroinvertebrates were visible in the sediments. Small invertebrates (0–2 cm depth) were collected from the site as an aggregation of visible food sources, using a 1-mm mesh sieve ($n = 5$, 25 May 2005). Microscopy revealed that the samples contained cumaceans, polychaetes of *Capitella* spp. (~1 cm length), and organic matter (i.e., a mixture of small invertebrates and biofilm). Large polychaetes were also collected by digging at the site ($n = 5$, 25 March 2006; $n = 15$, 28 March 2006; $n = 12$, 30 March 2006; $n = 3$, 18 April 2006; $n = 6$, 3 May 2006). All of the samples were dried and powdered for analyses of stable isotope ratios, total organic carbon content, total nitrogen content, and energy content.

Sediment samples (~1 mm depth) were collected on two days ($n = 6$, 16 March 2006; $n = 12$, 3 May 2006), and microphytobenthos was extracted by modifying the method of Couch (1989); the samples were spread on a tray to ~5 mm depth, a nylon screen (65 μm mesh) was laid over the sediment, and precombusted glass wool was placed over the screen. The tray was kept moist by spraying with filtered seawater and left in the dark at

ambient temperature (~20°C) overnight. The glass wool was removed and kept dry until stable isotope analyses.

Energy budget calculations

The energy budget of Western Sandpipers and the contribution of biofilm to daily energy requirement were estimated. Biofilm water content was calculated as the difference between biofilm mass before and after drying at 60°C for 24 h ($n = 6$). Energy content of biofilm was measured using a bomb calorimeter (CA-4PJ, Shimadzu, Kyoto, Japan; $n = 20$). Liquid paraffin was added to ensure complete combustion. Energy intake rate from biofilm (kJ/d) was estimated by multiplying the observed foraging action rate (actions/minute), defined as rate of contact of bill tip to the sediment surface (excluding when capturing small invertebrates), scraping biofilm mass per foraging action (mg wet mass per action; Elner et al. 2005), water content of biofilm (%), the energy content of biofilm (kJ/g dry mass), assimilation efficiency (%; Castro et al. 1989), available foraging time during tidal exposure in daytime (h/d), and the percentage of available foraging time spent foraging (%; Ntiamoa-Baidu et al. 1998). Daily energy requirement (kJ/d) was estimated by adding daily fattening rate (0.4 g/d, equal to 16 kJ/d, assuming 100% fat deposition to avoid overestimation of biofilm contribution; Warnock and Bishop 1998, Williams et al. 2007) to 2.8× basal metabolic rate (BMR; kJ/d; Castro et al. 1992). BMR was calculated using an allometric equation for nondesert environments (Tieleman and Williams 2000) and body mass (Williams et al. 2007).

Photopigment analyses

High performance liquid chromatography (HPLC) was carried out using the technique of Méléder et al. (2003), adapted from Mantoura and Llewellyn (1983), allowing estimation of both actively producing microphytobenthic biomass (chlorophyll *a*) and higher level taxonomic composition of this biomass (accessory pigments). Pigments were extracted from sediment samples (0.04–2.4 mL: 0.2–12 cm² surface area, 2 mm depth) and stomach contents, filtered through glass microfiber filters using 90% acetone (24 h at 4°C in the dark), and diluted in ammonium acetate. The spectra of biofilm and stomach contents were compared over the 400–800 nm spectral range and equivalent pigments identified.

To estimate the contribution of microphytobenthos to total carbon and nitrogen in surface sediments (biofilm), chlorophyll *a* (chl *a*) content of the biofilm was determined using spectrophotometry ($n = 10$; Lorenzen 1967). Chl *a* content was then converted into carbon and nitrogen contents using the carbon : chl *a* mass ratio of 40 (de Jonge 1980) and the Redfield ratio (carbon : nitrogen atomic ratio of 106:16; Redfield 1932). The calculated values were then divided by total organic carbon and total nitrogen contents in the biofilm.



PLATE 1. Western Sandpipers (*Calidris mauri*) grazing surficial biofilm on the Roberts Bank mudflat, British Columbia, Canada. For their feeding behavior, see movie files in the Appendices. Photo credit: T. Kuwae.

Stable isotope analyses

Droppings from Western Sandpipers were pretreated prior to stable isotope analyses to remove potential metabolites, such as uric acid, urea, and ammonium. Subsamples of each powdered sample (~5 mg) were placed in microtubes, mixed with a 1.4 mL 1:1 chloroform:methanol solution, centrifuged for 15 min at $1300 \times g$, and the supernatant eliminated. This treatment was repeated four times, and the solvent-treated sample was then freeze-dried. The samples were subsequently acidified using 1 mol/L HCl to eliminate carbonates (Cloern et al. 2002) because of the sediment content in Western Sandpiper droppings. All other samples used for stable isotope analyses were acidified in the same way.

Stable isotope ratios of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$), total organic carbon content, and total nitrogen content were measured using a Delta Plus Advantage mass spectrometer (Thermo Electron, Bremen, Germany) coupled with an elemental analyzer (Flash EA 1112, Thermo Electron) and a Delta Plus mass spectrometer (Thermo Finnigan, Bremen, Germany) coupled with an elemental analyzer (EA 1110, CE Instruments, Milan, Italy). Stable isotope ratios are expressed in δ notation as the deviation from standards in parts per thousand (‰) according to the following equation: $\delta^{13}\text{C}, \delta^{15}\text{N} = [R_{\text{smp}}/R_{\text{std}} - 1] \times 10^3$, where $R = {}^{13}\text{C}/{}^{12}\text{C}$ or ${}^{15}\text{N}/{}^{14}\text{N}$. Pee Dee Belemnite and atmospheric nitrogen were used as the isotope standards of carbon and nitrogen, respectively. The analytical precision in

the Delta Plus Advantage mass spectrometer system based on the standard deviation of the internal reference replicates was 0.15‰ for $\delta^{13}\text{C}$ and 0.14‰ for $\delta^{15}\text{N}$.

The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of small invertebrates was estimated using the general nature of a slight enrichment in ${}^{13}\text{C}$ by 1‰ (DeNiro and Epstein 1978) and a stepwise enrichment in ${}^{15}\text{N}$ by 3‰ (Minagawa and Wada 1984). We used 3‰ per trophic level as a conservative value for the ${}^{15}\text{N}$ fractionation factor to avoid overestimation of biofilm contribution.

Diet assessment: three-source mixing model

Contribution of potential food sources to the diet of Western Sandpipers was evaluated using the computer program IsoError (Phillips and Gregg 2001), which calculates estimates and confidence intervals of source percentage contributions to a mixture by linear mixing models to partition three sources with $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. Since the percentage of possible selective feeding of microphytobenthos from biofilm was unknown, we were unable to determine the unique $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for biofilm. Therefore, we used the isotopic values of biofilm in the full range of selective feeding of microphytobenthos from biofilm (0–100%). The values for biofilm were calculated using linear interpolation between the isotopic values of microphytobenthos (100%) and the estimated values of biofilm without microphytobenthos (0%). The values for biofilm without microphytobenthos were calculated using the fraction of microphytobenthos to total carbon and nitrogen in biofilm, determined by the method described in the previous section.

TABLE 1. Estimated energy budget of Western Sandpipers and the contribution of biofilm to daily energy requirements.

Measurements	Value (mean \pm SD) [†]	Reference
Foraging action rate (actions/min); <i>A</i>	121 \pm 42 (10)	This study
Scraping biofilm mass (mg wet mass/action); <i>B</i>	2.6	Elner et al. (2005)
Biofilm energy content (kJ/g dry mass); <i>D</i>	0.83 \pm 0.12 (20)	This study
Biofilm energy ingesting rate (kJ/min); <i>E</i> = <i>A</i> \times <i>B</i> \times (1 - <i>C</i>) \times <i>D</i>	0.12 \pm 0.04 [‡]	This study
Assimilation efficiency (%); <i>F</i>	75	Castro et al. (1989)
Available foraging time (h/d); <i>G</i>	12.6	This study
Foraging time in <i>G</i> (%); <i>H</i>	80	Ntiamoa-Baidu et al. (1998)
Biofilm mass ingesting rate (g wet mass/d); <i>I</i> = <i>A</i> \times <i>B</i> \times <i>G</i> \times <i>H</i>	190 \pm 66	
Biofilm energy intake rate (kJ/d); <i>J</i> = <i>E</i> \times <i>F</i> \times <i>G</i> \times <i>H</i>	54 \pm 19	Warnock and Bishop (1998), Williams et al. (2007)
Fattening rate (kJ/d); <i>K</i>	16	Castro et al. (1992), Tieleman and Williams, (2000), Williams et al. (2007)
Field metabolic rate (kJ/d); <i>L</i>	92	
Daily energy requirement (kJ/d); <i>M</i> = <i>K</i> + <i>L</i>	108	
Contribution of biofilm energy intake to daily energy expenditure (%); <i>N</i> = <i>J</i> / <i>M</i>	50 \pm 18	

[†] Sample sizes are in parentheses.

[‡] Mean and SD were calculated using bootstrap sampling (a value was randomly chosen from raw data for each of the three variables, *A*, *C*, and *D*); then 1000 simulations were run and the mean and SD were generated from these 1000 values.

We used samples of bird droppings to represent the isotopic signature of Western Sandpiper diets. Droppings are likely to be enriched in indigestible diet components relative to stomach contents (Sponheimer et al. 2003). For Western Sandpipers, indigestible components are primarily comprised of invertebrate hard parts (e.g., jaws and setae of the polychaete *Nereis*), as indicated by the difference in isotopic compositions of stomach contents and droppings. Therefore, use of droppings to represent diet provides an extremely conservative estimate of the contribution of biofilm to Western Sandpiper diets.

RESULTS AND DISCUSSION

Feeding behavior on biofilm

Telephoto video recordings show sandpipers not only pecking invertebrate prey from the surface and probing but also grazing biofilm (Appendices A and B). The behavior accompanying biofilm ingestion is distinct from pecking and probing as well as from surface tension transport (a feeding mode that uses the surface tension of the water surrounding a prey item to transport the prey from the bill tip to the mouth) found in other shorebirds (Rubega 1997). During grazing, the sandpiper advances relatively slowly compared to more rapid darting while pecking or probing. First, surficial biofilm is collected between an \sim 3-mm opening of the bill tips, possibly with assistance from the tongue. Secondly, the bill tips close to \sim 1 mm and, while retaining a detached bolus of biofilm, are raised from the sediment. Thirdly, the bill repeatedly opens and closes with accompanying throat movements and the bolus moves back and forth. Here, the bolus is subject to bidirectional movement between coarse denticles on the interior of the upper bill and dense spines and papillae of the tongue (Elner et al. 2005); these actions may serve to

mechanically extract or sort food types. Finally, the sandpiper's throat movements indicate swallowing of the bolus. The four-phase sequence is completed within 0.3 s, and chains of double impressions from the bill tips are evident on the sediment after the bird has passed. As tongue actions are obscured, the mechanism for transporting material is not discernable, but appears

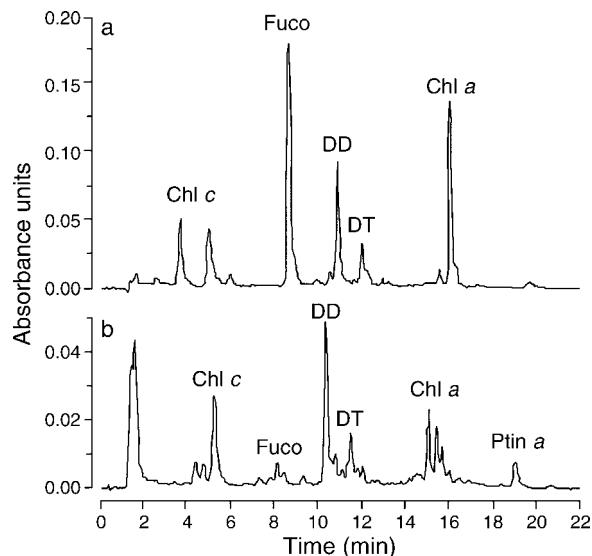


FIG. 1. Examples of HPLC chromatograms at 440 nm, 90% acetone extract, of (a) a sediment-biofilm sample and (b) stomach contents of Western Sandpipers collected on Roberts Bank, Fraser River estuary, British Columbia, Canada, in April/May, 2003 and 2004. Key to abbreviations: Chl *c*, chlorophyll *c*; Chl *a*, chlorophyll *a*; DD, diadinoxanthin; DT, diatoxanthin; Fuco, fucoxanthin; and Ptin *a*, phaeophytin *a* (a photopigment degradation product). One absorbance unit corresponds to the depreciation of the light intensity by 90% of the incident light.

distinct from surface tension feeding, where fluid is moved unidirectionally from bill tip to buccal cavity (Rubega 1997), and is more analogous to a suction system driven by a lingual pump (Zweers and Vanden Berge 1997).

Foraging rate and energy value

Surface foraging as determined by video image analysis was 137.1 ± 39.6 actions/min (mean \pm SD; $n = 10$), and probing occurred at a rate of 6.9 ± 9.0 actions/min ($n = 10$). The success rate for surface foraging actions was $13.3 \pm 9.7\%$ (16.2 ± 11.2 visible invertebrates/min, $n = 10$), and $22.7 \pm 28.1\%$ for probing (3.5 ± 5.0 visible invertebrates/min, $n = 6$). The relatively low mean capture rate for visible surface prey is consistent with sandpipers expending most foraging effort on biofilm (120.8 ± 41.5 actions/min, $n = 10$). Further, these observations show that biofilm intake can account, on average, for 50% (54 kJ/d) of the daily energy requirement of a sandpiper during migration (108 kJ/d; Table 1). However, if we consider the potential for nocturnal foraging by Western Sandpipers (Warnock and Takekawa 1996), available foraging time increases to 16.9 h and biofilm intake could then account for up to a mean of 68% (73 kJ/day) of daily energy requirement.

Biofilm as a component of natural diet

Consistent with observations of foraging behavior, visual and photopigment analyses of stomach contents reveal microphytobenthos and biofilm in addition to macro- and meiofauna. The 97 stomachs examined were variable in bulk, on average $75.6 \pm 26.9\%$ full, with a mean content mass of 0.43 ± 0.23 g. Seventy-six stomachs (78.4%) contained undigested parts of invertebrates, such as jaws and setae of *Nereis*, but these prey remnants contributed only a mean of $8.6 \pm 6.0\%$ to total stomach content volume. In comparison, sediment, including broken microphytobenthos, was present in all stomachs, with a mean sediment to total stomach content volume of $76.1 \pm 13.1\%$. Unbroken microphytobenthos occurred in 59 stomachs (61%), contributing a mean of $1.1 \pm 1.2\%$ to total stomach content volume. The same 23 photopigments were identified from both biofilm and stomach contents (Fig. 1), indicating that microphytobenthos present in biofilm is degraded in the stomach. These major pigments suggest that the dominant taxonomic group of microphytobenthos was diatomaceous. Degradation products (e.g., pheophytin *a*) typically found in the feces of algal grazers (Cartaxana et al. 2003), were detected in stomachs at higher levels than present in biofilms, further evidence that microphytobenthos is broken down by Western Sandpipers. Between 0.2 mg and 2.5 mg of chl *a* were present per stomach, corresponding to the amount of chl *a* present on 0.2–12 cm² of mudflat (this study). Therefore, a Western Sandpiper stomach can contain, at any one time, the equivalent of 0.2–12

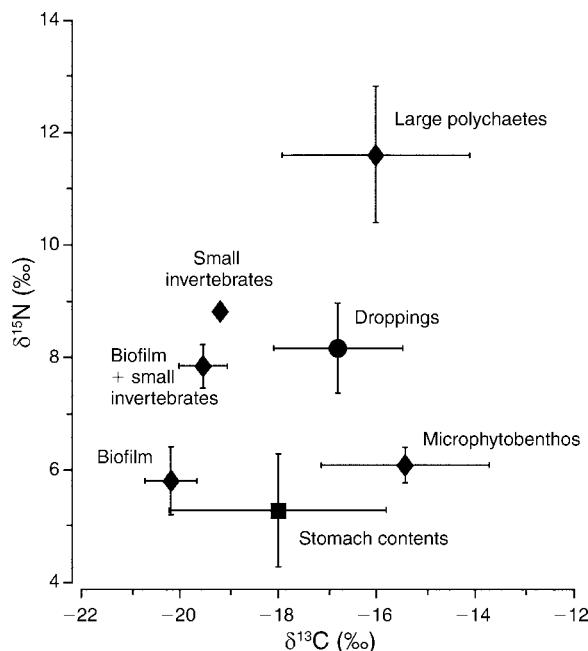


Fig. 2. Carbon and nitrogen stable isotopic ratios of droppings (circle; $\delta^{13}\text{C}$, $n = 107$, $\delta^{15}\text{N}$, $n = 105$; May 2005) and stomach contents (square; $n = 9$, collected in April/May 2003 and 2004) from Western Sandpipers. Diamonds indicate biofilm ($n = 20$; collected in May 2005), small invertebrates (mainly the polychaete *Capitella* and cumaceans), the mixture of biofilm and small invertebrates ($n = 5$; collected in May 2005), microphytobenthos ($\delta^{13}\text{C}$, $n = 18$; $\delta^{15}\text{N}$, $n = 17$; collected in March/May, 2006), and large polychaetes (mainly *Nereis*, *Glycera*, and *Eteone*; $\delta^{13}\text{C}$, $n = 41$; $\delta^{15}\text{N}$, $n = 38$; collected in March–May 2006) from Roberts Bank, Fraser River estuary, British Columbia, Canada. Symbols indicate mean \pm SD. The value of small invertebrates is estimated using the general enrichment in stable isotope signatures per trophic level.

cm² of scooped-up biofilm. The defecation rate recorded (0.50 droppings/min, $n = 117$) is high compared with other shorebirds (range, 0.047–0.52 droppings/min; Gonzalez et al. 1996, Zharikov and Skilleter 2002), suggestive of rapid passage of material through the gut. Together with the small stomach volume (<1 mL) and observations on sediment ingestion and microphytobenthos prevalence, this finding is indicative of microphagy.

We determined the stable isotopic ratios ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of potential food sources, stomach contents, and droppings of Western Sandpipers to estimate the relative contribution of each food source to Western Sandpiper diet (Fig. 2). Stomach content signatures are closest to those of biofilm and microphytobenthos, with extensive overlap, indicating that these sources comprise the majority of ingested material while invertebrates contribute comparatively little. The source and stomach content data are consistent with a low contribution of small invertebrates to assimilated food items in sandpiper diets, given the trophic enrichment of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values and the proximity of their $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures to those of droppings. Also, the shift from a

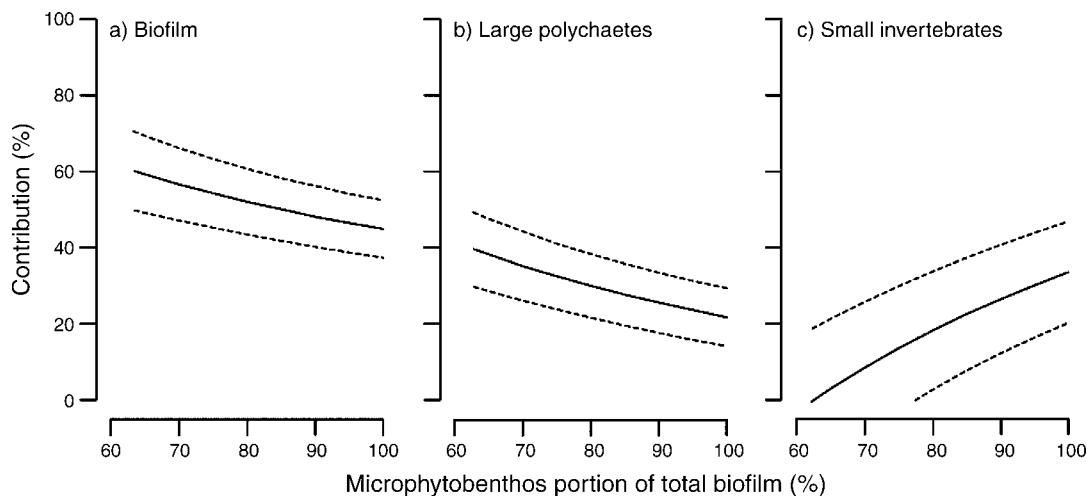


FIG. 3. Contributions of three food sources: (a) biofilm (microphytobenthos plus other sediment organic matter), (b) large polychaetes, and (c) small invertebrates to the diet of Western Sandpipers, varying with the percentage of microphytobenthos relative to total biofilm (x -axis). Solid lines and broken lines indicate means and 95% confidential intervals, respectively. Estimates were calculated using isotopic compositions of droppings as extreme conservative estimators of diet. Given the difference in isotopic compositions of stomach contents and droppings from Fig. 2, the actual contribution of biofilm is likely to be much higher. Percentage contribution of three food sources are shown for microphytobenthos to total biofilm values ranging from 65% to 100% because values <65% microphytobenthos predicted unrealistic (<0%) contributions of small invertebrates to the diet.

biofilm- and microphytobenthos-dominated signature in the stomach contents to a relatively invertebrate-dominated signature in the droppings suggests that biofilm and microphytobenthos are digested to a greater extent than invertebrates. The error bars indicate considerable individual variation within the dropping and stomach content values, which may reflect intra-species partitioning of diet, perhaps based, variously, on age, sex, and morphology (Durell 2000, Mathot and Elnor 2004), and a study of diet in relation to these parameters is underway.

We further examined the relative dietary contributions of biofilm, small invertebrates, and large polychaetes using a three-source mixing model (Fig. 3). Although biofilm comprised between $7.0 \pm 1.9\%$ (carbon basis, $n = 10$) and $11.0 \pm 4.1\%$ (nitrogen basis, $n = 10$) microphytobenthos, the diet models consistently indicated that consumed biofilm was at least 65% microphytobenthos, indicating selective feeding via the tongue-bill structures and bolus movements. Such selective feeding on microphytobenthos has been demonstrated in biofilm-grazing shrimp (Abreu et al. 2007). Further, regardless of the percentage of the sandpipers' selective feeding on microphytobenthos from biofilm, the calculated contribution of biofilm was consistently higher (45–59% by mass) than other potential food sources.

Implications of biofilm grazing

Revealing sandpipers as primary consumers and potential competitors with grazing invertebrates calls into question the trophic position of shorebirds in intertidal ecosystems. At least one other calidrid

sandpiper, Dunlin (*C. alpina*), also has tongue and bill morphology (Elnor et al. 2005) suggestive of biofilm grazing ability. Further, enigmatic observations of sediment (up to 60% of total contents) in shorebird alimentary tracts (Reeder 1951) could indicate biofilm consumption in other species. Shorebirds may be expected to select biofilm of suitable quality through a well-developed sense of taste (van Heezik et al. 1983). Advantages of biofilm feeding could include a hedge to nutritional uncertainty for long-distance migrants, a mechanism reducing intra- and interspecies competition for food, and a source of abundant immediately consumable energy such as carbohydrates (Characklis and Marshall 1989).

The challenge of harvesting a thin layer of nutritional and non-nutritional material, such as biofilm, at a rate sufficient to accumulate an energetically sufficient daily intake is offset by its ubiquitous presence (Newman and Banfield 2002, Mathot et al. 2007). Biofilm develops most richly over muddy low-energy intertidal and estuarine areas, without intensive sediment resuspension by hydrodynamic forcing (de Jonge and van Beusekom 1995). Biofilm grazing on such sites may also be facilitated by high water content in the sediment, as compared to sandy substrates in which both biofilm and water contents are low. However, biofilm is not restricted to intertidal flats or estuaries, and its use by shorebirds in other semiaquatic situations, such as the shorelines of lakes and lagoons, appears possible.

Intertidal sediments are recognized to vary in habitat value for shorebirds (e.g., Piersma 1987, Danufsky and Colwell 2003), but the results of this study suggest that situations promoting biofilm may be particularly critical

to some shorebirds. Threats to biofilm, either directly from coastal development, or indirectly through changing hydrodynamic processes, as well as additional grazing pressures from invasives such as the gastropod *Batillaria* (Miura et al. 2006), are not currently considered in environmental assessment procedures. Adverse cascading trophic interactions could be triggered by direct competition between higher vertebrates and invertebrates, leading to decreases in biofilm availability as a food for shorebirds, potentially contributing to population-level declines (Clark and Butler 1999). Thus, conservation of biofilm should be an explicit consideration not only for native invertebrate species but also higher trophic level organisms that have overlapping food sources.

Overall, our findings underscore the importance of microbial biofilms to ecosystem processes (Battin et al. 2003) and the physical and functional integrity of the intertidal system (Emmerson et al. 2001, Lundkvist et al. 2007). Further work on the dynamics of biofilm production and consumption by shorebirds, as well as the dynamics of sediment-stabilizing exopolymeric substances (mucopolysaccharide) produced mainly by benthic diatoms (Lundkvist et al. 2007), is necessary considering the estimated scale of shorebird grazing. A stop-over site such as the 6000-ha intertidal mudflat of Roberts Bank hosts over 1×10^6 Western Sandpipers over an ~15-day period during northward migration (Butler et al. 1987; M. Lemon and R. W. Butler, unpublished data). With each sandpiper ingesting 190 g wet mass of biofilm material per day (Table 1), approximately seven times mean body mass, $\sim 19.0 \times 10^3$ kg wet mass would be consumed per day by an average flock of 100 000 sandpipers. Also, future research should investigate the extent of biofilm grazing by other shorebird species in relation to the broader scale distribution of suitable biofilm. In particular, the greater abundance of small shorebirds ("peeps"), such as the Western Sandpiper, along the Flyways of the Americas relative to the East Asian-Australasian Flyways and the African-Eurasian Flyways (Wetlands International 2006) may reflect either differential patterns in biofilm availability or interspecific differences in the ability to use biofilm as a food resource.

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APPENDIX A

Normal speed mode replay of a Western Sandpiper biofilm-feeding on the Roberts Bank mudflat, the Fraser River estuary, British Columbia, Canada (*Ecological Archives* E089-033-A1).

APPENDIX B

Slow mode replay (1/4 speed) of a Western Sandpiper biofilm-feeding on the Roberts Bank mudflat, the Fraser River estuary, British Columbia, Canada (*Ecological Archives* E089-033-A2).