

AGRICULTURAL LANDS SUBSIDIZE WINTER DIET OF THE DUNLIN AT TWO MAJOR ESTUARIES

KEITH A. HOBSON^{1,5}, GARY L. SLATER², DAVID B. LANK³, RUTH L. MILNER⁴ AND RACHEL GARDINER³

¹Environment Canada, 11 Innovation Blvd., Saskatoon, SK S7N 3H5, Canada

²Ecostudies Institute, P.O. Box 703, Mount Vernon, WA 98273

³Simon Fraser University, Department of Biology, Burnaby, BC, Canada

⁴Washington Department of Fish and Wildlife, P.O. Box 1100, LaConner, WA 98257

Abstract. On the western coast of North America, several estuaries provide shorebirds with important winter and stopover habitat. These habitats include not only aquatic estuarine resources but also adjacent upland agricultural lands. The extent to which shorebirds use estuarine vs. upland habitats at these stopover sites is difficult to quantify but crucial to designing strategies for their conservation. We measured stable isotopes ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$) in whole blood of Dunlins (*Calidris alpina*) and their prey from two major estuaries in north Puget Sound, Washington, USA, to estimate their relative use of estuarine vs. upland agricultural zones. We identified four isotopically distinct dietary inputs (agriculture high in ^{15}N , other agriculture, marsh/marine, and freshwater plume). Isotopic sampling and modeling was informed by movements and habitat use derived from radiotelemetry. This isotopic structure allowed us to conclude that these Dunlins obtained about 62% of the protein in their diet from agricultural lands and 38% from the estuary. Our results underline the urgent need to combine management of estuaries and upland agricultural areas in strategies for shorebird conservation.

Key words: agricultural lands, carbon-13, diet, estuary, nitrogen-15, nutrients, Pacific Dunlin.

Las Tierras Agrícolas Subsidiarian la Dieta Invernal de *Calidris alpina pacifica* en Dos Grandes Estuarios

Resumen. En la costa oeste de América del Norte, varios estuarios brindan a las aves playeras hábitats importantes para el invierno y las paradas. Estos hábitats incluyen no sólo los recursos acuáticos del estuario, sino también las tierras agrícolas adyacentes. El grado con el que las aves playeras usan los hábitats del estuario vs. los de tierra firme en estos sitios de parada es difícil de cuantificar pero es crucial para diseñar estrategias para su conservación. Medimos isótopos estables ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$) en la sangre de *Calidris alpina* y en sus presas en dos grandes estuarios en el norte de Puget Sound, Washington, EUA, para estimar su uso relativo de las zonas del estuario vs. las agrícolas de tierra firme. Identificamos cuatro consumos de la dieta distintivos en términos isotópicos (agricultura alta en ^{15}N , otra agricultura, pantano/marino y penacho de agua dulce). El muestreo y modelado isotópico fue establecido a partir de movimientos y uso de hábitat derivado de radio telemetría. Esta estructura isotópica nos permitió concluir que *Calidris alpina* obtuvo cerca de 62% de la proteína de su dieta de las tierras agrícolas y 38% del estuario. Nuestros resultados subrayan la necesidad urgente de combinar el manejo de los estuarios y de las áreas agrícolas de tierra firme en las estrategias de conservación de las aves playeras.

INTRODUCTION

Coastal estuaries are among the most productive ecosystems on earth and provide important habitat for an array of organisms that exploit the gradient between terrestrial, freshwater, and marine resources (Mitsch and Gosselink 2000). Shorebirds, many of which aggregate in large numbers, are a conspicuous component of these systems and rely on them seasonally, especially as refueling sites during migration to and from their breeding grounds and when on the wintering grounds. Coastal estuaries also have been greatly altered through a wide spectrum of human activities, including the diking and draining of wetlands for agriculture. Shorebirds'

use of converted agricultural lands bordering estuaries for feeding and roosting has been widely documented (Goss-Custard 1969, Gerstenberg 1979, Rottenborn 1996, Shepherd and Lank 2004, Evans Ogden et al. 2005, Conklin and Colwell 2007). This has led authors to call for greater conservation and management of these agro-estuarine complexes for shorebirds, especially as these regions remain under intense pressure from additional development not expected to benefit shorebirds (Shepherd et al. 2003, Evans Ogden et al. 2008). A more comprehensive understanding of the ecological role these regions play in providing habitat for shorebirds during

Manuscript received 14 July 2012; accepted 12 November 2012.

⁵E-mail: Keith.Hobson@ec.gc.ca

the nonbreeding season, particularly with respect to food resources, is required to advance the development of key management priorities in these highly altered environments.

Estuarine foodwebs are inherently complex, both temporally and spatially, because of the mixing of nutrients from terrestrial and estuarine/marine sources, and it is often difficult to evaluate the relative importance of these sources to biota. The measurement of naturally occurring stable isotopes of key elements has been used to trace the flux of nutrients in several systems including estuaries (reviewed by Michener and Schell 1994, Coffin et al. 1994). This approach is based on the fact that the relative abundance of stable isotopes of elements sequestered in primary productivity changes according to a variety of biogeochemical processes, and these isotopic signals can be passed on to higher-order consumers. Specifically, marine and terrestrial systems typically differ in baseline isotopic signatures of elements such as C, N, H, S, and O. Additionally, plants can differ significantly in isotopic signature in both terrestrial and marine systems because of differences in photosynthetic pathways. In agricultural systems, the foodweb's isotopic values are also spatially complex, reflecting various processes including fertilizer input, the effect of soil tillage and exposure, irrigation, and planted crop (Nadelhoffer and Fry 1994, Pardo and Nadelhoffer 2010). Despite the isotopic complexity of agro-estuarine systems, tracing the origin of dietary sources of nutrients from various regions within these systems remains possible, especially with a dual-isotope approach.

We investigated the origin of diet nutrients in a wintering population of the Dunlin (*Calidris alpina*) by measuring stable isotopes in whole blood and prey items across a gradient from upland terrestrial to marine habitats in the Skagit River and Stillaguamish River deltas, both of which have been identified as important stopover estuaries for migrating and wintering shorebirds along the Pacific Flyway (Drut and Buchanan 2000). Like many estuaries along the west coast of North America, they have been greatly modified by agriculture development, yet each supports large congregations of migrating and wintering shorebirds (>30 000 individuals in each season; Evenson and Buchanan 1997; R. Milner, unpublished data). The Dunlin is the most abundant wintering species at these sites, constituting 90% of the shorebird population (Evenson and Buchanan 1997). The area has been the focus of a long-term study of wintering Dunlins' use of estuarine and agricultural habitat, involving traditional observational surveys and radio telemetry. Results from this isotope study will improve our understanding of habitat use and furthers our understanding of nutrient pathways leading to birds in estuarine foodwebs. The objectives of our study were to identify which isotopically distinct foodweb components could be traced by a two-isotope ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$) approach and to specifically quantify the relative proportion of nutrients from upland vs. estuarine and marine sources that contribute to the diet of wintering Dunlins. We further evaluated how

seasonality influenced variation in the source of nutrients because some previous studies have recorded shifts in shorebirds; use of uplands through the winter (Evans Ogden et al. 2005).

METHODS

STUDY AREA

We recorded data on overwintering Dunlins and their prey from two adjacent estuaries in northern Puget Sound, Washington; the Skagit River delta ($48^{\circ} 19' \text{ N}$, $122^{\circ} 24' \text{ W}$) and the Stillaguamish River delta ($48^{\circ} 12' \text{ N}$, $122^{\circ} 22' \text{ W}$; Fig. 1). The Skagit River is the largest river flowing into Puget Sound, terminating in Skagit Bay, and its delta extends over ~32 670 ha. The smaller Stillaguamish River, approximately 15 km to the south, terminates in Port Susan Bay. Its delta covers 4330 ha. In both estuaries, over 85% of wetland habitats have been lost, almost entirely to agricultural development (Collins 2000).

The study area supports one of the northernmost populations of the Dunlin wintering on the Pacific Coast, averaging 20 000–30 000 individuals (R. Milner, Washington Department of Wildlife, unpubl. data; Evenson and Buchanan 1997).

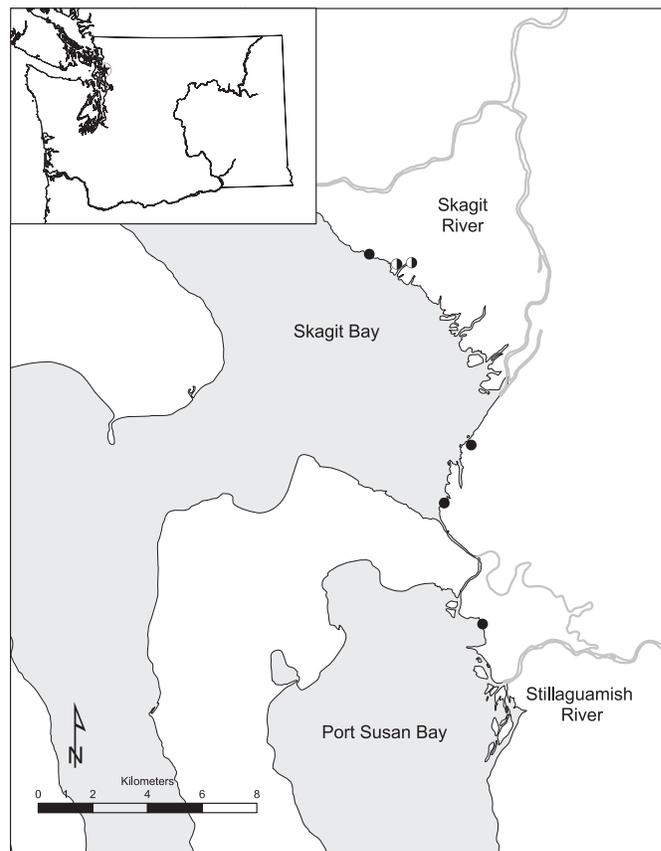


FIGURE 1. Map of study area and locations of captured Dunlins. Half-filled circles, locations of capture during the winters of both 2006–2007 and 2007–2008; filled circles, locations of capture during the winter of 2007–2008 only.

Dunlins typically arrive in the region from breeding grounds in October and remain until early April, when they depart for their breeding grounds (Paulson 1993).

BIRD CAPTURE AND SAMPLING

To obtain blood samples and attach radio transmitters, we captured Dunlins during winter 2006–2007 and 2007–2008. In the first year, captures, in mist nets positioned in agricultural and marsh habitats at sunrise and sunset (Fig. 1), were restricted to the Skagit River delta. We trapped Dunlins from 19 December 2006 to 9 February 2007, but individuals captured early in the season were only radio-marked, as our long processing time precluded both radio-marking and collecting blood from individuals. We obtained blood samples following radio-marking efforts from 4 January to 9 February 2007, with the exception of one blood sample collected on 19 December 2006. During the second winter, we expanded trapping to include south Skagit Bay and Port Susan Bay (Fig. 1) and captured birds with mist nets and a net gun. We also refined our processing technique, which allowed us to radio-mark and collect blood from the same individuals throughout the season. Dunlins were captured from 27 November 2007 to 7 February 2008 when tidal conditions were favorable (Fig. 2).

We banded each individual with a U.S. Fish and Wildlife band and measured its culmen length. We sexed the birds by culmen length (males <37.9 mm; unknown 37.9–39.7; females >39.7; Shepherd 2001) and distinguished first-year birds from adults by their buffy edges on the inner median coverts (Paulson 1993). We collected a 100- μ L blood sample via the brachial vein. Because Dunlins arrive at the study site no later than mid-October, and the rate of turnover of ^{13}C and ^{15}N isotopes in whole blood is about 21 days (Evans Ogden et al. 2004), the blood of birds we captured in this study had isotope values reflecting local conditions. Collected blood samples were frozen until lab analyses.

HABITAT USE OF THE DUNLIN

Details of radio-marking, tracking, and obtaining location data were presented by Slater et al. (2011). In brief, we tracked the radio-marked birds from fixed and variable points along dikes, roads, and walking transects across the estuaries. During the second winter, we supplemented these ground-based efforts with aerial tracking from fixed-wing aircraft, which allowed us to survey the expanded study area better and therefore greatly enhanced the probability of detection. Tracking by telemetry took place nearly daily within blocks stratified by river delta (Skagit vs. Stillaguamish), time of day (night vs. day), and tide stage (high vs. low), and aimed to cover agricultural and estuarine habitats similarly. We mapped habitats and categorized the birds' locations as either terrestrial, including cover crop, bare soil, crop residue, pasture, woody agriculture, and other agriculture, or estuarine, including mudflats and intertidal marsh. We calculated the mean habitat use of each radio-tagged bird, then combined data from individuals

to estimate the population-level habitat use, weighting each individual's contribution by the square root of n locations per bird (Aebischer et al. 1993).

PREY SAMPLING

Interpretation of stable-isotope values for dietary reconstructions requires obtaining all isotopically distinct dietary "end-point samples" of prey from the environment, against which blood values can be compared, following consideration of isotopic discrimination between diet and bird. We sampled from both the terrestrial and estuarine environments within the boundary of locations within which Dunlins were radio-tracked. To establish points for sampling within the terrestrial environment, we first defined two polygons of the primary agricultural regions Dunlins used in the Skagit and Stillaguamish river deltas. Within each polygon, we randomly selected >10 sampling points with the criterion that points were ≥ 500 m apart (Fig. 2). We visited each sampling point and collected 1 to 4 earthworms, a preferred prey of the Dunlin (Evans Ogden et al. 2007), dug from shallow holes (<10 cm deep).

Within the estuarine environment we established a pair of transects, roughly parallel to the tide line, in each estuary (four total) (Fig. 2). The rationale for placement of the transects in each estuary was based on two objectives: to ensure transects encompassed both the region where Dunlins were located via radio-telemetry and to sample the presumed freshwater/salinity gradient found in the estuary. We accomplished the first objective by identifying regions in each estuary where Dunlins foraged regularly on the basis of visual observations and radio-tracking. We addressed the second objective in two ways. First, we varied the proximity of each transect to the shoreline. The closest transect was established along the ecotone between emergent marsh and tidal flat, and the second transect was established approximately 1 km farther out on the tidal flat. We expected the transect farther from the shoreline to reflect more marine conditions. Second, we established transects perpendicular to a freshwater outflow, expecting the points closer to this outflow to reflect more freshwater conditions. Transects were approximately 2 to 3 km long, and we collected prey samples every 175 m by either collecting items off of the surface of the estuary (i.e., annelids) or digging a shallow hole (<10 cm deep) and collecting invertebrate prey (shrimp, mollusks, etc.). We collected samples only of invertebrates reported to be prey of the Dunlin. Samples were stored frozen until delivery to the lab.

STABLE-ISOTOPE ANALYSES

Blood samples were freeze-dried and powdered. Foodweb samples were cleaned with distilled water, freeze-dried, and powdered. Invertebrates were then subjected to a lipid-extraction step of a 2:1 chloroform:methanol soak and rinse. These samples were then dried in a fume hood for 48 hr. The samples were tested for the presence of carbonates by application of a few drops of 0.1 normal HCl. Samples showing any bubbling were then treated with this solution without being rinsed.

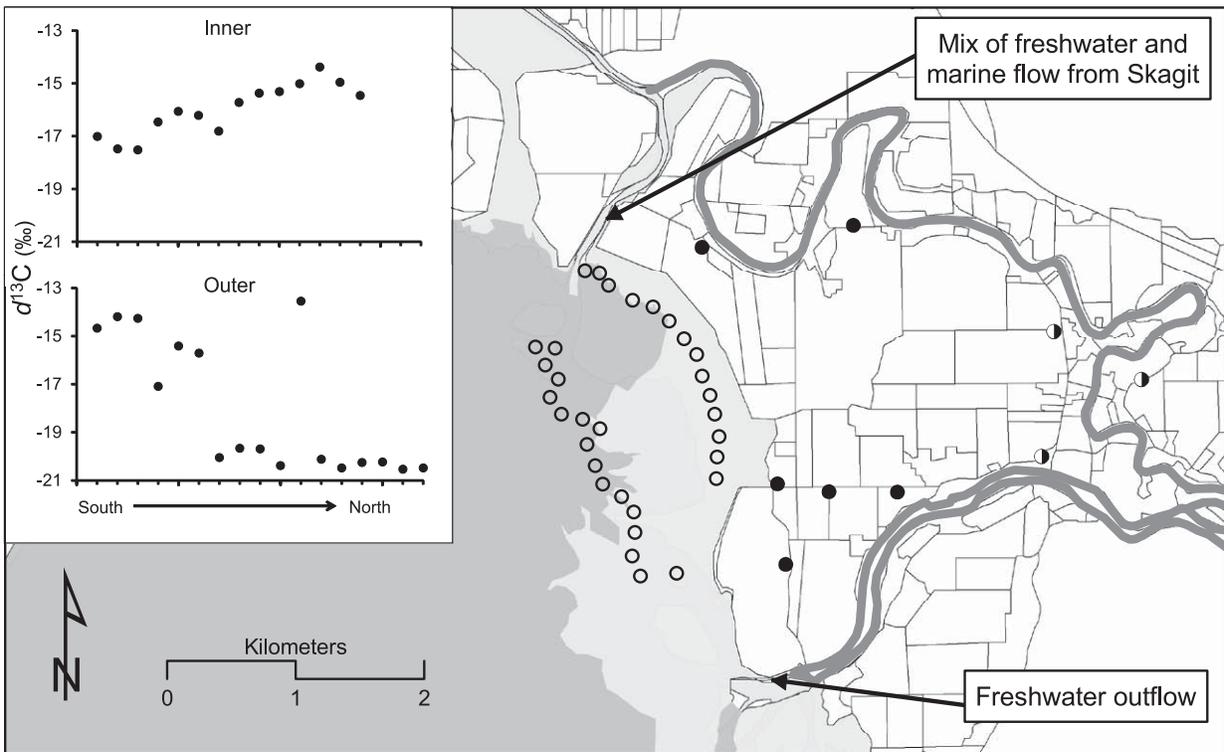
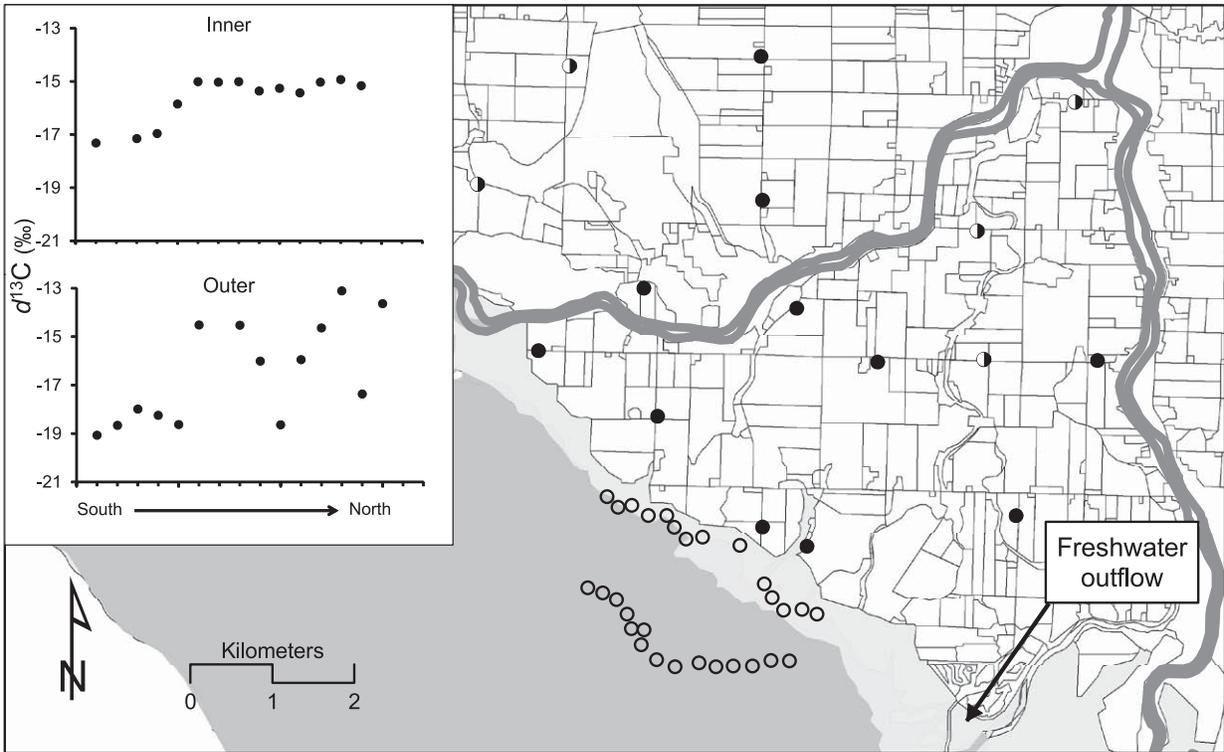


FIGURE 2. Locations of endpoint sampling in agricultural and estuarine environments of the Skagit River delta (upper) and the Stillaguamish River delta (lower). In the agricultural environment, half-filled circles indicate sampling locations omitted where Dunlin use was low. Rivers are represented by medium gray color, and land parcels are white with black border. In the estuary, open circles represent sampling locations. Dark gray color indicates tidal flats, light gray emergent marsh. Inset figures show the pattern of $\delta^{13}\text{C}$ values of prey along the inner and upper estuarine transects.

Treated powdered samples were weighed (1.0 ± 0.1 mg) into tin cups and analyzed with a Costech ECS4010 elemental analyzer coupled to a Delta V mass spectrometer with a ConFlo IV interface. Results were expressed in standard delta (δ) notation in parts per thousand deviation from the international standards Vienna Pee Dee Belemnite ($\delta^{13}\text{C}$) and atmospheric air ($\delta^{15}\text{N}$). We placed one internal laboratory standard (egg albumin for high-N samples and a Peagrain standard for low-N samples) between every five unknowns in each analytical run to correct for instrument drift. Using these replicate measurements within a run of standards, we estimate measurement precision to be of the order of $\pm 0.1\text{‰}$ for $\delta^{13}\text{C}$ and $\pm 0.3\text{‰}$ for $\delta^{15}\text{N}$. All analyses were conducted at the Stable Isotope Laboratory of the Department of Soil Science, University of Saskatchewan, Canada.

MIXING MODELS

Because our primary objective was to calculate the relative contribution of different habitats to the Dunlin's diet, we started by identifying isotopic endpoints of the foodweb representing the breadth of habitats the Dunlin used. Isotope values of prey were averaged for each sampling point along transects, so that the sampling point was considered the appropriate unit of dietary integration. This examination of how the isotope values differed by habitat and changed across the gradients we sampled enabled us to identify four unique/extreme endpoint isotopic signatures. We used these dietary endpoints, based on averages of these sampling-point values for each habitat, together with their SD, in a Bayesian mixing model (MixSIR, Moore and Semmens 2008) to quantify nutrients' relative inputs to diets.

For the second analysis, to evaluate the seasonality of the Dunlin's diet, we included only individuals captured during the second winter (2007–2008) because only during that winter did we capture birds throughout the winter. We divided isotope values into two groups; those of Dunlins captured before 10 January (early winter) and those of birds captured after 10 January (late winter). We selected this breakpoint for two reasons. First, it generally marked two periods of ecologically different habitat conditions in agricultural fields. In the agricultural fields of our study area, in late December winter rains typically saturate the soil and leave shallow pools of standing water. Second, the breakpoint coincided with a relatively long period in which no birds were captured. Most samples from the two periods were separated by a month; in the early-winter period, 44 of 45 samples were collected ≤ 23 December; in the late-winter period, 33 of 34 samples were collected ≥ 20 January. Because our previous analysis indicated that one of our four dietary inputs (freshwater) was minimal, we defaulted to using only three dietary endpoints in MixSIR. The mixing model run with two isotopes and three inputs gives a unique solution (vs. a probabilistic range of solutions).

Both of these approaches required the application of isotope-discrimination values for diet and blood derived

previously for this species from captive birds raised on an isotopically homogeneous diet (Evans Ogden et al. 2004; $\Delta^{13}\text{C}$: $+1.3\text{‰}$, $\Delta^{15}\text{N}$: $+2.9\text{‰}$).

RESULTS

CAPTURE AND SAMPLING

We captured 84 Dunlin during winter 2006–2007 and 87 in 2007–2008, obtaining a volume of blood sufficient for isotope analysis from 38 and 79 individuals, respectively. In 2006–2007, blood samples were extracted from 15 males, 9 females, and 14 individuals of unknown sex; 28 individuals were adults and 10 were in their first winter. In 2007–2008, blood samples were obtained from 34 males, 33 females, and 12 individuals of unknown sex; 27 individuals were adults and 52 were in their first winter. Measurements of $\delta^{15}\text{N}$ in Dunlins captured in 2006–2007 (13.1‰, 95% CI = 12.8 to 13.3‰, $n = 38$) and 2007–2008 (13.2, 95% CI = 13.1 to 13.3‰, $n = 79$) did not differ, but measurements of $\delta^{13}\text{C}$ were significantly lower in 2006–2007 (-18.6‰ , 95% CI = -18.0 to -19.2‰ , $n = 38$) than in 2007–2008 (-17.4 , 95% CI = -17.0 to -17.9‰ , $n = 79$).

Measurements of $\delta^{15}\text{N}$ in the blood of individuals captured before 10 January 2008 (early winter, $n = 45$, 13‰, 95% CI = 13.1 to 13.3‰) did not differ from those of individuals captured on or after 10 January (late winter, $n = 34$, 13.2‰, 95% CI = 13.0 to 13.4‰). Measurements of $\delta^{13}\text{C}$ for individuals captured before 10 January (-16.4‰ , 95% CI = -16.8 to -15.9‰) were significantly higher than for individuals captured on or after 10 January (-18.9‰ , 95% CI = -19.3 to -18.4‰).

HABITAT USE

In 2006–2007, we obtained 262 locations from 29 radio-tagged individuals (range 1–25), and in 2007–2008, we obtained 1132 locations from 69 radio-tagged individuals (range 1–41; Fig. 3). In both winters, Dunlins were more often located in estuarine habitats (2006–2007: mean (\pm SD) = $58 \pm 48\%$, $n = 29$; 2007–2008: mean = $76 \pm 46\%$, $n = 69$) than in terrestrial habitats (2006–2007: mean = $42 \pm 38\%$, 2007–2008: mean = $24 \pm 20\%$). In the estuary, Dunlins preferred tidal flats and low-elevation marsh habitats over high-elevation marsh (Table 1). They were rarely detected in terrestrial habitats other than agricultural cover types. Cover crops and fields of bare soil were the most commonly used agricultural habitats (Table 1).

ENDPOINT SAMPLING: TERRESTRIAL AND MARINE

We obtained samples of prey from 25 agricultural fields (16 from the Skagit River delta and 9 from the Stillaguamish River delta; Table 2). Values of $\delta^{15}\text{N}$ at the two agricultural sites did not differ (Skagit: mean = 7.5‰ , 95% CI = 6.3 to 8.7‰ , $n = 16$; Stillaguamish: mean = 6.8‰ , 95% CI = 4.1 to 9.6‰ , $n = 9$). Neither did those of $\delta^{13}\text{C}$ (Skagit: mean =

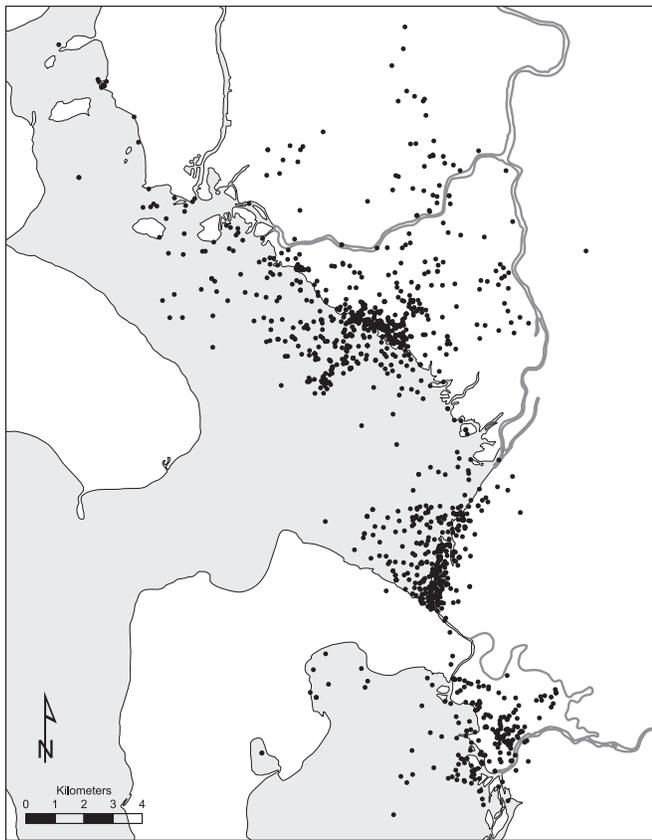


FIGURE 3. Locations of Dunlins recorded by ground-based observation and aerial tracking during the winters of 2006–2007 and 2007–2008 in the Skagit and Stillaguamish River deltas, Washington.

–25.4‰, 95% CI = –26.3 to –24.5‰, $n = 16$; Stillaguamish: mean = 24.7, 95% CI = –26.1 to –23.4‰, $n = 9$).

Location data from radio-marked birds allowed us to exclude sampling points in areas of low habitat use from consideration in our isotope model. We omitted five terrestrial points from the Skagit River delta and two points from the Stillaguamish River delta (Table 2, Fig. 2). Within the terrestrial environment, we found distinct regions of high (range 6.8–13.5‰) and low (range 2.5–4.9‰) values of $\delta^{15}\text{N}$ in prey (“high- ^{15}N ag” and “other ag,” respectively; Table 3).

We collected prey samples from 59 estuarine locations (28 in the Skagit River delta and 31 in the Stillaguamish River delta; Fig. 2, Appendix 1; available at <http://dx.doi.org/10.1525/cond.2013.120118>). Values of $\delta^{13}\text{C}$ in estuarine endpoint samples from each estuary varied strongly (Skagit: range 19.1–12.2‰; Stillaguamish: range –20.5 to –13.6‰; Fig. 2), presumably because of the effect of mixing of marine and freshwater sources, but there was little variation in $\delta^{15}\text{N}$ values (Skagit: range 7.4–13.6‰; Stillaguamish: range 8.5–11.3‰). In the Skagit River delta, the exterior transect generally showed, as predicted, a more marine signature than the interior transect

TABLE 1. Mean percent of habitat use (SD) of the Dunlin in estuarine and terrestrial habitats of the Skagit and Stillaguamish River deltas during the winters of 2006–2007 and 2007–2008. Population-level habitat use determined by weighting each individual’s contribution by the square root of locations per bird.

	2006–2007 $n = 29$	2007–2008 $n = 69$
Estuarine habitats		
Tidal flat	28 (28)	35 (23)
High marsh	0 (0)	6 (5)
Low marsh	30 (29)	35 (22)
Agricultural habitats		
Bare soil	25 (29)	7 (8)
Crop residue	1 (4)	2 (3)
Pasture	2 (5)	<1 (1)
Cover crop	11 (12)	12 (11)
Woody agriculture	<1 (1)	0 (0)
Other agriculture	1 (5)	1 (1)
Other habitats		
River	1 (5)	1 (1)
Urban and forest	0 (0)	0 (0)

(i.e., higher $\delta^{13}\text{C}$ values), but values did not differ significantly on the basis of overlapping 95% confidence intervals (Table 2). From north to south, prey samples from both the outer and inner transect had an increasingly terrestrial/freshwater signature, with the most terrestrial values closest to the freshwater outflow (Fig. 2). In the Stillaguamish River delta, patterns of prey $\delta^{13}\text{C}$ were more spatially complex. The interior transect had terrestrial/freshwater values increasing from north to south, but values along the exterior transect showed the opposite with more terrestrial/freshwater values to the north. We suspect that the differences found at the north end of the exterior and interior transects were due to the mixing of inflow and outflow waters in the channel at the northern part of the bay. This channel connects Skagit and Port Susan Bay and therefore the water moves in both directions with the tides. This channel may also receive a small amount of freshwater from a branch of the Stillaguamish River. In both estuaries, values of $\delta^{13}\text{C}$ in prey varied substantially more along the outer transects than along the interior transects.

The isotopic structure we detected in the prey transects from both estuaries helped us to inform dietary endpoints, and we defined endpoints into two groups: freshwater plume and marine/marsh. We defined the $\delta^{13}\text{C}$ endpoint of the freshwater plume as the samples of most negative estuarine prey equal to or below an assumed threshold of –19‰. This threshold was that derived by Yerkes et al. (2008) in their review of feather $\delta^{13}\text{C}$ values associated with terrestrial/freshwater and marine foodwebs. The marine/marsh $\delta^{13}\text{C}$ endpoint of –15‰

TABLE 2. Summary of stable-isotope values (mean \pm SD) for prey sampled in terrestrial (agriculture) and estuarine habitats of the Stillaguamish and Skagit River deltas. Shown are all agricultural data and those data reduced to only those regions Dunlins used, according to the results of radio tracking. In estuarine areas, inner and outer refer to the location of the sampling point along transects (see Fig. 2). LCI, 95% lower confidence interval; UCI, 95% upper confidence interval.

Endpoint (prey)	<i>n</i>	$\delta^{13}\text{C}$ (‰)	LCI	UCI	$\delta^{15}\text{N}$ (‰)	LCI	UCI
Terrestrial							
Stillaguamish	9	-24.8 ± 1.8	-26.1	-23.4	6.8 ± 3.6	4.1	9.6
Stillaguamish (reduced)	7	-24.9 ± 2.0	-26.7	-23.0	6.7 ± 4.1	2.9	10.5
Skagit	16	-25.4 ± 1.7	-26.3	-24.5	7.5 ± 2.3	6.3	8.7
Skagit (reduced)	11	-25.1 ± 1.8	-26.3	-23.9	7.9 ± 1.5	6.9	8.9
Ag (pooled)	25	-25.2 ± 1.8	-25.9	-24.4	7.3 ± 2.8	6.1	8.4
Ag (pooled/reduced)	18	-25.0 ± 1.8	-25.9	-24.1	7.4 ± 2.8	6.1	8.8
Estuarine							
Stillaguamish (inner)	14	-16.0 ± 1.0	-16.5	-15.4	10.0 ± 0.5	9.7	10.3
Stillaguamish (outer)	17	-18.0 ± 2.8	-19.5	-16.6	9.7 ± 0.6	9.4	10.0
Stillaguamish (pooled)	31	-17.1 ± 2.4	-18.0	-16.3	9.8 ± 0.6	9.6	10.1
Skagit (inner)	13	-15.7 ± 0.9	-16.2	-15.1	9.4 ± 1.0	8.8	10.0
Skagit (outer)	15	-16.2 ± 2.3	-17.5	-14.9	9.9 ± 1.7	8.9	10.8
Skagit (pooled)	28	-16.0 ± 1.8	-16.6	-15.3	9.6 ± 1.4	9.1	10.2
Estuary (pooled)	59	-16.6 ± 2.2	-17.1	-16.0	9.7 ± 1.1	9.5	10.0

TABLE 3. Isotopic values (mean \pm SD) of prey samples from transects used in the derivation of dietary endpoints for the MixSIR model. LCI, 95% lower confidence interval; UCI, 95% upper confidence interval.

Endpoint	<i>n</i>	$\delta^{13}\text{C}$ (‰)	LCI	UCI	$\delta^{15}\text{N}$ (‰)	LCI	UCI
High- ^{15}N agriculture	12	-25.1 ± 2.2	-27.7	-22.4	7.2 ± 0.7	6.4	8.1
Other agriculture	5	-26.3 ± 0.4	-26.8	-25.9	3.9 ± 0.8	2.9	4.9
Freshwater	11	-20.1 ± 0.5	-20.4	-19.8	9.8 ± 0.6	9.4	10.1
Marine/marsh	18	-14.4 ± 0.8	-14.8	-14.0	9.9 ± 1.5	9.2	10.7

was more arbitrary but clearly separated prey samples into an extremely enriched group (above -15‰) that were associated with these parts of the transects.

SOURCE OF NUTRIENTS TO THE DUNLIN

The identification of two viable terrestrial endpoints reflecting variation in ^{15}N sources (i.e., high- ^{15}N agriculture and “other agriculture”) and two isotopically distinct regions in the aquatic portion of the estuary, marine/marsh and freshwater plume, covered the range of isotopic values in prey encountered by the Dunlin and allowed the use of a two-isotope four-source mixing model to estimate relative inputs of these sources to the Dunlin. Isotope values for Dunlin blood fell mostly within this mixing polygon (Fig. 4), suggesting that we had captured most of the isotopic variance in the foodweb the Dunlin experienced in our area (note that Bayesian mixing models incorporate error in endpoint values so some excursion of data points outside this strict polygon based on mean endpoint values is expected). Using our mixing model, we estimated that Dunlins obtained a significantly greater portion

(62%) of their protein from agricultural lands and only about a third (38%) from the estuary (Table 4). Source contributions were dominated by single regions in both the terrestrial and estuarine environment; high- ^{15}N agriculture and marine/marsh; other agriculture and freshwater plume contributed very little (9.4%).

For the comparison of source contributions to Dunlins captured early in the winter (<10 January) vs. later in the winter during the winter of 2008–2009 (temporal distribution of sample, $\delta^{13}\text{C}$, and $\delta^{15}\text{N}$ values given in Fig. 5), we defaulted to using only three dietary endpoints as we were more interested in the directional seasonal changes in use of agricultural lands. We removed freshwater plume from the analysis because we previously showed its input was minimal for the whole sampled population. The mixing model MixSIR run with two isotopes and three inputs gives a unique solution (vs. a probabilistic range of solutions). That model predicted that Dunlins captured in the early-winter period ($n = 45$) received 73% of their protein from the marine/marsh region, 26% from high- ^{15}N and 0.2% from other agriculture. In contrast, those

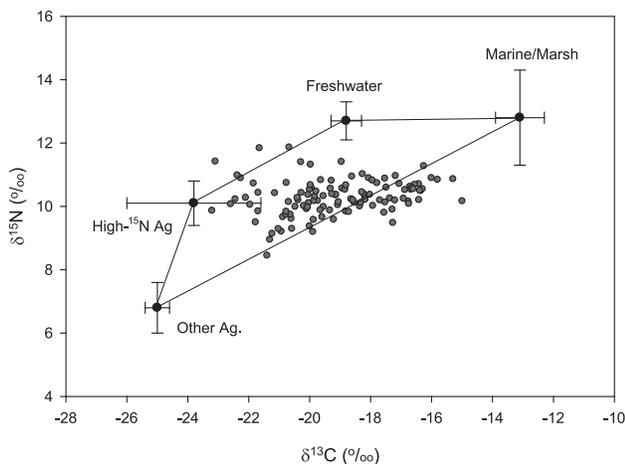


FIGURE 4. Minimum convex polygon used to estimate the source of nutrients used by Dunlins in the Skagit and Stillaguamish River deltas. Data are blood samples from individual birds. Endpoints have been adjusted with isotopic discrimination factors for conversion to equivalent blood values.

TABLE 4. Estimates of contributions of prey derived from agricultural and estuarine sources in the Stillaguamish and Skagit River deltas based on the Bayesian mixing model MixSIR.

Source	Mean percent	95% CI
High- ¹⁵ N agriculture	53.1	50.8 to 57.9
Other agriculture	9.2	5.9 to 11.1
Freshwater plume	0.2	0 to 0.6
Marine/marsh	38.0	35.7 to 39.1

captured in late winter ($n = 34$) received 48% of their protein from the marine/marsh source and 51% from high-¹⁵N agriculture and 0.5% from other agriculture.

DISCUSSION

Our extensive isotopic analysis of the Dunlin’s invertebrate prey together with samples of Dunlin blood throughout the areas known to be used by wintering birds allowed us to estimate the relative contribution of estuarine and agriculturally derived nutrients in the diet of Dunlins wintering at an important complex of estuaries along the Pacific coast. These analyses indicated that while Dunlins were found primarily in the marsh/marine zone of the estuary, presumably for foraging, they received nutrients to a large degree from adjacent agricultural lands with moderate to high $\delta^{15}\text{N}$ values. Seasonal variation in the degree of upland nutrients revealed that the proportion of nutrients from upland agricultural lands was also greater later in the winter. These results reaffirm the importance of these adjacent agricultural uplands in estuarine ecosystems but reveal our lack of understanding of the types

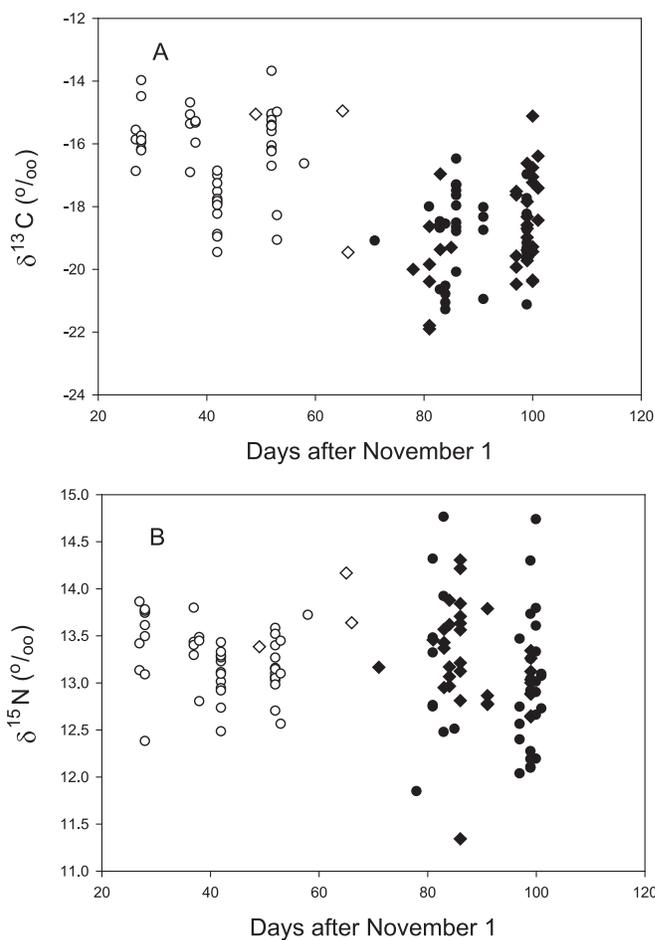


FIGURE 5. Isotopic ($\delta^{13}\text{C}$) values of whole blood of Dunlins captured during early winter (open symbols) and late winter (filled symbols) in 2006–2007 (diamonds) and 2007–2008 (circles) in the Skagit and Stillaguamish River deltas. Breakpoint between early and late winter periods is 10 January (day 71 after November 1). Larger values of $\delta^{13}\text{C}$ are associated with an estuarine diet.

of agricultural lands Dunlins prefer and why, or the processes involved in how they select these lands. Our results suggest the intriguing possibility that Dunlins may be using fields subsidized with significant agricultural N, since we expect higher $\delta^{15}\text{N}$ values in these regions due to processes such as the use of animal-based fertilizers and physical processes such as ammonification (Pardo and Nadelhoffer 2010).

In estuaries throughout the world, shorebirds specializing in the intertidal zone supplement their diet with prey from adjacent non-intertidal habitat, particularly agricultural lands. For example, at the nearby Fraser River estuary, Canada, wintering Dunlins obtain, on average, 38% their dietary nutrients from agricultural sources (Evans Ogden et al. 2005), and studies of shorebirds using estuaries in Europe also show several species acquiring prey in agricultural lands (reviewed in Evans Ogden et al. 2007). In northern Puget Sound estuaries, we found Dunlins obtained, at times, a greater proportion

of their nutrients from uplands (overall 62%) than from estuarine sources, emphasizing the importance of these habitats to their conservation.

We also found that sources of the Dunlin's diet from estuarine and agricultural habitats were not consistent over the winter, as the birds received a greater proportion of nutrients from upland agricultural lands later in the wintering period. This dietary switch is temporally aligned with the Pacific Northwest's winter rains, which generally begin to saturate soils and create areas of standing water from late December to early January. Dunlins strongly prefer intertidal flat and marsh habitats (Shepherd and Lank 2004, Slater et al. 2011). Smart and Gill (2003) suggested that when a shorebird with a strong preference for intertidal habitat chooses to forage on non-intertidal habitats, it does so either because it has discovered more profitable resources in non-intertidal habitats or because of deteriorating or fluctuating conditions in intertidal habitats (i.e., increased risk of starvation). Certainly, Dunlins may be taking advantage of supplemental foraging locations made available by winter rains, particularly at high tides when estuarine habitats are unavailable. However, we are unable to discount the possibility that the birds switch because of increased risk of starvation, from factors ranging from depletion of invertebrate prey (Colwell and Landrum 1993), perceived predation risk (Dekker and Ydenberg 2004, Ydenberg et al. 2010), or weather, in particular rain, which likely increases availability of invertebrate prey on agricultural lands and decreases its availability in estuarine habitats (Warnock 1994, Colwell and Dodd 1997). In some situations where rainfall is sufficient to create standing water and/or appropriate permanent wetlands are available, Dunlins may forsake coastal estuaries altogether and winter entirely in agricultural landscapes inland (Warnock et al. 1995, Kelly 2001, Long and Ralph 2001, Sanzenbacher and Haig 2002, Taft and Haig 2006). Regardless of the specific reason why Dunlins switch foraging habitats in northern Puget Sound, the degree to which they obtain nutrients from this region marks the importance of agricultural habitats and likely allows the region to support larger populations of shorebirds than estuaries without adjacent agricultural lands.

Clearly, management of uplands adjacent to estuaries used by shorebirds should form an important part of the overall strategy for management of these species. Although research has revealed some broad characteristics of uplands shorebirds use that could be managed for (Evans Ogden et al. 2007), more information on shorebirds' use of agricultural fields is needed. In particular, we now need to know which crops or land-use practices adjacent to estuaries important to shorebirds are most compatible with opportunities for shorebirds' foraging. For example, which chemical treatments of agricultural areas might create high- ^{15}N prey, which Dunlins appear to prefer, or promote invertebrates' abundance without untoward consequences for sustaining shorebird populations? Equally important is identifying the microhabitat characteristics within fields that

shorebirds select, as within individual fields apparently compatible with shorebird foraging, shorebirds appear to rely on specific regions rather than using the entire field evenly. Understanding these considerations also will help planning and management related to new threats, such as sea-level change, which may force shorebirds to rely more heavily on terrestrial habitats associated with estuaries.

CONCLUSION

Our approach to investigating sources of N and C for Dunlins wintering in this estuarine complex demonstrated that the birds tended to split their dietary inputs between the marine- or marsh-dominated regions of the estuary and the upland agricultural regions. They tended not to rely on freshwater-derived nutrients. This may be due to the relatively small influence of the freshwater plume in this area or to the lower productivity of this zone. Unfortunately, we were unable to distinguish isotopically between dietary contributions of the marsh and marine components of the estuary. This was due primarily to the higher values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of marsh regions. The use of other isotopes, especially of ^{34}S , could help to distinguish between input to the Dunlin's diet from marine and marsh areas, and we encourage further research (Fry et al. 1982, Peterson and Howarth 1987). Finally, because (1) the unique integrative nature of the stable-isotope approach to reconstructing diets of consumers is particularly well suited to deciphering nutrient inputs to consumers in complex agro-estuarine complexes and because (2) numerous species of shorebirds are associated with such habitats during winter and migration stopovers, we encourage the continued use of this approach to illuminate shorebirds' habitat use and requirements throughout their annual cycles.

ACKNOWLEDGMENTS

Research was funded by the SeaDoc Society, Seattle City Light, Washington Department of Fish and Wildlife, Pacific Coast Joint Venture, U.S. Forest Service International Program, The Nature Conservancy, and an operating grant by Environment Canada to KAH. We thank Chantal Gryba for assistance with preparation of stable-isotope samples and Myles Stocki for performing the isotope analyses at the University of Saskatchewan. Two anonymous reviewers provided constructive comments that improved the manuscript.

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