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Resource partitioning among five species of waterfowl (*Anas* spp.) at an autumn migratory stopover: Combining stable isotope and mercury biomarkers.

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Abstract

The Saskatchewan River Delta (SRD) is North America's largest inland delta and an important stopover site for waterfowl in the Central Flyway. However, little is known about their basic feeding ecology at this site and how species segregate or overlap in resource use. We used stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotopes and mercury concentrations ([Hg]) in liver tissue to trace use of local nutrient sources by five waterfowl species and tested for differences in diets among species, sexes and age groups. Macrophytes were the dominant food source for Northern Pintail (*Anas acuta* Linnaeus 1758) and American Wigeon (*A. americana* Gmelin 1789) with median proportions of 0.86 and 0.98, respectively. There was also evidence of partitioning of resources, as Blue-winged Teal (*A. discors* Linnaeus 1766) and Green-winged Teal (*A. carolinensis* Gmelin 1789) consumed invertebrates, as did a subset of Mallards (*A. platyrhynchos* Linnaeus 1758), suggesting that these birds might minimize competition for resources during the short staging period in the SRD when waterfowl densities are high. Other isotopes or tracers, such as [Hg] that varied among sources (0.03 to 0.20 $\mu\text{g/g}$ dry weight) and waterfowl species (0.22 to 3.19 $\mu\text{g/g}$ dry weight), can be used for further refining dietary estimates.

Keywords: MixSIAR, stable isotope mixing model, wetland food web, *Anas* spp., dabbling ducks, macrophytes, invertebrates

Introduction

Animal migration confers considerable physiological demands, such as the need to provision fuel through lipid accumulation or synthesis and protein catabolism, which are well demonstrated by migrating waterfowl (Davidson and Evans 1986; Klaassen et al. 2007, 2012). During autumn migration, many waterfowl stage in productive habitats, such as wetlands, where they build lipid reserves to fuel their subsequent journey. Wetland quality, as characterized by the abundance, composition and spatiotemporal dynamics of food sources, is an important factor influencing waterfowl populations (Davis and Smith 1998; Taft and Haig 2005; Hartke et al. 2009). Understanding waterfowl dietary needs during stopover or refueling periods, including which habitats best provide these requirements, is critical for successful waterfowl conservation and management (Havera 1999).

Submerged and emergent aquatic macrophytes and invertebrates are common foods for waterfowl during migration (Martin and Uhler 1939; Tidwell et al. 2013). Seeds and fruits of submerged macrophytes can be one of the most important sources of plant carbohydrates and lipids used during this period, sustaining thousands of individuals (Chura 1961; Hay 1974), while invertebrates provide primarily protein (Brochet et al. 2012). Tracking the flow of energy in stopover sites used by migratory birds is necessary because populations of waterfowl in an entire flyway may be affected by the availability of food (Myers 1983; Arzel et al. 2009) and habitat quality may also affect the timing of migration (Schneider and Harrington 1981). High quality foraging habitats are therefore important to maximize birds' body condition for successful migration (Drobney and Fredrickson 1979; Reid et al. 1989).

Conventional approaches for investigating consumers' nutrient sources, such as gut content analysis or visual observation, have limitations because they provide only information on

ingested materials that may not be assimilated (Duffy and Jackson 1986). Additionally, soft-bodied invertebrates that are an essential part of duck diet may be difficult to observe because they are digested more rapidly (Swanson and Bartonek 1970). Stable isotopes of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) in animal tissues and potential dietary sources have provided a complementary understanding of trophic ecology and nutrient sources (Hobson 1999; Post 2002). This method tracks nutrient sources over longer periods and gives information on assimilated nutrients. The amount of isotopic change, or discrimination, as source diet is converted to proteins in consumer tissue is low for $\delta^{13}\text{C}$ (typically $< 1\text{‰}$), making it an important ecological tracer of diet and connections to primary productivity (Hobson 1999; Inger and Bearhop 2008), whereas nitrogen undergoes isotopic discrimination at a rate of 2-5 ‰ from diet to consumer (Hobson 1999; Post 2002), making it a useful indicator of trophic position. Ecosystem differences in $\delta^{15}\text{N}$ values at the base of the food web, however, can confound the interpretation of $\delta^{15}\text{N}$ (Vander Zanden and Rasmussen 1999). For instance, amphipods that are considered primary consumers can have highly variable $\delta^{15}\text{N}$ values (Hobson et al. 2005). Use of other elements such as mercury (Hg), which is known to biomagnify in food webs (Watras and Bloom 1992; Atwell et al. 1998; Lavoie et al. 2013), may also help indicate trophic position in complex aquatic habitats where baseline $\delta^{15}\text{N}$ values may vary, but to date Hg has been used rarely to infer trophic position or to delineate food web structure (Zhang et al. 2012) and dietary source proportions (Soto et al. 2016).

The Saskatchewan River Delta (SRD) in central Canada, North America's largest inland delta, is an important autumn stopover site for waterfowl with thousands flocking there annually to stage (Bellrose and Kortwright 1976; Baschuk et al. 2012). Because many of these birds originate from elsewhere (Slattery 2008) and densities are high, there is potential for diet

segregation associated with differences in morphology and physiology (Gurd 2008; Brochet et al. 2012). Past information on nutrient sources used by these birds is based on gut content analysis and observational methods (Dirschl 1969). Here, we studied nutrient flow in this important waterfowl habitat using $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and Hg concentration ([Hg]) to unravel information that will be important to wetland conservation and management efforts in the SRD.

We aimed to determine general dietary sources to, and trophic position of, waterfowl using the SRD during their autumn migration. We were interested specifically in the relative contributions of detritus- and algal-based pathways to the diet of waterfowl as mediated through consumption of invertebrates, and how diets differed among species, sexes and age classes (Nudds and Kaminski 1984). We predicted that aquatic macrophytes would dominate waterfowl diet as recorded in other systems (Wersal et al. 2005) and because of known seasonal shifts of birds to this source in the autumn (Dirschl 1969; Brochet et al. 2012). We anticipated that different species would use different resources, with American Wigeon (*Anas Americana* Gmelin 1789) having the most herbivorous diet (Knapton and Pauls 1994), and expected more variation among species than within species (Nudds and Bowlby 1984). These comparisons were expected to provide insights into how birds maximize energy gains during short periods when resources are high.

Materials and Methods

Sample collection

We collected 118 hunter-killed birds comprising five species from Grassy Point in the SRD (53.75° N 102.443° W, Figure 1) in September and October of 2013. We sampled Mallard (*A. platyrhynchos* Linnaeus 1758, $n = 56$), Northern Pintail (*A. acuta* Linnaeus 1758, $n = 25$),

American Wigeon ($n = 13$), Green-winged Teal (*A. carolinensis* Gmelin 1789, $n = 4$) and Blue-winged Teal (*A. discors* Linnaeus 1766, $n = 20$). Birds were sexed and aged based on a wing identification manual (USFWS 1997) with assistance from trained personnel at the Canadian Wildlife Service in Saskatoon, Canada. At the time of collection, the study wetlands were open water with vegetation interspersed at the periphery. Aquatic macrophytes such as sedges (*Carex* spp.), reed grass (*Phragmites australis* Cav. Trin. ex Steud.), and cattails (*Typha* spp.) dominated the emergent zone of the wetlands. Following dissection in the field, we removed liver tissue, stored it in sterile individual Ziploc bags and transported it frozen to the Aquatic Food Webs Lab in the Toxicology Center at the University of Saskatchewan.

To assess the contribution of the different nutrient sources using stable isotopes, we selected liver tissue because it has a rapid turnover rate and short half-life. The half-life of liver tissue varies as a function of body size, as noted for avian blood (Carleton and Martinez Del Rio 2005) and for birds the size of waterfowl (~ 0.3 to 1.2 kg, Nudds et al. 1981) we expected an average half-life of 5-9 days (Boecklen et al. 2011). This tissue therefore provided information on the diet of individuals most likely associated with the local stopover site (Hobson and Clark 1992a, 1992b, Hobson 1999) which is important in this study system because the individuals sampled could have been migrants or residents (Asante 2016). Stopover duration for migrating waterfowl is typically 20 to 30 days (O'Neal et al. 2012), so the short turnover time of liver increases the likelihood that isotopic values represent local diet.

In the laboratory, liver samples were thawed, and washed with de-ionized water prior to preparation for isotope analysis. Samples were then oven dried and powdered. Lipids were extracted from a subset of samples ($n = 8$) to assess the utility of lipid correction equations in estimating lipid-free tissue $\delta^{13}\text{C}$ values, as variation in tissue lipid content can affect bulk tissue

$\delta^{13}\text{C}$ values and can be misinterpreted as dietary or habitat shifts (McConnaughey and McRoy 1979). Powdered liver samples were soaked in a 2:1 chloroform: methanol solution for one week and then dried under a fume hood and re-analyzed. Upon extraction, C:N ratios ranged from 3-4 and $\delta^{13}\text{C}$ values did not differ from values obtained using a generic correction equation for aquatic organisms ($\delta^{13}\text{C}_{\text{corrected}} = \delta^{13}\text{C}_{\text{untreated}} - 3.32 + 0.99 \times \text{C:N}$; $t_7 = 0.75$, $p = 0.479$; Post et al. 2007). Un-extracted samples had C:N ratios, a proxy for lipid content, which ranged from 4-6. Therefore, we applied the correction equation to the remainder of the un-extracted liver samples.

We collected potential diet items for waterfowl from the site where birds were harvested, and other nearby locations in the SRD where birds are known to forage. Invertebrates were collected using a dip net (150 μm mesh), coarsely live-sorted and stored in plastic vials. Aquatic macrophytes were handpicked and stored in Ziploc bags. While we analyzed only leaf tissues of macrophytes, seeds may be the dominant food source for many of our study species and seeds may differ isotopically from leaves. While we lack data for our study system, differences observed elsewhere between leaves and seeds tend to be small (e.g. $<0.3\text{‰}$ for $\delta^{13}\text{C}$, Herzsuh et al. 2010) and variation between plant parts in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ is smaller than variation among different dietary sources (Marco-Méndez et al. 2015). All samples were transported on ice to the laboratory and stored frozen (-20°C). In the laboratory, samples were thawed, counted and identified. Invertebrates were identified to the family level (Merritt and Cummins 2008) and individuals of the same family were grouped together for isotope analysis. Samples tended to be dominated numerically (550 of 804 individuals collected) by a group of omnivorous amphipods (Family Dogielinotidae). Macrophyte samples were thawed, counted and identified to the species level based on Lahring (2003).

Stable Carbon and Nitrogen Isotope Analysis

All tissue samples were dried in an oven for 72 hours at 50 °C, weighed into tin capsules (0.9-1.1 mg for animals and 3.5-4.5 mg for plants) and shipped to the Stable Isotope Facility at the University of California Davis for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analysis. Samples were analyzed using a PDZ Europa ANCA-GSL Elemental Analyzer interfaced to a PDZ Europa (20-20) isotope ratio mass spectrometer (Sercon Ltd, Cheshire UK). During analysis, at least two secondary isotopic reference materials were included; Glutamic acid ($\delta^{13}\text{C} = -28.8\text{‰}$, $\delta^{15}\text{N} = -4.3\text{‰}$) and Bovine Liver ($\delta^{13}\text{C} = -21.7\text{‰}$, $\delta^{15}\text{N} = +7.7\text{‰}$). The standards were calibrated against NIST reference materials (IAEA N1, USGS-40). The isotope values were expressed relative to international standards Vienna PeeDee Belemnite (V-PDB) and atmospheric nitrogen for carbon and nitrogen respectively. Based on replicate measurements of standards ($n = 20$) within runs, we estimated measurement error (1 SD) to be 0.1 ‰ and 0.2 ‰ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analysis, respectively.

Total Hg Analysis

Total Hg concentrations in liver tissue, invertebrates and aquatic macrophytes were determined by atomic absorption spectroscopy on a DMA-80 (Milestone, Inc., Shelton, Connecticut, USA; Haynes et al. 2006). Because of very high [Hg] in Blue-winged Teal livers (see Results), to explore Hg bioaccumulation further we also analysed a subset of muscle samples ($n = 9$) from this species that covered the range of liver [Hg]. For all [Hg] analyses, TORT-3 (lobster hepatopancreas, certified concentration: 0.29 $\mu\text{g/g}$, NRC, Ottawa, Ontario, Canada) and DORM-4 (dogfish muscle, certified concentration: 0.41 $\mu\text{g/g}$ NRC, Ottawa, Ontario, Canada) were used as certified reference materials. Mean recovery ($\pm$ SD) of Hg was $107 \pm 10\%$ ($n = 57$) for TORT-3 and $98 \pm 6\%$ ($n = 18$) for DORM-4.

Statistical Analysis

We used a multivariate analysis of variance in R 3.2.3 (R Core Team 2015) to evaluate whether observed $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ values and [Hg] differed among food sources, and in liver tissue to test for differences according to age (hatch year or after hatch year), sex and species (including all two- and three-way interactions). Where we found significant differences (determined using Wilks λ), we used univariate approaches followed by Tukey's Honest Significant Difference post-hoc test. We first tested for homogeneity of variances using Bartlett's test, which found differences in variance among some groups, but as sample sizes were similar ($n = 1\text{--}13$ per group), the model should be robust to this violation of assumptions. Differences were considered significant at $p < 0.05$.

Mixing Models

We constructed models to predict the proportional contribution of each of four food sources to waterfowl diet in the SRD: macrophytes and three groups of invertebrates (herbivores, carnivores and omnivores based on Merritt and Cummins 2008). Herbivores were composed of the families Corixidae and Physidae, omnivores were comprised of the crustaceans Cambaridae, Gammaridae, and Dogielinotidae, and carnivores were composed of the families Notonectidae, Dytiscidae, and Aeshnidae. To represent uncertainties in sources and diet-tissue discrimination factors, we used the MixSIAR mixing model programmed in R 3.2.3 (Parnell et al. 2013; Stock and Semmens 2013; R Core Team 2015). This uses Markov chain Monte Carlo (MCMC) methods in a Bayesian framework to estimate the relative contribution of sources (prey items) to

a mixture (consumer). It provides probability densities for dietary source proportions in mathematically undetermined systems (Parnell et al. 2010).

We used a concentration- dependent model (Phillips and Koch 2002; Phillips et al. 2014) because waterfowl are omnivorous (Baschuk et al. 2012; Tidwell et al. 2013) and there are large differences in %C and %N among sources (Table 1). We used diet-tissue discrimination factors of $+1.1 \pm 0.2$ ‰ for $\Delta^{13}\text{C}$, and $+4.0 \pm 0.2$ ‰ for $\Delta^{15}\text{N}$ for liver tissue (Ogden et al. 2004). Discrimination factors for [Hg] were calculated based on the knowledge that [Hg] increases by 4.7 ± 4.7 (SD) times per trophic level (Lavoie et al. 2013). Therefore, we multiplied the [Hg] of each of the sources by 4.7, and then subtracted each source [Hg] from the resulting hypothetical 'consumer' [Hg] to obtain source-specific discrimination factors ($[\text{Hg}_{\text{consumer}}] - [\text{Hg}_{\text{source}}]$). For example, the mean macrophyte [Hg] was $0.01 \mu\text{g/g}$ dry weight. Multiplying by 4.7 yields an expected [Hg] of $0.05 \mu\text{g/g}$ dry weight for a consumer feeding solely on macrophytes.

To assess overall transfer of Hg through the food web and compare with other Hg biomagnification studies (reviewed by Lavoie et al. 2013), we also calculated a Trophic Magnification Slope (TMS), or the incremental increase in Log Hg per unit increase in $\delta^{15}\text{N}$, for these waterfowl food webs. The TMS is the slope of the Log Hg vs. $\delta^{15}\text{N}$ regression, and ranges from -0.19 to 0.48 worldwide (Lavoie et al. 2013). Using Ordinary Least Squares Regressions, we calculated the slope with and without prey items included.

The MixSIAR mixing model parameterization included three chains, a chain length of 50,000, burn in of 25,000, and thin of 25, resulting in 3000 posterior draws. We included both residual and process error (Parnell et al. 2010), and report results as medians with 95% credible intervals (CrIs). Because Mallard $\delta^{15}\text{N}$ values were strongly bimodal, we divided individuals into two groups: Mallards (L) with $\delta^{15}\text{N} < +11$ ‰ and Mallards (H) with $\delta^{15}\text{N} > +11$ ‰. This

bimodality in liver $\delta^{15}\text{N}$ was apparent in Mallards that had feather $\delta^2\text{H}$ consistent with long-term residence in the SRD, as well as those that had feather $\delta^2\text{H}$ suggesting they had moulted elsewhere (Asante 2016).

To compare the posterior estimates of source contributions to each species' diet, we calculated pairwise Bhattacharyya Coefficients (BC, Bhattacharyya 1943; van den Boogaart et al. 2014), which indicate overlap between two Dirichlet distributions (Rauber et al. 2008; Bond and Diamond 2011). Pairwise comparisons with $\text{BC} > 0.60$ were indicative of significant dietary overlap (Bond and Diamond 2011; Jardine et al. 2015).

Results

Potential waterfowl food sources were highly variable in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values but macrophytes differed isotopically and in [Hg] relative to invertebrates (Wilks' $\lambda = 0.191$, $F_{9,46} = 5.02$, $p < 0.001$). Invertebrates were generally ^{15}N -enriched and ^{13}C -depleted, and had higher [Hg] compared with macrophytes (Table 1). Based on univariate analysis, there were significant differences in $\delta^{13}\text{C}$ ($F_{3,21} = 5.90$, $p = 0.004$) and $\delta^{15}\text{N}$ ($F_{3,21} = 9.34$, $p < 0.001$), but not [Hg] ($F_{3,21} = 2.72$, $p = 0.070$) among sources. Macrophytes and omnivorous invertebrates were differentiated from herbivorous invertebrates by $\delta^{13}\text{C}$ values, and further separation between omnivorous invertebrates and all other sources was achieved with $\delta^{15}\text{N}$ values (Table 1). Though carnivorous invertebrates and herbivorous invertebrates were not statistically distinct from one another based on any of our tracers, we retained them as separate categories in the mixing model.

There was no effect of age class or sex on $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and [Hg] (age class: Wilks' $\lambda = 0.970$, $F_{3,94} = 0.99$, $p = 0.40$; sex: Wilks' $\lambda = 0.97$, $F_{3,94} = 0.98$, $p = 0.41$). All two- and three-way interactions were also not significant (all $p > 0.45$). There were significant overall differences

among species (Wilks' $\lambda = 0.05$, $F_{15,260} = 34.45$, $p < 0.001$, Figure 2). Univariate analysis found significant differences in $\delta^{13}\text{C}$ ($F_{5,112} = 10.67$, $p < 0.001$), $\delta^{15}\text{N}$ ($F_{5,112} = 41.06$, $p < 0.001$) values, and [Hg] among species ($F_{5,112} = 85.59$, $p < 0.001$). Post-hoc tests showed three groups based on $\delta^{13}\text{C}$ values, four groups based on $\delta^{15}\text{N}$ values, and two based on [Hg] (Table 2).

Overall, the slope of the relationship between Log [Hg] and $\delta^{15}\text{N}$ (known as the TMS) was only 0.06 ± 0.02 SE ($r^2 = 0.09$, $F_{1,116} = 11.27$, $p = 0.001$) when only waterfowl livers were included, and 0.14 ± 0.02 SE ($r^2 = 0.35$, $F_{1,141} = 75.16$, $p < 0.001$) for the entire food web including prey items (Figure 3). The high concentrations in Blue-winged Teal (mean = $3.3 \mu\text{g/g}$ dry weight, range = 1.4 to $5.7 \mu\text{g/g}$) positioned them above the best-fit line in the TMS regression, with higher concentrations than expected based on their $\delta^{15}\text{N}$ values (Figure 3). The subset of Blue-winged Teal muscle samples analyzed did not, however, have high [Hg] (mean = $0.8 \mu\text{g/g}$ dry weight, range = 0.4 to $1.2 \mu\text{g/g}$) and these concentrations were only weakly correlated with [Hg] in liver ($r = 0.33$, $p = 0.380$).

Despite the overall dominance of macrophytes as a food source, we generally found very little overlap in estimated diet among species. Macrophytes had the greatest median contribution to the diet of all species, particularly so for American Wigeon and Northern Pintail (range of medians: 30-98%; Table 3), as evidenced by their relative position near this source in dual isotope space (Figure 2). Omnivorous invertebrates were the least important for all species (range of medians: 1-9%), with the exception of Blue-winged Teal, where they were the second most important diet group (32%; Table 3). The two teal species were most closely aligned isotopically with invertebrate prey (Figure 2) and had correspondingly high contributions from these sources. Mallards (L) and Green-winged Teal relied heavily on herbivorous invertebrates (36% and 28%, respectively), while Mallards (H) consumed 41% carnivorous invertebrates

(Table 3). All pairwise dietary comparisons had a $BC < 0.38$ with the exception of Blue-winged and Green-winged Teal ($BC = 0.608$), and Northern Pintail and Mallards (L) ($BC = 0.594$; Table 4).

Discussion

By estimating dietary composition of five species of waterfowl in the SRD using multiple tracers, our results demonstrate the importance of macrophytes prior to autumn migration. We also found considerable resource partitioning among species (Chesson 2000). This likely resulted from birds maximizing energy gains during short periods when resources were high (Staddon 2013). While source tracing with stable isotopes, especially $\delta^{15}\text{N}$, can be confounded by variation in isotopic baselines, our study was the first to use [Hg] as a tracer in Bayesian mixing models, allowing for potentially greater resolution of dietary and trophic patterns evident in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ data. The inclusion of other trace elements as dietary tracers or prior information in these models (Soto et al. 2016) can considerably expand resolving power in dietary studies.

The significant differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values among consumers resulted from them feeding on different dietary sources ($\delta^{13}\text{C}$) and at different trophic positions ($\delta^{15}\text{N}$), demonstrating resource partitioning in this waterfowl assemblage composed primarily of surface and dabbling feeders (Bellrose and Kortwright 1976; Johnsgard 2012; Wersal et al. 2005). This agrees with previous studies in France where high winter waterfowl densities led to resource partitioning among sympatric Mallards, Northern Pintails and Eurasian Teals (*A. crecca* Linnaeus 1758) (Guillemain and Fritz 2002). The cause of this partitioning has been variously explained by habitat structure (Nudds et al. 2000) coupled with varying phenotypic characters such as body size (Green 1998) and bill morphology (Nudds et al. 1994). These morphological

characters allow partitioning along depth and prey size gradients (Guillemain et al. 2002), both of which could be responsible for our observed dietary patterns. Larger species such as Mallards are able to reach the bottom of deeper waters and therefore forage over a broader range of water depths. This range may explain the two isotopically distinct groups of Mallards because $\delta^{15}\text{N}$ in prey is higher for plankton compared with the benthos (Vander Zanden and Rasmussen 1999). Smaller species such as Blue-winged and Green-winged teal are more likely to feed in the shallows but are able to switch among prey of varying sizes because of their higher lamellar density (Nudds and Bowlby 1984). Most prior studies on the relationship between diet and bill morphology focused on a single prey type (e.g. seeds, Brochet et al. 2012). We show that in addition to partitioning within prey types, partitioning also occurred across prey types.

Since all species in this study were hunted in a single wetland within the SRD, and densities of waterfowl in this delta are high during the autumn (Schmutz 2001; Slattery 2008), it is possible that some species opted to eat other foods instead of competing with conspecifics for the same food resource. Waterfowl are plastic in their foraging abilities and can show great overlap in prey choice when food abundance is high (Nummi and Vaananen 2001) but show evidence of divergence when resources decline (Guillemain et al. 2002). While we invoke competition as the driver for resource partitioning, our study was conducted during a period when resources were likely abundant, as a large summer flood preceded the autumn sampling, creating extensive habitat availability across the study area (MacKinnon et al. 2016). As such, the dietary divergence we observed may be a conservative estimate of resource partitioning, representing the ‘ghost of competition past’ (Connell 1980) or may simply reflect different energy needs among species associated with different migration distances (Bellrose and Kortwright 1976). Evidence for true competition may be more apparent on the wintering

grounds, where food tends to be scarce (Guillemain et al. 2002), conditions that might intensify competition and result in even stronger dietary divergence.

Generalist behavior in this waterfowl assemblage would have been evident in greater overlap in isotope space among species. In addition to our approach using BC, dietary overlap can also be assessed using ellipse areas in dual isotope space (Jackson et al. 2011; Layman et al. 2012). The advantage of the BC approach is the incorporation of dietary isotopic variation, whereas ellipse approaches consider only variation in the consumers. Using the BC approach, we found considerable dietary segregation among species in the SRD. While this segregation was based more strongly on phylogeny (species identity) than sex or age in our analysis (Nudds and Kaminski 1984), our sample sizes were small for many age-sex-species combinations, and the characterization of dietary patterns using isotopes is sensitive to sample size (Jackson et al. 2011).

Variation in the fundamental niche of an organism can affect population stability, among-species competition and the fitness of the entire population (Bolnick et al. 2002). We observed variation in diet among individuals within a species in Mallards, with two very clear groups separated by liver $\delta^{15}\text{N}$ values. While our mixing model suggested these two groups were feeding on different sources, [Hg] values were similar, suggesting that differences in $\delta^{15}\text{N}$ values could simply be attributed to differences in isotopic baseline (Hobson et al. 2005) rather than true differences in trophic position.

The high [Hg] in Blue-winged Teal result from feeding on omnivorous invertebrates that had high [Hg] and also higher $\delta^{15}\text{N}$ values than the other sources. Yet the high [Hg] values in liver relative to muscle and the weak correlation between the two tissues suggest short-term bioaccumulation of Hg in liver that is not representative of long-term diet. The disparity between

liver and muscle [Hg] in this species was also observed by Gerstenberger (2004) who recorded higher [Hg] in liver than in muscle of several waterfowl species, consistent with the role of liver as a detoxifying organ. The high [Hg] may also simply be due to a large proportion of inorganic Hg. Ruelas-Inzuna et al. (2009) reported only 26% methyl Hg in Blue-winged Teal muscle compared with 61% in Lesser Scaup (*Aythya affinis* Eyton 1838) and 100% in Shoveler (*A. clypeata* Linnaeus 1758), and demethylation is known to occur in waterbird livers (Eagles-Smith et al. 2009) which may explain generally lower % methyl Hg in livers (~35%) relative to muscle (~66%) reported elsewhere for seabirds (Kim et al. 1996). Our low overall TMS for the waterfowl-only food web compared to global averages (TMS of 0.16 ± 0.11 , Lavoie et al. 2013) stems in part from this wide variation in Log [Hg] for a given $\delta^{15}\text{N}$ value and the confounding effects of liver Hg storage, and is a limitation of measuring only total [Hg] as opposed to methyl [Hg] that exhibits stronger links with trophic level. Further development of trace elements as tracers (Soto et al. 2016) will rely on obtaining more precise estimates of diet to tissue enrichment or depletion for each element of interest.

We found that staging waterfowl relied heavily on aquatic macrophytes, which are high in carbohydrates and so are an important energy source (Knapton and Pauls 1994; Wersal et al. 2005; Johnsgard 2012). American Wigeon and Northern Pintail were the most herbivorous, a result consistent with the literature (DUC 2014; Miller 1987). Carbohydrates are used preferentially in powering metabolism, while proteins are more likely to be used in tissue synthesis (Tieszen and Fagre 1993; Voigt et al. 2008). Liver plays a role in both metabolism that mainly involves carbohydrates, and anabolism and allocation to tissues and organs, which mainly involves proteins, and is why we selected it as an indicator of recent diet.

Our results underline the importance of the SRD as a key stop over site for migratory waterfowl prior to autumn migration and how these birds partition resources. The heavy dependence on macrophytes as an energy source points to wetland vegetation as vital for fuel during autumn migration. While the productive nature of the SRD has long been recognized (Bellrose and Kortwright 1976; Smith 1996; Schmutz 2001) this study adds important detail on the dietary contribution of various energy pathways to the food webs supporting waterfowl during their time in the SRD. Therefore, developing a system to trace nutrient flow in this important waterfowl habitat using $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and [Hg] values will further wetland research, conservation and management efforts in the SRD.

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Draft

Table 1. Stable carbon and nitrogen isotope values (‰), mercury concentrations and elemental composition of four dietary sources for migratory waterfowl at Grassy Point, Saskatchewan River Delta, Canada. Source categories sharing common superscripts within a column are not significantly different.

Source	$\delta^{13}\text{C}$, ‰ (SD)	$\delta^{15}\text{N}$, ‰ (SD)	[Hg], $\mu\text{g g}^{-1}$ (SD)	%C (SD)	%N (SD)
Macrophytes	-26.8 (3.3) ^a	+4.3 (2.8) ^a	0.008 (0.007) ^a	36.2 (8.0)	2.6 (0.8)
Omnivorous invertebrates	-28.0 (1.9) ^a	+11.3 (1.3) ^b	0.160 (0.240) ^a	37.5 (4.7)	7.4 (2.2)
Herbivorous invertebrates	-32.5 (1.4) ^b	+6.4 (1.7) ^a	0.166 (0.032) ^a	49.4 (3.7)	9.4 (1.0)
Carnivorous invertebrates	-29.7 (1.6) ^b	+6.8 (2.2) ^a	0.173 (0.075) ^a	50.7 (4.3)	10.0 (1.2)

Draft

Table 2 Stable carbon and nitrogen isotope values (‰) and mercury concentrations in liver tissue of five migratory waterfowl at Grassy Point, Saskatchewan River Delta, Canada. Species sharing common superscripts within a column are not significantly different.

Species	$\delta^{13}\text{C}$, ‰ (SD)	$\delta^{15}\text{N}$, ‰ (SD)	[Hg], $\mu\text{g g}^{-1}$ (SD)
Mallard (low $\delta^{15}\text{N}$)	-28.0 (1.2) ^{ab}	+8.6 (0.9) ^a	0.57 (0.38) ^a
American Wigeon	-25.6 (1.5) ^c	+10.9 (2.5) ^{bc}	0.22 (0.09) ^a
Northern Pintail	-26.5 (2.1) ^c	+9.7 (1.5) ^{bc}	0.55 (0.30) ^a
Green-winged Teal	-29.7 (1.0) ^a	+11.4 (1.2) ^{bcd}	0.59 (0.20) ^a
Blue-winged Teal	-28.5 (1.7) ^a	+12.2 (1.2) ^{cd}	3.32 (1.12) ^b
Mallard (high $\delta^{15}\text{N}$)	-26.9 (0.9) ^{bc}	+13.2 (0.8) ^{cd}	0.75 (0.35) ^a

Draft

Table 3 Dietary source proportion estimates for five migratory waterfowl at Grassy Point, Saskatchewan River Delta, Canada.

Species	Carnivorous inverts Median (95% CrI)	Herbivorous inverts Median (95% CrI)	Omnivorous inverts Median (95% CrI)	Macrophytes Median (95% CrI)
Mallard (low $\delta^{15}\text{N}$)	2% (0%-7%)	36% (25-49%)	3% (0-7%)	58% (44-69%)
American Wigeon	0% (0-2%)	0% (0-1%)	1% (0-2%)	98% (97-99%)
Northern Pintail	2% (0-9%)	7% (1-18%)	4% (0-8%)	86% (74-93%)
Green-winged Teal	19% (1-66%)	28% (1-55%)	9% (1-45%)	30% (4-90%)
Blue-winged Teal	9% (2-63%)	13% (3-26%)	32% (2-52%)	39% (21-57%)
Mallard (high $\delta^{15}\text{N}$)	41% (2-67%)	4% (1-13%)	5% (1-54%)	45% (25-61%)

Draft

- 1
- Table 4** Pairwise comparisons of Bhattacharyya Coefficients [medians and lower (LCL) and upper confidence limits (UCL)]
- 2
- estimating overlap in dietary source proportions for five migratory waterfowl at Grassy Point, Saskatchewan River Delta, Canada.
- 3
- Median coefficients ≥ 0.60 (in bold text) indicate significant overlap for the given species pair.

Species	Green-winged Teal Median (LCL-UCL)	Mallard (high $\delta^{15}\text{N}$) Median (LCL-UCL)	Mallard (low $\delta^{15}\text{N}$) Median (LCL-UCL)	Northern Pintail Median (LCL-UCL)	American Wigeon Median (LCL-UCL)
Blue-winged Teal	0.608 (0.238-0.945)	0.267 (0.110-0.482)	0.323 (0.130-0.551)	0.382 (0.156-0.590)	0.035 (0.011-0.089)
Green-winged Teal	.	0.310 (0.064-0.728)	0.337 (0.091-0.758)	0.299 (0.062-0.883)	0.026 (0.004-0.317)
Mallard (high $\delta^{15}\text{N}$)	.	.	0.167 (0.051-0.390)	0.243 (0.076-0.495)	0.056 (0.013-0.158)
Mallard (low $\delta^{15}\text{N}$)	.	.	.	0.594 (0.188-0.856)	0.104 (0.026-0.237)
Northern Pintail	.	.	.	.	0.232 (0.069-0.568)

4

Figure legends

Figure 1 Grassy Point (indicated by solid circle) in the Upper Saskatchewan River Delta, Saskatchewan, where sources and waterfowl were collected (53, 75° N 102, 443° W).

Figure 2 Isotope bi-plots of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (‰) for dietary sources (solid symbols; diamond = macrophytes, triangle = herbivorous invertebrates, square = carnivorous invertebrates, circle = omnivorous invertebrates) and American Wigeon (open circles), Northern Pintail (open diamonds), Green-winged Teal (shaded triangles), Blue-winged Teal (open triangles), Mallards Low $\delta^{15}\text{N}$ (open squares) and Mallards High $\delta^{15}\text{N}$ (shaded squares). Source values were adjusted by estimated diet-tissue discrimination factors (+1.1‰ for $\delta^{13}\text{C}$ and +4.0‰ for $\delta^{15}\text{N}$).

Figure 3 Log [Hg] vs. $\delta^{15}\text{N}$ (‰) for liver tissue of waterfowl and their prey from Grassy Point in the Saskatchewan River Delta. Symbols as in Figure 2. Best-fit regression lines are shown for waterfowl only (solid line; $y = 0.055x - 0.786$, $r^2 = 0.089$) and waterfowl + prey items (hatched line; $y = 0.136x - 1.776$, $r^2 = 0.348$).

Figure 2. Isotope bi-plots of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (‰) for dietary sources (solid symbols; diamond = macrophytes, triangle = herbivorous invertebrates, square = carnivorous invertebrates, circle = omnivorous invertebrates) and American Wigeon (open circles), Northern Pintail (open diamonds), Green-winged Teal (shaded triangles), Blue-winged Teal (open triangles), Mallards Low $\delta^{15}\text{N}$ (open squares) and Mallards High $\delta^{15}\text{N}$ (shaded squares). Source values were adjusted by estimated diet-tissue discrimination factors (+1.1‰ for $\delta^{13}\text{C}$ and +4.0‰ for $\delta^{15}\text{N}$).

Figure 3 Log [Hg] vs. $\delta^{15}\text{N}$ (‰) for liver tissue of waterfowl and their prey from Grassy Point in the Saskatchewan River Delta. Symbols as in Figure 2. Best-fit regression lines are shown for waterfowl only (solid line; $y = 0.055x - 0.786$, $r^2 = 0.089$) and waterfowl + prey items (hatched line; $y = 0.136x - 1.776$, $r^2 = 0.348$).