

## Western Painted Turtles (*Chrysemys picta bellii*) in a Highly Urbanized System: Unexpected Variation in Resource Use Among Age Classes and Sexes

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**ABSTRACT:** Understanding patterns of resource use is an important aspect of the conservation and management of animal populations. We used stable isotopes of carbon and nitrogen from nail samples of Western Painted Turtles (*Chrysemys picta bellii*) to examine isotopic niches for individuals in an urban population. Western Painted Turtles are omnivorous, so we predicted that there would be little isotopic niche variation by sex, location, or age class. In contrast to our prediction, isotopic niche size varied up to three times among groups; females inhabiting marsh habitat had the largest niche, whereas females inhabiting creek habitat had the smallest. Isotopic niches overlapped 26–77%, with the least overlap between adult males and females, indicating niche partitioning by sex. Body size and location also contributed to the diversity of resource use. Isotopic mixing models indicated that all turtles consumed low proportions of a variety of prey items, but there were differences among groups. Turtles inhabiting creek habitat consumed higher proportions of chironomid larvae, whereas those in marsh habitat consumed higher proportions of crayfish and amphipods. Our findings indicate that urban turtles take advantage of a wide range of prey, and that aquatic systems with high productivity and diversity are well suited for maintaining turtle populations.

**Key words:** Dietary overlap; Emydidae; Isotopic niche; Mixing model; Stable isotope analysis

URBANIZATION is an increasing conservation threat for reptile species worldwide. Urban environments experience extensive habitat loss, have dense road networks (Steen and Gibbs 2004), and support subsidized predators (Marchand and Litvaitis 2004). Consequently, urban environments can negatively affect the fitness of long-lived species, such as turtles, through increased physiological stress, impeded movements, and reduced availability of key resources (Birnie-Gauvin et al. 2016). These factors are potentially exacerbated in peripheral populations (e.g., northern range limits), where the climate is extreme, and reptiles exhibit increased sensitivity to environmental changes (Lesbarrères et al. 2014). In northern areas, the cool, short growing seasons alter turtle life-history traits, including slower somatic growth rates (Lovich et al. 1998), older age at maturity (Cagle 1954), increased body size (Ashton and Feldman 2003), and larger clutch sizes (Christiansen and Moll 1973), compared to southern conspecifics. The slow rate of recruitment, combined with any increased risk of adult mortality in urban areas, could cause northern populations of turtles to be disproportionately affected by urbanization. Despite this concern, most studies on freshwater turtles at their northern range limits have been conducted in relatively pristine areas with little urban development.

Painted Turtles (*Chrysemys picta*) occur broadly across Canada, and reach the northern limit of the species' range throughout the southern portion of most Canadian provinces (Brooks 2007; Ernst and Lovich 2009). This species is one of the best studied in North America (Ernst and Lovich 2009; Lovich and Ennen 2013); however, most research occurs on populations closer to the core of the geographic range in the United States. Painted Turtles occur in a wide variety of slow-moving water bodies and are thought to be fairly tolerant of pollution (Ernst and Lovich 2009), but few

studies have specifically examined populations in urban areas (e.g., Conner et al. 2005; Eskew et al. 2010; Winchell and Gibbs 2016). Various aspects of the ecology and life history of painted turtles have been described in Canadian populations, with most focusing on Midland Painted Turtles (*C. p. marginata*) subspecies (e.g., Taylor and Nol 1989; Rollinson and Brooks 2007; Carrière et al. 2008; Rollinson et al. 2008). Few studies have examined the ecology of the western subspecies (*C. p. bellii*), which has a larger body size, and reaches higher latitudes than Midland Painted Turtles.

Trophic relationships and resource use (e.g., diet, habitat) are fundamental aspects affecting turtle population ecology. For example, in *C. picta*, resource use influences geographic variation in life-history characteristics such as clutch size (Gibbons and Tinkle 1969), somatic growth rate (Gibbons 1967; Knight and Gibbons 1968), and body size (MacCulloch and Secoy 1983; Lindeman 1996). Thus, the availability of key resources is inherently linked to turtle population size and productivity. Correspondingly, information on resource use can be important for the conservation and management of urban populations, where the surrounding environment has changed as a result of anthropogenic influences. Such changes can affect prey availability, potentially altering what resources are available to turtles in urban areas (Moll 1977). To examine dietary aspects of resource use in turtles, researchers had historically used techniques such as stomach flushing (e.g., Legler 1977; Parmenter 1980; Lindeman 1996; Cooley et al. 2003), fecal analysis (e.g., McKnight et al. 2015), or dissection (e.g., Knight and Gibbons 1968; Hart 1982). These three methods provide good short-term information on items recently ingested by individuals, but can be biased by capture time (McKnight et al. 2015), and by prey items that digest at different rates (e.g., aquatic vegetation vs. invertebrates; Kelly 2000). Therefore, these methods have limitations in understanding resource use over longer time scales and might not be effective for examining

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small-bodied individuals because of their small gape size (MacCulloch and Secoy 1983) and potential for injury.

Analyses of stable isotopes in tissues enable insight into the long-term resource use and isotopic niche of aquatic species. Ratios of the stable isotopes of carbon and nitrogen provide information about consumer energy source ( $\delta^{13}\text{C}$ ) and trophic position ( $\delta^{15}\text{N}$ ) with only a small tissue sample (e.g., Peterson and Fry 1987; Post 2002; Newsome et al. 2007).  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values for consumers enable the examination of the isotopic niche, providing insight into the relative trophic position of species within their food web, as well as their location within the habitat (Layman et al. 2007; Newsome et al. 2007). Additionally, the size of a consumer's isotopic niche can be quantified and used as a proxy for resource-use diversity (Layman et al. 2007; Jackson et al. 2011). This information can then be used to compare resource use within and among groups of interest statistically (Jackson et al. 2011; Guzzo et al. 2013). Stable isotope analysis (SIA) provides the potential to identify the long-term contributions of different dietary components with the use of isotopic mixing models (Fry 2006; Semmens et al. 2009; Phillips 2012; Phillips et al. 2014). Although SIA has become a common tool in aquatic ecology, it is less commonly used to study turtles. Much of the existing isotope literature focuses on sea turtles (e.g., Godley et al. 1998; Reich et al. 2007; McClellan et al. 2010; Seminoff et al. 2012), and several have reconstructed adult diet composition (e.g., Wallace et al. 2009; Lemons et al. 2011). However, only a few recent studies have used SIA to examine resource use and isotopic niches in freshwater turtles (e.g., Bulte and Blouin-Demers 2008; Hofmeister et al. 2013; Pearson et al. 2013; Aresco et al. 2015; Ferronato et al. 2016).

Here, we examine the isotopic resource use and niche breadth of an urban population of Western Painted Turtles in Saskatchewan, Canada, by examining stable isotope ratios of carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) in nail samples. Our study population occupies a small aquatic system located in the city of Regina near the northern range limit for the western subspecies. Based on data from other populations, we hypothesized that Western Painted Turtles in our study area would be generalist omnivores. Consequently, we predicted that they would consume a diverse range of prey and expected little isotopic variation based on sex group (male, female, subadult) or capture location of individuals. To test this prediction, we examined factors potentially influencing isotopic ratios and niche size, and applied Bayesian mixing models to identify primary diet items. Our overall objective was to understand resource use better and to inform ongoing conservation efforts for this urban population.

#### MATERIALS AND METHODS

We studied a population of Western Painted Turtles in Wascana Creek, an urban water body in Regina, Saskatchewan, Canada ( $50^{\circ}25'\text{N}$ ,  $104^{\circ}35'\text{W}$ ; datum = WGS84; Fig. 1). Wascana Creek is relatively shallow and macrophyte rich; it begins southeast of Regina and flows west through the city before entering the Qu'Appelle River. Within city limits, the system consists of a narrow creek, with a widened, man-made lake (average 8 m deep) and marsh (average 2 m deep) section at the core. Four major roadways intersect the study

area. The surrounding shoreline primarily consists of urban green space with activity trails, parkland, golf courses, and vegetated shores, which are entirely surrounded by residential and commercial development (Fig. 1). At the creek inflow on the eastern limit of the study area, patches of cattail (*Typha* spp.) line the shore and surrounding area, and residential development is typically farther from the shoreline (~150 m). Telemetry data, collected as part of a separate examination of habitat use, showed no turtle movement between the marsh (east) and creek (west) habitats because of a water control structure that acts as a barrier between the two habitat areas (Marchand 2017). Therefore, marsh and creek turtles were treated as two disjunct groups for our analysis.

#### Sample Collection

**Turtle nail sampling.**—Turtles were captured by a combination of hand capture, dip nets, and baited hoop traps from April to September 2015 and 2016. For each turtle, midline carapace length (CL;  $\pm 1$  mm) was measured with tree calipers (Haglof Sweden), and mass ( $\pm 1$  g) was measured with a digital scale. The sex of each individual was determined by observing secondary sex characteristics (Ernst and Lovich 2009). If secondary sex characteristics were not visible, they were classified as subadults. Over the course of 2 yr, 77 individuals (38 females, 24 males, 15 subadults) were captured and notched with a unique identifier along the marginal scutes of the carapace (Cagle 1939).

Upon capture, all nails on both hind feet were cleaned with isopropyl alcohol wipes. Samples were trimmed from the end of each nail with pet nail clippers, and stored in 1.5-mL centrifuge tubes at  $4^{\circ}\text{C}$ . Nail samples in *Trachemys scripta*, a species closely related to Western Painted Turtles, had a turnover rate (i.e., lag time between incorporation) of approximately 6 mo for  $\delta^{15}\text{N}$ , and more than 6 mo for  $\delta^{13}\text{C}$  (Aresco et al. 2015). Similarly,  $\delta^{15}\text{N}$  turnover rates are estimated to be 5–7 mo for muscle, liver, and blood of *T. scripta* (Seminoff et al. 2007). Because the metabolism of ectotherms decreases during the overwintering period, nail samples were assumed to provide long-term information on isotopic values accumulated over the active season.

**Sampling the food web.**—In May 2016, we sampled potential aquatic prey items from 16 randomly selected locations within the study area (Fig. 1). These locations were generated in ArcGIS v.10.3.1 (Environmental Systems Research Institute, Redlands, CA) with the use of the Random Points function in the Data Management Toolbox. Samples were collected by a combination of hand nets, seine nets, and minnow traps. Prey types were selected to represent potential diet items that were available to the turtles in both the creek and marsh habitat areas. Prey items that were collected included amphipods (Order Amphipoda,  $n = 39$ ), aquatic vegetation ( $n = 67$ ), chironomid larvae (Family Chironomidae,  $n = 58$ ), Virile Crayfish (*Orconectes virilis*,  $n = 28$ ), damselfly nymphs (Order Odonata,  $n = 34$ ), Fathead Minnows (*Pimephales promelas*,  $n = 77$ ), Brook Stickleback (*Culaea inconstans*,  $n = 44$ ), and water beetles (Order Hemiptera,  $n = 60$ ). Our experimental design focused on general functional groups of prey rather than particular species. Consequently, other than fish and crayfish, we did not identify potential prey to species (most

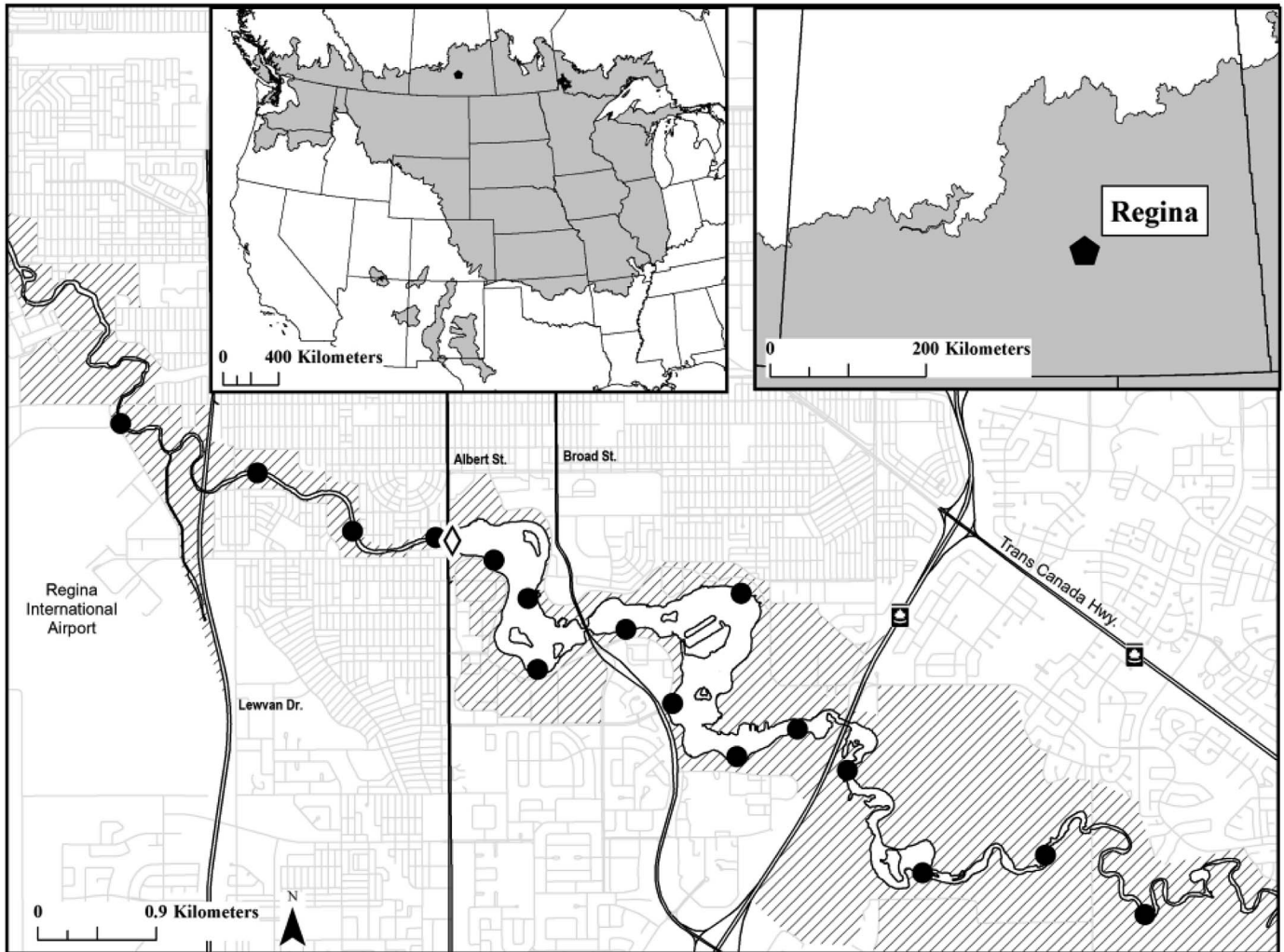


FIG. 1.—Study site of Western Painted Turtles (*Chrysemys picta bellii*) in Regina, Saskatchewan, showing the barrier (white diamond) between the creek (west) and marsh (east) habitat areas, and 16 randomly selected sites (black circles) where prey items were sampled in May 2016. Land use includes major roads (black), residential roads (gray), and urban green spaces (hatched area). Insets depict the North American range of Western Painted Turtles, and the provincial range with the location of the study site indicated (black pentagon).

prey categories contain multiple representative species). Samples were stored in 150-mL containers on ice, and transferred to a freezer at  $-20^{\circ}\text{C}$  until they were processed. For SIA, samples were thawed and sorted, and muscle tissue was dissected from crayfish and fish samples. Muscle tissue was used in SIA of fish and crayfish as it is readily digested by the turtles, in contrast to hardened structures (e.g., crayfish claws), which can be found undigested in fecal material. In addition, muscle tissue captures incorporation of short-term resource use of the consumer over several months (Perga and Gerdeaux 2005).

#### Stable Isotope Analysis

We weighed 0.4–0.7 mg from nail samples and placed it into tin capsules for isotope-ratio mass spectrometry (IRMS). To generate the appropriate mass, whole samples were taken from one nail for adults, and up to five nails for subadults. Prey samples, other than fish and crayfish, were rinsed with distilled water and dried whole in a drying oven at  $50^{\circ}\text{C}$  for approximately 5 d. Dried samples were ground into a fine powder with a mortar and pestle and stored in glass

scintillation vials. Muscle samples dissected from fish and crayfish were similarly dried, and ground to a fine powder with the use of a dental amalgamator. Lipids were extracted from the invertebrate and fish muscle samples with a 2:1 chloroform–methanol solution, following Doucette et al. (2011). Lipids were not extracted from the remaining samples because of their low lipid content (Bulte and Blouin-Demers 2008; Pearson et al. 2013). Each sample represented tissue from one prey item. Where possible, a minimum of 10 randomly selected samples were used for each prey item per habitat area representing multiple sample locations. All ground samples were weighed (vegetation, 3–5 mg; invertebrate/fish muscle tissue, 0.4–0.7 mg) and placed into tin capsules for IRMS.

Carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) SIA were performed with a Thermo Finnigan Delta V IRMS at the Institute of Environmental Change and Society at the University of Regina, Saskatchewan, Canada. Laboratory standards of bovine liver and wheat, as well as duplicates for each eight-sample interval were used for quality control. Stable isotope values are expressed as delta ( $\delta$ ) in per mil (‰), measured

TABLE 1.—Stable isotope values and niche metrics based on  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  for an urban population of Western Painted Turtles (*Chrysemys picta bellii*) in disjunct creek and marsh habitats in Regina, Canada. Values are reported for males (M), females (F), and subadults (S) as means  $\pm$  1 SD (min, max); body sizes, measured as straight-line carapace length (CL), are provided for each life-history stage. Niche dimensions are reported as standard ellipse area ( $\text{SEA}_c$ —corrected for small sample size).

| Location | Sex | <i>n</i> | CL (mm)      | $\delta^{13}\text{C}$ (‰)      | $\delta^{15}\text{N}$ (‰)   | C:N            | $\text{SEA}_c$ (‰ <sup>2</sup> ) | $\text{SEA}_c$ (‰ <sup>2</sup> ) |
|----------|-----|----------|--------------|--------------------------------|-----------------------------|----------------|----------------------------------|----------------------------------|
| Marsh    | F   | 32       | 217 $\pm$ 20 | -27.0 $\pm$ 0.8 (-28.6, -24.9) | 10.4 $\pm$ 0.7 (8.4, 12.15) | 3.7 $\pm$ 0.06 | 1.89                             | 1.95                             |
|          | M   | 19       | 183 $\pm$ 23 | -27.1 $\pm$ 0.7 (-28.4, -26.1) | 9.8 $\pm$ 0.5 (9.0, 10.7)   | 3.7 $\pm$ 0.09 | 0.96                             | 1.02                             |
|          | S   | 10       | 112 $\pm$ 40 | -28.0 $\pm$ 1.1 (-30.1, -26.0) | 10.0 $\pm$ 0.8 (8.4, 11.4)  | 3.7 $\pm$ 0.08 | 1.72                             | 1.94                             |
| Creek    | F   | 6        | 207 $\pm$ 11 | -27.1 $\pm$ 0.3 (-27.6, -26.9) | 11.2 $\pm$ 0.9 (9.4, 11.9)  | 3.7 $\pm$ 0.09 | 0.61                             | 0.76                             |
|          | M   | 5        | 193 $\pm$ 6  | -26.5 $\pm$ 0.9 (-27.4, -25.1) | 11.2 $\pm$ 0.3 (10.8, 11.6) | 3.7 $\pm$ 0.12 | 0.82                             | 1.09                             |

against the international standards of atmospheric nitrogen and Vienna-PeeDee Belemnite for nitrogen and carbon, respectively (Bond and Hobson 2012). Replicate samples had standard deviations of  $<0.2\%$  for both  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ .

**Inter- and intragroup isotopic variation.**—Turtle isotope data were grouped by capture location (marsh vs. creek), and sex/age class (female, male, or subadult). We used linear regression analyses to examine relationships between CL and both  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ . We used two-way analyses of variance (ANOVA) in R (v3.3.1, R Foundation for Statistical Computing, Vienna, Austria) to examine potential variation in isotope ratios based on sex, location, sample month, and the interaction between sex and sample years to ensure data from both years could be combined.

**Niche size and overlap.**—We used isotopic niche analysis with standard ellipses in the package Stable Isotope Bayesian Ellipses in R (SIBER; Jackson et al. 2011) to determine if isotopic resource use was the same across sex groups within each of the two habitat categories. Quantitative metrics of niche dimension (standard ellipse area; SEA) were calculated for each sex within the marsh and the creek habitat. The core isotopic niche area for each group was determined with the use of a 40% confidence interval standard ellipse around sampled individuals in each sex/location group. This value was then corrected for small sample sizes ( $\text{SEA}_c$ ; Jackson et al. 2011). Isotopic niche overlap metrics between groups within each location were measured using the methods of Stasko et al. (2015), with values  $>60\%$  indicating significant overlap in dietary resources (Mathur 1977; Guzzo et al. 2013). Isotopic niche size was compared with the use of a Bayesian framework ( $\text{SEA}_b$ ; Jackson et al. 2011) that tested the probability that the  $\text{SEA}_b$  of one group differed from another occupying the same habitat area.

**Isotopic mixing model analysis.**—To examine how potential prey items contribute to isotopic variation, we used the Bayesian Stable Isotope Mixing Model Analysis package in R (MixSIAR; Stock and Semmens 2013). This allowed examination of potential turtle prey items accounting for isotopic values from the consumers, prey, and trophic enrichment factors, using an uninformative prior (i.e.,  $\alpha = 1, 1, 1, \dots$ ). The isotopic values of sampled prey items were grouped to represent creek or marsh habitats. The mixing model calculated the proportion of each prey species for each turtle group with the mean ( $\pm 1$  SD)  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  for each of the eight potential prey items, and the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values for each individual turtle, within each habitat area. Consumers tend to be enriched in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  relative to their prey (Post 2002); to account for this we used a trophic

enrichment factor of  $0.23\%$  for  $\delta^{13}\text{C}$  and  $2.3\%$  for  $\delta^{15}\text{N}$ . These values were previously determined in the lab for nail samples from *T. scripta* (Aresco 2005; Aresco et al. 2015). Model convergence was verified at 200,000 iterations with the use of Gelman-Rubin, and Geweke diagnostics (Stock and Semmens 2013).

## RESULTS

### Turtle Nail Samples

We collected 77 nail samples representing 66 turtles from the marsh (32 females, 19 males, 15 subadults), and 11 turtles from the creek (6 females, 5 males) ranging in size from 49 to 270 mm CL. The majority of the nail samples were collected during 2016 ( $n = 63$ ), with 14 samples collected during 2015. Values of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in the turtle nails varied among and within the two study areas (Table 1). Mean  $\delta^{13}\text{C}$  ranged from  $-26.5 \pm 0.9\%$  in the creek males to  $-28.0 \pm 1.1\%$  in the marsh subadults. Mean  $\delta^{15}\text{N}$  varied from  $9.8 \pm 0.5$  in the marsh males, to  $11.2 \pm 0.9\%$  in the creek females. Overall, mean  $\delta^{15}\text{N}$  values for the marsh turtles were lower than values for individuals of the same sex in the creek.

### Inter- and Intra-group Isotopic Variation

There was a weak, but statistically significant, relationship between body size and both  $\delta^{13}\text{C}$  ( $F_{1,75} = 8.66$ ,  $R^2 = 0.10$ ,  $P < 0.01$ ) and  $\delta^{15}\text{N}$  values ( $F_{1,75} = 6.52$ ,  $R^2 = 0.08$ ,  $P = 0.01$ ). Nail tissue was slightly  $^{13}\text{C}$  and  $^{15}\text{N}$  enriched as body size increased (Fig. 2); although the relationships are weak and variable,  $\delta^{13}\text{C}$  increased  $0.01\%$  per centimeter, and  $\delta^{15}\text{N}$  increased  $0.005\%$  per centimeter. ANOVAs indicated an effect of sex ( $F_{2,66} = 5.94$ ,  $P < 0.01$ ) and the interaction between sex and year ( $F_{3,66} = 7.11$ ,  $P < 0.01$ ) on  $\delta^{13}\text{C}$ , and an effect of sex ( $F_{2,66} = 4.88$ ,  $P = 0.01$ ), and site ( $F_{1,66} = 19.6$ ,  $P < 0.01$ ) on  $\delta^{15}\text{N}$ . Tukey's post hoc tests indicated a difference in  $\delta^{13}\text{C}$  between subadults and both females ( $P < 0.01$ ) and males ( $P = 0.02$ ), between subadults sampled in 2015 and 2016 ( $P < 0.01$ ), and a difference in  $\delta^{15}\text{N}$  between females and subadults ( $P = 0.02$ ). Subadults had distinct isotopic values between years and could not be pooled for subsequent analyses. To ensure that adult samples from the 2015 and 2016 field seasons could be combined for analysis, we completed a paired *t*-test among individuals that had nail samples taken in both field seasons. No significant differences were detected between years for marsh ( $\delta^{13}\text{C}$ :  $t_{29} = 0.41$ ,  $P = 0.68$ ;  $\delta^{15}\text{N}$ :  $t_{29} = 1.86$ ,  $P = 0.07$ ) and creek turtles ( $\delta^{13}\text{C}$ :  $t_6 = 1.18$ ,  $P = 0.28$ ;  $\delta^{15}\text{N}$ :  $t_6 = -1.61$ ,  $P = 0.16$ );

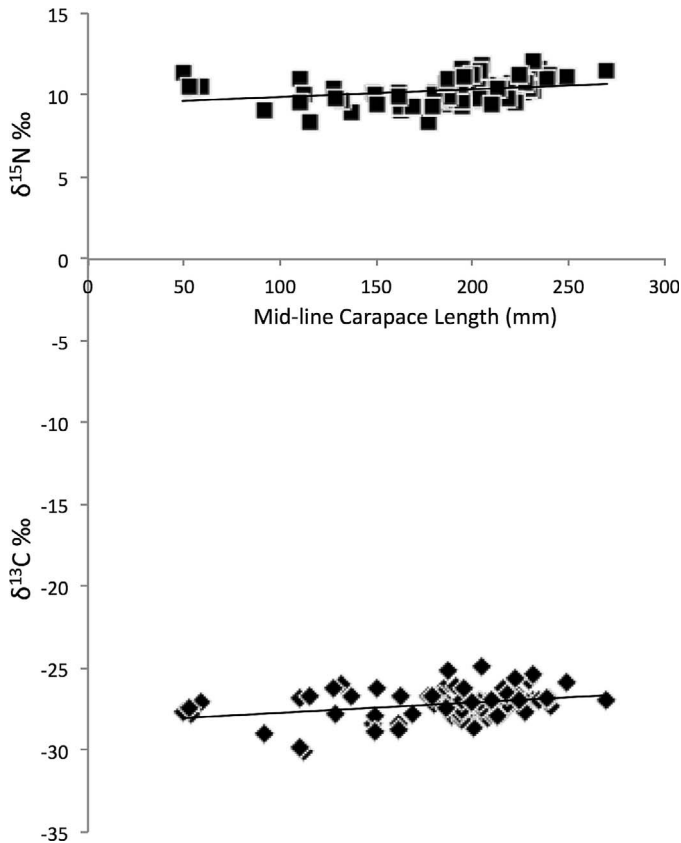


FIG. 2.—Regression analysis depicting the relationship between  $\delta^{13}\text{C}$  (diamonds;  $R^2 = 0.10$ ) and  $\delta^{15}\text{N}$  (squares;  $R^2 = 0.08$ ), and midline carapace length (in millimeters; a proxy for body size) in an urban population of Western Painted Turtles (*Chrysemys picta bellii*) in Regina, Canada.

therefore, adult nail samples ( $n = 62$ ) from the 2015 and 2016 field seasons were pooled for analyses.

#### Niche Size and Overlap

Because of the difference noted between 2015 and 2016 for subadults, isotopic niche analysis was conducted separately for this group for the 2 yr to compare isotopic niche size and overlap. The  $\text{SEA}_c$  for subadults was  $1.18\text{‰}^2$  and  $1.94\text{‰}^2$  in 2015 and 2016, respectively. Although there can be a higher degree of uncertainty in the  $\text{SEA}_c$  when examining small sample sizes (Jackson et al. 2011), between the 2 yr, there was no isotopic overlap in either the convex hull or  $\text{SEA}_c$ , and the Bayesian probability of subadults in 2015 having a smaller isotopic niche than subadults in 2016 was 87% (Fig. 3). Subadults sampled in 2015 were removed from the following niche-size comparisons and mixing model analyses.

Isotopic niche size ( $\text{SEA}_c$ ) varied across locations and sexes with the smallest  $\text{SEA}_c$  in the creek females ( $0.76\text{‰}^2$ ) and the largest in the marsh females ( $1.97\text{‰}^2$ ; Fig. 4). Within the marsh, female  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  ranged  $\sim 4\text{‰}$  among individuals, males ranged  $\sim 1\text{‰}$ , and subadults ranged  $\sim 2\text{‰}$  and  $\sim 3\text{‰}$  in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , respectively. Within the creek, female isotopic values ranged  $1\text{‰}$  in  $\delta^{13}\text{C}$  and  $2\text{‰}$  in  $\delta^{15}\text{N}$ , and males ranged  $2\text{‰}$  in  $\delta^{13}\text{C}$  and  $1\text{‰}$  in  $\delta^{15}\text{N}$ . Isotopic niche overlap within the marsh habitat varied among groups; there was 36% isotopic niche overlap between females and males, and a high probability that the female isotopic niche

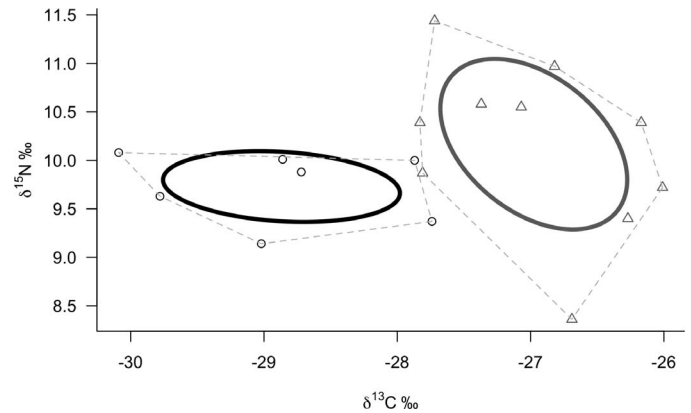


FIG. 3.— $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values for subadult Western Painted Turtles (*Chrysemys picta bellii*) captured during 2015 (black circles) and 2016 (gray triangles) in Regina, Canada. The 40% confidence interval standard ellipses represent the core isotopic niche for each field season. The dashed lines indicate the convex hull of the data points within each field season.

size was larger than that of males ( $P_{F > M} = 99\%$ ). There was 77% isotopic niche overlap between females and subadults, with a low probability that the isotopic niche size of females was larger than that of subadults ( $P_{F > S} = 45\%$ ). There was 55% isotopic niche overlap between males and subadults with a high probability that the isotopic niche of subadults was larger than that of males ( $P_{M < S} = 95\%$ ). Within the creek habitat, there was 26% isotopic niche overlap between females and males, with a low probability that the female isotopic niche was larger than the males ( $P_{F > M} = 45\%$ ).

#### Isotopic Mixing Model Analysis

Eight potential prey items were collected from random sites representing the creek and the marsh habitat. Mean isotope values for each prey item differed between the marsh and the creek habitats (Table 2). Overall, mean  $\delta^{15}\text{N}$  values in potential prey items were lower in the marsh habitat than values found for the same prey item in the creek. Biplots were created to represent the isotopic food web structure in the marsh and creek habitats (Fig. 5). Among the mean prey isotope values, there was a difference of 3‰ in  $\delta^{13}\text{C}$  and 2‰ in  $\delta^{15}\text{N}$  in the marsh, and a difference of 4‰ in  $\delta^{13}\text{C}$  and 6‰ in  $\delta^{15}\text{N}$  in the creek. All prey items were represented in the mixing models for turtles found in both marsh and creek habitats (Fig. 6), indicating that the turtles consume a variety of prey sources in varying proportions. The prey item that was estimated to be the most highly represented was crayfish for marsh females and subadults, amphipods for marsh males, and chironomid larvae for both creek males and females.

#### DISCUSSION

Our isotope data indicated that Western Painted Turtles as a population were generalist omnivores, but adult males and females and subadults did not use the same resources. Overall, turtles in our study generally had broad isotopic niches and consumed a wide variety of prey in low proportions. Niche sizes varied as much as three times among sex and age classes, however, and niche overlap ranged from resource use partitioning to significant overlap

TABLE 2.—Stable isotope values for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  from potential prey items sampled at 16 randomly selected sites within creek and marsh habitats of Regina, Canada. Values are reported as means  $\pm$  1 SD, and were used in mixing models for the diet of Western Painted Turtles (*Chrysemys picta bellii*). Other than crayfish and fish species, all other categories of prey might have contained multiple representative species.

| Prey item          | Location | n  | $\delta^{13}\text{C}$ (‰) | $\delta^{15}\text{N}$ (‰) |
|--------------------|----------|----|---------------------------|---------------------------|
| Amphipods          | Marsh    | 25 | $-26.6 \pm 0.9$           | $6.5 \pm 0.8$             |
|                    | Creek    | 14 | $-25.1 \pm 0.9$           | $8.3 \pm 0.6$             |
| Aquatic vegetation | Marsh    | 49 | $-26.8 \pm 2.2$           | $5.5 \pm 2.4$             |
|                    | Creek    | 18 | $-26.3 \pm 2.4$           | $6.7 \pm 1.4$             |
| Chironomid larvae  | Marsh    | 45 | $-29.1 \pm 2.1$           | $7.3 \pm 1.7$             |
|                    | Creek    | 13 | $-27.7 \pm 2.0$           | $9.1 \pm 0.8$             |
| Crayfish           | Marsh    | 23 | $-26.9 \pm 1.0$           | $8.9 \pm 1.3$             |
|                    | Creek    | 5  | $-26.3 \pm 0.3$           | $11.4 \pm 0.2$            |
| Damsel fly nymphs  | Marsh    | 19 | $-29.7 \pm 0.8$           | $8.3 \pm 0.6$             |
|                    | Creek    | 15 | $-27.0 \pm 0.7$           | $10.4 \pm 0.3$            |
| Fathead minnows    | Marsh    | 58 | $-27.9 \pm 1.2$           | $11.0 \pm 0.9$            |
|                    | Creek    | 19 | $-28.2 \pm 1.6$           | $12.0 \pm 0.8$            |
| Stickleback        | Marsh    | 29 | $-27.1 \pm 1.4$           | $11.4 \pm 0.7$            |
|                    | Creek    | 15 | $-26.4 \pm 0.9$           | $12.8 \pm 0.8$            |
| Water beetles      | Marsh    | 45 | $-28.7 \pm 3.5$           | $7.2 \pm 1.7$             |
|                    | Creek    | 15 | $-29.0 \pm 2.8$           | $7.0 \pm 1.7$             |

(26–77%; Jackson et al. 2012; Guzzo et al. 2013). In contrast, previous studies with short-term diet analyses have found little to no variation in diet among sex and age classes (Lindeman 1996; Rowe and Parsons 2000; Rowe and Bowen 2005), with the exception of shifts in overall diet choice attributable to ontogeny (more carnivorous to more herbivorous; Gibbons 1967) or location across the geographic range (Ernst and Lovich 2009). This indicates that although Painted Turtles are omnivorous, there might be further selection for particular resources among subgroups. Therefore, future studies should emphasize potential variation by examining isotopic resource use among sex and age classes.

Regardless of the habitat area, males and females had different isotopic niches, with low and nonsignificant overlap (<60% based on Guzzo et al. 2013). In the marsh habitat, niche partitioning by sex was attributable to  $^{15}\text{N}$  enrichment and larger isotopic niche size in females, indicating that they were consuming more diverse prey items from higher trophic levels than males (Peterson and Fry 1987; Post 2002). This inference was further supported by the mixing model analysis, which showed that females consumed a higher proportion of crayfish, and males consumed a higher proportion of insect larvae. In the creek habitat, both males and females had small isotopic niches that were essentially nonoverlapping (26%), with males being more  $^{13}\text{C}$  enriched than females. The  $^{13}\text{C}$  enrichment indicates variation in prey carbon source, such as among littoral, benthic, or terrestrial prey by sex (Post 2002; Fry 2006). Terrestrial foraging is rare in Painted Turtles (Sexton 1959; Moldowan et al. 2015), but direct inputs to the creek from the surrounding landscape are large and might provide terrestrial prey sources. In summary, male and female Western Painted Turtles use different isotopic resources as evidenced by variation in trophic levels as well as feeding locations within the habitats. This finding raises the possibility of previously undocumented sex differences in habitat use and/or trophic level at other locations, another potential avenue of future research for this species.

Vegetation is abundant in our study system, but isotopic data indicated that turtles consumed a high proportion of

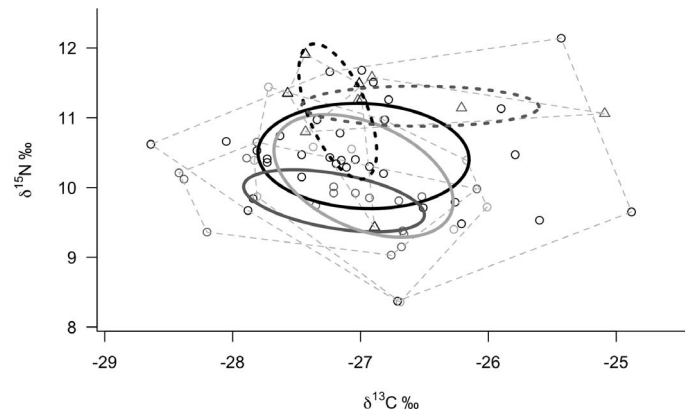


FIG. 4.— $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values for Western Painted Turtles (*Chrysemys picta bellii*) in marsh (circle) and creek (triangle) habitat of Regina, Canada. Standard ellipses indicate the 40% confidence interval representing the core isotopic niche for each group (solid line = marsh; dotted line = creek; black = female; dark gray = male; light gray = subadult). Dashed lines (light gray) indicate the convex hull of the outer data points for each group.

animal prey. In addition, our subjects did not display an ontogenetic shift in diet, such that subadults are carnivorous and adults are herbivorous as described by Gibbons (1967). In fact, subadults had a high degree of isotopic niche overlap with adult females (77%). Traditional stomach content analyses of a Western Painted Turtle population in the nearby Qu'Appelle River found similar dietary patterns with turtles consuming high proportions of crayfish and benthic invertebrates (amphipods, hemipterans, and odonates), and low proportions of vegetation and fish (MacCulloch and Secoy 1983). Previous studies on diet have suggested that Painted Turtles in northern populations exhibit higher degrees of carnivory to facilitate increased growth rates, attain larger body sizes, and produce larger clutches, when challenged with shorter active seasons than their southern conspecifics (Gibbons 1967; MacCulloch and Secoy 1983; Lindeman 1996; Cooley et al. 2003). At the northern limit of the species range, an herbivorous diet might not provide sufficient energy resources in comparison to a carnivorous diet with high mass-specific caloric content (Cooley et al. 2003).

Turtles and their prey from the creek habitat were more  $^{15}\text{N}$  enriched in comparison to the marsh. Typically, this variation would indicate a change in resource use (i.e., trophic level) among individuals of the same species. This pattern could also be attributable to anthropogenic influence on the baseline isotopic values in the system (Cabana and Rasmussen 1996; Hofmeister et al. 2013; Pearson et al. 2013; Ferronato et al. 2016). Hofmeister et al. (2013) found that *Chrysemys picta* had elevated  $\delta^{15}\text{N}$  in tail tissue at sites with high agricultural influence, which was correlated to similarly elevated baseline  $\delta^{15}\text{N}$  values in duckweed (*Lemna* sp.) from the same habitat. A similar study examining the isotopic composition of Eastern Long-necked Turtles (*Chelodina longicollis*) found that suburban sites had higher  $\delta^{15}\text{N}$  values than more natural sites (Ferronato et al. 2016). Varying degrees of urban development surround both habitat areas in our study, with inflows to the system through storm-water drains and runoff; however, there are comparably more inflows per surface area, decreased natural buffers (urban parks), and increased channelization in the creek habitat

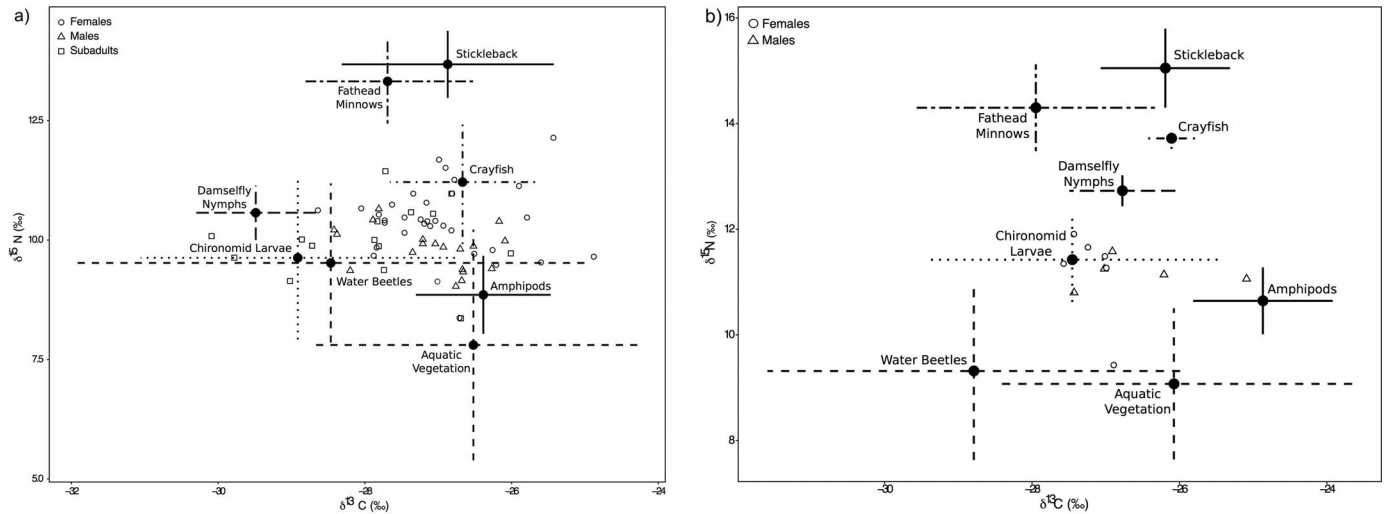


FIG. 5.— $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  biplot of nail tissue samples from Western Painted Turtles (*Chrysemys picta bellii*), and their potential prey items from (a) marsh and (b) creek habitats of Regina, Canada. Stable isotopes values are plotted as individual data points for nail samples from turtles of different life-history stages (see legend), and mean  $\pm 1$  SD for prey items, accounting for trophic enrichment factors (0.23‰ for  $\delta^{13}\text{C}$  and 2.3‰ for  $\delta^{15}\text{N}$ ).

compared to the marsh habitat. This could lead to the creek habitat being more  $^{15}\text{N}$  enriched because of increased impact from high nitrogen sources (e.g., fertilizer runoff). Overall, our data indicate site-specific variation on a relatively small spatial scale, reiterating the need to tailor sampling of prey items accordingly across study areas to verify whether variation among groups is caused by baseline shifts or changes in resource use. Within our study, the isotopic variation among turtles within the marsh and creek habitats appears to be a result of  $^{15}\text{N}$  enrichment acting upon the creek as opposed to variation in prey choice between the two habitat areas. This interpretation is further supported by the similarities in prey consumption observed in the mixing model analysis.

Isotopic niche variation across subgroups was the largest in subadult turtles. Subadults showed large differences in  $\delta^{13}\text{C}$  compared to adults, and also a temporal shift of 4‰ across years. Changes of 0–1‰ in  $\delta^{13}\text{C}$  typically indicate a change in consumer energy source (i.e., location within habitat; Peterson and Fry 1987). Little is known about the

life histories of subadult turtles because of their cryptic nature. A study on Painted Turtles in Michigan, however, found an ontogenetic shift in habitat use such that subadult turtles more commonly inhabit shallower areas than adults (Congdon et al. 1992). Correspondingly, we expected subadults potentially to exhibit more depleted  $\delta^{13}\text{C}$  compared to the adults to reflect the carbon source variation between littoral and benthic habitat areas. This trend was evident when we combined both subadult sample years. When the 2 yr were separated, however, we found a distinct temporal difference between  $\delta^{13}\text{C}$  values, with samples in 2016 being more  $^{13}\text{C}$  enriched than 2015. Temporal differences in isotopic niche could indicate variation in prey and/or habitat use for subadults across years; however, increased somatic growth might also contribute to subadult variation. Compared to their adult counterparts, young Desert Box Turtles (*Terrapene ornate luteola*) and Loggerhead Sea Turtles (*Caretta caretta*) have faster carbon turnover rates because of their rapid growth rates (Reich et al. 2008; Murray and Wolf 2013). As such, examining

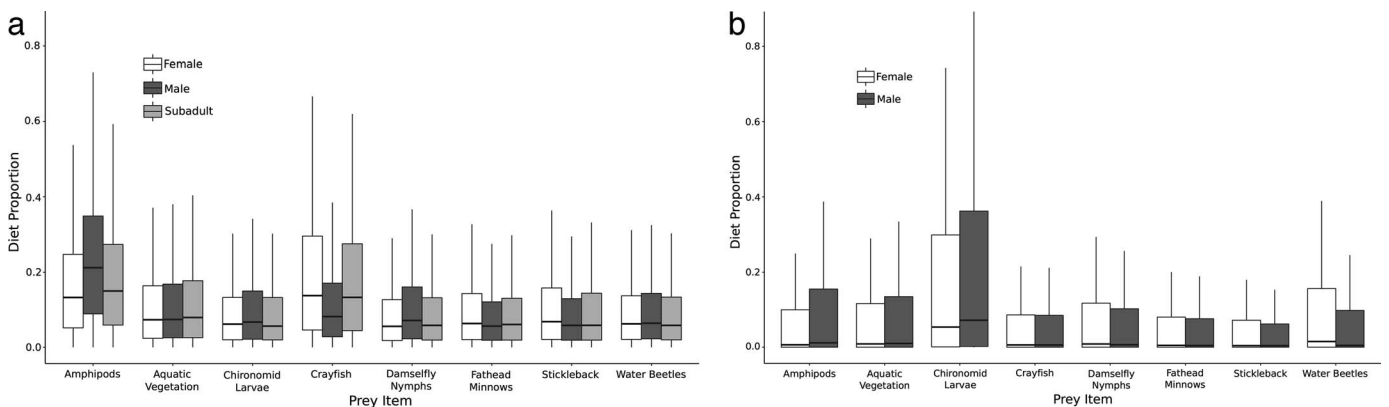


FIG. 6.— Box-whisker plots showing the range of estimated proportions of eight potential prey items in the diet of female (open), male (dark gray), and subadult (light gray) Western Painted Turtles (*Chrysemys picta bellii*) in marsh (a) and creek (b) habitats of Regina, Canada. Boxes indicate interquartile range, bars indicate the median, and whiskers indicate the confidence-interval range of feasible solutions determined by MixSIAR (Stock and Semmens 2013).

carbon isotope patterns in subadult turtles might more readily capture temporal changes within the habitat. Somatic growth rates of turtles decrease with age, with little to no growth following sexual maturity (Ernst and Ernst 1973; Congdon et al. 2013). In contrast to values for adults, subadult isotopic values might be more susceptible to temporal isotopic variation attributable to increased metabolic turnover. Future studies should examine isotopic and metabolic turnover in the lab and in the field, to further understand the variation between subadult and adults, and the influence of potential extrinsic factors (e.g., yearly isotopic baseline variation).

### Management Implications

Our data indicate that Western Painted Turtles in Regina are largely carnivorous, and take advantage of a wide variety of animal prey items (primarily aquatic invertebrates), supporting previous studies on freshwater turtle populations in northern environments (e.g., Cooley et al. 2003). As generalist omnivores occupying a relatively small spatial scale, we expected to see little isotopic variation among subgroups; however, in contrast we found a diversity of isotopic niche ranges, niche sizes, and niche overlap values among the turtle subgroups. Thus, not all turtles in the population are the same, which raises the possibility of a wide range of different responses by individuals and subgroups to environmental stressors or habitat modifications. This potential for variation has typically not been identified using short-term diet analyses (e.g., MacCulloch and Secoy 1983) highlighting the need for additional stable isotope research. Where turtles occur in urban areas, it might be necessary to account for the different needs of males, females, and subadults when developing long-term strategies to sustain those populations.

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