



## Isotopic niche in the eastern long-necked turtle, *Chelodina longicollis* (Testudines: Chelidae), along a natural-urban gradient in southeastern Australia

Bruno O. Ferronato<sup>1</sup>, Thiago S. Marques<sup>2</sup>, Neliton R.F. Lara<sup>2</sup>, Luiz A. Martinelli<sup>2</sup>, Luciano M. Verdade<sup>2</sup>, Plinio B. Camargo<sup>2</sup>, John H. Roe<sup>3</sup> & Arthur Georges<sup>1</sup>

<sup>1</sup>Institute for Applied Ecology, University of Canberra, Bruce, Canberra, ACT, 2601, Australia

<sup>2</sup>Isotopic Ecology Laboratory, Center for Nuclear Energy in Agriculture, University of São Paulo, Piracicaba 13416-000, Brazil

<sup>3</sup>Department of Biology, University of North Carolina, Pembroke, NC 28372, USA

Urbanisation is one of the most common threats to many native species, while others are capable of taking advantage of urban areas and even expanding their niche in urban-natural systems. The analysis of stable isotopes of carbon and nitrogen in a tissue sample provides data that can elucidate food web dynamics and trophic ecology of an animal. Our study aimed to evaluate variation in food resource exploitation in the freshwater turtle *Chelodina longicollis* along a habitat gradient (natural, rural, and suburban areas), and intraspecific niche variation among demographic groups (juvenile, adult male, adult female). We found that isotopic composition of *C. longicollis* varied along the habitat gradient, with  $\delta^{15}\text{N}$  levels highest in suburban environments, intermediate in rural areas, and lowest in the nature reserve.  $\delta^{13}\text{C}$  values were higher in suburb and rural turtles compared to those on the nature reserve. Besides some intraspecific differences in  $\delta^{13}\text{C}$  as evidence of demographic partitioning of the foraging niche, demographic groups apparently feed on the same trophic level within habitats. Our study included samples from small juveniles (<10 cm) and helped to cover a gap of understanding in intraspecific niche for *C. longicollis*. Future research should evaluate the reasons turtles in suburban areas are enriched in  $\delta^{15}\text{N}$ , either because they are foraging on different trophic levels or because they are feeding on prey enriched in nitrogen.

**Key words:** diet, generalist species, intraspecific variation, isotopic ecology, nitrogen

### INTRODUCTION

Urbanisation is one of the most common threats to native wildlife (Blair & Launer, 1997; McKinney, 2002, 2006; Chase & Walsh, 2006). Habitat loss and simplification, roads, sealed surfaces, buildings and exotic vegetation are some of the features that reduce biodiversity in urban areas (McKinney, 2002; Roe et al., 2006; Shochat et al., 2006; Deng et al., 2009). Many native species have been extirpated as a result of urban development (Blair & Launer, 1997; McKinney & Lockwood, 1999). In addition, an increase in interspecific niche overlap and potential competition can occur when species inhabit altered habitats (Luiselli, 2006; Pearson et al., 2013). However, many generalist species have been taking advantage of urban ecosystems (McKinney & Lockwood, 1999; Newsome et al., 2010). Examples of generalist taxa that have been successful in urbanised systems include birds (house sparrow *Passer domesticus*, European starling *Sturnus vulgaris*), mammals (house mouse *Mus musculus*, Norway rats *Rattus norvegicus*), amphibians (bullfrog *Rana catesbeiana*), reptiles (house gecko *Hemidactylus mabouia*, Geoffroy's side

necked turtle *Phrynops geoffroanus*), and invertebrates (Argentine ant *Linepithema humile*) (McKinney, 2002, 2006, 2008; Holway & Suarez, 2006; Hamer & McDonnell, 2008; Ferronato et al., 2009).

Resource partitioning and niche expansion are common strategies demographic groups within populations use to avoid competition and maximise coexistence (Tucker et al., 1996; Souza & Abe, 1998; Bolnick, 2001). There are several examples of intraspecific variation in resources use in reptiles. Studies on lizards (Schoener, 1968; Paulissen, 1987), crocodylians (Tucker et al., 1996; Plat et al., 2006; Marques et al., 2013) and freshwater turtles (Georges, 1982; Tucker et al., 1995; Souza & Abe, 1998) demonstrated that juveniles and adults can feed on different prey, with larger individuals ingesting larger prey sizes (Schoener, 1968; Souza & Abe, 1998; Platt et al., 2006). According to Schoener (1977), differences in diet among age-classes are more common than differences in activity pattern or habitat use.

Stable isotopes are a key tool in describing nutrient flow in food webs and ecosystems (Peterson & Fry, 1987; Ometto et al., 2005; Newsome et al., 2007). As animals incorporate the isotopic composition into

Correspondence: Bruno O. Ferronato (brunoferronato@hotmail.com)

their bodies via consumption and tissue synthesis, isotopes can be used to quantify bionomic elements of their niche (Newsome et al., 2007). The use of carbon ( $^{13}\text{C}/^{12}\text{C}$ ) and nitrogen ( $^{15}\text{N}/^{14}\text{N}$ ) isotope ratios, denoted as  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , can help ecologists to postulate the bionomic and scenopoetic dimensions of the niche, as  $\delta^{13}\text{C}$  traces the energy sources for consumers and  $\delta^{15}\text{N}$  is an indicator of trophic positions of organisms in the food web (Newsome et al., 2007; Turner et al., 2010). Recent advances in analytical tools have made possible the simultaneous analysis of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , also described as the isotopic niche (Layman et al., 2007a; Newsome et al., 2007; Jackson et al., 2011). Isotopic niche analysis offers time and space-integrated representations of the trophic ecology of organisms (Layman et al., 2007b; Newsome et al., 2007).

*Chelodina longicollis* (Shaw, 1794) is an Australian freshwater turtle that inhabits a broad range of habitats, including permanent riverine waterholes, lakes, farm dams, and shallow temporary wetlands (Kennett et al., 2009). Recent investigations of *C. longicollis* inhabiting suburban and natural areas have demonstrated that individuals from suburban regions tend to grow faster and are more abundant than the ones from natural habitats (Roe et al., 2011). *C. longicollis* is an opportunistic carnivore, with a diet consisting of planktonic and benthic macro-invertebrates and carrion (Chessman, 1984; Georges, et al. 1986). Although a study has shown that the diet composition of different sizes and sexes of *C. longicollis* varied relatively little, the composition of juveniles' diet was underrepresented, especially for turtles less than 10 cm (Chessman, 1984). Consequently, the complete picture of intraspecific variation in *C. longicollis* foraging ecology remains unclear. In addition, diet composition can vary according to location, as differences in local abundance of prey can be expected (Georges et al., 1986), and no information on *C. longicollis* feeding habits are available in rural or altered wetlands.

Here, we use stable isotope analysis of carbon and nitrogen for an examination of differences in diet resource exploitation for *C. longicollis* along a habitat gradient (natural, rural, and suburban areas), and among demographic groups (juvenile, adult male, and adult female). Our goal was to determine how the generalist *C. longicollis* responds to local environmental variation, including anthropogenic habitat alteration, via the assimilation of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  isotopes in its diet.

## METHODS

### Study area

We studied turtles between October 2012 and February 2013 in Canberra, Australian Capital Territory (ACT), southeastern Australia. For detailed information on the study sites see Rees et al. (2009) and Roe et al. (2011). The study sites consisted of three types of habitat: natural, rural and suburb, along a single watershed gradient. The natural site was the Mulligans Flat Nature Reserve. This nature reserve has 703 ha and is located on the outskirts of urban sprawl, consisting of woodlands, grasslands, several ponds and the upper tributaries of

Ginninderra Creek (Rees et al. 2009). We sampled turtles in four ponds in the nature reserve (one large: 6.35 ha and three smaller ponds: 0.66, 0.41 and 0.14 ha). These ponds are surrounded by native grasslands.

The rural site was the Ginninderra Experiment Station that belongs to the CSIRO (Commonwealth Scientific and Industry Research Organisation). The area is drained by ephemeral streams and has some ponds, part of the area has native grasses and eucalypt trees, while others have been cleared and cropped or sown to pasture (Webster & Butler 1976). We sampled turtles in two ponds in the rural site (2.75 and 2.44 ha). These ponds are surrounded by pasture for livestock.

The suburban site consists of large areas of residential and industrial development, including high road densities and managed suburban green spaces such as parks, golf courses, sport ovals and gardens. In addition, there are several suburban water bodies, including two large reservoirs (each 25 ha in surface area), several smaller golf course and storm water drainage ponds, and many streams that connect with the Ginninderra creek drainage (Rees et al., 2009; Roe et al., 2011). We sampled animals in four ponds in the suburbs (one large reservoir: 25 ha, a golf course pond: 1.26 ha, a canal: 0.62 ha and a storm water drainage pond: 0.30 ha). These ponds are surrounded by manicured gardens and lawns.

### Sampling methodology

Turtles were trapped using crab traps modified with a snorkel that allowed traps to sit on the bottom in up to two metres of water while allowing turtles to surface for air. Traps were baited with sardines and cow liver enclosed in a wire mesh so that turtles could not feed on the content. We set four traps per pond, except for one large reservoir in the suburban area where we set six traps. We trapped turtles once per month (five consecutive days of trapping per month), including four ponds each in the suburban and natural site, and two ponds in the rural site (see above). All turtles captured were marked with unique codes by notching the shell (Kennett & Georges, 1990), and released at the same site of capture.

We measured maximum carapace length (CL) and midline plastron length (PL) with calipers ( $\pm 0.1$  mm) and body mass with a scale ( $\pm 5$  g). We considered turtles with a  $\text{CL} < 145$  mm as juveniles, as this represents the best estimate on minimum size at maturity (Kennett & Georges, 1990); those with  $\text{CL} > 145$  mm were classified as males or females based on external morphological features (see Kennett & Georges, 1990). In total, we sampled 40 individuals (12 juveniles, 11 adult males and 17 adult females) of *C. longicollis* in the nature reserve, 30 individuals (9 juveniles, 9 adult males and 12 adult females) in the rural site, and 44 individuals (12 juveniles, 15 adult males and 17 adult females) in the suburban site.

Turtles' claws (the terminal 5 mm of the claw in adults and juveniles) were clipped and stored for later analysis. We used claw samples because they represent a mix of old and new tissues, reflecting temporal dietary time sequences (Bowen et al., 2005; Ethier et al., 2010).

**Table 1.** Stable isotope composition ( $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ ) of demographic groups in the eastern long-necked turtle (*Chelodina longicollis*) among different habitats in the Australian Capital Territory, Australia. (Superscripts indicate similarities or differences among demographic groups within columns, revealed by Bonferroni test).

		$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)
Nature Reserve	Juveniles	4.1±0.9 <sup>A</sup> (2.6–5.8)	-27.3±1.0 <sup>ABC</sup> (-28.9– -25.6)
	Males	5.0±1.1 <sup>A</sup> (3.9–7.1)	-27.4±0.9 <sup>AB</sup> (-28.8– -26.1)
	Females	5.3±0.9 <sup>A</sup> (3.7–6.6)	-28.9±1.4 <sup>A</sup> (-30.9– -26.3)
Rural	Juveniles	7.5±0.6 <sup>BC</sup> (6.6–8.7)	-26.4±0.5 <sup>BC</sup> (-27.1– -25.2)
	Males	8.4±1.1 <sup>B</sup> (6.7–10.2)	-24.8±1.9 <sup>A</sup> (-27.2– -21.6)
	Females	8.0±1.2 <sup>B</sup> (7.0–11.3)	-26.5±0.9 <sup>AB</sup> (-27.9– -24.9)
Suburb	Juveniles	9.5±0.9 <sup>BCD</sup> (8.4–12.1)	-25.4±1.1 <sup>BC</sup> (-26.8– -23.2)
	Males	9.7±0.6 <sup>CD</sup> (8.2–10.8)	-25.9±0.9 <sup>BC</sup> (-27.5– -24.1)
	Females	10.3±0.9 <sup>D</sup> (8.2–11.7)	-25.5±1.4 <sup>BC</sup> (-28.9– -22.9)

Samples were manually cleaned to remove contaminants from the environment and fragmented into very small pieces. The resultant material was weighed and put inside small tin capsules. The isotopic composition of carbon and nitrogen was determined by “on-line” combustion of the samples by CF-IRMS (Continuous Flow–Isotope Ratio Mass Spectrometers), using an elemental analyser Carlo Erba (CHN-1110) interfaced to an isotope ratio mass spectrometer Delta Plus in the Isotopic Ecology Laboratory (CENA, University of São Paulo, Brazil). The isotopic composition of carbon and nitrogen was calculated with the following equation:

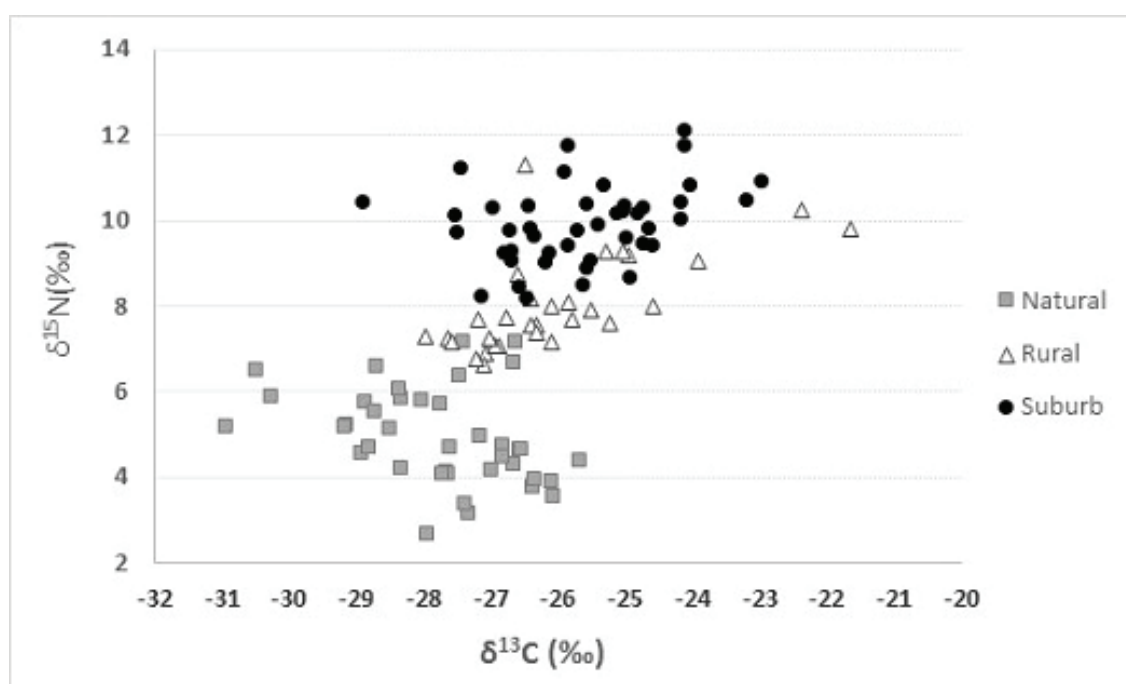
$$\delta^{\text{B}} \text{C} \text{ or } \delta^{\text{5}} \text{N} = \left( \frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \right) * 1000$$

where R is the molar ratio  $^{13}\text{C}/^{12}\text{C}$  or  $^{15}\text{N}/^{14}\text{N}$  in the sample

and standard, expressed as delta ( $\delta$ ) per mil (‰) (see Fry, 2006). The standards used for nitrogen and carbon were Pee Dee Belemnite (PDB) and atmospheric nitrogen (AIR), respectively. The analytical error of isotope measurements was estimated at 0.3‰ for  $\delta^{13}\text{C}$  and 0.4‰ for  $\delta^{15}\text{N}$  using repeated measures of internal standard (sugarcane).

#### Analytical methodology

We tested the residuals for normality using the Anderson Darling test and for homoscedasticity using Levene’s test prior to statistical analyses. We performed a factorial ANOVA (Zar, 1999) to test if turtles’ carapace length was different among our study sites, demographic groups (adult male, adult female) and its interaction. We performed an ANCOVA (Zar, 1999) to detect possible differences involving  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values among



**Fig. 1.** Isotopic compositions ( $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ ) of eastern long-necked turtles (*Chelodina longicollis*) inhabiting natural, rural and suburban ponds in the Australian Capital Territory, Australia.

**Table 2.** Estimates of Bayesian standard ellipse areas (SEA<sub>B</sub>) (%<sup>2</sup>) in different demographic groups and habitats in eastern long-necked turtle (*Chelodina longicollis*) in the Australian Capital Territory, Australia (superscripts indicate similarities or differences among groups). *p* values: Nature reserve- F x J: *p*=0.320, F x M: *p*=0.585, M x J: *p*=0.732; Rural- F x J: *p*=0.940, F x M: *p*=0.367, M x J: *p*=0.033; Suburb- F x J: *p*=0.814, F x M: *p*=0.949, M x J: *p*=0.734

	Nature Reserve		Rural		Suburb	
	Mean	Median	Mean	Median	Mean	Median
Juveniles	4.89 <sup>A</sup>	4.62	1.93 <sup>A</sup>	1.8	3.24 <sup>A</sup>	3.05
Males	3.83 <sup>A</sup>	3.60	4.42 <sup>B</sup>	4.09	2.52 <sup>A</sup>	2.42
Females	4.06 <sup>A</sup>	3.91	3.76 <sup>AB</sup>	3.56	4.43 <sup>A</sup>	4.26

study sites (natural, rural and suburban areas), among demographic groups (juvenile, adult male, adult female) and its interaction, considering the carapace length as a covariate. Differences among sites and demographic groups were evaluated by Bonferroni Test (Zar, 1999). All statistical analyses were performed using Minitab 16. The measurements are expressed as mean±standard deviation and range.

We then applied Bayesian standard ellipse (SEAB) to measure niche widths between demographic groups in each site (natural, rural and suburban areas) (Jackson et al., 2011). Probability values for differences between groups were obtained by calculating the proportion of the total number of simulations (10,000) where one group had a larger SEA than the other. For more details see Jackson et al. (2011). We also measured niche overlap through the overlapping areas of corrected standard ellipses of demographic groups using SIAR package (Stable Isotope Analysis in R) for R (R Development Core Team 2009; Parnell et al., 2010).

## RESULTS

Turtle size varied among study sites ( $F_{2,80}=3.7, p=0.027$ ), with larger individuals in the rural area [ $19.3\pm 2.3$  cm (14.5–23.9 cm)], which differed significantly in carapace length from nature reserve turtles [ $17.9\pm 2.6$  cm (13.3–22.6 cm)], but both were similar to suburban turtles

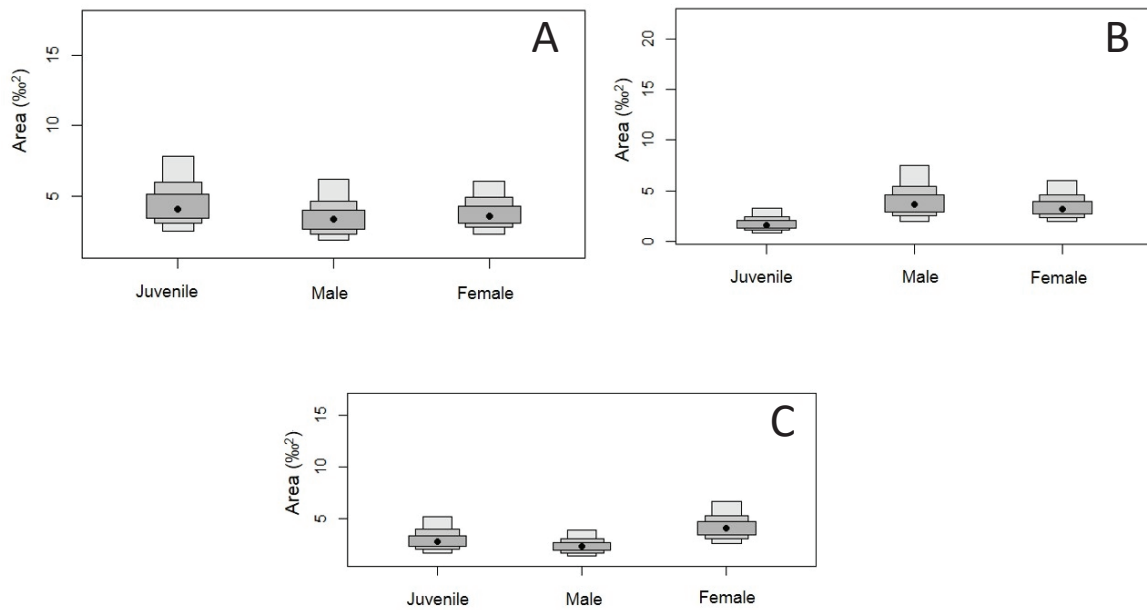
[ $18.9\pm 2.1$  cm (14.7–24.2 cm)]. Furthermore, there was a difference in carapace length between the demographic groups [ $F_{1,80}=14.3, p<0.001$ ; adult males:  $17.6\pm 1.8$  cm (14.5–20.9 cm); adult females:  $19.4\pm 2.4$  cm (13.3–24.2 cm)], but there was no interaction between factors ( $F_{2,80}=0.4, p=0.639$ ).

We observed significant differences in  $\delta^{15}\text{N}$  values among sites ( $F_{2,112}=255.7, p<0.001$ ; Fig. 1), with  $^{15}\text{N}$  values highest in suburban environments [ $9.9\pm 0.9\%$  (8.2–12.1‰)], intermediate in rural areas [ $8.0\pm 1.1\%$  (6.6–11.3‰)], and lowest in the nature reserve [ $4.9\pm 1.1\%$  (2.6–7.1‰)] evidenced by the Bonferroni test. However, we found no significant differences in  $\delta^{15}\text{N}$  among demographic groups ( $F_{2,112}=0.4, p=0.672$ ) (Table 1). In addition, there was no interaction between study area and demographic groups ( $F_{2,112}=1.2, p=0.297$ ) and no relationship between  $\delta^{15}\text{N}$  and carapace length ( $p=0.188, r=0.91$ ).

We observed significant differences in  $\delta^{13}\text{C}$  among sites ( $F_{2,112}=27.30, p<0.001$ ; Fig. 1), with nature reserve turtles less enriched in  $^{13}\text{C}$  [ $-27.7\pm 1.2\%$  (-30.9– -25.6‰)] than rural [ $-26.0\pm 1.4\%$  (-27.9– -21.6‰)] and suburban turtles [ $-25.6\pm 1.2\%$  (-28.9– -22.9‰)] evidenced by the Bonferroni test. We also found significant differences in  $\delta^{13}\text{C}$  among demographic groups ( $F_{2,112}=4.82, p=0.010$ ), where adult males presented higher values compared to juveniles in rural environments, but both were similar to females (Table 1). There was interaction between the

**Table 3.** Relative degree of niche overlap among demographic groups in the eastern long-necked turtle (*Chelodina longicollis*) by corrected standard ellipse from different habitats in the Australian Capital Territory, Australia. Values are the percent of individuals in each group (rows) that are encompassed by the convex hull of demographic comparison (columns) for each area.

Metric, site		Juvenile	Male	Female
Niche overlap (% individuals)				
Natural	Juvenile	-	80.62	38.82
	Male	60.78	-	49.67
	Female	31.26	53.06	-
Rural	Juvenile	-	18.98	31.51
	Male	48.42	-	19.24
	Female	83.64	20.02	-
Suburb	Juvenile	-	60.55	34.42
	Male	48.2	-	36.72
	Female	53.34	71.48	-



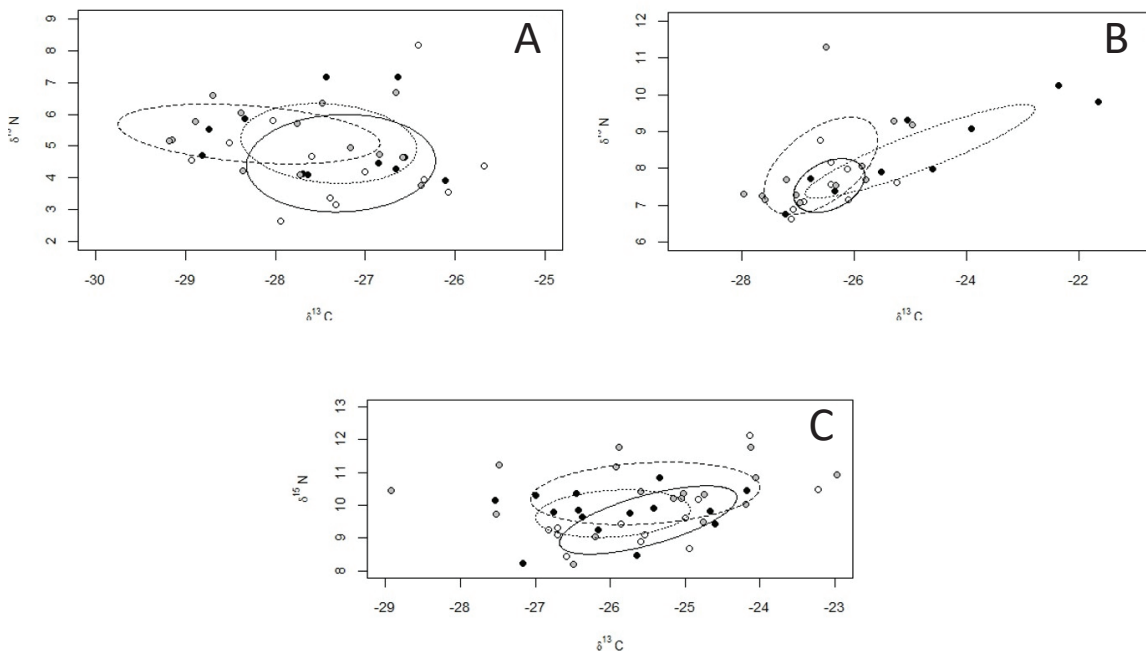
**Fig. 2.** Estimates of Bayesian standard ellipse areas (SEAB) in juvenile, adult male and adult female eastern long-necked turtles (*Chelodina longicollis*) in nature reserve (A), rural (B) and suburban (C) habitats in the Australian Capital Territory, Australia. Black dots correspond to the mean SEAB for each group, and shaded boxes representing the 50%, 75% and 95% credible intervals from dark to light grey.

study sites and demographic groups ( $F_{2,112}=3.4$ ,  $p=0.012$ ) and no relationship between  $\delta^{13}\text{C}$  and carapace length ( $p=0.119$ ,  $r=0.67$ ).

Niche widths using SEAB were similar for all demographic groups in each habitat type, except in the rural area where juveniles showed smaller niche width than males (Fig. 2; Table 2). Demographic groups showed less variability in the degree of niche overlap in the nature reserve and suburban area, while niche overlap was more variable in the rural area, with juveniles and females encompassing a smaller degree of males' niche (Fig. 3; Table 3).

## DISCUSSION

The present results suggest that *C. longicollis* had distinctive isotopic composition within the different environments. Considering intraspecific variation among demographic groups, our results demonstrate that within each habitat, juveniles, adult males and adult females seem to feed on the same trophic level, as differences in  $\delta^{15}\text{N}$ , an indicator of trophic positions, were not superior to the threshold limit of 3.4‰ (DeNiro & Epstein, 1981).



**Fig. 3.** Isotopic niche (corrected standard ellipses) use of eastern long-necked turtle (*Chelodina longicollis*) in a nature reserve (A), rural (B), and suburban (C) habitats in the Australian Capital Territory, Australia (Juveniles: white dots with tick line; adult males: black dots with smaller dashed lines; adult females: grey dots with larger dashed lines).

The technique represents another perspective on feeding ecology studies in chelonians, as the isotopes of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  reflect the diet over long periods in turtles, contrary to the more common techniques such as stomach flushing that represent a snapshot of recently consumed items (Seminoff et al., 2007).

Variation in  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values in the tissue of *C. longicollis* inhabiting natural, rural and suburban sites could reflect local dietary variation among sites. *Chelodina longicollis* is a carnivorous and opportunistic species (Chessman, 1984; Georges et al., 1986). Nature reserve turtles could be feeding primarily on macroinvertebrates, which is the bulk of their diet in natural settings (Chessman, 1984; Georges et al., 1986). In addition to macroinvertebrates, suburban turtles could also be scavenging on dead carp (*Cyprinus carpio*) or other non-native fish, which are abundant in the suburban lakes but not present in Mulligans Flat Nature Reserve (Lintermans, 2000), and such behaviour was observed by radio-tagged suburban turtles during a concurrent telemetry study (Ferronato et al., 2016). Consistent with this explanation, a long-necked turtle from South America, *Phrynops geoffroanus*, has a completely different diet in urban and natural habitats (Fachin-Teran et al., 1995; Souza & Abe, 2000; Ferronato et al., 2013).

Alternatively, isotopic variability in turtle tissues may reflect differences in the isotopic composition of prey tissues, rather than dietary differences among habitats (Hoeinghaus & Zeug, 2008). An enrichment in  $\delta^{15}\text{N}$  has been observed in painted turtles (*Chrysemys picta*) inhabiting rivers and ponds in an agricultural landscape compared to a pristine pond, which was caused by differences in baseline  $\delta^{15}\text{N}$  values among sites, and not due to differences in turtles' trophic position between habitats (Hofmeister et al., 2013). Besides statistical differences in  $\delta^{13}\text{C}$  values among sites in the present study, the differences were small and turtle tissues from all the study sites could reflect the isotopic composition of the aquatic macrophytes in their local habitat. Boon & Bunn (1994) and Deegan & Ganf (2008) reported  $\delta^{13}\text{C}$  values for aquatic macrophytes in Australia with a range of -24‰ to -30‰, which are within the range of our findings. Further analysis of turtle stomach contents, isotopic composition of prey, and external sources of nitrogen input in this system are in need to elucidate the cause of variation in  $\delta^{15}\text{N}$  in our study.

Chessman (1984) demonstrated that adult male and female *C. longicollis* overlap in their diet in the Murray River, with some degree in feeding habit differences between adults and juveniles. However, differences in diet between adult and juvenile turtles were minimal and limited by small sample sizes for turtles below 10 cm carapace length (Chessman, 1984). Our results demonstrate, through nitrogen assimilation, that males, females and juveniles overlap in their diet in all habitats studied. Such findings reiterate the generalist and opportunistic feeding behaviour of *C. longicollis* (Chessman, 1984; Georges et al., 1986), and help to cover the gap in the diet of small juveniles' *C. longicollis*. Although some studies have demonstrated

that hatchlings can reflect their maternal isotopic composition (Jenkins et al., 2001; Frankel et al., 2012), our investigation covered a wide range of juvenile sizes, which did not include hatchlings. In addition, turtles are likely feeding on the same trophic level within habitats. A shift in trophic level generally represents a mean value of 3.4‰ for  $\delta^{15}\text{N}$  (DeNiro & Epstein, 1981), but differences between  $\delta^{15}\text{N}$  for juveniles and adult females in the nature reserve, representing the widest range in  $\delta^{15}\text{N}$  among samples in our study, were approximately 1.3‰.

Considering our results of niche width and niche overlap in  $\delta^{13}\text{C}$ -  $\delta^{15}\text{N}$  space, we observed that turtles in natural, rural and suburban habitats showed a high degree of overlap among the demographic groups, except for juveniles and males in the rural site which showed low overlap and significant differences in niche width. The primary driver of this variation was  $\delta^{13}\text{C}$ , and not  $\delta^{15}\text{N}$  (see Results), which could be related to use of space and basal sources of energy than to trophic position in the food web (Newsome et al., 2007). These findings likely reflect similarity in the variety of resources consumed among individuals in each study site, as pointed out previously.

Although caution has been noted in interpreting  $\delta^{15}\text{N}$  results from turtles in an agricultural landscape without  $\delta^{15}\text{N}$  baseline values (Hofmeister et al., 2013), the ultimate reasons for differences in  $\delta^{15}\text{N}$  values in turtles along urban-natural habitats remains unclear in the present study. One possible cause of  $\delta^{15}\text{N}$  spatial variation among sites relates to nitrogen enrichment of prey (Hoeinghaus & Zeug, 2008), and ultimately turtles. In this scenario, isotopic enrichment in predator and prey tissues could be a result of variation in nutrient cycling in food webs, or differences in sources of input among study sites. Future research should focus on comparing denitrification rates among habitats (Hofmeister et al., 2013), nutrient cycling and food web dynamics (Jefferies, 2000; Polis et al., 1997), as well as sources of anthropogenic nitrogen output into the systems (Harrington et al., 1998; Groffman et al., 2004). An alternative explanation for the observed  $\delta^{15}\text{N}$  spatial variation relates to differences in feeding habits and food availability for turtles among sites. Future studies should include sampling more replicates within each habitat type and focus on using complementary techniques to evaluate turtle diet. Thus, stomach flushing and fecal analysis used together can increase our understanding of feeding habitats and intraspecific variation in turtles while quantifying resource availability and isotope composition of prey among study sites.

## ACKNOWLEDGEMENTS

We would like to thank Fiona Dyer for sharing her expertise in some topics in this study and Ross Thompson for reviewing this manuscript. Research was conducted in accordance with Animal Ethics guidelines and wildlife permits as they apply in the ACT. The study was funded by the Institute for Applied Ecology, the ACT Herpetological Association and Turtles Australia Inc. BOF was sponsored by an Endeavour International Postgraduate Research Scholarship and a W.J. Weeden Scholarship and the

Institute for Applied Ecology. TSM has a post-doctoral scholarship from FAPESP (Procs. No. 2013/11032-0). L.M.V., P.B.C. and L.A.M. hold CNPq Productivity Scholarships.

## REFERENCES

- Blair, R.B. & Launer, A.E. (1997). Butterfly diversity and human land use: species assemblage along an urban gradient. *Biological Conservation* 80, 113–125.
- Bolnick, D.I. (2001). Intraspecific competition favours niche width expansion in *Drosophila melanogaster*. *Nature* 410, 463–466.
- Boon, P.I. & Bunn, S.E. (1994). Variations in the stable isotope composition of aquatic plants and their implications for food web analysis. *Aquatic Botany* 48, 99–108.
- Bowen, G.J., Wassenaar, L.I. & Hobson, K.A. (2005). Global application of stable hydrogen and oxygen isotopes to wildlife forensics. *Oecologia* 143, 337–348.
- Chase, J.F. & Walsh, J.J. (2006). Urban effects on native avifauna: a review. *Landscape Urban Planning* 74, 46–49.
- Chessman, B.C. (1984). Food of the snake-necked turtle, *Chelodina longicollis* (Shaw) (Testudines : Chelidae) in the Murray Valley, Victoria and New South Wales. *Australian Wildlife Research* 11, 573–578.
- Deegan, B.M. & Ganf, G.G. (2008). The loss of aquatic and riparian plant communities: implications for their consumers in a riverine food web. *Austral Ecology* 33, 672–683.
- Deng, J.S., Wang, K., Hong, Y. & Qi, J.G. (2009). Spatio-temporal dynamics and evolution of land use change and landscape pattern in response to rapid urbanisation. *Landscape Urban Planning* 92, 187–198.
- DeNiro, M.J. & Epstein, S. (1981). Influence of the diet on the distribution of nitrogen isotopes in animals. *Geochimica et Cosmochimica Acta* 45, 341–351.
- Ethier, D.M., Kyle, C.J., Kyser, T.K. & Nocera, J.J. (2010). Variability in the growth patterns of the cornified claw sheath among vertebrates: implications for using biogeochemistry to study animal movement. *Canadian Journal of Zoology* 88, 1043–1051.
- Fachin-Teran, A., Vogt, R.C. & Gomez, M.F.S. (1995). Food habits of an assemblage of five species of turtles in the Rio Guapore, Rondonia, Brazil. *Journal of Herpetology* 29, 536–547.
- Ferronato, B.O., Marques, T.S., Souza, F.L., Verdade, L.M. & Matushima, E.R. (2009). Oral bacterial microbiota and traumatic injuries of free-ranging *Phrynops geoffroanus* (Testudines, Chelidae) in southeastern Brazil. *Phyllomedusa* 8, 19–25.
- Ferronato, B.O., Piña, C.I., Molina, F.C., Espinosa, R.A. & Morales, V.R. (2013). Feeding habits of Amazonian freshwater turtles (Podocnemididae and Chelidae) from Peru. *Chelonian Conservation and Biology* 12, 119–126.
- Ferronato, B.O., Roe, J.H. & Georges, A. (2016). Urban hazards: spatial ecology and survivorship of a turtle in an expanding suburban environment. *Urban Ecosystems* 19, 415–428.
- Frankel, N.S., Vander Zanden, H.B., Reich, K.J., Williams, K.L. & Bjorndal, K.A. (2012). Mother-offspring stable isotope discrimination in loggerhead sea turtles *Caretta caretta*. *Endangered Species Research* 17, 133–138.
- Fry, B. (2006). *Stable isotope ecology*. New York: Springer Science & Business Media.
- Georges, A. (1982). Diet of the Australian freshwater turtle *Emydura krefftii* (Chelonia: Chelidae), in an unproductive lentic environment. *Copeia* 1982, 331–336.
- Georges, A., Norris, R.H. & Wensing, L. (1986). Diet of the freshwater turtle *Chelodina longicollis* (Testudines: Chelidae) from the coastal dune lakes of the Jervis bay territory. *Australian Wildlife Research* 13, 301–308.
- Groffman, P.M., Law, N.L., Belt, K.T., Band, L.E. & Fisher, G.T. (2004). Nitrogen fluxes and retention in urban watershed ecosystems. *Ecosystems* 7, 393–403.
- Hamer, A.J. & McDonnell, M.J. (2008). Amphibian ecology and conservation in the urbanising world: a review. *Biological Conservation* 141, 2432–2449.
- Harrington, R.R., Kennedy, B.P., Chamberlain, C.P., Blum, J.D. & Folt, C.L. (1998).  $\delta^{15}\text{N}$  enrichment in agricultural catchments: field patterns and applications to tracking Atlantic salmon (*Salmo salar*). *Chemical Geology* 147, 281–294.
- Hoeinghaus, D.J. & Zeug, S.C. (2008). Can isotope ratios provide for community-wide measures of trophic structure? Comment. *Ecology* 89, 2353–2357.
- Hofmeister, N.R., Welk, M., Freedberg, S. (2013). Elevated levels of  $\delta^{15}\text{N}$  in riverine painted turtles (*Chrysemys picta*): trophic enrichment or anthropogenic output? *Canadian Journal of Zoology* 91, 899–905.
- Holway, D.A. & Suarez, A.V. (2006). Homogenisation of ant communities in Mediterranean California: the effects of urbanisation and invasion. *Biological Conservation* 127, 319–326.
- Jackson, A.L., Inger, R., Pamell, A.C. & Bearhop, S. (2011). Comparing isotopic niche widths among and within communities: SIBER—Stable Isotope Bayesian Ellipses in R. *Journal of Animal Ecology* 80, 595–602.
- Jefferies, R.L. (2000). Allochthonous inputs: integrating populations changes and food-web dynamics. *Trends in Ecology & Evolution* 15, 19–22.
- Jenkins, S.G., Partridge, S.T., Stephenson, T.R., Farley, S.D. & Robbins, C.T. (2001). Nitrogen and carbon isotope fractionation between mothers, neonates, and nursing offspring. *Oecologia* 129, 336–341.
- Kennett, R.M. & Georges, A. (1990). Habitat utilisation and its relation to growth and reproduction of the eastern long-necked turtle, *Chelodina longicollis* (Testudinata: Chelidae), from Australia. *Herpetologica* 46, 22–33.
- Kennett, R., Roe, J., Hodges, K. & Georges, A. (2009). *Chelodina longicollis* (Shaw 1794)—Eastern long-necked turtle, common long-necked turtle, common snake-necked-turtle. Conservation Biology of Freshwater turtles and Tortoises. *Chelonian Research Monographs* 5, 031.1–031.8.
- Layman, C.A., Quattrochi, J.P., Peyer, C.M. & Allgeier, J.E. (2007a). Niche width collapse in a resilient top predator following ecosystem fragmentation. *Ecology Letters* 10, 937–944.
- Layman, C.A., Arrington, D.A., Montaña, C.G. & Post, D.M. (2007b). Can stable isotope ratios provide for community-wide measures of trophic structures? *Ecology* 88, 42–48.
- Lintermans, M. (2000). The status of fish in the Australian Capital Territory: a review of current knowledge and management requirements. Technical Report 15, Environment ACT, Canberra. 112p.
- Luiselli, L. (2006). Food niche overlap between sympatric

- potential competitors increases with habitat alteration at different trophic levels in rain-forest reptiles (omnivorous tortoises and carnivorous vipers). *Journal of Tropical Ecology* 22, 695–704.
- Marques, T.S., Lara, N.R.F., Bassetti, L.A.B., Piña, C.I., Camargo, P.B. & Verdade, L.M. (2013). Intraspecific isotopic niche variation in broad-snouted caiman (*Caiman latirostris*). *Isotopes in Environmental and Health Studies* 49, 325–335.
- McKinney, M.L. & Lockwood, J.L. (1999). Biotic homogenisation: a few winners replacing many losers in the next mass extinction. *Trends in Ecology & Evolution* 14, 450–453.
- McKinney, M.L. (2002). Urbanisation, biodiversity and conservation. *BioScience* 52, 883–890.
- McKinney, M.L. (2006). Urbanisation as a major cause of biotic homogenisation. *Biological Conservation* 127, 247–260.
- McKinney, M.L. (2008). Effects of urbanisation on species richness: a review of plants and animals. *Urban Ecosystems* 11, 161–176.
- Newsome, S.D., del Rio, C.M., Bearhop, S. & Phillips, D.L. (2007). A niche for isotopic ecology. *Frontiers in Ecology and the Environment* 5, 429–436.
- Newsome, S.D., Ralls, K., Job, C.V.H., Fogel, M.L. & Cypher, B.L. (2010). Stable isotopes evaluate exploitation of anthropogenic foods by the endangered San Joaquin kit fox (*Vulpes macrotis mutica*). *Journal of Mammalogy* 91, 1313–1321.
- Ometto, J.P.H., Flanagan, L.B., Martinelli, L.A. & Ehleringer, J.R. (2005). Oxygen isotope ratios of waters and respired CO<sub>2</sub> in Amazonian forest and pasture ecosystem. *Ecological Applications* 15, 58–70.
- Parnell, A.C., Inger, R., Bearhop, S. & Jackson, A.L. (2010). Source partitioning using stable isotopes: coping with too much variation. *PLoS ONE* 5, e9672.
- Paulissen, M.A. (1987). Optimal foraging and intraspecific diet differences in the lizard *Cnemidophorus sexlineatus*. *Oecologia* 71, 439–446.
- Pearson, S.H., Avery, H.W., Kilham, S.S., Velinsky, D.J. & Spotila, J.R. (2013). Stable isotopes of C and N reveal habitat dependent dietary overlap between native and introduced turtles *Pseudemys rubriventris* and *Trachemys scripta*. *PLoS ONE* 8, e62891.
- Peterson, B.J. & Fry, B. (1987). Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics* 18, 293–320.
- Platt, S.G., Rainwater, T.R., Finger, A.G., Thorbjarnarson, J.B., et al. (2006). Food habits, ontogenetic dietary partitioning and observations of foraging behaviour of Morelet's crocodile (*Crocodylus moreletii*) in northern Belize. *The Herpetological Journal* 16, 281–290.
- Polis, G.A., Anderson, W.B. & Holt, R.D. (1997). Toward an integration of landscape and food web ecology: the dynamic of spatially subsidized food webs. *Annual Review of Ecology and Systematics* 28, 289–316.
- R Development Core Team. (2009). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna.
- Rees, M., Roe, J. H. & Georges, A. (2009). Life in the suburbs: behaviour and survival of a freshwater turtle in response to drought and urbanisation. *Biological Conservation* 142, 3172–3181.
- Roe, J.H., Gibson, J. & Kingsbury, B.A. (2006). Beyond the wetland border: estimating the impact of roads for two species of water snakes. *Biological Conservation* 130, 161–168.
- Roe, J. H., Rees M. & Georges, A. (2011). Suburbs: Dangers or drought refugia for freshwater turtle populations? *The Journal Wildlife Management* 75, 1544–1552.
- Schoener, T.W. (1968). The *Anolis* lizards of Bimini: resource partitioning in a complex fauna. *Ecology* 49, 704–726.
- Schoener, T.W. (1977). Competition and niche. In: *Biology of the Reptilia* (eds C. Gans & D.W.Tinkle) pp. 35–136. New York: Academy Press.
- Seminoff, J. A., Bjorndal, K. A., Bolten, A.B. (2007). Stable carbon and nitrogen isotope discrimination and turnover in pond sliders *Trachemys scripta*: insights for trophic study of freshwater turtles. *Copeia* 3, 534–542.
- Shochat, E., Warren, P.S., Stanley, H.F., McIntyre, N.E. & Hope, D. (2006). From patterns to emerging processes in mechanistic urban ecology. *Trends in Ecology & Evolution* 21, 186–191.
- Souza, F.L. & Abe, A.S. (1998). Resource partitioning by the Neotropical freshwater turtle, *Hydromedusa maximiliani*. *Journal of Herpetology* 32, 106–112.
- Souza, F.L. & Abe, A.S. (2000). Feeding ecology, density and biomass of the freshwater turtle, *Phrynops geoffroanus*, inhabiting a polluted urban river in south-eastern Brazilian. *Journal of Zoology* 252, 437–446.
- Tucker, A.D., Fitzsimmons, N.N. & Gibbons, J.W. (1995). Resource partitioning by the estuarine turtle *Malaclemys terrapin*: trophic, spatial, and temporal foraging constraints. *Herpetologica* 51, 167–181.
- Tucker, A.D., Limpus, C.J., McCallum, H.I. & McDonald, K.R. (1996). Ontogenetic dietary partitioning by *Crocodylus johnstoni* during the dry season. *Copeia* 1996, 978–988.
- Turner, T.F., Collyer, M.L. & Krabbenhoft, T.J. (2010). A general hypothesis-testing framework for stable isotopes ratios in ecological studies. *Ecology* 91, 2227–2233.
- Webster, R. & Butler, B.E. (1976). Soil classification and survey studies at Ginninderra. *Australian Journal of Soil Research* 14, 1–24.
- Zar, J.H. (1999). *Biostatistical analysis*. Upper Saddle River: Prentice-Hall Inc.

Accepted: 26 November 2015