Plasticity and specialisation in the isotopic niche of African clawless otters foraging in marine and freshwater habitats

Authors: Rowan K. Jordaan[1] (rowan.jordaan@zoology.up.ac.za)

Michael J. Somers[1, 2] (Michael.Somers@up.ac.za)

Grant Hall[1] (Grant.Hall@up.ac.za)

Trevor McIntyre[1, 3] (mcintt@unisa.ac.za)

Affiliations: [1]Mammal Research Institute, Department of Zoology and Entomology, University of Pretoria, Pretoria, South Africa

[2]Eugène Marais Chair of Wildlife Management, Centre for Invasion Biology, University of Pretoria, Pretoria, South Africa

[3]Department of Life and Consumer Sciences, University of South Africa, South Africa

Abstract

Individual-level behavioural plasticity resulting from differences in environmental conditions is prevalent in many organisms and may result in phenomena such as dietary- or habitat specialisation. The isotopic niche of African clawless otters, *Aonyx capensis*, occupying different habitats was investigated with the use of stable isotope techniques. Stable isotope analyses revealed that African clawless otter isotopic niche varied between, as well as within, individuals and varied when compared to conspecifics occupying different habitats. Some otters varied their isotopic niche and foraging areas temporally, whilst others did not. The isotopic niche of African clawless otters in a coastal habitat overlapped substantially with

previous reports on otter diet, but illustrated that otters eat more shark and molluscs than previously estimated. In freshwater habitats, not all otters had trout in their isotopic niche, although this prey item was abundantly available in the study area. Our results suggest that the African clawless otters can exhibit substantial behavioural plasticity. Such evident adaptability is likely to benefit otters and allow for extended use of non-pristine environments affected by human disturbance when sufficient quantities of prey remain available.

Keywords

Behavioural plasticity; Diet; Isotope analysis; Trout; Conflict; Africa

Acknowledgements

Funding was provided by the Department of Science and Technology, through the National Research Foundation (South Africa). Greg Hofmeyr and Gerry Camacho provided samples from southern coastline and the general Lydenburg area respectively. We are thankful to Millstream Farm, its Directors, shareholders and staff for permission to do the work on their property. Coastal fieldwork was aided by the Nature's Valley Trust and numerous assistants.

Introduction

Adaptive phenotypic plasticity occurs when a genotype varies its phenotype across varying environments to maintain a high performance across the entire environmental gradient (West-Eberhard, 2003; Bateson & Gluckman, 2011). Environmental conditions (e.g. rainfall, temperature, primary productivity and snow cover) and geographic positions (e.g. altitude, latitude) influence the development and expression of behaviour. As a result, behavioural plasticity (being inclusive of adaptive phenotypic plasticity) should be important in novel and variable environments (Snell-Rood, 2013). Differences in environmental and geographic conditions result in variations in food supply resulting in differences in diet, feeding habits and

body size between different populations of conspecific carnivores (Iriarte et al. 1990; Zhou et al. 2011; Bojarska & Selva 2012).

At an individual level, patterns of behavioural plasticity may differ between individuals within the same population, with differences arising as a result of past environmental conditions (i.e. experience), genotype and the interaction of these factors (Piersma & Drent, 2003; Nussey et al., 2007; Ghalambor et al., 2010). Dietary specialisation represents an adaptive response to intra-specific competition and a decrease in food resource availability (Glasser, 1982). When present, individual-level dietary specialisation can impact a species' population and community ecology (Bolnick et al., 2003; Estes et al., 2003; Newsome et al., 2009). Examples of aquatic mammal species displaying inter-individual variations in diet and dietary preferences include sea otters (*Enhydra lutris*) and killer whales (*Orcinus orca*) (Ford et al., 1998; Estes et al., 2003; Tinker et al., 2007; Tinker et al., 2008). In the case of killer whales, two distinct dietary specialisations (fish and marine mammals) occur in a sympatric population in the coastal waters around British Columbia and southeastern Alaska (Ford et al., 1998). Southern sea otter (*E. lutris nereis*) individuals follow one of three dietary specialisations varying in energy and rarity (Estes et al., 2003; Tinker et al., 2007). This high degree of individual specialisation in California sea otters likely reflects limited prey availability associated with recovering otter populations (Estes et al., 2003).

According to studies based primarily on scat (spraint) analysis, African clawless otters (*Aonyx capensis*) feed predominantly on fish, frogs and crabs and occasionally other taxa such as insects (Rowe-Rowe & Somers, 1998; Somers & Nel, 2003). In marine locations, crabs dominate the diet of this species with fish present in lower proportions (van der Zee, 1981; Somers, 2000; Emmerson & Phillip, 2004; Jordaan et al., 2015). This pattern is also observed in freshwater locations (Rowe-Rowe, 1977b; Kruuk & Goudswaard, 1990; Somers & Purves, 1996; Somers & Nel, 2003) which suggests a preference for benthic and/or slow-moving prey.

It is important to note that fish dominate the diet when crabs are unavailable (Watson & Lang, 2003). When present in freshwater habitats, invasive trout is mostly considered an unimportant prey species (Butler & du Toit, 1994; Rowe-Rowe, 1977b), although otters are persecuted for foraging on farmed trout (de Vos 2018). The important components in the diet of *A. capensis* vary between nearby marine locations (Emmerson & Philip, 2004), as well as between seasons at marine sites (Somers, 2000) and inland river systems (Rowe-Rowe, 1977b). However, long-term population-level dietary stability is evident in the diet of coastal populations (Jordaan et al. 2015). *Aonyx capensis* can be found in a large range of habitats and landscapes that range from semi-deserts to evergreen forests, pristine to polluted and from sea level to areas more than 3000 metres above sea level (Rowe-Rowe, 1991; Rowe-Rowe & Somers, 1998; Ponsonby & Schwaibold, 2018). Little is known about their population ecology, their role in the local species communities or if individual dietary specialisation is present in this species.

Predator diets can be inferred in various ways including through fatty acid signature analysis (e.g. Iverson et al., 1997; Walton et al., 2000; Hooker et al., 2001), gut content analysis (e.g. Norbury & Sanson, 1992; Ford et al., 1998), direct observations (e.g. Ford et al., 1998; Somers, 2000), and by bio-logging approaches (e.g. Tinker et al., 2007). The use of scat analysis is also common (Angerbjörn et al., 1994; Gorgadze, 2013; Day et al., 2015) and has often been used for dietary studies on African clawless otters (e.g. Rowe-Rowe, 1977a; Butler & Du Toit, 1994; Somers, 2000; Watson & Lang, 2003; Emmerson & Phillip, 2004). However, spraint analysis does not indicate the importance of prey items based on their biomass or energy consumed (Somers, 2000), and tends to underestimate prey taken in large amounts (Englund, 1965; Jenkins et al., 1979; Carss & Parkinson, 1996) Spraint analysis also favours prey species that are made up of large amounts of indigestible material, while underrepresenting prey made up mostly of soft tissue (Somers, 2000; Emmerson & Philip, 2004). Despite these

shortcomings, when interpreted with suitable caution, scat analysis remains a comparatively cost effective, simple and important means of determining carnivore diets (Klare et al., 2011).

Many of the limitations associated with spraint analysis can be overcome with the use of techniques such as stable isotope analysis (SIA). This method has been used to investigate numerous diets and isotopic niches including those of southern sea otter, *E. lutris` nereis*, and recently Neotropical otter, *Lontra longicaudis* (Angerbjörn et al., 1994; Grey et al., 2001; Newsome et al., 2009; Carrasco et al., 2019). Various animal tissues are used in SIA including red blood cells and blood plasma (Hilderbrand et al., 1996), bone collagen (Angerbjörn et al., 1994), muscle tissue (Grey et al., 2001), teeth (Carrasco et al., 2019) and vibrissae (Lübcker et al., 2016; Lerner et al., 2018). Vibrissae are potentially the tissue with the most benefits as they are comparatively non-invasive to sample and provide multiple data points providing a temporal component due to assimilation of nutrients and slow growth rate of vibrissae (Lübcker et al., 2016). These results suggest that this is a suitable method of diet and isotopic niche analysis.

We used SIA on sequentially sectioned vibrissae to determine the isotopic niche, as well as inter-individual and temporal variation in the isotopic niche of African clawless otters occupying coastal and freshwater habitats. We hypothesised that the isotopic niche would vary between individuals occupying different sites (Rowe-Rowe, 1977b; Somers, 2000; Emmerson & Philip, 2004) but would not vary temporally or between individuals within sites (Jordaan et al., 2015).

Methods

Study Sites

Southern coast (coastal habitat)

This study area is situated along the Indian Ocean between Keurboomstand (34.0131° S, 23.4151° E) and Port Elizabeth (33.9608° S, 25.6022° E) in the Western Cape and Eastern Cape provinces respectively (Fig. 1). The area has a mild climate and receives an average rainfall of 900mm at the coast, which increases to 1100mm on top of the escarpment and continues to increase to the Tsitsikamma Mountains behind the plateau (Arden-Clarke, 1986). Vegetation in the area is mostly dry Afromontane forest, dry scrub forest and mountain fynbos, where not disturbed by human settlements (Hanekom et al., 1989). The mean annual ocean temperature is 16.9ºC (Schumann et al., 1995). The majority of the coastline is made up of rocky shores with small stretches of sandy beaches and the population densities of invertebrates are high (van der Zee, 1979; Arden-Clarke, 1986).

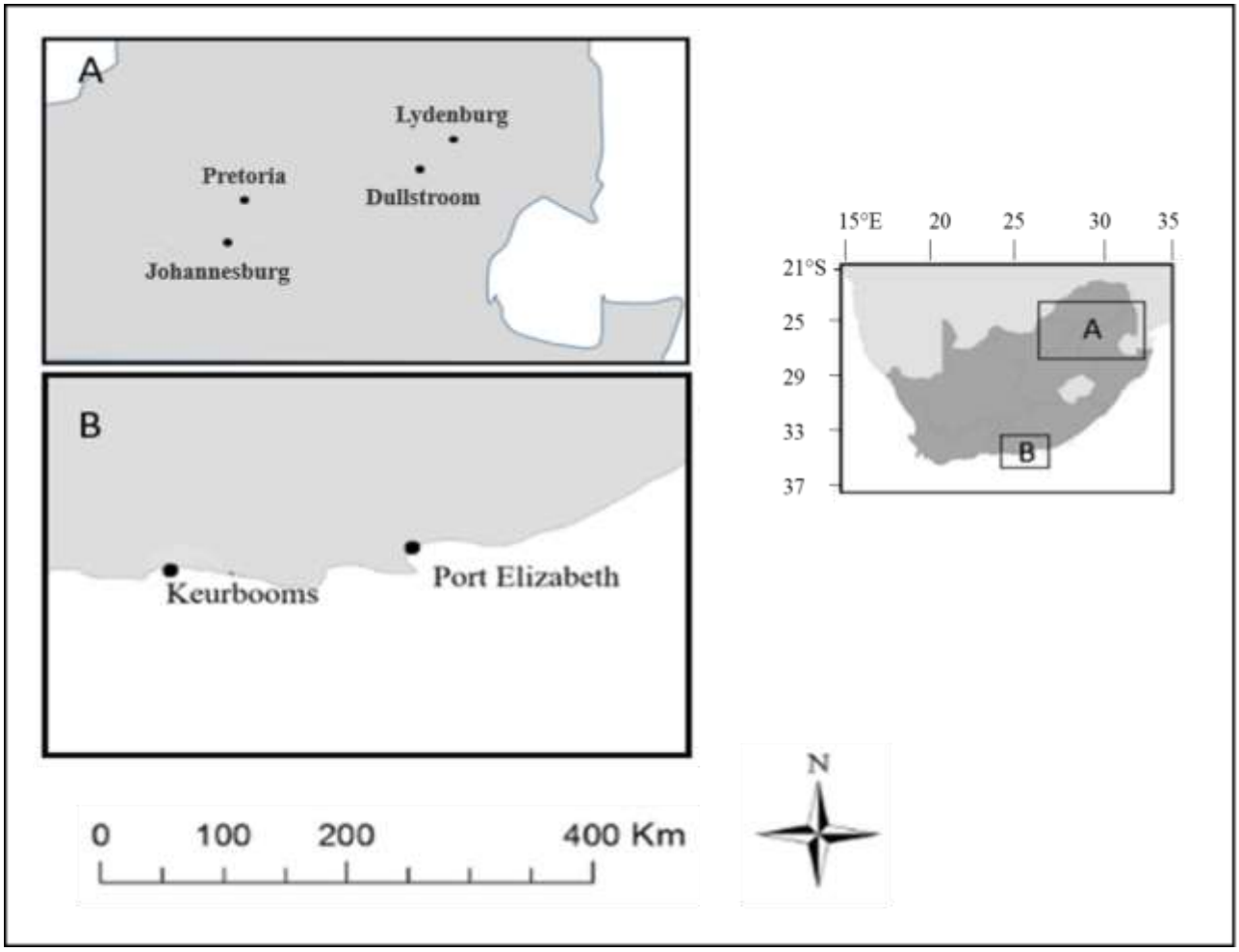

Figure 1. Freshwater and coastal study areas located in the Mpumalanga province (A) and on the southern coastline (B) of southern Africa respectively. The insert represents the location of the study areas in relation to the rest of South Africa (dark shading) and southern Africa.

Highlands Meander (freshwater habitat)

The Highlands Meander region encompasses the five towns of Belfast, Dullstroom, Machadodorp, Lydenburg and Waterval-Bovan (Fig. 1). The area has a warm, temperate climate with an average temperature of 13.1°C and mean annual rainfall of 894mm. This area comprises numerous rivers that have been dammed at intervals to provide year-round recreational fishing. This area has the largest concentration of stocked trout dams and rivers in South Africa (approximately 1200) (KPMG, 1999). Interactions between owners/anglers and

trout predators (incl. otters) are evident as measures have been put in place to reduce trout predation (Jordaan pers. obs.).

Millstream Farm (25.4520° S, 30.0919° E) is a fly-fishing farm near Dullstroom and consists of a series of 13 weirs (~2m deep) along the Witpoort River and eight dams (~6m deep) which are runoff filled. The river, as well as any overflow from the dams, flows into a large lake (~12m deep). The lake, dams and weirs are surrounded by natural vegetation including large reed beds (*Phragmites spp.*). The river is continuously polluted by upstream sewage works and is known to contain high levels of *Escherichia coli* bacteria while dams are fed by ground- and rainwater and have lower levels of *E. coli*. (Millstream staff pers. Comm.). Holidaymakers frequent the farm with the main activity being fly-fishing. The dams and weirs are stocked year-round with rainbow trout, *Oncorhynchus mykiss*, of varying weights with the smallest stocking weight being 300g.

Sampling Procedure

Vibrissae samples were collected opportunistically from different sources and from various sites, while a few samples were obtained through the capture and chemical restraint of otters. The longest vibrissa from each animal was sampled, with this typically being located furthest from the nose. A single vibrissa was sampled from each of five otters live-trapped at Millstream Farm and one otter live-trapped at Keurboomstrand. Otters were caught using trap-door carnivore cage traps (1.5m x 0.7m x 0.8m), baited with fresh spraints and trout (Millstream Farm) and spraint only (Keurboomstrand). Traps were set overnight and checked at sunrise. Otters captured at Millstream Farm were transported in the trap to a nearby veterinary facility (Sterkspruit Veterinary clinic) and were chemically immobilised upon arrival by the veterinarian in attendance. Immobilisation was achieved using a pre-calculated, weight dependant, dosage of medetomidine and ketamine administered via a dart syringe fired through

a mechanically pressurised blowgun. The single otter captured at Keurboomstrand was immobilised in the trap by an on-site veterinarian. Once immobilised, a single vibrissa was cut as close to the skin as possible. Atipamizole was administered to reverse the effects of the medetomidine, and otters were provided with water and appropriate substrate material and allowed to recover inside the trap. Once recovered, otters were released at the site of capture around sunset.

Vibrissae samples from otters in coastal habitats were obtained from four otter carcasses recovered by staff at Bayworld (Port Elizabeth Museum) in Port Elizabeth as part of their marine mammal stranding responses. Three samples from otters in the greater Highlands Meander were obtained from local conservation authorities (Mpumalanga Tourism and Parks Authority) – these having been collected opportunistically from otters trapped on nearby farms. Vibrissae were collected between August 2012 and March 2015 (coastal individuals) and between April 2015 and May 2016 (freshwater individuals). All samples are summarised in Table 1. All field research was done with permission from the University of Pretoria Animal Ethics Committee (project number: EC034-15).

At both freshwater and coastal sites, potential dietary items were sampled and frozen. Sampling was done both opportunistically and during dedicated searches. Opportunistic sampling included obtaining the remains of prey that an otter was feeding on, from fisherman's bycatch and from dead prey washed up on the beach. Dedicated searches during March 2015 in the coastal habitat involved snorkelling in rock pools and capturing prey items that have previously been recorded in otter diet. Prey items in the freshwater habitat were collected between April 2015 and May 2016 and were captured using different techniques, including, but not exclusively, angling (fly fishing) for trout capture, rock flipping for crab and frog capture and stick baiting for crab capture. In total, 74 dietary samples from 11 taxa and 54 samples from four taxa were collected from the coastal and freshwater habitat respectively.

Table 1. Overall vibrissae length, mean δ 13C, δ 15N and C/N ratios and sex of African clawless otters (SE in parenthesis). Sample size (n) refers to the number of segments that were sampled from each vibrissa. PE = Port Elizabeth. All otters sampled were adults.

Individual	*n*	Length (cm)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	C/N	Sex	Location
Coastal							
OT1	24	6.3	-12.84 (0.04)	14.42 (0.04)	3.73 (0.01)	M	Keurboomstrand
OT2	8	3.5	-15.46 (0.09)	7.89 (0.09)	3.64 (0.03)	M	PE
OT3	24	10.8	-15.30 (0.30)	16.08 (0.16)	3.69 (0.01)	M	PE
OT4	18	5.6	-14.59 (0.40)	16.71 (0.22)	3.65 (0.01)	M	PE
OT5	29	11.2	-13.57 (0.03)	14.60 (0.05)	3.72 (0.01)	F	PE
Mean (SE)	20.60	7.48	-14.35 (1.13)	13.94 (3.52)	3.69 (0.04)		
Freshwater							
OT6	16	6	-16.11 (0.12)	18.62 (0.29)	3.66 (0.01)	F	Millstream Farm
OT7	23	7.3	-17.10 (0.13)	13.38 (0.36)	3.60 (0.01)	M	Millstream Farm
OT8	27	5.3	-17.57 (0.15)	10.33 (0.07)	3.68 (0.01)	M	Lydenburg
OT9	20	6.9	-18.55 (0.14)	10.34 (0.07)	3.74 (0.01)	M	Lydenburg
OT10	19	6.2	-15.71 (0.07)	14.59 (0.33)	3.73 (0.01)	M	Millstream Farm
OT11	25	7.5	-18.21 (0.15)	9.80 (0.06)	3.73 (0.01)	M	Millstream Farm
OT12	16	5.9	-16.42 (0.09)	12.27 (0.25)	3.65 (0.01)	M	Millstream Farm
Mean (SE)	20.63	6.49	-16.58 (1.77)	12.97 (2.96)	3.69 (0.05)		

The details of the prey species collected are summarised in Table 2. Crabs collected at Millstream Farm were separated into two groups namely "crab1", originating from waters connected to the river system (i.e. weirs); and "crab2" originating from waters separated from the river system (i.e. dams). Crabs were separated into these two sub-populations per source, since pollution levels are known to differ between the two systems (Millstream management team pers. comm.). The differences in pollution levels have resulted in notable variation in the $\delta^{13}C$ and $\delta^{15}N$ ratios of these crabs due to the effect pollution has on basal isotopic ratios (Table 2). The remaining prey items did not show any variation between the two systems and were therefore not separated in the analyses.

Isotope analysis

All samples were processed and analysed for $\delta^{13}C$ and $\delta^{15}N$ at the Stable Isotope Laboratory located in the Mammal Research Institute, University of Pretoria, South Africa. Vibrissae were rinsed with a 2:1 chloroform: methanol solution to remove any surface contaminants (as described by Newsome et al., 2009), placed in an Ultrasonic Bath (Branson 1200, Branson Ultrasonics, Danbury, U.S.A.) for 5 min before being dried at 70^{o}C for 48 hours. To obtain sequential isotopic ratios, cleaned vibrissae were subsampled, with the use of a scalpel, into approximately 0.4 mg segments and sealed into toluene cleaned tin capsules for analysis.

Prey items were prepared for analysis by removing their soft and edible muscle tissue and drying this in an oven (Labotec Oven Dryer Model 323, Labotec, Midrand, South Africa). Once dried, this tissue was homogenised with a mortar and pestle. Lipids in the homogenised samples were removed by rinsing the samples in a 2:1 chloroform: methanol solution. Lipids were removed since they are depleted in $\delta^{13}C$ when compared to other compounds and therefore yield inaccurate results (O'Leary, 1981). Approximately 0.5 mg of rinsed sample was

Table 2. Mean δ 13C, δ 15N and C/N ratio values of likely prey species collected in both freshwater and coastal habitats (SE in parenthesis). Some groups comprise of several species. Additional parentheses indicate the location prey was collected (R = River and D = Dams).

Group name	Common name	Species name	*n*	δ^{13}C (‰)	δ^{15}N (‰)	C/N
Coastal						
Mussel	Brown mussel	*Perna perna*	46	-15.93 (0.11)	7.95 (0.13)	4.65 (0.05)
	Mediterranean mussel	*Mytilus galloprovincialis*				
	Black mussel	*Choromytilus meridionalis*				
Crab	Shore crab	*Cyclograpsus punctatus*	4	-14.69 (0.76)	12.24 (1.41)	4.97 (0.77)
Squid	Cuttlefish	*Sepia spp.*	6	-15.75 (0.24)	13.40 (0.40)	3.84 (0.10)
	Root-mounted jellyfish	*Eupilema inexpectata*				
	Common octopus	*Octopus vulgaris*				
Fish	Rock Sucker	*Chorisochismus dentex*	7	-15.45 (0.23)	12.88 (0.29)	4.17 (0.09)
	Cape Salmon/Geelbek	*Atractoscion aequidens*				
Shark	Leopard catshark	*Poroderma pantherinum*	2	-14.42 (0.11)	13.96 (0.82)	3.78 (0.04)
	Striped catshark	*Poroderma africanum*				
Freshwater						
Crab1 (R)	River Crab	*Potamonautes spp.*	11	-17.67 (0.22)	21.28 (0.98)	4.02 (0.04)
Crab2 (D)	River Crab	*Potamonautes spp.*	10	-20.84 (0.47)	7.23 (0.36)	4.28 (0.09)
Trout	Rainbow Trout	*Oncorhynchus mykiss*	24	-17.56 (0.19)	7.05 (0.16)	4.11 (0.06)
Frog	Common River Frog	*Amietia angolensis*	3	-20.51 (1.13)	5.50 (0.83)	4.29 (0.04)
Fish	Various	*Barbus spp.*	6	-15.32 (0.75)	18.90 (0.15)	4.10 (0.03)

weighed into tin capsules. The samples were combusted at 1020°C in an elemental analyser (Flash EA, 1112 Series, Thermo™, Thermo Fisher Scientific) and $\delta^{13}C$ and $\delta^{15}N$ isotope values were determined with the use of a continuous-flow isotope ratio mass spectrometer (Delta V Plus, Thermo Finnigan). Results are presented using standard delta notation in parts per thousand (‰) relative to an international standard: Vienna Pee Dee Belemnite (VPDB) for $\delta^{13}C$ and atmospheric N_2 (Air) for $\delta^{15}N$ (Coplen, 1994).

Data analyses

To infer the isotopic niche of African clawless otters, SIMMR (Stable Isotope Mixing Model in R), a stable isotope mixing model (Parnell, 2016) was used in the R environment (R Core Team, 2018). SIMMR uses a Bayesian framework and discrimination factors (the isotopic ratios change as they are incorporated from prey to consumers' tissue) to solve mixing models for stable isotope data. Vibrissae trophic discrimination factors for African clawless otters have not been determined, so 'standard' discrimination factors commonly used for carnivores and southern sea otters were used instead (Newsome et al., 2009; Newsome et al., 2010). The trophic discrimination factors used were 2.2‰ for carbon and 3.5‰ for nitrogen, along with standard deviations of 0.7‰ and 0.6‰ for carbon and nitrogen respectively (Newsome et al., 2010). The mean $\delta^{13}C$, $\delta^{15}N$ and C/N ratios for all vibrissae and potential prey samples collected are summarised in Tables 1 and 2. Isotopic space plots and box plots were produced in SIMMR. Isotopic space plots display the input data on a bivariate plot where the isotopic space is occupied by potential prey species displayed as coloured cross-hairs. Otter isotopic values are displayed within this space as various symbols. Boxplots depict the relative proportions of isotope niche that are made up by the various prey items. Box lines represent the 25, 50, 75 and confidence intervals.

Prey groups were grouped for analyses due to the similarity of taxa, as well as their economic importance (i.e. trout), as well as minimal variation in carbon and nitrogen isotopic values. More detail on these groups and the prey species within them are reported in Table 2. Time series line-graphs were drawn up in Microsoft Excel (2013) where both the carbon and nitrogen isotope values of each vibrissa were plotted. The tip of the vibrissae represents the oldest growth while the base represents the more recent growth.

Results

Coastal individuals

Sequential plotting of $\delta^{13}C$ and $\delta^{15}N$ values for each segmented vibrissa shows variation between individuals as well as variation within individuals (Fig. 2). Vibrissae $\delta^{13}C$ and $\delta^{15}N$ values were similar in OT1 ($\delta^{13}C$: -12.84‰ and $\delta^{15}N$: 14.42‰) and OT5 ($\delta^{13}C$: -13.57‰ and $\delta^{15}N$: 14.60‰), showing minimal variation along the length of the vibrissae (Table 1). Similarly, vibrissae $\delta^{13}C$ and $\delta^{15}N$ values for OT2 do not vary considerably over the length of the vibrissae and are lower than those of OT1 and OT5. The results for OT3 and OT4 show considerable variation in vibrissae $\delta^{13}C$ and $\delta^{15}N$ values over the length of the vibrissae.

Moving from the tip to the base of the vibrissa for OT3, the general trend of $\delta^{13}C$ ratios was to decrease (increase in negativity) while $\delta^{15}N$ ratios increased. The highest $\delta^{13}C$ and $\delta^{15}N$ values measured were -13.05‰ and 17.61‰ while the lowest were -18.26‰ and 14.76‰ respectively. When assessing OT4, there is no general trend when moving from the tip to the base. The highest and lowest $\delta^{13}C$ values measured were -12.44‰ and -18.17‰ respectively. $\delta^{15}N$ values were also extremely variable along the vibrissae and showed no clear pattern.

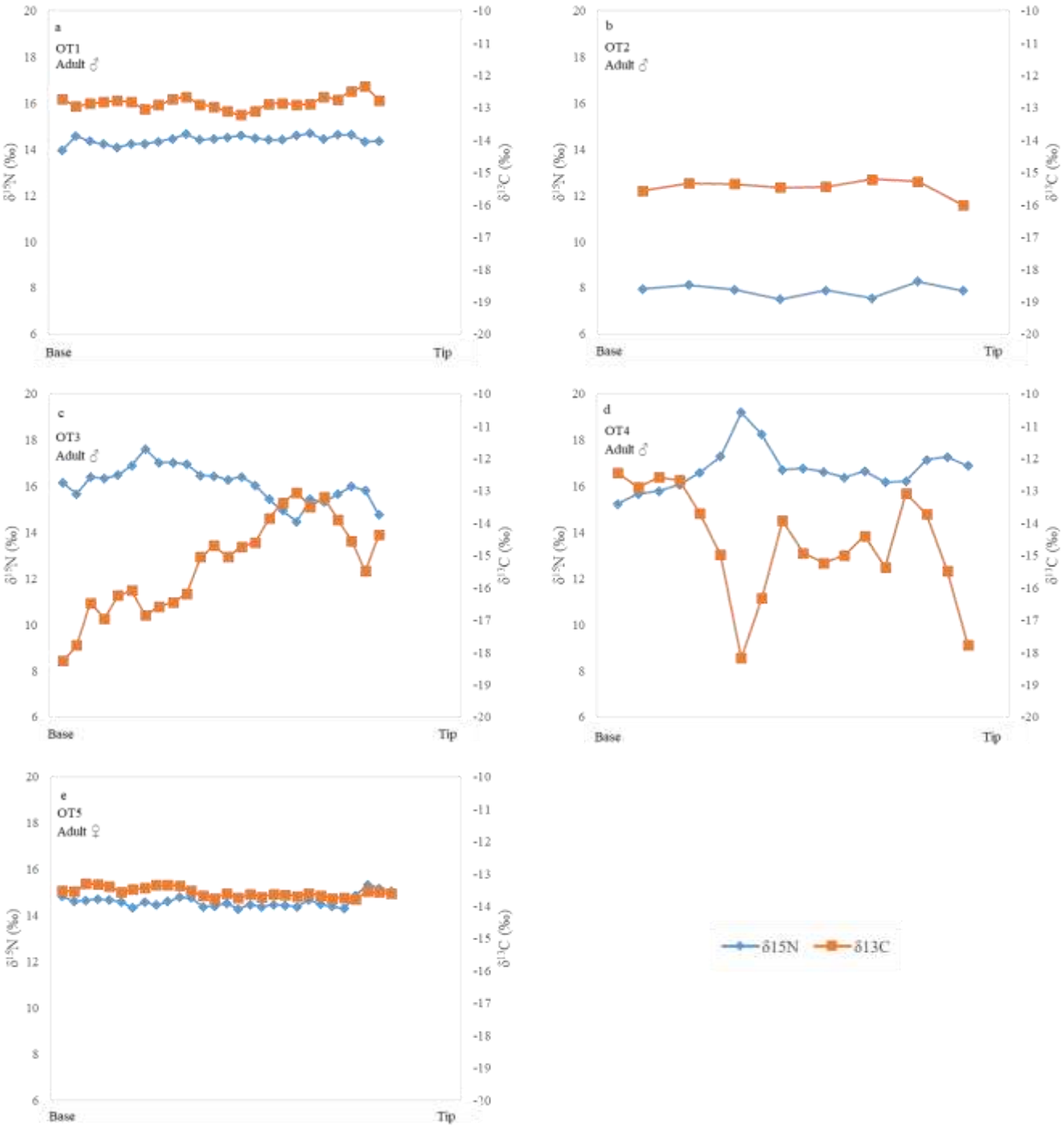

Figure 2. The δ13C and δ15N values in sequential vibrissae segments obtained for five African clawless otters from coastal areas. Otters reflected here are OT1-OT5 (a-e respectively), and additional information such as sex and age class are displayed in each plot. Base and tip denote the orientation of the vibrissae with the tip representing the oldest growth and the base the most recent. Information regarding the length (cm), the number of samples, mean isotopic values, with SE, can be found in Table 1 while the origin of each is discussed in the text.

The bivariate plot shows all isotopic values obtained from the segmented vibrissae from OT1-OT5 and potential coastal prey items (Fig. 3). All isotopic values for OT1 and OT5 are clustered closely together and fall within the isotopic space created by the prey items while none of the isotopic values from OT2 fall within the same space, although they are closely clustered to each other. The majority, but not all isotopic values from OT3 and OT4 fall within this same space, these values are, however, not as closely clustered and are more scattered when compared to OT1, OT2 and OT5.

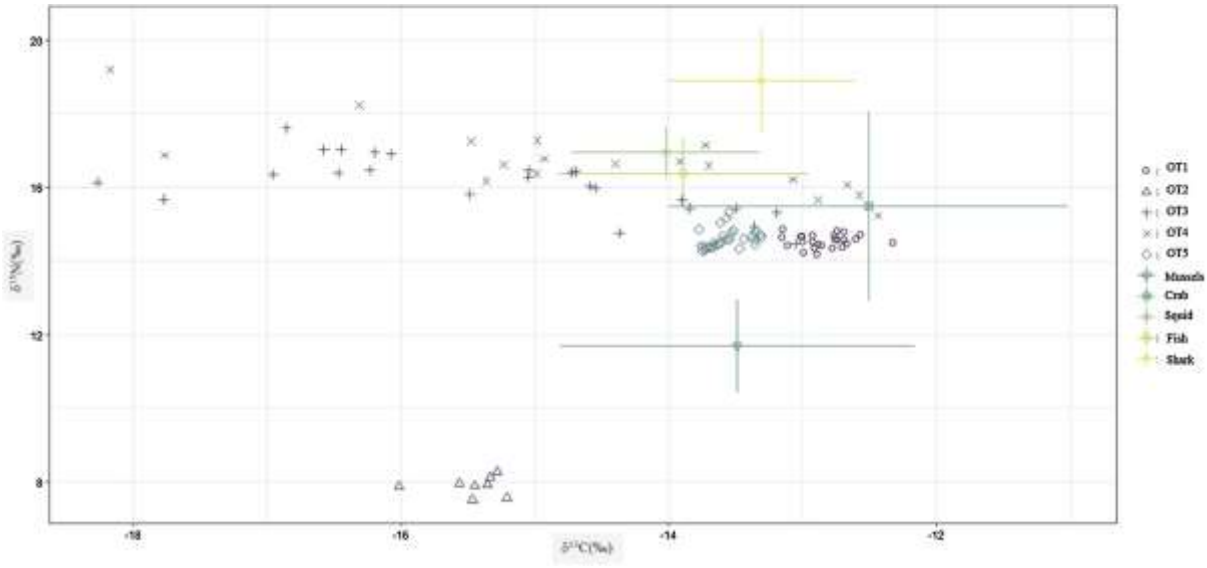

Figure 3. Bivariate isotopic space of δ13C and δ15N showing five African clawless otters and their potential prey species in a marine environment. The isotopic space occupied by potential prey species is displayed as coloured cross-hairs. Otter isotopic values are displayed as various symbols and were obtained from sequential analysis of vibrissae (hence the occurrence of replicas of the same symbol). Details regarding the colour and symbol allocated to each prey species or otter can be found in the key.

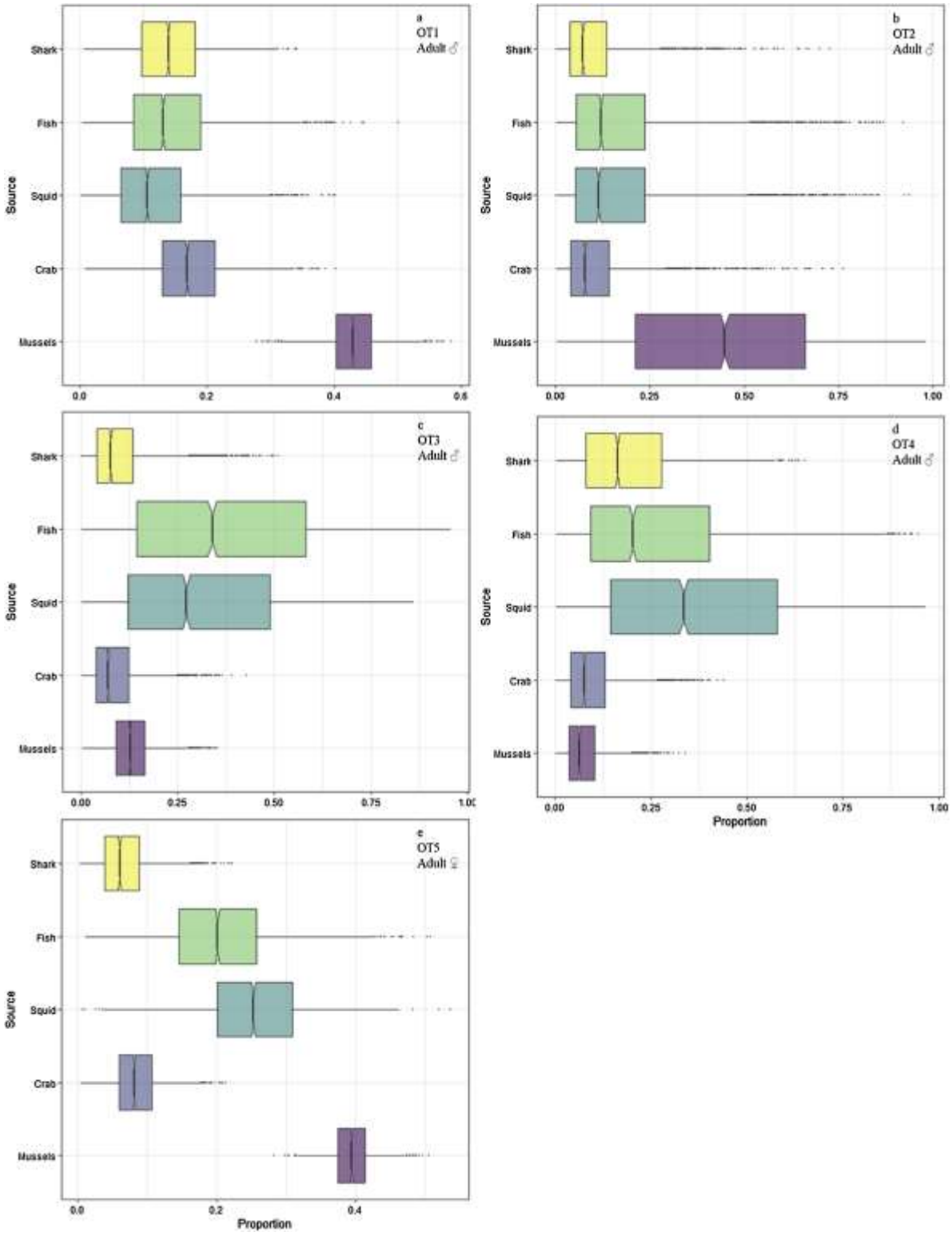

Figure 4. The proposed dietary contribution of potential prey species for five coastal African clawless otters. Otters reflected here are OT1-OT5 (a-e respectively), and additional information such as sex and age class is displayed in each plot. Proportions were obtained through the analysis of otter and potential prey isotopic values in SIMMR. Box lines represent the 25, 50, 75 and confidence intervals.

Figure 4 displays modelled proportions of prey consumed by each otter. SIMMR analyses produced mean values for prey and suggests that mussels ($\bar{x}$ = 0.43 ± 0.04) dominated the isotopic niche of OT1, followed by crab ($\bar{x}$ = 0.17 ± 0.06), shark ($\bar{x}$ = 0.14 ± 0.06) and fish ($\bar{x}$ = 0.14 ± 0.08). The mean isotopic niche proportions of OT2 are dominated by mussels ($\bar{x}$ = 0.44 ± 0.26), fish ($\bar{x}$ = 0.18 ± 0.16) and squid ($\bar{x}$ = 0.17 ± 0.16). The mean proportions of squid ($\bar{x}$ = 0.31 ± 0.21), fish ($\bar{x}$ = 0.37 ± 0.25) and mussels ($\bar{x}$ = 0.13 ± 0.06) make up the isotopic niche of OT3. Squid ($\bar{x}$ = 0.37 ± 0.25), fish ($\bar{x}$ = 0.27 ± 0.21) and shark ($\bar{x}$ = 0.19 ± 0.14) are the mean proportions that are suggested to make up the isotopic niche of OT4. Finally, the isotopic niche of OT5 is suggested to be made up mostly by mean proportions of mussels ($\bar{x}$ = 0.39 ± 0.03), followed by squid ($\bar{x}$ = 0.25 ± 0.08) and fish ($\bar{x}$ = 0.20± 0.08).

Freshwater individuals

As was the case for the coastal population, the sequential plotting of $\delta^{13}C$ and $\delta^{15}N$ values for each segmented vibrissae shows variation between individuals as well as variation within individual otters' samples in freshwater areas (Fig. 5). Otters OT8 ($\delta^{13}C$: -17.57‰ and $\delta^{15}N$: 10.33‰), OT9 ($\delta^{13}C$: -18.55‰ and $\delta^{15}N$: 10.34‰) and OT11 ($\delta^{13}C$: -18.21‰ and $\delta^{15}N$: 9.80‰) have similar and stable mean $\delta^{13}C$ and $\delta^{15}N$ values that do not vary considerably over the length of the vibrissae (Table 1). The remaining otters (means in parenthesis) have $\delta^{13}C$ and $\delta^{15}N$ values that vary over the length of the vibrissae (Fig. 5), these otters are OT6 ($\delta^{13}C$: -16.11‰ and $\delta^{15}N$: 18.62‰), OT7 ($\delta^{13}C$: -17.10‰ and $\delta^{15}N$: 13.38‰), OT10 ($\delta^{13}C$: -15.71‰ and $\delta^{15}N$: 14.59‰) and OT12 ($\delta^{13}C$: -16.42‰ and $\delta^{15}N$: 12.27‰).

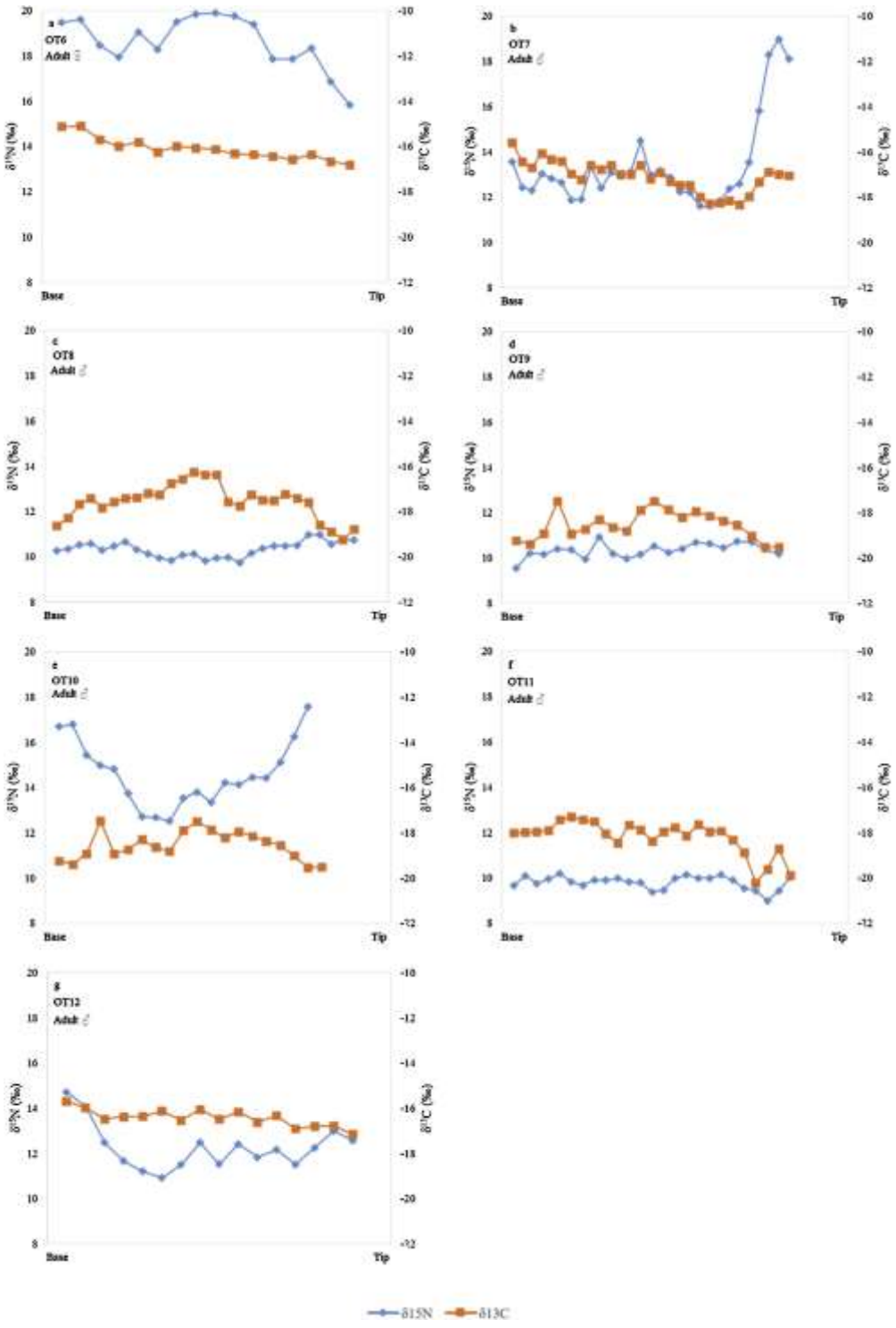

Figure 5. The δ13C and δ15N values in sequential vibrissae segments obtained for eight African clawless otters from freshwater areas. Otters reflected here are OT6-OT12 (a-g respectively), and additional information such as sex and age class is displayed in each plot. Base and tip denote the orientation of the vibrissae with the tip representing the oldest growth and the base the most recent. Information regarding the length (cm), the number of samples, mean isotopic values, with SD, can be found in Table 1 while the origin of each is discussed in the text.

While moving from the tip to the base of the vibrissae for the otters that have varying $\delta^{13}C$ and $\delta^{15}N$ values, there does not appear to be any general trends but rather periods where $\delta^{15}N$ was either high or low (Fig. 5). The highest values of $\delta^{15}N$ recorded for OT6, OT7, OT10 and OT12 were 19.89‰, 18.97‰, 17.56‰ and 14.71‰ respectively while their lowest $\delta^{15}N$ values were 15.82‰, 11.60‰, 12.51‰ and 10.92‰. Unlike the coastal population, $\delta^{13}C$ does not appear to be correlated with $\delta^{15}N$ with no general trends between these two ratios being observed. The mean $\delta^{15}N$ values (SE) for OT8, OT9 and OT11 were 14.45‰ (0.14), 10.33‰ (0.07), 10.34‰ (0.07) and 9.80‰ (0.06) respectively.

All the vibrissae isotopic values for OT8, OT9 and OT11 are closely clustered together while the isotopic values of OT6, OT7, OT10 and OT12 are more dispersed (Fig. 6). The highest mean proportions of trout are in the isotopic niche of OT10 ($\bar{x}$ = 0.43 ± 0.08) and OT12 ($\bar{x}$ = 0.45 ± 0.08) (Fig. 7). The highest proportions of crab from dams (Crab2) were found in the isotopic niche of OT7 ($\bar{x}$ = 0.34 ± 0.10), OT8 ($\bar{x}$ = 0.43 ± 0.08), OT9 ($\bar{x}$ = 0.59 ± 0.10) and OT11 ($\bar{x}$ = 0.46 ± 0.09), and the highest proportion of crab from the river (Crab1) was found in the isotopic niche of OT6 ($\bar{x}$ = 0.38 ± 0.07) (Fig. 7). Frog appears in the isotopic niche of all otters with mean proportions between 0.11 and 0.40. Fish features in the isotopic niche of some otters, namely OT6 ($\bar{x}$ = 0.24 ± 0.08), OT7 ($\bar{x}$ = 0.09 ± 0.05), OT10 ($\bar{x}$ = 0.16 ± 0.07) and OT12 ($\bar{x}$ = 0.08 ± 0.04), but is virtually absent from the isotopic niches of OT8, OT9 and OT11 with means less than 0.02 (Fig. 7).

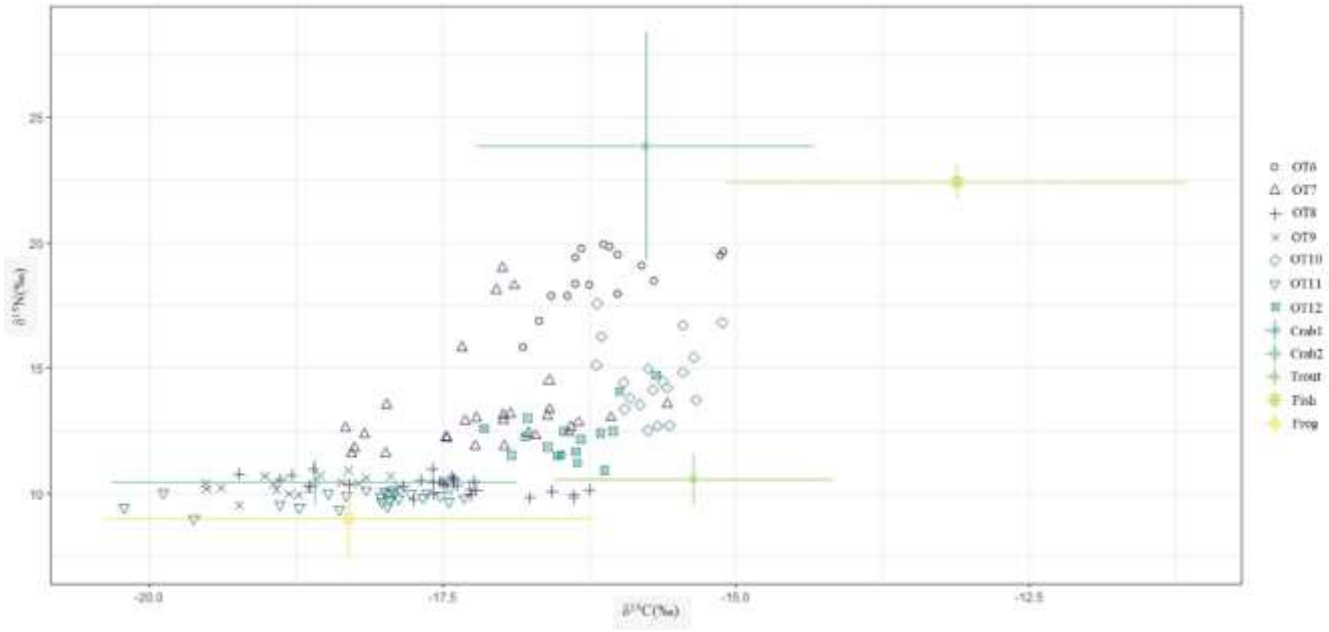

Figure 6. Bivariate isotopic space of δ13C and δ15N showing eight African clawless otters and their potential prey species in a freshwater environment. The isotopic space occupied by potential prey species is displayed as coloured cross-hairs. Otter isotopic values are displayed as various symbols and were obtained from sequential analysis of vibrissae (hence the occurrence of replicas of the same symbol). Details regarding the colour and symbol allocated to each prey species or otter can be found in the key.

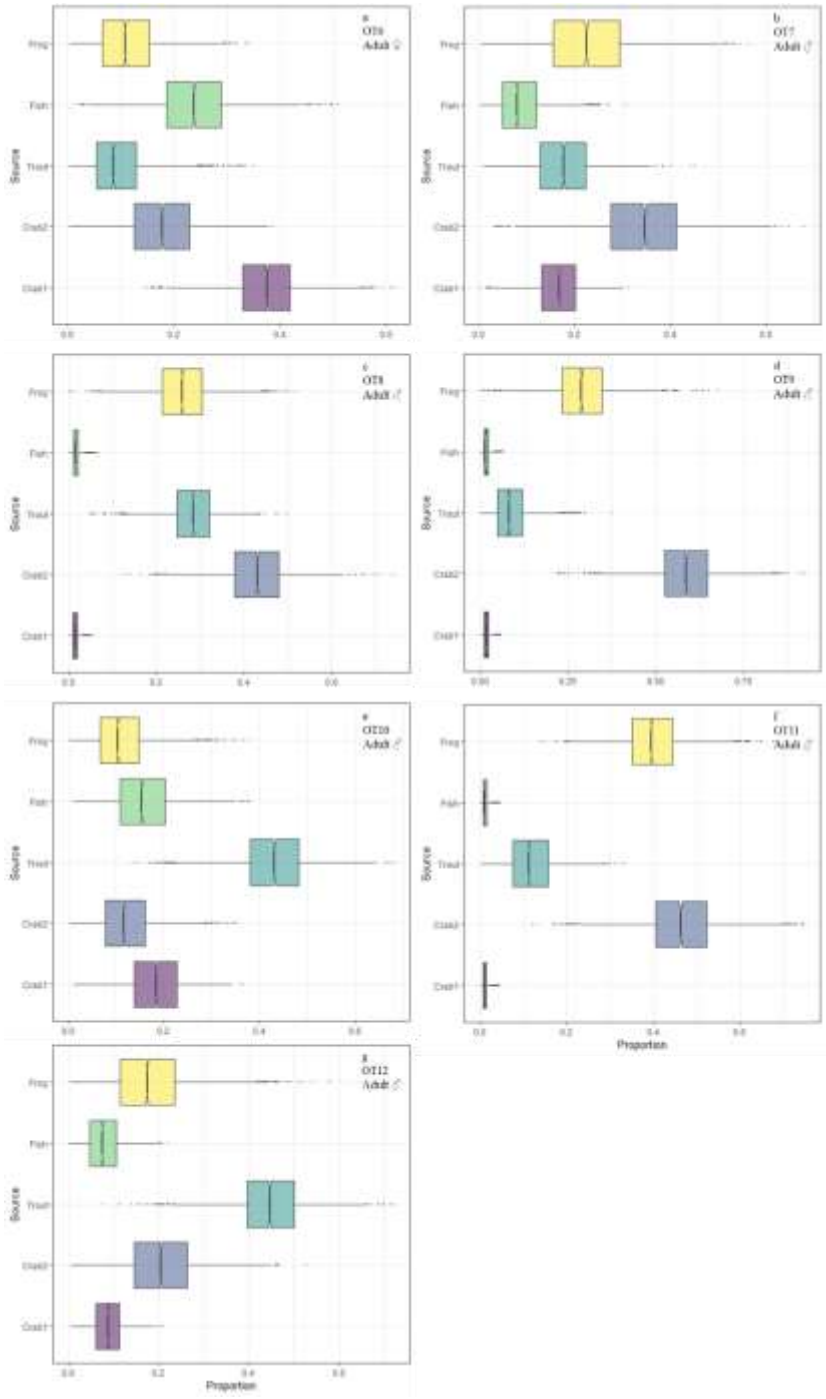

Figure 7. The proposed dietary contribution of potential prey species for eight African clawless otters from freshwater areas. Otters reflected here are OT6-OT12 (a-g respectively), and additional information such as sex and age class are displayed in each plot. Details on otter ID, sex and age class is displayed in each plot. Proportions were obtained through the analysis of otter and potential prey isotopic values in SIMMR. Box lines represent the 25, 50, 75 and confidence intervals.

Discussion

Intra- and inter-population isotopic niche variation is evident in African clawless otters. Within all the otters investigated, there were several individuals that displayed isotopic niche stability that did not vary temporally either in their isotopic niche or foraging habitat (OT1, OT2, OT5, OT8, OT9 and OT11). However, there were also otters that showed substantial temporal variation in isotopic niche and foraging habitat (OT3, OT4, OT6, OT7, OT10 and OT12).

The proportions of crab, fish and trout making up otter isotopic niche in freshwater habitats were similar to previous diet studies using spraint analyses (Butler & Du Toit, 1994; Somers & Purves, 1996; Watson & Lang, 2003). The results suggest that coastal populations of African clawless otters have higher proportions of mussels, squid and sharks in their isotopic niche when compared to previous spraint analyses dietary studies (Fig. 4) (Van der Zee, 1981; Somers, 2000; Jordaan et al., 2015). The remaining prey item proportions appear to be similar between individuals. Sharks are likely to be an infrequent prey item that has not been documented in previous studies (Van der Zee, 1981; Somers, 2000; Jordaan et al., 2015) but has been observed (Jordaan pers. obs.). One reason that these prey items (mussels, squid and sharks) have been under-represented or omitted from the results of previous studies may be due to the otters not consuming the hard, indigestible, parts resulting in little or no evidence of these items in spraints. Another is that the biomass of the invasive Mediterranean mussel has drastically increased since invading in the mid 1970's and has provided an additional source of food to higher predators such as the endangered African Black Oystercatcher, *Haematopus moquini* (Branch & Steffani, 2004; Bownes & McQuaid, 2006). The increase in availability of such a sedentary species may have therefore facilitated a shift in diet, resulting in the higher presence of mussel in otter isotopic niche.

In the coastal population, long-term trophic level isotopic niche stability was displayed by OT1, OT2 and OT5. This is consistent with what has been described previously for the

population-level diet of otters in a coastal habitat (Jordaan et al., 2015). However, the remaining otters (OT3 and OT4) displayed temporal isotopic niche variation, as is depicted by variation in $\delta^{15}N$ along the length of the vibrissae.

$\delta^{13}C$ values vary between coastal and freshwater habitats with coastal habitats having greater $\delta^{13}C$ values than freshwater habitats (Farquhar et al., 1989; Clementz & Koch, 2001). These variations in $\delta^{13}C$ and $\delta^{15}N$, therefore, suggest that OT3 and OT4 were probably feeding in coastal, estuarine and freshwater habitats and not restricting foraging to coastal habitats such as suggested by the data from OT1 and OT5. Higher $\delta^{15}N$ values were recorded when OT3 and OT4 were evidently foraging in freshwater habitats. One likely explanation for this is not that these otters were feeding at a higher trophic level in the freshwater habitat, but that there is more pollution in the freshwater habitat causing the basal level of N in this habitat to be higher than in the coastal habitat (Minagawa & Wada, 1984; Hobson & Welch, 1992). Although OT2 did not vary its isotopic niche and foraging habitat, its $\delta^{13}C$ values reflect those of a freshwater habitat and δ15N values suggest that this otter fed at a low trophic level. OT2 was found dead (cause of death unknown) on a coastal beach near a river mouth in Blue Horizon Bay, Port Elizabeth. We therefore consider it likely that this otter spent the period depicted in his vibrissa foraging in a nearby freshwater environment and was washed onto the beach post-mortem. Alternatively, OT2 ventured into the coastal environment and died before any isotopic traces of the coastal environment could be assimilated into his vibrissa.

The results obtained from African clawless otters occupying a human modified, freshwater habitat (Millstream Farm) suggest that these otters show dietary behaviour that varies between and within individuals. (Fig. 5; Fig. 7). Individual isotopic niche is either dominated by crab (OT6, OT7, OT8; OT9 and OT11) or by trout (OT10 and OT12), followed by lower proportions of trout and crab respectively, with frogs providing the smallest part of their isotopic niche. Frogs are, however, more prominent in the isotopic niche of the two otters

sampled outside of Millstream Farm (OT10 and OT11). Temporal isotopic niche variation (OT6, OT7, OT10, OT12), as well as temporal stability in diet (OT8, OT9, OT11) is evident in this population (Fig. 5). The results also suggest that some of these otters were site specific and did not move much between systems (OT8 and OT11), while others (OT6, OT7, OT10, OT12) moved around between different areas and water systems differentiated by different levels of pollution.

The high proportions of crab and a low proportions of trout in the isotopic niche of some African clawless otters are similar to what was found in trout stocked areas (Rowe-Rowe, 1977b; Butler & Du Toit, 1994). Conversely, the relatively high proportion of fish is not surprising as fish has been found to be present, and occasionally prominent, in the diet of African clawless otters foraging in non-trout waters (Rowe-Rowe, 1977b; Somers & Purves, 1996; Watson & Lang, 2003). However, proportions of trout in some of the African clawless otter isotopic niches (OT10 and OT12) are much higher than what has been previously determined in trout waters (Rowe-Rowe, 1977b; Butler & Du Toit, 1994). Some African clawless otters, although exposed to trout, do not appear to feed on trout but have an isotopic niche that is similar to African clawless otters foraging in non-trout waters (Rowe-Rowe, 1977b; Somers & Purves, 1996).

Temporal variation in isotopic niche is thought to largely be due to the inaccessibility of crabs during winter months and the reduced locomotion ability of fish (non-trout spp.) in winter months (Rowe-Rowe, 1977b). The mobility of trout, on the other hand, would be greatest (i.e. harder to catch) during winter months when water was coldest, while mobility would be greatly reduced (i.e. easier to catch) in the summer months when water is at its warmest (Jonsson & Jonsson, 2009). These results suggest that this species has a preference for benthic and/or slow-moving prey. This seemingly high proportion of trout and a low proportion of crab in the isotopic niche suggests that these otters can exploit the more active

trout, possibly through different foraging strategies. The higher proportion of frogs in the isotopic niche of otters from Lydenburg may be indicative of seasonal variation or of higher frog abundance here due to comparatively better water quality (Boyer & Grue, 1995), although this was not tested. The presence of sewage pollution in the river system did not seemingly influence isotopic niche and/or movement patterns. Therefore, variation in the isotopic niche of African clawless otters could be attributed to the factors mentioned above and possibly related to the differences in prey availability between the different sites that these otters visited (Rowe-Rowe, 1977b; Somers, 2000; Emmerson & Phillip, 2004).

These isotope data suggest that phenotypic plasticity is present in populations of African clawless otters foraging in freshwater and marine environments. Evidence presented suggests that there is variation in isotopic niche between- and within individuals in the same population, as well as between populations occupying different habitats. Different habitats can have varying levels of predator pressure, climate, and resource availability, resulting in the occurrence of location-linked differences in predator diet (Reznick & Endler, 1982; Reznick & Yang, 1993; Smith & van Buskirk, 1995). Such dietary variation between populations occupying different habitats has been observed in numerous predator species (Iriarte et al., 1990; Angerbjörn et al., 1994; Ford et al., 1998; Zhou et al., 2011; Bojarska & Selva, 2012), including European, *Lutra lutra* (Clavero et al., 2003) and Neotropical, *L. longicaudis* (Rheingantz et al., 2017) otters. Dietary variation, associated with phenotypic plasticity, would have been selected for in harsh environments hence the presence of within-species dietary variation found today (Hoffmann & Parsons, 1997; Badyaev, 2005). Dietary variation may also be attributed to short-term behavioural plasticity (Brown, 1996), or may be the result of differences in past environmental conditions (i.e. experience), differences in genetic make-up, and the interaction of both of these factors (Piersma & Drent, 2003; Nussey et al., 2007; Ghalambor et al., 2010).

Previous studies on otter diet in South Africa used spraint analysis to infer and compare diet between sites and seasons (Rowe-Rowe, 1977b; Van der Zee, 1981; Somers, 2000; Somers and Nel, 2003; Emmerson & Phillip, 2004; Jordaan et al., 2015), but these studies were unable to differentiate the diet and isotopic niche of individuals within sites. The presence of population-level intraspecific isotopic niche variation for African clawless otters in this study demonstrates that stable isotopes can be used to compare isotopic niche between individuals within sites as well as between sites. Intraspecific dietary specialisation leads to individual-level specialisation, which there is evidence for in individual otters in the different habitats in this study. Individual-level dietary specialisation may arise due to seasonal variation of prey, differences in foraging strategies, individual sex/maturity status, age, size of the predator and/or prey availability (Ford et al., 1998; Grey, 2001; Meynier et al., 2008). Additionally, the energy content of available prey items, relative to the energetic costs of prey capture likely further influence the development of dietary specialisation (Meynier et al., 2008), while limited prey resources may support the development of individual specialisation as a means of competition avoidance (Tinker 2007).

Although these findings highlight how useful stable isotope techniques are when compared to more traditional methods (i.e. spraint analysis), they also show some limitations. One limitation is that not all samples were collected at the same location during the same period, complicating direct comparisons due to non-uniform distribution of prey species (Fry & Wainright, 1991; Hemminga & Mateo, 1996). It is also possible that samples from all potential prey items were not collected, since our prey sampling approach was not an exhaustive one. This is evident in the case of OT2 as this otter falls out of the proposed bivariate isotopic space (Fig. 3), the resulting conclusion on its diet (Fig.4) is therefore made with caution. However, our prey samples generally exceeded the diversity of prey items previously recorded for African clawless otters (e.g. Somers & Nel, 2003; Emmerson & Phillip, 2004;

Jordaan et al., 2015), suggesting that the prey items sampled in this study to be appropriately representative. We further acknowledge that the sample size is small, and inferences are, therefore, made with caution. However, compared to other stable isotope mixing models, SIMMR accounts well for natural variations in the stable isotope composition of prey and consumers and the under determination of potential resources (Phillips & Greg, 2003; Moore & Semmens, 2008; Parnell et al., 2010).

In conclusion, the results presented here illustrate substantial intra- and inter-individual isotopic niche variation in African clawless otters sampled from coastal and freshwater populations. These differences can be due to differences in the available prey base and suggest substantial dietary plasticity in this species. African clawless otters are evidently opportunistic and adaptable foragers (Somers & Purves, 1996; Emmerson & Phillip, 2004; Newsome et al., 2009; Jordaan et al., 2015), although some individuals evidently also specialise in specific prey types. Finally, these results illustrate the usefulness of stable isotope techniques, particularly when applied to inert tissues such as vibrissae, to further inform our understanding about carnivore diets and temporal trends in diets.

References

Angerbjörn, A., Hersteinsson, P. Lidén, K., Nelson, E. 1994. Dietary variation in arctic foxes (*Alopex lagopus*) – an analysis of stable carbon isotopes. Oecologia 99, 226-232.

Arden-Clarke, C.H.G. 1986. Population density, home range size and spatial organization of the Cape clawless otter, *Aonyx capensis*, in a marine habitat. J. Zool. Lond. 209, 201-221.

Badyaev, A.V. 2005. Stress-induced variation in evolution: from behavioural plasticity to genetic assimilation. Proc. R. Soc. Brit. 272, 877-886.

Bateson, P., Gluckman, P. 2011. Plasticity, Robustness, Development and Evolution. Cambridge University Press, Cambridge.

Bojarska, K., Selva, N. 2012. Spatial patterns in brown bear *Ursus arctos* diet: the role of geographical and environmental factors. Mamm. Rev. 42(2), 120-143.

Bolnick, D.I. Svanback, R. Fordyce, J.A. Yang, L.H. Davis, J.M. Hulsey, C.D., Forister, M.L. 2003. The ecology of individuals: Incidence and implications of individual specialization. Am. Nat. 161, 1-28.

Bownes, S.J., McQuaid, C.D. 2006. Will the invasive mussel *Mytilus galloprovincialis* Lamarck replace the indigenous *Perna perna* L. on the south coast of South Africa? J. Exp. Mar. Biol. Ecol. 338, 140-151.

Boyer, B., Grue, C. E. 1995. The Need for Water Quality Criteria for Frogs. Environ. Health Perspect. 103, 352-357.

Branch, G.M., Steffani, C.N. 2004. Can we predict the effects of alien species? A case-history of the invasion of South Africa by *Mytilus galloprovincialis* (Lamarck). J. Exp. Mar. Biol. Ecol 300, 189-215.

Brown, A.C. 1996. Behavioural plasticity is a key factor in the survival and evolution of the

macrofauna on exposed sandy beaches. Rev. Chil. Hist. Nat. 69, 469-474.

Butler, J.R.A., Du Toit, J.T. 1994. Diet and conservation of Cape clawless otters in eastern Zimbabwe. S. Afr. J. Wildl. Res. 24, 41-47.

Carrasco, T.S., Botta, S., Machado, R., Simões-Lopes, P.C., Carvalho-Junior, O., Ott, P.H., Colares, E.P., Secchi, E.R. 2019. Isotopic niche of the Neotropical otter, *Lontra longicaudis* (Carnivora, Mustelidae), in different coastal aquatic systems in southern Brazil. Hydrobiologia 835, 83-100.

Carss, D.N., Parkinson, S.G. 1996. Errors associated with otter *Lutra lutra* faecal analysis in assessing general diet from spraints. J. Zool. Lond. 238, 301-307.

Clavero, M., Prenda, J., Delibes, M. 2003. Trophic diversity of the otter (*Lutra lutra* L.) in temperate and Mediterranean freshwater habitats. J. Biogeogr. 30, 761-769.

Clementz, M.T., Koch, P. L. 2001. Differentiating aquatic mammal habitat and foraging ecology with stable isotopes in tooth enamel. Oecologia 129, 461-472.

Coplen, T.B. 1994. Reporting of stable hydrogen, carbon, and oxygen isotopic abundances. Pure Appl. Chem. 66, 273-276.

Day, C.C. Westover, M.D., McMillan, B.R. 2015. Seasonal diet of the northern river otter (*Lontra canadensis*): What drives prey selection? Can. J. Zool. 93, 197-205.

de Vos, M. 2018. Human-predator conflict in the South African fly-fishing industry: fish survival probabilities and stakeholder perceptions. Unpublished MSc dissertation, University of Pretoria, South Africa.

Emmerson, W., Philip, S. 2004. Diets of Cape clawless otters at two South African coastal localities. Afr. Zool. 39: 201-210.

Englund, J. 1965. Studies on the food ecology of the Red fox *(Vulpes vulpes)* in Sweden. Viltrevy 3, 377-484.

Estes, J.A., Riedman, M.L., Staedler, M.M., Tinker, M.T., Lyon, B.E. 2003. Individual variation in prey selection by sea otters: patterns, causes and implications. J. Anim. Ecol. 72, 144-155.

Farquhar, G.D., Ehleringer, J.R., Hubick, K.T. 1989. Carbon isotope discrimination and photosynthesis, in: Briggs, W. R. (Ed.), Annual review of plant physiology and plant molecular biology vol 40. Annual Reviews, Palo Alto, pp. 503-538.

Ford, J.K.B., Ellis, G.M., Barrett-Lennard, L.G., Morton, A.B., Palm, R.S., Balcomb, K.C. 1998. Dietary specialization in two sympatric populations of killer whales (*Orcinus orca*) in coastal British Columbia and adjacent waters. Can. J. Zool. 76, 1456-1471.

Fry, B., Wainright, S.C. 1991. Diatom sources of carbon-13 rich carbon in marine food webs. Mar. Ecol. Prog. Ser. 76, 149-157.

Ghalambor, C.K., Angeloni, L.M., Carroll, S.P. 2010. Behavior as phenotypic plasticity, in: D. F., & Fox, C. W. (Eds.), Westneatv, Evolutionary Behavioral Ecology, Oxford University Press, New York, pp 90-107.

Glasser, J.W. 1982. A theory of trophic strategies: the evolution of facultative specialists. Am. Nat. 119, 250-262.

Gorgadze, G. 2013. Seasonal diet of the otter (*Lutra lutra*) on the Alazani River (Georgia). Hystrix. 24, 157-160.

Grey, J. 2001. Ontogeny and dietary specialization in brown trout (*Salmo trutta* L.) from Loch Ness, Scotland, examined using stable isotopes of carbon and nitrogen. Ecol. Freshw. Fish 10, 168-176.

Hanekom, N., Southwood, A., Ferguson, M. 1989. A vegetation survey of the Tsitsikamma Coastal National Park. Koedoe 32, 47-67.

Hemminga, M.A., Mateo, M.A. 1996. Stable carbon isotopes in seagrasses: variability in ratios and use in ecological studies. Mar. Ecol. Prog. Ser. 140, 285-298.

Hilderbrand, G.V., Farley, S.D., Robbins, C.T., Hanley, T.A., Titus, K., Servheen, C. 1996. Use of stable isotopes to determine diets of living and extinct bears. Can. J. Zool. 74, 2080-2088.

Hobson, K.A., Welch, H.E. 1992. Determination of trophic relationships within a high Arctic marine food web using $\delta^{13}C$ and $\delta^{15}N$ analysis. Mar. Ecol. Prog. Ser. 84, 9-18.

Hoffmann, A.A., Parsons, P.A. 1997. Extreme environmental change and evolution. Cambridge University Press.

Hooker, S.K., Iverson, S.J., Ostrom, P., Smith, S.C. 2001. Diet of northern bottlenose whales inferred from fatty-acid and stable isotope analyses of biopsy samples. Can. J. Zool. 79, 1442-1454.

Iriarte, J.A., Franklin, W.L., Johnson, W.E., Redford, K.H. 1990. Biogeographic variation of food habits and body size of the America puma. Oecologia 85, 185-190.

Iverson, S.J., Frost, K.J., Lowry, L.F. 1997. Fatty acid signatures reveal fine scale structure of foraging distribution of harbor seals and their prey in Prince William Sound, Alaska. Mar. Ecol. Prog. Ser. 151, 255-271.

Jenkins, D., Walker, J.G.W., McCowan, D. 1979. Analysis of otter *(Lurra lutra)* faeces from Deeside, N.E. Scotland. J. Zool. Lond. 187, 235-244.

Jonsson, B., Jonsson, N. 2009. A review of the likely effects of climate change on anadromous Atlantic salmon *Salmo salar* and brown trout *Salmo trutta*, with particular reference to water temperature and flow. J. Fish. Biol. 75, 2381-2447.

Jordaan, R.K., McIntyre, T. Somers, M.J., Bester, M.N. 2015. An assessment of spatial and temporal variation in the diet of Cape clawless otters (*Aonyx capensis*) in marine environments. Afr. J. Wildl. Res. 45, 342-353.

Klare, U., Kamler, J.F., McDonald, D.W. 2011. A comparison and critique of different scat-analysis methods for determining carnivore diet. Mam. Rev. 41, 294-312.

KPMG. 1999. Mpumalanga tourism growth plan. Unpublished report. Nelspruit: Mpumalanga Tourism Authority.

Kruuk, H., Goudswaard, P.C. 1990. Effects of changes in fish populations in Lake Victoria on the food of otters *(Lutra maculicollis* Schinz and *Aonyx capensis* Lichtenstein). Afr. J. Ecol. 28, 322-329.

Lerner, J.E., Ono, K., Hernandez, K.M., Runstadler, J.A., Puryear, W.B., Polito, M.J. 2018. Evaluating the use of stable isotope analysis to infer the feeding ecology of a growing US gray seal (*Halichoerus grypus*) population. PLoS ONE 13:e0192241.

Lübcker, N., Condit, R., Beltran, R.S., de Bruyn, P.J.N., Bester, M.N. 2016. Vibrissal growth parameters of southern elephant seals *Mirounga leonina*: obtaining fine-scale, time-based stable isotope data. Mar. Ecol. Prog. Ser. 559, 243-255.

Meynier, L., Pusineri, C., Spitz, J., Santos, M.B., Pierce, G.J., Ridoux, V. 2008. Intraspecific dietary variation in the short-beaked common dolphin *Delphinus delphis* in the Bay of Biscay: importance of fat fish. Mar. Ecol. Prog. Ser. 354, 277-287.

Minagawa, M., Wada, E. 1984. Stepwise enrichment of ^{15}N along food chains: Further evidence and the relation between ^{15}N and animal age. Geochim. Cosmochim. Acta 48, 1135-1140.

Moore, J.W., Semmens, B.X. 2008. Incorporating uncertainty and prior information into stable isotope mixing models. Ecol. Lett. 11, 470-480.

Newsome, S.D., Tinker, M.T., Monson, D.H., Oftedal, O.T., Ralls, K., Staedler, M.M., Fogel, M.L., Estes, J.A. 2009. Using stable isotopes to investigate individual diet specialization in California sea otters (*Enhydra lutris nereis*). Ecology 90, 961-974.

Newsome, S.D., Bentall, G.B., Tinker, M.T., Oftedal, O.T., Ralls, K., Estes, J.A., Fogel, M.L. 2010. Variation in □^{13}C and □^{15}N diet-vibrissae trophic discrimination factors in a wild population of California sea otters. Ecol. Appl. 20, 1744-1752.

Norbury, G.L., Sanson, G.D. 1992. Problems with measuring diet selection of terrestrial, mammalian herbivores. Aust. J. Ecol. 17, 1-7.

Nussey, D.H., Wilson, A.J., Brommer, J.E. 2007. The evolutionary ecology of individual phenotypic plasticity in wild populations. J. Evol. Biol. 20, 831-844.

O'Leary, M.H. 1981. Carbon isotope fractionation in plants. Phytochemistry 20, 553-567.

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.

Parnell, A. 2016. SIMMR: A Stable Isotope Mixing Model. R package version 0.3

Phillips, D.L., Gregg, J.W. 2003. Source partitioning using stable isotopes: coping with too many sources. Oecologia 136, 261-269.

Piersma, T., Drent, J. 2003. Phenotypic flexibility and the evolution of organismal design. Trends Ecol. Evol. 18, 228-233.

Ponsonby, D.W., Schwaibold, U. 2018. Country otter, city otter: The distribution patterns of two otter species in an urbanized area of Gauteng, South Africa. Afr. J. Ecol. 1-7.

R Core Team. 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rheingantz, M.L., Santiago-Plata, V.M., Trinca, C.S. 2017. The Neotropical otter *Lontra longicaudis*: a comprehensive update on the current knowledge and conservation status of this semiaquatic carnivore. Mamm. Rev. 47, 291-305.

Reznick, D.N., Endler, J.A. 1982. The impact of predation on life history evolution in Trinidadian guppies (*Poecilia reticulata*). Evolution 36, 160-177.

Reznick, D., Yang, A.P. 1993. The influence of fluctuating resources on life history: patterns of allocation and plasticity in female guppies. Ecology 74, 2011-2019.

Rowe-Rowe, D.T. 1977a. Variation in the predatory behaviour of the clawless otter. Lammergeyer 23, 22-27.

Rowe-Rowe, D. T. 1977b. Food ecology of otters in Natal, South Africa. Oikos 28, 210-219.

Rowe-Rowe, D. T. 1991. Status of otters in Africa. Habitat 6, 15-20.

Rowe-Rowe, D.T., Somers, M.J. 1998. Diet, foraging behaviour and coexistence of African otters and the water mongoose. Symp. Zool. Soc. Lond. 71, 215-227.

Schumann, E.H., Cohen, A.L., Jury, M.R. 1995. Coastal sea surface temperature variability along the south coast of South Africa and the relationship to regional and global climate. J. Mar. Res. 53, 231-248.

Smith, D.C., van Buskirk, J. 1995. Phenotypic design, plasticity, and ecological performance in two tadpole species. Am. Nat. 145, 211-233.

Snell-Rood, E.C. 2013. An overview of the evolutionary causes and consequences of behavioural plasticity. Anim. Behav. 85, 1004-1011.

Somers, M.J. 2000. Seasonal variation in the diet of Cape clawless otters (*Aonyx capensis*) in a marine habitat. Afr. Zool. 35, 261-268.

Somers, M.J., Purves, M.G. 1996. Trophic overlap between three semi-aquatic carnivores: Cape clawless otter, spotted-necked otter and water mongoose. Afr. J. Ecol. 34, 158-166.

Somers, M.J., Nel, J.A.J. 2003. Diet in relation to prey of Cape clawless otters in two South African rivers. Afr. Zool. 38, 317-326.

Tinker, M.T., Costa, D.P., Estes, J.A., Wieringa, N. 2007. Individual dietary specialization and dive behaviour in the California sea otter: Using archival time-depth data to detect alternative foraging strategies. Deep Sea Res. Part II: Top. Stud. Oceanogr. 54, 330-342.

Tinker, M.T., Estes, J.A., Bentall, G. 2008. Food limitation leads to behavioral diversification and dietary specialization in sea otters. Proc. Nat. Acad. Sci. of the USA 105, 560-565.

van der Zee, D. 1979. Food and status of the Cape clawless otter, Aonyx capensis Schinz, in the Tsitsikamma Coastal National Park, South Africa. M.Sc. thesis, University of Pretoria, South Africa.

van der Zee, D. 1981. Prey of the Cape clawless otter (*Aonyx capensis*) in the Tsitsikamma Coastal National Park, South Africa. J. Zool. Lond. 194, 467-483.

Walton, M.J., Henderson, R.J., Pomeroy, P.P. 2000. Use of blubber fatty acid profiles to distinguish dietary differences between grey seals *Halichoerus grypus* from two UK breeding colonies. Mar. Ecol. Prog. Ser. 193, 201-208.

West-Eberhard, M.J. 2003. Developmental Plasticity and Evolution. New York: Oxford University Press.

Watson, L.H., Lang, A.J. 2003. Diet of Cape clawless otters in Groenvlei Lake, South Africa. S. Afr. J. Wildl. Res. 33, 135-137.

Zhou, Y.B., Newman, C., Xu, W.T., Buesching, C.D., Zalewski, A., Kaneko, Y., Macdonald, D.W. Xie, Z.Q. 2011. Biogeographical variation in the diet of Holarctic martens (genus Martes, Mammalia: Carnivora: Mustelidae): adaptive foraging in generalists. J. Biogogr. 38, 137-147.