ORIGINAL PAPER

Ontogenetic shift in diet of a large elapid snake is facilitated by allometric change in skull morphology

M. Patterson[1] · A. K. Wolfe[2] · P. A. Fleming[1] · P. W. Bateman[2] · M. L. Martin[1] ·
E. Sherratt[3] · N. M. Warburton[1]

Abstract

As snakes are limbless, gape-limited predators, their skull is the main feeding structure involved in prey handling, manipulation and feeding. Ontogenetic changes in prey type and size are likely to be associated with distinct morphological changes in the skull during growth. We investigated ontogenetic variation in diet from stomach contents of 161 Dugite specimens (*Pseudonaja affinis*, Elapidae) representing the full range of body size for the species, and skull morphology of 46 specimens (range 0.25–1.64 m snout-vent-length; SVL). We hypothesised that changes in prey type throughout postnatal ontogeny would coincide with distinct changes in skull shape. Dugites demonstrate a distinct size-related shift in diet: the smallest individuals ate autotomised reptile tails and reptiles, medium-sized individuals predominantly ate mammals, and the largest individuals had the most diverse diet, including large reptiles. Morphometric analysis revealed that ~40% of the variation in skull shape was associated with body size (SVL). Through ontogeny, skulls changed from a smooth, bulbous cranium with relatively small trophic bones (upper and lower jaws and their attachments), to more rugose bones (as a likely reflection of muscle attachment) and relatively longer trophic bones that would extend gape. Individual shape variation in trophic bone dimensions was greater in larger adults and this likely reflects natural plasticity of individuals feeding on different prey sizes/types. Rather than a distinct morphological shift with diet, the ontogenetic changes were gradual, but positive allometry of individual trophic bones resulted in disproportionate growth of the skull, reflected in increased gape size and mobility of jaw bones in adults to aid the ingestion of larger prey and improve manipulation and processing ability. These results indicate that allometric scaling is an important mechanism by which snakes can change their dietary niche.

Keywords Trophic · Squamata · Geometric morphometrics · Allometric scaling · Post-natal growth · Micro-CT

✉ M. Patterson
    31566976@student.murdoch.edu.au

1   Harry Butler Institute, Murdoch University, Perth, Murdoch, WA 6150, Australia

2   Behavioural Ecology Lab, School of Molecular and Life Sciences, Curtin University, Perth, Bentley, WA 6102, Australia

3   School of Biological Sciences, The University of Adelaide, Adelaide, SA 5005, Australia

Introduction

Snakes (Squamata, Serpentes) of the clade Macrostomata are "bulk-feeders", specialising in capturing and swallowing whole relatively large prey (Bellairs 1969; Kardong 2012a, 2012b; Scanferla 2016). The maximum size of prey they can consume is limited by the maximum gape of their jaws (Dwyer and Kaiser 1997; Hampton 2011). Snake skulls have several adaptations for ingesting large prey, including enhanced jaw mobility though modified mandibular articulation and the absence of the mandibular symphysis, and a strengthened braincase that protects the brain during prey manipulation (Bellairs 1968, 1969; Kardong 2012a, 2012b; O'Malley 2005; Romer and Parsons 1986). Further adaptations have been reported for specialist diets. For example, snakes that prey on frogs often have broad heads, robust upper jaw bones and long quadrate and supratemporal bones (Andjelković et al. 2016; Dwyer and Kaiser 1997; Hampton 2011), while snakes that specialise in eating fish have longer, narrower skulls and jaws to cope with the physical demands of striking at prey underwater (Andjelković et al. 2016; Hampton 2011; Vincent et al. 2007).

To understand dietary specialisation among species, one approach is to investigate the impact of diet changes during ontogeny. Many snake species demonstrate a dramatic shift in diet during their postnatal growth (Natusch and Lyons 2012; Vincent et al. 2007). For example, the elapid, *Pseudonaja textilis*; and the pythons, *Morelia viridis* and *M. spilota* exhibit an ontogenetic diet shift from small reptilian prey as juveniles to large mammalian prey as adults (Natusch and Lyons 2012; Shine 1989, 1991). Such dietary shifts involve different challenges in prey capture, handling and ingestion (Cundall and Greene 2000; Scanferla 2016) that are expected to impose different functional demands on the skulls (and associated musculature) as they grow (Hampton 2011). Such a dietary shift may be facilitated by the proportional growth of the skull elements with increasing head and body size (i.e., allometry), or be associated with a distinct morphological shift (i.e., a significant change in the relationship between size and shape). Therefore allometry, the study of size and its consequences, is a powerful tool to explore the relationship between diet and morphology during ontogeny.

Australian brown snakes (Elapidae; *Pseudonaja* spp.) are known to feed mainly on reptiles when they are small juveniles, while larger individuals take a greater proportion of mammalian prey (Shine 1989; Wolfe et al. 2017). Here, we investigate the relationship between diet (as determined by stomach contents) and skull morphology (examined using linear and landmark-based morphometric techniques) of Dugites, *Pseudonaja affinis* (Günther 1872) across a range of body sizes to:

(1) Quantify ontogenetic changes in diet in terms of the number, type, and size of prey taken;
(2) Characterise how cranium shape changes with body size during ontogeny;
(3) Characterise how trophic bone dimensions change during ontogeny; and
(4) Examine the relationship between change in diet and morphology of the skull.

Materials and methods

Study species

The Dugite, *Pseudonaja affinis* (Günther 1872) is a venomous elapid commonly found throughout southwest Western Australia and in the southeast of South Australia (Bush

et al. 2010; Maryan and Bush 1996). It is an opportunistic diurnal forager (Shine 1989; Shine and Schwaner 1985). It can be found in a wide variety of habitats, including coastal dunes, shrublands, semi-arid woodlands and wet sclerophyll forests, and often shows a preference for disturbed urban habitats and those associated with agriculture (Maryan and Bush 1996). Neonatal snout-to-vent length (SVL) ranges from 0.19 to 0.23 m, while adults average 1.09 m SVL (maximum 1.63 m) with little to no sexual size dimorphism (Maryan and Bush 1996; Shine 1989, 1994a, 1994b).

A total of 568 specimens, previously examined by Wolfe et al. (2017), were analysed for diet through stomach content analysis: the majority of specimens held by the Western Australian Museum had been collected between 1910 and 2015, while a further 20 specimens were opportunistically collected during 2014–2015 as road-kill from around the Perth region in Western Australia (under a Department of Parks and Wildlife Regulation 17 license #SF009895). We recorded the SVL and body mass of each Dugite. For snakes > 0.40 m in length, sex was determined either by probing or dissection of the sub-caudal scales (Wolfe et al. 2017). For juvenile snakes < 0.40 m, sex was determined by extracting the gonads and examining histological slices under a microscope (Bloom and Fawcett 1975; Dellmann 2006; Jacobson 2007).

Do Dugites show a significant ontogenetic shift in diet?

To quantify diet of Dugites, stomach contents were examined by gross dissection. The stomachs of 568 specimens were removed via mid-ventral midline abdominal incision. Stomachs were opened lengthwise, and all contents were removed. A total of 151 specimens had items present in their stomachs. Prey items were identified to the lowest possible taxon by physical examination, prey orientation in the stomach was recorded, and each prey item was measured (head length, snout-vent length for reptiles or head-body length for mammals) and weighed (±0.001 g), and we recorded whether or not it had an intact tail.

To test whether the number of prey taken was influenced by snake size, we examined the relationships between the number of prey found in each of 151 Dugite stomachs with the snake SVL using the *glm* function in 'lme4' (Bates et al. 2014) in the R statistical environment (R Core Team 2018).

To test whether the type of prey taken was influenced by snake size, for 293 individual prey items collected from 151 Dugite stomachs, we modelled the relationship between the snake SVL and prey type (reptile tails only, reptile bodies with or without their tails, and mammals) as dependent variables in separate binomial models using Generalized Linear Models (GLMs) with the *glm* function in 'lme4' in R. We then used the 'ggeffect' package (Lüdecke 2019) in R to predict the likelihood of each prey type being found in Dugite stomachs against snake SVL.

To test whether the size of prey taken was influenced by snake size, we examined the relationships between prey size (SVL, head length, and body mass) with snake SVL and prey type (with an interaction term between snake SVL and prey type, as indicated by the lowest AIC value) using the *glm* function in 'lme4'. We then tested the slopes between prey size and snake size for each prey type separately.

We compared the orientation of prey taken by snakes upon dissection of stomachs (head-first or tail-first; snakes with multiple prey in both orientations were excluded from this analysis) using a Pearson's Chi test, with expected values calculated as

an equal proportion of head-first or tail-first prey across three snake size categories (SVL < 0.5 m, 0.5–1.0 m, 1.0–1.5 m).

Do Dugites show an ontogenetic change in skull morphology?

A total of 46 specimens were available to study skull morphology (sampling subject to specimen damage) and micro-Computed Tomography (micro-CT) was used to make digital models of the skulls. In preparation for scanning, intact heads were removed from the body and placed in 10% buffered formalin for at least a week before being scanned. Heads were scanned using a Skyscan 1176 In Vivo Micro-CT at 65 kV, 381 mA with a resolution of 18 μm (Centre for Microscopy, Characterisation and Analysis facility; Harry Perkins Institute of Medical Research; Nedlands, WA). Details of the micro-CT processing steps are available in Appendix 2. The micro-CT data was first imported into NRecon v.1.7.1.0 (Micro Photonics Inc., USA) for slice reconstruction and then CT-Analyser v.1.17.7.2 (Micro Photonics Inc., USA) was used to visualise the reconstructed slices and produce 3D surface models of the skulls. Each 3D surface model was then imported into MeshLab v.2016.12 (Cignoni et al. 2008), where they were simplified to ease manipulation for landmarking using Quadric Edge Decimation to reduce the number of vertices (to ~ 500,000 vertices). Landmarks were placed onto the skull models using IDAV Landmark Editor v.3.6 (Wiley et al. 2007).

Two landmarking schemes were used to capture shape variation of the skull across specimens. The first scheme consisted of 61 landmarks placed on the cranium (Fig. 1a-c), while the second landmarking scheme consisted of 32 landmarks placed on the trophic bones (Fig. 1d-f) from which 14 inter-landmark distances (linear dimensions) were calculated (Table 1). These cranium versus trophic bone datasets facilitate complementary analysis of shape changes through postnatal ontogeny on two principal regions of the skull; the cranium being relatively fixed and immovable (Fig. 1a-c), and the trophic bones being articulated and highly mobile (Fig. 1d-f).

Landmarks digitised on the cranium were analysed using geometric morphometrics, a method that preserves the geometry of objects (typically represented by landmark configurations) and permits statistical analysis of shape (e.g., Adams et al. 2013). Landmark coordinates were standardised to Procrustes shape coordinates by aligning the landmark configurations using generalised Procrustes superimposition (Rohlf and Slice 1990), taking object symmetry into account (Klingenberg et al. 2002) using the *bilat.symmetry* function in 'geomorph' package v.3.0.6 (Adams et al. 2018) in R.

To examine ontogenetic allometry in shape of the cranium, we measured the strength and statistical significance of the relationship between cranium shape and body size using a multivariate regression of the Procrustes shape coordinates (dependent variable) against $\log_{10}$-transformed SVL (independent variable) (Klingenberg 2016). To account for possible sexual dimorphism, sex was included in the regressions as a fixed factor. To visualise the allometric relationships between shape and size, a regression score was calculated and plotted against $\log_{10}$-transformed SVL (Drake and Klingenberg 2008). The regression score is a univariate summary of the multivariate regression vector (Drake and Klingenberg 2008). These analyses were carried out using the *procD.lm* function in 'geomorph' in R.
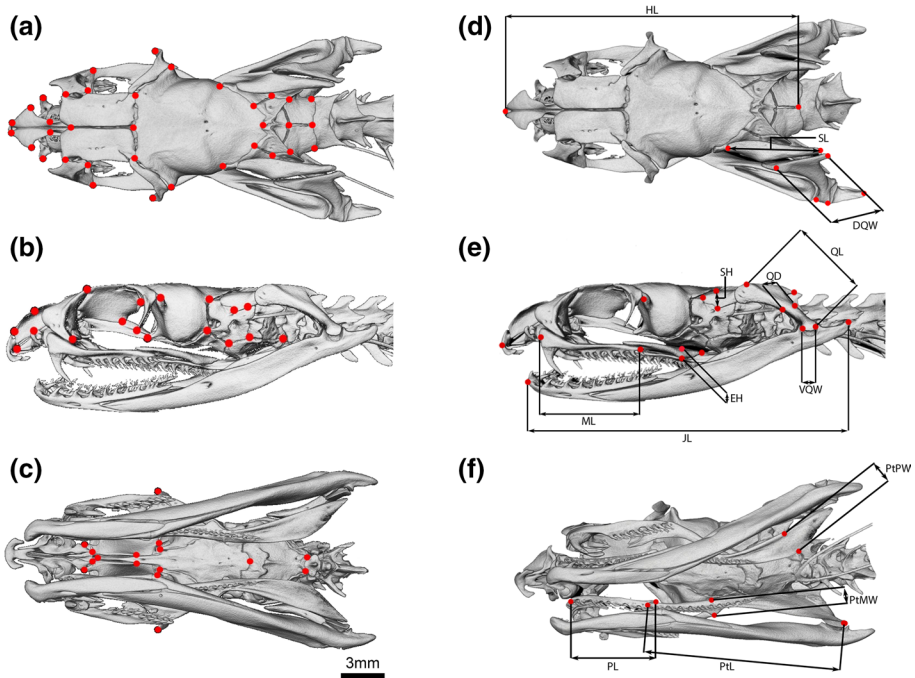
Fig. 1 3D models of Dugite (*Pseudonaja affinis*) skulls in **a**, **d** dorsal, **b**, **e** lateral, and **c**, **f** ventral views showing landmark locations and linear measurements taken. Models a-c depict the locations of the 61 landmarks used for examining cranium shape. Models d-f depict the locations of the linear measurements taken on trophic bones (plus head length) and landmarks placed there (see **Table 1** for descriptions of each linear measurement)

Do Dugites show an ontogenetic change in relative dimensions of trophic bones?

Linear dimensions were measured from the digital skull models to capture the size of the main trophic bones identified in previous studies: the pterygoid, palatine, maxilla, quadrate, mandible (compound and dentary), ectopterygoid and supratemporal bones (Andjelković et al. 2016; Camilleri and Shine 1990; Cundall and Rossman 1984; Dwyer and Kaiser 1997; Hampton 2011; Palci et al. 2016; Vincent et al. 2007). A total of 14 linear measurements were obtained digitally by calculating distances between landmarks pairs using the *interlmkdist* function in 'geomorph' in R (Table 1, Fig. 1d-f). Prior to analyses, the linear measurement data was transformed into log-shape ratios to standardise them for scale in order to quantify the shape variation across the range of specimens (Mosimann and James 1979). This transformation involves dividing each of the measures by a standard size variable (geometric mean of all variables), which quantifies the overall size of the specimen, and then log-transforming the measurements, and is equivalent to the scaling step of the Procrustes superimposition described above (Mosimann and James 1979).

Ontogenetic variation in trophic bone morphology was examined as above for cranial shape using a multivariate regression of the log-shape ratios against $\log_{10}$-transformed SVL (independent variable), while taking into account sex as a fixed factor. A series of reduced major axis (RMA) regression of individual $\log_{10}$-transformed linear measurements against $\log_{10}$-transformed SVL and $\log_{10}$-transformed head length

Table 1 Table of linear measurements of Dugite (*Pseudonaja affinis*) skulls as depicted in Fig. 1, with descriptions of each

Abbrev	Description
HL	Head length; anterior tip of premaxilla to exoccipitals
JL	Jaw length; anterior tip of dentary to posterior edge of compound bone
DQW	Dorsal quadrate width; anterior to posterior end of dorsal portion of quadrate (where it articulates with supratemporal)
QD	Quadrate diameter; diameter of quadrate at central point along length of quadrate
VQW	Ventral quadrate width; outer edge to inner edge of ventral portion of quadrate, perpendicular to length of jaw (where articulates with mandible)
QL	Quadrate length; dorsal edge (articulates with supratemporal) to ventral edge (articulates with mandible) of quadrate
ML	Maxilla length; straight line distance from anterior tip to posterior tip of maxilla
EL	Ectopterygoid length; straight line distance from anterior tip to posterior tip of ectopterygoid
EH	Ectopterygoid height, dorsal edge to ventral edge of ectopterygoid at bony protrusion towards posterior end
PtL	Pterygoid length; straight line distance from anterior tip to posterior tip
PtMW	Pterygoid medial width; width of pterygoid at point where it meets with ectopterygoid
PtPW	Pterygoid posterior width; width at back of pterygoid where bone fans out flat, taken at widest fanning out point
PL	Palatine length; straight line distance from anterior to posterior end of palatine
SL	Supratemporal length; straight line distance from anterior end to posterior end of supratemporal
SH	Supratemporal height; dorsal edge to ventral edge of supratemporal

were performed separately to explicitly calculate the allometric slopes of the trophic bones. RMA regression was chosen over ordinary least-squares (OLS) regression as it accounts for measurement error commonly found in morphological data (Vincent et al. 2006a). These analyses were carried out using the 'lmodel2' package v.1.7–3 (Legendre 2008) in R.

Is there a change in skull morphology coinciding with an ontogenetic change in diet?

Preliminary examination of diet data revealed that there was a shift in the primary prey item when snakes reached ~ 0.80 m SVL. Therefore, we divided the data into two size classes (< 0.80 m SVL and > 0.80 m SVL) to test whether there is a change in the allometric slope of skull shape coinciding with this dietary shift (i.e., a distinct change in growth trajectory at ~ 0.80 m SVL). We performed a MANCOVA, evaluating a model with SVL and size class as interacting independent factors, implemented with *procD. lm* function in 'geomorph' in R, which is designed for high-dimensional data (Collyer et al. 2015). This approach performs a multivariate regression for the two size classes separately, estimating the slope and intercept for each group; if the interaction term is significant, this indicates the two classes have a different allometric slope, and thus supports the hypothesis of a morphological shift.

Results

Do Dugites show a significant ontogenetic shift in diet?

Number and type of prey taken

151 of the 568 dissected snakes had prey present, with the remainder having empty stomachs. Dugite stomachs contained an average of 0.50 ± 1.30 range 0–12 prey items, with a significant positive relationship between prey number and snake SVL (estimate 0.75 ± 0.15, $t = 5.10$, $p < 0.001$; Fig. 2a).

Prey included 59 reptile tails only, in addition to 114 reptiles with tails representing at least 18 species. Of the 114 reptiles with tails, 37% had lost their tail either before or

Fig. 2 **a** The number of prey items in the stomachs of 568 Dugites (*Pseudonaja affinis*) (diamonds are 151 stomachs with prey, + represent empty stomachs) shown as a function of snake size (snout-vent length). **b** Predicted probability estimated using *ggpredict* that a snake with prey in its stomach had consumed reptiles (either their tails only, or whole animals) or mammals
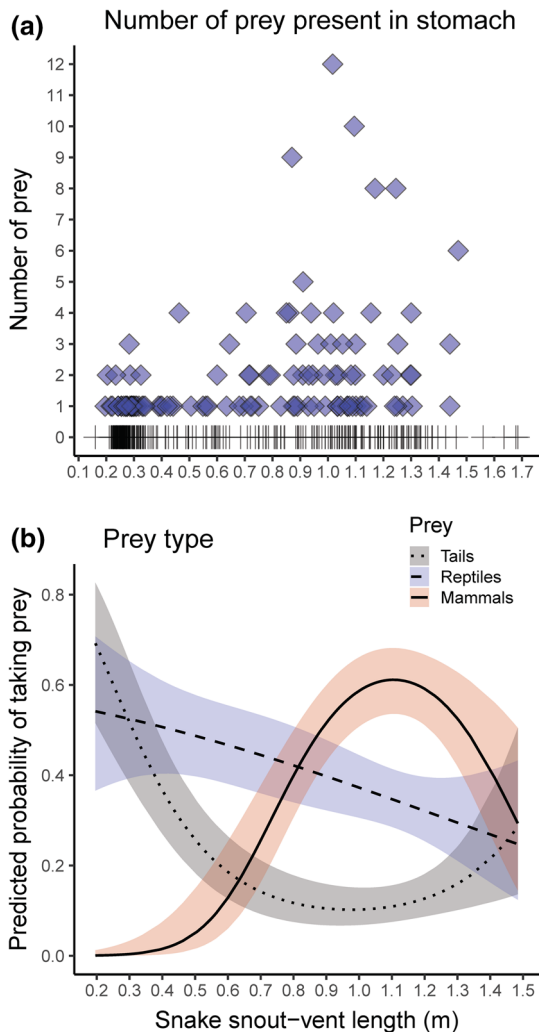
during prey handling, but their autotomised tail was also present in the snake's stomach. The most common smallest reptile species that could be identified was *Acritoscincus tri-lineatus* (m_b 0.73 ± 0.57 range 0.09–1.50 g, n = 10) and the largest species consumed was *Tiliqua rugosa* (m_b 24.10 ± 18.66, range 2.43–49.60 g, n = 7).

There were 120 mammal prey recorded representing at least 4 species. At least 67% of mammal prey items were confidently identified as the introduced house mouse *Mus musculus*, while 13% of mammal prey were juvenile rodents that could not be identified to species through macroscopic study and some of these therefore could also have been house mice. There were two specimens each of introduced rats (*Rattus norvegicus* and *Rattus rattus*) and the only native mammal species identified (categorised as *Notomys mitchelli*).

The smallest snakes only consumed lizard tails or small lizards (Fig. 2b). Intermediate-sized snakes were most likely to have consumed mammals, while the largest snakes, which showed a greater diversity of diet, had also fed on larger lizards.

Size of prey taken

The size of reptile prey taken by Dugites increased with snake size. Head length (p < 0.001, Fig. 3b) and mass (p = 0.001, Fig. 3c) of reptile prey taken by Dugites increased with size of the snake, but the pattern for prey SVL with snake size did not reach statistical significance (p = 0.151, Fig. 3a). There was a negative relationship between mammal prey head-body length and snake size (p = 0.004, Fig. 3a), although these data were strongly influenced by a single adult mouse taken by a 0.33 m SVL Dugite, and five juvenile mice taken by a 1.48 m SVL Dugite. The relationships between mammal prey head length (p = 0.127, Fig. 3b) and mass (p = 0.416, Fig. 3c) with snake size were not statistically significant.

Prey handling

There was evidence of a change of prey handling as Dugites increased in body size. Reptiles had largely been ingested either head-first (n = 57 snakes) or tail-first (n = 29 snakes), with only n = 7 snakes having prey of both orientations in their stomachs (Fig. 4). Small snakes (< 0.5 m SVL, n = 26) were significantly more likely to have ingested reptile prey tail-first ($\chi^2_1 = 23.45$, p < 0.001), while the largest snakes (1.0–1.5 m SVL, n = 31) were significantly more likely to have ingested reptile prey head-first ($\chi^2_1 = 8.49$, p = 0.004) (Fig. 4). The orientation of reptile prey ingested by the intermediate size category (0.5–1.0 m SVL, n = 29) was not significantly different from the expected proportions calculated as the average of all snakes ($\chi^2_1 = 2.79$, p = 0.095) (Fig. 4).

Do Dugites show an ontogenetic change in cranium shape?

Multivariate regression of Procrustes residuals representing cranium shape against SVL revealed 44% of the variation was associated with body size ($R^2 = 0.44$, P < 0.001). There was a small but significant difference between the sexes in this relationship ($F_{2,45} = 5.00$, p < 0.001). Cranium shape variability between individuals remained consistently low throughout our ontogenetic series while remaining closely correlated with body size (Fig. 5a).

Changes in shape of the anterior cranium (snout) with an increase in SVL were relatively slight, with the nasals, and to a lesser degree the frontals, becoming more

Fig. 3 Relationships between the **a** body size, **b** head length, and **c** mass of individual prey items with the snout-vent length of Dugites (*Pseudonaja affinis*) that these prey were collected from. Note that data for body length and head length were only available for intact reptiles and mammals
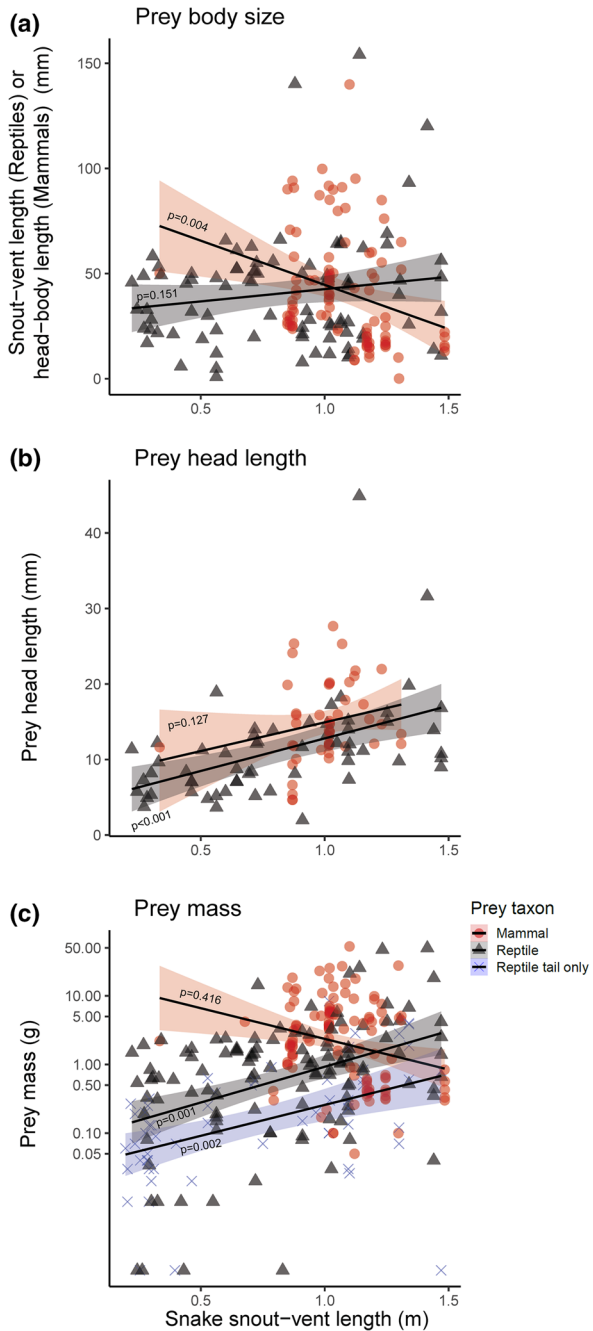
elongated and compressed in larger snakes (Fig. 6). By contrast, substantial changes in the shape of the posterior cranium (braincase) were evident with increasing SVL, specifically in the supraoccipital and occipital bones (located posterior to the parietal

Fig. 4 The orientation of prey taken by Dugites (*Pseudonaja affinis*) grouped into three size classes (*n* is number of snakes in each class). Asterisks indicate level of significance **p < 0.01, ***p < 0.001 for a Pearson's Chi test testing hypothesis that head-first or tail-first feeding strategies were in equal frequency across the different snake body size categories; note that snakes with multiple prey in both orientations were excluded from this analysis
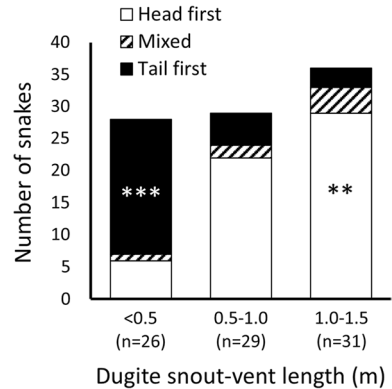
bone on dorsal surface), which change from smooth and wide in juvenile individuals to a more compressed, ridged and elongated form in adults (Fig. 6).

Do Dugites show an ontogenetic change in relative dimensions of trophic bones?

Multivariate regression of the trophic bone dimensions against SVL revealed 37% of the variation was associated with SVL ($R^2 = 0.37$, P < 0.001). There was a small but significant difference between the sexes in this relationship ($F_{2, 45} = 5.44$, p < 0.001) (Fig. 5b). In contrast with cranium shape, trophic bone variability between individuals appeared to increase during postnatal ontogeny (Fig. 5b).

When examining each bone individually, RMA regressions revealed that ectopterygoid height (EH) scaled isometrically while all other measurements scaled with significant negative allometry against SVL (Fig. 7a, Appendix Table 1a). Against head length, most trophic bone measurements scaled with significant positive allometry, the only exception was quadrate diameter (QD), which scaled isometrically (Fig. 7b, Appendix Table 1b).

The trophic bones changed from short and slender in juveniles to elongate and robust in adults (Fig. 8). Dimensions that had the highest contribution to the multivariate regression score were jaw length, ectopterygoid height, quadrate diameter, and pterygoid length (Fig. 8), revealing that these dimensions experienced the most dramatic change with increasing body size, relative to all other dimensions. Jaw length had the most positive regression score, indicating that the lower jaw increased to the greatest degree during development relative to the other trophic bones (Fig. 8). Conversely, the ectopterygoid height had the most negative regression score, indicating that height of the ectopterygoid grew at a lower rate relative to the other trophic bones (Fig. 8). Additionally, there was a gradual backwards rotation of the quadrate bone.

Is there a change in skull morphology coinciding with an ontogenetic change in diet?

For cranium shape, the interaction term between SVL and size class category was marginally significant (MANCOVA, $F_{1,46} = 1.81$, p = 0.015), indicating a slight difference in the direction of the regression trajectories between larger snakes (> 0.8 m) and smaller
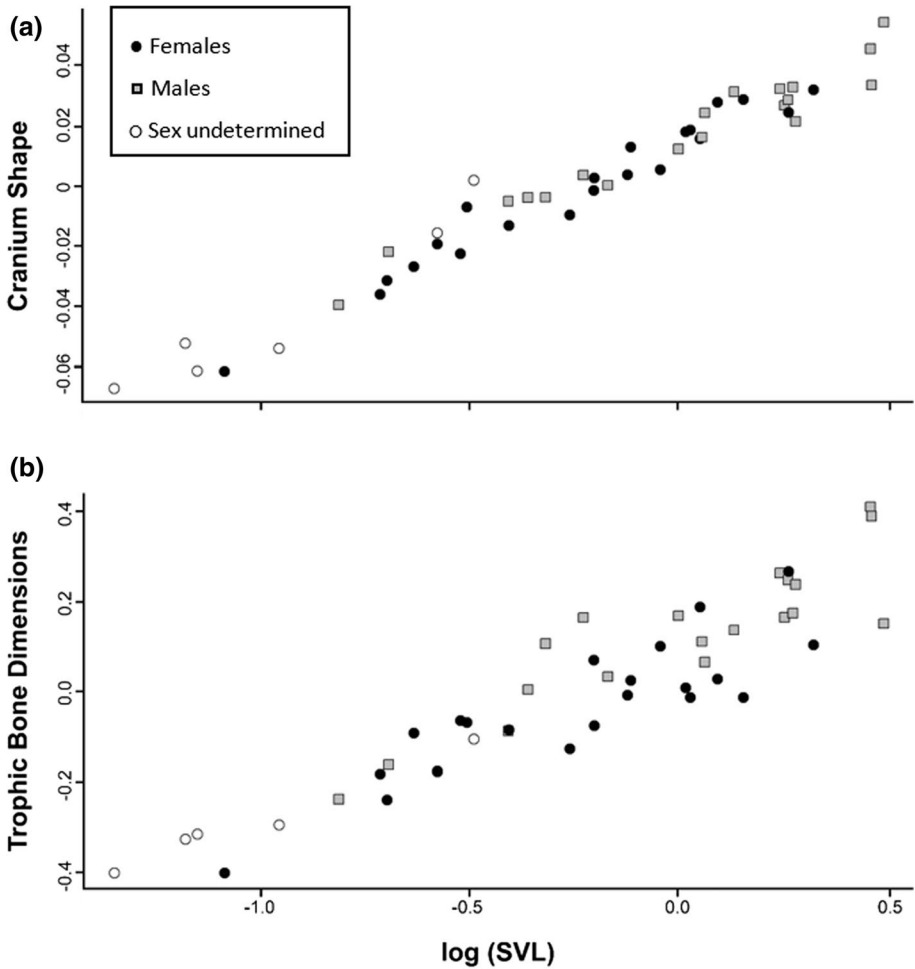
Fig. 5 Multivariate Regressions demonstrating ontogenetic allometry of the skull against body size (log$_{10}$-transformed SVL; m) for 46 Dugite (*Pseudonaja affinis*) specimens. **a** Regression scores for cranium shape (Procrustes residuals), **b** Regression scores for trophic bone dimensions (log-shape ratios)

snakes (< 0.8 m) (Table 2). This suggests weak support for the hypothesis that there was a morphological shift in cranium shape associated with a change in diet at around this body size. For trophic bone dimensions, the interaction term was not significant ($F_{1,46} = 1.77$, p = 0.089), indicating that the larger snakes (> 0.8 m) did not have a different allometric trajectory in comparison to the smaller snakes (< 0.8 m) (Table 2), rejecting the hypothesis of a distinct morphological shift with the ontogenetic diet change.

Fig. 6 Changes in cranium shape through ontogenetic growth for 46 Dugite (*Pseudonaja affinis*) specimens relative to body size (SVL). Lollipop diagrams show landmark positional changes representing change in cranium morphology from a juvenile shape (red dot) to adult shape (end of yellow lollipop tail) presented in **a** dorsal, **b** lateral and **c** ventral views. Length of lollipop tail indicates the magnitude of change, and angle indicates direction of change. For reference, layered underneath is an image of the smallest juvenile specimen (landmark positions approximate). The largest magnitude of change that can be seen is medial translation of the posterior-dorsal landmarks indicating the negative allometry of the size of the cranium relative to the trophic regions of the skull
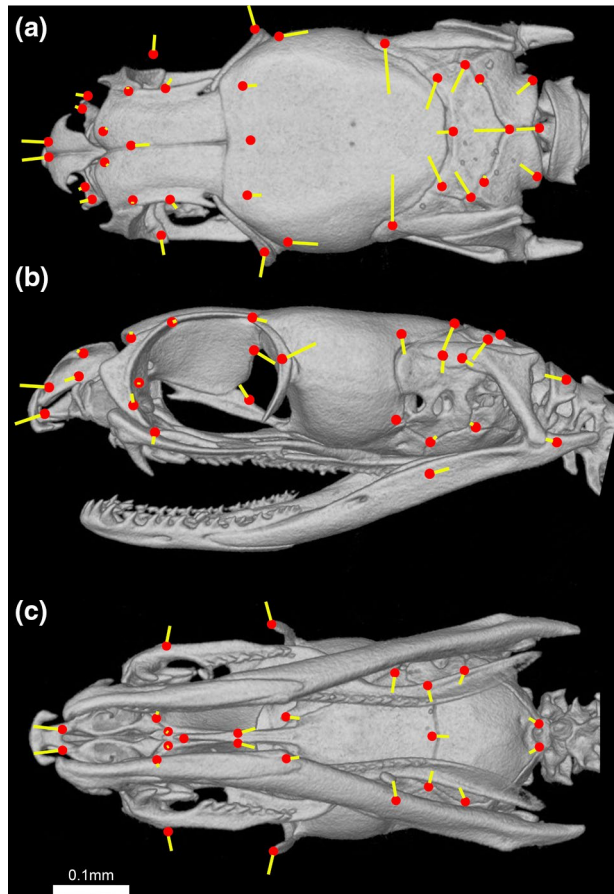
Discussion

Dugites demonstrate both a change in prey size and prey type as snakes grew larger, shifting from autotomised reptile tails to small reptiles, and later to mammals and larger reptiles. This ontogenetic change in diet would result in different challenges in terms of prey handling. We also quantified significant changes in both the cranium and trophic bones of Dugites during ontogeny. A significant proportion of variation in cranium shape (44%) and relative dimensions of trophic bones (37%) was associated with increasing body size in Dugites. There was also significant positive allometry of trophic bone dimensions with head size, indicating that these bones grow relatively larger as head size increases. These morphological changes were consistent with the ontogenetic change in diet in Dugites.
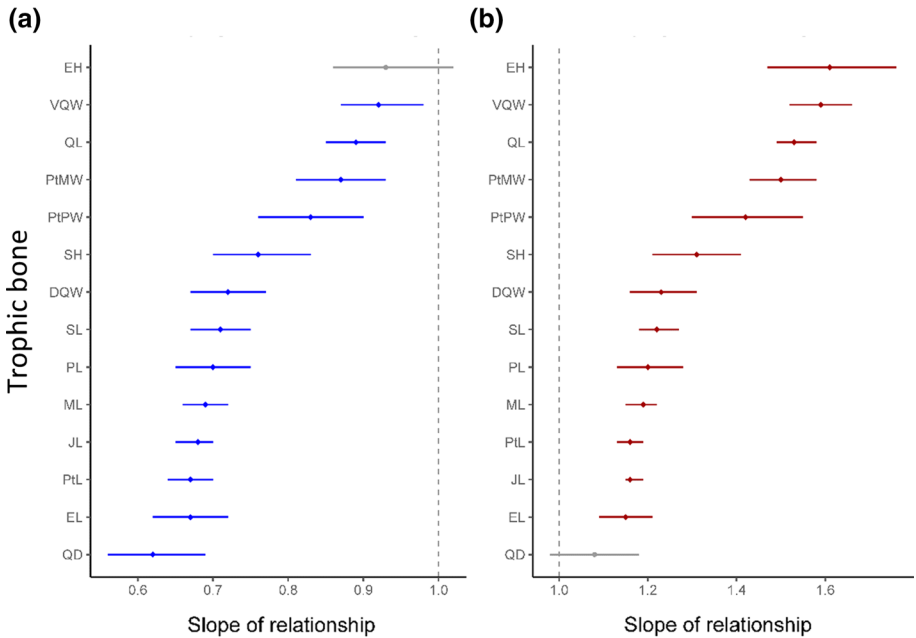
Fig. 7 Slope estimates from RMA regressions of trophic bone dimensions against $\log_{10}$-transformed **a** SVL and **b** head length for 46 Dugite (*Pseudonaja affinis*) specimens, showing the slope (allometric coefficient) and 95% confidence intervals. Colours show isometry (grey symbols) and negative (blue) or positive (red) allometry. Abbreviations are indicated in Table 1. Further details of these regressions are shown in Table A1

Ontogenetic change in diet

Rather than a distinct shift in diet, we found a gradual change in prey type and prey size taken by Dugites as they grow larger. Autotomised reptile tails and small reptiles were the main prey of juveniles and small adults (< 0.8 m SVL), but the proportion of these two prey types decreased as Dugites increased body size. Conversely, the proportion of mammals and larger reptiles in the diet of Dugites increased with body size, becoming the primary prey of large adults (> 0.8 m SVL). Notably, small reptile prey remained in the diet in even the largest animals, albeit in small proportions, rather than a complete prey-type switch.

A gradual change in diet to increasing prey size and/or diversity, rather than a complete prey switch, is the norm for most snakes as they grow (e.g. Bryant et al. 2012a; de Queiroz et al. 2001; Hampton 2011; Shine 1989, 1991; Vincent et al. 2004) (Table 3). Less frequently, species of snakes have a distinct shift in prey type as they grow (Table 3). For example, the green tree python (*Morelia viridis*) exhibits a shift from reptiles to birds and mammals, with individuals > 0.75 m SVL almost exclusively feeding on mammals (Natusch and Lyons 2012). Similarly, some colubrid watersnakes (e.g., *Nerodia erythrogaster* and *N. fasciata*) switch their diet quite abruptly from small fish to large frogs once they reach a particular body size (> 0.5 m SVL) (Mushinsky et al. 1982; Vincent et al. 2007).
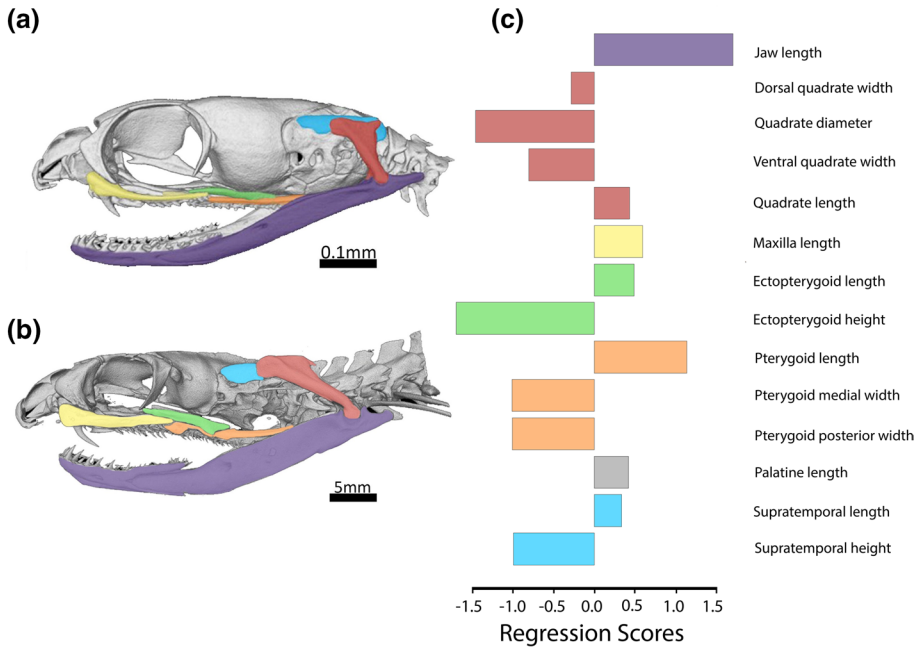
Fig. 8 Changes in linear dimensions of the trophic bones through ontogenetic growth for 46 Dugites (*Pseudonaja affinis*) specimens relative to body size (SVL), as given by their regression score. The main trophic bones of the skull are illustrated on a **a** juvenile and **b** adult. **c** A barplot of the regression scores for the log-shape ratios of the trophic bones, coloured as in (**a**) and (**b**), illustrates how each measurement changes relative to all others, and with increasing body size (log-transformed SVL)

Table 2 Results of MANCOVA testing for a distinct change in the allometric trajectory of skull shape with snout-vent length (SVL) between smaller (n = 21, < 0.8 m SVL) and larger (n = 25, > 0.8 m SVL) Dugite (*Pseudonaja affinis*) size classes

Term	DF	SS	MS	R^2	F	p
Cranium shape						
SVL	1	0.039	0.039	0.44	35.81	0.001
Size class	1	0.002	0.002	0.03	2.23	0.008
SVL x size class	1	0.002	0.002	0.02	1.81	0.015
Residuals	42	0.046	0.001	0.51		
Total	45	0.089				
Trophic bone dimensions						
SVL	1	1.486	1.486	0.37	26.99	0.001
Size class	1	0.168	0.168	0.04	3.05	0.006
SVL x size class	1	0.098	0.097	0.02	1.77	0.089
Residuals	42	2.313	0.055	0.57		
Total	45	4.064				

A significant interaction term indicates differences in the regression slope of each size class

Change in prey size and type, be it gradual or abrupt, can require changes in hunting behaviour and/or prey handling. Species that completely shift their prey type must often develop a new feeding strategy or behaviour to suit the different prey type (Eskew et al.

Table 3 Examples of ontogenetic shifts in diet and skull shape in the Macrostomata

Ontogenetic diet change	Ontogenetic skull morphology change	Species	Reference
General increase in prey size and diversity	No significant change in relative skull dimensions	*Agkistrodon piscivorous* *Morelia spilota*	Vincent et al. (2004) Shine (1991)
Distinct shift (increase) in prey size with SVL	Not examined	*Nerodia rhombifera* *Nerodia cyclopion* *Morelia s. imbricata*	Mushinsky et al. (1982) Mushinsky et al. (1982) Bryant et al. (2012a)
	Positive allometry of trophic bones, jaw muscles and gape relative to head length	*Nerodia fasciata*	Vincent et al. (2007)
Distinct shift in prey size and type with SVL	Not examined	*Nerodia erythrogaster* *Thamnophis validus* *Pseudonaja textilis*	Mushinsky et al. (1982) de Queiroz et al. (2001) Shine (1989)
	Positive allometry of trophic bones and gape relative to head length	*Morelia viridis*	Natusch and Lyons (2012)
	Cranium shape change from smooth surfaces to rugose bones with additional surface area for muscle attachment	*Pseudonaja affinis*	Present study
	Increasing robustness and positive allometry of trophic bones relative to head length; rotation of quadrate		

2009; Lind and Welsh 1994). Even where the change in diet is gradual, a change in behaviour may be advantageous. As prey get larger, they often become bulkier and therefore require different handling to manipulate and ingest. Prey may also become physically stronger or more robust, which can incur more risk of damage to the predator. From our sample of gut contents in Dugites, as well as prey type selection, there was evidence of a change in prey handling technique/feeding behaviour during ontogeny. Smaller Dugites swallowed reptile prey tail-first, while larger snakes swallowed reptiles head-first. Coupled with the greater incidence of tails-only in small snakes, this suggests that small snakes fail to take lizards before they effectively autotomise their tail (Bateman and Fleming 2009), while larger and older snakes have better prey handling. It would be an interesting future study to examine ontogenetic changes in prey handling behaviour.

Allometric change in skull morphology

Many previous studies of snake diet have inferred changes in skull morphology, such as larger trophic bones, gape size, jaw mobility and muscular strength, as a requirement for handling and ingesting the larger prey items as the snakes grow (Bryant et al. 2012; de Queiroz et al. 2001; Mushinsky et al. 1982; Shine 1989, 1991). Very few studies have quantified changes in skull morphology. Those studies that have demonstrated ontogenetic changes in both diet and skull morphology predominantly examined scaling patterns and changes in trophic morphology relative to body size (Hampton 2011; Natusch and Lyons 2012; Vincent et al. 2004, 2007), but few studies have quantified scaling patterns of trophic structures relative to head size (Vincent et al. 2007) (Table 3).

In Dugites, relative to body size, the shape of the cranium changed as the animals grew. The nasals and frontals became more elongated and compressed (frontals to a lesser degree), while the braincase changed from a rounded, smooth and broad form in juveniles to a much more compressed, narrow and ridged form with a distinct mid-sagittal crest forming on the parietal bone in adults. The posterior section of the cranium is where some of the major trophic elements (e.g., quadrate, supratemporal) attach to, and therefore the more obvious changes in shape seen here could reflect the importance of the roles these bones play in feeding (Vincent et al. 2007). These observations are consistent with previous studies showing that the general size and shape of back of the cranium is possibly linked to the ingestion of prey through interactions with these mobile trophic bones (Cundall and Gans 1979; Dwyer and Kaiser 1997).

The trophic bone dimensions relative to body size scaled with significant negative allometry, indicating that juvenile snakes had larger heads relative to their body size when compared with larger, adult snakes. This negative allometry of head size and trophic elements with body size has been reported in numerous snake species (King 2002; King et al. 1999; Vincent et al. 2006a, 2004), but also more generally in other vertebrates (e.g. Birch 1999; Meyers et al. 2018; Richard and Wainwright 1995). The skull morphology in smaller (<0.8 m SVL) Dugites is dominated by the relative size of the braincase and orbits, and thus the negative allometry of the head relative to body size seems best to reflect constraints imposed by the early development of the brain and sensory organs (Forsman 1991; King 2002; Vincent et al. 2006b, 2006c). Furthermore, juveniles presumably require a minimum head size to enable feeding.

In contrast to the relationship with overall body size, there was positive allometry for trophic bones in Dugites relative to head length. This corroborates other studies (Rossman 1980; Vincent et al. 2007, 2006c; Young 1989). Positive allometry of trophic bones

with head size is linked to ontogenetic changes in diet found in numerous snake species (Table 3) as larger trophic structures are strongly correlated with an increase in overall gape size. Elongation of the trophic bones is associated with increased jaw mobility (Cundall and Greene 2000; Scanferla 2016), and is likely to assist with the capture and ingestion of larger, bulkier prey consumed by larger snakes (Vincent et al. 2006c). In particular, elongation of the mandible and pterygoid, elongation and rotation of the quadrate, and extension and enlargement of the supratemporal have previously been linked with the ingestion, manipulation and processing of large prey in other snake species that have known shifts in diet (Cundall and Greene 2000; Hampton 2011; Scanferla 2016; Vincent and Mori 2007).

Ontogenetic allometry is the predictable change in a trait with increasing body size during growth — the key word being predictable — such that a linear regression can estimate this relationship (Huxley 1932). While we hypothesised that the change in diet at ~0.8 m SVL would coincide with a distinct change in the allometric trajectory of skull shape to facilitate feeding on large prey, evidenced by a significant difference in the regression slopes of the two size classes, this was not supported by our data. Instead, we found positive allometry ('hyperallometry') — i.e. disproportionate growth rates in particular dimensions of these bones relative to head size — that is likely to facilitate larger Dugites having a greater capacity to take larger prey.

The smaller trophic bones (relative to the size of the head) in juvenile Dugites limits the maximum prey size that they could ingest, restricts prey handling ability, and would likely increase handling time required to manipulate and swallow prey items (Shine 1991; Vincent et al. 2006c; Werner and Gilliam 1984). Small trophic bones also impact on the overall skull kinesis, as small bones will have a limited range of movements compared with the longer, more developed jaw bones seen in larger snakes (Cundall and Greene 2000). This seems to correlate with the diet of juvenile Dugites, which feed almost exclusively on the autonomised tails of skinks and geckos, prey items that are likely to require very little effort to manipulate and swallow.

Trophic bone size variance appears to increase with body size, which could reflect the greater range of prey sizes and types ingested by larger snakes and suggests greater phenotypic (feeding) plasticity for larger snakes (Aubret et al. 2004; Clifton et al. 2020). While gape is difficult to measure in preserved specimens (Jayne et al. 2018; King 2002; Vincent et al. 2007), positive allometry of the trophic bones suggests that larger Dugites have relatively larger gapes than do smaller Dugites, allowing for ingestion of larger, bulkier prey items. This pattern coincides with the observed increase in proportion of mammals and larger reptile prey consumed by larger Dugites.

As well as the ingestion of prey, gape size is also likely to constrain the size of prey that a snake has the capacity to envenomate, and thus may also be related to prey choice through this mechanism (Cundall and Greene 2000; Hampton 2011). As a note, while the introduced house mouse (*Mus musculus*) was the most common mammal prey we found, Dugites have co-evolved with native rodents (Geffen et al. 2011) that have presumably formed an important part of their native diet. This suggests that there is likely to always have been selection for ingestion of bulker mammal prey in Dugites.

Snakes (along with lizards) display a unique pattern of modularity in the skull compared with other vertebrates (Watanabe et al. 2019). The kinetic skull of snakes is expected to be especially modular (Andjelković et al. 2017; Rhoda et al. 2021), with weak statistical covariation between bones. Diversity of complex structures, like the skull, is facilitated by their modular structure, where bones can change in shape and size relatively independently in response to evolutionary selection for different functions (e.g. Albertson et al. 2005) or developmental plasticity resulting from use (e.g. Aubret et al. 2004). Diet appears to be

the main factor driving diversity in skull morphology among snakes and other squamates (Watanabe et al. 2019). Differential growth pattern of skull bones, resulting in allometric shape changes of the skull, is the mechanism by which much of this variation is attained (Da Silva et al. 2018; Sherratt et al. 2019). Since many ecological attributes of snakes are intrinsically linked to body size (Shine 1994a), studies of allometry are therefore powerful in understanding the ecological success and evolutionary diversity of snakes.

Data Availability Not Applicable.

Code Availability Not Applicable.

Declarations

Conflict of interest The authors have no conflict of interest to declare.

Ethical Approval This study used data that came from road-killed specimens collected from roads in Western Australia under the Department of Parks and Wildlife's Regulation 17 license (#SF009895).

Consent to participate Not Applicable.

Consent for Publication All authors have read and commented on the final version of this manuscript and consent to publication in its present form.

References

Adams DC, Rohlf FJ, Slice DE (2013) A field comes of age: geometric morphometrics in the 21st century. Hystrix 24:7–14

Adams, D.C., Collyer, M.L., Kaliontzopoulou, A. and Sherratt, E. 2018. Geomorph: software for geometric morphometric analyses, R package v. 3.0. 6: Vienna, Austria: R Foundation.

Albertson RC, Streelman JT, Kocher TD, Yelick PC (2005) Integration and evolution of the cichlid mandible: the molecular basis of alternate feeding strategies. Proc Natl Acad Sci 102:16287–16292

Andjelković M, Tomović L, Ivanović A (2016) Variation in skull size and shape of two snake species (*Natrix natrix* and *Natrix tessellata*). Zoomorphology 135:243–253

Andjelković M, Tomović L, Ivanović A (2017) Morphological integration of the kinetic skull in *Natrix* snakes. J Zool 303:188–198

Aubret F, Shine R, Bonnet X (2004) Adaptive developmental plasticity in snakes. Nature 431:261–262

Bateman PW, Fleming PA (2009) To cut a long tail short: a review of lizard caudal autotomy studies carried out over the last twenty years. *J. Zool.* Lond 277:1–14

Bates D, Maechler M, Bolker B, Walker S, Christensen RHB, Singmann H, Dai B, Grothendieck G, Eigen C, Rcpp L (2014) Package 'lme4.' R Foundation for Statistical Computing, Vienna

Bellairs A (1968) Reptiles. Hutchinson and Co, London, UK

Bellairs, A. (1969) Feeding and cranial mechanics. In *The Life of Reptiles* (A. Bellairs, ed), pp. 116–184. Weidenfeld and Nicolson, London, UK

Birch JM (1999) Skull allometry in the marine toad, *Bufo marinus*. J Morphol 241:115–126

Bloom W, Fawcett DW (1975) A textbook of histology. W. B. Saunders Company, Philadelphia

Bryant GL, De Tores PJ, Warren KA, Fleming PA (2012a) Does body size influence thermal biology and diet of a python (*Morelia spilota imbricata*)? Austral Ecol 37:583–591

Bryant GL, DeTores P, Warren KA, Fleming PA (2012b) Does body size influence thermal biology and diet of a python (*Morelia spilota imbricata*)? Austral Ecol 37:583–591

Bush B, Maryan B, Browne-Cooper R, Robinson D (2010) Field guide to the reptiles and frogs of the Perth region. Western Australian Museum, Perth, AU

Camilleri C, Shine R (1990) Sexual dimorphism and dietary divergence: differences in trophic morphology between male and female snakes. Copeia 1990:649–658

Cignoni, P., Callieri, M., Corsini, M., Dellepiane, M., Ganovelli, F. and Ranzuglia, G. 2008. Meshlab: an Open-Source Mesh Processing Tool. Paper presented at the 6th Eurographics Italian Chapter Conference, Salerno, Italy, 2008.

Clifton IA, Chamberlain JD, Gifford ME (2020) Role of phenotypic plasticity in morphological differentiation between watersnake populations. Integr Zoo 15:329–337

Collyer ML, Sekora DJ, Adams DC (2015) A method for analysis of phenotypic change for phenotypes described by high-dimensional data. Heredity 115:357–365

Cundall D, Gans C (1979) Feeding in water snakes: an electromyographic study. J Exp Zool 209:189–207

Cundall D, Greene HW (2000) Feeding in snakes. In: Schwenk K (ed) Feeding: Form, Function and Evolution in Tetrapod Vertebrates. Academic Press, San Diego, CA, pp 293–333

Cundall D, Rossman DA (1984) Quantitative comparisons of skull form in the colubrid snake genera *Farancia* and *Pseudoeryx*. Herpetologica 40:388–405

Da Silva FO, Fabre A-C, Savriama Y, Ollonen J, Mahlow K, Herrel A, Müller J, Di-Poï N (2018) The ecological origins of snakes as revealed by skull evolution. Nature Comm 9:1–11

de Queiroz A, Henke C, Smith HM, Guyer C (2001) Geographic variation and ontogenetic change in the diet of the Mexican Pacific lowlands garter snake, *Thamnophis validus*. Copeia 2001:1034–1042

Dellmann HD (2006) Dellmann's Textbook of Veterinary Histology. Blackwell Publishing, Iowa, USA

Drake AG, Klingenberg CP (2008) The pace of morphological change: historical transformation of skull shape in St Bernard dogs. Proc Royal Soc Biol Sci 275:71–76

Dwyer CM, Kaiser H (1997) Relationship between skull form and prey selection in the thamnophiine snake genera *Nerodia* and *Regina*. J Herpetol 31:463–475

Eskew EA, Willson JD, Winne CT (2009) Ambush site selection and ontogenetic shifts in foraging strategy in a semi-aquatic pit viper, the Eastern cottonmouth. J Zool 277:179–186

Forsman A (1991) Adaptive variation in head size in *Vipera berus* L. populations. Biol J Linnean Soc 43:281–296

Geffen E, Rowe KC, Yom-Tov Y (2011) Reproductive rates in Australian rodents are related to phylogeny. PLoS ONE 6:e19199

Günther A (1872) II.—Seventh account of new species of snakes in the collection of the British Museum. Annals Mag Nat Hist 9:13–37

Hampton PM (2011) Comparison of cranial form and function in association with diet in natricine snakes. J Morphol 272:1435–1443

Huxley JS (1932) Problems of Relative Growth. Methuen & Co., Ltd, London, UK

Jacobson ER (2007) Infectious Diseases and Pathology of Reptiles: Color atlas and text. CRC Press, Boca Raton, FL

Jayne BC, Voris HK, Ng PKL (2018) How big is too big? Using crustacean-eating snakes (Homalopsidae) to test how anatomy and behaviour affect prey size and feeding performance. Biol J Lin Soc 123:636–650

Kardong KV (2012a) Vertebrates: comparative anatomy, function, evolution, 6th edn. McGraw-Hill, New York

Kardong KV (2012b) Verterbrates: comparative anatomy, function, evolution, 6th edn. McGraw-Hill, New York

King RB (2002) Predicted and observed maximum prey size–snake size allometry. Funct Ecol 16:766–772

King RB, Bittner TD, Queral-Regil A, Cline JH (1999) Sexual dimorphism in neonate and adult snakes. J Zool 247:19–28

Klingenberg CP (2016) Size, shape, and form: concepts of allometry in geometric morphometrics. Dev Genes Evol 226:113–137

Klingenberg CP, Barluenga M, Meyer A (2002) Shape analysis of symmetric structures: quantifying variation among individuals and asymmetry. Evolution 56:1909–1920

Legendre, P. 2008. Model II Regression Users Guide, R Edition, 2008.

Lind AJ, Welsh HH Jr (1994) Ontogenetic changes in foraging behaviour and habitat use by the Oregon garter snake, *Thamnophis atratus hydrophilus*. Anim Behav 48:1261–1273

Lüdecke D (2019) Package ggeffects: tidy data frames of marginal effects from regression models. J Open Source Soft 3:772

Maryan B, Bush B (1996) The dugite or spotted brown snake (*Pseudonaja affinis*). Herpetofauna 26:22–34

Meyers JJ, Nishikawa KC, Herrel A (2018) The evolution of bite force in horned lizards: the influence of dietary specialization. J Anat 232:214–226

Mosimann JE, James FC (1979) New statistical methods for allometry with application to Florida red-winged blackbirds. Evolution 33:444–459

Mushinsky HR, Hebrard JJ, Vodopich DS (1982) Ontogeny of water snake foraging ecology. Ecology 63:1624–1629

Natusch DJ, Lyons JA (2012) Relationships between ontogenetic changes in prey selection, head shape, sexual maturity, and colour in an Australasian python (*Morelia viridis*). Biol J Lin Soc 107:269–276

O'Malley B (2005) Snakes In *Clinical Anatomy and Physiology of Exotic Species: structure and function of mammals, birds, reptiles and amphibians*. Elsevier Saunders, Edinburgh, New York, pp 77–93

Palci A, Lee MSY, Hutchinson MN (2016) Patterns of postnatal ontogeny of the skull and lower jaw of snakes as revealed by micro-CT scan data and three-dimensional geometric morphometrics. J Anat 229:723–754

R Core Team. 2018. R: A language and environment for statistical computing.

Rhoda D, Polly PD, Raxworthy C, Segall M (2021) Morphological integration and modularity in the hyperkinetic feeding system of aquatic-foraging snakes. Evolution 75:56–72

Richard B, Wainwright P (1995) Scaling the feeding mechanism of largemouth bass (*Micropterus salmoides*): kinematics of prey capture. J Exp Biol 198:419–433

Rohlf FJ, Slice D (1990) Extensions of the Procrustes method for the optimal superimposition of landmarks. Syst Biol 39:40–59

Romer AS, Parsons TS (1986) The Vertebrate Body. Saunders College Publishing, Florida

Rossman CE (1980) Ontogenetic changes in skull proportions of the diamondback water snake, *Nerodia rhombifera*. Herpetologica 36:42–46

Scanferla A (2016) Postnatal ontogeny and the evolution of macrostomy in snakes. Royal Soc Open Sci. https://doi.org/10.1098/rsos.160612

Sherratt E, Sanders KL, Watson A, Hutchinson MN, Lee MSY, Palci A (2019) Heterochronic shifts mediate ecomorphological convergence in skull shape of microcephalic sea snakes. Integr Comp Biol 59:616–624

Shine R (1989) Constraints, allometry, and adaptation: Food habits and reproductive biology of Australian brownsnakes (*Pseudonaja*: Elapidae). Herpetologica 45:195–207

Shine R (1991) Why do larger snakes eat larger prey items? Funct Ecol 5:493–502

Shine R (1994a) Allometric patterns in the ecology of Australian snakes. Copeia 1994:851–867

Shine R (1994b) Sexual size dimorphism in snakes revisited. Copeia 1994:326–346

Shine R, Schwaner T (1985) Prey constriction by venomous snakes: a review, and new data on Australian species. Copeia 1985:1067–1071

Vincent SE, Mori A (2007) Determinants of feeding performance in free-ranging pit-vipers (Viperidae: *Ovophis okinavensis*): key roles for head size and body temperature. Biol J Lin Soc 93:53–62

Vincent SE, Herrel A, Irschick DJ (2004) Ontogeny of intersexual head shape and prey selection in the pitviper *Agkistrodon piscivorus*. Biol J Lin Soc 81:151–159

Vincent S, Dang P, Herrel A, Kley N (2006a) Morphological integration and adaptation in the snake feeding system: a comparative phylogenetic study. J Evol Biol 19:1545–1554

Vincent SE, Moon BR, Shine R, Herrel A (2006b) The functional meaning of "prey size" in water snakes (*Nerodia fasciata*, Colubridae). Oecologia 147:204–211

Vincent SE, Vincent PD, Irschick DJ, Rossell JM (2006c) Do juvenile gape-limited predators compensate for their small size when feeding? J Zool 268:279–284

Vincent SE, Moon BR, Herrel A, Kley NJ (2007) Are ontogenetic shifts in diet linked to shifts in feeding mechanics? Scaling of the feeding apparatus in the banded watersnake *Nerodia fasciata*. J Exp Biol 210:2057–2069

Watanabe A, Fabre A-C, Felice RN, Maisano JA, Müller J, Herrel A, Goswami A (2019) Ecomorphological diversification in squamates from conserved pattern of cranial integration. Proc Natl Acad Sci 116:14688–14697

Werner EE, Gilliam JF (1984) The ontogenetic niche and species interactions in size-structured populations. Annu Rev Ecol Syst 15:393–425

Wiley, D.F., Amenta, N., Alcantara, D.A., Ghosh, D., Kil, Y.J., Delson, E., Harcourt-Smith, W., Rohlf, F.J., St. John, K., Hamann, B., Motani, R., Frost, S., Rosenberger, A.L., Tallman, L., Disotell, T. and O'Neill, R. 2007. University of California, Davis: Institute for Data Analysis and Visualization.

Wolfe AK, Bateman PW, Fleming PA (2017) Does urbanization influence the diet of a large snake. Current Zoology 64:311–318

Young BA (1989) Ontogenetic changes in the feeding system of the red-sided garter snake, *Thamnophis sirtalis parietalis*. I. Allometric analysis. J Zool 218:365–381

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.