Causes and consequences of body armour in the group-living lizard, *Ouroborus cataphractus* (Cordylidae)

by

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DECLARATION

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ABSTRACT

Cordylidae is a family of predominantly rock-dwelling sit-and-wait foraging lizards endemic to southern Africa. The significant variation in spine length and extent of osteoderms among taxa makes the family an excellent model system for studying the evolution of body armour. Specifically, the Armadillo lizard (*Ouroborus cataphractus*) offers an ideal opportunity to investigate the causes and consequences of body armour. Previous studies have hypothesised that high terrestrial predation pressure, resulting from excursions to termite foraging ports away from the safety of the shelter, has led to the elaboration of body armour and a unique tail-biting behaviour. The reduction in running speed associated with heavy body armour, in turn, appears to have led to the evolution of group-living behaviour to lower the increased aerial predation risk.

In this thesis, a comparative and integrative approach is used to provide more insight into the conditions under which body armour could have evolved in *O. cataphractus* and the consequences of body armour for life-history traits. Chapter 2 attempts to investigate how competitive and predatory pressures affect the activity patterns of *O. cataphractus*. Analysis of activity patterns, obtained via remote camera trapping techniques, show low levels of activity during summer in *O. cataphractus*, resulting from increased competition for food and high predation pressure. In contrast, a shift in activity to spring, when food availability is relatively high, appears to override the negative effects of body armour and group-living behaviour in *O. cataphractus*. Chapter 3 tests the hypothesis that body armour serves as protection against attacks from predators during foraging excursions away from the safety of the shelter. The relatively high skin toughness, due to the presence of thick osteoderms in the dermis, protects *O. cataphractus* against most terrestrial predators, while the skin toughness values for other cordylid lizards are well-below the bite forces of potential mammalian predators.

The remaining chapters focus strongly on the feeding behaviour of *O. cataphractus* and how it is linked to body armour. Chapter 4 investigates the consequences of a reduction in running speed for the diet and tests for the presence of compensatory alternative performance capacities (i.e. increased bite force or jaw closing velocity). A comparative phylogenetic analysis shows that the possession of body armour affects the proportion of evasive prey items that can be included into the diet, thereby restricting the prey spectrum of heavily armoured taxa, such as *O. cataphractus,* to slow-moving prey (e.g. Coleoptera). Although the results indicate a relatively

high bite force in *O. cataphractus*, the primary selection pressure seems to be tail-biting behaviour, rather than the consumption of hard-bodied Coleoptera (Chapter 5). Bite force, however, trades-off with jaw closing velocity in lizards. A novel lingual prehension mode, exclusive to *O. cataphractus* (Chapter 6) appears to have evolved in response to the force-velocity trade-off. Given the slow nature of lingual prehension, increased prey capture efficiency appears to be the main selection pressure (Chapter 7), rather than miminsing exposure to predators by reducing the time spent in the open.

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GENERAL INTRODUCTION

Cordylidae is a family of scinciform lizards endemic to sub-Saharan Africa and is comprised of two subfamilies: the oviparous Platysaurinae, and the viviparous Cordylinae. While the former is morphologically conserved, the latter underwent rapid cladogenesis, resulting in a high degree of morphological disparity (Stanley *et al*., 2011; Stanley, 2013). Cordylid lizards are predominantly rock-dwellers, though some species have adapted to a terrestrial lifestyle, and are present in a variety habitats ranging from lowland regions to mountain tops at high altitude (Mouton & Van Wyk, 1997). They are considered typical sit-and-wait foragers (reviewed in Whiting, 2007) and have a largely insectivorous diet (e.g. Mouton *et al*., 2000a; Van Wyk, 2000; Clusella-Trullas & Botes, 2008). Cordylidae are characterised by their variation in body armour, including the extent and arrangement of osteoderms and length of keratinous spines (Losos *et al*., 2002; Stanley, 2013). This variation in morphology ranges from an almost complete absence of body armour in *Platysaurus*, *Hemicordylus*, and *Pseudocordylus* to elaborated body armour in *Smaug* and *Ouroborus* (Stanley *et al*., 2011; Stanley, 2013).

A remarkable example of elaborated body armour is present in the Armadillo lizard (*Ouroborus cataphractus*). Sharp spines and rugose scales cover the entire body of this species, especially the postcranial, lateral and caudal regions are heavily spinose. In addition to the body armour, individuals will grasp the tail between the jaws when threatened and roll up into an impenetrable ball, with the spiny tail and legs protecting the soft under parts (Mouton *et al*., 1999). The general consensus is that armour in *O. cataphractus* evolved as an antipredator mechanism during foraging excursions away from the safety of the rock-crevice (Mouton, 2011). Although a wide range of invertebrate prey are included in the diet, especially during spring when food abundance is high, analysis of stomach contents has revealed that the southern harvester termite (*Microhodotermes viator*) constitutes the most important prey item (Mouton *et al*., 2000a; Shuttleworth *et al*., 2008). Individuals sporadically visit the foraging ports of *M. viator* nests, located some distance away from the crevice, where they feed on this abundant food source (Effenberger, 2004; Mouton, 2011). Sit-and-wait foragers typically take up an ambush position as close as possible to the refuge to facilitate a fast retreat in case of confrontation with a predator. The chances of outrunning a predator are thus highly affected by distance to the rock crevice (Cooper, 1997). For rock-dwelling species such as *O.*

cataphractus, which always retreats to a specific rock crevice, distance becomes a critical factor during foraging. Venturing away from the refuge implies an increased risk of mortality to predation for sit-and-wait foragers and this in turn will favour the evolution of alternative antipredator adaptations (Kacoliris *et al*., 2009; Zani *et al*., 2009), such as body armour in the case of *O. cataphractus*.

Body armour, however, has several disadvantageous side-effects. Firstly, it negatively affects running speed in lizards (Losos *et al*., 2002; Bergmann *et al*., 2009) and escape-response performance in fish (Andraso & Barron, 1995). Secondly, armour appears to be predator-specific. In the case of cordylid lizards, armour presumable does not protect against the sharp beaks and talons of birds of prey (Mouton & Flemming, 2001). Consequently, individuals are vulnerable to aerial predation during general maintenance behaviour at the rock crevice. It has been proposed that the group-living behaviour displayed in *O. cataphractus* is a direct consequence of the trade-off between body armour and running speed (Mouton, 2011). Group-living behaviour is not a unique phenomenon in the Cordylidae, but has evolved convergently among several members of the family (reviewed in Mouton, 2011). Although the causal factors underlying group-living behaviour are species-specific, individuals in *O. cataphractus* clearly gain from enhanced vigilance (Hayward, 2008). The response time of group-living individuals to a threat from an aerial predator is significantly faster than that of solitary individuals (Hayward, 2008). This suggest that group-living in this species evolved to reduce the aerial predation risk (Effenberger, 2004; Mouton *et al*., 2005; Mouton, 2011), as seen in social mongoose species (Stankowich *et al*., 2014).

Group-living behaviour should greatly increase intraspecific competition for food in this sit-and-wait forager (Shuttleworth *et al*., 2013). Moreover, individuals remain close to the rock-crevice as a result of increased vulnerability to aerial predation (Mouton & Flemming, 2001; Losos *et al*., 2002), leading to a decrease in foraging efficiency at the rock-crevice (Shuttleworth *et al*., 2013). Without the use of a food source away from the highly competitive environment close to the crevice, the effects of competition for food among group members may become detrimental (Mouton, 2011). Exploiting termites away from the communal rock crevice during summer and shifting activity to periods of high overall food availability are hypothesised to be the compensatory mechanisms deployed by *O. cataphractus* to counteract the negative consequences of competition for food (Visagie, 2001; Shuttleworth *et al*., 2008; Mouton, 2011; Shuttleworth *et al*., 2013).

The link between exploiting termites away from the crevice, development of heavy body armour and eventually the evolution of group-living behaviour, appears to resemble a

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complex event which has reinforced itself through a feedback loop (Shuttleworth *et al*., 2013). Hence, *O. cataphractus* provides an ideal opportunity to investigate the causes and consequences of body armour. The main aim of this study was to employ a comparative and integrative approach to provide more insight into the circumstances under which body armour evolved and the consequences of body armour for the life history traits of *O. cataphractus*. I designed six collective chapters, with information from each chapter being used to justify the hypotheses and predictions of the other chapters.

Chapter 2 attempts to investigate how competitive and predatory pressures affect the activity patterns of *O. cataphractus* and a closely-related and sympatric species *Karusasaurus polyzonus*. To corroborate and elaborate on the findings of Visagie (2001), remote camera traps were used to record long-term activity patterns in the two species. Additionally, I used camera trapping and placement of replica lizard models to investigate predator dynamics.

Chapter 3 attempts to test the hypothesis that body armour serves as protection against attacks from predators during foraging excursions away from the safety of the shelter. As armour will be ineffective against the attacks by birds of prey (Mouton & Flemming, 2001), it would have evolved to reduce terrestrial predation pressure. To test this hypothesis, I compared the toughness of the skin of various cordylid lizards to the actual bite forces of several mongoose species and investigated whether inter- and intraspecific variation in skin toughness had a morphological basis.

Chapter 4 investigates the consequences of body armour for feeding behaviour in cordylid lizards. The possession of body amour might inhibit individuals, not only from using running speed as an escape strategy (Losos *et al*., 2002; Bergmann *et al*., 2009), but also from benefitting from running speed during prey capture. Using a phylogenetic approach, I investigated whether alternative performance capacities (i.e. increased bite force or jaw closing velocity) can evolve to compensate for costs associated with impaired locomotor performance. In addition, I tested whether the possession of body armour affected the proportion of evasive and hard prey items that can be included into the diet.

Following Chapter 4, I investigated the constraint of rock-dwelling behaviour on head morphology (Herrel *et al*., 2001a; Lappin *et al*., 2006; Revell *et al*., 2008) and consequently bite force in Chapter 5. The ability to generate a high bite force might be advantageous for *O. cataphractus* as it could increase the potential prey spectrum and/or be a valuable aid for tail-biting behaviour. I thus expected morphological changes in head configuration to allow individuals to maintain or improve their bite force under the constraint of crevice-dwelling behaviour.

During summer, when reliance on termites is the highest (Shuttleworth *et al*., 2008), visibility to aerial predators might be particularly high due to the low vegetative cover. Individuals are thus expected to minimise the time spent outside the rock crevice and I expected adaptations that ensure a rapid feeding event. In Chapter 6, I investigated the morphological and/or behavioural specialisations of the feeding apparatus that allow *O. cataphractus* to minimise the duration of feeding bouts and maximise the intake of termites. For this purpose, I examined prey prehension in cordylid lizards. Subsequently, I tested the contribution of specific prey capture mechanisms to prey capture efficiency in Chapter 7.

The final chapter is dedicated to summarise the key findings of this thesis and attempts to put the results into perspective to help unravel the unique life-history traits of *O. cataphractus*.

<div align="right"># CHAPTER 2</div>

EFFECTS OF PREDATION RISK, COMPETITION AND WEATHER CONDITIONS ON THE ACTIVITY PATTERNS OF *OUROBORUS CATAPHRACTUS* AND *KARUSASAURUS POLYZONUS*[*]

ABSTRACT

Fluctuations in weather conditions, such as ambient temperature, have considerable impact on the activity levels of ectothermic organisms. Under certain conditions, however, inactivity might have a selective advantage over activity, as it increases survival by reducing exposure to predators and lessens intraspecific competition for shared food resources. Consequently, the effect of weather conditions on the activity patterns of ectotherms might be influenced by competitive and predatory pressures. Using remote camera traps, I recorded long-term activity patterns in two closely related sympatric cordylid lizards, *Karusasaurus polyzonus* and *Ouroborus cataphractus*. The former species is a solitary, fast-moving lizard, while the latter is a heavily armoured lizard that permanently lives in groups. The significant interspecific difference in antipredator morphology and degree of sociality allowed me to unravel the effects of predation, competition and weather conditions on the activity patterns of the two species. My results demonstrate that activity in *K. polyzonus* predominantly occurs during summer, when ambient temperatures are favourable enough to permit activity. Unsurprisingly, activity strongly related to temperature in this species. In contrast, a peak in activity during spring, coinciding with high food availability, was observed in *O. cataphractus*. Activity during summer is limited and restricted to early morning and late-afternoon. High activity peaks, however, were observed after occasional summer rainfall. Contrary to *K. polyzonus*, none of the weather variables related to activity. The selective inactivity displayed by *O. cataphractus* appears to be a survival strategy related to the high intraspecific food competition and increased predation risk experienced during summer.

INTRODUCTION

In ectothermic organisms, activity provides the opportunity for thermoregulation, feeding and social interactions, such as mating and territory defence (Huey, 1982). It is generally assumed that favourable weather conditions, especially ambient temperature, promote activity in ectotherms (Rose, 1981; Grant & Dunham, 1988; Peterson *et al*., 1993), as the majority of physiological processes operate optimally when an organism reaches its preferred body temperature (Avery *et al*., 1982; Hertz *et al*., 1983; Van Damme *et al*., 1991; Bauwens *et al*., 1995; Xiang *et al*., 1996). Furthermore, variation in precipitation could stimulate activity, as is it responsible for shifts in food availability, especially in (semi-)arid environments (e.g. Reddy & Venkataiah, 1990; Vasconcellos *et al*., 2010). Several selection pressures, however, including predation risk and intraspecific competition for similar dietary resources, have been proposed to act against continuous activity (Simon & Middendorf, 1976; Rose, 1981; Huey, 1982). Consequently, the influence of weather variables on activity is strictly regulated by competitive and predatory pressures and activity patterns should therefore reflect the selective advantage of the positive and negative components of activity, as postulated by Rose (1981). While the effect of the interaction between abiotic and biotic factors on niche use has been shown in *Anolis* lizards (Lopez-Dariaz *et al*., 2012), little data are available to test how weather conditions shape activity patterns under competitive and predatory pressures in ectothermic organisms.

In this Chapter, I investigate the activity patterns of two closely related cordylid lizards, *Ouroborus cataphractus* and *Karusasaurus polyzonus*. The two species have an overlapping distribution (Bates *et al*., 2014) and co-occur along the west coast of South Africa. This semi-arid region is characterised by a peak in vegetative growth and arthropod abundance during spring, resulting from winter rainfall, followed by a long period of drought (Desmet & Cowling, 2004). *Ouroborus cataphractus* and *K. polyzonus* utilise similar microhabitats and are often observed occupying the same shelter sites (Effenberger & Mouton, 2006). However, the two species differ greatly in antipredator morphology and degree of sociality. *Karusasaurus polyzonus* is a relatively fast, lightly armoured lizard and has a strictly solitary lifestyle (Visagie, 2001). In contrast, *O. cataphractus* is a slow-moving, heavily armoured lizard that usually lives in groups of two to six individuals, although larger groups of up to 60 individuals occur in suitable environments (Mouton *et al*., 1999; Effenberger & Mouton 2007).

The apparent opposite lifestyles of the two cordylid lizards make them excellent study organisms to examine the effect of weather conditions on activity patterns under competitive and predatory pressures. An examination of the effect of weather conditions on the activity patterns of *K. polyzonus* and *O. cataphractus*, however, requires emphasis on the relationship between the life history traits on the one hand, and predation and competition on the other hand. The two species display a sit-and-wait foraging strategy (Cooper *et al.,* 1997, Mouton *et al*., 2000a) typically observed in cordylid lizards (Whiting, 2007). Sit-and-wait foragers usually take up an ambush position close to the shelter (e.g. rock crevice) from which they chase prey over short distances. As a result, intraspecific competition for similar food resources is a major cost for a group-living sit-and-wait forager compared to a solitary sit-and-wait forager (Mouton, 2011), especially in larger groups (Mouton *et al*., 2000a). Additionally, in cordylid lizards, species either possess body armour consisting of keratinous spines and osteoderms in the dermis, or are adapted for a high sprinting capacity (Losos *et al*., 2002). The possession of body armour can serve as protection against attacks by small terrestrial mammals directly (Chapter 3) or might prevent extraction from shelters by predators (Cooper *et al*., 2000). Running speed is more effective against birds of prey, as body armour is assumingly ineffective against the beaks and talons of these aerial predators (Mouton & Flemming, 2001). Degree of body armour, however, is incompatible with running speed (Losos *et al*., 2002). Consequently, heavily armoured cordylid lizards appear to compensate for their reduction in running speed by remaining close to the rock shelter during general maintenance behaviour (Losos et al. 2002). In the case O. cataphractus, this would further increase the level of intraspecific competition for food (Mouton, 2011).

For a solitary species, such as *K. polyzonus,* that relies on running speed as an antipredator strategy, I hypothesise that favourable weather conditions (i.e. high temperatures) permit activity and that temporal fluctuations in activity patterns are resulting from short-term variation in weather conditions. I predict that activity will be strongly related to temperature in this species. For a group-living heavily armoured species, such as *O. cataphractus,* I hypothesise that the low food availability and resultant increase in intraspecific competition during summer causes seasonal fluctuations in activity patterns. I predict that activity will peak in spring when food availability is the highest, followed by a prolonged period of inactivity during summer when food availability is the lowest, as proposed by Visagie (2001). Furthermore, in contrast to *K. polyzonus*, I predict that activity in *O. cataphractus* will not be strongly affected by short-term variation in weather conditions. In addition, I test the hypothesis that the vulnerability of *O. cataphractus* to aerial predation further enhances selection for a

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seasonal peak in activity as the chance of getting caught by a predator during summer is higher than during spring due to the overall lower number of active lizards. I predict that the frequency of visits by predators at the rock crevice will be similar throughout the year, but that an activity peak in spring will dilute the predation risk, hence more attacks are to be expected on lizards during summer.

MATERIALS AND METHODS

STUDY SITE

The study site, located 20 km north of Lambert's Bay, Western Cape, South Africa was restricted to an isolated area of c. 0.02 km2 consisting of scattered sandstone outcrops. Leipoldtville Sand Fynbos vegetation, consisting of perennial grass (*Cladoraphis cyperoides*) and dwarf shrubs (e.g. *Galenia africana*, *Zygophyllum morgsana*) is present on the coastal plains (Mucina & Rutherford, 2006). This arid region is characterised by extensive vegetative ground cover (mainly annual Asteraceae) and a peak in arthropod abundance during spring (i.e. August to October) resulting from winter rainfall, followed by a long period of drought (Desmet and Cowling 2004, Mucina and Rutherford 2006, Desmet 2007). Annual rainfall is low (less than 200 mm), but the close proximity of the study site to the Atlantic Ocean (< 10 km) reduces the aridity greatly.

ACTIVITY DATA

To assess temporal and seasonal patterns in lizard activity, remote camera traps (Reconyx PC900 HyperFire, Reconyx Inc., Wisconsin, USA) were used. Compared to focal observations, camera trapping is a less dependent and labour-intensive method than focal observations to obtain activity data. Consequently, activity patterns can be inferred from data collected from an extended continuous period of time, instead of being inferred from data collected from a short period of time, typical for research methods (e.g. Beuchat, 1989; Lister & Aguayo, 1992; Hailey & Coulson, 1996). In addition, observer presence could potentially alter the behaviour of organisms or predators thereof (e.g. Sugerman & Hacker, 1980; Kerr *et al.*, 2004). The extreme sit-and-wait strategy deployed by these lizards and their rock-dwelling lifestyle provides a unique opportunity to monitor activity of a fixed group or individual lizards using remote camera trapping. Cameras were mounted onto sand-colour painted metal poles,

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80 cm above ground and positioned 1-2 m from a rocky outcrop inhabited by either one or both species. First, lizards were allowed to acclimatise to the new camera stimuli. For this purpose, lizard activity was continuously recorded at ten rocky outcrops for four weeks, after which all cameras were positioned at different rocky outcrops. After three months (i.e. from 1 January 2013 till 31 March 2013), the majority of the lizards in the area were acclimatised to the camera traps. Following the acclimatisation period, five rocky outcrops were selected, while the remaining five randomly placed cameras monitored the activity of predators in the surrounding environment. Rocky outcrops were selected based on the following criteria: (1) the rocky outcrop was required to be inhabited by both *O. cataphractus* and *K. polyzonus*, (2) the rocky outcrop was required to be a loose standing rock and (3) the rocky outcrop was required to have a simple crevice, with an opening on only one side of the rock. Furthermore, to account for the influence of group-size on activity in *O. cataphractus* (Visagie, 2001), rocky outcrops were selected that were inhabited by a different number of individuals. From 1 April 2013 till 31 March 2014, the cameras were programmed to take photographs every five minutes, from 07.00 h till 20.00 h (Fig. 2.1). Predator activity was recorded by infrared sensors throughout the day. Camera traps were checked every 4-8 weeks to ensure minimal disturbance.

Each day was divided into 24 consecutive half-an-hour intervals, with each interval being represented by six images. For each interval, I recorded the highest number of 'active' lizards. A lizard sitting with its body exposed outside the crevice, either mobile or immobile, was counted as 'active'. In this sense, activity includes a range of behaviours, including thermoregulation, feeding, mating and territory defence. The numbers of active individuals were summed up and divided by the total number of intervals (i.e. 24) to obtain a daily index of activity. In *O. cataphractus*, the total number of intervals was multiplied with the total group size in order to take the degree of sociality into account. This method allowed me to obtain an index of activity score ranging from 0 to 1, with 0 being no lizards active during the day and 1 being all lizards active during the entire day. Note that no discrimination was made between individuals within groups of *O. cataphractus* as I was interested in interspecific variation in activity patterns, rather than individual variation in activity.

METEOROLOGICAL DATA

Weather data were obtained from the Nortier weather station, 10 km south of the study site. Weather variables included temperature, humidity, rainfall, wind speed and barometric pressure. Additionally, the percent cloud cover was visually estimated from camera images.

PREDATION PRESSURE ESTIMATE

To test whether attack rates by predators on lizards differed between seasons, I made use of replica models (e.g. Schneider *et al.*, 1999; Diego-Rasilla, 2003; Vervust *et al.*, 2007; Daly *et al.*, 2008). A two-fold latex mould was constructed from an adult preserved specimen of *O. cataphractus*. Two 75 mm steal cut nails were inserted into the mould to provide weight and steadiness. Next, polyurethane foam (Alcolin, Cape Town, South Africa) was sprayed into the mould and was allowed to dry in an incubator at 35°C for at least one hour. Models were removed from the mould, excess foam was cut away and models were spray-painted. In order to match the colouration of the models to the population they represent, spectrophotometric data were obtained from Truter (2011) and models were painted accordingly. Models of *O. cataphractus* were deployed during spring (September 2012) and summer (March 2013). In total 200 models were placed: half of the models on a rock surface outside lizard shelters and half on a ground surface, with at least 2 meters between consecutive models. The bottom-side of each model was provided with a number and the GPS-coordinates of all models were taken so no visual objects that facilitate recovery (e.g. flags) had to be used. All models were recovered eight days post placement and checked for signs of attack. A model was considered to be "attacked" when it exhibited at least one mark by either bird (beak or claw marks) or mammal (teeth marks). Marks made by arthropods (mandibles) and rodents (incisors) were not counted as predator attacks.

In addition, I calculated the frequency of predator occurrence for each month. As not all camera traps were operational throughout the year, I standardised the monthly frequency of predator occurrence by dividing the number of observations of predators for each month by the number of camera trapping days (i.e. monthly sum of the total number of operational cameras per day).

STATISTICAL ANALYSES

A time-series analysis was performed using the activity data as dependent variable to determine the effect of weather variables on lizard activity. Because of the correlated nature of weather variables, prior to statistical analyses, a principal components analysis (PCA) was performed on a correlation matrix with $\log_{10}$-transformed mean temperature, mean wind speed and mean barometric pressure, $\log(1 + p)$-transformed total rainfall, and arcsine transformed

mean cloud cover and mean humidity. The resulting PC-scores were retained and used as input for subsequent analyses. Firstly, I tested for stationarity of the data by examining each variable separately for the presence or absence of a unit root (i.e. indication of non-stationarity) using the Augmented Dickey-Fuller test. Non-stationary time-series indicate that the values increase or decrease over time. As such, they violated the assumptions of the statistical estimation theory and are unsuitable for regression analysis (Granger & Newbold, 1974; Philips, 1986). Secondly, the relationship between each independent variable (i.e. PC-scores) and the dependent variable (i.e. lizard activity) was investigated by performing ordinary least squares regressions. Since weather, and potentially activity, tend not to change drastically from one day to the next, weather and activity variables from one day to the next might be highly correlated. Consequently, the Durbin-Watson statistic was used to test for autocorrelation in the residuals from the regression analysis. The Durbin-Watson statistic ranges from 0 (positive autocorrelation) to 4 (negative autocorrelation), with a value of 2 indicating that no autocorrelation in present in the sample (Durbin, 1970). If the Durbin-Watson statistic was below or above 2, the autocorrelation function (ACF) and partial autocorrelation function (PACF) of the residual series were examined and the correct model was included in the regression analysis. A slow decay of the ACF and censoring of the PACF indicates an autoregressive [AR(p)] model, while a slow decay of PACF and censoring of the ACF indicates a moving-average [MA(p)] model. A slow decay of both ACF and PACF indicates an autoregressive-moving-average [ARMA(p,q)] model. The regression coefficients and significance level were used to determine the strength of the effects of weather conditions on lizard activity. The principal component analysis was conducted in the Statistical Package for the Social Sciences (SPSS) Statistics 17.0.1 (SPSS Inc., Chicago, IL, USA). Time-series analyses were conducted using Eviews version 8.1 (Quantitative Micro Software, Irvine, CA, USA).

To test for differences in predation pressure between the seasons, the frequency of attacks on replica lizard models was calculated and compared between dry and rain season, as well as between models placed on a rock surface and on a sand substrate. Therefore, contingency table analyses were used, conducted in R 3.1.1 (R Development Core Team, 2014).

RESULTS

ACTIVITY PATTERNS

Five rocks inhabited by both *O. cataphractus* and *K. polyzonus* were selected for analysis of activity patterns. For *O. cataphractus*, rocks were selected to encompass a range of group-sizes (rock 1: $n = 4$, rock 2: $n = 14$, rock 3: $n = 28$, rock 4: $n = 3$, rock 5: $n = 9$ individuals). The large size of rocky outcrop 3 did not permit accurate detection of the activity of *K. polyzonus*, hence this individual was excluded from analysis. Despite the occurrence of missing data due to battery failure or damage caused by larger mammals, activity data were recorded during 280 days (range: 236-349 days) on average per rock. The camera trapping yielded a total of 223,860 photographs that served as input for my analyses of activity patterns.

In *K. polyzonus*, activity was low to absent from late autumn until spring, but increased during late spring and peaked during the dry summer months (Fig. 2.2, Fig. 2.3). During summer, lizards were active throughout the day, except around mid-day when temperatures became too high (Fig. 2.2). In *O. cataphractus*, an opposite pattern was present. During winter, days of inactivity were alternated by mid-day activity (Fig. 2.4, Fig. 2.5). Individuals were predominantly active from late-August till the beginning of November (Fig. 2.5). During summer, lizards remained inactive most of the time. When active though, activity was restricted to early morning and late afternoon / early evening (Fig. 2.4). However, during January and March, several peaks of high activity were detected, coinciding with occasional summer rainfall (Table 2.1, Fig. 2.5, Fig. 2.7).

EFFECT OF WEATHER CONDITIONS ON ACTIVITY PATTERNS

The principal component analysis conducted on the weather variables yielded three axes explaining 82.6% of the total variation (Table 2.2). The first axis was negatively correlated with temperature and positively with cloud cover and humidity. This axis represents a gradient from hot dry days typically observed during summer to cold cloudy days characterising winter. The second axis was negatively correlated with barometric pressure and represents thermal lows present in arid environments during the warm season. The third axis was positively correlated with wind speed and represents a gradient from windy to wind-still days (Table 2.2). None of the principal component data or activity data were stationary (i.e. no increase or decrease over time) (Table 2.3) and could therefore directly be used as input for ordinary least

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squares regression analysis with activity as dependent variable and the PC-scores as independent variables. Ordinary least squares regression revealed a statistically significant relationship between the index of activity and PC1 in *K. polyzonus* (Table 2.4, Fig. 2.6). In contrast, no statistically significant relationship between the index of activity and the principal component scores could be detected in *O. cataphractus* (Table 2.4).

PREDATION PRESSURE

Remote camera trapping indicated the presence of several terrestrial and aerial predators that can be classified as potential predators of the two species (Fig. 2.8, Fig. 2.9). Terrestrial predators were observed in the study area on 239 days, while aerial predators were detected by the camera traps on 44 days. Small grey mongoose (*Galerella pulverulenta*) and meerkat (*Suricata suricatta*) were responsible for the majority of the observations, while large grey mongoose (*Herpestes ichneumon*) and yellow mongoose (*Cynictis penicillata*) were only occasionally detected. Despite limited detection of aerial predators by camera traps placed near the ground surface, several predatory bird species were recorded. These include jackal buzzard (*Buteo rufofuscus*), African harrier-hawk (*Polyboroides typus*), southern pale chanting goshawk (*Melierax canorus*), rock kestrel (*Falco rupicolus*) and pied crow (*Corvus albus*). Terrestrial predators were active at similar rates throughout the year, with an activity peak in June and October (Fig. 2.10). Aerial predators were also detected at similar rates, despite limited camera trap detectability, but a peak in aerial predator activity (mainly *P. typus*) was observed in May (Fig. 2.10).

Replica models of *O. cataphractus* placed on sand substrate and rock surface were attacked at similar rates during each season (spring: Fisher Exact Test; $P = 0.68$, summer: $\chi^2 = 1.59$, $P = 0.21$). However, there was a seasonal effect on the proportion of attacked models depending on the substrate. Models placed on sand substrate were attacked more often during summer than during spring ($\chi^2 = 8.87$, $P = 0.003$, Fig. 2.12), but the frequency of attacks on models was similar on a rock surface ($\chi^2 = 0.80$, $P = 0.37$).

DISCUSSION

Favourable weather conditions have been widely assumed to regulate activity in ectothermic organisms (Rose, 1981; Grant & Dunham, 1988; Peterson *et al.*, 1993). Competitive and predatory interactions, however, can exert strong selection pressure on

organisms and might influence how weather variables affect activity patterns (Rose, 1981). My results uncovered that weather conditions affect the activity patterns differently in *Ouroborus cataphractus* and *Karusasaurus polyzonus*, two lizard species that differ significantly in their anti-predator morphology and degree of sociality. Consistent with my prediction, temperature plays an important role in determining the activity of *K. polyzonus* (Fig. 2.6). Consequently, this species' activity appears to be restricted to hot days traditionally associated with summer. In contrast, *O. cataphractus* shows a prolonged period of inactivity during this time of the year. I hypothesise that the increased competition for food at the rock-crevice resulting from group-living behaviour (Mouton, 2011; Shuttleworth *et al*., 2013) and the low overall food availability are the selection pressures driving inactivity. In addition, the cost of foraging outside the crevice might be high during summer given the intense predation pressure (Fig. 2.12). In *Anolis nebulosus*, for example, low levels of activity are hypothesised to be an adaptation to reduce predation risk (Lister & Aguayo, 1992). Although I could not discriminate between attacks from aerial and terrestrial predators, it highly unlikely that terrestrial predators, which rely mainly on smell, would attack a replica model. Hence, aerial predators, which are presumably unaffected by body armour (Mouton & Flemming, 2001), appear to pose a significant threat during summer.

Organisms are known to alter their feeding and foraging behaviour when faced with relatively high predation risk or in a highly competitive environment (Sih, 1993), because this could lead to reduced growth rates (Downes, 2001), body condition (Martin & Lopez, 1999; Rands & Cuthill, 2001) and fecundity (Skelly & Werner, 1990). In case of *O. cataphractus*, it appears that a number of mechanisms evolved to counter potential energy shortages during summer. Firstly, *O. cataphractus* has a low resting metabolic rate compared to other cordylid lizards (Mouton *et al*., 2000b). Secondly, and most importantly, dietary specialisation in feeding on southern harvester termites (*Microhodotermes viator*) appears to have evolved to reduce intraspecific competition for food during summer (Mouton, 2011). Individuals belonging to larger groups of *O. cataphractus* have been reported to consume more termites than individuals belonging to small groups during summer (Shuttleworth *et al*., 2008). The clumped nature of *M. viator* makes this a profitable food source during summer. Additionally, the location of termite foraging ports some distance away from the lizard shelters aids in reducing the intra-group competition for food at the rock crevice. Although the predation risk is higher during this time of the year, the clumped nature of termites minimizes the number of foraging excursions individuals need to undertake. Remote camera traps positioned at termite foraging ports show that *O. cataphractus* exploits termites in the late-afternoon and evening

during summer (Fig. 2.13). These findings are consistent with the activity peaks of *O. cataphractus* during this time period (Fig. 2.4) and indicate that foraging excursions might only be made when predator activity or visibility is lower, as suggested by Visagie (2001). Despite overall low activity during summer, several peaks in activity were observed in all groups after summer rainfall. The stimulating effect of rainfall on activity in arthropods inhabiting arid environments (Whitford & Ettershank, 1975; Dean, 1992) could have exerted an overriding effect on the tendency to remain inactive.

Although I did not explicitly test for variation in activity among groups, no visible differences in activity patterns related to group-size were detected in *O. cataphractus*. On the one hand, one could speculate that individuals belonging to small groups should experience less competition for food than individuals belonging to large groups (Mouton *et al*., 2000a) and can therefore afford to be more active during periods of low food availability. However, individuals belonging to small groups benefit less from enhanced vigilance (Hayward 2008), and consequently, activity might be costly despite lower competition for food. On the other hand, large groups appear to consume more termites during summer (Shuttleworth *et al*., 2008). This suggests that individuals belonging to large groups should visit termite foraging ports more frequently during summer. Variation in foraging behaviour away from the crevice might have been present, but this could unfortunately not be detected by the camera trapping method. More data are required to test the effect of group-size on foraging behaviour in *O. cataphractus*.

The finding that *O. cataphractus* displays a peak in activity during late-winter and spring are in concert with my hypothesis and corroborate the results from a study by Visagie (2001). The lower aerial predation pressure resulting from extensive vegetation cover during spring (Desmet, 2007) and protective effect of armour against terrestrial predation pressure (Chapter 3) allows individuals to make frequent foraging excursions away from the crevice, despite a high frequency of terrestrial predator occurrence. The abundance of arthropods allows individuals to build up energy reserves for summer. Flemming & Mouton (2002) recorded fat bodies of *O. cataphractus* weighing five times more than those of other cordylid lizards. In addition, *O. cataphractus* has the lowest preferred body temperature range of all cordylids evaluated to date (Truter *et al*., 2014). Although thermoregulation may be compromised in group-living individuals as a result of competition for suitable basking places (Truter, 2011), it might have evolved as an adaptation to feeding at lower ambient temperatures. Given the thermal dependence of prey capture behaviour and digestive efficiency (Greenwald, 1974; Avery *et al*., 1982; Van Damme *et al*., 1991; McConnachie & Alexander, 2004), a lower

optimal body temperature would allow individuals to capture and process prey efficiently during late winter and spring.

The restriction of *O. cataphractus* to the winter rainfall zone (WRZ) of South Africa (Chase & Meadows, 2007), and especially to areas with high solar radiation (Shuttleworth *et al*., 2013) concurs with my findings. A winter to late-spring peak in arthropod availability, associated with the flowering of annuals (Mucina & Rutherford, 2006) appears to be sufficient to counteract the negative consequences of heavy armour and group-living behaviour (Shuttleworth *et al*., 2013). This is further supported by the presence of larger group-sizes in the coastal areas compared to inland areas where the seasonal changes in vegetation are less strong (Shuttleworth, 2006). *Karusasaurus polyzonus*, on the contrary, has a much larger distribution area (Bates *et al*., 2014) and predominates the dry Succulent Karoo area east of the distribution of *O. cataphractus*. The differences in diet among the two closely related species (Chapter 4, 5), coupled with dissimilarities in physiology (e.g. optimal body temperature: Clusella-Trullas *et al*., 2007; Truter *et al*., 2014), morphology (e.g. body armour: Losos *et al*., 2002, Chapter 4) and behaviour (e.g. sociality: Mouton *et al*., 1999) allows the two species to coexist in the same habitat and supports their current sympatric distributions.

In summary, my results show a strong relationship between temperature and the activity levels of *K. polyzonus*, but no such relationship was present in *O. cataphractus.* While *K. polyzonus* is predominantly active during summer, the activity levels of *O. cataphractus* peak during spring or immediately following a summer rainfall event. These findings suggest that selective inactivity might be an adaptation to reduce the cost of intraspecific competition for food during the summer, resulting from low food availability and an increased aerial predation risk. In contrast, the effects of competition for food and aerial predation risk during summer are significantly less in the solitary fast-moving *K. polyzonus*, hence individuals can remain active during this time of the year.

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Table 1.1: Summary of weather variables indicating the climatic conditions experienced by lizards at the Lambert's Bay field site from April 2013 till March 2014.

	Mean temperature (°C)	Humidity (%)	Barometric pressure (hPa)	Wind Speed (m/s)	Cloud cover (%)	Rainfall (mm)
Apr-13	19.1 ± 4.4	61.6 ± 21.0	1006.5 ± 3.9	3.04 ± 0.99	23.2 ± 33.5	10.4
May-13	17.2 ± 4.1	68.6 ± 26.4	1006.7 ± 3.1	2.65 ± 1.12	34.4 ± 33.8	8.8
Jun-13	14.3 ± 2.9	74.1 ± 19.8	1008.5 ± 4.9	3.37 ± 1.53	44.9 ± 41.0	19.2
Jul-13	14.3 ± 3.2	74.3 ± 18.1	1010.1 ± 4.8	2.70 ± 0.91	27.7 ± 36.8	18.2
Aug-13	14.3 ± 3.2	71.3 ± 19.4	1008.2 ± 3.5	3.18 ± 0.99	45.9 ± 41.1	17.6
Sep-13	14.9 ± 2.7	70.9 ± 14.0	1009.4 ± 4.4	3.36 ± 1.04	33.9 ± 39.3	12
Oct-13	16.9 ± 2.1	70.8 ± 12.9	1006.3 ± 3.7	3.58 ± 1.29	45.9 ± 41.1	1.4
Nov-13	20.9 ± 3.4	62.9 ± 14.1	1002.7 ± 3.2	3.75 ± 1.04	33.9 ± 39.3	0.2
Dec-13	21.5 ± 2.3	64.9 ± 13.4	1002.7 ± 3.2	3.48 ± 1.15	30.1 ± 38.2	0
Jan-14	21.7 ± 2.5	69.6 ± 12.1	1000.5 ± 3.5	3.48 ± 0.93	24.8 ± 34.2	11.6
Feb-14	22.8 ± 4.1	66.4 ± 16.1	1000.2 ± 2.0	3.41 ± 1.15	18.4 ± 27.0	0
Mar-14	19.5 ± 2.3	70.2 ± 13.4	1003.3 ± 2.4	3.22 ± 1.08	20.2 ± 31.0	13.2

Table entries are monthly means ± standard deviation and total monthly rainfall.

Table 2.2: Summary of the results from a principal component analysis performed on the weather variables. Three principal components, explaining 82.6 % of the total variation, were retained from the PCA analysis. The factor coordinate correlations and eigenvalues of the variables are shown.

	PC1	PC2	PC3
Temperature	**-0.845**	0.403	-0.165
Humidity	**0.827**	0.032	-0.249
Barometric pressure	0.247	**-0.860**	0.358
Wind speed	-0.536	0.105	**0.722**
Cloud cover	**0.742**	0.422	0.108
Rainfall	0.542	0.497	0.526
Total variation explained	43.158	22.324	17.125
Eigenvalue	2.589	1.339	1.027
Values in bold represent loading scores greater than 0.70			

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Table 2.3: Results of Augmented Dickey Fuller (ADF) unit root tests conducted on the individual and averaged activity time-series, as well as on the principal components. Stationarity of the data (i.e. no increase or decrease over time) occurs when the t-statistic value is below the critical value.

	t-statistic	Critical value (5%)	P-value
Ocat 1	-8.461623	-3.427616	**< 0.0001**
Ocat 2	-6.429166	-3.429834	**< 0.0001**
Ocat 3	-8.717480	-3.423136	**< 0.0001**
Ocat 4	-6.809813	-3.428503	**< 0.0001**
Ocat 5	-8.762222	-3.423799	**< 0.0001**
Kpol 1	-5.009573	-3.428349	**0.0003**
Kpol 2	-10.23611	-3.429834	**< 0.0001**
Kpol 3	-7.779813	-3.428503	**< 0.0001**
Kpol 4	-5.362042	-3.424726	**0.0001**
Ocat avg	-8.077829	-3.422865	**< 0.0001**
Kpol avg	-5.568293	-3.423669	**< 0.0001**
PC1	-10.77794	-3.422218	**< 0.0001**
PC2	-12.66853	-3.422218	**< 0.0001**
PC3	-14.35100	-3.422218	**< 0.0001**

Statistically significant P-values are indicated in bold.

Table 2.4: Results from an ordinal least squares analysis on individual and species average activity time-series showing the relationship between each independent variable (i.e. PC-scores) and the dependent variable (i.e. lizard activity).

	Regression Coefficient	*P*-value	Durbin Watson statistic	Terms included in model
Ocat 1 * PC1	-0.0040	0.51	2.08	AR(1)
Ocat 1 * PC2	0.0022	0.71	2.08	AR(1)
Ocat 1 * PC3	0.0017	0.72	2.08	AR(1)
Ocat 2 * PC1	-0.0019	0.61	2.02	AR(1), AR(2)
Ocat 2 * PC2	-0.0008	0.98	2.02	AR(1), AR(2)
Ocat 2 * PC3	0.0022	0.48	2.02	AR(1), AR(2)
Ocat 3 * PC1	-0.0038	0.50	2.05	AR(1), AR(2)
Ocat 3 * PC2	-0.0017	0.75	2.05	AR(1), AR(2)
Ocat 3 * PC3	0.0087	0.06	2.05	AR(1), AR(2)
Ocat 4 * PC1	-0.0020	0.85	2.04	AR(1), AR(2)
Ocat 4 * PC2	-0.0062	0.54	2.04	AR(1), AR(2)
Ocat 4 * PC3	-0.0046	0.61	2.04	AR(1), AR(2)
Ocat 5 * PC1	0.0007	0.88	1.97	AR(1), AR(2)
Ocat 5 * PC2	-0.0034	0.41	1.98	AR(1), AR(2)
Ocat 5 * PC3	0.0054	0.14	1.98	AR(1), AR(2)
Kpol 1 * PC1	-0.0215	0.12	2.10	AR(1), AR(2)
Kpol 1 * PC2	-0.0170	0.20	2.10	AR(1), AR(2)
Kpol 1 * PC3	-0.0141	0.20	2.10	AR(1), AR(2)
Kpol 2 * PC1	-0.0454	**0.002**	1.96	AR(1), AR(2)
Kpol 2 * PC2	0.0112	0.43	1.95	AR(1), AR(2)
Kpol 2 * PC3	0.0018	0.89	1.96	AR(1), AR(2)
Kpol 3 * PC1	-0.0431	**0.002**	2.04	AR(1), AR(2)
Kpol 3 * PC2	-0.0057	0.68	2.05	AR(1), AR(2)
Kpol 3 * PC3	-0.0056	0.64	2.05	AR(1), AR(2)
Kpol 4 * PC1	-0.0405	**0.002**	2.08	AR(1), AR(2)
Kpol 4 * PC2	-0.0147	0.24	2.09	AR(1), AR(2)
Kpol 4 * PC3	-0.0043	0.70	2.09	AR(1), AR(2)
Ocat avg * PC1	-0.0025	0.59	2.04	AR(1), AR(2)

Table 2.4: Continued

Ocat avg * PC2	-0.0011	0.80	2.04	AR(1), AR(2)
Ocat avg * PC3	0.0046	0.23	2.05	AR(1), AR(2)
Kpol avg * PC1	-0.0286	**0.001**	2.00	AR(1), AR(2), AR(3)
Kpol avg * PC2	-0.0063	0.49	2.00	AR(1), AR(2), AR(3)
Kpol avg * PC3	-0.0045	0.56	2.11	AR(1), AR(2)

Statistically significant *P*-values are indicated in bold. Durbin-Watson statistic after inclusion of the model terms is presented. Legend: AR: autoregressive.

Figure 2.1: Camera trap photographs illustrating the type of image used to calculate the activity index of *Ouroborus cataphractus* (top) and *Karusasaurus polyzonus* (bottom).
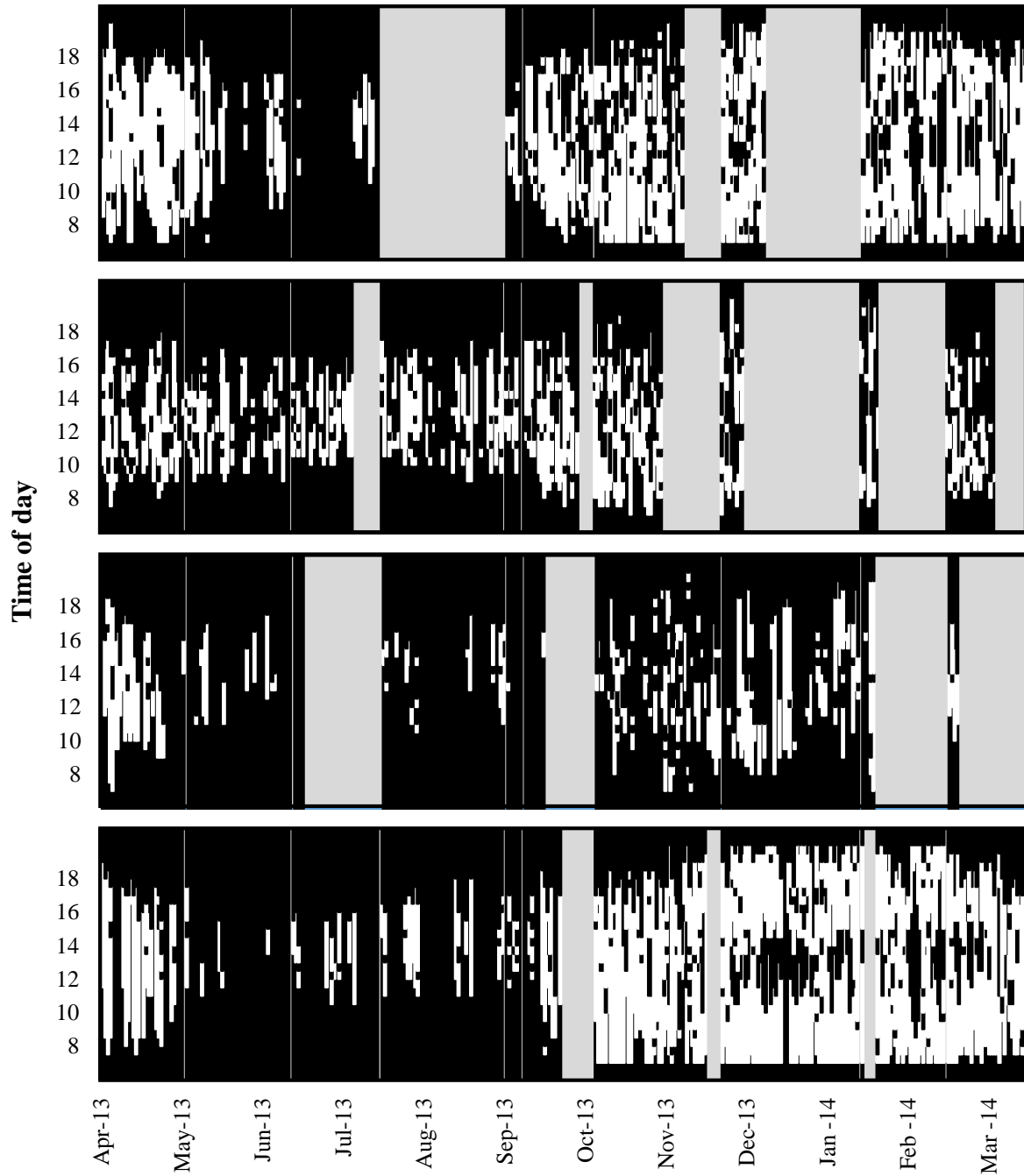
Figure 2.2: Length of daily activity (represented by the white bars) of four *Karusasaurus polyzonus* individuals from April 2013 till March 2014. Missing data are indicated by grey bars.

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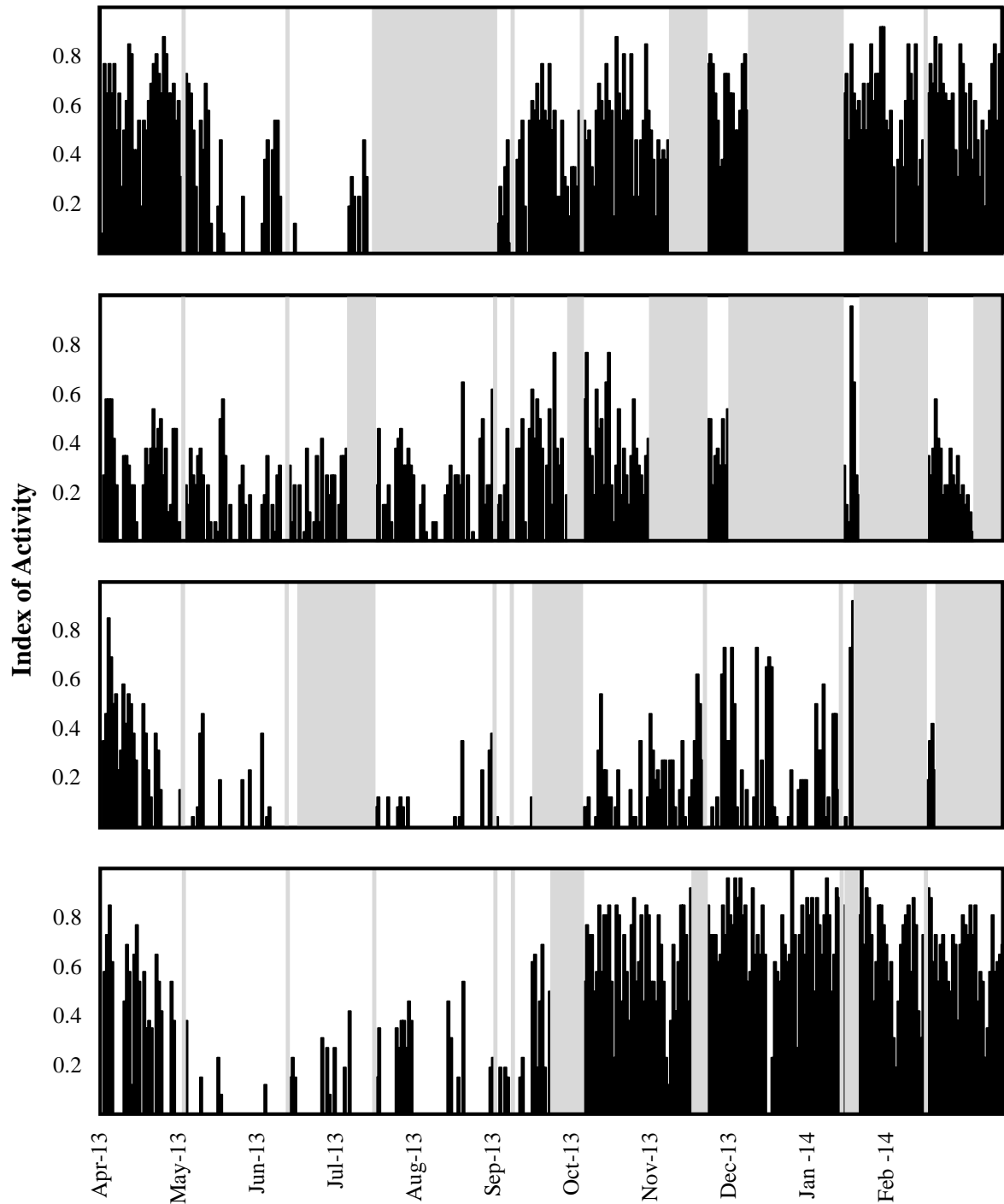
Figure 2.3: Index of activity of four *Karusasaurus polyzonus* individuals from April 2013 till March 2014. Missing data are indicated by grey bars.

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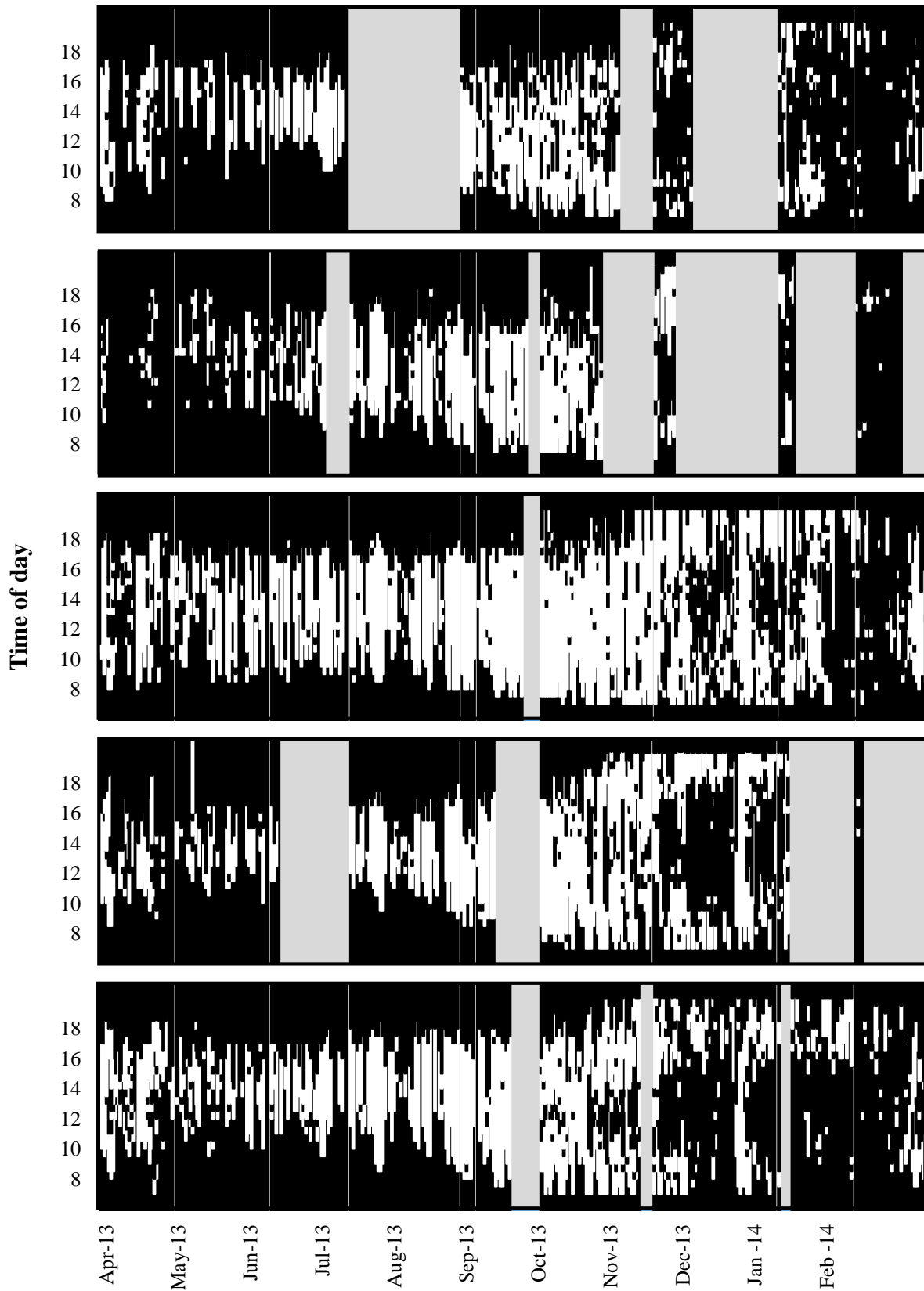
Figure 2.4: Length of daily activity (represented by the white bars) of five *Ouroborus cataphractus* groups from April 2013 till March 2014. Missing data are indicated by grey bars.
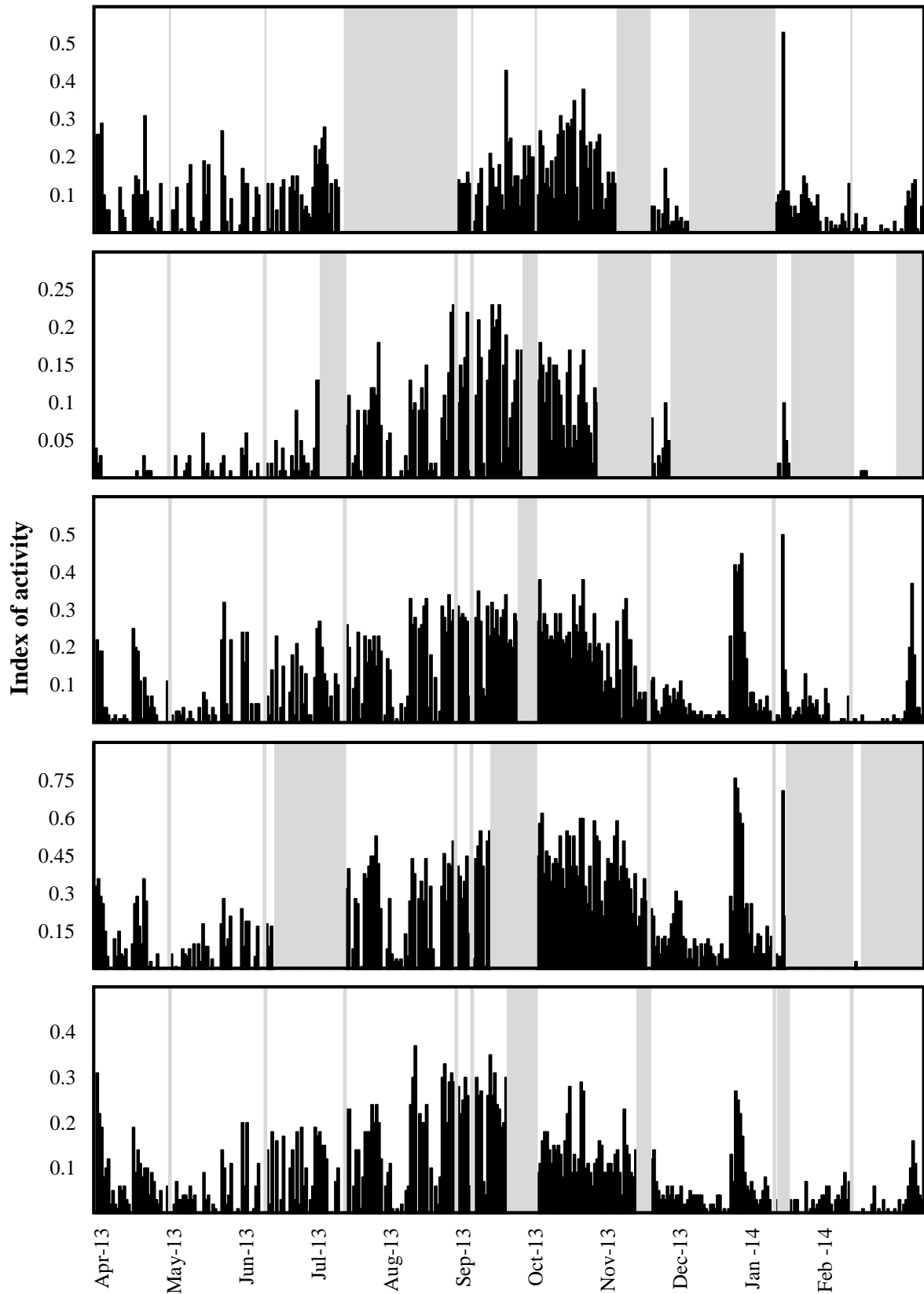
Figure 2.5: Activity pattern of five *Ouroborus cataphractus* groups from April 2013 till March 2014. Missing data are indicated by grey bars.

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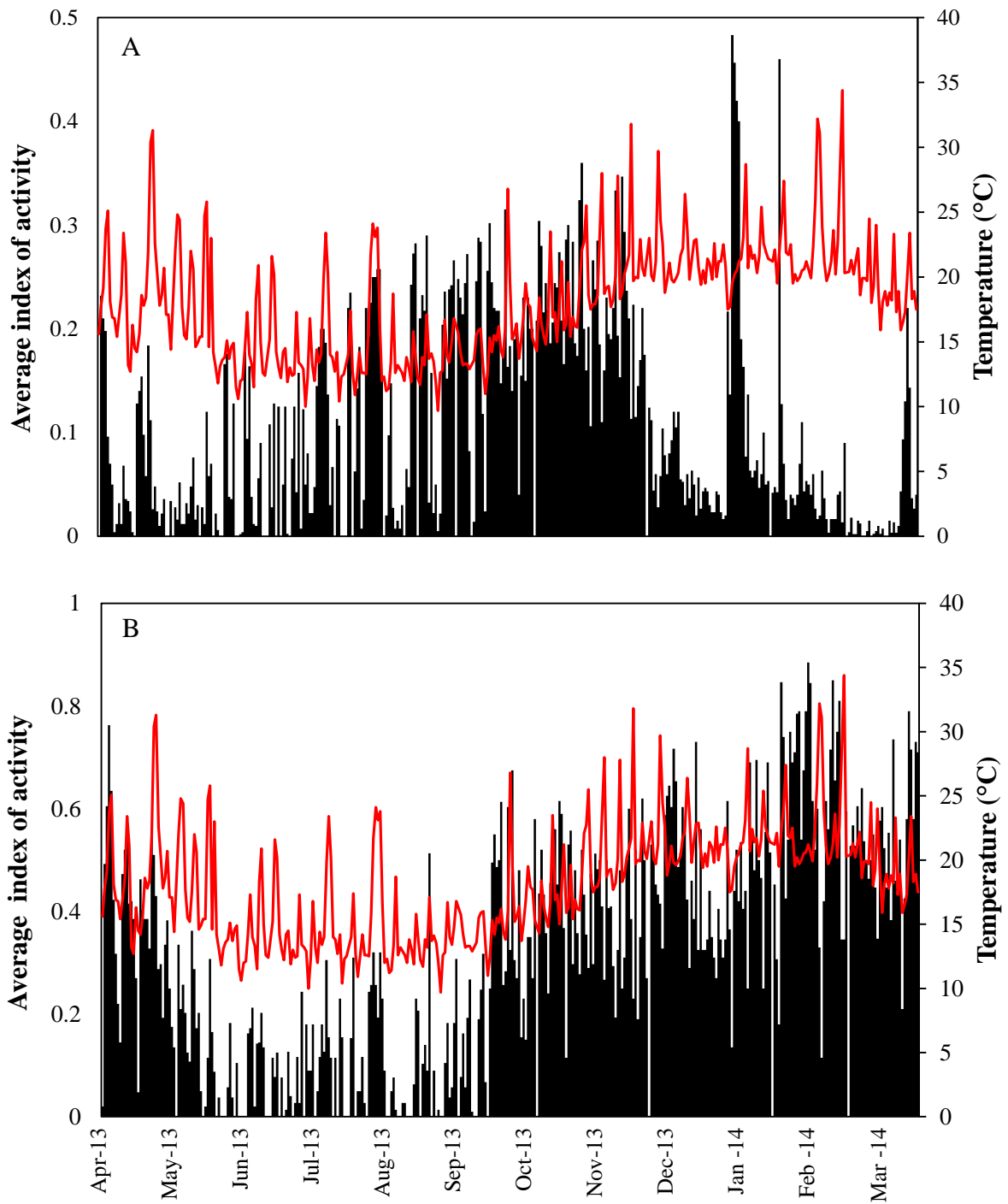
Figure 2.6: Average index of activity of *Ouroborus cataphractus* (A) and *Karusasaurus polyzonus* (B). Temperature, represented by the red line, is superimposed.

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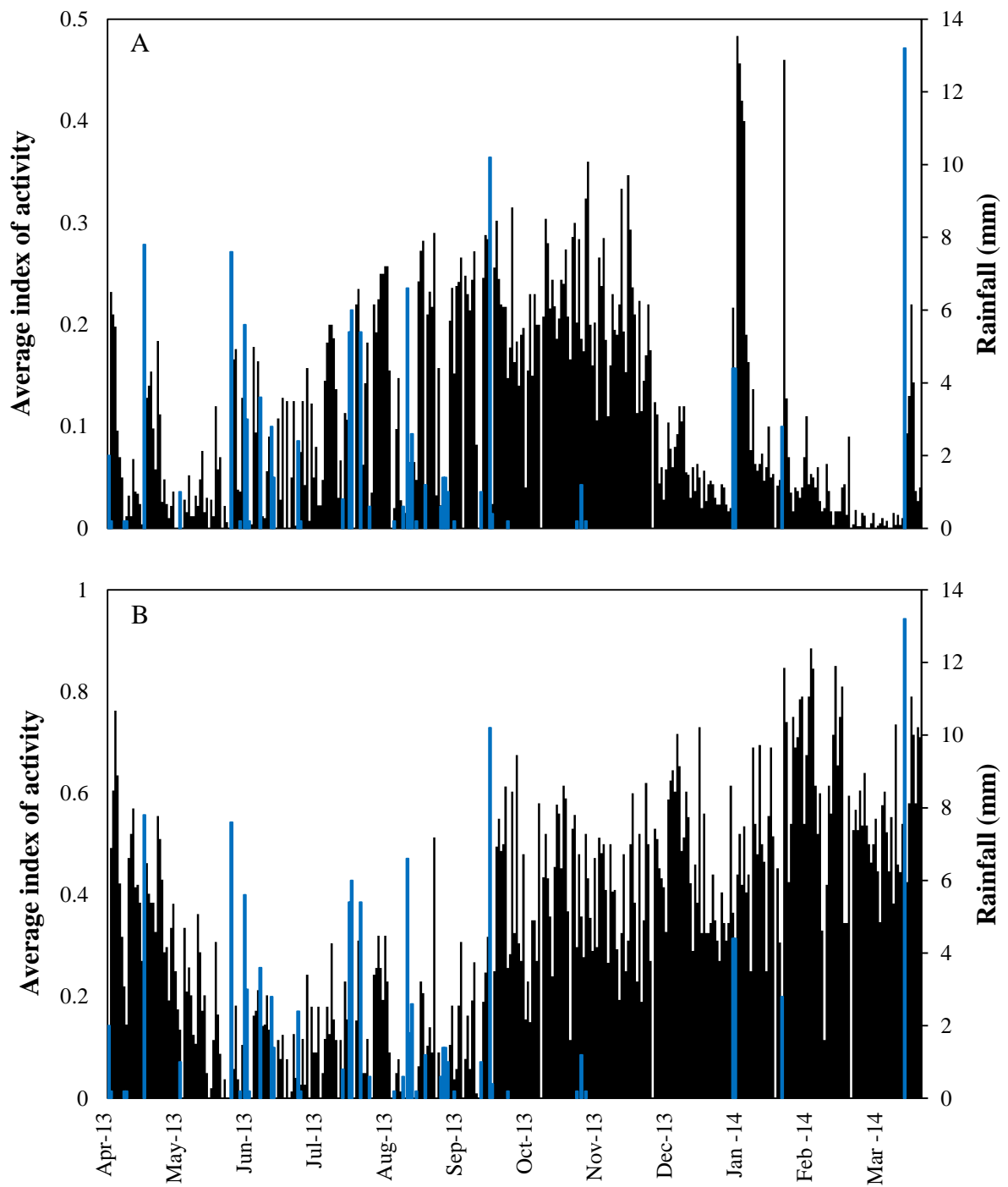
Figure 2.7: Average index of activity of *Ouroborus cataphractus* (A) and *Karusasaurus polyzonus* (B). Total rainfall, represented by the blue bars, is superimposed.

Figure 2.8: Camera trap photographs illustrating examples of terrestrial predators present in the habitat. Top: small grey mongoose (*Galerella pulverulenta*), bottom: meerkat (*Suricata suricatta*).

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Figure 2.9: Camera trap photographs illustrating examples of aerial predators present in the habitat. Top and bottom: African harrier-hawk (*Polyboroides typus*), bottom: pied crow (*Corvus albus*).
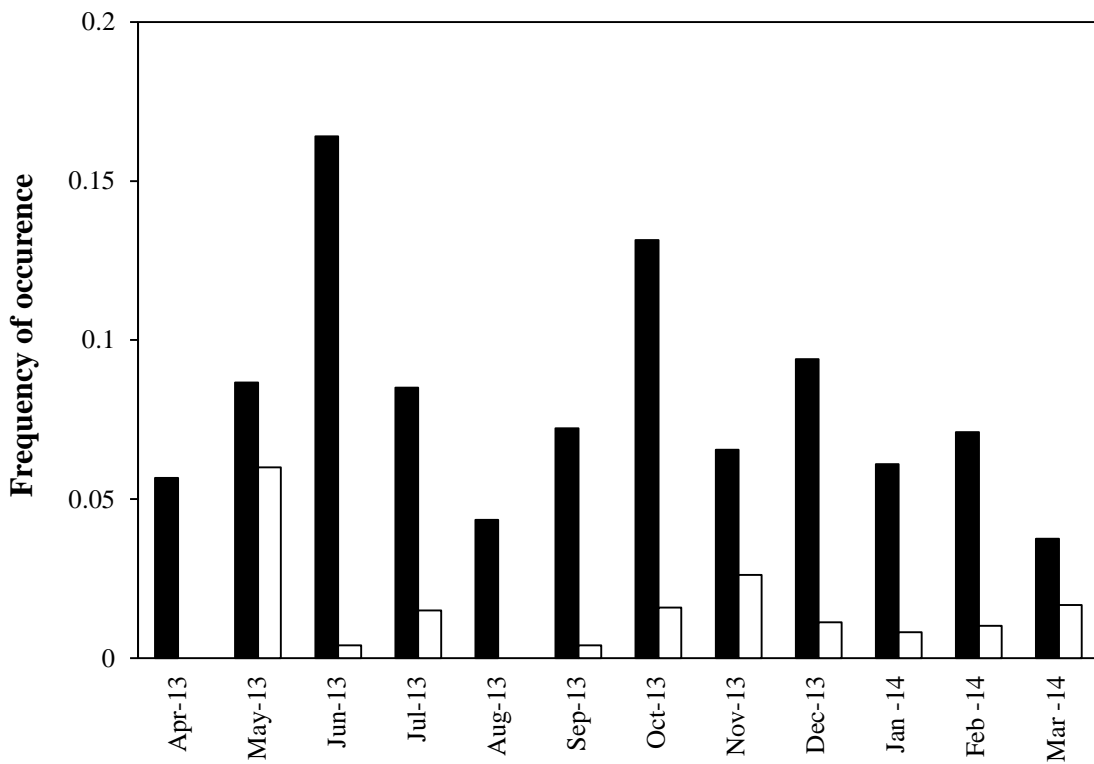
Figure 2.10: Monthly frequency of occurrence of aerial (□) and terrestrial (■) predators. Terrestrial predators were particularly active in June and October, while aerial predators were mainly observed in May.
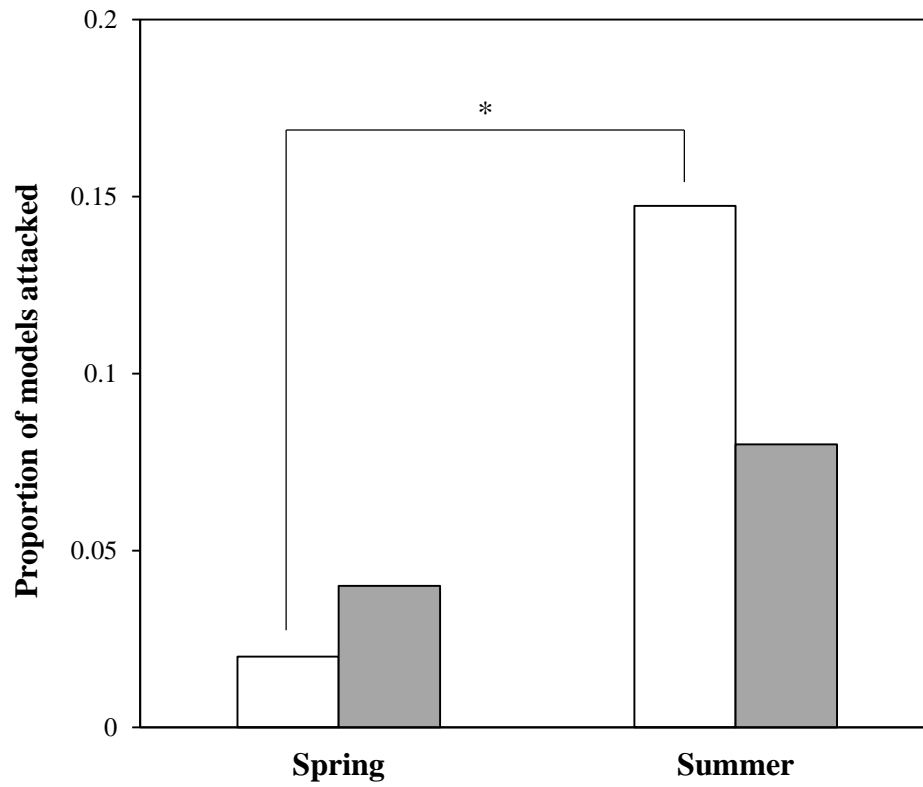
Figure 2.11: Differences in frequency of attacks on models placed on sand substrate in the open (□) and on a rock surface near crevices (■) during both seasons. The asterisk indicates a statistically significant difference in predation pressure.

Figure 2.12: Photographs showing the placement of replica models of *Ouroborus cataphractus* on sand substrate to illustrate the differences in ground cover between spring (A) and summer (B).
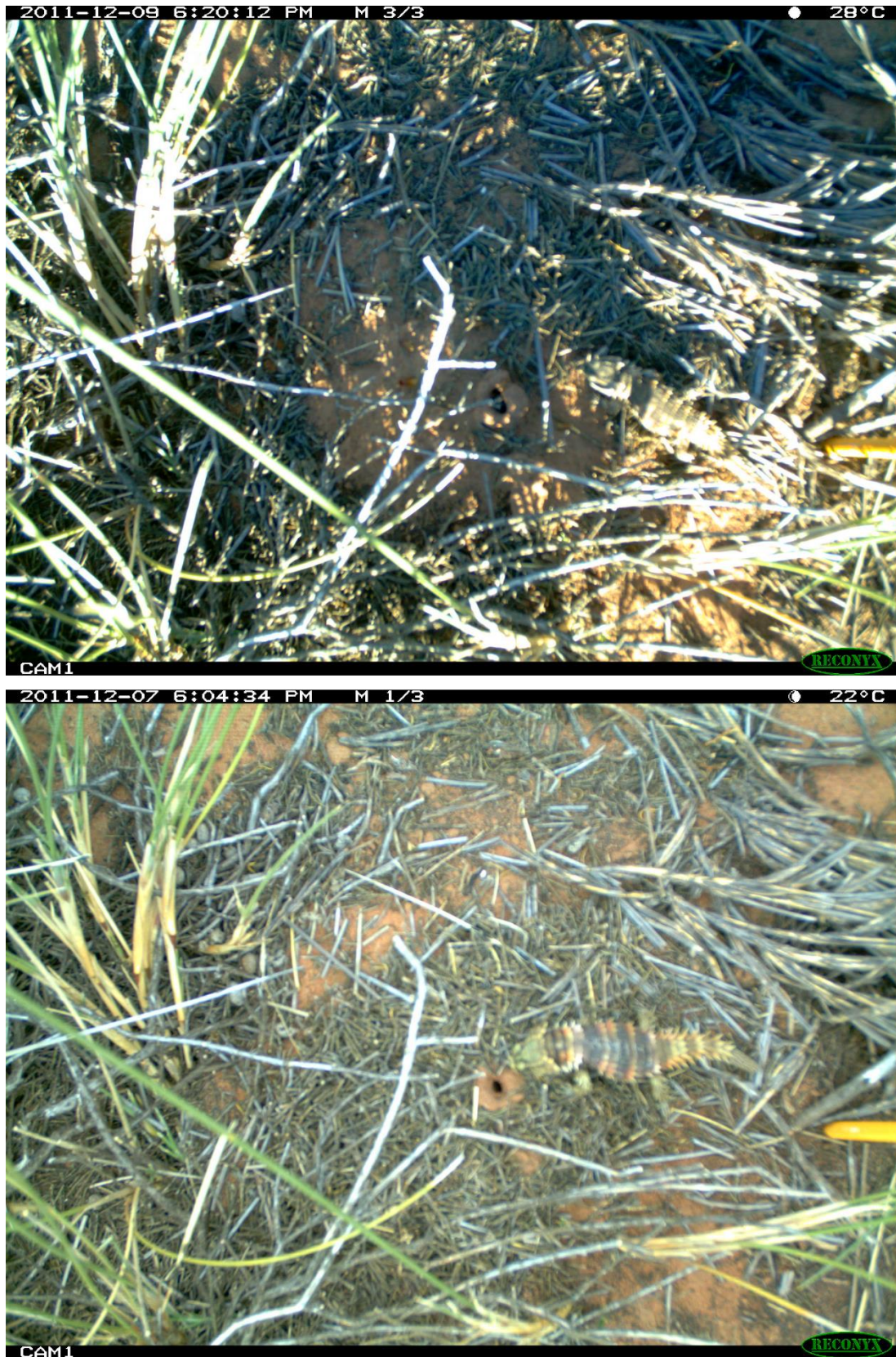
Figure 2.13: Camera trap photographs showing *Ouroborus cataphractus* at an active termite foraging port during the evening hours (summer).

CHAPTER 3

FUNCTIONAL BASIS FOR VARIATION IN BODY ARMOUR IN CORDYLID LIZARDS[*]

ABSTRACT

In the animal kingdom, several morphological antipredator strategies exist to avoid being preyed upon. One of the morphological traits that evolved multiple times among vertebrates is body armour, including carapaces, thickened keratinised scales and plates of dermal bone. While it is generally assumed that body armour provides protection against a predatory attack, little data are available to supports this hypothesis. Cordylid lizards provide an opportunity to test the hypothesis that body armour serves an antipredator function, as considerable variation in the degree of body armour is present within the family. The armadillo lizard (*Ouroborus cataphractus*) is of particular interest, as its heavy armour has been proposed to serve as protection against terrestrial predators during foraging excursions away from safety. Experiments were conducted to test whether the bite forces of four species of mammalian predators were high enough to penetrate the skins of *Karusasaurus polyzonus*, *Namazonurus peersi*, *Cordylus cordylus* and *Cordylus macropholis*, as well as that of *O. cataphractus* individuals originating from three localities that differed in their predator diversity. Moreover, I investigated whether variation in skin toughness was associated with concomitant changes in degree of epidermal (i.e. β-keratin) and dermal (i.e. osteoderm) armour. My results show that the thick osteoderms in the dermis of two out of three *O. cataphractus* populations serve as protection against bites from at least two small terrestrial predators. In contrast, the skin toughness values for the four other cordylid lizards tested in this study were well-below the bite forces of the mammalian predators. I discuss alternative causes of body armour, such as thermoregulation or predation by snakes in cordylid lizards.

INTRODUCTION

Prey animals exhibit a variety of morphological, physiological and behavioural mechanisms to actively defend themselves against attacks from predators (reviewed in Edmunds, 1974; Cloudsley-Thompson, 1994; Caro, 2005). One of the defensive mechanisms that evolved in vertebrates is body armour, including carapaces, thickened keratinised scales (i.e. scutes) and plates of dermal bone (i.e. osteoderms). Its presence in a variety of animals, most notably in armadillos, pangolins and turtles, indicates that convergent evolution has played a major role in shaping body armour. While the general consensus is that body armour serves as protection from predators, there is little direct evidence supporting the predation hypothesis, as pointed out by a number of authors (e.g. Reimchen, 1994; Superina & Loughry, 2011). The defensive function of body armour in prey is often inferred from dietary studies of predators or from geographical overlap of predator and prey (Hagen & Gilbertson, 1973; Currey, 1988; Delany & Abercrombie, 1986; Aresco & Dobie, 2000; Caro & Shaffer, 2010).

Recently, studies have begun to use mechanical and functional morphological approaches to determine the antipredator properties of armour (Stayton, 2009; Hu *et al*., 2011, Meyers *et al.*, 2012). For instance, Hu *et al.* (2011) showed that the theoretical breaking force of a turtle carapace is sufficient to withstand bites of predators with the same body mass. Similarly, Meyers *et al.* (2012) conducted penetration experiments, in which they showed that the scales of *Arapaima* fish were sufficiently strong to withstand the bite force of the red piranha (*Pygocentrus natteri*). However, the non-comparative nature of these studies does not permit us to ascertain the role of predators in shaping the evolution of body armour. The scarcity of studies on the evolution of body armour can be attributed to the fact that, in most cases, the presence of body armour characterises all the species within a particular family (Endler, 1986). Because of that, the closest non-armoured relatives often differ significantly in other morphological and behavioural traits, making comparative studies difficult to conduct (Losos *et al*., 2002). For this reason, studies are needed that examine how variation in degree of body armour influences predation risk both between and within species using a closely related set of taxa with varying antipredator morphologies.

Cordylid lizards (Squamata: Cordylidae) provide such an opportunity, as species vary considerably in their degree of body armour (Losos *et al*., 2002; Stanley *et al*., 2011). The body armour of Cordylidae consists of a combination of dermal armour (i.e. osteoderms), epidermal armour (i.e. β-keratin) and keratinous spines. The primary function of body armour appears to be to prevent extraction from rock crevices, by strengthening the contact with the rock surface

(Cooper *et al*., 1999, 2000). In the Armadillo lizard (*Ouroborus cataphractus*), however, body armour has been hypothesised to serve as protection against attacks from predators during foraging excursions away from the safety of its shelter (Mouton, 2011; Shuttleworth *et al*., 2013). Because body armour, in this specific case, will not be effective against the talons and beaks of predatory birds, it would have evolved to reduce terrestrial predation pressure (Mouton & Flemming, 2001). Thick skin, sharp spines and tail-biting behaviour (Mouton *et al*., 1999) would render *O. cataphractus* a challenging prey item for small mammalian predators. Hence, I firstly hypothesise that the body armour of *O. cataphractus* serves as an impenetrable barrier against attacks by terrestrial mammals. I predict that the force required by terrestrial mammals to penetrate the skin of *O. cataphractus* will exceed their respective bite forces, while the force required to penetrate the skin of other cordylid lizards will be lower than the bite forces of the mammalian predators. Secondly, I hypothesise that the degree of skin toughness in *O. cataphractus* relates to predator diversity. I predict that the skin toughness of individuals inhabiting habitats with a larger number of terrestrial mammal species will be higher than that of individuals inhabiting low-predation habitats. Lastly, I hypothesise that inter- and intraspecific differences in skin toughness have a morphological basis. I predict that skin toughness will be determined by the combined action of dermal armour (i.e. osteoderms) and epidermal armour (i.e. β-keratin). In addition, I determined whether any variation in skin toughness and morphology among populations of *O. cataphractus* could be due to the presence of 'cryptic' species by using genetic data to examine the phylogenetic relationships among several populations.

Investigating the role of predation in shaping body armour is important because the possession of body armour leads to several consequences. For instance, it greatly reduces the sprinting capacity in cordylid lizards (Losos *et al*., 2002), swimming speed in sticklebacks (Bergstrom, 2002) and has been linked to plantigrade locomotion in mammals (Lovegroove, 2001). The impaired locomotion might render individuals vulnerable to predators that are unaffected by the body armour and this, in turn, should lead to compensatory behavioural antipredator tactics (Losos *et al*., 2002). Hence, understanding the antipredator benefits of armour allow me to explain such behaviours.

MATERIALS AND METHODS

SKIN TOUGHNESS

To test whether body armour provides protection against the bites of predatory mammals, the amount of force required by four species of mongoose commonly found in areas inhabited by cordylid lizards (*Galerella pulverulenta*, *Cynictis penicillata*, *Suricata suricatta* and *Herpestes ichneumon;* see Fig. 3.1) to penetrate the skins of several cordylid species was measured and compared to the bite forces of the respective species. Skins of previously frozen, unpreserved adult specimens of several cordylid species obtained from various studies were used to estimate skin toughness. These include: *Ouroborus cataphractus*, *Karusasaurus polyzonus*, *Namazonurus peersi*, *Cordylus macropholis* and *Cordylus cordylus.* The specimens of *O. cataphractus* belonged to three populations that differed in their predator composition, whereas individuals of the other species all belonged to single populations (Table 3.1; Fig. 3.2). Prior to the experiments, the skins were defrosted and fixed individually on a platinum-cure silicone rubber (Soma Foama, Smooth-On, Inc., Easton, Pennsylvania) layer using a silicone adhesive (Skin Tite, Smooth-On, Inc., Easton, Pennsylvania). This was done to imitate the soft tissue underlying the osteoderm and β-keratin layer.

Next, the upper jaws of adult specimens of *G. pulverulenta* ($n = 2$), *C. penicillata* ($n = 1$), *S. suricatta* ($n = 1$) and *H. ichneumon* ($n = 1$) were mounted on a force transducer (model 9203, Kistler Inc., Winterthur, Switzerland) and connected to a charge amplifier (model 5995A, Kistler Inc., Winterthur, Switzerland). Skulls were selected based on the intactness of the upper canines. Polyurethane foam (PUR MC5, Vosschemie GmbH, Uetersen, Germany) was used to attach the force transducer to the upper section of the skull in order to spread the force evenly across the surface (Fig. 3.3). The upper jaws were placed on the lizard skins and pressure was slowly increased by making use of a lever press. The force required to penetrate the skin was recorded and considered as an estimate of skin toughness. Because of the small body size of the lizards, for each skin, only two force measurements were recorded per predator skull.

Following Thomason (1991), the bite force at the canine was estimated for the four species of mongoose. Pictures were taken of the lateral and ventral view of skulls of *G. pulverulenta* ($n = 8$), *C. penicillata* ($n = 6$), *S. suricatta* ($n = 2$) and *H. ichneumon* ($n = 2$). The cross-sectional area of the masseter-pterygoideus (M) and temporalis (T) muscle complex, as well as the in-lever moments arm for the masseter (I_m) and temporalis (I_t) about the temporomandibular joint were estimated from these images.

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Bite force was subsequently estimated using the formula

$$Bf = (T * I_t + M * I_m)/I_o$$

with I_o being the centre of the upper canine to the out-lever moment arm. The resulting value was multiplied by three estimates of maximal isometric force generated by mammalian muscles: 250 KPa (Cleuren *et al*., 1995; Herzog, 1995), 300 KPa (Thomason, 1991) and 370 KPa (Weijs & Hillen, 1985; Koolstra *et al*., 1988; Christiansen & Adolfssen, 2005) to get an estimate range of maximal bite forces.

<div style="text-align:center">SKIN MORPHOLOGY</div>

A small fragment of dorsal skin (2-5 mm²) was excised from each of the specimens used for the skin toughness experiment. Following standard techniques (Humason, 1979), the skin tissues were decalcified for 24 hours in 3% nitric acid, dehydrated and embedded in paraffin wax. Transverse sections (8-10 μm) were prepared and stained using Masson-Goldner trichrome (Goldner, 1938), and Meyer's haematoxylin, phloxine, alcian blue and orange G (Dane & Herman, 1963). The morphology of the lizard skin was examined under a Leica DM LB light microscope (Leica Microsystems, Wetzlar, Germany). Photographs were taken at 40X magnification with a Leica EC3 camera (Leica Microsystems AG, Heerbrugg, Switzerland) and digitised using tpsDIG v2.0 (Rohlf, 2004). Five histological sections with intact dermal and epidermal layers were selected per individual. For each section, the thickness of the osteoderm and β-keratin layer was measured at ten randomly chosen intervals. The average osteoderm and β-keratin thickness was calculated per section to account for fluctuations in skin structure and the highest average value for the five histological sections was retained for further analysis. The maximal thickness of the osteoderm layer gave an indication of the degree of dermal armour, while the maximal thickness of the β-keratin layer gave an indication of the degree of epidermal armour. The sum of the maximal thickness of osteoderm and β-keratin layer gave an indication of the degree of total body armour. Degree of dermal, epidermal and total body armour was compared between all species, as well as between the populations of *O. cataphractus* making use of an analysis of variance (ANOVA) with subsequent Bonferroni *post-hoc* test. To test whether there was a relationship between skin toughness and body armour, Pearson correlation analyses were performed for the species and population mean values of skin toughness and degree of dermal, epidermal and total body armour.

PHYLOGENETIC TREE

Ouroborus cataphractus specimens were collected from 4 localities (*n* = 50) and were complemented with additional samples acquired from the South African National Biodiversity Institute (SANBI) (*n* = 6). Total genomic DNA was extracted from tail tissue using a MACHEREY-NAGEL NucleoSpin Tissue kit, following the manufacturer's protocol. A partial mitochondrial DNA (mtDNA) locus, namely ND2, was amplified and sequenced for all specimens. The primer pair vMet3 (5' GTCCATACCCCGAAAATGTTG 3') and vTrp3 (5' GCTCTTATTTAGGGCTTTGAA 3') (Daniels *et al*., 2004) was used to amplify the partial ND2 gene fragment. The PCR profile used was 95 ˚C for 2 min, 95 ˚C for 30 s, 55 ˚C for 40 s, 72 ˚C for 1 min. The last three steps were repeated for 32 cycles followed by a final extension of 10 min at 72 ˚C. PCR products were visualised on a 1% agarose gel containing ethidium bromide, and sequencing was performed by Macrogen Inc. (Seoul, Korea) on an automated DNA analyser (ABI 3730XL; Applied Biosystems, Foster City, CA). A closely related species, *K. polyzonus,* was used as outgroup (Stanley *et al*., 2011). Sequences for *K. polyzonus* generated by Engelbrecht *et al.* (2011) were obtained from GenBank (accession numbers: JF834001, JF833979 and JF833985).

All DNA sequences were aligned and edited using BioEdit Sequence Alignment Editor 7.0.5.3 (Hall, 1999). As ND2 is a protein coding locus, all sequences were translated into amino acids using EMBOSS Transeq (http://www.ebi.ac.uk/emboss/transeq) to confirm the sequence framework and to check for the presence of stop codons. A DNA substitution model was obtained for each of the three codon positions using JModeltest (Posada, 2008), implementing the Akaike information criterion corrected (AICc) (Burnham & Anderson, 2002). A Bayesian Inference analysis was performed using MrBayes version 3.2.2 (Ronquist *et al*., 2012), partitioning the gene fragment by codon. Five chains were run, sampling every 2000 generations for a total of 4 million generations. The first 25% was discarded as burn-in. Nodes were considered well supported if they had a posterior probability (p*P*) greater than or equal to 0.95. The resulting tree was visualised using FigTree version 1.4 (Rambaut, 2012).

The phylogenetic approach in this study was merely to confirm that the three populations of *O. cataphractus* used for the skin toughness experiments and analysis of skin morphology belong to the same species. Inferring relationships among populations of *O. cataphractus* would require a larger sample size and higher number of genetic markers.

RESULTS

SKIN TOUGHNESS

Despite the low sample size, an analysis of variance revealed statistically significant differences in skin toughness between the species (*G. pulverulenta*: $F_{4,42} = 51.20$, $P < 0.001$; *S. suricatta*: $F_{4,42} = 24.10$, $P < 0.001$; *C. penicillata*: $F_{4,42} = 36.80$, $P < 0.001$; *H. ichneumon*: $F_{4,42} = 61.84$, $P < 0.001$) and between the populations of *O. cataphractus* (*G. pulverulenta*: $F_{3,18} = 14.15$, $P < 0.001$; *S. suricatta*: $F_{3,18} = 11.36$, $P = 0.001$; *C. penicillata*: $F_{3,18} = 9.70$, $P = 0.001$; *H. ichneumon*: $F_{3,18} = 14.60$, $P < 0.001$). The skin of *O. cataphractus* was significantly stronger than those of the other species (Bonferroni *post-hoc* test: all $P < 0.001$; Table 3.2; Fig. 3.4), but no differences could be detected between the other cordylid species (Bonferroni *post-hoc* test: all $P = 1$; Table 3.2; Fig. 3.4). Within *O. cataphractus*, the specimens from Namaqua National Park had the lowest skin toughness values, followed by those from the Cederberg. Individuals belonging to the Lambert's Bay population had the toughest skin (Table 3.2; Fig. 3.4).

The range of average predator bite force differed significantly between the species. *Galerella pulverulenta* (range: 24.93 - 36.90 *N*) and *C. penicillata* (range: 27.69 - 40.98 *N*) had the lowest bite force, followed by *S. suricatta* (range: 39.89 - 59.03 *N*) and *H. ichneumon* (range: 61.01 - 90.29 *N*). Analysis of skin toughness data combined with the theoretical bite force of four mammal predators revealed that, with the exception of the Namaqua National Park population, most *O. cataphractus* individuals would be able to withstand an attack from *Galerella pulverulenta* and *C. penicillata* (Fig. 3.4). Only individuals of the Lambert's Bay populations would be able to withstand an attack from *S. suricatta,* while the bite force of *H. ichneumon* would be sufficiently strong to penetrate the skin of all cordylid lizards tested in this study (Fig. 3.4).

SKIN MORPHOLOGY

The degree of dermal ($F_{4,43} = 10.11$, $P < 001$), epidermal ($F_{4,43} = 8.71$, $P < 0.001$) and total body armour ($F_{4,43} = 11.46$, $P < 0.001$) differed significantly between the species. The degree of dermal armour and total body armour was significantly greater in *O. cataphractus* than in the other species (Bonferroni *post-hoc* test: *P*-value range from 0.18 - 0.002; Table 3.2), but no differences could be detected in dermal and total body armour between the other cordylid species (Bonferroni *post-hoc* test: all $P = 1$; Table 3.2).The degree of epidermal

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armour in *O. cataphractus* was similar to that of *K. polyzonus* (Bonferroni *post-hoc* test: $P = 0.88$) and *C. cordylus* ($P = 0.63$), but greater than that of *C. macropholis* ($P < 0.001$) and *N. peersi* ($P = 0.005$) (Table 3.2; Fig. 3.5, Fig. 3.6). Statistically significant differences in degree of dermal ($F_{2,19} = 25.17$, $P < 0.001$) and total body armour ($F_{2,19} = 23.47$, $P < 0.001$) were also present among the populations of *O. cataphractus*. The degree of dermal and total body armour of the individuals from Namaqua National Park was lower than that of individuals from the Cederberg population. Individuals from the Lambert's Bay population had the highest degree of dermal and total body armour (Bonferroni *post-hoc* test: all $P < 0.001$; Table 3.2). The three populations, however, had a similar degree of epidermal body armour ($F_{2,19} = 2.25$, $P = 0.13$).

Regardless of the predator species considered, skin toughness was correlated with degree of dermal armour ($r = 0.94 - 0.97$, all $P \leq 0.001$, Fig. 3.7A) and degree of total body armour ($r = 0.97 - 0.99$, all $P < 0.001$, Fig. 3.7C). Skin toughness was also correlated with the degree of epidermal armour ($r = 0.82 - 0.86$, all $P \leq 0.03$, Fig. 3.7B).

PHYLOGENETIC TREE

The Bayesian Inference analysis for the ND2 locus strongly supported the monophyly of *O. cataphractus* ($pP = 1$; Fig. 3.8). The maximum uncorrected sequence divergence between the localities was 2.3%. Hence, any variation in skin toughness and skin morphology among populations could not due to the presence of 'cryptic' species.

DISCUSSION

Predation is one of the major selective forces driving the evolution of morphology in organisms (Reimchen, 1994). Yet, despite the general assumption that the presence of defensive structures, such as bony plates, carapaces and keratinised scales, serve as protection against predators, few studies have attempted to experimentally test whether variation in antipredator morphology relates to predation risk (Spence *et al.*, 2013). In the present study, I investigated variation in body armour and skin toughness in a number of cordylid lizards and examined whether mammalian predation risk acts as the agent of selection.

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FUNCTIONAL SIGNIFICANCE OF BODY ARMOUR IN *O. CATAPHRACTUS*

The thick osteoderms in the dermis of *Ouroborus cataphractus* serve as protection against bites from small terrestrial predators as the force required to penetrate the skin is higher than the actual bite forces of three out of four mongoose species. This finding supports the hypothesis that body armour in this species evolved as a defence mechanism against terrestrial predators when exploiting termites as a food source away from the safety of the shelter (Mouton, 2011; Shuttleworth *et al.*, 2013). However, there appears to be significant variation in the degree of body armour and skin toughness within the three populations and a complex relationship between antipredator morphology and predation risk seems to be present. While the body armour of individuals belonging to the Cederberg and Lambert's Bay populations are sufficiently strong to resist an attack from *Galerella pulverulenta* and *Cynictis penicillata*, individuals from Lambert's Bay are also able to withstand bites from *Suricata suricatta*. These findings are only partially in accordance with the presence of the specific predators in the habitat, as the body armour of individuals from the Lambert's Bay population does not serve as an impenetrable barrier against bites from the large *Herpestes ichneumon*. However, it must be noted that the occurrence of *H. ichneumon* is rather rare compared to the other species (Chapter 2).

In contrast to the Cederberg and Lambert's Bay individuals, the relatively thin osteoderms of the Namaqua National Park individuals do not appear to serve as protection against any of the mammalian predators. A possible explanation is that only *G. pulverulenta*, which is absent at Namaqua National Park, poses a predation risk. In sticklebacks, for example, rapid evolutionary transitions from multiple to a small number of bony plates have been observed in the absence of predators (Le Rouzic *et al.*, 2011). However, in the case of *O. cataphractus*, this is unlikely because all four species of mongoose appear to include reptiles in their diet (Du Toit, 1980; Stuart, 1981; Avenant & Nel, 1992; Cavallini & Nel, 1995; Avenant & Nel, 1997; Nel & Kok, 1999). It must however be noted that no dietary data are available from localities where both *O. cataphractus* and predators are present. More dietary data would, however, be required to verify the possibility that only *G. pulverulenta* poses a predation risk. A more plausible explanation is a change in direction from selection for body armour to selection for running speed, resulting from differences in habitat among populations. The Cederberg locality lies on the transition zone between the Mountain Fynbos and Succulent Karoo biome and is relatively densely vegetated by non-succulent (mainly Asteraceae), as well as karroid succulent shrubs (e.g. *Crassula*, *Ruschia*, *Euphorbia, Tylecodon*) (Taylor, 1996;

Mucina & Rutherford, 2006; Fig. 3.9). The Lambert's Bay habitat consists of deep sands, covered with scattered vegetation consisting of medium tall to shrubs (e.g. *Galenia africana*, *Zygophyllum morgsana*) and perennial grass (*Cladoraphis cyperoides*) (Fig. 3.9). Annuals and geophytes provide additional ground cover during spring (Mucina & Rutherford, 2006; Desmet, 2007, Chapter 1). In contrast, the habitat where the Namaqua National Park individuals were collected, consists of quartz patches interspersed with open-canopy dwarf succulent shrubs (e.g. *Dicrocaulon*, *Jacobsenia*, *Monilara*) (Mucina & Rutherford, 2006; Fig. 3.9). In the former two habitats, vegetation cover is provided either throughout the year (Cederberg) or during peak activity (Lambert's Bay, see Chapter 1). Rocky outcrops and bushy vegetation provide the ideal habitat for *G. pulverulenta* (Cavallini & Nel, 1990) and it is thus highly likely that this species poses the greatest threat. In the latter case, the individuals from Namaqua National Park would be highly visible to aerial predators throughout the year and selection should favour running speed over body armour. The trade-off between degree of body armour and running speed (Losos *et al.*, 2002), might thus have resulted in selection for reduced body armour in more open habitats.

Lastly, the intraspecific variation in body armour in *O. cataphractus* might be the result of other factors not related to predation. For example, in sticklebacks, dissolved calcium concentration appears to be the primary agent of selection for dermal plates (Spence *et al.*, 2013). Likewise, cycles of resorption and deposition of minerals in the bone cortex resulting from fluctuations in food availability (Curtin *et al.*, 2005; Mouton, 2011) might underlie variation in body armour in *O. cataphractus*. However, the Lambert's Bay population has the highest lizard density (Shuttleworth, 2006) and presumably experiences the highest competition for food, but has the strongest and thickest skin.

ALTERNATIVE CAUSES OF BODY ARMOUR IN CORDYLID LIZARDS

In contrast to *O. cataphractus*, the skin toughness values for the four other cordylid lizards tested in this study were well-below the bite forces of the mammalian predators. Nonetheless, body armour in these species could still serve an antipredator function, either to prevent extraction from crevices as hypothesised by Cooper *et al*. (2000), to protect the vital organs against venomous bites from snakes, or both. Several snake species include (cordylid) lizards in their diet (e.g. Branch & Burger, 1991; Whiting, 2002; Shine *et al*., 2006a, b; Shine *et al*., 2007; Parusnath, 2012) and are in many cases able to access lizards inside rock crevices. The combination of osteoderm and spines would render individuals difficult to extract from

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their crevice and difficult to subdue or constrict by snakes. A number of alternative explanations for body armour have been reported in literature, but it is highly unlikely that these were the primary selection pressure promoting the evolution of body armour in cordylid lizards. For example, Song *et al.* (2011) suggested that the multi-layer dermal armour of bichirs (*Polypterus senegalus*) serves as protection against biting attacks from conspecifics. Although I did not test for intersexual variation in body armour, the fact that both males and females possess body armour suggests that this is probably not the case in cordylid lizards. Another possibility is that body armour, especially osteoderms, may participate in thermoregulation, as proposed for crocodilians (Seidel, 1979). Mouton & Flemming (2001) found that the osteoderms in melanistic, cold-adapted populations of *Cordylus* species were consistently thinner than those of non-melanistic populations. The thermoregulatory advantage of body armour thus requires further investigation.

In summary, my results show that while the body armour in *O. cataphractus* clearly serves as protection against terrestrial mammals in at least two populations, the function of osteoderms in the other cordylid lizards remains unclear. Future studies should investigate alternative causes of body armour, such as thermoregulation or snake predation. Moreover, it would be interesting to investigate whether osteoderms in *O. cataphractus* exhibit an allometric growth as juveniles would be vulnerable to mammalian predators.

Table 3.1: Species of mammalian predators present in the habitat of the respective species based on camera trapping data and recent distribution records.

Species	*Galerella pulverulenta*	*Cynictis penicillata*	*Suricata suricatta*	*Herpestes ichneumon*
Ouroborus cataphractus				
Lambert's Bay	X	X	X	X
Namaqua National Park	-	?	X	-
Cederberg	X	-	-	?
Karusasaurus polyzonus	X	X	X	X
Namazonurus peersi	X	X	X	-
Cordylus macropholis	X	X	X	X
Cordylus cordylus	X	-	-	X

Legend: present (X), absent (-), equivocal (?)

Table 3.2: Summary of skin toughness and degree of body armature of three populations of *Oubororus cataphractus* and four additional cordylid lizards.

Species	n	Skin toughness (N)				Degree of body armature (mm)		
		Galerella pulverulenta	*Cynictis penicillata*	*Suricata surictta*	*Herpestes ichneumon*	Dermal armour	Epidermal armour	Total body armour
Ouroborus cataphractus								
Lambert's Bay	7	58.2 ± 6.5	59.6 ± 5.7	59.9 ± 6.8	75.6 ± 7.9	0.470 ± 0.069	0.066 ± 0.005	0.531 ± 0.065
Namaqua National Park	5	35.1 ± 2.1	35.7 ± 8.2	31.2 ± 6.7	50.2 ± 5.2	0.218 ± 0.038	0.049 ± 0.009	0.264 ± 0.043
Cederberg	9	49.0 ± 9.5	47.8 ± 11.7	46.0 ± 13.5	61.7 ± 9.1	0.330 ± 0.024	0.068 ± 0.024	0.393 ± 0.077
Karausasaurus polyzonus	12	17.0 ± 2.4	16.9 ± 3.8	18.3 ± 5.1	22.6 ± 4.5	0.138 ± 0.025	0.048 ± 0.010	0.180 ± 0.024
Namazonurus peersi	5	12.6 ± 0.6	13.2 ± 2.9	13.7 ± 2.7	17.6 ± 2.0	0.166 ± 0.030	0.030 ± 0.009	0.194 ± 0.025
Cordylus macropholis	5	17.7 ± 1.5	18.8 ± 4.1	16.0 ± 2.6	25.1 ± 3.2	0.176 ± 0.064	0.022 ± 0.008	0.167 ± 0.071
Cordylus cordylus	4	13.2 ± 1.5	15.7 ± 3.1	15.9 ± 5.6	21.2 ± 3.3	0.117 ± 0.028	0.045 ± 0.003	0.156 ± 0.020

Table entries are the mean ± standard deviation.

Figure 3.1: Photographs illustrating the four species of mongoose present in the habitats of the cordylid lizards used in this study. All photographs were taken at the Lambert's Bay site using remote camera trapping. Top left: small grey mongoose (*Galerella pulverulenta*), top right: meerkat (*Suricata suricatta*), bottom left: yellow mongoose (*Cynictis penicillata*), bottom right: large grey mongoose (*Herpestes ichneumon*).
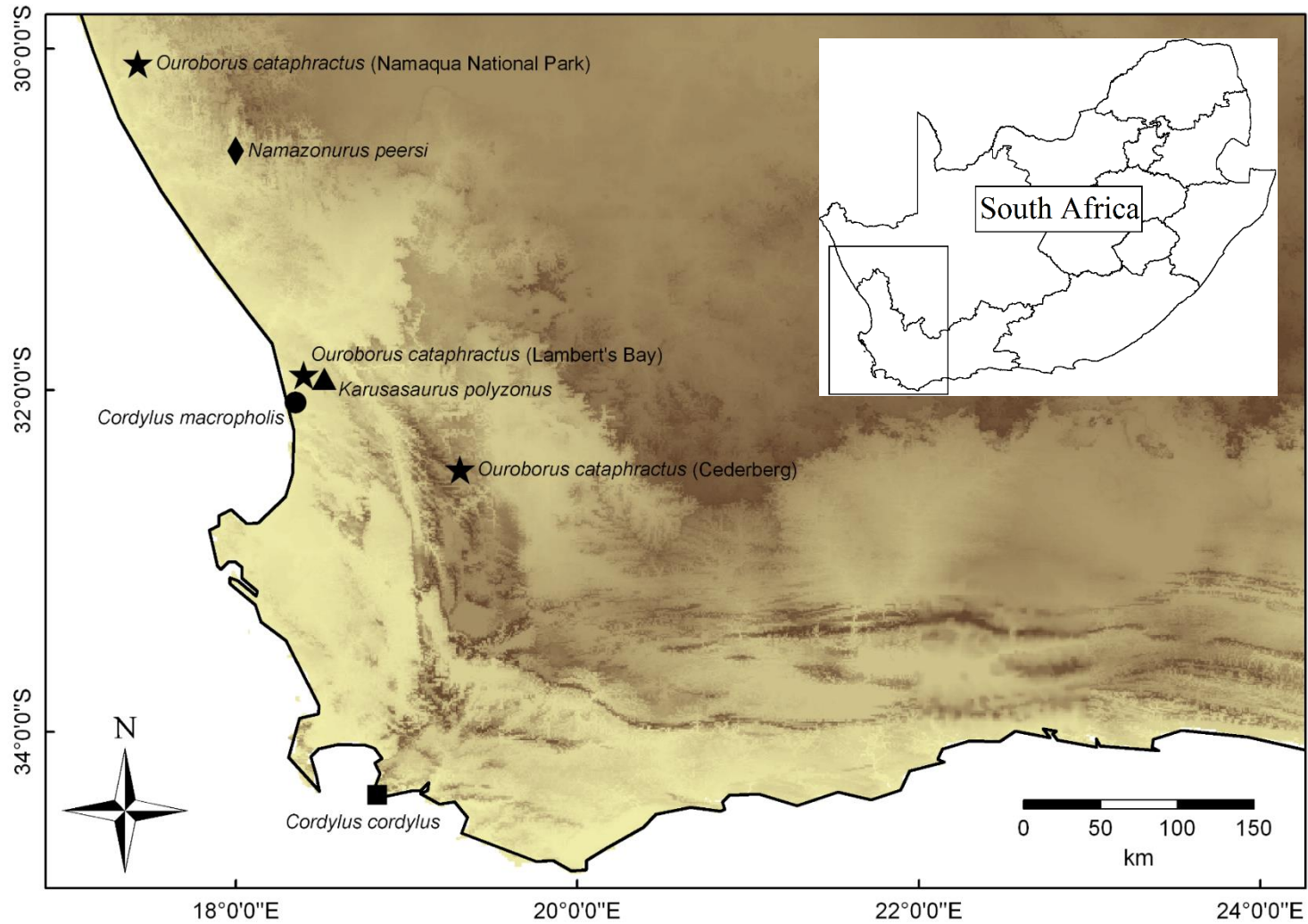
Figure 3.2. Sampling localities for all cordylid lizards used during this study.
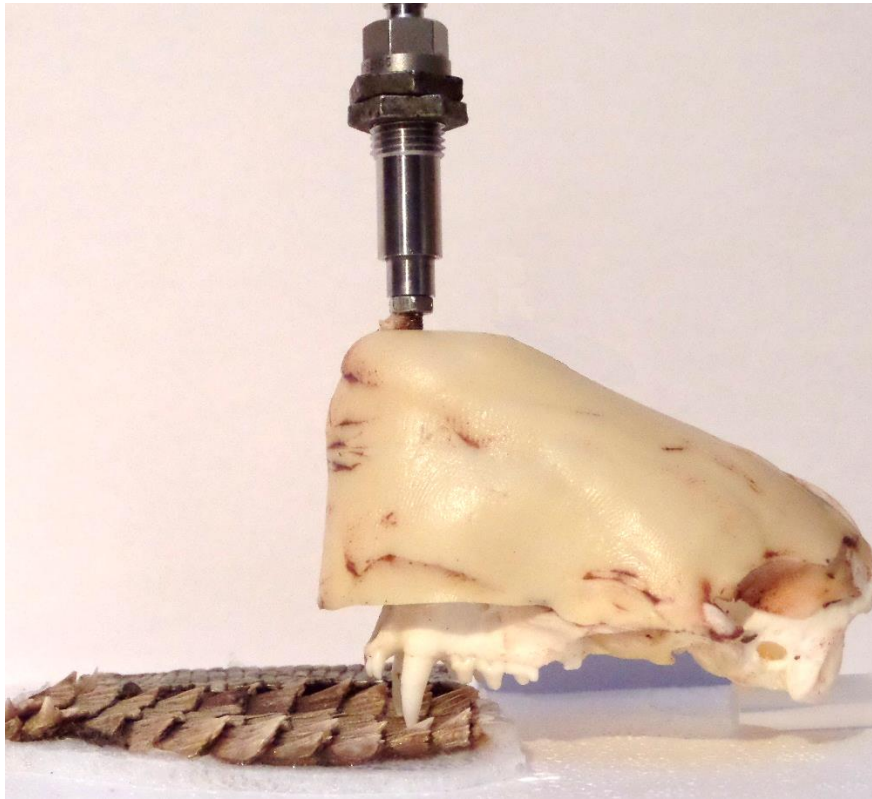
Figure 3.3: Experimental set-up for measuring the force needed for penetrating the skin. The upper jaws of various mongoose species were placed on the skins of cordylid lizards and pressure was slowly increased by making use of a lever press. The force required to penetrate the skin was recorded and considered as an estimate of skin toughness.
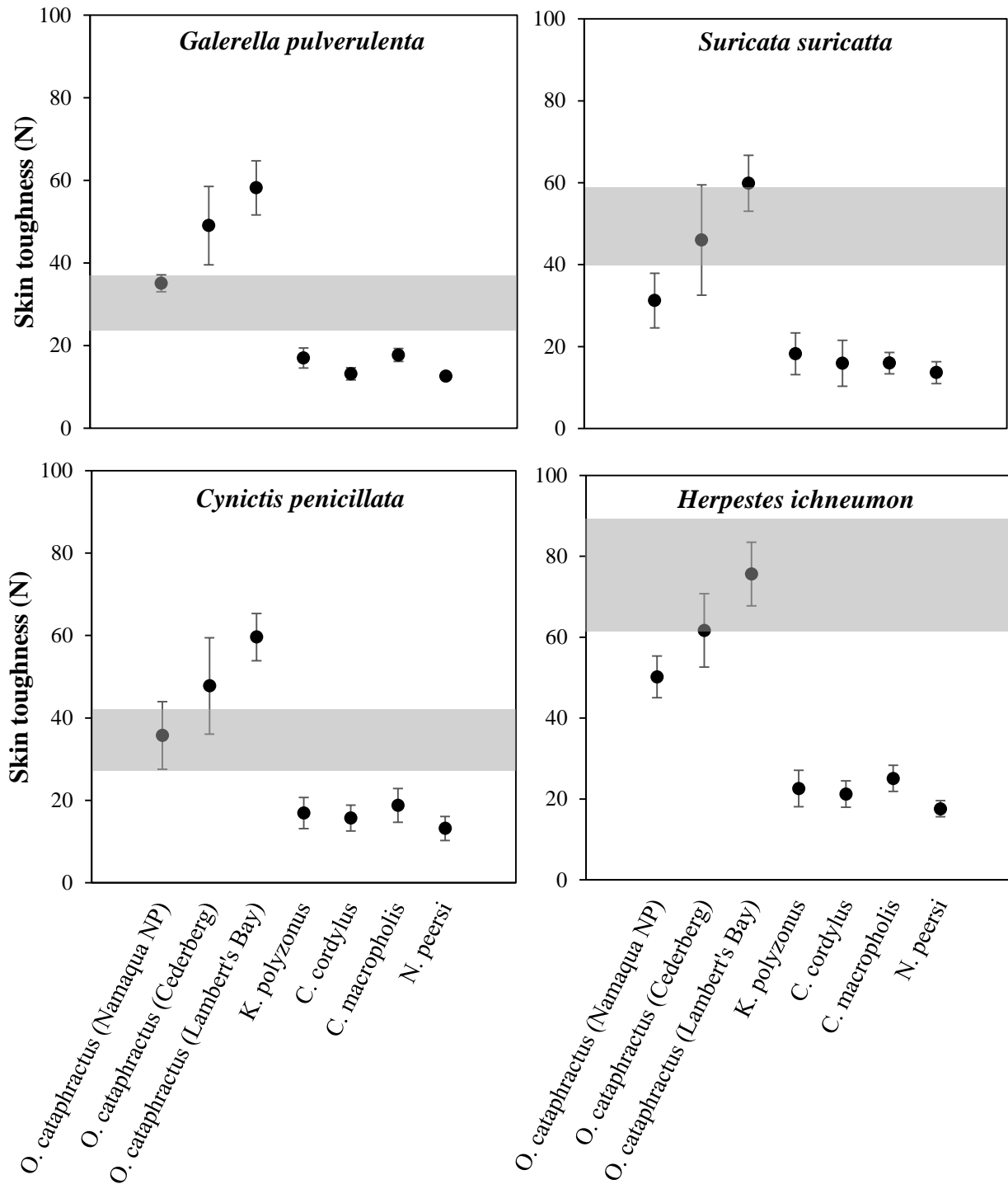
Figure 3.4: Graph illustrating the average skin toughness values (± SD) of *Karusasaurus polyzonus*, *Namazonurus peersi*, *Cordylus cordylus*, *Cordylus macropholis* and three populations of *Ouroborus cataphractus* and four additional cordylid lizards. Grey shading indicates the range of average bite forces of the respective mongoose species.

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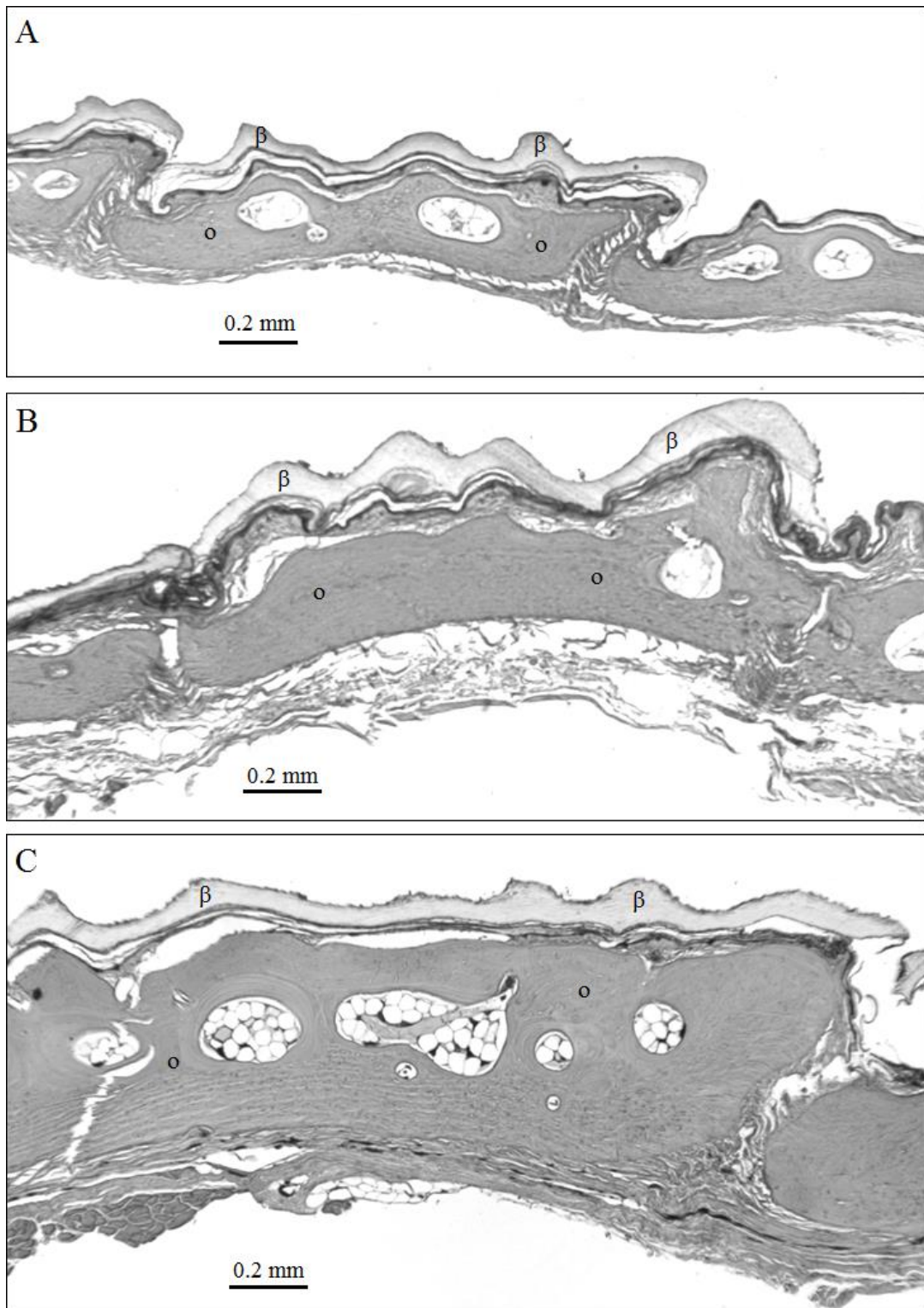
Figure 3.5: Transverse sections (10 µm) through the dorsal skin of *Ouroborus cataphractus*. Individuals from Namaqua National Park (A) had the thinnest osteoderm layer, followed by those of the Cederberg (B). Individuals from Lambert's Bay (C) had the thickest osteoderm layer. Legend: o, osteoderm; β, β-keratin**.**
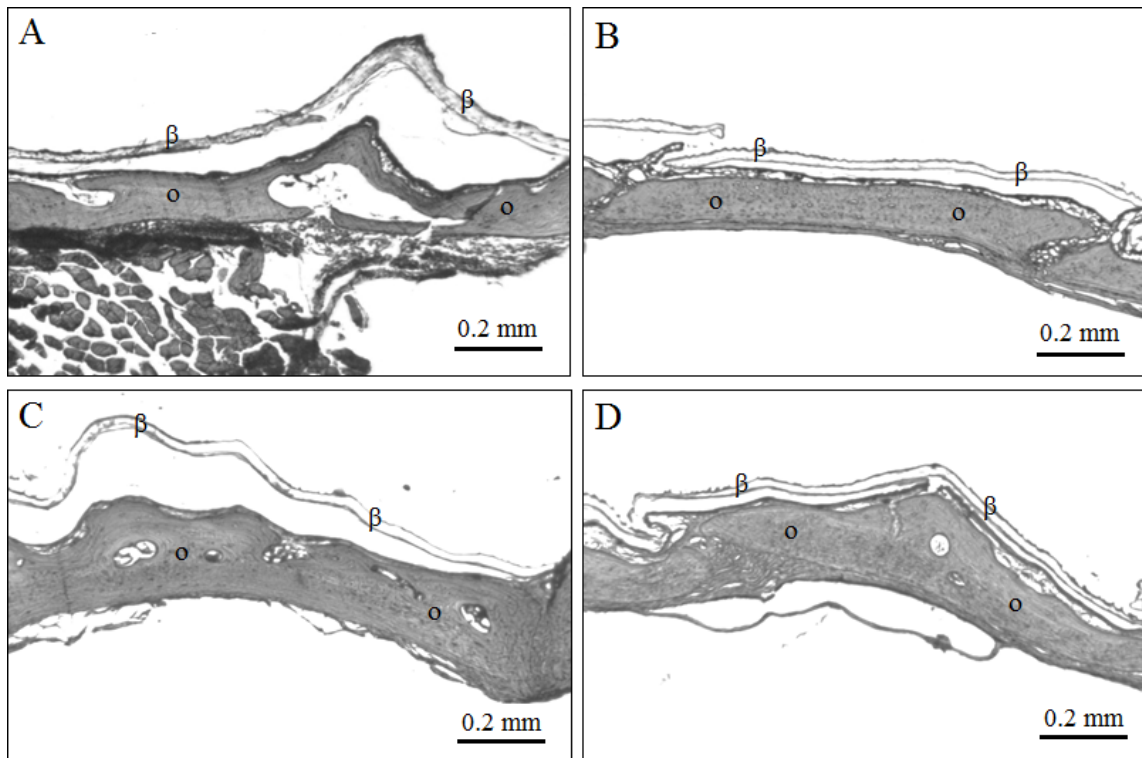
Figure 3.6: Transverse sections (8 µm) through the dorsal skin of *Namazonurus peersi* (A), *Karusasaurus polyzonus* (B), *Cordylus macropholis* (C) and *Cordylus cordylus* (D). Legend: o, osteoderm; β, β-keratin.
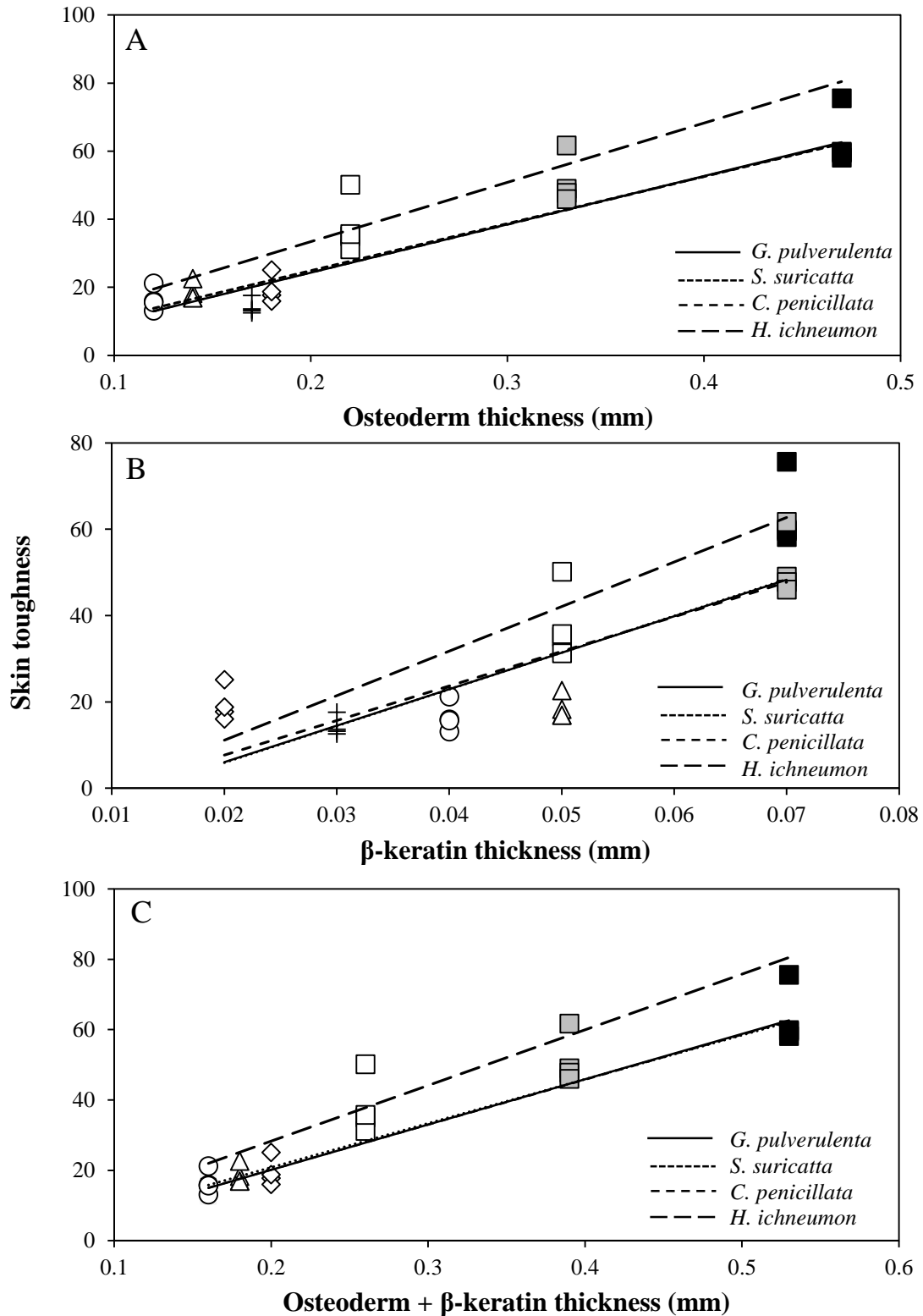
Figure 3.7: Graphs illustrating the relationship between skin toughness and degree of dermal armour (A), degree of epidermal armour (B) and degree of total body armour (C) for *Ouroborus cataphractus* (Lambert's Bay: ■, Cederberg: ▨, Namaqua National Park: □), *Karusasaurus polyzonus* (△), *Cordylus macropholis* (◇), *Namazonurus peersi* (+), *Cordylus cordylus* (○).
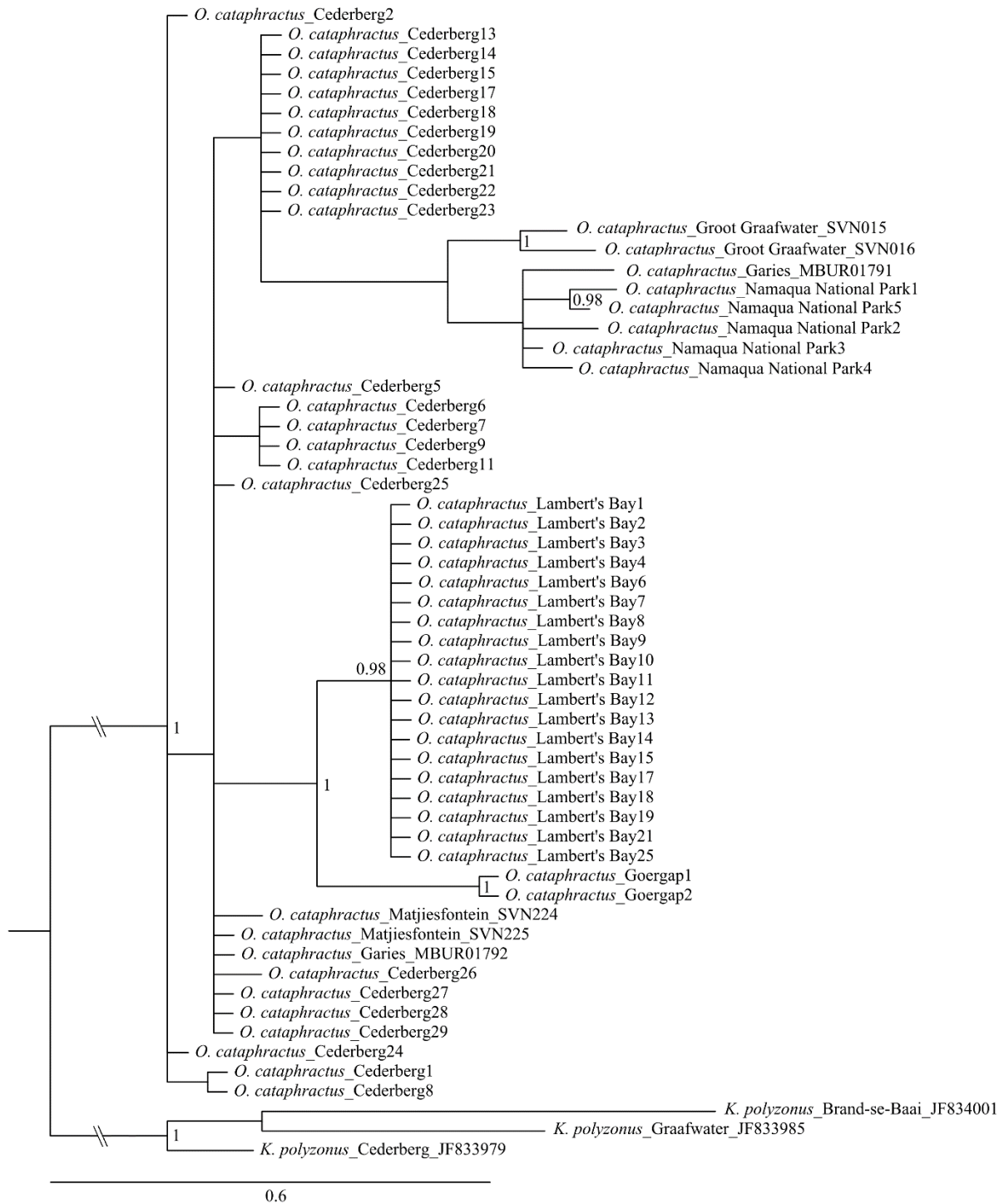
Figure 3.8: A Bayesian inference phylogram representing the ND2 partial gene fragment showing the monophyly of *Ouroborus cataphractus*. Nodal support is represented by posterior probability values ≥ 95% .
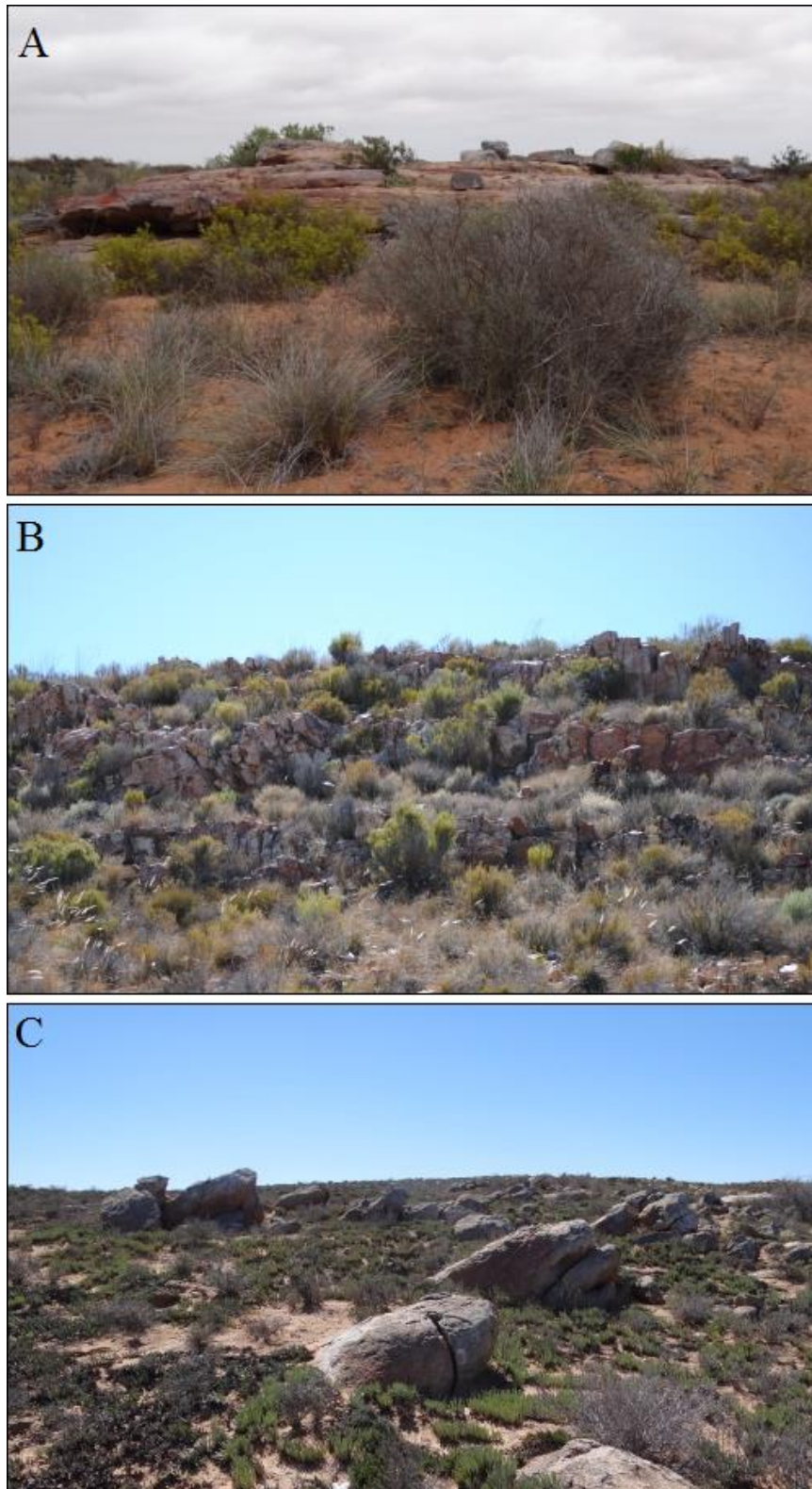
Figure 3.9: Photographs illustrating the differences in habitat between populations of *Ouroborus cataphractus*. The sites at Lambert's Bay (A) and in the Cederberg (B) were characterised by succulent plant growth, as well as small shrubs and grasses, while the ground surface of the site in Namaqua National Park (C) was only sparsely covered with succulents. All photographs were taken in April (i.e. end of summer).

<div align="right">

CHAPTER 4

</div>

CONSEQUENCES OF BODY ARMOUR FOR FEEDING BEHAVIOUR IN CORDYLID LIZARDS[*]

<div align="center">

ABSTRACT

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Sprinting capacity is an important component of prey capture as it allows organisms to rapidly reduce the distance between themselves and their prey. In some cases, however, the possession of antipredator defences that impair locomotor performance, such as body armour, might inhibit individuals from benefitting from running speed during prey capture. Here, I investigate whether alternative performance capacities can evolve to compensate for costs associated with impaired locomotor performance making use of Cordylidae, a family of lizards that vary significantly in degree of body armour. My results provide no evidence for correlated evolution between performance capacities such as jaw closing velocity (to increase prey capture efficiency) or increased bite force (to increase the prey spectrum) and degree of body armour. Instead, the possession of body armour appears to be costly in terms of energy acquisition as it limits the proportion of evasive prey than can be captured. I propose that different selection pressures act upon cranial traits that affect performance capacity, especially bite force, and that these selection pressures might differ between taxa with varying degrees of body armour. Furthermore, I suggest that behavioural adjustments and habitat use appear to be means used by heavily armoured taxa to reduce costs associated with body armour.

INTRODUCTION

An organism's ability to perform ecologically relevant behaviours such as prey capture may have significant effects on survival, growth, reproduction, and consequently, fitness (Arnold, 1983; Pough, 1989; Wainwright, 1994). The most frequently examined correlate of prey capture, and arguably the most crucial, is locomotor performance (reviewed in Higham, 2008). The speed of a predator attained during prey capture will determine the outcome of a feeding event and a high sprinting or swimming capacity is especially vital to predators preying on evasive prey (Rice & Westneat, 2005; Higham, 2007a, 2007b, 2008; Higham *et al*., 2007; Collar *et al.,* 2008; Edwards *et al*., 2013). Under certain circumstances, however, locomotor performance becomes impaired and organisms might no longer be able to benefit from the advantages of a high sprinting capacity during prey capture. For example, animals possessing antipredator defences, such as spines and osteoderms, appear to be relatively slow as body armour makes rapid locomotion difficult due to the added mass and inflexibility (Andraso & Barron, 1995; Losos *et al*., 2002; Bergmann *et al.*, 2009). The possession of such antipredator defences should therefore be costly and natural selection should favour the evolution of performance capacities and behaviours that provide solutions for the reduction in sprinting capacity.

Girdled lizards (Squamata: Cordylidae) provide an opportunity to test the hypothesis that alternative performance capacities evolve to compensate for costs associated with impaired locomotor performance. Cordylidae is a relatively small (i.e. between 80 – 90 species) family of scinciform lizards, which contains morphologically and ecologically distinct and diverse taxa (Stanley *et al*., 2011). They are considered strict sit-and-wait foragers (Cooper *et al*, 1997; Mouton *et al.,* 2000a; Whiting, 2007) and utilize a variety of microhabitats, though most taxa are predominantly rock-dwelling (Mouton & Van Wyk, 1997). Extensive variation in antipredator morphology is present within Cordylidae, ranging from an almost complete lack of body armour (i.e. spines and osteoderms) to elaborated body armour (Stanley *et al*., 2011). Losos *et al*. (2002) showed a clear trade-off between degree of body armour (i.e. spine length) and running speed, with heavily armoured species being worse sprinters than lightly armoured species. Whether running speed or armour is favoured appears to be dictated by the relative importance of aerial and terrestrial predation, which, in turn, is determined by habitat and microhabitat use (Mouton & Flemming, 2001). Species that spend the majority of their activity time on large rock surfaces appear to lack body armour to facilitate escape and to reduce the likelihood of getting captured by visually orientated predators (e.g. birds of prey; Mouton &

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Flemming, 2001; Losos *et al*., 2002). Lizard species that spend the majority of their activity close to crevices or in terrestrial habitats appear to be more heavily armoured to protect themselves against extraction risk from terrestrial predators. An additional cost of armour is the increased vulnerability to aerial predators during general maintenance behaviour outside the crevice, because armour does not serve as protection against birds of prey (Mouton & Flemming, 2001). As a consequence, heavily armoured taxa are not only slow, but are also forced to remain close to their shelters (Losos *et al*., 2002), which in turn would result in lower encounter rates with potential prey.

I hypothesise that fast-moving lightly armoured taxa rely on sprinting capacity to capture prey, while armoured taxa evolved alternative performance capacities to compensate for costs associated with the trade-off between armour and running speed. Firstly, as aerial predation risk inhibits heavily armoured taxa to chase prey over long distances, they could instead rely on a fast strike and benefit from a high jaw closing velocity to increase prey capture success. Under this scenario, I predict correlated evolution between degree of body armour and jaw closing ratio-lever towards short jaw closing in-levers (i.e. high jaw closing velocity: McBrayer, 2004; Metzger & Herrel, 2005; McBrayer & Corbin, 2007; Verwaijen & Van Damme, 2007). Furthermore, I predict that diet will not be related to degree of body armour under this scenario. Secondly, an increase in bite force could allow heavily armoured taxa to shift their dietary niches towards slow-moving hard-bodied prey as bite force increases the prey spectrum available for intake and reduces prey handling time for harder prey (Verwaijen *et al.,* 2002; Aguirre *et al*., 2003; Marshall *et al*., 2012). Here, I predict correlated evolution between degree of body armour and jaw closing ratio-lever towards long jaw closing in-levers (i.e. high bite force: Herrel *et al*., 2001a, 2007, 2010; Vanhooydonck *et al.*, 2007). Moreover, I predict that heavily armoured species will have a higher proportion of hard prey items in the diet. Alternatively, interspecific variation in feeding performance could merely be the result of variation in diet, independent of the degree of body armature. Variation in diet could, for instance, have resulted from differences in habitat use between lightly and heavily armoured taxa. Therefore, I test for correlated evolution between diet and degree of body armour on the one hand and diet and performance capacities on the other hand.

MATERIALS AND METHODS

MORPHOLOGY

Morphological traits were measured in 27 species representative of all clades and body plans. Data on antipredator morphology were taken from Losos *et al.* (2002) and additional measurements were performed on live specimens and preserved specimens belonging to the Ellerman Collection of the University of Stellenbosch, South Africa. Briefly, spine length, measured at the tail, front leg, hind leg, side of the trunk, neck, and head was quantified and used to describe body armour. All spine measurements were transformed using the 'log1p' function in R due to the absence of certain spines in specific body regions in a number of taxa. A phylogenetic regression analysis was performed using the phyl.resid function in the R package PHYTOOLS (Revell, 2009) to generate seven size-corrected spine measurements. Because spine measurements are highly correlated (Losos *et al.*, 2002), the residual spine measurements were subjected to a phylogenetic principal components analysis (PCA), performed using the phyl.pca function in PHYTOOLS (Revell, 2009).

Jaw mechanics were described by calculating the jaw closing lever ratio of the lower jaw. The right lower jaw was dissected out of preserved lizards and high resolution (4288 x 2848 pixels) photographs were taken of the lingual side of the lower jaw with a digital camera (Nikon D300, Nikon Corporation, Tokyo, Japan) mounted on a tripod. Images were digitised using tpsDIG v2.0 (Rohlf, 2005) and the distance between the tip of the jaw and the jaw-joint (i.e. jaw out-lever), as well as the distance between the coronoid process and jaw-joint (i.e. closing in-lever) was calculated. The ratio of the closing in-lever to the jaw out-lever was used to calculate the mechanical advantages of jaw closing.

PERFORMANCE MEASURES

To relate variation in jaw closing lever-ratio to variation in performance traits directly related to feeding, *in vivo* measurements of jaw closing velocity and bite force were collected for a subsample of species. Feeding experiments were conducted for eleven species (Fig. 4.1) using the protocol described in Chapter 6. Briefly, a prey item was placed approximately 10 cm in front of a lizard's shelter and the feeding behaviour was recorded using at 240 fps using a high-speed camera (Casio Exilim EX-FH25, Casio Computer Co., Ltd, Tokyo, Japan). Because of the effect of prey type, size and speed on the modulation of prey capture kinematics

(Montuelle *et al*., 2010), immobilised crickets were chosen as prey item and the predator-prey size ratio was standardised by corresponding the size of the prey equal to half of the predator's head size.   For *Ouroborus cataphractus*, only sequences during which the prey was apprehended using jaw prehension were used (see Chapter 6). Scaled image sequences of trials during which the head was perfectly perpendicular to the camera lens were imported in tpsDIG v2.0. A landmark was placed on the tip of the upper jaw and on the tip of the lower jaw and the (x, y) coordinates of the upper and lower jaw landmarks were used to calculate gape distance. All data were filtered with a fourth-order zero-phase shift Butterworth filter with a cut-off frequency of 100 Hz (Winter, Sidwall & Hobson, 1974). Consequently, jaw closing velocities were calculated from the smoothened data by differentiation of the displacement profile. The highest values for each individual were retained for further analyses.

Bite force was measured using the set-up described in Herrel *et al*. (1999). The apparatus consists of an isometric force transducer (model 9203, Kistler Inc., Switzerland) connected to a portable charge amplifier (model 5995A, Kistler Inc., Switzerland). Before the start of the bite performance experiments, lizards were allowed to freely thermoregulate to obtain their optimal body temperature. Next, lizards were induced to bite onto two parallel metal plates located at the free ends of the bite force apparatus. The distance between the bite plates was adjusted according to the size of the lizards to eliminate the effect of gape size on bite performance (Anderson *et al.*, 2008). Bite force was recorded five times for each individual and the maximum bite force of each individual was retained for analyses. Prior to phylogenetic analyses, bite force and jaw closing velocity were regressed against snout-vent length.

All specimens were collected under permit numbers AAA007-00026-0056 (Western Cape), AAA007-00340-0035 (Western Cape), 0056-AAA041-00030 (Western Cape), 01/14638 (Free State), FAUNA 570/2013 (Northern Cape) or were obtained from commercial dealers. The performance experiments were approved by the Research Ethics Committee of the Faculty of Science, Stellenbosch University (Ethical clearance number: SU-ACUM12–00024) and are in accordance with the ethical guidelines set by the American Society of Ichthyologists and Herpetologists.

<div align="center">DIET</div>

Dietary data were taken from published accounts of stomach contents (Mouton *et al*., 2000a; Van Wyk, 2000; Fell, 2005; Chapter 5). In addition, the stomachs of *Hemicordylus robertsi*, *Pseudocordylus microlepidotus*, *Cordylus cordylus*, *Cordylus niger* and *Cordylus macropholis* were dissected to supplement the literature data with new dietary data. All dietary

data were obtained from specimens resembling the localities of those used to obtain spine, lower jaw and performance measures. Specimens collected during different times of the year were selected to account for potential seasonal variation in diet. All remains were identified to order level, with the exception of larvae (i.e. Lepidoptera, Diptera, Coleoptera), which were treated separately due to their sedentary nature and Formicidae, which were grouped separately from Hymenoptera due to the non-evasive nature. Next, all prey items were classified into two arbitrary categories: 'hard' (i.e. Coleoptera, Diplopoda and Scorpiones) and 'evasive' (i.e. Hymenoptera, Orthoptera, Blattaria, Diptera, Lepidoptera, Embioptera), based on published accounts of prey hardness and personal observations of evasiveness (Herrel *et al*., 1999, 2001b; Aguirre *et al*., 2003; Vanhooydonck *et al.*, 2007; Broeckhoven, 2011; Chapter 5). Subsequently, the proportion each category (i.e. 'hard' or 'evasive') represented in the diet was calculated. All proportions were arcsin transformed before statistical analyses (Sokal & Rohlf, 1995).

PHYLOGENETIC TREE

Partial gene fragments generated by Stanley *et al.* (2011), representing three mitochondrial (12S, 16S, ND2) and three nuclear (PRLR, MYH2, KIF24) DNA gene regions, were downloaded from GenBank for 27 ingroup taxa representing the Cordylidae. Three outgroup taxa were selected, namely *Cordylosaurus subtesselatus*, *Gerrhosaurus nigrolineatus* and *Matobosaurus validus*. All sequences were aligned and edited in MEGA version 6 (Tamura *et al*., 2013). JModeltest (Posada, 2008) was used to obtain the best-fit substitution model for each gene fragment, using the AICc criteria. In order to attain an ultrametric tree with relative divergence times between the in-group taxa, the BEAST package version 2.1.3 (Bouckaert *et al.*, 2014) was employed. The models obtained for all six loci, along with their parameters, were used for specifying the site models in BEAUti. As I was interested in relative, rather than absolute node ages, a relaxed lognormal clock model was selected, estimating around the clock rate of 1.0. The birth-death model was selected as tree prior. The Markov Chain Monte Carlo (MCMC) was run for 20 million generations, sampling every 2000 generations. Tracer version 1.6 (Rambaut *et al*., 2013) was employed to assess the chain convergence before discarding the first 10% as burn-in using TreeAnnotator version 2.1.2 (available within the BEAST software package), while the remaining 9001 trees were summarised as a maximum clade credibility tree. Finally, FigTree version 1.4 (Rambaut, 2012), was used to visualize the resulting tree.

PHYLOGENETIC ANALYSES

An independent contrast (IC) approach was used (1) to test if there is correlated evolution between degree of body armour and jaw mechanics, (2) to confirm that differences in jaw mechanics underlie differences in bite force and jaw closing velocity and (3) to examine if bite force or jaw closing velocity are responsible for shifts in the proportion of hard and evasive prey in the diet or if variation in diet results from differences in degree of body armour. Prior to the calculation of independent contrasts, I determined whether branch length transformation was appropriate for the constructed phylogeny. The fit of the gradual Brownian motion model in which phenotypic covariance is proportional to shared branch length (Felsenstein, 1985) was evaluated against the non-phylogenetic model in which shared phylogenetic history has no relation to covariance (using Pagel's lambda; Pagel, 1997, 1999) and a punctuational model in which phenotypic covariance among species is proportional to the number of shared lineage splitting events (using Pagel's kappa; Pagel, 1997, 1999). The function 'fitContinuous' in the R package GEIGER (Harmon *et al*., 2008) and the Akaike information criterion with correction for sample size (AICc) was used to evaluate the fit of the models (Burnham & Anderson, 2002). Next, the presence of correlated evolution between morphological and performance traits, as well as diet, was tested for. For this purpose, independent contrasts (Felsenstein, 1985) were calculated using the GEIGER package in R (Harmon *et al*., 2008). The appropriate branch length transformation, if required, was applied prior to the calculation of independent contrasts.

RESULTS

Our phylogeny (Fig. 4.1) is in reasonable accord with Stanley *et al*.'s (2011) phylogeny of Cordylidae. However, the phylogenetic positioning of the *Karusasaurus-Ouroborus* clade is more basal in my analysis. Despite the fact that this placement is in accordance with a revised squamate classification by Pyron *et al*. (2013), this could be due solely to the lower number of taxa included in the phylogenetic analysis.

The 27 species of cordylid lizards studied here show marked interspecific variation in spine length (Table 4.1, Fig. 4.1), partially resulting from the size difference between species: the snout-vent length of *Smaug giganteus* was approximately three times greater than that of the smaller *Cordylus* species. All results were therefore based on (phylogenetically) size-corrected residuals. A phylogenetic principal component analysis conducted on the

phylogenetic size-corrected spine measurements resulted in one principal component explaining 78 % of the variation in spinocity. This principal component, coined degree of body armour, was retained for subsequent analyses.

Brownian motion was the best model fit for degree of armour indicating that closely related species are more similar in their antipredator morphology than distantly related species. In contrast, a model that showed no phylogenetic structure fitted the jaw closing lever-ratio, performance measures and dietary data best. Branch length transformation was therefore applied prior to the calculation of independent contrasts. The independent contrasts of size-corrected bite force and jaw closing lever-ratio were positively correlated ($r = 0.69$, $F_{1, 10} = 9.27$, $P = 0.01$), while the contrasts of size-corrected jaw closing velocity and jaw closing lever-ratio were negatively correlated ($r = -0.89$, $F_{1, 9} = 35.42$, $P < 0.001$). Moreover, a trade-off was present between independent contrasts of size-corrected bite force and jaw closing velocity ($r = -0.65$, $F_{1, 9} = 67.67$, $P = 0.02$; Fig. 4.2). There was no correlation between the contrasts of degree of body armour and jaw closing lever-ratio ($r = 0.27$, $F_{1, 25} = 1.91$, $P = 0.18$). The proportion of hard prey in the diet was not dependent on the degree of body armour (IC; $r = 0.39$, $F_{1, 7} = 1.27$, $P = 0.30$) or absolute bite force (IC; $r = 0.05$, $F_{1, 7} = 0.02$, $P = 0.90$), but was correlated with absolute jaw closing velocity (IC; $r = 0.73$, $F_{1, 6} = 6.67$, $P = 0.04$). The proportion of evasive prey in the diet, in contrast, depended on the degree of body armour (IC; $r = -0.75$, $F_{1, 7} = 8.94$, $P = 0.02$) (Fig. 4.3), but not on absolute bite force (IC; $r = 0.22$, $F_{1, 7} = 0.36$, $P = 0.57$) or jaw closing velocity (IC; $r = -0.17$ $F_{1, 6} = 0.18$, $P = 0.69$).

DISCUSSION

ARMOUR AND PREY CAPTURE IN CORDYLID LIZARDS

The data presented in this study provide no support for the hypothesis that bite force or jaw closing velocity increases with degree of body armour in cordylid lizards. This suggests that body arrmour might be a costly trait in terms of energy acquisition. Indeed, the diet of heavily armoured taxa appears to be limited to slow-moving prey items. The increased vulnerability to aerial predators appears to force heavily armoured taxa to remain close to the shelter (Mouton & Flemming, 2001; Losos *et al*., 2002). While fast-moving species can afford to chase prey for a longer time, the increased aerial predation risk in heavily armoured slow-moving species will prohibit individuals from chasing after evasive prey for an extended time. The relationship between the proportion of evasive prey items in the diet and degree of body

armour supports this hypothesis. It must be noted that although my experimental design only allowed lizards to strike from a short distance, all species, regardless of their degree of body armour, used running to chase prey during general feeding events. However, more behavioural data are required to test the hypothesis that giving-up time declines with increasing degree of body armour.

Rather than alternative performance capacities, it is possible that heavily armoured species adapted alternative behaviours to overcome some of the problems associated with armour. For instance, in some populations of *Ouroborus cataphractus*, activity and feeding are mainly restricted to spring time when vegetative cover provided by annuals is high and food availability peaks (Visagie, 2001; Chapter 2). Similarly, the heavily armoured *Smaug giganteus* hibernates during winter, but extensive activity and feeding occurs during summer when grass provides cover (Van Wyk, 1992, 2000). In both cases, vegetative cover provides protection against visually orientated predators such as birds of prey, thereby lowering the aerial predation risk associated with armour. Alternatively, in *Cordylus macropholis*, a well-armoured species that inhabits the succulent plant *Euphorbia caput-medusae* (Bauwens *et al*., 1999), individuals do not have to forage away from their retreat sites as these succulents normally harbour a high number of prey (Nieuwoudt, 2001).

<div align="center">PERFORMANCE CAPACITIES AND DIETARY NICHE</div>

Performance capacities are no compensatory means used to counteract the negative effect of armour on running speed, hence they might be related to variation in diet (i.e. the proportion of hard or evasive prey). The finding that bite force does not correlate with the proportion of hard prey in the diet partially rejects this hypothesis. This is surprising, given that an increase in bite force makes exploitation of hard prey more profitable (Herrel *et al*., 2001a; Verwaijen *et al*., 2002; but see Chapter 5). Nevertheless, I argue that the absolute bite forces observed in most cordylid lizards well exceed the hardness of most available prey. For instance, with the exception of very hard Coleoptera (Chapter 5), most arthropod prey consumed by lizards do not exceed 10 N (Herrel *et al*., 2001a). Thus, bite force should only have a limited effect on dietary niche partitioning. These findings suggest that additional selection pressures might act on bite force in lizards. A first possibility is that in some species, bite force might serve a purpose of antipredator defence, rather than an aid in prey handling. For example, some lightly armoured species, such as *Hemicordylus robertsi* and *Pseudocordylus microlepidotus* rely on flight to escape from predators (Losos *et al*., 2002), but appear to be very aggressive

when captured (Branch, 1998; Broeckhoven & Mouton, personal observations). Prey capture in these lightly armoured taxa will be facilitated by their locomotor capabilities (Losos *et al*., 2002), thereby allowing investment in characters that enhance bite force such as an increase in length of jaw closing in-lever. The role of bite force in defence against predators might not only be confined to lightly armoured taxa. The relatively high bite force in *O. cataphractus* appears be related to the unique tail-biting behaviour displayed by this species (Mouton *et al*., 1999), as suggested in Chapter 5. Secondly, head height might be constrained by rock-dwelling behaviour as the possession of a tall head and associated use of wider crevices might increase extraction risk imposed by predators (Cooper *et al*., 1999, 2000; Herrel *et al*., 2001b; Lappin *et al*., 2006; Revell *et al*., 2007; Kohlsdorf *et al*. 2008; Kaliontzopoulou *et al*., 2012; Chapter 5). A reduction in head height could in turn decrease bite force, as there will be less area available for muscle attachment (but see Chapter 5). The relatively low number of terrestrial cordylid lizards, however, makes an examination of the effect of lifestyle (i.e. rock-dwelling versus terrestrial) on head morphology, and consequently bite force, rather difficult. Additional selection pressures acting on bite force will directly influence jaw closing velocity as there is a trade-off between the two. Although force-velocity trade-offs have been reported in a number of invertebrate and vertebrate taxa (Westneat, 1994; Levinton & Allen 2005; Herrel *et al*., 2009), this is the first empirical demonstration of such a trade-off in the lizard feeding apparatus. The positive relationship between the proportion of hard prey in the diet and jaw closing velocity, seems to be shaped by *S. giganteus*, because this species not only got the highest jaw closing velocity, it also includes a high proportion of Coleoptera in the diet (Table 4.1, Table 4.2).

In conclusion, my data show that degree of body armour affects the dietary niche of cordylid lizards, especially the proportion of evasive prey available for intake. The absence of correlated evolution between armour and bite force or jaw closing velocity rejects the hypothesis that alternative performance capacities can evolve to compensate for a reduction in sprinting capacity. Instead, selection on performance capacities, such as bite force, appears to depend largely on the ecology and life history of the predator and prey.

Table 4.1: Summary of morphological and performance data from 27 species of cordylid lizards considered in this study.

Species	Morphometric data			Performance			Diet	
	SVL	Armour (phylPCA)	Jaw closing lever-ratio	Jaw closing velocity (cm s^{-1})	Bite force (N)		% Hard prey	% Evasive prey
Pl. intermedius	94.6 ± 3.7 (4)	-1.25595 (4)	0.332 ± 0.007 (3)	64.6 ± 7.1 (3)	9.6 ± 0.2 (2)			
Pl. capensis	79.8 ± 0.5 (5)	-1.32537 (5)	0.328 (1)					
S. giganteus	177.2 ± 9.6 (5)	1.33993 (5)	0.279 ± 0.002 (2)	94.0 ± 16.8 (3)	79.1 ± 6.8 (2)		81.1	2.2
S. mossambicus	121.8 ± 3.9 (2)	0.32627 (2)	0.386 (1)	54.0 ± 10.9 (2)	58.6 ± 2.1 (2)			
S. depressus	115.3 ± 6.0 (10)	1.07375 (10)	0.324 ± 0.005 (2)					
Ni. coeruleopunctatus	73.9 ± 2.8 (5)	0.16703 (5)	0.344 ± 0.013 (4)					
Ch. anguina	79.3 ± 2.8 (2)	-0.76095 (2)	0.286 ± 0.010 (3)					
Ps. microlepidotus	146.3 ± 14.7 (6)	-0.82172 (6)	0.349 ± 0.017 (5)	69.1 ± 10.5 (3)	101.0 ± 21.5 (3)		53.2	32.5
Ps. subviridis	115.5 ± 7.8 (4)	-0.41923 (4)	0.392 ± 0.017 (3)					
Ps. spinosus	84.3 ± 3.7 (5)	-0.31030 (5)	0.391 (1)					
Na. peersi	78.0 ± 5.6 (5)	1.19893 (5)	0.322 ± 0.018 (5)	63.3 ± 2.9 (3)	20.8 ± 1.3 (5)		39.5	5.6
Na. namaquensis	68.7 ± 6.2 (3)	1.62461 (3)	0.324 ± 0.008 (2)					
O. cataphractus	113.7 ± 3.7 (5)	2.10153 (5)	0.375 ± 0.011 (4)	50.5 ± 4.9 (6)	54.1 ± 15.3 (22)		53.7	0
K. polyzonus	104.4 ± 6 (8)	0.58071 (8)	0.337 ± 0.015 (5)	59.6 ± 12.3 (6)	31.5 ± 6.7 (36)		35.9	34.3
K. jordani	105.2 (1)	1.57735 (1)	0.335 (1)					

Table 1. Continued

H. nebulosus	67.9 ± 6.2 (3)	1.00105 (3)	0.307 ± 0.010 (2)				
H. capensis	93.4 ± 3.1 (4)	-0.05555 (4)	0.355 ± 0.026 (2)				
H. robertsi	100.0 ± 1.4 (4)	-0.83817 (4)	0.347 ± 0.005 (4)	60.7 ± 0.9 (3)	39.2 ± 4.1 (4)	29.3	43.6
Co. macropholis	68.5 ± 2.9 (5)	1.19429 (5)	0.311 ± 0.011 (4)		11.3 ± 2.2 (15)	73.3	0
Co. mclachlani	61.4 ± 2.4 (3)	1.26854 (3)	0.318 ± 0.006 (4)				
Co. minor	62.0 ± 2.5 (5)	1.27335 (5)	0.315 ± 0.014 (3)				
Co. niger	83.3 ± 2.6 (6)	0.50617 (6)	0.357 ± 0.010 (6)	51.6 ± 4.6 (3)	19.9 ± 2.4 (5)	36.8	36.0
Co. cordylus	82.8 ± 5.1 (5)	0.83817 (5)	0.357 ± 0.014 (5)	54.8 ± 3.3 (4)	19.0 ± 7.2 (19)	40.0	31.6
Co. oelofseni	60.7 ± 0.7 (5)	1.03227 (5)	0.319 ± 0.009 (6)				
Co. tropidosternum	71.0 ± 1.0 (4)	0.95418 (4)	0.338 (1)	65.2 ± 5.7 (2)	21.0 ± 1.2 (3)		
Co. vittifer	83.3 ± 7.2 (5)	1.04299 (5)	0.356 ± 0.018 (4)				

Table entries are mean values ± standard deviation for snout-vent length (SVL), jaw closing lever-ratio, bite force and jaw closing velocity.

Degree of body armour is based on a phylogenetic principal component analysis performed on seven phylogenetically size-corrected spine measurements. Samples sizes are shown between brackets.

Table 4.2: Stomach contents of nine species of cordylid lizards. Total number of prey items are presented per prey type according to taxonomical classification. The number of stomachs examined per species is indicated in brackets.

	S. giganteus[1] (140)	*Ps. microlepidotus*[*] (22)	*Na. peersi*[4] (127)	*O. cataphractus*[2] (39)	*K. polyzonus*[3] (53)	*H. robertsi*[*] (24)	*Co. macropholis*[*] (15)	*Co. niger*[*] (11)	*Co. cordylus*[*] (18)
Araneidae	9	0	12	5	1	2	1	1	0
Blattaria	0	4	1	0	2	5	0	0	0
Chilopoda	4	2	1	0	2	2	0	0	0
Coleoptera	908	39	117	24	46	96	22	7	8
Coleoptera larvae	0	0	18	0	2	0	0	0	0
Diplopoda	21	1	2	4	0	0	0	0	2
Diptera	0	0	2	0	6	3	0	1	0
Diptera larvae	0	0	0	0	1	0	0	0	1
Embioptera	0	0	0	0	0	1	0	0	0
Formicidae	113	0	83	3	12	55	1	0	1
Heteroptera	34	8	14	5	4	22	4	3	3
Hymenoptera	0	7	3	0	25	132	0	5	8
Isopoda	0	0	0	0	0	2	0	0	0
Isoptera	0	0	10	10	10	0	0	1	1
Lepidoptera	0	7	6	0	7	1	0	0	0
Lepidoptera larvae	29	0	25	2	4	6	2	1	0
Mantodea	1	1	0	0	0	0	0	0	0
Neuroptera	0	0	0	0	0	0	0	0	0
Orthoptera	25	7	5	0	4	1	0	0	1
Phasmodea	0	0	0	0	1	0	0	0	0
Scorpionidae	0	1	0	1	0	0	0	0	0
Solifugidae	1	0	0	0	1	0	0	0	0
Thysanoptera	0	0	2	0	0	0	0	0	0
Total number of prey	1145	77	301	54	128	328	30	19	25

[*]this study; [1]Van Wyk (2000); [2]Mouton *et al*. (2000); [3]Broeckhoven & Mouton (2014); [4]Fell (2005)
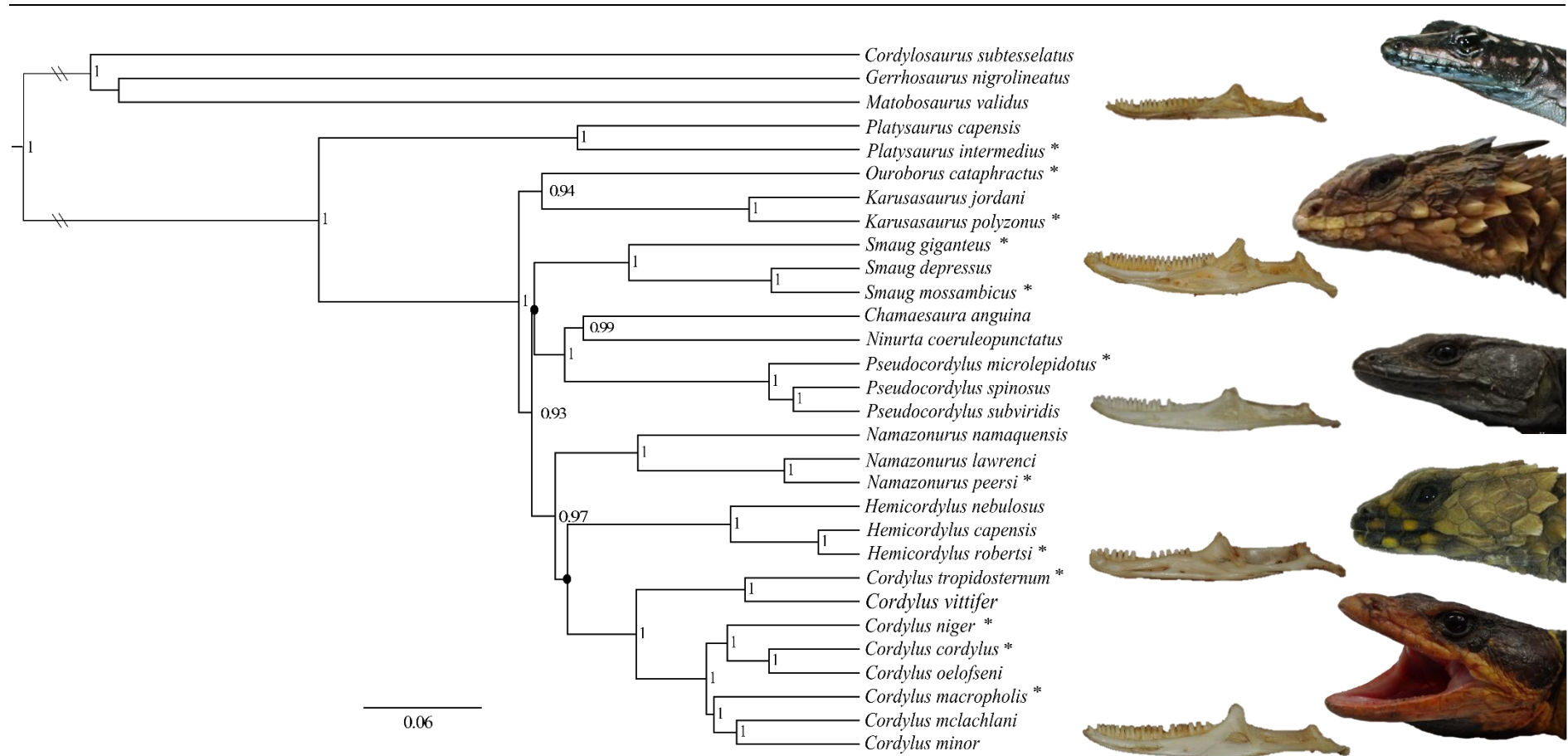
Figure 4.1: The maximum clade credibility tree attained for the BEAST analysis indicating relative divergence times. Posterior probabilities are indicated at each node, while black circles indicate nodes with no support. The asterisk indicates species that are used for the performance experiments. Images on the right illustrate the variation in body armour and jaw morphology in Cordylidae. From top to bottom: *Platysaurus intermedius*, *Smaug giganteus*, *Hemicordylus robertsi*, *Ouroborus cataphractus*, *Pseudocordylus microlepidotus*.
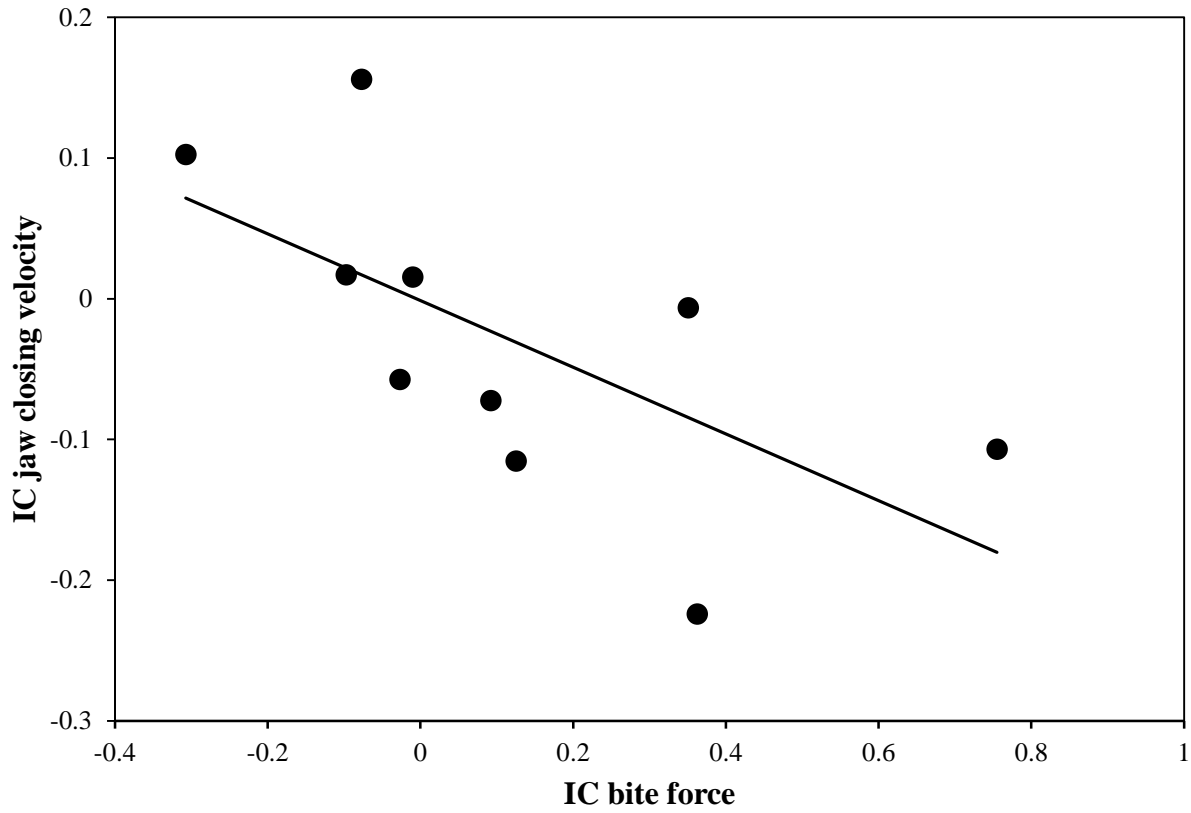
Figure 4.2: Graph illustrating the relationship between the independent contrasts of residual bite force and residual jaw closing velocity. Bite force and jaw closing velocity trade-off in the cordylid lizards.
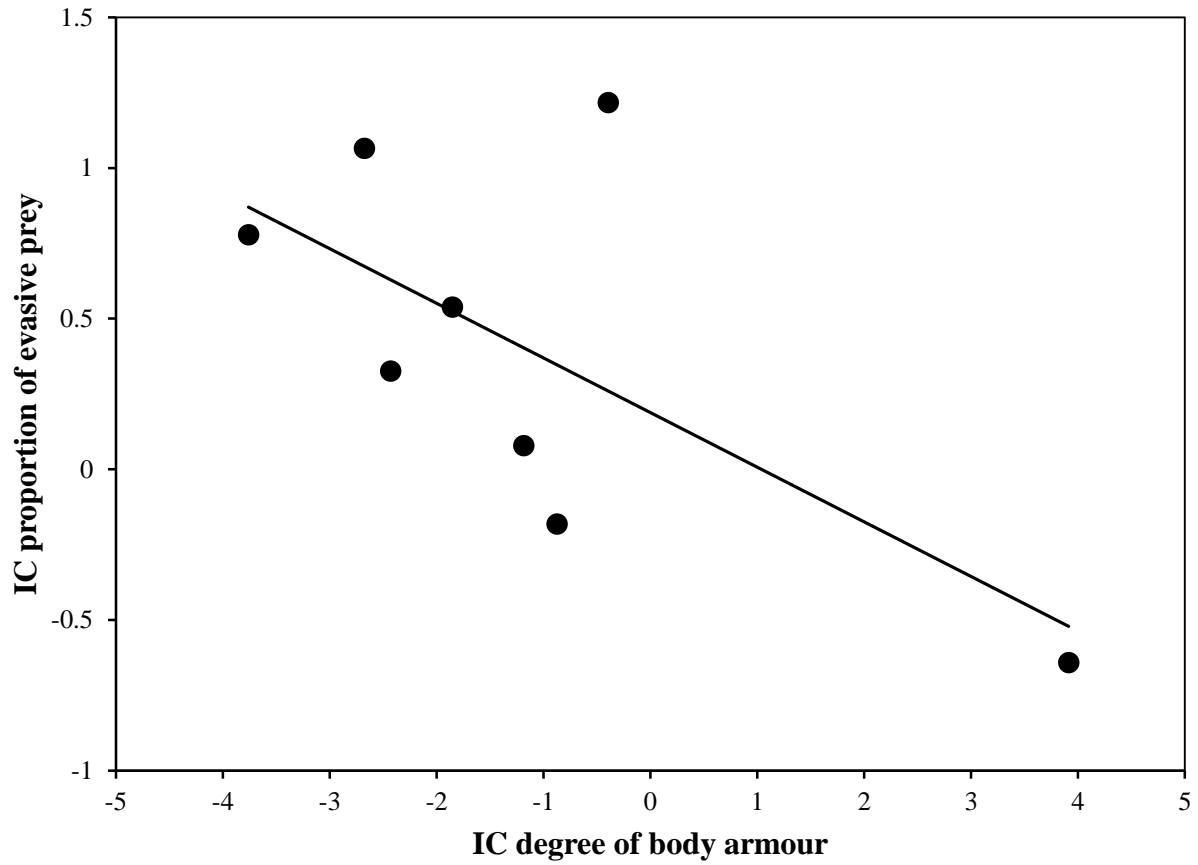
Figure 4.3: Graph illustrating the relationship between the independent contrasts of body armour and proportion of evasive prey in the diet. An increase in degree of body armour is paralleled by a decrease in the proportion of evasive prey in the diet.

MORPHOLOGICAL AND ECOLOGICAL CORRELATES OF BITE FORCE IN THE ROCK-DWELLING LIZARD *OUROBORUS CATAPHRACTUS* AND *KARUSASAURUS POLYZONUS*[∗]

ABSTRACT

Rock-dwelling lizards are hypothesised to be highly constrained in the evolution of head morphology and consequently bite force. As the ability to generate a high bite force might be advantageous for a species' dietary ecology, morphological changes in head configuration that allow individuals to maintain or improve their bite force under the constraint of crevice-dwelling behaviour are to be expected. I addressed this issue by examining head morphology, bite force and a number of dietary traits in the rock-dwelling cordylid lizards *Ouroborus cataphractus* and *Karusasaurus polyzonus*. My results show that *O. cataphractus* has a larger head and higher bite force than *K. polyzonus*. While in *K. polyzonus*, head width, lower jaw length and jaw closing-in lever are the best predictors of bite force, head height is the main determinant of bite force in *O. cataphractus*. Although the observed difference in bite force between the species does not seem to be related to dietary patterns or prey handling, the prey spectrum available for intake was greater in *O. cataphractus* compared to *K. polyzonus*. I discuss the influence of interspecific differences in antipredator morphology on head morphology and bite force in these rock-dwelling species.

INTRODUCTION

The cranial system of vertebrates is a complex integrated system and is responsible for an array of functions and behaviours, including feeding, drinking, chemoreception, display and defense (Bels *et al*., 1993; Schwenk, 1995, 2000). For an organism, it is often not possible to optimise these functions simultaneously, as the selective pressures on the different components of the cranial system are in many cases conflicting, leading to functional trade-offs (e.g. fish: Westneat, 1994; turtles: Herrel *et al*., 2002; birds: Herrel *et al*., 2009). Moreover, not only is the cranial system affected by conflicts between internal components, it is also subjected to external selective pressures imposed by environmental factors (Vitt *et al.*, 1997; Vanhooydonck & Van Damme, 1999; Herrel *et al*., 2001a; Lappin *et al*., 2006; Kohlsdorf *et al.*, 2008; Revell *et al*., 2008; Vanhooydonck *et al*., 2010; Barros *et al*., 2011).

In lizards, the evolution of head morphology has been hypothesised to be influenced by habitat use. To illustrate, head size appears to be constrained in burrowing skinks (Barros *et al*., 2011), as selection for a large head will increase burrowing time and consequently predation risk (Vanhooydonck *et al*., 2011). In lacertid lizards, a large head seems to negatively affect climbing performance by shifting the lizard's centre of mass away from the substrate (Vanhooydonck & Van Damme, 1999; Vanhooydonck *et al*., 2007). Similarly, the use of crevices by rock-dwelling species appears to constrain head morphology (Herrel *et al*., 2001a; Lappin *et al*., 2006; Revell *et al*., 2008; Kaliontzopoulou *et al*., 2012; but see Kohlsdorf *et al*., 2008), as a tall head prohibits individuals from using narrow crevices, thereby increasing the risk of becoming extracted by predators (Cooper *et al*., 1999, 2000).

The constraints of crevice-dwelling behaviour on head morphology might impair bite performance, as changes in form, size and relative position of cranial and muscular elements greatly affect bite force (Herrel *et al.* 1999, 2001a, b, 2006, 2007; Lappin & Husak, 2005; Brecko *et al.*, 2008). A reduction in bite force, in turn, can have major consequences for a species' diet, as it may limit the proportion of hard prey available for intake (Herrel *et al*., 1999, 2001b; Aguirre *et al*., 2003; Marshall *et al*., 2012) or affect prey selection through prolonged handling duration for harder and/or larger prey (Andrews & Bertram, 1997; Verwaijen *et al*., 2002). A study by Kaliontzopoulou *et al.* (2012) showed that although microhabitat divergence between species is reflected in their head morphology, a rock-dwelling lifestyle does not constrain bite force or diet.

These findings suggest that the presence of morphological changes in head configuration serve to maintain bite force, thereby allowing rock-dwelling species to utilise similar dietary resources as terrestrial species.

In this chapter, I aim to provide more insight into the relationships between head morphology, bite force and several aspects of dietary ecology in the rock-dwelling cordylid lizards, *Ouroborus cataphractus* and *Karusasaurus polyzonus* (Fig. 5.1). The two species are closely related (Stanley *et al.,* 2011) and are considered strict rock-dwelling sit-and-wait foragers (Mouton & Van Wyk, 1997). They co-occur throughout their distribution range (Bates *et al*., 2014) and are often observed sharing the same rock crevices (Effenberger & Mouton, 2007; Chapter 2). *Ouroborus cataphractus* and *K. polyzonus*, however, strongly differ in several aspects of their behaviour and morphology. *Ouroborus cataphractus* is a group-living, heavily armoured species that will grip its tail between the jaws when threatened by predators and roll up into an impenetrable ball, with the spiny tail and legs protecting the soft underparts (Mouton *et al.,* 1999; Losos *et al*., 2002; Mouton, 2011). During summer, individuals sporadically visit the foraging ports of the southern harvester termite, *Microhodotermes viator* (Shuttleworth *et al*., 2008). It has been hypothesised that the heavy armour and tail-biting behaviour in *O. cataphractus* are direct consequences of exploiting termites away from the safety of the crevice and the terrestrial predation pressure associated with these foraging excursions (Mouton, 2011). Despite the use of a clumped food source (i.e. termites) away from the communal crevice, competition between group-members appears to remain high (Mouton *et al*., 2000a; Shuttleworth *et al*., 2008). The concurrence of a short activity peak with high arthropod abundance during spring appears to counteract the negative effects of group-living behaviour, thereby allowing individuals to survive summer (Flemming & Mouton, 2002; Shuttleworth *et al*., 2013). In contrast, *K. polyzonus* is a strictly solitary, moderately armoured species that does not display tail-biting behaviour. *Karusasaurus polyzonus* has an overall generalistic diet (De Waal, 1978; Branch & Bauer, 1995; Branch, 1998) and is active throughout the year (Visagie, 2001; Chapter 2). Furthermore, whereas in the group-living *O. cataphractus* males display territorial polygyny (Effenberger & Mouton, 2007), only low levels of intraspecific aggression have been observed in *K. polyzonus* (Broeckhoven & Mouton, personal observations).

The first aim of the study is to investigate which morphometric variables contribute to bite force in the two rock-dwelling species. I predict that (1) head width and length of the jaw closing

in-lever will be better predictors of bite force than head height, which is constrained by crevice dwelling-behaviour and that (2) the effect will be more pronounced in *O. cataphractus* because a higher bite force is presumably beneficial for tail-biting behaviour. The second aim is to examine whether bite force influences aspects of the dietary ecology of the two species. I predict that (3) an increase in bite force will increase the number of hard-bodied prey items than can be consumed and reduce the time needed to process a given prey item.

MATERIALS AND METHODS

MORPHOLOGY AND BITE FORCE

A sample of 39 *O. cataphractus* (19 males and 20 females) and 36 *K. polyzonus* (16 males and 20 females) was used to describe external head morphology and to quantify bite force. For each individual caught, the following measurements were taken: snout-vent length (SVL); head length (HL), measured from the posterior edge of the parietal bone to the tip of the snout; head width (HW), measured at the widest part of the head; head height (HH), measured posterior to the orbits at the highest part and lower jaw length (LJL), measured from the back of the retro-articular process to the tip of the lower jaw; jaw out-lever (JOL), measured from the quadrate to the tip of the lower jaw and snout-length (SL), measured from the coronoid to the tip of the lower jaw. The length of the jaw opening in-lever (OIL) was estimated by subtracting the jaw out-lever from the lower jaw length. The jaw closing in-lever (CIL) was given by subtracting the snout-length from the jaw out-lever. All measurements were taken with a precision of 0.01 mm using digital callipers (Mitutoyo Ltd., Sakato, Japan).

Bite force (BF) was measured *in vivo* making use of a force transducer (model 9203, Kistler Inc., Winterthur, Switzerland), mounted on a purpose-built holder and connected to a portable charge amplifier (model 5995A, Kistler Inc., Winterthur, Switzerland). A description of the device used to measure bite force can be found in Herrel *et al.* (2001b). As bite force is affected by body temperature (Anderson *et al*., 2008), lizards were kept individually in cloth bags and placed in an incubator for at least 1 h prior to each bite force measurement in order to obtain their optimal body temperatures: 30°C for *O. cataphractus* (Truter *et al*., 2014) and 34°C for *K. polyzonus* (Clusella-Trullas *et al.,* 2007). Bite force was recorded by inducing the lizards to bite onto two metal plates,

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which were moved further apart when testing larger individuals to remove any effects of gape angle on bite force. Measurements were repeated five times for each animal, with an interval of at least 30 min. The maximal value acquired during the five trials was considered to be the maximal bite force for that individual.

Individuals used to measure bite force were collected under permit number AAA007-00026-0056 (Western Cape). The protocol used to measure bite force was approved by the Research Ethics Committee of the Faculty of Science, Stellenbosch University (Ethical clearance number: SU-ACUM12–00024) and is in accordance with the ethical guidelines set by the American Society of Ichthyologists and Herpetologists.

DIET AND PREY HARDNESS

Dietary data for 37 specimens of *O. cataphractus* (24 males and 13 females) were taken from Mouton *et al.* (2000a). To obtain dietary data for *K. polyzonus*, the stomachs of 53 specimens (24 males and 29 females) were dissected using preserved material in the Ellerman Collection of the University of Stellenbosch. All specimens came from the same geographical area and stomach contents belonged to individuals caught at different times of the year to rule out any possibility of seasonal variation in diet. All remains were identified to order level, with the exception of larvae (i.e. Lepidoptera, Diptera, Coleoptera), which were treated separately due to their sedentary nature and Formicidae, which were grouped separately from Hymenoptera due to the non-evasive nature. Additionally, all prey items were classified into functional categories according to their hardness based on previous measurements of prey hardness (Herrel *et al*., 1999, 2001b; Aguirre *et al*., 2003; Broeckhoven, 2011): hard (Coleoptera, Diplopoda and Scorpiones), intermediate (Orthoptera, Solifugae and Hymenoptera) or soft (all other categories).

In order to assess the effect of bite force on the potential prey spectrum, the mean and maximal bite force of the two species (males and females separately) was compared to the hardness of various prey items. Prey hardness was measured by pushing the flattened top of a screw, mounted on a force transducer (model 9203, Kistler Inc., Winterthur, Switzerland), onto the prey until the exoskeleton showed mechanical failure (see Herrel *et al.,* 2001b for a description of the set-up). The force required to crush the hardest parts of the arthropod was considered the prey hardness. The experimental procedure was limited to Coleoptera, Diplopoda and Scorpiones, as

dietary niche partitioning is most likely to occur for these tougher prey categories. In total, 149 specimens belonging to 11 species were collected in areas inhabited by both lizard species.

FEEDING EXPERIMENTS

Feeding experiments were conducted using a subset of lizards from both species (*O. cataphractus*: 4 males, 10 females; *K. polyzonus:* 7 males, 6 females). Each lizard was presented with five equally sized field crickets (*Gryllus assimilis;* length approximately 23 mm). Each cricket was introduced into the lizard's enclosure, approximately 10 cm from the opening of the shelter. Lizards were left undisturbed and feeding behaviour was digitally recorded at 120 frames per second using a Casio Exilim EX-FH25 high-speed video camera (Casio Computer Co., Ltd., Tokyo, Japan). Per individual, the prey handling duration, calculated as the difference between the time of jaw closing onto the prey to the onset of the swallowing phase, was determined. Average prey handling duration was calculated per individual to avoid pseudoreplication.

DATA ANALYSIS

Prior to the analysis, all data were $\log_{10}$-transformed in order to meet the assumptions of the statistical tests. Given the inter- and intraspecific differences in body size, all morphological and performance variables were regressed against snout-vent length and residuals were calculated. To test for inter- and intraspecific variation in head morphology, a multivariate analysis of variance (MANOVA) was used, with species, sex and their interaction as model effects. Univariate *F*-tests were performed to test for differences in relative bite force and relative head dimensions between the species and sexes. Analyses of variance were conducted with SPSS Statistics v. 17.0.1 (SPSS Inc., Chicago, IL, USA). The significance level was accepted at $P < 0.05$ and adjusted using a sequential Bonferroni correction (Rice, 1989)

To test which combination of head morphometrics best explained variation in bite force, I applied the Akaike's model selection procedure for regression models (Burnham & Anderson, 2002) on the combined dataset, as well as for each species-sex group separately. Residual bite force was used as the dependent variable and all residual head morphometrics were used as independent variables. The relative importance of each individual morphometric was given by the sum of the Akaike weights for that specific variable across all models with Delta AIC < 4

(Burnham & Anderson, 2002). By employing a method based on multimodel inference, thereby considering all possible combinations of predictor variables, several issues associated with multicollinearity could be avoided (Graham, 2003). The model selection techniques were carried out in R v. 3.1.1 (R Development Core Team, 2014) using the MuMIn package (Barton, 2013).

To test for inter- and intraspecific differences in prey use, three descriptors were used. First, the use index (UI) (Jover, 1989; Carretero, 2004) was used to describe the dominance of each prey in the stomachs examined, by combining the percentage of stomachs containing a given prey with the percentage of its numeric abundance. In order to make the index values independent of sample size, I calculated UI' = (UI/n) × 100, with n being the number of individuals (Ruiz, 1985). Using the graphical approach proposed by Carretero & Llorente (2001), the UI' were compared between sexes of the same species and between species of the same sex. Second, Pianka's index (Pianka, 1973), applied on the UI' values, was calculated for each combination of species-sex groupings to determine the degree of similarity in prey use. Third, the proportion of hard prey represented in the diet was compared between the sexes and species using a Chi-Squared test. Finally, an association between bite force and dietary niche was tested for using a Mantel test with 10000 randomisations in PASSaGE v. 2 (Rosenberg & Anderson, 2011). A distance matrix was generated for log$_{10}$-transformed bite force using Euclidean distances and for dietary niche (being 1 - Pianka's niche overlap; Kaliontzopoulou *et al*., 2012).

To test for differences in prey handling duration, an ANOVA with species, sex and their interaction as model effects was used. In addition, to examine whether differences in bite force between the species and sexes contribute to prey handling duration, bite force was incorporated as a covariate in the analysis. Furthermore, as the bite force exerted upon a prey item depends on the gape angle (Dumont & Herrel, 2003; Anderson *et al*., 2008; Williams *et al*., 2009), which in turn is determined by head morphology, the maximal gape angle was estimated for each individual used during the feeding experiments. Maximal gape height (GH$_{max}$) and distance from the tip of the snout to the quadrate (i.e. jaw out-lever, JOL) was measured in a number of previously frozen but not preserved specimens of *K. polyzonus* (n = 7) and *O. cataphractus* (n = 5). Next, maximal gape angle (GA$_{max}$) was calculated with the formula:

$$GA_{max} = \cos^{-1}\left[1 - \left(\frac{GH_{max}^2}{2*JOL^2}\right)\right]$$

For each individual, the predicted gape angle was calculated from the obtained regression equations of the form $GA_{max} = a * JOL + b$. To investigate whether inter- and intraspecific differences in gape angle contribute to prey handling duration, an ANCOVA was conducted with gape angle as a covariate.

RESULTS

MORPHOLOGY AND BITE FORCE

The results of a MANOVA performed on the residual head dimensions revealed statistically significant species effects (Wilk's Lambda = 0.086; $F_{8,64} = 85.15$; $P < 0.001$) as well as sex effects (Wilk's Lambda = 0.641; $F_{8,64} = 4.49$; $P < 0.001$). The species–sex interaction effect was not statistically significant (Wilk's Lambda = 0.867; $F_{8,64} = 1.23$; $P = 0.30$). Univariate F-tests indicated that species differed significantly in bite force and head morphology, but the length of the closing and opening in-lever was similar in both sexes (Table 5.1, Table 5.2). *Ouroborus cataphractus* individuals had relatively larger heads and higher bite forces than *K. polyzonus* individuals, but had shorter snout lengths. In both species, males had larger heads for a given body size than females and bit considerably harder (Table 5.1, Table 5.2).

Regression models performed on the residual data showed that although head length, width and height were important predictors of bite force across sexes and species, clear differences were present between the four groups (Table 5.3). In *O. cataphractus*, head height explained most of the variation in bite force, whereas an opposite trend was present in *K. polyzonus*. Indeed in *K. polyzonus*, head width and lower jaw length were important predictors of bite force in males, while closing in-lever was an important predictor of bite force in females (Table 5.3).

DIET

Examination of 182 prey items showed that the diet of both species consisted predominantly of Coleoptera (40 - 68 %). Analysis of both prey indices revealed high overlap in diet between sexes in *K. polyzonus*, but less overlap was present between the sexes in *O. cataphractus* (Table 5.4). Graphical comparison of use indices showed that the consumption of

Coleoptera was higher in *O. cataphractus* males, while the consumption of Diplopoda was higher in *O. cataphractus* females (Fig. 5.2). Male and female adults consumed prey from the three hardness classes in similar proportions, but statistically significant differences were present between the species (Table 5.4). However, there were no differences between species or sexes in the proportion of hard prey, except between males (Table 5.4). Analysis of prey hardness data revealed that all prey items could theoretically be crushed by *O. cataphractus* males, as well as by females when maximal bite force was considered. A number of Coleoptera, Diplopoda and Scorpiones, however, exceeded the maximal bite force measured in *K. polyzonus* (Table 5.5; Fig. 5.3). No statistically significant association was present between bite force and dietary niche (Mantel test; $P = 0.96$).

FEEDING EXPERIMENTS

Analysis of variance performed on the mean prey handling duration revealed no difference between species ($F_{2,24} = 2.25$; $P = 0.15$) or sexes ($F_{2,24} = 0.87$; $P = 0.36$). No statistically significant interaction effect between sex and species was present ($F_{3,23} = 2.97$; $P = 0.1$). Mean prey handling duration for crickets were similar in the two species (*O. cataphractus*: 28.6 ± 8.7 s; *K. polyzonus*: 22.3 ± 9.4 s). Prey handling duration was negatively correlated with $\log_{10}$-transformed bite force ($r = -0.66$; $P = 0.01$; Fig. 5.4A) and positively correlated with $\log_{10}$-transformed gape angle ($r = 0.72$; $P = 0.004$; Fig. 5.4B) in *O. cataphractus*, as well as in *K. polyzonus* (bite force: $r = -0.57$; $P = 0.04$; gape angle: $r = -0.62$; $P = 0.02$; Fig. 5.4A, Fig. 5.4B). Prey handling duration differed significantly between the species when bite force ($F_{3,23} = 15.85$; $P = 0.001$) or gape angle ($F_{3,23} = 18.26$; $P < 0.001$) was introduced as a covariate in the analysis but no difference could be detected between the sexes (bite force: $F_{3,23} = 0.72$; $P = 0.41$; gape angle: $F_{3,23} = 0.18$; $P = 0.68$). All interaction effects were non-significant (all $P > 0.05$).

DISCUSSION

MORPHOLOGICAL CORRELATES OF BITE FORCE

The finding that head width, lower jaw length and closing in-lever are important predictors of bite force in *Karusasaurus polyzonus* is in accordance with my prediction that head dimensions

that are not constrained by crevice-dwelling behaviour contribute the most to bite force in rock-dwelling species. In contrast, head height is the best predictor of bite force in *Ouroborus cataphractus* and significant inter- and intraspecific variation in head height is present in the two species. While an increase in any head dimension can improve bite force as more space is provided to accommodate musculature, an increase in head height could further enhance bite force by increasing the physiological cross-section of the jaw-adductor muscles (Herrel *et al*., 2001a).

Although head height is a strong predictor of bite force in other rock-dwelling lizards (Herrel *et al.*, 2001a; Lappin *et al*., 2006), the greater relative head height in *O. cataphractus* compared to *K. polyzonus* appears to contradict the hypothesis that this dimension is constrained by crevice-dwelling behaviour. Differences in habitat use or predation pressure might be responsible for interspecific variation in head height, as suggested by Herrel *et al*. (2001a) for rock-dwelling xenosaurids. However, this does not seem to be the case here, as both species co-occur throughout most of their range (Bates *et al*., 2014) and are subjected to similar predation pressures. The observed interspecific difference in head height is possibly related to the antipredator morphology of the two species. To avoid extraction, cordylid lizards typically position their bodies parallel with the crevice opening, thereby using the tail to block access to the lizard's side by the predator (Cooper *et al*., 2000). The heavy armour of *O. cataphractus*, and more specifically the presence of long tail spines, might allow an increase in head height without increasing extraction risk, thereby enabling individuals to achieve a higher bite force. Support for this hypothesis is provided by the fact that cordylid lizards that lack spines (e.g. *Platysaurus*) appear to have relatively flat heads (Losos *et al*., 2002). Moreover, intersexual differences in body size and consequently spine length in *O. cataphractus* (Broeckhoven, unpublished data) would surely have strengthened a potential liberating effect of armour on head height in males. Given the role of bite force during agonistic encounters (Lailvaux *et al*., 2004; Huyghe *et al*., 2005; Husak *et al*., 2009), this could have facilitated the evolution of a polygynous mating system in *O. cataphractus* (Effenberger & Mouton, 2007).

INFLUENCE OF BITE FORCE ON DIETARY NICHE PARTITIONING

Based on the observed inter- and intraspecific variation in bite force, one would expect dietary divergence in type and hardness of prey consumed (Herrel *et al.*, 1999, 2001a, b, 2006;

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Verwajien *et al*., 2002; Brecko *et al*., 2008; Vanhooydonck *et al*., 2010; Kaliontzopoulou *et al*., 2012)*.* In contrast to my expectations, there appears to be a considerable amount of overlap between species and sexes, no difference in consumption of hard prey between *O. cataphractus* and *K. polyzonus* (except in males) and no association between bite force and dietary niche overlap. In the latter case, the detection of a significant association might have been restricted by the fact that preserved specimens were used for dietary analyses and prey availability might have changed radically since the specimens were collected. However, a comparison of recently collected faecal pellets to the stomach contents used for this study shows similar prey use in both species (Broeckhoven, unpublished data).

Our finding that prey hardness (1) is positively correlated with prey size and (2) differs between similarly-sized prey items, might confound interpretations especially given the fact that Coleoptera dominate the diet in both species. Therefore, using a more theoretical approach (i.e. comparison of potential prey spectra) might be a more reliable way of assessing the biological relevance of bite force to the observed dietary patterns, rather than using an arbitrary assignment of prey into functional groups. Indeed, comparison of bite force to the hardness of various prey items shows that while *O. cataphractus* is able to crush the whole array of tested prey items, the force needed to crush several Coleoptera and Diplopoda falls outside the range of the bite forces recorded for *K. polyzonus*. In case of *O. cataphractus*, the ability to exploit the hardest prey present in the habitat might be vital to survival. Firstly, the heavy armour and associated decrease in running speed (Losos *et al.,* 2002) presumably complicates the ability to catch evasive prey, thereby restricting the diet of *O. cataphractus* to slow-moving (and hard-bodied) prey. Secondly, the fact that *K. polyzonus* is unable to exploit very hard prey could lower interspecific competition for food and aid in the coexistence of the two species.

The observation that an increase in bite force reduces prey handling duration is consistent with the results from previous studies (Herrel *et al*., 1999; Verwaijen *et al.,* 2002). Prey handling is an ecologically relevant measure, as a prolonged handling duration might expose individuals to a greater predation risk (Andrews *et al*., 1987) or kleptoparasitism (Verwaijen *et al*., 2002). In this regard, an increase in bite force should ultimately reduce the time an individual spends outside the rock crevice and consequently limit exposure to predators. Nevertheless, prey handling duration is similar in the two species, despite clear differences in bite force. An explanation for this may be related to the effect of gape angle on prey handling duration. Given the larger gape angle in *K.*

polyzonus compared to *O. cataphractus*, processing of large crickets in *O. cataphractus* seems to occur at maximal gape size, thereby constraining bite force (Dumont & Herrel, 2003; Williams *et al*., 2009). The similar duration values obtained for the two species could thus be attributed to the counteractive effects of bite force and gape angle on prey handling duration. It must, however, be noted that prey handling experiments were conducted using an orthopteran, a prey item that is not present in the diet of *O. cataphractus*. A repetition of the feeding experiments using prey types that are consumed by both species (e.g. coleopterans) might provide more insight into the role of bite force on prey handling duration.

In conclusion, my data show a close association between head morphology, bite force and diet in two rock-dwelling cordylid lizards. The evolution of heavy armour in *O. cataphractus* appears to have counteracted the constraining effects of crevice-dwelling behaviour on head height, thereby enabling individuals to improve their bite force. A high bite force would not only have been beneficial for the tail-biting behaviour displayed by this species, it also influences the ability to exploit some of the hardest prey available in the habitat.

Table 5.1: Variation in head morphology and bite force in *Ouroborus cataphractus* and *Karusasaurus polyzonus*. Morphological variables and bite force are shown for males and females separately.

	Ouroborus cataphractus		*Karusasaurus polyzonus*	
	Males	Females	Males	Females
Snout-vent length (mm)	105.03 ± 6.02	100.00 ± 4.13	101.04 ± 7.30	105.30 ± 6.34
Head length (mm)	29.08 ± 1.67	27.05 ± 1.23	25.78 ± 1.82	26.08 ± 1.37
Head width (mm)	27.54 ± 2.02	24.81 ± 1.24	21.68 ± 2.01	21.42 ± 1.53
Head height (mm)	14.01 ± 0.89	12.92 ± 0.60	12.81 ± 1.14	12.72 ± 0.84
Lower jaw length (mm)	28.64 ± 1.41	26.52 ± 1.49	26.48 ± 1.81	26.37 ± 1.55
Jaw out-lever (mm)	26.08 ± 1.64	23.93 ± 1.24	24.44 ± 1.64	24.43 ± 1.44
Snout length (mm)	17.54 ± 2.20	15.76 ± 1.15	17.81 ± 1.25	17.69 ± 1.02
Opening in-lever (mm)	2.56 ± 0.78	2.59 ± 0.49	2.03 ± 0.54	1.94 ± 0.68
Closing in-lever (mm)	8.54 ± 1.46	8.17 ± 0.58	6.64 ± 0.77	6.73 ± 0.63
Bite force (N)	58.01 ± 13.34	40.09 ± 7.39	33.19 ± 13.63	30.15 ± 6.31

Table entries are means ± standard deviation.

Table 5.2: Results of univariate *F*-tests comparing residual head morphometric variables and residual bite force between the species (SP) *Ouroborus cataphractus* and *Karusasaurus polyzonus* and sexes (SEX).

	SP		SEX		SP * SEX	
	$F_{2,72}$	*P*	$F_{2,72}$	*P*	$F_{3,71}$	*P*
Head length	86.16	**<0.001**	9.62	**0.003**	1.31	0.26
Head width	283.9	**<0.001**	21.08	**<0.001**	0.57	0.45
Head height	45.28	**<0.001**	20.22	**<0.001**	0.26	0.62
Lower jaw length	29.89	**<0.001**	19.89	**<0.001**	0.10	0.75
Jaw out-lever	10.81	**0.002**	24.19	**<0.001**	0.22	0.64
Snout length	18.65	**<0.001**	12.21	**0.001**	0.06	0.81
Opening in-lever	12.26	**0.001**	0.02	0.90	0.39	0.54
Closing in-lever	60.14	**<0.001**	0.03	0.87	0.01	0.96
Bite force	183.4	**<0.001**	43.57	**<0.001**	0.02	0.88

Table entries are the *F*-ratio values from each test. Significant effects (after sequential Bonferroni corrections) are indicated in bold.

Table 5.3: Results of the model selection procedure for bite force fitted to the combined dataset as well as to each group separately. The most important variables are indicated in bold.

		Head length	Head width	Head height	Lower jaw length	Jaw out-lever	Snout length	Open in-lever	Close in-lever	R²	AICc
All	BM	1.60	1.28	1.62	-1.38					0.75	-190
	VI	**1**	**0.97**	**1**	0.51	0.40	0.49	0.22	0.33		
OCM	BM			1.63						0.34	-66.0
	VI	0.07	0.14	**0.91**	0.08	0.07	0.18	0.06	0.11		
OCF	BM			3.45	-2.36					0.45	-57.3
	VI	0.11	0.26	**1**	**0.91**	0.13	0.18	0.07	0.23		
KPM	BM		1.15		2.52					0.65	-47.2
	VI	0.11	**0.66**	0.10	**0.90**	0.05	0.12	0.10	0.19		
KPF	BM								0.80	0.17	-50.2
	VI	0.19	0.08	0.08	0.16	0.12	0.10	0.08	**0.57**		

Legend: AICc: Akaike Information Criterion for small samples; BM: regression coefficients for the best model; VI: variable importance sum of the Akaike weights for each individual variable; OCM: *O. cataphractus* males; OCF: *O. cataphractus* females; KPM: *K. polyzonus* males; KPF: *K. polyzonus* females.

Table 5.4: Dietary niche overlap for each combination of species-sex groupings and results from the comparison of proportions of prey hardness categories found in the stomachs.

| | | Prey hardness | | | | |
| | | Global proportion | | | Proportion hard prey | |
	Diet overlap	X^2	P		X^2	P
OCM-OCF	0.80	0.18	0.91		0.18	0.67
KPM-KPF	0.96	2.86	0.24		1.53	0.22
OCM-KPM	0.80	10.8	**0.01**		5.91	**0.02**
OCF-KPF	0.76	6.33	**0.04**		0.66	0.42
OCM-KPF	0.87	10.4	**0.01**		0.17	0.68
OCF-KPM	0.70	5.90	**0.05**		2.68	0.10

Significant effects are indicated in bold. Legend: OCM: *O. cataphractus* males; OCF: *O. cataphractus* females; KPM: *K. polyzonus* males; KPF: *K. polyzonus* females

Table 5.5: Variation in prey length and hardness in a number of hard-bodied prey items present in the habitat of *Ouroborus cataphractus* and *Karusasaurus polyzonus*.

Prey	*n*	Length (mm)	Hardness (N)
Coleoptera			
Prionorhinus canus	5	19.57 ± 1.13	27.28 ± 5.10
Psammodes sp. 1	21	22.63 ± 1.55	44.87 ± 9.20
Psammodes sp. 2	17	23.77 ± 2.20	43.35 ± 9.77
Psammodes sp. 3	25	16.47 ± 1.24	18.62 ± 3.93
Scarabaeus rugosus	8	25.12 ± 4.65	48.99 ± 5.48
Stenocara dentata	18	12.88 ± 1.61	36.26 ± 11.8
Thermophilum sp.	9	18.73 ± 1.80	20.48 ± 3.23
Trigonopus sp.	23	16.63 ± 1.76	14.10 ± 2.59
Diplopoda			
Diplopoda sp.	7	48.42 ± 12.8	37.73 ± 13.5
Scorpiones			
Opistacanthus sp.	8	30.92 ± 5.36	28.34 ± 9.86
Opistophthalmus sp.	8	24.17 ± 6.36	11.59 ± 5.54

Table entries are means ± standard deviation.
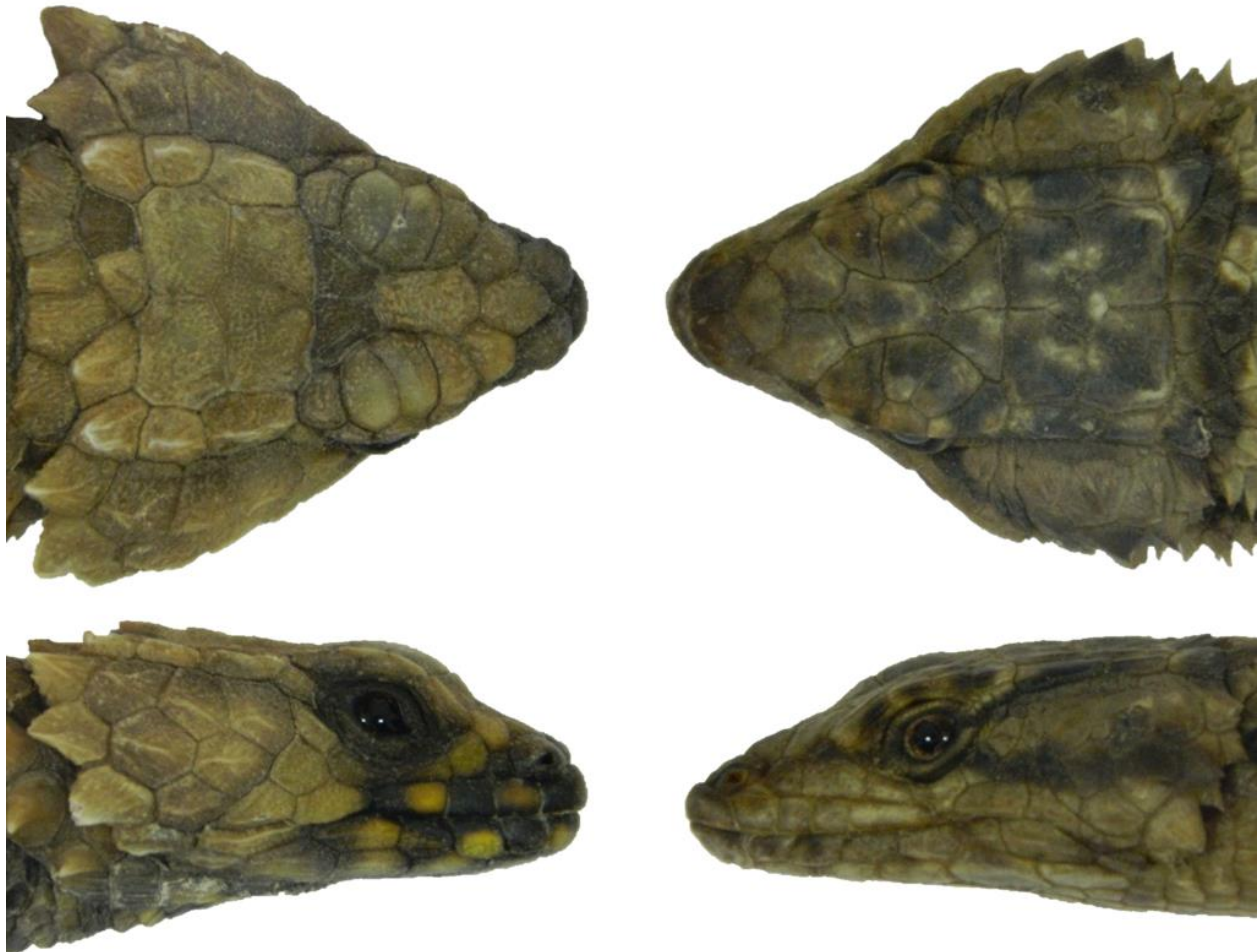
Figure 5.1: Photographs of the heads of *Ouroborus cataphractus* (left) and *Karusasaurus polyzonus* (right) in dorsal (top) and lateral (bottom) view to illustrate differences in head shape.
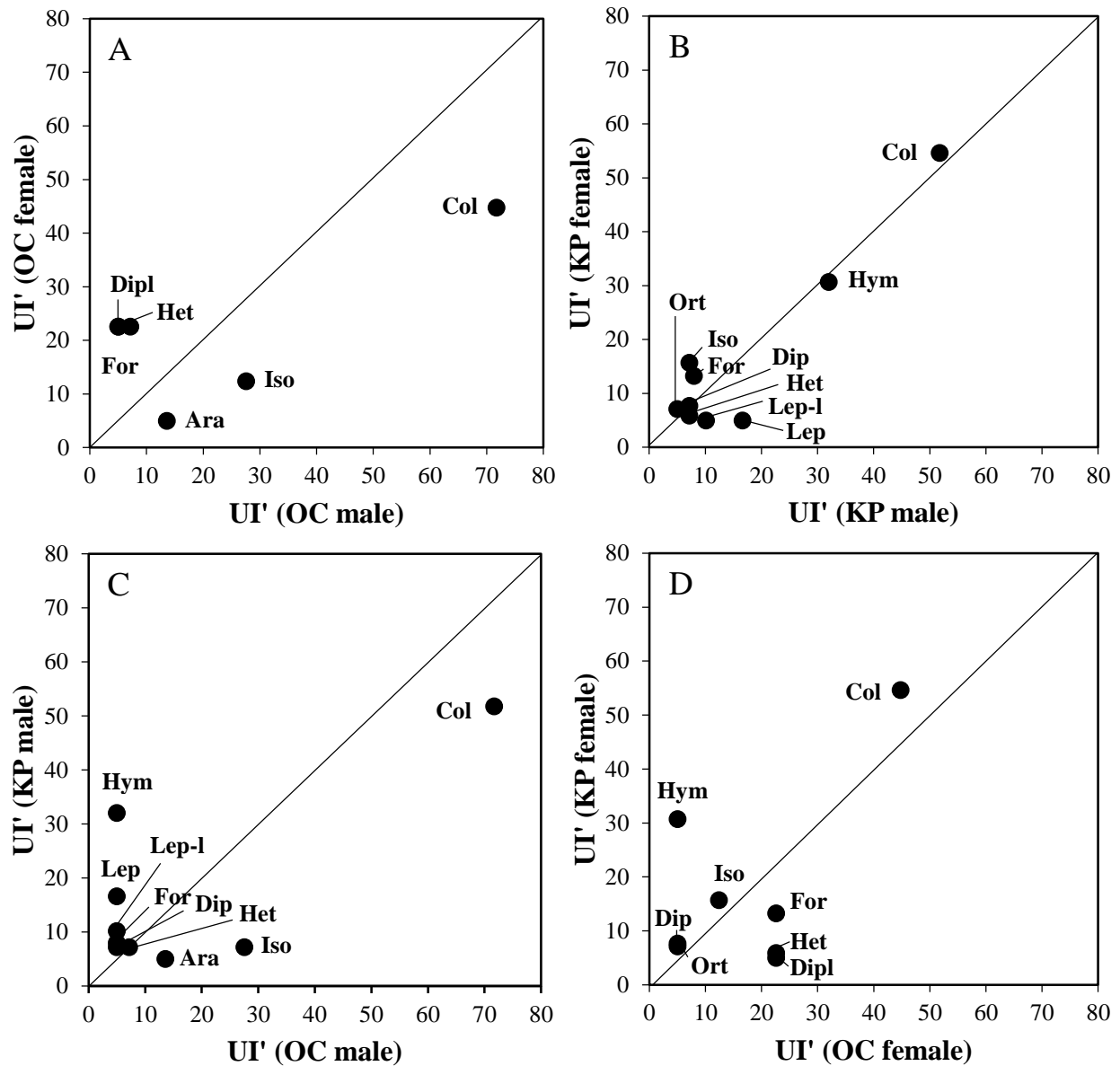
Figure 5.2: Graphical comparison of use indices for each prey type consumed between the sexes in (A) *Ouroborus cataphractus* and (B) *Karusasaurus polyzonus*, as well as between (C) males and (D) females of both species. Equal prey use by each group is represented by the continuous line. Legend: OC = *O. cataphractus*; KP = *K. polyzonus*; Ara = Araneidae; Col = Coleoptera; Dipl = Diplopoda; Dip = Diptera; For = Formicidae; Het = Heteroptera; Hym = Hymenoptera; Iso = Isoptera; Lep = Lepidoptera; Lep-l = Lepidoptera larvae; Ort = Orthoptera.

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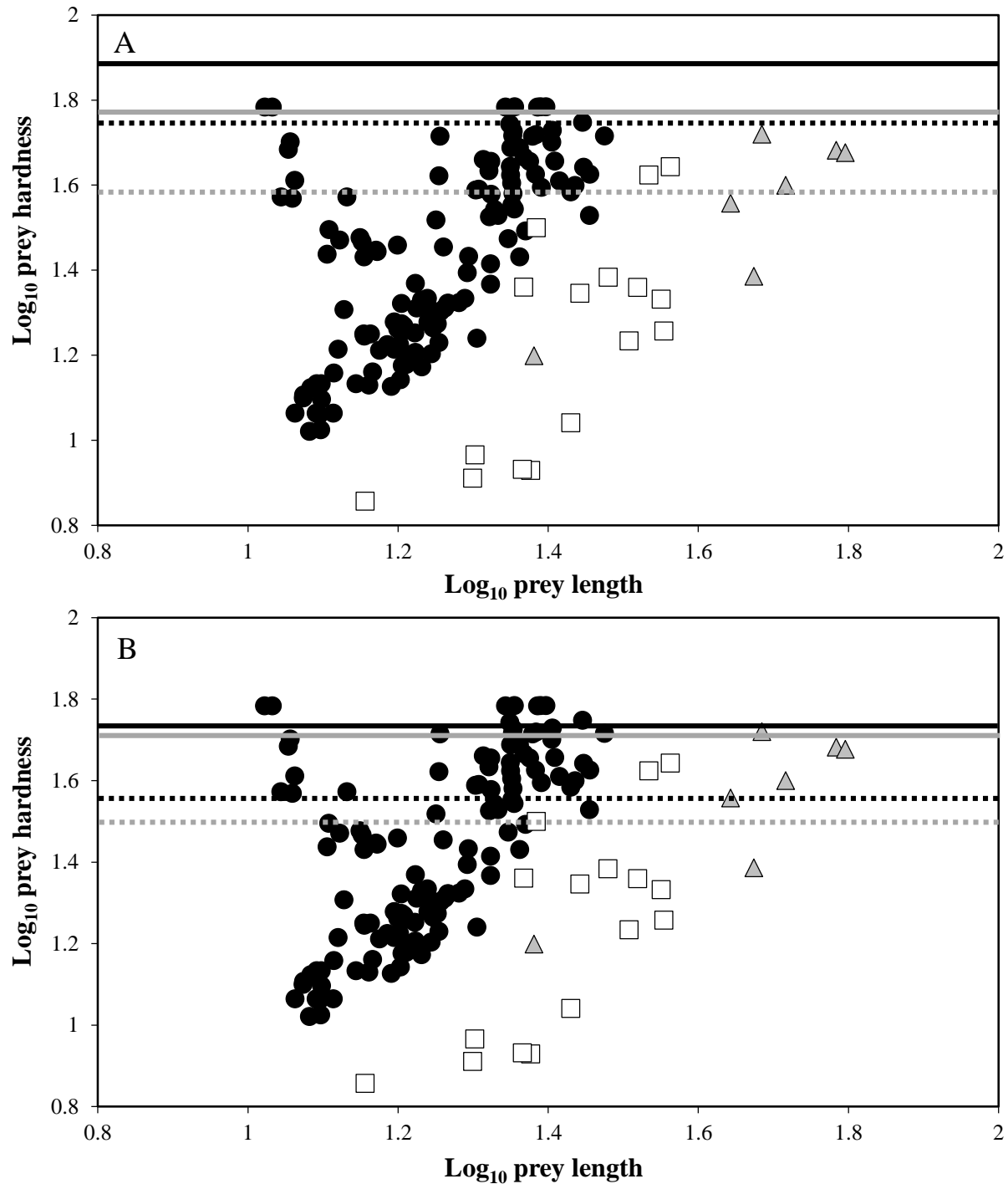
Figure 5.3: Scatterplots illustrating the relationship between prey length and prey hardness in Coleoptera (●), Diplopoda (▲) and Scorpiones (□). The maximum (solid line) and average (dotted line) bite forces of males (black) and females (grey) of *Ouroborus cataphractus* (A) and *Karusasaurus polyzonus* (B) are superimposed.

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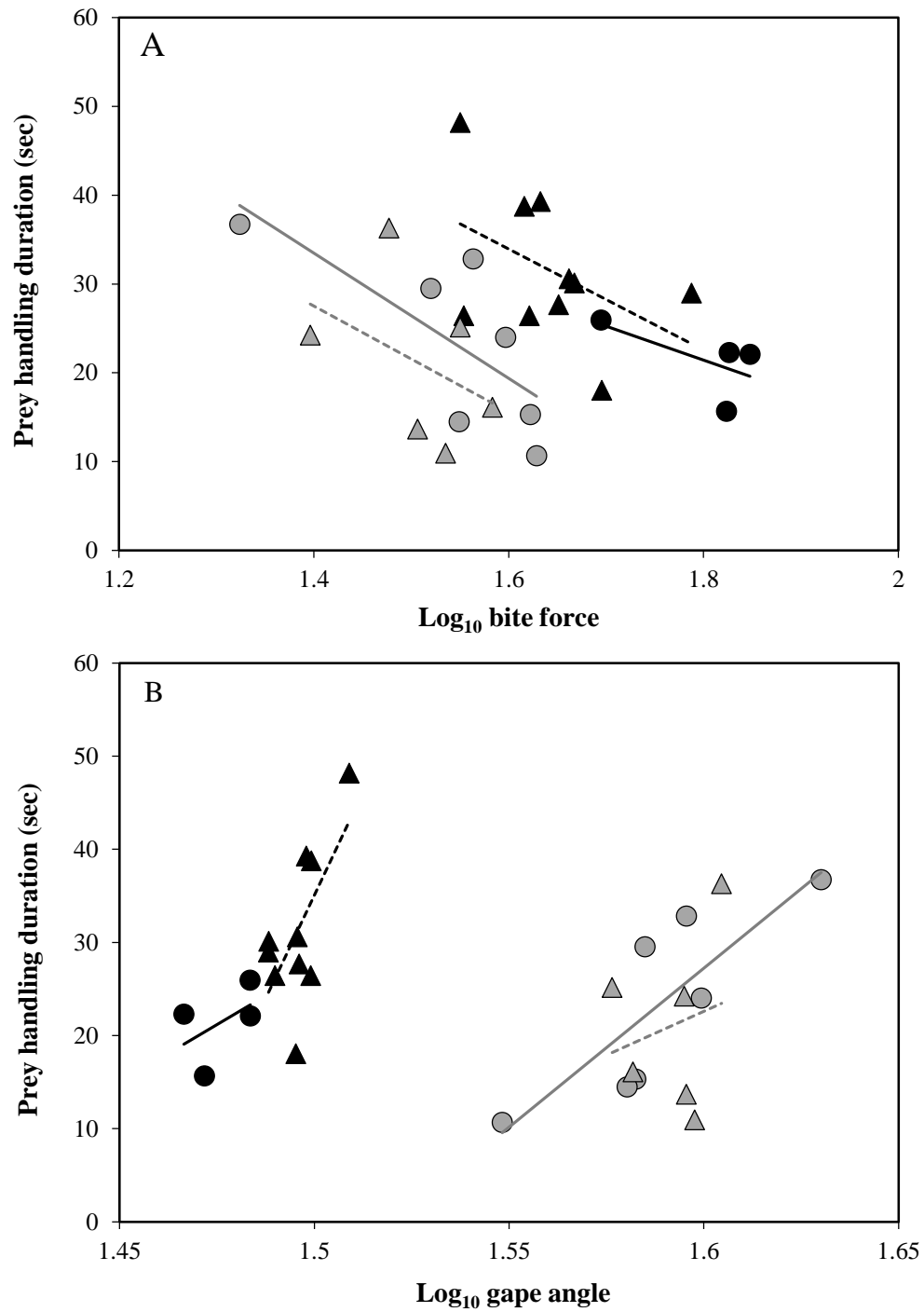
Figure 5.4: Scatterplots illustrating the relationship between (A) bite force and prey handling duration and (B) gape angle and prey handling duration in male (circles, continuous line) and female (triangles, dashed lines) *Ouroborus cataphractus* (black) and *Karusasaurus polyzonus* (grey).

<div align="right">**CHAPTER 6**</div>

INFLUENCE OF DIET ON PREHENSION MODE IN CORDYLID LIZARDS﹡

ABSTRACT

Lizards exhibit a variety of mechanisms to capture prey, including lingual prehension, jaw prehension and lingual pinning. Despite being the topic of numerous studies, the link between prehension mode and diet remains poorly understood, especially in clades where multiple prehension modes are present. I addressed this issue by comparing the feeding behaviour and tongue morphology of a termite-eating specialist (*Ouroborus cataphractus)* with that of a closely related dietary generalist (*Karusasaurus polyzonus).* I used high-speed videography to test the effect of prey species (termite versus small cricket) and prey size (small versus large cricket) on prehension mode. In addition, I included several other cordylid lizards representing the major clades in the family into my analysis to examine whether the prehension modes present in *O. cataphractus* characterise all cordylid species or whether they represent isolated occurrences. Finally, I investigated the morphology of the tongue in Cordylidae, with emphasis on *O. cataphractus* and *K. polyzonus*, using light and scanning electron microscopy techniques. My data showed that the consumption of termites in *O. cataphractus* has resulted in the evolution of a novel lingual prehension mode, during which the ventral surface of the tongue is used to apprehend prey. This is in contrast to other lizards, which use the dorsal surface of the tongue to contact prey. Moreover, I demonstrated that this novel lingual prehension mode is accompanied by distinct morphological elaborations of the tongue surface. None of the other cordylid lizards tested in my study used lingual prehension during prey capture, except *K. polyzonus* which used the tongue in a very small percentage of feeding trials. Overall, this study suggests that dietary specialisation might underlie the evolution of novel prehension mechanisms in lizards.

INTRODUCTION

Prey capture is undoubtedly one of the most important behaviours in lizards, critical for energy intake. Consequently, the feeding apparatus is expected to be subjected to stringent selective pressures and to be well adapted for efficient prey handling. Yet, despite the wide range of diets, lizards are limited in their prey capture repertoire as only three prehension mechanisms are recognised: jaw prehension, lingual prehension and lingual pinning followed by jaw prehension (Reilly & McBrayer, 2007). Prey prehension has been extensively investigated in lizards and considerable interest has been generated in understanding the complexity of the different prehension modes (Schwenk & Throckmorton, 1989; Schwenk, 2000; Wagner & Schwenk, 2000; Bels, 2003; Reilly & McBrayer, 2007). However, little insight has been gained into the selective pressures underlying shifts between prehension modes, especially in clades where multiple modes are present, such as Scinciformata (Urbani & Bels, 1995; Smith *et al*., 1999; Reilly & McBrayer, 2007; Montuelle *et al*., 2009).

Theoretical and empirical analyses suggest that increasing prey size favours jaw prehension, while small prey size favours lingual prehension (Bramble & Wake, 1985; Urbani & Bels, 1995; Smith *et al*., 1999). However, the fact that prehension mode does not appear to be exclusively elicited by prey size (e.g. Smith *et al.*, 1999), renders the proximate basis for selecting a specific prehension mode of special interest. The functional relationship between prey and prehension mode should be most obvious in species with a narrow or specialised diet (Greene, 1982; Schwenk, 2000). While a study by Meyers & Herrel (2005) showed that specialised diets can indeed result in the use of specific prehension modes, no data are available to test the effect of dietary specialisation on the modulation of prehension mechanisms in Scinciformata.

In this Chapter, I examine prey capture behaviour in the cordylid lizard *Ouroborus cataphractus*. *Ouroborus cataphractus* is an obligate termite-eater and individuals sporadically visit the foraging ports of the southern harvester termite (*Microhodotermes viator*), some distance away from the lizard's permanent rock shelter (Mouton *et al*., 2000a; Shuttleworth, Mouton *et al*., 2008; Mouton, 2011). The evolution of heavy armour and the tail-biting behaviour displayed by *O. cataphractus* presumably resulted from harvesting termites out in the open away from the safety of their rock shelters and the terrestrial predation pressure associated with these foraging excursions (Mouton, 2011; Chapter 3). During summer, when protection offered by vegetative

cover is limited and reliance on termites high (Shuttleworth *et al.*, 2008), predation pressure may be particularly intense (Chapter 2). Individuals are thus expected to restrict their activity away from the safety of the crevice and selection should act on phenotypic characters that reduce feeding time, for example, by increasing the rapidity or efficiency of prey prehension.

The aims of my study are threefold: (1) to examine the effect of prey species and prey size on prehension mode by comparing prey prehension between *O. cataphractus* and closely related species, *Karusasaurus polyzonus* (Stanley *et al.*, 2011), a species with a generalist diet (including *M. viator*; Chapter 5) and that occurs sympatrically with *O. cataphractus* (Bates *et al*., 2014); (2) to present data on feeding behaviour in Cordylidae as no information on prehension mode in this family is available [the genus *Zonosaurus*, cited by Urbani & Bels (1995) as an example for Cordylidae, in fact belongs to Gerrhosauridae (Raselimanana *et al.*, 2009)]; (3) to examine whether differences in prey prehension are accompanied by concomitant changes in tongue morphology.

MATERIALS AND METHODS

STUDY ORGANISMS AND EXPERIMENTAL PROTOCOL

Prey prehension was tested in 20 adult *Ouroborus cataphractus* (mean snout-vent length: 103.1 ± 6.3 mm) and 21 adult *Karusasaurus polyzonus* (mean snout-vent length: 105.2 ± 6.1 mm). Each lizard was isolated in an enclosure (90 x 40 x 40 cm), which contained a shelter consisting of two 20 x 20 cm terracotta tiles separated by two wooden strips. A heat lamp (60 W) provided 10 h of light per day and created a thermal gradient of $28 - 35$ °C. This thermal range includes the optimal body temperatures of both species (Clusella-Trullas *et al.*, 2007; Truter *et al*., 2014). All lizards were fed house crickets (*Acheta domestica*) and harvester termites (*Microhodothermes viator*) twice a week. Water was provided *ad libitum.*

After an acclimatisation period of approximately four weeks, a prey item was introduced into the lizard's enclosure, 10 cm from the opening of the shelter. Lizards were left undisturbed and feeding behaviour was digitally recorded at 240 frames per second using a Casio Exilim EX-FH25 high-speed video camera (Casio Computer Co., Ltd., Tokyo, Japan). A fixed grid, placed immediately behind the lizard, was used as a reference frame. To test the effect of prey species on prehension mode, similarly sized southern harvester termites (size range: $8.5 - 11.5$ mm) and house

crickets (size range: 8 - 11 mm) were used. Each lizard was presented with at least 10 termites and an equal number of crickets. To test the effect of prey size on prehension mode, all individuals of *O. cataphractus* and a subsample of 10 *K. polyzonus* were presented with five large house crickets (size range: 15.5 – 19.5 mm). Confounding effects of prey evasiveness and orientation on feeding behaviour (Smith, 1984; Schwenk & Throckmorton, 1989; Urbani & Bels, 1995; Smith *et al.*, 1999; Montuelle *et al*., 2010) were removed by gently squeezing the thorax of all prey items before placing them perpendicular to the lizard's head.

To address whether the prehension modes present in *O. cataphractus* characterise all cordylid lizards or whether they represent isolated occurrences, prey prehension was experimentally tested for in five additional species, representing the major clades in the family (Stanley *et al.*, 2011). As it has been hypothesised that a switch in prehension mode in Scinciformata is mediated by the predator/prey size ratio (Schwenk & Throckmorton, 1989; Urbani & Bels, 1995; Smith *et al.*, 1999), only the largest members of the respective clades were used for analysis. These include *Platysaurus imperator* (n = 2); *Smaug giganteus* (n = 3), *Pseudocordylus microlepidotus* (n = 3), *Hemicordylus capensis* (n = 3) and *Cordylus cordylus* (n = 4). Prey prehension was investigated using the protocol described above, but behavioural experiments were limited to small crickets as (1) *P. imperator* and *S. giganteus* occur outside the distribution range of the southern harvester termite and familiarity with this prey item or lack thereof might confound interpretation (Schwenk, 2000) and (2) most Scinciformata appear to favour jaw prehension when preying on large crickets (Urbani & Bels, 1995; Smith *et al*., 1999).

All specimens were collected under permit numbers AAA007-00026-0056 (Western Cape), AAA007-00340-0035 (Western Cape), 0056-AAA041-00030 (Western Cape), 01/14638 (Free State) or were obtained from commercial dealers. The feeding behaviour experiment was approved by the Research Ethics Committee of the Faculty of Science, Stellenbosch University (Ethical clearance number: SU-ACUM12–00024) and is in accordance with the ethical guidelines set by the American Society of Ichthyologists and Herpetologists.

STATISTICAL ANALYSIS

Firstly, the frequency of a specific prehension mode was calculated for each prey species and prey size and compared between *O. cataphractus* and *K. polyzonus* by making use of

contingency table analyses. Secondly, a quantitative analysis was performed on the sequences in which the lateral side of the lizard's head was perpendicular to the camera. Each feeding sequence was evaluated using frame-by-frame video analysis with VirtualDub 1.9.11 (Lee, 2010) and subsequently analysed with tpsDig 2.16 (Rohlf, 2010). The following kinematic variables were extracted: (1) gape opening time, calculated as the time between the start of jaw opening and the time when maximum gape is reached; (2) gape closing time, calculated as the time between the start of jaw closing and the completion of jaw closing; (3) gape cycle time, calculated as the time between the start of jaw opening and the completion of jaw closing; (4) time to prey contact, calculated as the time between the opening of the jaws and the contact with the prey; (5) maximum gape angle, calculated as the maximum angle between the vertex, upper jaw tip and lower jaw tip; (6) maximum gape distance, calculated as the maximal linear distance between the upper and lower jaw tips; (7) head angle at jaw opening, calculated as the angle between the axis of the head and the neck at the start of jaw opening; (8) tongue reach distance, calculated as the maximal linear distance between tongue tip and lower jaw tip.

Mean values were calculated for each individual lizard to avoid pseudoreplication. Prior to statistical analyses, all kinematic variables were $\log_{10}$-transformed to meet the assumptions of the statistical tests. Firstly, a principal component analysis (PCA) with varimax rotation was performed to reduce the number of duration variables. Secondly, a multivariate analysis of variance (MANOVA) was performed on the principal components to test for potential prehension mode, prey type and interaction effects. As timing of jaw movements are affected by prey size (Montuelle *et al*., 2010), I conducted all analyses for each prey item separately using univariate *F*-tests on the principal component scores coupled to Bonferroni *post-hoc* tests. The significant level was corrected using a sequential Bonferroni correction (Rice, 1989). Finally, a PCA was performed on the duration variables for all cordylid species combined. However, no mean values were calculated for the other five cordylid species because of the low number of individuals used during the experiments.

Contingency table analyses were conducted in R 2.15.2 (R Development Core Team, 2012); variance analyses were conducted in SPSS Statistics 17.0.1 (SPSS Inc., 2008). The significance level was accepted at *P* < 0.05.

MORPHOLOGY OF THE TONGUE

Tongue morphology was investigated making use of standard light and scanning electron microscopy techniques. The tongue was excised from one adult individual of *O. cataphractus*, *K. polyzonus* and *H. capensis,* respectively*,* using preserved material in the Ellerman Collection of the University of Stellenbosch. These were then examined for surface elaborations using a Euromex ZE 1671 stereomicroscope (Euromex Microscopen BV, Arnhem, The Netherlands). Next, tongue tissue was prepared for paraffin histology using standard techniques (Humason, 1979). Serial transversal and longitudinal 10 μm sections were stained using Harris' hematoxylin-eosin. The morphology of lingual papillae was examined under a Leica DM BL light microscope (Leica Microsystems, Wetzlar, Germany). For scanning electron microscopy, tongue tissue samples were taken from individuals which had died in captivity. All tissue was fixed for 24 h in 2.5% glutaraldehyde (pH 7.4 in phosphate buffer), washed in the same phosphate buffer and subsequently submerged in 25% HCl. After rinsing with distilled water, the samples were dehydrated in a graded alcohol series, critical-point dried in carbon dioxide for 1.5 h using a Polaron E3000 critical point dryer apparatus (Polaron Equipment Ltd., Watford, UK) and coated three times with 3 nm gold in an Edwards S150 sputter coater (Edwards High Vacuum Ltd., Sussex, UK). Samples were examined using a LEO-1430 VP scanning electron microscope (Leo Electron Microscopy Ltd., Cambridge, UK).

RESULTS

PREY PREHENSION EXPERIMENTS

In *Ouroborus cataphractus*, prey was captured by means of (1) lingual prehension, (2) jaw prehension and (3) jaw prehension with tongue protrusion. During lingual prehension, the tongue was protracted, followed by the dorsal curling of the tongue and retraction of the prey into the buccal cavity using the ventral side of the fore-tongue (Fig. 6.1A). Jaw prehension was characterised by the movement of the head towards the prey and prehension was accomplished with the jaws (Fig. 6.1B). In the third mode, the tongue was used to make initial contact with the prey, but the head moved towards the prey immediately following prey contact and prehension

was carried out by the jaws (Fig. 6.1C). Although the tongue was protruded during this prehension mode, it was not used to pin the prey to the substrate and jaw closing only occurred after the tongue had been retracted in the buccal cavity. There was a strong association between prey species (termite versus small cricket) and prehension mode ($\chi^2 = 251.59$, d.f. = 2; $P < 0.001$) and between prey size (small versus large cricket) and prehension mode ($\chi^2 = 55.71$, d.f. = 2; $P < 0.001$). Termites were apprehended exclusively with the tongue (Table 6.1), while large crickets were apprehended exclusively using the jaws with or without tongue protrusion (18.6 % and 81.4% of the cases respectively). A mixture of the three prehension modes was present when feeding on small crickets (Table 6.1).

In *Karusasaurus polyzonus*, jaw prehension was the main prehension mode (Fig. 6.2A). In 1.8% of the trials, however, the tongue was involved, either to pin the prey to the substrate, followed by movement of the head towards the prey and prehension with the jaws (Fig. 6.2B) or to drag the prey into the buccal cavity (Fig. 6.2C). There was no association between prehension mode and prey species (Fisher's Exact Test, $P = 0.13$) or prey size (Fisher's Exact Test, $P = 1$). Jaw prehension was predominant during all feeding trials (Table 6.1).

In all five other cordylid lizards, jaw prehension was exclusively used when feeding on small crickets; no other prehension modes were observed.

QUANTITATIVE ANALYSIS OF KINEMATICS

Data for lingual prehension in *K. polyzonus* during termite feeding trials ($n = 1$) and lingual pinning during small cricket feeding trials ($n = 2$) were excluded from statistical analysis due to their low frequency of occurrence. A principal components analysis on the $\log_{10}$-transformed kinematic data for *O. cataphractus* and *K. polyzonus* resulted in two significant principal components which explained 78.9 % of the variation. The first component (PC1; Eigenvalue = 4.28) consisted exclusively of duration variables, while the second component (PC2; Eigenvalue = 1.98) was defined by high scores for head angle at jaw opening, maximum gape angle and maximum gape distance (Table 6.2). A MANOVA performed on the two principal components revealed a strong effect of prey type (Wilk's Lambda = 0.542; $F_{4,222} = 19.90$; $P < 0.001$) and prehension mode (Wilk's Lambda = 0.145; $F_{8,222} = 45.04$; $P < 0.001$). The interaction effect was not statistically significant (Wilk's Lambda = 0.958; $F_{6,222} = 0.812$; $P = 0.56$). Univariate F-tests

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revealed that only PC1 differed significantly between the prehension modes during termite trials (Table 6.3). Lingual prehension in *O. cataphractus* was slower than lingual pinning in *K. polyzonus* (Bonferroni *post-hoc* test; $P < 0.001$) and both modes were slower than jaw prehension in *K. polyzonus* (*post-hoc* test; $P < 0.001$). The differences in duration of prey capture resulted from the prolonged opening phase associated with lingual prehension or pinning (Table 6.4, Fig. 6.3). For small cricket trials, univariate *F*-tests showed that both PC1 and PC2 differed significantly between the prehension modes (Table 6.3). The duration of lingual prehension in *O. cataphractus* was similar to that of jaw prehension with tongue protrusion (*post-hoc* test; $P = 1$), but both modes were slower than jaw prehension (*post-hoc* test; both $P < 0.001$). Jaw prehension in *K. polyzonus* was significantly faster than any of the prehension modes used by *O. cataphractus* individuals (*post-hoc* test; all $P \leq 0.001$). PC2 scores were higher for jaw prehension in *O. cataphractus* than for jaw prehension in *K. polyzonus* (*post-hoc* test; all $P = 0.02$). Differences in duration between the prehension modes when feeding on large cricket were similar to those observed for small crickets. Jaw prehension in *O. cataphractus* was faster than jaw prehension with tongue protrusion (*post-hoc* test; $P = 0.002$), but both modes were slower than jaw prehension in *K. polyzonus* (*post-hoc* test; both $P < 0.001$).

A principal component analysis on the combined species data set containing only duration variables resulted in one principal component, explaining 86.3% of the variation (Eigenvalue = 3.45). This principal component differed significantly between the species/modes (ANOVA, $F_{8,97} = 56.31$; $P < 0.001$). A Bonferroni *post-hoc* test revealed that the jaw movements of *K. polyzonus*, *Hemicordylus capensis* and *Cordylus cordylus* were among the fastest of all cordylid lizards tested (Table 6.5). The duration of jaw prehension in *O. cataphractus* was similar to that of *Smaug giganteus*, *Pseudocordylus microlepidotus* and *Platysaurus imperator* (*post-hoc* test; $P = 0.56 – 1$; Table 6.5).

MORPHOLOGY OF THE TONGUE

The dorsal surface of the fore-tongue was covered with short, flat topped, non-glandular papillae, similar to those observed in other Scinciformata (Schwenk, 1988; Wassif, 2002). These papillae extended towards the lateral margins of the ventral side of the fore-tongue (Fig. 6.4, Fig. 6.5), but were not present on the highly keratinised tines underlying the tongue tip (McDowell,

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1972). The central zone of the ventral side of the fore-tongue was free of papillae in *K. polyzonus* and *H. capensis*, but provided with finger-like surface elaborations in *O. cataphractus.* These surface elaborations covered the entire ventral side of the fore-tongue and tongue tip, excluding a part of the tines (Fig. 6.4, Fig. 6.5). The surface epithelium of the fore-tongue consisted of keratinised, stratified squamous epithelium without glands.

DISCUSSION

DIETARY SPECIALISATION AND PREHENSION MODE IN *O. CATAPHRACTUS*

The alternation between prehension modes based on prey size and prey species in *Ouroborus cataphractus* is consistent with observations for other Scinciformata (Urbani & Bels, 1995; Smith *et al.*, 1999; Reilly & McBrayer, 2007). Although functional characteristics of prey (e.g. size, evasiveness, orientation) are hypothesised to be important mediating factors in switches between prehension modes in Scinciformata (Smith *et al.*, 1999; Montuelle *et al.*, 2009), lingual prehension is rarely predominantly used for a specific prey species in these groups (Urbani & Bels, 1995; Smith *et al.*, 1999; Reilly & McBrayer, 2007). In contrast, *O. cataphractus* in my study used the tongue to apprehend termites in all cases. When feeding on similarly sized crickets, lingual prehension was no longer dominant, but instead a mixture of three prehension modes was present.

Although the presence of multiple prehension modes in *O. cataphractus* conforms to the general condition in Scinciformata (Urbani & Bels, 1995; Smith *et al.*, 1999; Reilly & McBrayer, 2007; Montuelle *et al.*, 2009), lingual prehension in *O. cataphractus* differs significantly from lingual prehension in other lizards. In squamates using the tongue during prey capture, lingual prehension is characterised by the exposure of the dorsal surface of the tongue to the prey (Gorniak *et al.*, 1982; Bels, 1990; Delheusy *et al.*, 1994; Smith *et al.*, 1999; Schwenk, 2000), while in *O. cataphractus*, the dorsal pad of the tongue curls as the tongue protrudes and the ventral side of the fore-tongue makes contact with the prey. Deviations from the general lingual prehension mode have, to date, only been observed in the extreme ant-eating specialist *Moloch horridus*, which uses the tongue tip to contact prey (Meyers & Herrel, 2005). Furthermore, in contrast to Scinciformata that lack any type of surface elaborations (Schwenk, 2000), lingual prehension in *O. cataphractus* is accompanied by structural elaborations of the ventral surface of the fore-tongue. As no glands

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are present, I speculate that these surface elaborations have limited adhesive properties, and are mainly used to increase friction when making contact with the smooth dorsal surface of the termite.

<div align="center">PREHENSION MODE AND FORAGING STRATEGY IN CORDYLIDAE</div>

Although, in my study, *Karusasaurus polyzonus* occasionally used lingual prehension to either pin the prey to the substrate or to drag the prey into the buccal cavity, jaw prehension predominated feeding trials. Moreover, lingual prehension and lingual pinning in *K. polyzonus* differ kinematically from jaw prehension and no clear transition between the three modes is present. The low incidence of lingual prehension in *K. polyzonus* is consistent with the lack of prominent tongue surface elaborations and suggests that lingual prehension is of little importance in this species.

Despite the fact that the protocol used in this study eliminated all those factors that are hypothesised to favour jaw prehension over lingual prehension, such as a high prey evasiveness, large prey size and low predator/prey ratio (Smith, 1984; Schwenk & Throckmorton, 1989; Urbani & Bels, 1995; Smith *et al*., 1999), none of the cordylid species evaluated, other than *O. cataphractus* and *K. polyzonus*, used lingual prehension or lingual pinning during feeding trials. Given the conservative nature of prey prehension mode in lizard clades in general, the fact that my selected species represent all the major clades in the family, and the novel nature of lingual prehension in *O. cataphractus* (and to a lesser extent *K. polyzonus)*, one can safely assume that jaw prehension is the ancestral condition in Cordylidae. This is no surprise, since jaw prehension is highly compatible with the rock-dwelling sit-and-wait foraging lifestyle of most cordylid lizards (Cooper *et al*., 1997; Mouton & Van Wyk, 1997). The increased exposure to avian predators associated with a rock-dwelling nature (Mouton & Flemming, 2001) makes individuals particularly vulnerable away from the safety of the rock shelter. Selection should thus act to increase the speed and precision of prey capture, by favouring jaw prehension (Urbani & Bels, 1995; Smith *et al*., 1999; Montuelle *et al*., 2010).

In contrast, most extant members of the Scincidae and Gerrhosauridae are ground-dwelling active foragers (Cooper *et al*., 1997) and include mobile as well as sedentary prey into the diet (Vitt & Pianka, 2007). It seems that the retention of multiple prehension modes in these families enables individuals to switch between prehension modes depending on the functional

characteristics of the prey (Urbani & Bels, 1995; Smith *et al*., 1999; Montuelle *et al*., 2009). Ground-dwelling active foragers can easily take up an ambush position close to a clumped food source, thereby rendering small prey highly profitable. The rock-dwelling sit-and-wait foraging lifestyle of *O. cataphractus*, however, should result in strong selective pressures favouring prehension mechanisms that increase the consumption rate of termites in order to make foraging excursions away from the safety of the shelter profitable. At present, it remains unclear why specialisation on termites required the evolution of a novel prehension. I hypothesise that in *O. cataphractus*, other factors such as increasing prey capturing efficiency or limiting the ingestion of extraneous material might be more important than speed. It must also be noted that jaw prehension is still maintained in *O. cataphractus* as this species remains a typical rock-dwelling cordylid that consumes mainly large prey items in addition to termites (Mouton *et al*., 2000a).

In summary, my data suggest that dietary specialisation might underlie the evolution of novel prehension mechanisms in lizards. Moreover, prey prehension, foraging mode and lifestyle (terrestrial versus rock-dwelling) appear to be highly intercorrelated traits, and further research investigating the link between these traits could provide more insight into the evolution of feeding in lizards.

Table 6.1: Comparison of prehension mode between *Ouroborus cataphractus* and *Karusasaurus polyzonus* for two prey species (southern harvester termite and house cricket) and two prey sizes (small and large cricket).

Prey	*Ouroborus cataphractus*				*Karusasaurus polyzonus*			
	n	Lingual	Jaw w/ tongue protrusion	Jaw	*n*	Lingual	Lingual pinning	Jaw
termite	254	100	-	-	206	0.5	2.4	97.1
cricket (small)	180	37.2	22.2	40.6	178	1	-	99
cricket (large)	97	-	18.6	81.4	51	-	-	100

Values represent the percentage of the trials that resulted in the specific prehension mode. Number of feeding trials per prey item is indicated.

Table 6.2: Results of a principal component analysis performed on the kinematic data describing prey capture in *Ouroborus cataphractus* and *Karusasaurus polyzonus.*

	Principal component	
	1 (53.8%)	2 (25.1%)
Gape opening time	**0.972**	0.098
Gape closing time	**0.880**	0.088
Gape cycle time	**0.979**	0.099
Time to prey contact	**0.939**	0.177
Maximum gape angle	0.266	**0.885**
Maximum gape distance	0.167	**0.886**
Head angle at jaw opening	-0.168	**0.766**
Tongue reach distance	-0.639	0.049

Values in bold represent loading scores greater than 0.70. The percentage of variation explained by each principal component is noted in parentheses.

Table 6.3: Results of ANOVA analysis showing differences in prey capture kinematics (principal component scores) between the prehension modes for three prey items.

		F	P
Termite	PC1	$F_{2,43} = 146.04$	**<0.001**
	PC2	$F_{2,43} = 0.38$	0.69
Small cricket	PC1	$F_{3,42} = 54.48$	**<0.001**
	PC2	$F_{3,42} = 4.09$	**0.01**
Large cricket	PC1	$F_{2,27} = 44.69$	**<0.001**
	PC2	$F_{2,27} = 3.09$	0.06

Table entries are the F-ratio values from each test. P-values in bold are significant after sequential Bonferroni corrections were applied.

Table 6.4: Summary of the kinematic variables describing prey capture in *Ouroborus cataphractus* and *Karusasaurus polyzonus* feeding on termites, small crickets and large crickets.

Prey * Species	Prehension mode	n	Gape opening (ms)	Gape closing (ms)	Gape cycle (ms)	Time to prey contact (ms)	Max. gape angle (deg.)	Max. gape distance (cm)	Tongue reach (cm)	Head angle (deg.)
Termite										
O. cataphractus	Lingual	78	205.4	38.6	243.8	161.6	24.0	0.62	0.58	147.0
			± 47.7	± 12.8	± 51.1	± 48.0	± 4.7	± 0.08	± 0.10	± 7.2
K. polyzonus	Jaw	81	71.2	16.0	87.1	74.0	22.1	0.58	0	150.6
			± 20.4	± 4.5	± 21.0	± 21.3	± 4.8	± 0.13		± 9.3
	Lingual	1	108.3	12.5	120.8	116.7	17.1	0.62	0.65	137.7
	Lingual	4	100.0	17.7	117.7	91.7	22.3	0.60	0.37	144.6
	pinning		± 12.3	± 5.2	± 12.9	± 11.3	± 1.5	± 0.08	± 0.13	± 6.8
Small cricket										
O. cataphractus	Lingual	27	221.7	32.3	244.1	184.3	32.3	0.78	0.60	155.1
			± 47.6	± 8.5	± 49.0	± 44.3	± 6.1	± 0.12	± 0.12	± 7.2
	Jaw	9	130.6	26.4	157.4	128.2	34.0	0.82	0	153.3
			± 40.3	± 5.1	± 38.9	± 38.4	± 6.63	± 0.08		± 5.8
	Jaw	19	165.4	36.2	192.3	158.1	33.9	0.78	0.30	156.4
	w/ tongue		± 49.0	± 37.0	± 50.0	± 48.6	± 5.62	± 0.09	± 0.17	± 6.9

Table 6.4: Continued

Small cricket									
K. polyzonus	Jaw	89	66.9	14.2	81.1	70.0	25.1	0.70	0	154.3
			± 18.5	± 3.9	± 19.9	± 19.3	± 4.83	± 0.12		± 9.9
	Lingual	2	64.6	39.6	104.2	58.3	18.9	0.70	0.31	136.1
			± 20.6	± 20.6	± 0.1	± 11.8	± 2.72	± 0.33	± 0.11	± 2.3
Large cricket									
O. cataphractus	Jaw	34	174.8	27.3	201.8	173.0	33.8	0.96	0	158.1
			± 51.6	± 6.6	± 55.1	± 49.9	± 3.53	± 0.07		± 10.3
	Jaw	13	209.6	36.5	247.1	188.8	34.1	0.96	0.41	162.0
	w/ tongue		± 60.2	± 11.2	± 64.3	± 56.4	± 6.45	± 0.15	± 0.18	± 7.8
K. polyzonus	Jaw	40	71.1	16.7	87.8	76.8	31.0	0.89	0	155.8
			± 14.8	± 3.3	± 15.8	± 16.0	± 5.0	± 0.17		± 7.1

The number of sequences analysed per prehension mode is indicated. Values are means ± standard deviation.

Table 6.5: Summary of the duration variables describing prey capture in *Platysaurus imperator, Smaug giganteus, Pseudocordylus microlepidotus, Hemicordylus capensis* and *Cordylus cordylus* feeding on small crickets.

Species	n_1	n_2	Gape opening time (ms)	Gape closing time (ms)	Gape cycle time (ms)	Time to prey contact (ms)
Pl. imperator	10	4	104.2 ± 10.8	20.8 ± 3.4	125 ± 12.3	101 ± 11.0
S. giganteus	34	15	94.2 ± 35.7	25 ± 14.3	119.2 ± 45.9	98.1 ± 37.1
Ps. microlepidotus	32	13	100 ± 33.1	27.2 ± 5.5	127.2 ± 31.8	108.3 ± 38.6
H. capensis	29	15	50.3 ± 13.8	18.1 ± 3.0	63.3 ± 15.6	48.9 ± 13.3
C. cordylus	40	16	42.7 ± 12.0	15.1 ± 4.3	57.8 ± 11.5	49.7 ± 12.8

The number of feeding trials performed per species (n_1), as well as the number of sequences analysed (n_2) is indicated. Values are means ± standard deviation.
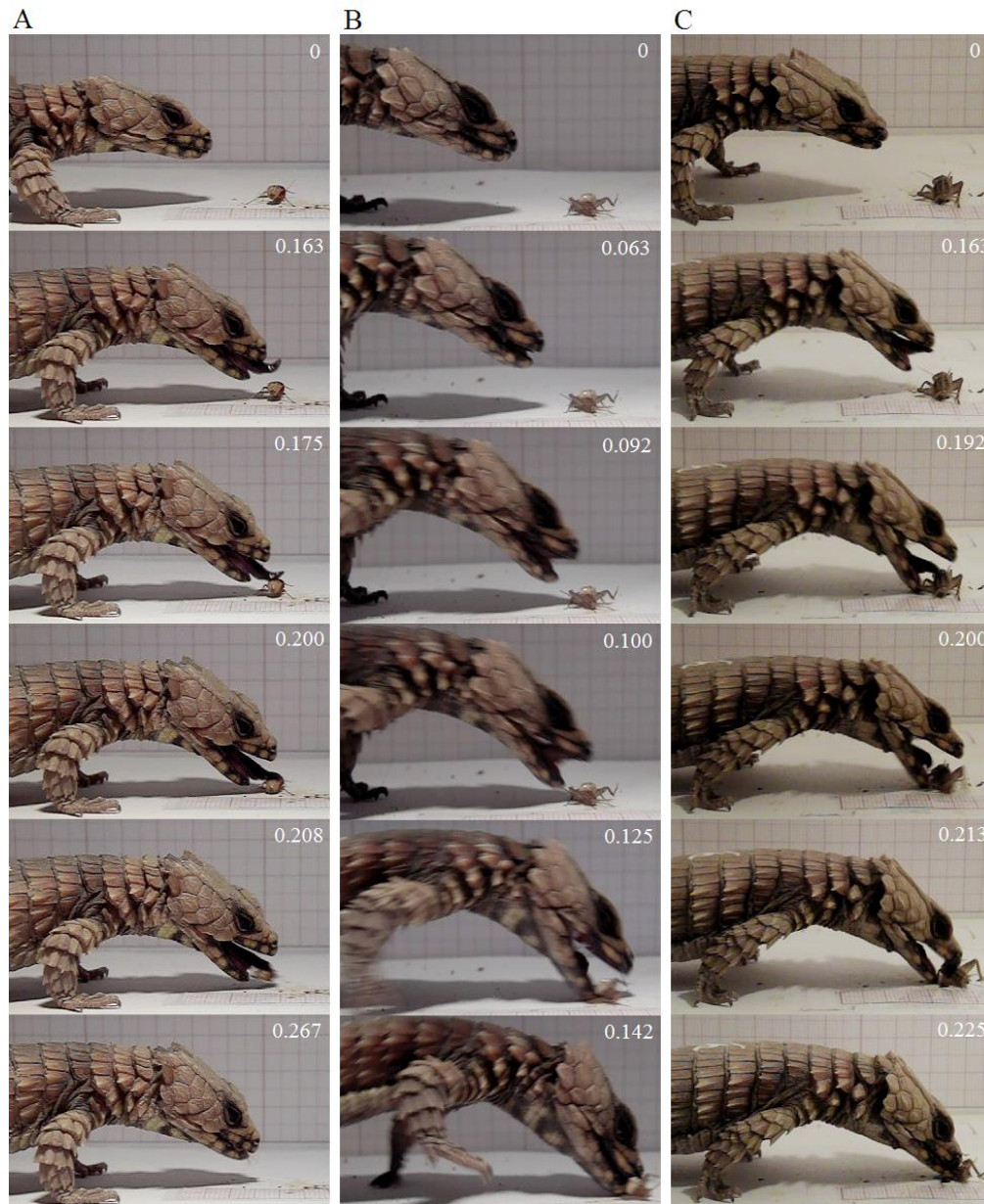
Figure 6.1: Figure illustrating the different prehension modes in *Ouroborus cataphractus*. Time (s) from the onset of mouth opening is indicated in the upper right of each frame. (A) Lingual prehension, during which the ventral surface of the fore-tongue is used to lift the prey into the buccal cavity. (B) Jaw prehension. (C) Jaw prehension with tongue protrusion, during which the tongue is protruded, but immediately retracted following prey contact. The jaws are used to capture the prey after the tongue has been retracted into the buccal cavity.

Figure 6.2: Figure illustrating the different prehension modes in *Karusasaurus polyzonus*. Time (s) from the onset of mouth opening is indicated in the upper right of each frame. (A) Jaw prehension. (B) Jaw prehension preceded by pinning of the prey with the tongue against the substrate. (C) Lingual prehension, during which the ventral surface of the fore-tongue is used to drag the prey into the buccal cavity.
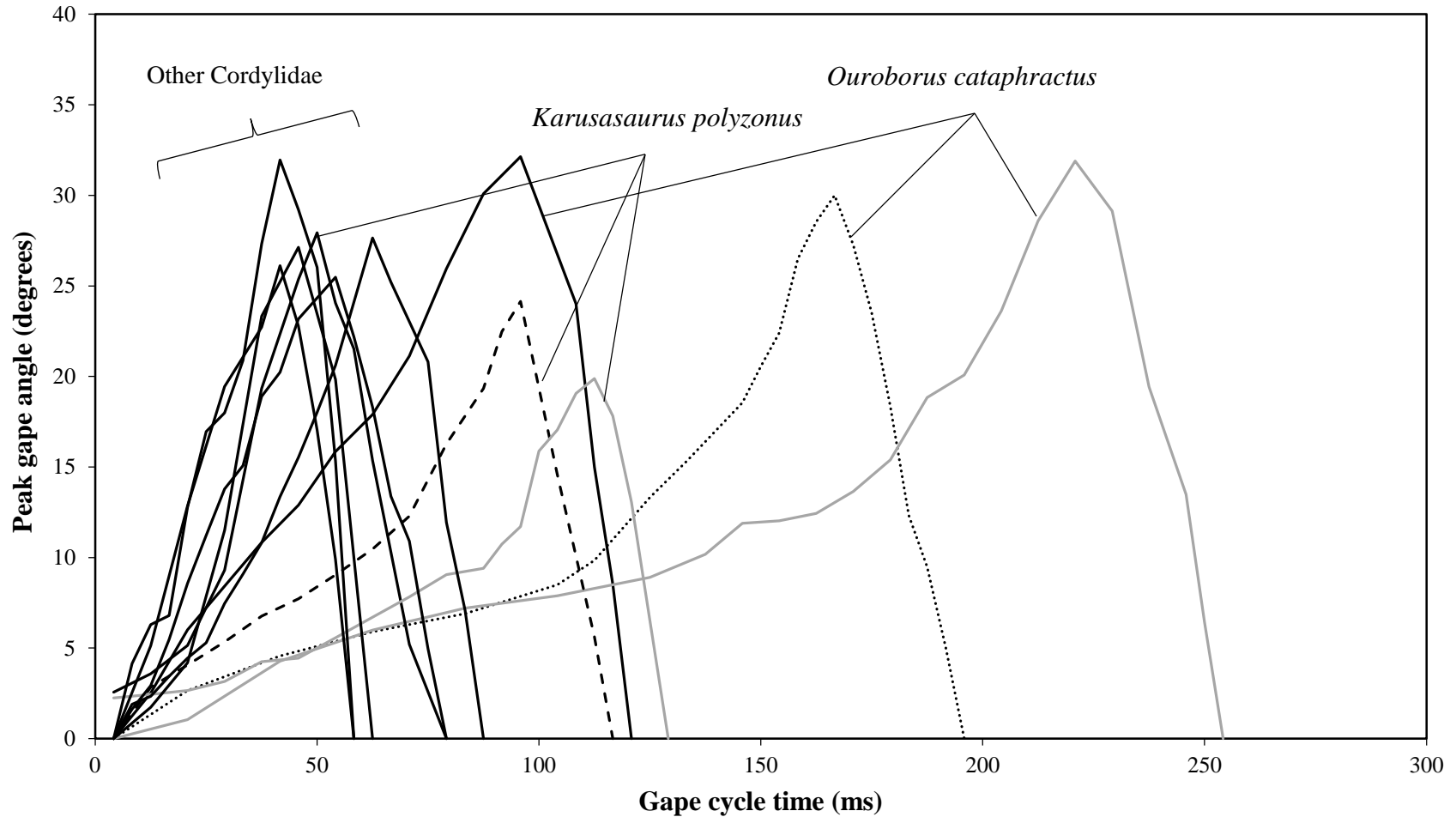
Figure 6.3: Representative kinematic profile of jaw opening and closing phases during prey capture, depicting the different prehension modes observed in *Ouroborus cataphractus* and *Karusasaurus polyzonus*: jaw prehension (black solid line), lingual prehension (grey solid line), lingual pinning (black dashed line) and jaw prehension with tongue protrusion (black dotted line).
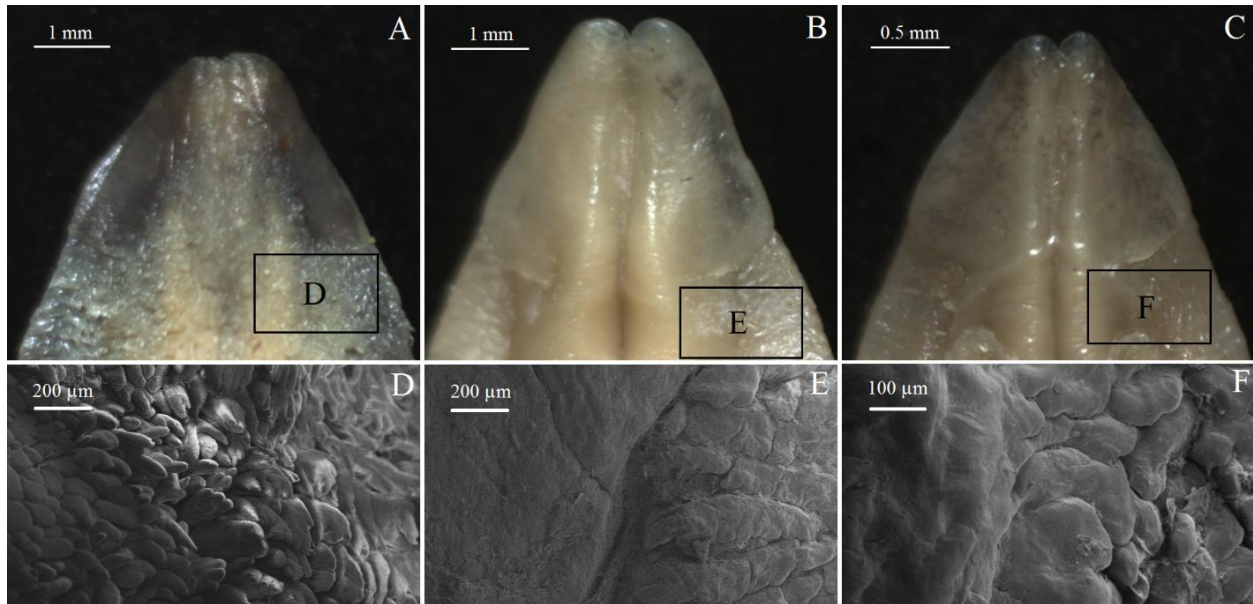
Figure 6.4: Morphology of the ventral surface of the fore-tongue. The ventral surface of the fore-tongue is provided with papillae in *Ouroborus cataphractus* (A), but non-papillose in *Karusasaurus polyzonus* (B) and *Hemicordylus capensis* (C). Low magnification scanning electron microscopy shows the presence of finger-like surface elaborations in *O. cataphractus* (D), but absence in *K. polyzonus* (E) and *H. capensis* (F).
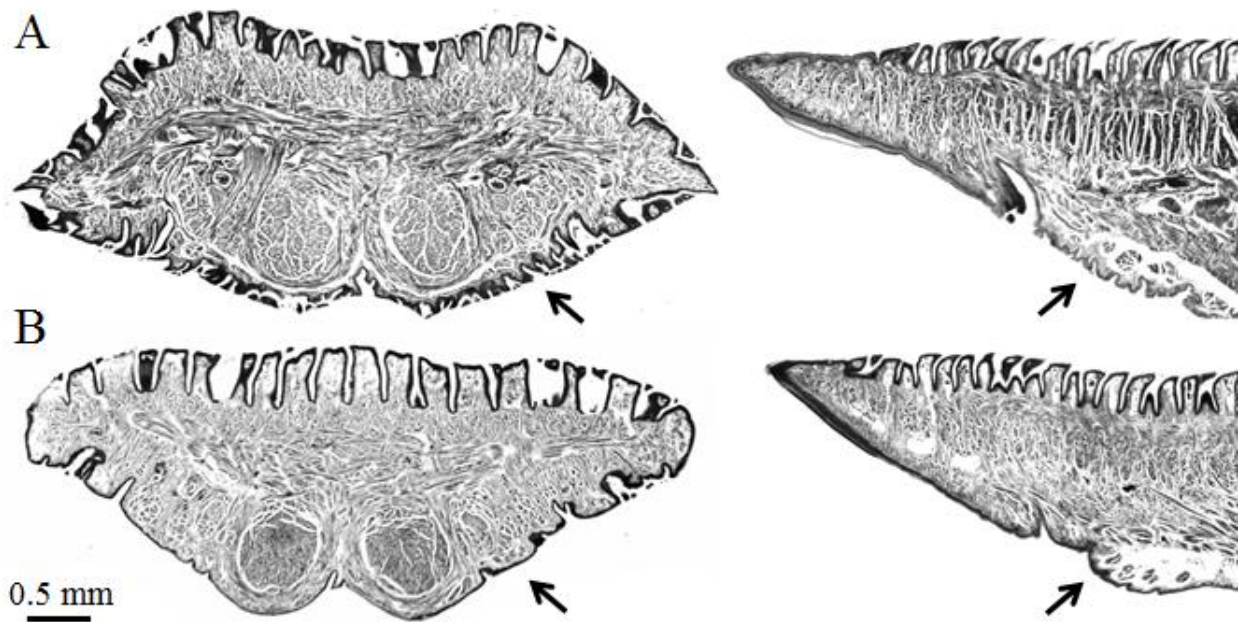
Figure 6.5: Transverse (left) and longitudinal (right) sections (10 µm) through the fore-tongue of *Ouroborus cataphractus* (A) and *Karusasaurus polyzonus* (B). Short apical papillae with broad bases cover the dorsal surface, as well as the lateral part of the ventral surface of the tongue. The centre of the ventral tongue surface (indicated by arrow) is smooth in *K. polyzonus*, while surface elaborations are present on the centre of the tongue in *O. cataphractus.*

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CHAPTER 7

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FUNCTIONAL MECHANISMS UNDERLYING PREY CAPTURE EFFICIENCY IN THREE CORDYLID LIZARDS[*]

ABSTRACT

Prey capture is one of the most important behaviours in organisms as it directly determines energy acquisition, which in turn is vital for fitness and survival. The contribution of specific prey capture mechanisms to prey capture efficiency, however, remains largely unresolved, especially in groups where a variety of mechanisms is present, such as lizards. Using three sympatric cordylid lizards as model organisms, I investigated (1) the effect of lingual prehension on prey capture efficiency when feeding on small prey (i.e. termites) and (2) whether in species that use jaw prehension to capture prey, prey capture efficiency decreases with increasing predator-prey size ratio. Prey capture efficiency, defined as the proportion of termites that was captured at first attempt, was higher in the species using lingual prehension than in the similarly-sized species using jaw prehension. In contrast, the two species using jaw prehension had a similar prey capture efficiency, despite differences in body size. The observed variation in prey capture efficiency between lingual and jaw prehension is discussed in the light of foraging mode in lizards.

INTRODUCTION

The diet of an organism is determined by a set of successive factors, consisting of the ability to encounter, detect, recognise, and ultimately, capture prey (Ferry-Graham *et al*., 2002). The proficiency in capturing prey is the most crucial step as it directly determines prey capture success, and thus energy intake. Although multiple feeding behaviours can result in effective prey capture, they might not contribute equally to energy gain if specific morphological or behavioural capabilities lead to a higher prey capture efficiency than others (Ferry-Graham *et al*., 2001). As an increase in prey capture efficiency can increase an individual's fitness (e.g. by allowing more energy to be obtained per unit effort), investigating the functional mechanisms underlying prey capture efficiency becomes crucial for the understanding of the evolution of the feeding behaviour.

Lizards form an especially interesting group with regard to prey capture efficiency because of the presence of multiple prehension modes (Reilly & McBrayer, 2007). Of particular interest is the apparent evolutionary reappearance of lingual prehension in specific clades/taxa (Vidal & Hedges, 2009; Chapter 6). Theoretical evidence suggests that the use of lingual prehension could improve prey capture efficiency for relatively small prey (i.e. in proportion to the lizard's body size), due to the adhesive or frictional capacity of the tongue (Schwenk, 2000; Chapter 6). Indeed, lingual prehension appears to improve prey capture success in species that have the ability to use multiple prehension modes (Smith *et al*., 1999). No data, however, are available to test the advantages of lingual prehension for prey capture efficiency at an interspecific level, which, in turn, might provide useful information on the conditions under which lingual prehension could have evolved in lizards.

In this Chapter I investigate the effect of prehension mode and body size on prey capture efficiency in three closely-related cordylid lizards that occur sympatrically in the arid western parts of South Africa. I hypothesise that in similarly-sized species, lingual prehension is more efficient than jaw prehension when feeding on small prey, such as termites. To test this hypothesis, I compare prey capture efficiency between *Karusasaurus polyzonus* and *Ouroborus cataphractus*. Although the two species have a generalist diet, the Southern harvester termite (*Microhodotermes viator*) is an important prey item in the diet *O. cataphractus* (Mouton *et al*., 2000a; Shuttleworth *et al*., 2008). While in the latter species, the consumption of termites seems to have resulted in the evolution of a lingual prehension, *K. polyzonus* uses predominantly jaw prehension to capture

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termites (Chapter 6). My second hypothesis is that in species using jaw prehension, prey capture efficiency decreases with increasing predator-prey size ratio. For this purpose, I test prey capture efficiency in an additional cordylid lizard that uses jaw prehension, but differs in snout-vent length from the two species: *Namazonurus peersi*. I predict that *N. peersi* (smaller species) will be more efficient than *K. polyzonus* (larger species) in capturing prey, but no difference will be present between *N. peersi* and *O. cataphractus*. By measuring prey capture efficiency in a number of species, I aim to obtain a better understanding of the circumstances under which lingual prehension could have evolved.

MATERIALS AND METHODS

HUSBANDRY

Prey capture efficiency was calculated for adult specimens of *O. cataphractus* ($n = 17$, size range: 94 – 121 mm), *K. polyzonus* ($n = 18$, size range: 94 – 114 mm) and *N. peersi* ($n = 4$, size range: 76 – 82 mm). Lizards were acclimatised to captive conditions for at least four weeks before the start of the feeding experiments. During this period, they were fed house crickets (*Acheta domestica*), mealworms (*Tenebrio molitor*) and southern harvester termites (*M. viator*) twice a week. Water was provided *ad libitum*. All lizards were kept separately in an enclosure measuring $90 \times 40 \times 40$ cm provided with a shelter. A thermal gradient of 28–35°C was created to allow individuals to maintain their optimal body temperature (Truter *et al*., 2014) as feeding behaviour is highly affected by body temperature in lizards (Van Damme *et al*., 1991).

EXPERIMENTAL PROCEDURE

Following habituation, each individual was presented with 10 termites placed in a petri dish (15 cm diameter) approximately 30 cm from the entrance of the lizard's shelter. Four series of 10 termites were presented to each lizard in a consecutive order, yielding a total of 40 termites per individual. A high-speed video camera (model Exilim EX-FH25, Casio Computer Co., Ltd., Tokyo, Japan) was positioned outside the enclosure to record feeding behaviour digitally at 120 frames per second. Prey capture efficiency was defined as the proportion of termites that was

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successfully captured at first attempt. Individual termites that were not attacked were excluded from the analysis. In addition, the snout-vent length (SVL) of each individual lizard was measured and served as an estimate of body size.

All specimens were collected under permit numbers 0035-AAA007-00340 (Western Cape) and FAUNA 570/2013 (Northern Cape). The feeding behaviour experiment was approved by the Research Ethics Committee of the Faculty of Science, Stellenbosch University (Ethical clearance number: SU-ACUM12–00024) and is in accordance with the ethical guidelines set by the American Society of Ichthyologists and Herpetologists.

STATISTICAL ANALYSES

Firstly, prey capture efficiency was compared between the two similarly-sized species *O. cataphractus* (lingual prehension) and *K. polyzonus* (jaw prehension). The effect of species and size on prey capture efficiency was analysed with a generalised linear model (GLM) for binomial response in R v. 3.1.1 (R Development Core Team, 2014) using the 'glm' function. The number of termites that was captured successfully at first attempt, as well as the number of termites that was missed at first attempt was specified in a 2-vector response variable. Species (fixed factor) and $\log_{10}$-transformed SVL (covariate) were the predictors, as well as the interaction between species and size. The data were checked for overdispersion and, if necessary, this was corrected for by adding an overdispersion parameter ("family = quasibinomial") to the model. Secondly, prey capture efficiency was compared between the two species that use jaw prehension but differ in SVL and between *N. peersi* and *O. cataphractus* using a GLM according to the above mentioned procedure (only species was included as fixed factor).

RESULTS

Prey capture efficiency differed statistically significantly between the two similarly-sized species *O. cataphractus* (lingual prehension) and *K. polyzonus* (jaw prehension) (quasibinomial GLM, $t = -3.683$, $P < 0.001$; Fig. 7.1). Neither the effect of SVL on prey capture efficiency (quasibinomial GLM, $t = 0.931$, $P = 0.36$), nor the interaction effect (quasibinomial GLM, $t =$

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0.567, $P = 0.58$) was statistically significant. *Karusasaurus polyzonus* and the smaller species, *N. peersi,* has a similar prey capture efficiency (quasibinomial GLM, $t = -0.995$, $P = 0.33$). For comparison, lingual prehension in *O. cataphractus* was more efficient than jaw prehension in *N. peersi* (quasibinomial GLM, $t = -2.767$, $P = 0.01$).

DISCUSSION

Our data show that lingual prehension contributes significantly to prey capture efficiency, when compared to similarly- and smaller-sized species that uses jaw prehension, in at least one species *O. cataphractus*. These findings collaborate with the observation that lingual prehension increases prey capture success in species that have the ability to switch between prehension modes (Smith *et al*., 1999). In contrast to my hypothesis, the effect of predator-prey size ratio does not seem to influence prey capture efficiency in species using jaw prehension. Although this could be due to the smaller sample size of *N. peersi* individuals and resulting loss of statistical power, the similarity in prey capture efficiency between *K. polyzonus* and *N. peersi* could potentially be due to the presence of alternative mechanisms involved in prey capture. Several feeding mechanisms, including mesokinesis (Frazzetta, 1983; Schwenk, 2000) and an increased jaw closing velocity (McBrayer & Corbin, 2007) have been proposed to enhance the precision of a prehensile bite in species using jaw prehension and might be present in *K. polyzonus*.

Assuming that an increase in predator-prey size ratio does not lead to a decrease in prey capture efficiency in species using jaw prehension, which selective pressures could favour the evolution of lingual prehension in *O. cataphractus*? I propose that the evolution of lingual prehension is interrelated with foraging mode, as suggested in Chapter 6. Termites constitute an important prey item for *O. cataphractus* (Mouton *et al*., 2000a; Shuttleworth *et al*., 2008). Because of their clumped and often temporally and spatially unpredictable nature (Dean, 1992), they are partially unavailable to sit-and-wait foragers who detect prey visually as they pass by (Huey & Pianka, 1981). Maximising food intake when the opportunity arises should therefore favour the evolution of an efficient prehension mechanism that maximises prey capture efficiency, especially in a species such as *O. cataphractus* that relies heavily on termites when overall food availability is low (Shuttleworth *et al*., 2008). Similar circumstances might have favoured the evolution of lingual prehension in the sit-and-wait foraging clade Iguania. Iguania, deeply nested within a clade

of actively foraging species that exclusively use jaw prehension (i.e. Episquamata), are characterised by a unique lingual prehension mode (Reilly & McBrayer, 2007; Vidal & Hedges, 2009). Specialisation on ants in this clade (Schwenk, 2000) might have played a central role in the evolution of lingual prehension. In active foragers, the need for an increased efficiency when preying on a clumped food source should be less than in sit-and-wait foragers as they can move through the habitat in search for patchy prey. Another possibility is that actively foraging taxa that use jaw prehension, such as Lacertidae and Teiidae, are often characterised by long, narrow skulls (McBrayer & Corbin 2007, but see Edwards *et al*., 2013) that should facilitate the capture of small prey. As a result, these taxa can consume or even specialise on ants and termites (Pianka, 1986), without the use of lingual prehension. In contrast, sit-and-wait foragers have shorter, broader skulls (McBrayer & Corbin, 2007), presumably to increase bite force, as the evasive prey items they mostly encounter are often quite hard (McBrayer, 2004). Given that bite force trades-off with jaw-closing velocity (Herrel *et al*., 2009; Chapter 4), prey capture efficiency for small prey might be impaired in some sit-and-wait foragers, thereby favouring the evolution of alternative prehension mechanisms.

In conclusion, my results show a clear advantage of lingual prehension for prey capture efficiency in *O. cataphractus*. Further investigation of alternative mechanisms that influence prey capture (e.g. cranial kinesis, jaw kinematics) in a phylogenetic context, as well as the effect of foraging mode should shed more light on the evolution of feeding behaviour in lizards.
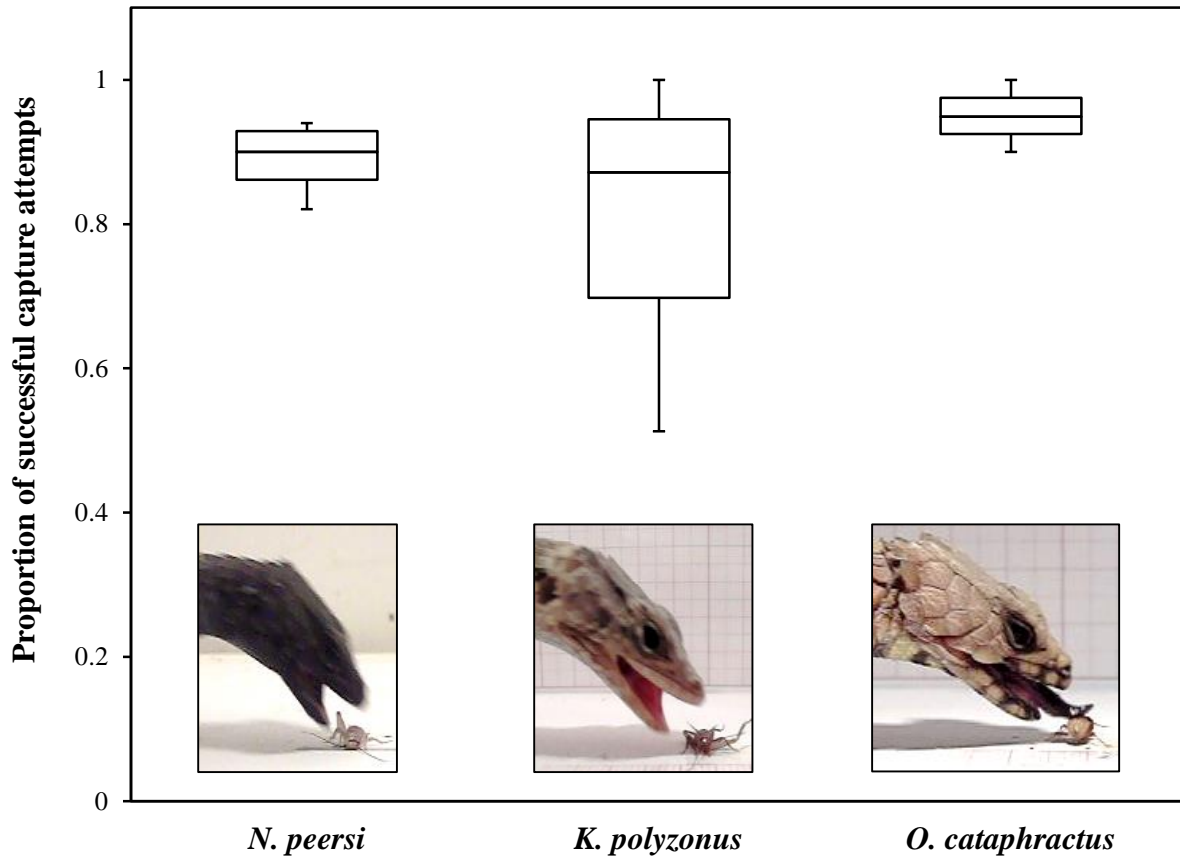
Figure 7.1: Figure illustrating the proportion of termites that was successfully captured at first attempt in *Namazonurus peersi* (smaller species, jaw prehension), *Karusasaurus polyzonus* (larger species, jaw prehension) and *Ouroborus cataphractus* (larger species, lingual prehension). The median value is shown by the horizontal line in each box plot, the top and bottom of the box plot show the 25th and 75th percentiles respectively and the whiskers show the interquartile range of the data. Images showing the different prehension modes are adapted from Broeckhoven & Mouton (2013).

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CHAPTER 8

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CONCLUSIONS

The findings of the six chapters of this thesis, combined with extensive previous research on *Ouroborus cataphractus*, allow me to produce a possible secenario of the evolutionary history of this remarkable species. Specifically, these findings support the central role of termitophagy in the evolution of heavy armour and consequently group-living behaviour in *O. cataphractus* (Fig. 8.1). Termites constitute an important food source in lizards (Schwenk, 2000; Vitt & Pianka, 2007), especially in (semi-)arid environments (Huey & Pianka, 1981; Ricklefs *et al*., 1981; Abensperg-Traun, 1994). Because of their clumped nature, termites are mostly encountered by species that actively search for prey (Huey & Pianka, 1981; Magnusson *et al*., 1985; Bergallo & Rocha, 1994), whereas sit-and-wait foragers would only encounter termites occasionally (i.e. when close to the lizard's vantage point). The unique combination of adaptations that allow the regular use of termites in a sit-and-wait forager, such as *O. cataphractus*, suggests that frequent exploitation of termites might have played a crucial role during morphological and behavioural evolution.

Rock-dwelling sit-and-wait foraging species might benefit from high sprinting capacities to rapidly reduce the distance between themselves and their prey (Huey *et al*., 1984; Miles *et al*., 2007; McBrayer & Wylie, 2009). Running speed, however, can only be used efficiently up to a certain distance away from the shelter. Given that the chance of outrunning a predator decreases with increasing distance to the refuge (Cooper, 1997), venturing away from the refuge would pose an increased risk of predation and alternative antipredator adaptations are to be expected (Kacoliris *et al*., 2009; Zani *et al*., 2009). The elaborated body armour and tail-biting behaviour of *O. cataphractus* seems to have evolved to protect individuals from attacks by predators when away from the safety of the shelter (Chapter 3). Body armour, however, will most likely not protect against the sharp beaks and talons of the large birds of prey present in the habitat. This is important, as the exploitation of termites away from the shelter in the absence of vegetative cover would result in an equal predation risk (i.e. equal susceptibility to aerial and terrestrial predation) and selection will act against elaboration of body armour. In contrast, exploitation of termites in the presence of vegetative cover would lower the aerial predation risk (i.e. visibility predators is

impaired), rendering mainly terrestrial predation pressure important. I speculate that only under these conditions, i.e. exploiting termites away from the refuge under vegetative cover, could the body armour of *O. cataphractus* have evolved. Once evolved, body armour was advantageous and consequently selected for.

However, a central question underpinning the discussion about the evolution of body armour remains: what is the driver for the origin of dependence on termites in *O. cataphractus*? A change from a moister to a drier climate occurred in the western parts of South Africa between 10 and 5 Mya as indicated by a major change in vegetation composition (Dupont *et al*., 2011). The low food requirements of the southern harvester termite (*Microhodotermes viator*) would have allowed them to thrive in this drier environment (Coaton, 1958). While the harvester termite *Hodotermes mossambicus* is largely dependent on grass, *M. viator* can subsist on limited food such as leaves and twigs derived from Karroid vegetation (Coaton, 1958). The divergence time of *O. cataphractus*, which was dated back to the late Miocene, approximately 6 Mya (Broeckhoven, Diedericks & Mouton, unpublished data), is highly consistent with this hypothesis and suggests that the ancestor of *O. cataphractus* relied on termites due to the desertification of the western parts of South Africa. Furthermore, the establishment of the winter rainfall regime during the Pliocene (i.e. between 5.3 – 2.6 Mya; Chase & Meadows, 2007) and intensification of seasonally arid conditions c. 3 Mya (deMenocal, 2004), would have resulted in a prolonged period of low food availability during the summer-autumn period and would have increased their dependence on termites.

Although the elaborated body armour provides protection against terrestrial predators, *O. cataphractus* is particularly vulnerable to aerial predation during general maintenance behaviour as the heavy armour makes a speedy retreat difficult (Losos *et al*., 2002). Group-living behaviour in this species appears to have evolved to increase vigilance (Hayward, 2008). The high competition for food associated with group-living behaviour, as well as the reduction in perching distance (Losos *et al*., 2002) in this sit-and-wait forager, would, in turn, have increased the need for termites, especially during summer (Shuttleworth *et al.*, 2008).

The relationship between termitophagy, body armour and group-living behaviour seems to be best illustrated by an example of two populations that were studied in this thesis. In the Cederberg, *O. cataphractus* inhabits rocky outcrops in a habitat relatively densely vegetated by dwarf to medium shrubs throughout the year (Mucina & Rutherford, 2006). The diet of Cederberg

individuals consists of 96 % termites (Broeckhoven & Mouton, unpublished data) and their body armour provides protection against the only terrestrial mammalian predator (i.e. *Galerella pulverulenta*) they would encounter during foraging excursions (Chapter 3). Group sizes are small and typically contain only two to four individuals (Shuttleworth, 2006; Broeckhoven & Mouton, personal observations). The conditions experienced by the Cederberg population could be regarded as the primary selective forces favouring the morphological and behavioural evolution of *O. cataphractus*. On the contrary, the habitat along the west coast of South Africa (e.g. Lambert's Bay) is characterised by scattered vegetation, but ground cover is provided by annuals during spring (Mucina & Rutherford, 2006). Individuals thus restrict their activity to spring when flowering plants provide cover and attract a high abundance of arthropods (Chapter 2). The latter allows individuals to store energy to survive summer (Flemming & Mouton, 2002). In addition, high food availability during spring would allow for group sizes much larger than those recorded in the Cederberg (up to 60 individuals; Mouton *et al*., 1999; Effenberger & Mouton, 2007). During summer, when food availability is low and the aerial predation risk high, the cost of foraging should be relatively high and individuals consequently reduce their activity (Chapter 2). The exploitation of termites during this time of the year seems to be a requirement to overcome the negative effects of competition for food, especially in large groups (Shuttleworth *et al*., 2008). However, vegetative cover is low during summer in this area and individuals seem to restrict their foraging excursions to late afternoon / early evening when the visibility of aerial predators is lower (Chapter 2). Dietary analysis corroborates these findings: the diet of Lambert's Bay individuals consists of only 25% termites (of which 20% during summer; Broeckhoven & Mouton, unpublished data). In contrast to the Cederberg, this coastal habitat has a higher number of terrestrial predators, hence an elaboration of body armour (i.e. thicker osteoderms) is present (Chapter 3).

The possession of body armour has major consequences for the feeding behaviour of *O. cataphractus*. For instance, the proportion of evasive prey items is low to absent in heavily armoured cordylids (Chapter 4). Given that Coleoptera and Hymenoptera constitute the most important prey categories (Chapter 4), a reduction in running speed would have restricted the diet of *O. cataphractus* (especially coastal populations) to slow-moving, hard-bodied Coleoptera (Mouton *et al*., 1999; Chapter 5). The relatively high bite force of *O. cataphractus* does not seem to have evolved in response to a shift to relatively hard prey items (Chapter 4), but tail-biting

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behaviour is more likely the main selection pressure favouring the relatively high bite force of *O. cataphractus* (Chapter 5). The similar bite forces of Cederberg and Lambert's Bay individuals during spring, despite large variation in diet, suggests these conclusions (Broeckhoven & Mouton, unpublished data). Surprisingly, the rock-dwelling nature does not seem to constrain bite force in *O. cataphractus* as the elongated tail spines would allow for an increase in head height (i.e. best predictor of bite force), without increasing an individual's risk of getting extracted from the crevice by predators (Chapter 5). A trade-off, however, exists between bite force and jaw-closing velocity in lizards (Chapter 4). The fact that prey capture efficiency isn't greatly impaired suggests that the novel lingual prehension mode in *O. cataphractus* appears to have evolved in response to the force-velocity trade-off (Chapter 6). Because of the slow nature of lingual prehension, I hypothesise that lingual prehension is a consequence of an increased bite force, and an adaptation to increase prey capture efficiency for termites (Chapter 7).

In conclusion, the integrative nature of the life-history characteristics of *O. cataphractus* seems to have resulted in a feedback loop which reinforced itself throughout the evolution, resulting in a species with a remarkable, yet complex, biology. Many questions, however, should be addressed in future research. Firstly, a comparative inter-population analysis should be conducted to further investigate the relationship between habitat use, predation risk and antipredator morphology. Secondly, the foraging behaviour of *O. cataphractus* requires more attention, especially in relation to group-living behaviour. Thirdly, the sensory means by which *O. cataphractus* locates termites given the lack of prey chemical discrimination in this sit-and-wait forager should be examined. Lastly, an exploration of alternative functions of body armour in cordylid lizards should contribute to a better understanding of the causes and consequences of body armour in general. Specifically, the role of thermoregulation and predation by snakes should be considered in order to explain patterns of body armour evolution.
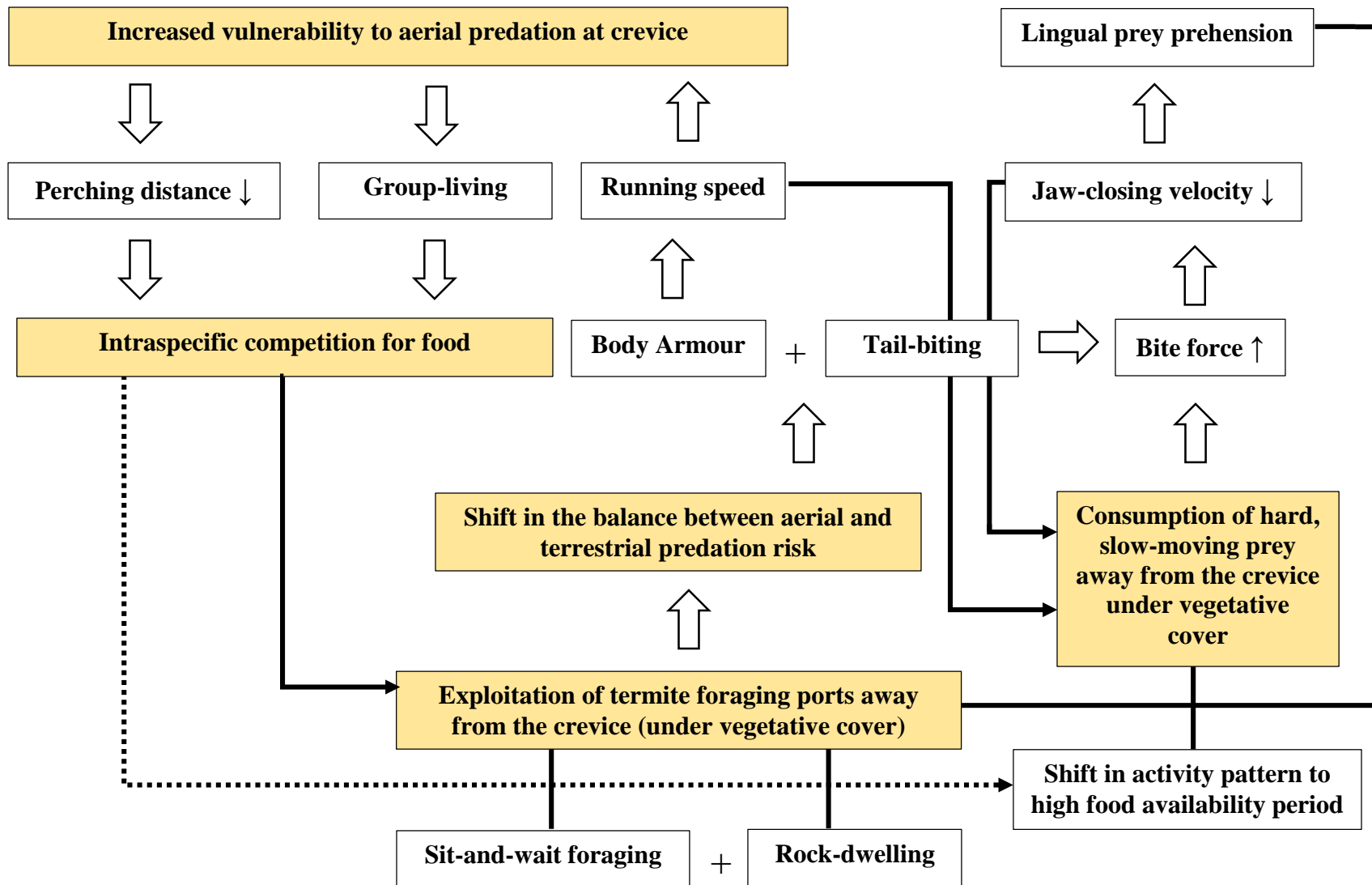
Figure 8.1: Possible scenario representing the evolution of body armour and its consequences in *Ouroborus cataphractus*. The arrows inside the box indicate an increase (↑) or decrease (↓) in trait value, while the arrows between boxes indicate causal effects.

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