Bite force and cranial bone strain in four species of lizards

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Abstract

In vivo bone strain data provide direct evidence of strain patterns in the cranium during biting. Compared to mammals, *in vivo* bone strains in lizard skulls are poorly documented. This paper presents strain data from the skulls of *Anolis equestris*, *Gekko gecko*, *Iguana iguana* and *Salvator merianae* during transducer biting. Analysis of variance was used to investigate effects of bite force, bite point, diet, cranial morphology and cranial kinesis on strain magnitudes. Within individuals the most consistent determinants of variance in bone strain magnitudes are gage location and bite point, with the importance of bite force varying between individuals. Inter-site variance in strain magnitudes—strain gradient—is present in all individuals, and varies with bite point. Between individuals within species, variance in strain magnitude is driven primarily by variation in bite force, not gage location or bite point, suggesting that inter-individual variation in patterns of strain magnitude is minimal. Between species, variation in strain magnitudes is significantly impacted by bite force and species membership, as well as by interactions between gage location, species, and bite point. Independent of bite force, species differences in cranial strain magnitudes may reflect selection for different cranial morphology in relation to feeding function, but what these performance criteria are is not clear. The relatively low strain magnitudes in *Iguana* and *Uromastyx* compared to other lizards may be related to their herbivorous diet. Cranial kinesis and the presence or absence of postorbital and supratemporal bars are not important determinants of inter-specific variation in strain magnitudes.

Introduction

Data on *in vivo* bone strain magnitudes are crucial for understanding the relationships between animal behavior and skeletal design—form-function relationships. Bone tissue yields in the range of 4000 to 12,000 $\mu\varepsilon$ (Currey, 2004), suggesting that skeletal form should be adapted to keep strain magnitudes below these values, but attempts to identify more restrictive rules on maximum strain magnitudes have been unsuccessful. The theory of dynamic strain similarity, that similar safety factors to failure are maintained "by allometrically scaling the magnitude of the peak forces applied to them during vigorous locomotion" (Rubin and Lanyon, 1984), does not explain the diversity of safety factors in tetrapod limb bones associated with taxonomic, ontogenetic, physiological, and functional diversity (Biewener, 1993; Blob et al., 2014; Kawano et al., 2016; Main and Biewener, 2004). In part this reflects the fact that repetitive loading of bones during cyclic behaviors lowers the strain magnitudes at which bone fails—human and bovine femoral bone loaded at 9,000 $\mu\varepsilon$ in tension fails after 10 cycles, but it will fail at only 6,000 $\mu\varepsilon$ after 10,000 cycles (Zioupos et al., 2001). The maximum strain magnitudes to which limb bones should be adapted probably vary with the frequency with which a given behavior is employed, predicting variation in bone safety factors across taxa, ages, behaviors or—the focus of this study—functional systems.

To date, the majority of studies relating strain magnitudes to skeletal design have focused on the limb skeleton, which has one predominant function—transmission, amplification and resistance of muscle and substrate reaction forces during locomotion (Biewener, 2003; Main and Biewener, 2004). In contrast, the bones of the skull perform many functions, suggesting that optimality criteria driving skull evolution may be more diverse than those driving limb bone shape. This diversity in skull function has been invoked to explain the wide variation in strain magnitudes recorded from different parts of the skull during feeding. In mammals, the neurocranial and circumorbital skeletons experience much lower strain magnitudes during feeding than the zygomatic bone or mandible (Hylander et al., 1991b; Ross and Metzger, 2004). Strain magnitudes in the brow ridges of cercopithecine monkeys during feeding are absolutely low (always $< 500\ \mu\varepsilon$ and usually $< 200\ \mu\varepsilon$), and lower than those recorded simultaneously elsewhere in the facial skeleton (Hylander et al., 1991b). Indeed, strain magnitudes in primate mandibles are 3.5-6.5 times higher than those strains recorded from the circumorbital region simultaneously or during similar behaviors (Ross and Metzger, 2004). These data contradict the idea that the facial skeleton is optimized for "maximum strength with minimum material" during feeding; i.e., the bones of the brow ridges and other regions of the skull that experience low strain magnitudes during feeding could be significantly reduced in size, or their shapes changed, without compromising their strength during feeding (Hylander and Johnson, 1997; Hylander et al., 1991a; Ross and Metzger, 2004).

This conclusion may at first seem counter-intuitive: don't low bone strain magnitudes during feeding suggest that a structure is well designed, not poorly designed for resisting feeding forces? Hylander and colleagues noted several problems with this line of reasoning (Hylander and Johnson, 1997; Hylander et al., 1991b). It does not explain the diversity of strain magnitudes (and inferred safety factors) recorded from the facial skeleton during feeding: if low strained areas of the skeleton are well designed, must that mean that high strained areas are poorly designed? And if so, why has selection not provided mechanisms to reduce those strain magnitudes? After all, there are costs to moving bony structures during locomotion and feeding, costs to building them during development, and costs to maintaining and repairing them during life. Indeed, larger bones than necessary are not necessarily better as they can fail at lower stresses than smaller bones because their larger volume increases the probability that they accumulate microcracks that can grow into larger deficits that might cause bone yield or failure (Currey, 2002; Weibull, 1951).

Hence, if two regions of bone experience very different bone strain magnitudes during the same behavior, they are by definition not optimized for maximum strength with minimum material during that behavior, leading to the conclusion that either this optimality criterion is not equally important in both bones or regions, or the experimenter has not captured the full range of behaviors generating strain in that bone (Grőning et al., 2013). Perhaps the most difficult strain data to collect *in vivo* are those associated with infrequent traumatic events, such as blows or bites during predation or intra-specific agonistic interactions, which have been hypothesized to be important determinants of skull design in primates (Carrier, 2011; Hylander and Johnson, 1997; Hylander et al., 1991b; Hylander and Ravosa, 1992). In the absence of *in vivo* strain data across the complete range of animal behaviors, and estimates of their frequency and ecological importance (Ross et al., 2016), some progress can be made by assuming that behaviors associated with relatively high strain magnitudes are likely to impose greater demands on skeletal design than behaviors associated with lower strain magnitudes—bone size and shape are expected to be more closely adapted to resist high strain than low strain magnitude loading regimes. This expectation applies not only across behaviors—different gaits (Biewener et al., 1983a; Biewener et al., 1983b; Blob and Biewener, 1999); biting versus chewing, licking and yawning (Hylander, 1981; Ross et al., 2016)—but also between different phases of the same behavior. For example, the shapes of limb bones are expected to be more closely adapted to dissipating forces associated with stance phase than swing phase of the gait cycle (Biewener, 2003), and mandible shape to dissipating forces associated with the power stroke than the opening phases of the gape cycle (Hylander et al., 1987).

Invoking this line of reasoning, skull bones that experience relatively low strain magnitudes during feeding must be designed to perform non-feeding functions, such as: insulating the visual system from unwanted displacements (Cartmill, 1972; Cartmill, 1980; Heesy, 2005; Ravosa et al., 2000b; Ross, 1995a; Ross, 1996); augmenting areas for muscle attachment (Ross, 1995b); providing a rigid framework to maintain the volume of the respiratory passages (Ross, 1995b; Ross, 2001; Ross and Metzger, 2004); and protecting the brain and sense organs from “infrequent non-masticatory traumatic loads” (Carrier, 2011; Hylander and Johnson, 1992; Hylander and Johnson, 1997; Hylander et al., 1991a; Ravosa et al., 2000b). Areas such as the mammalian zygoma, which experiences high strain magnitudes during feeding, are expected to show closer matches between form and the mechanical demands of feeding (Behrents et al., 1978; Herring and Teng, 2000; Herring et al., 1996; Hylander et al., 1991b; Hylander and Ravosa, 1992; Lieberman et al., 2004; Ravosa, 1991; Ross, 2001; Ross, 2008; Ross et al., 2011; Ross and Hylander, 1996; Ross and Metzger, 2004; Thomason et al., 2001).

All of the examples given above come from mammals, predominantly primates: among non-avian reptiles, strain data have only been extensively sampled from the skull of *Alligator* (Metzger et al., 2005; Porro et al., 2013), with limited data from the crania of *Varanus* and *Uromastyx* (Porro et al., 2014; Smith and Hylander, 1985). Consequently, the magnitude and determinants of variation in strain magnitudes across the crania of non-mammalian tetrapods are poorly understood, and we have little idea how the apparent principles of skull design in mammals might or might not apply to other tetrapod clades. This paper presents *in vivo* bone strain data from the frontal, parietal and maxilla of four species of lepidosaur: the insectivorous anole, *Anolis equestris* Merrem 1820 (Dalrymple, 1980; Lister, 1981); the insectivorous gekkonid, *Gekko gecko* Linnaeus 1758; the herbivorous iguanid, *Iguana iguana* Linnaeus 1758; and the omnivorous teiid, *Salvator* (previously *Tupinambis) merianae* Dumeril and Bibron 1839 (Colli et al., 1998). The data are used to address general questions regarding patterns of strain in non-mammalian crania. Do individuals in the same species share common patterns of strain magnitudes? How do bite force, bite point, species-specific cranial morphology and cranial kinesis impact strain magnitudes in lizard crania? What drives variation in strain magnitudes within and between individuals and species?

We then address hypotheses regarding the effects of diet, cranial morphology and kinesis on lizard cranial strain magnitudes. Herbivorous lepidosaurs, like *Iguana* in our study, have light skulls, short snouts, large jaw elevator (adductor) muscles, and high bite forces (Herrel et al., 2007;

Metzger and Herrel, 2005; Stayton, 2006). Light skulls suggest that, when bite force is controlled for, *the crania of our herbivorous species*—Iguana—*might experience higher strains compared with the other species studied here.* Arguing against this prediction, Porro et al. (2014) reported low strain magnitudes in the herbivorous lizard *Uromastyx*, suggesting that this might reflect adaptation for avoiding fatigue failure of repetitively loaded bone (see above), assuming that herbivorous lizards perform more feeding cycles per day than carnivorous or omnivorous lizards. There are no data that we know of that speak to the validity of that assumption, but Porro et al.'s hypothesis *predicts that* Iguana *should experience lower strains than the non-herbivorous species in our sample*.

Our data also allow us to make preliminary assessments of the impact of important variants in cranial architecture in lepidosaurs: the presence or absence of bars of bone and cranial kinesis. *Gekko gecko* displays streptostyly, mesokinesis, and metakinesis, and lacks the supratemporal (ST) and postorbital (PO) bars possessed by the other three species (Herrel et al., 1999; Metzger, 2002); *Salvator* may be streptostylic and mesokinetic (Barberena et al., 1970; Smith, 1980); *Anolis equestris* is streptostylic (AH, personal observations); and *Iguana iguana* is akinetic (Throckmorton, 1976). The precise effects of these interspecific differences in cranial morphology and kinesis are difficult to predict because of uncertainty regarding deformation regimes in lizard crania. One possibility is that *the absence of ST and PO bars will result in higher strain magnitudes,* either because there is less bone to absorb muscle and bite forces, or because the cranium is less rigid overall*. Kinesis might be expected to be associated with lower strain magnitudes* because strain energy is dissipated in viscoelastic sutural tissues. This might be associated with large differences in strain magnitudes between frontal and parietal bones, on either side of the mesokinetic joint.

To control for effects of bite force and bite point, this study focuses on strain magnitudes recorded during transducer biting. Although transducer biting is not normal feeding behavior, it does allow the effects of bite force to be controlled while testing hypotheses regarding the effects of species-specific morphology on variation in bone strain magnitudes. Bite force impacts cranial bone strain magnitude through interactions with bite point, not only because the torques acting on the cranium change with bite point, but also because of relationships between bite point and bite force. In mammals, variation in bite force with bite point has been shown to be described by the constrained lever model which predicts that, because of constraints against tensile forces in the biting side jaw joint, bite forces at the most posterior (distal) teeth are lower than those in the middle of the postcanine tooth row (Greaves, 1978; Spencer, 1995; Spencer, 1998; Thompson et al., 2003). Similar predictions have been made for "reptiles" (Druzinsky and Greaves, 1979). Here we evaluate whether the constrained lever model applies to lepidosaurs and test the hypothesis that there are significant interactions between bite point and bite force in their effects on bone strain magnitudes in the lizard cranium.

In sum, this study uses *in vivo* bone strain magnitude data collected simultaneously from multiple cranial sites, from multiple individuals from four lizard species with diverse cranial morphologies to address the following questions:

- Controlling for bite force and bite point, do lizards show strain gradients—variation in strain magnitudes—across the cranium during biting?
- Do individuals in the same species share common patterns of variation in strain magnitude across the cranium when bite force and bite point are controlled statistically?
- When bite force and bite point are controlled, what are the effects of diet, species-specific cranial morphology and cranial kinesis on variation in strain magnitudes in lizard crania?

Materials and Methods

Animal care

All experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of Chicago and the *Salvator* experiments were approved by the University of Antwerp Ethics Committee. Four adult *Anolis equestris*, *Gekko gecko*, and *Iguana iguana* were purchased through commercial dealers and housed in individual enclosures (152 x 61 x 61 cm) in the Carlson Animal Resources Center at the University of Chicago. The housing room was maintained at appropriate ranges of ambient temperature (24 – 28 °C) and humidity (50 – 80%). Each enclosure was equipped with a heat lamp to provide a basking spot (38 °C) during the day and a UVB light to ensure proper vitamin D3 production. Animals were fed with crickets, worms, mice, and/or fruit every other day, and fresh water was provided daily. Three adult *Salvator merianae* were obtained through commercial dealers and housed in individual cages (120 x 80 x 80 cm) in a temperature-controlled room set at 25 °C in the Functional Morphology Laboratory, Department of Biology, University of Antwerp, Belgium. A basking spot at higher temperature (45°C) was available, the animals were fed with mice and/or fruit twice weekly, and water was available ad libitum. Jaw length, a biomechanically relevant size variable for the feeding system, was measured from the tip of the retroarticular process to the tip of the jaw at the symphysis *in vivo* or from 3d reconstructions of CT scans of the animals post mortem (Table 2).

Bone strain data

Bone strain data were recorded using stacked delta rosette strain gages (SA-06-030WY-120, Micromeasurements, Raleigh, NC) wired, insulated, and gas sterilized using procedures described previously (Ross, 2001; Ross et al., 2011). Following anesthesia through an intramuscular injection of a mixture of ketamine and dexmedetomidine (respectively 50 mg/kg and 200 µg/kg body mass) (Chai et al., 2009), < 1 cm^2 of skin overlying each gage site was removed, the periosteum elevated, the bone degreased with chloroform, and the gage bonded to the surface of the bone using cyanoacrylate adhesive. The lead wires were either tunneled under the skin to the nuchal region (*Salvator*) or run outside the skin to the nuchal area where they were sutured to the skin (other taxa). Strain gage sites are shown in Fig. 1 and Fig. S1 and include the mandible site in one *Salvator*. After placement of EMG electrodes (EMG data not presented in this study) the animals were returned to temporary housing cages for at least 12 hours prior to data recording. Instrumentation effects were tested using pairwise comparisons between bite forces of individual animals before and after placement of strain gages and EMG electrodes.

Data collection

The animals were manually restrained and simultaneous bite force and bone strain data were collected while the animals bit on a calibrated bite force transducer described previously (Herrel et al., 1999). Bite point (anterior midline; anterior one-third of non-midline tooth row, middle third and posterior third on left and right sides) was recorded on video tape or on the voice track of a tape recorder. Voltage changes in the strain gages were conditioned and amplified on Vishay 2310 bridge-amplifiers and the data acquired at 1 KHz through a National Instruments DAQ board run by MiDAS data acquisition software package (Xcitex, Cambridge, MA, USA) or the analog data collection module in a Vicon MX T40 (Vicon, Los Angeles, CA, USA), system, and were saved to a server for subsequent analysis.

Bite force data analysis

To assess whether our subjects are representative of their wider populations, bite forces recorded at the anterior midline bite point in the experimental animals were compared with those collected using the same methods from non-experimental, conspecific, captive *Anolis*, *Iguana*, *Gekko* and *Salvator* housed: in Prague, Czech Republic; Miami, USA; Paris, France; the University of Antwerp, Belgium; and the University of Tulane, New Orleans, USA (Fig. 2A). *Salvator* data were also collected from 125 semi-wild animals in a conservation breeding program (Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis, reg. 1-35-94-1088-8) in the Jacarezário, Universidade Estadual Paulista (Rio Claro, São Paulo, Brazil). There the lizards are kept in groups of 5–10 individuals in outdoor pens (5 m x 10 m or 2 m x 2 m) with free access to water, ground shelters, and to shaded and sunny areas for thermoregulation. In spring and summer the animals are fed three times a week with ground beef, fruits, and/or one day old chickens.

The simple lever model predicts that bite forces will increase at progressively posterior bite points, whereas the constrained lever model predicts that bite forces will increase as the bite point moves posteriorly, except at the most posterior bite points, where they will decrease. To determine which of these models best explains bite force distributions along the toothrow, a Jonckheere-Terpstra (Jonckheere, 1954; Terpstra, 1952) test for an ordered difference in bite force medians was used, within each species and on each side of the tooth row.

Bone strain data analysis

In IGOR Pro 4.0 (WaveMetrics, Inc., Lake Oswego, OR, USA) custom written software was used to convert the strain data from volts to microstrain ($\mu\varepsilon = 10^{-6}\ \Delta L/L$) using shunt calibration files recorded during the experimental sessions, and to calculate the magnitudes of maximum (ε_1) and (ε_2) minimum principal strains (Hibbeler, 2000). The peak magnitudes of these variables during each bite were extracted to IBM SPSS Statistics for Windows, Version 24 (IBM Corp., Armonk, NY, USA) for statistical analyses.

Univariate ANOVAs were used to investigate the factors driving variance in bone strain magnitudes at different sites in the cranium in the four lizard species. Separate models were calculated: across gage locations and bite points within each individual; across gage locations, bite points and individuals within each species; and then across all factors, including species membership. Species membership was treated as a random factor, i.e., the set of species from the clade Lepidosauria was randomly chosen with respect to the hypotheses. Because of the wide diversity in size and cranial design, the long branches joining them, and the fact that only four species were sampled, the species were assumed to be independent; i.e., phylogeny was not taken into account. Bite point (seven "levels" or locations: anterior midline; anterior, middle and posterior on left and right sides) was treated as a fixed factor because it has precisely defined locations replicated across individuals, because bite point effects apply only to those locations (assuming we sampled the tooth row densely enough), and because variation in bite point within each of the seven locations is random. Gage location was also treated as a fixed factor, with three locations (parietal, frontal, maxilla) because similar/homologous sites were sampled across species, and because we assume there is no variation in gage sites between individuals (Doncaster and Davey, 2007). The validity of this assumption depends on the level of precision one is prepared to accept: certainly the gages were placed on homologous bones in very similar places. The effects of this variation will emerge at the level of inter-individual variation within species. The degree to which the gage sites are "homologous" across species is debatable.

Both bite force and animal size (jaw length) are plausible covariates of inherent interest (Doncaster and Davey, 2007): bite force is a covariate of muscle forces and joint reaction forces; and jaw length is a not only an indicator of the size of the skeleton resisting these forces, but it is also a covariate of muscle size (Metzger and Herrel, 2005). The effects of kinesis and herbivory on cranial strain magnitudes were estimated by comparing measured principal strain magnitudes and estimated marginal means from the ANOVAs, which remove the effects of bite force (as a covariate) and bite point (random effect). Frontal and parietal gage sites were compared to assess the effects of mesokinesis; strains from *Iguana* were compared with those recorded from other, non-herbivorous taxa to test for effects of diet and its associated suite of skull modifications. Type III sums of squares were used because of the interactions between factors. Interaction terms were included in the models: interaction effects are represented with an ‘X’; e.g., gage location X species interaction effects. ANOVAs were run in SPSS using the General Linear Models menu.

RESULTS

Bite force is affected by instrumentation, size and bite point

The bite forces of the *Anolis* and *Gekko* subjects (Table S1) fell within the range of values obtained from non-experimental animals but the bite forces of the captive *Salvator* were lower than those of similarly sized semi-wild animals (Fig. 2A). Our experimental *Iguana* individuals were smaller than the non-experimental animals for which data were available, making meaningful comparisons impossible. Effects of instrumentation (strain gage and EMG electrode placement) on bite force magnitudes were estimated in one *Anolis,* one *Iguana,* and four *Gekko* using a univariate ANOVA. Controlling for bite point there were significant decreases in bite force associated with instrumentation in all individuals except one *Gekko* and the *Iguana* (Table 1).

In all species, an independent samples test rejected the null hypothesis that bite force is the same at all bite points in the tooth row (Table 2, Fig. 2B, Fig. S2). Jonckheere-Terpstra tests for an ordered difference in bite force medians reveal that there is a significant effect of bite point on bite forces on both sides in all species except for right bites in *Gekko* and *Iguana*. The constrained lever model predicted rank order of bite force magnitude on both left and right sides in *Gekko* and *Anolis*, and on left sides in *Iguana* and *Salvator*, whereas the simple lever model only predicted rank order of bite forces in *Anolis* (both sides), *Gekko* (left side), and *Iguana* (left side). These results suggest that either model may apply to lepidosaurs, they confirm the effects of bite point on bite force (and plausibly bone strain magnitudes), and they necessitate the inclusion of bite point as a factor in the ANOVA analyses reported below. Bivariate correlations between $\log_{10}$ bite force and $\log_{10}$ jaw length between individuals within species are not significant, but across all individuals and bite points both mean and maximum bite forces are correlated with jaw length at $P < 0.007$ (r_{mean}, 0.825; r_{max}, 0.885) (Fig. S2). These analyses suggest that animal size and bite force are correlated, so bite force was included in our analyses as a covariate and jaw length was excluded.

Gage location and bite point drive variance in cranial bone strain magnitudes within individuals

Strain magnitude data for all individuals are given in Table S2. Within each individual, ANOVA was used to test for effects of gage location, bite point, and bite force on mean ε_1 and ε_2 magnitudes separately. In one *Gekko* (1398975), one *Iguana* (1392969), two *Anolis* (1386575, 1386576), and one *Salvator* (# 3), bite force was not a significant covariate with either mean ε_1 or ε_2 magnitude. In two *Salvator* (# 1 and 4) bite force was not a significant covariate with mean ε_2 magnitude but it was with mean ε_1 magnitude. In the rest of the individuals (two *Anolis*, two *Iguana*, two *Gekko*) bite force was a significant covariate with both mean ε_1 and ε_2 magnitudes. In most individuals bite point had a significant impact on means of both ε_1 and ε_2 magnitudes, the exceptions being two *Salvator*, one *Anolis* and one *Gekko*, in which bite point affected mean ε_1 but not ε_2 magnitudes. In most individuals, gage location also had a significant effect on both mean ε_1 and ε_2 magnitudes, and in all individuals it had an effect on either mean ε_1 or ε_2 magnitudes. Bite point X gage site interactions were significant in all individuals except the individuals where gage site or bite point effects were not significant.

In summary, within individuals the most consistent determinants of variance in cranial bone strain magnitudes are strain gage location, bite point and their interaction, with the importance of bite force varying between individuals. These results reveal that inter-site variance in bone strain magnitudes—strain gradient—is present in all the individuals studied here, and the nature of this gradient varies with bite point.

Bite force drives variance in cranial bone strain magnitudes between individuals within species

Within each species ANOVAs were used to model the effects of individual, gage location, bite point, and bite force on mean ε_1 and ε_2 magnitudes separately. Within all species, individual was not a significant factor and bite force was a significant covariate of both mean ε_1 and ε_2 magnitudes. In *Gekko* and *Iguana,* gage location did not affect inter-individual variation in either mean ε_1 or ε_2 magnitudes, and in *Anolis* and *Salvator* gage location only had a significant effect on inter-individual variation in mean ε_1 magnitudes. Bite point had a significant effect only on ε_1 magnitudes in *Salvator* and ε_2 magnitudes in *Anolis*. Thus, the most consistent determinant of variance in cranial bone strain magnitudes within species is bite force, not individual, gage location or bite point. These results reveal that the strain gradients documented in the previous section are consistent across individuals within species.

Species membership, bite force, and diet, but not kinesis, drive variance in cranial bone strain magnitudes between species

ANOVA was used to model the effects of species membership, bite force (covariate), bite point, and gage location on mean ε_1 and ε_2 magnitudes (Table 3). Mean ε_1 and ε_2 magnitudes were significantly impacted by species membership, bite force, gage location X species interaction effects, and bite point X gage location X species interactions. ε_1 magnitudes were also impacted by bite point X gage interactions. Independently, bite point and gage location did not significantly affect strain magnitudes.

The estimated marginal means from this ANOVA (Table 3) falsify the hypothesis that our herbivorous species (*Iguana*) experiences higher strains than the other taxa. Indeed, the reverse is true. Pairwise comparisons reveal that, controlling for bite force and bite point, *Iguana* has significantly lower, not higher, estimated marginal mean ε_1 strain magnitudes than the other three species, and significantly lower mean ε_2 strain magnitudes than *Anolis* and *Gekko*. Nor do the data support the hypothesis that absence of the ST and PO bars is associated with higher cranial strain magnitudes or the presence of kinesis is associated with lower strain magnitudes: *Gekko* (kinetic) and *Anolis* (akinetic) do not differ from each other in estimated principal strain marginal means, but both experience significantly higher estimated mean ε_2 strain magnitudes than *Iguana* and *Salvator* (both akinetic).

The effect of mesokinesis on cranial strain magnitudes was also tested by comparing marginal means from an ANOVA of principal strain magnitudes at frontal and parietal gage sites, accounting for the effects of bite force (as a covariate) and bite point (random effect) (Table 5). There is no effect of the presence of mesokinesis on the ratios of principal strains at the frontal gage site to that at the parietal gage site. In the akinetic *Iguana*—the largest species—principal strains in the frontal were nine to fourteen times larger than in the parietal, whereas in both the akinetic *Anolis* and the highly kinetic *Gekko*, strains in the frontal were 1.5 to 2.6 times larger than in the parietal. Hence, principal strain magnitudes are always several times higher in the frontal than in the parietal, regardless of the presence or absence of mesokinesis between frontal and parietal bones.

DISCUSSION

The data presented here have some limitations: not all gage combinations were recorded from all animals, manual restraint of the *Salvator* appears to have deformed the cranium, requiring us to exclude some of the data; the data are from transducer biting, not feeding; and instrumentation may have resulted in lower bite forces in some individuals. Nevertheless, these data represent a significant advance in our understanding of *in vivo* cranial function of lizards during feeding.

Bite force data may corroborate the constrained lever model in lepidosaurs

The distribution of bite forces across bite points in these lepidosaurs is explained at least as well, and in some cases better, by the constrained lever model than by the simple lever model. The constrained lever model proposes that balancing side (non-biting side) muscle activity must be reduced during biting at the most posterior bite points in order to avoid tensile forces in the biting side jaw joint (Druzinsky and Greaves, 1979; Greaves, 1978; Spencer, 1995; Spencer, 1998; Thompson et al., 2003). This model predicts lower bite forces at the most posterior (more distal) teeth than in the middle of the postcanine tooth row, a prediction broadly consistent with the data presented here. Corroboration of this hypothesis in lepidosaurs would suggest that, as in mammals, models of muscle recruitment during biting by lepidosaurs should take into account effects of both bite point and joint reaction forces (Curtis et al., 2010; Shi et al., 2012). It also implies that, if the constrained lever model applies broadly across amniotes, then sensorimotor mechanisms modulating bite point-specific muscle recruitment might also be similarly distributed. In mammals, sensory afferents from muscle spindles and the periodontal ligament connecting the teeth to the mandible are essential for feed-forward and feed-back regulation of bite force, respectively (Komuro et al., 2001; Ottenhoff et al., 1992a; Ottenhoff et al., 1992b; Trulsson, 2006): the roles of spindle afferents and afferents in intra-cranial, intra-mandibular, and craniomandibular joints for modulation of bite force in lepidosaurs and *Caiman* (McIntosh et al., 2002*)* remain to be evaluated.

An alternative explanation for the decrease in bite force at the most posterior bite points is the effect of gape distance on the part of the jaw elevator muscle length tension curves. The bite force transducer plates were a constant distance apart in all trials, so that, depending on the axis of rotation of the jaw, or jaw/quadrate system, at more posterior bite points the jaw elevator muscles may have been most highly stretched. It is possible that this stretched the muscles beyond the optimal region of their length tension curves, resulting in lower maximum bite forces. Future studies of the effects of bite point on bite force should control for this effect to determine whether gape effects or the constrained lever model best explain the lower bite forces at the most posterior bite points. Investigation of the location of the axis of rotation of lizards would be of interest in this regard (cf. Iriarte-Diaz et al., 2017).

Determinants of strain magnitude in lepidosaur crania

We asked whether, when bite force and bite point are statistically controlled, do lizards show strain gradients—variation in strain magnitudes—across the cranium during biting? If lizard crania were optimized for maximum strength during feeding with minimum material then strain magnitudes would be fairly uniform across the cranium during feeding, and the crania of all species would experience similar strain magnitudes during feeding. Of course, biting on different regions of the toothrow must necessarily be associated with different strain magnitudes in different parts of the cranium because torques and compressive, tensile and shearing forces vary with bite point. However, once these factors are taken into account (by eliciting powerful bites across a range of bite locations *in vivo*), if the crania are optimized for maximum strength during feeding with minimum material there should not be strain gradients—differences in strain magnitudes across gage locations. In fact, within individuals, the most consistent determinants of variance in bone strain magnitudes during transducer biting are strain gage location, bite point, and gage location X bite force interactions: cranial bone strain magnitudes vary across the cranium; i.e., there are strain "gradients".

We also asked whether individuals in the same species share common patterns of variation in strain magnitude across the cranium when bite force and bite point are statistically controlled. Indeed, between individuals, within species, the most consistent determinant of variance in cranial bone strain magnitudes is bite force, not gage location, individual, or bite point. The importance of bite force in driving cranial bone strain magnitudes between individuals (and species, see below) is not surprising: higher bite forces exert larger compressive, tensile, and shearing forces at gage sites, as well as larger bending and twisting moments about the gage sites, and must also be associated with higher muscle and joint reaction forces. Moreover, the fact that gage location is not a significant determinant of inter-individual variation in strain magnitudes suggests that the patterns of strain recorded from these individuals—including the variation between gage locations—are consistent representations of species-specific patterns.

Finally, we asked: when bite force and bite point are controlled, what are the effects of diet, species-specific cranial morphology and cranial kinesis on variation in strain magnitudes in lizard crania? Between species, *in vivo* bone strain magnitudes in the cranium are significantly impacted by bite force and species membership independently, as well as by gage location X species, gage location X bite point (ε_1 magnitudes), and gage location X bite point X species interaction effects. Bite point and gage location did not significantly affect interspecific variation in strain magnitudes independent of these interaction effects. Species level differences in cranial strain magnitudes (independent of bite force) argue against the idea that selection designs all lizard crania to a common optimality criterion of maximum strength during feeding for minimum material. This interspecific variation may reflect selection for different cranial morphology:feeding function relationships—different cranial "designs"—but what the specific performance criteria might be is not clear. *Iguana* displayed lower overall ε_1 strain magnitudes than the other three species, and *Iguana* and *Salvator* displayed lower ε_2 strain magnitudes than *Anolis* and *Gekko* (Table 4). The only other species-level effects are species X bite force and species X bite force X bite point interaction effects.

Possible species-level effects on strain magnitudes include the presence of supratemporal and postorbital bars, as well as varying degrees of kinesis. Previous bone strain studies of kinesis in *Varanus exanthematicus* used single element gages to measure strain across the top of the frontoparietal suture (mesokinetic hinge joint) and rosette gages to record strain of several hundred microstrain from the frontal bone rostral to the joint during feeding sequences (Smith and Hylander, 1985). In the present study, strains recorded from the frontal and parietal bones on either side of the frontoparietal suture were recorded during transducer biting, not feeding. Strain magnitudes were uniformly higher in the frontal than in the parietal, often by two orders of magnitude, regardless of the presence or absence of mesokinesis, or supratemporal or postorbital bars (Table 5). This suggests that the distribution of strain magnitudes in the roof of the lepidosaur cranium are not significantly different between those animals with and without mesokinesis, arguing against the hypothesis that kinetic crania have lower frontal and parietal stress and strain magnitudes.

Another possible source of interspecific variation in strain magnitudes is dietary effects. Herbivorous lepidosaurs have lighter skulls, shorter snouts, larger jaw elevator muscles, and higher bite forces (Herrel et al., 2007; Metzger and Herrel, 2005; Stayton, 2006). We hypothesized that this might predict *higher* strains in the crania of our herbivorous species—*Iguana*—than the other species, but the opposite was true: *Iguana* had the lowest strains of all the species. Low strains may represent increased resistance to fatigue effects in *Iguana* crania, as suggested for *Uromastyx*

(Porro et al., 2014), a hypothesis that would be supported if *Iguana* are shown to chew more frequently than non-herbivorous lepidosaurs. Fatigue effects explain strain magnitudes in a wide variety of situations (reviewed by Ross and Metzger, 2004), so their importance for lepidosaur cranial form would not be surprising. Interactions between diet and overall size (see below) would not be unexpected—herbivorous lizards might both chew more and be larger. However, size related effects on diet and feeding behavior, including number of chewing cycles per day, are certainly complex in mammals (Ross et al., 2009), and better data are needed before fatigue effects on lizard cranial form can be advocated.

Salvator merianae and *Iguana iguana* are larger than *Anolis equestris* and *Gekko gecko* and it is possible that overall cranial size impacts strain magnitudes in the cranium. Ravosa et al. noted a negative allometry of peak principal strains in pairwise comparisons within cercopithecine primates (*Macaca* and *Papio*) and galagos (*Otolemur crassicaudatus* and *O. garnetti*) (Ravosa et al., 2000a), a pattern replicated in lemurids *Eulemur fulvus* and *Varecia variegata* (Ross, 2008) (unpublished data). Allometry of circumorbital strain magnitudes could be due to allometry of the external forces acting on the cranium, allometry in optimality criteria (i.e., differences in the optimality criteria influencing form at different body sizes), or allometry of the extent to which optimality criteria actually matter for cranial design (Ross and Metzger, 2004). Choosing between these explanations will require more data than are currently available, especially from studies of taxa with a wider range and finer gradation of diets.

Notably, gage location and bite point do not drive interspecific variation in cranial strain magnitudes independently of interaction effects with bite point and/or species membership, and bite point does not impact strain magnitude variation independently of gage location. This suggests that strain magnitudes are influenced by species-specific factors other than cranial morphology, such as patterns of muscle recruitment and associated joint reaction forces (Porro et al., 2011). Analysis of EMG data collected during these experiments will be of interest.

Strain magnitudes in the lepidosaur parietal and frontal are similar to or higher than those in the maxilla.

Our results reveal that the strain magnitudes in the frontal and parietal bones of the lizards studied here are usually higher than those in the maxilla. The presence of high biting strains in the parietal and frontal bones indirectly overlying the braincase suggests that the morphology (size and shape) of these bones may be more optimized for maximum strength with minimum material during feeding than are the bones of the braincase in mammals (Table 6). Where known, the calvarial bones of mammals experience lower strains than the facial skeleton (Behrents et al., 1978; Herring and Teng, 2000; Thomason et al., 2001), suggesting that the calvarial strength needed to protect their relatively enlarged brains against infrequent impact loads exceeds that necessary for a feeding system optimized for maximum strength with minimum material . In support of this hypothesis, it is noteworthy that mammals with relatively smaller brains (*Ovis*) experience higher strain magnitudes in the calvaria during feeding (ε_1 up to 635 με) (Thomason et al., 2001) than primates (Behrents et al., 1978), reflecting increased influence of feeding system design criteria on the calvaria.

The *Alligator* cranium may also be less well optimized for maximum strength with minimum material, as some parts of the cranium appear to experience higher strains than others (Metzger et al., 2005; Ross and Metzger, 2004). It is possible that the aquatic habits of *Alligator* alleviate selective pressure to minimize skull mass, or maybe the need to quickly move the head laterally to capture prey in an aquatic environment imposes other design constraints on the cranium (Busbey, 1995; Metzger et al., 2005). Certainly the cross-sectional shape of the snout is not optimal for resisting bending moments associated with high magnitude bite forces, suggesting that the mechanical needs of moving through an aquatic environment trump those associated with resisting biting stresses (Erickson et al., 2012). To compensate for this sub-optimal cross-sectional shape, alligators exhibit an extensive hard palate and overlapping scarf joints between some of the bones (Busbey, 1989; Busbey, 1995). However, it is important to note that the majority of the cranial bone strain data from *Alligator* (Metzger et al., 2005; Porro et al., 2011) and lizards (presented here) were collected during transducer biting, whereas the majority of mammal data were collected during feeding. Strong conclusions about the biological significance of differences in strain magnitudes between these clades must await better controlled experiments.

Conclusions

In vivo bone strain data from the crania of four species of lizards reveal that, like mammals and alligators, bone strain magnitudes vary across the cranium of lepidosaurs. Although the mammal data were collected primarily during feeding, and those from alligators and lizards are mostly collected during transducer biting, the regional variability in bone strain magnitudes indicates that cranial design in tetrapod skulls is not dominated by the criterion of maximum strength with minimum material during feeding. The data presented here also suggest that there are species-specific patterns of variation in cranial bone strain magnitudes that are not obviously related to patterns of cranial kinesis, or to the presence or absence of postorbital and supratemporal bars, and future work should consider these features in the context of overall cranial architecture. Strain magnitudes are larger in the frontal than the parietal, and usually larger in the frontal than in the maxilla. This may reflect differences in optimality criteria between lizard and mammal crania, but better data are needed to confirm these clade level differences.

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Competing interests

The authors declare no competing interests.

Author contributions

CFR designed the study, collected and analyzed the data, drafted the manuscript and figures. LBP collected data, prepared figures and commented on the manuscript. AH collected data and commented on the manuscript. SE and MJF designed and funded the study, and commented on the manuscript.

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TableS

Table 1. Tests of instrumentation effects.

			Pre-instrumentation bite force (N)				Post-instrumentation bite force (N)				Instrumentation effect		Significant effect?	
Species	**individual**	**bite point**	**n**	**Mean**	**Max.**	**Std. Dev.**	**n**	**Mean**	**Max.**	**Std. Dev.**	**Absolute (N)**	**% of pre-instr.**	**F^1**	**P**
Anolis	1386575	anterior midline	4	36.31	47.07	7.894	13	26.39	38.07	8.959	-9.93	-27.33	27.451	0.000
Jaw length (mm)	44.79	L anterior	4	65.73	67.13	2.281	13	35.04	47.87	6.963	-30.69	-46.70		
		L middle	6	94.50	117.78	16.117	1	60.59	60.59		-33.91	-35.88		
		R anterior	3	62.95	65.38	2.143	8	32.76	47.45	15.187	-30.19	-47.96		
		R posterior	1	87.24	87.24	.	8	71.61	101.61	19.276	-15.63	-17.92		
Gekko	1398971	anterior midline	5	11.33	13.50	1.777	10	5.58	6.68	0.988	-5.75	-50.73	101.017	0.000
Jaw length (mm)	32.31	L anterior	6	15.16	17.67	1.850	21	7.11	12.70	2.659	-8.05	-53.08		
		L posterior	6	13.08	14.56	1.277	11	7.59	12.54	3.120	-5.50	-42.01		
		R anterior	3	11.95	13.79	1.861	7	6.78	17.05	1.789	-5.17	-43.28		
		R posterior	5	15.73	17.05	0.888	15	9.09	14.46	3.635	-6.65	-42.25		
Gekko	1398972	L anterior	4	7.09	9.59	2.539	5	4.13	4.76	0.566	-2.96	-41.70	13.470	0.003
Jaw length (mm)	26.89	R posterior	1	9.21	9.21		5	4.82	5.19	0.326	-4.39	-47.68		
Gekko	1398973	anterior midline	4	12.10	17.84	6.568	6	15.33	18.52	2.510	3.23	26.65	0.238	0.628
Jaw length (mm)	30.16	L anterior	12	14.48	19.05	5.080	2	11.51	16.15	6.568	-2.97	-20.52		
		L posterior	10	19.00	24.96	4.512	6	14.50	18.84	3.387	-4.50	-23.67		
		R posterior	4	8.18	15.54	5.151	2	10.91	12.56	2.335	2.73	33.31		
Gekko	1398974	anterior midline	2	6.31	9.10	3.951	2	7.99	10.03	2.884	1.67	26.53	16.017	0.001

Jaw length (mm)	31.50	L posterior	5	18.91	20.74	1.128	6	13.41	16.35	1.918	-5.49	-29.06		
		R anterior	1	15.15	15.15	.	3	9.16	12.03	4.202	-5.99	-39.55		
		R posterior	1	13.43	13.43	.	5	9.85	11.45	1.178	-3.58	-26.65		
Iguana	1398975	anterior midline	2	20.44	21.89	2.051	1	20.28	20.28		-0.17	-0.81		
Jaw length (mm)	44.16	L anterior	10	18.99	34.26	12.346	7	23.46	34.40	11.613	4.46	23.50	0.117	0.735
		L posterior	8	19.98	30.86	8.217	2	29.04	29.14	0.148	9.06	45.34		
		R posterior	7	33.00	45.99	10.809	4	17.66	35.18	11.783	-15.34	-46.48		

[1] F-tests of the effect of instrumentation based on linearly independent, pairwise comparisons among estimated marginal means.

Abbreviations: max., maximum; N, newtons; pre-instr., pre-instrumented

Table 2. Summary of Jonckheere-Terpstra tests for ordered differences in bite force.

Species	Rank order model	Left bites	Right bites
Anolis	*Bite point effect*[1]	<0.001	<0.001
	Simple lever[2]	<0.001	<0.001
	Constrained lever[3]	<0.001	<0.001
Gekko	*Bite point effect*[1]	0.003	ns
	Simple lever[2]	0.008	ns
	Constrained lever[3]	0.008	0.007
Iguana	*Bite point effect*[1]	<0.000	ns
	Simple lever[2]	<0.001	ns
	Constrained lever[3]	<0.001	ns
Salvator	*Bite point effect*[1]	<0.000	0.008
	Simple lever[2]	ns	ns
	Constrained lever[3]	<0.001	ns

p values are probability that null hypotheses are correct; i.e.,

[1] that median bite forces are the same across bite points

[2] that bite forces are the same across simple lever model ranks

[3] that bite forces are the same across constrained lever model ranks.

Table 3. ANOVA models of determinants of $\log_{10}$ principal strain magnitudes in *Gekko*, *Anolis*, *Iguana* and *Salvator;* species, random factor; bite point and gage location, fixed factors; bite force, covariate.

		$\log_{10} \varepsilon_1$						**$\log_{10} \varepsilon_2$**					
Source		**Type III Sum of Squares**	**f**	**Mean Square**	**F**	**Sig.**	**Partial Eta Square**	**Type III Sum of Squares**	**f**	**Mean Square**	**F**	**Sig.**	**Partial Eta Square**
Intercept	Hypothesis	278.13	1.00	278.13	204.648	0.000	0.984	183.09	1.00	183.09	48.797	0.005	0.939
	Error	4.49	3.31	1.36				11.84	3.16	3.75			
$\log_{10}$ bite force	Hypothesis	10.63	1.00	10.63	110.294	0.000	0.079	13.25	1.00	13.25	114.254	0.000	0.090
	Error	123.61	1283.00	0.10				133.92	1155.00	0.12			
Bite point	Hypothesis	3.67	6.00	0.61	2.455	0.053	0.376	5.11	6.00	0.85	1.157	0.395	0.391
	Error	6.10	24.50	0.25				7.95	10.81	0.74			
gage	Hypothesis	21.10	5.00	4.22	2.704	0.149	0.729	31.88	3.00	10.63	2.484	0.202	0.655
	Error	7.85	5.03	1.56				16.81	3.93	4.28			
species	Hypothesis	20.78	3.00	6.93	5.479	0.045	0.758	54.00	3.00	18.00	8.523	0.023	0.843
	Error	6.64	5.25	1.26				10.06	4.76	2.11			
Bite point * gage	Hypothesis	24.42	25.00	0.98	2.393	0.018	0.712	17.07	18.00	0.95	1.388	0.236	0.548
	Error	9.88	24.20	0.41				14.10	20.63	0.68			
Bite point * species	Hypothesis	6.80	17.00	0.40	1.017	0.474	0.411	8.88	11.00	0.81	1.244	0.320	0.393
	Error	9.74	24.78	0.39				13.68	21.08	0.65			
gage * species	Hypothesis	9.38	5.00	1.88	6.332	0.000	0.505	17.33	4.00	4.33	9.377	0.000	0.597
	Error	9.18	31.00	0.30				11.71	25.35	0.46			
Bite point * gage * species	Hypothesis	10.67	22.00	0.48	5.032	0	0.079	14.80	20.00	0.74	6.383	0.000	0.100
	Error	123.61	1283.00	0.10				133.92	1155.00	0.12			

Table 4. Species-level estimated marginal means of principal strains across all gage sites from ANOVA in Table 3.

	ε_1 estimated marginal means					ε_2 estimated marginal means				
species	mean ($\mu\varepsilon$)	$\log_{10}$ mean[a, c]	Std. Error	Lower Bound	Upper Bound	mean ($\mu\varepsilon$)	$\log_{10}$ mean[b, d]	Std. Error	Lower Bound	Upper Bound
Anolis	360.6	2.557	0.031	2.496	2.619	463.4	2.666	0.035	2.598	2.734
Gekko	457.1	2.66	0.036	2.590	2.730	482.0	2.683	0.039	2.606	2.760
Iguana	157.8	2.198	0.016	2.167	2.230	90.6	1.957	0.018	1.922	1.991
Salvator	452.9	2.656	0.039	2.580	2.733	157.0	2.196	0.139	1.923	2.469

a. Covariates evaluated at $\log_{10}$ bite force = 1.36.

b. Covariates evaluated at $\log_{10}$ bite force = 1.33.

c. Pairwise comparisons: *Iguana* ε_1 estimated marginal mean differs from all others at $p < 0.001$

d. Pairwise comparisons: *Iguana* and *Salvator* ε_2 estimated marginal means differ from *Anolis* and *Gekko* at $p < 0.001$

Table 5. Estimated marginal means from ANOVAs of principal strain magnitudes at frontal and parietal gage sites across *Gekko*, *Anolis* and *Iguana*: Species, random factor; bite point and gage location, fixed factors; bite force, covariate.

Species	principal strain	gage sites	mean ($\mu\varepsilon$)	frontal:parietal ratio	Mean ($\log_{10}$)	Std. Error	Lower Bound	Upper Bound
Gekko	ε_1	frontal	727.8	2.1	2.86	0.064	2.737	2.987
		parietal	343.6		2.54	0.064	2.411	2.661
	ε_2	frontal	-709.6	2.6	2.85	0.072	2.710	2.992
		parietal	-273.5		2.44	0.072	2.297	2.578
Anolis	ε_1	frontal	445.7	1.8	2.65	0.055	2.540	2.757
		parietal	252.3		2.40	0.063	2.278	2.525
	ε_2	frontal	-538.3	1.5	2.73	0.062	2.609	2.853
		parietal	-356.5		2.55	0.071	2.413	2.691
Iguana	ε_1	frontal	389.9	9.1	2.59	0.027	2.538	2.645
		parietal	42.7		1.63	0.028	1.576	1.685
	ε_2	frontal	-306.9	14.1	2.49	0.031	2.427	2.548
		parietal	-21.8		1.34	0.031	1.277	1.398

Table 6. Summary of *in vivo* cranial bone strain data from tetrapods.

				ε_1		ε_2		γ_{max}	
Species	**Individual**	**Gage location**	**behavior**	**mean**	**max**	**mean**	**max**	**mean**	**max**
Primates[1]		**Frontal bone**[2]							
Papio anubis	1	Dorsal interorbital	mastication	161	215	-51	-68	212	283
Hylander et al., 1991			incision	167	230	-42	-60	209	290
Macaca fascicularis	5A	Dorsal interorbital	mastication	292	462	-119	-189	411	651
Hylander et al., 1991			incision	235	298	-68	-88	303	386
	6	Dorsal interorbital	mastication	148	210	-37	-52	185	263
			incision	216	311	-49	-75	266	386
	2A	Dorsal interorbital	mastication	133	227	-72	-120	204	347
			incision	189	270	-70	-98	259	369
	4	Dorsal interorbital	mastication	51	91	-21	-35	72	126
			incision	62	86	-23	-32	85	118
Aotus trivirgatus	1	Dorsal interorbital	mastication	168	356	-145	-307	313	654
Ross and Hylander 1996			incision	78	105	-177	-245	255	346
	2	Dorsal interorbital	mastication	35	114	-105	-194	140	248
			incision	81	89	-168	-340	216	425
Otolemur garnetti	1	Dorsal interorbital	mastication	361	587	-383	-634	745	1221
Otolemur crassicaudatus	2	Dorsal interorbital	mastication	183	378	-315	-698	498	1076
Ravosa et al., 2000	3	Dorsal interorbital	mastication	312	354	-209	-235	642	918
Eulemur fulvus	Ba (exp 76)	Dorsal interorbital	mastication	62	110	-69	-147	130	256
Ross, unpublished	Be (exp 78)	Dorsal interorbital	mastication	34	104	-40	-145	73	225
	Ma (exp 79)	Dorsal interorbital	mastication	44	109	-53	-136	96	237
Varecia variegata	D (Exp 97)	Dorsal interorbital	mastication	23	88	-44	-156	65	242
Ross, unpublished	B (exp 94)	Dorsal interorbital	mastication	21	39	-137	-369	148	393
Sus scrofa	147	frontal	mastication	124		-74		198	
Herring and Teng, 2000	154	frontal	mastication	21		-27		48	
	157	frontal	mastication	47		-58		105	
	158	frontal	mastication	52		-31		83	

		162	frontal	mastication	18		-25		43	
		164	frontal	mastication	30		-66		96	
		165	frontal	mastication	54		-69		123	
Procavia capensis		H2	Dorsal interorbital	mastication	273		-159		432	
	Lieberman et al., 2004	H3	Dorsal interorbital	mastication	16		-231		247	
Ovis[3,4]		1	frontal	mastication	515	635	-469	-575	984	1210
	Thomason et al., 2001	2	frontal	mastication	271	356	-115	-358	386	714
		3	frontal	mastication	351	492	-235	-339	586	831
		4	frontal	mastication	412	504	-325	-412	737	916
		5	frontal	mastication	227	345	-210	-312	437	657
		2	maxilla	mastication	711	955	-29	-147	740	1102
		3	maxilla	mastication	204	257	-170	-379	374	636
		4	maxilla	mastication	479	593	-32	-49	511	642
		5	maxilla	mastication	280	424	-96	-143	376	567
Alligator		e64	frontal	biting	631	1388	-216.00	-391	858	1778
	Metzger et al ., 2005									
Anolis		1380233	frontal	biting	379	582	-306	-939	681	1368
	This study	1380234	frontal	biting	993	2091	-682	-1386	1675	3458
		1386575	frontal	biting	475	742	-863	-1266	1307	1762
		1386576	frontal	biting	842	1321	-1195	-2303	2036	3624
Gekko		1398971	frontal	biting	739	1887	-692	-1460	1431	3246
	This study	1398972	frontal	biting	662	817	-634	-808	1295	1550
		1398973	frontal	biting	424	575	-419	-619	840	1103
		1398974	frontal	biting	940	2063	-842	-1208	1774	2987
Iguana		1390109	frontal	biting	457	1188	-384	-930	841	2037
	This study	1392969	frontal	biting	517	845	-492	-822	1008	1393
		1398975	frontal	biting	451	886	-295	-572	744	1130
Salvator		1	frontal	biting	1004	1998	-278	-809	1256	2807
	This study	3	frontal	biting	454	777	-218	-359	668	1135
		4	frontal	biting	231	521	-287	-1226	509	1738

Notes

[1] For each individual we used experiment with largest values; within experiments cycle with largest value of ε_1 was chosen.

[2] Strain magnitudes in these regions were even lower than the dorsal interorbital region.

[3] Row with largest ε_1 or ε_2 value

[4] Thomason et al. used single element gages, so mandibular "ε_1" and "ε_2 " underestimate principal strains.

Figures

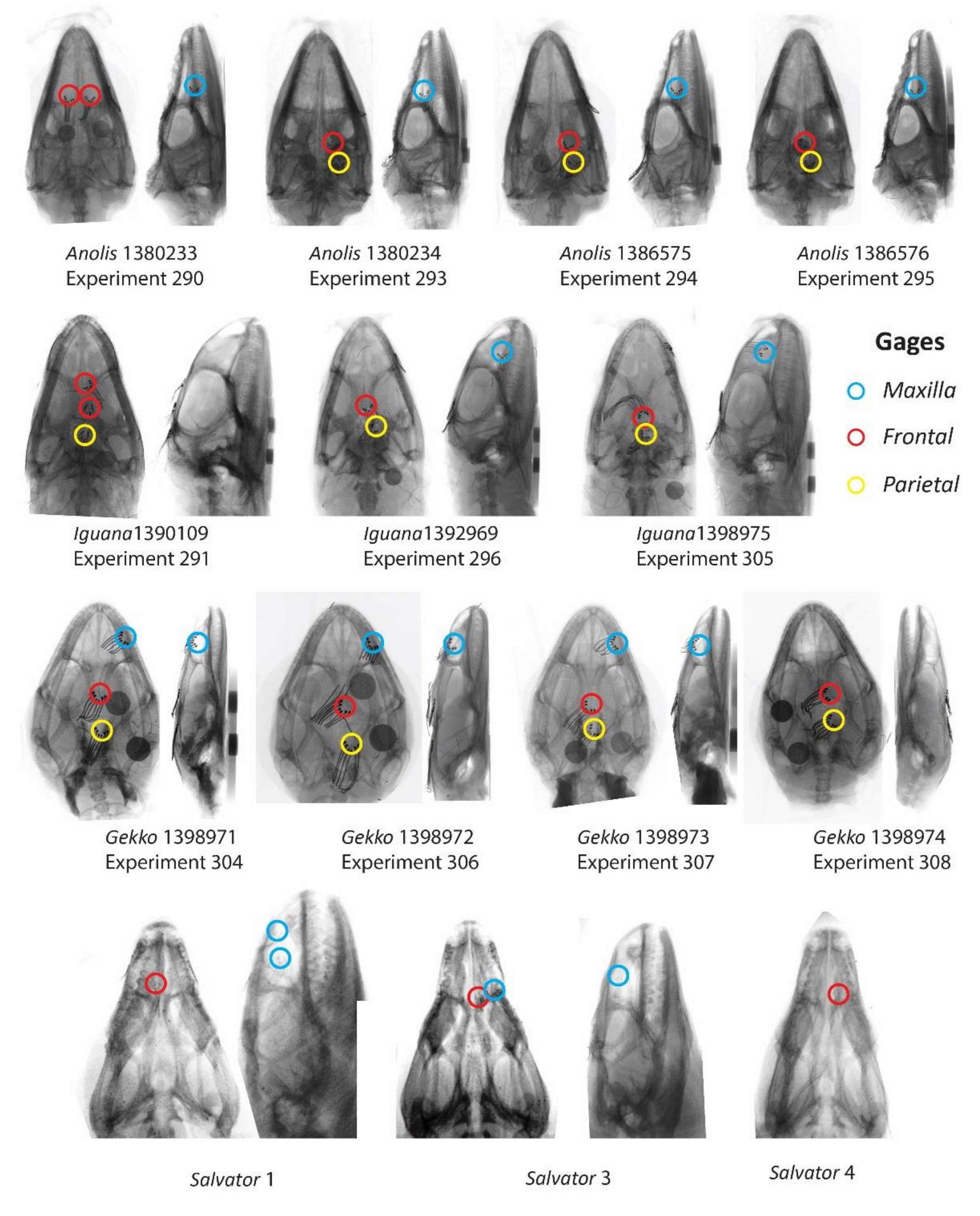

Figure 1. Strain gage locations in all experimental individuals. Radiographs of *Anolis, Iguana* and *Gekko* were made *post mortem, Salvator* radiographs are stills from videoradiographic sequences made during the recording sessions. Images not to scale: lower jaw lengths are given in Table 2. Exp.: Experiment numbers, before instrumentation (P) or after the animals were instrumented with gages and EMG electrodes (I). Dorsoventral views are from the top so that the animal's right is to the right.

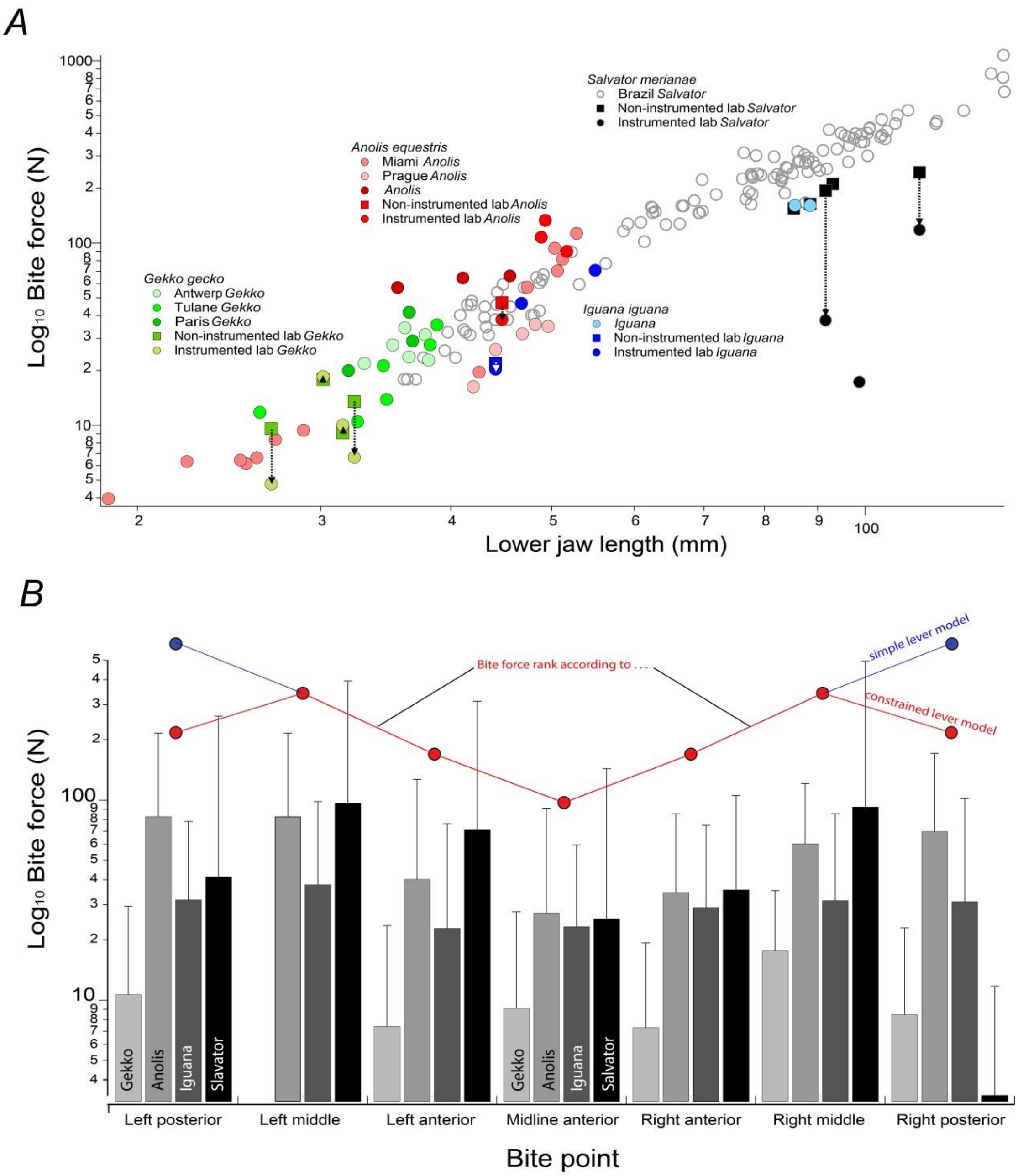

Figure 2. Bite force data from experimental animals and conspecifics. (A) Maximum bite force in newtons (N) at anterior bite point plotted against lower jaw length (mm), colored by species. Dashed arrows, intra-individual decreases in bite force associated with instrumentation. (B) Bite force (N) by species and bite point. Bars, species means; whiskers, species maxima. Red lines and markers illustrate the rank order of bite force predicted by the simple and constrained lever models. Two models were run for left and right sides separately: one predicting that posterior bite points would be associated with bite forces higher than middle bite points (as predicted by a simple lever model); and one predicting that posterior bite points forces would fall below middle but above anterior bite forces (constrained lever model).

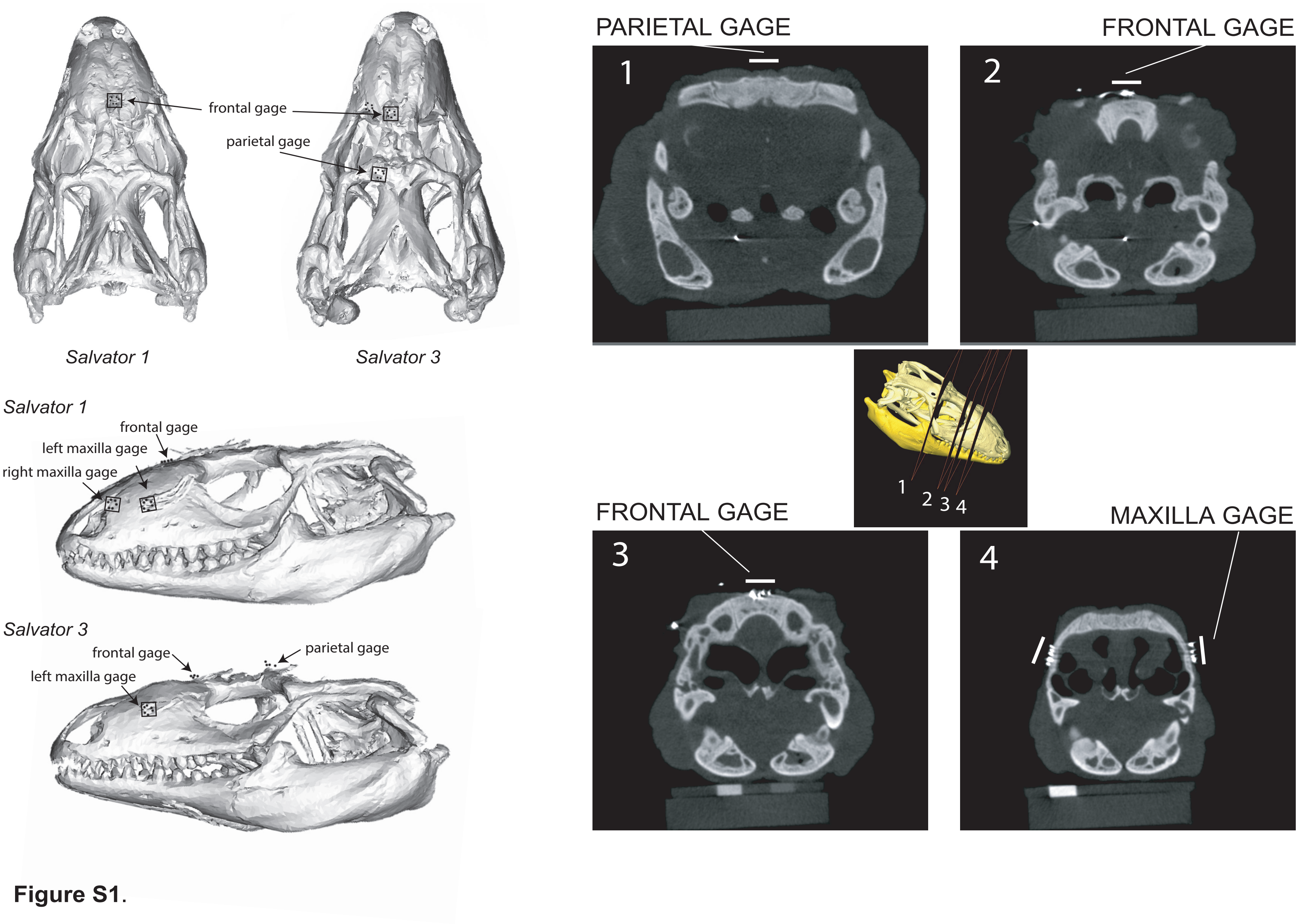

Figure S1.

Strain gage locations in *Salvator* illustrated on reconstructions of CT scans of two of the experimental individuals. White lines overlie gage locations, revealed by metallic densities of solder dots and gage elements.

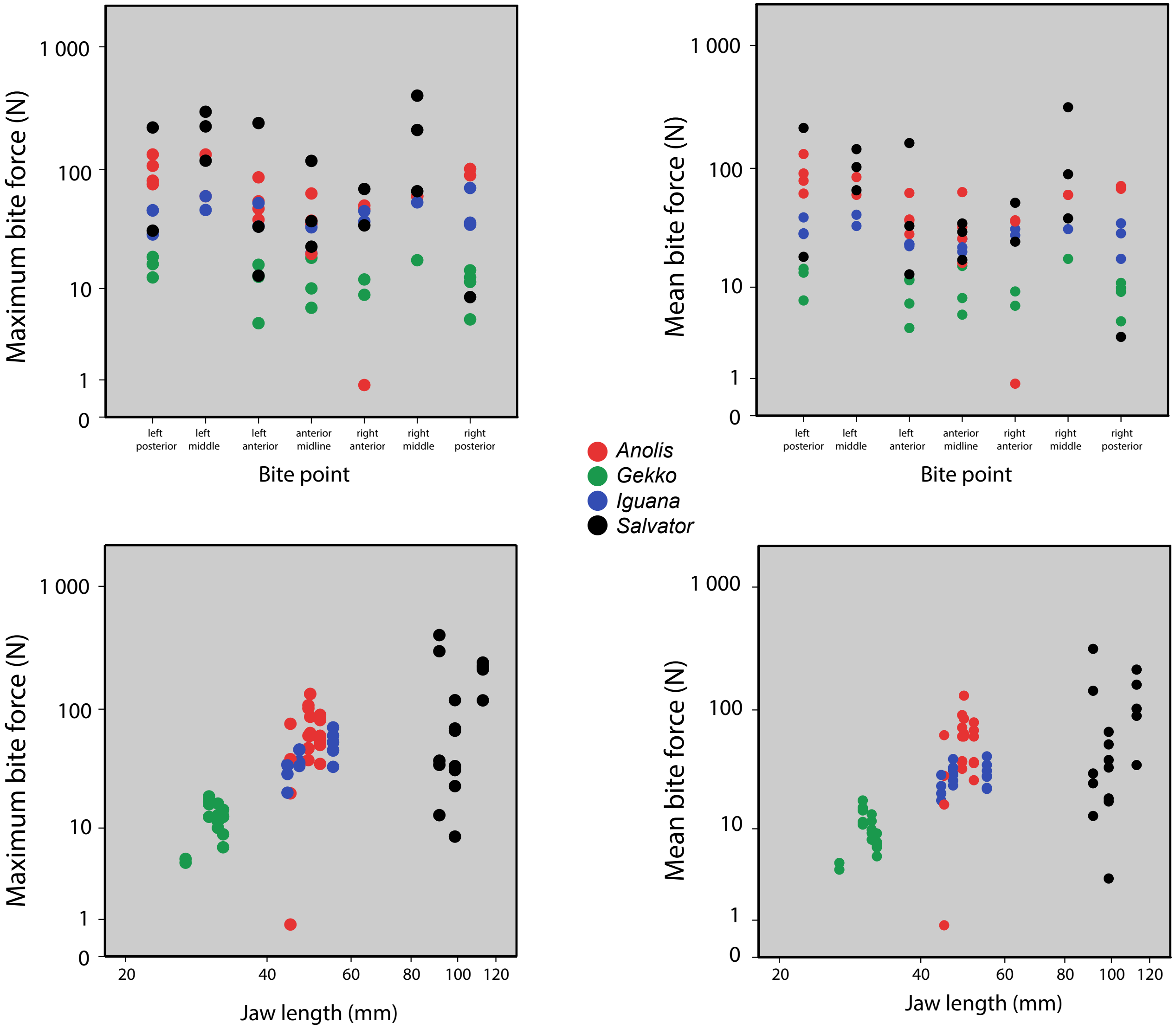

Figure S2.

Top row: Maximum and mean bite force by species and bite point.
Bite forces at the posterior bite points are lower than those at the middle bite points, as predicted by the constrained lever model.

Bottom row. Maximum and mean bite force for each individual, plotted against jaw length;
both correlations are significant at $P < 0.007$; means, $r = 0.825$; max, $r = 0.885$.

Table S1. Mean and maximal bite forces recorded from individuals and species.

Click here to Download Table S1

Table S2. Bite force and strain data

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