

Beyond body size: muscle biochemistry and body shape explain ontogenetic variation of anti-predatory behaviour in the lizard *Salvator merianae* (Squamata: Teiidae).

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Abstract

Anti-predatory behaviour evolves under strong action of natural selection because the success of individuals avoiding predation essentially defines their fitness. Choice of anti-predatory strategies is defined by prey characteristics as well as environmental temperature. An additional dimension often relegated in this multilevel equation is the ontogenetic component. In tegus *Salvator merianae*, adults run away from predators at high temperatures but prefer fighting when it is cold, while juveniles exhibit the same flight strategy within a wide thermal range. Here we integrate physiology and morphology to understand ontogenetic variation in the temperature-dependent shift of anti-predatory behaviour in these lizards. We compiled data for body shape and size and quantified enzyme activities in hindlimb and head muscles, testing the hypothesis that morpho-physiological models explain ontogenetic variation in behavioural associations. Our prediction is that juveniles exhibit body shape and muscle biochemistry that enhance flight strategies. We identified biochemical differences between muscles residing mostly on the LDH: CS ratio, being hindlimb muscles more glycolytic than jaw musculature. Juveniles, which often use evasive strategies to avoid predation, have more glycolytic hindlimb muscles and are much smaller when compared to adults being 1-2 years old. Ontogenetic differences in body shape were identified but marginally contributed for behavioural variation between juvenile and adult tegus, and variation in anti-predatory behaviour in these lizards reside mainly on associations integrating body size and muscle biochemistry. Our results are discussed in the ecological context of predator avoidance by individuals differing in body size living at temperature-variable environments, where restrictions imposed by the cold seem compensated in specific phenotypes.

Keywords: *Tegu lizards, morphology, muscle biochemistry, behavioural shift, temperature, ontogeny, predation.*

Introduction

Evolution of anti-predatory behaviour has been traditionally explained under the focus of natural selection because the success of organisms to avoid and/or chase predators likely affects their survival in natural environments (Lima and Dill, 1990; Lind and Cresswell, 2005; Magurran et al., 1993; Ydenberg and Dill, 1986). When facing a predator and after choosing among possible anti-predatory strategies, animals likely adjust characteristics and intensity of the elected behavioural response according to the perceived level of predation risk (Brown et al., 2006; Greene, 1988; Martín and López, 2003; Ydenberg and Dill, 1986). Many factors may influence the choice and intensity of the elected anti-predatory tactic, such as local conditions (i.e. environmental temperature, amount of light/ period of day and vegetation cover/ terrain characteristics - Savino & Stein 1989; Christensen & Persson 1993; Brodie & Russell 1999; Shine et al. 2000, 2003; Schulte et al. 2004; Durso & Mullin 2014) or type and density of predators or their form of attack (Greene, 1988; Langkilde et al., 2004; Relyea, 2001; Seyfarth et al., 1980). The anti-predatory response is still dependent of prey's condition, such as its stage in the reproductive cycle or the nutritional status (Brown and Shine, 2004; Burger et al., 1989; Shine et al., 2000), or of its phenotype characteristics. For example, in *Thamnophis ordinoides* the degree of aggressiveness towards a predator is associated with differences of color patterns (Brodie III, 1989; Brodie III, 1992), and these characteristics may be established during embryo development, as observed Hagman and collaborators (2015) in the snake *Natrix natrix*.

From an eco-morphological perspective (based on Arnold's paradigm - Arnold 1983; Garland & Losos 1990), the focus on anti-predatory behaviour is supported in morphological and physiological associations that directly influence performance and, in the last instance, individual's fitness. For fighters, predictions of biomechanical models derived for biting performance in squamates and fishes sustain that larger individuals and those with bigger heads will bite harder (Broeckhoven and Mouton, 2014; Herrel et al., 2001a; Herrel et al., 2002a; Herrel et al., 2002b; Verwaijen et al., 2002). Individuals having such characteristics may therefore accept higher risks when facing a predator, a trend demonstrated even intra-specifically in males of the lizard *Lacerta monticola* (López et al., 2005). However, survival may also be granted by running away from a given predator: how fast an animal can flee is frequently sustained by morphological associations, such as the variation in limb proportions observed in

many lizard species (see Losos 1990a, 1990b; Bonine & Garland 1999; Irschick 2000; Melville & Swain 2000; McElroy & Reilly 2009 for some examples) that seems essential for escaping success. Survival rates of hatchling lizards, for example, is positively correlated with sprint speeds and stride lengths, although this metrics is also benefitted by increased body sizes (Miles, 2004; Warner and Andrews, 2002). Given that morphological features favoring combat against a predator do not necessarily enhance the performance of running away, a trade-off between fight and flight abilities may be expected (as observed in Anderson & Vitt 1990; López & Martín 2002; Cameron et al. 2013 in a sexual selection context). In fact, such trade-off has been corroborated by data obtained in tegu lizards: individuals exhibiting higher bite forces are those having impaired escape responses because they run slower (Herrel et al., 2009).

The expected trade-off between fight and flight abilities likely resides also on physiological traits, for example related to metabolic and structural profiles of skeletal muscles. Increased proportions of slow-oxidative fibers – fueled by aerobic metabolic pathways – might enhance endurance but would also require improved oxygen delivery capacity and increased mitochondrial scope in skeletal muscles (Bennett, 1991; Gleeson and Harrison, 1986; Johnston, 1981). In contrast, muscle fibers having a glycolytic nature are fueled by anaerobic metabolic pathways, and contract quickly but also fatigue rapidly (Bennett, 1991; Gleeson and Harrison, 1986; Johnston, 1981). The relative contributions of aerobic and anaerobic profiles, established both by relative proportions of different fiber-types composition and by the activity of specific enzymes, may be coupled with changes in muscle mass, contraction speeds, and elastic and force properties that determine muscle function and, ultimately, define organismal performance (Ashley-Ross and Barker, 2002; Baldwin, 1982; Bonine et al., 2001; Choi and Park, 1996; Gleeson et al., 1980; James et al., 2005; McBrayer and White, 2002; Pasi and Carrier, 2003; Putnam and Bennett, 1983; Seibel et al., 1998; Throckmorton and Saubert, 1982). As a consequence, we expect associations between the metabolic scope of different muscle groups and the performance exhibited in specific activities where these are involved, which in the context of anti-predatory behaviour may encompass both combat strategies, such as biting, and the escape response of running away.

The morphological and physiological relationships influencing the choice of a given anti-predatory strategy are not static and, especially in vertebrate ectotherms,

these may vary according to the thermal conditions where predator-prey interactions occur. For example, locomotion and metabolism are in general reduced at low temperatures (Bennett 1980, 1990; Huey 1982; but see Angilletta 2009; James 2013 for reviews), and animal's sensorial perception may also be restricted in such conditions (Van Damme et al., 1990). Accordingly, shifts between flight and fight behaviours in response to variation in body temperature have been demonstrated in several squamate lineages (Citadini and Navas, 2013; Crowley and Pietruszka, 1983; Hertz et al., 1982; Mautz et al., 1992; Polčák and Gvoždík, 2014; Schieffelin and De Queiroz, 1991). For example, the agamid lizard *Trapelus pallida* exhibits a temperature-dependent shift from evasive to aggressive behaviours that seems explained by increased thermal sensitivity of muscles used for sprinting in comparison to those involved in biting (Herrel et al. 2007). Such temperature-dependent shift in behaviour has also an ontogenetic dimension: adults of tegu lizards (*Salvator merianae*) run away from a predator at high temperatures but choose the combat when it is cold, while juveniles exhibit the same flight strategy within a wide range of thermal conditions (Barros et al., 2010). Given that ontogenetic development encompasses modifications at different levels of organization, it is plausible to predict that morpho-physiological parameters could at least partially explain the differences between adult and juvenile tegus in the anti-predatory responses exhibited at lower temperatures.

The disregard of ontogenetic components in studies focusing on temperature-dependent shifts of anti-predatory behaviour seems counterintuitive in face of the effects of juvenile's survival for the perpetuation of populations in natural habitats. Here we fill this gap by integrating physiological and morphological traits to test the hypothesis that multivariate models explain the ontogenetic variation in thermal relationships of anti-predatory behaviour reported in *Salvator merianae*. Based on this hypothesis, we derive two predictions: (1) that enhanced glycolytic scopes in the limb musculature of juveniles compensate the impairment imposed by small body sizes and allow these individuals to flee from predators even at limiting thermal conditions involving low temperatures, and (2) that modifications of shape, involving for example relative limb proportions or jaw length, also explain differences in anti-predatory behaviour between the ontogenetic classes of tegu lizards. To test this prediction, we combine behavioural results for recently-hatched juveniles and adult tegus reported in Barros et al. (2010) with new data for body morphometry and muscle biochemistry obtained in hindlimb and head muscles sampled from the same individuals used by

Barros et al. (2010). The integrative approach used here explores interactions among the multiple dimensions that likely determine if a given prey will fight or flight when facing a predator.

Material and Methods

The dataset assembled to test our hypothesis is detailed in this section following three main categories - morphology, muscle biochemistry, and behaviour. We focused on anti-predatory responses exhibited by juvenile and adult tegus at 22.5°C – from a previous study (Barros et al., 2010) we know that, as body temperature decreases, at 22°C most adults shift from running away to turning aggressive but juveniles remain evasive, and therefore at this thermal condition the behavioural differences focused here turn prominent (see our supplementary material for detailed analyses showing differences on the anti-predatory behaviour between ontogenetic classes at lower temperatures).

1. *Composed dataset*

Animals used and tissue samples obtained

In this study we combined results on anti-predatory behaviour previously published by Barros et al. (2010) with new data of morphology and muscle biochemistry obtained for the same individuals studied before. All individuals of *Salvator merianae* used in this study were obtained from a captive population reared outdoors in large enclosures at Universidade Estadual Paulista in Rio Claro (São Paulo, Brazil; permit from IBAMA number 02001-000412/94-28). Tegu lizards are found both in natural and urban areas in Brazil, and these animals are found at the same University campus where the outdoor enclosures for captive colonies locate; we assume therefore that animals used in our study were reared under equivalent conditions of natural populations, being even exposed to the same occasional predators (i.e. birds and mammals). After obtaining behavioural data published by Barros et al. (2010), we measured those 17 recently-hatched juveniles (89.81 ± 19.13 g) and 10 adults of 1-2 years old (871.05 ± 209.85 g) of *Salvator merianae* and sampled muscle tissues. Tegus were killed by overdose of anesthetic (0.5 ml solution of xylazine and ketamine, 2:1 ratio) that was injected intraperitoneally in juveniles and in adults was inoculated directly into the tail vein in order to enhance anesthetic effects. Animals recently euthanized were immediately measured (see below), and then tissue samples were removed for biochemical assays, which were performed in two muscles: the *adductor externus superficialis* (AESM) from the head, and the *iliofibularis* (ILIM), adjacent to

the femur. The AESM is involved in jaw movements including bite (McBrayer and White, 2002; Schwenk, 2000), while the ILIM is recruited to push the body during locomotion (Bonine et al., 2005; Jayne et al., 1990). These two muscles are anatomically similar because they both have a central region mainly composed by slow-oxidative fibers that is surrounded by a region where most fibers are glycolytic or exhibit intermediate metabolic characteristics (Gleeson et al., 1980; Putnam et al., 1980; Throckmorton and Saubert, 1982). Samples used in the biochemical assays were dissected from the right side of individuals, being immediately frozen in liquid nitrogen and then stored at -80°C . All the procedures were authorized by IBAMA permits (186/2006, number 14109-1/RAN IBAMA) and approved by the Ethics Committee for the Use of Animals in Research of the University of São Paulo (CEUA, protocol number 06.1.1390.53.5).

Morphology

Seven morphological traits of body size and shape were measured in all euthanized tegus before muscle dissection. Body mass (MASS) was recorded in a digital scale, and a digital caliper (Mitutoyo CD-15B; ± 0.01 mm) was used to measure snout-vent-length (SVL), *humerus* and *radius* lengths (HUML and RADL, respectively), *femur* and *tibia* lengths (FEML and TIBL, respectively), and upper jaw length (JAWL, measured from tympanic aperture to the tip of the snout). For the statistical analyses all morphological variables were \log_{10} -transformed, and scaling effects were explored through SMA regressions (see below on *Data Analysis* section).

Muscle Biochemistry

Biochemical properties of hindlimb and head muscles were assessed based on maximum activity of two metabolic enzymes: lactate dehydrogenase (LDH) was used as an indicator of oxygen-independent pathways, while citrate synthase (CS) represented the muscle aerobic metabolism (Eme et al., 2009; Fields et al., 2008; Kirkton et al., 2011; Kohlsdorf et al., 2004; Norton et al., 2000; Rosa et al., 2009; Seibel et al., 1998; Souza et al., 2004; Vetter and Lynn, 1997). Enzyme activities were measured using a spectrophotometer Beckman DU-70 under saturating and non-inhibitory substrate conditions, following Souza et al. (2004), as further detailed. As aforementioned, we performed all analyses with results obtained at 22.5°C because this is the temperature where ontogenetic differences in the anti-predatory responses exhibited by tegu lizards

are prominent (Barros et al., 2010). In order to minimize possible errors derived from enzymatic activity accessed solely at 22.5°C and with the aim of also evaluating effects of temperature variation on muscle biochemistry, we performed biochemical assays at three temperatures (18, 27 and 36°C) and constructed thermal-sensitivity curves for LDH and CS, which were then used to estimate activities of these two enzymes at 22.5°C. First we pooled individual values obtained from biochemical assays into individual regression curves and inferred a linear relationship between temperature and biochemical activity of LDH and CS for each ontogenetic category in both muscles at the thermal experimental interval accessed (see supplementary material for additional details – Tables S3 and S4 and Figs. S3 and S4). Subsequently, fitted values at 22.5°C were estimated from each individual regression through a simple linear equation ($y=a+bx$). These procedures were implemented using the software TableCurve2D. Enzyme activities of LDH and CS at 22.5°C were then used for calculation of a LDH:CS ratio in each muscle, used as an indirect measurement of glycolytic or oxidative tissue's potential (Gleeson and Harrison, 1986).

In order to measure enzyme activities of LDH and CS in head and limb muscles, a cross-sectional fraction 1mm thick of AESM and ILIM was extracted of each muscle sample previously obtained from euthanized tegus and stored frozen at -70°C. These fractions included all fibers (white and red) composing the muscles, which are parallel-fibered tissues. Each sample from each individual was homogenized in nine volumes of buffer Imidazole-HCl-20 mM pH 7.4, with 1mM PMSF (phenyl-methyl-sulphonyl-fluoret), 2 mM EDTA, and Triton X-100-0.1%. Cellular and mitochondrial membranes were lysed using a sonicator U-200Scontrol (IKA LaborTechnik) with 50% of cycle and amplitude of 0.5 in three periods of 10 seconds at 4°C. Samples were then centrifuged at 1500·RPM in 4°C for 2·minutes, and the supernatant was diluted according to each enzyme protocol. Specifically, activity of LDH was estimated as the absorbance of NADH to 340nm. The assay consisted of 633 µL Imidazole-HCl-100 mM (pH 7.0); 35 µL DTT (dithio-treitol)-100 mM; 7 µL NADH-0.15 mM; 7µL homogenate (diluted 1:40) and 18 µL of pyruvate-40 mM. Pyruvate was omitted in the control. In the case of CS, enzyme activity was measured as the reduction of DTNB (dithio-tris-nitro-benzoic acid) at 412nm. The assay consisted of 522 µL Tris-HCl-100 mM (pH 8.0); 70 µL DTNB-0.1 mM; 50 µL acetyl-CoA-3 mM; 28 µL homogenate (diluted 1:30) and 30 µL of oxaloacetic acid-20mM. Oxaloacetic acid was omitted in the control. All reactions were performed at a final volume of 0.7·mL and in duplicate. Maximum enzyme

activity was set within a range of 90 seconds intervals, and mean values between duplicates were used in the analyses. Results were expressed in μmol of substrate converted to product per minute per gram of wet mass.

Behaviour: establishment of a Total Behaviour Score (TBS)

We used the original dataset from Barros et al. (2010) comprising 27 tegus (10 adults and 17 juveniles) to establish a 'Total Behaviour Score' (TBS). As explained before, we focused on anti-predatory responses exhibited at 22.5°C because at this temperature adult tegus shift from running away to confronting the predator, and therefore behavioural variation between juveniles and adults is more prominent. To construct the TBS, we assigned different weights to each of the five anti-predatory behaviours reported by Barros et al. (2010), giving positive values to aggressive behaviours and negative values to escape behaviours as follows: bite = 2, defensive posture = 1, no response/immobility = 0, walk = -1, and run = -2 (modified from Citadini & Navas 2013). In order to differentiate the most aggressive individuals, we granted 5 extra points as a positive bonus to the TBS of individuals that bit or assumed a defensive posture within the first 20 seconds of each duplicate test. In contrast, 5 points were suppressed from the calculated TBS of individuals that exhibited escape strategies after all stimuli imposed at each duplicate test. As a result, the final TBSs varied from 50 positive points (i.e. 40 points in a hypothetical individual that bit in all trials added with 10 extra points from the positive bonus if the individual was aggressive within the first 20 seconds in both trials) to 50 negative points (i.e. minus 40 points in a hypothetical individual that ran away in both trials, suppressed with 10 extra points for being evasive in all stimuli). The normality of TBS was confirmed through a Shapiro-Wilk normality test ($W=0.9419$; $p=0.1357$). A detailed table including the total set of responses is provided in the supporting information.

2. Data Analyses

All analyses were performed in R (version 3.1.0) using RStudio (0.98.501). As aforementioned, statistical analyses were employed in three complementary steps. First, we assessed the physiological disparity between juveniles and adults regarding muscle biochemistry. Then we analyzed allometric differences between juvenile and adult tegus. Finally, we combined morphological and physiological traits to test for

associations with anti-predatory behavioural responses exhibited at 22.5°C, which were carried out using two complementary approaches: a canonical correspondence analysis (CCA) to test associations of each behavioral response separately, and comparisons among complex linear models that combined morphological and physiological traits to explain variation in TBS. Implementation of the analyses performed in each step is further detailed in three corresponding topics.

Physiological disparity between juveniles and adults

Differences between juveniles and adults regarding enzyme activities in head and hindlimb muscles were tested using a two-factor analysis of variance (ANOVA). Specifically, we used as dependent variables either LDH activity, CS activity or the LDH: CS ratio, while muscle and age class were included as independent variables in the model, assuming interactions between terms. A *post-hoc* test was performed, with “muscle-type” as a fixed factor across age classes, using the R-package *phia* (Martinez, 2013).

Morphological differences between age classes and construction of SHAPE matrix

We first tested for differences between juveniles and adult tegus using a Student's T-test on SVL. Subsequently, we investigated allometric differences between age classes testing for significant associations between SVL and the remaining morphological measurements. We tested for significant differences in slope and elevation of regression curves using Standard Major Axis (SMA) regressions implemented in the R-package *smatr3* (Falster et al., 2006; Warton et al., 2012), where AGE was included as a covariate. After these allometric analyses, we performed between-group regressions because slopes were similar within-ages for all traits; such approach aimed to separate size-effects from shape variation (Berner, 2011; Reist, 1985). From this analysis, we retained the regression residuals of the size-corrected *humerus* (named RHUML), *radius* (named RADL), *femur* (named RFEML), *tibia* (named RTIBL), and upper jaw length (named RJAWL), which were combined into a multivariate matrix named SHAPE.

Integrative Analyses: canonical correspondence analysis and model comparisons

In order to identify relationships between morpho-physiological traits and anti-predatory behaviour in a multivariate framework, we first explored how studied traits

were related by performing a CCA (Legendre and Legendre, 1998) and testing the axes significance using the R-package *vegan* (Oksanen et al., 2013). In this step, we used the frequencies of each behavioural category separately considering the individual values (see detailed table in supplementary material – Table S1.B), which totaled 27 observations. We used the ratio between LDH: CS activity of muscles and the SHAPE components matrix as traits. In addition, we included logarithmized MASS to the model as a proxy of body size. We chose MASS instead of SVL because muscle potential is also dependent of cross-section area and the total amount of muscle fibers, which relate to total volume, more than length, of the animal. As a complementary step of data analyses, we selected the morpho-physiological traits that most contributed to the variation observed in significant canonical axes and performed a multiple linear model using TBS as a dependent variable. We started by fitting a complex model [lm (TBS ~ ILIM ratio + AESM ratio + SIZE + RJAWL + RHUML + RTIBL)], and used a backward elimination to select the best models (Gotelli and Ellison, 2011). The significance level for retention of a given variable in the model was set to 0.05, and Akaike information criterion with a correction for finite sample sizes (AICc) was used to confirm the model with better fit, following Burnham & Anderson (2002).

Results

This study integrates physiological and morphological traits for understanding variation in a temperature-dependent shift of anti-predatory behaviour that results in the differences among ontogenetic classes of tegus. The first step of data analyses here consisted of evaluating the physiological disparity between juveniles and adults. The main differences on muscles' profiles reside on the LDH: CS ratio. Specifically, the ILIM is proportionally 3-fold more glycolytic than the AESM, and juveniles have hindlimb muscles proportionally 20% more glycolytic than adults, as detailed in *post-hoc* tests (see Table 1 for absolute values and Table 2 for descriptive statistics). We have also detected differences between muscles in maximum LDH activity (approximately 2.5-fold higher in hindlimb than head muscles), but ontogeny does not contribute for such differences. Maximum CS activity does not differ in relation to ontogeny or between muscles.

The second step of data analyses in this study consisted of testing for morphological differences between age classes. This analysis corroborated that juveniles are much smaller than adults on SVL ($F_{1,25}=659.6$, $p<0.001$). Regarding body shape, we also identified differences in slope elevation between age classes in the size-corrected traits: juveniles have proportionally shorter upper jaws than adult tegus (Table 3).

The final step of data evaluation consisted of integrative analyses based on two complementary approaches. Results from the CCA indicated that increased MASS and higher values of the LDH: CS ratio in the AESM are positively correlated to aggressive behaviour of tegus in the CCA1, while increased values of the LDH: CS ratio in ILIM and a relative shorter upper jaw are associated with evasive strategies on CCA2 (Fig. 1). Humerus and tibia lengths also slightly contributed to the second axis (Table 4; Fig. 1). The second approach, based on comparisons of AICc, supports a best-fit model where MASS explains anti-predatory behaviour together with the LDH: CS ratio in the ILIM (Table 5; Figs. 2, 3), while components of SHAPE had no association with variation in TBS values. These results suggest that baby tegus, which more often escape from predators adopting evasive strategies, exhibit higher glycolytic scopes on hindlimb muscles when compared to young adults.

Discussion

The present study provides an integrative explanation for the ontogenetic variation of a temperature-dependent shift in the anti-predatory behaviour of tegu lizards. According to our results, the ontogenetic differences in the temperature-dependent shift of anti-predatory behaviour previously reported in tegus (Barros et al. 2010) reside on multidimensional associations that integrate body size with muscle biochemistry. In the ecological context of predator avoidance in natural habitats, adults are big and therefore may rely on strong bites during combat, a strategy inefficient in juveniles, which are comparatively smaller than adults. When tegus emerge from their shelters early in the morning to feed and/or thermoregulate, they experience body temperatures that may vary from 18-26°C depending of the season, reaching 33-37°C later in the day (Sanders et al., 2015), the later range encompassing their preferential temperatures (Cecchetto and Naretto, 2015). Therefore, these animals may in fact face predators when their body temperatures are below their preferred range. Restrictions imposed by environmental thermal conditions may be compensated in juveniles by phenotypes that enhance escaping, as further discussed.

Enzyme activities on hindlimb and head muscles

Results from this study confirm that muscles employed for different activities in tegus are metabolically distinct despite having similar fiber type architectures: ILIM, involved in locomotion, is proportionally more glycolytic than AESM, used to bite. Aerobic metabolism provides only a portion of the total energy used to sustain high levels of activity in lizards, and most of the available energy is derived from anaerobic metabolism (Bennett & Dawson 1972; Bennett & Licht 1972; but see Bennett 1991 for a review). The relative proportion of aerobic and anaerobic pathways is however an important factor in these animals, and can be used for understating how the metabolic balance between two pathways influences muscle function. In tegus, AESM is activated during fast and high amplitudes that characterize crushing bites, as well as other head muscles, but is the only one active during slow closing bites (McBrayer and White, 2002). In this case, an equilibrated ratio between LDH and CS activities in the jaw muscle may reflect the important role of slow-oxidative fibers in this muscle for joint stabilization and prey manipulation (Throckmorton and Saubert, 1982), activities common to juveniles and adults.

The results for enzyme activity in the hindlimb muscle differ from those obtained for the jaw muscle: juvenile tegus have a hindlimb muscle that is proportionally more glycolytic than that of adults, a trend that contributes for understanding why recently-hatched tegus rely on running away from a predator regardless of thermal conditions (Barros et al. 2010). Although energetic demands would favor during growth a reduction in the levels of aerobic metabolism coupled with increased anaerobic rates (for discussions on scaling-effects, see Somero & Childress 1980; Childress & Somero 1990; Norton et al. 2000; and Moyes & Genge 2010 for a review), in juveniles locomotion may face increased selection pressures triggered by predation rates, summed to their developmental demands (Kirkton et al., 2011). In that sense, phenotypes that enhance escaping, such as hindlimb muscles that are comparatively more glycolytic, may compensate for restrictions imposed by environmental conditions involving low temperatures. Adults of *Salvator merianae*, in contrast, exhibit more equilibrated LDH: CS ratios in their hindlimb muscles, a balance that may grant suitable use of aerobic pathways during locomotion. Adult tegus are active foragers that course long distances in their natural habitats (Winck et al., 2011), and they depend on enlarged home ranges for feeding and maintaining large body sizes (Blanco et al. 2011 and references therein). An equilibrium between aerobic and anaerobic pathways may not only sustain efficient locomotion of adults through their territories, but likely contributes for buffering muscle pH and enhancing lactate metabolism for glycogen re-synthesis after intense locomotion (Curtin et al., 1997; Gleeson, 1996; Passarella et al., 2008).

Ontogenetic variation of anti-predatory behaviour: integration between morphology, physiology and ecology

Recently-hatched tegus are small and rely more often on escape behaviours when facing a predator (Barros et al. 2010). These little individuals have enhanced glycolytic capacity in their hindlimb muscles, which likely contributes for successfully running away from an aggressor. The combination of an enhanced anaerobic metabolism in hindlimb muscles with the preference for strategies that involve escape is likely effective in animals that are too small for successfully injuring a predator (Barros et al., 2010), especially because access to energetic storages in glycolytic pathways is quick and produces powerful sprints (Bennett, 1991; Bennett and Licht, 1972; Childress and Somero, 1990; Somero and Childress, 1980). As aforementioned, increased LDH:

CS ratios in hindlimb muscles of juvenile tegus seem particularly relevant at low temperatures, when enzyme activities may be impaired for sustaining energetic demands of continuous activity but combating the predator is not an efficacious choice.

The multilevel equation explaining evolution of anti-predatory behaviour in *Salvator merianae* is not composed though solely by morphological proportions and biochemical scopes changing through ontogeny. Tegu lizards are born in the summer (late December-January in South America) with approximately 15 g, and face an increase of approximately 5 to 7-fold of body mass in the subsequent 5 months, before experiencing their first dormancy period in the winter (Souza et al., 2004). After dormancy, which involves partial loss of body mass, animals restart growing as soon as they retake feeding, and in the end of the first-year cycle young adults may weight 300 g (Souza et al., 2004). This ontogenetic change in body mass is coupled with a remarkable differentiation in coloration (see supplementary material), and the cast of predators attacking juveniles and adults may be also variable (A Abe, pers. com.). The cryptic coloration of baby tegus, together with the observation that they are rarely observed moving around in their natural habitats (A Abe, pers. com.), corroborates the hypothesis of ontogenetic differences in the tegus' preferred strategy for avoiding predation: juveniles rely on camouflage and avoid direct interactions with predators, remaining restricted to small territories until reaching a body size that grants effective bites and allows expansion of home ranges (Winck et al. 2011; A Abe, pers. com.).

The behavioural shifts addressed here meet the ecology of tegu lizards regarding daily variation in body temperatures as well as seasonal changes involving dormancy periods. Early in the morning, T_B of tegus is mainly influenced by temperature changes inside their shelters and, because this parameter is variable along the year (Sanders et al., 2015; Tattersall et al., 2016), at hotter seasons the lizards may avoid emerging from their shelters if T_B is too low. During the night, T_B decreases very slowly specially in the summer, when nocturnal temperatures are higher, an effect more prominent in larger tegus due to thermal inertia (Tattersall et al., 2016). At colder months, however, the nighttime minimum T_B in tegus remains low but maximum daily voluntary temperatures also decline, a pattern related to dormancy (Sanders et al., 2015). During this period, although tegus remain most time inside their burrows, they also emerge for briefly periods, and their T_{BS} persist between 18 and 22°C (Andrade, 2016; Sanders et al., 2015). Such condition represents a critical situation for survival, as animals may face predators when their T_{BS} are below preferred temperatures and locomotion could be

impaired. Therefore, the behavioral differences between newborn and young adult tegus at 22.5°C are ecologically relevant.

Body temperatures may change fast in small lizards due to their body size, but newborn tegus also rely on camouflage to escape from predators (see Fig. S1; A Abe, pers. com.). Consistently, our results indicate that recently-hatched juveniles exhibit an enhanced metabolic capacity that is compatible with their propensity to run regardless of thermal conditions (Barros et al. 2010). Adult tegus may also face predators when emerging from shelters during the winter, but warming might be slow due to their large body size. The preference of adult tegus for aggressive behaviours when facing predators presumably compensates for restrictions in escaping abilities imposed by specific environmental conditions that impair locomotion (Barros et al., 2010; Brodie III and Russell, 1999; Citadini and Navas, 2013; Crowley and Pietruszka, 1983; Hertz et al., 1982). Increased body sizes often enhance biting forces, likely reducing the threshold for the individual to engage an aggressive encounter because the animal will combat predators while minimizing its risk of injury (see Huyghe et al. 2005; Herrel et al. 2009; Barros et al. 2010 for some examples). Besides body size, bite performance is also affected by variation in body shape: wider and higher heads accommodate larger jaw muscles and increment jaw closing in-lever lengths, all of which improve bite performance (Herrel et al., 2001a; Herrel et al., 2001b; Vanhooydonck et al., 2007). Defensive postures potentially followed by effective bites also demand coordination between jaw, neck and forelimb, as jaw movements must be synchronized with body motion to grant suitable head velocity and jaw opening angles (Montuelle et al., 2009; Montuelle et al., 2012). For example, in teiids and cordyliforms strikes on prey or during combat involve forelimb extension at the elbow joint to elevate the neck in the late phase of jaw opening (Montuelle et al., 2012). Because of these relationships, is not surprising that some morphological measurements in *Salvator merianae*, as relatively longer upper jaws and shorter humerus, have appeared as factors associated with fight behaviour in the CCA. Associations with body shape seem however less apparent in our dataset than those involving body size and muscle biochemistry, a trend that may reside on the fact that our sample was composed by young adults. The largest tegus used in our study are on average 900g and 300 mm in terms of body size, representing a growth stage of 1-2 years old, at which tegus are not completely sexually mature. Older animals already reproducing reach up to 5-8 kg (A Abe, pers. com.). These may become more aggressive, having enlarged head muscles and cranial structure, which enhances bite

performance (see Herrel et al., 2009). Although we could not distinguish sexes in our sample, in general the males of *Salvator merianae* show a stronger degree of integration between the cranium, mandible and mandibular muscles, suggesting a sexual selection pressure linked to combat (Fabre et al., 2014). Our study provides evidence for differences already identifiable between newly born animals and young adult tegus that experienced their first cycle of dormancy, and the contrast among ontogenetic classes may turn even more prominent when reproductive animals are considered.

Extreme size disparity, in our study represented by recently-hatched juveniles and young adults, imposes differences in prey-predator relationships between ontogenetic classes. As discussed here, absolute levels of locomotor output and variation in continuous activity and running bursts depend on morphology and physiology, and the influence of environmental thermal conditions in the preference for a given anti-predatory strategy is coupled with such phenotypic associations. In this study, we show that body size influences the anti-predatory strategy adopted by tegu lizards, and also describe the relative ratio between LDH and CS activity in hindlimb muscles as an important factor for understanding behavioural differences between ontogenetic classes. Other morpho-physiological components, as longer upper jaw and increased LDH: CS ratios in head muscles, may also contribute for the shift favoring more aggressive behaviours when temperatures are low, although such effect seems less apparent in our dataset. Organismal traits are often studied under compartmentalized approaches that dissect morphology from physiology in studies of behavioural variation that usually relegate ontogeny. However, we showed that these dimensions interact in a complex equation that underlies ontogenetic variation in the thermal-dependent shift of anti-predatory behaviour in tegu lizards.

Acknowledgements

We acknowledge the members of the Laboratory of Evolution of Tetrapods (LET - FFCLRP/USP) for comments on early drafts of this manuscript. We also acknowledge two anonymous reviewers that significantly contributed for improving our article.

Funding

This study was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo-Brazil [2005 / 60140-4 to T.K., 2008/ 57712-4 to J.E.C.], and fellowships from Fundação de Amparo à Pesquisa do Estado de São Paulo-Brazil [2007 / 54609-5 and 2011/24171-3 to F.C.B.], and CNPq [INCT in Comparative Physiology to A.A. and to J.E.C.].

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Tables

Table 1. Mean values with standard deviation for morphological and physiological traits examined tegu lizards; juvenile and adult tegus are separated in two groups. Enzyme activity is given in $\text{U.g}_{\text{tissue}}^{-1}$; morphological variables are given in mm, and body mass is given in g.

	Adults (n=10)	Juveniles (n=17)
PHYSIOLOGY		
Ileofibularis LDH activity	790.89 ± 114.34	863.25 ± 139.30
Ileofibularis CS activity	8.24 ± 1.95	7.16 ± 1.00
ILIM ratio	100.77 ± 26.05	123.28 ± 28.99
Adductor LDH activity	386.06 ± 45.36	319.14 ± 39.23
Adductor CS activity	8.77 ± 1.56	9.49 ± 1.36
AESM ratio	44.93 ± 7.63	33.99 ± 4.49
MORPHOLOGY		
Mass	871.05 ± 209.85	89.81 ± 19.13
Snout-vent-length	285.40 ± 17.73	141.36 ± 10.06
Upper jaw length	68.60 ± 3.31	37.39 ± 1.70
Femur length	49.50 ± 3.72	23.44 ± 2.31
Tibia length	65.20 ± 4.98	28.71 ± 2.44
Humerus length	38.00 ± 2.98	17.24 ± 1.56
Radius length	43.70 ± 2.21	21.19 ± 1.66

Table 2. Statistical results from the analyses of variance (ANOVA) performed with enzyme activity as a dependent factor and MUSCLE and AGE as categorical predictors. *P*-values significant at the 0.05 level are indicated in bold.

	Df	LDH activity			CS activity			LDH/CS ratio		
		SS	F	P	SS	F	P	SS	F	P
MUSCLE	1	819437	86.936	< 0.001	1.430	0.700	0.407	15590	38.209	< 0.001
AGE	1	28202	2.992	0.090	3.230	1.585	0.214	754	1.848	0.180
MUSCLE:AGE	1	61071	6.479	0.014	10.160	4.987	0.030	3524	8.636	0.005
Interactions										
Adductor (J vs A)	1	28202	2.992	0.180	3.229	1.585	0.428	754	1.848	0.360
Iliofibularis (J vs A)	1	32962	3.497	0.135	7.350	3.607	0.127	3191	7.821	0.015
Residuals	50									

SS: sums of squares for ANOVA; F: exact or approximate *F*-value; P: exact or approximate *P*-value (in case of interaction, p-adjusted value by Bonferroni); Df: degrees of freedom; J: juveniles; A: adults.

Table 3. Results of *Standard major axis* (SMA) regressions performed on morphological traits to examine between-age differences in tegu lizards. *P*-values significant at the 0.05 level are indicated in bold.

Morphological measurements vs SVL	BETWEEN GROUPS TEST				
	JAWL	FEML	TIBL	HUML	RADL
H ₀ : slope are equal	0.3825	0.7030	0.8935	0.9782	0.4821
Estimate (slope)	A = 0.796 [0.501, 1.266]; J = 0.634 [0.469, 0.855]	A = 1.229 [0.789, 1.911]; J = 1.372 [0.902, 2.085]	A = 1.245 [0.823, 1.884]; J = 1.193 [0.710, 1.981]	A = 1.251 [0.675, 2.316]; J = 1.239 [0.870, 1.765]	A = 0.828 [0.434, 1.581]; J = 1.073 [0.717, 1.608]
H ₀ : no difference in elevation	0.0319	0.2241	0.7478	0.5277	0.8016
Estimate (elevation)	A = 0.157 [-0.305, 0.618]; J = 0.102 [-0.271, 0.476]	A = -1.481 [-2.519, -0.443]; J = -1.411 [-2.252, -0.571]	A = -1.200 [-2.230, -0.170]; J = -1.182 [-2.016, -0.348]	A = -1.471 [-2.495, -0.446]; J = -1.435 [-2.264, -0.606]	A = -0.789 [-1.708, 0.129]; J = -0.802 [-1.545, -0.059]
Df	1	1	1	1	1

See Materials and methods section for abbreviations of variables. Df: degrees of freedom.

Table 4. Correlations between the morpho-physiological traits and behavioural responses identified in the canonical correspondence analysis (CCA) with ANOVA results for maximum permutations (9999). Numbers in bold indicate statistically significant differences for models. See Materials and methods section for abbreviations of variables.

	Canonical variables			
	CCA1	CCA2	CCA3	CCA4
<i>Behavioral responses</i>				
BITE	1.108	-1.296	0.624	-0.352
DEFENSIVE.POSTURE	0.860	-0.274	-0.052	0.067
IMMOBILE	0.767	0.497	-0.012	-0.068
WALK	-0.576	0.107	0.128	0.049
RUN	-0.729	-0.154	-0.139	-0.056
<i>Morpho-Physiological Traits</i>				
AESM ratio	0.613	0.329	0.673	0.154
ILIM ratio	-0.524	0.536	-0.024	-0.010
MASS	0.939	0.217	0.106	0.176
RJAWL	0.054	-0.476	-0.182	0.393
RFEML	0.015	-0.124	-0.176	-0.311
RTIBL	-0.072	-0.180	-0.275	0.491
RHUML	-0.185	0.222	-0.419	0.609
RRADL	0.042	0.028	-0.302	0.012
Importance of constrained components (0.66 of variation)				
	CCA1	CCA2	CCA3	CCA4
Eigenvalue	0.540	0.097	0.016	0.005
Proportion Explained	0.819	0.148	0.025	0.008
Cumulative Proportion	0.819	0.967	0.992	1.000
<i>F</i> -statistic	23.352	4.205	0.711	0.231
<i>P</i> -value	0.001	0.012	0.558	0.915

Table 5. Results of tests of multiple linear model with TBS as dependent variable and SIZE, SHAPE components, and LDH: CS enzymatic activity ratio of muscles as independent variables. The best model (M5) was selected from a complex model (M1) by backward step and AICc values. Standard errors are reported in parentheses adjacent to the coefficients. Numbers in bold indicate statistically significant associations for a given model.

	<i>Dependent variable: TBS</i>				
	(M1)	(M2)	(M3)	(M4)	(M5)
AESM ratio	-0.339 (0.566) p = 0.556	-0.339 (0.552) p = 0.547			
ILIM ratio	-0.200 (0.114) p = 0.094	-0.203 (0.106) p = 0.070	-0.212 (0.104) p = 0.054	-0.240 (0.099) p = 0.024	-0.234 (0.098) p = 0.026
MASS	36.756 (9.088) p = 0.001	36.716 (8.861) p < 0.001	32.728 (5.930) p < 0.001	32.416 (5.908) p < 0.001	32.542 (5.885) p < 0.001
RJAWL	275.536 (275.278) p = 0.329	262.537 (232.313) p = 0.272	286.597 (225.703) p = 0.218	173.753 (191.407) p = 0.374	
RHUML	-135.906 (135.713) p = 0.329	-137.402 (131.558) p = 0.309	-120.210 (126.698) p = 0.354		
RTIBL	-9.799 (104.283) p = 0.927				
Constant	-60.745 (22.923) p = 0.016	-60.314 (21.924) p = 0.012	-63.021 (21.168) p = 0.007	-59.037 (20.702) p = 0.010	-60.058 (20.596) p = 0.008
Observations	27	27	27	27	27
R ²	0.709	0.708	0.703	0.691	0.680
AICc (dAICc)	237.3 (11.7)	233.2 (7.6)	230.0 (4.3)	227.7 (2.1)	225.6 (0.0)
Weight	0.002	0.015	0.076	0.236	0.671
F-Statistic (Df)	8.104 (6, 20)	10.204 (5, 21)	13.031 (4, 22)	17.149 (3, 23)	25.498 (2, 24)

Figures

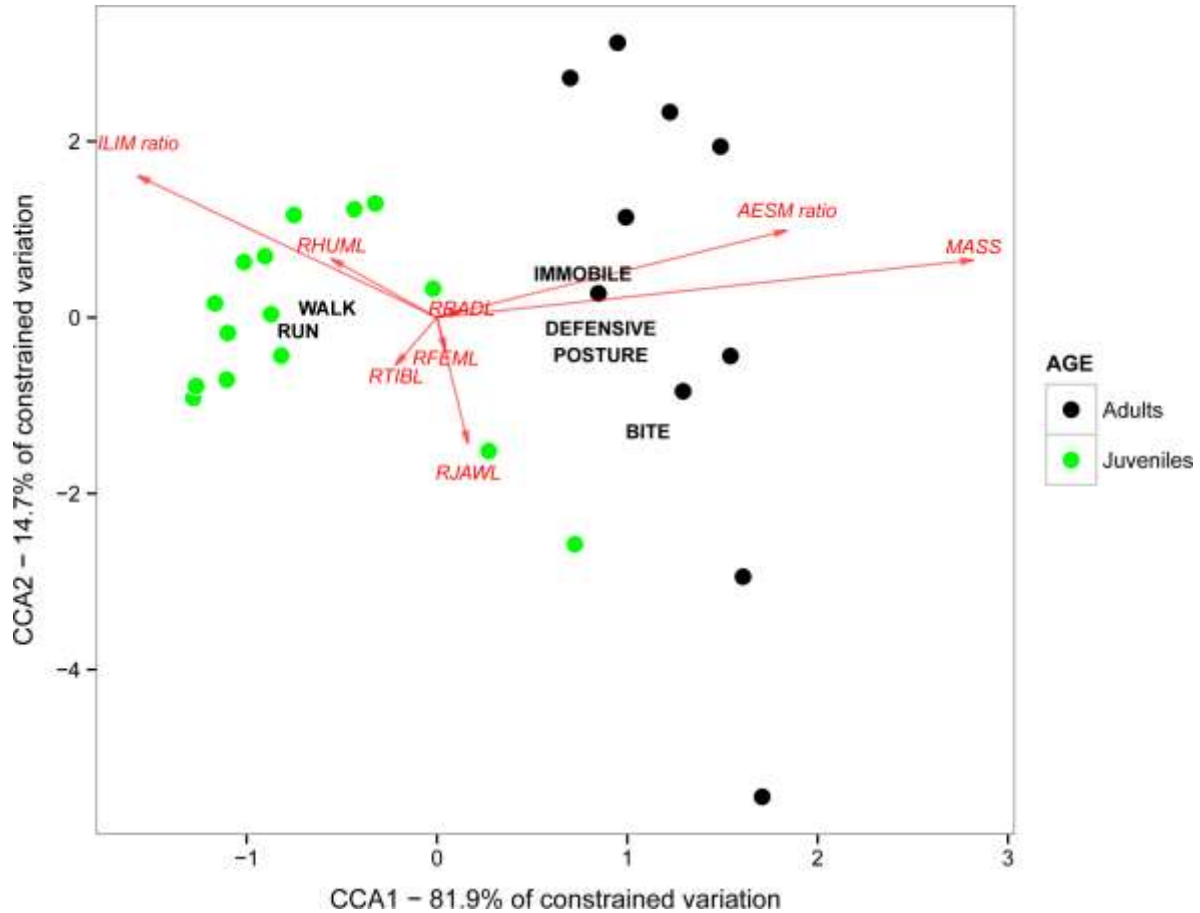


Figure 1. Scatterplot of the first and second principal canonical axis for juveniles and adults of *Salinator merianae* obtained from CCA of morpho-physiological traits and behavioural responses. N=27 observations.

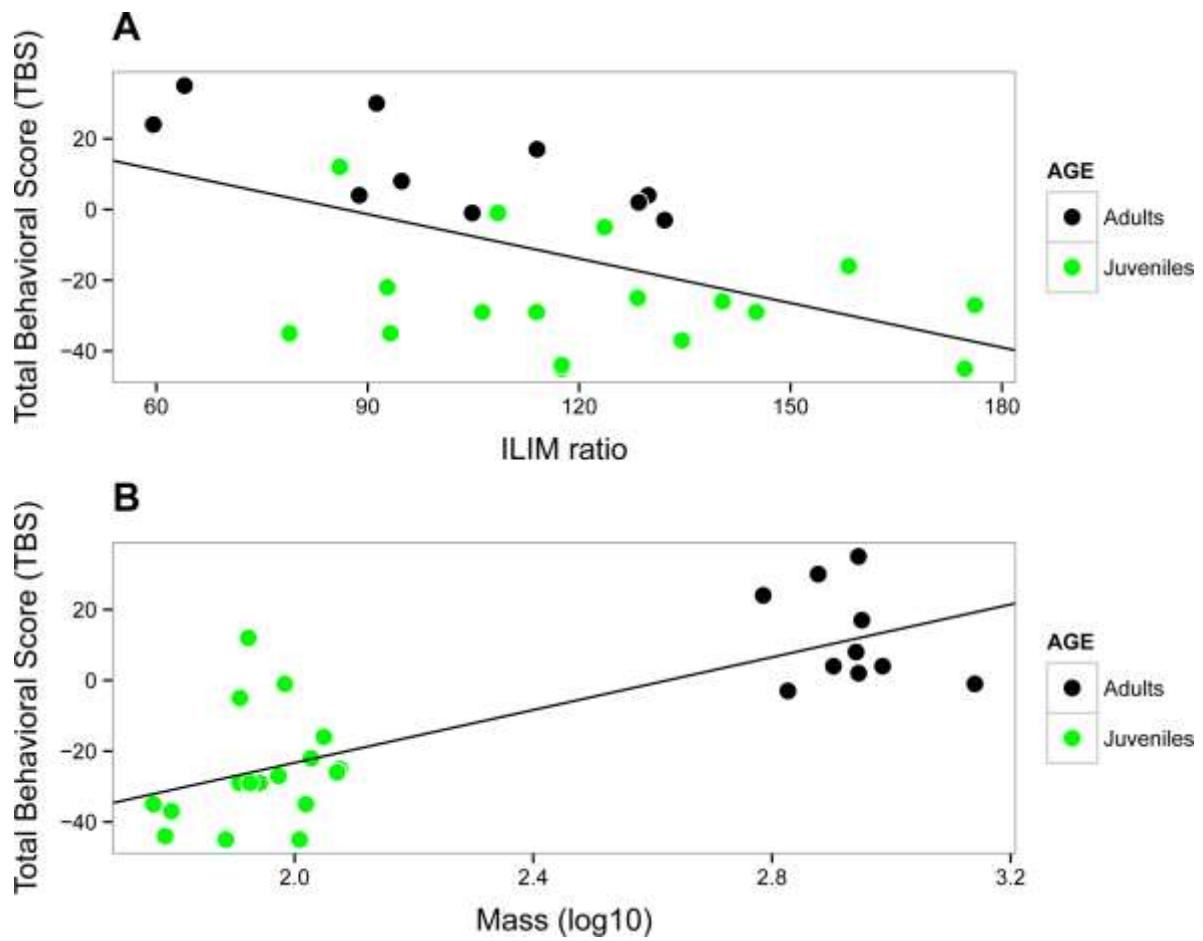


Figure 2. Graphs illustrating the correlation between behavioural index and morpho-physiological factors in tegu lizards. (A) TBS vs LDH:CS activity ratio on ILIM. (B) TBS vs MASS. N=27 individuals.

List of Abbreviations

AESM = *adductor externus superficialis* muscle

AEMS ratio = enzymatic activity ratio between lactate dehydrogenase and citrate synthase on *adductor externus superficialis* muscle

AICc = Akaike information criterion with a correction for finite sample sizes

CS = citrate synthase

FEML = *femur* length

HUML = *humerus* length

ILIM = *iliofibularis* muscle

ILIM ratio = enzymatic activity ratio between lactate dehydrogenase and citrate synthase on *iliofibularis* muscle

JAWL = upper jaw length

LDH = lactate dehydrogenase

LDH: CS ratio = enzymatic activity ratio between lactate dehydrogenase and citrate synthase enzymes

MASS = logarithmized body mass

RADL = *radius* length

TBS = total behaviour score

TIBL = *tibia* length

Table S1. Behavioural responses for each individual at different temperatures, data published in Barros et al. (2010). The ‘latency time’ indicates the time until the individual adopted an aggressive response during application of 10 stimuli; dashes represent animals that did not exhibit aggressive responses. TBS indicates the total behaviour score considering both tests. A = adult tegus; J = juvenile tegus. (A) 18.0°C; (B) 22.5°C; (C) 27.0°C; (D) 31.5°C and (E) 36.0°C.

Individual	Behavioural Test 1						Behavioural Test 2						TBS
	Bite	Defensive posture	Walk	Run	Immobile	Latency time	Bite	Defensive posture	Walk	Run	Immobile	Latency time	
A01	0	7	1	0	2	13	0	0	6	0	4	–	5
A02	0	6	3	0	1	13	0	0	7	0	3	–	1
A03	1	6	0	1	2	12	2	7	0	0	1	2	27
A04	0	7	1	0	2	11	0	5	1	0	4	57	15
A05	0	5	3	0	2	24	0	3	1	0	6	40	4
A06	0	2	2	0	6	26	0	1	3	0	6	51	-2
A07	0	0	4	0	6	–	0	0	2	0	8	–	-6
A08	0	0	4	0	6	–	0	0	3	0	7	–	-7
A09	0	9	0	0	1	2	0	10	0	0	0	5	29
A10	0	0	1	0	9	–	0	0	1	0	9	–	-2
J01	0	0	5	5	0	–	0	0	2	4	4	–	-30
J02	0	0	3	0	7	–	0	1	8	0	1	84	-10
J03	0	0	3	7	0	–	0	0	4	5	1	–	-36
J04	0	3	6	0	1	13	0	0	6	1	3	–	-6
J05	0	2	6	2	0	34	0	4	2	0	4	8	-1
J06	0	4	1	5	0	23	0	0	5	1	4	–	-14
J07	0	0	3	5	2	–	0	0	1	9	0	–	-37
J08	0	0	3	1	6	–	0	0	9	1	0	–	-21
J09	0	1	0	9	0	95	0	0	2	6	2	–	-31
J10	0	0	9	0	1	–	0	0	7	0	3	–	-16
J11	0	0	6	4	0	–	0	0	7	1	2	–	-28
J12	0	4	2	4	0	44	0	0	4	1	5	–	-12
J13	0	2	6	0	2	77	0	0	8	0	2	–	-12
J14	0	0	9	0	2	–	0	0	8	1	1	–	-19
J15	0	6	4	0	0	16	0	0	3	4	3	–	-4
J16	0	0	0	10	0	–	0	0	3	6	1	–	-40
J17	0	0	5	2	3	–	0	0	8	1	1	–	-19

Table S1. B

Individual	Behavioural Test 1						Behavioural Test 2						TBS
	Bite	Defensive posture	Walk	Run	Immobile	Latency time	Bite	Defensive posture	Walk	Run	Immobile	Latency time	
A01	0	8	0	0	2	56	0	0	4	0	0	–	4
A02	0	6	2	1	1	40	0	3	1	1	0	55	2
A03	1	9	0	0	0	4	4	6	0	0	4	3	35
A04	0	9	0	0	1	13	0	5	0	0	0	3	24
A05	0	7	0	0	3	23	0	7	2	0	0	4	17
A06	0	7	0	0	3	22	0	1	0	0	0	90	8
A07	0	2	0	0	8	–	0	1	6	0	0	24	-3
A08	0	3	0	0	7	–	0	0	4	0	0	–	-1
A09	1	9	0	0	0	2	0	9	0	0	0	17	30
A10	0	0	2	0	8	–	0	4	2	0	0	54	4
J01	0	0	1	7	2	–	0	0	3	2	0	–	-22
J02	0	5	5	0	0	24	0	1	7	0	0	2	-1
J03	0	0	5	5	0	–	0	0	3	6	0	–	-35
J04	0	0	8	2	0	–	0	0	6	3	0	–	-29
J05	0	0	5	5	0	–	0	0	7	1	0	–	-29
J06	1	4	0	5	0	4	0	8	0	1	0	13	12
J07	0	0	3	7	0	–	0	0	2	8	0	–	-45
J08	0	2	2	6	0	35	0	0	5	4	0	–	-25
J09	0	0	3	7	0	–	0	0	2	8	0	–	-45
J10	0	0	6	4	0	–	0	0	7	0	0	–	-26
J11	0	1	1	8	0	35	0	0	6	4	0	–	-35
J12	0	1	7	2	0	58	0	1	6	3	0	15	-16
J13	0	0	5	5	0	–	0	0	8	2	0	–	-37
J14	0	0	8	2	0	–	0	0	6	3	0	–	-29
J15	1	9	0	0	0	33	0	0	9	1	0	–	-5
J16	0	0	1	9	0	–	0	0	5	5	0	–	-44
J17	0	0	4	6	0	–	0	0	2	2	0	–	-27

Table S1. C

Individual	Behavioural Test 1						Behavioural Test 2						TBS
	Bite	Defensive posture	Walk	Run	Immobile	Latency time	Bite	Defensive posture	Walk	Run	Immobile	Latency time	
A01	0	0	10	0	0	–	0	0	10	0	0	–	-30
A02	0	0	2	7	1	–	0	0	5	3	2	–	-27
A03	2	7	0	1	0	4	2	8	0	0	0	3	31
A04	0	0	10	0	0	–	0	0	10	0	0	–	-30
A05	0	0	9	0	1	–	0	1	9	0	0	22	-17
A06	0	0	6	1	3	–	0	0	5	0	5	–	-13
A07	0	0	8	1	1	–	0	0	3	0	7	–	-13
A08	0	0	5	5	0	–	0	0	6	0	4	–	-26
A09	0	5	0	0	5	4	0	4	2	0	4	57	12
A10	0	0	10	0	0	–	0	0	6	0	4	–	-21
J01	0	0	0	6	4	–	0	0	3	4	3	–	-23
J02	1	2	7	0	0	5	0	2	7	0	1	5	2
J03	0	0	0	10	0	–	0	0	2	5	3	–	-37
J04	0	0	1	9	0	–	0	0	3	7	0	–	-46
J05	0	2	1	7	0	66	0	1	4	3	2	43	-22
J06	2	2	0	6	0	14	0	2	2	6	0	82	-13
J07	0	0	1	9	0	–	0	0	2	8	0	–	-47
J08	0	0	0	10	0	–	0	0	2	8	0	–	-48
J09	0	0	0	10	0	–	0	0	1	9	0	–	-49
J10	0	0	3	2	5	–	0	0	6	1	3	–	-15
J11	0	0	0	10	0	–	0	0	3	7	0	–	-47
J12	0	5	0	5	0	23	0	1	4	6	0	–	-25
J13	0	6	4	0	0	40	0	0	1	9	0	–	-22
J14	0	0	5	5	0	–	0	1	5	4	0	15	-27
J15	1	8	0	1	0	13	0	8	2	0	0	2	24
J16	0	0	0	10	0	–	0	0	0	10	0	–	-50
J17	0	0	0	8	2	–	0	0	3	7	0	–	-38

Table S1. D

Individual	Behavioural Test 1						Behavioural Test 2						TBS
	Bite	Defensive posture	Walk	Run	Immobile	Latency time	Bite	Defensive posture	Walk	Run	Immobile	Latency time	
A01	0	0	10	0	0	–	0	0	6	4	0	–	-34
A02	0	0	4	4	2	–	0	0	4	3	3	–	-22
A03	0	8	0	0	2	3	1	3	3	1	2	12	18
A04	0	1	7	0	2	24	0	0	2	0	8	–	-8
A05	0	0	4	5	1	–	0	0	7	1	1	52	-23
A06	0	0	4	3	3	–	0	0	8	2	0	–	-27
A07	0	0	7	1	2	–	0	0	2	0	8	–	-11
A08	0	0	9	1	0	–	0	0	9	1	0	–	-32
A09	0	2	6	0	2	4	0	1	8	0	1	45	-6
A10	0	0	9	0	1	–	0	0	9	1	0	–	-25
J01	0	0	3	7	0	–	0	0	1	9	0	–	-46
J02	0	4	3	3	0	6	0	3	7	0	0	54	-4
J03	0	0	0	10	0	–	0	0	0	10	0	–	-50
J04	0	0	0	10	0	–	0	0	1	9	0	–	-49
J05	0	0	1	9	0	–	0	2	2	6	0	12	-31
J06	2	1	2	5	0	14	0	0	2	8	0	–	-25
J07	0	0	0	10	0	–	0	0	0	10	0	–	-50
J08	0	0	0	10	0	–	0	0	1	9	0	–	-49
J09	0	0	2	8	0	–	0	0	3	7	0	–	-45
J10	0	2	3	5	0	13	0	0	2	8	0	–	-29
J11	0	0	0	10	0	–	0	0	1	9	0	–	-49
J12	0	0	1	9	0	–	0	0	0	10	0	–	-49
J13	0	0	3	7	0	–	0	0	7	3	0	–	-40
J14	0	0	0	10	0	–	0	0	0	10	0	–	-50
J15	1	3	0	6	0	27	0	0	0	10	0	–	-32
J16	0	0	1	9	0	–	0	0	0	10	0	–	-49
J17	0	0	2	8	0	–	0	0	1	8	1	–	-40

Table S1. E

Individual	Behavioural Test 1						Behavioural Test 2						TBS
	Bite	Defensive posture	Walk	Run	Immobile	Latency time	Bite	Defensive posture	Walk	Run	Immobile	Latency time	
A01	0	0	9	1	0	–	0	0	6	4	0	–	-35
A02	0	0	4	6	0	–	0	0	3	6	1	–	-36
A03	0	6	3	1	0	2	1	6	1	0	2	11	18
A04	0	0	10	0	0	–	0	0	8	1	1	–	-25
A05	0	0	7	3	0	–	0	0	8	1	1	–	-28
A06	0	0	10	0	0	–	0	0	9	1	0	–	-31
A07	0	0	8	0	2	–	0	0	5	0	5	–	-13
A08	0	0	10	0	0	–	0	0	8	2	0	–	-32
A09	0	5	5	0	0	21	0	0	8	0	2	–	-8
A10	0	0	6	4	0	–	0	0	5	5	0	–	-39
J01	0	0	0	10	0	–	0	0	2	8	0	–	-48
J02	0	0	9	1	0	–	0	0	9	1	0	–	-32
J03	0	0	0	10	0	–	0	0	0	10	0	–	-50
J04	0	0	1	9	0	–	0	0	4	6	0	–	-45
J05	0	0	7	3	0	–	0	0	0	10	0	–	-43
J06	0	0	0	10	0	–	0	0	6	4	0	–	-44
J07	0	0	0	10	0	–	0	0	0	10	0	–	-50
J08	0	0	1	9	0	–	0	0	1	9	0	–	-48
J09	0	0	0	10	0	–	0	0	1	9	0	–	-49
J10	0	0	0	10	0	–	0	0	5	5	0	–	-45
J11	0	0	2	8	0	–	0	0	0	10	0	–	-48
J12	0	0	3	7	0	–	0	0	0	10	0	–	-47
J13	0	0	5	5	1	–	0	0	5	5	0	–	-40
J14	0	0	0	10	0	–	0	0	1	9	0	–	-49
J15	0	0	1	9	0	–	0	0	0	10	0	–	-49
J16	0	0	0	10	0	–	0	0	1	9	0	–	-49
J17	0	0	0	10	0	–	0	0	1	9	0	–	-49

Table S2. Statistical results for ANOVA testing differences between juveniles and adults of tegu lizards in terms of anti-predatory behaviour.

	Df	TBS		
		SS	F	P
TEMP	4	9192.7	10.393	< 0.001
AGE	1	4310.4	19.493	< 0.001
TEMP:AGE	4	1633.8	1.847	0.124
Interactions				
18.0°C (J vs A)	1	4310.4	19.493	< 0.001
22.5°C (J vs A)	1	8790.4	39.753	< 0.001
27.0°C (J vs A)	1	1418.9	6.417	0.063
31.5°C (J vs A)	1	3451.1	15.607	< 0.001
36.0°C (J vs A)	1	3411.3	15.427	< 0.001
Residuals	125			

SS: sums of squares for ANOVA; F: exact or approximate *F*-value; P: exact or approximate *P*-value (in case of interaction, p-adjusted value by Bonferroni); Df: degrees of freedom; J: juveniles; A: adults.

Table S3. AICc results and estimated values for CS and LDH enzymatic activity at 22.5°C, describing similarities between best-fit models and a simple linear regression equation ($y=a+bx$) in juveniles of tegu lizards in the adductor and ileofibularis muscles.

Baby Tegus	Equation	Rank	r ²	F	RSS	AICc	ΔAICc	y-value (at 22.5°C)	95 Conf -	95 Conf +	Scatter plot
Enzyme: CS	$y=a+b/\ln x$	1	0.730	132.78	138.48	21.50	0.00	9.98	9.47	10.50	FigS4. (A)
Muscle: Adductor	$y=a+bx$	41	0.712	121.06	148.02	21.70	0.20	9.49	8.92	10.06	FigS4. (B)
Enzyme: CS	$y=a+bx\ln x$	1	0.803	199.90	94.46	20.35	0.00	7.10	4.27	9.93	FigS4. (C)
Muscle: Ileofibularis	$y=a+bx$	3	0.803	199.14	94.75	20.36	0.01	7.16	4.33	9.99	FigS4. (D)
Enzyme: LDH	$y=a+bx^2\ln x$	1	0.892	405.44	99.60	43.18	0.00	300.88	279.35	322.42	FigS4. (E)
Muscle: Adductor	$y=a+bx$	29	0.879	355.09	99.84	43.53	0.35	319.14	297.32	340.95	FigS4. (F)
Enzyme: LDH	$y=a+bx^2\ln x$	1	0.865	312.99	235.45	49.40	0.00	818.39	757.67	879.11	FigS4. (G)
Muscle: Ileofibularis	$y=a+bx$	23	0.854	286.46	250.63	49.62	0.23	863.25	802.98	923.51	FigS4. (H)

Table S4. AICc results and estimatee values for CS and LDH enzymatic activity at 22.5°C, describing similarities between best-fit models and a simple linear regression equation ($y=a+bx$) in adult tegus in the adductor and ileofibularis muscles.

Adult Tegus	Equation	Rank	r ²	F	RSS	AICc	ΔAICc	y-value (at 22.5°C)	95 Conf -	95 Conf +	Scatter plot
Enzyme: CS Muscle: Adductor	$y=a+bx^{0.5}\ln x$	1	0.655	53.05	190,509.62	20.51	0.00	8.85	8.04	9.67	FigS5. (A)
	$y=a+bx$	10	0.654	52.85	214,249.03	20.52	0.01	8.77	7.95	9.60	FigS5. (B)
Enzyme: CS Muscle: Ileoibularis	$y=a+bx^3$	1	0.606	43.07	1,514,638.40	23.09	0.00	7.73	6.37	9.08	FigS5. (C)
	$y=a+bx$	15	0.581	38.76	1,634,451.50	23.28	0.19	8.24	6.93	9.55	FigS5. (D)
Enzyme: LDH Muscle: Adductor	$y=a+bx^2$	1	0.824	131.24	220,319.65	43.61	0.00	370.55	330.16	410.93	FigS5. (E)
	$y=a+bx$	13	0.819	127.01	226,334.43	43.69	0.08	386.06	346.63	425.49	FigS5. (F)
Enzyme: LDH Muscle: Ileoibularis	$y=a+bx^2$	1	0.881	208.10	642,230.05	46.82	0.00	757.10	688.15	826.06	FigS5. (G)
	$y=a+bx$	22	0.873	192.82	686,673.64	47.02	0.20	790.89	722.22	859.57	FigS5. (H)



Fig. S1. Differences in color pattern between (A) juvenile tegus (recently-hatched individuals) and (B) adult (1 year-old, 800 g) individual of *Salvator merianae*.

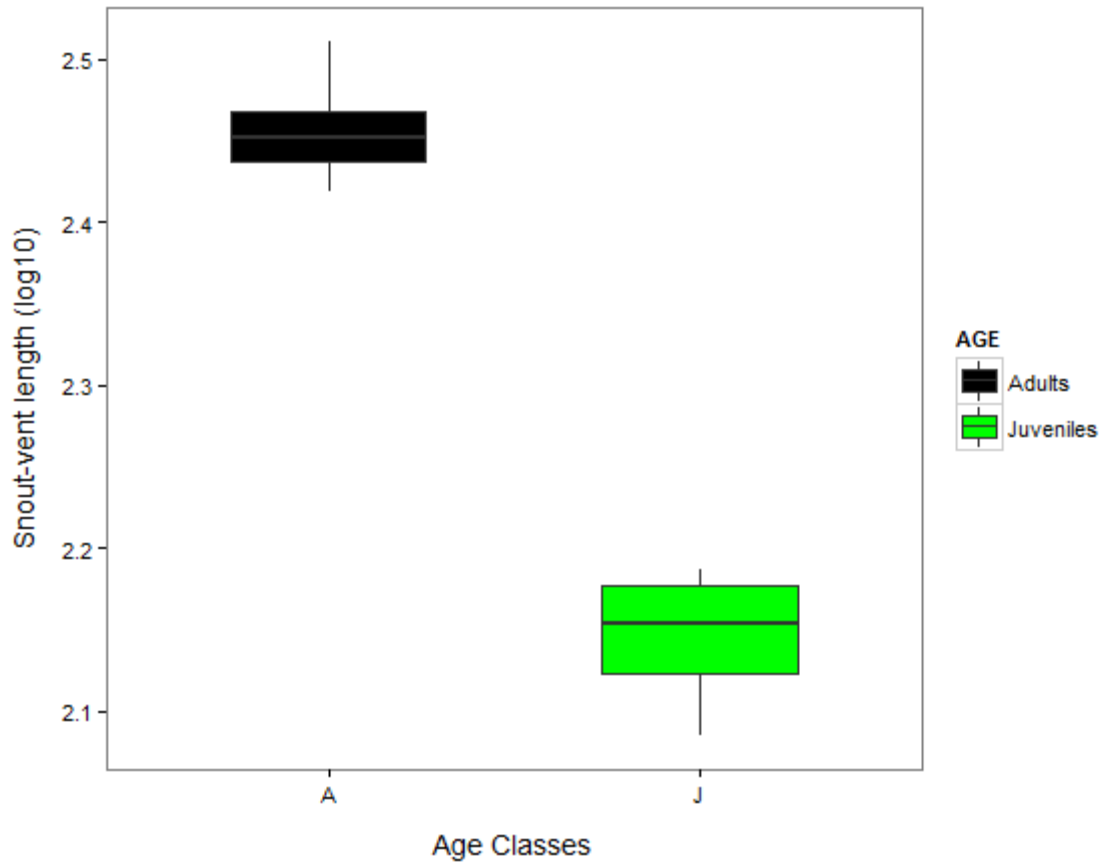


Fig. S2. Boxplot of SIZE differences between juveniles and adults of tegu lizards.

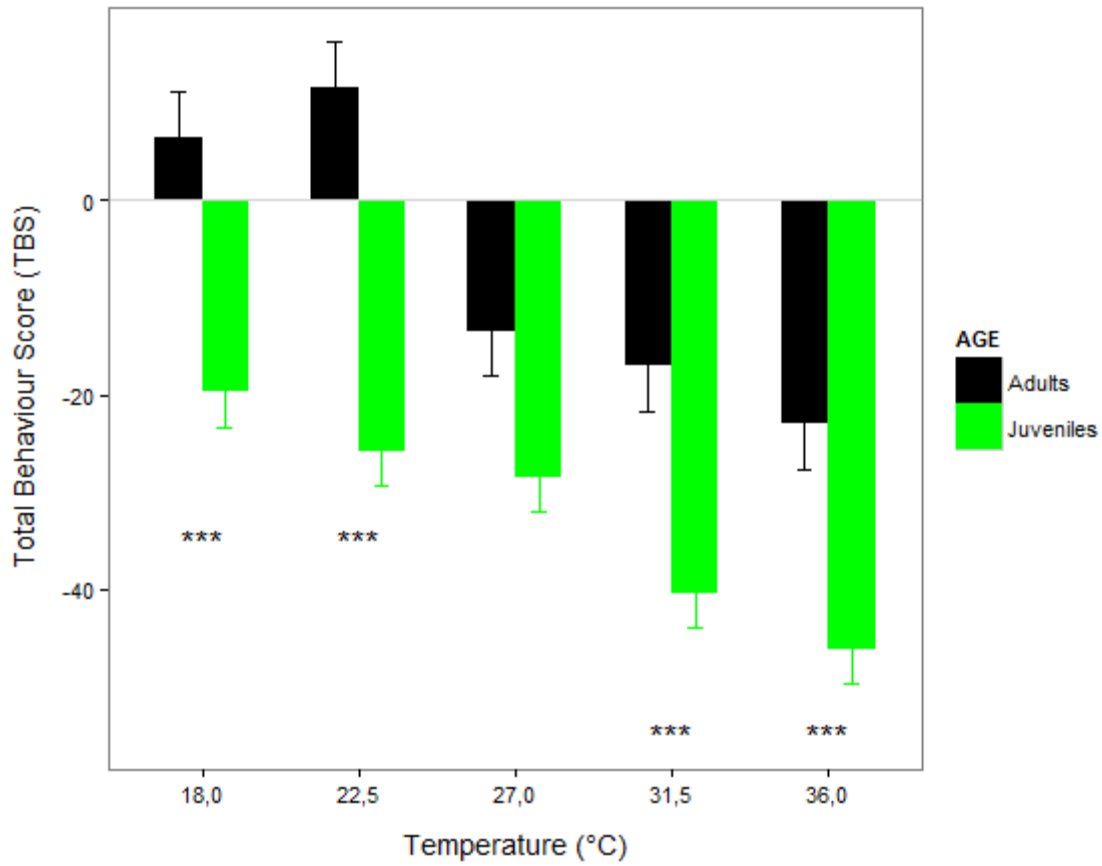
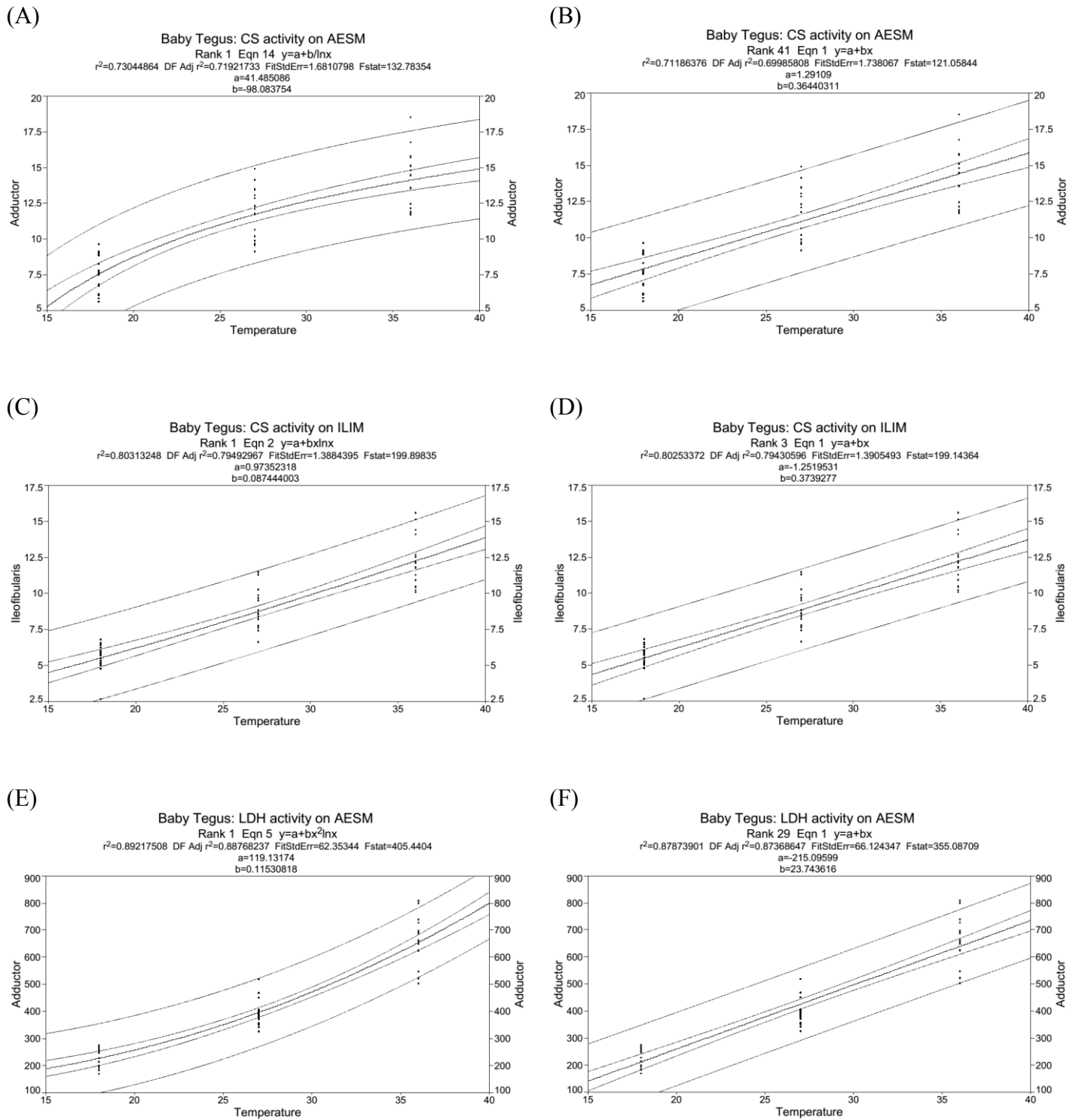


Fig. S3. Barplot of TBS means in different temperatures for young and adult tegus. Positive values represents aggressive behaviour and negative values are related to evasive tactics. ‘***’ represents significance level at 0.05.



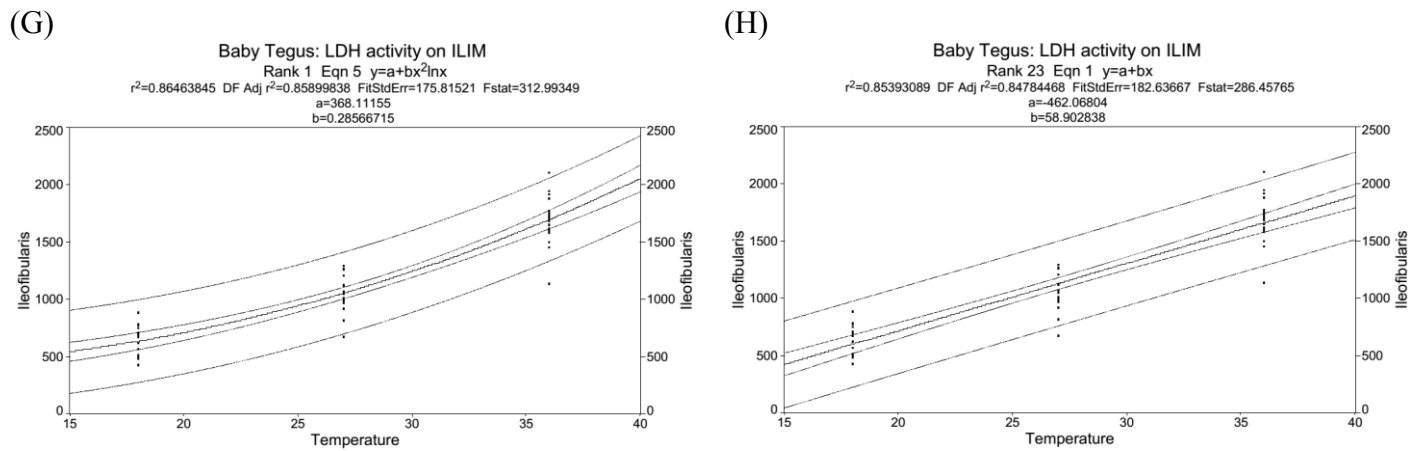
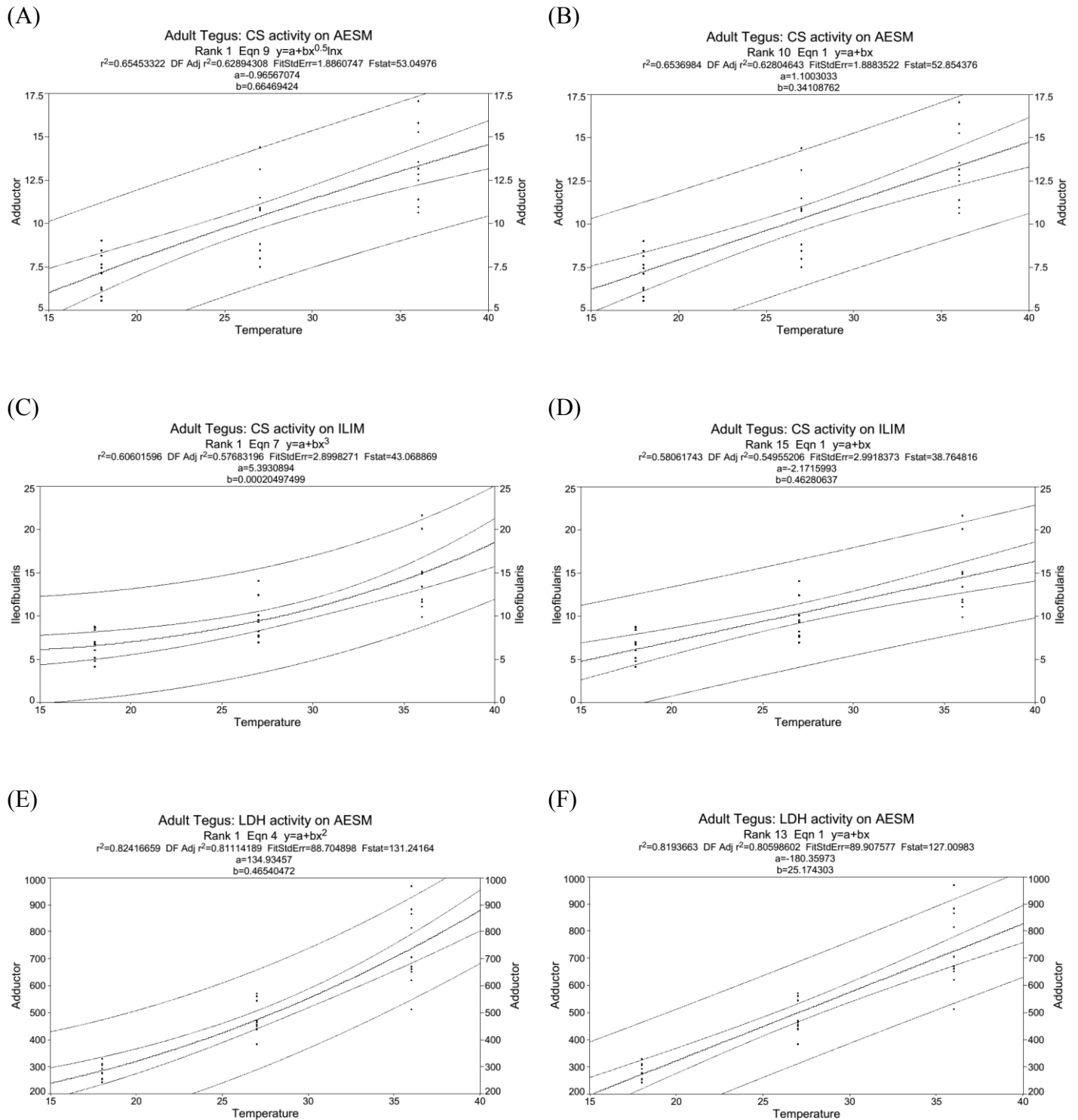


Fig. S4. Graphs comparing best-fit models and a simple linear regression equation ($y=a+bx$) describing enzyme activity relationships at three experimental temperatures (18, 27 and 36°C) in juveniles of tegu lizards in the adductor and ileofibularis muscles. Internal lines represent confidence intervals at 95% and external lines the predicted values at 95%.



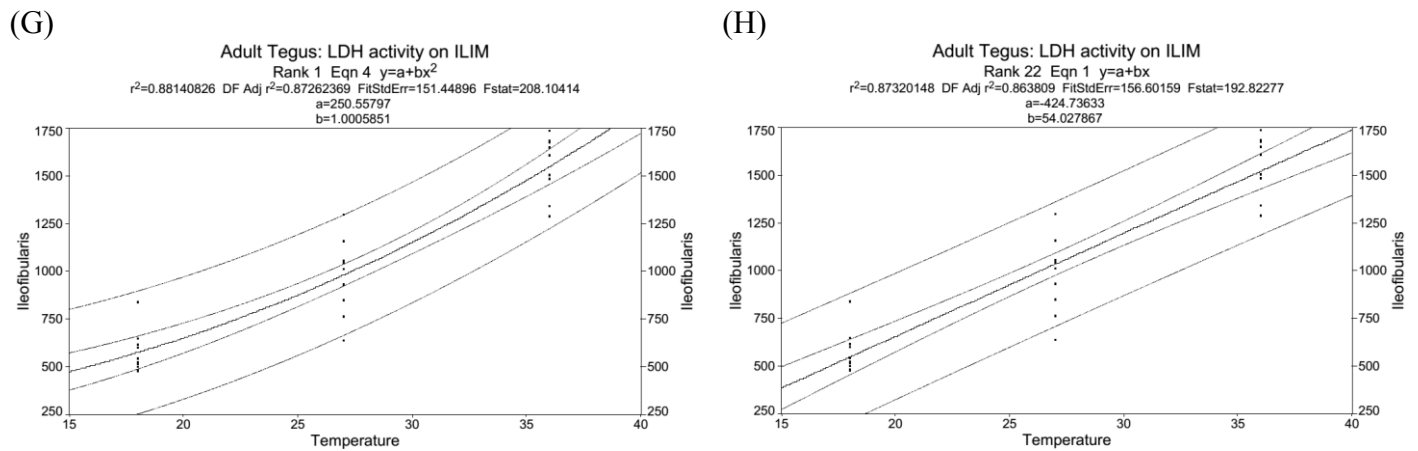


Fig. S5. Graphs comparing best-fit models and a simple linear regression equation ($y=a+bx$) describing enzyme activity relationships at three experimental temperatures (18, 27 and 36°C) in adults of tegu lizards in the adductor and ileofibularis muscles. Internal lines represent confidence intervals at 95% and external lines the predicted values at 95%.