# **Behavioural Processes**

# Daily running trials increase sprint speed in endangered lizards (Gallotia simonyi) --Manuscript Draft--

| Manuscript Number:    |  |  |  |
|-----------------------|--|--|--|
| Article Type:         | Research Paper   |  |  |
| Keywords:             | training; running trials; endangered lizard; Gallotia simonyi  |  |  |
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| Abstract:             | Due to increasing number of animal species in danger of extinction, captive breeding of individuals has become a necessary procedure for many recovery programs. As specimens born and raised in captivity during several generations may not develop some behavioral and performance aptitudes properly, training is a useful method to apply before releasing them into the wild. We present here the results of experiments aiming to detect the effect of daily running trials in young males of the endangered lizard ( Gallotia simonyi ) from El Hierro (Canary Islands). We made individuals run in a racetrack twice every day, for five days a week between the end of July and the end of September. We filmed all running trials and calculated running speed for each individual dividing the distance run by the time used. Running speed did not correlate with body condition of the lizards but there was variation in running speeds of some individuals with similar body conditions. Mean running speed of lizards used in the experiments significantly increased along the whole trial period. Mean running speed did not change significantly in a control group, participating twice in running trials, one at the beginning and the other at the end of the experimental period. From these results we suggest that locomotor training contributed to increasing final running speeds of experimental lizards. Based on these results, training schedules have been recommended to be implemented for lizards kept in captivity before they are reintroduced to the wild. |  |  |
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La Laguna, March, 8th 2021

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Prof. Olga Lazareva

Department of Psychology, Drake University, 316 Olin Hall, Des Moines, Iowa, 50311, USA

Dear Prof. Lazareva:

Fax: 34 22 31 83 11

In the accompanying files we are enclosing the text, figures and table of a manuscript entitled "Daily running trials increase sprint speed in endangered lizards (*Gallotia simonyi*)", that we are submitting to be considered for its publication in Behavioural Processes. The manuscript is within the area of behavioral biology and conservation.

The manuscript contains only material that is original, it has not been published or submitted elsewhere and is approved by all authors.

The work includes a study that is part of actions being performed within the recovery plan of the species and therefore the local institution (Cabildo of El Hierro) gave us the permit to carry out the experimental trials. We were coordinated with the official agency responsible for the conservation effort for this species.

The main result is that lizards performing daily trials increased their final running speed in comparison with those at the beginning and compared with a control group. Running speed is a crucial behaviour relevant as an antipredator method once the animals are reintroduced into the natural environment.

The enclosed work is novel basically because it is the first time that this anti-predator training has been performed in lizards of a critically endangered species before their reintroduction into the wild. The result of this work (together with that reported in our previous publication: Burunat et al., 2018) has led to implement a new policy before reintroducing lizards into the wild and the method used could be of general interest to conservation researchers and/or policy makers working on lizards or other small terrestrial vertebrates.

I look forward to hearing from you.

Sincerely,

Dr. Miguel Molina-Borja

Highlights

## Highlights

Running trials were performed by lizards kept in a Breeding Center.

Daily running speed of lizards significantly increased along the trial period.

Running speed did not change significantly in a control group.

We recommended to apply this training to lizards before reintroduction to the wild.

Due to increasing number of animal species in danger of extinction, captive breeding of individuals has become a necessary procedure for many recovery programs. As specimens born and raised in captivity during several generations may not develop some behavioral and performance aptitudes properly, training is a useful method to apply before releasing them into the wild. We present here the results of experiments aiming to detect the effect of daily running trials in young males of the endangered lizard (Gallotia simonyi) from El Hierro (Canary Islands). We made individuals run in a racetrack twice every day, for five days a week between the end of July and the end of September. We filmed all running trials and calculated running speed for each individual dividing the distance run by the time used. Running speed did not correlate with body condition of the lizards but there was variation in running speeds of some individuals with similar body conditions. Running speed of lizards used in the experiments significantly increased along the whole trial period. Mean running speed did not change significantly in a control group, participating twice in running trials, one at the beginning and the other at the end of the experimental period. From these results we suggest that locomotor training contributed to increasing final running speeds of experimental lizards. Based on these results, training schedules have been recommended to be implemented for lizards kept in captivity before they are reintroduced to the wild.

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| Daily running trials increase sprint speed in endangered lizards ( <i>Gallotia</i> simonyi)   |
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ABSTRACT

Due to increasing number of animal species in danger of extinction, captive breeding of individuals has become a necessary procedure for many recovery programs. As specimens born and raised in captivity during several generations may not develop some behavioral and performance aptitudes properly, training is a useful method to apply before releasing them into the wild. We present here the results of experiments aiming to detect the effect of daily running trials in young males of the endangered lizard (Gallotia simonyi) from El Hierro (Canary Islands). We made individuals run in a racetrack twice every day, for five days a week between the end of July and the end of September. We filmed all running trials and calculated running speed for each individual dividing the distance run by the time used. Running speed did not correlate with body condition of the lizards but there was variation in running speeds of some individuals with similar body conditions. Running speed of lizards used in the experiments significantly increased along the whole trial period. Mean running speed did not change significantly in a control group, participating twice in running trials, one at the beginning and the other at the end of the experimental period. From these results we suggest that locomotor training contributed to increasing final running speeds of experimental lizards. Based on these results, training schedules have been recommended to be implemented for lizards kept in captivity before they are reintroduced to the wild.

Keywords: training, running trials, endangered lizard, Gallotia simonyi

#### 1. Introduction

Many animal species are in danger of extinction generally due to habitat alteration or destroy and to direct human effect (UICN, Baillie et al., 2004). Therefore, many recovery programs were and are carried out to assist against declining populations (Morris Gosling and Sutherland, 2000). Part of these recovery programs include breeding and rising of individuals from the endangered populations in controlled situations of laboratory or outdoor enclosures. For some much-endangered populations, individuals are bred in captivity during several generations before several them are released into the wild. Against a common opinion, after a few generations in captivity animals may lose certain behavioural capacities that are fundamental for survival (Wallace, 2000; Brokordt et al., 2006). The effect of experience on the development of antipredator behaviour, for example, can have practical importance when captive-bred individuals will participate in reintroduction programs of the considered species (Kleiman, 1989). Therefore, it is very important to put into practice management actions to help those individuals to be released to acquire or ameliorate behavioural capacities that will increase their chances of surviving once in the wild. A variety of these actions has been used previously in reintroduction programs of several endangered species (see Morris Gossling and Sutherland, 2000, for a revision).

Especially important for reintroduced individuals are the capabilities to find and consume adequate food and their antipredator aptitudes. For example, survival was higher in young lion tamarins released into the wild after they could acquire higher locomotor capabilities (Stoinski and Beck, 2004). Providing individuals with predator training has also become a fundamental part of many recovery programs for endangered species (Griffin et al., 2000; Griffin, 2004) as for example in black-footed ferrets (Miller et al., 1994), bustards (van Heezik et al., 1999), fish (Mirza and Chivers, 2000; Brown and Laland, 2001; Kelly and Magurran, 2003), and prairie dogs (Shier and Owings, 2007).

Running speed is a crucial factor as an antipredator strategy in lizards (Greene, 1988) and finding quickly a refuge may mean the difference between surviving and not a predatory attack (Martín and López, 1999). Running performance is also important for capturing prey and defending territories (Garland et al., 1990); and running speed may be a crucial parameter for younger individuals to be able to escape from aggression of older conspecifics (Alberts,1994). On the other hand, locomotor performance relates to foraging behaviour in some lacertids (Huey et al., 1984) and to social dominance in several lizard species (Perry et al., 2004, Husak and Fox, 2006). In fact, such studies suggest that lizards in the wild may attain higher speed in social interactions rather than when escaping from predators and of course when capturing prey.

The opportunities for animals kept in captivity to run at high speeds are limited, even when kept in outdoor enclosures. Young lizards kept in this type of enclosures may perform short runs when fleeing from a conspecific or as a reaction to occasional external noise, but these moments are much spaced in time and the distances over which they may run are limited. Nevertheless, many lizards do not escape for long distances but exhibit a succession of short bursts alternated with pauses (Avery et al., 1987; Braña, 2003), that also apply to *Gallotia* lizards (own unpublished observations). On the other hand, locomotor performance has been shown to vary intra- and inter-individually in several reptiles (Garland, 1985, 1994), including juveniles of some *Gallotia* species (Vanhooydonck et al., 2001). Losos et al. (2002) already suggested that motivation may partially account for the variation among lizard runs. Therefore, low locomotor performance of some individuals, together with restricted captive conditions, may severely limit the possibility for animals to develop appropriate locomotor capacities before releasing them into the wild during reintroduction programs.

Gallotia simonyi is an extremely endangered lacertid species, endemic of El Hierro Island, the smallest and western most of the Canaries. A small population still lives in a north-western high inland cliff (Pérez-Mellado et al., 1999), and lizards born and raised in captivity (Rodríguez-Domínguez and Molina-Borja, 1998) have begun to be reintroduced in two new sites of the island since several years ago (Consejería Medio Ambiente, Canarian Government, 1997: http://www.gobiernodecanarias.org/medioambiente/piac/temas/biodiversidad/medidas-y-factores/flora-fauna/conservacion-especies/proyectos-life-naturaleza/reintroduccion-lagarto-hierro/). Recent data show that initial reintroduction of *G. simonyi* in two localities of El Hierro has not been successful (Trujillo, 2008).

Survival of reintroduced *G. simonyi* individuals may depend crucially on their locomotor abilities, as some predators (mainly kestrels and some cats) are still present in the localities where lizards are being reintroduced (though a control program for cats is being carried out there). Cejudo and Márquez (2001) showed that both adult and juvenile *G. simonyi* captive specimens had slower sprint speed, measured in a range of different body temperatures, than wild individuals of the similar body-sized *G. stehlini* (from Gran Canaria Island).

Therefore, we wanted to test if long-term daily trials of running could enhance the locomotor capacities in young El Hierro lizards kept in semi-captive conditions. If this proved to be true, lizards could then be trained before releasing them into the wild, selecting for reintroduction those individuals having the higher running speed for their body length. We predicted that continuous daily trials should increase running speed of the experimental lizards in comparison to non-trained individuals.

#### 2. Methods

#### 128 2.1. Individuals and maintenance

We used young individuals of El Hierro giant lizard (*G. simonyi*) bred in the Centre for the Reproduction and Research of this species in Frontera (El Hierro) as part of the recovery program for the species. We used ten five-year old males (Snout-vent length –SVL- and Body mass –BM- data in Table 1) that were candidates to be released into the wild in the following years. Lizards were held together in an outdoor terrarium (4 x 3 m) with soil as substrate, natural plants and a wire mesh covering the top. They were fed three times a week with natural food including leaves and fruits of local plants as well as adult and larvae insects. Staff personnel supplied food from outside the terrarium and many hiding places (bark logs and stones) were available throughout the substrate. During their stay in captivity and during the experiments the animals were cared for in accordance with guidelines published by Animal

#### 2.2. Experimental procedure

Behaviour (ASAB/ABS 2012; Anim. Behav. 83:301–309).

Each lizard was grabbed smoothly by hand and put in a racetrack (Huey et al., 1981;  $0.3 \times 3 \text{ m}$ ) twice per day, one in the morning (11.30-12.00 h) and another in the afternoon (16.00-16.30 h), five times a week. The racetrack was in a darkened room and it was illuminated with two Reptistar fluorescent lamps (Sylvania, F18W 6500 K, emitting partly in the near ultraviolet range). The floor of the track was covered with a 3 mm thick cork plate.

In each trial, an animal was put at one end of the track and made to run by chasing it with the hand. Between the end of July and the end of September 2001, for each lizard we performed 58 trial sessions (morning and afternoon) along 29 days. Trials were filmed with a video-camera (Panasonic NV-DS15, mini DV) placed on top of the track with its lens perpendicular to it and with its visual field covering the whole length of the racetrack. After each trial, we cleaned the bottom cork plate with a diluted alcohol solution and allowed it to dry before starting a new trial.

Before each trial, we warmed every lizard (by putting it under an incandescent light bulb) until reaching a cloacal temperature around 32 °C; this temperature is in the middle of the selected –preferred- body temperatures of this species (Márquez et al., 1997). Measurement of cloacal temperature was made by gently inserting a quick-reading mercury thermometer (Miller and Weber,  $\pm$  0.1 °C precision). We also measured temperature at the bottom of the track at the beginning of each trial.

Running speed for each lizard was calculated dividing the distance traveled by the time used in doing it (calculated from the timed frames of the videotape, 1s accuracy); this method provides much better results than considering intervals (Gomes et al., 2017). We did not want to measure only maximal sprinting speed so we included in the analyses speed calculations for all experimental animals (Losos et al., 2002). As a control group, we used a different set of ten young male lizards that participated in two running trials, one at the beginning of the experimental period and another at the end. Individuals of this control group had similar SVL to experimental animals and running experiments were performed with identical lab conditions as for the experimental group.

2.,3. Data analyses

Running speed and cloacal temperature data from each experimental lizard were stored in computer files and analysed using SPSS 22.0 statistical package. We calculated individual body condition as the residuals of log BM to log SVL and analyzed its relationship with the corresponding running speed of each specimen, separately at the beginning and at the end of the experimental period. Before other analyses, we firstly compared running speeds and cloacal temperatures from morning and afternoon trials and, after confirming that there was no significant difference (Wilcoxon test, Z = -0.392, p = 0.695 and Z = -1.745, p = 0.081, respectively), we used all measurements along the 29 consecutive days. As data did not fulfill normality and homoscedasticity requirements, we used generalized linear models (GLM) using running speed as the dependent variable and SVL, body mass and environmental temperature as covariates, and individual's code as a random variable. We used Spearman rho as correlation statistic. Significance level was set at  $\alpha = 0.05$ .

#### 3. Results

3.1. Running speed and body temperature

In the different trials along the whole experimental period individuals did have slightly different cloacal temperatures (maximal range between 32.7 - 33.5 °C), but there was no significant difference among them (ANOVA,  $F_{1,9} = 1.682$ , p = 0.09). On the other hand, there was no significant relationship between cloacal temperatures and running speeds (Spearman rank correlation coefficient, rho = 0.12, p = 0.76). Temperature inside the track was between 28 and 31 °C in different trials; however, it did not significantly correlate to running speeds of individuals (rho = 0.04, p = 0.43)

3.2. Temporal change in running speeds

Running speeds increased significantly along the time between the first and last trials (Fig. 1 and Table 1). Mean values were 115.02 cm/s ( $\pm$  6.69 SE, range 36.14-222.33) and 164.9 cm/s ( $159.36\pm8.31$  SE, range 74.99-319.89) during the first and last five-day experimental periods, respectively. There was a significant difference in running speeds between individuals of the experimental group (Fig. 2 and Table 1). The control group in turn did not significantly change its mean running speed at the end of the experimental period ( $134.7\pm20.9$  cm/s) in relation to that of the beginning ( $126.9\pm28.3$  cm/s; Z=-1.42, p=0.15).

Body condition of the studied lizards did not significantly change between the beginning and the end of experiments (Z = -0.153, n = 10, p = 0.87). On the other hand, neither at the beginning nor at the end of the experimental periods, running speeds did show a significant relationship to individual's body condition (r = -0.201, n = 10, p = 0.57 and r = 0.079, n = 10, p = 0.83, respectively (Fig. 3).

#### 4. Discussion

4.1. Body temperatures and running speeds

We initially proved that lizard body temperature did not vary significantly along experimental trials and that the very small differences among specimen temperatures did not significantly affect running speeds. This is an evidence that lizards were close to the plateau of the speed-temperature curve, but since environmental temperature was slightly lower than preferred temperature (Márquez et al., 1997), there is still space to increase (Angilletta, 2006). Additionally, the significant difference among lizard running speeds reflects interindividual variation in this trait that is independent from body temperature; this agrees with interindividual variation in maximal sprint speed found in *Zootoca vivipara* (Artacho et al., 2013).

Our results show that mean sprinting speeds of the specimens studied are somewhat below the figures given for adult males of the same species by Cejudo and Márquez (2001). However, the range of SVL is lower for our specimens than the adult males studied by those authors; therefore, the sprint speeds calculated in our study are in between those for juveniles and smaller adult males in the same range of body temperatures (Table 1 and see Table 2 of Cejudo and Márquez, 2001). Interestingly, Marquez et al. (1997) reported higher preferred temperature for juveniles than for adults and this suggests that juveniles may thermally compensate such slower speeds.

In other species higher sprinting speeds have been documented for phrynosomatid (Bonine and Garland, 1999) and anolid (Calsbeek and Irschick, 2007) lizards with much smaller SVL or body mass than *G. simonyi*. For example, a mean speed of 4.62 m/s was reported for *Crotaphytus collaris* with a mean body mass of 22.3 g (Bonine and Garland, 1999).

4.2. Training effects

We also have shown a significant increase of running speed of experimental lizards along the experimental study period. The control group did not change its mean running speed, so we suggest that the running training during the trials have contributed to the increased speed of the experimental group at the end of the test period.

This result contrasts with previous studies that reported no significant changes with training in different lizard species. Thus, running endurance (and several respiratory and metabolic measurements) did not significantly change with training in the lizard *Sceloporus occidentalis* (Gleeson, 1979) and even a reduction in sprinting speed of trained lizards (*Amphibolurus nuchalis*) was found (Garland et al., 1987). In the case of *Sceloporus occidentalis*, the experimental period lasted 6 and 8 weeks in two studies and measurements of experimental animals were made at 2-week intervals. More recently, *Anolis* lizards that were exercise-trained three times a week for 8 weeks did not increase sprint speed but increased their endurance (Husak et al., 2015). Lailvaux et al. (2019) did not detect any effect of 9 weeks of training on sprint speed of green anoles. The procedures used in all these works included shorter training period and therefore were different from our experimental procedure, where animals run every day and for 29 successive trials along the whole experimental period. However, Gleeson (1979) stated in his results that, after 6 weeks, trained animals run longer and slightly farther (significant difference) than untrained ones.

Our results then show that training can influence locomotor speed in a squamate and would agree with other results for juvenile estuarine crocodiles where exercise training increased maximum rate of oxygen consumption and locomotor endurance (Owerkowicz and Baudinette, 2008). Therefore, *G. simonyi* would be an exception within other squamates where no effect of training was reported (Garland et al., 1987; Conley et al., 1995; Thompson, 1997) and would not support the suggestion of Gleeson (1979) that these vertebrates would be "metabolically inflexible".

Logistic difficulties at El Hierro reproduction Centre rendered it impossible to handle daily the lizards from the control group like those from the experimental one, as it would have been

necessary in a strict control group. Therefore, we cannot completely discard the possibility that everyday manipulation of the experimental lizards (handling and heating them before the running trials) could influence their sprinting speeds. The lizards in the installations of the Centre for Reproduction and Research at El Hierro are handled for veterinary controls at least once a year (Martínez-Silvestre et al., 2004) and are habituated to some human interactions that, on the other hand, are performed considering all recommended rules of Guidelines for use of animals in research (Greenberg, 1994). The occurrence or not of breeding success has been considered one important method of several proposed to quantify the effect of anthropogenic stressors on wild animals (Tarlow and Blumstein, 2007). An index of *G. simonyi* individuals being in good health and not stressed is the reproductive success of females, laying eggs every year in the usual seasonal times and with egg number and viable hatched offspring having normal figures for these big lizards (Rodríguez-Domínguez and Molina Borja, 1998).

It could be argued that every-day handling could have had a stressor effect on *G. simonyi* specimens and, therefore, could have influenced long-term changes in running speeds.

However, continuous exposure to stressful factors usually produces some sort of habituation. Thus, the levels of blood corticosterone (a hormone known to be affected by stressful situations) in *Urosaurus ornatus* after chronic stress (animals kept three weeks in captivity) was much lower than those for acute stress (Moore et al., 1991). Similarly, in a study of marine iguanas, groups habituated to the presence of tourists did have low corticosterone levels in comparison with control groups (Romero and Wikelski, 2002). In a work analyzing the stressful effects of different research protocols on the scincid *Eulamprus heatwolei*, Langkilde and Shine (2006) showed that one of the treatments (putting the lizards in a racetrack for testing locomotor speed) was one of the several factors producing a transient increase in corticosterone levels. However, each lizard was sprinted only four times, which is not a chronic treatment. Therefore, daily manipulation of our experimental animals should be expected to induce habituation rather than sensitization, and thus we do not attribute the effect found on running speeds to stress caused by frequent lizard handling.

## 4.3. Importance of running speed for survival

 Running speed is a crucial factor as antipredator tactic in lizards (Greene, 1988; Martín and López, 1999) and several speed measurements relate to limb sizes. For example, a phylogenetic study of 13 lacertids showed that maximal speed correlated positively with relative hind limb length (Bauwens et al., 1995) and sprint performance correlated positively with hind limb lengths in phrynosomatid lizards (Bonine and Garland, 1999) and in *Psammodromus algirus* (Zamora-Camacho et al., 2014). Extrapolated results from the only published data in four *Gallotia* species (Márquez et al., 1997) showed that those having relatively longer hind limbs to

their SVL also have higher maximum sprint velocities (Vanhooydonck et al., 2002). Moreover, juveniles of *G. simonyi* had slower sprint performance (absolute speed) than those of *G. stehlini*, a similar sized lizard from Gran Canaria Island (Cejudo and Márquez, 2001). As larger *Gallotia* species show relatively shorter hind limbs than smaller ones (Molina-Borja and Rodríguez-Domínguez, 2004), maximal speed could be a restraining factor in the larger, more endangered, Canarian lizard species.

On the other hand, Garland (1985) found that, after 22 months in captivity, *Amphibolurus* lizards were 12% slower than field-fresh animals, and sprint speed affects individual survival, above all in juvenile individuals (Husak, 2006a). Considering all the above data, a low sprinting speed could be a restraining factor limiting the surviving capabilities of large *G. simonyi* lizards in the field. Therefore, locomotor training is an important aspect to consider for lizard maintenance in captivity, above all for those individuals that are going to be released into the wild after passing several years in captive conditions. As far as we know, there has been no attempt to do this kind of training for endangered lizards, but different types of training have proved to be a useful method in other endangered vertebrates born in captivity and afterwards being released into the wild (review in Wallace, 2000).

Due to restriction of number of animals available for experiments at the Breeding Center, we could not measure running speeds for a higher number of males and the number of available females was very low to be included in the study. However, the analysis of female running capabilities deserves future attention, considering that sexual dimorphism has already been shown in performance traits of other lizards (Cullum, 1998); moreover, females suffer locomotion performance restrictions when pregnant (Van Damme et al., 1989; Cooper et al., 1990; Le Galliard et al., 2003; Husak, 2006b), but also compensate for that in the field by becoming more cryptic. On the other hand, pregnant female lacertids are obliged to decrease preferred temperature due to the O<sub>2</sub> requirements of the embryos (Braña, 1993; Carretero et al., 2005).

Finally, considering the results of our experiments and as the objective of the experimental study was to provide suggestions for the management and recovering of the species, in the last reintroductions to the natural habitat, training schedules have been implemented for lizards kept in captivity before they are reintroduced to the wild. As lizards from the control group could not be handle every day in the same way as the experimental group, we recommended that the whole experimental procedure (handling, heating, and forcing to run) should be applied to lizard candidates to be released into the wild.

|                            | 353        | None.  |
|----------------------------|------------|--|
| 1<br>2                     | 354        | Funding  |
| 3<br>4                     | 355        | This research did not receive any specific grant from funding agencies in the public,              |
| 5<br>6                     | 356        | commercial, or not-for-profit sectors.   |
| 7<br>8<br>9<br>10<br>11    | 357<br>358 | Acknowledgements   |
|                            | 359        | We thank Enrique Font and Miguel A. Carretero for the revision of an earlier version of the        |
| 12<br>13                   | 360        | manuscript. The Area de Medio Ambiente of Cabildo Insular de El Hierro (local island               |
| 14<br>15                   | 361        | institution) for its permission to use the installations for experiments at the Breeding Centre of |
| 16                         | 362        | Frontera. We are grateful to Juan P. Pérez and Alfonso Quintero that helped in the everyday        |
| 17<br>18                   | 363        | lizard maintenance labours. We also thank Leslie Smith for the revision of the English.            |
| 19<br>20                   | 364        |  |
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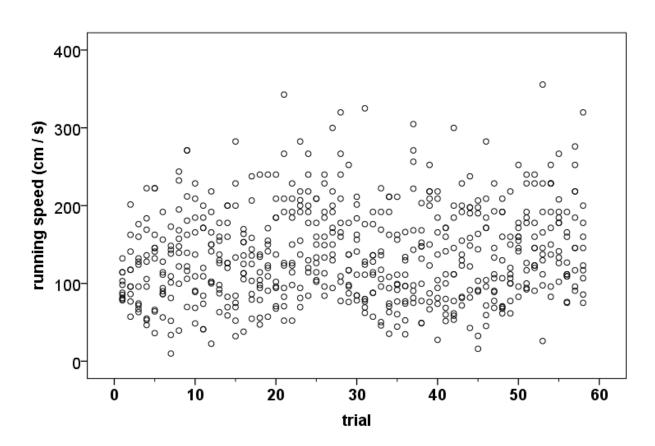
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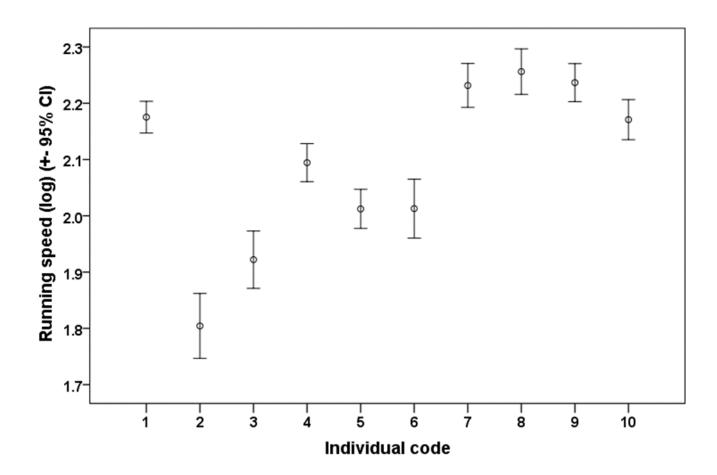
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- Fig. 1. Evolution of sprinting speeds (Y axis) for each experimental lizard in every trial day along the study period (X axis). A different code for every lizard is not included to clarify the figure.
- Fig. 2. Mean (+ 95% CI) running speed of each of the lizards used in the experiments during the whole experimental period.
- Fig. 3. Scatter plots of body condition (as residuals of log<sub>10</sub> (BM) on log<sub>10</sub> (SVL) to running speeds of lizards at the beginning and at the end of experimental trials.



González-Ortega et al., Fig. 1



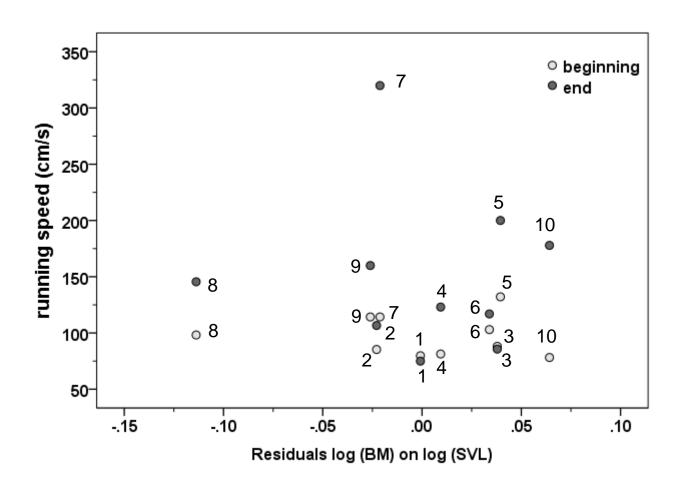


Table 1.- Statistics of GLM applied to running speed, showing the effect of every factor considered. Significant variables in bold.

| Origin      | numerator df | denominator df | F       | р      |
|-------------|--------------|----------------|---------|--------|
| Time        | 57           | 18.042         | 5.860   | <0.001 |
| Lizard ID   | 9            | 111.820        | 111.448 | <0.001 |
| Temperature | 1            | 208.696        | 2.046   | 0.154  |
| body mass   | 1            | 182.508        | 2.399   | 0.123  |
| SVL         | 1            | 89.535         | 0.812   | 0.370  |
|             |              |                |         |        |