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Insular geckos provide experimental evidence on refuge selection priorities by ectotherms

José João Pereira[a,b], Evandro P. Lopes[a,c], Miguel Á. Carretero[a,c,1], Raquel Vasconcelos[a,c,*,1]

[a] CIBIO Centro de Investigação em Biodiversidade e Recursos Genéticos, InBIO Laboratório Associado, Universidade do Porto, Campus de Vairão, Rua Padre Armando Quintas, 4485-661, Vairão, Portugal

[b] Departamento de Biologia, Faculdade de Ciências da Universidade do Porto, Rua do Campo Alegre 1021/1055, 4169-007, Porto, Portugal

[c] FECM Faculdade de Engenharias e Ciências do Mar, Uni-CV Universidade de Cabo Verde, CP 163, Mindelo, São Vicente, Cabo Verde

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ABSTRACT

In small sedentary ectotherms, patterns of spatial use result from the interplay between multiple, often conflicting factors, including abiotic and biotic interactions. Evaluating the costs and benefits of these pressures is crucial to make correct behavioural decisions in terms of fitness. The insular São Vicente's wall gecko Tarentola substituta provides a relatively simple model system to study these questions as it inhabits arid rocky habitats where refuges are limited, density of conspecifics is high, and terrestrial predators are almost absent. In the field, adults tend to find diurnal shelter under mid-sized rocks, frequently in male-female couples, while juveniles occupy small rocks which are thermally suboptimal. A lab experiment was conducted to determine the roles of ecological (shelter size and temperature) and social (conspecifics) factors in refuge selection. Single and pair combinations of geckos of different age and sex classes were allowed to select among four refuges: cold small, hot small, cold large, or hot large rock. Based on previous studies, we hypothesized that larger and thermally buffered rocks would be the preferred refuges, and that adult male-female pairs under the same rock would be more frequent than other combinations.Geckos primarily selected larger shelters, trading off the presence of conspecifics against thermal quality. In social terms, sex, adult condition and size-related disparity shaped the patterns of aggregation, resulting in lower aggregation frequencies between adults and juveniles and even between juveniles of different sizes. These results reasonably match field observations suggesting selection of rocks as diurnal retreats according to their thermal properties, and social aggregations mainly involving adult males and females but not juveniles. Overall, this combined evidence provides insights on the spatial ecology of geckos, and likely other ectotherms, under conditions of low predation, limited resources and high intraspecific competition, such as those prevailing on island systems.

1. Introduction

Refuge use by small sedentary ectotherms is influenced by several biotic and abiotic factors (Penado et al., 2015). Competition with conspecifics, or predator avoidance, and type and availability of refuges are some of the conditioning factors in small ectotherms fitness (Downes and Shine, 1998; Huey et al., 1989b). Among ectotherms, geckos stand out due to their key ecological role in food webs of arid habitats (Cloudsley-Thompson, 1991), especially on islands (Vitt and Caldwell, 2014). Furthermore, islands, especially arid ones, provide simplified systems of study, with scarcity of terrestrial predators, scarcity of interspecific competitors and low habitat complexity (Whittaker and Fernández-Palacios, 2007).

Use of refuges is crucial to prevent predation, but their selection is also constrained by abiotic factors. In particular, temperature is important for ectotherms since it can determine the rate of behaviours such as running, foraging, reproduction and survival (Angilletta, 2009; Huey, 1982). Active ectotherms aim to be close to their performance optimum (e.g. running), but when inactive they still need to prevent attaining critical temperatures while enabling digestion and possible behavioural interactions. Indeed, refuges play a role in aiding ectotherms to balance their thermal necessities with other biological functions in order to survive and thrive (Huey and Kingsolver, 1989). Diurnal reptiles select for basking sites and display postures enhancing

* Corresponding author at: CIBIO Centro de Investigação em Biodiversidade e Recursos Genéticos, InBIO Laboratório Associado, Universidade do Porto, Campus de Vairão, 4485-661, Vairão, Portugal.

E-mail address: raquel.vasconcelos@cibio.up.pt (R. Vasconcelos).

[1] These authors contributed equally to this work.

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heat gain or loss to keep body temperatures within a narrow range optimizing their biological functions (Huey, 1982). However, nocturnal geckos have limited possibilities for thermoregulation, attaining low body temperatures (Huey et al., 1989a; Kearney and Prevadec, 2000), although, conversely, they are more selective in their diurnal retreats to prevent overheating (Angilletta et al., 1999).

In addition, social and agonistic interactions may occur at the intraspecific level under refuges (Downes and Shine, 1998), which may influence the individual refuge selection. Geckos are frequently territorial sit-and-wait foragers, and hence prone to strong intraspecific interactions (Pianka and Vitt, 2003). Male lizards are much more aggressive than females towards juveniles and other adults (Cooper et al., 2015), and may actually hurt or display threating behaviour in cases of intraspecific male competition (Bohórquez-Alonso et al., 2014) or territorial defence (Marcellini, 1974). This suggests a prominent role of intraspecific competition for refuge in geckos, for determining individual fitness in these environments. Thus, refuge availability may pose social constraints among nocturnal geckos, since refuge choice becomes critical when gecko populations are close to carrying capacity, i.e, high gecko densities and limited refuges (Huey, 1982; Kearney, 2002; Shah et al., 2004). This scenario is particularly suited to arid islands (Vasconcelos et al., 2012a), contrary to continental or temperate areas. In the latter areas, factors such as thermal restriction or biotic interactions with other species usually keep populations at lower sizes and, hence, less limited by refuges, turning the refuge selection a multifactorial problem. Therefore, analysing the retreat-site features on islands may shed light upon the underlying social and ecological factors related to shelter choices that can then be applied to more complex systems. Beyond the intrinsic behavioural interest, this inference will be valuable in conservation terms considering the significance of microhabitat features for species distribution and abundance (Shah et al., 2004).

Summarizing, due to the high diurnal temperatures, adequate retreat-site choice of island geckos becomes crucial, since provides: (1) shelter from predators; (2) heat source for subsequent activity period (Huey et al., 1989a); (3) protection from over-heating (Kearney, 2002; Vasconcelos et al., 2012a); and (4) conditions within operative physiological ranges (Autumn et al., 1995).

The present study follows an experimental approach, based on previous field and lab experience (Carretero et al., 2016, Vasconcelos et al. 2012, 2017), to infer the roles of ecological and social factors on the refuge selection by a sedentary ectotherm under a context of low predation pressure and high interspecific competition. As model organism we use the nocturnal wall gecko *Tarentola substituta* endemic of São Vicente Island, Cabo Verde. This rock-dwelling gecko spends the daylight hours inactive under refuges, with preference for medium to big-sized rocks (Vasconcelos et al., 2012a). Conspecifics have been observed aggregating predominantly in adult male and female couples, sharing mid-size rocks within and outside the reproductive season (Vasconcelos et al., 2012a, 2017). This suggests persistent competition for optimal refuges between adults (mainly males) and juveniles (Vasconcelos et al., 2012a).

Here, we address the following questions: 1) Does thermal environment influence refuge selection in *T. substituta*? 2) Does refuge quality affect aggregation behaviour? 3) Does the presence of conspecifics influence aggregation or agonistic behaviour? We predict that geckos will select retreat-sites in a non-random fashion according first to their sex and body size, second to the refuge size and only third to the thermal properties of the refuge. The high density of conspecifics leads us to believe that this will take precedent over other factors, while rock size will follow since it has already been shown its importance for this species (Vasconcelos et al. 2012). Results in this experimental simplified system are expected to contribute for the understanding of refuge selection, and will also shed light on the understudied social behaviour of geckos and other small sedentary ectotherms. In a more applied way, we aim to establish a robust framework for the management and conservation of this endemic reptile and other species with similar requirements.

2. Methods

2.1. Study species

Tarentola substituta (Squamata, Phyllodactylidae) is an endemic nocturnal, medium-sized (average adult snout-vent length, SVL, of 51.60 ± 3.64 mm) flattened gecko with a long tail, adhesive pads, and a large head with a pointed snout (Joger, 1984; Vasconcelos et al., 2012b). Adult males of the species are larger and heavier considering absolute SVL and body mass in comparison to adult females (Schleich, 1987). It is a rock-dwelling species which exclusively uses rocks as retreat-sites during high diurnal temperatures, with temperatures under refuges ranging between 22 and 41 °C depending on the hour of the day and size of the rock (Vasconcelos et al., 2012a). The species remains active throughout the year (Schleich, 1987). It is locally very abundant and exclusively occurs across the vast arid areas of São Vicente Island, avoiding the scarce sub-humid or sandy areas (Vasconcelos et al., 2013).

2.2. Experiment description

A total of 45 individuals were collected from a single population in Calhau (16° 51′ 11.2″ N, 24° 52′ 11.8″ W), in equal number of males, females and juveniles. The collection period (October 30, 2017) fell outside the reproductive season to prevent interaction with copulation and egg-laying. Habitat use and thermal ecology have already been assessed during the same period (Vasconcelos et al., 2012a; Carretero et al., 2016). Geckos were transported to the laboratory, where they were identified and placed in individually tagged cloth bags to reduce stress. The SVL of each individual (Ind) was measured to the nearest 1 mm with a ruler and its body mass weighted to the nearest 0.001 g (Table A1 in Appendixes), using a digital scale (Nahita Electric balance series 5153). Every individual was classified as adult or juvenile based on its SVL (juvenile ≤ 45 mm) and on the presence of evident secondary sexual characters (e.g. enlarged cloacal spurs in males) (Vasconcelos et al., 2012a). Adult individuals were sexed considering the presence of evident primary (e.g. hemipenises in males; eggs or follicles in females) and secondary sexual characters (Vasconcelos et al., 2012a). Additionally, males also presented higher body mass and larger body size in contrast to adult females (Schleich, 1987; see below). Individuals were consequently divided into three classes: males (M), females (F) or juveniles (*J*). All geckos were individually marked on the ventral side using a temporary marker pen and photographed (dorsal and ventral sides) on top of millimetre paper for additional identification.

The refuge selection experiment tests were conducted from October 31 to November 4, 2017. Experiments consisted of single individuals and pair combinations of geckos placed inside a terrarium (1 × 0.3 × 0.4 m), with two sets of refuge options: a high-quality refuge, a medium-sized rock following the definition by Vasconcelos et al. (2012a) – herein designated as big rock (20 × 10 cm) – and a low-quality refuge – small rock (7 × 4 cm) herein – placed close and far from a heat source produced by a 150 W infrared lamp (Fig. 1). The experiment was conducted in a laboratory at the University of Cabo Verde deprived of natural light and maintained at 21 °C, which was ensured by monitoring the air temperature with a digital thermometer (HIBKO 14, precision ± 0.1 °C) before each round of 10 tests. A total of 520 refuge selection tests, randomized for time, terrarium, class or test type in R (code = function (n) sample (1:45, n, replace = T)), with n = 10 in case of single tests and n = 20 in case of pair tests) were performed. The initial test list was then manually corrected in order to avoid the same animal to perform consecutive tests. Tests were planned *a priori* to encompass a period of five days (October 31 to November 4, 2017), totalling 100–130 tests per day using 10 terraria simultaneously.

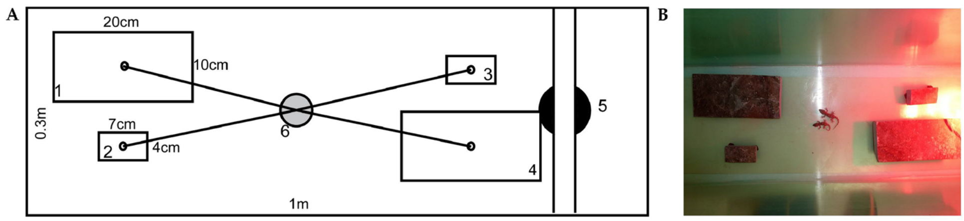

Fig. 1. Layout of the terraria used in the experiment. A) The available refuge centroids are equidistant to the opaque cup where the tested animals were placed: 1. cold medium-sized rock; 2, cold small-sized rock; 3, heated small-sized rock; 4, heated medium-sized rock; 5, 150 W infrared lamp; 6, opaque plastic cup. B) Photography of a F/J test after removing the cup.

Tests were conducted from 8 a.m. to 8 p.m., with 20 tests performed per hour in total, using 10 terraria. Every day, at 7 a.m., 1 h before removing the individuals from their individually marked bags, we turned on the lamps of each terrarium to create a gradient between 20 and 50 °C (Carretero et al., 2016). Multiple randomized replications for all combinations of pairs of gecko classes (male–male, M/M; male–female, M/F; male–juvenile, M/J; female–female, F/F, female–juvenile, F/J; juvenile–juvenile, J/J), for individual geckos, terraria and time intervals were tested. Indices for size disparity in SVL (SVL_I) and mass (W_I) were calculated as (SVL Ind1-SVL Ind2)/ (SVL Ind1 + SVL Ind2) and (W Ind1-W Ind2)/ (W Ind1 + W Ind2), respectively.

Experimental tests were conducted by observers who were unaware of the experimental treatment. All individuals were initially placed inside an opaque plastic cup on standby for two minutes in the middle of the terrarium, before the beginning of the test. The test started as the cup was manually and blindly lifted lasting 45 min, during which geckos were left completely undisturbed. At the end of this time, every rock in each terrarium was lifted and the position of each individual was recorded (under refuge – big or small rocks – or outside refuge – on the walls, ground; on the hot or cold side of the terrarium). It was also registered if there was aggregation. After each test, individuals were placed back inside their bags and the following test would ensue. The test was considered not valid in the few cases when geckos escaped from the terrarium or a technical problem happened and removed from the analyses. In total, we performed 455 valid lab tests (of the 520 planned ones), which encompassed 89 single animal tests and 366 pair tests. Table A2 contains the details of the experiments and describes the variables used.

Four data-loggers (i-buttons Thermochron temperature loggers DS1923, Maxim Integrated Products) were placed beneath each rock of and rotated between randomly chosen terraria to monitor temperature (to the nearest 0.01 °C) and relative humidity (to the nearest 0.01%) during the experiments, ensuring the differences between the four types of refuges (See Table A3).

2.3. Ethical note

Individuals were kept in captivity for five days. While not tested, geckos were kept inside individually tagged cloth-bags to minimise stress (Anonymous, 2018). Geckos were released back in the collecting site after the experiments. Male/female individuals found together in the wild were retrieved to the respective original rocks. Collecting permit was provided by S. Araújo from ‘Direcção Nacional do Ambiente’ and experiments followed the ethical guidelines of Uni-CV, the ABS/ASAB guidelines for ethical treatment of animals and in Anonymous (2018).

2.4. Statistical analysis

Biometric variables were log-transformed to ensure normality by means of Shapiro-Wilk tests and compared between classes using ANOVA (SVL and W) or ANCOVA (W with SVL as covariate). Temperature and humidity under the four refuges were compared by means of ANOVA for repeated measures with refuge size and refuge temperature as independent variables. The effect of the different factors (e.g.: temperature, Temp; refuge type, Ref; presence of a conspecific, Agg; class of the individual and/or conspecific, Class/ Test; differences in SVL, SVL_I, and mass, W_I, see Table A2) shaping refuge selection of *T. substituta* in a controlled environment (constant air temperature and light conditions) were tested using Generalized Linear Mixed-Models (GLMM) using the glmer R-package from the lmer4 library with binomial as family. In single animal tests, the dependent variable was the presence or absence of an individual under a rock, Out, and rock size (big/small), class of the individual (M/F/J), and temperature (hot/cold) were classified as independent variables, even though other combinations were also tested (see Table A4). Also, SVL and W were used as continuous predictors. For the pair tests, the dependent variables were aggregation and presence outside the refuge. Rock size, temperature, SVL_I, W_I and test type (Table A2) were used as independent variables, while in the models using temperature as dependent variable, aggregation, SVL_I, W_I, refuge and test type were used as independent variables (see Tables 1 and A4). In all tests, the variable Ind was used as random factor. Model evaluation were performed according Burnham and Anderson (2003) and Zuur et al. (2009). The models with the lowest AIC score, lower variable complexity and most explanatory significant results were selected among the multiple tested models. The significance level was set at $P = 0.05$ in all cases.

3. Results

3.1. Individual and refuge differences

Juveniles, females and males SVL measured 37.7 ± 0.1, 51.1 ± 0.1, and 53.3 ± 0.1 mm, respectively; and weighted 1.51 ± 0.01, 4.55 ± 0.01, and 4.96 ± 0.01 g, respectively (average ± SD). These measurements did not deviate from normality (Shaphiro-Wilks, tests $P > 0.2$). Beyond the obvious result that juveniles were smaller and lighter than adults, in our sample males were marginally longer than females (ANOVA $F_{1,28} = 3.90$, $P = 0.05$) but neither heavier in absolute terms (ANOVA $F_{1,28} = 0.99$, $P = 0.33$) nor heavier for the same length than them (ANCOVA $F_{1,26} = 01.52$, $P = 0.23$).

From the four data loggers, temperatures registered were (average ± SD): small/ hot refuge (30.03 ± 3.20 °C); big/hot refuge (30.28 ± 3.46 °C); small/cold refuge (26.31 ± 1.98 °C); big/cold refuge (26.37 ± 2.06 °C). Hot rocks were consistently much hotter than cold rocks across tests regardless their size while small rocks were slightly hotter without no interaction between both factors (ANOVA hot/cold $F_{1,440} = 706.53$, $P = 10^{-6}$; big/small $F_{1,440} = 7.32$, $P = 0.007$; hot/cold*big/small $F_{1,440} = 2.82$, $P = 0.09$). As for humidity, hot rocks were always less humid than cold rocks, big rocks were more humid than small rocks, but humidity differences were more contrasted between big hot and small hot rocks (ANOVA hot/cold $F_{1,440} = 706.53$, $P = 10^{-6}$; big/small $F_{1,440} = 7.32$, $P = 0.007$; hot/cold*big/small $F_{1,440} = 2.82$, $P = 0.09$).

3.2. Refuge use

Most individuals were found under refuges, namely 692 times (Fig. 2). Individuals were registered outside refuge (in a wall or ground) 131 times (one case in single tests: F = 1; and 130 times in pair tests: F/F = 15, F/J = 31, J/J = 43, M/F = 12, M/J = 23, M/M = 6; Fig. 2).

Table 1
Most relevant models for single (S) and pair tests (P), with the respective AIC, and coefficients (Coef), standard deviation (SD) and *P*-values of significant outputs (* stands for *P*-values < 0.050, n.s. for non-significant outputs, and 1 | Ind stands for the variable used as random factor). Table A2 provides details on the variables used in the models.

Code	Variables	AIC	Output	*P*-value		Coef	SD
S1	Ref ˜ Temp + (1 | Ind)	9.2	Temp[‡]	0.000	*	−334.1	15.5
S2	Ref ˜ SVL * W + (1 | Ind)	12.8	All	n.s.			
S3	Ref ˜ Temp * W + (1 | Ind)	13.2	Temp[‡]	0.000	*	−2138.4	37.3
			W[‡]	0.000	*	4935.5	123.6
			Temp W[‡]	0.000	*	−4934.0	123.7
S4	Ref ˜ W + (1 | Ind)	13.6	W	0.676			
S5	Ref ˜ SVL + (1 | Ind)	13.9	SVL	0.781			
S6	Ref ˜ Class + (1 | Ind)	16.7	All	n.s.			
S7	Ref ˜ Class * Temp + (1 | Ind)	17.2	All	n.s.			
P1	Out ˜ Temp * W_I + (1 | Ind1_2)	312.8	Temp W_I	0.033	*	−1.6	0.7
			W_I	0.021	*	1.8	0.8
			Temp W_I	0.663			
P2	Out ˜ Temp * SVL_I + (1 | Ind1_2)	318.4	Temp	0.021	*	−1.6	0.7
			SVL_I	0.046	*	4.5	2.2
			Temp SVL_I	0.648			
P3	Out ˜ Test * SVL_I * W I + (1 | Ind1_2)	614.5	F/J	0.070			
			J/J	0.282			
			M/F	0.660			
			M/J	0.339			
			M/M	0.400			
			SVL_I	0.193			
			W_I	0.057			
			F/J SVL_I	0.048	*	−71.2	36.0
			J/J SVL_I	0.492			
			M/F SVL_I	0.458			
			M/J SVL_I	0.067			
			M/M SVL_I	0.349			
			F/J W_I	0.072			
			J/J W_I	0.284			
			M/F W_I	0.158			
			M/J W_I	0.124			
			M/M W_I	0.431			
			SVL_I W_I	0.061			
			F/J SVL_I W_I	0.039	*	383.6	185.8
			J/J SVL_I W_I	0.157			
			M/F SVL_I W_I	0.089			
			M/J SVL_I W_I	0.044	*	375.3	186.6
			M/M SVL_I W_I	0.716			
P4	Agg ˜ Temp * W I * SVL_I + (1 | Ind1_2)	646.9	All	n.s.			
P5	Out ˜ Test + (1 | Ind1_2)	661.3	F/J	0.034	*	0.7	0.3
			J/J	0.000	*	1.2	0.3
			M/F	0.890			
			M/J	0.094			
			M/M	0.106			
P6	Agg ˜ Test * Temp * SVL_I + (1 | Ind1_2)	661.4	F/J	0.008	*	3.0	1.1
			J/J	0.064			
			M/F	0.007	*	2.5	0.9
			M/J	0.950			
			M/M	0.023	*	2.5	1.1
			Temp	0.143			
			SVL_I	0.031	*	22.8	10.6
			F/J Temp	0.750			
			J/J Temp	0.538			
			M/F Temp	0.200			
			M/J Temp	0.725			
			M/M Temp	0.108			
			F/J SVL_I	0.014	*	−28.7	11.7
			J/J SVL_I	0.024	*	−34.7	15.4
			M/F SVL_I	0.016	*	−35.5	14.7
			M/J SVL_I	0.103			
			M/M SVL_I	0.091			
			Temp SVL_I	0.277			
			F/J Temp SVL_I	0.433			
			J/J Temp SVL_I	0.442			
			M/F Temp SVL_I	0.969			
			M/J Temp SVL_I	0.531			
			M/M Temp SVL_I	0.099			
P7	Agg ˜ Test * W I * SVL_I + (1 | Ind1_2)	703.3	F/J	0.540			
			J/J	0.243			
			M/F	0.577			
			M/J	0.044	*	−18.2	9.1
			M/M	0.395			
			SVL_I	0.716			
			W_I	0.007	*	−16.6	6.2
			F/J SVL_I	0.704			
			J/J SVL_I	0.635			
			M/F SVL_I	0.330			
			M/J SVL_I	0.028	*	136.4	62.2
			M/M SVL_I	0.594			
			F/J W_I	0.162			
			J/J W_I	0.001	*	25.4	7.9
			M/F W_I	0.065			
			M/J W_I	0.014	*	45.0	18.3
			M/M W_I	0.095			
			SVL_I W_I	0.017	*	195.4	81.5
			F/J SVL_I W_I	0.024	*	−189.8	84.3
			J/J SVL_I W_I	0.004	*	−286.6	100.5
			M/F SVL_I W_I	0.289			
			M/J SVL_I W_I	0.002	*	−411.3	134.0
			M/M SVL_I W_I	0.365			
P8	Agg ˜ W I * SVL_I + (1 | Ind1_2)	707.0	All	n.s.			
P9	Agg ˜ Test * Temp + (1 | Ind1_2)	724.5	F/J	0.081			
			J/J	0.859			
			M/F	0.191			
			M/J	0.078			
			M/M	0.150			
			Temp	0.093			
			F/J Temp	0.010	*	−1.7	0.6
			J/J Temp	0.628			
			M/F Temp	0.024	*	−1.5	0.7
			M/J Temp	0.410			
			M/M Temp	0.448			
P10	Temp ˜ Ref * SVL_I * W_I + (1 | Ind1_2)	750.4	All	n.s.			

(*continued on next page*)

Table 1 (*continued*)

Code	Variables	AIC	Output	*P*-value		Coef	SD
P11	Temp ~ Ref * SVL_I + (1 | Ind1_2)	777.8	All	n.s.			
P12	Temp ~ Ref * W_I + (1 | Ind1_2)	784.2	All	n.s.			
P13	Temp ~Test * SVL_I+ (1 | Ind1_2)	838.8	F/J	0.012	*	1.7	0.7
			J/J	0.258			
			M/F	0.315			
			M/J	0.320			
			M/M	0.962			
			SVL_I	0.987			
			F/J SVL_I	0.075			
			J/J SVL_I	0.554			
			M/F SVL_I	0.172			
			M/J SVL_I	0.472			
			M/M SVL_I	0.516			
P14	Temp ~ Agg* SVL_I + (1 | Ind1_2)	842.7	All	n.s.			
P15	Temp ~ Agg* W_I + (1 | Ind1_2)	848.6	All	n.s.			
P16	Temp ~Test * Agg+ (1 | Ind1_2)	901.9	F/J	0.626			
			J/J	0.446			
			M/F	0.486			
			M/J	0.812			
			M/M	0.600			
			Agg	0.092			
			F/J Agg	0.009	*	−1.7	0.6
			J/J Agg	0.629			
			M/F Agg	0.023	*	−1.5	0.7
			M/J Agg	0.409			
			M/M Agg	0.446			
P17	Temp ~Test + (1 | Ind1_2)	906.4	All	n.s.			

ǂ Significance due to the single observation: a juvenile using the hot and small refuge.

n total, the big rocks were selected as refuge 646 times (Pairs: F/F = 104, F/J = 105, J/J = 86, M/F = 85, M/J = 81, M/M = 99; Fig. 2). As for the smaller rocks, individuals used them as refuge 44 times in total, with only one occurrence (a juvenile) in the single animal tests.

Comparing the selection of hot versus cold side of the terraria (Temp), there were 387 geckos observations on the colder side of the terraria, of which 37 times in the single animal tests and 350 times for the pair tests. As for the hot side, it was chosen 350 times in total, 51 times in the single animals tests and 299 in the pair tests. Each diferent gecko class had a similar number of observations in the colder and in the hotter area of the terrarium.

3.3. *Aggregation*

During pair tests, individuals were found alone under a rock 560 times and aggregated under the same rock in 172 cases (F/F = 30, F/J = 32, J/J = 36, M/F = 24, M/J = 16, M/M = 34; see Fig. 2).

3.4. *Interactions between refuge use, temperature and aggregation*

GLMM models revealed complex relationships between rock size and temperature when geckos selected for refuges individually which added to the effect of aggregation when geckos were tested in pairs (Table 1). In some of the models there was evidence that sexual maturity and size disparity modulated the results.

Specifically, for the single animal tests, the results confirmed a clear pattern for seeking refuge under bigger rocks, with no clear preference of temperature (Fig. 2). Results also showed no significant differences in the GLMM models among gecko classes, sizes or weights, although juveniles seem to prefer hot rocks (model AS10 in Table A4 and raw data, available at 10.6084/m9.figshare.7880357). In pair tests

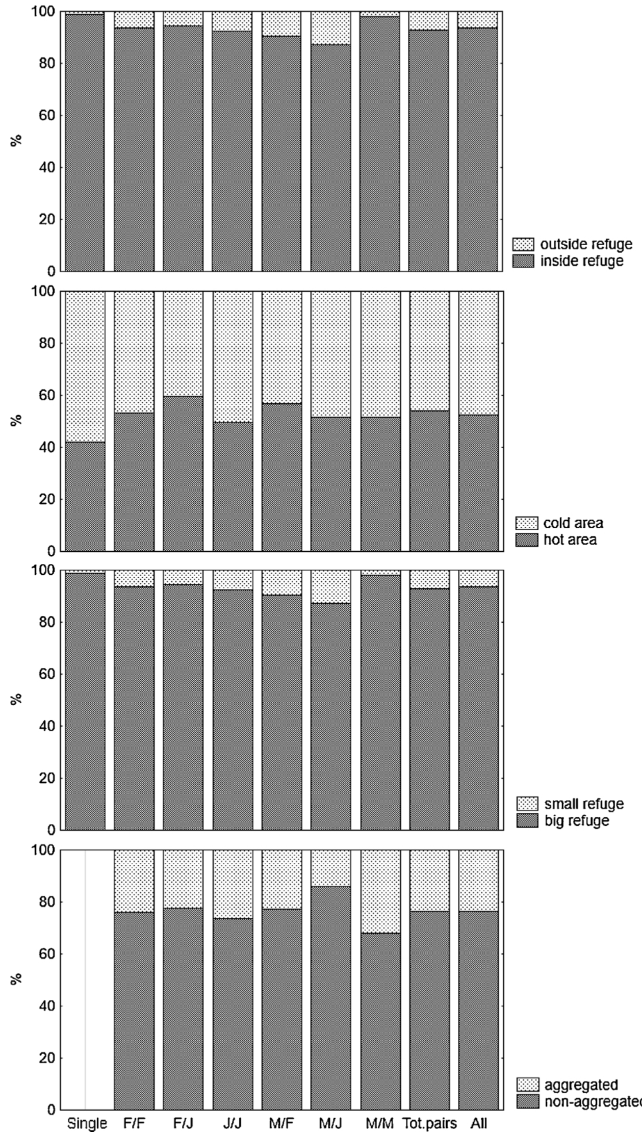

Fig. 2. Test results. Percentage of individuals found under or outside refuges, on the hot or cold side of the terraria, using small or big refuges, and aggregated or not, considering the type of test (single, pairs, and in all tests).

(Table 1), the GLMM showed that the percentage of individuals found outside refuges was significantly associated to juveniles (both in J/J and adult/juvenile tests), cold temperatures, to larger differences of SVL and weights between tested individuals, with a degree of interaction among these variables (Fig. 2; Table 1 and raw data). However, no significant values were detected in the GLMM test for refuge types among gecko classes in pair tests (Fig. 2), although smaller rocks were significantly more used as refuges in the colder side of the terrarium (Table A4 and raw data).

Males and juveniles aggregated less than other pairs (Fig. 2). As expected, such trend was significantly mediated by SVL and/or weight disparities in GLMM models (Table 1). In fact, low aggregation between both adult classes and juveniles and even between juveniles was mediated by disparities in SVL and/or body mass (Table 1). M/F tests seem to only interact significantly with SVL_I. The variable W_I *per se* was also significant for aggregation. Lastly, the combinations F/J and M/F differed from the remaining results for aggregation since when accounting for temperature, in the first case also when using W_I as covariant, the interaction was significant (Table 1). In both cases, geckos aggregated more when they were in the colder area of the terrarium (check raw data).

4. Discussion

The results supported a clear refuge selection by *T. substituta*. As such, geckos selected refuges based on size, temperature and presence of conspecifics. However, these individuals seem to prioritize one factor (refuge size) above the others. Remarkably, lab results reasonably matched with our systematic observations in the field (Vasconcelos et al., 2012a), proving the realism or the experiment and providing insights on the organization of the spatial priorities for this species and for other sedentary ectotherms. In particular, our models suggest that geckos prioritize refuge size having their thermal properties a subsidiary role while social aggregations tend to be lower between males and juveniles, with size disparity between individuals having an important influence.

4.1. Under or outside refuge?

The study species seeks refuge during daytime in order to fulfil its thermal requirements as a nocturnal ectotherm and find protection from aerial predators (Vasconcelos et al., 2012a). As expected, most geckos tended to use the refuges provided in the experiment as diurnal retreat. Likely, this reflects the secretive behaviour and foraging strategy of *Tarentola* geckos (Lisičić et al., 2012; Penado et al., 2015; Vroonen et al., 2012) but also the resemblance between artificial refuges and those commonly used in the field (Vasconcelos et al., 2012a). In fact, in single tests, there is only one case registered of a female outside the refuge during the experiment. Furthermore, they tended to select big refuges regardless their sex and size. More importantly, this trend did not seem to be majorly affected by either the thermal properties of the rocks or the presence of conspecifics. This suggests that refuge size takes precedence on the other two factors. Geckos may find other resources under large-sized rocks (see below) that they cannot find under small ones, while ensuring a better thermal microhabitat even under similar radiation (Penado et al., 2015; Schlesinger and Shine, 1994a; Vasconcelos et al., 2012a).

Interactions between adults (of either sex) and juveniles are among the tests with higher number of cases with individuals found outside refuges, where juveniles are the ones found the most outside of the refuge. Indeed, larger individuals have been observed to dominate smaller conspecifics (Downes and Shine, 1998; Penado et al., 2015; Stamps, 1977b; Stamps and Tanaka, 1981; Williams and McBrayer, 2007), promoting the possibility of agonistic interactions and competition for optimal retreat-sites. Under laboratory conditions, larger male velvet geckos *Oedura lesueurii* forced smaller subordinate males to suboptimal retreat-sites (Downes and Shine, 1998). The present experimental design only provided a single refuge of each type, which was expected to force the subordinate individual to use a suboptimal refuge. However, here, large rocks were apparently optimal in either cold or hot side of the terraria since the experimental design avoided temperatures close to critical maximum. Therefore, one of the apparent optimal refuges was available, which could have been selected instead of staying outside refuges. Nevertheless, in such a small area as a terrarium, the dominance effect might have led juveniles out of refuges. The findings of both Vasconcelos et al. (2012a) and our own field observations indicate a tendency for juveniles to be found under small rocks, which may suggest a competitive displacement to these sub-optimal refuges. Remarkably, in those tests between two adult males, the percentage of animals outside refuges was the lowest. This suggests that, in situations of balance between males similar in size and mass, staying under a refuge may represent a temporary state while geckos establish dominance. However, intriguingly juvenile–juvenile tests yielded the highest number of cases outside refuges. Apparently, intraspecific competition starting earlier than sexual maturity may have resulted in clear subordinate and dominant juveniles mediated by size differences (even though they were harder to detect by us due to the smaller SVL and weight differences between juveniles) or individual aggressive/ passive behaviour (Civantos, 2000; Stamps, 1977a; Stamps and Tanaka, 1981). All the above is well supported by the models regarding Out variable, where size and/or weight disparities significantly interacted with M/J, F/J and J/J tests.

4.2. Big or small rocks, hot or cold refuges?

There was a clear preference, in all pair combinations, for the larger rock regardless the temperature, with no clear differences among test types. Also, in single tests, such preference stands, and only a juvenile was registered using a small rock. This may be attributed to the larger area of protection that a big rock offers, mainly shielding the animal from predators (Croak et al., 2008; Penado et al., 2015; Shah et al., 2004), but also due to its better thermal and hydric properties (buffered temperature variation and higher humidity values, see data-logger tests and Vasconcelos et al., 2012a). Nonetheless, São Vicente's wall gecko has few terrestrial predators and big rocks were chosen despite the temperature it yielded for most tests (both cold and hot sides of the terraria were chosen in roughly the same proportions; Fig. 2). Thus, preference for larger rocks may rely on other factors. Probably, large refuges harboured or attracted more potential prey beneath them (Hódar et al., 2006). Literature on nocturnal geckos and diurnal lizards frequently supports a preference for warmer refuges by day (Downes and Shine, 1998; Shah, 2002; Langkilde and Shine, 2004; Shah et al., 2004; Aguilar and Cruz, 2010), underlying its importance in aspects such improved locomotor and digestive performance. Notwithstanding, the environmental context is important to understand the thermoregulatory behaviour of this species. In such extreme conditions as those in São Vicente, low temperatures and humidity do not pose a constraint, as these individuals are mainly active at night, preferred temperatures are relatively low, and are quite resistant to dehydration (Carretero et al., 2016). In the study area, geckos have a clear need of refuge during high daily temperatures but avoided those attaining extreme temperatures risking overheating and possibly death (Vasconcelos et al., 2012a). This risk is higher when smaller rocks are chosen, and, in fact, dead juveniles have been occasionally found in the field (pers. obs.). Despite the data-loggers recording similar mean temperatures under each refuge (˜30 °C), hot big rocks were preferred over the smaller hot ones and smaller rocks are significantly more used as refuges in the colder side of the terrarium. Due to its size, the smaller rock provides less heterogeneous temperatures (from centre to periphery) compared to the larger one, while offers a poorer shelter from the extreme heat from the infra-red lamp. It is also important to highlight that significant differences in temperature of the shared refuges were registered in females/juveniles, and males/females tests.

4.3. Aggregation

Although the overall levels of aggregation observed in the laboratory apparently approached those expected by chance (0.25%), some pair combinations clearly deviated from that pattern. In particular aggregation between males and juveniles was much lower than expected by chance. This contrasts with juvenile-juvenile and male-male combinations whose aggregation percentage was higher than expected by chance. This pattern is expectable if agonistic interactions between males and juveniles occurred, and juveniles were expelled from the best available refuges, as observed in previous studies on this species (Vasconcelos et al., 2012a, 2017). In fact, the significant interactions of the aggregation events in male-juvenile, female-juvenile and juvenile-juvenile with size-related disparities (either SVL or weight) are a strong indication that differences in size may drive dominance for refuge use, suggesting the possibility of hierarchies even before sexual maturity, as suggested above. The high number of male-male aggregations was, however, unexpected since, in natural conditions, according to our field observations, dominant male geckos do not tolerate other males under the same retreat-site similarly to what was seen in other species

(Downes and Shine, 1998; Schlesinger and Shine, 1994b). A tentative explanation is that the short duration of test did not provide enough time for the adult males to establish dominance status, and therefore they were still interacting to determine which one would stay. It is also worth noting that the experiment was conducted out of the species reproduction period (Vasconcelos et al., 2012a), which could promote a less aggressive responses between males. Nevertheless, our results clearly evidence that territoriality and social interactions in this species extend beyond mating, likely involving other ecological aspects such as foraging, thermoregulation and defence (Mouton et al., 2000; Cooper et al., 2000).

In principle, male-female pairs should have yielded the highest aggregation frequency as a way to increase reproductive success and reduce agonistic interactions (Vasconcelos et al., 2017). However, during the sampling period, geckos were not reproducing. Moreover, one must consider that those pairs were randomly chosen and did not reflect the possibility of stable pairs in nature. Thus, males or females would not be necessarily prone to aggregate because they were unfamiliar. In fact, aggregation events were lower that observed in nature during the same period (32–38%, Vasconcelos et al., 2017). Remarkably, during a mark-capture-recapture census performed during the time of this experiment, two male-female pairs were found together under different rocks after four days (unpublished results) suggesting some degree of pair fidelity resilient to the interruption of reproduction and to perturbation. This aspect deserves further investigation since such stable social structure is uncommon among reptiles (Bull, 1988).

5. Conclusions

Overall, despite the simplicity of this laboratory setup, our results confirmed suggestions of previous field studies unravelling the complexity of behavioural decisions in refuge use by sedentary ectotherms. Decision-making abilities are needed to trade-off between multiple, often conflicting pressures encompassing social interactions, thermoregulation, predator avoidance and foraging. In this arid island system, geckos almost lack competitors and predators and are likely approaching carrying capacity of the system in terms of refuges and food. In these conditions, competition with conspecifics apparently plays a dominant role and ensuring a quality refuge has priority. Although the study we conducted was out of the reproductive season, our unpublished field observations also suggest some stability on the social relations between adult males and females (see Barry et al., 2014, for a similar case), which may carry non-reproductive benefits for both partners which deserves further attention.

Data accessibility

All data necessary for the replication of this study is present in Appendixes and on-line files, including raw data sets, in figshare (available at 10.6084/m9.figshare.7880357).

Declarations of interest

None.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.beproc.2019.03.008.

References

Aguilar, R., Cruz, F.B., 2010. Refuge use in a Patagonian Nocturnal Lizard, *Homonota darwini*: the role of temperature. J. Herpetol. 44 (2), 236–241. https://doi.org/10.1670/08-270.1.

Angilletta, M.J., 2009. Thermal Adaptation: A Theoretical and Empirical Synthesis. Oxford University Press.

Angilletta Jr, M.J., Montgomery, L.G., Werner, Y.L., 1999. Temperature preference in geckos: diel variation in juveniles and adults. Herpetologica 55 (2), 212–222.

Anonymous, 2018. Guidelines for the treatment of animals in behavioural research and teaching. Anim. Behav. 135, I–X. https://doi.org/10.1016/j.anbehav.2017.10.001.

Autumn, K., Nardo, D.F., De, 1995. Society for the study of amphibians and reptiles behavioral thermoregulation increases growth rate in a nocturnal lizard. J. Herpetol. 29 (2), 157–162.

Barry, M., Shanas, U., Brunton, D.H., 2014. Year-round mixed-age shelter aggregations in Duvaucel’s geckos (*Hoplodactylus duvaucelii*). Herpetologica 70, 395–406.

Bohórquez-Alonso, M.L., Martínez-Cotrina, J., Molina-Borja, M., 2014. Morphological and behavioural correlates of contest success in male yellow-headed geckos, *Gonatodes albogularis*: sequential assessment or self-assessment? Behaviour 151 (11), 1535–1554. https://doi.org/10.1163/1568539X-00003199.

Bull, C.M., 1988. Mate fidelity in an Australian lizard *Trachydosaurus rugosus*. Behav. Ecol. Sociobiol. 23 (1), 45–49. https://doi.org/10.1007/BF00303057.

Burnham, K.P., Anderson, D.R., 2003. Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach. Springer, New York.

Carretero, M.A., Lopes, E.P., Vasconcelos, R., 2016. An ecophysiological background for biogeographic patterns of two island lizards? Sci. Nat. 103 (11–12), 97. https://doi.org/10.1007/s00114-016-1422-8.

Civantos, E., 2000. Home-range ecology, aggressive behaviour, and survival in juvenile lizards, *Psammodromus algirus*. Can. J. Zool. 78 (9), 1681–1685. https://doi.org/10.1139/cjz-78-9-1681.

Cloudsley-Thompson, J.L., 1991. Ecophysiology of Desert Arthropods and Reptiles. Springer, Berlin.

Cooper Jr, W., Van Wyk, J.H., Mouton, P.L.F., Al-Johany, A.M., Lemos-Espinal, J.A., Paulissen, M.A., Flowers, M., 2000. Lizard antipredatory behaviors preventing extraction from crevices. Herpetologica 394–401.

Cooper, W.E., Dimopoulos, I., Pafilis, P., 2015. Sex, age, and population density affect aggressive behaviors in Island lizards promoting cannibalism. Ethology 121 (3), 260–269. https://doi.org/10.1111/eth.12335.

Croak, B.M., Pike, D.A., Webb, J.K., Shine, R., 2008. Three-dimensional crevice structure affects retreat site selection by reptiles. Anim. Behav. 76 (6), 1875–1884. https://doi.org/10.1016/j.anbehav.2008.08.011.

Downes, S., Shine, R., 1998. Heat, sex or safety? Using habitat selection experiments to identify a lizard’s priorities. Anim. Behav. 55 (5), 1387–1396.

Hódar, J.A., Pleguezuelos, J.M., Villafranca, C., Fernández-Cardenete, J.R., 2006. Foraging mode of the Moorish gecko *Tarentola mauritanica* in an arid environment: inferences from abiotic setting, prey availability and dietary composition. J. Arid Environ. 65 (1), 83–93. https://doi.org/10.1016/j.jaridenv.2005.08.006.

Huey, R.B., 1982. Temperature, physiology, and the ecology of reptiles. Biology of the Reptilia. https://doi.org/10.1016/j.dsr.2014.07.003.

Huey, R.B., Kingsolver, J.G., 1989. Evolution of thermal sensitivity of ectotherm performance. Trends Ecol. Evol. 4 (5), 131–135. https://doi.org/10.1016/0169-5347(89)90211-5.

Huey, R.B., Niewiarowski, P.H., Kaufmann, J., Herron, J.C., 1989a. Thermal biology of nocturnal ectotherms: is sprint performance of geckos maximal at low body temperatures? Physiol. Zool. 62 (2), 488–504. https://doi.org/10.1086/physzool.62.2.30156181.

Huey, R.B., Peterson, C.R., Arnold, S.J., Porter, W.P., Huey, R.B., 1989b. Hot rocks and not so hot rocks: retreat site selection by garter snakes and its thermal consequences. Ecology 70 (4), 931–944. https://doi.org/10.2307/1941360.

Joger, U., 1984. Die Radiation der Gattung *Tarentola* in Makaronesiem (Reptilia: Sauria: Gekkonidae). Courier Forschungsinstitut Senckenberg 71, 91–111.

Kearney, M., 2002. Hot rocks and much-too-hot rocks: seasonal patterns of retreat-site selection by a nocturnal ectotherm. J. Therm. Biol. 27 (3), 205–218. https://doi.org/10.1016/S0306-4565(01)00085-7.

Kearney, M., Prevadec, M., 2000. Do nocturnal ectotherms thermoregulate? A study of the temperate gecko *Christinus marmoratus*. Ecology 81 (11), 2984–2996. https://doi.org/10.1890/0012-9658(2000)081[2984:DNETAS]2.0.CO;2.

Langkilde, T., Shine, R., 2004. Competing for crevices: interspecific conflict influences retreat-site selection in montane lizards. Oecologia 140 (4), 684–691. https://doi.

org/10.1007/s00442-004-1640-1.
Lisičić, D., Drakulić, S., Herrel, A., Dikić, D., Benković, V., Tadić, Z., 2012. Effect of competition on habitat utilization in two temperate climate gecko species. Ecol. Res. 27 (3), 551–560. https://doi.org/10.1007/s11284-011-0921-5.
Marcellini, D.L., 1974. Acoustic behavior of the gekkonid lizard, *Hemidactylus frenatus*. Herpetologica 30 (1), 44–52. https://doi.org/10.2307/3891412.
Mouton, Le, P., F.N, Geertsema, H., Visagie, L., 2000. Foraging mode of a group-living lizard, *Cordylus cataphractus* (Cordylidae). Afr. Zool. 35 (1), 1–7. https://doi.org/10.1080/15627020.2000.11407185.
Penado, A., Rocha, R., Sampaio, M., Gil, V., Carreira, B., Rebelo, R., 2015. Where to "rock"? Choice of retreat sites by a gecko in a semi-arid habitat. Acta Herpetol. 10 (1), 47–54. https://doi.org/10.13128/Acta.
Pianka, E.R., Vitt, L.J., 2003. Lizards: Windows to the Evolution of Diversity Vol. 5 University of California Press.
Schleich, H.H., 1987. Herpetofauna caboverdiana. Spixiana 12, 1–75.
Schlesinger, C.A., Shine, R., 1994a. Choosing a rock: perspectives of a bush-rock collector and a saxicolous lizard. Biol. Conserv. 67 (1), 49–56. https://doi.org/10.1016/0006-3207(94)90008-6.
Schlesinger, C.A., Shine, R., 1994b. Selection of diurnal retreat sites by the nocturnal gekkonid lizard *Oedura lesueurii*. Herpetologica 50 (2), 156–163.
Shah, B., 2002. Why Do Thick-Tailed Geckos (*Underwoodisaurus Milii*) Aggregate? University of Sydney, Sydney.
Shah, B., Shine, R., Hudson, S., Kearney, M., 2004. Experimental analysis of retreat-site selection by thick-tailed geckos *Nephrurus milii*. Austral Ecol. 29 (5), 547–552. https://doi.org/10.1111/j.1442-9993.2004.01388.x.
Stamps, J.A., 1977a. A field study of the ontogeny of social behavior in the lizard *Anolis aeneus*. Behavior 66 (1), 1–30. https://doi.org/10.1163/156853978X00396.
Stamps, J.A., 1977b. The relationship between resource competition, risk, and aggression in a tropical territorial lizard. Ecology 58 (2), 349–358. https://doi.org/10.2307/1935609.
Stamps, J., Tanaka, S., 1981. The influence of food and water on growth rates in a tropical lizard (*Anolis aeneus*). Ecology 62 (1), 33–40. https://doi.org/10.2307/1308184.
Vasconcelos, R., Santos, X., Carretero, M.A., 2012a. High temperatures constrain microhabitat selection and activity patterns of the insular Cape Verde wall gecko. J. Arid Environ. 81, 18–25. https://doi.org/10.1016/j.jaridenv.2012.01.013.
Vasconcelos, R., Perera, A., Geniez, P., Harris, D.J., Carranza, S., 2012b. An integrative taxonomic revision of the *Tarentola* geckos (Squamata, Phyllodactylidae) of the Cape Verde Islands. Zool. J. Linn. Soc. 164 (2), 328–360. https://doi.org/10.1111/j.1096-3642.2011.00768.x.
Vasconcelos, R., Brito, J.C., Carranza, S., Harris, D.J., 2013. Review of the distribution and conservation status of the terrestrial reptiles of the Cape Verde Islands. Oryx 47 (1), 77–87. https://doi.org/10.1017/S0030605311001438.
Vasconcelos, R., Rocha, S., Santos, X., 2017. Sharing refuges on arid islands: ecological and social influence on aggregation behaviour of wall geckos. PeerJ 5, e2802. https://doi.org/10.7717/peerj.2802.
Vitt, L.J., Caldwell, J.P., 2014. Herpetology. An Introductory Biology of Amphibians and Reptiles, 4th ed. Academic Press.
Vroonen, J., Vervust, B., Fulgione, D., Maselli, V., Van Damme, R., 2012. Physiological colour change in the Moorish gecko, *Tarentola mauritanica* (Squamata: Gekkonidae): effects of background, light, and temperature. Biol. J. Linn. Soc. 107 (June), 182–191. https://doi.org/10.1111/j.1095-8312.2012.01915.x.
Whittaker, R.J., Fernández-Palacios, J.M., 2007. Island Biogeography: Ecology, Evolution, and Conservation. Oxford University Press, New York.
Williams, S.C., McBrayer, L.D., 2007. Selection of microhabitat by the introduced Mediterranean gecko, *Hemidactylus turcicus*: influence of ambient light and distance to refuge. Southwest. Nat. 52 (4), 578–585. https://doi.org/10.1894/0038-4909(2007)52[578:SOMBTI]2.0.CO;2.
Zuur, A.F., Ieno, E.N., Walker, N., Saveliev, A.A., Smith, G.M., 2009. Mixed Effects Models and Extensions in Ecology with R. Springer, New York.