THE EFFECTS OF HABITAT FRAGMENTATION ON THE THERMAL PLASTICITY OF THE AEGEAN WALL LIZARD *(PODARCIS ERHARDII, LACERTIDAE)*.

by

Vincent Deem

A thesis submitted
in partial fulfillment of the requirements
for the degree of
Master of Science
(Natural Resources and Environment)
at the University of Michigan
April 2015

Faculty advisors:

Associate Professor Johannes Foufopoulos, Chair
Assistant Professor Ines Ibáñez

Abstract

Global climate change is altering the ecology of organisms across all major biomes and is likely to contribute to a rapidly increasing number of species extinctions. The effects of climate change are further exacerbated in fragmented landscapes, where isolated populations are known to be losing genetic diversity. This loss of genetic diversity is thought to impact the physiological flexibility (termed 'plasticity') that a species needs to survive the warmer, more fluctuating temperatures that are associated with global climate change.

In this study we examined the thermal plasticity of adult male Aegean wall lizards (*Podarcis erhardii,* Lacertidae) occurring on Cycladic land bridge islands (Aegean Sea, Greece). Populations were sampled from three different size islands ranging from 0.01 km^2 to 448 km^2. Previous studies have shown that *P. erhardii* exhibits a predictable gradient in genetic diversity correlating with island area and time since isolation. After collection, lizards were acclimated under identical thermal lab conditions for three weeks after which lizards were divided into control and treatment groups. Treatment groups were subjected to an elevated temperature regime for three weeks corresponding to local conditions under a warming climate while control groups were left under the initial cooler lab conditions. Thermal preference (T_p) and critical thermal maximum (CT_{max}) were quantified after the initial three week lab acclimation period and then again after a three week experimental manipulation period. Changes in these parameters were then used as measures of thermal plasticity. Overall conclusions from this study indicate that (i.) *P. erhardii* has surprisingly rigid thermal preferences, and (ii.) level of genetic impoverishment is not related to the extent of thermal plasticity in the species. Understanding how global warming might impact reptile populations isolated in fragmented landscapes will be critically important for evaluating a population's extinction risk and aid in guiding appropriate management decisions.

Acknowledgements

I would like to acknowledge all the people who helped make my research possible. I'd like to thank Scott Brenton for his help and support in the field as well as Hayden Hedman and Nick Louis for their help not only in the field but throughout my entire graduate school career. Thank you Dr. Ines Ibáñez for being on my committee and for all your help with my data analysis and writing. To my advisor, Dr. Johannes Foufopoulos, thank you for giving me this experience which has allowed me to grow as a person and of course for all your help in the field and at school. I'd also like to acknowledge Dr. Panayiotis Pafilis and the University of Athens for their support in Greece and Dr. Barry Sinervo and Dr. Matthew E. Gifford for their advice on experimental design. Thank you to the University of Michigan's School of Natural Resources and Environment and Rackham Graduate School for providing funding without which this research would not have been possible. Lastly I'd like to thank my parents for all their love in support during my time in graduate school and in everything I do.

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1. Introduction

Global climate change is altering the ecology of organisms across all major biomes and is likely to contribute to a rapidly increasing number of species extinctions (Wilson et al., 2005, Parmesan 2006). Future predictions from climate models vary somewhat, but in general there is a consensus that most regions will experience not only shifts in mean temperature (mostly towards hotter conditions) but also an increase in climate variability (Houghton et al., 2001; IPCC, 2013). Organisms can respond to these environmental changes either by shifting their geographic range to locations with more favorable climates (Buckley et al, 2008) or by adjusting to new environmental conditions through behavioral adaptation, physiological plasticity, or evolutionary (i.e. gene frequency) change (Seebacher, 2005). Recent research indicates that species are already shifting their ranges due to climate change and that local population extinctions are already occurring (McLaughlin et al, 2002, Thomas et al, 2004, Sinervo et al, 2010). Because habitat fragmentation is limiting the dispersal ability of many organisms and evolutionary change may not happen fast enough, acclimation to altered climatic conditions may prove the main process allowing species survival. However, loss of genetic diversity in reduced/isolated populations may negatively impact the thermal acclimation ability of a species (West-Eberhard, 1989). Previous studies have shown other forms of plasticity have been linked to heterozygosity and this link may also hold true for thermal acclimation responses (Dobzhansky, 1947; Lerner, 1954; Gillespie, 1989). By using

naturally isolated island populations of different sizes and levels of genetic diversity, this study attempts to shed light on how organisms will respond to future climate change through acclimation and how the genetic diversity of the population may play a role in this process.

Phenotypic plasticity is the differential expression of more than one form of morphology, physiological state, and/or behavior produced by a single genotype in response to environmental conditions (West-Eberhard, 1989; Scheiner, 1993). Phenotypic plasticity, unlike developmental plasticity or irreversible acclimation (Aubret and Shine, 2010), is considered a plastic response that is reversible and repeatable within an individual's lifetime (Seebacher, 2005).

Being able to physiologically respond to changes in the thermal environment is a particularly crucial phenotypic response due to the pervasive effects of temperature on physiological function (Haynie, 2001). The laws of thermodynamics determine both the direction and rate of biochemical reactions and therefore rate processes will fluctuate with changes in temperature unless buffered by compensatory responses. The ability to reversibly change rate processes to compensate for environmental variability and maintain constant rates in spite of a changing thermal environment are characteristics found in individuals of many species (Seebacher, 2005).

The direction and degree of plasticity to environmental factors (reaction norm) is genetically variable and subject to selection. (West-Eberhard, 1989). The genetic basis of plasticity has been described by three

different models that are not mutually exclusive. Model 1- Overdominance: Proposes that plasticity is a function of homozygosity (Scheiner, 1993). Overdominace is also described as heterozygous advantage in which heterozygotes possess adaptive values higher than homozygotes and are capable of responding very rapidly to alterations in the environment (Dobzhansky, 1947). Model 2- Pleiotropy: Plasticity is a function of the differential expression of the same gene in different environments and the expression of an allele in one environment is potentially independent of its expression in a different environment. Model 3- Epistasis: Plasticity is due to genes that determine the magnitude of a response to environmental effects which interact with genes that determine the average expression of the character. The trait mean and the trait plasticity are potentially independent characters (Scheiner, 1993).

This study will attempt to investigate the mechanism stated in Model 1 by examining the relationship between thermal plasticity and genetic diversity. Elucidating this relationship is of particular importance as climate change impacts are further exacerbated by the fact that many wildlife populations today are suffering from inbreeding depression and reduced genetic richness as a result of human-caused fragmentation of their natural habitats (Stork et al, 1999; Keyghobadi et al, 2005; Dixo et al, 2009.) The loss of genetic diversity seen in reduced/isolated populations may impact the physiological plasticity that a species needs to survive the warmer, more fluctuating temperatures that are associated with global climate change. We hypothesize that populations

suffering from inbreeding depression will have a reduced ability to acclimate to increased and more variable temperature regimes compared to populations with greater genetic diversity under the assumptions of the heterozygous advantage of Model 1. Previous studies have reported mixed results showing no correlation (Scheiner, 1993), a negative correlation (Yampolsky and Kalabushkin, 1991; Yampolsky and Scheiner, 1994), or a positive correlation (Dobzhansky, 1947) between heterozygosity and other forms of phenotypic plasticity.

Ectotherms (such as reptiles) depend on environmental temperature heterogeneity and behavioral responses to regulate their own internal body temperature within a specific range (i.e. thermal preference). Regulating body temperature within this range is required to maintain optimal physiological functions, particularly for ectotherms which are less able to buffer body temperature against ambient temperature through physiological mechanisms (Aubret and Shine, 2010). Many reptile species are at an increasing risk of extinction due to climate change, as temperatures are projected to increase faster than species can adapt or shift their ranges to cooler latitudes or altitudes (Buckley et al, 2013). As climate warms, the hours of activity for reptiles become restricted since higher temperatures force them to seek refuge in cooler places to avoid overheating (Sinervo et al, 2010). This can greatly reduce the time they can spend on vital activities such as searching for food and mates. Thus, given the current climatic trajectory, understanding how global warming might impact wildlife populations as a function of the

population's genetic variability, will be critically important for appropriate biodiversity management along fragmented landscapes.

This study examines a set of Greek island lizard populations that were subject to natural habitat fragmentation as the result of rising sea levels. Populations of the Aegean Wall lizard (*Podarcis erhardii)* in this region exhibit a predictable level of genetic diversity that varies according to island size and age (Hurston et al, 2009). We examined the thermal plasticity of *P. erhardii* in one large, genetically diverse island (Naxos), one intermediate sized island (Irakleia) and one smaller satellite islet (Aspronissi) with impoverished genetic diversity. In particular, we tested the effects of reduced heterozygosity on the thermal plasticity of this species. We hypothesize that the larger, more genetically diverse lizard population will exhibit a more plastic response to experimentally elevated thermal conditions and would be better able to cope with increased heat stress than the more genetically depauperate islet populations. Understanding how global warming might impact reptile populations isolated in fragmented landscapes will be critically important for evaluating a population's extinction risk and aid in guiding appropriate management decisions.

2. Methods

2.1 Study Locations

Podarcis erhardii (Lacertidae) lizards were collected from three different Cycladic islands that varied greatly in terms of size. The Cyclades archipelago (Aegean Sea, Greece) is a landbridge island system composed of over 200 islands and islets (Fig. 1). These islands formed one large land mass during the last ice age but have since been separated from each other by rising sea levels in the last 18,000 years (Foufopoulos and Ives, 1999). This provides an ideal system for the study of the long-term effects of habitat fragmentation on wildlife populations.

Naxos, with 448 km^2 the largest island, is characterized by a diverse Mediterranean landscape. Vegetation in most places consists of low, summer-deciduous, thorny shrubs known as 'phrygana'. Agriculture is widespread throughout the island's lowlands while the interior of the island is steep and mountainous. The second island, Irakleia (18km^2), lies approximately 6 km south of Naxos. Like Naxos, Irakleia is covered mostly by phrygana but given the relatively small size, fewer areas are devoted to agriculture. Aspronissi, the third island, is a small rock island 0.0102 km^2 in area approximately 1.5 km off of Naxos's western shore. It is characterized by large granodiorite boulders and low thick plant cover at its center. The island also serves as a predator-free nesting ground for several species of sea birds. All sampling sites were located 15-30m above sea-level and were separated by no more than 24 km and as a result are subject to the same climatic conditions.

2.2 Study Organism

The Aegean wall lizard, *Podarcis erhardii,* (Lacertidae, Reptilia) is a small, highly differentiated species reaching a maximum snout to vent length (SVL) of up to 75 mm (Valakos et al, 2008). *P. erhardii* is widely distributed across the Southern Balkan Peninsula and is found on most of the islands across the western portion of the Aegean Sea. It typically occurs in arid, stony places where it shelters in low, dense vegetation. This species is an excellent model organism for the study of the long-term effects of fragmentation due to its widespread distribution on landbridge islands and its weak over-water dispersal abilities. Lack of substantial vegetation for rafting, the cold waters of this region, relatively large inter-island distances, and the lizard's very poor floating abilities argue for little overwater dispersal; the existence of numerous morphologically distinct island subspecies, as well as pronounced genetic differentiation demonstrate that very little if any gene flow occurs between islands (Hurston et al, 2009).

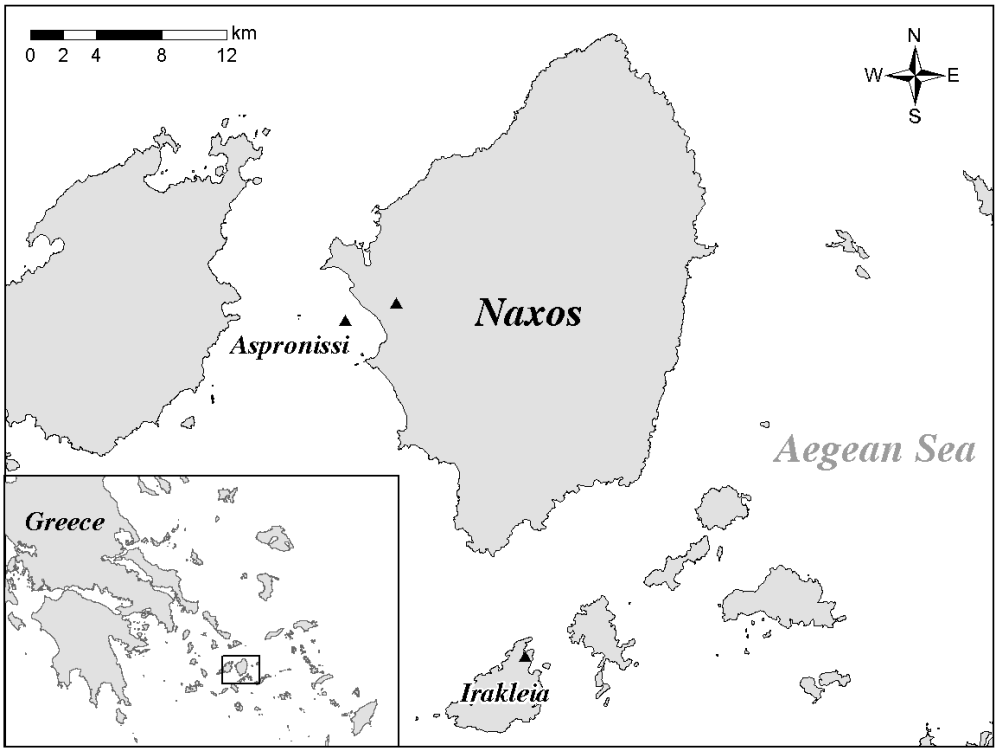

Figure 1. Map of the central Cyclades. Triangle markers indicate locations sampled.

2.3 Field Data

2.3.1 Environmental temperatures

To compare thermal environments, environmental temperature data were collected at each of the study locations (Naxos: E25.38° N37.05°; Irakleia: E25.47° N36.86; Aspronissi: E25.35° N37.05°) using HOBO data loggers (Onset, Model U23-003). Six loggers were deployed at each location with each logger having two probes, resulting in 12 total temperature readings per site. Each logger probe was inserted into a hollow lizard model constructed from a 1.5 cm diameter PVC pipe cut to approximately 8 cm in length and painted, corresponding to the approximate size and color of an adult *P. erhardii*. The data loggers were then placed in three different

microhabitats; two in full shade, two in mixed sun and shade (e.g., under partial vegetation cover) and two in full sun. Temperature was recorded at 5 min intervals over 2-8 sampling days at each of the study sites (Irakleia: 5/17-5/18/2014; Aspronissi: 5/21-5/23/2014; Naxos: 5/29-6/5/2014).

2.3.2 Collection of Specimens & Field Body Temperatures

Twenty lizards were captured from each of the study locations (N_{total} = 60). Collection took place between May-June, 2014. Lizards were captured using a string noose attached to a telescoping fishing pole. Each animal's activity was noted before capture and coded as one of three possible categories; basking, hiding, or moving. Field body temperature (T_b) was measured by inserting a glass rapid-read cloacal thermometer (Miller & Weber Model T-6000) approximately 5 mm into the cloaca. Care was taken not to touch the lizard's abdomen to prevent altering the lizard's core temperature. Substrate temperature was then measured by touching the tip of the cloacal thermometer to the approximate location of the lizard's capture. Air temperature was also measured by shading the thermometer and holding it 5 cm above the point where substrate temperature was measured.

2.4 Housing

After collection, all lizards were brought back to a lab on Naxos. Lizards were housed individually under standardized conditions in plastic terrariums (~32 x 17 x 9 cm) with screen lids. To create a thermal gradient in

the terrariums, a 40W incandescent light bulb with an aluminum reflector shield, hanging at a height of approximately 25 cm was placed at one end of the terrarium. A rock for basking was placed under the light and at the opposite end of the terrarium an additional rock shelter was provided to create a cooler refuge. Timers were used to turn the basking lights on at 06:00 and off at 18:00 to maintain 12-12h day/night cycles. Lizards were fed meal worms (*Tenebrio sp.)* until satiation every other day during the study and provided water in a small dish *ad libitum*.

2.5 Experimental Procedure

The optimal temperature range for biochemical and physiological activities often corresponds to the body temperature selected by a lizard, and can be estimated by measuring its thermal preference (T_p) in a laboratory thermal gradient (Li et al, 2009). The critical thermal maximum (CT_{max}) is the highest tolerable temperature of a species (Leal and Gunderson, 2012; Sinervo et al, 2010) and has been identified experimentally as the temperature at which an individual can no longer right itself after being turned on its back (i.e. loss of righting response) (Lutterschmidt and Hutchison, 1997; Yang et al, 2008). To measure these two parameters, all lizards were acclimated to initial lab conditions for three weeks to get baseline T_p and CT_{max} data, followed by the splitting of each population into treatment (12) and control (8) groups to evaluate each population's response to increased temperatures.

2.5.1 Thermal Preference

Thermal preference (T_p) was measured using a long melamine coated particle board corridor divided into two lanes to allow the measurement of two lizards simultaneously. Each corridor lane measured L x W x H ≈ 150cm x 20cm x 42cm, and 1.5-2 cm of sand was placed on the corridor floor as substrate. A thermal gradient was created in each corridor lane by hanging a 100W incandescent light bulb approximately 16 cm above the corridor floor at one end. Ice packs were placed on the outside of the corridor at the opposite end to create a broad thermal gradient (Cold End vs. Hot End: 24.7 ± 0.1°C versus 47.6 ± 0.2°C [means ± SE]).

Lizards were kept at room temperature and not fed for at least 8 hours prior to the beginning of measurements to avoid any effects of food on an individual's T_p. Lizard body temperatures were measured using a 0.8 mm ∅ thermocouple (Omega Engineering Model 5SC-TT-T-40-36) inserted approximately 5 mm into the cloaca and secured with a small piece of medical tape. The tip of the thermocouple wire was coated with a thin layer of epoxy prior to cover any sharp edges and protect the animal. The other end of the thermocouple was then plugged into a digital thermometer (Omega Engineering Model HH506A). This setup allows for a constant reading of the lizard's body temperature during the entirety of the trial without impeding the animals movement (Sinervo, 1990). With the thermocouple inserted, lizards were then placed into the center of the corridor and allowed to acclimate for approximately 10 min. As the lizard thermoregulated by moving between the

hot and cold ends of the corridor, body temperature readings were recorded at 2 min intervals for 60 min. These temperature measurements were then averaged to obtain an individual's T_p. Individual T_p's were further averaged to estimate mean population T_p.

2.5.2 Critical Thermal Maximum

CT_{max} was measured the day after T_p using the same thermocouple and digital thermometer setup as described in the T_p study (Section 2.5.1). With the thermocouple inserted, lizards were placed into an 8 L plastic bucket. A 100W incandescent light bulb with an aluminum reflector shield was then suspended approximately 40 cm above the bottom of the bucket to gradually heat up the lizard (Kaufmann and Bennett, 1989). Lizards were heated slowly from their resting temperature (usually 30-35°C) to 40°C after which they were flipped onto their back. After an animal righted itself, heat was applied again until the internal body temperature increased to 40.5°C, after which the lizard was flipped again onto its back. This was repeated at 0.5°C intervals until a body temperature of 41.5°C was reached, after which flipping took place at 0.2°C intervals. When the lizard could no longer right itself, its body temperature was recorded as the lizard's CT_{max} (Kaufman and Bennett, 1989; Lutterschmidt and Hutchison, 1997; Yang et al, 2008; Li et al, 2009). The lizard was then quickly removed from the heat source and its torso gently submerged into a container of room temperature water to aid in lowering the

lizard's internal temperature. After the lizard's body temperature was lowered to at least 35°C, the lizard was placed back into its terrarium and kept at room temperature for the remainder of the day until the basking lights came on the following morning. All lizards fully recovered from the trial after a few minutes.

2.5.3 Thermal manipulation

After baseline measurements of T_p and CT_{max} were completed, a random subset of 12 lizards from each population were placed under increased temperature conditions. The remaining lizards were kept under the initial thermal environment as a control group. Ambient temperatures in the treatment group were elevated by replacing the 40W light bulbs with 60W light bulbs. This resulted in a temperature increase of approximately 5°C under the basking light and 3°C at the cool end of the terrarium. Basking lights were kept on for the same 12-12hr day/night cycle as before in both treatment and control groups. To quantify terrarium temperatures between the two groups, six HOBO data loggers (Onset, Model U23-003) were placed in terrariums (three in control terrariums and three in treatment terrariums) with each logger having two probes, resulting in 12 total temperature readings. Each logger probe was inserted into a hollow lizard model as described in Section 2.31. One probe was placed directly under the basking light while the other was placed under the rock shelter at the opposite end of the terrarium to measure the thermal gradient between the warmest and the coolest spot in the terrariums. Temperature

was recorded at 10 min intervals over the three week experimental treatment. The thermal data recorded between the hours of 07:00-17:00 from each day of the treatment was then averaged to get the mean temperature of the hot and cold ends of the terrariums. At the end of the three-week treatment period, T_p and CT_{max} were re-measured for both the treatment and control groups as described previously in Sections 2.5.1 and 2.5.2 (see Table1, Fig. 2.).

Table 1. Temperatures of terrariums (°C) during the 3 week experimental manipulation.

	Mean		Min		Max	
	Treatment	Control	Treatment	Control	Treatment	Control
Basking	49.3 ± 0.1	44.3 ± 0.2	43.28	27.95	54.23	51.42
Shelter	34.9 ± 0.1	32.1 ± 0.1	30.72	27.9	38.09	35.48

Data are expressed as means ± SE

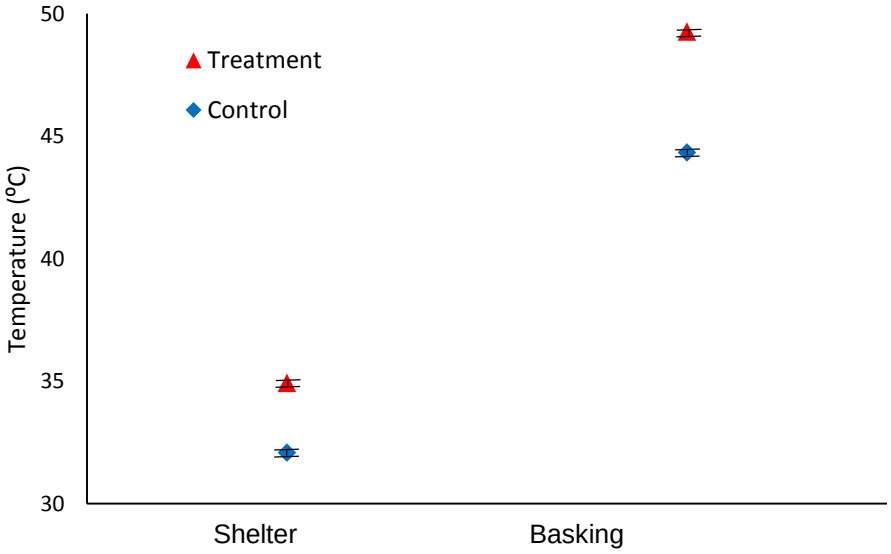

Figure 2. Data (ºC) for average thermal conditions experienced by treatment and control groups of lizards during a 3 week experiment period. Lizards were kept in individual terrariums with thermal gradients differing between groups. Temperatures are plotted as means ± SE.

2.6 Analytical Methods

Intrapopulation comparisons were made between initial and post-experiment measurements and between treatment and control groups, as well as interpopulation comparisons between islands. We used R statistical software package (Version 3.1.3 for Windows) to analyze data (R Core Team, 2015). All dependent variables were assumed to follow a normal distribution. Linear models (ANCOVA) were created to compare across groups. Model intercepts were compared and considered statically significant if estimated intercepts did not overlap in their 95% confidence intervals.

To compare the thermal environments of sampling sites, models were created for mean, maximum, and minimum temperatures for each microhabitat (full sun, intermediate sun/shade, full shade) for each study site. Mean, maximum and minimum temperature values for each site were calculated and used as the dependent variable. Site was set as a fixed effect. Due to logistical issues, site data could not be recorded for the three sites on the same days and therefore weather station statistics (mean, maximum, or minimum) obtained online from recordings from the Naxos weather station were used as covariates.

To determine differences in field body temperatures (T_b) across sites, T_b was used as the dependent variable and study site and activity were set as fixed effects. A positive correlation was found between T_b and mass, so mass was included as a covariate along with substrate temperature. When analyzing

T_p and $CT_{max,}$ treatment and island were set as fixed effects and mass was included as a covariate. We used a paired t-tests to compare initial T_p and CT_{max} to post T_p and CT_{max} respectively.

3. Results

3.1 Field Data

The three study sites were found not to differ significantly in minimum, mean, or maximum temperature in any of the three microhabitats measured (full sun, intermediate sun/shade, full shade) (see Fig. 3, Appendix Table 6.). This indicates that thermal conditions on the 3 study islands do not differ in any significant way from each other (see Appendix for full model outputs). No significant differences in T_b were detected across sites. A positive correlation was found between T_b and lizard mass (T_b vs. mass: Pearon's r = 0.426, $p < 0.001$), but mass was found not to be a significant predictor of T_b ($p > 0.05$) in the model. Substrate temperature was found to be a significant predictor of T_b in the model ($t_{(1,59)} = 5.202$, $p < 0.001$). An across island comparison of T_b showed that while ground temperature had a strong effect on T_b, island of origin and type of activity did not. There was however a weakly significant Island by activity interaction (see Table 2).

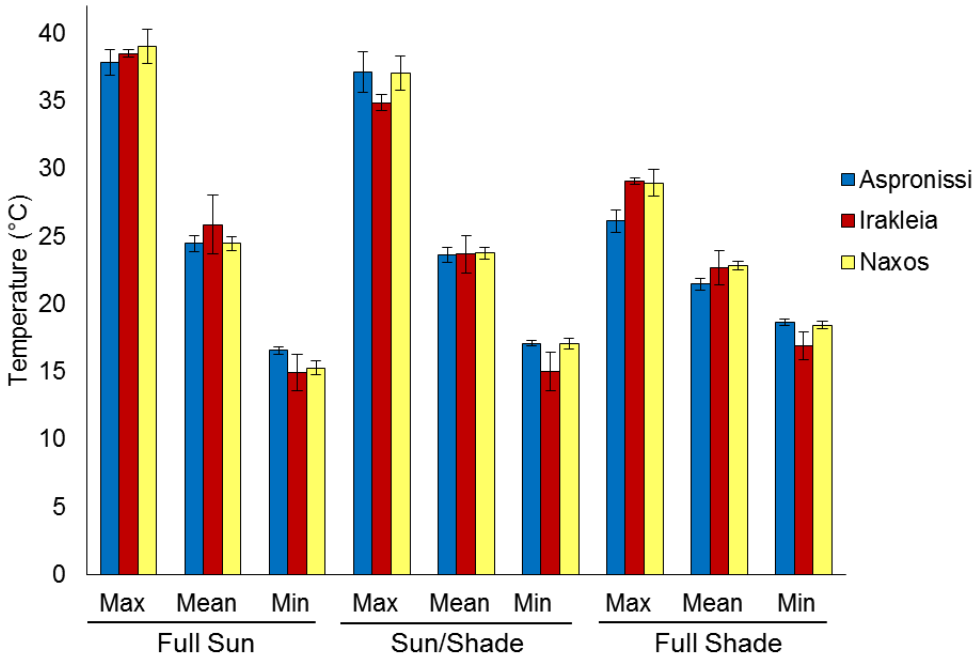

Figure 3. Microhabitat temperature data from the three study islands. Temperature data was collected using six data loggers were placed in three different microhabitats; two in full sun, two in intermediate sun and shade and two in full shade. No significant differences were found between islands. Bars represent means ± SE

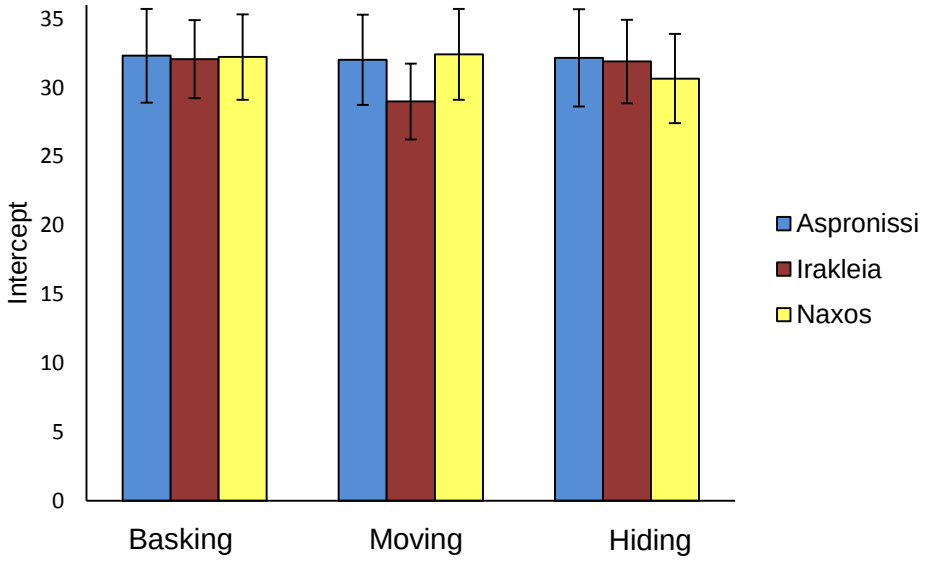

Figure 4. Model intercepts for field body temperatures of *P. erhardii* from three different Cycladic islands. Analysis reveals no significant difference in T_b between islands or between types of lizard activity. Intercepts are plotted ± SE

Table 2. Dependent Variable: Field Body Temperature (T_b)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Intercept	123.062	1	123.062	30.288	<.0001	0.368
Mass	6.586	1	6.586	1.645	0.206	0.032
Ground Temp	95.388	1	95.388	23.824	<.0001	0.323
Island	16.389	2	8.194	0.645	0.601	0.367
Activity	17.700	2	8.850	0.756	0.556	0.385
Island * Activity	28.867	2	14.433	3.605	0.034	0.126

3.2 Laboratory Measurements

3.2.1 Thermal Preference

T_p was significantly correlated with animal mass ($r = 0.26$, $p = 0.045$, $n = 60$, Pearson); consequently mass was retained in all models comparing T_b across islands. An analysis of covariance model that included island and mass showed that different island populations did not differ from each other in terms of initial T_p (Table 4A). A parallel analysis on post treatment T_p that investigated the effects of island, treatment, and their interaction while accounting for lizard mass also did not find any significant differences (Table 4B). This lack of strong differences between islands or treatments can also be seen in Fig. 5 that shows no difference in the model intercepts between the different groups. Lastly, we investigated patterns in thermal preference by comparing for each island group T_p before and after treatment. A paired t-test was used to compare initial and post T_p for both treatment and control groups from each island. The direction of change was inconsistent and non-

significant between islands with the exception of the Irakleia treatment group which showed a weak increase in T_p ($t_{(11)}$ = -2.42, p = 0.034; Fig. 6).

Table 3. Descriptive statistics for T_p and CT_{max} (°C). Initial values were recorded after the first 3 week lab acclimation period under standardized conditions. Post experiment values were recorded after the second 3 week period where treatment groups were exposed to elevated ambient temperature conditions.

Island	Initial T_p		Post T_p	
	Treatment	Control	Treatment	Control
Aspronissi	35.3 ± 0.3 (12)	35.7 ± 0.2 (8)	35.1 ± 0.5 (12)	35.4 ± 0.3 (8)
Irakleia	34.6 ± 0.5 (12)	35.1 ± 1.0 (8)	36.0 ± 0.4 (12)	36.1 ± 0.4 (8)
Naxos	35.7 ± 0.4 (12)	35.4 ± 0.3 (8)	35.7 ± 0.4 (12)	34.6 ± 0.6 (7)
	Initial CT_{max}		Post CT_{max}	
	Treatment	Control	Treatment	Control
Aspronissi	41.4 ± 0.4 (12)	41.6 ± 0.4 (8)	43.0 ± 0.4 (12)	43.5 ± 0.6 (8)
Irakleia	43.3 ± 0.4 (12)	43.0 ± 0.4 (8)	43.9 ± 0.3 (12)	43.8 ± 0.4 (8)
Naxos	42.1 ± 0.3 (12)	42.4 ± 0.5 (8)	43.4 ± 0.4 (12)	43.6 ± 0.7 (6)

Data are expressed as mean ± SE. Numbers in parentheses are sample sizes

Table 4A. Dependent Variable: Tp - initial

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	9.783[a]	3	3.261	1.417	0.247	0.071
Intercept	1185.023	1	1185.023	514.974	<.0001	0.902
Mass	5.192	1	5.192	2.256	0.139	0.039
Island	0.403	2	0.202	0.088	0.916	0.003
Error	128.863	56	2.301			
Total	75023.011	60				
Corrected Total	138.646	59				

a. R^2 = .071; Adj. R^2 = .021

Table 4B. Dependent Variable: Tp - post

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Intercept	1264.529	1	1264.529	662.62	<.0001	0.926
Mass	7.203E-6	1	7.20E-6	<.0001	0.998	0.000
Island	6.651	2	3.326	1.249	0.409	0.471
Treatment	0.72	1	0.72	0.252	0.666	0.111
Island * Treatment	5.729	2	2.865	1.484	0.236	0.054

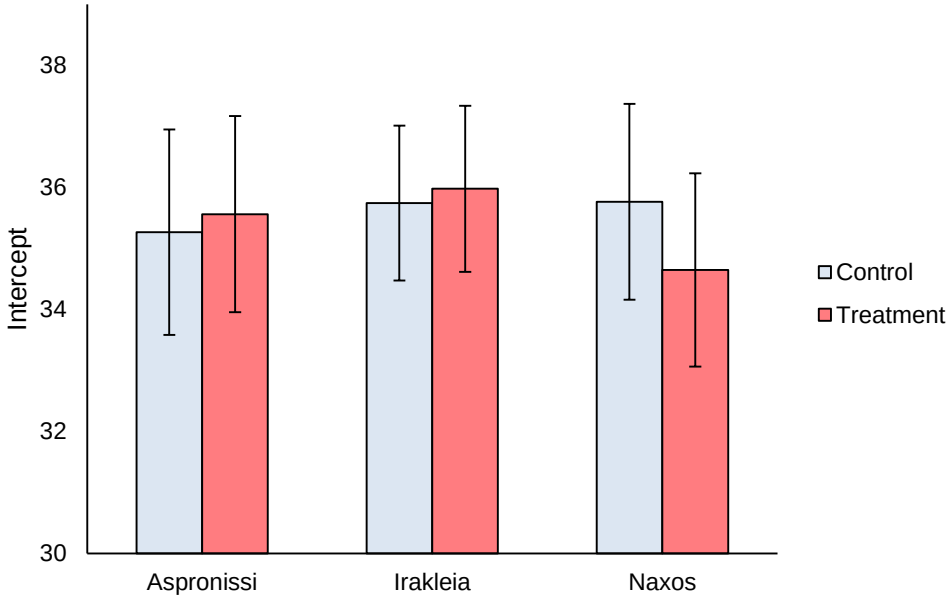

Figure 5. Model intercepts for thermal preferences (T_p) of Aegean wall lizards from the 3 study populations following the 3 week experimental treatment. No significant differences were found between treatment and control groups or between islands. Model intercepts are plotted ± SE.

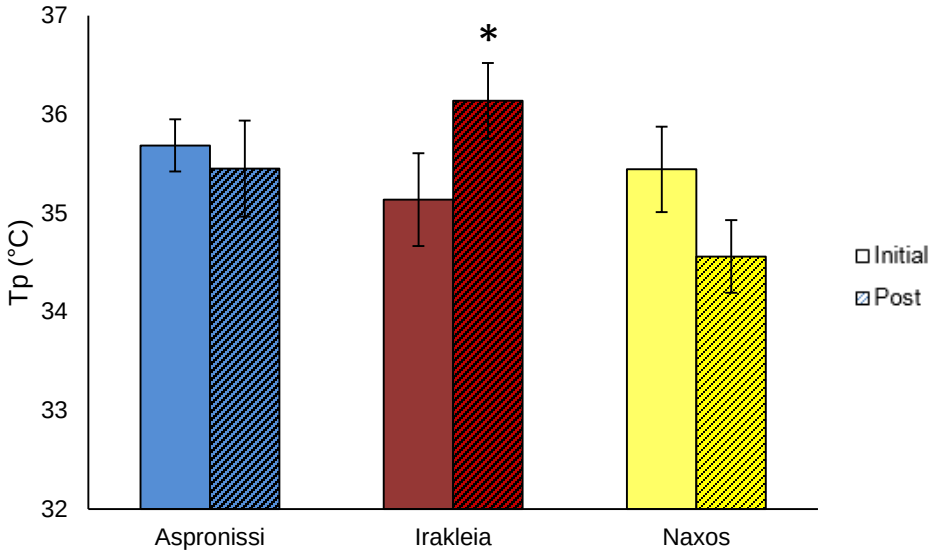

Figure 6. Thermal preferences (T_p) of treatment groups of Aegean wall lizards from 3 different sample islands. A paired t-test was used to compare initial and post T_p. Analysis shows Tp increased after the experiment in the Irakleia treatment group ($t_{(11)}$ = -2.42, p = 0.034). Means are plotted ± SE. An asterisk denotes a significant difference between initial and post values.

3.2.2 Critical Thermal Maximum

To evaluate the effects of island origin on critical thermal maxima we conducted separate analyses for initial and post experiment CT_{max} data. Because lizard mass was found to be strongly associated with CT_{max} (r = -0.531, p <0.0001, Pearson) it was included as a covariate in all analyses. For initial CT_{max} no significant differences between islands were found, although lizard mass remained a highly significant predictor of CT_{max} in the model ($F_{1, 59}$ = 8.062, p = 0.006, ANCOVA) (Table 5A).

For post-experiment CT_{max} we found no significant differences between islands or treatment and control groups (Table 5B.) though mass was a marginally significant predictor of CT_{max} in the model ($F_{1, 58}$ = 3.990, p = 0.051, ANCOVA). This lack of strong differences between islands or

treatments can also be seen in Fig. 7 that shows no difference in the model intercepts between the different groups. A paired t-test was used to compare initial and post CT_{max} for both treatment and control groups from each island. In general there was an increase in the CTmax during the second, post-experiment measurement (see Table 3, Fig. 8.). These increases were significant for the Aspronissi treatment group; $t_{11} = -4.32$, $p < 0.01$, paired t-test) and nearly significant in the Irakleia and Naxos treatment group ($t_{11} = -2.06$, $p = 0.063$; ($t_{10} = -2.14$, $p = 0.058$, paired t-test). These increases were in general present but not as pronounced in the control groups and were significant only for the Aspronissi population ($t_7 = -3.44$, $p = 0.011$). CT_{max} post experiment was correlated with CT_{max} before ($r = 0.437$, $p = 0.001$, n=58, Pearson) and were in general higher (with the most pronounced increases in the animals that had lower initial CT_{max}) (see Fig. 9).

Table 5A. Dependent Variable: Initial CT_{max}

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	35.297a	3	11.766	8.54	<.0001	0.314
Intercept	2229.956	1	2229.956	1618.583	<.0001	0.967
Mass	11.107	1	11.107	8.062	0.006	0.126
Island	3.584	2	1.792	1.301	0.28	0.044
Error	77.152	56	1.378			
Total	107461.39	60				
Corrected Total	112.45	59				

a. R2= .314; Adj. R2 =.277

Table 5B. Dependent Variable: CT_{max} Post

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Intercept	2081.609	1	2081.609	1092.924	0.000	0.955
Mass	7.696	1	7.696	3.99	0.051	0.073
Island	0.177	2	0.089	0.154	0.859	0.014
Treatment	0.558	1	0.558	2.880	0.232	0.590
Island * Treatment	0.387	2	0.194	0.100	0.905	0.004

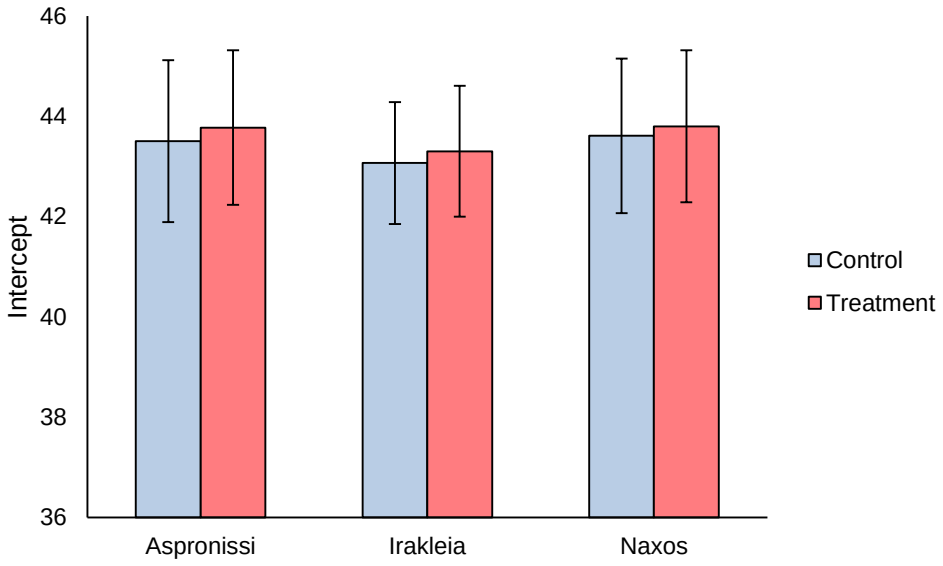

Figure 7. Model intercepts for critical thermal maxima (CT_{max}) of Aegean wall lizards from 3 different Cycladic islands after the 3 week experimental treatment. No significant differences were found between treatment and control groups or between islands. Intercepts are plotted ± SE.

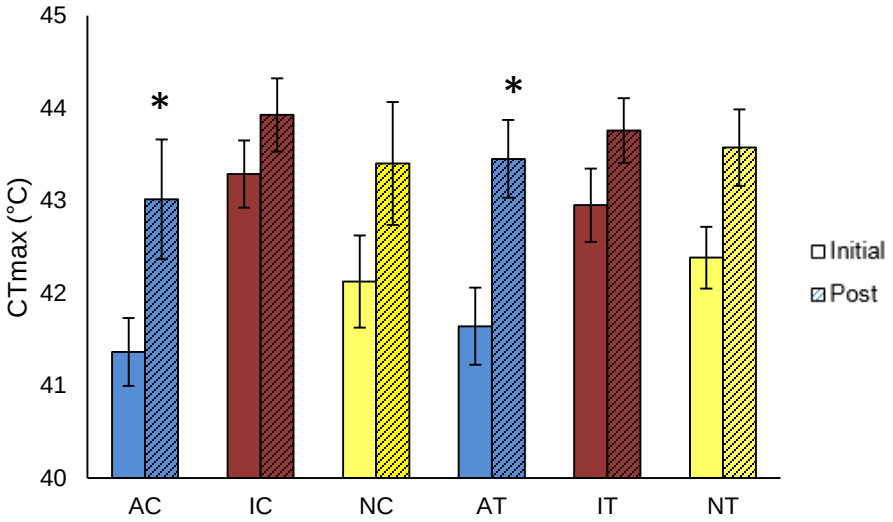

Figure 8. Critical thermal maxima (CT_{max}) of treatment groups of Aegean wall lizards from 3 different sample islands. A paired t-test was used to compare initial and post $CT_{max.}$ Analysis shows CT_{max} increased significantly in the Aspronissi treatment groups (t_{11} = -4.32, $p < 0.01$) and nearly significantly in the Irakleia and Naxos treatment group (t_{11} = -2.06, $p = 0.063$; t_{10} = -2.14, $p = 0.058$) respectively. The control group for Aspronissi also showed a significant increase in CTmax (t_7 = -3.44, $p = 0.011$). Means are plotted ± SE. An asterisk denotes and significant difference between initial and post values. AC = Aspronissi control, IC = Irakleia control, NC = Naxos control, AT = Aspronissi treatment, IT = Irakleia treatment, NT = Naxos treatment.

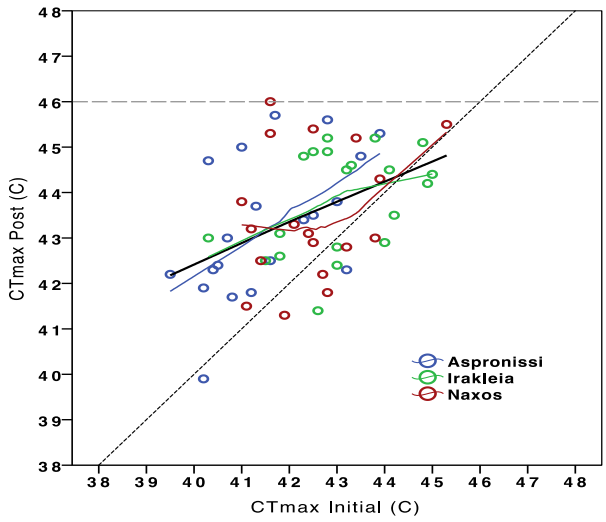

Figure 9. Scatter plot showing CT_{max} before and after the thermal manipulation experiment. The two measurements were highly correlated to each other (r = 0.437, $p < 0.001$, Pearson). Most animals increased their CT_{max} performance, as evidenced by the fact that most of the dots lay above the dotted diagonal 1:1 line. These increases were most pronounced for those animals that had initially low initial CT_{max}. Overall no CT_{max} values were above the 46°C line (horizontal broken line) suggesting that this temperature represents an inflexible upper thermal limit for the species. Loess lines fitted to the different subgroups (heavy black line for all data) using an Epanechnikov kernel.

4. Discussion

The impact of climate change on terrestrial ectotherms will to a large extent be determined by the acclimation, adaptation, dispersal, and behavioral plasticity of these organisms (Deutsch, 2008). However climate change will not be acting in a vacuum. Habitat fragmentation and other anthropogenic forces will further exacerbate climate effects in our increasingly human-dominated world. Teasing apart the effects of these forces on wildlife populations, both individually and in interaction, will be crucial for forecasting a species' extinction risk and in guiding appropriate biodiversity management.

Both thermal preference and maximum thermal tolerance of reptiles have been found to be influenced by internal and external factors, including acclimation regime, photoperiod, geography, age, sex, and physiological state (Patterson and Davies, 1978; Lutterschmidt and Hutchison, 1997; Yang et al, 2008). This means that in order to compare individuals from different localities, all individuals must first be acclimated under standardized conditions to yield biologically meaningful results (Yang et al, 2008). This was accomplished in this study by establishing an initial three week lab acclimation period after which baseline T_p and CT_{max} were measured.

We did not observe any significant differences between the island populations in T_p either before or after the experimental heating manipulation and once a known covariate (mass) was included in the analyses. The inter-population similarity in initial T_p is not surprising considering the physical

proximity of the three islands and the fact that the prevailing microclimatic conditions are very similar. Furthermore, the general lack of difference in the post T_p measurements, suggests that even drastic changes in the thermal environment, also do not appear to affect temperature preferences in *P. erhardii*. Nonetheless, a before-and-after analysis within each island revealed that the Irakleia treatment groups showed a weakly significant increase in T_p following exposure to elevated thermal conditions. However, because this shift was relatively modest, and different island populations responded in opposing directions, this pattern cannot be considered robust and needs to be interpreted with caution. Instead our results paint a broad picture of non-consistent inter-island differences in the manner lizards' preferred temperatures respond to warming conditions.

A first analysis of initial CT_{max} indicated that significant inter-island differences exist. However, a more detailed analysis revealed that CT_{max} is strongly and negatively related to body size and given that average lizard body size differs among the 3 islands, follow-up analyses showed that these observed inter-island differences in CT_{max} could ultimately be attributed to differences in body size. Our results therefore indicate that while it is possible that any effects of climate change will be felt differently by each of the three island populations in terms of their CT_{max}, any of these differences are going to be modest and largely mediated by inter-island differences in average body size.

A paired t-test indicates that the post CT_{max} values for the treatment groups from all three islands were significantly higher that the initial values. Nonetheless, no significant differences were found in post CT_{max} values between the treatment and control groups. This indicates that the observed increases in CT_{max} occurred irrespective of experimental treatment and suggests that they are best attributed either to conditions in captivity or, more likely, to accommodation to the CT_{max} measurement procedure. Other possible explanations are that changes in seasonality or physiological state (transitioning from breeding to post breeding status) during the six weeks in the lab may have been responsible for the observed response. We observed a general lack of difference in post CT_{max} between treatment and control groups. This can be possibly attributed to the thermal manipulation not having been of sufficient length to alter the lizards' physiology, although other studies conducted on a similar timeframe have yielded statistically significant results (Kaufmann and Bennett, 1989; Yang et al, 2008; Li et al, 2009).

Previous studies in other species have shown T_p and CT_{max} tend to rise as acclimation temperature increases (Kaufmann and Bennett, 1989; Yang et al, 2008). However, this effect isn't always consistent. Other studies have found that acclimation temperature does not alter T_p. Licht (1968) found this to be the case in *Anolis carolinensis* while Wheeler (1986) found that *Cordylus jonesi* and *Lacerta lilfordi* did not differ in T_p, but lizards of both species showed significantly higher metabolic rates when acclimated to 20°C compared to 30°C. Still even more unusual trends have been observed. Li et al

(2009) acclimated three species of *Eremia* lizards (Lacertidae) to three different temperatures, 28°C, 33°C, and 38°C. Lizards acclimated to 28°C and 38°C overall selected lower body temperatures than those acclimated at 33°C, while lizards acclimated at high temperatures were less tolerant of low temperatures and vice versa (Li et al, 2009). When exposing *Sceloporus occidentalis* to high ambient temperatures, Wilhoft and Anderson (1960) reported that this could actually reduce T_p, the opposite of what would be expected in a compensatory response. The changes observed in T_p in this study, while most not being statically significant, showed similar mixed results. The Aspronissi lizards showed almost no change in T_p between initial and post values while the Irakleia population showed slight increases in T_p in both treatment and control groups. The Naxos treatment group actually showed a decrease in T_p after the experimental treatment. Further study of the effects of thermal acclimation on T_p between these island populations is needed to discern if any of these observed trends are biologically meaningful.

The lack of change in CT_{max} and T_p in response to the increased thermal conditions provides important information on the species' thermal biology and physiological plasticity. Our data indicate that *P. erhardii* can operate under a range of cooler-than ideal field conditions but that it has a very consistent preference for T_p of ca. 35°C and cannot tolerate temperatures greater than 46°C. While the species is therefore characterized by an ability to deal with a fairly broad range of low temperatures in particular, its preferred temperature appears to be surprisingly rigid and not apt to change under

altered temperature conditions. The lack of inter-population differences in either T_p or CT_{max} at the beginning of the study is perhaps not surprising considering the similarity in the thermal environments on the three islands. However the lack of post-experiment differences in T_p or CT_{max} between lizards coming from these islands indicates that differences in genetic diversity do not seem to affect a species' thermal plasticity. Our results indicate that a loss of genetic diversity due to habitat fragmentation does not affect the thermal plasticity of *P. erhardii*. As a result, the overdominance model of phenotypic plasticity is not supported by this study as there seems to be no link between heterozygosity and phenotypic plasticity. This finding is in agreement with most other studies that have examined the genetic basis of plasticity (Schlichting and Levin, 1986; Santiago et al, 1989, Scheiner et al, 1991; Scheiner and Lyman, 1991; Weber and Scheiner, 1992). Provided that these results hold true for other ecothermic species, this would be positive news for conservation managers concerned about the interactive effects of habitat fragmentation and the concomitant loss of genetic diversity and the ability of wildlife populations to respond to climate change.

5. Appendix

Table 6. Environmental temperatures. Data loggers were placed in three different micro-habitats; two in full sun, two in intermediate sun and shade (e.g., under partial vegetation cover) and two in full shade. No significant differences were detected between islands.

Site Means		Df	Sum Sq	Mean Sq	F	Sig.
Full Sun	Island	3	3.328	1.6638	0.603	0.568
	Mean WS	1	0.295	0.2951	0.107	0.751
	Residuals	9	24.842	2.7602		
Sun/Shade	Island	3	0.036	0.0181	0.011	0.989
	Mean WS	1	0.66	0.6598	0.405	0.54
	Residuals	9	14.666	1.6296		
Full Shade	Island	3	4.221	2.1103	1.961	0.196
	Mean WS	1	0.035	0.0351	0.033	0.861
	Residuals	9	9.684	1.076		
Site Max		Df	Sum Sq	Mean Sq	F	Sig.
Full Sun	Island	2	3.09	1.547	0.196	0.8251
	Max WS	1	27.1	27.103	3.44	0.0966
	Residuals	9	70.9	7.878		
Sun/Shade	Island	2	8.4	4.201	0.373	0.699
	Max WS	1	5.14	5.137	0.456	0.517
	Residuals	9	101.42	11.269		
Full Shade	Island	2	18.56	9.278	1.394	0.297
	Max WS	1	1.14	1.142	0.172	0.688
	Residuals	9	59.92	6.658		
Site Min		Df	Sum Sq	Mean Sq	F	Sig.
Full Sun	Island	2	4.458	2.229	1.433	0.2882
	Min WS	1	5.351	5.351	3.44	0.0966
	Residuals	9	13.999	1.555		
Sun/Shade	Island	2	7.12	3.56	3.021	0.0991
	Min WS	1	3.088	3.088	2.621	0.1399
	Residuals	9	10.605	1.178		
Full Shade	Island	2	4.441	2.2205	3.239	0.0872
	Min WS	1	0.468	0.4682	0.683	0.4299
	Residuals	9	6.17	0.6855		

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