Universidad de La Laguna

Departamento de Botánica, Ecología y Fisiología Vegetal

Facultad de Ciencias
Sección BIOLOGÍA

Aproximación al estudio de ciclos de vida de coleópteros de la superficie del suelo como indicadores ecológicos en un gradiente altitudinal en la isla de Tenerife

Approximation to the study of life-cycles of soil-surface beetles as ecological indicators in an altitudinal gradient on the island of Tenerife

Trabajo de Fin de Grado

Daniel Basilio Suárez Ramos

Grado en Biología. Universidad de La Laguna.

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Datos Personales

Nº DNI o pasaporte: 45340678L	Nombre y Apellidos: Daniel Basilio Suárez Ramos
Teléfono: 630739415	Dirección de correo electrónico: alu0100765380@ull.edu.es

SOLICITA la defensa y evaluación del Trabajo Fin de Grado

TÍTULO

Aproximación al estudio de ciclos de vida de coleópteros de la superficie del suelo como indicadores ecológicos en un gradiente altitudinal en la isla de Tenerife

Autorización para su depósito, defensa y evaluación

D./Dña. Antonio de los Santos Gómez	
Profesor/a del Departamento de Botánica, Ecología y Fisiología Vegetal	
y D./Dña.	
Profesor/a del Departamento de	
autorizan al solicitante a presentar la Memoria del Trabajo Fin de Grado	
Fdo.: Antonio de los Santos	Fdo.:

La Laguna, a 4 de julio de 2016

Firma del interesado/a

Daniel Basilio

SR/A. PRESIDENTE DE LA COMISIÓN DE GRADO DE LA FACULTAD DE BIOLOGÍA

Documentación a adjuntar:
- Un ejemplar en formato electrónico de la Memoria conforme a las normas de presentación establecidas en el Anexo I del Reglamento para la elaboración y defensa del TFG.
- Informe-evaluación de los tutores en sobre cerrado y firmado.

Ejemplar para la Secretaría

1. Abstract

Climate-related variation in life-cycle, abundance and altitudinal distribution of some soil-surface beetle species was studied. We performed a sampling from the late winter and spring period in 2016 along the Güímar valley. Results were compared with results of previous sampling years. Four strata were defined and two sampling quadrats were selected in each altitudinal stratum. Hydrothermal environment and activity-density, body weight and number of eggs were analyzed by simple linear regressions and ANOVA. It was shown changes in the population parameters both seasonal and yearly as altitudinal. It was shown displacement to summit strata of *Carabus abbreviatus* population, previously in the cloudy montane, where also certain species of *Calathus* are absent, and a new population has appeared (*Orthomus berytensis*). Likewise, a temperature increase was shown among the sampling years in each stratum. There's an increase in activity-density in *Hegeter amaroides* and *Hegeter tristis*. Also, mean number of eggs in *C. abbreviatus* females increased. There was significant regression between temperature and activity-density following a temporal variation in *H. amaroides* and *H. tristis*, as well as significant regression between rainfall and mean number of eggs in *C. abbreviatus*. Those conclusions are an approximation of beetle's population state but have to be reviewed by a one-year life-cycle study.

Key-words: Coleptera, environmental correlate, life-cycle

Resumen

Se ha estudiado la variación de los ciclos de vida, la abundancia y la distribución altitudinal de algunas especies de escarabajos de superficie del suelo en relación con el clima. Hemos llevado a cabo un muestreo durante el invierno tardío y la primavera de 2016 a lo largo del Valle de Güímar. Los resultados se comparan con resultados de muestreos de años anteriores. Se definieron cuatro estratos y se seleccionar dos cuadratines en cada estrato altitudinal. Se analizaron mediante regresiones simples lineares y ANOVA el ambiente hidrotermal y densidad-actividad, peso corporal y número de huevos. Se han observado cambios en los parámetros poblacionales tanto estacionales como interanuales y altitudinales. Se ha visto un desplazamiento de la población de *Carabus abbreviatus* al estrato de cumbre, anteriormente en el montano húmedo, donde algunas especies de *Calathus* están ausentes y una nueva población ha aparecido (*Orthomus berytensis*). Además, se ha observado un incremento de la temperatura durante los años de muestreo en cada estrato. Hay un incremento en la densidad-actividad de *Hegeter amaroides* y *Hegeter tristis*. Además, el número medio de huevos en hembras de *C. abbreviatus* ha aumentado. Existe correlación entre la temperatura y la densidad-actividad de *H. amaroides* y *H. tristis*, así como entre la pluviometría y el número de huevos de *C. abbreviatus*. Estas conclusiones son una aproximación al estado de las poblaciones de escarabajos pero deben ser revisadas con un estudio de ciclo anual completo.

Palabras clave: ciclo de vida, Coleoptera, correlación ambiental

2. Introduction

2.1. Background

Traditionally, several studies have been done in order to describe life cycles of Coleoptera, mainly on soil-surface beetles (Barlow, 1973; de Zordo, 1979; Gilbert, 1956; Houston, 1981; Jorum, 1985; Refseth, 1984). *Sensu* Matalin (2007), we consider life-cycle as the change of the active and resting periods of the development stages during the vegetation season (annual rhythm) and the periodic changes in the rate of the gonad development in response to the key environmental factors, ensuring reproduction (gonad cycle). Larsson (1939) was the first author who classified life-cycle patterns in three different reproductive types and correlated them with species distribution. However, Thiele (1977) suggested that life-cycle patterns should be classified, at least, in five annual rhythms types. Those classifications try to explain soil-surface beetles adaptations to seasonal changes of the environment.

It is known that insect life-cycle processes affected by climate and weather include lifespan duration, fecundity, diapause, dispersal, mortality and genetic adaptation (Patterson *et al*., 1999). As insects play important roles in ecosystems services, important changes in their abundance and diversity will potentially alter the services they provide (Cornelissen, 2011). Thus, this group may be particularly suitable as environmental and ecological bioindicators, especially soil-surface beetles, which have shown to be sensitive to human induced environmental changes (Gerlach *et al.*, 2013, Rainio & Niemelä, 2003). In the last years, some authors used soil-surface beetles as bioindicators for their researches. Wang *et al*. (2014) hypothesized that ground beetles (Coleoptera: Carabidae) may optimize and synchronize their life cycle with seasonal changes of the environment. Also, Kaltsas *et al*. (2012) used darkling beetles (Coleoptera: Tenebrionidae) as environmental bioindicators as long as the number of species occurring and their respective abundances are very high. In this context, Canary Islands represent a suitable study area due to the great biodiversity and the altitudinal gradient, with a remarkable climate variation among stratum. It is known that in oceanic islands, biodiversity is determined mainly by the island's altitude, because it is clearly related with the number of altitudinal bioclimatic strata (Oromí *et al*, 2015). Tenerife is the highest island in this archipelago and, so for that, it's the most representative area to accomplish a research of soil-surface beetle life-cycle through an altitudinal gradient. Also, because of the high environmental diversity, organisms developed adaptive processes with a high ecological valence (de los Santos & Arbelo, 2000).

Shelford (1931) postulated that the rate of an organism growth is limited by an element excess or deficiency. Hutchinson (1957) defined the fundamental niche as a set of points in an abstract n-dimensional N space. He proposed Gaussian curves as a good model to represent the response of an organism to certain parameters. In this way, de los Santos (2009) suggested that beetle populations could show particular altitudinal distributions in terms of their physiological optima (hydric and thermal) and tolerance limits. Also, Pozgai & Littlewood (2001) told that most species' distribution is dependent on climatic factors such as temperature and precipitation. Particularly, darkling beetles are well distributed among arid and semi-arid terrestrial ecosystems (de los Santos & Arbelo, 2000). The reactions of these beetles to heat are largely behavioural while their responses to water shortage are primarily physiological (Cloudsley-Thompson, 2001). In contrast, ground beetles seem to be well distributed among higher altitudes, characterized by a higher level of humidity. Machado (1976) described that the humidity is probably the most decisive limiting factor for most of the carabids and, since the laurel forest is the most humid environment in the Canary Islands, the vast majority of the carabid fauna is bound to it. As beetles show adaptive processes linked with the environment in which they live, there should be size patterns that suggest morphological adaptations to face climatic fluctuations. In this context, allometry links ecological processes with body dimensions. Allometry was used as an indirect method for biomass assessment, developing a quick estimation with length-dry mass regressions (Baumgärtner & Rothhaulpt, 2003), and to determine assemblage vulnerability (de los Santos, 2013). It is known that assemblages could be disturbed by fluctuations as down hyperthermal conditions or/and upward hypothermal conditions (de los Santos *et al.*, 2014). Shukla (1993) referred "short-term" to fluctuations with time scales varying from a month (30 days) to a few years (1000 days) from a given initial situation. Several authors suggested that temperature could influence life history traits (Refseth, 1980, Doyen & Tschinkel, 1974) and differences in the body sizes (Wise, 1981).

2.2. Justification

This research was accomplished in order to study the relationship between environmental changes, especially climatic, and organisms' life-cycle. In this way, climate-related variation in life-cycle, abundance and altitudinal distribution of some soil-surface beetle species was studied within a framework of short-term climatic fluctuations, seasonality and elevation gradients during late winter and spring of 2016.

3. Objectives

The main objective of this final degree project is to determine how soil-surface beetles react to changes in the environment, particularly focusing on adaptive responses of their life cycles during a limited period of time between late winter and early summer.

Our attention is focalized into a group of species that lives in an environment of definable and quantifiable variability, involved in interaction processes, adjustment and regulation, which is percept as a sequence of births and deaths, or as flows of matter and energy, and whose results define models of ecological succession and natural evolution.

In this context, we establish the following hypothesis: changes in the environment, especially climatic, have influenced soil-surface Coleoptera life-cycle, thus altering the main variables that define their life-cycle (activity-density, number of eggs and body weight) through the years of study in the last 30 years.

Particularly, we can establish more concrete hypotheses:

1. There's evidence of short-term thermal fluctuations during the years of study in the last 30 years
2. Some species changed their altitudinal distribution during the years of study in the last 30 years
3. There are advances or delays of activity periods for a same species during the years of study in the last 30 years
4. There are asynchronies between the maximum activity-density for a same species during the years of study in the last 30 years
5. Ovarian maturation varies for a same species in different years following a temporal variation
6. Mean body weight varies for a same species in different years following a temporal variation

4. Materials and methods

4.1. Fieldwork

The research was carried out through an altitudinal gradient, along the Güímar valley, in the southeast of the island of Tenerife (Fig. 1). The soil-surface beetle sampling started at sea level and extended up to 2.200 m a.s.l. This research was accomplished since 5th March until 5th June 2016.

Among this gradient, four representative strata were defined and, on each stratum, two quadrats were established, except for slope of Arafo.

SITE	ZONE	STRATA	QUADRAT	ELEVATION
1	Badlands of Güímar	Basal stratum	1	50
1	Badlands of Güímar	Basal stratum	2	70
2	Ravine of Badajoz	Cloudy montane strata	3	600
2	Ravine of Badajoz	Cloudy montane strata	4	700
3	Slope of Arafo	Summer-xeric montane strata	5	1.400
4	Izaña	Summit strata	6	2.100
4	Izaña	Summit strata	7	2.200

Table 1: Main features of plots among the altitudinal gradient.

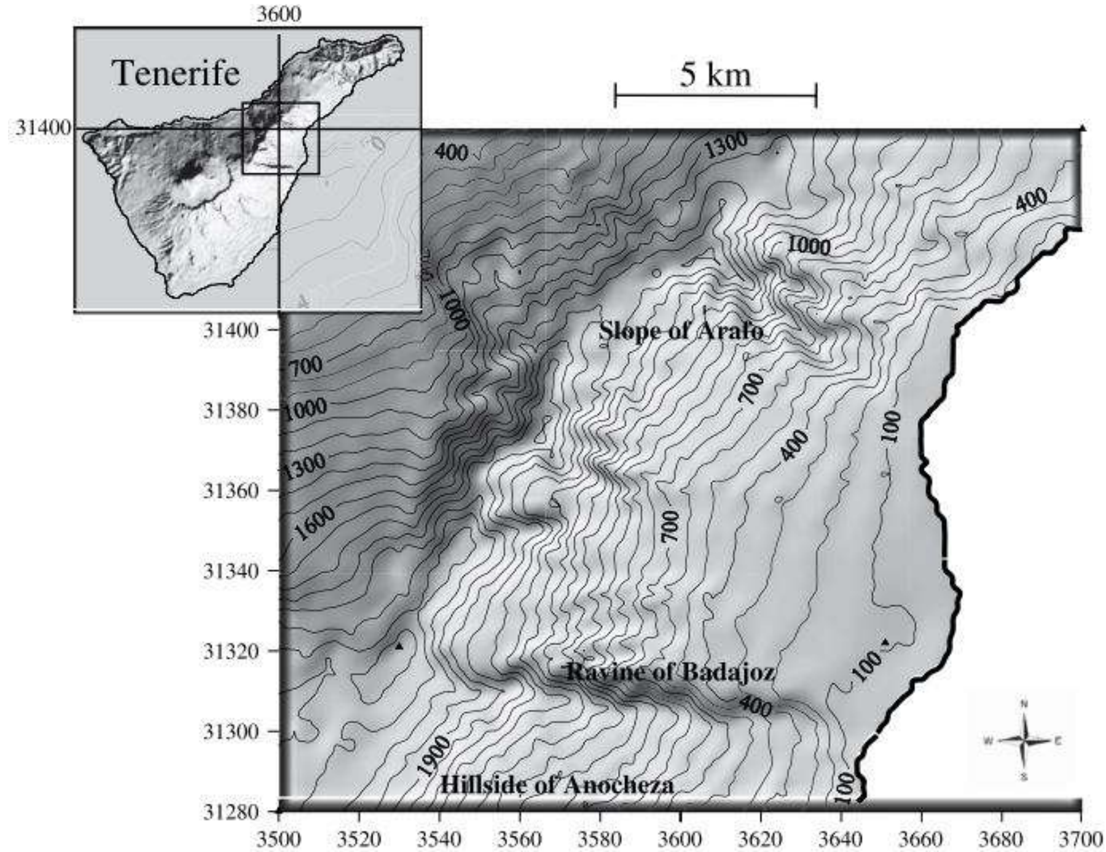

Fig. 1: Study site location. The numbers in abscissas and ordinates represent Universal Transverse Mercator (UTM) coordinates, which are used to identify locations. Extracted from de los Santos, 2009.

A grid of 10 pitfall traps was laid in each quadrat. Pitfall trapping is probably the most frequently used method for sampling ground-dwelling arthropods, especially Coleoptera and Arachnida (Cheli & Corley, 2010; de los Santos *et al.*, 1982; Luff, 1975). The spatial layout of those traps followed a random distribution in order to minimize the number of variables that affect on beetles activity. Slopes, rocky surface and dense vegetation were avoided. This layout is supported by a contagious distribution of soil-surface beetles' populations.

Pitfall traps were constructed with a plastic glass shaped container (139 mm high, maximum diameter 118 mm, minimum diameter 84 mm) (Fig. 2). Each trap incorporates a funnel that prevents beetles from escaping the trap (Luff, 1975). It also has two sieves that separate beetles by their size. The first sieve (6 mm mesh, 105 mm diameter) retains big sized beetles while the second sieve (3 mm mesh, 95 mm diameter) retains medium sized beetles. In the bottom of the trap, smallest beetles were found. This separation is possible due to soil-surface beetle positive geotropism. This layout prevents the loss of information linked to interspecific relationships such as predation (de los Santos *et al.*, 1982). Those traps are dug so that they are matched to the soil surface. Then, traps are covered with a stony slab that protects it from insolation and precipitation.

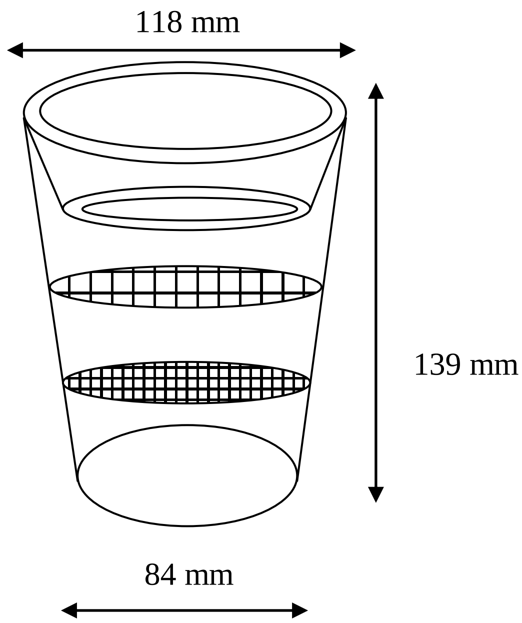

Fig. 2: Pitfall traps dimensions.

Traps were emptied weekly; soil-surface beetles were identified at species level, counted and after, released within 2 m of each trap, except a fraction of the specimens, which were chosen randomly and taken to the laboratory for further analysis.

Chicken liver was used as bait. Killing-preserving agents weren't used due to the potential impacts associated to removing a large portion of the arthropod fauna of an area through kill pitfall trapping (Weeks & McIntyre, 1997).

During the sampling period, temperatures and rainfall were recorded using a maximum-minimum thermometer and a pluviometer placed on the soil surface at each site.

4.2. Laboratory processing

The catch was transferred into 4% formaldehyde until further dissection, as optimal storage method since it does not alter the estimate of body size and body mass (Knapp, 2012). Body measurements (length, width and height of head, pronotum and abdomen) were measured under a dissecting microscope with an ocular calibrated micrometer.

Then, beetles were dissected in order to determine sex and ovarian maturation. Testis maturation was not assessed. Reproductive condition was determined by dissecting females, examining the ovaries and counting the number of mature eggs in the oviducts. The elytra and wings were displaced laterally and the dorsal body wall was cut around the edges and folded back to reveal the ovaries (Barlow, 1973, Refseth, 1984). Four reproductive categories were recognized: (1) “immature” females without egg, (2) “maturing” females with mature and immature egg, (3) “mature” females with mature egg and (4) “spent” females without egg. “Spent” and “immature” categories can be distinguished respectively by the presence or absence of “corpus luteum”.

Subsequently, each individual was dried in an oven at 60ºC for 24 h, and afterwards cooling in a dessicator and then was weighed on an electronic balance to determine mean body weight to the nearest 0,01 mg (de los Santos, 2013).

4.3. Data processing

Activity-density pattern was calculated for every Carabidae, Tenebrionidae, Staphylinidae and Silphidae species. This parameter provides a good estimate of the role of a species in an ecosystem, not only depending on its frequency but also on its mobility (Thiele, 1977). It is calculated dividing the number of individuals caught by the product of multiplying the number of traps times the day that have passed.

$$\text{Activity-density} = \frac{Number\ of\ individuals}{Number\ of\ traps \cdot Days} \cdot 100$$

10 representative species were chosen. Those species were *Hegeter amaroides* Solier, 1835 (Coleoptera: Tenebrionidae), *Hegeter lateralis* Brullé, 1838 (Coleoptera: Tenebrionidae), *Hegeter proximus* Lindberg, 1950 (Coleoptera: Tenebrionidae), *Hegeter tristis* (Fabricius, 1792) (Coleoptera: Tenebrionidae), *Arthrodeis obesus* (Brullé, 1838) (Coleoptera: Tenebrionidae), *Carabus abbreviatus* Brullé, 1835 (Coleoptera: Carabidae), *Platyderus alticola* (Wollaston, 1864) (Coleoptera: Carabidae), *Calathus ascendens* Wollaston, 1862 (Coleoptera: Carabidae), *Calathus depressus* Brullé, 1838 (Coleoptera: Carabidae) and *Orthomus berytensis* (Reiche & Saulcy, 1854) (Coleoptera: Carabidae).

Statistical analyses were done only for *Hegeter amaroides, Hegeter tristis, Hegeter lateralis* and *Carabus abbreviatus* for being the most representative species and due to the lack of information of the rest of the species for parameters such as mean body weight or mean number of eggs in females. All statistical calculations were performed using the Statistical Package for the Social Sciences version 18.

5. Results

5.1. Population parameters patterns

Activity-density patterns for every chosen species are given, following a temporal variation. Spatial variations and altitudinal distributions for the selected species are implicit in the plots. Those species are grouped according to the strata they belong. In abscissas, temporal period it's represented (2 weeks per month during the sampling period). In ordinates, activity-density values are represented.

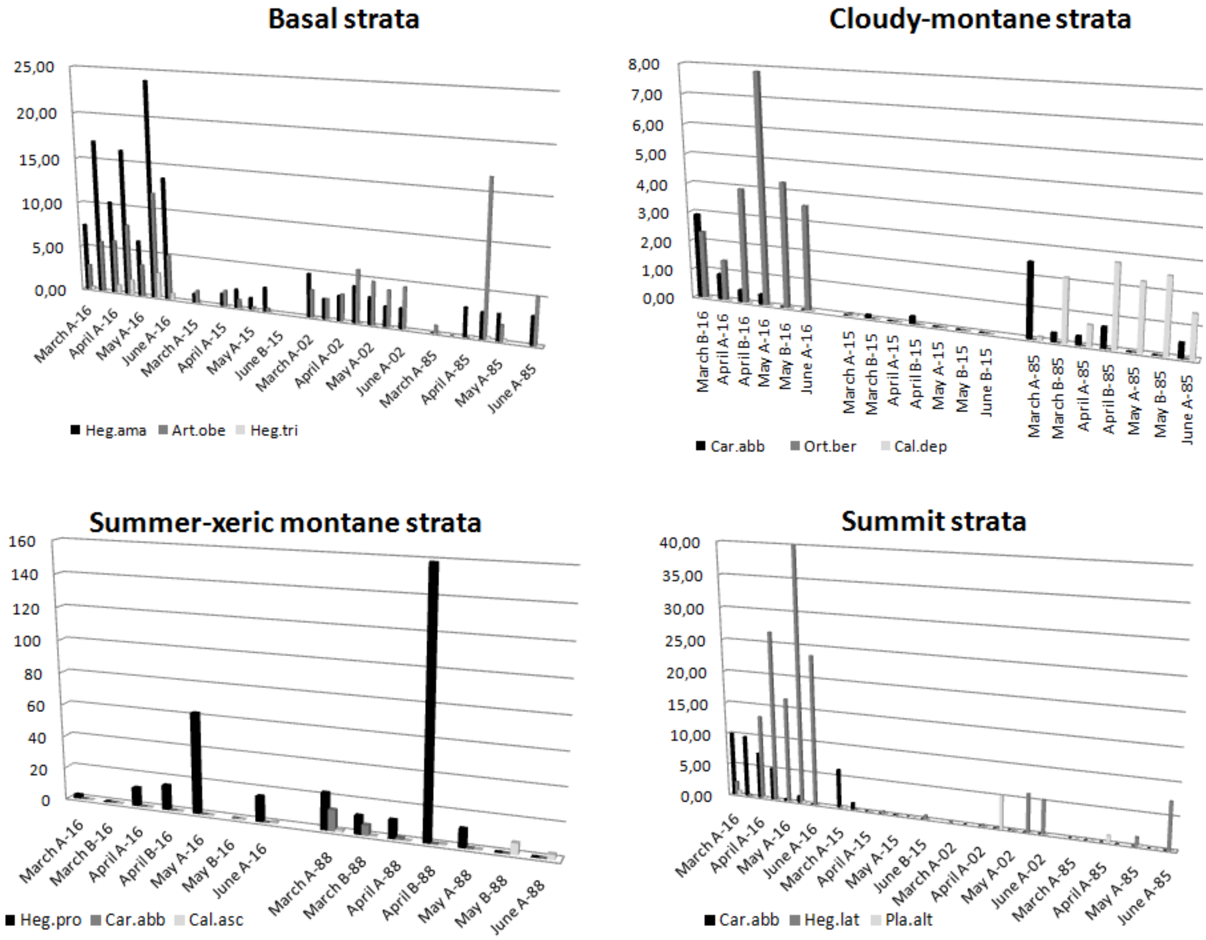

Fig 3: Activity-density patterns for selected species (*Hegeter amaroides*, *Arthrodeis obesus* and *Hegeter tristis* in the basal strata; *Carabus abbreviatus*, *Orthomus berytensis* and *Calathus depressus* in the cloudy-montane strata; *Hegeter proximus*, *Carabus abbreviatus* and *Calathus ascendens* in the summer xeric montane strata; *Carabus abbreviatus*, *Hegeter lateralis* and *Platyderus alticola* in the summit strata).

In these plots we can observe yearly variations in the activity-density patterns. In the basal strata, activity-density pattern for *Hegeter amaroides* seems to be greater in 2016 than in the previous years. Plus, *Arthrodeis obesus* presents a more homogenous distribution among the study period; while in 1985 is found mainly since April, in 2016 they're active since March.

In the cloudy-montane strata, *Calathus depressus* is absent in 2016 during the study period while a new populations has appeared (*Orthomus berytensis*). *Carabus abbreviatus* population's has reduced their activity-density in 2015 and 2016, especially during spring. In the summer xeric montane strata, *Carabus abbreviatus* is absent in 2016 during the study period and the activity-density pattern for *Calathus ascendens* has been bounded to just one period (early June). In the summit strata, activity-density pattern for *Hegeter lateralis* seems to be greater in 2016 than in the previous years. Also, *Carabus abbreviatus* has reached the summit strata in 2015 and, in 2016, their activity-density is greater than in the cloudy-montane strata.

Subsequent analyses will be done only for *Hegeter amaroides, Hegeter tristis, Hegeter lateralis* and *Carabus abbreviatus* for being the most representative species and due to the lack of information of the rest of the species for parameters such as mean body weight or mean number of eggs in females. Thus, having this overview of how species have changed among the last 30 years, it is possible to represent population parameters ("Act-dens" – activity-density; "Egg" – mean number of eggs in females; "Weight" – mean body weight) and climatic variables ("T_{min}" – minimum temperature; "T_{max}" – maximum temperature; "Plu" – pluviometry) to assess whether there's a correlation between population parameters and climatic variables.

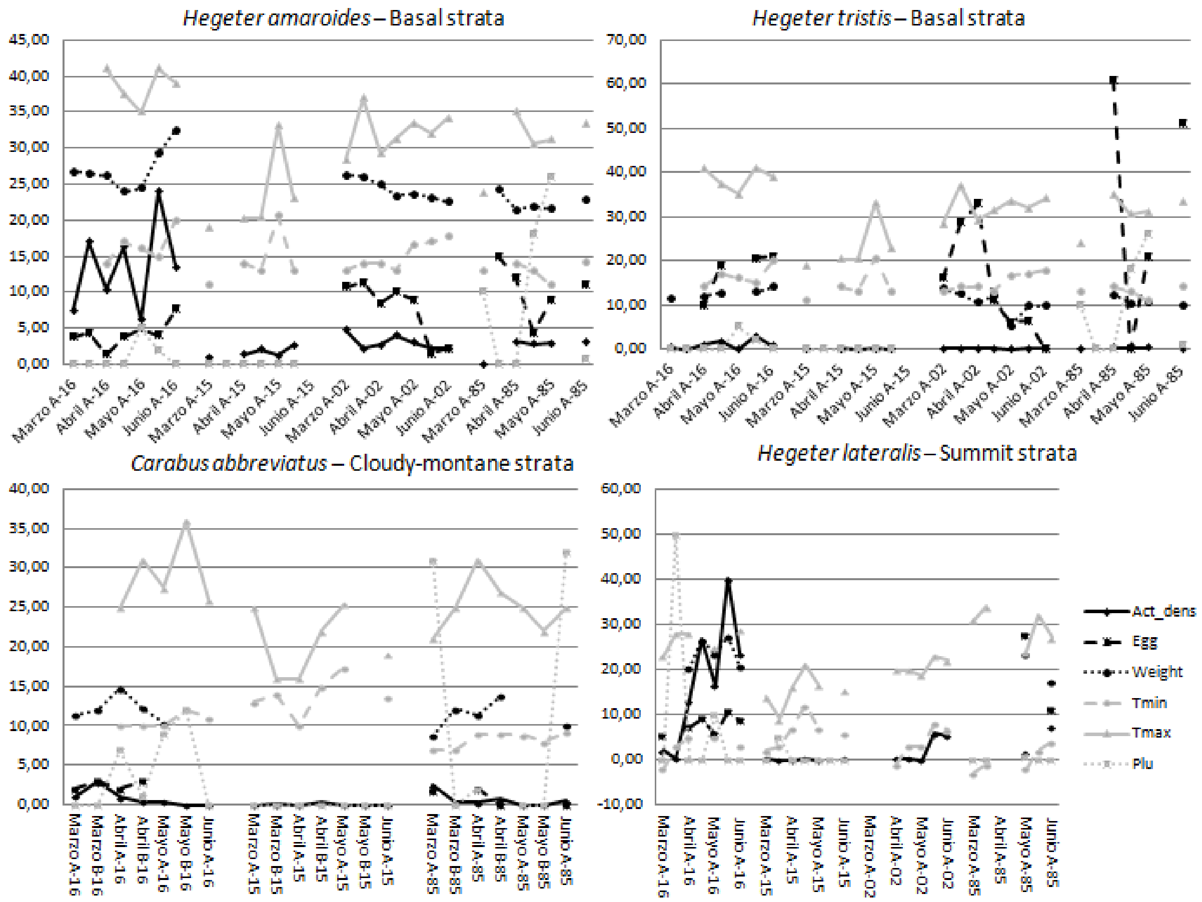

Fig 4: Population parameters parameters (black lines) (“Act-dens” – activity-density (continuous line); “Egg” – mean number of eggs in females (dashed line); “Weight” – Mean body weight (dotted line) and climatic variables (grey lines) (“T_{min}” – minimum temperature (dashed line); “T_{max}” – maximum temperature (continuous line); “Plu” – pluviometry (dotted line)) in *Hegeter amaroides* (up, left), *Hegeter tristis* (up, right), *Hegeter lateralis* (down, right) and *Carabus abbreviatus* (down, left).

These plots give a perspective of differences in the climatic variables among the years of study. In the basal strata there’s a progressive increase in the maximum and minimum temperature while there’s a decrease in the rainfall. It seems to be a positive relationship between mean body weight and maximum temperature in *Hegeter amaroides* as heavier individuals match with high maximum temperature periods. In *Hegeter tristis*, it seems to be a progressive reduction in the mean number of eggs in females in the study period among the studied years. In the summit strata, *Hegeter lateralis* presents higher values of activity-density when rainfall is lower. In the cloudy-montane strata, mean number of eggs in *Carabus abbreviatus* females match with high rainfall periods. In this way, it seems to be a correlation between population parameters and climatic variables.

5.2. Relationship between population parameters and climatic variables

The relationship between population parameters (“Act_dens” – activity-density; “Egg” – mean number of eggs in females; “Weight” – mean body weight) and climatic variables (“T_{min}” – minimum temperature; “T_{max}” – maximum temperature; “Plu” – pluviometry) is assessed through a simple linear regression.

STRATA	SPECIES	Dependent Variable	Variable Entered	R	R Square	Adjusted R Square	F	Sig.	Beta	t	Sig.
BASAL	*Hegeter amaroides*	Act_dens	Tmin	0,195	0,038	-0,026	0,593	0,453	0,195	0,770	0,453
			Tmax	0,660	0,435	0,398	11,569	**0,004**	0,660	3,401	**0,004**
			Plu	0,328	0,108	0,063	2,415	0,136	-0,328	-1,554	0,136
		Egg	Tmin	0,387	0,149	0,064	1,757	0,214	-0,387	-1,326	0,214
			Tmax	0,448	0,200	0,120	2,504	0,145	-0,448	-1,582	0,145
			Plu	0,101	0,010	-0,056	0,156	0,699	0,101	0,395	0,699
		Weight	Tmin	0,225	0,051	-0,044	0,535	0,481	0,225	0,732	0,481
			Tmax	0,513	0,263	0,190	3,572	0,088	0,513	1,890	0,088
			Plu	0,385	0,149	0,092	2,617	0,127	-0,385	-1,618	0,127
BASAL	*Hegeter tristis*	Act_dens	Tmin	0,163	0,027	-0,022	0,547	0,468	0,163	0,739	0,468
			Tmax	0,584	0,341	0,308	10,341	**0,004**	0,584	3,216	**0,004**
			Plu	0,079	0,006	-0,060	0,094	0,764	-0,079	-0,306	0,764
		Egg	Tmin	0,231	0,053	-0,020	0,731	0,408	-0,231	-0,855	0,408
			Tmax	0,052	0,003	-0,074	0,035	0,855	0,052	0,187	0,855
			Plu	0,382	0,146	0,003	1,023	0,351	-0,382	-1,012	0,351
		Weight	Tmin	0,093	0,009	-0,068	0,112	0,743	-0,093	-0,335	0,743
			Tmax	0,353	0,125	0,057	1,853	0,197	0,353	1,361	0,197
			Plu	0,501	0,251	0,144	2,348	0,169	-0,501	-1,532	0,169
CLOUDY-MONTANE	*Carabus abbreviatus*	Act_dens	Tmin	0,455	0,207	0,158	4,185	0,058	-0,455	-2,046	0,058
			Tmax	0,062	0,004	-0,058	0,062	0,807	-0,062	-0,248	0,807
			Plu	0,233	0,054	0,005	1,094	0,309	0,233	1,046	0,309
		Egg	Tmin	0,239	0,057	-0,179	0,242	0,648	0,239	0,492	0,648
			Tmax	0,369	0,136	-0,080	0,631	0,472	0,369	0,794	0,472
			Plu	0,816	0,666	0,610	11,955	**0,014**	-0,816	-3,458	**0,014**
		Weight	Tmin	0,322	0,104	-0,046	0,695	0,436	0,322	0,834	0,436
			Tmax	0,252	0,063	-0,093	0,405	0,548	0,252	0,637	0,548
			Plu	0,066	0,004	-0,120	0,035	0,857	0,066	0,186	0,857
STRATA	*Hegeter lateralis*	Act_dens	Tmin	0,050	0,003	-0,060	0,041	0,842	0,050	0,202	0,842
			Tmax	0,637	0,406	0,369	10,953	**0,004**	0,637	3,310	**0,004**
			Plu	0,177	0,031	-0,043	0,419	0,529	-0,177	-0,647	0,529
		Egg	Tmin	0,294	0,086	-0,142	0,378	0,572	-0,294	-0,615	0,572
			Tmax	0,331	0,109	-0,113	0,491	0,522	-0,331	-0,701	0,522
			Plu	0,276	0,076	-0,078	0,495	0,508	-0,276	-0,704	0,508
		Weight	Tmin	0,096	0,009	-0,238	0,038	0,856	-0.096	-0,194	0,856
			Tmax	0,637	0,406	0,257	2,732	0,174	-0,637	-1,653	0,174
			Plu	0,043	0,002	-0,164	0,011	0,919	0,043	0,106	0,919

Table 2: R, R squared, adjusted R squared, regression values of the analysis of the variance and regressions coefficients values. (Abbreviations: F – ANOVA F-test; Sig – probability; Beta – standardized coefficients; t- Student’s test; bold values indicate statistical significance at the 95% level or higher)

At a 95% level of confidence, there's positive correlation between activity-density and maximum temperature for every *Hegeter* species tested (p = 0,004 < 0,05). Also, there's possitive correlation between mean number of eggs in females and pluviometry in *Carabus abbreviatus* (p = 0,014 < 0,05). Particularly, as *Carabus abbreviatus* has reached the summit strata in the last 10 years, an ANOVA is performed to test whether the population parameters are the same in the cloudy-montane strata and in the summit strata.

Dependent Variable	Source	df	Mean Square	F	Sig.
Egg	Strata	1	7,939	10,965	**0,009**
Act_dens	Strata	1	53,345	5,253	**0,048**
Weight	Strata	1	872,512	1,901	0,201

Table 3: Tests of Between-Subjetcs Effects of the difference in *Carabus abbreviatus* population parameters in 2016 in the cloudy-montane and summit strata. (Abbreviations: df – degrees of freedom; F – ANOVA F-test; Sig – probability; bold values indicate statistical significance at the 95% level or higher)

At a 95% level of confidence, there's evidence of a difference in the mean number of eggs in females (p = 0,009 < 0,05) and in the activity-density (p = 0,048 < 0,05) between the cloudy-monate and the summit strata in the year 2016. The mean number of eggs in females in the summit strata is lower than in the cloudy-montane strata while the activity-density is lower in the cloudy-montane strata.

Ovarian maturation stages are represented in the following plots. The numbers in abscissas represent the sampling period when *Carabus abbreviatus* was caught (week 2 until week 7). The numbers in ordinates represent weekly ovarian maturation stages in percentages.

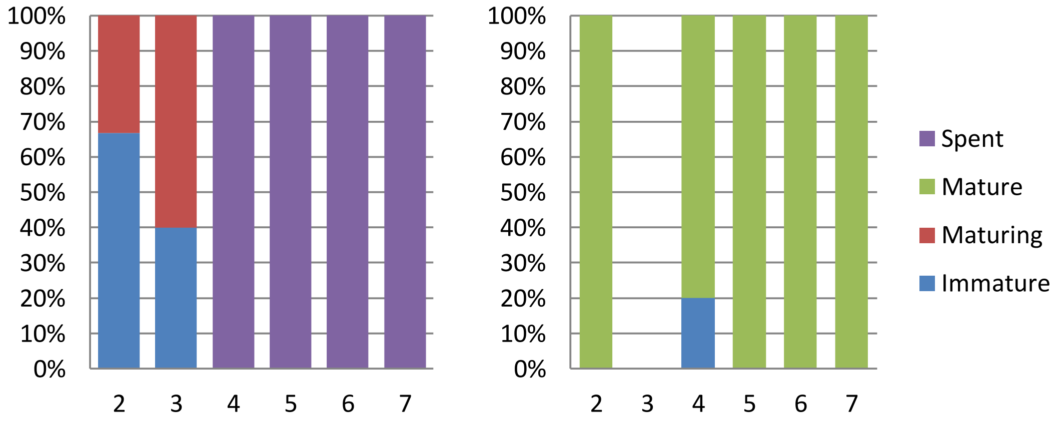

Fig 5: Ovarian maturation of *Carabus abbreviatus* females in the summit strata (left) and the cloudy-montane strata (right).

Oviposition period seems to be shorter in the summit strata, as females of *Carabus abbreviatus* mature later and spent females appear earlier. Also, mean number of eggs in females is lower in the summit strata.

5.3. Changes of population parameters

An ANOVA is performed to test whether population parameters ("Act_dens" – activity-density; "Egg" – mean number of eggs in females; "Weight" – mean body weight) varied among the years of study during the last 30 years. For this analysis, *Carabus abbreviatus*, *Hegeter amaroides*, *Hegeter tristis* and *Hegeter lateralis* were chosen.

STRATA	SPECIES	Dependent Variable	Source	df	Mean Square	F	Sig.
BASAL	*Hegeter amaroides*	Act_dens	Year	2	236,678	15,223	**0,000**
		Egg	Year	2	34,285	3,333	0,063
		Weight	Year	2	35,639	8,153	**0,004**
BASAL	*Hegeter tristis*	Act_dens	Year	2	2,757	11,392	**0,002**
		Egg	Year	2	469,938	1,697	0,224
		Weight	Year	2	794,766	1,921	0,189
CLOUDY-MONTANE	*Carabus abbreviatus*	Act_dens	Year	1	10,835	0,192	0,676
		Egg	Year	1	4,946	6,619	**0,042**
		Weight	Year	1	536,445	1,536	0,263
SUMMIT	*Hegeter lateralis*	Act_dens	Year	1	306,612	1,837	0,233
		Egg	Year	1	10,006	2,445	0,179
		Weight	Year	1	42,642	3,927	0,104

Table 4: Tests of Between-Subjetcs Effects. It is tested the variation of population parameters among the years of study during the last 30 years. (Abbreviations: df – degrees of freedom; F – ANOVA F-test; Sig – probability; bold values indicate statistical significance at the 95% level or higher).

At a 95% level of confidence, there's statistical significance of changes in the activity-density in the years of study during the last 30 years in *Hegeter amaroides* ($p = 0,000 < 0,05$) and *Hegeter tristis* ($p = 0,002 < 0,05$). There's statistical significance of changes in the mean number of eggs in females in *Carabus abbreviatus* ($p = 0,042 < 0,05$) in the mean body weight in *Hegeter amaroides* ($p = 0,004 < 0,05$). All variables that changed significantly, increased.

5.4. Analyses of climatic variables

An Analysis of Variance (ANOVA) was performed in order to test whether climatic variables ("T_{min}" - minimum temperature; "T_{max}" - maximum temperature; "Plu" - pluviometry) changed among the years of study during the last 30 years in the study area. Tests of Between-Subjetc Effects are given for every stratum. Plus, for those strata which had more than two yearly climatic register, a table of Multiple Comparisons is given.

STRATA	Dependent Variable	Source	df	Mean Square	F	Sig.
Basal	Tmin	Year	2	14,854	3,847	**0,047**
	Tmax	Year	2	875,536	101,758	**0,000**
	Plu	Year	1	164,571	2,859	0,117
Cloudy-montane	Tmin	Year	1	15,621	18,104	**0,002**
	Tmax	Year	1	48,010	3,342	0,097
	Plu	Year	1	1063,143	2,031	0,180
Summer-xeric montane	Tmin	Year	1	3,260	1,118	0,315
	Tmax	Year	1	283,393	11,055	**0,008**
	Plu	Year	1	6648,193	1,237	0,292
Summit	Tmin	Year	3	149,454	7,300	**0,002**
	Tmax	Year	3	127,472	3,199	**0,050**
	Plu	Year	2	153,882	1,106	0,356

Table 5: Tests of Between-Subjetcs Effects. It is tested the variation of climatic variables among the years of study during the last 30 years in the study area. (Abbreviations: df – degrees of freedom; F – ANOVA F-test; Sig – probability; bold values indicate statistical significance at the 95% level or higher)

Dependent Variable	Year	Year	Mean Difference	Std.Error	Sig.
Tmax	1985	2002	15,6571	1,71755	**0,000**
	1985	2016	-8,0000	1,85516	**0,001**
	2002	2016	-23,6571	1,71755	**0,000**
Tmin	1985	2002	-2,1429	1,15059	0,084
	1985	2016	-3,4000	1,24278	**0,016**
	2002	2016	-1,2571	1,15059	0,084

Table 6: Table of Multiple Comparisons between 1985, 2002 and 2016 in the basal strata. Bold values indicate statistical significance at the 95% level or higher.

Dependent Variable	Year	Year	Mean Difference	Std.Error	Sig.
Tmax	1985	2003	-3,9800	2,86171	0,182
	1985	2015	-12,0000	2,73988	**0,000**
	1985	2016	-2,8000	2,86171	0,342
	2003	2015	-8,0200	2,73988	**0,009**
	2003	2016	1,1800	2,86171	0,685
	2015	2016	9,2000	2,73988	**0,004**
Tmin	1985	2003	8,8400	3,99248	**0,041**
	1985	2015	10,4333	3,82251	**0,014**
	1985	2016	3,0000	3,99248	0,463
	2003	2015	1,5933	3,82251	0,682
	2003	2016	-5,8400	3,99248	0,162
	2015	2016	-7,4333	3,82251	0,069

Table 7: Table of Multiple Comparisons between 1985, 2003, 2015 and 2016 in the summit strata. Bold values indicate statistical significance at the 95% level or higher

At a 95% level of confidence, these tests provide strong information of thermal differences among years and strata. In the basal strata, there's statistical difference in maximum temperature between 1985, 2002 and 2016 ($p = 0{,}000 < 0{,}05$), while minimum temperature is statistically different between 1985 and 2016 ($p = 0{,}016 < 0{,}05$). In the cloudy montane strata, minimum temperature is statistically different between 1985 and 2016 ($p = 0{,}002 < 0{,}05$). In the summer-xeric strata, there's statistical difference in maximum temperature between 1988 and 2016 ($p = 0{,}008 < 0{,}05$). In the summit strata, maximum temperature and minimum temperature varied among the years of study during the last 30 years (minimum temperature was statistically different in 1985 from 2003 ($p = 0{,}041 < 0{,}05$) and 2015 ($p = 0{,}014 < 0{,}05$) years and maximum temperature was statistically different in 2015 from the rest of the years).

6. Discussion

In the years of study during the last 30 years, there has been a temperature increase. This kind of short-term thermal fluctuations affects life-cycle strategies of some species of beetles. *Sensu* Hutchinson (1957) and Shelford (1931), a deviation of the climatic optima would affect biological efficiency. Thus, it has been demonstrated that some species changed their altitudinal distribution or population parameters due to climatic fluctuations that affected their biological efficiency.

During the 1980's, the populations of *Carabus abbreviatus* were restricted to the middle climatic zone (600 – 1.700 m). This population reached their maximum activity-density between 1200 and 1500 m (de los Santos, 2009). However, during the 2010's, the populations of *C. abbreviatus* decreased in the middle climatic zone and increased in the summit strata. There wasn't a significant decrease in the activity-density in the cloudy-montane strata following yearly variations. However, if the study is extended and data are recorded in autumn and winter, these changes may be significant as *C. abbreviatus* populations have their phenological peak during autumn and early winter. It is known that *C. abbreviatus* feeds on darkling beetles larvae, so the prey abundance in the summit strata may explain this change in the distribution in the last 10 years. Also, the increase in temperature in the cloudy-montane strata may have influenced in the decrease of *C. abbreviatus* populations. However, in the summit strata, the humidity in the summit strata may affect their tolerance response as they suffer a strong hydric stress. Plus, if temperatures continue rising, *C.*

abbreviatus won't have an upper zone to establish so, if the adaptive rate is lower than the ecosystem change rate, this species will become extinct.

In the cloudy-montane strata, the absence of *Calathus depressus* during late winter and spring could be explained because of the temperature increase. During the 1980's it was a well-distributed species among the altitudinal gradient but, in the last years, it wasn't found during the sampling period, but, to confirm this absence, we have to record data in autumn and winter, as *C. depressus* populations have their phenological peak during autumn and early winter. This displacement would have stimulated *Orthomus berytensis* to colonize Ravine of Badajoz. This is a riparian species found in the coastal zone, never in forests, where their dispersal capability is reduced (Machado, 1992). The colonization of this species may be explained by a progressive warming of the study area.

Hegeter populations in the basal strata have increased their activity-density pattern due to a temperature increase. Regression analyses demonstrated that these populations are mainly affected by the maximum temperature. During 1985, *Hegeter* species were found mainly during spring and summer. However, in 2016, we've found *Hegeter* since March, which may indicate a variation in their phenology. *Hegeter lateralis* populations did not show a significant variation in any of their population parameters, although a temperature increase in the summit strata was shown. This may indicate that beetles in the summit strata are adapted to short-term fluctuations.

Transformations of the life-cycle have an adaptive value as well as variability of the life-cycle allows species not only to survive, but also to establish itself under new living conditions (Trushitsyna & Matalin, 2016). In this context, studying life-cycles will indicate adaptive responses as a result of environmental changes, especially climatic fluctuations. However, it's crucial to understand that those results are partial, as we need to have a global view of a one-year life cycle to get more representative conclusions, especially for those species which presents phenological peak during autumn and early winter. Also, it's important to understand that testing relationship between climatic variables and population parameters following a fine-scale will be more representative, supported by this broad-scale project.

7. Conclusions

From these empiric evidences, fieldwork experience and statistical analyses some interesting conclusions are obtained:

1. There's evidence of short-term thermal fluctuation during the years of study in the last 30 years in the study area
2. Minimum temperature and/or maximum temperature have increased during the years of study in the last 30 years in every stratum
3. Temperature is one of the main factor that affects life-cycle strategies of soil-surface beetles
4. During the years of study in the last 30 years, in the study area, some species (mainly ground beetles) have changed their distribution along the altitudinal gradient
5. A global view of a one-year life-cycle will be more representative and conclusion will be more representative of the soil-surface beetles species status

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