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To Maria, Jacinto and Manuela

Thank you for making my life such a wonderful and exciting ride!

Rhinella schneideri, (Werner, 1894)
On the cover – *Rhinella granulosa*, (Spix, 1824)

“All over the world the wildlife that I write about is in grave danger. It is being exterminated by what we call the progress of civilization. (…) Does a creature have to be of direct material use to mankind in order to exist? By and large, by asking the question "what use is it?" you are asking the animal to justify its existence without having justified your own.”

Gerald Durrell

“We can't leave people in abject poverty, so we need to raise the standard of living for 80% of the world's people, while bringing it down considerably for the 20% who are destroying our natural resources.”

Jane Goodall

ABSTRACT

Amphibians across the world are threatened by climate change. This work deals with the analysis of thermal tolerance and sensitivity and their latitudinal variation at the community level, with the intent of examining the prediction that tropical amphibians are at higher risk of extinction due to global warming than temperate species since their environmental temperatures are closer to their upper thermal limits.

To test this prediction, two larval amphibian communities were selected from contrasting latitudes: subtropical (Argentina) and temperate Mediterranean (Iberian Peninsula) climates. In both locations, the following key parameters were obtained: 1) environmental pond temperatures (Thab), by monitoring ponds at different locations using water dataloggers; 2) critical thermal maximum, using a dynamic method called CTmax or knockdown temperature, to assess how close environmental temperatures are from their upper thermal limit; and 3) optimum temperature (Topt), by analysing tadpole’s maximum swimming speed at different temperatures and building thermal performance curves (TPCs), to determine how changes in environmental temperatures will affect the ability to perform ecologically relevant functions and therefore their general fitness.

Warming Tolerance (WT) (WT=CTmax-Thab) and Thermal Safety Margins (TSM) (TSM=Topt-Thab) were also calculated for all species.

Analyses of CTmax and optimal performance temperature indicate that species have adapted their critical and optimal temperatures to cope with environmental conditions. Species exposed to higher maximum or average temperatures usually have higher CTmax or optimum temperatures, respectively. In addition, there is a significant positive correlation between these traits.

Results also show that Argentinean subtropical species, although having higher CTmax and optimum temperature values, have lower WT and narrower TSM. Therefore, these species generally appear to be in greater extinction risk than temperate species from the Iberian Peninsula, under predicted scenarios of rising temperatures and climate change.

Keywords: thermal tolerance, thermal sensitivity, performance, global warming, amphibian decline.

RESUMO

Este trabalho aborda a questão de como os organismos irão lidar com a ameaça do aquecimento global. Seleccionaram-se os anfíbios como objecto de estudo por estes serem o grupo de vertebrados terrestres mais ameaçado e por potencialmente serem altamente sensíveis aos efeitos da subida da temperatura. A sua ectotermia, fase larvar aquática e mobilidade limitada poderão aumentar o impacto que o aquecimento global terá nos anfíbios e nas suas populações. Pretendeu-se abordar esta complexa questão através de uma análise de duas comunidades, procurando desvendar se espécies de anfíbios sujeitas a regimes térmicos distintos, por exemplo comunidades de anfíbios tropicais "versus" temperadas, diferem no risco de extinção face aos desafios impostos pelos cenários previstos de mudança climática.

Analisaram-se a tolerância e a sensibilidade térmicas, ao nível específico e de toda a comunidade, e a sua variação latitudinal. Examinou-se a previsão de que os anfíbios tropicais se encontram sujeitos a um risco de extinção mais elevado devido ao aquecimento global do que as espécies temperadas, uma vez que as temperaturas ambientais a que estão sujeitos estão mais perto dos seus limites térmicos superiores.

De modo a testar esta previsão, seleccionaram-se duas comunidades de larvas de anfíbios, abrangendo os climas subtropical (Argentina) e temperado mediterrânico (Península Ibérica). Para cada comunidade, foram obtidos os seguintes parâmetros-chave: temperaturas ambientais das charcas; temperatura crítica máxima (CTmax) de uma selecção de espécies, para determinar a proximidade do seu limite térmico máximo às temperaturas ambientais; e curvas de "performance" térmica (TPCs) durante a fase larvar, de modo a perceber como as temperaturas ambientais afectam a sua capacidade de realizar funções ecologicamente relevantes e, portanto, a sua "fitness" geral.

Para a comunidade subtropical, seleccionaram-se áreas de estudo no norte da Argentina. Foram incluídas charcas da região do "El Gran Chaco", províncias de Formosa e Chaco, e também da parte norte da província de Corrientes, abragendo uma área geográfica entre 24-27ºS e 58-61ºW. Estas regiões caracterizam-se por um regime sazonal de precipitação, concentrada durante o verão austral, o que condiciona os anfíbios a reproduzirem-se num período especialmente quente e húmido. O "El Gran Chaco" é , inclusive, uma das regiões mais quentes da América do Sul.

As áreas de estudo para a comunidade temperada de anfíbios situaram-se na Península Ibérica, onde os girinos foram recolhidos em Portugal e Espanha, cobrindo uma distribuição norte-sul desde Oviedo, Astúrias (43ºN), até Doñana, Andaluzia (37ºN), e uma distribuição este-oeste desde Granada, Andaluzia (3ºW), até Verdizela, na costa portuguesa (9ºW). A maioria dos anfíbios da Península Ibérica reproduz-se com temperaturas mais frias, durante o Outono, Inverno e/ou Primavera; apenas as espécies que vivem a altitudes mais elevadas se reproduzem no início do Verão.

De modo a estimar a tolerância ao aquecimento e as margens de segurança térmicas, são necessários dados ambientais sobre a temperatura da água das charcas.

Para a monitorização dos perfis térmicos dos habitats de reprodução, colocaram-se "dataloggers" para água no fundo das charcas (zona fria) e na margem (zona quente), de modo a obter todo o gradiente térmico existente. No total, foram monitorizadas 20 charcas.

No total, estudaram-se 19 espécies quanto à sua tolerância e sensibilidade térmicas: 9 da comunidade subtropical na Argentina (*Pseudis limellum, Pseudis paradoxa, Scinax acuminatus, Scinax nasicus, Elachistocleis bicolor, Trachycephalus venulosus, Hypsiboas raniceps, Leptodactylus latinasus, Leptodactylus bufonius* e *Physalaemus albonotatus*) e 9 da comunidade temperada da Península Ibérica (*Pelobates cultripes, Pelodytes ibericus, Alytes cisternasii, Alytes dickhilleni, Rana temporaria, Rana iberica, Hyla meridionalis, Hyla arborea* and *Epidalea calamita*).

Os limites máximos de tolerância térmica foram determinados usando um método dinâmico designado por CTmax ou temperatura de "knockdown". Foram testados 15-20 girinos por espécie, em laboratório. Os animais estiveram no mínimo 4 dias à temperatura de aclimatização de 20°C e foram mantidos em recipientes de plástico, a uma densidade larvar semelhante, com um fotoperíodo de 12D:12L e alimentadas "ad libitum". Determinou-se o CTmax aquecendo os girinos a uma taxa de 1 ºC/min. Colocou-se cada larva em 400 ml de água sem cloro numa taça de vidro de 700 ml parcialmente submergida dentro de um recipiente de 2000 ml. A água no recipiente maior foi aquecida uniformemente por um agitador magnético Agimatic-N (P-Selecta). Cada teste começou à temperatura de aclimatização. Monitorizou-se a temperatura da água com um termómetro digital (digi-thermo) (± 0,1°C). Registou-se a perda de "righting response" (LRR) e o início dos espasmos (OS). Uma vez atingido o OS, o girino foi colocado em água fria para permitir a sua recuperação.

Para estimar a sensibilidade térmica foram construídas curvas de "performance" térmica para cada espécie (TPCs). Estas TPCs foram baseadas na capacidade locomotora medida como "burst swimming" nos girinos. Utilizou-se um canal de metacrilato, no qual as larvas nadaram a temperatura controlada. Cada girino foi colocado no canal e estimulado com uma vareta fina até que produzisse um "burst swim". Os testes foram gravados com uma câmara de video digital, colocada 2 m acima do canal. Foram testadas 15-20 larvas por espécie, com aclimatização igual à do CTmax, às temperaturas de 20°, 24°, 28°, 32°, 35° e 38°C para as espécies argentinas e de 10°, 15°, 20°, 24°, 28°, 32° e 35°C para as espécies ibéricas. Algumas espécies argentinas não toleram aclimatização a 20°C (por exemplo, alguns Leptodactilídeos) pelo que se considerou que as temperaturas inferiores a 20°C pudessem estar fora do intervalo de "performance" locomotora natural para esta comunidade. O mesmo argumento justifica a temperatura máxima testada nas comunidades paleárticas.

A relação entre tolerância térmica, sensibilidade e temperatura de habitat foi examinada num contexto filogenético, de modo a determinar até que ponto os padrões observados poderão derivar estritamente de um fundo genético comum entre espécies próximas. Como tal, foi usado o teste TFSI ("test for serial independence")

para caracteres contínuos e o método de PIC (“phylogenetically independent contrasts”) para determinar se cada caracter estava significativamente associado à sua história filogenética e para corrigir as correlações encontradas tendo em conta a sua filogenia.

As espécies de anfíbios da Argentina apresentaram, em geral, um valor de CTmax mais elevado que as espécies da Pensínsula Ibérica. À escala global, para as 19 espécies estudadas, os limites térmicos máximos de tolerância reflectiram as condições ambientais. Tanto antes como depois de corrigir pela filogenia, obteve-se uma correlação significativa entre as temperaturas ambientais e os limites térmicos máximos de tolerância.

Quanto à temperatura óptima de “performance”, os resultados foram semelhantes aos das análises dos limites térmicos máximos. Em geral, as espécies argentinas possuem um valor óptimo de temperatura mais elevado que as espécies ibéricas. Porém, existe muita variabilidade para este caracter nas espécies da Península Ibérica. Com base nessa variabilidade, foi possível discriminar dois grupos: espécies que se reproduzem na Primavera e princípios de Verão, com valores de óptimo bastante semelhantes aos das espécies argentinas; e espécies que se reproduzem no Outono/Inverno e/ou em habitats frios (ribeiras ou montanha), com valores de óptimo claramente inferiores aos das espécies subtropicais.

Os resultados indicaram também que as espécies adaptaram a sua temperatura óptima de modo a lidar com as condições ambientais locais, pelo que existe uma correlação significativa entre temperatura óptima e temperatura média ambiental.

Tanto para o Ctmax como para a temperatura óptima de “performance”, espécies expostas a temperaturas ambientais mais elevadas possuem valores também mais elevados para estes dois caracteres.

As espécies de anfíbios da Argentina, apesar de possuírem valores de CTmax e de temperatura óptima mais elevados, têm menor tolerância ao aquecimento e margens de segurança térmica mais estreitas que as espécies da Península Ibérica. Portanto, num cenário de mudança climática, em que se prevê um aquecimento global, as espécies de anfíbios subtropicais estarão em maior risco de extinção que os anfíbios de regiões temperadas.

Palavras-chave: tolerância térmica, sensibilidade térmica, aquecimento global, performance, anfíbios.

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1. INTRODUCTION

1.1. State of the art

Temperature affects virtually all physiological processes. It determines both rates of chemical reactions (Hochochka and Somero, 2002) and many ecological interactions (Dunson and Travis, 1991). Therefore, it is expected that environmental temperature changes associated with global warming will have broad ecological consequences for species and communities (Southward et al., 1995; Pearson and Dawson, 2003; Case et al., 2005). Recent reports, using meta-analyses, have presented comprehensive synthesis of the impact of climate change on a range of species (Parmesan and Yohe, 2003; Root et al., 2003). However, extensive debate still exists on whether the observed biological changes can be conclusively linked to an anthropogenic effect on climate or contrarily it may be better attributed simply to sampling bias (Jensen, 2003).

To assess how climate change will really impact organims, deep knowledge is required on three main key issues: a) the current conditions and future climatic scenarios; b) how close organisms are to their thermal tolerance in nature; and c) to know the degree to which organisms are able to adjust or acclimatize their thermal sensitivity (Stillman, 2003; Gilman et al., 2006). One can expect that organisms with the greatest risk of extinction from rapid climatic change are those with a low tolerance to warming, limited acclimation ability and reduced dispersal, incapacitating them to avoid/adjust to new challenging conditions.

Ectotherms comprise most of the terrestrial biodiversity and are expected to be especially vulnerable to global warming since their basic physiological functions, development and behaviour are strongly affected by temperature. In these organisms, most physiological processes proceed rapidly over a range of body temperatures defining a thermal performance curve or TPC (Huey and Stevenson, 1979). This thermal sensitivity curve rises gradually from a minimum critical temperature (CTmin), to an optimum temperature (Topt), and then falls rapidly to a critical thermal maximum (CTmax). Critical thermal limits define the thermal tolerance range of an organism. Temperatures either below or above the range of tolerance result in impaired physiological function (Hillman et al., 2009).

Impacts of global warming on biodiversity are often assumed to be geographically dependent, being predicted to be smaller in the tropics relatively to those in temperate regions (Root et al., 2003; Parmesan, 2007) because the projected rate of climate warming in the tropics is lower than the one expected for higher latitudes (IPCC, 2007a). However, this prediction based on absolute temperature change may be misleading due to several factors associated with behaviour, physiology and ecology of organisms.

First, there are wide indications that thermal tolerance in different groups of ectotherms is related to the magnitude of temperature variation they normally experience (Janzen, 1969; Addo-Bediako et al., 2000; Ghalambor et al., 2006), thereby increasing with latitude. Most evidences suggest that temperate zone species should have relatively broad thermal tolerances (e.g. the difference between critical thermal minimum [CTmin] and maximum [CTmax]) than tropical ones, primarily because they are much more cold tolerant. Some available compilations of body temperatures are consistent with the prediction that their variability is reduced in the tropics and increases with latitude, for example in salamanders (Feder and Lynch, 1982), lizards (van Berkum, 1988) and crabs (Stillman and Somero, 2000).

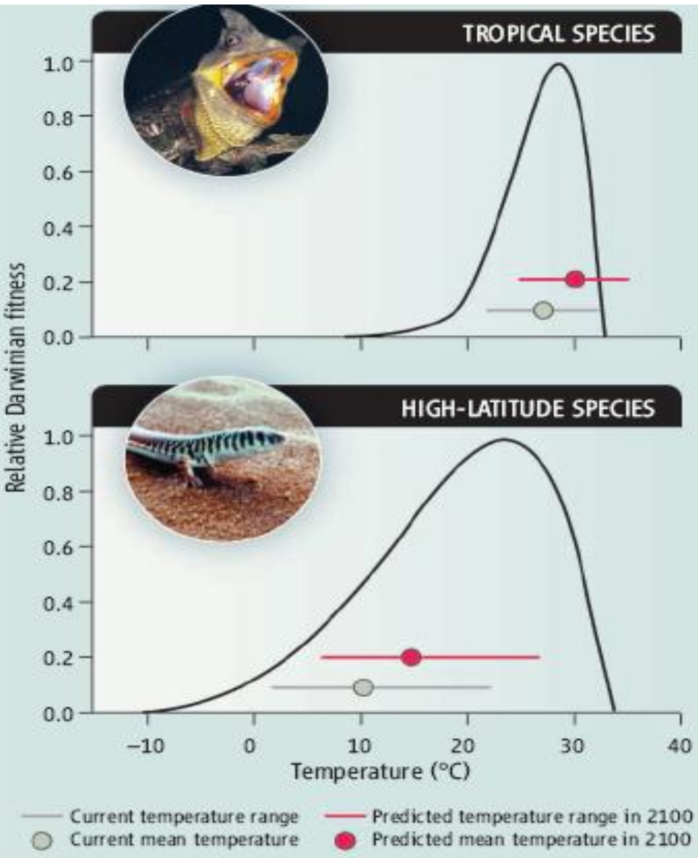

Fig.1- Survival in a warmer climate. Data from diverse terrestrial ectotherms suggest that tropical species living in stable aseasonal climates, such as the Amazonian lizard *Enyalioides palpebralis* (top inset), have narrower thermal tolerances than do higher-latitude species such as the temperate lizard *Nucras tessellate* (bottom inset), and also live in climates that are closer to their physiological optima. The former may thus be highly vulnerable even to modest climate warming (in Tewksbury et al., 2008; Copyright © 2008 by the American Association for the Advancement of Science, Science).

Second, the impacts of environmental warming should be more negative on thermally specialized animals (Ghalambor et al., 2006) and/or on those that have a lower acclimation capacity (Stillman, 2003). From previous works, tropical ectotherms appear to be thermal specialists with lower acclimation capacity than higher-latitude species (van Berkum, 1988; Addo-Bediako et al., 2000; Hoffmann et al., 2003; Ghalambor et al., 2006; Gilman et al., 2006; Calosi et al., 2008; Deutsch et al., 2008).

Third, tropical ectotherms which are exposed to higher temperatures throughout the year might be expected to express higher heat tolerance. However, it seems that critical thermal maximum of terrestrial ectotherms does not vary much across latitude (Addo-Bediako et al., 2000; Ghalambor et al., 2006; Deutsch et al., 2008; Tewksbury et al., 2008). Since many tropical ectotherms live in environments where body temperatures are close or even above optimal temperatures for performance (Deutsch et al., 2008), any small increment in temperature may have catastrophic consequences. These evidences suggest these species, constituting the richest faunistic biodiversity of the world, would be at a greater risk of extinction due to global warming comparing to species from higher latitudes. Nevertheless, a realistic evaluation of a higher geographic impact on the tropical ectotherms, due to predicted increase in temperatures, is lacking. This will need to combine both accurate knowledge of specific tolerance of organisms and the environmental temperature they are exposed to.

A vast majority of the research on thermal adaptation and thermal tolerance has been conducted on a few well-studied model-organisms, such as *Drosophila* sp., undergoing experimental stress under laboratory conditions (Hoffmann and Parsons, 1997; Hoffmann et al., 2003). In spite of this being a fundamental first step, the interest in producing information on focal threatened taxa, and specially those distributed across sensible habitats (e.g., amphibians in tropical and mountain areas), is rising as conservation actions will demand a higher level of knowledge of the physiological ecology and genetics of species in these particular systems.

Recently, Deutsch et al. (2008) provided a first quantitative assessment of this prediction, employing a physiological framework which integrated fitness curves, reflecting thermal tolerance of terrestrial insects from around the world, with projected geographic distribution of climate change, for the next century. To assess latitudinal variations in global warming impact on ectotherms, assuming that their basic physiological functions, such as locomotion, growth, development and reproduction, are heavily dependent on environmental temperature, two operative metrics were defined:

1. Warming tolerance (WT=CTmax–Thab), as the difference between the organism’s critical thermal maximum and the current temperature of its environment. This metric is related to the average warming that an ectotherm can tolerate before environmental temperatures reach its upper tolerance limit. The prediction is that tropical species would have lower WT than temperate or high-latitude species.

2. Thermal safety margin (TSM=Topt–Thab), as the difference between the organism’s thermal optimum (Topt) and its current environmental temperature (Thab). The prediction was that tropical species would have a narrower TSM because they live at environmental temperatures closer to their physiological optimum. Therefore, any small increase in temperature could trigger a decrease in their performance. On the other hand, species from temperate or high-latitude are exposed to cooler

temperatures than their optimal and, therefore, global warming may even increase their fitness and population growth rates.

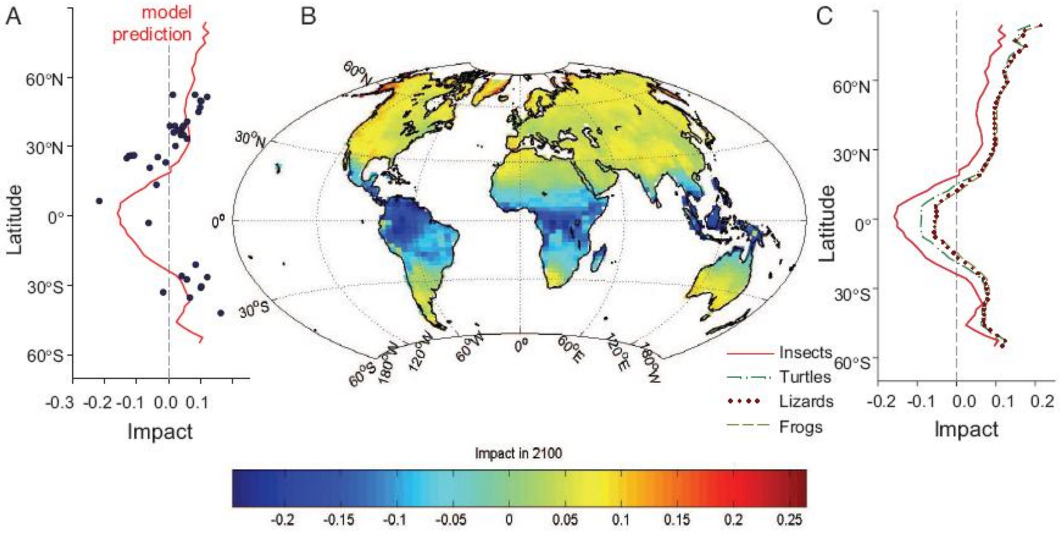

Fig.2- Predicted impact of warming on the thermal performance of ectotherms in 2100. (A) Impact versus latitude for insects using thermal performance curves fit to intrinsic population growth rates measured for each species (black circles) and for a global model (red line) in which performance curves at each location are interpolated from empirical linear relationships between seasonality and both warming tolerance and thermal safety margin. (B and C) Results from the simplified conceptual model are shown globally for insects (B) for which performance data are most complete, and versus latitude for three additional taxa of terrestrial ectotherms: frogs and toads, lizards, and turtles (C), for which only warming tolerance was available (in Deutsch et al., 2008, fig. 3, © 2008 by The National Academy of Sciences of the USA).

Their results (figure 2) show that tropical insects and ectothermic vertebrates (reptiles and amphibians), despite being subjected to a lower increase in air temperatures, will suffer a large decrease in their fitness since they are currently living very close to their optimal temperature. Regarding species from higher latitudes, currently living in cooler temperatures, a changing climate may even benefit them.

However, this approach is opened to several criticisms. The major shortcomings of this approach are: first, habitat and body temperatures were estimated using only air temperature measurements of their habitats and thus disregarding the potential of thermoregulation of most ectotherms; and second, their estimates of CTmax were pooled from literature sources that did not control by acclimation and other factors that may induce bias on CTmax estimates (see Methodology 2.2.).

1.2. Objectives and study organisms

The main objective of this thesis is to assess whether tropical amphibians species (living normally under higher environmental temperatures) are at higher risk of extinction than temperate species (living normally under colder environmental temperatures) when facing global warming, since they are expected to exhibit both lower warming tolerance (WT=CTmax–Thab) and narrower thermal safety margins (TSM=Topt–Thab).

Amphibians are considered the most endangered group of vertebrates since near one third of all extant species are threatened with extinction (Stuart et al., 2004). They have a number of physiological, ecological and life-history characteristics that make them highly susceptible to environmental change such as ectothermy, permeable skin and complex life-cycles (with metamorphosis), the last one presumed to be an adaptation to the sequential occupation of temporary wetlands and terrestrial environments (Wells, 2007). All of these traits also determine an important dependence on environmental factors and, in addition, can explain the geographic pattern of amphibian species richness. The tropics hold much higher species richness, containing more than 85 % of the current amphibian species (Duellmann, 1999; Wells, 2007; Stuart et al., 2008). Particularly, the Neotropical realm of Central and South America alone concentrates the highest diversity of amphibians on earth with 2916 recognized species, 49 % of world total (IUCN, 2006; Stuart et al., 2008).

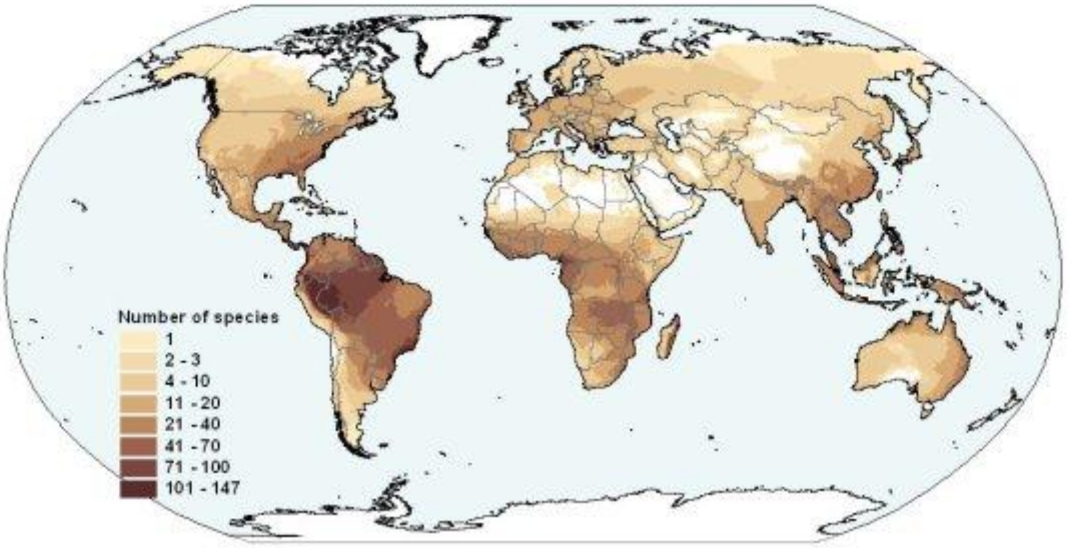

Fig.3- Global diversity of amphibian species (in Global Amphibian Assessment, IUCN, 2006).

Many of the reported cases of amphibian declines and extirpation of local populations have been caused by the pathogenic fungus *Batrachochytrium dendrobatidis* (Stuart et al., 2004; Lips et al., 2006; Wake and Vredenburg, 2008), or similar emergent diseases. Although it is unclear the extent to which global warming could have triggered devastating chytridiomicosis outbreaks (Rohr et al.,2008), there is mounting evidence that epidemic diseases may be driven by changes in the thermal environment (Pounds et al., 2006; Raffel et al., 2006; Bosch et al., 2007; Ruthig, 2008). So far, there has been no report on amphibian population decimation directly attributable to warming. Nevertheless, there is an obvious risk that the direct effects of increasing temperatures and other manifestations of climate change can cause a second wave of extinctions in the mid/long-term.

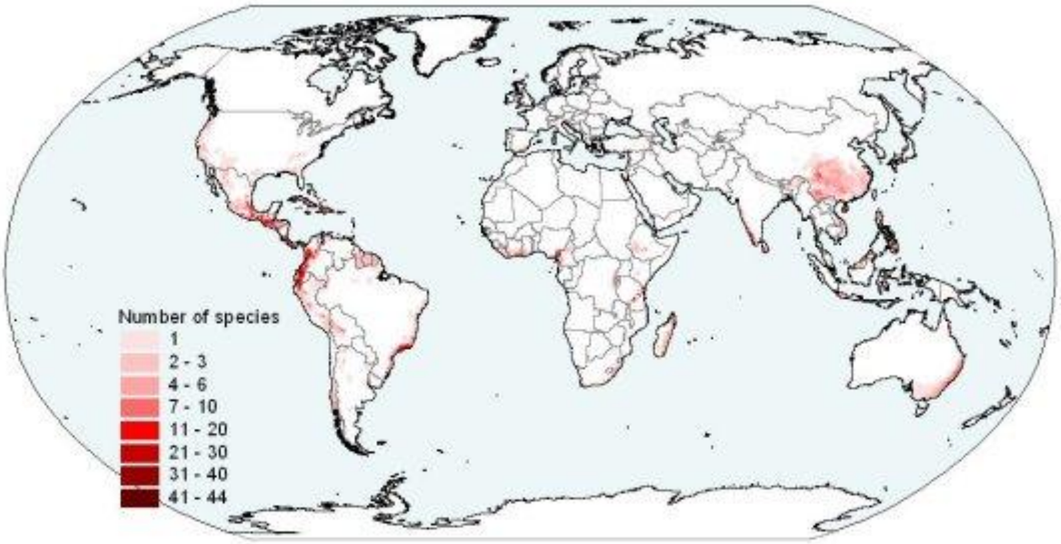

Fig. 4- Global distribution of threatened amphibians (in Global Amphibian Assessment, IUCN, 2006). See Global Amphibian Assessment for detailed maps of critical areas.

This work will look at the larval stage of amphibians. For several reasons, tadpoles can be considered a model organism for studying thermal tolerances in ectotherms, in general, and in amphibians, in particular:

1) The tadpole phase is a growth and developmental period and all analyzed traits are independent of reproductive condition;

2) Tadpoles are aquatic and their body temperatures are the same as the temperature of surrounding environment (Spotila et al., 1992);

3) Tadpoles living in water can not suffer the correlated dehydration response of terrestrial stages, when heating. However, they can suffer other stressors associated with increased temperature such as a decrease in water dissolved oxygen and subsequently in their aerobic performance (see Pörtner and Knust, 2007) or an increase in osmotic stress (Gómez-Mestre and Tejedo, 2003, 2004) that may interact with thermal tolerances (Re et al. 2006);

4) Although tadpoles are capable of behaviourally regulating their body temperatures through selection amongst a range of available temperatures (Noland and Ultsch, 1981; Wollmuth et al., 1987; Hutchison and Dupré, 1992), their physical environments eventually limit the extent of their thermoregulation (Huey, 1974; Wu and Kam, 2005). This is particularly dramatic for tadpoles occupying temporary ponds because these are typically shallow and with small water volume and can suffer intense heating, especially those receiving direct sunlight, with important daily thermal gradients and without thermal stratification.

Fig.5- Tadpole of *Pseudis paradoxa*, (Linnaeus, 1758). Foto by Ricardo Reques.

In ponds located in tropical environments with a wet summer breeding season, tadpoles may be exposed to temperatures over 40°C. During heating waves, tadpoles may not be able to escape from dangerous temperatures before ponds dry completely, even though they are capable of behavioural thermoregulation (Wells, 2007), unless they can reach metamorphosis and jump to land. Here, a wider range of microhabitats can be found and possibly they will be able to select a more thermally favourable microclimate (Navas et al, 2007). The expected increase in global mean temperature, together with more frequent extreme hot events, such as heat waves, will accentuate this condition (IPCC, 2007b). On the other hand, global warming is predicted to shorten pond hydroperiods in many areas such as Central America and Australia due to parallel shortage in rainfalls (IPCC, 2007b) which may potentially increase local extinction of amphibian populations.

Most of the literature concerning temperature effects and responses has focused groups of ectothermic vertebrates other than amphibians (fish and reptiles). However, thermal physiology research in amphibians, in spite of seminal contributions in the 50s - 70s (Brattstrom, 1959, 1962 and 1968; Lillywhite, 1970; Hutchison, 1961; Heatwole et al., 1965; Mahoney and Hutchison, 1969), has been intensively developed specially in later decades (see reviews in Rome et al., 1992; Hutchison and Dupré, 1992; Ultsch et al., 1999; Wells, 2007; Navas et al., 2008; Hillman et al., 2009).

1.3. Thermal tolerance studies

The analysis of thermal tolerances in amphibians was initially developed by Brattstrom (1968) in anurans, and Hutchison (1961), in salamanders. Interestingly, Brattstrom's study included comparative data of CTmax for 53 species of frogs from a latitudinal and altitudinal gradient in North and Central America. He found that CTmax varied both at the species and population levels.

For most anuran larvae, CTmax was determined to fall between 38°C and 42°C (Ultsch et al., 1999). The differences in CTmax among populations may be a response to interdemic thermal habitat through local genetic adaptation in thermal tolerance. A number of studies have demonstrated within species variation in heat tolerance (e.g., Hutchison, 1961; Brattstrom, 1968 and 1970; Delson and Whitford, 1973; Miller and Packard, 1977; Hoppe, 1978; Hertz et al., 1979; Garland and Adolph, 1991; Meffe et al., 1995; Schwarzkopf, 1998; Gvoždík and Castilla, 2001; Winne and Keck, 2005; Huang and Tu, 2008). However, few studies have performed common garden experiments designed to distinguish between genetic and acclimation induced differences in physiology (Garland and Adolph, 1991). In amphibians some evidences have been reported that CTmax may adaptively differ between populations (Skelly and Freidenburg, 2000; Chen et al., 2001; Wu and Kam 2005; C. Navas, unpublished data).

Other factors should be taken into account. Ontogeny may affect CTmax, which drops 3°C-4°C when larvae are close to metamorphic climax (Floyd, 1983), requiring deeper analysis of maximum heating during this risk-sensitive phase. Acclimation to higher temperatures may affect the estimate of CTmax, increasing its value up to 4°C (Brattstrom, 1968; Navas et al., 2008). It is recognized that CTmax exhibits a phylogenetic signal and differences between amphibian lineages can be found both in adult stages (Navas et al., 2008), and tadpoles, (H. Duarte and J.P. do Amaral, unpublished data).

Finally, some controversy exists whether CTmax is dependent on latitude. Analysis on insects revealed no geographical trend (Addo-Bediako et al., 2000). In amphibians, the analysis of Brattstrom (1968) data set is inconclusive: Snyder and Weathers (1975) found a significant decline in CTmax with increasing latitude ($r=0,70$; $p<0,05$) whereas the re-analysis of Ghalambor et al. (2006) showed that the trend was not significant ($p > 0,70$).

Since estimates of CTmax are susceptible to error due to several factors, including variations in end-points (Lutterschmidt and Hutchison, 1997), acclimation temperature (Navas et al., 2008), or even differences in researcher perceptions, it is top-priority to standardize all the measurements in order to provide testable comparisons.

1.4. Thermal sensitivity studies

The study of thermal sensitivity and optimal temperature in locomotor performance has been largely developed in ectotherms in general (e.g. Bauwens et al., 1995; Claussen et al., 2000) and in amphibians in particular (Rome et al., 1992; Whitehead et al., 1989; Tejedo et al., 2000; Wilson, 2001; Gomes et al., 2002). Maximum sprint speed is an ecologically relevant index of organismal performance capacity and has been employed as a good proxy to estimate optimal temperatures in ectotherms since it may correlate with fitness (Jayne and Bennett, 1990; Le Galliard et al., 2004; Husak, 2006).

Two hypotheses have been suggested to explain the evolution of thermal sensitivity using thermal performance curves. The ‘‘warmer is better’’ (or

‘’thermodynamic constraint’’) hypothesis states that the maximal performance of organisms with high optimal temperatures should be higher than that of organisms with low optimal temperatures (Huey and Kingsolver, 1989; Savage et al., 2004). The ‘’Jack-of all-temperatures is a master of none’’ (Huey and Hertz, 1984) hypothesis assumes a trade-off between maximal performance and the breadth of the performance curve (Levins, 1968; Huey and Slatkin, 1976). Few interespecific analyses have tested these hypotheses and also whether generalist/specialist species predominate either in tropical or temperate communities.

In this work, tadpoles from 19 different species were tested for critical thermal maximum (CTmax) and for maximal locomotor performance to obtain their upper thermal limit and an estimation of their optimum temperature, respectively. Optimum temperature and CTmax are likely to be co-adaptive traits (Huey and Bennett, 1987; Angilletta, 2009), therefore their relationship was also analysed. Species tested include 10 from the Argentinean community (*Pseudis limellum, Pseudis paradoxa, Scinax acuminatus, Scinax nasicus, Elachistocleis bicolor, Trachycephalus venulosus, Hypsiboas raniceps, Leptodactylus latinasus, Leptodactylus bufonius* and *Physalaemus albonotatus*) and nine from the Iberian community (*Pelobates cultripes, Pelodytes ibericus, Alytes cisternasii, Alytes dickhilleni, Rana temporaria, Rana iberica, Hyla meridionalis, Hyla arborea* and *Epidalea calamita*). Pond water (environmental) temperatures were also measured *in situ* and used to calculate Warming Tolerance (WT) and Thermal Safety Margins (TSM). WT and TSM results allow an evaluation of the vulnerability to warming for the species from both Argentinean and Iberian communities and will help to determine which group of species is more threatened facing global warming.

2. METHODOLOGY

2.1. Field work

2.1.1. Study areas

Fig.6- Study areas. a) location of monitored ponds in the Iberian Peninsula (temperate community); b) location of monitored ponds in Argentina (subtropical community). See table 1 and Annexe 1 for pond details.

Regarding the subtropical community from northern Argentina, sampled ponds were located in the El Gran Chaco region, in the provinces of Formosa and Chaco, and also in the northern part of the province of Corrientes, ranging from 24ºS to 27ºS and 58ºW to 61ºW (see figure 6). The El Gran Chaco region is one of the warmest areas of South America, characterized by a seasonal regimen of precipitation concentrated during the austral summer, thus, amphibians from this region breed in a hot humid season.

The study area for the temperate amphibian community was situated in the Iberian Peninsula (see figure 6). Sampled populations were spread throughout Portugal and Spain, spanning a north-south distribution from Oviedo, Asturias (43ºN), to Doñana, Andalusia (37ºN), and an east-west distribution from Granada, Andalusia (3ºW) to the Portuguese coast, at Verdizela (9ºW). Amphibian species from the Iberian Peninsula (temperate community) differ from the subtropical species because they breed at cooler environmental temperatures, during autumn, winter and/or spring; only species living in higher mountain areas breed in the early summer.

2.1.2. Monitoring environmental temperatures

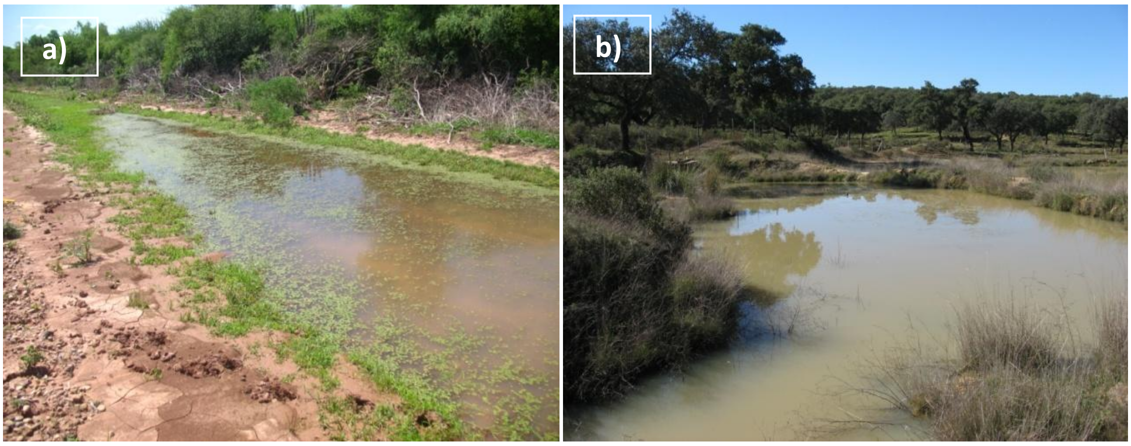

Fig.7- a) A pond from Gran Chaco reagion, Formosa, Argentina. b) Zacallon 1 pond from Las Navas, Andaluzia, Spain

Thermal profiles of breeding habitats were monitored using temperature dataloggers. In each pond where tadpoles were collected, two dataloggers were placed: one at the bottom, considered to be the coolest zone; and the other on the shoreline, the hottest zone of the pond. This procedure was used to obtain the pond's thermal profile and the range of temperatures tadpoles are exposed to.

In total, 20 ponds were monitored for both communities, Table 1 (see also Annexe I).

Table 1- Pond location and geographic coordinates.

Pond	Geographic coordinates	Location
1. CH. PELODYTES CENTRO	37° 47' 29,34'' N 06° 04' 41,94'' W	Las Navas, Sevilla, Andaluzia, Spain
2. LOS LLANOS	37° 46' 56,81'' N 06° 05' 03,03'' W	Las Navas, Sevilla, Andaluzia, Spain
3. RIBEIRA NISA	39° 18' 47,40'' N 07° 23' 06,21'' W	Ribeira de Nisa, Portalegre, Portugal
4. TOBA SUR ABRIL	37° 59' 39,88'' N 04° 54' 07,82'' W	Córdoba, Andaluzia, Spain
5. TOBA SUR	37° 59' 39,88'' N 04° 54' 07,82'' W	Córdoba, Andaluzia, Spain
6. TOBA PRINCIPAL	37° 59' 39,88'' N 04° 54' 07,82'' W	Córdoba, Andaluzia, Spain
7. VERDIZELA	38° 34' 32,70'' N 09° 08' 39,48'' W	Verdizela, Lisboa, Portugal
8. ZACALLON 1	37° 47' 28,40'' N 06° 04' 39,77'' W	Las Navas, Sevilla, Andaluzia, Spain
9. ZACALLON 2	37° 47' 28,36'' N 06° 04' 40,65'' W	Las Navas, Sevilla, Andaluzia, Spain
10. POZO DE LA NIEVE	37° 22' 43,79'' N 02° 51' 45,30'' W	Pozo de la Nieve, Granada, Andaluzia, Spain
11. FUENTE BOLICHE	36° 56' 51,81'' N 02° 50' 57,35'' W	Fuente del Boliche, Almeria, Andaluzia, Spain
12. COLOR CUNETA OVIEDO	43° 17' 42,69" N 05° 16' 26,78" W	Oviedo, Asturias, Spain
13. CUBILLA OVIEDO	42° 59' 04,98" N 05° 55' 33,78" W	Oviedo, Asturias, Spain
14. CECOAL	27° 29' 32,87'' S 58° 45' 34,97'' W	Corrientes, Corrientes, Argentina
15. DERMATONOTUS FRAGA	23° 45' 36.33'' S 62° 08' 06,62'' W	Teniente Fraga, Formosa, Argentina
16. FONTANA	25° 19' 00,01'' S 59° 41' 43,69'' W	Comandante Fontana, Formosa, Argentina
17. LA MORACHA ORILLA	24° 33' 25,75'' S 60° 28' 00,35'' W	Las Lomitas, Formosa, Argentina
18. LA MORACHA CENTRO	24° 33' 25,75'' S 60° 28' 00,35'' W	Las Lomitas, Formosa, Argentina
19. LEPIDOBATRACHUS	24° 13' 41,25'' S 61° 56' 38,32'' W	Reserva Formosa, Formosa, Argentina
20. PERICHON	27° 25' 20,25'' S 58° 44' 29,75'' W	Corrientes, Corrientes, Argentina

2.2. Comparative evaluation of the upper thermal maxima and Warming Tolerance

Some controversies exist with the employment of different tolerance indices since this diversity inherently reduces the possibility of comparisons among population and species (Berrigan, 2000; Cooper et al., 2008).

To avoid these problems, all upper thermal tolerances were determined by applying the same dynamic method known as CTmax or knockdown temperature (Huey et al., 1992; Lutterschmidt and Hutchison, 1997) which involves increasing the test temperature until an end-point is reached, usually the onset of muscular spasms. Recording of CTmax was done by the same person to reduce any possible bias.

Additionally, some artefacts are associated with CTmax estimates. For instance, basic experimental design such as increased rates of temperature change, cumulative heat shock or very long assays can reduce or inflate the estimates (Czajka and Lee, 1990; Cooper et al., 2008; Chown et al., 2009).

Acclimation also alters heat resistance, often in predictable ways. Higher acclimation temperature induces higher heat resistance (Levins, 1969; Worthen and Haney, 1999; Hoffmann et al., 2003; Navas et al. 2008). In order to avoid bias, acclimation temperature and heating rate used were the same for all studied species from both communities.

A random sample of 15 tadpoles per species was analyzed, with larval developmental stages between 25-39 Gosner's (Gosner, 1960). Acclimation temperature was set to 20ºC, for a minimum of 4 days. Acclimated individuals were maintained in plastic containers, at a similar larval density, with 12D:12L photoperiod and fed *ad libitum*.

CTmax was determined by heating tadpoles at a rate of 1°C/min. Tadpoles were individually placed in 400 ml of dechlorinated tap water in a 700 ml glass bowl that was partially submerged within a larger 2000 ml beaker (see figure 8). The water in the larger beaker was heated by an Agimatic-N (P-Selecta) magnetic stirrer ensuring a uniform heating of the water in the inner trial beaker. Each trial started at the same temperature as the acclimation temperature. Water temperature was monitored with an electric thermometer (digi-thermo) (± 0.1°C).

Fig.8- CTmax testing. Overall look of equipment used.

Loss of righting response (LRR) and the onset of spasms (OS) were recorded. OS was defined as the end-point for CTmax determination following Lutterschmidt and Hutchison (1997) recommendations. Once reached the end-point, the tadpole was quickly removed from the beaker and placed in cold water in order to recover.

Tested individuals were weighed, to the nearest 0,001g, after excess skin water was gently removed, by placing larvae on a piece of mesh over blotting paper, staged following Gosner's (1960) and photographed on a scale to estimate body and total lengths.

Statistical analyses of CTmax and WT included analyses of variance, for inter and intraspecific comparisons, and correlations, to determine covariation of traits. Since not all assumptions were met, nonparametric tests were used in most cases (Spearman's rank R correlation and Kruskal-Wallis ANOVA & Median test). A significance level of α=0,05 was used for all statistical tests. Statistical analyses were computed using STATISTICA 7.0 software (StatSoft 2000). All data appear as means ± 1 SE or SD.

2.3. Evaluation of thermal sensitivity of locomotor performance and Thermal Safety Margins

Thermal performance curves (TPCs) were obtained in order to estimate thermal sensitivity. TPCs were based on maximal locomotor performance by measuring tadpoles maximal burst swimming speed. Locomotor performance is considered to be a proxy, representing maximum physiological performance, and a critical variable in fleeing from predators (e.g. Feder, 1983; Werner and McPeek, 1994; Watkins 1996).

To determine maximum swimming speed, burst swimming of tadpoles was estimated. Tadpoles were placed on a thermally controlled and opened cross section methacrylate tube and gently prodded with a thin stick to stimulate swimming. Each trial was recorded using a digital camera installed around 2 m above the tube (see figure 9). Tadpoles were acclimated for at least 4 days at 20°C and 9-38 individuals per species were tested (see table 5).

TPCs were defined using 6-7 temperatures within the natural range recorded in the ponds (see Annexe I). For Argentinean species, the test temperatures were 20°, 24°, 28°, 32°, 35° and 38°C and for the Iberian species 10°, 15°, 20°, 24°, 28°, 32° and 35°C. Temperatures were randomized and tadpoles were tested on consecutive days, one temperature per day. Prior to swimming, tadpoles were submitted for half an hour to the test temperature.

Previous analysis with a Chacoan frog community (northern Argentina) revealed that a few species could not tolerate acclimation at 20°C (some Leptodactilids from the Cavicola group, *Leptodactylus* sp.). This situation matched the thermal profiles measured in the ponds so temperatures lower than 20°C were considered to be out of the natural thermal range for the subtropical Argentinean community. A similar argument was used to select the maximum temperature tested for the Iberian community.

Swimming recordings were analyzed with software Measurement in Motion v3.1 (http://www.motion.com/products/measurement/index.html) that provided tadpole swimming speed at each video recording frame.

After the swimming trials, all tested tadpoles were staged and wet weighed to the nearest 0,001 g. Dorsal and lateral pictures were taken and digitized for morphometric measurements of total length, head-body length, head-body height, tail muscle height, total tail height, total tail area, tail muscle area, head-body width, and tail muscle width (see Dayton et al., 2005) to conduct further morphofunctional analyses, not included in this thesis.

A simple Gaussian model was applied to the data (Angilletta, 2006) in order to estimate optimum temperatures for swimming performance. Statistical analyses of

Topt and TSM were similar to those described for the analyses of CTmax and WT (see Methodology 2.2.).

Fig.9- Swimming performance testing. Overall aspect of equipment assemblage.

2.4. Phylogenetic analyses

The relation of thermal tolerance and sensitivity to habitat temperature was examined within a phylogenetic context to determine the extent to which the observed patterns might derive strictly from phylogenetic relatedness among species (see Stillman and Somero, 2000).

Different phylogenetic techniques may yield different results, especially when the number of species involved is low, since they are based on contrasting evolutionary assumptions (Martins et al. 2002). The use of at least two phylogenetic approaches with different assumptions has been recommended (Martins et al., 2002), in addition to a nonphylogenetic analysis (Garland et al., 1999).

Data were tested for serial independence (TFSI) on continuous characters, to determine whether a trait was significantly associated with its phylogenetic history, using the software Phylogenetic Independence v2.0 (Abouheif, 1999; see also http://www.biology.mcgill.ca/faculty/abouheif/protocols.html).

Diagnosis was based on a measurement of the autocorrelation of each trait across phylogeny, in the form of a *C*-statistic, resulting from similarity between adjacent phylogenetic observations. Topology and associated numerator distribution were randomized 1,000 times and the *C*-statistic was calculated for each randomized topology to build the null hypothesis. Observed *C*-statistic was compared to the randomized distribution to calculate its level of significance.

If the former analyses revealed that examined traits (CTmax, Topt and Thab) exhibited significant phylogenetic autocorrelation, the coevolution of all traits will require a correction for phylogeny since data is not independent. In order to avoid this bias, correlations between traits were obtained using the method of phylogenetically independent contrasts (PIC) as described by Felsenstein (1985), with PDTREE module (PDAP package by Midford et al., 2003) for *Mesquite* (v. 2.6) software (Maddison and Maddison, 2009).

PIC is a method of correcting for phylogeny. It requires that both the tree topology and the branch lengths are known, and that characters are allowed to be modelled by Brownian motion on a linear scale. Given these conditions, the phylogeny specifies a set of contrasts among species, contrasts that are statistically independent and can be used in regression or correlation studies (Felsenstein, 1985).

Using independent contrasts, PDTREE also allows the estimation of ancestral states (values at internal nodes or at any point along a branch) and their standard errors (Garland et al., 1999). In most cases, PIC is mathematically and statistically equivalent to generalized least-squares (GLS) models (Garland et al., 2005).

Previous works have used similar analysis to evaluate evolutionary trends amongst traits in amphibians (see Gvozdik and van Damme, 2008, for an example with European newts).

For these analyses, three phylogenetic trees were constructed based on Frost et al (2006), including only the studied species: one for each community and one combining all species from both communities (see Annexe II). All branch lengths were set equal to one.

3. RESULTS

3.1. Monitoring environmental temperatures

Thermal profiles of the ponds were obtained from the dataloggers placed in the water. Figure 10 shows the output of a selected datalogger. By examining the output, it is possible to identify periods of stormy and clouded days associated with rains (marked in green) and periods where the pond was entirely dry (marked in red), with the sensor presumably measuring air temperature. This information was taken into account while estimating maximum, average and minimum temperatures for the ponds.

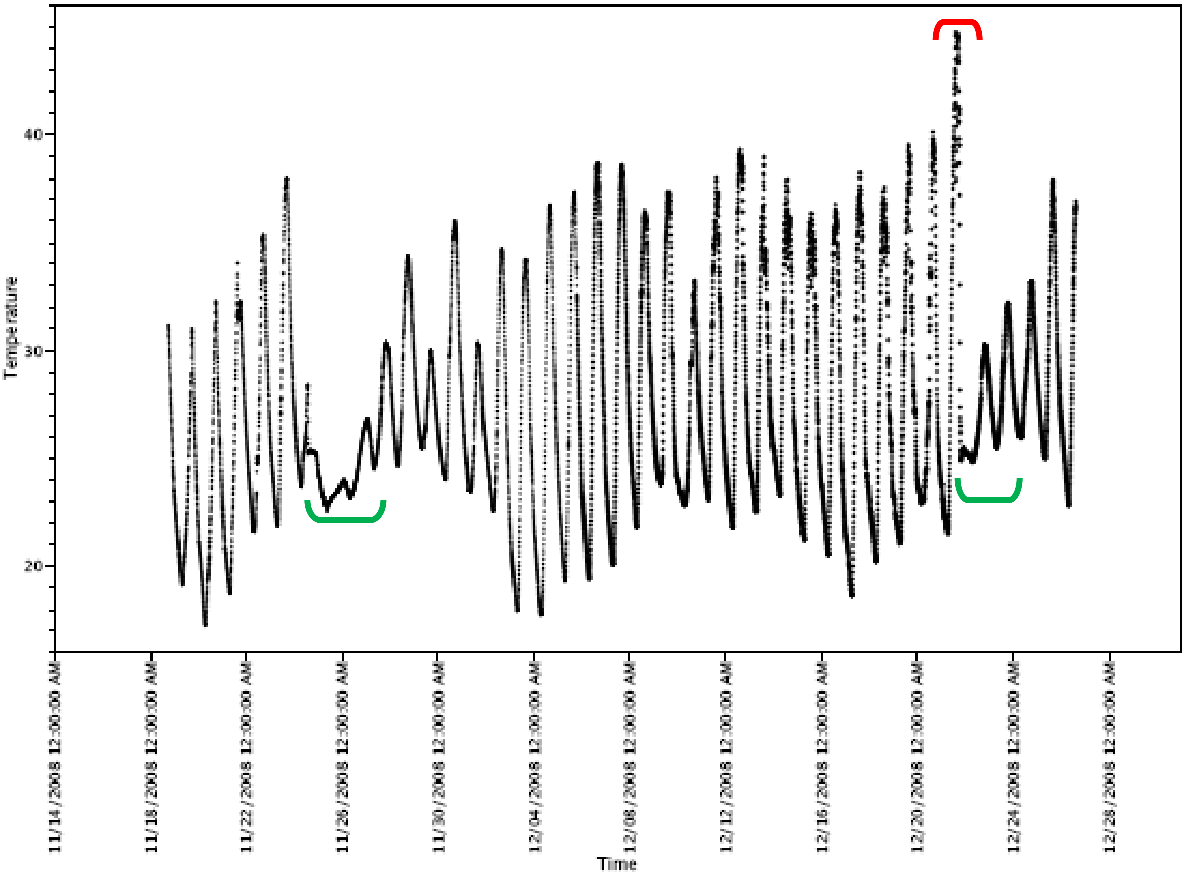

Fig.10- Temporary pond at Comandante Fontana (Formosa province, Argentina, 25° 19' 00,01'' S, 59° 41' 43,69'' W) depth range (max-min) between 25-0 cm depth, 18th November-26th December 2008.

Table in Annexe I provides data on all the ponds monitored and their water temperatures.

Figure 11 shows both shoreline and maximum depth water temperatures for a selected pond, during three consecutive days. Two conclusions can be derived from these observations: first, during the coldest time of the day there is no apparent difference between the two sites while during the warmest there is a clear thermal divergence, with higher values at the shoreline; second, both sites cool at the same rate but differ on their heating rate.

This situation has several biological implications. Tadpoles cannot escape the minimum temperature because the pond's water is thermally homogeneous. During the warmest part of the day, tadpoles can behaviourally thermoregulate selecting amongst a range of temperatures, thus avoiding, into some extent, somewhat undesirable and dangerously high temperatures.

There is a significant difference between the two communities regarding both their Average (Kruskal-Wallis test: $H_{1,20}$=13,00; p=0,0003; N=20) and Maximum Temperature (ANOVA: $F_{1,20}$=13,79032; p=0,000072; N=20) with higher temperatures occurring in the subtropical community (maximum: 38,24°C, SD=2,85°C; average: 26,66°C, SD=1,5°C). In the temperate Iberian community, habitat temperatures were generally cooler (maximum: 28,28°C, SD=4,67°C; average: 13,60°C, SD=3,07°C).

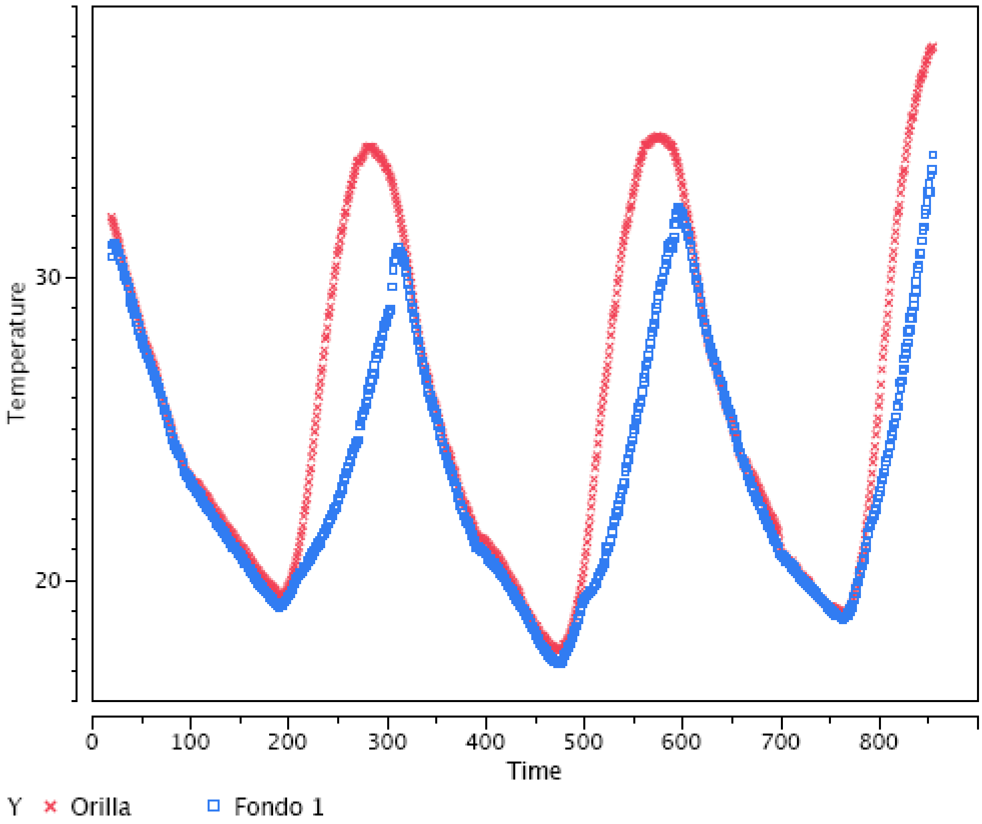

Fig.11- The same pond as in figure 3 but with two different temperature records: at maximum depth (25 cm depth, blue) and at the shore (3 cm depth, red) between 18th-21st November 2008.

For each species, average and maximum temperatures were scored for all ponds monitored where each particular species was collected (Table 2). These values were used in the calculation of species thermal safety margins and warming tolerance, respectively.

Table 2- Average and maximum temperatures per species and per community (Iberian Peninsula (IP) and Argentina (ARG).

Species	Community	Average Temperature		Maximum Temperature	
		Pond	T ºC	Pond	T ºC
A. cisternasii	IP	Toba Sur Abril	10,64	Toba Sur Abril	24,16
A. dickhilleni	IP	Fuente Boliche	12,52	Fuente Boliche	25,13
E. calamita	IP	LosLlanos	14,58	Toba Sur	34,16
P. cultripes	IP	Zacallon 1	10,88	Ch.Pelodytes	35,54
P. ibericus	IP	Toba Principal	18,11	Ch.Pelodytes	35,54
H. arborea	IP	Verdizela	21,06	Verdizela	31,37
H. meridionalis	IP	Toba Principal	18,11	Toba Sur	34,16
R. iberica	IP	Ribeira Nisa	14,02	Ribeira Nisa	29,05
R. temporaria	IP	Cubilla Oviedo	12,21	Cubilla Oviedo	24,47
E. bicolor	ARG	CECOAL	24,17	CECOAL	34,16
H. raniceps	ARG	Perichon	25,38	Perichon	38,21
L. bufonius	ARG	Fontana	27,32	Fontana	40,09
L. latinasus	ARG	La MorachaOrilla	27,43	La MorachaOrilla	38,73
L. limellum	ARG	Perichon	25,38	Perichon	38,21
P. albonotatus	ARG	Fontana	27,32	Fontana	40,09
P. paradoxa	ARG	Perichon	25,38	Perichon	38,21
S. acuminatus	ARG	Perichon	25,38	Perichon	38,21
S. nasicus	ARG	Perichon	25,38	Perichon	38,21
T. venulosus	ARG	La MorachaOrilla	27,43	La MorachaOrilla	38,73

To estimate Warming Tolerance, maximum temperature was selected instead of average temperature because it better reflects the risk that high environmental temperatures represent to the tadpoles (in disagreement with Deutsch et al., 2008 who used mean annual temperature for calculating both Warming Tolerance and Thermal Safety Margins). For instance, if habitat temperature rises above CTmax value, even if it happens only once, it will be fatal for the species survival. Therefore it is considered to be more realistic to estimate warming tolerance using the highest habitat temperature found because it represents the most extreme situation that the species was exposed to during this monitoring period.

To estimate Thermal Safety Margins, average temperature of the pound was used. It is argued that, when comparing optimal temperatures with habitat temperatures, it seems more realistic to use average temperature of the pond rather than the highest temperature recorded because it represents, to some extent, the temperature tadpoles where exposed to during all their larval phase. Maximum temperature can represent a temperature tadpoles were only occasionally exposed to during their larval stage.

3.2. Upper thermal maxima and Warming Tolerance

In total, 19 species were tested for CTmax, 10 from the Argentinean community and 9 from the Iberian Peninsula. Results are shown in Table 3.

Table 3- Community of origin, sample size, average CTmax response and respective SE of all species studied.

Community	Species	*n*	CTmax	SE	Community	Species	*n*	CTmax	SE
ARG	Ebi	15	41,66	0,19	IP	Eca	15	39,73	0,10
ARG	Hra	17	41,18	0,17	IP	Har	15	40,02	0,12
ARG	Lbu	30	43,29	0,15	IP	Hme	15	39,83	0,10
ARG	Lla	15	42,47	0,16	IP	Pcu	14	39,41	0,11
ARG	Lli	14	41,88	0,12	IP	Pib	30	36,98	0,09
ARG	Pal	15	41,12	0,19	IP	Aci	15	38,24	0,17
ARG	Ppa	31	42,25	0,08	IP	Adi	15	37,62	0,12
ARG	Sac	16	43,01	0,17	IP	Rib	15	35,42	0,05
ARG	Sna	15	42,59	0,17	IP	Rte	15	37,21	0,19
ARG	Tve	14	41,87	0,11					

ARG: Argentina community, IP: Iberian Peninsula community. Species - Ebi: *Elaschistocleis bicolor*; Hra: *Hypsiboas raniceps*; Lbu: *Leptodactylus bufonius*; Lla: *Leptodactylus latinasus*; Lli: *Pseudis limellum*; Pal: *Physalaemus albonotatus*; Ppa: *Pseudis paradoxa*; Sac: *Scinax acuminatus*; Sna: *Scinax nasicus*; Tve: *Trachycephalus venulosus*; Aci: *Alytes cisternasii*; Adi: *Alytes dickhilleni*; Eca: *Epidalea calamita*; Har: *Hyla arborea*; Hme: *Hyla meridionalis*; Pcu: *Pelobates cultripes*; Pib: *Pelodytes ibericus*; Rib: *Rana iberica*; Rte: *Rana temporaria*. Both CTmax and SE were expressed in ^{0}C.

Species differ significantly in CTmax value within both the Iberian community (Kruskal-Wallis test: $H_{8,149}$=126,8174; p<0,001) and the Argentinean community ($F_{9,171}$)=25,529, p<0,0001), see also Annexe III.

Using the mean CTmax for each species (Table 3), a significant difference between the two communities was found (Kruskal-Wallis test: $H_{1,19}$=13,500; p=0,0002; N=19). Argentinean community (42,13°C, SD=0,73°C) has a value of CTmax almost 4ºC higher than the Iberian community (38,27°C, SD=1,59°C), as shown in figure 12. Nevertheless, the Iberian community displayed more variation in CTmax (Levene's test: $F_{1,17}$=6,8906; p=0,0177; N=19).

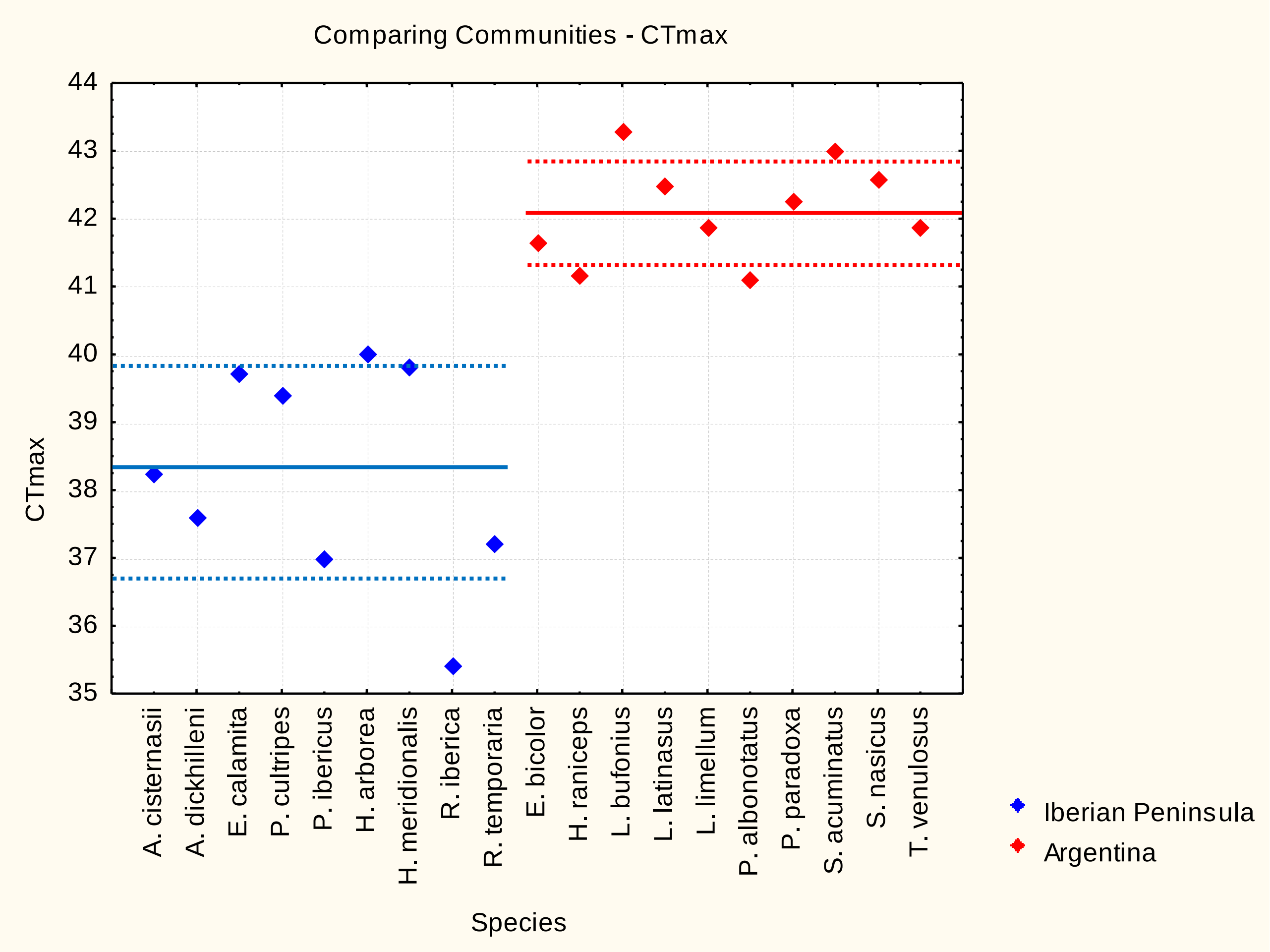

Fig.12– Estimated mean value of CTmax (solid lines) and SD (dotted lines) for each community.

Warming Tolerance is shown in Table 4. There is a significant difference between the two communities in WT (Kruskal-Wallis test: $H_{1,19}$=5,6067; p=0,0179; N=19; Argentina: 3,85 ^{o}C, SD=1,64^{o}C; Iberian Peninsula: 7,88 ^{o}C, SD=4,39^{o}C).

Table 4- Warming Tolerance (WT) for all species from both communities.

Iberian Peninsula		**Argentina**	
Species	WT	Species	WT
A. cisternasii	14,08	*E. bicolor*	7,50
A. dickhilleni	12,49	*H. raniceps*	2,97
E. calamita	5,57	*L. bufonius*	3,21
P. cultripes	3,87	*L. latinasus*	3,75
P. ibericus	1,44	*L. limellum*	3,67
H. arborea	8,65	*P. albonotatus*	1,04
H. meridionalis	5,67	*P. paradoxa*	4,04
R. iberica	6,37	*S. acuminatus*	4,80
R. temporaria	12,74	*S. nasicus*	4,38
		T. venulosus	3,14

Iberian species have higher warming tolerance (figure 13), and continue to show more variation than the Argentinean community (Levene's test: $F_{1,17}$=11,6626; p=0,003299; N=19).

Interestingly, species from Argentinean community although having higher values of CTmax generally have lower warming tolerance. There is a slight negative correlation, although not significant, between CTmax and WT (Spearman's r_s=-0,379; p=0,1096).

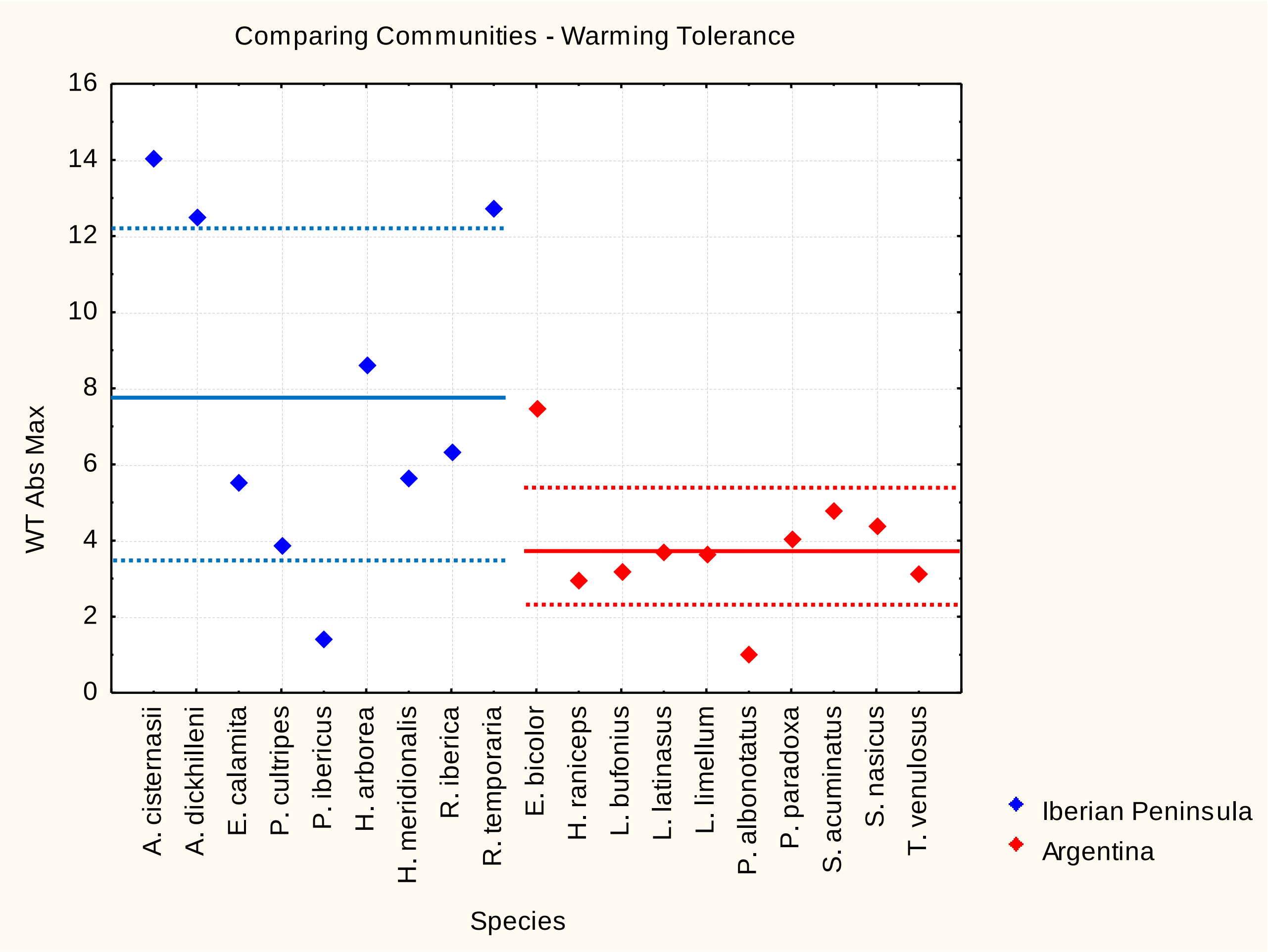

Fig.13– Estimated mean value of Warming Tolerance (WT) (solid lines) and SD (dotted lines) for each community.

There is a strong correlation between maximum habitat temperature and CTmax (Spearman's r_s=0,758; p=0,000169), as shown in figure 14. This implies that species exposed to higher temperatures also have higher CTmax values. When considering both communities separately, no significant correlations were found (Iberian Peninsula: Spearman's r_s=0,21; p=0,5874 and Argentina: Spearman's r_s=0,098; p=0,7884).

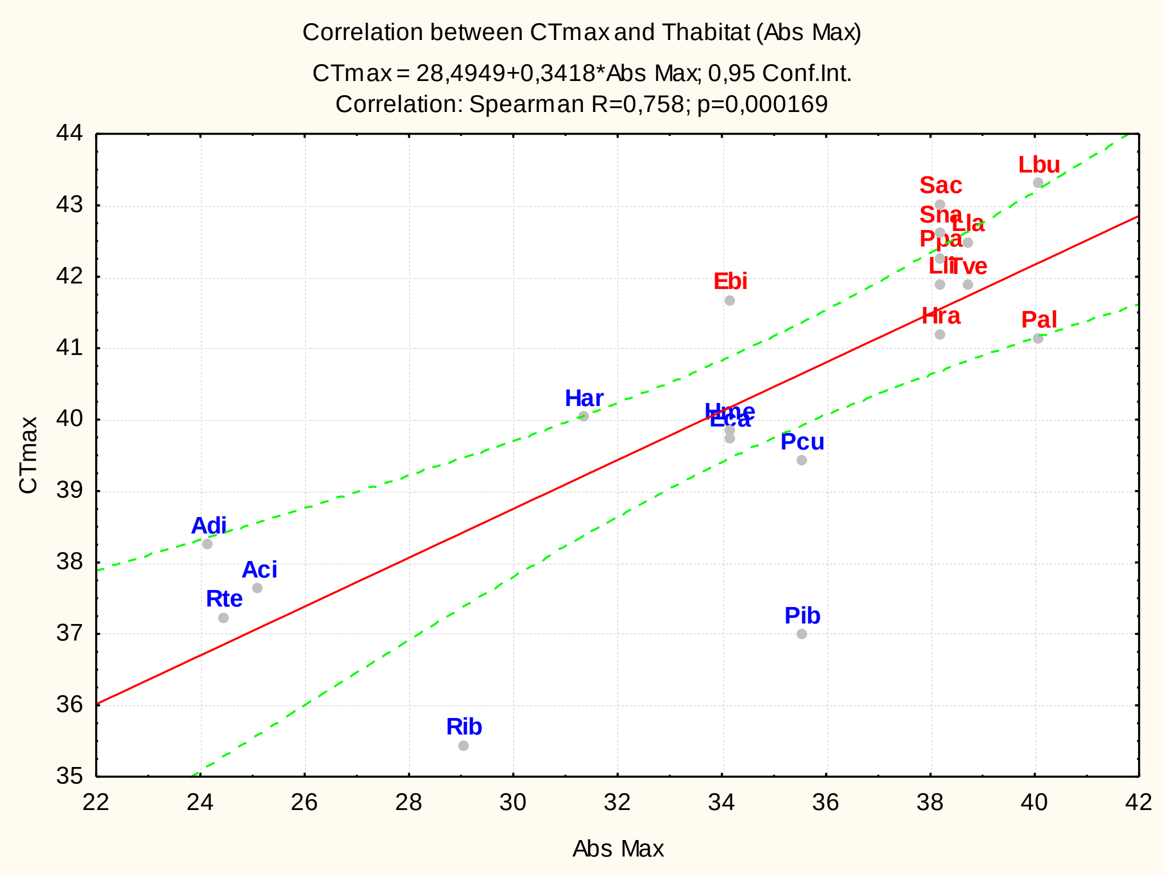

Fig.14– Correlation between maximum habitat temperature (Abs Max) and CTmax. Green lines represent 95% confidence intervals. Argentinean community in red and Iberian community in blue. Species - Ebi: *Elaschistocleis bicolor*; Hra: *Hypsiboas raniceps*; Lbu: *Leptodactylus bufonius*; Lla: *Leptodactylus latinasus*; Lli: *Pseudis limellum*; Pal: *Physalaemus albonotatus*; Ppa: *Pseudis paradoxa*; Sac: *Scinax acuminatus*; Sna: *Scinax nasicus*; Tve: *Trachycephalus venulosus*; Aci: *Alytes cisternasii*; Adi: *Alytes dickhilleni*; Eca: *Epidalea calamita*; Har: *Hyla arborea*; Hme: *Hyla meridionalis*; Pcu: *Pelobates cultripes*; Pib: *Pelodytes ibericus*; Rib: *Rana iberica*; Rte: *Rana temporaria*.

Regarding CTmax, a phylogenetic signal was obtained for the combined tree of the two communities (C=0,541211; p=0,011) but phylogenetic autocorrelations were not found when analysing either the Iberian community (C=0,170625; p=0,12) or Argentinean community (C=0,089523; p=0,3730) separately. For maximum habitat temperature, a phylogenetic signal was obtained for all three combinations (Argentina: C=0,144591; p=0,033; Iberian Peninsula: C=0,454365; p=0,033 and combined communities: C=0,69786; p=0,001).

This suggests that the tip correlation between CTmax and maximum habitat temperature may be affected by phylogenetic history and therefore, the use of phylogenetic independent contrasts is justified (Rheindt et al., 2004).

After correcting for phylogeny, in none of the three possible cases (Iberian community, Argentinean community and the combination of both) a correlation was found. However, the p-value for the combined tree was 0,1097, close to statistical significance (figure 15).

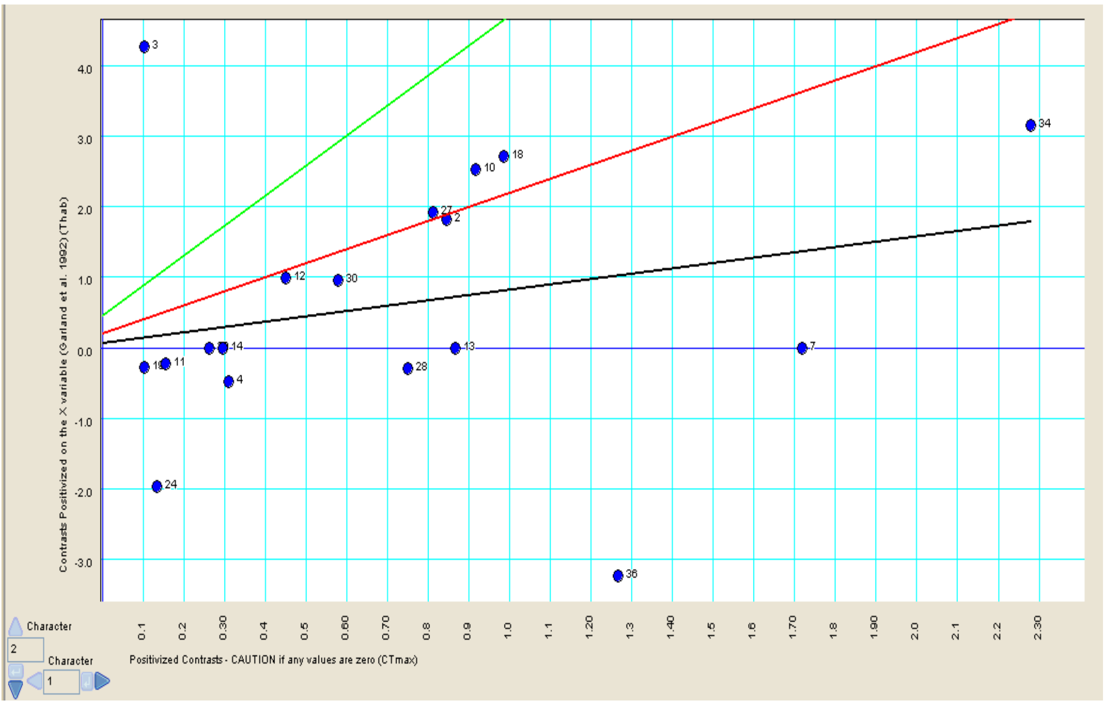

Fig.15– Plot of contrasts vs. positivized contrasts for combined tree for both Argentina and Iberian Peninsula communities (CTmax, Thabitat). Number of contrasts: 18; Pearson Product-Moment Correlation Coefficient: 0,379; Two tailed p-value: 0,10977; Regression lines through origin: Black is ordinary least squares (OLS), Green is major axis (MA) and Red is reduced major axis.

3.3. Thermal sensitivity and Thermal Safety Margins

In total, 18 species were tested for thermal sensitivity, 9 from the Argentinean community and 9 from the Iberian Peninsula. Results are shown in Table 5.

Table 5- Community origin, sample size, estimated optimum temperature (Opt) and temperature of highest performance (T) of all species studied.

Community	Species	*n*	Opt	T	Community	Species	*n*	Opt	T
ARG	Ebi	19	31,02	28	IP	Eca	20	35,61	35
ARG	Hra	18	36,14	35	IP	Har	14	36,09	35
ARG	LEP	9	38,04	35	IP	Hme	15	37,64	35
ARG	Lli	16	33,09	28	IP	Pcu	20	37,98	35
ARG	Pal	18	36,17	38	IP	Pib	38	26,93	24
ARG	Ppa	21	30,56	28	IP	Aci	20	22,82	24
ARG	Sac	19	35,81	38	IP	Adi	19	23,00	24
ARG	Sna	20	35,03	38	IP	Rib	14	26,64	28
ARG	Tve	20	39,09	38	IP	Rte	14	23,70	24

ARG: Argentina community, IP: Iberian Peninsula community. Species - Ebi: *Elaschistocleis bicolor*; Hra: *Hypsiboas raniceps*; LEP: *Leptodactylus* sp.; Lli: *Pseudis limellum*; Pal: *Physalaemus albonotatus*; Ppa: *Pseudis paradoxa*; Sac: *Scinax acuminatus*; Sna: *Scinax nasicus*; Tve: *Trachycephalus venulosus*; Aci: *Alytes cisternasii*; Adi: *Alytes dickhilleni*; Eca: *Epidalea calamita*; Har: *Hyla arborea*; Hme: *Hyla meridionalis*; Pcu: *Pelobates cultripes*; Pib: *Pelodytes ibericus*; Rib: *Rana iberica*; Rte: *Rana temporaria*. Both Topt and T are expressed in ^{o}C.

The symbol "T" shown in Table 5 refers to the treatment temperature at which tadpoles had the best overall swimming performance and Topt is the estimated optimum temperature (see also figure 16 and Annexe IV).

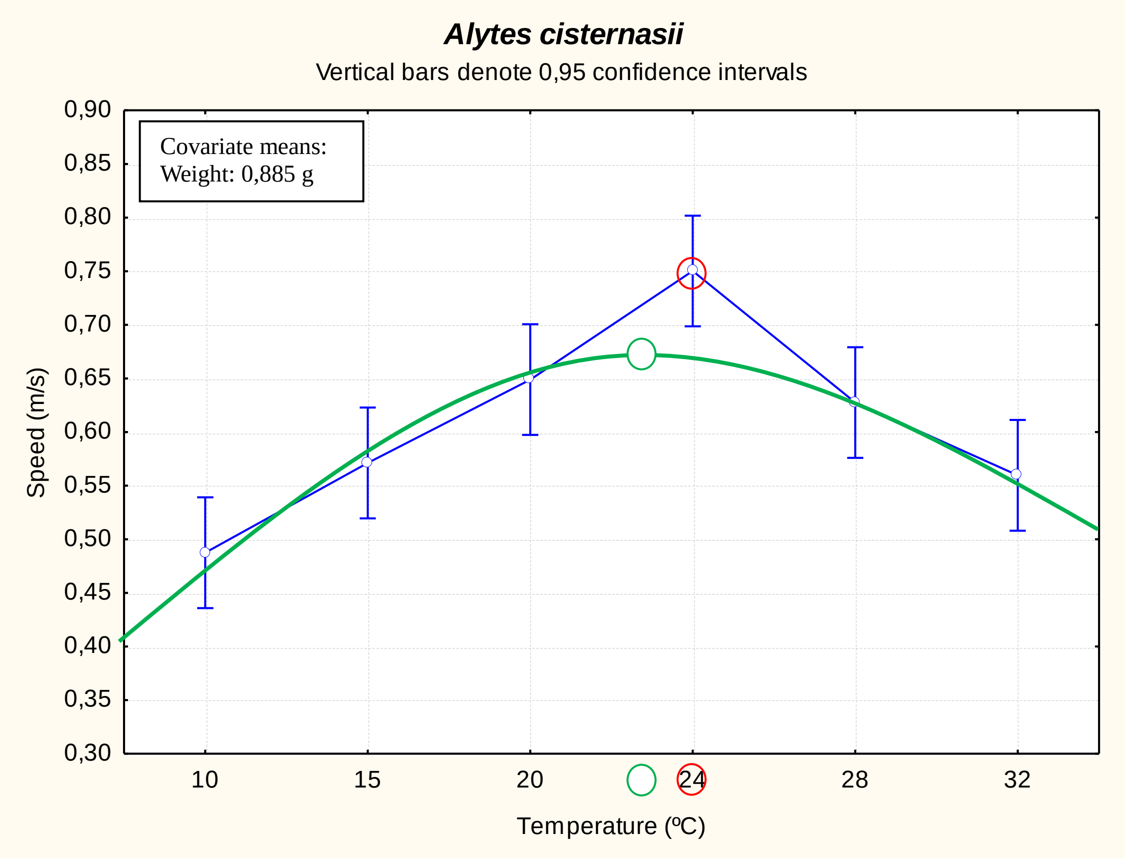

Fig.16–Swimming speed of *Alytes cisternasii* (blue), by treatment temperature. Best overall swimming performance (red circle) and estimated Optimum temperature and performance curve (green circle and line).

Optimum temperatures were higher in the Argentinean community, although not statistically different from those of the Iberian Peninsula community (Kruskal-Wallis test: $H_{1,18}$=2,387914; p=0,1223; N=18; Argentina: 34,99^{o}C, SD=2,93^{o}C; Iberian Peninsula: 30,05^{o}C, SD=6,63^{o}C), as shown in figure 17. Variance in optimum temperature was higher for the Iberian community (Levene's test: $F_{1,16}$=22,12609; p=0,000239; N=18).

If the comparison is made using T temperature (from Table 5), a significant difference is found (Kruskal-Wallis test: H=4,421337; p=0,0355; N=18) which confirms the tendency found in the previous test.

Interestingly, Iberian species Topt values can be differentiated into two groups: a *cool* Topt group that encompases *Alytes cisternasii*, *Alytes dickhilleni*, *Pelodytes ibericus*, *Rana iberica* and *Rana temporaria;* and a *warm* Topt group, containing *Epidalea calamita*, *Pelobates cultripes*, *Hyla arborea* and *Hyla meridionalis*.

These two groups differ significantly in their optimum temperature (Kruskal-Wallis test: $H_{1,9}$=6,000; p=0,0143; N=9). The *cool* group also differs significantly from the Argentinean community (Kruskal-Wallis test: $H_{1,14}$=9,000; p=0,0027; N=14). Average value of the *warm* group (36,82°C, SD=1,16°C) did not differ from the one found for the Argentinean community (Kruskal-Wallis test: $H_{1,13}$=0,5952; p=0,4404; N=13).

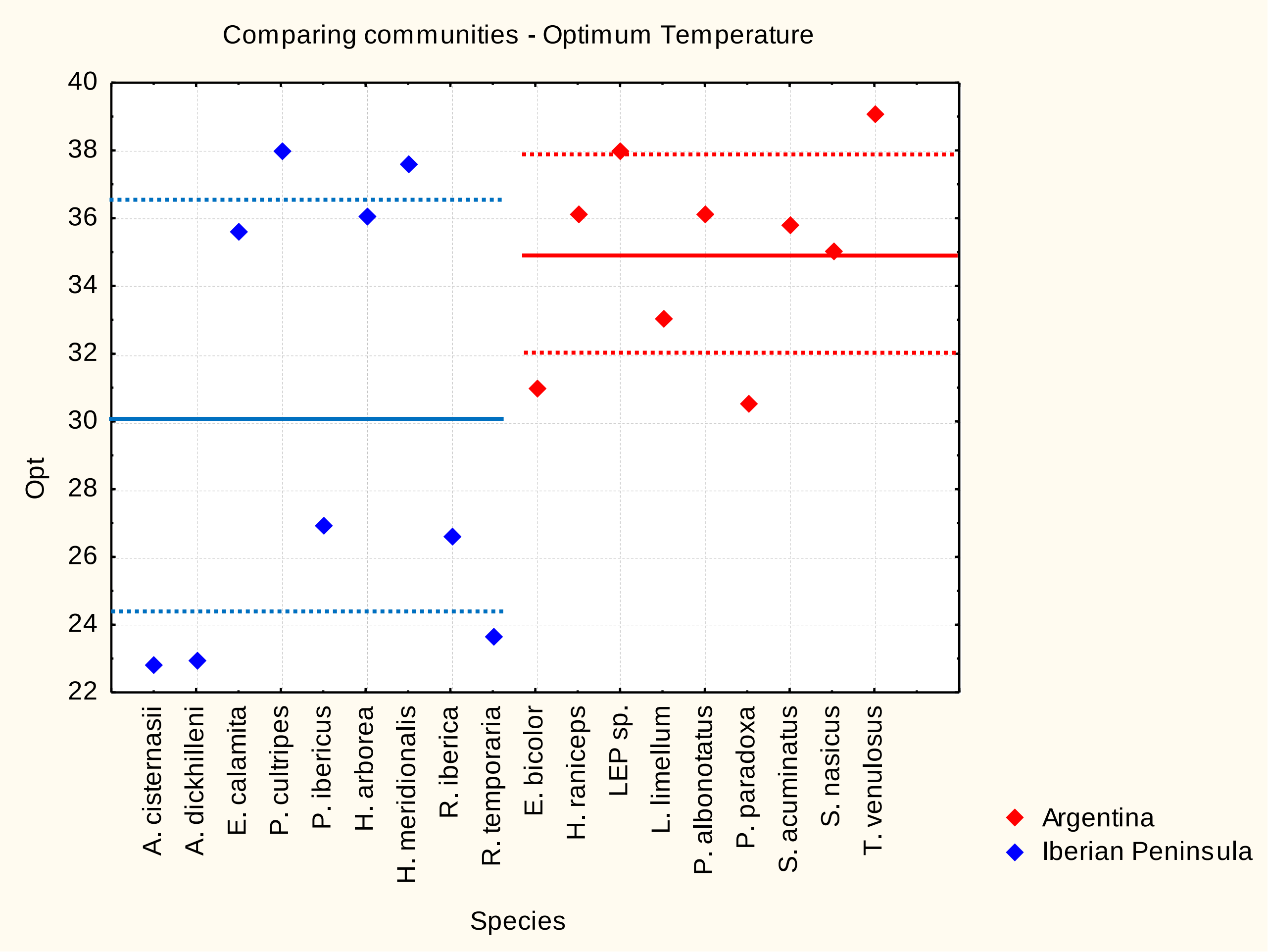

Fig.17– Estimated Optimum Temperature (Opt) (solid lines) and SD (dotted lines) for each community.

Thermal Safety Margins appears in Table 6. There is a significant difference between the two communities ($F_{1,18}$=11,2286; p=0,0041; N=18), with the Iberian community having higher value of TSM (13,51 ^{o}C; SD=3,34^{o}C) than that found for Argentina species (9,09^{o}C; SD=2,13^{o}C), as shown in figure 18.

Similar to CTmax and WT, the species from Argentinean community, although having higher values of optimum temperature, generally have narrower thermal safety margins. There is no significant correlation between Optimum temperature (Topt) and TSM (Spearman's r_s=0,292; p=0,2396).

Table 6- Thermal Safety Margins (TSM) for all species from both communities.

Iberian Peninsula		**Argentina**	
Species	TSM	Species	TSM
A. cisternasii	12,18	*E. bicolor*	6,85
A. dickhilleni	10,48	*H. raniceps*	10,76
E. calamita	14,55	*Leptodactylus* sp.	10,72
P. cultripes	16,93	*L. limellum*	7,71
P. ibericus	8,82	*P. albonotatus*	8,85
H. arborea	15,03	*P. paradoxa*	5,18
H. meridionalis	19,53	*S. acuminatus*	10,43
R. iberica	12,62	*S. nasicus*	9,65
R. temporaria	11,49	*T. venulosus*	11,66

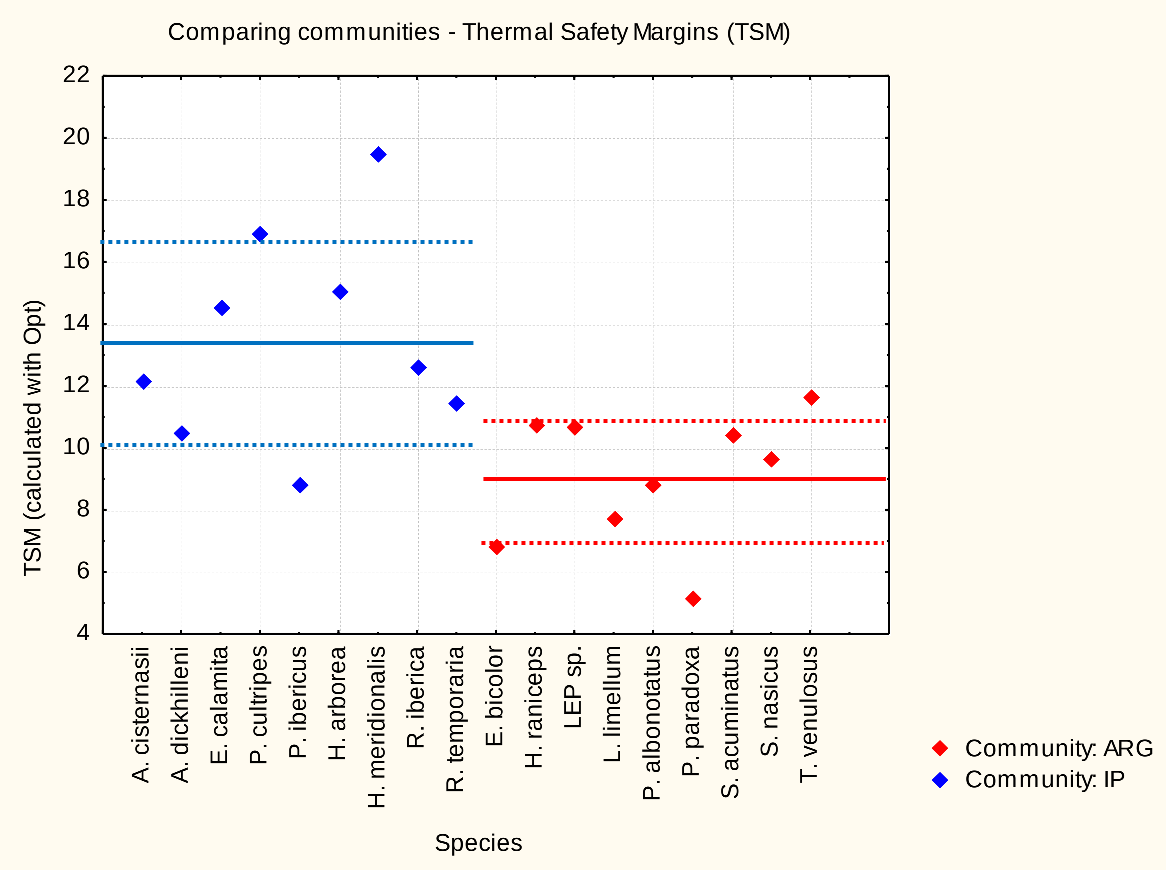

Fig.18– Thermal Safety Margins (TSM) (solid lines) and SD (dotted lines) for each community, using estimated optimum temperature.

Average habitat temperature and optimum temperature exhibit a strong correlation (Spearman's r_s=0,6977; p=0,001286, figure 19). This suggests that species exposed to higher average temperatures have a higher optimum temperature. Considering both communities separately, there was also a significant correlation (Iberian Peninsula: Spearman's r_s=0,857; p=0,00314 and Argentina: Spearman's r_s =0,853; p=0,00344).

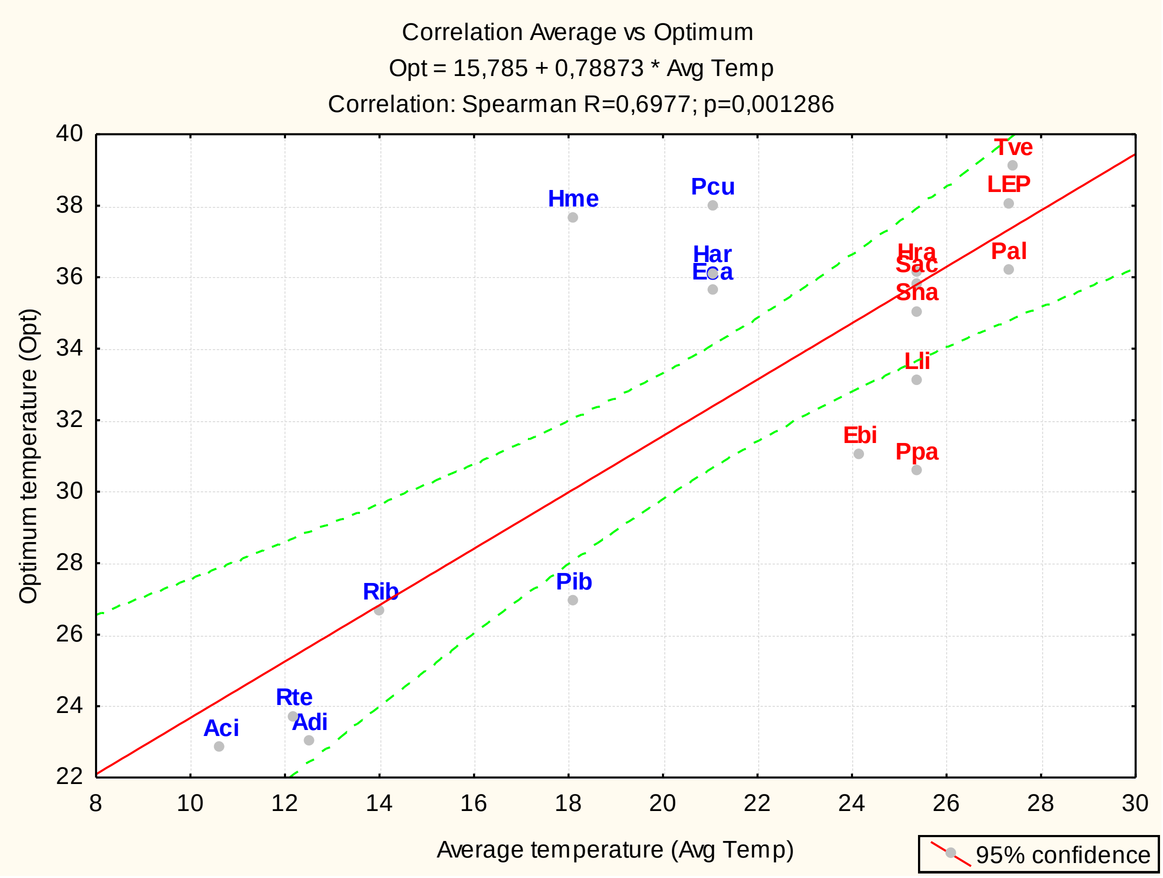

Fig.19– Correlation between average habitat temperature (Avg Temp) and Optimum temperature (Opt). Green lines represent 95% confidence intervals. Argentinean community in red and Iberian community in blue. Species - Ebi: *Elaschistocleis bicolor*; Hra: *Hypsiboas raniceps*; LEP: *Leptodactylus* sp.; Lli: *Pseudis limellum*; Pal: *Physalaemus albonotatus*; Ppa: *Pseudis paradoxa*; Sac: *Scinax acuminatus*; Sna: *Scinax nasicus*; Tve: *Trachycephalus venulosus*; Aci: *Alytes cisternasii*; Adi: *Alytes dickhilleni*; Eca: *Epidalea calamita*; Har: *Hyla arborea*; Hme: *Hyla meridionalis*; Pcu: *Pelobates cultripes*; Pib: *Pelodytes ibericus*; Rib: *Rana iberica*; Rte: *Rana temporaria*).

Optimum temperature, Topt, showed a phylogenetic signal either for the combined tree of the two communities (C=0,726445; p=0,001) and for the Iberian community (C=0,661175; p=0,021). For the Argentinean community no phylogenetic signal was found (C=0,013542; p=0,186). Average habitat temperature also exhibited a phylogenetic signal either for the combined tree of the two communities and for both communities separately (Argentina: C=0,447386; p=0,048; Iberian Peninsula: C=0,427766; p=0,049 and combined communities: C=0,628466; p=0,001).

Significant phylogenetic independent contrasts correlations between Topt and average habitat temperature were found for the Iberian community and the combined communities (Pearson Product-Moment´s r=0,760; p=0,0175 and r=0,590; p=0,00784, respectively), after correcting for phylogeny (figure 20), but regarding the Argentinean community, no correlation was found between optimum temperature and average habitat temperature.

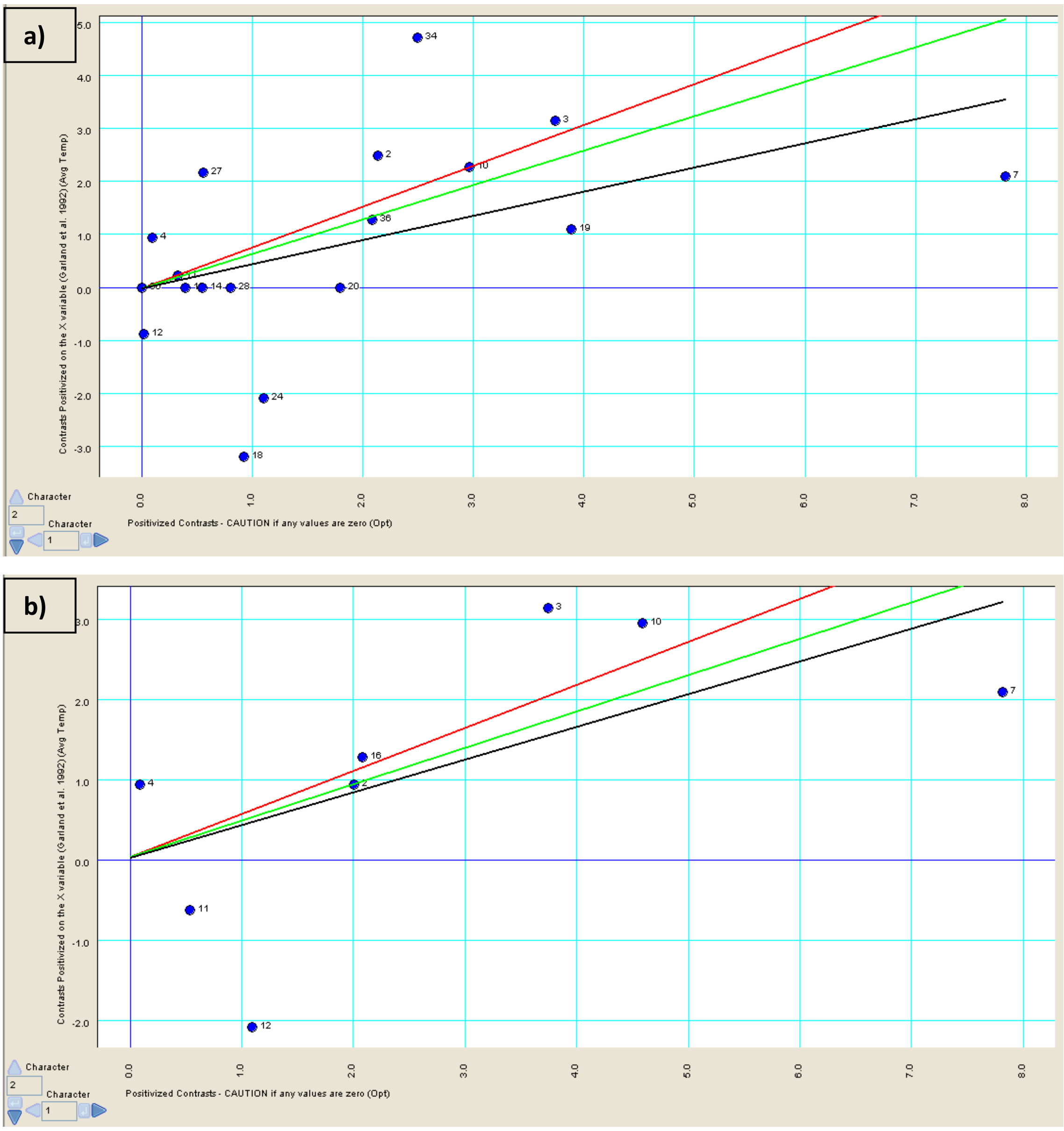

Fig.20– a) Plot of contrasts vs. positivized contrasts for combined tree (Opt, Thabitat). Number of contrasts: 18; Pearson Product-Moment Correlation Coefficient: 0,590; Two tailed p-value: 0,00784. b) Plot of contrasts vs. positivized contrasts for Iberian tree (Opt, Thabitat). Number of contrasts: 8; Pearson Product-Moment Correlation Coefficient: 0,760; Two tailed p-value: 0,0175. Regression lines through origin: Black is ordinary least squares (OLS), Green is major axis (MA) and Red is reduced major axis.

3.4. CTmax vs Optimum Temperature and Warming Tolerance vs Thermal Safety Margins

Following the previous results, CTmax and Optimum Temperature were tested to see if there was any relation between these two characters. *Leptodactylus bufonios* and *Leptodactylus latinasus* were considered to have the same optimum temperature, also when calculating TSM.

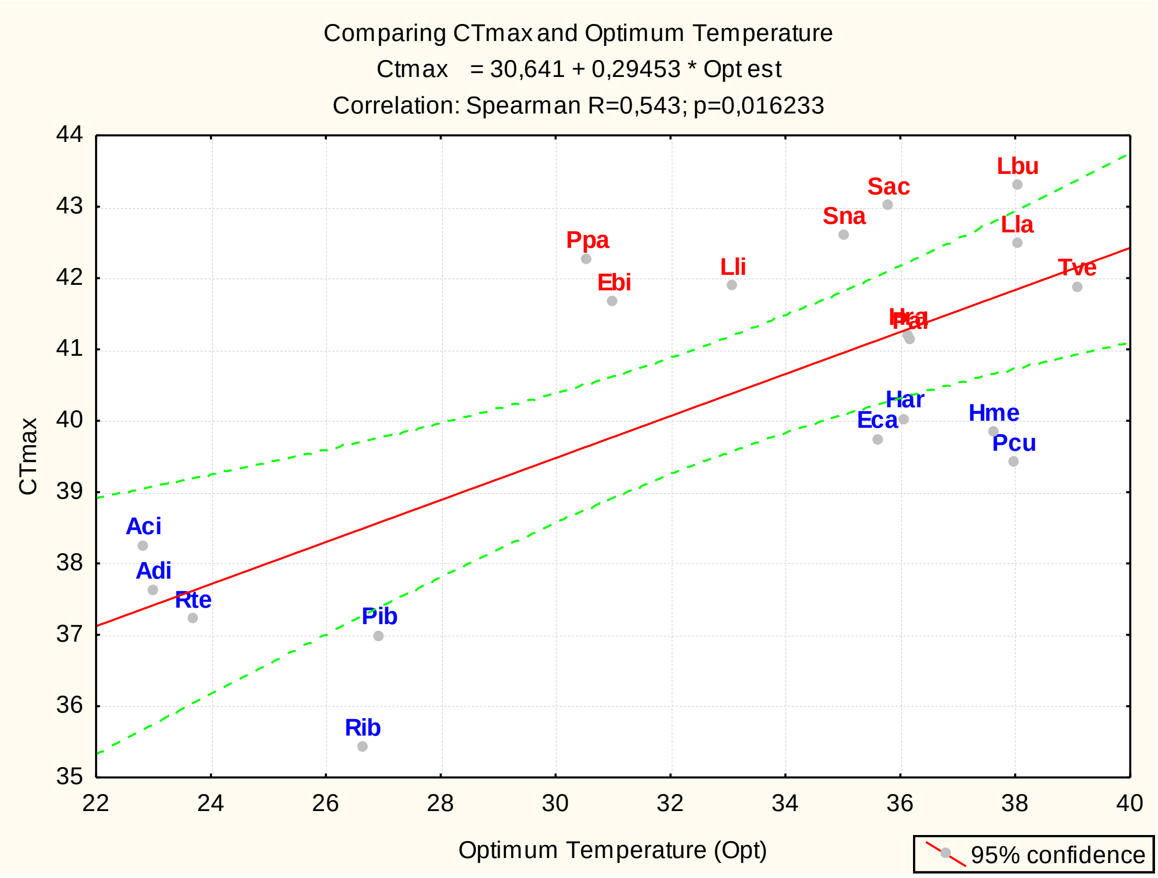

Fig.21– Correlation between Optimum Temperature (Opt) and CTmax. Argentinean community in red and Iberian community in blue. Species - Ebi: *Elaschistocleis bicolor*; Hra: *Hypsiboas raniceps*; Lbu: *Leptodactylus bufonius*; Lla: *Leptodactylus latinasus*; Lli: *Pseudis limellum*; Pal: *Physalaemus albonotatus*; Ppa: *Pseudis paradoxa*; Sac: *Scinax acuminatus*; Sna: *Scinax nasicus*; Tve: *Trachycephalus venulosus*; Aci: *Alytes cisternasii*; Adi: *Alytes dickhilleni*; Eca: *Epidalea calamita*; Har: *Hyla arborea*; Hme: *Hyla meridionalis*; Pcu: *Pelobates cultripes*; Pib: *Pelodytes ibericus*; Rib: *Rana iberica*; Rte: *Rana temporaria*).

A significant tip correlation was found (Spearman's r_s=0,543; p=0,016233), figure 21. This correlation suggests that a species with higher optimum temperature also have higher CTmax. However, for both Argentinean and Iberian communities separately, no correlation was found (Spearman's R=0,061; p=0,8675 and R=0,567; p=0,1116 respectively).

PIC correlations for both the Iberian community and the combined communities were found to be significant (figure 22), whereas no correlation was found between CTmax and optimum temperature for the Argentinean community.

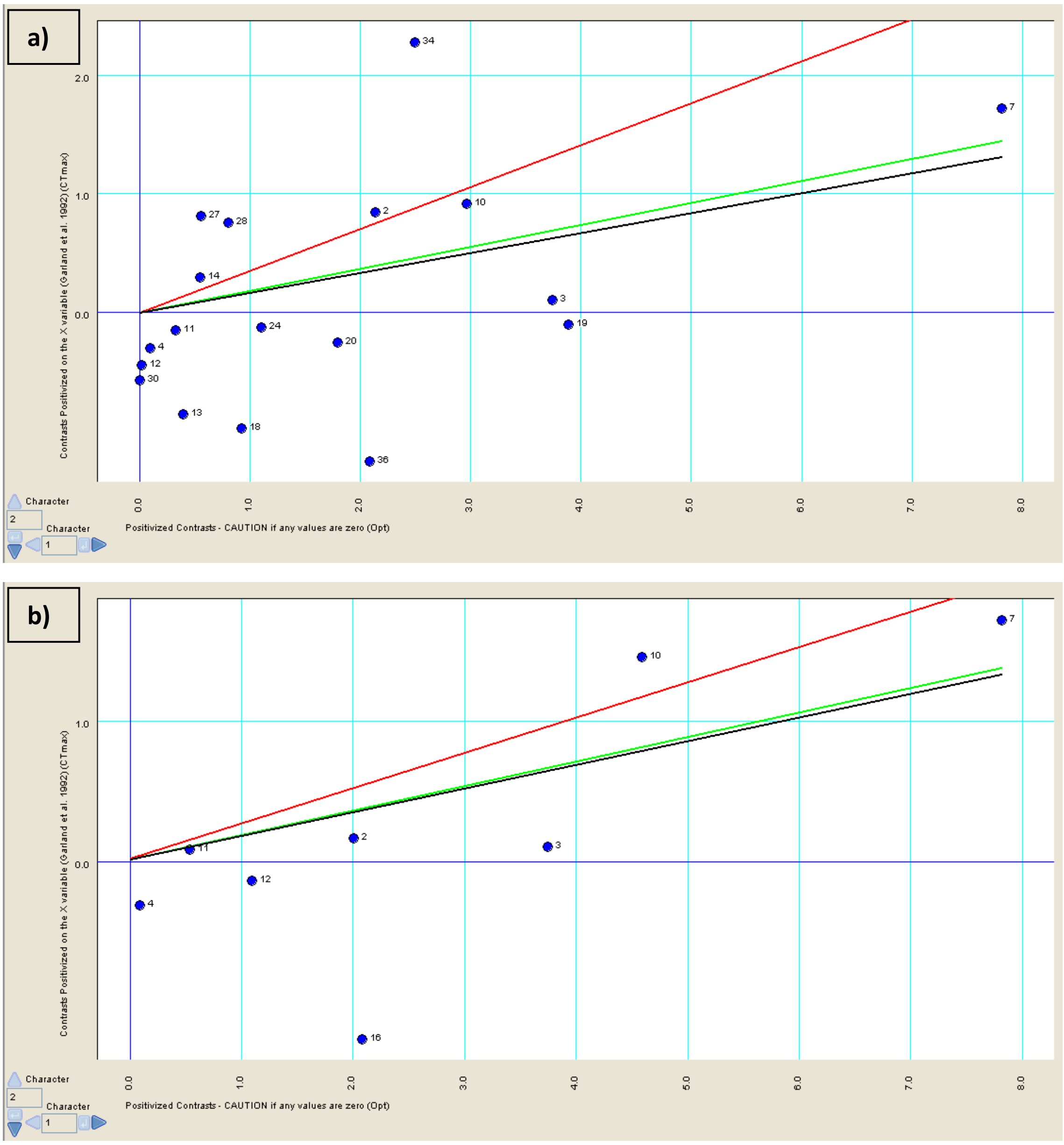

Fig.22– a) Plot of contrasts vs. positivized contrasts for combined tree (Opt, CTmax). Number of contrasts: 18; Pearson Product-Moment Correlation Coefficient: 0,474; Two tailed p-value: 0,0404. b) Plot of contrasts vs. positivized contrasts for Iberian tree (Opt, Thabitat). Number of contrasts: 8; Pearson Product-Moment Correlation Coefficient: 0,671; Two tailed p-value: 0,04775. Regression lines through origin: Black is ordinary least squares (OLS), Green is major axis (MA) and Red is reduced major axis.

Relationship between thermal TSM and WT was also examined, with no correlation found, as shown in figure 23. Similar results were obtained for the Iberian Peninsula and Argentina separately (Spearman's r_s=-0,216; p=0,5755 and r_s=-0,48; p=0,16 respectively). Both TSM and WT vary independently.

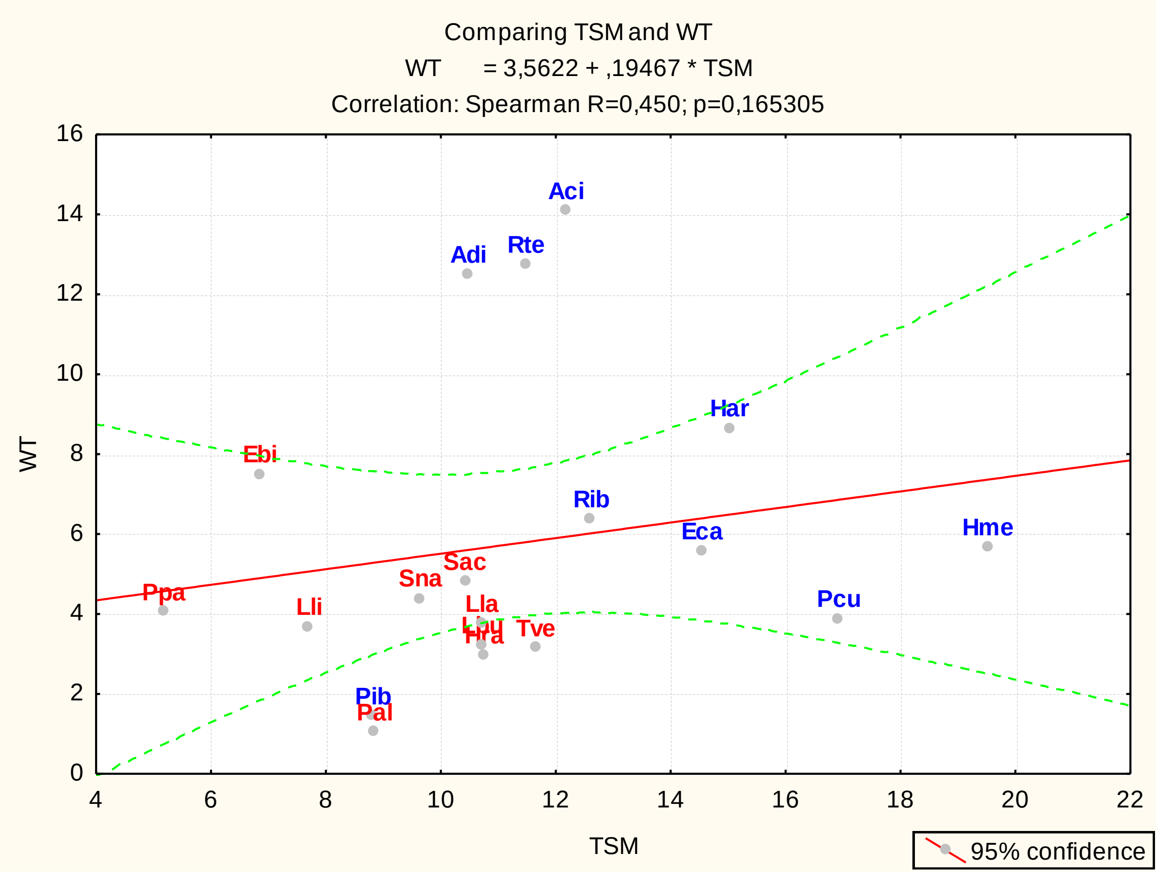

Fig.23– Correlation between Thermal Safety Margins (TSM) and Warming Tolerance (WT). Argentinean community in red and Iberian community in blue. Species - Ebi: *Elaschistocleis bicolor*; Hra: *Hypsiboas raniceps*; Lbu: *Lepdactylus bufonius*; Lla: *Leptodactylus latinasus*; Lli: *Pseudis limellum*; Pal: *Physalaemus albonotatus*; Ppa: *Pseudis paradoxa*; Sac: *Scinax acuminatus*; Sna: *Scinax nasicus*; Tve: *Trachycephalus venulosus*; Aci: *Alytes cisternasii*; Adi: *Alytes dickhilleni*; Eca: *Epidalea calamita*; Har: *Hyla arborea*; Hme: *Hyla meridionalis*; Pcu: *Pelobates cultripes*; Pib: *Pelodytes ibericus*; Rib: *Rana iberica*; Rte: *Rana temporaria*).

For TSM, in none of the three possible cases a phylogenetic signal was found (Argentina: C=-0,106475; p=0,192; Iberian Peninsula: C=0,250598; p=0,325 and combined tree: C=-0,026773; p=0,224). For WT, a phylogenetic signal was found for both Argentina (C=0,295549; p=0,018) and the combined tree (C=0,436341; p=0,008) but not for the Iberian Peninsula (C=0,216807; p=0,134). Nevertheless, in none of the possible three cases, the PIC correlation was found to be significant.

4. DISCUSSION

4.1. Thermal tolerance

CTmax values were clearly higher for the summer-breeding subtropical Argentinean species than for the Iberian species, which breed at much cooler water temperatures. This suggests the hypothesis that tolerance to heating may have evolved through thermal selection.

Results also revealed that CTmax is correlated with maximum habitat temperature at the tip level. Since both CTmax and Thab showed phylogenetic autocorrelation, a phylogenetic control was mandatory (Rheindt et al. 2004). However, the non-significant PIC correlation revealed that these traits have not coevolved. Differences between species and contrast correlations are likely to be of biological significance and, in this case, where traits are correlated and their phylogenetic contrasts are not, it points at a correlated evolution only during an early adaptive radiation in the history of a clade and not later (Price, 1997). Nevertheless, the p-value's proximity to statistical significance can be alternatively explained by a low statistical power (type II error) since the addition of more species from the present analysed communities, and also from Sweden, revealed that the PIC analysis' outcome exhibits a positive and highly significant p-value (H. Duarte, M. Tejedo and M. Katzenberger, unpublished data).

Summarizing, maximal thermal tolerance limits reflected habitat conditions and differences found in CTmax between communities and species should be more related to local adaptation to habitat temperatures than to their phylogenetic history. Similar results were found in crabs inhabiting temperate and tropical waters (Stillman and Somero, 2000).

Janzen (1967) stated that it can be assumed that animals and plants are evolutionarily adapted to, and/or have the ability to acclimate to, the temperatures normally encountered in their temporal and geographic habitat (microhabitat). Since environmental temperatures vary with latitude, traits that have evolved as adaptations to habitat temperatures, such as CTmax, should also depend on latitude. This theory is still controversial.

Previous works, such as Addo-Bediako et al. (2000, on insects) and Ghalambor et al. (2006, re-analysis of Brattstrom (1968) data set on amphibians) revealed no geographical trend on thermal tolerance. However, our results tend to agree with Snyder and Weathers (1975) re-analysis of Brattstrom (1968) data set which found a significant decline in CTmax with increasing latitude, since CTmax values were higher for Argentinean species than for Iberian species. Recent data from a Swedish community of amphibians (around 60^{o}N) indicate that these species exhibit the lowest CTmax values when compared to both the Argentinean and the Iberian species (H. Duarte, M. Tejedo and M. Katzenberger, unpublished data), thus supporting the hypothesis that CTmax is negatively related to latitude.

4.2. Thermal sensitivity

When comparing both communities for their optimum temperature, no significant differences were found, although the mean was higher for the subtropical Argentine community. The lack of differences is the result of the high heterogeneity in optimal thermal values for the Iberian species. Within the Iberian community, two groups were found: one comprising species with cool optimum temperatures (Aci, Adi, Rte, Rib and Pib) and a second group that displayed warmer optimal temperatures (Eca, Hme, Har, Pcu) that did not differ from the values expressed in the subtropical community.

These groups of Iberian species largely differ in their breeding phenology and aquatic habitats. The cold species group includes species living at cool ponds represented by both autumn-winter breeders (Aci, Pib) and species that breed either at high mountains (Adi, Rte) or at cool streams (Rib). The high temperature group included species that either develop at warm pond environments, with the breeding season occurring at mid-late Winter or early Spring (Eca, Hme, Har) or that, although breeding in Autumn or Winter, have a very long larval period reaching metamorphosis at mid or late Spring and therefore exposed to higher temperatures (Pcu), (Ferrand de Almeida et al., 2001; Malkmus, 2004; García-París et al., 2004).

Argentinean species included in this work breed during austral summer and they all came from pond habitats around the same altitudinal level (despite being in different regions). Rainfall is low (750-900 mm annually) and concentrated in a short rainy season during the austral summer (Duellman, 1999), the period in which most of the Chacoan amphibians breed (Cei, 1980). Chacoan wetlands inhabited by amphibians include basically temporary ponds and low flooded rivers and streams. Therefore, Argentinean breeding sites were less variable in thermal regimen than breeding habitats from the Iberian Peninsula.

Our results indicate that habitat is acting as a selective force and species have adapted their optimum temperature to cope with environmental temperatures. Both Argentine and warm-breeders from the Iberian community have evolved a higher thermal optimum. Species with lower optimum temperature values tend to have lower TSM values. In the Iberian Peninsula, species with cool optimum temperatures (Aci, Adi, Rte, Rib and Pib) might be more affected by rising temperatures than species with warmer optimal temperatures (Eca, Hme, Har, Pcu). In the Argentinean community, species with lower optimum temperature (e.g. *Pseudis* sp.) seem to be more vulnerable to rising temperatures. In the future, community structure of the Iberian Peninsula could change and be constituted only by average and warm optimum temperatures species. Argentinean's community structure could also change and only be composed by warm optimum temperature species. To determine if this predicted shifts in community structure, regarding hot-cold species, will also be accompanied by changes in the proportions of specialist/generalist species still requires further research.

It is still unclear whether there are cold optimum temperature species in the subtropical Chacoan community. Recently, some additional results on CTmax and habitat temperature have been reported for two species of *Melanophryniscus* sp. (species not included in this work), winter stream-breeding species from Misiones province, nearby the Grand Chaco region, in North Eastern Argentina, where rains are distributed evenly throughout the year. Both species show much lower CTmax values (*M. krauczuki*, 39,93°C and *M. devincenzii*, 39,16°C) and cooler environmental temperature (around 18°C of average habitat temperature) than the studied Chacoan summer-breeding species (mean 42,13^{o}C) (F. Marangoni, unpublished data).

Optimum temperature is positively correlated with CTmax (this study and Huey, 2009) and with average habitat temperature (this study). These results are in agreement with previous works which state that optimum temperature and CTmax are likely to be co-adaptive traits (Huey and Bennett, 1987; Angilletta, 2009). Presumably, Argentinean winter-breeding species may have lower values for optimum temperature. If so, they should fall between the group of cold-breeding Iberian species and the Argentinean summer-breeding species, regarding their CTmax and optimum temperature values.

Assuming that cool breeders from the tropical areas have lower Topt, we can expect more variation in Topt and CTmax in the tropics than our results with the summer breeder species reveal. Independently of the community of origin, there should be a strong influence of species breeding periods (seasonality) in both CTmax and optimum temperature values. This would be consistent with theoretical expectations (Janzen, 1967; Ghalambor et al., 2006) and with Deutsch et al. (2008) results which consider seasonality a strong predictor of Warming Tolerance (WT) and also of Thermal Safety Margins (TSM). Future research on these species could confirm this suggestion.

4.3. Evaluating vulnerability to warming temperatures

Having a high value for CTmax or optimum temperature does not necessarily reflect a high warming tolerance (WT) or broader thermal safety margins (TSM), respectively. Argentinean species have higher CTmax values and higher optimum temperature, in general, but they have lower WT and narrower TSM. This is due to the already high environmental temperatures they experience. Nevertheless, groups of species that differ in habitat and breeding phenology can have similar values of TSM and WT (Huey et al, 2009) and phylogenetic signal was very weak for both WT and TSM.

For evaluating species' vulnerability to warming, the plot WT vs TSM (figure 23) provides valuable information. Species with low WT and narrower (lower value) TSM are expected to be more threatened than species with high WT, broader TSM or both.

All species from Argentina have WT lower than 8°C and TSM lower than 12°C. Only one species from the Iberian Pensisula, *Pelodytes ibericus*, has values within this range.

Based on this information, subtropical taxa, or at least summer-breeding subtropical species of amphibians (which includes most amphibian species from the Chaco region), would be subjected to higher risk of extinction under a scenario of rising temperatures in the upcoming years. This can also be true for species from all seasonal lowlands of South America and possibly other tropical and subtropical species of Africa and Australia, which breed during the wet season, in the austral Summer (Cogger, 1983; Lambiris, 1989; Channing and Howell, 2006).

Whether global warming will also affect these species more negatively or not, there is still limited information. Global warming is more than just rising temperatures; it also implies deep changes in rainfall amount, expected to be reduced in large areas of Central America, Mediterranean basin, South and Central Africa and Australia (IPCC, 2007a, b). Increase in temperature, and correlated raise in evapotranspiration, can be accompanied by a reduction in the amount of rain determining shorter pond hydroperiods. Therefore, the outcome pattern will be a strong reduction in pond durability and thus species with long larval period or low developmental plasticity will have to shift their breeding habitats to more permanent ones (see Newman, 1992; Wells, 2007).

Although results may give a first idea of how changes in temperature will affect these species, further information about traits with important relation to pond duration is required. For instance, in many cases, the key question may be not what temperature can species tolerate but how fast can they develop and metamorph to escape from the water before ponds dry. This is true, for example, for species that breed in temporary and ephemeral ponds.

Some species from Chaco that breed in ephemeral ponds (habitats with high environmental temperatures) already have high development and growth rates. Considering that temperatures will rise and pond duration may be reduced, what is their physiological limit to their development and growth rates or, in other words, what is the minimum amount of time required to reach metamorphosis and survive?

Larval amphibian growth and differentiation are largely dependent on temperature (Smith-Gill and Berven, 1979). Size and time to metamorphosis are considered fitness related traits and there is a strong selection on both. Size at metamorphosis determines differential survival at the terrestrial stage and shorter larval period in desiccating ponds may help to avoid catastrophic mortalities (Reques and Tejedo, 1997; Altweeg and Reyer, 2003). Alternatively, selection may favour phenotypic plasticity due to the heterogeneity of pond environments (Tejedo and Reques, 1994; Laurila et al., 2006). The analysis of temperature-induced plasticities in development and growth rates is badly needed (see Kaplan and Phillips, 2006).

Species may be forced to change their breeding seasonality to be able to cope with expected climate changes. In the Iberian Peninsula, species breeding in late Spring may be forced to advance reproduction to early Spring or even to late winter, depending on the extent of habitat changes, because their current breeding season will be too hot and/or dry for them. The situation in tropical regions may be more critical because wet season coincides with Summer. Opportunities for breeding will occur on a much lesser regular basis than they currently occur. If they are able to shift their reproductive season, or at least their reproductive habits (changing breeding locations from ephemeral and temporary to permanent ponds), species may have overlapping breeding periods which would mean high density of tadpoles on the ponds, resulting in a stronger competition for resources and increased predation pressure (more predators are found in permanent pond, e.g. Wellborn et al, 1996).

Another way of predicting what will happen is to understand what occurred in similar periods of Earth's history, when climate was warmer than it currently is. It would be interesting to know what happened in the interglacial periods, during the Pleistocene.

In the Eemian period (third interglacial period in Northern Europe), ranging from around 130,000 to 114,000 years ago, temperatures were 3ºC to 5ºC higher than they are today and it is the nearest time known that matches temperatures expected for the next 100 or 200 years (Larsen, 2009). Mammalian fauna such as the Common Hippopotamus (*Hippopotamus amphibius*, Linnaeus, 1758), a species whose current distribution only embraces tropical reagions, reached as far as Northwestern and Central Europe (van Kolfschoten, 2000). This raises the question: if there was tropical fauna inhabiting modern temperate reagions during the interglacial periods, what happened in the current tropical reagions during those periods? Unfortunately, there is currently no sufficient information for the tropic regions to make predictions based on paleontological records.

As mentioned, at a given time, Earth already had temperature values similar to those predicted to be reached in the next 100 or 200 years. The magnitude of the changes is really important but so is the time they take to occur. Species have the capability of coping with changes through adaptive evolution, plasticity, migration (less likely or even inexistent in most amphibians) and habitat niche shift (Parmesan, 2006).

Expected rate of current global warming can be too high for species to evolve accordingly. Therefore, if neither the plasticity of important traits such as CTmax, optimum temperature, development and growth rates or habitat niches shift cannot buffer the effect of rising temperatures, species could be facing extinction during the next couple of centuries. The biodiversity loss would be huge if tropical amphibian species are confirmed to be more threatened than temperate species.

5. ACKNOWLEDGMENTS

I'm still giving my first steps in the scientific world and this thesis is one of them. The whole process of gathering data and writing was a constant test to my capability as a young scientist and this work allowed my skills as a researcher to flourish while also learning new things. On a personal level, all the travelling I did to accomplish this work and all the people I met along the way really helped me to get a bigger picture of the world we all share. The possibility of meeting different people and cultures, together with the passion and admiration for wildlife and the natural world, makes my life meaningful and, in particular, made this thesis such an enrichening experience.

For these reasons, I want to especially thank:

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7.ANNEXES

Annexe I. Monitored ponds

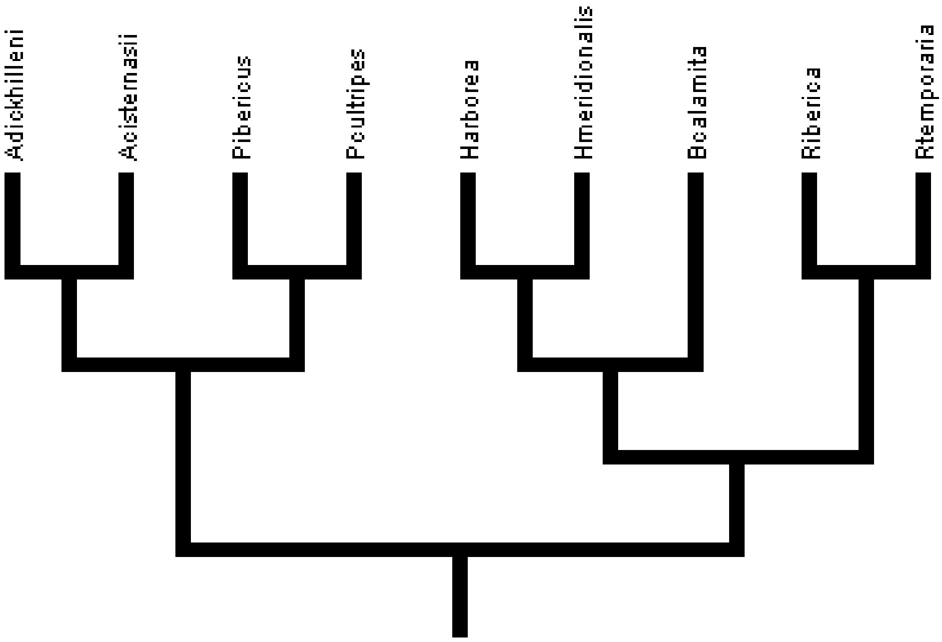
Information about monitored ponds, including measured temperatures and number of days sampled.

Pond	Days	Maximum		Overall	Minimum	
		Abs	Mean	Mean	Abs	Mean
1. CH. PELODYTES CENTRO	98	35,54	17,45	12,42	4,73	9,20
2. LOS LLANOS	80	28,36	21,05	14,58	5,04	9,86
3. RIBEIRA NISA	43	29,05	16,92	14,02	10,26	12,23
4. TOBA SUR ABRIL	138	24,16	13,05	10,64	1,44	8,68
5. TOBA SUR	176	34,16	16,18	13,45	1,44	11,30
6. TOBA PRINCIPAL	12	31,98	25,99	18,11	9,97	12,25
7. VERDIZELA	8	31,37	28,94	21,06	13,27	14,60
8. ZACALLON 1	191	33,74	12,80	10,88	2,09	9,28
9. ZACALLON 2	223	23,97	13,08	11,84	2,84	10,76
10. POZO DE LA NIEVE	92	21,90	14,92	10,28	0,00	6,93
11. FUENTE BOLICHE	345	25,13	13,62	12,52	2,33	11,43
12. COLOR CUNETA OVIEDO	200	23,84	16,39	14,76	6,84	13,58
13. CUBILLA OVIEDO	120	24,47	15,62	12,21	3,32	9,31
14. CECOAL	13	34,16	30,13	24,17	18,05	20,37
15. DERMATONOTUS FRAGA	7	41,36	37,21	27,69	21,29	23,22
16. FONTANA	34	40,09	35,01	27,32	17,23	21,98
17. LA MORACHA ORILLA	3	38,73	35,96	27,43	19,84	21,52
18. LA MORACHA CENTRO	14	34,60	30,66	26,14	15,08	22,41
19. LEPIDOBATRACHUS	19	40,57	33,90	28,51	20,32	24,73
20. PERICHON	46	38,21	32,10	25,38	12,49	21,75

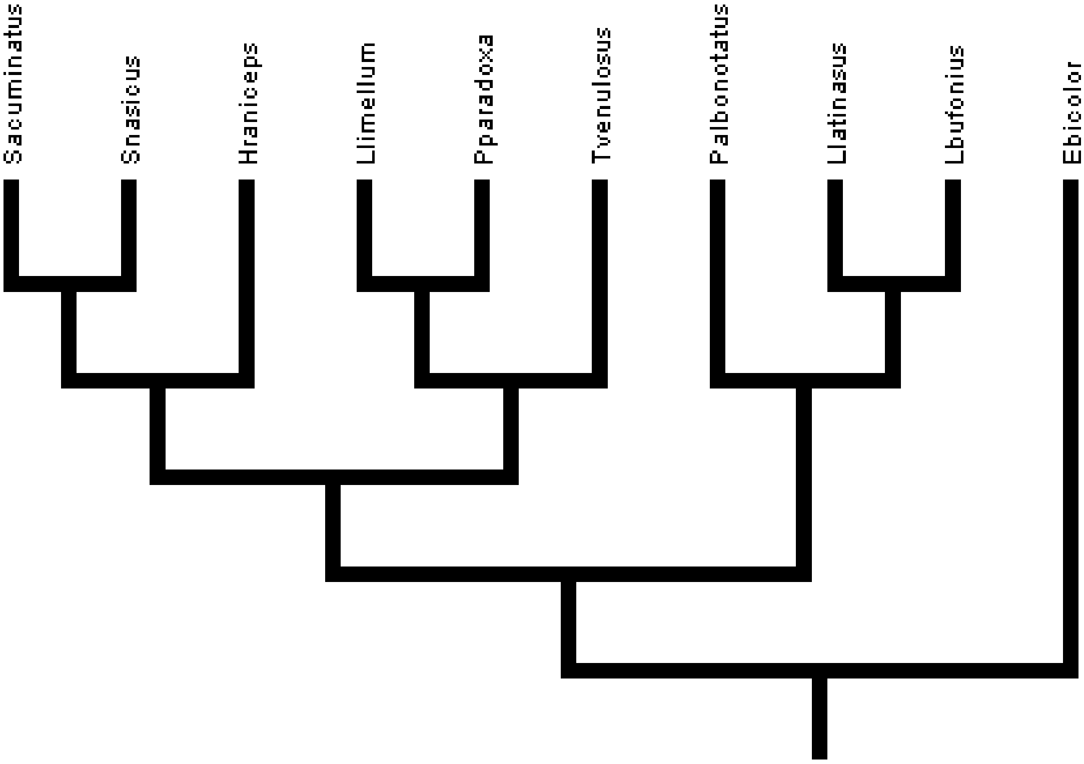
Annexe II. Phylogenetic trees

Phylogenetic trees: a) Iberian species; b) Argentinean species and c) combined tree with both Argentinean and Iberian species (all species studied in this work).

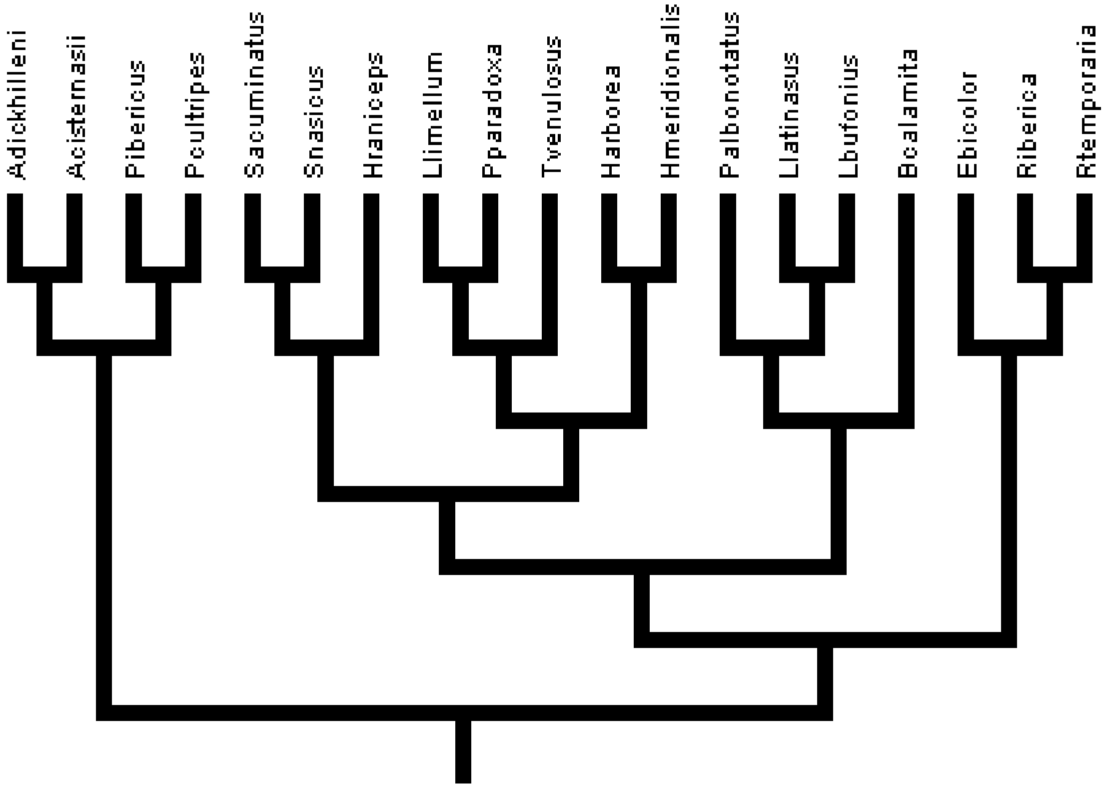
a)



b)



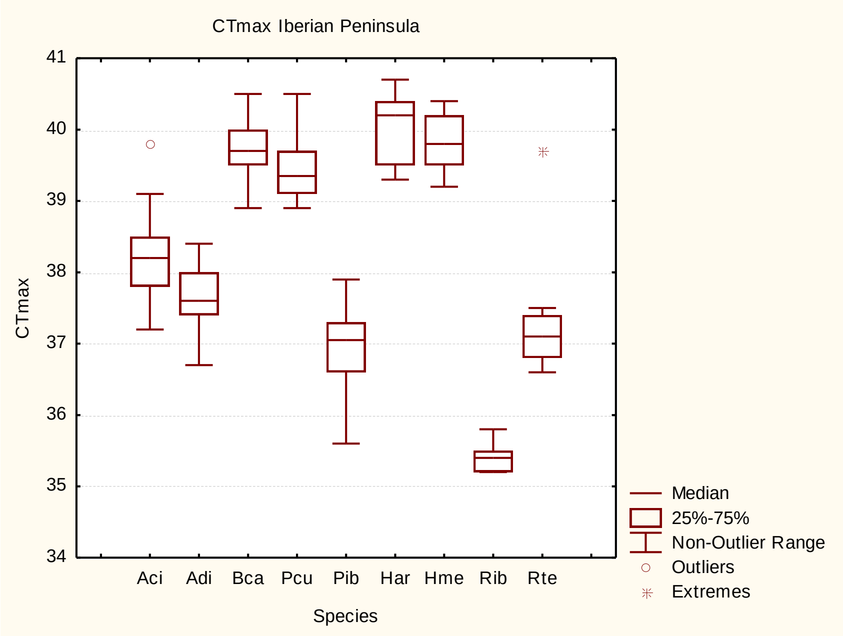
c)


Adickhilleni
Acisternasii
Pibericus
Pcultripes
Sacuminatus
Snasicus
Hraniceps
Llimellum
Pparadoxa
Tvenulosus
Harborea
Hmeridionalis
Palbonotatus
Llatinasus
Lbufonius
Bcalamita
Ebicolor
Riberica
Rtemporaria

Annexe III. CTmax

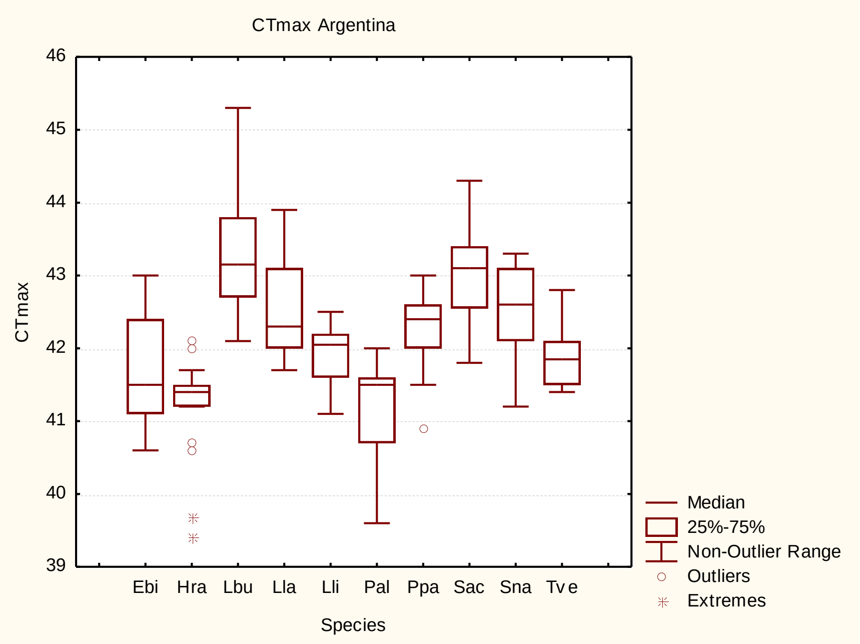
Box and whiskers plots for CTmax of species.

Iberian Peninsula



Species: (Aci: *Alytes cisternasii*; Adi: *Alytes dickhilleni*; Bca: *Epidalea calamita*; Har: *Hyla arborea*; Hme: *Hyla meridionalis*; Pcu: *Pelobates cultripes*; Pib: *Pelodytes ibericus*; Rib: *Rana iberica*; Rte: *Rana temporaria*).

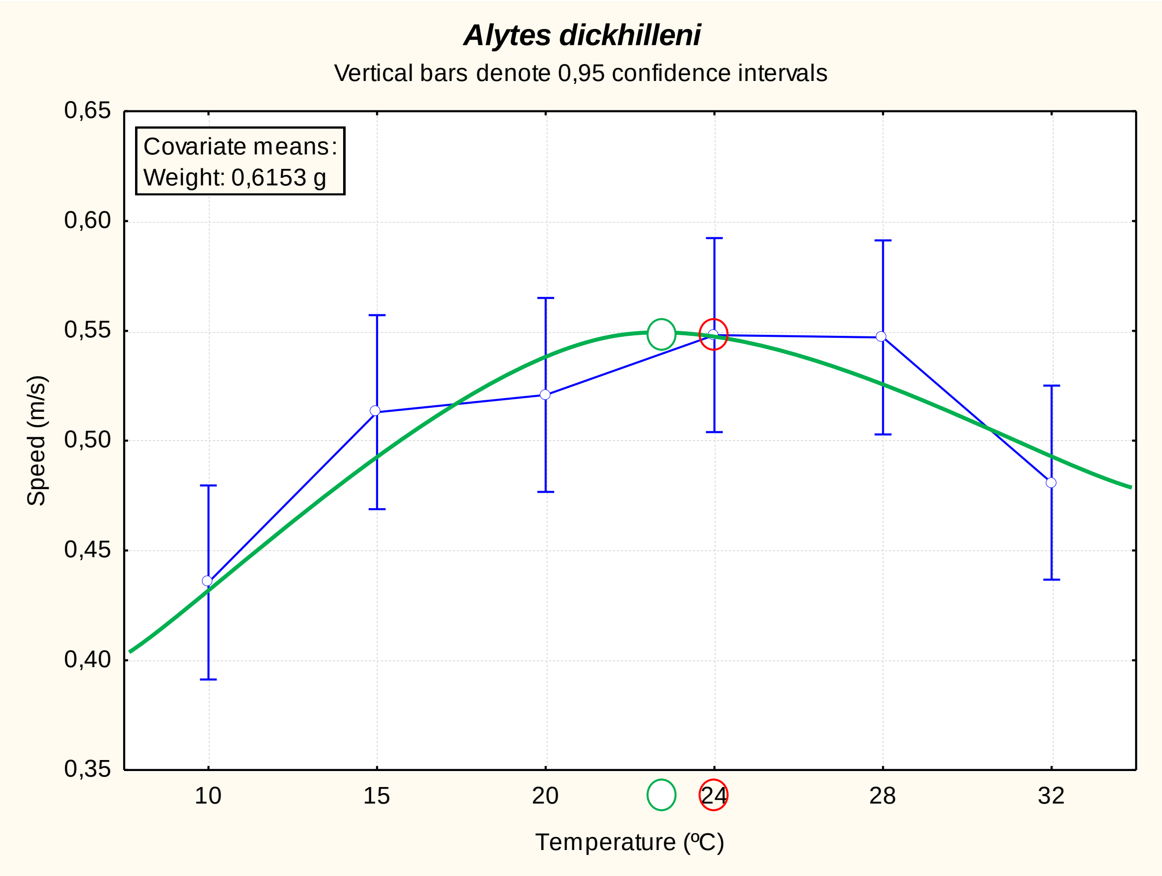
Argentina

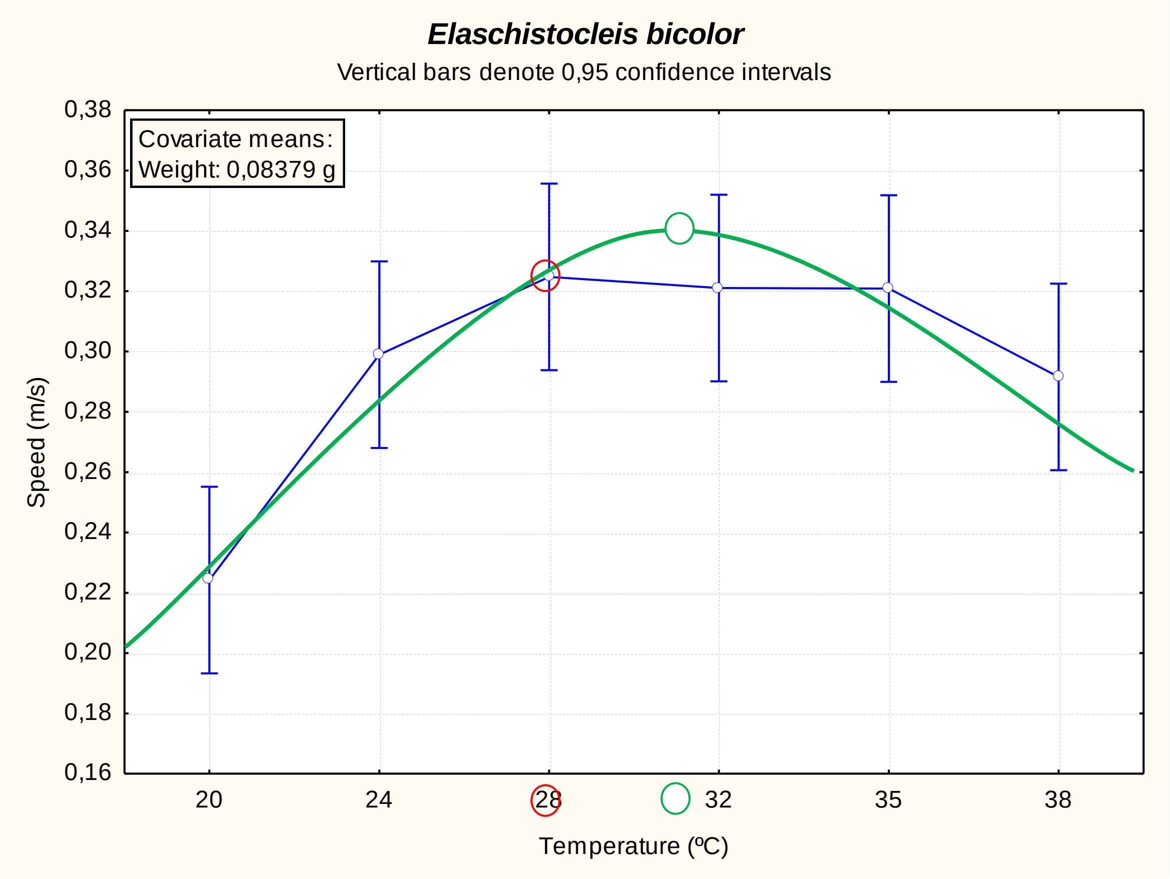


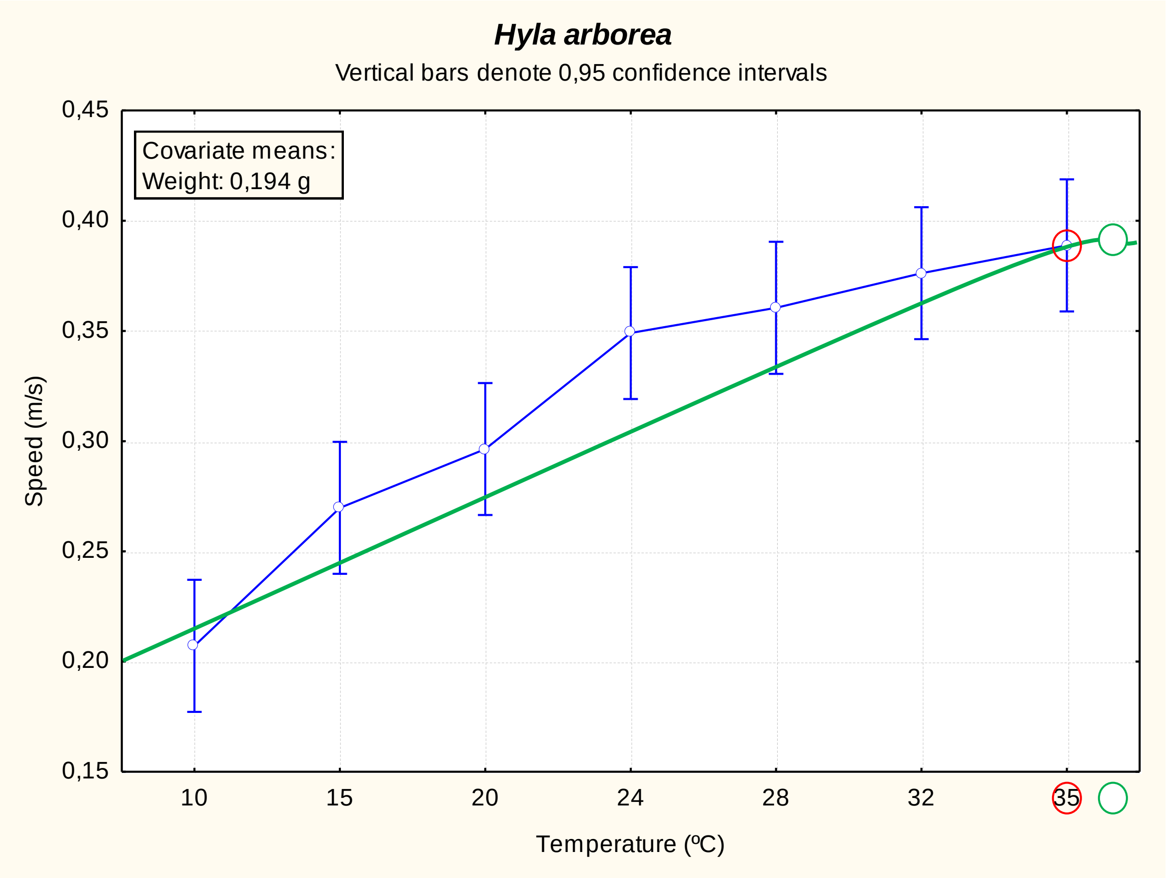
Species: (Ebi: *Elaschistocleis bicolor*; Hra: *Hypsiboas raniceps*; Lbu: *Lepdactylus bufonius*; Lla: *Leptodactylus latinasus*; Lli: *Pseudis limellum*; Pal: *Physalaemus albonotatus*; Ppa: *Pseudis paradoxa*; Sac: *Scinax acuminatus*; Sna: *Scinax nasicus*; Tve: *Trachycephalus venulosus*).

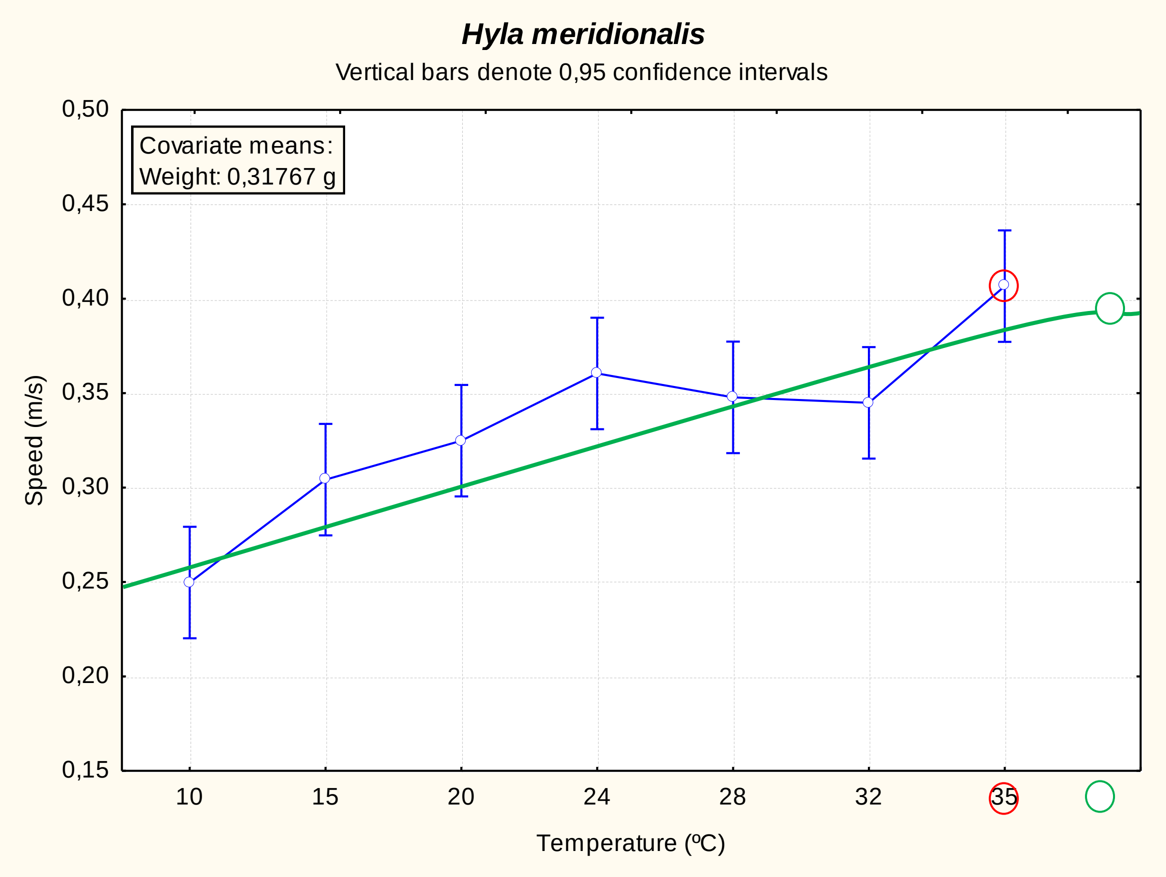
Annexe IV. Thermal performance curves

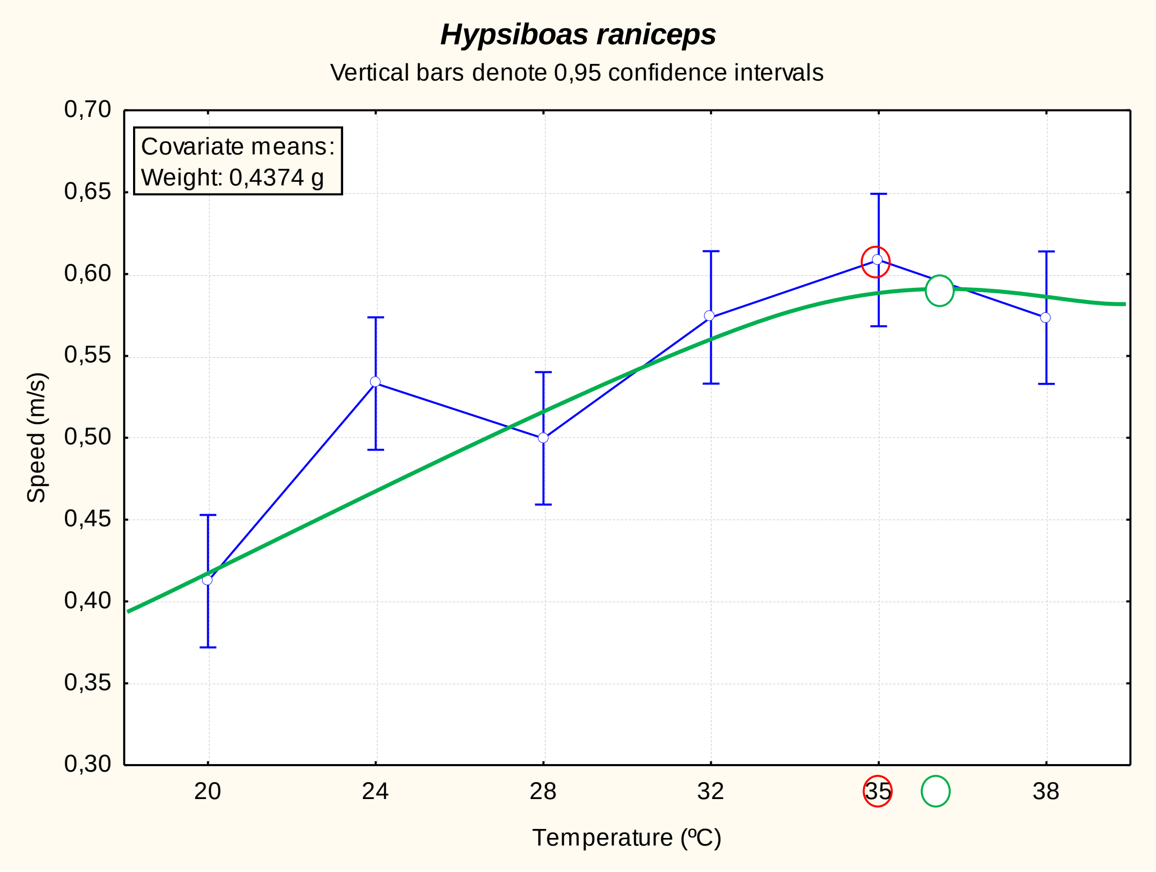
Thermal performance curves with estimated optimum temperature for all species included in this study. Best overall swimming performance (red circle) and estimated Optimum temperature and performance curve (green circle and line).

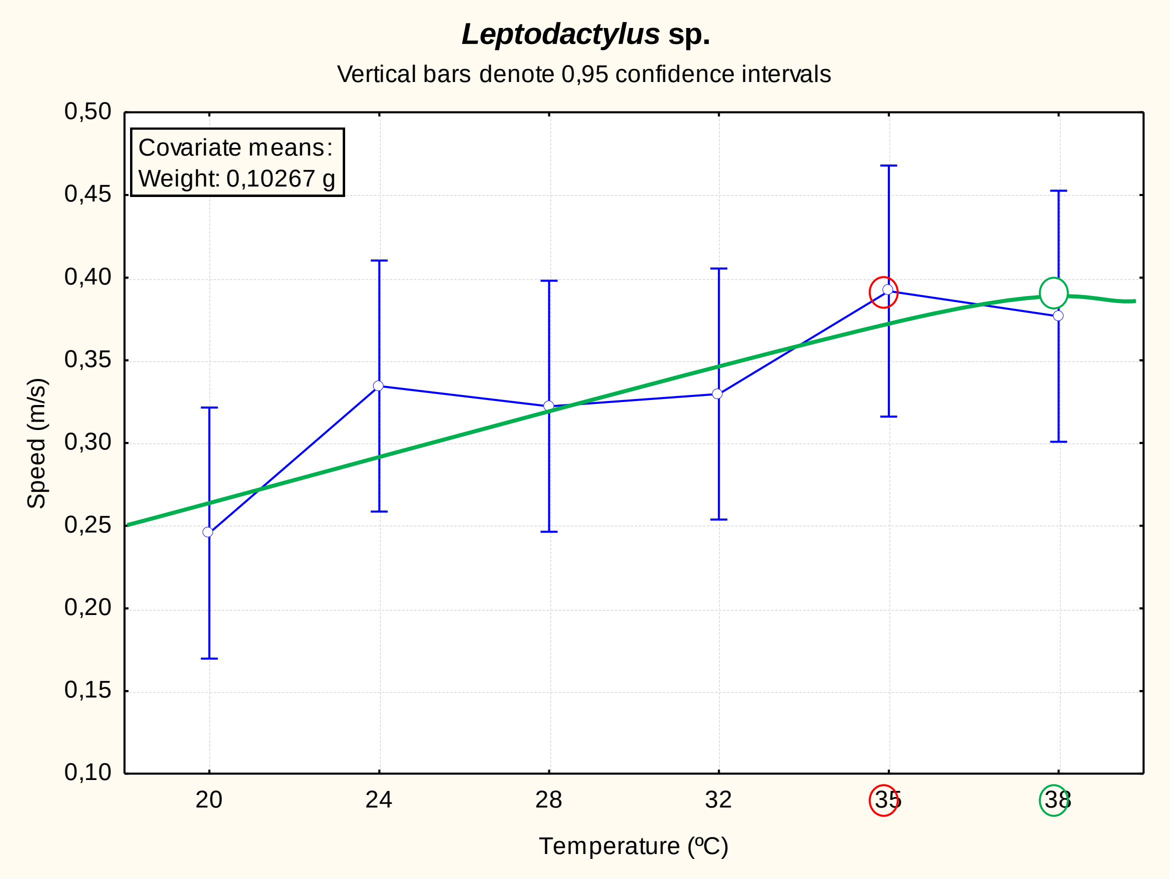


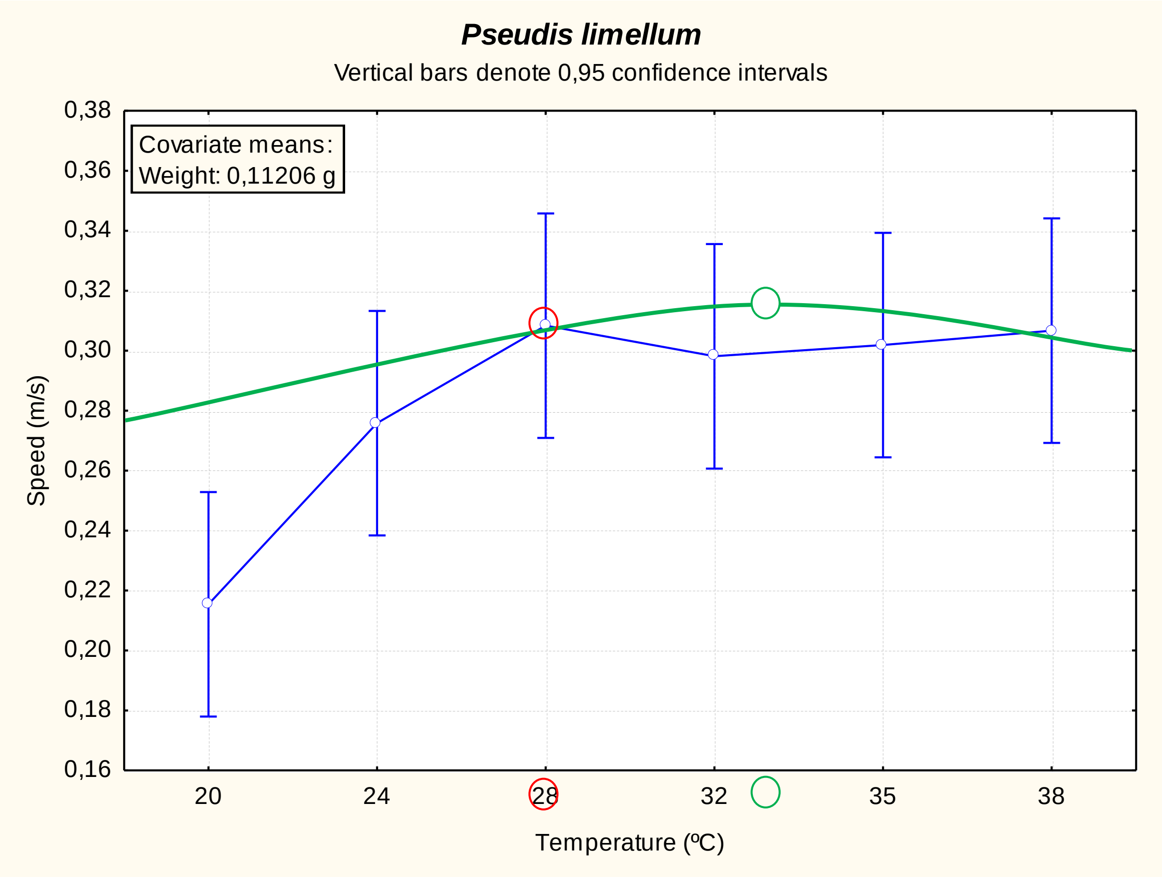

Elaschistocleis bicolor
Vertical bars denote 0,95 confidence intervals
Covariate means:
Weight: 0,08379 g
Speed (m/s)
0,38
0,36
0,34
0,32
0,30
0,28
0,26
0,24
0,22
0,20
0,18
0,16
20
24
28
32
35
38
Temperature (ºC)

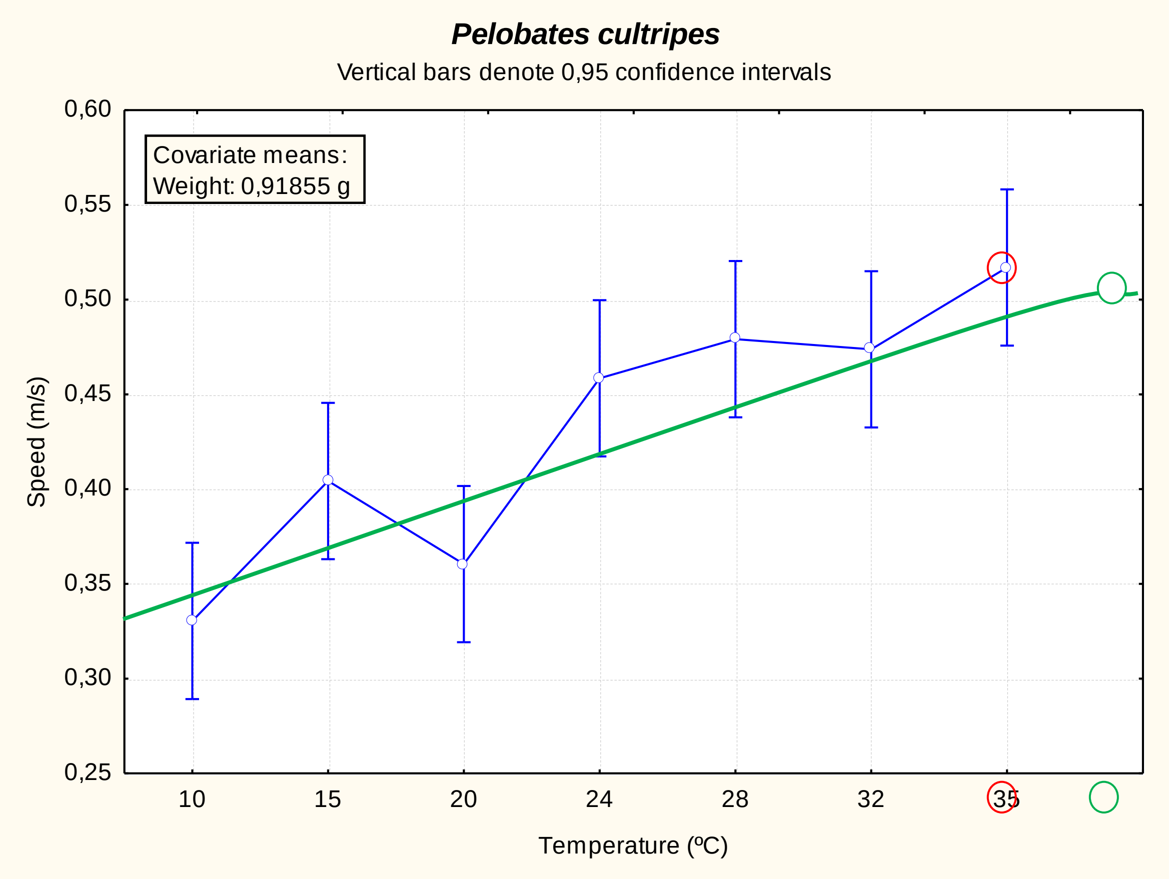

Hyla arborea
Vertical bars denote 0,95 confidence intervals
Covariate means:
Weight: 0,194 g
Speed (m/s)
0,45
0,40
0,35
0,30
0,25
0,20
0,15
10
15
20
24
28
32
35
Temperature (ºC)


Hyla meridionalis
Vertical bars denote 0,95 confidence intervals
Covariate means:
Weight: 0,31767 g
Speed (m/s)
0,50
0,45
0,40
0,35
0,30
0,25
0,20
0,15
10
15
20
24
28
32
35
Temperature (ºC)

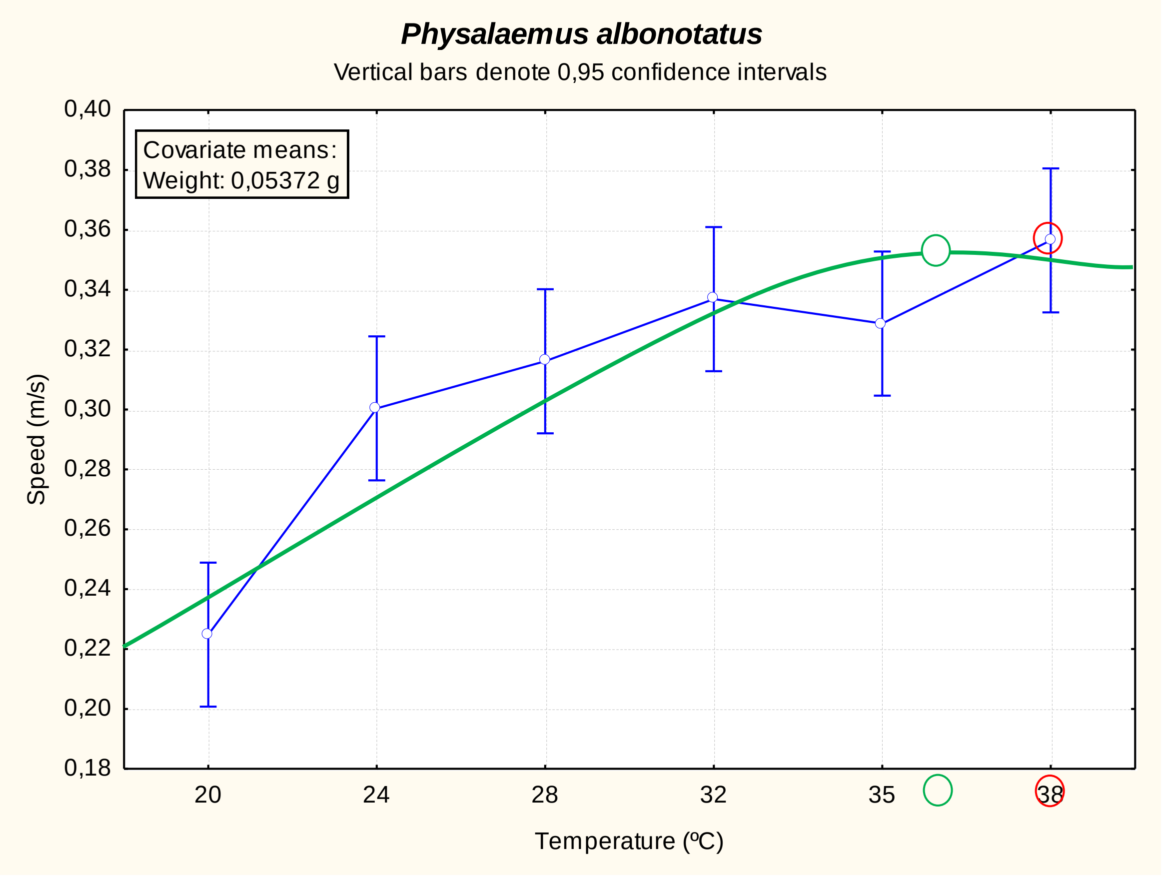

Hypsiboas raniceps
Vertical bars denote 0,95 confidence intervals
Covariate means:
Weight: 0,4374 g
Speed (m/s)
0,70
0,65
0,60
0,55
0,50
0,45
0,40
0,35
0,30
20
24
28
32
35
38
Temperature (ºC)


Leptodactylus sp.
Vertical bars denote 0,95 confidence intervals
Covariate means:
Weight: 0,10267 g
Speed (m/s)
0,50
0,45
0,40
0,35
0,30
0,25
0,20
0,15
0,10
20
24
28
32
35
38
Temperature (ºC)

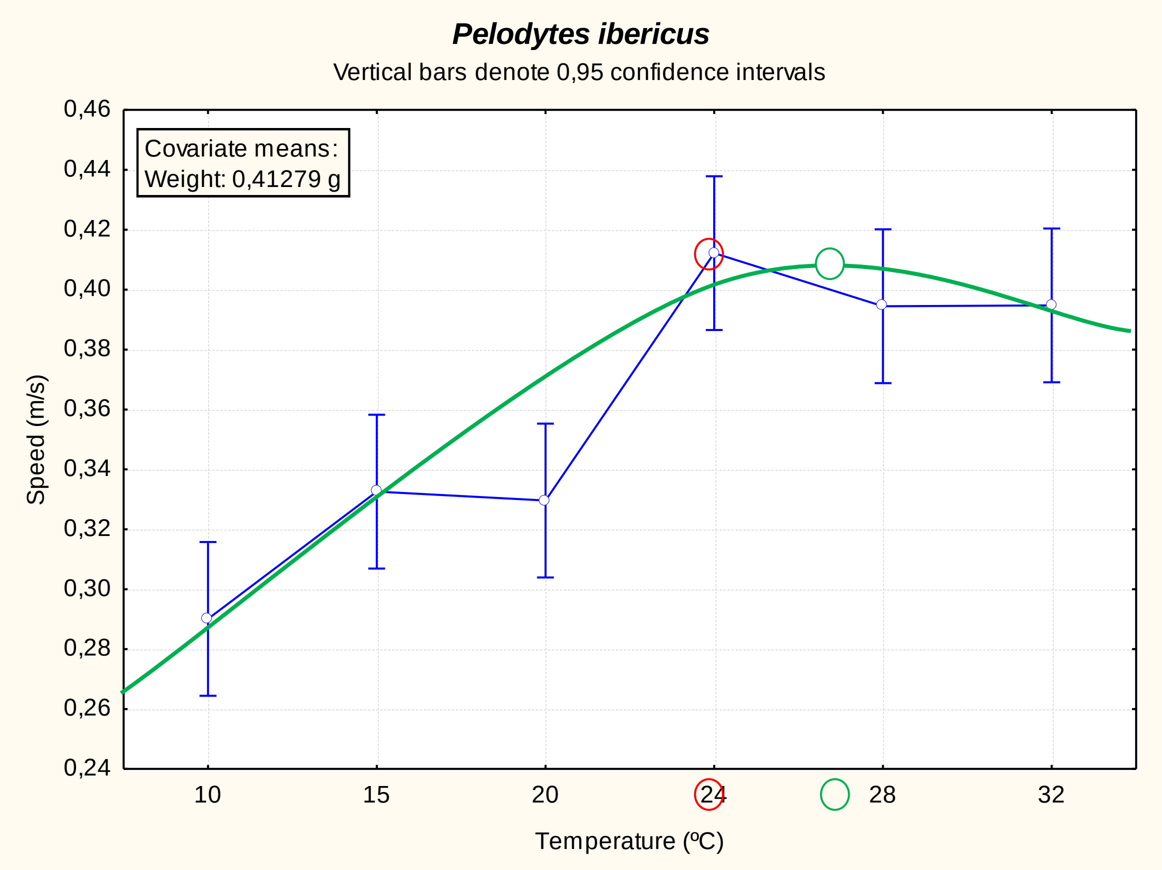

Pseudis limellum
Vertical bars denote 0,95 confidence intervals
Covariate means:
Weight: 0,11206 g
Speed (m/s)
0,38
0,36
0,34
0,32
0,30
0,28
0,26
0,24
0,22
0,20
0,18
0,16
20
24
28
32
35
38
Temperature (ºC)

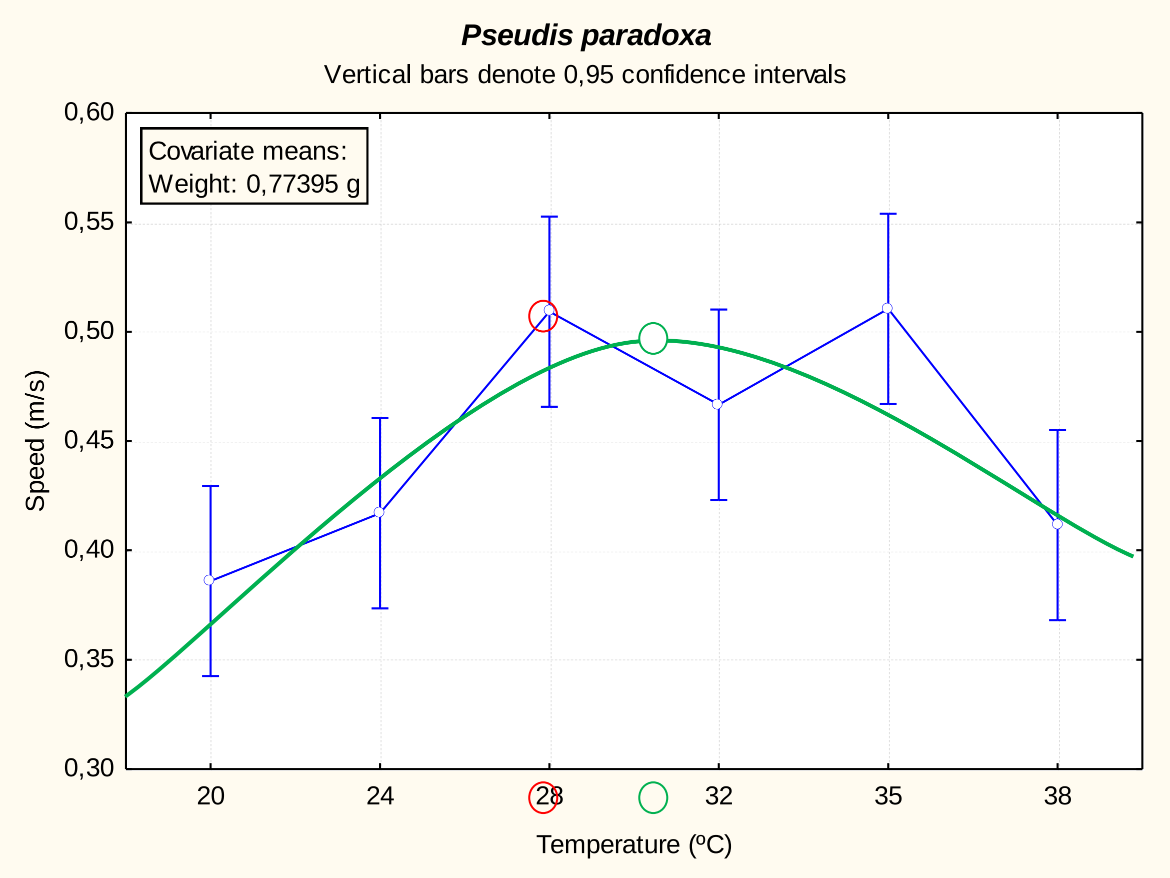


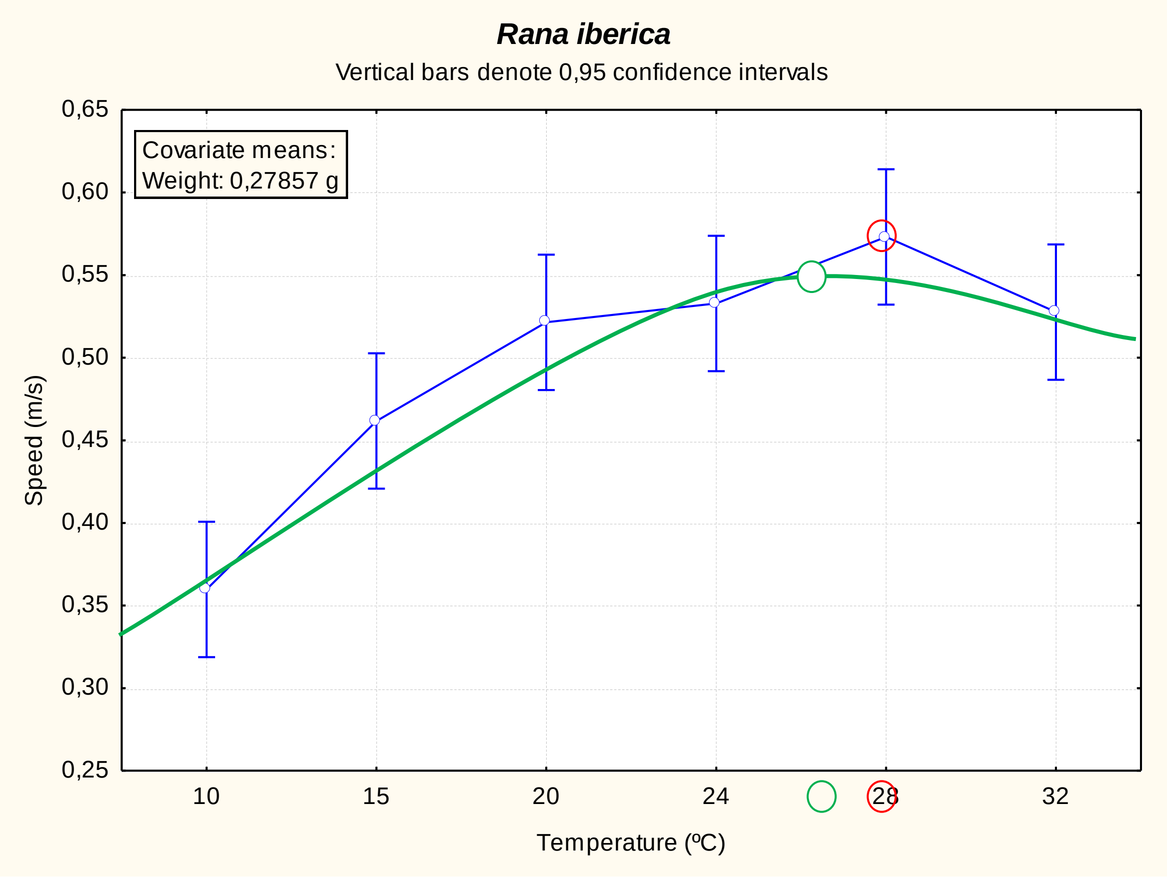
Pelobates cultripes
Vertical bars denote 0,95 confidence intervals
Covariate means:
Weight: 0,91855 g
Speed (m/s)
0,60
0,55
0,50
0,45
0,40
0,35
0,30
0,25
10
15
20
24
28
32
35
Temperature (ºC)

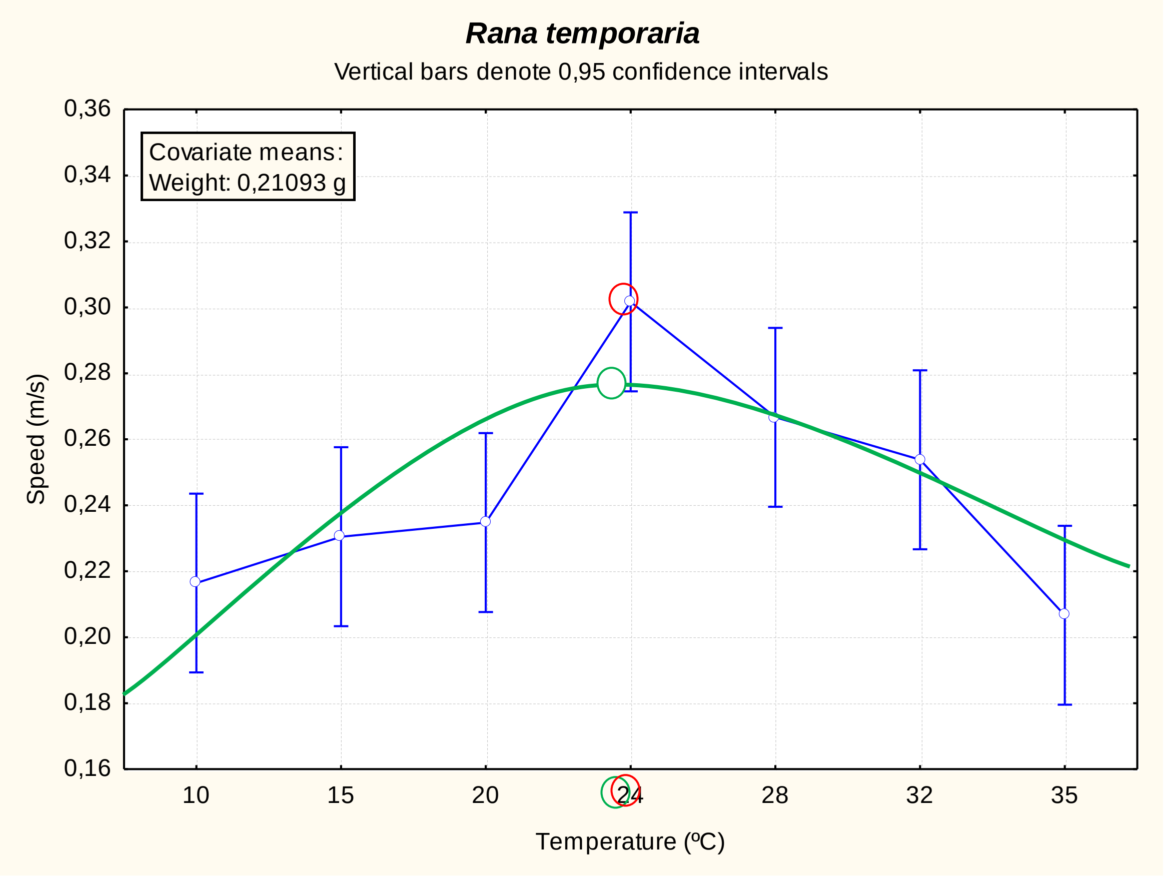


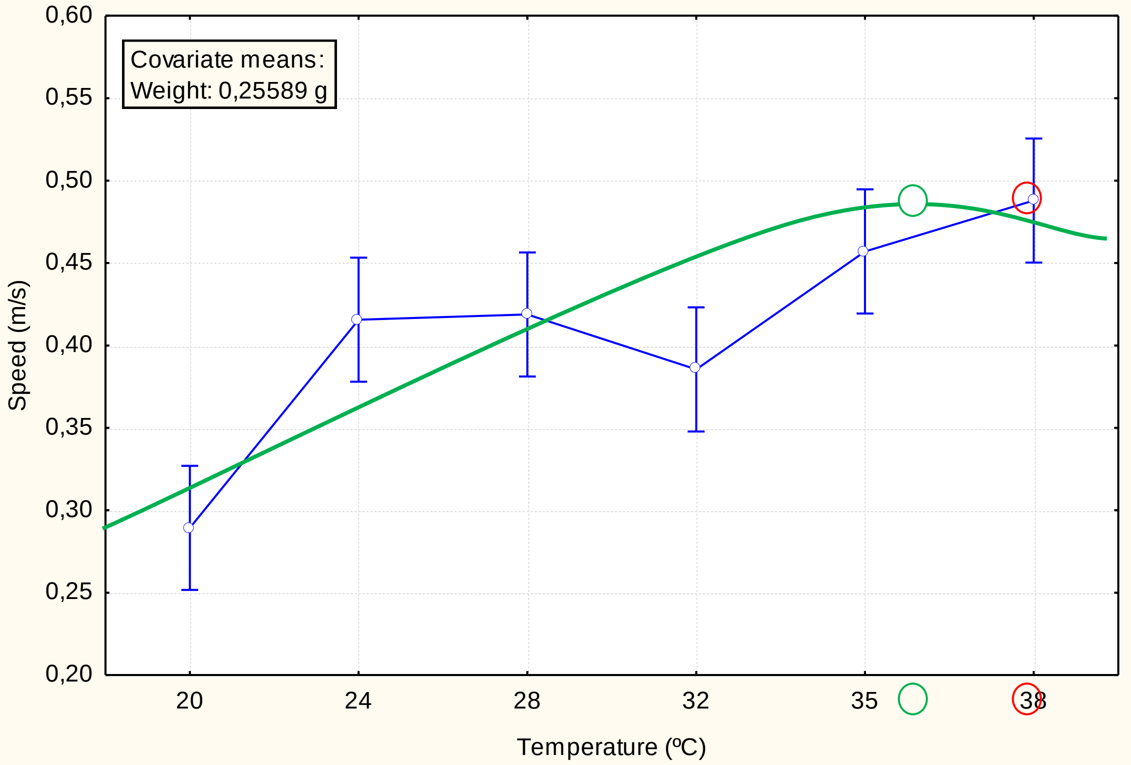
Physalaemus albonotatus
Vertical bars denote 0,95 confidence intervals
Covariate means:
Weight: 0,05372 g
Speed (m/s)
0,40
0,38
0,36
0,34
0,32
0,30
0,28
0,26
0,24
0,22
0,20
0,18
20
24
28
32
35
38
Temperature (ºC)


Pelodytes ibericus
Vertical bars denote 0,95 confidence intervals
Covariate means:
Weight: 0,41279 g
Speed (m/s)
0,46
0,44
0,42
0,40
0,38
0,36
0,34
0,32
0,30
0,28
0,26
0,24
10
15
20
24
28
32
Temperature (ºC)


Pseudis paradoxa
Vertical bars denote 0,95 confidence intervals
Covariate means:
Weight: 0,77395 g
Speed (m/s)
0,60
0,55
0,50
0,45
0,40
0,35
0,30
20
24
28
32
35
38
Temperature (ºC)

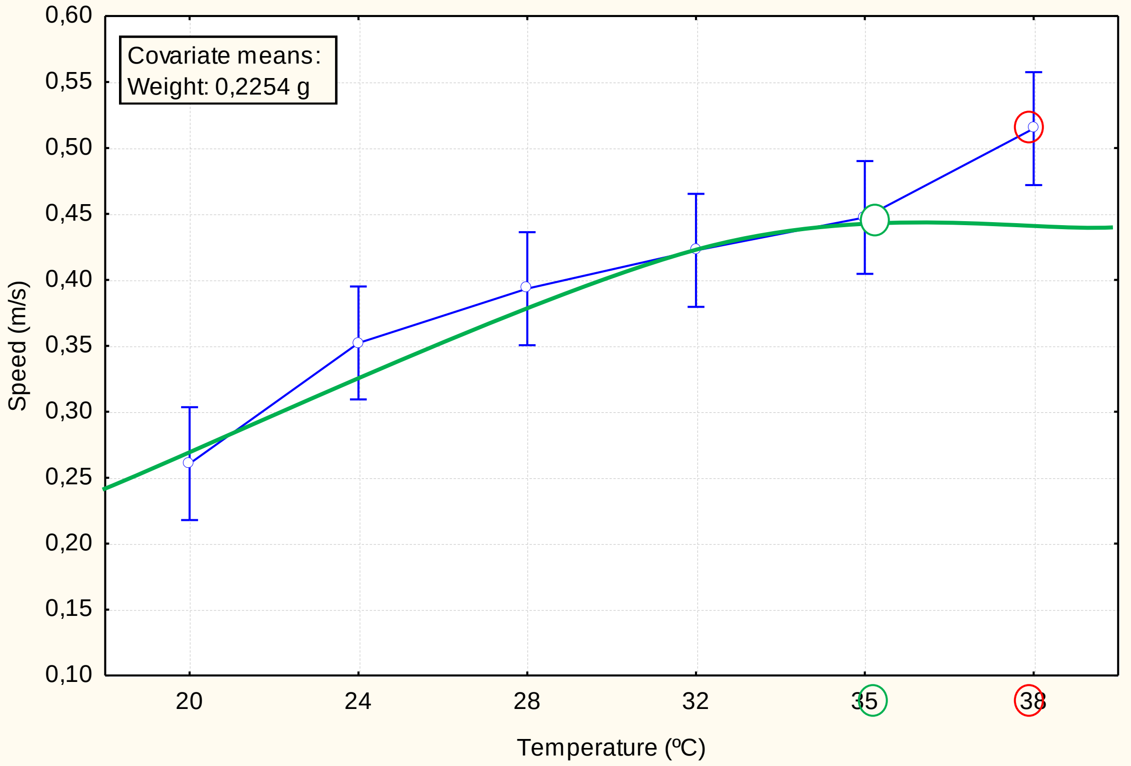

Rana iberica
Vertical bars denote 0,95 confidence intervals
Covariate means:
Weight: 0,27857 g
Speed (m/s)
0,65
0,60
0,55
0,50
0,45
0,40
0,35
0,30
0,25
10
15
20
24
28
32
Temperature (ºC)


Rana temporaria
Vertical bars denote 0,95 confidence intervals
Covariate means:
Weight: 0,21093 g
Speed (m/s)
0,36
0,34
0,32
0,30
0,28
0,26
0,24
0,22
0,20
0,18
0,16
10
15
20
24
28
32
35
Temperature (ºC)



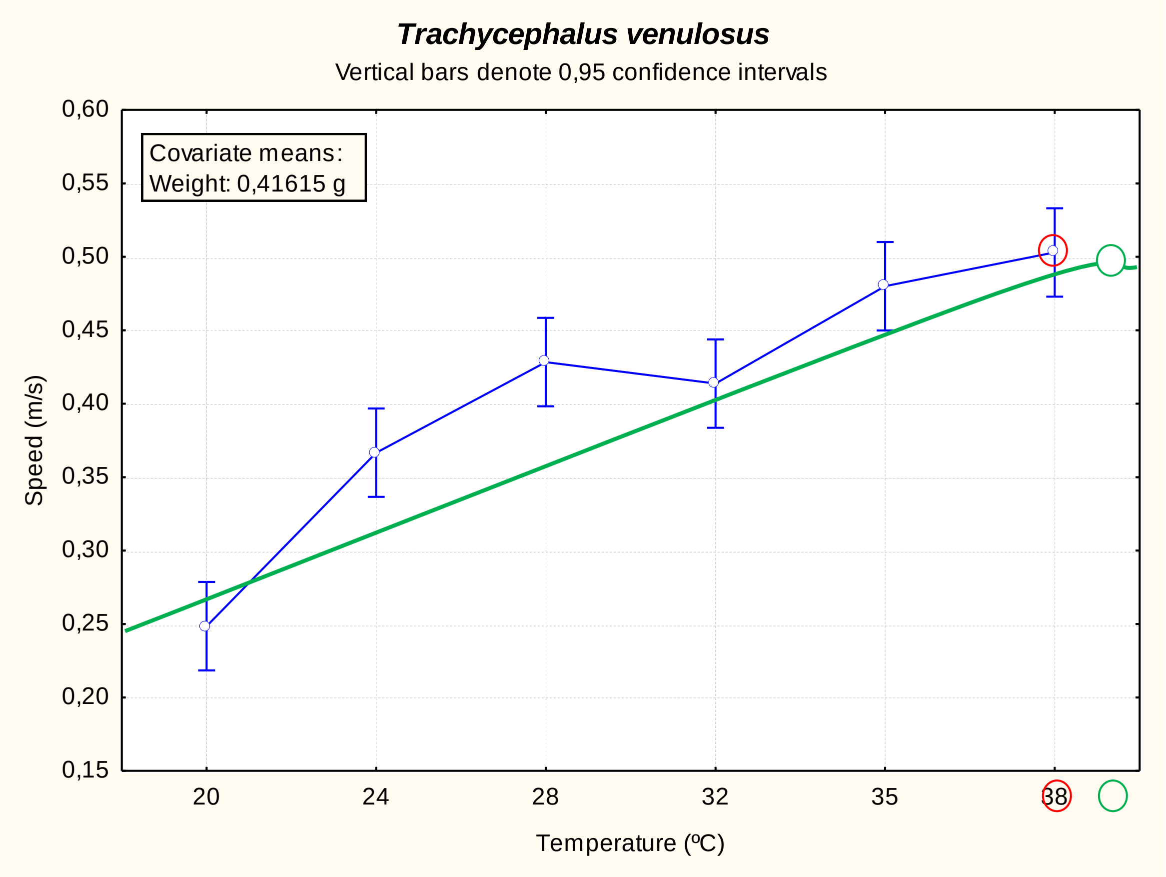
Scinax acuminatus

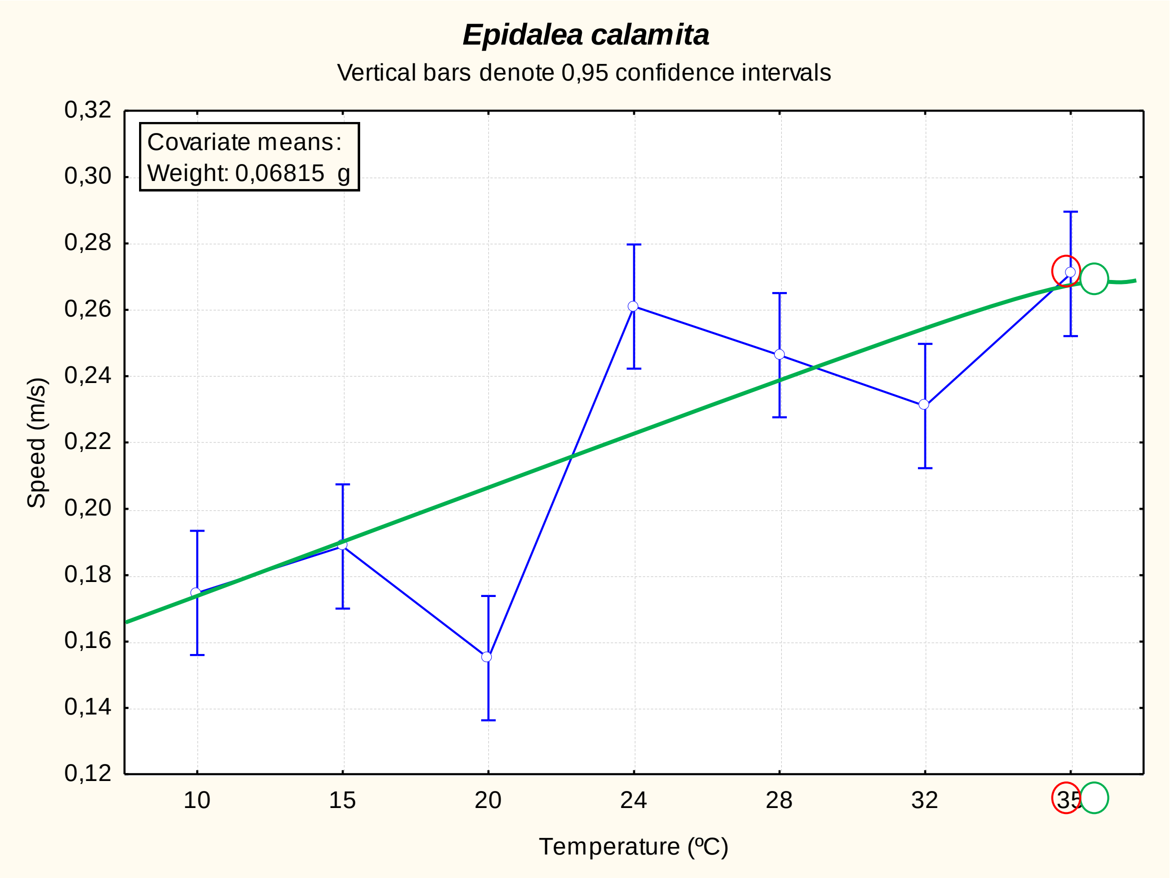
Vertical bars denote 0,95 confidence intervals



Scinax nasicus

Vertical bars denote 0,95 confidence intervals


Trachycephalus venulosus
Vertical bars denote 0,95 confidence intervals
Covariate means:
Weight: 0,41615 g
Speed (m/s)
0,60
0,55
0,50
0,45
0,40
0,35
0,30
0,25
0,20
0,15
20
24
28
32
35
38
Temperature (ºC)


Epidalea calamita
Vertical bars denote 0,95 confidence intervals
Covariate means:
Weight: 0,06815 g
Speed (m/s)
0,32
0,30
0,28
0,26
0,24
0,22
0,20
0,18
0,16
0,14
0,12
10
15
20
24
28
32
35
Temperature (ºC)