

**Reduction of the metabolic level by phenotypic plasticity involved in cave
colonization by the Pyrenean newt *Calotriton asper*.**

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Running title: Metabolic plasticity for cave colonization

Abstract

To test the hypothesis whether a lower metabolic rate is expected in cave organisms compared to surface ones due to an adaptation to food scarcity in subterranean environments, we measured the oxygen consumption rates of individuals from hypogean (i.e. subterranean) and epigean (i.e. surface) populations of the troglomorphic (i.e. living both at the surface and underground) newt *Calotriton asper*. We found that epigean individuals exhibit higher rates than hypogean ones and showed that when we acclimated epigean *C. asper* to cave conditions, these individuals reduced their oxygen consumption. We compared this metabolic plasticity to the obligate cave salamander *Proteus anguinus* as well as two epigean species: one Urodel (*Ambystoma mexicanum*) and a Fish (*Gobio occitaniae*). As predicted, we find differences between hypogean and epigean species, and that the troglomorphic *C. asper* exhibited in-between performances. We argue then that this shift of the metabolic level observed between non-acclimated and acclimated epigean *C. asper* is not directly due to the food availability in our experiments but to a stasis of the temperature. We then discuss that this adjustment of the metabolic level under a temperature close to the thermal optimum may further allow individuals to cope with the food limitations of the subterranean environment.

Keywords: adaptation, phenotypic plasticity, metabolic rate, Amphibians, cave

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Introduction

In temperate regions, caves are generally characterized -among other features- by a food scarcity compared to surface habitats (Juberthie and Decu, 1994; Hüppop, 2000 Poulson, 2001; Culver and Pipan, 2008; Romero, 2009). To cope with the limitation, theories on adaptation to cave life suggest that many characters have evolved in cave dwellers, such as an increase of a food-finding ability, a starvation resistance and a reduction of energy demand (Hervant and Renault, 2002). As part of a strategy of energetic optimisation, a low basal metabolic rate has been observed in many cave species (summarised in Hüppop, 2000).

To date, most of studies have compared metabolic rates between closely related hypogean and epigean species, or within cave species to estimate their adaptation level (Mejía-Ortíz and López-Mejía, 2005). Due to the phylogenetic distance, such comparative studies are thus limited in terms of evolutionary conclusions, while the involved physiological processes that result in metabolic depression are still controversial (Culver and Pipan, 2008; Romero, 2009; Poulson, 2011). In nature, few opportunities allow us to broach these evolutionary questions. The Pyrenean newt *Calotriton asper* offers a rare chance since is troglophilic (i.e. living mainly aboveground but also in underground habitats). Cave-dwelling populations have been strongly genetically isolated from epigean ones (Mila et al., 2010; Valbuena-Ureña et al., 2018) while any troglomorphic traits (i.e. eye regression, skin depigmentation) have been observed in cave-dwelling populations. Comparing physiological traits between a hypogean and an epigean population, Issartel et al. (2010) concluded that the colonisation of the cave environment by *C. asper* implied a fasting adaptation with acquisition of a lower basal metabolic rate. However the evolutionary process of this acquisition is still not clear. Plasticity may be

important for cave-life adaptation since metabolic rate can be highly plastic in animals, and can vary across time within individuals (i.e. owing to seasonal changes), as well as among individuals (i.e. intra-population differences in energy demands) and among populations (i.e. owing to local adaptation) (Fox et al., 2019).

Here, we investigated whether phenotypic plasticity may shift down the routine metabolic rates of epigean *C. asper* acclimated to cave conditions, driven by climatic stasis of the subterranean environment. Then, to provide a frame of comparison of the potential shift level, we compare data to a cave-dwelling population of *C. asper*, to the troglotic (i.e. obligate or strictly subterranean animals) urodel *Proteus anguinus*, and two epigean aquatic species (the urodel *Ambystoma mexicanum* and the benthic fish *Gobio occitaniae*).

Material and Methods

Study Species

The Pyrenean newt *Calotriton asper* is a brook newt, endemic to the French, Andorran, and Spanish Pyrenees. It lives mainly in rivers and lakes at altitudes between 400 and 2500 m but several cave populations have been found (Clergue-Gazeau and Martinez-Rica, 1978; Mila et al. 2010). Although lungs are not present in the adult phase, they are entirely aquatic (Clergue-Gazeau, 1971). Individuals are sexually mature at 6 years old, and can live up to 25 years, measuring then 6 to 16 cm total length and weighing 5-13 g (Guillaume and Cavagnara, 1998; Miaud and Guillaume, 2005). Depending on water temperature, reproduction occurs in spring and summer for epigean populations while in cave populations, reproduction can occur throughout the year; however eggs are been rarely observed in nature (Clergue-Gazeau, 1971; Guillaume and Cavagnara, 1998).

The proteus *Proteus anguinus* is the only obligately cave-adapted vertebrate in Europe that probably colonized caves approximately 20 Myr ago, exhibiting troglomorphic characteristics such as

skin depigmentation, eye degeneration and neoteny (Bulog, 1994). Found mainly in Slovenia and Croatia, a breeding programme was started at the end of the 1950s in a cave in France, in order to produce individuals without the need to collect them in the wild. The founder individuals came from successive imports from the Piuka cave river near the town of Planina in Slovenia (Vandel and Bouillon, 1959; Juberthie, 1991). Reproductions were observed since 1959 (Juberthie, 1991). Until now, it was the only successful programme, allowing the collection of information on its development and life cycle while quite any data was collected on natural populations (Bulog, 1994). Individuals are sexually mature at 15 years old. Adults measure between 20-35 cm total length and weigh 7-19 g. Their mean lifespan is estimated to 68 years and their maximal lifespan to more than 100 years (Voituron et al., 2010).

The axolotl *Ambystoma mexicanum* is a neotenic urodel which has become widespread in laboratories and aquariums throughout the world. However, in the wild *A. mexicanum* is close to extinction in the last remaining habitats, which are limited on 2 mountain lakes at altitudes more than 2000 m near Mexico City (Bride et al., 2008). While the common wild form is generally pigmented, a form with pigmented eyes but depigmented body also occurs. Individuals are sexually mature after 2 years, measure up to 40 cm total length and weigh up to 50 g.

The gudgeon *Gobio occitaniae* is a benthic fish locally present in southwestern France (in the Garonne-Dordogne river drainage). They live in lotic clear water systems, and feed mainly upon small invertebrates (Keith et al., 2011). Adults are 12-15 cm long, and reach sexual maturity around 2 years.

Animal Origin and Maintenance

Epigeal *Calotriton asper* were captured by hand from Cailla Brook, (600–1000m, longitude: 42.805993°, latitude: 2.190989°), a first group in November 2006 then a second group in October 2015 (thus after the breeding season). They were maintained in the cave of the “Station d’Ecologie Théorique and Experimentale, i.e. SETE” (Ariège, France), where the temperature is 11.5°C ± 0.5 throughout the year. The first group (collected in 2006) was kept under total darkness for 8 years before

the tests. The second group (collected in 2015) was exposed to alternating 12h day/12h night photic cycle for one year. To mimic the day phase, we used full spectrum daylight fluorescent lamps.

Hypogean *Calotriton asper* were captured by hand in the cave of Labouiche (43.01231°, 1.342365°), a first group in November 2006 then a second group in November 2015. These were maintained in the cave of the SETE. The first group (collected in 2006) was kept in total darkness for 8 years. The second group (collected in 2015), was in total darkness before a first tests series, and then one year under alternating 12h day/12h night before a second tests series.

Proteus anguinus were maintained in the cave of SETE in total darkness. Eight were exposed to 12h day/12h night alternance in the cave of SETE during the study.

Ambystoma mexicanum (eyes pigmented and depigmented body) were supplied from a private breeder. They were maintained also in the cave of SETE and exposed to under 12h day/12h night conditions for several months before being tested.

Gobio occitaniae were fished from the Vère in Cahuzac (43.984917°, 1.911782°). They were sampled using electrofishing on a 100m river stretch, and 20 adults were brought to the laboratory. Electrofishing was done under the authorisation of local authorities ('Arrêté préfectoral' from the Tarn, French department). They were kept in tanks, exposed to a 12h day/12h night in the cave of SETE tens days before being tested.

Animals used in this study were fed outside the measurement period *ad libitum* with live chironomid larvae once a week.

Experimental set up

Twenty individuals from each species (and populations) were tested for each treatment (constant dark / 12:12 day:night cycle; excepted *P. anguinus* exposed to light, N=8). They were isolated in a 10 L plastic transparent chamber filled with water from the cave river (10.13 ± 0.19 ppm O₂, $9.90 \pm 0.21^\circ$ C), where they were free to move on the bottom and utilize a hiding place. During a 3-day

acclimatization period, the water was continuously renewed. At the start of the measurement period, the flow was cut and the dissolved oxygen was measured using the FireSting fiber-optical oxygen meter (PyroScience company). We monitored the oxygen concentration of each chambers every 30 minutes for up to 15 days; tests were stopped if the oxygen concentration decreased down to 4 ppm so as not to provide a physiological stress.

Statistical analyzes

The variation of the oxygen concentrations during time were analyzed using simple linear regression analysis. The difference on the oxygen consumption level between species or populations or treatments among the same species were analyzed using the Steel-Dwass-Critchlow-Fligner bilateral Test for multiple comparisons by pairs.

The differences on the oxygen consumption level between epigean *C. asper* just caught from nature and then after 1 year in the cave were analyzed using the Wilcoxon unilatéral Test.

The differences on the consumption level exhibited by individuals (Hypogean *C. asper* or *P. anguinus*) first in darkness, versus just exposed to a day/night alternance, versus after one year under a day/night alternance, were analysed using the Friedman Test.

Results

Oxygen concentrations for all individuals decreased linearly over the time of measurement (Table 1). Since we fixed a lower ppm threshold to end the test, metabolic rate was monitored from 6 to 15 days (Table 2). The differences were due to differences between species, to differences of their average body mass (e.g. *A. mexicanum* are 3 to 4 times heavier than *C. asper*) and individuals within the same species. Indeed, when we corrected the oxygen concentrations by the body mass of tested individuals ($Variable = \frac{oxygen\ concentration}{Body\ mass}$) the slopes of the curves showed significant differences between species or populations and treatments among *C. asper* (Table 3).

Epigean *C. asper* recently caught exhibited higher rates than hypogean *C. asper* (Fig. 1). However, the rates of individuals from the same epigean populations after several years in the cave in darkness were reduced to levels close to the hypogean individuals. The oxygen consumption rate of the cave *P. anguinus* were lower than others species, but surprisingly the difference was not significant compared to the epigean *A. mexicanum*. Clearly, the oxygen consumption rates of *G. occitaniae* were higher than those of the Urodelan species.

Moreover, when individuals from the epigean population of *C. asper* were maintained in the cave under a day/night photic cycle for one year, their oxygen consumptions significantly decreased (Fig. 2). On the other hand, we did not note any significant changes with individuals from the hypogean population exposed to a day/night alternance even after one year (Fig. 3). On the contrary, exposure to the photic cycle increased the rate of *P. anguinus* and the difference became significant after one year (Fig. 4).

Discussion

Our experimental set up allowed us to compare the oxygen consumption rate of various benthic Urodela and a fish in water, with a high realistic degree since measurements were made in conditions mimicking natural ones, continuously without restraints during several days. We thus assume that in this study the measured oxygen consumption rates are rather good approximations of the routine metabolic level.

Since we fed *ad libitum* all tested individuals, we can argue that measured differences cannot be driven directly by the food availability. It is generally conceded that in caves in Europe, food availability is a limiting factor (Juberthie and Decu, 1994; Hüppop, 2000 Poulson, 2001; Culver and Pipan, 2008). Even if very few studies assess this parameter focused on the Pyrenees (Gers 1998) and Dinaric Karst (Pipan and Culver, 2003; Simon et al., 2007; 2010), the food limitation seems theoretically true for

190 amphibians having the size of *C. asper* and *P. anguinus*, which are the biggest species from the cave
191 fauna. Indeed, in Europe, troglobitic and troglophilic species are mostly invertebrates. There is no
192 stygobitic (i.e. aquatic troglobitic) fish (Greenwood, 1967), all come from caves in the tropics and sub-
193 tropics (Weber et al, 1998). Recently (2015), a cave population of loaches (*Barbatula*) was discovered
194 in Danube-Aach system in Southern Germany (Behrmann-Godel et al., 2017). This population, hosting
195 individuals with reduced eyes, enlarged barbels and pale body coloration, is of recent origin but
196 genetically divergent from surface populations residing in the same drainage. Probably, the most
197 crucial step towards a successful permanent colonization of the subterranean environment by fishes
198 is the adaptation to a low food supply in amount, space and time (Weber et al., 1998). Among
199 Amphibians, Suwannapoom et al. (2018) reported recently the discovery of a new troglophilous genus
200 and species of microhylid frog from a limestone cave in the tropical forests of western Thailand.
201 Excepting *P. anguinus* in Europe, only seven other Urodelan troglobitic species were known around
202 the world, all from North America; and except *C. asper*, only salamanders from the genus *Hydromantes*
203 are considered as troglophilic in Europe, even if many other Urodelans are often observed in caves
204 (Durand, 1998). All troglobitic and troglophilic forms are without functional lungs and breathe through
205 gills and/or the skin (Durand, 1998). *Hydromantes* sp. lack lungs and an aquatic stage and because of
206 their specific physiology, when external conditions become harsh, *Hydromantes* move underground
207 close to the entrances looking for a suitable microclimate and take advantage from epigeous prey
208 which often show high abundances in subterranean environments during hot seasons (Lunghi et al.,
209 2018). Unlike what usually occurs in temperate region where cave habitats exhibit high temporal and
210 spatial heterogeneity of food availability (Hüppop, 2005), some tropical caves are characterized by a
211 potentially high level of nutriment (Deharveng, 2005). In these systems, troglomorphism is not linked
212 to the food availability (Salin et al., 2010) and should not require a genetically-determined reduced
213 metabolism allowing low energy (Spicer, 1998). In some cases (e.g. *Poecilia mexicana*) cavernicolous
214 individuals have higher routine metabolic rates than relatives from surface habitats, such that overall
215 reductions in energetic demands are primarily driven by reductions in body size (Passow et al. 2015).

In some crayfish species, the decrease of the oxygen consumption rate does not necessarily depend solely on the availability of food, but also of other important factors as the rate of dissolved oxygen in the water (Mejía-Ortíz and López-Mejía, 2005). In general, since epigean *C. asper* lives in mountain brooks, the level of dissolved O₂ in water may not be so much different compared to the cave river and close to the saturation, except locally.

In the same way, in our study a lack of food cannot explain the shift of the metabolism, which seems due to other drivers. The light has no effect on the metabolic level of *C. asper*. This is not so surprising in this species which is nocturnally active. On the contrary, the effect of the light is immediate in *P. anguinus* who avoid it. The metabolic rate increases probably due to the stress and also to the production of melanin since individuals increased dark coloration. Therefore, the reduction of the metabolic level in cave acclimated *C. asper* can be more likely ascribed to the temperature. The developmental thermic optimum of both hypogean and epigean *C. asper* forms is between 12-15°C which is slightly above the temperature range observed in nature in caves (Clergue-Gazeau, 1971; Guillaume, 2000). Thus, the shift of the metabolic level observed in *C. asper* acclimated to the cave may be more logically due to the thermic stasis within a favorable range close to the thermic optimum. The situation is probably similar for *P. anguinus*, whose optimum is 16°C, level never reached in nature (Durand, 1998). There is evidence that *C. asper* can use behavioral adjustments to keep the body temperature in their specific temperature range and may select temperatures in order to compensate for low opportunities of favorable temperatures through contrasted seasonal climatic variations (Trochet et al., 2018). The stasis of the underground climate offers to the stenothermal ectotherms *C. asper*, conditions close to the homeothermy nearby its thermic optimum, and thus taking refuge into caves may be an efficient alternative strategy to face seasonal changing environments occurring outside.

The time frame for the evolution of traits in cave animals is poorly documented, however recent studies in the fish *Astyanax mexicanus* suggested that changes accompanying cave colonization can be established rapidly at the morphological and behavioural level (McGaugh et al., 2019) and that many

cave-related traits can appear within a single generation by phenotypic plasticity (Bilandzija et al., 2020). Phenotypic plasticity allows a quick response to environmental conditions and may favour the colonization of heterogeneous habitats (Pigliucci, 2001), however there is little evidence of such mechanism involved in the colonization of caves. For example, cave populations of the freshwater amphipod *Gammarus minus* have become generalists or omnivorous by including animal protein in their diet (MacAvoy et al., 2016). Differences in life history traits in surface and cave forms have been observed in fishes (Romero and Green, 2005; Riesch et al., 2009). In *C. asper*, the darkness delays the metamorphosis (Clergue-Gazeau, 1971). The non-obligate cave salamander *Plethodon albagula* also exhibits plasticity in growth, body size, and development, which may be adaptive and a function of extreme variation in surface environmental conditions (Taylor et al. 2015). On the contrary, in the epigeous fire salamander *Salamandra salamandra*, Manenti and Ficetola (2012) studying predation performance, suggested that plasticity plays a minor role during acclimatization to underground conditions.

Colonization of caves is generally viewed either as accidental (entrapment or refugium) or as actively advantageous, with the potential advantages including, for example, environmental stability, exploitation of empty niches, and protection from predation (Holsinger 2000; Romero and Green 2005). Some recent data suggest that *C. asper* probably recolonized the Pyrenees from distinct glacial refugia (Lucati et al., unpublished). During this process, some populations may have colonized caves to avoid climatic changes occurring outside (Guillaume, 2001; Miaud and Guillaume, 2005). Considering the present study, these events could have been favored through a plasticity of the metabolic rate due to an adjustment to the temperature in caves, that may further allow individuals to cope the food limitations in such an environment. Indeed, in animals, physiological traits, although acting in the short-term, have long-term basis driven by evolutionary processes, meaning that initial advantages could lead to further cumulative advantages (Fox et al., 2019). Additionally, the stasis of the underground climate may offer to *C. asper* constant favorable conditions close to its thermic optimum and thus a selective advantage compared to individuals exposed to changing environments through

time (such as those above ground), as well in short-term to face dramatic and rapid variation, as with global climate changes.

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Author Contributions

OG conceived and designed the experiments, collected the Urodelan species, performed the experiments, analyzed the data, prepared figures and tables, authored and reviewed drafts of the paper, approved the final draft. MD performed the experiments, analyzed the data, and reviewed drafts of the paper. AR collected *G. occitaniae*, analyzed the data, and reviewed drafts of the paper. OC managed the ethic and legal issues, collected the Urodelan species, and reviewed drafts of the paper. AT analyzed the data and reviewed drafts of the paper.

Animal Ethics

All required French permits relating to animal experimentation on the species used in this study were been obtained (Animal experimentation accreditation n°A09583 for the lab and n° A09-1 (2001)-A09-2 (2007)-A09-3(2011) - A09-5(2013) for the experimenters, n° 09-19, 09-273, 09-295 for Animal caretakers and handlers to use wildlife in scientific purposes.

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296 **Field Study Permissions**

297 All required French permits (permits no. 2017-04; 2007-11-1342; 2016-s-01) relating to an
298 authorization of capture, marking, transport, detention, use and release of protected amphibian
299 species; and animal experimentation permits were been obtained and the project was approved by
300 the National Council for Nature Protection the 19th March 2007 and the Regional Scientific Council
301 for the Natural Heritage of the Region Languedoc-Roussillon-Midi-Pyrénées the 5th April 2016.

302

303 **Data availability statement**

304 Upon acceptance, data will be deposited to the Dryad public repository.

305

306 **Literature cited**

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436

Tables

N° individual	N°1	N°2	N°3	N°4	N°5	N°6	N°7	N°8	N°9	N°10	N°11	N°12	N°13	N°14	N°15	N°16	N°17	N°18	N°19	N°20
Just caught epigean <i>C. asper</i>																				
R ²	0,974	0,996	0,981	0,989	0,928	0,974	0,980	0,959	0,960	0,985	0,996	0,969	0,885	0,990	0,987	0,959	0,993	0,990	0,992	0,960
F	33103	231221	44609	81539	8764	25874	32792	15518	16036	42552	162142	21244	5238	65614	50400	17463	101581	76045	92300	16051
Pr > F	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001
Epigean <i>C. asper</i> 8 years in cave																				
R ²	0,990	0,975	0,981	0,939	0,977	0,988	0,974	0,993	0,974	0,980	0,972	0,981	0,972	0,967	0,979	0,987	0,892	0,977	0,981	0,987
F	685890	256894	339030	101169	208519,54	394250,654	182641,849	735554,405	270818	357227	243776	368613	249647	208429	339974	544376	51772	268258	324303	479585
Pr > F	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001
Hypogean <i>C. asper</i> 8 years in cave																				
R ²	0,982	0,971	0,932	0,984	0,965	0,990	0,996	0,991	0,980	0,998	0,995	0,983	0,989	0,998	0,996	0,994	0,965	0,992	0,846	0,992
F	36889	22036	9154	40066	18439	66654	170341	72204	32011	347199	144946	37636	61866	421641	175756	109391	18492	80844	3683	86653
Pr > F	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001
<i>P. anguinus</i>																				
R ²	0,975	0,904	0,971	0,965	0,955	0,984	0,913	0,990	0,987	0,996	0,980	0,976	0,983	0,988	0,988	0,981	0,996	0,979	0,951	0,991
F	27937	6702	23555	19614	15102	45324	7550	73430	52756	187104	34209	28665	40642	58936	61718	36419	183738	38536	15832	86436
Pr > F	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001
<i>A. mexicanum</i>																				
R ²	0,945	0,977	0,991	0,988	0,981	0,997	0,985	0,999	0,937	0,994	0,986	0,920	0,991	0,996	0,999	0,984	0,980	0,994	0,905	0,991
F	5839	14398	35784	28721	19259	102234	24590	400379	5115	60073	23365	3925	51494	123753	516416	28205	24099	84588	4630	52814
Pr > F	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001
<i>G. occitaniae</i>																				
R ²	0,987	0,991	0,989	0,970	0,983	0,967	0,971	0,974	0,983	0,990	0,963	0,989	0,988	0,997	0,990	0,999	0,988	0,993	0,975	0,955
F	18154	26738	20380	7535	11470	5868	6516	7375	11470	268858	72026	238505	246676	1097507	279526	3672028	235612	422279	117600	62750
Pr > F	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001

Table 1. Linear regression analysis (R² coefficient of determination, F Fisher 's test) of the oxygen consumption per gram of body mass explained by the time for twenty individuals from various species.

Species	<i>C. asper</i>				<i>P. anguinus</i>	<i>A. mexicanum</i>	<i>G. occitaniae</i>
	Epigean		Hypogean				
	Just caught	Acclimated to cave	Just caught	Acclimated to cave			
Average lasting in days	14.4 ± 1.3	15.3 ± 1.7	15.5 ± 1.8	15.5 ± 1.9	15.3 ± 0.9	8.4 ± 1.3	6,0 ± 0.6
Average mass in grams	8.4 ± 1.6	9.5 ± 1.7	7.4 ± 2.1	7.2 ± 2.3	11.8 ± 3.7	32.4 ± 5.9	14.5 ± 6.1

444 Table 2. Average duration of tests and body mass of tested individuals (N=20). Since we find
 445 significative differences between individuals, sexes, populations, species and time, the oxygen
 446 consumption have been obviously corrected by the body mass.

447

448

Epigean <i>C. asper</i> acclimated to cave		Hypogean <i>C. asper</i>	<i>P. anguinus</i>	<i>A. mexicanum</i>	<i>G. occitaniae</i>
Just caught epigean <i>C.</i>					
<i>asper</i>	0.004	0.004	< 0.0001	< 0.0001	0,008
Epigean <i>C. asper</i>					
acclimated to cave		0.923	0.002	0.019	< 0.0001
Hypogean <i>C. asper</i>			< 0.0001	< 0.0001	< 0.0001
<i>P. anguinus</i>				0,689	< 0.0001
<i>A. mexicanum</i>					< 0.0001

449 Table 3. Comparison of the oxygen consumption level in ppm per gram of body mass per second for *G.*
 450 *occitaniae*, epigean *C. asper* just caught from nature, epigean *C. asper* acclimated 8 years to the cave,
 451 hypogean *C. asper* maintained 8 years in the cave, *A. Mexicanum*, and *P. anguinus*. The table gives the

452 P-values for multiple comparisons by pairs with the Steel-Dwass-Critchlow-Fligner bilateral Test
453 (N=20).
454

Figures

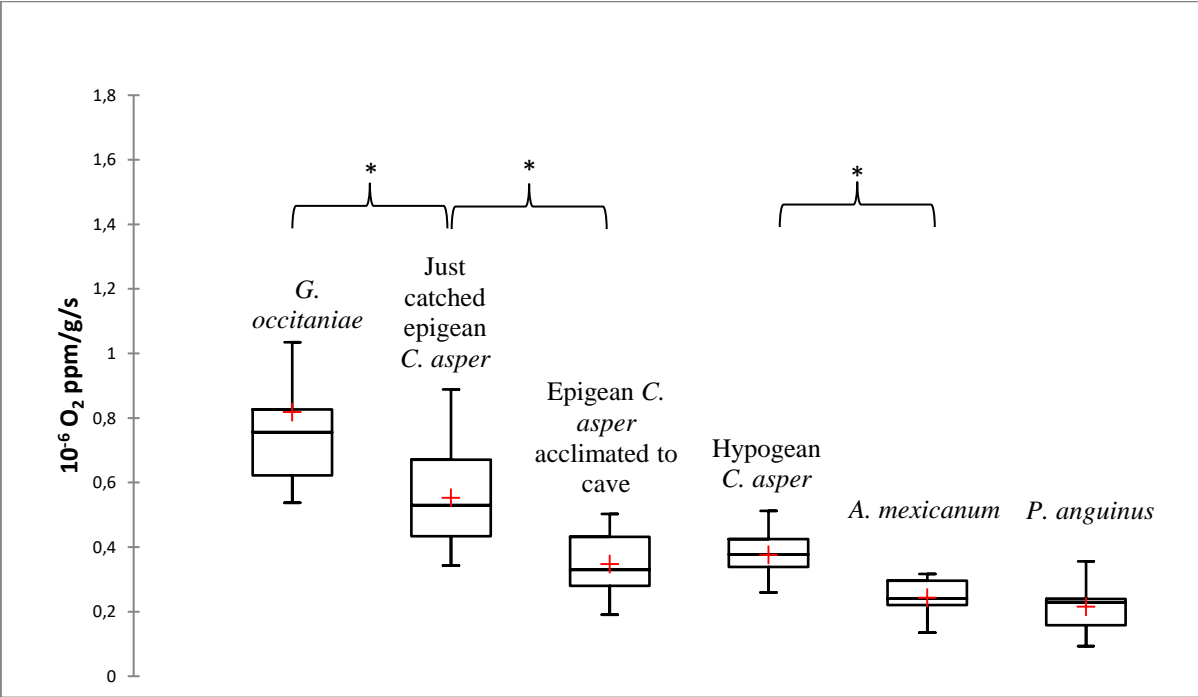


Fig. 1. Comparison of the oxygen consumption level in ppm per gram of body mass per second for *G. occitaniae*, epigean *C. asper* just caught from nature, epigean *C. asper* acclimated 8 years to the cave, hypogean *C. asper* maintained 8 years in the cave, *A. Mexicanum*, and *P. anguinus*. * indicates that the difference is significative. N=20; the p-values are given in table 3.

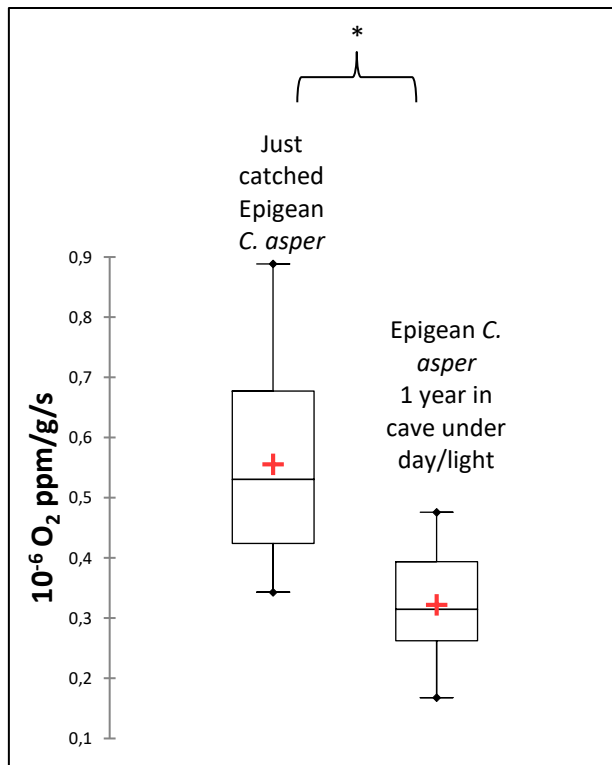


Fig. 2. Comparison of the oxygen consumption level in ppm per gram of body mass per second for epigean *C. asper* first just caught from nature and then after 1 year in the cave under a day/nigth exposure. * indicates that the difference is significative. (N=20; Wilcoxon Test p-value < 0,0001)

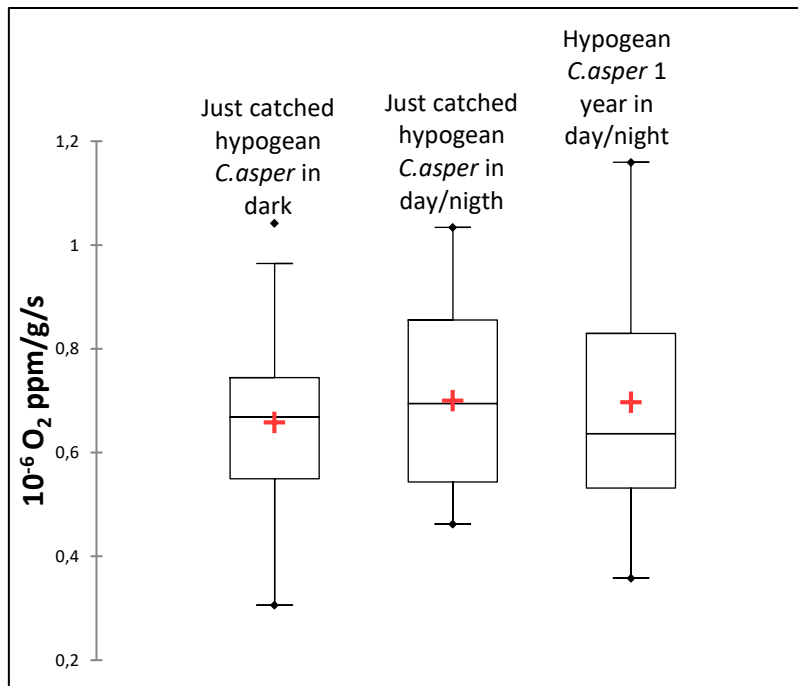
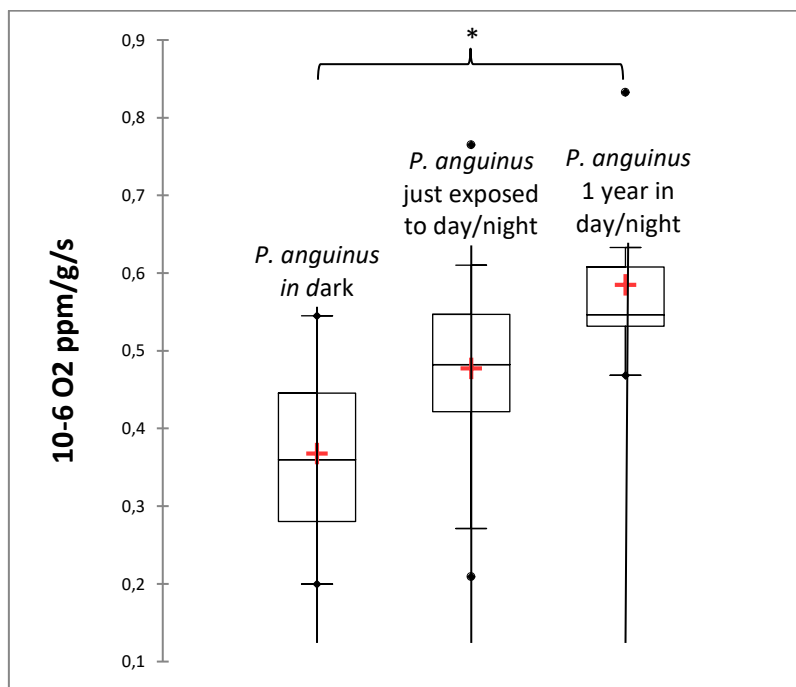


Fig. 3. Comparison of the oxygen consumption level in ppm per gram of body mass per second for hypogean *C. asper* first in darkness, then under a day/nigh alternance. The differences are not significant (N=20; Friedman Test p-value 0.584)

478



479

480

481 Fig. 4. Comparison of the oxygen consumption level in ppm per gram of body mass per second for
 482 *P. anguinus* first in darkness, then under a day/nigth alternance. * indicates that the difference is
 483 significative (N=8; Friedman Test p-value 0.018).