Surface newt *Calotriton asper* acclimation to cave conditions improved their foraging ability in darkness

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Caves in temperate regions are characterized by food scarcity compared to surface habitats. Therefore, hypotheses on adaptation to cave life suggest that many characteristics, such as resistance to starvation, reduced energy demand, and increased food-finding ability, have evolved among cave dwellers to cope with food frugality. To test the hypothesis involving increased food-finding ability, the prey detection performances of three surface and three subterranean populations of the Pyrenean newt, *Calotriton asper*, were compared. First, the rapidity of surface individuals in capturing prey at short distances inside a restricted aquarium under dark and light conditions and their score for capturing prey in a larger aquarium under similar conditions were measured. Simultaneously the scores obtained from individuals in cave populations in darkness were compared. Individuals from one of the surface population were maintained in darkness for approximately five years and retested. The surface *C. asper* individuals captured prey faster at short distances under light conditions than in total darkness; however, the differences were not significant. The scores of the surface *C. asper* individuals in the large aquarium were significantly better in darkness than in light conditions. In addition, the scores of cave populations at short distances in darkness were better than those of epigean populations in light conditions; however, the differences were not significant. Finally, the scores of surface individuals maintained in darkness for five years improved. To explain these results, it should be noted that surface *C. asper* populations forage at night using non-visual cues (e.g., chemical and mechanical cues), further suggesting that vision may somewhat inhibit other senses. This ability to forage at night may be favorable for cave colonization. Moreover, some hypogean populations, when the use of prey-detecting non-visual senses has developed, may start to exhibit adaptivity.

KEYWORDS

newts, food-feeding, detection performance, cave, darkness

Introduction

In temperate regions, caves are characterized by food scarcity, among other features, compared to surface habitats (Juberthie and Decu, 1994; Hüppop, 2000; Culver and Pipan, 2009; Romero, 2009). To cope with frugality, theories on adaptation to cave life suggest that many characteristics have evolved in cave dwellers, such as increased food-finding ability, resistance to starvation, and reduced energy demand (Hervant and Renault, 2002). The Pyrenean newt, *Calotriton asper*, inhabits surface brooks and cave rivers. Previous studies have correlated a fasting adaptation (Issartel et al., 2010), a lower metabolic level in cave populations, and a decreased surface population with their acclimation to cave conditions for years (Guillaume et al., 2020). However, few studies on their food-finding ability have been conclusive. *Calotriton asper* can develop efficient foraging activity in darkness; however, this has only been observed in captivity and not in nature (Uiblein et al., 1992), although the species may exhibit nocturnal activity, as established by some observations reported in the gray literature (for instance Nicol, 1990; Dalibard, 2021). Previous preliminary observations made on some individuals of unknown origin (Uiblein et al., 1992) showed that *C. asper* could detect prey using only chemical and/or mechanical cues but concluded that vision dominates the foraging behavior of *C. asper* at short distances with improved performance in light conditions than in darkness. This study investigated the possible variation in the food-feeding ability between surface and cave populations and its possible improvement when surface *C. asper* populations are maintained in cave conditions for years.

Materials and methods

Animal origin and maintenance

Adult *Calotriton asper* individuals were captured from three surface and three hypogean populations (the locations of these populations are indicated in **Table 1**). They were placed in the "Station d'Ecologie Théorique et Expérimentale, SETE" (Ariège, France) cave in aquaria filled with water from the cave river (10.13 ± 0.19 ppm O_2, $11.5 \pm 0.25°C$), and marked using Nanotec transponders. Individuals from surface populations were exposed to alternating 12 h day/night photic cycles, whereas those from cave populations were maintained in total darkness. They were fed live blood worms (prey usually found in the diet of *C. asper*) *ad libitum* once a week (Clergue-Gazeau, 1969; Montori, 1991). The first series of tests was performed up to 3 weeks after capture with twenty individuals for each population. The twenty individuals from surface population 1 were maintained in total darkness for five years and retested. These were identified by their transponder so their scores at the

first series and the second one five years later could have been individually compared.

Experimental setup

Individuals were subjected to a one-week fast before testing. They were isolated in a small $30 \times 15 \times 20$ cm aquarium filled with 4.5 L water. Following a 2 h acclimatization period, one live blood worm was introduced to the center of the aquarium, and observations were done remotely with a camera connected to a recorder. The time to worm capture was recorded; failure to capture the prey after 2 h was also recorded. Then, the newts were fed *ad libitum* and were replaced with others. Twenty individuals from each population were tested successively. Tests were repeated after with the same individuals identified by their transponders inside a larger $80 \times 30 \times 20$ cm aquarium filled with 22.5 L water. Finally, each individuals from surface populations was tested successively (after a one-week fast between each test) four time (one in a small aquarium in light, one in a small aquarium in darkness, one in a larger aquarium in light, one in a larger aquarium in darkness) except for individuals from the surface population & who were tested again two more time (one in a small aquarium in dark five years later, one in a larger aquarium in dark five years later), and each individuals from cave populations, was tested two times (one in a small aquarium in dark, one in a larger aquarium in dark).For individuals from surface populations, tests under the light condition (spectrum daylight fluorescent lamps True-light ®Natural Daylight 5500 K/Color Rendering 1 A, Ra > 90) were conducted during the day, and those in darkness were conducted at night. Observations in darkness were made using infrared light [420TVL, 0.5 lux/f2.0, 0, Lux (15m IR ON), 24 LED's IR].

Statistical analysis

Twenty individuals from each population were examined. The variations in time required for prey capture under

TABLE 1 Name and location of the three surface and three cave *C. asper* populations where individuals used in the study were obtained.

	Name of the stream or cave	Coordinates	
		Longitude	Latitude
Surface population 1	Cailla	42.805°	2.190°
Surface population 2	Olhadoko	42.990°	−0.947°
Surface population 3	Ribaui	42.790°	1.342°
Cave population 1	Betharram	43.100°	−0.198°
Cave population 2	Labouiche	43.012°	1.342°
Cave population 3	Bernard	42.999°	1.534°

various conditions (aquarium size, in darkness or light conditions) were not normally distributed, and considering the limited sample size, data were thus analyzed using non-parametric tests. The scores of the same identified animals tested under various conditions were compared using the Wilcoxon unilateral test (XLstat software). Differences between populations were analyzed using the Friedman test (XLstat software). $P < 0.05$ were considered statistically significant.

Animal ethics

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

Field study permissions

All the required French permits (permit no. 2017-04, 2007-11-1342, and 2016-s-01) for the capture, marking, transport, detention, use, and release of protected amphibian species, and animal experimentation were obtained. The project was approved by the National Council for Nature Protection on March 19, 2007, and the Regional Scientific Council for the Natural Heritage of the Region Languedoc-Roussillon-Midi-Pyrénées on April 5, 2016.

Results

For the three surface populations, the time to prey capture in the small aquarium was shorter under light conditions than in darkness, although the differences were not significant (Figure 1). In contrast, the time to prey capture in the larger aquarium was significantly longer under light conditions than in darkness for the three surface populations (population 1, $P = 0.32$; population 2, $P = 0.021$; population 3, $P = 0.022$).
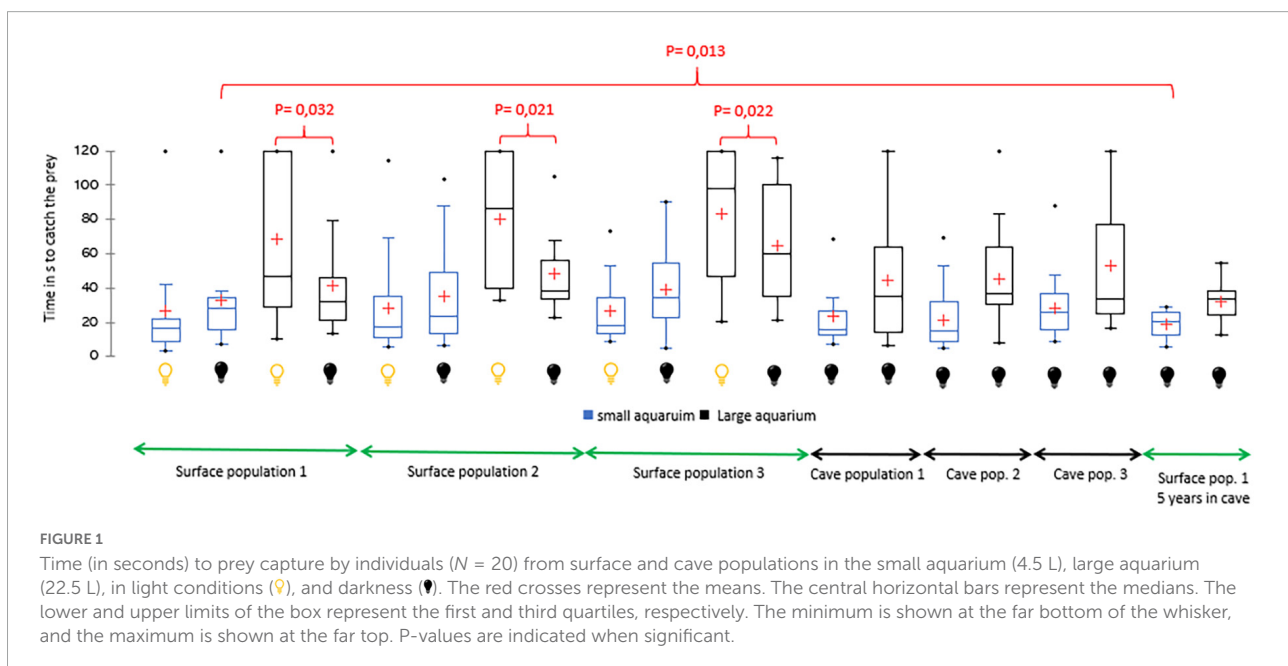
The scores of cave populations in the small aquarium in darkness were better than those of the epigean populations in light and darkness; however, in all cases, the differences were not significant.

The scores of the three surface populations in the large aquarium in darkness did not differ from those of the cave populations.

The scores of surface population 1 subjected to darkness for five years improved compared to the previous tests: (i) a significant difference was observed in the small aquarium in darkness ($P = 0.013$), (ii) an insignificant difference was observed in the small aquarium in light conditions, and (iii) an insignificant difference was observed in the large aquarium in darkness.

Discussion

The study findings herein confirmed that surface *C. asper* individuals could develop efficient foraging activity in darkness at night; however, they exhibited an improved speed for prey capture in light at a short distance than in darkness.

FIGURE 1
Time (in seconds) to prey capture by individuals ($N = 20$) from surface and cave populations in the small aquarium (4.5 L), large aquarium (22.5 L), in light conditions (💡), and darkness (🔦). The red crosses represent the means. The central horizontal bars represent the medians. The lower and upper limits of the box represent the first and third quartiles, respectively. The minimum is shown at the far bottom of the whisker, and the maximum is shown at the far top. P-values are indicated when significant.

Nevertheless, these values were not higher than those of individuals from cave populations in darkness. Moreover, surface *C. asper* individuals captured prey more rapidly in total darkness at a long distance. In another study with individuals from epigean populations, vision was highly dominant, considerably inhibiting extra-optical prey perception (Uiblein et al., 1995). These results prompted a question: given that multimodal sensory information for foraging is widespread throughout the animal kingdom, can vision blur other senses in *C. asper* and decrease efficiency when combined? Relatively little evidence of such processes exists. For instance, in the pit viper, visual and infrared information is effective in prey targeting; however, interference between the two modalities can occur, causing a reduction in performance (Chen et al., 2012). Moreover, Uiblein et al. (1995) hypothesized that the change in the foraging style of surface *C. asper* was related to the prey encountered in darkness. Similar behavior has been observed in fire salamanders, where larvae from cave-breeding populations have a better predation performance in complete darkness than in light conditions (Manenti and Ficetola, 2013); they exhibit a more active search strategy in cave conditions, giving up the standard sit-and-wait strategy of epigeans, which is less efficient in caves due to prey scarcity and difficulty in detecting preys (Manenti et al., 2013). In surface *C. asper* populations, the shift toward an efficient strategy to forage in the dark may be favored by a predisposition to night foraging. The present study was conducted only with adults, and similar experiments on youngers also could have been interesting to really assess the potential of the species to survive in caves, however, in the majority of the populations, larvae and juveniles are rarely observed, especially among caves populations, and the spontaneous reproduction of this species in captivity is still hard to obtain (Clergue-Gazeau, 1971, 1976; Montori, 1988; Guillaume, 2000). Another important finding in this study was that surface *C. asper* individuals had an improved ability to capture in the dark when maintained in cave conditions for years. The mechanism by which this is achieved is unclear; however, some species, such as zebrafish (*Danio rerio*), have been found to learn to forage in the dark (Carrillo and McHenry, 2016). Foraging plasticity is considered a facilitating trait for colonization and adaptation to new habitats (Crispo, 2008), especially when experiencing challenging pressures, such as in caves (Manenti et al., 2013). Furthermore, plasticity might be a dominant process in the first steps of colonization because it contributes to the persistence of settlers, allowing for rapid adaptive responses, which can, in a subsequent phase, accompany local adaptations (Crispo, 2007). A strong genotypic differentiation exists between the surface and cave *C. asper* populations, suggesting a lack of gene flow between them for a long time and the existence of local adaptive phenotypic divergence for multiple traits (Milá et al., 2010; Valbuena-Ureña et al., 2018; Lucati et al., 2020). However, the contribution of plasticity in the processes of genotypic diversification should

be clarified. In particular, the question of whether adaptive evolution proceeds through the genetic fixation of plastic phenotypes must be addressed. Finally, this study supports the hypothesis that surface *C. asper* individuals can colonize caves through plastic traits and that surface and cave populations may have fragmented and diverged during the postglacial climatic warming where conditions in low valleys were unfavorable, except in caves (Guillaume, 2001).

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

This animal study was reviewed and approved by Ministére de L'Enseignement Supérieur et de la Recherche N°068.

Author contributions

OG conceived and designed the experiments, collected the urodelan species, performed the experiments, analyzed the data, prepared figure and table, authored, and reviewed drafts of the manuscript.

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Conflict of interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer RM declared a past collaboration with the author OG.

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