The first ecological study on the oldest allochthonous population of European cave salamanders (*Hydromantes* sp.)

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Abstract. The introduction of allochthonous species often represents a serious problem for ecosystems and native species. Usually, these cases involve common widespread species that show high adaptability and strong competitiveness against local species. Within amphibians, the introduction of allochthonous species mainly deals with anurans, while cases involving caudata are few and poorly studied. We report the first assessment of an introduced population of European plethodontid salamanders. This population is located in the French Pyrenees and represents the oldest allochthonous *Hydromantes* population. We reconstructed the history of its introduction and collected data on the ecology and feeding habits of this population. Our results show that this population is stable and reproductive, showing strong similarities to Italian mainland species of *Hydromantes*. This study provided the base for further studies focused on this allochthonous population of European cave salamanders.

The introduction of allochthonous species is often detrimental to the environment (Nori et al., 2011; Gürtler et al., 2017). This is particularly true for invasive species, which are leading causes of biodiversity loss worldwide (Doherty et al., 2016; Ward-Fear et al., 2016). Introductions may be deliberated (i.e., to create food stock, to introduce control agents for pests, to perform research), but they can also occur naively or accidentally (Sket, 1997; Franch et al., 2007; Dyer et al., 2017). Regarding amphibians, in a few cases species were introduced as food resources, while the pet trade is considered the main source of allochthonous species (Kraus, 2009). Among the most invasive amphibian species there are *Lithobates catesbeianus* and *Xenopus laevis*. The well-known literature relating to such introduced species report that they represent one of the major causes of biodiversity loss, directly affecting both native species and biotopes (Lillo et al., 2005; Ficetola et al., 2007; Ficetola et al., 2010). *L. catesbeianus* is often infected by the fungus *Batrachochytrium dendrobatidis*, so it may also represent one of the major vectors promoting the spread of chytridiomycosis (Garner et al., 2006). Furthermore, as in the case of the green frogs of the genus *Pelophylax*, introduced species can breed with native species, promoting the spread of hybrids and thus representing a serious threat to biodiversity (Holsbeek et al., 2008; Maletzky et al., 2008; Meilink et al., 2015).

Along with these numerous and well-known introductions of frog species, there are a few understudied cases related to the introduction of salamander species in Europe as in the case of information relating to the introduction of European plethodontid salamanders (genus *Hydromantes*; see Wake, 2013). European plethodontids are terrestrial salamanders which lack lungs and an aquatic stage (Lanza et al., 2006a). The distribution of these species is almost confined

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to Italy; only one has a small range in a portion of the French Provence (Lanza et al., 2006a). Three of eight species (*H. strinatii*, *H. ambrosii* and *H. italicus*) are distributed in mainland Italy, while the other five (*H. flavus*, *H. imperialis*, *H. supramontis*, *H. sarrabusensis* and *H. genei*) exclusively inhabit Sardinia island (Lanza et al., 2006b; Chiari et al., 2012). The biogeographic factors that determined such a distribution are unclear and still debated (Macey, 2005; Lanza et al., 2006a; Carranza et al., 2008; Wake, 2013). Despite their vernacular name, European cave salamanders are mainly epigeous species which exploit several types of environments, such as forests, bare rocks and even dry stone-walls (Lanza et al., 2006a; Manenti, 2014; Costa, Crovetto and Salvidio, 2016). However, because of their specific physiology (Spotila, 1972), when external conditions become too harsh, they move underground looking for a suitable microclimate (Ficetola, Pennati and Manenti, 2012; Lunghi, Manenti and Ficetola, 2014a). In such environments, these salamanders tend to occupy areas in which adequate microclimatic conditions are realized (i.e. relatively cold temperature and high moisture) and where prey are likely to be more abundant (Salvidio et al., 1994; Ficetola, Pennati and Manenti, 2013; Lunghi, Manenti and Ficetola, 2014a; Lunghi, Manenti and Ficetola, 2017). Recent studies show that the microhabitat features at which *Hydromantes* salamanders are recorded during summer periods, approximate well the body conditions and the tolerance to the climatic limiting factors (Lunghi et al., 2016). Indeed, the high sensitivity of these salamanders forces them to select different parts of the subterranean environment according to seasonality (Lunghi, Manenti and Ficetola, 2015), as they quickly reach thermal equilibrium with their surrounding environment (Lunghi et al., 2016). *Hydromantes* carefully chose underground sites also because such environments represent safe places in which they can breed and escape most of their natural predators (Lanza et al., 2006a; Lunghi et al., 2014b; Salvidio et al., 2017).

Hydromantes species have been introduced six times beyond their natural range. An experiment of artificial ex situ syntopy between *H. italicus* and *H. ambrosii* was done in 1983 in an Italian cave located outside the range of both mentioned species (Forti et al., 2005; Lanza et al., 2006a). The species successfully persisted in the new location, giving birth to viable and fertile hybrids (Forti et al., 2005; Cimmaruta et al., 2013). There is no data on their spreading to surrounding sites available. A transplantation experiment of *H. ambrosii* individuals in a cave within the range of *H. strinatii* was performed during the summer of 1991 (Cimmaruta et al., 1999). The experiment established an allochthonous population of *H. ambrosii* in syntopy with *H. strinatii* (Cimmaruta et al., 1999). Another case deals with *H. italicus* in a population recently established in an artificial site in central Germany (Veith M., pers. comm.). Two cases deal with *H. strinatii*: a population was established in the Italian Karst (North-East Italy) and still persists (Lanza et al., 2006a) and another population was recently discovered in a natural cave of west-central France, 500 km far from the natural range of the species (Lucente et al., 2016). The last case represents the most ancient and interesting case of the *Hydromantes* salamander translocations. In 1970, at least 20 individuals belonging to a rearing established in the Subterranean Laboratory of the Scientific Station of Moulis (Fr) were released in a mine in the French Pyrenees (Pascal et al., 2003). Progenitors were caught from about fifteen localities in France and Italy (from Maritime Alps to Tuscan Apennines). At the time of collection, those *Hydromantes* were all considered to belong to the same species (*H. italicus*); however, individuals were probably sampled from all three of the mainland species (*H. strinatii*, *H. ambrosii* and *H. italicus*) (Guillaume and Durand, 2003).

Ecological studies on these allochthonous populations may produce new data reporting on how species adapt to a novel area. In this study,

we report the first status assessment of the oldest introduced population of *Hydromantes* salamanders, with emphasis on their ecology and feeding habits.

The allochthonous population of *Hydromantes* inhabits a mine situated in Parc naturel régional des Pyrénées Ariégeoises, France. The mine has a total development of 39 m. The inner environment shows a general uniform morphology, having an average width (± SD) of 1.57 ± 0.04 m and average height of 2.04 ± 0.04 m. In the first 18 m of the mine, seepage of water created a pool which held several larvae of the fire salamander (*Salamandra salamandra*) and had the following physical characteristics: pH = 8, hardness = 15°dH, $NO_2 = 0$ and $NO_3 = 10$. Nearly no information is present in the literature about this population; the only available information being contained in the breeding books of the Subterranean Laboratory of the Scientific Station of Moulis. From 1966 to 1970 several individuals of the *Hydromantes* species were kept in captivity inside the laboratory to perform studies on their breeding behavior. In 1967 at least 90 adults were reared in the laboratory (Durand, 1967; Durand, 1970; Lanza et al., 2006a). It is not possible to quantify how many individuals died or were fixed in alcohol between 1966 and 1970 (books of subterranean laboratory report with security the fixing of 34 adults and 40 eggs). In 1970 the CNRS aborted the study program on *Hydromantes*, so that year likely represents when individuals were released inside the mine. The only available information reports the release of about 20 individuals (Guillaume and Durand, 2003); however, considering that in 1970 no further individuals were fixed in alcohol, we do not know if all the other remaining *Hydromantes* from the laboratory were also released in the mine.

The inner environment of the mine was divided into portions of 3 m-length (hereafter sector), such subdivision allows a reliable data collection of microclimatic features (Lunghi, Manenti and Ficetola, 2015) and roughly represents the known home range of mainland *Hydromantes* species (Salvidio et al., 1994). During the first half of August 2016 we visited the mine twice, in late morning, and in each sector we recorded data on temperature and humidity using a TDP92 thermo-hygrometer (accuracy: 0.1°C and 0.1%) adopting precautions to avoid influence on cave microclimate (Lopes Ferreira et al., 2015), while the average incident light of sectors was estimated using a PCE 170 light meter (minimum recordable light: 0.01 lux). In each sector we also recorded the abundance of *Meta menardi* spiders, because this species is considered to be a good proxy of prey availability (Manenti, Lunghi and Ficetola, 2015).

In each sector two surveyors dedicated 3.5 min each to assess the presence of *Hydromantes* by Visual Encounter Survey (Crump and Scott, 1994), an efficient method used to detect caudata species (Flint and Harris, 2005). For each individual, we recorded the position (distance from the mine entrance) and we took biometrics (SVL and weight). Individuals were divided into three groups: we considered juveniles all *Hydromantes* with SVL ⩽ 40 mm, while within adults we separated males and females basing on the

presence of secondary sexual characters of males (Lanza et al., 2006a).

During the first survey, we randomly selected 26 individuals (3 juveniles, 11 males and 12 females; 65% of observed population, see Results) to perform stomach flushing to check for prey items stored in their stomachs (Salvidio, 1992). Stomach flushing was performed using a 5-ml syringe connected to a soft-plastic pipe with a 0.1 mm diameter. The plastic pipe was inserted into the mouth and 5 ml of water was gently injected into their stomach. Reflux was collected in a graduated pipette using a small funnel (Salvidio et al., 2012). Contents of the pipettes were examined with an optic microscope and all recognizable prey items were classified at least until the taxonomic level of order. For each individual, we counted the number of recognizable items with the following equation:

$$\sum_{i=1}^{n} i = a + b + c$$

where: *a*) is the number of integer items; *b*) is the number of lone heads; *c*) is the difference between lone abdomens and lone heads (only when abdomens > heads) and *i* represents the number of taxonomic order.

European cave salamanders have a high detectability during their underground phase (Lunghi, Manenti and Ficetola, 2015); thus, giving that we observed most of the individuals present in the mine in that time, we used the program Past to identify age classes within adult *Hydromantes*.

We used a binomial Generalized Linear Mixed Model (GLMM) to identify if occurrence of cave salamanders was related to both environmental and biological features. As the dependent variable we used the salamanders' presence observed in each sector. We used temperature, humidity and minimum light as environmental variables, while abundance of *Meta* spiders as a biological variable and sector identity as a random factor. All possible models were built and ranked following AICc while nested models and models with AICc higher than the simplest were not considered (Richards, Whittingham and Stephens, 2011).

Finally, we used Linear Mixed Models (LMM) to identify if age class or sex influenced the distribution of cave salamanders inside the mine. We used the respective abundance of sexes and age classes as dependent variables, while distance from mine entrance was used as an independent variable.

GLMM and LMM were performed in the R environment using packages lme4, nlme, MuMIn and MASS (Venables and Ripley, 2002; Bates et al., 2015; Bartoń, 2016; Pinheiro et al., 2016; R Core Team, 2016).

Hydromantes presence was positively related to sector temperature (table 1), with salamanders tending to occupy areas in which temperatures were warmer ($B = 606.5$, $\chi^2 = 12.84$, $P < 0.001$). Occupied sectors showed a temperature range of 12.2–12.9°C, while humidity fluctuated between 93.5 and 95.5% and minimum light ranged from 0 to 8.47 lux. Within

Table 1. The top five AICc models related to *Hydromantes* distribution. Presence of species was considered a dependent variable, while humidity, illuminance (Lux), temperature, and *Meta* abundance were considered independent variables.

Independent variables included into the model				df	AICc	Δ-AICc	Weight
Humidity	Lux	*Meta*	Temp				
			712.3	3	9.4	0	0.802
−1285			579.4	4	14.7	5.24	0.058
	−298		724.1	4	14.7	5.24	0.058
		−421.20	716.8	4	14.7	5.24	0.058
				2	18.3	8.91	0.009

Table 2. Biometrics of *Hydromantes*. For each group (adult males, adult females and juveniles) the average weight (± SD), maximum and minimum SVL and average SVL (± SD) of each identified age class are shown.

	Weight ± SD (g)	SVL min (mm)	SVL max (mm)	Age class1 (proportion)	Average ± SD (mm)	Age class2 (proportion)	Average ± SD (mm)
Males	2.91 ± 0.44	50	65	0.57	52.62 ± 1.65	0.43	62.66 ± 2.06
Females	4.14 ± 0.95	45	75	1	66.59 ± 5.52	−	−
Juveniles	1.17 ± 0.32	30	45	1	36.2 ± 6.43	−	−

our surveys the maximum number of observed *Hydromantes* was 40 (9 juveniles, 14 males and 17 females). Based on SVL size, we identified two age classes for adult males and one for adult females and juveniles (table 2). Juveniles significantly occupied sectors closest to the mine entrance ($F_{1,37} = 21.06$, $P < 0.001$), while within adults, sex did not have any influence on their distribution ($F_{1,29} = 2.64$, $P = 0.161$). Considering the 26 individuals which underwent stomach flushing, none had an empty stomach and only 6 of them (3 males and 3 females) regurgitated prey items which were not possible to identify due to their advanced state of digestion. Within the other sampled *Hydromantes* (77%) we identified 82 prey items belonging to 6 taxonomic orders: Diptera (92%), Araneae (3%), Coleoptera (2%), Isopoda (1%), Hemiptera (1%) and Trichoptera (1%).

The Pyrenean *Hydromantes* population showed a preference for relative warmer temperature during the study period (table 1). The features of the occupied sectors matched those observed in Italian mainland species during the same period, where salamanders occupied sectors close to the cave entrance, but deep enough to show specific microclimate (Lunghi, Manenti and Ficetola, 2015). Even spatial distribution of

individuals followed what was already observed in Italian species, with juveniles occupying sectors close to the main entrance, which are areas where prey is likely to be more abundant (Ficetola, Pennati and Manenti, 2013). The studied mine has a gate at the entrance which limits environmental influences from external surrounding areas, promoting suitable conditions for *Hydromantes* within the first few meters.

Based on our observations the current population is comprised of approximately 22% juveniles and 78% adults with a similar proportion of both sexes (Salvidio, 2008). Considering all adults, the difference in dimensions of the two sexes falls within the size gap which normally occurs in *Hydromantes* mainland species (Salvidio and Bruce, 2006). However, looking at the two different groups of males, we could clearly see that the size of the second group was definitively bigger (around 20%), so they probably represent an older group/generation. Release of *Hydromantes* in the French Pyrenees happened about 50 years ago (Guillaume and Durand, 2003) thus, considering the available information on *Hydromantes* life span, this population has likely reached at least the fourth/fifth generation (Lanza et al., 2006a). Evaluating all information obtained by this study, the Pyrenean

Hydromantes population seems to be dynamic and reproductive.

Our study represents the premier data on the diet of an allochthonous population of *Hydromantes*. These salamanders show a generalist and opportunistic diet, hunting on any available prey nearby using their protrusible tongue (Deban and Richardson, 2011). Their diet includes several invertebrate orders, making them able to feed on a large number of different prey items (Salvidio, 1992; Vignoli, Caldera and Bologna, 2006; Salvidio et al., 2012). Observed stomach contents of this allochthonous population of *Hydromantes* allows us to hypothesize that its feeding behavior basically remained the same of that of the mainland Italian species (Salvidio, 1992; Vignoli, Caldera and Bologna, 2006). In fact, more than 90% of their summer diet was composed by dipterans (mostly *Limonia nubeculosa* adults), a taxon which often shows high abundances in subterranean environments during hot seasons (Salvidio et al., 1994; Manenti, Lunghi and Ficetola, 2015). The only Hemiptera found within prey items belong to the family Veliidae. Riffle bugs are generally found in bodies of water or on emergent vegetation; however, is it possible to find some individuals on plants away from water (Epler, 2006). Therefore, it is possible that *Hydromantes* preyed on this bug directly from the body of water present in the first few meters of the mine.

This study provides new insights on a poorly studied phenomenon, the introduction of a European salamander in areas outside of their native range. Currently, most of the existing reports on such phenomenon deal with the olm (*Proteus anguinus*), one of the most intriguing species which has been relocated on different occasions, mainly for scientific purposes, in some Italian, French and German localities; however, the studies of its impact on the native cave communities remain partial and confined in the grey literature (Dolce and Pichl, 1982). Our assessment of the allochthonous population of *Hydromantes* represents a first step to understand if these salamanders may be detrimental for native communities or not. The data obtained from stomach content analysis revealed the capability to capture a wide range of native invertebrates that enter the mine during the summer and the predation pressure of this allochthonous salamander population may affect the dynamics of the autochthonous invertebrate community. The results clearly show that the studied population can persist at the site of release with abundances similar to those observed, for example, in *H. italicus* in its natural range (Lunghi, Manenti and Ficetola, 2014a; Lunghi, Manenti and Ficetola, 2015). Although such population is often considered to be composed only by *H. strinatii* individuals (Raffaëlli, 2007; Lucente et al., 2016), our study emphasizes the fact that founders of the Pyrenean *Hydromantes* population potentially belonged to all three mainland species, and therefore may be genetically unique. This study provided the first ecological data on this allochthonous *Hydromantes* population, representing the foundation for further studies which will deeply investigate the ecology, the genetic structure and the potential impact of these animals on local species.

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