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# Nesting of cave salamanders (*Hydromantes flavus* and *H. italicus*) in natural environments

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**Abstract.** The reproductive biology of European salamanders of the genus *Hydromantes* (subgenus *Speleomantes*) is poorly known. The lack of information is related to their cryptic behaviour and habitat, which make detection extremely difficult, especially during breeding-related activities. Most of the information on *Hydromantes* reproduction refers to observations in captivity and in semi-natural conditions, while observations in the wild are extremely limited. We report on the first observations of brooding and maternal care in *Hydromantes italicus* and *H. flavus* in natural environments. In both species, brooding females and eggs were found in small fissures within the aphotic zone of caves where microclimates are stable. After hatching, the females of both species attended continuously to their newborns. Available information on breeding biology suggests high similarity amongst species, but more studies, under both natural and controlled conditions, are required to better understand the complex breeding behaviour of Hydromantes salamanders and evaluate interspecific variation.

Key words. Amphibia, Caudata, Plethodontidae, biospeleology, brooding behaviour, parental care, reproduction.

## Introduction

Salamanders belonging to the genus *Hydromantes* Gistel, 1848 require environments with distinctive features, such as very high moisture and relatively cool temperatures (Lanza et al. 2006, Ficetola et al. 2012). However, these animals live in Mediterranean regions, where these conditions can be found in epigean environments only during some periods of the year (usually from late autumn to early spring; Salvidio et al. 1994, Cimmaruta et al. 1999, Lanza et al. 2006, Vignoli et al. 2008). Therefore, the European species of *Hydromantes* (subgenera *Speleomantes* and *Atylodes*; see Vieites et al. 2011 for justification) spend a substantial part of their life in underground environments (both edaphic and hypogean), where they find suitable conditions also during dry periods (Lanza et al. 2006) and where breeding is supposed to occur as well.

Unfortunately, their underground habits severely limit the possibilities to gather knowledge of their behaviour and life history, for which reason the breeding biology of these salamanders is scarcely known. For instance, most of the information on their breeding behaviour stems from captive individuals, while there are almost no published observations of nesting in natural conditions (Lanza et al. 2006). The very limited information obtained from the wild mostly refers to *Hydromantes* (*Atylodes*) *genei*. The first data on *Hydromantes* egg deposition in nature were reported by Stefani & Serra (1966), who observed a female *H. genei* with ten eggs, found in a small crevice in a cave wall. Both the female and the eggs were preserved in formalin, precluding subsequent behavioural studies. More recently, Papinuto (2005) observed one female *H. genei* with eggs in a shot hole of an abandoned mine and followed the egg development until hatching. For the other species, information on reproduction and subsequent parental care essentially refers to individuals maintained in terraria or under semi-natural conditions (reviewed in Lanza et al. 2006; see also Oneto et al. 2010, 2013).

In this context, field observations are extremely valuable, because of possible differences between captive and wild be-

haviour (Ficetola & De Bernardi 2005). With no information on nesting being available for most of *Hydromantes* species, it is difficult to know whether the few available data are idiosyncratic of some species/situations. In this study, we report the first observations on brooding and subsequent mother-offspring relationships in two *Hydromantes* species (the Italian cave salamander *H. italicus* and the Monte Albo cave salamander *H. flavus*) under natural conditions.

## Materials and methods

### Study area

The study area for *Hydromantes italicus* is the "Canale della Radice" Valley in the Apuan Alps (Tuscany, between 44°0'48" N, 10°18'6" E and 43°59'0" N, 10°18'26" E). In this area, five cave-like structures (mostly abandoned mines) were investigated in early July 2013, and the presence of cave salamanders was assessed using visual encounter surveys (Crump & Scott 1994, Ficetola et al. 2012). For *H. flavus*, the study site was the Gana 'e Gortoe cave (Mount Albo, Sardinia, 40°34'42" N, 9°41'37" E) where visual encounter surveys of cave salamanders were performed during speleological exploration activities. To limit external influences, all observations were conducted without touching or otherwise disturbing the subject animals. After the first detection of eggs, sites were repeatedly revisited until newborns appeared. During the study stints of *H. italicus*, air temperature and humidity were recorded using a Lafayette TDP92 hygrometer, while maximum and minimum illuminance was measured with a EM882 multifunction Environmental Meter (PCE Instruments; minimum illuminance 0.01 lux) within 3 m from the breeding site. Furthermore, we recorded morphological features of the observation sites surrounding breeding spots (maximum width and height of the cave).

## Results

### *Hydromantes italicus*

We found a brooding female of *H. italicus* with her egg clutch in an abandoned mine on 5 July 2013. The breeding site was a small shot hole nearly cylindrical in shape (dia-


Figure 1. Female *Hydromantes italicus* with her eggs. A) 5 July 2013; B) 5 August; C) 19 August. The mother continues to stay in the nest with her offspring; D) 11 September. Photos A+C: E. Lunghi, B+D: © Francesco Bacci.

meter: 4 cm; depth: 13 cm), 22.4 m from the cave entrance and 1.15 m above the floor. In this section, the cave is a nearly straight gallery with maximum height of 1.8 m and width of 1.9 m. At least seven eggs were visible above the mother's body (Figs. 1a–b). The egg envelopes were transparent and fitted with a peduncle. According with our ethic policy, the embryos and female were not touched or disturbed. The photic zone of the cave extended from the entrance to 11.5 m into the interior, and the breeding site was in complete darkness (illuminance < 0.01 lux). The female with her eggs was repeatedly observed between 8 July and 19 August (Figs. 1a–c). During the first observation we noted that the embryos had abundant yolk available and were at an advanced developmental stage, as indicated by the eyes being clearly visible through the envelope (Fig. 1a). During subsequent observations the yolk appeared reduced and the development advanced, as was proved by the appearance of well-developed legs with elongated digits (Figs. 1b–c). On 11 September, we found eight newborns close to the female, still inside the hole; all the newborns were very similar in size (Fig. 1d). The mother remained perched atop her young while the newborns stayed between the mother's body and the bottom of the hole (Fig. 1d). Temperature and humidity remained extremely constant during the whole period (temperature: mean ± SD = 14.7°C ± 0.4; relative humidity: 92.7% ± 0.3).

### *Hydromantes flavus*

On 17 August 2011, we found a brooding female keeping an unknown number of eggs in a small cavity (width < 10 cm) located about 1.5 m above the floor in the zone named "Manù labyrinth" of the Gana 'e Gortoe cave on Mount Albo. This zone is 700 m from the currently open entrance to the cave, and almost 100 m from a currently close entrance named "Base Camp"; in this section, the maximum width of the cave was 3.5 m and the maximum height was approximately 5 m. The site is in the aphotic zone of the cave (illuminance = 0 lux). During the following two visits (28 August and 4 September), the mother was observed with four newborns (Fig. 2). The mother remained in the breeding spot also in this instance, with the hatchlings perching on her back or staying very close to her (Fig. 2b).

## Discussion

Our results represent the first observations in the wild of nesting, egg-brooding, and post-hatching parental care in the plethodontid salamanders *Hydromantes flavus* and *H. italcus*. For *H. flavus*, we are not aware of any information on nesting, while for *H. italicus* the few available pieces of information stem from captive individuals (cf. Lanza et al. 2006). In our observation, females of both *H. flavus* and *H. italicus* used small holes in vertical walls for laying their eggs, and the breeding spots were very similar to the ones previously observed in *H. genei* (Stefani & Serra 1966, Papinuto 2005). The eggs had been deposited in small fissures and cavities with just a small opening, where the females could easily protect them. The shape and structure of nesting holes are not the only features shared by the three species. All breeding sites were located in cave sectors characterized by cool temperature and high ambient moisture and high humidity levels. For instance, Papinuto (2005) found *H. genei* with eggs nearly at the end of a tunnel (13 m long) in a zone with constant temperatures (16–18°C). Similarly, we found *H. flavus* and *H. italicus* eggs relatively far from the entrance in totally dark areas with stable microclimatic features. The selection of nesting sites is extremely important, as the wrong choice can cause breeding failure. From these observations it is possible to

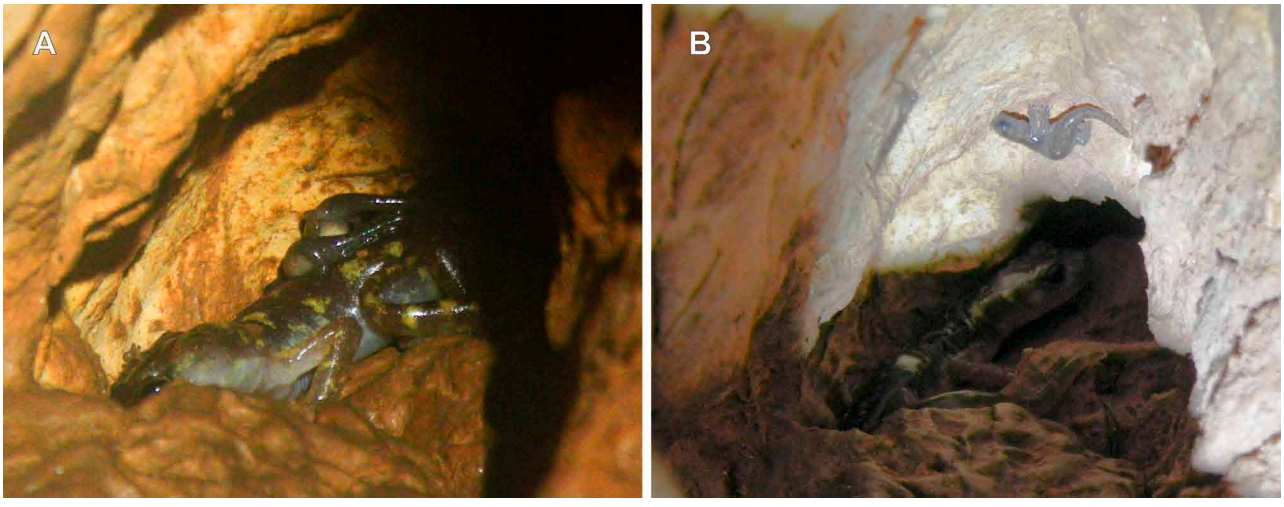


Figure 2. Female *Hydromantes flavus* with her hatchlings at the same site where the eggs were first found on 17 August 2011. A) Recently hatched individuals (28 August), with some in close contact with their mother; B) 4 September 2011. Photos: S. Manca.

hypothesize that *Hydromantes* females lay eggs in sites with specific geomorphological and suitable, constant microclimatic features throughout the year. Adult *Hydromantes* are generally found in relatively deep sectors of caves where humidity is high and temperature rather low (Salvidio et al. 1994, Ficetola et al. 2013): A very high level of humidity obviously prevents the desiccation of skin and eggs. Furthermore, microclimatic conditions are more stable in deeper sectors. Such stability may be extremely important, as egg development is slow and may require nearly one year (Lanza et al. 2006, Oneto et al. 2010). Within the deep areas of caves with a suitable microclimate, salamanders require small cavities in vertical walls, which are likely to reduce the risk of detection by potential predators. A small hole may also increase the female's chances of successfully protecting her eggs (e.g., against predators, cannibalism, or through skin secretions that might protect eggs from fungi or bacteria; see Lanza et al. 2006, Oneto et al. 2010). A small crevice may allow mothers to keep their offspring safe during the first days after hatching, protecting them against predators like the orb-weaver spider *Meta menardi*, or from intraspecific aggression (Lanza et al. 2006, Pastorelli & Laghi 2006, Oneto et al. 2013). While finding *Hydromantes* individuals in suitable caves is rather easy, nesting sites have been discovered only a few times, probably because of their specific geomorphological features that make detection extremely difficult.

*Hydromantes* are the only European amphibians in which the pachytene spermatocytes do not degenerate during the cold season; this peculiarity might allow them to mate throughout the year (Lanza et al. 2006). However, the available observations from the field or semi-natural conditions suggest that eggs hatch in late summer, both in the present study (before 11 September for *H. italicus*; before 28 August for *H. flavus*) and from previous ones (e.g., 18 August for *H. genei*, Papinuto 2005; 22 September in *H. strinatii*, Oneto et al. 2010). Late summer/early autumn may be a particularly suitable season for newborns, as there are favourable meteorological conditions (i.e., lower temperatures and high levels of ambient moisture from rainfall and humidity) that allow them to exit caves and temporarily expand their home ranges (Lanza et al. 2006). Both *H. italicus* and *H. flavus* newborns kept some residual yolk after hatching (Fig. 2b) and remained close to, or above, the mother in the nest (Figs. 1d, 2b). During this period, the female apparently continued to attend the nest, often maintaining physical contact with the hatchlings (Figs. 1d, 2). In *H. flavus*, during the first observation after hatching, several hatchlings were perched on the body of the female, without contact to the substrate (Fig. 2a). These behavioural expressions are in agreement with the published observations on *H. strinatii* (Oneto et al. 2010, 2013). In summary, our observations in wild conditions suggest similar nesting behaviours among *Hydromantes* species for multiple parameters, including the selection of nesting sites, hatching period, and the presence of parental care after hatching. Nevertheless, interspecific differences are likely to exist, as is shown by the observation of ovoviparity in at least one species (*H. sarrabusensis*, Lanza & Leo 2001), but remain poorly understood. These salamanders show extremely complex behaviours, including prolonged brood attendance and parental care after hatching, yet our knowledge of *Hydromantes* breeding biology remains extremely limited, and for most of the species is based on very few, unreplicated observations. More studies, both under natural and controlled conditions, are required to better understand the factors promoting such complex behaviours and evaluate the degree of interspecific variation.

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**References**

Cimmaruta, R., G. Forti, G. Nascetti & L. Bullini (1999): Spatial distribution and competition in two parapatric sibling species of European plethodontid salamanders. – Ethology Ecology & Evolution, **11**: 383–398.

Crump, M. L. & N. J. Scott (1994): Visual Encounter Surveys. – pp. 84–92 in: Heyer, W. R., M. A. Donnelly, R. W. McDiarmid, L. C. Hayek & M. S. Foster (eds): Measuring and monitoring biological diversity: standard methods for Amphibians. – Smithsonian Institution Press, Washington.

Ficetola, G. F. & F. De Bernardi (2005): Interspecific social interactions and breeding success of the frog *Rana latastei*: a field study. – Ethology, **111**: 764–774.

Ficetola, G. F., R. Pennati & R. Manenti (2012): Do cave salamanders occur randomly in cavities? An analysis with *Hydromantes strinatii*. – Amphibia-Reptilia, **33**: 251–259.

Ficetola, G. F., R. Pennati & R. Manenti (2013): Spatial segregation among age classes in cave salamanders: habitat selection or social interactions? – Population Ecology, **55**: 217–226.

Lanza, B. & P. Leo (2001): Prima osservazione sicura di riproduzione vivipara nel genere *Speleomantes* (Amphibia: Caudata: Plethodontidae). – Pianura, **13**: 317–319.

Lanza, B., C. Pastorelli, P. Laghi & R. Cimmaruta (2006): A review of systematics, taxonomy, genetics, biogeography and natural history of the genus *Speleomantes* Dubois, 1984 (Amphibia Caudata Plethodontidae). – Atti del Museo Civico di Storia Naturale di Trieste (suppl.) **52**: 5–135.

Oneto, F., D. Ottonello, M. V. Pastorino & S. Salvidio (2010): Posthatching Parental Care in Salamanders Revealed by Infrared Video Surveillance. – Journal of Herpetology, **44**: 649–653.

Oneto, F., D. Ottonello, M. V. Pastorino & S. Salvidio (2013): Nuovi dati sul comportamento di cura parentale nei caudati: *Hydromantes* (*Speleomantes*) *strinatii* (Amphibia, Plethodontidae). – pp. 204–207 in: Scillitani, G., C. Liuz-

zi, L. Lorusso, F. Mastropasqua & P. Ventrella (eds): Atti IX Congresso Nazionale della Societas Herpetologica Italica, Bari-Conversano 26–30 settembre 2012. – Pineta Publ., Conversano (Bari).

Papinuto, S. (2005): Sul ritrovamento ed il monitoraggio di una nidiata di *Speleomantes genei* (Temminck & Schlegel, 1838) (Amphibia Urodela Plethodontidae) in una galleria mineraria dell'Inglesiente (Sardegna sud-occidentale). – Sardegna Speleologica, **22**: 3–6.

Pastorelli, C. & P. Laghi (2006): Predazione su *Speleomantes italicus* (Amphibia, Caudata, Plethodontidae) da parte di *Meta menardi* (Arachnida, Araneane, Metidae). – Riassunti 6° Congresso Nazionale della Societas Herpetologica Italica. Stilgrafica, Roma.

Salvidio, S., A. Lattes, M. Tavano, F. Melodia & M. V. Pastorino (1994): Ecology of a *Speleomantes ambrosii* population inhabiting an artificial tunnel. – Amphibia-Reptilia, **15**: 35–45.

Stefani, R. & G. Serra (1966): L'oviparità in *Hydromantes genei* (Temm. et Schl.). – Bollettino di Zoologia, **33**: 283–291.

Vieites, D. R., S. N. Roman, M. H. Wake & D. B. Wake (2011): A multigenic perspective on phylogenetic relationships in the largest family of salamanders, the Plethodontidae. – Molecular Phylogenetics and Evolution, **59**: 623–635.

Vignoli, L., F. Caldera & M. A. Bologna (2008): Spatial niche of the Italian cave salamander, *Speleomantes italicus* (Dunn, 1923) (Plethodontidae, Amphibia), in a subterranean system of Central Italy. – Italian Journal of Zoology, **75**: 59–65.