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Posthatching Parental Care in Salamanders Revealed by Infrared Video Surveillance

FABRIZIO ONETO, DARIO OTTONELLO, MAURO VALERIO PASTORINO, AND SEBASTIANO SALVIDIO^{1,2}

Gruppo Speleologico Ligure "Arturo Issel", Villa Comunale ex Borzino CP 21, I-16012 Busalla (GE), Italy

¹Dipartimento per lo Studio del Territorio e delle sue Risorse (DIPTERIS), Università di Genova, Corso Europa 26, I-16132 Genova, Italy

ABSTRACT.—Posthatching parental care is known in amphibians for frogs and caecilians but, thus far, has never been reported for salamanders. Here, we describe the parental behavior of a female Northwest Italian Cave Salamander, *Speleomantes strinatii*, from egg deposition to nest site abandonment. The female was kept in seminatural conditions and filmed in complete darkness by an infrared video camera. In November 2007, the female laid nine eggs in a small depression of the terrarium floor, displaced the clutch with hind limbs, and showed antipredator behaviors toward a conspecific female and an intruding Roof Rat (*Rattus rattus*). During egg brooding, the female remained in contact with the clutch for about 98% of the time. In September 2008, two young hatched and shared the nesting site for six weeks with the female, which attended the nesting site for 87% of the time. Hatchlings repeatedly climbed over the female's body, lying on her for hours. The female walked out of the nesting site with a young on its back twice. These prolonged skin contacts between parent and offspring should be considered as the first certain case of young attendance in salamanders. This behavior may be related to increased survival of hatchlings during their first weeks of life, when young are particularly vulnerable to predation, skin infection, and dehydration.

Amphibians exhibit a diverse array of pre- and posthatching parental behaviors that have independently evolved in different taxonomic groups (Lehtinen and Nussbaum, 2003). In amphibians, parental care can increase offspring survival by reducing predation, desiccation, infection, yolk sedimentation of the eggs, and, in aquatic egg-laying species, increasing the dispersion of embryos' metabolites in the surrounding medium (Nussbaum, 2003). In amphibians, posthatching parental behavior has been described in frogs (Anura) in which attending, brooding, feeding, transport, and defense of tadpoles and transport of froglets has been documented (Lehtinen and Nussbaum, 2003). Also, posthatching parental behavior has been described in caecilians (Gymnophiona) in which female parents may provide trophic resources to hatchlings although specialized skin tissues (Kupfer et al., 2006). Unambiguous evidence for posthatching parental behavior is still lacking in salamanders (Urodela), where documented parental behavior is limited to attendance and defense of eggs (Nussbaum, 2003; Wells, 2007). For example, in aquatic salamanders such as *Desmognathus fuscus*, *Desmognathus ochrophaeus*, and *Desmognathus ocoee*, females sometimes remain in the nest with hatched larvae for a few days before the larvae make their way to the water (Wells, 2007). In terrestrial salamanders such as *Plethodon cinereus* and *Aneides aeneus* hatchlings remain with the female up to four weeks (Mathis et al., 1995), and in *Ensatina*, newly hatched salamanders have been observed remaining in close proximity to the female for some time (D. Wake, pers. comm.). However, the interactions occurring between the female parent and offspring and, in particular, the female role during the posthatching period have never been described and analyzed in detail and, thus, remain enigmatic.

In this study, the parental behavior of *Speleomantes strinatii*, one of the seven species of plethodontid salamanders found in southern Europe (Lanza et al., 2005; Lanza, 2006), was investigated by means of infrared video from the moment of egg deposition to the abandonment of the nest site. Continuous recordings were obtained in complete darkness and in seminatural conditions. In this way, cryptic behaviors were observed with little disturbance of the studied animals.

MATERIALS AND METHODS

Study Species.—*Speleomantes strinatii*, the Northwest Italian Cave Salamander, is a completely terrestrial plethodontid (total length < 115 mm) endemic to southeast France and northwest Italy (Lanza, 2006). Nest sites and egg clutches of *S. strinatii* have not been observed in the wild. In captivity, females produced 6–14 relatively large eggs (Durand, 1970; Salvidio et al., 1994) that were guarded by the female for up to 10 months (Durand, 1970). During brooding, the female coils around its eggs and defends them against approaching conspecifics (Durand, 1970). Occasionally, attending females eat their own eggs, and Durand (1970) suggested that cannibalistic behavior was directed toward nondeveloping eggs.

Experimental Site.—This study was conducted inside the Biospeleological Station of San Bartolomeo located at 390 m above sea level near the village of Savignone, 25 km north of Genoa (Liguria, northwest Italy). The Biospeleological Station is a 34-m long underground horizontal tunnel excavated during World War II for use as an air raid shelter. Since 1987, the entrance was closed with an iron gate and the station dedicated to ecological research on the resident *S. strinatii* population (e.g., Salvidio et al., 1994; Salvidio and Pastorino, 2002). An experimental terrarium used to study the animals' behavior was located about 20 m from the entrance, where solar illumination is completely lacking (0 lux, measured by Deltha Ohm Luxmeter HD 8366, Padova, Italy), air temperature has an

²Corresponding Author. E-mail: salvidio@dipteris.unige.it

annual mean of 9.7°C (range 6.7–12.5°C), and the relative air humidity is constantly around 95% (Salvidio et al., 1994). To record the parental behavior of *S. strinatii*, two apparently gravid females, caught in a nearby cavity in June 2007, were kept inside the experimental terrarium that consisted of open Plexiglas (70 × 50 × 30 cm) with about 7 cm of plaster on the floor. To prevent the salamander from escaping, the upper borders were turned inside and down. Small depressions (13 × 10 × 4 cm) were molded into the plaster to provide nesting sites. Leaf litter and soil samples were regularly introduced into the terrarium to increase prey availability. Furthermore flying insects entered spontaneously through the open upper side of the terrarium.

Infrared Recording and Behavior Analysis.—A hard-wired waterproof Sony SuperHAD™ camera equipped with a Charge Coupled Device (CCD) optical sensor and with a built-in infrared 12-led illuminator (RE-BCC6L, Digital Surveillance Equipment, DSE, Torino, Italy) was suspended 1.2 m over the terrarium and connected to a computer. Video recording at 12.5 frames per second was conducted in complete darkness. Recording was continuous during the entire experiment, although some electrical black-outs occurred. Recorded sequences were saved on the computer as 60-min packages and were viewed at normal speed or in slow motion using commercial software developed by DSE (Torino, Italy). Continuous video sequences allowed for the interpretation and analysis of the animals' behavior, even if the quality of each video frame was relatively low. After egg deposition, the infrared camera was moved closer to the nesting site, and live flies caught inside the cave were introduced to the terrarium to increase feeding opportunities. During the experiment, the focal salamanders were never touched or manipulated. Video sequences were usually examined once per week, but at the end of the experiment all sequences were analyzed, and the duration (min) of each behavior was calculated by using the counter visible on individual frames. We defined a behavior as "partial skin contacts" when a part of the hatchling's body was touching the female while other parts were still in contact with the substrate (see Fig. 1E in which both hatchlings are in partial contact with the female). "Complete skin contacts" were those in which hatchlings climbed over the female's body without any physical contact with the substrate (see Fig. 1F). In this study, only complete skin contacts lasting more than 5 min were analyzed.

RESULTS

On 6 November 2007, one of the two focal females laid a clutch of nine eggs inside a small depression on the terrarium floor. During the first week after deposition, the female was constantly in direct contact with the clutch, often turning and displacing the eggs with her hind limbs. Ten days after egg laying, the brooding female began to leave the nesting site for relatively short periods (from 3–46 min). Five days after egg deposition, the other, nonbrooding female housed in the terrarium began to intrude into the nesting site, and on 16 November, the first physical contact between the two salamanders occurred. Subsequently, the intrusions of the nonbrooding

female increased in frequency, and the attending female was observed intercepting, biting, and repelling the conspecific from the clutch (Fig. 1A, B). During the brooding period, the female remained within the nest chamber or in contact with the eggs for about 98% of the time.

On 4 and 9 February 2008, a Roof Rat (*Rattus rattus*) intruded the terrarium by walking on the infrared camera wire and closely approached, possibly touching the salamander (Fig. 1C), which remained coiled around her eggs throughout these encounters (Fig. 1D). Because these intrusions occurred frequently, we released the nonbrooding female from the terrarium, covered it with an opaque lid to protect the brooding female, and attempted to capture the Roof Rat using live traps. When the lid was removed in the spring 2008, only two of the nine eggs remained. A few days before hatching, the female pushed the remaining eggs into a small cavity in the plaster floor and hid in it. On 22 September 2008, 45 weeks after egg deposition, the first hatchling emerged from the hole followed five days later by the second one. This asynchrony in hatching resulted in different body sizes of the animals, with the first young larger than the second, allowing visual recognition of the two hatchlings (e.g., Fig. 1E). The three focal animals shared the nesting site for six weeks. During the first week posthatching, the female remained motionless inside the nesting site for about 97% of the time, but nest site attendance decreased to 77% by the sixth week (Table 1). Overall the female remained inside the nest chamber 87% of time. During this period, both hatchlings engaged in partial or complete skin contact with the female parent. They often spontaneously climbed on the mother's body, in particular on her trunk, head, legs, and tail, resting motionless without having any physical contact with the substrate for hours (Fig. 1F; Table 1). Overall, complete contacts were performed six times by the first hatchling and 13 times by the second one, with no statistically significant differences in the number of events between the two (Friedman repeated-measures ANOVA blocked by week, $S_1 = 1.80$, $P = 0.180$ adjusted for ties). The total time spent in complete contact with the female's body was 1,486 and 1,715 min for the first and the second hatchling, respectively (Table 1). The mean time per event was 244 min (SE = 111, range 19–799) for the first and 133 min (SE = 55, range 5–595) for the second young, and there was no significant difference between the hatchlings (Friedman repeated-measures ANOVA blocked by week, $S_1 = 0.67$, $P = 0.414$). During the sixth week posthatching, the first hatchling climbed over the female parent twice for relatively extended periods (Table 1). These two prolonged complete contacts occurred 41 and 42 days after hatching and took place just before two observations of the female walking out of the nesting site carrying the young on her back. In the first case, the transport lasted about 5 min, in the second about 6 min. Finally, during the sixth week posthatching, the female began to frequently leave the nesting site (Table 1). At that time, both hatchlings began exiting the nesting cavity on their own for the first time. Forty-two days posthatching, the three focal animals escaped from the terrarium, and video recording was terminated.

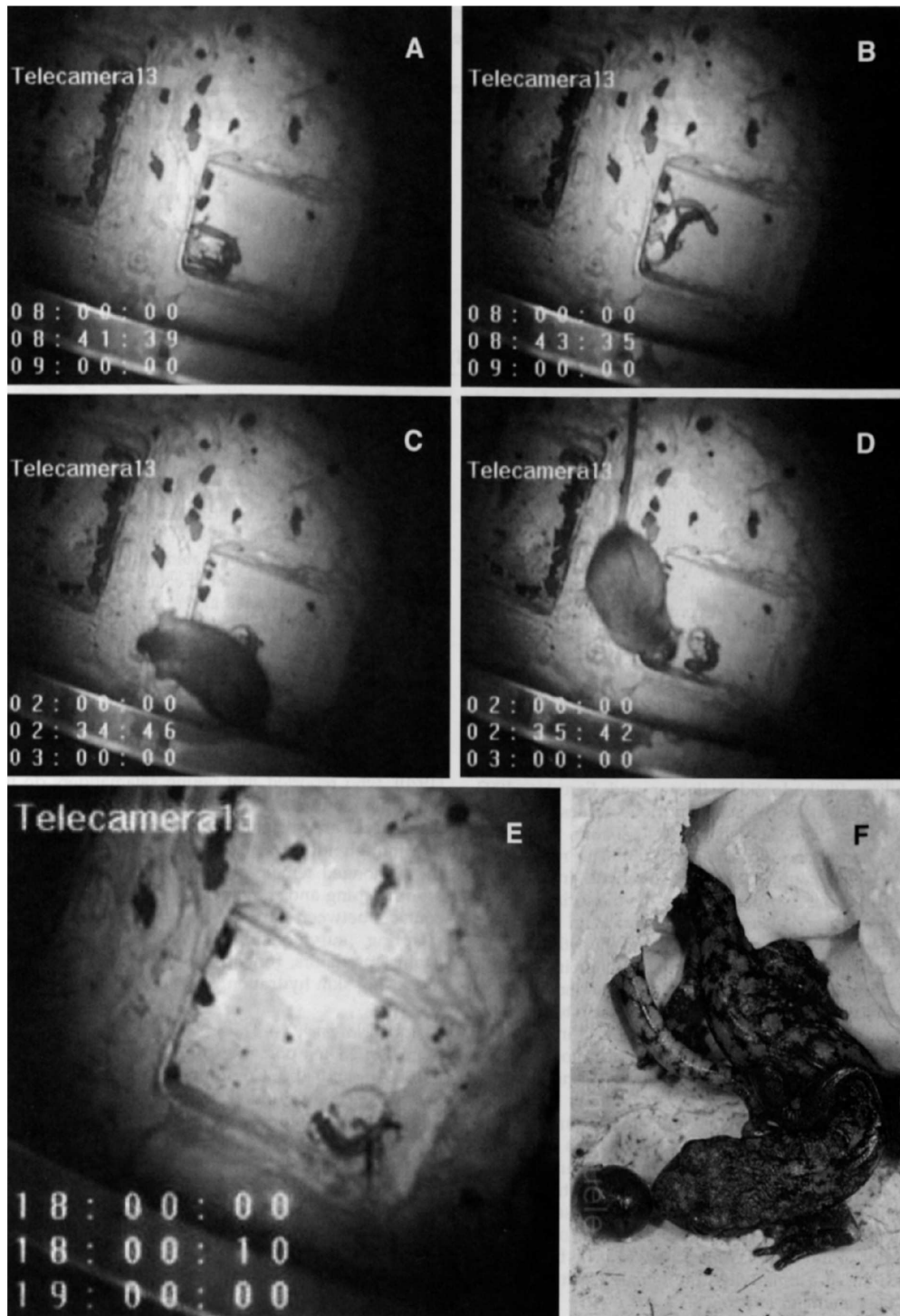


FIG. 1. Stills from infrared video footage showing antipredator behavior of the attending *Speleomantes strinatii* female toward and intruding conspecific (A, B) and a Roof Rat (C, D); (E) particular of a still from infrared video footage showing partial contacts between female and hatchlings: the first hatchling has the jaw in contact with the female's back, whereas the second one has the jaw in contact with the female's tail; (F) photograph showing a complete contact between the first hatchling and the female (photo by E. Biggi).

TABLE 1. Number of complete skin contacts between hatchlings and parental female, and number of nest chamber desertions by the female. In parenthesis, the mean duration in min \pm SE; only events lasting more than 5 min were analyzed.

| | First week | Second week | Third week | Fourth week | Fifth week | Sixth week | Total |
|---------------------------------------|-------------------|-------------------|------------------|------------------|-------------------|--------------------|------------|
| Complete contacts by first hatchling | 2 (878 \pm 500) | 0 | 0 | 0 | 2 (79 \pm 28) | 2 (529 \pm 21) | 6 (1,486) |
| Complete contacts by second hatchling | 2 (975 \pm 110) | 2 (571 \pm 318) | 2 (64 \pm 39) | 3 (55 \pm 15) | 4 (50 \pm 5) | – | 13 (1,715) |
| Nest site desertions by female | 2 (50 \pm 55) | 2 (140 \pm 55) | 2 (110 \pm 64) | 3 (150 \pm 43) | 2 (305 \pm 240) | 12 (319 \pm 210) | 23 (1,074) |

DISCUSSION

The infrared video recording of the brooding behavior of the female *S. strinatii* revealed an array of activities that included egg turning and displacing, aggressive attacks toward approaching conspecifics, egg attendance in presence of a potential mammalian predator, long-term sharing of the nesting site with hatchlings, recurring skin contact with offspring, and hatchling transport. These behaviors, with the exception of the repeated skin contact and transport of young by the female, were already known in salamanders (Nussbaum, 2003; Wells, 2007), and in particular in *Speleomantes* (Durnad, 1970). Aggressive defense of the eggs and terrestrial nesting sites by brooding females have been described for members of at least four families: Plethodontidae (Jaeger and Forester, 1993; Mathis et al., 1995), Salamandridae (Thiesmeier and Hornberg, 1998), Hynobiidae (Nussbaum, 2003), and Ambystomatidae (Nussbaum, 2003). The results presented here confirm these observations and provide new directions for future research on the social behavior of plethodontids. For instance, it would be highly interesting to experimentally verify whether *Speleomantes* females actively defend hatchlings in the same way they defend their eggs. This information would be relevant to the study of social behavior and territoriality of salamanders (Mathis et al., 1995) and in assessing possible variations in risk-taking and in young recognition (Forester et al., 2008) during the extended period of *Speleomantes* parental care.

Six weeks posthatching, the female transported a young salamander on its back twice. This surprising behavior was unknown for salamanders until now. It is possible that the female intentionally transported hatchlings from the nesting cavity, but it seems more parsimonious to suggest that the transfer occurred as a result of incidental contact between the female and hatchling at a time when the female elected to leave the nest chamber to forage.

In plethodontids, egg brooding by the attending female is an energetically expensive parental investment (e.g., Forester, 1981; Ng and Wilbur 1995). The studied *S. strinatii* female remained inside the nesting site after hatching for a percentage of time similar to that spent attending the developing eggs (87% and 98% of the time, respectively). This relatively long post-

hatching nest attendance likely incurs a cost and might be considered as a true parental investment (Trivers, 1972). During its prolonged nest attendance, the female rarely fed but probably succeeded in taking some prey when wandering inside the open terrarium. In fact, the female did not appear emaciated after 10.5 months of brooding (see the detail of the tail in Fig. 1F). That brooding plethodontid females occasionally leave their clutch to forage in close proximity of the nesting site has been described in *Desmognathus*, *Bolitoglossa*, and *Plethodon* (Nussbaum, 2003). However, the hatchlings of *S. strinatii* did not abandon the nesting site for about six weeks after hatching and were never seen feeding on small invertebrates present inside the terrarium. Thus, our results corroborate the observation of Durand (1970), who reported that young salamanders do not feed autonomously for a period ranging from four to eight weeks after hatching.

Although not explicitly tested by the current study, the prolonged female attendance at the nesting site after hatching and the repeated and long-lasting skin contacts between parent and offspring may increase offspring survivorship by providing protection against predators, fungal or bacterial infections, and increasing skin hydration.

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