Successful reproduction of the mole salamander *Ambystoma talpoideum* in captivity, with an emphasis on stimuli environmental determinants

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ABSTRACT - Generating and promoting evidence-based husbandry protocols for urodeles, commonly known as newts and salamanders, is urgently needed because most of the up-to-date ex situ programs are focused on frogs and toads than Urodela. Data on biology, life history, ecology and environmental parameters are lacking for many species and are needed to establish suitable husbandry and breeding conditions in captive environments. Two adult females and two adult males, of the mole salamander *Ambystoma talpoideum* successfully reproduced in captivity. It was found that reproduction of this species depends on various complex stimuli: including natural photoperiod 12:12, rainwater (acidic to neutral pH) and an aquarium full of various debris. Additionally high temperature variations ranging from 2 °C to 17 °C (a decrease followed by an increase) between November and February showed that it is possible to breed adults in aquariums provided the right stimuli are applied at the right moment of time in winter. *A. talpoideum* shows an explosive breeding mode as previously reported for the whole genus *Ambystoma*.

INTRODUCTION

Since the 1980s, the current global amphibian extinction crisis has been discussed and acknowledged (Wake, 1991; Raffaëlli, 2013; Pasmans et al., 2014; Hernandez, 2016a,b, 2017). Habitat destruction and overexploitation of the urodela group for human consumption and terrarium hobbyists accounted for 54% of the population decline observed since 1980 (Hernandez, 2016). 46% were suffering from the disease: chytridiomycosis or "chytrid" caused by two devastating fungus, *Batrachochytrium dendrobatidis* (Bd) and *Batrachochytridium salamandrivorans* (Bs). No solution has been found to date to save infected populations in the wild, and contamination continues all over the world (Pasmans et al., 2014). Many assessment programs were established but efforts are insufficient due to a large global extinction at an international scale. Moreover, North and Central America as well as Asia are the most severely affected and threatened places in the world (Raffaëlli, 2013; Hernandez, 2016a). Thus, maintaining in captivity (ex situ) small amphibian populations of potentially threatened or endangered populations providing from these regions is becoming essential today (Raffaëlli, 2013; Pasmans et al., 2014; Hernandez, 2016a,b). However, the husbandry requirements for keeping newts and salamanders in captivity are complex. For many species requiring captive breeding programs the husbandry requirements are just unknown because too little is known about their ecology and habitat. Some salamanders simply fail to breed in captivity and hence it is essential to collect every appropriate parameters and protocols that would successfully lead to a result. All these data are needed for conservation purposes (Hernandez, 2017). In this paper one of the first successes in breeding the mole salamander *Ambystoma talpoideum* in captivity is described with an emphasis on the environmental determinant stimuli involved. These data may assist in improving breeding these salamanders under artificial conditions.

A. talpoideum is endemic to the south-eastern and central United States, from southern Carolina to northern Florida and east to eastern Texas as well as south-eastern Oklahoma. The range extends north in the Mississippi valley to southern Illinois. There are a number of disjoint populations in Virginia, North and South Carolina, Georgia, Alabama, Tennessee and Kentucky (Shoop, 1964; Petranka, 1998). It inhabits woodlands and forested habitats including upland conifer-hardwood forests, pine flatwoods and bottomland hardwood forests and is often found in expansive floodplains in areas near gum and cypress ponds (Shoop, 1960; Semlitsch, 1983; Petranka, 1998). The mole salamander is listed as Least Concern by the IUCN while it is a species of special concern in North Carolina and Tennessee. Special permits are required to conduct any activity involving this species in these states. Populations are threatened by habitat destruction or its intensive degradation. This fossorial species shows an important geographic variation involving differences in the mode of egg lying or deposition and its history life suggests that these groups are genetically separated (Petranka, 1998; Raffaëlli, 2013). Atlantic Coastal Plain populations deposit eggs singly in ponds whereas Gulf Coastal Plain populations lay egg in small clusters that are often placed on the same twig (Semlitsch & Walls, 1990). The species is known to show maturity at a total length of 8-12 cm (TL) and may live up to 6-8 years in its natural habitat (Raymond and Hardy, 1990; Williams & MacGowan, 2004). In natural conditions *A. talpoideum* breeds in winter from December to the end of March when temperatures are above freezing (Hardy & Raymond, 1980).

METHODS

On April 2016, 4 adult specimens comprising two females and two males were acquired in a pet store from Paris, animals originating from a special importation from South Carolina. *A. talpoideum* is a stocky salamander with a short body and a large head, with a total length (TL) of about 10-13 cm (see Figs. 2 & 3). The four adult specimens were obtained in Paris in January 2016 from the pet trade and maintained in a terrestrial terrarium (Exo Terra) in a porch between 16-22 °C up to October 2016 and with a natural circadian rhythm. The terrarium measured 90x45x45 cm (LxWxH) with no lamp, the substrate consisted of 30 cm of leaf litter and humus from pine and oak forests. Moss (*Kindbergia praelonga*) and oak leaves were placed in the terrarium to provide refuges and a stable humidity of about 75 to 85 %. Small crickets (*Acheta domestica*) and earthworms (*Dendrobaena veneta*) were the main food supply. The salamanders were found to be nocturnal with little activity. However, on October 2016, mole salamanders increased nocturnal activity and were sprayed with water twice a day. They were then placed in an aquatic aquarium of about 60x40x40 cm furnished with debris, e.g. gravel, rocks, oak leaves and branches and placed in the garden at temperatures between 2-17 °C for 4 months. The water depth of the aquarium was about 25-30 cm. All water used was rainwater obtained from empty tanks or water collected in a natural spring. Substrate used was gravels, rocks as well as oak leaves and branches. *Elodea* sp. were also present. No filter was used. Water was acidic to neutral with a pH from about 6.3 to 7.8 during the reproduction period. At the beginning of February 2017, temperatures dropped to 2 °C at night and 8 °C during daytime. The aquarium was sheltered under a covered porch to avoid the intense morning freeze which gave temperatures of around 14-18 °C which were gradually increased. Partial water changes of 5-10 % were performed every two-three weeks.

Food comprising earthworms (*Dendrobaena veneta*) and small aquatic invertebrates such as Chironimidae, *Gammarus* spp., *Asellus aquaticus* and *Lumbricus terrestris* all supplied weekly. On February 11th at 9h00 in the morning, eggs were found singly deposited on branches and on leaves of *Elodea* sp. (see Fig. 1). To avoid predation from the adults, eggs were removed from the aquarium one day after the egg laying and maintained under identical parameters at 16-18 °C in a separate tank of 60x45x45 cm until hatching an air pump (Tetra whisper 10”) was used during all the protocols to oxygenate water. Oak leaves were added to avoid fungus such as *Saprolegnia* spp. Adults were left aquatic all year. Adult males did not show any aggressive behaviour. Larvae were raised at 18-25 °C from February to July in small tanks of about 40x40x40 cm containing about 10-20 larvae each and fed with mosquitoes larvae, *Gammarus* sp., zooplankton and Chironimidae. They became terrestrial at 4-5 months and were maintained in a wet atmosphere in the same kind of tanks than the one described for the adults. Six were still aquatic in August and showed paedomorphism, which is commonly known for this species (Petranka, 1998; Raffaëlli, 2013).

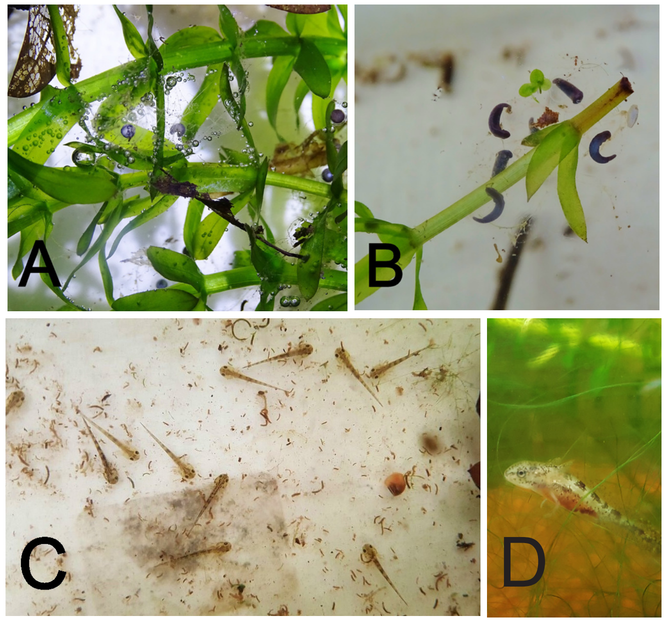

Figure 1. Life cycle of *A. talpoideum* in captivity: **A**. Eggs (2 days). **B.** Eggs with embryo (10 days) **C.** Young larvae with external gills after hatching (22 days). **D.** Larva with legs (30 days).

RESULTS

In late December 2016, males were observed more active and showed colourful blue spots on the body and a large blue-grey line on the tail. On a wet February 9th, 2017 at temperatures between 6.8-7.2 °C one male engaged in a nudging ritual with a female. The male repeatedly encircled the female, and then deposited two spermatophores on rocks and branches. Two days later on February 11th at a temperature of 12.5°C, the female laid 63 eggs singly attached to plants and branches (Fig. 1). The second female laid 71 eggs on February 19th at 11.8 °C with a second laying observed on March, 6th at 14.3 °C of 65 eggs. During 2016-17, the two females therefore deposited a total of 199 eggs. However, egg viability until hatching amounted to 85.9 % giving a total of 171 larvae.

One of these was consumed by congeners and a high rate of cannibalism was subsequently observed when more than 20-30 larvae were housed in the same tank. Another group of larvae was maintained under the same conditions than the adults at 17-22 °C with a diet consisting of *Enchytraeus*, *Gammarus*, *Chironomus* and mosquitoes larvae. For this group, the larval phase lasted approximately 120 to 150 days (Figs. 1 & 2). High larval mortality was observed when summer temperatures increased to 32 °C. A total of 38 animals were raised to the sub-adult stage.

DISCUSSION

The females in this study deposited a total of 199 eggs with egg viability 85.9 % indicating this species is useful for breeding programmes. Attention to food variety may be a key factor in captive reproduction with dietary supplements such as vitamins added to food before the reproductive period. This has been previously reported for the Salamandridae family (Hernandez, 2016b, 2017). This study confirms that the southern Carolina populations

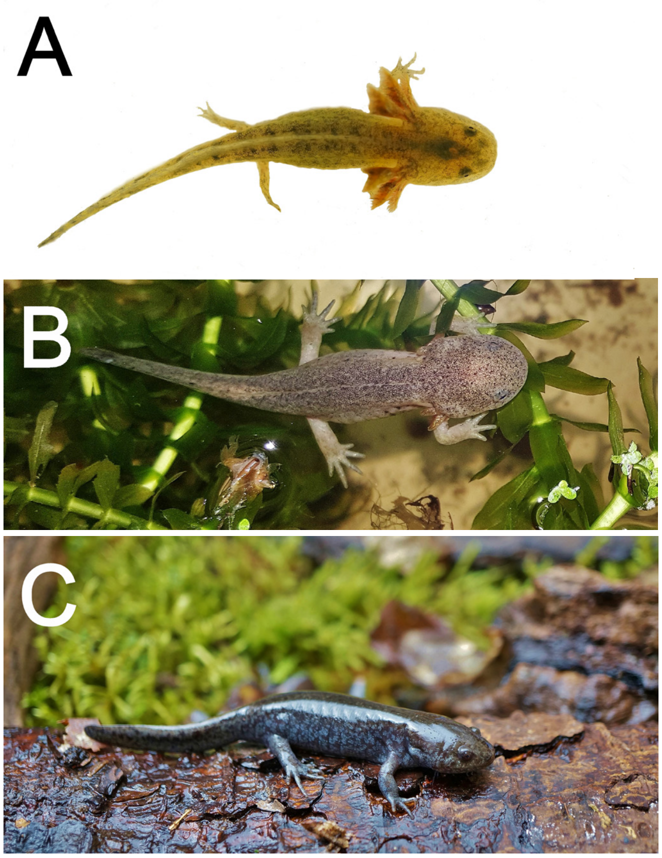

Figure 2. **A.** Larvae near metamorphosis after 60 days; **B.** Larvae near metamorphosis after 110 days; **C.** Adult male after one year.

deposit eggs singly on vegetation and rocks a reproductive mode that corroborates the origin of the adults, which is most of the time unknown for salamanders imported through the pet trade. The origin of the salamanders is important for conservation purposes in captivity (Pasmans et al., 2014; Hernandez, 2016a,b). Additionally, many of the larvae here showed paedomorphism as previously reported in this species (Petranka, 1998; Jackson & Semlitsch, 1993; Doyle & Whiteman, 2008; Raffaëlli, 2013; Walls et al., 2013).

However, the reproductive behaviour documented here is not yet complete and more data is required, for example long-term observations of reproduction. Certain species of salamander exhibit breeding behaviours and phenology wildly out of sync with wild populations and it would be interesting to know the extent of behavioural divergence of captive animals differ from wild counterparts. A new study including patterns of migration, courtship and oviposition in relation with the wild populations would be useful. Important stimuli identified for *A. talpoideum* reproduction could be further investigated and include a natural photoperiod 12:12; rainwater use (acidic to neutral pH) in an aquarium full of debris (such as branches, rocks and oak leaves; see Fig. 3); and high temperature variations from 2 °C up to 17 °C with temperatures in December close to freezing (2-4 °C). Adults housed in an aquarium at the right time in winter and where all the reported stimuli are present could successfully be bred. This choice of parameters may be useful for ex situ programs involving other related taxas such as *A. maculatum*, *A. opacum* and *A. tigrinum* that occur in sympatry in south-east America. Finally, it has been shown that *A. talpoideum* presents an explosive breeding mode as previously reported for the whole genus *Ambystoma*.

Generating and promoting evidence-based husbandry protocols for Urodela is urgently required since to date most of the ex situ breeding programs concern frogs and toads. For many species data on biology, life history, ecology and environmental parameters are lacking but are needed to establish appropriate husbandry conditions. Moreover, some salamanders fail to thrive and breed in captivity. This is especially true for salamanders originating from the neotropical and tropical zones. The observations reported in this paper indicate captive breeding of *A. talpoideum* depends on various and complex stimuli that must mimic natural conditions as much as possible. The results obtained are potentially useful to inform captive breeding programmes. However, it should be emphasised that captive bred populations of amphibians do not necessarily protect genetic diversity and are not themselves useful stock for reintroduction to nature. This problem is a major concern in terms of conservation.

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