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Notes on reproduction and development of *Pleurodeles nebulosus* (Caudata: Salamandridae) in captivity

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ABSTRACT - The reproduction of captive *Pleurodeles nebulosus* was followed during five breeding seasons. The breeding season lasted from the onset of autumn to early spring. Most eggs were laid in October, although oviposition occurred throughout the breeding season. Each season a female would produce one or more clutches of 19-224 eggs. Diameter of the ova was 1.63-1.65 mm. Eggs hatched after 8-12 days, and at hatching the larvae were in Harrison stages 37-38. It took 73-108 days for the larvae to reach metamorphosis and the metamorphs measured 25.6-32.1 mm in standard length. A modest correlation was found between standard length at metamorphosis and larval development time. Juveniles, which were kept aquatic, reached maturity after 9-10 months, whereas juveniles, which were reared on land, did not reach maturity until approx. 20-22 months after metamorphosis.

INTRODUCTION

The ribbed newts of the genus *Pleurodeles* consist of three species. While *P. waltl* is found both on the Iberian Peninsula and in northern Morocco, the distribution of the two other species is limited to North Africa. *P. nebulosus* is distributed across northern Tunisia and Algeria, except on the Edough Peninsula and surrounding lowland areas of north-eastern Algeria, where it is replaced by *P. poireti* (Carranza & Wade, 2004).

P. waltl is a well-studied species (e.g., Schleich et al., 1996, Salvador & Garía-París, 1999, García-París et al., 2004), whereas both *P. nebulosus* and *P. poireti* remain poorly known. Although Samraoui et al. (2012) provided some information on the natural history and conservation of *P. poireti*, almost all research into these two smaller species has focused on *P. nebulosus*, and most studies made on *P. poireti* before the work of Carranza & Wade (2004) actually refer to *P. nebulosus*. In this study data obtained from captive newts during five consecutive breeding seasons from 2012 to 2016 is compared with the available data from the field and other captive breedings.

MATERIALS AND METHODS

The newts used in this study were the offspring (F2 and F3) of individuals collected in the vicinity of Tabarka, Tunisia. Eight newts were used in the study. They were kept in groups consisting of two females and two males in each tank. The tanks were standard aquaria measuring 60 x 30 x 35 cm. At a height of about 17 cm from the bottom a glass shelf covering a third of the bottom area was glued in place with silicone. This constituted the land area of the tank and allowed the water part to cover the entire bottom area of the tank. The shelf was covered with a 2 cm thick sheet of dark grey foam rubber, which was always kept slightly moist. As hiding places for the newts pieces of cork bark were placed on top of the foam. A piece of driftwood allowed the newts to easily reach the land area when leaving the water. The water level was kept at a height of 15-16 cm. To allow for easy cleaning no substrate was used in the water part of the tank and aside from the piece of driftwood no other decorations were used. Various aquatic plants filled the water part, such as aquatic mosses of the genera *Taxiphyllum* and *Vesicularia*, hornwort (*Ceratophyllum demersum*) and Brazilian waterweed (*Egeria densa*). Over the summer filamentous green algae would occasionally appear in the tank. These were not removed as the newts would often use them for egg-laying. The water in each tank was filtered by a large air-driven sponge filter, which was cleaned about once a month. The tanks were lighted with fluorescent light year round for 12 hours a day. As they were placed near a window, the tanks also received some ambient light. Water temperature ranged from 20-26 °C in summer to 15-20 °C in winter. When terrestrial the newts were primarily fed with buffalo worms (*Alphitobius diaperinus*) and earthworms, whereas in the water the main foods were defrosted bloodworms (*Chironomidae*) and live *Tubifex* sp.

As the adult newts would often consume their own eggs, they were removed from the tanks as soon as they appeared and were placed in small water filled plastic boxes. To prevent infection, a small amount of methylene blue was added to the water with the eggs. Upon hatching the larvae were moved to densely planted aquaria ranging in size from 25 to 63 litres. The larvae were fed once a day with live brine shrimp naupliae (*Artemia* sp.) and banana worms (*Panagrellus nepenthicola*). As the larvae got bigger the brine shrimp and banana worms were gradually replaced by Tubifex, chironomid larvae and water fleas (*Daphnia* spp.).

Eggs and newly hatched larvae were measured to the nearest 0.01 mm by photographing them next to a stage micrometer, whereas metamorphosed juveniles were measured to the nearest 0.1 mm using dial callipers. To restrain movement of the juveniles they were placed in small plastic bags while measurements were made.

RESULTS

The breeding season began when the newts entered the water in early autumn. The newts would leave the water regularly and stay on land for up to a week at a time. From late spring the newts stayed on land where they would remain until the following autumn, signalling the end of the breeding season. To indicate the onset of the mating period the males developed brown nuptial pads on the underside of the humeral and their cloacas swelled slightly. After a short time in the water both sexes also developed low tail fins, just barely discernible in the females. Amplexus was observed within a day after the newts entered the water and would continue sporadically during the entire breeding season.

Oviposition and eggs

Oviposition occurred from September to March (Fig. 1), and appeared to be triggered by a water change. The earliest recorded clutch was deposited on 23 September 2015, the latest on 4 March 2016. The most prolific month was October, in which five of the 13 recorded clutches were deposited. Eggs were deposited several times during the breeding season. The number of eggs in each clutch was highly variable and ranged from 19 to 224 eggs; averaging 75.4±55.862 eggs (mean ± 1 SD; N = 13). Eggs were deposited on aquatic plants, mosses, algae, driftwood and even on the filter sponge, either singly or in clumps of 2-32 eggs. Average number of eggs per clump in each clutch ranged from 2.9±1.552 to 11.8±7.786 eggs (mean ± 1 SD; N = 8 and 10, respectively). A total of 120-355 eggs were produced in each tank during a breeding season. Assuming they each produced an equal number of eggs, each female laid 60-178 eggs during a breeding season. Considering the largest observed clutch size, this is obviously an underestimation. The maximum number of eggs produced by a female must therefore be at least 224 eggs.

In the freshly laid eggs (Fig. 2) the diameter of the ovum ranged from 1.63 to 1.65 mm and averaged 1.64±0.007 mm, whereas the diameter of the perivitelline chamber measured 2.19-2.39 mm with an average of 2.283±0.059 mm. The largest outer capsule was slightly oval and its largest diameter ranged from 4.74 to 6.25 mm with an average of 5.41±0.490 mm (mean ± 1 SD; N = 10).

Egg development and hatching

At temperatures of 16-23 °C the eggs hatched after 8-12 days. Hatching did appear to be somewhat dependent on temperature, so that eggs kept at 21-23 °C would hatch after 8 days, whereas eggs kept at 16-20 °C took up to 12 days to hatch. At time of hatching the larvae (Fig. 3) had a total length of 7.90-8.94 mm, averaging 8.365±0.352428 (mean ± 1 SD, N = 10). They were whitish-yellow with darker pigment arranged into two distinct stripes on each side of the body. The fins were clear to slightly milky. The

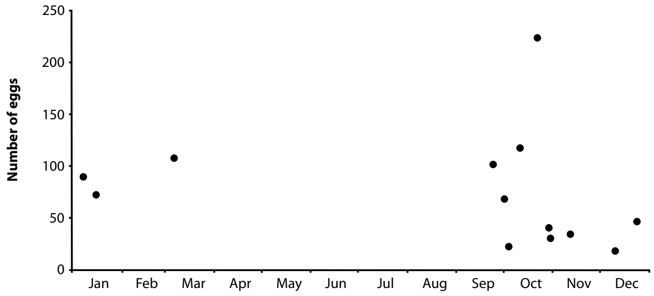

Figure 1. Time of oviposition in *P. nebulosus*. Oviposition occurred from September to March but peaked in October. Each dot (●) represents a clutch of eggs.

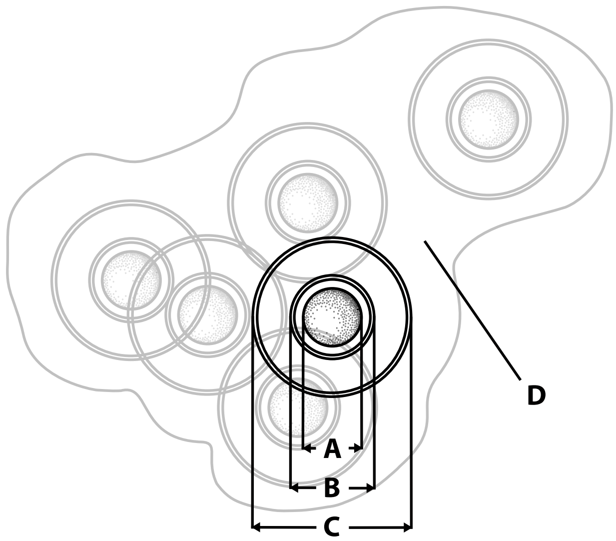

Figure 2. Schematic drawing of a clump of eggs showing the measurements taken: **A**. diameter of the ovum; **B**. diameter of the perivitelline chamber; **C**. diameter of the largest outer capsule; **D**. viscous jelly

Figure 3. Newly hatched larva. Note the large balancers, branching of the gill filaments and beginning pigmentation.

size difference among the larvae was also reflected in their stage of development. All the larvae had three distinct gills on each side of the head. The gill filaments varied in length and would in some larvae reach the base of the forelimb; in others they would not. Branching was just beginning. In the smallest larvae only the tiniest sprout could be discerned on largest of the gill filaments, whereas sprouts were clearly visible on all gill filaments in the largest larvae. The balancer was slightly shorter than the gill filaments in the smallest larvae, but just as long and very slightly club-shaped in the larger larvae. Forelimb buds were visible in all larvae, although more distinctly bud-shaped in the largest larvae. This corresponds to Harrison stages 37-38 (Harrison, 1969).

Larval development and metamorphosis

At a temperature of 17-20 °C it took 73-108 days for the larvae (Fig. 4) to reach metamorphosis, with an average of 86.90±10.621 days (mean ± 1 SD; N = 17). The standard length (as measured from the tip of the snout to the posterior end of the vent) at metamorphosis was 25.6-32.1 mm with an average of 28.89±1.75 mm (mean ± 1 SD; N = 17). Although the smallest individual took the shortest time to reach metamorphosis and the largest took the longest, there was only a modest correlation between standard length at metamorphosis and larval development time (Spearman Rank Correlation Coefficient (r_s) = 0.472; N = 17). Tail length was far more variable than standard length and the tail length to total length ratio ranged from 0.421-0.501, averaging 0.463±0.021 (mean ± 1 SD; N = 17). This was probably due to the larvae biting each other's tail tips off, which would often happen during feeding if the larvae were kept at high densities. No deaths resulted from this, though, and the tails healed fast.

Figure 4. Large larva at an age of 85 days. Total length is 62 mm.

Figure 5. Juvenile with a total length of 61 mm, one month after metamorphosis

After metamorphosis the juveniles (Fig. 5) were either reared in small terraria on moist foam rubber with pieces of cork bark for cover or in densely vegetated aquaria with relatively low water levels (up to 20 cm). Growth was much faster in the aquaria, where most of the juveniles would reach maturity just 9-10 month after metamorphosis. Most juveniles reared on land were not mature until 12 months later, i.e., at a postmetamorphic age of approximately 20-22 months. Mortality was, possibly due to drowning, much higher when the juveniles were raised in water than when raised on land. However, this was not quantified.

DISCUSSION

Pasmans & Bogaerts (2001) observed that in captivity *P. nebulosus* would mate in the autumn, while Ben Hassine et al. (2012) found that mating begins with falling temperatures in October and lasts until the beginning of March. This is consistent with my observations, although mating was observed to begin slightly earlier, which can probably be attributed to the earlier onset of autumn at the more northern latitudes at which the newts were kept.

Almost nothing is known about oviposition in the wild. Blanc (1935) witnessed egg-laying in January and in February Pasmans et al. (2002) found larvae, ranging in size from 1 to 3 cm, in a ditch, which led them to speculate that oviposition occurred in December and January. At least in captivity, oviposition took place during the entire breeding season but with a peak in October, suggesting that *P. nebulosus* is primarily an autumnal breeder. Out of 36 newts found by Pasmans et al. (2002) in February only one was observed in the water, which supports the hypothesis that the peak mating season of *P. nebulosus* under natural conditions probably lies in autumn and early winter. The annual egg production of 60-224 eggs per female is more or less in accordance with the 100-200 eggs noted by Ben Hassine et al. (2012).

Descriptions of the eggs of *P. nebulosus* are almost nonexistent. Ben Hassine et al. (2012) did report an average diameter of the ovum of just 1.2 mm, which is, however, much lower than the 1.64 mm observed in this study. At 20 °C Ben Hassine et al. (2012) found that it took 10 days for the eggs to hatch, whereas at 15-16 °C hatching was prolonged to 20 days. This is similar to my findings and corroborates the observed effect of temperature on egg development.

Under natural conditions larvae have been found from the beginning of December to the first half of April (Pasmans et al., 2002, Sicilia et al., 2009) and according to Ben Hassine et al. (2012) it takes the larvae 2-3 months from hatching to metamorphosis. This is slightly less, than what I have observed, but the time it takes the larvae to reach metamorphosis seems to be highly variable. Although there was only a modest correlation between size and development time, availability of prey/food could be a determining factor. As the larvae were kept at relatively high densities, some were probably better at obtaining food than others and as a result grew faster, eventually reaching metamorphosis earlier than their siblings.

Due to lack of data it is not possible to compare the reproductive biology of *P. nebulosus* to that of *P. poireti*. In comparison to the well-studied *P. waltl*, however, the similarities seem to outweigh the differences. In the lowlands of North Africa *P. waltl* breeds, similar to *P. nebulosus*, from October to April (Schleich et al., 1996). Despite its often much larger size (at least on the Iberian Peninsula, females of *P. waltl* may reach total lengths of up to 286 mm (González de la Vega, 1988) compared to the maximum total length for female *P. nebulosus* of 170.4 mm reported by Ben Hassine et al. (2012)) the eggs of *P. waltl* are only slightly larger than those of *P. nebulosus*. According to González de la Vega (1988) the ova of the eggs of *P. waltl* have diameters of 1.7-2.0 mm, just slightly larger than the ovum diameter of 1.63-1.65 mm in *P. nebulosus*. The reproductive advantage due to the larger size of *P. waltl* over the smaller *P. nebulosus* seems, therefore, to be limited to a much larger egg production with large females producing up to 1303 eggs annually (González de la Vega, 1988), perhaps even up to 2000 eggs (Schleich et al., 1996). This is a substantial egg production when compared to the annual production of 60-224 eggs, which I observed in *P. nebulosus*.

Larvae of *P. waltl* have been reported to hatch after 2-26 days (Gallien & Durocher, 1957; González de la Vega, 1988; Schleich et al., 1996), which is a much wider time span that I was able to observe in *P. nebulosus*. Despite similarities in ovum size larvae of *P. waltl* appear to hatch at a larger size than *P. nebulosus*. Reported sizes for newly hatched *P. waltl* range from 6.5-8 mm (González de la Vega, 1988) to 10-14 mm (Gallien & Durocher, 1957; Schleich et al., 1996).

It takes the larvae of *P. waltl* 3-5 months to reach metamorphosis (González de la Vega, 1988; Schleich et al., 1996). While this is similar to *P. nebulosus*, *P. waltl* seems to transform at a larger size. According to Schleich et al. (1996) newly metamorphosed *P. waltl* reach snout-vent lengths of 55-80 mm, although other authors report somewhat smaller sizes (e.g., González de la Vega, 1988). Growth in *P. waltl* after metamorphosis is fast and well-fed juveniles may reach sexual maturity within 6 months (Schleich et al., 1996), which is much faster than even my fastest developing *P. nebulosus*.

The knowledge of the reproductive biology of *P. nebulosus* obtained in this and other studies has been fairly predictable and hasn't revealed any surprises. However, much of the available data was, as in the present study, obtained from captive animals and may be biased by the conditions of captivity. To wholly understand the natural history of *P. nebulosus* more detailed studies on wild populations are needed.

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