Aquatic courtship and reproductive behaviour of the red-eyed grass snake *Natrix astreptophora* (Seoane, 1884)

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Resumen: Durante la temporada reproductiva de *Natrix astreptophora* en Osona, noreste de Catalunya (península ibérica), se documentaron por primera vez comportamientos de cortejo y cópula en un medio acuático. Las observaciones, realizadas en 2022 y 2024 mediante fototrampeo y observación directa, muestran que tanto el cortejo como la cópula tienen lugar en charcas estacionales, lo que sugiere un papel funcional de estos hábitats en la reproducción de la especie. Durante los eventos se observaron varios machos intentando cortejar a una misma hembra, sin comportamientos agresivos entre ellos. Además, la hembra mostró un comportamiento inédito al generar turbidez en el agua, probablemente como estrategia para ocultarse. Estos resultados aportan nuevos datos sobre la ecología reproductiva de *N. astreptophora* y destacan la importancia de conservar las charcas temporales como hábitats esenciales para su reproducción.

The courtship behaviour of *Natrix astreptophora*, an Iberian endemic grass snake, remains underexplored. Much of the existing literature on the natural history of Grass Snakes, particularly their sexual behaviour, is derived from studies of *Natrix natrix* and *Natrix helvetica* (Kabisch, 1999). These findings may not fully apply to *N. astreptophora*, which diverged from its European relatives 9.6–10.6 million years ago (Pokrant *et al.*, 2016). Thus, specific data on the reproductive ecology of the species remain scarce, and it is unclear whether such behaviours are widespread or population-specific.

In the Catalan County of Osona (42.0144º N, 2.2840º E), *N. astreptophora* shares its habitat with other sympatric species, including *N. maura*. While *N. maura* has been well-documented as water-dependent (Santos & Fernández Cardenete, 2015), *N. astreptophora* is often characterized as less reliant on aquatic habitats, as evidenced by observations of individuals far from water sources (Salvador, 1998). However, these conclusions may underestimate *N. astreptophora* reliance on temporary water bodies, particularly during critical life stages such as reproduction. Aquatic habitats may provide thermally stable conditions, as well as advantages for concealment or even predator evasion (Snickars *et al.*, 2004), critical during mating.

This research provides valuable insights into reproductive behaviour over extended observation periods. Understanding reproductive strategies in reptiles like *N. astreptophora* is essential for both ecological studies and conservation efforts. The objectives of this study are: 1) to document, for the first time, the courtship behaviour of *Natrix astreptophora*; and 2) to offer new insights into the reproductive ecology of this species.

The reproductive season for this *N. astreptophora* population in Osona, spans May to June, coinciding with peak activity levels. The female exhibits diurnal behaviour from March to October, transitinoning to nocturnal patter-

ns during the summer months. A reproductive strategy during the courtship of the species *N. astreptophora* inside a pond was recorded during June 2022 and May 2024. These observations are part of a long-term investigation into the reproductive behaviour of five sympatric species monitored over eight years.

The study was conducted in a fragmented semi-natural habitat comprising agricultural areas with three seasonal ponds. *Natrix astreptophora* was observed in all three ponds; however, the specific behaviours described were recorded at a small pond of approximately 20.47 m^2, characterized by shallow water and minimal vegetation. This pond retained water the longest or experienced the shortest dry periods among the three, consistently attracting the highest snake activity despite frequent human disturbance.

To monitor the reproductive behaviour of *Natrix astreptophora*, an adult female was tracked using reduced-dimensional camera traps and direct observations. Sexual dimorphism in this species is pronounced, with males typically measuring 50–70 cm by age 5–8, while females can exceed 100 cm by age 15 (Pleguezuelos *et al.*, 2018). The specimen in the study was confirmed to be an adult female, as the shed skin collected measured 1.10 m in length. Camera traps were placed strategically around the snake's known resting refuges and adjacent areas to capture key reproductive events such as courtship and copulation. Remotely controlled devices minimized observer interference, ensuring minimal disturbance and reducing potential behavioural bias. In addition, direct observations were conducted during periods of increased activity to complement the camera data. Data collection followed methodologies outlined by Serrano-Fochs (2019). All individuals were observed without interference, no animals were handled or captured. The study prioritized the preservation of natural behaviour, even at the cost of missing some recordings.

At 13:30 on June 3, 2022, the female was observed entangled with a male. A second male, similar in size to the first but noticeably smaller than the female, was also present at the pond. Both males actively searched for the female in the water, exhibiting frequent tongue-flicking and responding to water ripples and movements. The encounter was recorded for two and a half hours. No aggressive behaviour or competition was observed between the males (Video S1- courtship - http://www.herpetologica.org/BAHE/videos/H_3601-v1.mp4).

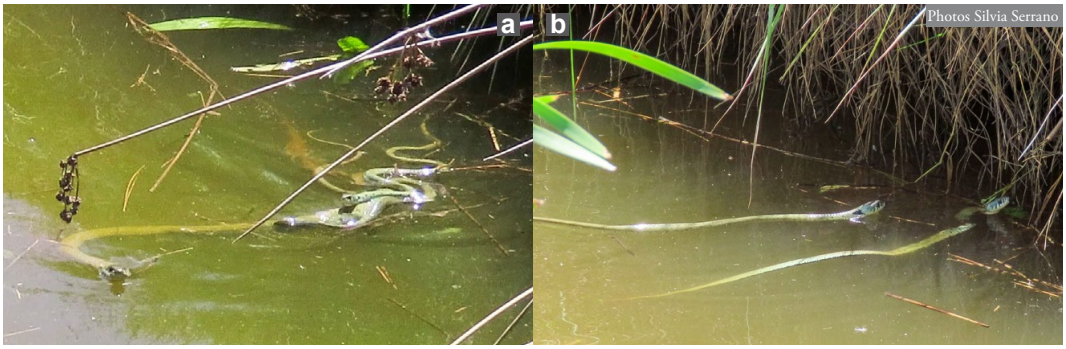

Figure 1: a) *Natrix astreptophora* female being courted by two males during a reproductive event (2022). b) Males of *Natrix astreptophora*, similar in size, searching for the female (2022).
Figura 1: a) Hembra de *Natrix astreptophora* siendo cortejada por dos machos durante un intento reproductivo (2022). b) Machos de *Natrix astreptophora*, de tamaño similar, buscando a la hembra (2022).

Figure 2: *Natrix astreptophora* female generating turbidity during 2022 reproductive event.
Figura 2: Hembra de *Natrix astreptophora* generando turbidez durante un evento reproductivo en 2022.

Courtship occurred in the water, with the males repeatedly attempting to coil their tails around the female's cloaca, presumably to initiate copulation. The female initially tolerated these attempts, allowing the males to come into close proximity (Figure 1). However, after a period of interaction, the female began to display convulsive movements to shake them off. Once she managed to evade the males, the female would retreat to a corner of the pond with vegetation, where she remained completely still. Despite this, the males relocated her with ease, and the cycle of approach, rejection, retreat, and rediscovery repeated.

As the interaction progressed, the female began to display more distinctive evasive behaviour. She deliberately rubbed her head and body against the muddy bottom of the pond, a movement that generated a dense cloud of sediment (Figure 2 / Video S2- turbidity - http://www.herpetologica.org/BAHE/videos/H_3601-v2.mp4). This cloud of turbidity likely served to conceal her. Once the water became murky, the female remained stationary. This behaviour was observed multiple times during the interaction, with the resulting turbidity persisting for several minutes before dispersing.

On May 30, 2024, the same female, identified by a distinct bite-like wound on one side that had left a dark, easily recognizable scar, was monitored again. At 3:41 p.m., two larger males were observed courting her, showing similar behaviour to 2022, but with fewer rejection interactions (Figure 3). Unlike in 2022, the female did not display turbidity-creating behaviour. Multiple copulations were documented on June 6, 7, 8, and 9, all among vegetation at the pond's edge, each lasting over an hour (Video S3- mating - http://www.herpetologica.org/BAHE/videos/H_3601-v3.mp4). Following copulation, the snakes remained motionless, seemingly resting, even during the

Figure 3: a) Female being courted by a male in 2022. b) *Natrix astreptophora* female being courted by a male in 2024. In both images, the male's size can be compared, with the male in 2022 being smaller.
Figura 3: a) Hembra de *Natrix astreptophora* siendo cortejada por un macho en 2022. b) Hembra de *Natrix astreptophora* siendo cortejada por un macho en 2024. En ambas imágenes puede compararse el tamaño relativo de los machos, siendo el de 2022 más pequeños.

afternoon. Only one male was observed with the female each day, and both males were never observed together with her. It remains unclear whether she copulated with the same male on consecutive days or alternated between them.

Additionally, on May 29, 2024, the copulation involving *Natrix maura* was recorded at the pond's edge within vegetation, eight days before the copulation of *Natrix astreptophora* on June 6, 2024, at the exact same location. During one *N. astreptophora* copulation, a *N. maura* was caught in between the female and male, waiting calmly before escaping the interaction.

The cycle of approach, rejection, retreat, and rediscovery repeated multiple times, which could indicate both male persistence and female evasion, possibly as a selective mate-rejection strategy or response to persistent advances (personal observation). Observations of two males courting a single female suggest the potential multi-paternity, although no aggressive or competition was noted, unlike in other *Natrix* species (Luiselli, 1996). Closely related *N. helvetica* shows multi-paternity in up to 90% of clutches (Meister *et al.*, 2012), genetic studies are needed to confirm this in *N. astreptophora*.

N. astreptophora shows similarities and differences with the sympatric *N. maura* (Salvador, 1998), however *N. maura* is strongly water-dependent (Santos & Fernández Cardenete, 2015). Overlapping niches and occasional hybridization (Gonzalez de la Vega *et al.*, 2021) suggest shared resources and reproductive strategies. Recent findings indicate *N. astreptophora* may rely more on aquatic habitats than previously thought highlighting the need for broader studies of its hydric requirements and behaviour.

The sediment-clouding behaviour observed in female *N. astreptophora* during courtship is a novel strategy potentially serving mate selection and/or predator evasion. This specific tactic of sediment clouding has not been previously documented, making it a unique addition to our understanding of snake behaviour in aquatic habitats. By generating turbidity, the female may obscure its location, evading persistent males and controlling reproductive interactions. This aligns with anti-predator behaviours in aquatic environments, where turbidity reduces detection risk (Snickars *et al.*, 2004). In low vegetation settings, turbidity may compensate the lack cover, providing a critical survival advantage. This new insight highlights

the complexity of reproductive and survival strategies in aquatic environments.

These findings underscore the ecological importance of preserving seasonal ponds and their surrounding landscapes, especially as climate change and habitat fragmentation threaten these environments. Understanding hydric and reproductive behaviour will be critical for effective conservation. Future studies should monitor multiple individuals under varied conditions, integrating genetic and environmental data to better understand these behaviours.

This study highlights the relevance of aquatic habitats in the reproductive behaviour of *Natrix astreptophora*. Observations from 2022 and 2024 show that courtship and copulation occurred in or near water, suggesting a functional role for aquatic settings during mating. These habitats may offer thermal stability, cover, and escape routes (Doody *et al.*, 2014), and possibly facilitate mate detection via pheromone dispersion (Aldridge *et al.*, 2005). While further studies are needed to quantify reproductive success in different pond types, these findings suggest that water presence may support critical courtship interactions.

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