

Successful reproduction of feral *Trachemys scripta* (Schoepff, 1792) in an inland wetland of the Veneto region, Le Basse del Brenta, Italy

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The introduction of exotic species has contributed to the global loss of biodiversity (Wilson, 1988). Exotic species can have multiple negative consequences for native biota, such as predation, parasitism, competition, diffusion of diseases, hybridisation and ecosystem modifications (Strayer et al., 2006). The pond slider *Trachemys scripta* (Schoepff, 1792), native to the Eastern and central United States of America, is considered one of the world's worst invasive alien species (Lowe et al., 2000). To date, as a result of their massive import as pets (Arvey and Servan, 1998; Bonin, 2004), slider turtles have been introduced outside their natural range throughout all continents except Antarctica and on some Oceanic Island (Ernst and Lovich, 2009).

In 2014, the European Union published Regulation (EU) No 1143/2014, declaring an action plan to prohibit the import, breeding and release of invasive species (European Parliament, 2014), including *T. scripta* (European Commission, 2016). However, in Italy, many individuals are still abandoned in natural or artificial ponds and rivers (Macchi et al., 2020). Nonetheless, not all pond sliders occurring in Europe are reproductively active (Cadi et al., 2004; Ficetola et al., 2009; Liuzzo et al., 2020). Bioclimatic models suggest that Italian coastal areas are suitable for reproduction of the sliders; this increases the risk of establishment of this long-lived species in areas where the European pond turtle *Emys orbicularis* (L., 1758) is still common (Ficetola et al., 2008). Hence, the early detection of *T. scripta* occurrence and the confirmation of its reproduction in new locations are key factors for the efficient development

and implementation of adequate conservation measures (Martins et al., 2018).

In the Veneto region, the first record of the alien slider turtle *Trachemys scripta elegans* (Wied-Neuwied, 1839) dates back to 1990, namely in Padova (Bonato et al., 2007). Despite this species being frequently reported in the Venetian lacustrine environments (i.e., in the urban parks of Mestre, in the Casale Oasis of Vicenza, at Badoere di Morgano in Treviso; Bonato et al., 2007), very limited data are available regarding the reproductive biology under natural conditions. To date, no evidence of reproducing populations of the locally abundant pond slider, *T. scripta*, are confirmed in the Veneto region (Bonato et al., 2007). In this study, we report on evidence of successful reproduction of a feral turtle individual in a protected area of the Veneto region where *T. scripta* and the endemic Italian Agile Frog *Rana latastei* Boulenger, 1879 coexist.

On 9 July 2020, at 21:30 h, a large *Trachemys scripta elegans* female was observed while attempting to excavate a nest with her hind limbs along the south-eastern shore of the lake in the local nature reserve "Le Basse del Brenta" (45.6928°N, 11.6928°E) (Fig. 1). The study area is of high conservation value and is included in the Special Area of Conservation (SAC) "Grave e zone umide della Brenta" (SAC IT3260018). This study site has an oceanic semi-continental climate. The annual average temperature is about 14 °C, with the average temperature of the warmest month (July) being of 24.3 °C. The annual average precipitation is 1228.4 mm, while the average annual solar radiation is 4814.7 MJ/m². Climatic parameters were calculated for a time series of 26 years (from 1994 to 2020) recorded at the ARPAV meteorological stations of Rosà and Cittadella. The reserve comprises semi-natural habitats, such as hay meadows, dry grasslands and managed woods, and freshwater habitats, including the main course of river Brenta and small eutrophic lakes.

Specifically, the nest of the adult female individual of

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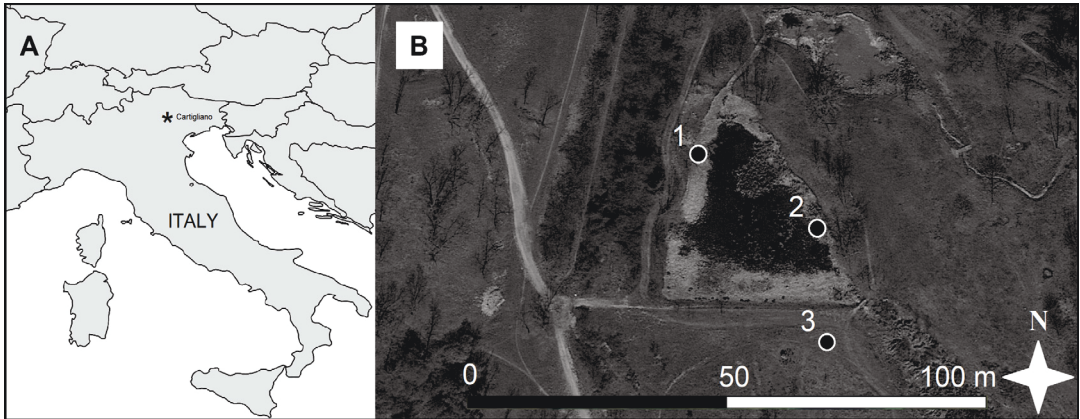


Figure 1. Territorial framework of the local nature reserve “Le Basse del Brenta” (A). The circles and IDs represent the Veneto locality where *Trachemys* individuals was collected (B).

T. s. elegans was located in a dry calcareous grassland characterised by shallow, skeletal soil and dominated by *Bothriochloa ischaemum* (L.) Keng and *Festuca myuros* L. (Fantinato et al., 2016). The nest site was located almost 1 m from a walking path and 15 m away from a eutrophic lake of 3094 m², newly created in 2017 after the flooding of a dismissed extraction quarry. Although recently created, in 2020 the lake already showed signs of high nature value, with the presence of reeds of *Typha latifolia* L. and submerged vegetation dominated by *Myriophyllum spicatum* L. and *Ranunculus penicillatus* (Dumort.) Bab (Masin and Scortegagna, 2011). From a zoological point of view, the study area is characterised by the presence of *Rana latastei*. This frog is endemic to the lowlands of Northern Italy and West Slovenia

(Sillero et al., 2014), and is classified as vulnerable by the IUCN, with populations declining because of habitat loss, fragmentation, and invasive species (Sindaco et al., 2009).

The *T. s. elegans* individual was collected, measured using a calliper, and weighed with a precision balance. The Straight Carapace Length (SCL), Carapace Width (CW), Carapace Height (CH) and Body Mass (BM) were recorded. The clutch was examined on 13 December 2020, at 15:00 h. Ten hatchlings and one infertile egg were found in relative proximity to the surface in brumation behaviour; hatchlings were placed in an aquarium for further care. Each hatchling’s yolk sac was not fully absorbed, the egg tooth still present (Fig. 2). The shells and epidermal keratinous scutes of



Figure 2. *Trachemys scripta elegans* while excavating the nest collected in the local nature reserve “Le Basse del Brenta” (A); Dorso-ventral view of hatchling individual with yolk sac not yet fully absorbed (B). Photographs by M. Liuzzo and E. Fantinato.

two hatchlings showed a few morphological anomalies with aberrant locomotor performance. About 24 h after hatching two out of the 10 juvenile turtles had died. Each plastral scute was covered with dark spots. All ten hatchlings had a vertical connection between the postorbital stripe and the orbito-cervical stripe behind the eye. The carapace lengths ranged from 28.85 to 30.15 mm, the carapace width from 21.81 to 28.10 mm and the carapace height from 13.75 to 16.52 mm. Body mass ranged from 3.00 to 6.00 g (Table 1). In the frame of nest monitoring, aimed at investigating the breeding success of *T. s. elegans* in this study area, another two yellow-bellied slider *Trachemys scripta scripta* (Thunberg in Schoepff, 1792) were collected manually and measured along the shore of the lake (Table 2). These adult females were inspected by inguinal palpation to detect the presence of oviductal eggs. However, no egg presence was confirmed. As the natural reserve was recently created, all individuals found in the lake were detained to be destined to detention centres as suggested by Macchi et al. (2020) in the national management plan.

This is the first evidence of an egg-laying feral *T. s. elegans* in the Veneto region. A dedicated monitoring program of this exotic species is thus desirable in order to verify whether the recorded breeding event was isolated, or if additional successful reproduction

is occurring. In its native distribution area, *T. scripta* occurs in a wide variety of different climate zones, ranging from humid subtropical to semi-arid climates (Ernst and Lovich, 2009). It is no surprise, then, that *T. scripta* can reproduce in the Mediterranean climate of southern Europe. However, our data provide additional evidence that *T. scripta* may reproduce in colder regions as well. Similar results were observed in central Slovenia where a nest with fertilised eggs has been documented (Vamberger et al., 2012).

Our data also suggests a late deposition clutch, or a clutch produced in a relatively cold fall as the hatchlings were observed in brumation behaviour during the inspection of the nest chamber. According to several authors (Servan et al., 1989; Andreas and Paul, 1998; Rössler, 2000; Novotny et al., 2004) the hatchlings could have hibernated in the nest chamber to emerge in spring. However, during very cold winters the nest might not ensure enough protection and many hibernating offspring may die (Schneeweiss, 2004). Moreover, *T. scripta* eggs that develop in dry incubation substrate generally produce smaller and lighter hatchlings than those in moist substrates (Congdon and Gibbons, 1990; Tucker and Paukstis, 1999). This observation was confirmed by our study, with the hatchlings' weight ranging from 3.00 g to 6.00 g. In their native range, hatchlings weigh between 4.40 g and 10.30 g

Table 1. Morphometric data of the ten *Trachemys scripta* hatched in a lake at the local nature reserve “le Basse del Brenta” (Veneto, Italy) in 2020. Shell measurements in millimetres, body mass in grams. HN = Hatchling number; SCL = Straight carapace length; CW = Carapace width; CH = Carapace height, BM = Body mass; Mean = Arithmetic mean; SD = Standard deviation.

HN	1	2	3	4	5	6	7	8	9	10	Mean	SD
SCL	27.94	27.2	29.57	29.83	29.03	28.61	28.64	28.59	30.15	29	28.85	0.87
CW	23.25	23.8	23.34	25.58	28.1	21.81	21.84	27.63	27.65	23.05	24.60	2.43
CH	15.35	13.75	16.18	13.8	16.22	15.6	15.46	16.52	15.35	15.08	15.33	0.93
BM	4.00	3.00	4.00	4.00	5.00	4.00	5.00	6.00	4.00	6.00	4.50	0.97

Table 2. Morphometric data of the three-adult female *Trachemys scripta* collected in a lake at the local nature reserve “le Basse del Brenta” (Veneto, Italy) in 2020. Shell measurements in millimetres, body mass in grams. SCL = Straight carapace length; CW = Carapace width; CH = Carapace height, BM = Body mass. The nesting turtle was highlighted with an asterisk.

ID	Subspecies	BM	SCL	CW	CH	Geographic coordinates
1	<i>T. s. scripta</i>	744	183.70	144.73	70.01	45.6926°N, 11.6897°E
2	<i>T. s. scripta</i>	884	162.50	134.20	62.05	45.6924°N, 11.6902°E
3	<i>T. s. elegans*</i>	2320	252.20	168.02	98.15	45.6921°N, 11.6902°E

($n = 151$; Tucker, 2000). Moreover, with an average carapace length of 28.85 (SD = 0.87) mm, our records showed that the hatchlings' body size is at the lower end of the species' range. For instance, the carapace length of hatchlings of wild populations in Illinois has been found to range between 25.40 mm to 40.00 mm (Tucker, 2000).

In optimal environments, invasive *T. scripta* can reach large population sizes. The presence of *T. scripta* may potentially alter ecosystem functioning through influencing resource availability, increasing habitat disturbance, or modulating environmental conditions (Hooper et al., 2005). Being omnivorous, slider turtles can also directly affect ecosystems by influencing aquatic food webs on different trophic levels (McCann and Hastings, 1997; Duffy, 2002). For instance, tadpoles of several European amphibians can chemically detect the presence of native predatory terrapins and modify their behaviour to reduce predation risk, but they are unable to appropriately respond to the presence of sliders. Therefore, sliders might capture and consume tadpoles more easily than native terrapins, and thus have a competitive advantage during foraging (Polocavia et al., 2009). In the Veneto region, this might have important consequences for the management and conservation of the endemic *Rana latastei*. Moreover, the release of sliders into natural ecosystems can also increase the risk of transmission of pathogens (such as nematodes and bacteria) to native taxa such as *Emys orbicularis* (e.g., Spinks et al., 2003; Hidalgo-Vila et al., 2009). Verifying the establishment of self-sustaining *T. scripta* populations in the Italian inland water bodies is crucial for implementing proper management activities, including effective eradication programs.

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