



Short communication

Native red foxes depredate nests of alien pond sliders: Evidence from molecular detection of prey in scats



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ABSTRACT

Predation by native species is a chief resistance factor that may counter the spread of alien organisms. Its comprehension plays therefore an important role to assess the impact of biological invasions and implement management. In this study, we show for the first time that red foxes (*Vulpes vulpes*) may depredate nests of alien pond sliders (*Trachemys scripta*). We set our work in a Mediterranean area of Southern Italy where both species are frequent. We observed that red foxes excavated pond slider nests to eat the eggs. We then used a molecular approach to demonstrate the presence of pond slider's DNA in the carnivore's scats, and found that pond slider's DNA occurred in over half of the scat sample collected during the oviposition season. Whether egg consumption by red foxes is widespread rather than only a local response and might eventually lead to population control of pond sliders needs further investigation.

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The impact of native predators on alien species may play a crucial role in countering biological invasions, yet such interactions are a complex, multifaceted process that is still largely unknown (Pintor and Byers, 2015). Observations on this important topic are therefore especially welcome for comprehending the ecology of biological invaders and support the development of effective management actions.

In this study, we present for the first time evidence that red foxes (*Vulpes vulpes*) may excavate and depredate nests of alien pond sliders *Trachemys scripta*. The latter is a freshwater turtle native to Northern and Central America (van Dijk et al., 2011), over many years massively imported as a pet (particularly its subspecies *T. scripta elegans*). Incautiously released by owners into freshwater ecosystems, the pond slider has established many feral populations worldwide (Ota et al., 2004; Ramsay et al., 2007; Ficetola et al., 2009). The species may compete with native turtles for food and basking sites (Salzberg, 2000; Cadi and Joly, 2003; Somma

et al., 2017), feed on aquatic animals and plants (Prévoit-Julliard et al., 2007; Teillac-Deschamps et al., 2009) and carry parasites and pathogens potentially harmful to wildlife (Hidalgo-Vila et al., 2008) and humans (Nagano et al., 2006). The subspecies *T. scripta elegans* is one of the “100 among the worst invasive species” listed by the IUCN Invasive Species Specialist Group (<http://www.iucngisd.org>). In the European Union, the pond slider is as an invasive species of Union Concern according to Regulation no. 1143/2014 of the European Parliament and of the Council of 22 October 2014 on the prevention and management of the introduction and spread of invasive alien species.

Opportunistic predators are especially likely to target alien organisms because their trophic niche reflects fluctuations over time of different food items and unlike specialists (Schoener, 1971) they are less subject to neophobia, exhibiting more audacity towards novel prey (Marples et al., 1998). The red fox, characterized by an outstanding ecological flexibility, is one of such predators (Soe et al., 2017). We set our study in a nature reserve of Southern Italy where pond sliders occur since 1990s and red foxes are common. Although no systematic monitoring of the turtle population is ongoing in the area, the pond slider does not seem to have expanded at least over the last decade (pers. obs.), perhaps because of biotic resistance. The nature reserve staff had reported anecdotally on

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depredation of nests of pond sliders by unknown carnivores, supposedly red foxes. We built on these preliminary observations and tested whether red foxes may prey upon pond sliders, especially its nests. We combined field observations and molecular detection of prey DNA in scats. DNA-based approaches overcome limits of morphological investigation of prey-predator interaction by making it possible to establish species identity for both actors, also from fecal or degraded biological material (Galimberti et al., 2015, 2016).

We carried out our work in the Astroni Crater Nature Reserve (Naples, Italy; 40°50' N, 14°8' E), a 247-ha site managed by the WWF, where the pond slider was first observed in 1995. Turtles occur in a 3.3 ha lake at ca. 10 m a.s.l., at the bottom of a dormant volcano's crater whose top altitude is 253 m a.s.l. The area is covered with deciduous mixed forest dominated by English oak (*Quercus robur*) at lower elevation, red oak (*Quercus rubra*), chestnut (*Castanea sativa*), hornbeams (*Ostrya carpinifolia*, *Carpinus orientalis*, *C. betulus*) and flowering ash (*Fraxinus ornus*) along the crater's slopes, and Mediterranean shrubland on top.

In April – July 2010 and 2016, during pond slider's oviposition season, we searched the entire crater bottom (118 ha) for nests where vegetation allowed access. Intact nests are difficult to spot because females level the soil with their plastron after egg laying so we focused on excavated nests. The predator often lay scats either on, or near the excavated nest. In 2016, we collected all scats morphologically assignable to red foxes retrieved at deposition sites and their surroundings. We placed scats in polyethylene bags and transported them to the laboratory immediately after collection, where they were stored at –20 °C. Although red fox scats are normally easy to recognize, some might be confused with those of domestic dogs of similar size. Free-ranging dogs occasionally occur in the study area, so we adopted molecular techniques to make sure that collected scats belonged to red foxes and then to test whether they contained DNA of pond sliders.

We first selected from previous studies two primer pairs for the specific detection of red fox and pond slider. We chose the primers Vv_F (5'-ACCTCCCGCACCATCAA-3'), and R Vv_R (5'-TGTTGCAATCTGTAGAATAAGGATA-3') targeting exclusively the mitochondrial region of 83 bp length of *Cytb* for red foxes (Knapp et al., 2016) and CO1-TSc-01-F (5'-GGGAAGTACTCGTGCCATTA-3') and CO1-TSc-01-R (5'-GGGCTAAATTTCCGGCTAAT-3') targeting the mitochondrial *CoxI* fragment of 178 bp for pond slider detection (Davy et al., 2015).

We then evaluated the amplification success of CO1-TSc-01-F/CO1-TSc-01-R primer pair for both *T. scripta scripta* and *T. scripta elegans* subspecies. Genomic DNA was extracted from fresh samples (25 mg) employed as positive controls using DNeasy Blood & Tissue Kit (QIAGEN®) following the manufacturer's instructions. Such samples encompassed two eggshells from pond slider nests and two tissue slices belonging to both subspecies. We carried out quantitative polymerase chain reactions (qPCR) following Bruno et al. (2017). We decided to perform a quantitative PCR since this method is more sensitive than the classical PCR visualized through agarose gel electrophoresis. Each 10- μ l reaction included 5 μ l of 2X master mix (EvaGreen SsoFast with Low ROX, BIO-RAD), 0.1 μ l [10 μ M] of each primer, 2 μ l of template and 3 μ l of water (Milli-Q). Thermal profile of PCR was 95 °C for 10' of initial denaturation, followed by 40 cycles of 10" at 95 °C and annealing of 1' at 60 °C. We ran all samples and standards in triplicate, and tested negative (no template) controls in triplicate for each amplification. A dissociation stage followed all essays, resulting in the generation of melting curves. We regarded amplification as positive for the tested species (i.e., red fox and pond slider) when Ct < 38 and Tm = 80 °C at both markers. Ct threshold was calculated from a LOD (Limit of Detection) assay.

We extracted genomic DNA from 200 mg of each fecal sample using the PowerFecal® DNA Isolation Kit (MOBIO) for red fox and

pond slider detection in scats, with a final elution volume of 60 μ l. We tested all egg and fecal samples for the presence of red fox and pond slider DNA using qPCR. Amplification conditions were, as previously described, 95 °C for 10' of initial denaturation, followed by 40 cycles of 10" at 95 °C and final annealing of 1' at 55 °C for *Cyt-b* fragment and at 60 °C for *CoxI* fragment. We ran all samples, standards and no-template controls in triplicate for each amplification. A dissociation stage followed all assays, resulting in the generation of melting curves. In this case too, amplification was considered positive for the tested species when Ct < 38 and Tm = 80 °C for CO1-TSc-01-F/CO1-TSc-01-R and Ct < 38 and Tm of 78 °C for Vv_F/Vv_R.

We found 25 pond slider nests, 24 of which had been excavated. They were located 36.54 m (median value; range 0.2–283.6 m) from the lakeshores and 1.2 m (range 0–9.9 m) from the nearest vegetation edge, in open sites; canopy closure of surrounding trees was on average 15 \pm 28%. The predator often left scats and footprints either on the destroyed nest or in its surroundings, which allowed us to establish it was a red fox. We achieved DNA extraction successfully for all scat samples we tested. None of the negative controls showed any amplification using the CO1-TSc-01-F/CO1-TSc-01-R or Vv_F/Vv_R primer pair. Scat samples' Ct were always < 38. We detected pond slider DNA in 52.3% (23) of the 44 collected scats, all of which confirmed to be from red foxes.

We showed that red foxes feed on eggs of alien pond slider and that this food item is frequent in scats during oviposition. Although red foxes may depredate nests of native turtles both in the fox's native and introduced ranges (Spencer, 2002; Kurz et al., 2011; Dawson et al., 2014), to our best knowledge ours is the first report of native red foxes preying upon an alien turtle. Molecular detection of prey in scats is necessary since the predator digests eggshells due to their proteic nature, so morphological examination of scat content is not a suitable approach to reveal their presence. Although we cannot tell whether molecular traces in scats reflected nest depredation rather than predation on adults or hatched juveniles, the latter is unlikely given the absence of macroscopic turtle remains in scats (pers. obs.).

Our study is preliminary because we only considered one site, where perhaps only one or few foxes performed nest excavation. Future work should establish the magnitude of this impact and test whether complex predator-prey interactions such as hyperpredation (Bate and Hilker, 2012) may lead red foxes to affect also native prey, such as e.g. European ponds *Emys orbicularis*, following the introduction of alternative prey.

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