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Diet of a restocked population of the European pond turtle *Emys orbicularis* in NW Italy

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Abstract. Recently several projects have been implemented for the conservation of the European turtle *Emys orbicularis*, but few aspects of the captive-bred animals released into the wild have been described. In this note we report about the trophic habits of a small restocked population of the endemic subspecies *E. o. ingauna* that is now reproducing in NW Italy. Faecal contents from 25 individuals (10 females, 11 males and 4 juveniles) were obtained in June 2016. Overall, 11 taxonomic categories of invertebrates were identified, together with seeds and plant remains. Plant material was present in 24 out of 25 turtle faecal contents, suggesting that ingestion was deliberate. There were no differences between the dietary habits of females and males, and the trophic strategy of adult individuals was characterised by a relatively high specialization on dragonfly nymphae. These findings suggest that captive bred turtles are adapting well to the wild and that restocked individuals assumed an omnivorous diet, a trophic behaviour typical of others wild turtle populations living in similar habitats.

Keywords. Captive-breeding, food habits, freshwater turtle, omnivory, restocking

In Europe the populations of the pond turtle *Emys orbicularis* (Linnaues, 1758) are declining and in many countries the species is considered threatened or even locally endangered (Fritz and Chiari, 2013). Therefore, many conservation projects have been implemented to improve the species' status, in particular by restocking captive-breed individuals, for example in Portugal (Teixeira et al., 2013), France (Cadi and Miquet, 2004; Thienpont et al., 2013) and Spain (Ayres et al., 2013). The outcomes of these restocking projects are monitored by means of different descriptors, and in particular by the estimation of the annual survival of captive-bred individuals released into the wild (Cadi and Miquet, 2004; Masin et al., 2015; Canessa et al., 2016) or by the extent of individual displacements and habitat use obtained by radiotracking (Mignet et al., 2014). In general, however, few data are available on the ecological adaption of captive-bred animals to their new environment. To assess this topic, we focused our interest on the feeding habits of a restocked population, an issue widely studied in wild individuals (e.g., Fritz, 2001; Ottonello et al., 2005; Zuffi et al., 2011; Balzani et al., 2016). In fact, captive bred animals are usually fed with commercial pellets, or with fresh or frozen food like fishes, shrimps, chironomidae (non-biting midges) and tenebrionidae (darkling beetles) larvae. Hence information about the feeding habits of captive-bred individuals after their release in the wild is of fundamental interest when evaluating their adaptation to their new environment. In this context, we use as case study a small restocked population of the endemic subspecies *E. o. ingauna* found in Liguria, NW Italy (Jesu et al., 2004). These individuals are part of a conservation project, that began in 1999 and is still ongoing (Ficetola et al., 2013; Canessa et al., 2016). Restocked animals were born in an outdoor facility (i.e., the “Centro Emys” situated in Leca di Albenga, NW Italy) from local genetically screened adults (Manfredi et al., 2013). Turtles were bred in the facility for 2-5 years until restocking; they were fed with commercial pellets in the first year, then with frozen shrimps and fish. Before restocking, all animals were marked by both scute notches (Servan et al., 1986) and subcutaneous pit tags, while their health status was evaluated by screening for blood and gastrointestinal pathogens in accordance to veterinarian protocols (Canessa et al., 2016).

Turtles were sampled in two semi-permanent clay ponds in the alluvial plain of the river Centa, in the Province of Savona (Liguria, NW Italy). For conservation purposes we are not giving exact localisations of the sites. In one pond, native turtles are still found, but the other pond was excavated in 2009 and hosts only restocked animals. In the surroundings of this latter site, three turtle nests with successfully hatched eggs were observed in November 2017 (Dario Ottonello, pers. obs.), giving the first evidence of reproduction in the wild of this restocked population.

Turtles were captured by unbaited fyke nets, from the 25th to the 26th of June 2016, and transported to the “Centro Emys”, situated less than 2 km from both sites, where they were checked for sex and their straight carapax length (SCL) measured. Only individuals with evident sexual characters were considered as adults (Zuffi and Gariboldi, 1995), while the others were considered as juveniles. Faecal samples were obtained from turtles kept in individual buckets for 24h. Then, samples were filtered on a sieve and prey remains stored in 70% ethanol. Prey items were identified under a dissecting microscope in the laboratory by at least two observers (AR; SS; MV), while plant remains could not be assigned to species or genus because they were highly fragmented. All turtles were returned to their capture site and no mortality was observed during the study.

The diet of female and male turtles was analysed by means of the frequency of occurrence (FO), defined as the proportion of faecal samples containing that category divided by the entire sample, by Shannon's diversity index (H) and by the Equitability index (H/H_{max}), this latter expressing how prey items are distributed among prey categories (Magurran and Gills, 2011). Therefore, we analysed differences between sexes, with and without plant material, by means of the analysis of similarity (ANOSIM) using Bray-Curtis dissimilarity index (Clarke, 1993). The population trophic strategy was estimated by the graphical method of Amundsen et al. (1996). This method projects each prey category on a plane delimited by an X axis which is given by the FO, and an Y axis represented by the prey-specific abundance (P_i). Prey-specific abundance is the percentage of each prey category *i*, considering the total number of prey items ingested only by those animals that ate that specific category (Amundsen et al., 1996; Ottonello et al., 2017). The distribution of prey categories in the plot describes the main trophic strategy of the focal population, as being specialised (upper quadrants of the graph) or generalist (lower quadrants) and with a high between (upper left quadrant) or within (lower right quadrant) population component (see Fig. 3 in Amundsen et al., 1996). Statistical analyses and ecological indexes were obtained by means of PAST software (Harper and Ryan, 2001).

During the study, 26 European pond turtles were captured: 10 females, 12 males and 4 juveniles, but no faecal contents could be obtained from one male (Table 1). Females were larger (mean SCL = 109.26 mm ± 21.89 SD) than males (mean SCL = 106.20 mm ± 7.00 SD), this difference being not significant (Welch test for unequal variances t = -0.44; P = 0.67). Overall, 11 invertebrate categories were found in the faecal contents of the pond turtles: 5 in females, 7 in males and 7 in juveniles (Table 1). Seeds and plant remains (mainly root and leaf fragments) were found in 23 out of 24 faecal contents, suggesting that ingestion was deliberate. Concerning animal items, all of them were aquatic with the exception of two ants, probably captured on the water surface. The most frequent prey categories were composed by dragonfly (Odonata) nymphae and fly larvae, that were mainly non-biting midges (Chironomidae). No vertebrate remains were found in the faecal contents of the study sample.

Shannon's diversity and Equitability indexes did not differ between females and males (Table 1: Shannon H, P = 0.09 and Equitability H/H_{max}, P = 0.08, both values obtained after 1000 permutations). Moreover, there were no differences in diet composition between females and males considering animal categories alone (ANOSIM, r = 0.02, P = 0.29) or when vegetal matter was included in the analysis (ANOSIM, r = - 0.04, P = 0.74). On the basis of these results, the trophic strategy of the adult population was examined after pooling females and males together (Ottonello et al., 2017). Figure 1 shows that the turtle population assumed a generalist diet for all categories, with the exception of dragonfly nymphaea, that were consumed by several relatively specialised individuals.

Our study highlights two main findings, the first that the overall dietary habits of captive-bred female and male *E. o. ingauna* released in the wild were similar, the second that adult and juvenile turtles behaved as omnivorous feeders, switching between a carnivorous and herbivorous diet within the same site and within a relative short time period. Concerning the absence of sex differences in diet, other studies found low variation between the adult male and female pond turtles. For example, Cicek and Ayaz (2011) and Ottonello et al. (2017) observed similar diets between the sexes of *Emys orbicularis* in Turkey and of *Emys trinacris* in Sicily, respectively. According to these authors, correspondence in food habits between sexes is explained by a similar use of microhabitats, an explanation that may by realistic also for the *E. o. ingauna* population analysed in this study.

In reptiles, herbivory tends to evolve in environments where food quality and quantity fluctuate temporally and spatially (Cooper and Vitt, 2002), as is the case in Mediterranean ponds, that are characterised by high seasonal variations in water level and water temperature. Also the diet of juveniles' captive-bred turtles appeared to contain many vegetable remains, but our small sample size hindered any quantitative comparison with the adults.

Another interesting result was that adult turtles seemed relatively specialised on dragonfly nymphae, a feature that could be due to the high availability of this kind of prey in the environments or to an overestimation bias due to the chitinous structure of their mouth parts, a feature that may facilitate retrieval and identification of this taxon in faecal contents. In any case, only sampling the potential prey community available to the turtles will provide information to better understand this issue.

The contemporary presence of plant and animal material in the diet of *E. o. ingauna* is not peculiar to this population, considering that it has already been described in other European pond turtle populations living in Mediterranean regions of Central Italy (Lebboroni and Chelazzi, 1991), Southern France (Ottonello et al., 2005), Northwestern Spain (Ayres et al., 2010) and Turkey (Cicek and Ayaz, 2011). Moreover, Ottonello et al. (2017) found plant remains in the congeneric *Emys trinacris* from Sicily, suggesting a widespread use of vegetation as supplementary food in turtles belonging to the genus *Emys*.

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Table 1. Prey categories, number of prey items, frequency of occurrence (FO in percentage) and diversity indexes for *E. o. ingauna* females, males and juveniles.

	Females (n =10)		Males (n = 11)		Adults total (n = 21)		Juveniles (n = 4)	
	Number	FO	Number	FO	Number	FO	Number	FO
Odonata nymphs	22	0.80	21	1.00	43	0.90	1	0.25
Diptera larvae	2	0.20	9	0.27	11	0.24	10	0.50
Heteroptera nymphs	1	0.10	3	0.18	4	0.14	0	-
Ephemeroptera Iarvae	0	-	1	0.09	1	0.05	4	0.75
Coleoptera adults	0	-	4	0.36	4	0.19	0	-
Plecoptera larvae	0	-	0	-	0	-	1	0.25
Imenoptera Formicidae	1	0.10	0	-	0	-	1	0.25
Tricoptera larvae	0	-	0	-	0	-	1	0.25
Oligochaeta	0	-	1	0.09	1	0.05	0	-
Isopoda	0	-	1	0.09	1	0.05	0	-
Gastropoda	1	0.10	0	-	1	0.05	0	-
Hexapoda indet.	2	0.20	1	0.09	3	0.14	2	0.50
Shannon Diversity (H)	0.927	-	1.375	-	1.356	-		
Equitability (H/H_{max})	0.517	-	0.707	-	0.589	-		
Seeds	152	0.60	126	0.36	278	0.48	9	0.75
Plant fragments	122	1.00	95	0.82	217	0.90	26	1.00

Figure Legends

Fig. 1. Amundsen's plot showing the trophic strategy of the *Emys orbicularis ingauna* restocked population. FO = frequency of occurrence, P*i* = prey-specific abundance (see text for definitions).

Fig. 1