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ORIGINAL ARTICLE

Factors driving semi-aquatic predator occurrence in traditional cattle drinking pools: conservation issues

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ABSTRACT

In several cases, human impact on water bodies and on their freshwater communities is detrimental, but in some cases the human activity may favour and enhance the biodiversity of small water bodies, as traditional cattle drinking pools. Despite their small size, small water bodies may constitute hot spot of biodiversity often representing the only lentic aquatic biotope in landscapes where superficial water lacks or flows in lotic environments like creeks and streams. Predators are good indicators of biodiversity in ponds and give information of food chain web complexity. In particular, semi-aquatic predators like amphibians and dragonflies may account for a substantial percentage of energy flow between aquatic and terrestrial ecosystems. In this study, we evaluated the conservation value of traditional cattle drinking pools building by assessing the factors determining the occurrence and distribution of the semi-aquatic predators. From April to August 2015, we investigated 30 distinct pools recording several abiotic and biotic environmental variables. We detected 4 semi-aquatic predators: Salamandra salamandra *larvae,* Triturus carnifex, Aeshna *sp. larvae and* Libellula *sp. larvae. Abiotic features played a major role in shaping the predator community that resulted linked to stable, with no dryness period, and large drinking pools. Invertebrate prey biomass was not particularly important, while vegetation cover and occurrence of unpalatable tadpoles were the most important biotic features of the pools. Our study provides novel evidence on the importance of cattle drinking pools management to preserve biodiversity especially in areas where traditional pastoral activity is disappearing.*

Key words: Pool; biodiversity; conservation; freshwater salamander; dragonfly.

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INTRODUCTION

Freshwater biodiversity is suffering a worldwide decline (Wood *et al.*, 2003; Smukler *et al.*, 2010; Vanderplank *et al.*, 2014) and too many organisms are currently facing high risk of extinction: from native crayfish to eels, salmons and hundreds of other less known animals and plants (Beebee and Griffiths, 2005; Denoel and Ficetola, 2008; Manenti *et al.*, 2014).

Smaller waters may be of great importance for biodiversity conservation. Species which are confined to small inland water bodies (*e.g.*, freshwater bivalves) cannot move simply between different pools of the same area or between different areas or catchment basins; therefore inland water bodies may be characterized by high levels of endemism or local genetic differentiation of freshwater species or populations (Downing, 2005). Thus, they are fragile ecosystems and their alteration may lead to the loss of entire populations and consequently to the dramatic loss of important genetic biodiversity (Janse *et al.*, 2015). Besides obligate aquatic dweller organisms (*e.g.*, aquatic crustaceans), small water bodies like ponds and springs host also many 'terrestrial' species that, more or less occasionally, feed in them (*e.g.*, water birds) and a high variety of semi-aquatic plants (*e.g.*, flooded woods or different riparian plants associated with the slopes of the water bodies) and animals (*e.g.*, beavers or amphibians). These semi-aquatic organisms play very important roles in driving and regulating the biomass flux between the terrestrial and the aquatic ecosystem and vice versa (Reinhardt *et al.*, 2013).

The flux of biomass and nutrients across neighbouring systems is a well-known property of most natural ecosystems (De Toledo *et al.*, 2009; Larson *et al.*, 2015). Neighbouring habitats, including small waters and their surrounding landscapes, are linked by fluxes of biomass and nutrients that can feed plants and other primary producers (Hocking and Reynolds, 2011), herbivores and aquatic predators (Reinhardt *et al.*, 2013). These fluxes through ecosystems may consistently affect the direct and indirect inter- and intra-specific interactions in the food chains (Piovia-Scott, 2011) and can contribute to the stability of the food web both in terms of composition and phenology (Huxel *et al.*, 2002). Therefore, any change in the dynamics of one ecosystem is likely to influence the structure and stability of adjacent ecosystems. A major role in maintaining the fluxes between aquatic and terrestrial habitats is played by semi-aquatic organisms and especially semi-aquatic predators (Sih, 1988; Zabala *et al.*, 2003).

Predators regulate populations of some aquatic invertebrates (Ranvestel *et al.*, 2004) and strongly affect the structure of the freshwater communities. In particular, semi-aquatic predators are predicted to affect the energy flow between aquatic and terrestrial habitats by consuming aquatic prey biomass during the larval phase and exporting biomass from aquatic to terrestrial habitats when metamorphosing. Thus, they directly provide energy at the predator trophic levels of the terrestrial habitat. Moreover, predators are really good indicators of the diversity and richness of the aquatic trophic web of small waters (Regester *et al.*, 2008; Kovac and Krocke, 2013). The occurrence and the population dynamics of small waters' semi-aquatic predators may be influenced by breeding phenology, cannibalism, predation, environmental heterogeneity and human disturbance (Limongi *et al.*, 2015). Although well studied, semi-aquatic predators are rarely examined at community level and in a conservational perspective. Understanding factors determining their occurrence is of primary importance to establish proper conservation managements. Salamanders and dragonflies are semi-aquatic predators that may account for a substantial percentage of energy flow between aquatic and terrestrial ecosystems since they may reach high abundances and their acquired energy is well transferred to other food web levels (Regester *et al.*, 2006, 2008). Salamanders and dragonflies show complex life cycles with aquatic larval stages and terrestrial adult stages and are an assorted component of many temperate terrestrial and aquatic ecosystems (Bulankova, 1997; Wells, 2007). Adult salamanders and dragonflies breed in freshwater environments where they deposit eggs or larvae, transferring part of the energy acquired in terrestrial habitats to the aquatic ones. Salamanders and dragonflies larvae are generally dominant aquatic predators where fish lack as it is often the case in small waters. Large salamander larvae may prey upon small dragonfly larvae and vice versa larger dragonfly larvae are often predators of salamander larvae; moreover, salamander larvae may be cannibalistic. Both dragonflies and salamanders larvae are considered keystone predators of small waters.

In several cases, human impact on fresh waters and on their communities may be detrimental (Triebskorn *et al.*, 2003; Revenga *et al.*, 2005) because of pollution and habitat destruction. However, in some cases the human activity may favour and enhance the biodiversity of small waters as in the case of traditional cattle drinking pools (Canessa *et al.*, 2013; Manenti *et al.*, 2013a). Despite their small size, they may constitute a hot spot of biodiversity often representing the only lentic aquatic biotope in landscapes where superficial water lacks or flow in lotic environments like creeks and streams. A growing body of evidence suggests that small ponds significantly contribute to the biodiversity of the entire landscapes (Razgour *et al.*, 2010; Stahlschmidt *et al.*, 2012), but few studies were made on the role played by cattle drinking pools on mountain landscapes. In this study, we evaluated the conservation value of these traditional pools by assessing the factors determining the occurrence and the distribution of the semi-aquatic predator community.

METHODS

Study area

The study area is situated in Northern Italy (Lombardy) in the Prealps and includes the western slopes of the Canto Alto Mountain in the low Brembana valley (lat.: 45.77 N long.: 9.66 E). Altitude ranges between 300 and 1000 m asl. The area is characterized by broadleaf mixed woods surrounded by grazing pastures. Pastures are traditionally managed and characterized by seasonal cattle grazing and traditionally several pools have been built in the area for cattle drinking. Some of them are filled by rainwater, while other have been realised catching already occurring springs. The other aquatic biotopes occurring in the study area are mainly formed by the springs that form streams and creeks tributary of the Brembo River.

Surveys

From April to August 2015 we performed extensive surveys of the area to localize cattle drinking pools. Every site was than surveyed at least 3 times (average 3.4 times, maximum 7 times). During each survey, we assessed the occurrence of the semi-aquatic predator species, in particular the occurrence of fire salamander larvae (*Salamandra salamandra* (Linnaeus, 1758)), of breeding adults and larvae of the Italian crested newt (*Triturus carnifex* (Laurenti, 1768)), and of dragonfly larvae of *Aeshna* sp. and *Libellula* sp.

All these organisms are generalist and opportunist top predators in small lentic habitats (Corbet, 2004; Wells, 2007). Fish or other top predators were not found in the study sites.

We recorded four abiotic features describing pool morphology and structure, which can be important for the occurrence of semi-aquatic predators: maximum area; maximum depth; average maximum illuminance at the middle hours in sunny days using a luxmeter PCE_M883; stability (absence of summer dryness). We recorded five biotic features of the sites: the biomass of potential invertebrate prey, the occurrence of aquatic vegetation, the percentage of cover of riparian vegetation and the occurrence of palatable and unpalatable tadpoles. The occurrence of palatable and unpalatable tadpoles was recorded by extensive deep netting of the pools and through tadpole recognition. Tadpoles of *Rana temporaria* Linnaeus, 1758 were considered palatable, while those of *Bombina variegata* (Linnaeus, 1758) and *Bufo bufo* (Linnaeus, 1758) unpalatable. In fact, *B. variegata* shows epidermal ven-

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omous glands already at the larval stage (Ambrogio and Mezzadri, 2014) and the toxic effect of *B. bufo* tadpoles has been reported (Gunzburger and Travis, 2005).

The percentage of riparian vegetation cover was estimated by considering a strip of 1 meter along the pool perimeter and assessing the presence of bushes at each meter of length. The biomass of the invertebrate prey was assessed using pipe sampling technique (Dodd, 2010). Samples were collected by thrusting a 0.20 m^2 circular pipe sampler through the water column and about 5 cm into the substrate. In all cases, the top of the sampler was above the water level. We used small nets (mesh size: 1 mm) to collect all animals from the sampler (Werner *et al.*, 2009; Dodd, 2010). We collected about one sample/5 m^2 (average: three samples per pool). Invertebrates were identified following standard keys (Ghetti, 1997), weighed with a G&G TS-B+G precision balance (precision 0.01 g) and immediately released.

Statistical analyses

A site is confidently 'occupied' if a species is detected at that site, but the lack of detection of a species during all sampling occasions does not necessarily mean that the species is absent (Mackenzie, 2006). This can lead to an underestimation of occupancy and might influence the results of analyses, increasing the risk of data over-interpretation, with type II errors being potentially significant. We used Presence 5.5 (Hines, 2006) to assess the probability of detection per visit. We used a series of constrained redundancy analyses (RDA) to evaluate the relative role of biotic and abiotic features on the multivariate structure (*i.e.*, species composition) of predator community. RDA is a canonical analysis, combining the properties of regression and ordination techniques, that allows evaluation of how much of the variation of one dataset structure (*e.g.*, community composition in a stream; endogenous dataset) is explained by independent variables (*e.g.*, habitat biotic and abiotic features; exogenous datasets) (Borcard *et al.*, 2011). We performed two RDAs using the vegan package (Oksanen *et al.*, 2005). In the first one, we considered as exogenous matrix one matrix composed of the abiotic environmental features, and we used the matrices of predator occurrence as endogenous. In the second one, we used the same endogenous matrix and we considered as exogenous the matrix composed by biotic features. We calculated the significance of explained variance by performing ANOVA-like permutation tests (10,000 permutations) (Borcard *et al.*, 2011).

We also used generalized linear mixed models (GLMMs) assuming binomial error distribution to relate presence/absence of predator species in each pool to the recorded variables. We built models representing all the possible combinations of independent variables, and we considered the model with the lowest AICc (Akaike Information Criterion) as the 'best AIC' model (Rolls, 2011). Models explaining the highest proportion of variation using the smallest number of predictors have smallest AICc values and are considered to be the 'best models'. As AICc may select overly complex models, we considered a complex model only if it showed AICc less than the AICc of all of its simpler nested models (Richards *et al.*, 2011). We calculated the Akaike weights, wi (AICc weights), representing the probability of the different models given the data (Lukacs *et al.*, 2007). All the models were checked for variance inflation factor (VIF). Variance inflation factor measures the impact of collinearity among the variables in a regression model; we considered models with a VIF value <5. Subsequently, for each variable we summed the AIC weights of all the models in which the variable was included, to obtain the probability for each variable to be included in the best model (Burnham and Anderson, 2002). We assessed significance of variables composing the best model using a likelihood ratio test; we performed all statistical analyses in the R 3.2.1 environment (R Development Core Team, 2013).

RESULTS

We investigated 30 distinct pools. The most common predator was the fire salamander *Salamandra salamandra;* its presence was recorded in 73.3% of the surveyed sites (occurrence, O=73.3%). The occurrence of the other predators was: for *Aeshna* sp. larvae 46.7%, for *Libellula* sp. larvae 16.7% and for the Italian crested newt 10%. Detection probability was high and the misdetection rate was <0.2 for all predators.

The cattle drinking pools average area was 17.27 [Standard Error (SE)=3.42] m^2. We recorded a diversified invertebrate community in the pools with 18 different invertebrate *taxa* including detrivorous, erbivorous, mesopredators, filter feeders and decay-feeders. On average the prey biomass for sampling was 0.03 (SE=0.01) g corresponding to 65.26 (SE=12.6) average prey items per sampling with average pools' prey biomass of 1.22 g m^{-2}.

Semi-aquatic predators community structure was significantly related to both abiotic and biotic habitat features (both permutation tests: P<0.01). The relationship between predators and abiotic features explained 35% of variation. The first RDA axis was represented by the gradient between small temporary (with dryness period) water bodies to large and stable ones (without dryness period), while the second axis was represented by pool illuminance level (Fig. 1). *S. salamandra* was strongly associated to stable and shady pools. The same combination of factors explain the presence of the *Aeshna* larvae, even though this link is weaker. *Libellula* dragonflies were associated to luminous and stable pools and *T. carnifex* to large and stable ones.

The relationship between pools biotic features and predators community explained 38% of the variation. The first axis was represented by aquatic vegetation occurrence while the second by unpalatable tadpoles (Fig. 2). *T. carnifex* and *Libellula* sp. were positively related to the abundance of the aquatic vegetation, while *S. salamandra* and *Aeshna* sp. larvae to unvegetated pools without unpalatable tadpoles.

The preference of every predator taxa was confirmed by the GLMMs analysis (Tab. 1). All the species showed significant association to larger pools.

DISCUSSION

In this study, we evaluated the distribution of top predator taxa in cattle drinking pools in order to assess the status and importance of these artificial habitats in an area where other lentic water bodies are scarce. We recorded different abiotic and biotic parameters including, as novel approach, the occurrence of palatable and unpalatable tadpoles. Our results indicate that small water body features, both biotic and abiotic, drive the community composition of the aquatic predators revealing a clear connection to their human management and conservation. In particular, even though all recorded predators were semi-aquatic and lived in water only at larval stage, they clearly avoided temporary pools. In hilly – mountain landscapes, where lentic pools generally lack, pastoral activity through the building of cattle drinking pools is the main source of lentic water bodies (Canessa *et al.*, 2013). The first sign of decay of these pools is their drying up in the warmest seasons; they become temporary, unstable and are rapidly filled by terrestrial sediment with a reduction of the pool area (Brusa *et al.*, 2011). This is mainly due to the substrate of the pools that, without managing, stops to be impermeable and let the water to filter away. The predator community was linked to larger and stable water bodies. Temporary pools were particularly avoided by fire salamander and *Aeshna* dragonflies while all species preferred larger pools. Odonata larval stages may last longer than the amphibian ones and this may explain the preference of dragonfly for more stable and larger pools, even if *Libellula* larvae are able to survive also in drying ponds (Rebora *et al.*, 2007). These results underline the importance of a proper management and pool status conservation to maintain the biodiversity and the complexity of the food web inside them. With the decreasing of the tradi-

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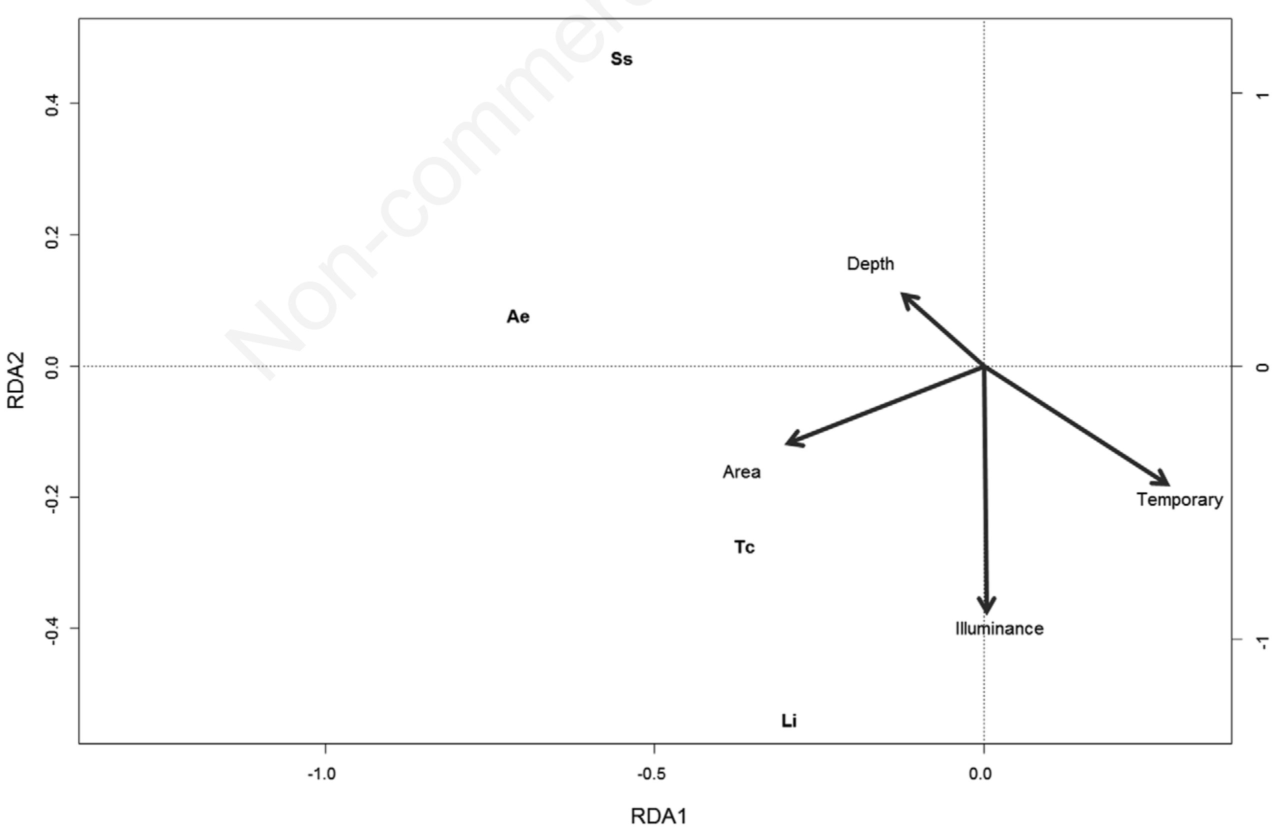

Fig. 1. Results of constrained redundancy analysis showing the relationship between habitat abiotic features and predators distribution. Grey arrows represent constraining variables. Ss, *Salamandra salamandra*; Tc, *Triturs carnifex*, As, *Aeshna* sp; Li, *Libellula* sp.

tional pastoral activity and the land abandonment, these biotopes risk to loss their function and disappear in the short -medium term.

Among biotic variables, we found scarce effect of invertebrate prey abundance, while a major role was played by vegetation cover that reflects ponds illuminance. This factor is negatively linked to *S. salamandra* occurrence and positive linked to *T. carnifex* and *Libellula* sp. The crested newt is known to choose vegetated sites for breeding as eggs are usually laid around the aquatic plants (Crucitti *et al.*, 2010; Ficetola *et al.*, 2010; Cinquegranelli *et al.*, 2015). *Libellula* sp. larvae, as confirmed by our results, often de-

Tab. 1. Results of GLMMs analysis showing the variables included in the best model selected on the basis of AIC weigh explaining the distribution of each species.

Species	Variables in the best model	B	X^2	P
Salamandra salamandra	Illuminance	-2.5	3.2	0.07
	Area	3.2	5.03	0.02
	Temporary	-7.0	12.2	<0.001
	Aquatic vegetation	-3.8	4.62	0.03
Triturus carnifex	Area Aquatic vegetation	7.5 8.9	6.7 9.98	0.01 <0.01
Aeshna sp.	Area	2.92	7.8	<0.01
	Temporary	-3.7	6.42	0.01
Libellula sp.	Illuminance Area Aquatic vegetation	3.3 4.3 4.2	4.1 6.0 5.5	0.04 0.01 0.01

GLMM, generalized linear mixed model.

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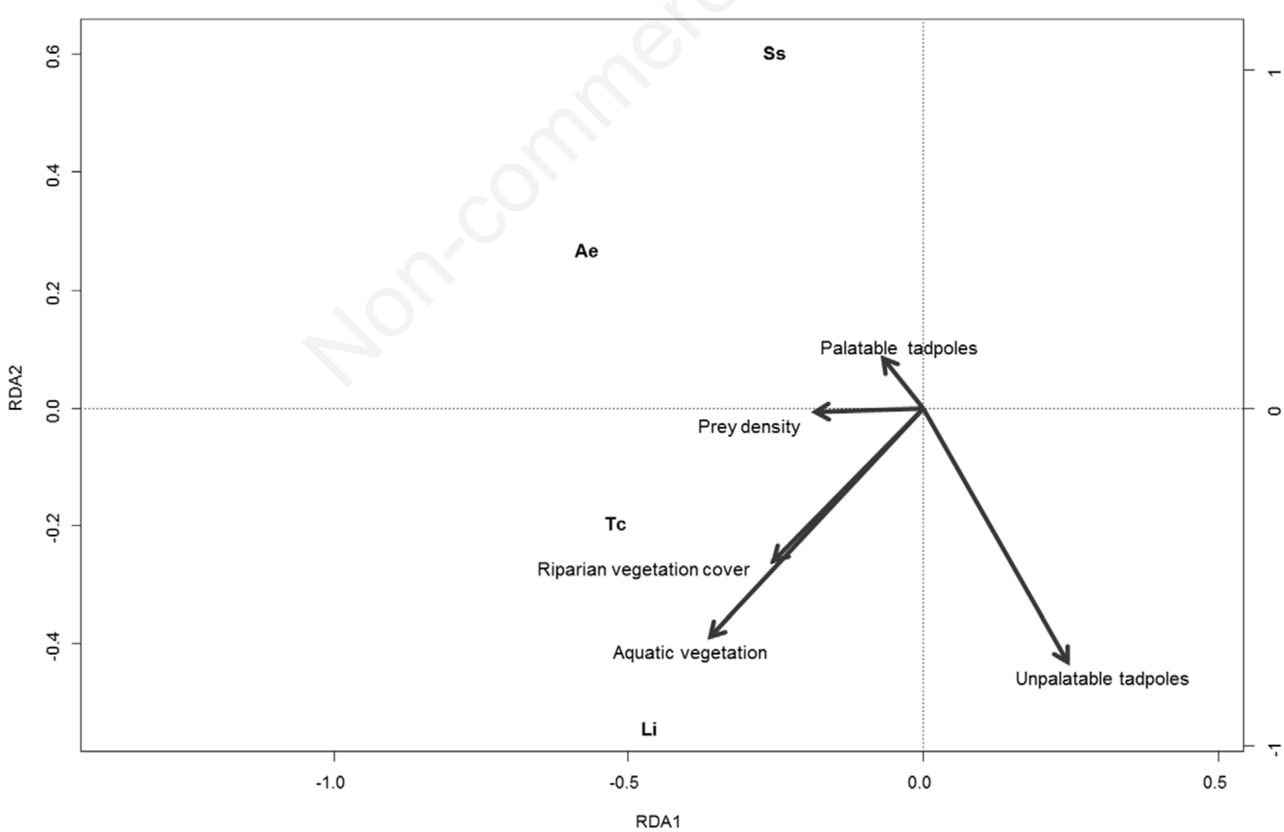

Fig. 2. Results of constrained redundancy analysis showing the relationship between biotic habitat features and predators distribution. Grey arrows represent constraining variables. Ss, *Salamandra salamandra*; Tc, *Triturs carnifex*, As, *Aeshna* sp; Li, *Libellula* sp.

velop in ponds well exposed to sunlight (Moore, 1987; Wissinger, 1989) even if aquatic plants may provide shelters for successful 'sit and wait' foraging of the *Libellula* larvae. Moreover, we observed that predators generally avoid pools where unpalatable tadpoles are present even if this relationship is not particularly strong, according to the RDA. In general it is considered more likely for salamanders to taste non palatable amphibian tadpoles than for insect predator larvae (Gunzburger and Travis, 2005) although a study found that *Aeshna* larvae avoid pools where *B. bufo* tadpoles are present (Henrikson, 1990).

In the analyzed small water bodies, the fire salamander is the most common predator. Usually *S. salamandra* breeds in lotic environments like creeks and streams and its presence in lentic pools is of particular relevance. Fire salamanders usually lay gilled larvae in epigeous running waters (Manenti *et al.*, 2009) even if populations have also specialized in using other aquatic habitats: epigeous stagnant waters such as ponds (Caspers *et al.*, 2009) and hypogeous (*i.e.*, subterranean) springs or pools (Manenti *et al.*, 2013b). The colonization of pools occurs when lotic habitats are nearby and can be extremely localised (Denoël and Winandy, 2014).

CONCLUSIONS

The good distribution of the top predators that we detected in cattle drinking pools reflects the diversification of the food web chain that can be achieved in these habitats (Bulankova, 1997). Our results underline that the conservation of these small artificial lentic water bodies is a major determinant for preserving the biodiversity of the area. The context of our study area is particularly favorable for studying the ecological role of artificial lentic habitats because the valley slopes and structure allow the existence of only rare natural ponds and standing waters. Although human activities are often detrimental for biodiversity survival, in some cases it can favor some natural processes and benefit some organisms (Canessa *et al.*, 2013). This is the case of traditional cattle drinking pools and both short- and long-term actions should be established to conserve these aquatic environments.

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