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RAPID COMMUNICATION

Vanishing herpetofauna: 30 years of species relaxation in a wetland remnant of the Po plain (Northern Italy)

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Abstract An analysis of a 30-year dataset (1985–2014) concerning a herpetofauna assemblage studied in a site of conservation concern (Northern Italy) is reported with the aim of evidencing a trend in species richness which may reveal the most sensitive ones (i.e., the first to go extinct locally). Our results point to a progressive loss of species (nested species relaxation) which were not replaced over the study period and consequently to a decline in species richness. Among the amphibians, two thresholds in mean species richness were evident in the mid and late 1990s, respectively, whereas reptiles showed a significant threshold only at the end of the 1990s. An inductive hypothesis to explain the abrupt relaxation in species richness in the mid and late 1990s may involve a combination of "pulse" or "press" perturbations at different scales. Moreover, although our long-term standardized study suffers from some limitations, our data clearly indicate a different species-specific sensitivity which could be useful to identify focal species of conservation concern. However, further comparisons with data set collected from long-term and wide-ranging studies are needed to confirm our temporal thresholds, in order to infer a more general model from this local pattern.

Keywords Long-term data set · Species richness · Thresholds · Relaxation · Sensitive species

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Introduction

The great flood plain of the Po river (Northern Italy) is one of the most densely populated European areas (Romano and Zullo 2015). After the end of World War II, this large region was land reclaimed and transformed, as a consequence of major industrial development and changes in agricultural economy which today are reflected by the intensive agricultural matrix characterizing the entire plain of the Po River (Provolo and Riva 2003). Research carried out in this area over the last decades has evidenced a decline and collapse of many biological groups (Groppali and Riccardi 2005). In particular, due to profound landscape transformations, many wetlands have become highly isolated and, consequently, faunal assemblages have experienced local extinctions (Ghezzi and Groppali 1987; Schiavo 2001; Crottini and Andreone 2007).

In this short communication, we report data from a long-term study (1985–2014) carried out in a site of conservation concern located in the plain of the Po River which was based on a standardized sampling design. Starting from the pattern of presence/absence of amphibians and reptiles, our aim was to analyze the general trend in richness at the assemblage level over a time span of three decades, emphasizing the progressive loss (local extinction) of species through a so-called process of nested species relaxation (Diamond 1972), i.e., small communities form proper subsets of large communities, so reflecting a non-random process of progressive species loss and disaggregation of assemblages after long-term environmental changes (Ulrich and Gotelli 2007; Baselga 2010). Moreover, we aimed at highlighting temporal thresholds suggesting causal hypotheses at different scales (global, regional, local; see Gaston and Blackburn 2000). To our knowledge, this is the first study carried out on such a topic of long-term environmental change in the Mediterranean region. Indeed, although many long-term monitoring projects have been carried out (e.g., ILTER: Kim 2006), research on progressive changes in species assemblages on large time spans (i.e., >20 years) is still scanty (Dennis and Taper 1994; Semlitsch et al. 1996; Meyer et al. 1998; Kaiser 2008; Schriever et al. 2009; Reading et al. 2010).

Methods

Study area

The "Lanca di Gerole" nature reserve is a Special Area of Conservation (code SCI IT20A0013 sensu 92/43/CEE "Habitat" Directive and code ZPS IT20A0402 sensu 79/409/CEE and 2009/147/EC "Bird" Directive; 476.1 ha) located in the Cremonese flood plain (Municipality of Motta Baluffi and Torricella del Pizzo; central sector of the Po plain; Supplementary materials S1). The reserve presents a stretch of open river flood plain where a patchy succession of hygrophilous river vegetation occurs (further details in Ferri and Soccini 2001).

Protocol and data analysis

From 1985 to 2014, a standardized and representative field study covering the entire study area was carried out (long-term monitoring; Magurran et al. 2010). The area was considered as a single sample unit ("Lanca di Gerole" wetland mosaic). A total of 16 permanent 250-m-long linear transects were randomly located and the distance among transects varied from 50 to 150 m. Data on sampling occurrence of amphibians and reptiles were collected with standardized methods (Heyer et al. 1994; Elzinga et al. 2001). As far as amphibians are concerned, visual encounter survey (V.E.S.) and audio encounter survey (A.E.S.) sessions (Heyer et al. 1994) were conducted from March to September in each transect, with an average of six visits per year for visual counts and one visit per year for audio counts. With regard to reptiles, we conducted time-constrained systematic sampling survey (S.S.S.) sessions (Dood 2010), with the goal of finding as many individuals and species as possible, mainly searching animals in suitable key habitat structures (Tews et al. 2004). Each visit to the 250-m-long transect was 45–60 min long. Sampling was carried out by a single researcher (VF), in order to exclude any inter-observer bias due to different detection by different observers.

Over 30 years, we carried out a total of 182 transect visits (total 588 km). Concerning the taxonomic diagnosis, we refer to Lanza et al. (2007) for amphibians and to Corti et al. (2011) for reptiles. Only native species were taken into consideration for data analysis (excluding *Trachemys scripta*, firstly recorded in 1998, and *Pelophylax kurtmuelleri*, firstly recorded in 2012; Uzzell et al. 2009) obtaining the sampling occurrence at species level for each assemblage (amphibians and reptiles), i.e., the yearly presence of at least an individual of a species in at least one transect (Hayek 1994; Schmidt 2003). We only considered occurrence data because, apparently, in elusive and hardly detectable taxa, a relatively high and unquantified error rates can negatively affect the abundance trends for which most studies were designed, mainly over long periods of time (Lotz and Allen 2007).

A value of species richness (i.e., number of species recorded/year) at the level of the entire assemblage and, separately, for amphibians and reptiles was obtained. Then, data were divided into 5-year sub-periods thus obtaining a value of mean species richness (and standard deviation). Since when data are analyzed year-by-year results can be affected by a bias due to false absence, we preferred to aggregate the information over a 5-year period, in order to reduce this source of error.

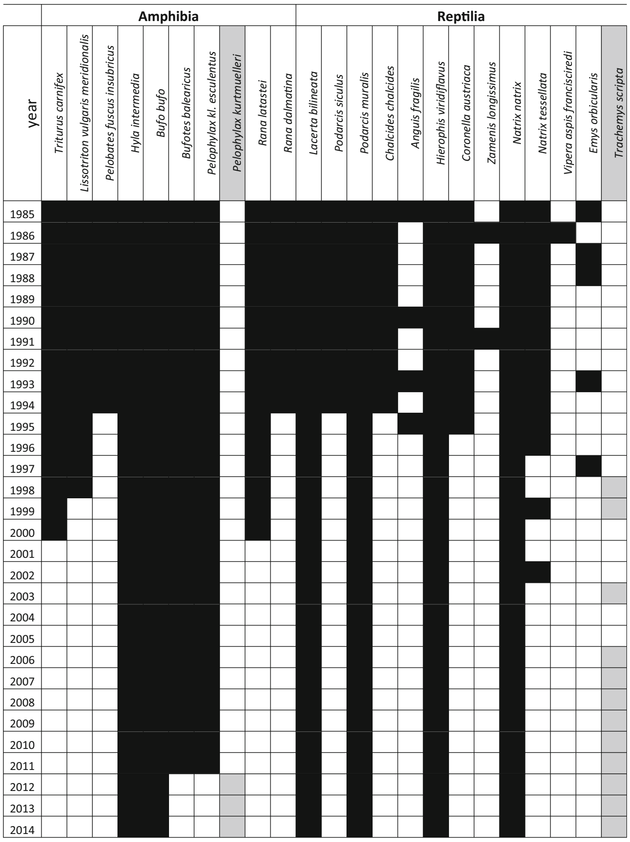
A non-parametric Friedman test was performed in order to determine whether the mean values among the 5-year periods were significantly different. Moreover, the Wilcoxon signed rank test was applied to verify whether the paired 5-year periods were significantly different. We used the SPSS 13.0 software for Windows (SPSS Inc. 2003). Alfa was set at the 0.05 level.

To assess the level of nested species relaxation in determining the long-term pattern in native herpetofauna assemblages, we performed a nestedness analysis by calculating with the NeD software (ecosoft/alwaysdata.net; Strona et al. 2014): (i) the matrix temperature (MT), which uses the Euclidian distances of unexpected empty or filled cells from the isocline (i.e., "diagonal line") that separates presences from absences in a perfectly nested matrix; (ii) the Brualdi and Sanderson Discrepancy (BR), which is a count of the number of discrepancies (absences or presence) that must be "corrected" to produce a perfectly nested matrix; (iii) the nestedness measure based on overlap and decreasing fills (NODF), which is the percentage of presences in inferior rows and in right columns that are in the same position (column or row) as the presences in, respectively, upper rows and left columns with higher marginal totals for all pairs of columns and rows. In order to test whether a matrix was significantly nested, we compared the nestedness value of the matrix under examination with those of a set of null matrices. This comparison is usually made using a Z value (Strona and Fattorini 2014), here computed on 50 simulated null matrices constructed using the "Proportional column and row totals (CE)" algorithm (Ulrich et al. 2009).

Results

A progressive loss of species, both in amphibians and reptiles, was observed over time (Table 1). In particular, a set of species continuously recorded since 1984 was not recorded in the successive years: *Pelobates fuscus insubricus*, *Rana dalmatina*, *Podarcis siculus*, and *Chalcides chalcides* were observed for the last time in 1994, *Coronella austriaca* in 1995, *Lissotriton vulgaris* in 1998, *Rana latastei* and *Triturus carnifex* in 2000, and *Bufotes balearicus* and *Pelophylax* kl. *esculentus* in 2011. Other species were not observed anymore after specific years: *Zamenis longissimus* (last observation: 1991), *Anguis fragilis* (1995), *Emys orbicularis* (1997), and *Natrix tessellata* (2002; Supplementary materials S2).

Table 1 Pattern of occurrence for amphibians and reptiles in the study area, 1985-2014. Presence (*black*), absence (*white*), *grey* (non-native species). Data for non-native species were not analyzed further. Raw data in Supplementary materials S2



	Amphibia										Reptilia												
year	*Triturus carnifex*	*Lissotriton vulgaris meridionalis*	*Pelobates fuscus insubricus*	*Hyla intermedia*	*Bufo bufo*	*Bufotes balearicus*	*Pelophylax kl. esculentus*	*Pelophylax kurtmuelleri*	*Rana latastei*	*Rana dalmatina*	*Lacerta bilineata*	*Podarcis siculus*	*Podarcis muralis*	*Chalcides chalcides*	*Anguis fragilis*	*Hierophis viridiflavus*	*Coronella austriaca*	*Zamenis longissimus*	*Natrix natrix*	*Natrix tessellata*	*Vipera aspis francisciredi*	*Emys orbicularis*	*Trachemys scripta*
1985	■	■	■	■	■	■	■	□	■	■	■	■	■	■	■	■	■	□	■	■	□	■	□
1986	■	■	■	■	■	■	■	□	■	■	■	■	■	■	□	■	■	■	■	■	■	□	□
1987	■	■	■	■	■	■	■	□	■	■	■	■	■	■	□	■	■	□	■	■	□	■	□
1988	■	■	■	■	■	■	■	□	■	■	■	■	■	■	□	■	■	□	■	■	□	■	□
1989	■	■	■	■	■	■	■	□	■	■	■	■	■	■	□	■	■	□	■	■	□	□	□
1990	■	■	■	■	■	■	■	□	■	■	■	■	■	■	■	■	■	□	■	■	□	□	□
1991	■	■	■	■	■	■	■	□	■	■	■	■	■	■	□	■	■	■	■	■	□	□	□
1992	■	■	■	■	■	■	■	□	■	■	■	■	■	■	□	■	■	□	■	■	□	□	□
1993	■	■	■	■	■	■	■	□	■	■	■	■	■	■	■	■	■	□	■	■	□	■	□
1994	■	■	■	■	■	■	■	□	■	■	■	■	■	■	□	■	■	□	■	■	□	□	□
1995	■	■	□	■	■	■	■	□	■	□	■	□	■	□	■	■	■	□	■	■	□	□	□
1996	■	■	□	■	■	■	■	□	■	□	■	□	■	□	□	■	□	□	■	■	□	□	□
1997	■	■	□	■	■	■	■	□	■	□	■	□	■	□	□	■	□	□	■	□	□	■	□
1998	■	■	□	■	■	■	■	□	■	□	■	□	■	□	□	■	□	□	■	□	□	□	▨
1999	■	□	□	■	■	■	■	□	■	□	■	□	■	□	□	■	□	□	■	■	□	□	▨
2000	■	□	□	■	■	■	■	□	■	□	■	□	■	□	□	■	□	□	■	□	□	□	□
2001	□	□	□	■	■	■	■	□	□	□	■	□	■	□	□	■	□	□	■	□	□	□	□
2002	□	□	□	■	■	■	■	□	□	□	■	□	■	□	□	■	□	□	■	■	□	□	□
2003	□	□	□	■	■	■	■	□	□	□	■	□	■	□	□	■	□	□	■	□	□	□	▨
2004	□	□	□	■	■	■	■	□	□	□	■	□	■	□	□	■	□	□	■	□	□	□	□
2005	□	□	□	■	■	■	■	□	□	□	■	□	■	□	□	■	□	□	■	□	□	□	□
2006	□	□	□	■	■	■	■	□	□	□	■	□	■	□	□	■	□	□	■	□	□	□	▨
2007	□	□	□	■	■	■	■	□	□	□	■	□	■	□	□	■	□	□	■	□	□	□	▨
2008	□	□	□	■	■	■	■	□	□	□	■	□	■	□	□	■	□	□	■	□	□	□	▨
2009	□	□	□	■	■	■	■	□	□	□	■	□	■	□	□	■	□	□	■	□	□	□	▨
2010	□	□	□	■	■	■	■	□	□	□	■	□	■	□	□	■	□	□	■	□	□	□	▨
2011	□	□	□	■	■	■	■	□	□	□	■	□	■	□	□	■	□	□	■	□	□	□	▨
2012	□	□	□	■	■	□	□	▨	□	□	■	□	■	□	□	■	□	□	■	□	□	□	▨
2013	□	□	□	■	■	□	□	▨	□	□	■	□	■	□	□	■	□	□	■	□	□	□	▨
2014	□	□	□	■	■	□	□	▨	□	□	■	□	■	□	□	■	□	□	■	□	□	□	▨

As far as the assemblage level is concerned, we observed a progressive decrease in the mean species richness (Fig. 1 and Supplementary materials S3), with a significant difference in averaged values (all the species: χ^2 = 22.706; amphibians: χ^2 = 24.352; reptiles: χ^2 = 23.065; d.f. = 5, $p < 0.001$; Friedman test).

When taking into account the whole herpetological assemblage and, in particular, the two amphibians abrupt thresholds were evidenced: the first between 1990 and 1994 and 1995–2000 (all herpetofauna: $Z = -2.041$, $p = 0.041$; only amphibians: $Z = -2.121$, $p = 0.034$) and the second between 1995 and 2000 and 2000–2004 (all herpetofauna: $Z = -2.070$, $p = 0.038$; only amphibians: $Z = -2.060$, $p = 0.039$; Wilcoxon signed rank test). Considering only reptiles, a significant difference was observed only between 1990 and 1994 and 1995–2000 ($Z = -2.032$, $p = 0.042$; Wilcoxon signed rank test; Fig. 1).

Native herpetofauna assemblage was significantly nested for all the used metrics (matrix size 357, fill 0.567; BR = 7, $z = -17.119$, $p < 0.001$; MT = 5.225, $z = -11.897$, $p < 0.001$: NODF = 85.329, $z = 12.961$, $p < 0.001$).

Discussion

A progressive decline in species richness was observed over the 30-year study period, without any temporal turnover pointing to species replacement (see Quian et al. 2005). However, such a decline was not constantly distributed among assemblages and over time. Actually, it was apparently more evident in amphibians (two significant thresholds: in the mid- and late 1990s), when compared to reptiles (only a significant threshold at the end of the 1990s).

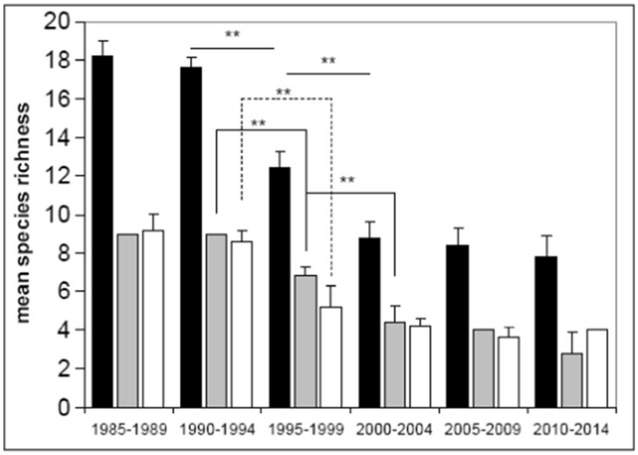


Fig. 1 Mean species richness (and standard deviation, s.d.) in the study area, subdivided into 5-year periods, for the entire native herpetofauna (*black*) and for amphibians only (*gray*) and reptiles only (*white*); ** = significant difference ($p < 0.01$)

The abrupt relaxation in species richness, which characterized the amphibians in the mid-1990s and both amphibians and reptiles in the late 1990s, may be due to a combination of processes acting at different scales.

At local scale, the study area was interested by exceptional flooding events. In fact, the water regime of the Po river shows a great annual variability with two maximal discharges in spring and autumn and two minimal discharges in winter and summer (average flow in Pontelagoscuro hydrometric station is 400 m^3/s in the lean regime, 1540 m^3/s in normal flow, and 9750 m^3/s in full regime; Zanchettin et al. 2008). Nevertheless, two flooding events (coinciding with the local collapse of species) were recorded in 1994 (flow rate >11,000 m^3/s; Marchi et al. 1995a, b) and in 2000 (>10,000 m^3/s as maximum range). Those events can be considered exceptional because since 1951, the flood plains of the Po had not been subjected to such hydrological disasters (Zanchettin et al. 2008). Therefore, it is conceivable that they induced a collapse of many local populations: for example, the flood of 1994 has probably led to the local disappearance of the most localized species, like *Pelobates fuscus insubricus*, *Podarcis siculus*, and *Chalcides chalcides*, no longer marked since that year. In the same way, the flooding events of 2000 probably induced the apparent collapse of local populations of *Rana latastei* and *Triturus carnifex.* In line with these findings, research carried out in other areas of Northern Italy (Tanaro river) showed that several species disappeared after inundation events (Giacoma and Gazzara 2002).

Nonetheless, further causes might be hypothesized at regional scale. For example, in the last two decades, the plain of the Po experimented a progressive expansion of intensive agriculture, with an increase in the use of synthetic chemicals and the reorganization of irrigation systems (Naldi et al. 2008). This has led to major seasonal fluctuations in the water basin caused by irrigation practices (with ponds undergoing water stress) and a progressive accumulation of nitrates, phosphates, herbicides, and other chemicals (e.g., Terbuthylazine and its metabolites, PBDE, PCB, synthetic estrogens, nonylphenol) in wet areas (Laini et al. 2011, 2012). The demographic collapse observed in amphibians at a local level could be due to this accumulation (Rouse et al. 1999; Collins and Halliday 2005; Du Preez et al. 2005; Relyea and Diecks 2008; Viganò et al. 2015).

Another factor which might be linked to the observed decrease in the number of amphibian species is the significant increase, at regional scale, of various predator species along the Po plain, such as native birds of prey (e.g., Ardeidae; Fasola and Alieri 1991; Fasola et al. 2007) and non-native fish species (e.g., *Silurus glanis*, *Micropterus salmoides*; Razzetti et al. 2001). As recently pointed out, pesticides and predators can exert a synergistic effect on wetland communities (Relyea 2003; Relyea and Hoverman 2008). Moreover, in the last decades, the Po plain underwent large urban sprawl with consequent habitat fragmentation. Such changes could be considered as a further factor affecting populations at a regional scale (Cushman 2006; Biaggini and Corti 2015), mainly when considering species which use different habitats in the different stages of their life cycle (“habitat split”: Becker et al. 2007; Glista et al. 2008). It has been observed that the proportions of both current and historic arable lands were negative determinants of amphibian occurrence and species richness (Van Buskirk 2005; Gardner et al. 2007; Piha et al. 2007). It cannot be ruled out, though, that the local decline observed might also be connected to the worldwide decline observed in amphibians and reptiles over the course of the twentieth century. The amphibian populations started to constantly decrease in 1960s while reptiles, after an initial decrease, showed a peak at the end of 1990s (Houlahan et al. 2000). These alterations were probably due to large scale factors such as changes in UV-B irradiation, emerging diseases, and climate change and their interaction (Gibbons et al. 2000; Gardner 2001; Whittaker et al. 2013; Petrovan and Schmidt 2016).

However, these spatial-scaled root causes could also have a different time-scaled pattern (Bender et al. 1984), making it difficult to define linear causal chains. Actually, it is probable that local flooding catastrophes acted as “pulse” events, i.e., single time-limited perturbations, different from regional and large-scale threats (urbanization, fragmentation, etc.) acting continuously over time (“press processes”; Gotelli and Ellison 2004). These differences may have implication in recovery times and on resilience of isolated populations (Battisti et al. 2016).

At a population level, a first set of species, which was continuously recorded, was not observed any longer after a specific time. This set included both species of high conservation concern (as *Pelobates fuscus insubricus*; Andreone and Luiselli 2000) and relatively common species (as *Podarcis siculus*). Our hypothesis is that these species either went locally extinct or, at least, were subjected to such a strong decrease in their density that their detection was made highly difficult. In addition, all these species did not show any tendency to recolonize the site. On the contrary, for another set of species which is more difficult to detect, there were only gaps in the records until a specific year, after which, however, they were not observed anymore.

Different species-specific ecological traits (e.g., local rarity, dispersal ability, trophic specialization, niche breadth) could be considered important intrinsic factors explaining the species sensitivity to external events (as catastrophic flooding, habitat changes, and landscape fragmentation) and, ultimately, their local extinctions. As a matter of fact, the low dispersal ability and high demographic fluctuation in amphibians, and the high trophic level, specialization, and intrinsic low population size in reptiles, appear to be the critical ecological traits exposing these species to extinctions in fragmented habitats (Henle et al. 2004; Ewers and Didham 2006; Trochet et al.

2014). For example, *Pelobate fuscus insubricus* is a highly specialized frog with fluctuating, rare, and declining populations in all the Po plain (Ferri and Schiavo 1988; Gentilli et al. 1996). Its situation shows a close analogy to that of *Rana latastei*, an endemic species with low adaptability to habitat changes (Andreone and Luiselli 2000; Barbieri and Bernini 2004). It should also be noted, however, that, in the Po plain, also more common species are subjected to critical isolation due to habitat fragmentation. This condition makes them more exposed to local demographic collapse (amphibians: *Triturus carnifex*, *Lissotriton vulgaris*; reptiles: *Coronella austriaca*, *Zamenis longissimus*, *Natrix tessellata*), as confirmed in surrounding sites (Ferri 1992; Scali 1993; Ildos and Ancona 1994; Bernini et al. 2004; Scali 2011; Di Cerbo and Biancardi 2013).

Understanding temporal dynamics of occurrence may be important to separate anthropogenic declines in species richness from natural fluctuations in extinction-prone groups, such as amphibians and reptiles (Storfer 2003; Salvidio 2009). Particularly in amphibians, it is difficult to use short-term studies as a basis for deciding if an assemblage is increasing or decreasing in the long term (Collins and Halliday 2005). In this sense, although our study shows some limitations (e.g., bias due to false-negatives, one-site survey, no comparison to other sites, discrete approach of trends; Schmidt 2003; MacKenzie 2005), we have obtained for the first time coarse-grained evidence for a long-term decline (with thresholds) in a herpetofauna assemblage, which apparently suggests a lack of fluctuation (and re-colonization). Moreover, analyses on large time ranges mainly utilize heterogeneous historical sources (anecdotic data, gray literature, field surveys with different methods and researchers), so that comparisons can be biased (Swetnam et al. 1999; Skelly et al. 2003). Instead, in our study, we compared standardized presence/absence data among years which were obtained using the same protocol and researcher, in order to limit the error arising from different inter-observer detectability, often occurring in long-term monitoring studies (Thomas 1996).

The interpretation of our data allowed us to postulate only a-posteriori hypotheses (inductive approach; Romesburg 1981; Guthery 2007). These should be tested through further research at different scales which could aim at disentangling local threats from regional or global ones and analyzing data using a continuous (year-by-year) approach. In this sense, since it has been highlighted that local research plays a role in building general models (Houlahan et al. 2000; Salafsky et al. 2002), our temporal trends and thresholds might be compared to those pertaining to other sites.

Finally, our data have practical implications for proactive conservation actions. For the first time, we have obtained evidence that species may undergo extinction in a nested relaxation process (Baselga 2010), scarcely reported for amphibians and reptiles after long-term environmental changes (e.g., Hecnar et al. 2002; Watling et al. 2009). An analysis of the ecological traits of the most sensitive species and the knowledge of the predictable order of deterministic extinctions could favor a future selection of lists of sensitive focal species for conservation planning (Battisti and Luiselli 2011; Beazley and Cardinal 2004).

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Conflict of interest The authors declare that they have no conflict of interest.

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