Land abandonment may reduce disturbance and affect the breeding sites of an Endangered amphibian in northern Italy

STEFANO CANESSA, FABRIZIO ONETO, DARIO OTTONELLO, ATTILIO ARILLO and SEBASTIANO SALVIDIO

Abstract Although human-related disturbance is usually detrimental for biodiversity, in some instances it can simulate natural processes and benefit certain species. Changes in the disturbance regime, both natural and human-driven, can affect species that rely on it. The Apennine yellow-bellied toad *Bombina variegata pachypus*, an amphibian endemic to peninsular Italy, has declined throughout its range in the last 3 decades. We sought to identify the drivers of the decline in the region of Liguria, at the north-western limit of its distribution. In 2009 and 2010 we surveyed sites where the species occurred until 2005 and related the persistence of breeding activity to the characteristics of sites. Populations had disappeared from 50% of the sites between 2005 and 2009. Current breeding sites have less aquatic and bank vegetation, fewer predators and better insolation. Frequent disturbance events (desiccation and floods) were related to reduced vegetation, which in turn may decrease predator densities and increase insolation. In this region disturbance is provided by natural factors or, in the case of artificial water bodies, by regular maintenance carried out by landowners. The widespread land abandonment in Liguria can disrupt disturbance regimes, interrupting the removal of vegetation, and thus rapidly reduce the suitability of artificial sites. This was confirmed in our study, with most abandoned breeding sites occurring in formerly cultivated areas. Possible short-term conservation actions include creating new ponds, maintaining artificial water bodies and clearing vegetation. However, long-term conservation may be more problematic as the land abandonment process is unlikely to be reversed.

Keywords Agriculture, *Bombina*, cultural landscapes, disturbance, insolation, land-use change, pond management, predation

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Introduction

Disturbance is a key component of landscapes and ecosystems, influencing the biodiversity they support (Petraitis et al., 1989). This influence varies in magnitude and type: some species can be negatively affected whereas others may benefit from intermediate or high levels of disturbance that increase trophic resources or reduce predation (Brawn et al., 2001; Rydgren et al., 2007). Although disturbance caused by human activities is usually associated with detrimental effects on wildlife, it can sometimes replace natural processes and benefit certain species (Pykala, 2000). Changes in long-standing human activities may thus have negative effects on biodiversity (MacDonald et al., 2000; Sirami et al., 2008). Therefore, the value of traditional practices that generate low or moderate disturbance (e.g. in agriculture) is increasingly being considered by conservation planners (Bignal & McCracken, 2000).

Such dynamics may play a role in the decline and extinction of some amphibian species for which habitat loss is one of the main drivers (Cushman, 2006). For example, agricultural ponds may provide suitable habitat for amphibian species, which can be affected by the disappearance of the ponds (Beja & Alcazar, 2003; Knutson et al., 2004). The Apennine yellow-bellied toad *Bombina variegata pachypus* (Anura, Bombinatoridae) is an example of a declining species potentially threatened by this type of habitat loss. This subspecies of *B. variegata*, endemic to the Italian Apennine Mountains and considered by some to be a separate species (Canestrelli et al., 2006; but see Hofman et al., 2007), is categorized as Endangered on the IUCN Red List (Andreone et al., 2009). Disappearances of local populations have been observed since the late 1970s in the northern and central Apennines (Stagni et al., 2004; Barbieri et al., 2004).

B. variegata is well adapted to small water bodies, including those associated with human activities, such as potholes and irrigation ponds (Barandun, 1990; Barandun & Reyer, 1997b). It shows plasticity in the number of breeding events and in the choice of spawning sites (Barandun & Reyer, 1997a; Guarino et al., 1998). It is usually associated with temporary water bodies that have limited canopy cover and high insolation, allowing high water temperatures and fast larval development, minimizing the threat of

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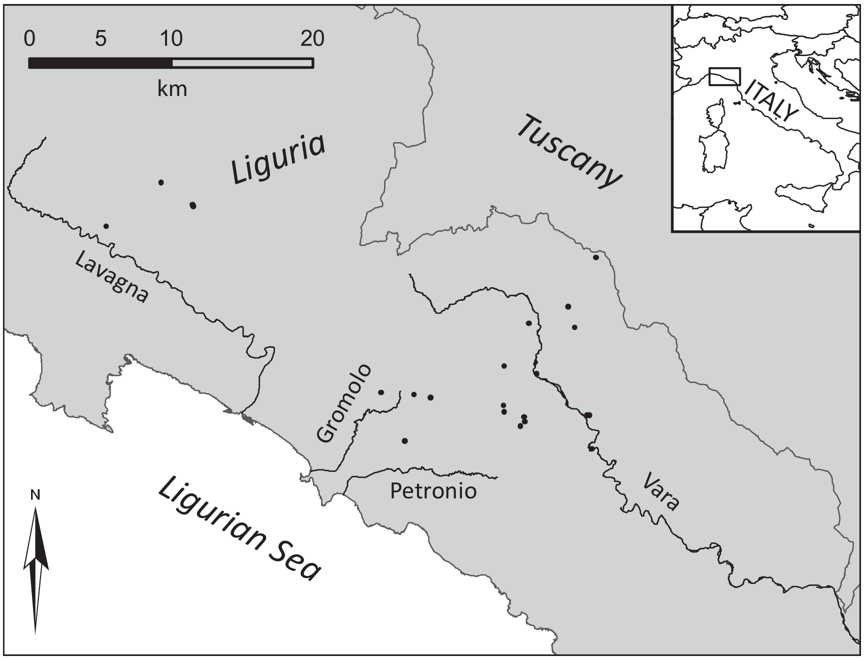

FIG. 1 The study area in eastern Liguria, showing the main rivers; the circles indicate the sites surveyed in 2009 and 2010. The inset indicates the location of the main map in north-western Italy.

desiccation while decreasing predatory pressure on tadpoles (Hartel et al., 2007). Suggested explanations for the decline of the species focus on the loss of temporary wetlands, particularly those related to agricultural activities (Mirabile et al., 2009). Mortality from chytrid fungus has also been observed for *B. v. pachypus* in the north-eastern Apennines (Stagni et al., 2004).

The region of Liguria in north-western Italy is the northernmost limit of the species' range (Doria & Salvidio, 1994). During 1998–2008 > 75% of the known populations in the area have disappeared (Arillo et al., 2009). In this study we investigated possible explanations for the regional decline of *B. v. pachypus*. We surveyed water bodies where the species was recorded in recent years and compared the ecological characteristics of current and abandoned breeding sites. We then related these patterns to aspects of natural and human-related disturbance, in particular those associated with land-use change in the region.

Methods

Study area and field methods

The study area covers c. 800 km^2 in eastern Liguria (Fig. 1). The landscape comprises cultivated areas, secondary grasslands, oak and pine forests, with forest regrowth in abandoned fields. We selected 21 sites where the species had been detected between 1998 and 2005, in three river catchments (Lavagna, Petronio and Vara) on the southern slopes of the Apennines at altitudes of 197–1,052 m (L. Ciuffardi, A. Raineri & L. Braida, pers. comm.). These included natural (pools in mountain brooks, small wetlands) and artificial sites (cattle and irrigation ponds, stone wash tubs). Although the small sample size limits the robustness of the study, it reflects the rarity of these species in the region.

We surveyed each site 2–6 times in 2009 and 2010 during late May–early August, which is the period of breeding and larval development (Doria & Salvidio, 1994). We followed recommended protocols to prevent transmission of infectious pathogens (Speare et al., 2004). Each survey was carried out by 2–3 observers during 08.00–12.00, in fair weather to maximize the chance of spotting individuals in the water. We were able to search sites thoroughly because of their small size. Adults, eggs and tadpoles can be reliably identified in the field (Doria & Salvidio, 1994). We categorized breeding status of sites as 'current' where eggs or tadpoles were sighted at least once and as 'abandoned' otherwise.

For each site we estimated the average daily hours of insolation during May–August, using data loggers for air temperature immediately above the water (FT-800/system micro-recorders, Econorma, Treviso, Italy). We calculated the number of hours between the initial increase in air temperature and the start of its final decrease of the day. Insolation was preferred over direct temperature measurements, to avoid potential confounding by seasonal effects. We counted newts (*Ichthyosaura alpestris* and *Triturus carnifex*) visually over the total extension of the water body. We estimated the density of invertebrate predators (Dytiscidae and Odonata) by dip-netting (width 20 × 20 cm nets, mesh size 0.5 mm), taking one 20 cm long sweep per m^2 of water surface. Overall densities of predators were then calculated over the entire surface area.

We counted flooding and desiccation events at each site during each study year. At artificial sites the count also

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included maintenance operations by landowners (drying, dredging and vegetation clearing). We visually estimated the mean proportion of aquatic and bank vegetation cover at a site as a proximate measure of disturbance (previously shown to affect habitat suitability for *B. variegata* in man-made sites in Germany; Warren & Buttner, 2008).

As potential indices of human presence in the area we calculated the elevation of sites and the road density (km km^{-2}) within a 200 m buffer around each site. We also calculated the proportion within this buffer covered by open-canopy vegetation types. This information was obtained from a categorical regional land-use map, based on Spot 4 and IRS-P6 images taken in 2006, with 20- and 23-m resolution, respectively (CORINE land cover; EEA, 2007). We aggregated agricultural, grassland and shrubland vegetation classes, which in Liguria are an indication of current or recent agricultural activities (Gentili et al., 2009). The buffer size was based on existing knowledge about *B. variegata* that suggests highly sedentary behaviour during and between breeding seasons (Hartel, 2008).

Statistical analyses

We performed two-dimensional non-metric multidimensional scaling (NMDS) of the site parameters (altitude, vegetation, insolation, predators and road density). NMDS is commonly applied in ecological studies for exploratory purposes; the relationship between the dissimilarity matrix and the Euclidean distance between sites is used to locate each site within an *n*-dimensional map in the ordination space (Minchin, 1987). Note that NMDS axis numbers, unlike metric ordination such as principal component analysis, do not reflect a trend in the variance explained (i.e. axis one does not necessarily explain more variability than axis two).

Dissimilarity matrices were based on the Bray–Curtis index (Bray & Curtis, 1957). The ordination was performed using the *Vegan* package for *R* (Oksanen et al., 2010). We assessed whether the direction and magnitude of changes for each variable matched the discrimination between current and abandoned breeding sites (function *envfit*). We then investigated the relationship between the breeding status of a site, its ecological characteristics and the mean frequency of disturbance events. We fitted a smooth surface to the *n*-dimensional ordination space using a generalized additive model approach (*ordisurf*; Oksanen et al., 2010). We calculated the maximum correlation with the ordination, assessing its significance through a permutation test ($n = 100{,}000$, $\alpha < 0.05$).

The effect of local parameters on breeding activity was then assessed using logistic regression. We used the Akaike information criterion (AIC) to compare models (Burnham & Anderson, 2002). We considered models with $\Delta AIC < 2$ ($\Delta AIC = AIC–AIC_{min}$) as equally supported, and models with $\Delta AIC < 5$ were considered as potentially supported. We compared the effect of individual covariates using multiplicative effect sizes. These reflect the change in the odds of breeding at a site across the range of a covariate, calculated as

$$E_i = \exp(\hat{\beta}_i * \text{range}_i)$$

where E_i is the multiplicative effect of variable i, $\hat{\beta}_i$ is the estimated logistic regression coefficient for that variable and range_i is the range of observed values for that variable. Finally, we calculated Pearson's correlation coefficients between environmental parameters and graphically assessed the relationship between these and the frequency of disturbance events at a site.

Results

We found evidence of breeding at 10 of the 21 sites surveyed. At another site (a recently abandoned stone wash tub) we found a single individual but no eggs or tadpoles. The species was absent from the remaining 10 sites. These observations were consistent between 2009 and 2010. Five of 10 natural sites and five of 12 artificial sites were categorized as current breeding sites. All abandoned sites except one were subject to < 2 disturbance events per year, whereas only three current sites out of 10 were entirely undisturbed.

The non-metric ordination converged adequately (stress = 4.57, $R^2 = 0.99$) and showed a relatively clear separation between current and abandoned breeding sites along the first axis (Fig. 2). In particular, abandoned sites appeared more homogeneous than current breeding sites. The density of predators and vegetation explained most of the overall variation (predators: $R^2 = 0.91$, $P < 0.001$; vegetation: $R^2 = 0.75$, $P < 0.001$). These factors also matched the site classification: current breeding sites had lower values of vegetation and predators (Fig. 2). The number of daily hours of sun exposure was higher in current breeding sites, although the overall variance explained was less significant ($R^2 = 0.31$, $P = 0.04$). Altitude was not significant ($R^2 = 0.21$, $P = 0.35$). Road density had a significant weight in the ordination ($R^2 = 0.51$, $P < 0.01$) but its vector of change did not match the main separation between current and abandoned breeding sites. Among artificial water bodies, however, current breeding sites were surrounded by high road densities (with lower densities of predators). The only exception was a stone wash tub, in a large cultivated area without roads, but still maintained by the landowners and hosting no predators.

The fitted surface for the frequency of disturbance events at a site showed a significant gradient of change across the ordination space ($R^2 = 0.57$, $P < 0.001$; Fig. 2a): current breeding sites were more disturbed, with the exception of

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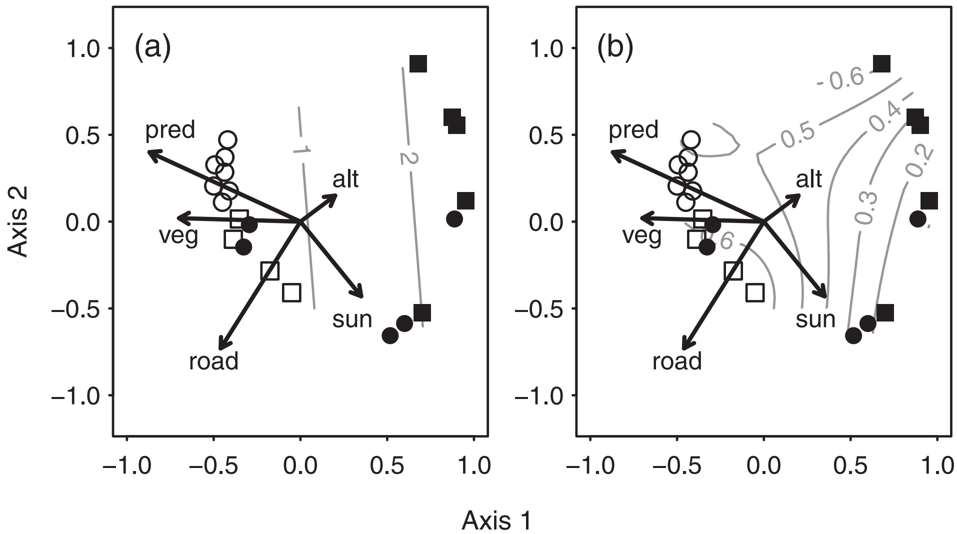

FIG. 2 NMDS of the ecological characteristics of surveyed sites. Filled and empty symbols represent current and abandoned sites, respectively; squares and circles represent natural and artificial sites, respectively. Arrows indicate the direction and magnitude of change for single covariates (alt, altitude; pred, density of predators; road, density of roads within 200 m of a site; sun, number of daily hours of insolation; veg, density of aquatic and riparian vegetation). Grey lines represent the fitted surface for (a) the mean frequency of disturbance events at a site per year and (b) the proportion of open-canopy vegetation types within a 200-m buffer.

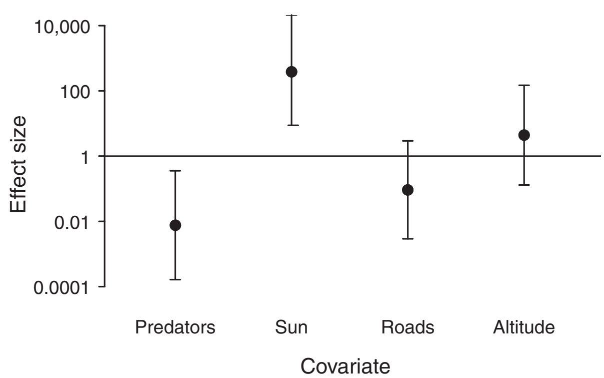

FIG. 3 Multiplicative effect sizes of the regression covariates on the probability of *Bombina variegata pachypus* breeding at a site. Bars correspond to 95% confidence intervals; covariates with intervals encompassing 1 (the horizontal line) can be interpreted as having no significant effect at α=0.05.

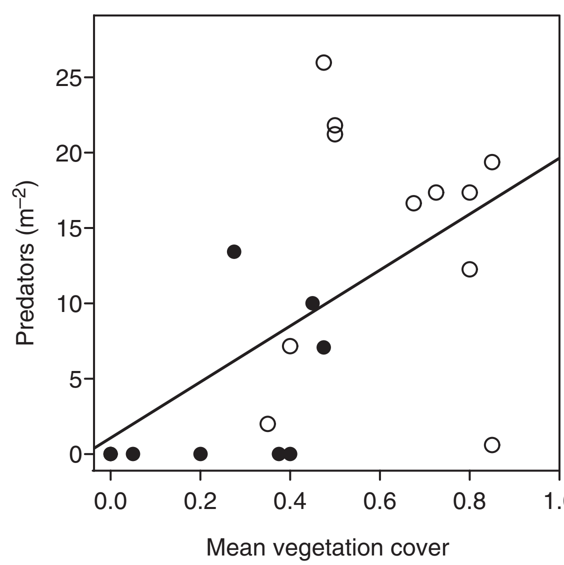

FIG. 4 Linear regression (the diagonal line) of the density of predators at a site against the mean proportion of vegetation cover. Filled and empty circles represent current and abandoned breeding sites, respectively.

two artificial sites infrequently maintained by the landowners but still used as breeding sites. Predators and vegetation also appeared to decrease with increasing disturbance, whereas sun exposure increased and road density and altitude showed almost no change. The proportion of open-canopy vegetation types within a 250-m buffer around each site followed a similar pattern: most abandoned sites were located in a part of the ordination space associated with high levels of the variable, corresponding to high densities of vegetation and predators and low insolation, as well as low levels of disturbance. The correlation with the ordination was significant ($R^2 = 0.42$, $P < 0.001$; Fig. 2b).

Logistic regression models confirmed this trend. The density of predators best described the permanence of breeding activity at a site (DIC = 20.7): the second-best model included the number of hours of sun exposure of the water body (ΔAIC = 4.8), whereas models including road density and altitude were essentially unsupported, with ΔAIC scores of 10.6 and 11.7, respectively. Multiplicative effect sizes suggested a negative effect of predation on the

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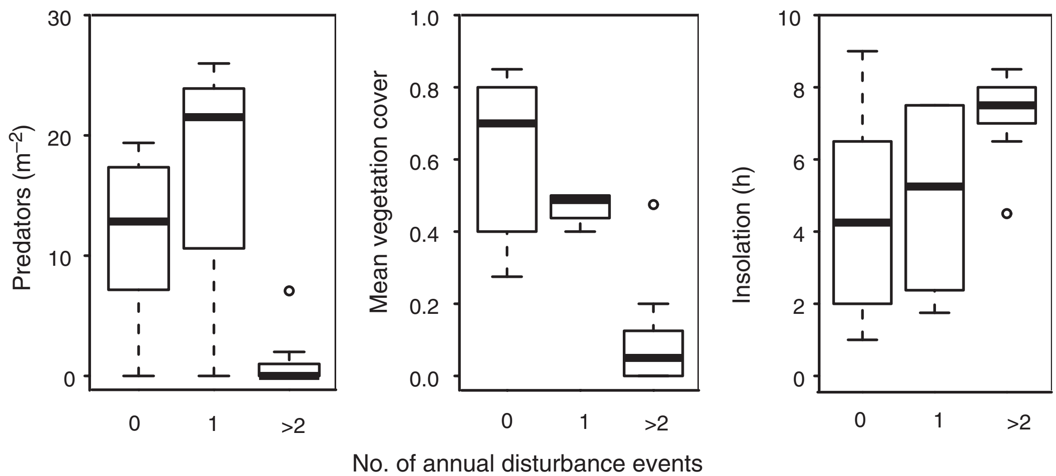

Fig. 5 Comparison of the density of predators, the mean proportion of vegetation cover and the number of daily hours of insolation at sites subject to increasing frequencies of annual disturbance events. Sample sizes were n = 10 for no events, n = 4 for one event, and n = 7 for two or more events.

odds of *B. v. pachypus* breeding at a site, whereas sun exposure had a positive effect (Fig. 3). Road density and altitude had negative and positive effects, respectively, but these were not significant.

We did not include a model for vegetation cover as this variable was significantly correlated with the density of predators at a site ($r = 0.72$, $P < 0.001$): a linear regression model confirmed the positive relationship (standardized regression coefficient: $\beta = 3.15$, $P = 0$). Densely vegetated sites were more likely to host high numbers of predators: current breeding sites tended to show lower values for both parameters (Fig. 4). The correlation between vegetation and sun exposure at a site was not significant ($r = -0.31$, $P = 0.13$). Sites with frequent disturbance events (two or more per year) had lower densities of predators and vegetation and greater insolation (Fig. 5). Sites subjected to one disturbance event per year did not show relevant differences compared to entirely undisturbed ones.

Discussion

More than 50% of the breeding sites recorded until 2005 were no longer occupied in 2009–2010, confirming the decline of *B. v. pachypus* in Liguria. Although the local extinction rate appears lower than reported elsewhere (75%; Arillo et al., 2009) we did not survey sites that had been destroyed (permanently dried or filled), and thus underestimated the overall decline. The local trend justifies the categorization of this subspecies as Endangered (note it is categorized as a separate species; IUCN, 2009) and matches observations across the northern part of the species' range (Barbieri et al., 2004). Local extinctions were consistent during the study period, although further monitoring is required to rule out recolonizations in the long term.

Locally, sites with fewer predators were more likely to maintain breeding. High densities of predators can negatively affect the survival of *B. v. variegata* tadpoles, especially in the early stages (Barandun & Reyer, 1997a; Vorndran et al., 2002). In our study the species also appeared to prefer unshaded water bodies, matching observations for *B. variegata* throughout Europe (Barandun & Reyer, 1997b; Hartel et al., 2007). Direct sunlight allows fast development of tadpoles, although there can be a trade-off against higher threats of desiccation (Barandun & Reyer, 1997a). However, we observed no desiccation events until late August, when most metamorphs are likely to have emerged. Therefore, this threat appears marginal in our study area, where *B. v. pachypus* relies less on ephemeral water bodies than in other parts of Italy (Mirabile et al., 2009) and than *B. v. variegata* (Vorndran et al., 2002; Hartel et al., 2007). We found no evidence of infection by *Batrachochytrium dendrobatidis*, although specific analyses are required to exclude this possibility as it has been reported for the species in the north-eastern Apennines by Stagni et al. (2004).

Abandoned sites also had higher densities of vegetation. Although shading can impair the development of tadpoles of *B. v. pachypus*, in our study the correlation between insolation and vegetation was too weak to entirely explain local extinctions. However, we found a positive correlation between vegetation and predator densities. Several studies have shown that scarce vegetation in agricultural ponds can reduce the density of invertebrate predators (Painter, 1998; Bang, 2001; Carchini et al., 2005). Newts also prefer ponds with submerged vegetation (Sebasti & Carpaneto, 2004). Therefore, vegetation may have an indirect effect on tadpole survival, increasing pressure of predation at a site.

Moreover, we found that the amount of vegetation and predator densities decreased at sites with a high frequency

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of disturbance events. Desiccation and floods eliminate aquatic plants, ultimately reducing the density of predators. In streams, peak flows also remove substrate from the bottom and banks of water bodies, further preventing plant regrowth. In natural sites, autumn and spring floods may wash away overwintering invertebrate larvae (Twisk et al., 2000). Sun exposure was also higher in more disturbed ponds, although the relationship was less marked: events that prevent the growth of vegetation may ensure better insolation, facilitating tadpole development. These results match those of Warren & Buttner (2008), who found that high proportions of bare ground (scarce vegetation) around ponds, resulting from human activities, increased the probability of occurrence of *B. v. variegata* in a system of ponds in Germany.

In our study area floods and desiccation are the main causes of disturbance of natural breeding sites of *B. v. pachypus*. Conversely, artificial ponds were traditionally maintained by landowners: in autumn and spring they were drained and the bottom and banks cleared of substrate and vegetation. These activities replaced the natural disturbance processes, making artificial sites suitable for tadpoles of *B. v. pachypus*, reducing sun exposure and vegetation cover (thus lowering predatory pressure), without the stochastic risk factors typical of natural sites (desiccation or floods). If maintenance ceases, predators may increase and negatively affect reproductive success.

Given the possible relationship between human activities and the persistence of *B. v. pachypus* at artificial sites in the region, land-use change can play a key role in the conservation of this species. Liguria has experienced widespread abandonment of previously cultivated inland areas since the 1960s, similar to most Italian mountain areas where open-canopy vegetation types are being progressively replaced by forests (Mazzoleni et al., 2004; Gentili et al., 2009). The traditional water sources are thus abandoned or progressively replaced by direct connections to water pipes, particularly for irrigation purposes. In our study area land-cover change does not appear a threatening factor in itself: most current breeding sites were located within forest areas. However, land abandonment may affect artificial sites through a change in the disturbance regime. Artificial sites in open landscapes and subject to little disturbance were less likely to host breeding populations: these were water bodies no longer maintained by landowners, where vegetation developed allowing predator densities to increase. Road density had limited influence on the breeding status of sites: this may also reflect the relevance of agricultural activities, and not just any human presence in the area.

Natural sites within agricultural landscapes (e.g. shallow wetlands or rainwater pools) are also threatened by the effects of land abandonment, in this case vegetation regrowth and subsequent shading. In addition, without consistent disturbance such as floods small natural wetlands will naturally tend to infill. In our study natural sites in open landscapes (indicating current or recent agricultural activity) all had low levels of disturbance, and were abandoned by the species between 2005 and 2009. Although *B. variegata* is known for its plasticity in the selection of spawning sites this has been demonstrated mainly within pond systems (Hartel et al., 2007; Warren & Buttner, 2008). In Liguria most such systems include a previously dense network of artificial water bodies; their degradation and disappearance may reduce connectivity, thus preventing dispersal and recolonization by these amphibians (Ficetola & De Bernardi, 2004). Conversely, complex natural sites with frequent disturbance (e.g. streams) may prove more resilient in the long term, provided that the water input remains constant.

Given these possible threats several management actions can be envisaged for the conservation of *B. v. pachypus* in Liguria. The loss of artificial sites may be prevented by promoting traditional agricultural practices and creating new ponds in cooperation with landowners. Regular maintenance, either by landowners or conservation agencies, can ensure high insolation and low predatory pressure. Shading by surrounding vegetation can threaten naturally disturbed sites in the medium–long term (> 10 years). In this case, local clearing of vegetation can improve insolation. Several of these actions are being implemented by managers of regional protected areas (Parco Regionale Monte-Marcello Magra) in cooperation with local landowners and the University of Genoa (Arillo et al., 2011). However, even with these actions land abandonment in the region is not likely to be reversed. Stochastic events coupled with a general loss of connectivity may be the main future threat, and this needs to be considered when evaluating long-term conservation plans.

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Biographical sketches

Stefano Canessa researches techniques for improving decision-making in monitoring and conservation of amphibians and other taxa, with particular attention to ex-situ programmes. Fabrizio Oneto and Dario Ottonello, naturalists and consultants in herpetology, carry out research and participate in in-situ and ex-situ conservation programmes for amphibians and reptiles in Italy. Attilio Arillo has initiated many research and conservation plans for wildlife, particularly regarding the use of traditional agriculture as a means of conserving Apennine amphibians. Sebastiano Salvidio is a herpetologist interested in the ecology and conservation of amphibians and reptiles in Italy and the Mediterranean area.

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