The increase of an amphibian population:
11 years of *Rana temporaria* egg-mass monitoring in 30 mountain ponds

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Mount Guglielmo in the Italian Alps is an ancient transhumance area where man-made ponds for watering cattle are an ancestral component of the landscape and represent the most important breeding sites for common frogs (*Rana temporaria*) in the study area. Egg-masses in 30 mountain ponds from the Mount Guglielmo were counted from 2005-2015 to monitor the population trends of *R. temporaria*, after a putative population decline due to lethal bacterial infections which affected the tadpoles from 2002 to 2006. Egg-mass counts were also used to understand how the recovery of ponds from the natural process of drying-out and the replacement of traditional ponds with pools waterproofed with polymers influence the population dynamics of *R. temporaria* and the suitability of the ponds as breeding sites. This *R. temporaria* population increased over the study period, suggesting that the population was able to withstand long lasting larval mortality. Population increase was also sustained by the recovery of some dried ponds, but the modern pools were less suitable than traditional ponds for *R. temporaria* reproduction. These results suggest that the persistence of the traditional practices related to transhumance (e.g. maintenance of man-made ponds) in the study area is probably of pivotal importance for the conservation of the local herpetofauna.

INTRODUCTION

Amphibians diseases are a major cause of the global amphibian decline crisis (Daszak *et al*., 1999), and are a primary factor determining the natural dynamics of wild populations (Anderson & May, 1986). To understand the real extent of the global amphibian decline crisis, biologists need to distinguish between natural population fluctuations and abnormal declines that may be directly or indirectly due to human actions (Pechmann *et al*., 1991; Gardner, 2001). Long-term studies, encompassing population fluctuations or cycles, can help biologists learn more about this problem (Meyer *et al*., 1998; Loman & Andersson, 2007). In any case, mortality events attributable to infectious diseases should be carefully monitored to assess its impact on amphibian populations.

In the present 11-year study, egg-mass counts from 30 mountain ponds were used to assess the population dynamics of *Rana temporaria Linnaeus*, 1758 on Mount Guglielmo massif (Italian Alps). The present study was initiated to quantify the demographic effects of a series of widespread mass die-offs affecting *R. temporaria* tadpoles at many ponds in the study area (Tiberti, 2011). Around 2002, die-offs of *R. temporaria* tadpoles were reported for the first time, often resulting in mass mortality of the entire tadpole population within a pond and affecting most ponds in the entire area (Tiberti, 2011). Other common amphibian species (*Bufo bufo* and *Triturus carnifex*; Tiberti in press) were apparently unaffected by the epidemics. Die-offs have been ascribed to bacterial infections caused by *Aeromonas* sp. (Tiberti, 2011), often associated to epidemics of red-leg disease (Rigney *et al*., 1978). *Aeromonas* sp. is an ubiquitous bacterial genus living in free waters, on the skin and in the digestive tract of...
amphibians without causing infections, and is considered an opportunistic pathogen, infecting immunodepressed hosts (Carey, 1993). Therefore, the spread of the epidemic could have been facilitated by other immunosuppressant density dependent (e.g. overcrowding) or environmental factors (see Meyer et al., 1998), or by the presence of other, not detected, pathogens (Tiberti, 2011). Despite not being able to determine the root causes, such die-offs have not been reported in the study area (at least with the same severity and spatial extent) since 2007.

Mount Guglielmo is an ancient area of alpine transhumance (the seasonal transfer of livestock to high altitude pastures). Due to its geology (dominated by calcareous rocks with many karst landforms), surface water is largely absent above 1000 m a.s.l. and water for cattle was traditionally obtained by constructing ponds. These anthropogenic habitats are an ancestral part of the cultural landscape (alpine transhumance dates back to Neolithic; Festi et al., 2014) and represent virtually the only surface aquatic habitat in high altitude pasturaleands in the study area. In this context traditional land use practices (e.g. pond construction and maintenance) sustain valuable ecosystems for amphibians. However modernization in the water supply for cattle has meant that some traditional ponds (dug into the ground, with a clay bottom) were replaced with large artificial pools (waterproofed with polymers sheets). Thus, a second objective of this study was to assess R. temporaria use of modern artificial ponds compared to traditional transhumance ponds.

**MATERIALS AND METHODS**

**Study area**

Mount Guglielmo (1957 m a.s.l.) is an isolated massif between the Trompia and Camonica valleys (Brescia Prealps, Italy) (fig. 1). In the study area there are 30 mountain man-made ponds for watering cattle, which sustain Bufo bufo and Triturus carnifex populations and are the most important breeding sites for R. temporaria in the study area. The mean altitude of the ponds was 1535 m a.s.l. (range: 1166-1863 m a.s.l.), mean surface area was 706 m2 (range: 140-3400 m2), and mean depth was 0.81 m (range: 0.2-2.0 m; values based on the maximum seasonal depths measured in May-July 2006). Nearby ponds (situated less than 1 km apart) were clustered into four groups (A-D; fig. 1), thus R. temporaria could probably disperse among ponds in the same group. Most of the ponds had a clay bottom, but ponds A2, C2, C3, D3, D6, and D8 (fig. 1) had an artificial substrate made of waterproof polymers.

**Egg-mass counts**

Obtaining accurate counts of R. temporaria egg-masses is possible because their clutch size is large (up to 4500 eggs; Nöllert & Nöllert, 1992), they are easily visible in shallow water, and laid simultaneously by numerous frogs in a confined area (Bernini & Razzetti, 2006). For 11 years (2005-2015), one to three surveys per pond were performed annually to count the egg-masses deposited in all mountain ponds in the study area (N = 30, fig. 1). Surveys were conducted just after the putative breeding season by walking along the entire edge of each pond. The date of the first survey was decided based on the weather conditions (e.g. persistence of the snow cover) and on the pond features (e.g. altitude and exposure) during the usual breeding period in the study area, which was highly predictable (between the 21 April and 10 May). Assuming that the time of reproduction should be the same in the ponds closer together, repeat surveys were needed when the egg-masses had not yet been laid in a certain area/group of ponds or when amplexing pairs were still observable in the ponds. The position and developmental stage (freshly laid or not) of the egg-masses was recorded at each survey to not underestimate counts when repeat surveys were conducted. Of 330 potential egg mass counts (30 sites for 11 years) over the study, only 15 were not conducted, when i) the ponds were still ice-covered and a second survey was not performed, or ii) when the survey was done after the eggs were already hatched, which rarely occurred. During the egg-mass surveys, the number of adult Triturus carnifex along the shorelines was counted because their presence can potentially affect the suitability of the ponds as a breeding site of R. temporaria (Tiberti, 2011). When surveys were repeated, the maximum number of Triturus carnifex was used as an index of abundance.

**Statistical analysis**

A linear mixed effects model (LME) was used to test if the observed trend in the time series of egg-mass counts was significant, including the effects of some potentially important covariates, and accounting for the repeated counts in the same ponds and groups of ponds. Transformed of annual egg-mass counts (log+1) were added to the model as a dependent variable, the group of ponds as a random effect and each pond as a nested
random effect. The year of the survey (from year 1 to 11), the pond altitude (centered at 1500 m a.s.l.), the pond area (m²), the number of adult newts along the shoreline, the substrate of the pond (artificial vs. natural), and the mean temperature and the precipitation during i) the breeding season (from 21 April to 10 May), ii) the previous hibernating season (November-April), and iii) the previous activity season (May-October), were added to the model as covariates. In particular, the counts of *Triturus carnifex* were added to the model as their presence can affect the suitability of the ponds as a breeding site for *R. temporaria* (Tiberti, 2011), while the climatic variables were added as they could affect the survival and the energy allocated to reproduction of overwintering frogs, the survival and fat reserves of the frogs at their feeding grounds, and the mildness of the climatic conditions during the breeding period (Meyer *et al*., 1998). Meteorological covariates were measured at the weather station of Pisogne, Brescia (45°49’02”N, 10°09’00”E, 842 m a.s.l.), 6.8 km from the peak of Mount Guglielmo. An “autoregressive moving average” class (corARMA) was used to model the temporal autocorrelation structure of the residuals, previously calculated with the function ACF (Autocorrelation Function) (Crawley, 2012). The LME was fitted by Maximum Likelihood following Zuur *et al.* (2009) using the nlme package of the statistical environment R version 3.1.1 (R Development Core Team, 2010). The MuMIn package (Bartoń, 2011) was used following Grueber *et al.* (2011) to select the best fitting models (ΔAICc < 4) among the models including all possible combinations of the fixed covariates. We report the 95% confidence intervals of the averaged parameter estimates and the relative importance of the covariates provided by the function model.avg of MuMIn. Linear regression was used to check if the used climatic covariates showed a significant trend during the study period.

**Figure 1.** Mount Guglielmo summit (triangle) bordered by the 1000 m a.s.l. contour line. Dotted lines delimit a 500 m buffer area around the numerated ponds belonging to the groups A-D.
RESULTS

The number of egg-masses oviposited in the study area increased significantly from 2005 to 2015 (fig. 2, tab. 1). The number of egg-masses oviposited in natural ponds (38.2 ± 58.5; mean ± sd) was significantly higher than artificial ponds (3.5 ± 11.2; table. 1). In 2014, a decline of the egg-mass counts was observed (fig. 2), although this could be partially influenced by missing data (4 missing counts from ponds B7, D5, D6, and D7 due to ice-covered conditions during the last survey, which, due to the impossibility of performing a second survey, made it impossible to account for the probable later ovipositions).

There was no evidence that climatic variables (mean temperature or precipitation) had a significant effect on oviposition (tab. 1) and there was not a relationship between climatic variables and egg-mass counts. Olenly precipitation during the hibernating season showed a significant increasing trend over the study period (tab. 2), but LME results exclude a causal relationship with the increase in the number of egg-masses in the study area (tab. 1).

Figure 2. Time series of the egg-mass counting in the whole Mount Guglielmo area and divided by groups of ponds (A-D).
DISCUSSION

Because the breeding biology of *R. temporaria* (e.g. aggregate breeding and single clutch per female) suggest that egg-mass counts provide an accurate estimate of the number of reproductive females (Crouch & Paton, 2000), the observed increase in the number of egg-masses likely indicates an increase of the number of breeding females, and probably of the effective population size over this 11-year study.

The climatic variables (mean temperatures and precipitations, tab. 1) were chosen as potentially important factors affecting the survival of hibernating *R. temporaria*, their survival/resource allocation during the activity period (Lardner & Loman, 2003) and the suitability of the breeding season. Climatic factors may affect effective population sizes of several amphibian species (McCaffery & Maxell, 2010), but the present results are consistent with those from other studies, where *R. temporaria* populations were relatively unaffected by a number of climatic variables (Elmberg, 1990; Meyer et al., 1998; Tattersall & Ultsch, 2008). Probably the effects of climatic factors are masked by the importance of density dependent regulation within the population. For example, an amphibian population experiencing a rapid expansion can be relatively unaffected by climatic factors, of course in the absence of catastrophic climatic events.

It is likely that the observed population increase was the results of the end of the epidemic (Tiberti, 2011). These results suggest that some populations of *R. temporaria* have the capacity to withstand high larval mortality. Unfortunately historical data on the abundance of *R. temporaria* before the observed larval mortality events are not available and it is impossible to determine if the observed increases represent post-epidemic recovery. Therefore, it is not possible to know how the observed population increases compares to population sizes before the epidemic. Female *R. temporaria* take 2-5 years to reach sexual maturity (Guarino et al., 2008). Therefore, the effects of the epidemic on the population size might not become evident for years. Such process may explain the pattern of ongoing decline observed in pond group D in 2005-2008 (only 9 egg-masses were counted in ponds group D in 2008) after the end of epidemic in 2006. It is possible that in group D the monitoring period included part of the putative decline.

The persistence of the studied population after high tadpole mortality could be explained in several ways. First *R. temporaria* has a high reproductive potential, which allows populations to recover from a small number of surviving individuals. Moreover this species is distributed widely (Kuzmin et al., 2009) and could re-colonize the extirpated habitats. Also *R. temporaria* longevity (6-15 years; Guarino et al., 2008) could explain the persistence of the studied population: adults can breed for multiple years and, if recruitment failure only occurs for a limited number of years, it is possible that adult persistence could buffer populations against tadpole mortality. Indeed, compared to the mortality of post-metamorphic stages, increased mortality in anuran larvae is less likely to affect the overall population growth rate and the population viability (Biek et al., 2002). However, recurrent epidemic events can sometimes cause persistent declines and local extinctions in *R. temporaria* populations (Teacher et al., 2010), suggesting not to rely too much on their capacity to withstand epidemics.

There are other factors potentially contributing to the observed trend in egg-mass counts. For example,
the sudden onset of *R. temporaria* egg-masses in group C is the result of the increased hydroperiod of a dry pond (C4, fig. 1) in an area where *R. temporaria* was usually absent, probably due to the presence of a particularly large population of Triturus carnifex, which could prevent the reproduction of *R. temporaria* (Tiberti, 2011). This new pond was quickly colonized by *R. temporaria*, which established a small population.

Without a regular maintenance (e.g. removal of vegetation and sediments and re-waterproofing of the bottom), most of the mountain ponds in the study area will probably disappear within a few years to decades, due to their progressive burial or to the infiltration of water at the clay bottom. Pond recovery is a common practice among the local farmers (nine pond restorations were detected in ten years) in the study area, where transhumance still produce an actual water demand for cattle. The survival of alpine transhumance is probably favorable for amphibians and their breeding habitats, preventing the progressive abandonment of the rural landscape, which is often viewed with concern for the likely negative consequences for biodiversity due to land use change (e.g. Hartel & von Wehrden, 2013). However, sometimes the modernization of agricultural practices has meant that traditional ponds - much more suitable as breeding sites for *R. temporaria* (tab. 1) - were replaced by artificial large pools with bottoms made of waterproofing polymers. These pools, due to their steep shore, may also become a lethal trap both for amphibians and other animals, such as small mammals, marmots, and reptiles which can be often found therein drowned (personal observation). This is somehow in contrast with the results from other study areas, where the widespread construction of artificial ponds - less prone to drying out than natural ones - produced a population increase in *R. temporaria* (Cooke, 1972). Probably artificial ponds can become more attracting for *R. temporaria* in a context of general loss of natural ones, but in the area of the Mount Guglielmo frogs can find both the habitats within a short distance, and their choice falls on natural ponds.

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**LITERATURE CITED**


