

Article

Newt life after fish introduction: extirpation of paedomorphosis in a mountain fish lake and newt use of satellite pools

Mathieu DENOËL,^{a,*} Patrick SCIMÈ,^b and Nicola ZAMBELLI^c

^aLaboratory of Fish and Amphibian Ethology, Behavioural Biology Unit, Department of Biology, Ecology and Evolution, University of Liège, 22 Quai van Beneden, 4020 Liège, Belgium, ^bVia Maestra 3, 6930 Bedano, Switzerland, ^cMuseo Cantonale di Storia Naturale, V. C. Cattaneo 4, 6901 Lugano, Switzerland

*Address correspondence to Mathieu Denoël. E-mail: Mathieu.Denoel@ulg.ac.be.

Received on 10 March 2015; accepted on 6 April 2015

Abstract

Fish introduction is one of the main causes of amphibian decline worldwide. It affects particularly rare aquatic phenotypes such as paedomorphs, which retain gills during the adult stage. In this context, we determined whether small wetlands, such as pools surrounding fished and fishless lakes, could sustain paedomorphic and metamorphic newts. To this end, we surveyed lakes known historically to sustain Alpine newts *Ichthyosaura alpestris* as well as 35 nearby pools. On the basis of the published records, the only known population exhibiting paedomorphosis in the Swiss Alps was found to be extirpated by salmonid introductions. However, the metamorphs persisted in peripheral pools, paedomorphosis was discovered at a new locality, and overwintering larvae were still present in one of the lakes. These results show the importance of conserving varied aquatic habitats such as pools in mountainous environments where the main resources can become unsuitable for amphibians because of fish introductions. Pools may also function as reservoirs in maintaining newt populations until programs to remove fish from lakes can be carried out. It is not known if paedomorphs could reappear after fish removal. However, the combined resilience of amphibians after fish removal and the genetic basis for paedomorphosis highlighted in other taxa by previous studies suggest that there is the potential to maintain this intraspecific case of diversity even after its disappearance.

Key words: alps, amphibian decline, fish introduction, mountain lake, Alpine newt, paedomorphosis, pool, wetland.

Fish introductions are widespread all over the world and are known to impact aquatic organisms such as amphibians (McGeoch et al. 2010). Many amphibian species, specifically from lotic habitats which were historically devoid of fish, are particularly vulnerable to introduced fish. They are now either absent from such disturbed habitats or reach low population sizes when co-existence is maintained (Knapp 2005). Long-term surveys also identified amphibian declines following introductions (Denoël et al. 2005), whereas resilience was observed after fish extirpation (Vredenburg 2004).

High-altitude lakes also face fish introductions, particularly in the framework of halieutic activities (Knapp et al. 2001a; Pilliod et al. 2010). Fish are usually not native to these habitats because of

their remoteness and because unpassable barriers, such as waterfalls, often prevent colonizations. In contrast, they have been predominantly inhabited by a variety of organisms such as amphibians (Grossenbacher 1988). Local differentiations and the presence of rare phenotypes such as paedomorphs (i.e., larvae that retain gills throughout adult stage) were historically reported in many of these high-altitude habitats (Denoël et al. 2001, 2005). However, fish introductions during the last decades have caused population declines and extirpation of the paedomorphic phenotype (Denoël et al. 2005).

Small water bodies, such as pools, sometimes of only a few square meter surface area, are potential habitats for amphibians

such as newts and salamanders (Jakob et al. 1999; Durand et al. 2008; Kopecky et al. 2010; Cayuela et al. 2011). For instance, ruts filled with water on unpaved forest roads are usually colonized by newts (Denoël and Demars 2008; Kopecky et al. 2010). Small wetlands, particularly vernal pools are considered to be favorable to amphibians as they are less expected to be stocked with fish (Zedler 2003). However, it is not known whether such small habitats can maintain newt populations and serve as a reservoir for paedomorphosis when the core population living in lakes is extirpated. This is such an important conservation concern to warrant realistic future action plans. The presence of not only nearby metamorphs but also particularly of paedomorphs could be essential for full resilience in their native main habitat following fish removal (for frog resilience see e.g., Knapp et al. 2001b, 2007; Vredenburg 2004;).

In the Alpine newt *Ichthyosaura alpestris*, almost all cases of paedomorphosis (the process by which a larva becomes a paedomorph) are found in the Italian and Balkan peninsulas, whereas this developmental process has been very rarely documented in the Western lineage of central Europe where *I. alpestris* is located (Denoël et al. 2001; Recuero et al. 2014). The rarity of reported cases in this clade suggests that it is particularly endangered. For instance, Ernst (1952) reported a single population in the Swiss Alps, at Pianca Lake; however, more recent visits have reported fish introductions and detrimental effects on Alpine newts (K. Grossenbacher, personal communication).

In this context, we investigated the occurrence of fish and Alpine newts and also compiled an index of newt abundance at the historically sampled lakes (Ernst 1952) and in neighboring pools. In addition, we assessed the contribution of some habitat variables on pool occupancy by newts. Through this approach, our objective was to show the importance of satellite water bodies either as adequate habitats for breeding or paedomorphosis or either as potential sources of individuals for future management work such as extirpating fish from lakes. In the perspective of persistence of the ability of these individuals to give birth to progenies following a paedomorphic developmental pathway, we expect that there would be hope for resilience of paedomorphosis.

Materials and Methods

The studied area is located around the Cima del Masnee between the Maggia and Verzasca Valleys in Canton Ticino, Switzerland (Figure 1). It includes three lakes studied by Ernst (1952): Lago Starlarèscio (also named Lago Starlarèsc da Sgióf and Lago del Starlarèsc in some maps; 1875 m elevation above sea level), Lago Pianca (named Laghetto di Pianca on recent maps; 1913 m), and Laghetto Masnee (named Laghetto Pianca by Ernst; 2,008 m) as well as the neighboring pools (i.e., small natural ponds located at the border and in the upper and lower shelves of the lakes) (Figure 2). Another lake, Lago Scimarmota (also named Lago Starlarèsc da Scimarmota; 1855 m) was also visited. Both Starlarèsc Lakes are part of the hydrographic basin of Verzasca Valley, whereas Pianca and Masnee Lakes are part of the hydrographic basin of Maggia Valley. The pools and lakes are situated in a mountainous environment at elevations ranging from 1790 to 2011 masl (Table 1).

After preliminary observations between 30 July and 2 August, 2013, the main study took place between 27 and 30 July, 2014. The lakes and pools were mapped in 2014 on the basis of recent orthoimages (Swiss Topo 2012, 0.5-m resolution) and a digital elevation model (Swiss Topo 2014, 0.5-m resolution) in using the Swiss

projection CH1903/LV03. WGS 1984 UTM Zone 32 coordinates were used to locate the central part of each pool. Field measurements, ground locations done with a GPS Garmin 64st and aerial photographs taken during the 2014 survey helped in designing the maps. The smallest pools (less than 1 m² or very shallow) and storm pools (i.e., those filled for a short period of time only after rains) were not included. All 35 studied pools were observed on both the 2010 and 2012 orthoimages (Swiss Topo). They can be considered to be “stable” aquatic habitats, i.e., formed during the ecological succession processes of lakes and shelves, and maintaining water consistently through years. Given the configuration of pools and lakes, pools and lakes are not connected by channels (no junctions were noticed during this study).

The maximum water depth was recorded in each pool. During the visit, the water levels were close to the maximum. The concentration of oxygen and the conductivity were recorded at a depth of 10 cm below the water surface in each wetland using a HQ30 Hach-Lange fleximetre (LDO outdoor sensor) and a WTW conductimeter (WTW 330i with Tetracon 325 sensor), respectively.

The main censuses were carried out visually in 2014 in all the water bodies (i.e., pools and lakes) of the area. Water was translucent making it possible to observe Alpine newts (*I. alpestris*, historically described as *Triturus alpestris* and *Mesotriton alpestris*). Each habitat was censused twice: once during the day and once during the night (after sunset) as newts are more easily seen at night in small aquatic habitats such as pools. To determine an index of abundance, newts were counted by walking slowly around the periphery of the water bodies, thus assessing newts present along the shorelines. The index was then expressed in numbers of newts per metre of shoreline. We used the highest value obtained (in all but one case higher at night, $n = 39$). In addition, the presence of overwintering larvae and paedomorphic newts were noted when relevant. Their adulthood was verified after catching them by dip netting on the basis of secondary sexual traits such as a developed cloaca (Denoël and Joly 2000). Snout-vent length (from the tip of the snout to the end of the cloaca) was also measured with a ruler to show size differences between adults and overwintering larvae. Fish occurrence was visually assessed in all habitats. Fishing was carried out with a stick in Pianca Lake in 2013. Additional observations by snorkelling, scuba diving, and trapping (1.5 L bottles) were carried out in Starlarèscio Lake in 2013 and 2014.

We tested for spatial autocorrelation in the dataset in using Moran's *I* to determine if nearby localities had similar newt density values (Dormann et al. 2007). Moran's *I* was calculated on the basis of a matrix of inverse distance weights to give more weight to nearby ponds. Then, the *I* values referring to the distance classes (25-m intervals) were each based on a different distance, to account for each pond being connected to all the ponds within the given distance classes. We plotted the standard residuals on a map to show their spatial distribution (Zuur et al. 2009). We computed a spatial general linear-mixed model (GLMM) with a penalized quasi-likelihood (PQL) method to test for the effect of environmental variables on the density of newts in the 35 pools. We used an exponential correlation structure in the method to take into account the spatial structure in the data. Preliminary analyses with Gaussian and Spherical correlation structure yielded identical results (data not shown). This procedure has good performance when the data are spatially autocorrelated (Dormann et al. 2007). We chose to include in the models only the variables showing large variations (i.e., distance to pools, distance to lakes, and maximum water depth). In this way, the number of descriptors was not too high given the number

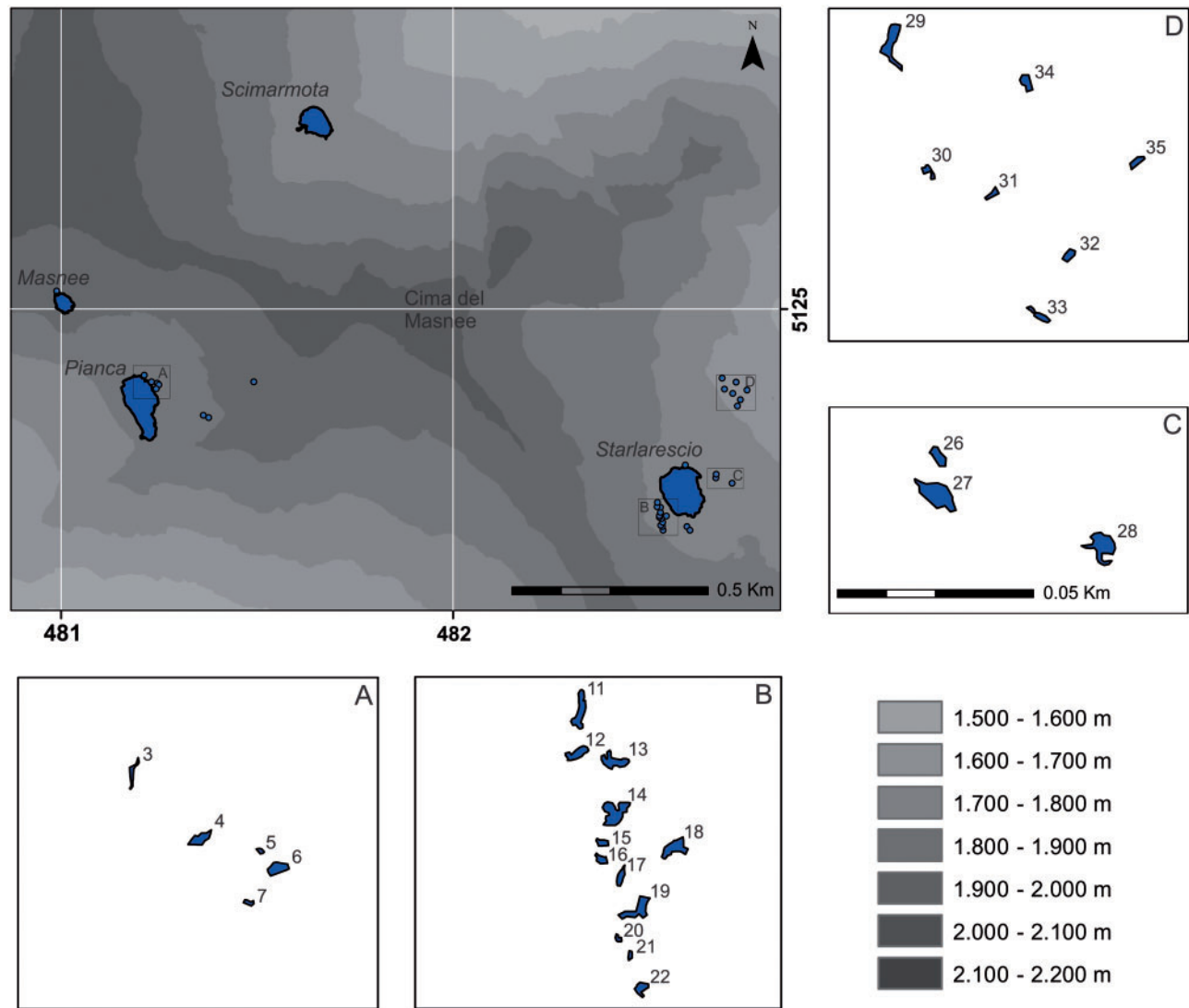


Figure 1. Configuration of pools and lakes around Cima del Masnee (Verzasca and Maggia Valleys, Canton Ticino, Switzerland). The gray scale refers to the elevation above sea level. The four groups of pools (A–D) are shown in detail (zoom 10 \times). See Table 1 for details on newt distribution and pool characteristics. Map coordinates: WGS84 (UTM, in km).

of sites (Ficetola et al. 2014). None of the studied variables were highly correlated (all $r < 0.46$) allowing their simultaneous use in the models. The distances to other aquatic bodies are used to determine the importance of habitat configuration, whereas water depth is a metric used for determining the suitability of pools (Cayuela et al. 2011; Denoël et al. 2013). Moreover, we run the models a second time in considering the same metrics, but in considering the distances to the nearest lakes or aquatic habitat inhabited by newts because nearby populations could have a more positive effect than only wetlands (Ficetola and de Bernardi 2004; Denoël and Lehmann 2006). Oxygen content, conductivity, and elevation above sea level had low variation, therefore, they were not included in the models (separate models showed they had no effects, all $P > 0.76$). Moreover, all ponds were well oxygenated (see results). The hydroperiod was not known, however, as all pools were also observed in 2010 and 2012 orthoimages and because they remained full with water during the summer periods, most could be considered permanent. The water temperature was not included in the models because it showed large diurnal and daily variations. Moran's I tests

were done with the spatial statistics tool in ArcGis 10.2. Mapping residuals and computing spatial GLMM were computed with `gstat`, `spdep`, and `mass` (including `nlme`) packages in R.3.1.2 (Venables and Ripley 2002; Pebesma 2004; Dormann et al. 2007; Bivand 2014; Pinheiro et al. 2014).

Results

There were 35 main pools in the area around Cima del Masnee. They were mainly located close to Starlarescio Lake ($n = 25$), whereas the other pools were around Pianca Lake and the nearby Masnee Lake ($n = 8$ and 2 , respectively; Figure 1, Table 1). Pools had a surface area (mean \pm SE) of 12.2 ± 2 m², a maximum water depth of 0.37 ± 0.03 m, an oxygen concentration of 8.3 ± 0.2 mg/L and a conductivity of 6.3 ± 0.3 μ S/cm. The minimum distance (mean \pm SE) was 15.2 ± 4.4 m between pools and 77 ± 14.8 m between pools and lakes. The most distant pools were 1790 m from each other.



Figure 2. Example of (A) a network of pools (#A, bordering Pianca Lake) and (B) a pool hosting an Alpine newt population (#6, Maggia Valley, Switzerland). See Figure 1 for the cartographic location of pools and lakes.

Fish were present in three of the four lakes (Starlarescio, Scimarmota, and Pianca) in both 2013 and 2014 surveys. Very few fish were recorded at Starlarescio (less than 10 minnows *Phoxinus phoxinus* in a single group, with a maximum density of 0.01 fish/m²), whereas large and small rainbow trouts *Oncorhynchus mykiss* were recorded and caught in Pianca Lake. In Scimarmota Lake, brook trouts *Salvelinus fontinalis* were abundant.

Newts were present in two of the four lakes: Starlarescio and Masnee. They were also found in 49% of pools (i.e., 17 out of 35): 40% of those around Starlarescio Lake ($n=25$) and 70% of those around Pianca and Masnee Lakes ($n=10$). Considering only pools that are within 100 m of the lakes, all five pools were inhabited close to Pianca Lake, one out of two pools close to Masnee Lake and only 5 out of 18 around Starlarescio Lake.

Metamorphic newts reached densities of 0.6 and 0.9 individuals per meter along the shores of Starlarescio and Masnee Lakes,

respectively. In pools, the average ($\pm SE$) densities were 0.12 ± 0.03 newts per meter when considering all 35 pools and 0.24 ± 0.06 newts (min–max: 0.04–0.72) per meter when considering the 17 pools in which newts were present. In terms of the local index of abundances, this represented a maximum of 237 and 177 newts counted along the shore in Starlarescio and Masnee Lakes, respectively, 1.7 ± 0.5 newts when considering all 35 pools and 3.6 ± 0.9 newts (min–max: 1–13) when considering the 17 pools in which newts were present.

Only one paedomorphic individual (one female) was found in Masnee Lake (snout-vent length: 46 mm). All the other adults found in the pools in Masnee and Starlarescio Lakes were metamorphs. In Masnee Lake, the metamorphs had a snout-vent length of 50.3 ± 0.9 mm ($n=26$). Overwintering larvae were recorded in Starlarescio, in Masnee and in one pool. In Starlarescio Lake, both small and large cohorts of overwintering larvae were found, but

Table 1. Characteristics of the studied pools and number of detected newts around Cima del Masnee (Verzasca and Maggia Valleys, Canton Ticino, Switzerland)

Pool	X	Y	Z	Area (m ²)	Depth (cm)	Lake	Lake Dist. (m)	Pool Dist. (m)	Newts
1	480,990	5,125,046	2,011	9	52	Masnee	6	3	0
2	480,991	5,125,040	2,010	3	39	Masnee	1	3	1
3	481,213	5,124,832	1,915	5	38	Pianca	3	21	1
4	481,230	5,124,813	1,915	11	56	Pianca	4	12	3
5	481,246	5,124,810	1,915	2	26	Pianca	16	4	2
6	481,250	5,124,806	1,915	12	33	Pianca	14	4	7
7	481,244	5,124,796	1,915	2	33	Pianca	5	8	1
8	481,363	5,124,728	1,982	4	36	Pianca	119	10	0
9	481,377	5,124,722	1,982	21	29	Pianca	131	10	0
10	481,492	5,124,813	2,051	52	29	Pianca	245	142	8
11	482,520	5,124,507	1,888	17	41	Starlarescio	17	4	2
12	482,520	5,124,495	1,888	11	70	Starlarescio	24	4	0
13	482,529	5,124,493	1,888	15	41	Starlarescio	17	4	0
14	482,529	5,124,480	1,888	26	29	Starlarescio	23	4	1
15	482,526	5,124,473	1,889	4	25	Starlarescio	32	2	0
16	482,526	5,124,468	1,889	5	45	Starlarescio	35	2	0
17	482,530	5,124,464	1,889	6	30	Starlarescio	33	4	0
18	482,544	5,124,471	1,888	17	12	Starlarescio	16	10	0
19	482,536	5,124,455	1,889	17	50	Starlarescio	33	4	0
20	482,530	5,124,447	1,889	2	46	Starlarescio	45	3	0
21	482,533	5,124,443	1,889	2	28	Starlarescio	46	3	0
22	482,536	5,124,435	1,889	7	18	Starlarescio	49	6	0
23	482,597	5,124,444	1,878	3	53	Starlarescio	25	6	0
24	482,604	5,124,435	1,878	15	43	Starlarescio	31	6	0
25	482,592	5,124,601	1,875	12	30	Starlarescio	4	76	0
26	482,671	5,124,568	1,869	11	30	Starlarescio	38	4	10
27	482,669	5,124,569	1,869	43	51	Starlarescio	34	4	4
28	482,713	5,124,556	1,868	32	18	Starlarescio	78	33	4
29	482,686	5,124,823	1,790	25	59	Starlarescio	238	25	0
30	482,697	5,124,790	1,790	5	23	Starlarescio	215	14	7
31	482,713	5,124,784	1,790	4	27	Starlarescio	217	14	1
32	482,733	5,124,768	1,790	6	21	Starlarescio	214	15	1
33	482,725	5,124,752	1,790	7	77	Starlarescio	197	15	1
34	482,722	5,124,813	1,790	8	40	Starlarescio	246	26	2
35	482,751	5,124,792	1,790	5	14	Starlarescio	243	26	0

X, Y, Z: Geographic coordinates (UTM Zone 32T, in m) and elevation above sea level (in m), Area: surface area, Depth: maximum water depth, Lake: closest lake, Lake Dist.: closest distance to a lake, Pool Dist.: closest distance to a pool, Newts: maximum number of adult Alpine newts seen in the pools. All data were recorded in July of 2014.

large individuals were rare (less than 10 recorded). Metamorphs, large and small overwintering larvae had a snout-vent length (mean \pm SE) of 50.7 ± 0.8 mm ($n = 30$), 38.3 ± 1.7 mm ($n = 6$), and 26.6 ± 0.3 mm ($n = 20$), respectively. Trapping in the deepest parts in 2014 and diving in 2013 in Starlarescio Lake gave similar observations on the rarity of large overwintering larvae (found by diving and not by trapping) and the absence of paedomorphs. Courtship behavior was only observed in Masnee Lake during the 2014 surveys (5 pairs), whereas the breeding had already ended in August 2013.

Newt densities were spatially autocorrelated (Moran's $I = 0.303$, $P < 0.001$). Spatial autocorrelation was significant at distances up to 75 m (25 m: $I = 0.251$, $P < 0.05$, 50 m, $I = 0.212$, $P = 0.05$, 75 m: $I = 0.208$, $P < 0.05$). Four out of 5 standard residuals were positive at Pianca Lake and all were negative on the upper shore of Starlarescio Lake (Figure 3). Newt densities were not significantly affected by any of the studied environmental variables: nearest distance to another pool (estimate = -0.001 ± 0.012 , $t_{31} = -0.113$, $P = 0.91$), nearest distance to a lake (0.001 ± 0.004 , $t_{31} = 0.375$, $P = 0.71$), and water depth of the pool (-0.026 ± 0.021 , $t_{31} = 0.375$, $P = 0.22$). Considering the nearest distances to pools

and lakes inhabited by newts gave similar nonsignificant results (to pools: estimate = -0.016 ± 0.012 , $t_{31} = -1.367$, $P = 0.18$; to lakes: 0.007 ± 0.004 , $t_{31} = 1.680$, $P = 0.10$).

Discussion

The presence of pools surrounding lakes allowed the local subsistence of newts after fish introduction in lakes; however, only the dispersal phenotype (the metamorph) was observed in these peripheral habitats. The rare phenotype disappeared following fish introduction in the historical locality; however, paedomorphosis remains present in the area. The persistence of metamorphic newts in satellite pools and of paedomorphosis in a nearby lake suggests the potential for conservation management procedures. On a broader scale, this shows the importance of alternative habitats for the survival of newt populations and possibly their endangered phenotypes.

Although paedomorphosis was reported in almost 100 populations of Alpine newts, very few cases are known to occur in the nominate subspecies of the Western lineage (i.e., in Northwestern and Central Europe) (Breuil 1986; Denoël et al. 2001; Recuero et al. 2014). In Switzerland, Ernst (1952) reported both metamorphs and

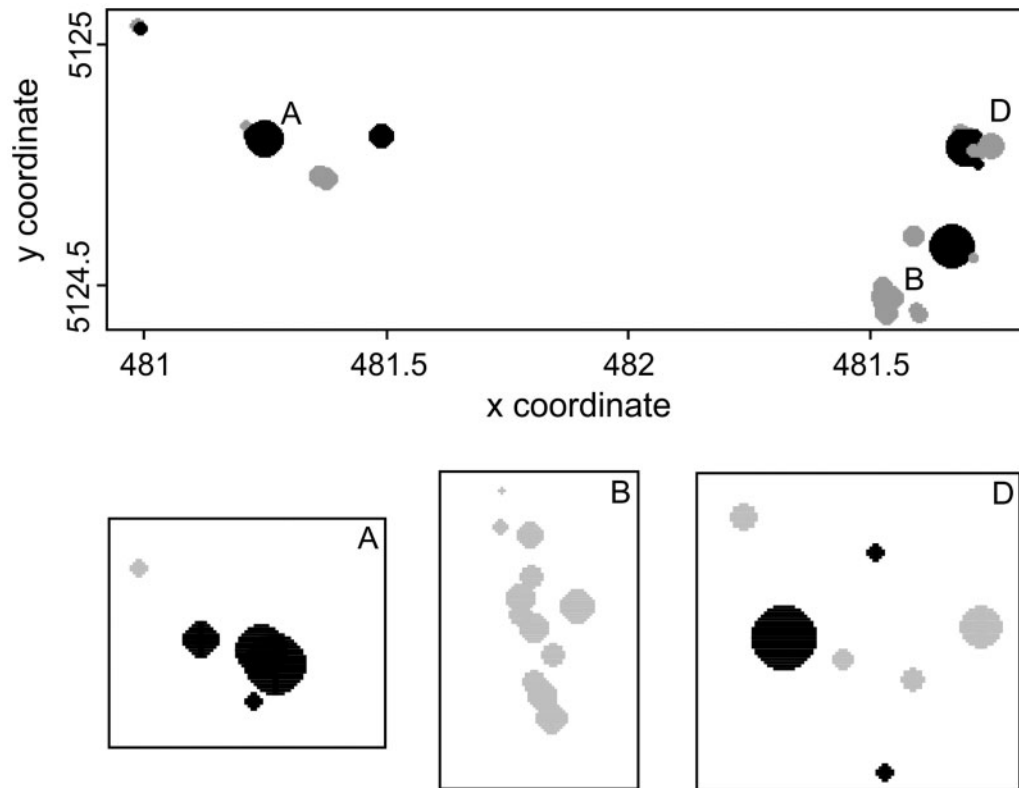


Figure 3. Standard residuals of newt densities versus their X and Y geographic coordinates (UTM, in km). The size of dots is proportional to the values of residuals. Gray: negative values, black: positive values. As some pools were superposed, the three main groups of pools (A, B, D) are shown in detail.

paedomorphs only in Pianca Lake in both 1949 and 1950. Although Ernst did not give census data or relative frequencies of the two phenotypes, he reported large number of adults (i.e., at least 100 in both dates). However, the present surveys (2013–2014), carried out during the same period of the year as those of Ernst (i.e., mid-summer), showed that the species got extirpated in this lake and that paedomorphosis is thus no longer present there, making the historically published case for Switzerland extinct. It is worth pointing out another case of paedomorphosis which was found in Formazza Valley in 1861, which is not that far from this population but now thought to be extinct (de Filippi 1861; Denoël et al. 2001; K. Grossenbacher, personal communication). These populations are located at the southern part of the distribution range of the *I. alpestris* taxon, Western clade (Andreone and Sindaco 1998; Roček et al. 2003; Meyer et al. 2009; Recuero et al. 2014).

The main change that occurred in Pianca Lake was the repeated introduction of salmonid fish such as rainbow trouts. Salmonids are newt predators (Orizaola and Braña 2006) associated with a particularly high decline in the paedomorphic phenotype (Denoël et al. 2005). Metamorphs most probably suffered successive fish introductions with some resilience between these events. Indeed, in August 1972, fish but no Alpine newts were reported by K. Grossenbacher in the lake itself, whereas the opposed pattern was found by M. Sartoris in the lake in 1989. Fish were then reintroduced after 1989 with reports of 150 introduced rainbow trouts in 2009, 200 in 2011, and 100 in 2012 (Ufficio della caccia e della pesca del Canton Ticino, personal communication) with a strong effect on newts as none were recorded in Pianca Lake during our study. No paedomorphs were mentioned in the lake subsequently by Ernst (1952) [i.e., after 1972 (K. Grossenbacher, personal communication)].

The longer persistence of metamorphs but not of paedomorphs is also consistent with the literature (Denoël et al. 2005). The present surveys (2013 and 2014) show that extirpation of metamorphs correlates with contact with introduced fish, but after a longer period than paedomorphs.

Both small and large overwintering larvae were recorded in 2013 and 2014 in Starlarescio Lake. This confirms historical observations of Ernst (1952) in Starlarescio and tends to support the fact that Ernst most probably made a correct distinction between gilled adult and larval stages (that he called “*erwachsenen Neotenen*” and “*neotenen Larven*”, respectively). The presence of two cohorts at this altitude suggests that the largest larvae may have spent 2 winters in the lake; however, skeletochronological analysis would be required to provide a more accurate estimate (Denoël and Joly 2000). Only a few large overwintering individuals (branchiate juveniles *sensu* Denoël and Joly 2001) were observed in the lake (i.e., at least 20 times less than adults), thus indicating that this is a limited phenomenon. It is yet interesting as it is not frequent (Grossenbacher 1979). Moreover, the presence of overwintering larvae and absence of paedomorphs has been reported in several Alpine lakes (Ernst 1952). Branchiate juveniles could thus either represent an intermediate developmental stage towards paedomorphosis (Denoël and Joly 2001) or an alternative strategy (or constraint) of increasing the duration of larval stage while maintaining sexual reproduction after metamorphosis. Comparing such different strategies across wetlands would thus help at understanding the evolution of alternative ontogenetic pathways (see also Whiteman 1994; Whiteman et al. 2012).

Fish were also introduced officially in Scimarmota Lake and unofficially in Starlarescio Lake (Ufficio della caccia e della pesca del

Canton, personal communication). Ernst (1952) found many Alpine newts (i.e., more than 100) in Starlarescio Lake but did not mention fish. Similar observations were reported in July 1993 (more than 50 seen in 1 h) and in spring 1995 (A. Catenazzi, personal communication). Newts are still present and in quite comparable densities in Starlarescio Lake than in Masnee Lake (where fish were never reported). The very low fish densities (only one small shoal of minnows) in Starlarescio Lake could explain the persistence of quite high abundance of newts in the lake. However, if the fish reproduced or additional fish were introduced, this could have fast detrimental consequences on the population as minnows constitute a high risk for Alpine newt populations (Schabetsberger et al. 1995).

Three out of the 4 studied lakes—including not only the lake historically inhabited by paedomorphic newts (i.e., Pianca Lake) but also Masnee and Starlarescio Lakes have a special status of protection, listed in the federal inventory of amphibian breeding sites of national importance for the 2 former and of cantonal importance for the later (Fossati and Maddalena 2003; Pellet et al. 2012). Fish introductions have nevertheless continued. Fortunately, no more fish stocking should occur in both Pianca and Starlarescio Lakes. In addition, measures should be taken so that fish are removed in these two lakes in the perspective of the possible newt resilience in Pianca Lake. This has been shown as a successful approach in mountain lakes to allow rapid recoveries of frogs after fish removal (Knapp et al. 2001b; Vredenburg 2004). For this purpose, the use of nets was shown to be efficient (Knapp and Matthews 1998), but the small size of some fish may make it a difficult task. At a broader extent, the previous successes of fish eradications to recreate favorable biotopes for amphibians in the mountains of North America (see e.g., Knapp et al. 2001b; Vredenburg 2004) show that active management should also be done in European Alpine lakes and in other habitats to allow the maintenance of species such as newts. Habitat restorations have also been shown to be the best strategy to promote the persistence of paedomorphs such as axolotls that are at the edge of extinction (Zambrano et al. 2007; Valiente et al. 2010; Merlín-Uribe et al. 2012). Varied reports show that fish stocking have been widespread in European lakes and continue nowadays, affecting amphibian populations globally (Denoël et al. 2005; Birck et al. 2013). It is thus urgent to launch conservation programmes at local and international scales. The new European legislation on invasive species could hopefully make such actions realistic (Genovesi et al. 2015).

The presence of satellite habitats such as permanent pools around lakes was beneficial as they were inhabited by newts in at least half of the cases. The presence of spatial autocorrelation in the data indicated that not all pool networks were equally inhabited by newts and that a metapopulation structure could explain the distribution of the newts. All the pools close to the shores of Pianca Lake (the fish lake where newt disappeared) were occupied, whereas those close to Starlarescio Lake were almost devoid of newts. Although much smaller than the lakes, these habitats allowed the persistence of Alpine newts around Pianca Lake.

The fact that newts occupy pools around fish lakes has great potential for conservation management, showing the importance of maintaining and creating such small aquatic habitats to sustain amphibian biodiversity (see e.g., Durand et al. 2008). Despite this essential feature, their small size makes them neglected in conservation action plans. In the studied system, several reasons show the importance of preserving them. First, it is known that newts can move among small nearby pools and disperse to farther wetlands (Perret et al. 2003; Kopecky et al. 2010) and that resilience is favored in the presence of neighbouring populations (Knapp et al.

2001b). The proximity between pools and fish lakes (here, such as Pianca Lake and the pools: 67 m on average) could thus promote the resilience of newts. Second, pools are often unlikely to be colonized by fish because of the usual absence of junctions between them and fish lakes and because they are small-size habitats that may not be favorable for long-term establishments of fish at such high elevations above sea level. This is yet not a general rule in Alpine habitats. Third, paedomorphosis has a genetic underpinning that is expressed depending on environmental cues (Semlitsch 1987; Voss et al. 2012). Artificial selection experiments in ambystomatids showed that selection of paedomorphic individuals increases the probability of larvae becoming paedomorphic in the next generations (Semlitsch and Wilbur 1989). As the effect sizes of alleles was observed to vary among species, it is not known whether the results obtained in ambystomatids can be translated in salamandrids such as Alpine newts (Johnson and Voss 2013). However, paedomorphic Alpine newts have been shown to react to environmental disturbance by following a metamorphic developmental pathway similarly to ambystomatids (see e.g., Denoël 2003). If in Alpine newts, paedomorphosis has additive effects and has not been fully counterselected during the generations of metamorph breeding in the pools or in the nearby lake, there might be some potential that progenies laid in the lake after fish extirpation could become paedomorphs. The fact that paedomorphosis was not expressed in the pools is not surprising as paedomorphs are known to prefer deeper waters and to metamorphose in the case of drying (Semlitsch 1987; Denoël 2003; Denoël and Ficetola 2014). During the years of selection for the metamorphic pathways in pools, it is yet likely that “metamorphic” alleles were thus favored over those delaying metamorphosis. Fourth, the observation of a paedomorph in Masnee Lake in 2014 (i.e., at only 22.5 m from Pianca Lake) shows that the process is still present locally and could thus spread through the dispersal morph, the metamorph, which could subsequently lay eggs, from which larvae could become paedomorphs. It is essential that future studies analyze resilience patterns of not only metamorphosis but also paedomorphosis to better understand the evolution of the polyphenism. Experiments and genetic studies are also waited on salamandrid newts to determine the mechanisms promoting the alternative developmental pathways.

Although densities could be similarly high in pools and in lakes, local abundances depend directly on the size of the wetlands. This means that the studied pools could not replace lake populations, which can support much higher population sizes, a trait that might be required for long-term viability. Newt population numbers were low in pools: maximum 13 versus up to 177 and 143 along the shores of Masnee and Starlarescio Lakes, respectively, as determined using the same visual counting protocol. Moreover, in the studied lakes, there were newts out of visual reach from the shoreline (M. Denoël, personal communication); thus, actual population sizes are proportionally much higher than in the pools where estimates are closer to the population size. Even if there are many pools and not all the newts were recorded during the surveys, only a maximum of 56 adults were observed across all observations. The loss of individuals from Pianca Lake cannot thus be fully balanced by survival in pools. Moreover, the presence of several large populations would be beneficial in the case of local crashes, such as those caused by occasional fish stocking. In addition, not all pools may be suitable for reproduction (Denoël et al. 2006; Knapp et al. 2007; Kopecky et al. 2010). Here, no local features explained the distribution of newts in pools. This may be due to the similarity of the studied pools, but also to the low population sizes in the pools. Local densities are

site-specific and then high newt densities can also occur in pools, particularly the more permanent, which offers valuable alternatives to larger habitats such as ponds or lakes (Denoël and Demars 2008; Kopecky et al. 2010). Comparing not only different pool networks across the distribution range of newts and over time but also determining associated reproductive outputs could then help to understand the specificities that make pools suitable for sustaining large newt populations.

Acknowledgments

We thank K. Grossenbacher and three anonymous reviewers for their constructive comments on the article, Alessandro Catenazzi, Kurt Grossenbacher, and Marina Sartoris for sharing their observations, Tiziano Maddalena, Davide Vezzano, and Patrick Wermelinger for field help, Francesco Ficetola and Laurane Winandy for statistical advice, G. Rimbaud for fish identification, the Ufficio della misurazione ufficiale e della geoinformazione (authorization UMG 23 September 2014), and the Centro Sistemi Informativi for providing digital maps (reference 5704002141). Capture and study permits were provided by the Ufficio della Natura e del Paesaggio and the Ufficio della caccia e della pesca (Canton Ticino, Switzerland). M.D. is a Senior Research Associate at Fonds de la Recherche Scientifique (F. R. S. -FNRS). This research was funded by a F.R.S.-FNRS grant (number J.008.13) and a Fonds Spéciaux de la Recherche F.S.R. grant of the University of Liège (number C11/23). This is a publication of the Applied and Fundamental Fish Research Center (AFFISH-RC) and of the Ocean and Freshwater Sciences Research Unit.

References

- Andreone F, Sindaco R, 1998. Erpetologia del Piemonte e della Valle d'Aosta. Atlante degli Anfibi e dei Rettili. Torino: Museo Regionale di Scienze Naturali.
- Birck C, Epailard I, Leccia MF, Crassous C, Morand A, et al., 2013. Sentinel lakes: a network for the study and management of mountain lakes in the French Alps and in Corsica. *Journal of Protected Mountain Areas Research* 5:63–69.
- Bivand R, 2014. spdep: Spatial dependence: weighting schemes, statistics and models. (17 March 2015).
- Breuil M, 1986. Biologie et différenciation génétique des populations du triton alpestre *Triturus alpestris* (Amphibia Caudata) dans le sud-est de la France et en Italie, PhD Thesis. Paris: Paris-Sud University.
- Cayuela H, Cheylan M, Joly P, 2011. The best of a harsh lot in a specialized species: breeding habitat use by the yellow-bellied toad *Bombina variegata* on rocky riverbanks. *Amphibia-Reptilia* 32:533–539.
- de Filippi F, 1861. Sulla larva del *Triton alpestris*. *Archivio Zoologico Italiano Napoli* 1:206–211.
- Denoël M, 2003. How do paedomorphic newts cope with lake drying? *Ecography* 26:405–410.
- Denoël M, Demars B, 2008. The benefits of heterospecific oophagy in a top predator. *Acta Oecologica* 34:74–79.
- Denoël M, Duguet R, Džukić G, Kalezić ML, Mazzotti S, 2001. Biogeography and ecology of paedomorphosis in *Triturus alpestris* (Amphibia, Caudata). *Journal of Biogeography* 28:1271–1280.
- Denoël M, Džukić G, Kalezić ML, 2005. Effect of widespread fish introductions on paedomorphic newts in Europe. *Conservative Biology* 19:162–170.
- Denoël M, Ficetola GF, 2014. Heterochrony in a complex world: disentangling environmental processes of facultative paedomorphosis in an amphibian. *Journal of Animal Ecology* 83:606–615.
- Denoël M, Joly P, 2000. Neoteny and progenesis as two heterochronic processes involved in paedomorphosis in *Triturus alpestris* (Amphibia: Caudata). *Proceedings of Royal Society of London B Biological Sciences* 267:1481–1485.
- Denoël M, Joly P, 2001. Size-related predation reduces intramorph competition in paedomorphic Alpine newts. *Canadian Journal of Zoology* 79:943–948.
- Denoël M, Lehmann A, 2006. Multi-scale effect of landscape processes and habitat quality on newt abundance: implications for conservation. *Biological Conservation* 130:495–504.
- Denoël M, Perez A, Cornet Y, Ficetola GF, 2013. Similar local and landscape processes affect both a common and a rare newt species. *PLoS ONE* 8:e62727.
- Denoël M, Whiteman HH, Wissinger SA, 2007. Foraging tactics in alternative heterochronic salamander morphs: trophic quality of ponds matters more than water permanency. *Freshwater Biology* 52:1667–1676.
- Dormann CF, McPherson JM, Araujo MB, Bivand R, Bolliger J et al., 2007. Methods to account for spatial autocorrelation in the analysis of species distributional data: a review. *Ecography* 30:609–628.
- Durand J, Windmiller B, Richards FP, 2008. Vernal pool identification and protection: current and future permitting implications. In: Goodrich-Mahoney JW, Abrahamson LP, Ballard JL, Tikalsky SM, editors. *Environment Concerns in Rights-of-Way Management 8th International Symposium*, Elsevier p, 479–492.
- Ernst F, 1952. Biometrische Untersuchungen an schweizerischen Populationen von *Triton alp. alpestris* (Laur.). *Revue Suisse de Zoologie* 59:399–476.
- Ficetola GF, De Bernardi F, 2004. Amphibians in a human-dominated landscape: the community structure is related to habitat features and isolation. *Biological Conservation* 119:219–230.
- Ficetola GF, Bonardi A, Mucher CA, Gilissen NLM, Padoa-Schiopoa E, 2014. How many predictors in species distribution models at the landscape scale? Land use versus LiDAR-derived canopy height. *International Journal of Geographical Information Science* 28: 1723–1739.
- Fossati A, Maddalena T, 2003. Strategia cantonale per lo studio e la protezione degli anfibi e dei rettili. Belinzona, Switzerland: Repubblica e Cantone Ticino.
- Genovesi P, Carboneras C, Vilà M, Walton P, 2015. EU adopts innovative legislation on invasive species: a step towards a global response to biological invasions? *Biological Invasions* 17: 1307–1311.
- Grossenbacher K, 1979. Untersuchungen zur Entwicklungsgeschwindigkeit der Larven von *Triturus a. alpestris* (Laurenti 1768), *Bufo b. bufo* (Linnaeus 1758) und *Rana t. temporaria* (Linnaeus 1758) aus Populationen verschiedener Höhenstufen in den Schweizer Alpen, PhD Thesis. Bern: Universität Bern.
- Grossenbacher K, 1988. Atlas de distribution des amphibiens de Suisse. Bâle: Ligue Suisse pour la Protection de la Nature.
- Jakob C, Morand A, Crivelli AJ, 1999. Amphibian communities in a mosaic of Mediterranean seasonally-flooded ponds: species richness and composition (Nature reserve of Roque-Haute, France). In: Miaud C, Guyétant R editors. *Current Studies in Herpetology*. Le Bourget du Lac: SEH. p, 221–225.
- Johnson CK, Voss SR, 2013. Salamander paedomorphosis: linking thyroid hormone to life history and life cycle evolution. In: Shi Y-B editor. *Current Topics in Developmental Biology*, vol. 103. *Animal Metamorphosis*. Burlington: Academic Press. p, 229–258.
- Knapp RA, 2005. Effects of nonnative fish and habitat characteristics on lentic herpetofauna in Yosemite National Park, USA. *Biological Conservation* 121:265–279.
- Knapp RA, Boiano DM, Vredenburg VT, 2007. Removal of nonnative fish results in population expansion of a declining amphibian (mountain yellow-legged frog *Rana muscosa*). *Biological Conservation* 135:11–20.
- Knapp RA, Corn PS, Schindler DE, 2001a. The introduction of nonnative fish into wilderness lakes: good intentions, conflicting mandates, and unintended consequences. *Ecosystems* 4:275–278.
- Knapp RA, Matthews KR, 1998. Eradication of nonnative fish by gill netting from a small mountain lake in California. *Restoration Ecology* 6:207–213.
- Knapp RA, Matthews KR, Sarnelle O, 2001b. Resistance and resilience of alpine lake fauna to fish introductions. *Ecological Monographs* 71:401–421.
- Kopecky O, Vojar J, Denoël M, 2010. Movements of Alpine newts *Mesotriton alpestris* between small aquatic habitats (ruts) during the breeding season. *Amphibia-Reptilia* 31:109–116.
- McGeoch MA, Butchart SHM, Spear D, Marais E, Kleynhans EJ, et al., 2010. Global indicators of biological invasion: species numbers, biodiversity impact and policy responses. *Diversity Distribution* 16:95–108.
- Merlín-Uribe Y, Contreras-Hernández A, Astier-Calderón M, Jensen OP, Zaragoza R, et al., 2012. Urban expansion into a protected natural area in

- Mexico City: alternative management scenarios. *Journal of Environmental Planning and Management* 56:398–411.
- Meyer A, Zumbach S, Schmidt B, Monney J-C, 2009. Les amphibiens et les reptiles de Suisse. Bern, Switzerland: Haupt.
- Orizaola G, Braña F, 2006. Effect of salmonid introduction and other environmental characteristics on amphibian distribution and abundance in mountain lakes of northern Spain. *Animal Conservation* 9:171–178.
- Pebesma EJ, 2004. Multivariable geostatistics in S: the gstat package. *Computers & Geosciences* 30:683–691.
- Pellet J, Borgula A, Ryser J, Zumbach S, 2012. Inventario federale dei siti di riproduzione degli anfibi di importanza nazionale. Valutazione dei siti di riproduzione e definizione dei valori soglia. Ufficio federale dell'ambiente (UFAM): Switzerland.
- Perret N, Pradel R, Miaud C, Grolet O, Joly P, 2003. Transience, dispersal and survival rates in newt patchy populations. *Journal of Animal Ecology* 72:567–575.
- Pilliod DS, Hossack BR, Bahls PF, Bull EL, Corn PS, et al., 2010. Non-native salmonids affect amphibian occupancy at multiple spatial scales. *Diversity Distribution* 16:959–974.
- Pinheiro P, Bates D, DebRoy S, Sarkar D, 2014. Linear and nonlinear mixed effects models. R package version 3.1.
- Recuero E, Buckley D, García-París M, Arntzen JW, Cogălniceanu D, et al., 2014. Evolutionary history of *Ichthyosaura alpestris* (Caudata, Salamandridae) inferred from the combined analysis of nuclear and mitochondrial markers. *Molecular Phylogenetics and Evolution* 81:207–220.
- Roček Z, Joly P, Grossenbacher K, 2003. *Triturus alpestris* (Laurenti, 1768) - Bergmolch. In: Grossenbacher K, Thiesmeier B editors. Handbuch der Reptilien und Amphibien Europas. Schwanzlurche IIA. Wiebelsheim: Aula. p, 607–656.
- Schabetsberger R, Jersabek CD, Brozek S, 1995. The impact of Alpine newts *Triturus alpestris* and minnows *Phoxinus phoxinus* on the microcrustacean communities of two high altitude karst lakes. *Alytes* 12:183–189.
- Semlitsch RD, 1987. Paedomorphosis in *Ambystoma talpoideum*: effects of density, food, and pond drying. *Ecology* 68:994–1002.
- Semlitsch RD, Wilbur HM, 1989. Artificial selection for paedomorphosis in the salamander *Ambystoma talpoideum*. *Evolution* 43:105–112.
- Valiente E, Tovar A, González H, Eslava-Sandoval D, Zambrano L, 2010. Creating refuges for the axolotl *Ambystoma mexicanum*. *Ecological Restoration* 28:257–259.
- Venables WN, Ripley BD, 2002. Modern applied statistics with S. New York: Springer.
- Voss SR, Kump DK, Walker JA, Shaffer HB, Voss GJ, 2012. Thyroid hormone responsive QTL and the evolution of paedomorphic salamanders. *Heredity* 109:293–298.
- Vredenburg VT, 2004. Reversing introduced species effects: experimental removal of introduced fish leads to rapid recovery of a declining frog. *Proceedings of National Academy of Sciences USA* 101:7646–7650.
- Whiteman HH, 1994. Evolution of facultative paedomorphosis in salamanders. *Quarterly Review of Biology* 69:205–221.
- Whiteman H, Wissinger S, Denoël M, Mecklin C, Gerlanc N, et al., 2012. Larval growth in polyphenic salamanders: making the best of a bad lot. *Oecologia* 168:109–118.
- Zambrano L, Vega E, Herrera LG, Prado E, Reynoso VH, 2007. A population matrix model and population viability analysis to predict the fate of endangered species in highly managed water systems. *Animal Conservation* 10:297–303.
- Zedler PH, 2003. Vernal pools and the concept of “Isolated wetlands”. *Wetlands* 23:597–607.
- Zuur AF, Ieno EN, Walker NJ, Saveliev AA, Smith GM, 2009. Mixed effects models and extensions in ecology with R. New York: Springer.