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ORIGINAL ARTICLE

Monitoring of the last stronghold of native pool frogs (*Pelophylax lessonae*) in Western Europe, with implications for their conservation

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Abstract

The original diversity of *Pelophylax* water frogs has been compromised by multiple biological invasions all over Western Europe. For the European pool frog (*P. lessonae*), the Joux Valley—a 30 km highland depression in northwestern Switzerland—stands as the last stronghold spared by exotic lineages. In order to manage *P. lessonae* in the valley, we combined traditional field surveys with environmental DNA metabarcoding and mapped the regional distribution of amphibian species. Both approaches concurred that *P. lessonae* persists at a single isolated site (Pontet). Continuous monitoring of this population throughout the spring and summer 2019 informed on their wintering quarters (most likely the forest litter immediately surrounding the breeding pond), as well as the timing of migration (end of April), breeding (June), and larval development (June–August). In parallel, we experimented the first use of drone technology for amphibian surveillance: 30 adults were individually counted during an aerial survey at the peak of the breeding season, confirming the small size of the population. Finally, we compared some biotic and abiotic properties among water bodies throughout the valley and flagged a few sites that were ecologically similar to Pontet. In a landscape dominated by pastoral activities where *Pelophylax* dispersal is virtually impossible, these could be candidates for future translocation efforts. Our study illustrates the application of next-generation monitoring techniques for the urgent management of threatened species and stresses the need for reevaluating the conservation status of *P. lessonae* in Western Europe, where it appears to subsist in low numbers at one last locality.

Keywords Biological invasion · Drone · Environmental DNA · Jura Mountains · *Pelophylax bergeri*

Introduction

Cryptic biological invasions represent an emerging challenge for wildlife authorities. Their management requires to survey morphologically similar and hybridizing taxa in order to set up conservation responses specific to the local populations under threat (Allendorf et al. 2001; Gaskin 2017; Morais and Reichard 2017). In a first step, genetic surveys are helpful to map native vs alien species ranges and investigate patterns of genetic admixture (Petit 2004; Crispo et al. 2011), notably to identify areas with the highest conservation value, i.e., where populations are free of exotic alleles. In a second step, gaining specific insights on the ecology, life history, and demography of the confirmed native populations, as well as refining their distributions at the local scale, shall provide much-needed information to properly channel management resources for protective actions (e.g., habitat restoration, connectivity, translocation programs). The latter step is particularly relevant, because native taxa may have different ecological niches than their cryptic alien counterparts (Morais and Reichard 2017). For instance, some of the Mediterranean herpetofauna of Europe thrive in northern invasive ranges, potentially due to a broader ecological tolerance than local species (Dubey et al. 2019a).

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In this study, we focus on the European pool frog (*Pelophylax lessonae*), a lowland anuran amphibian predominantly found in shallow vegetated ponds, which faces local declines due to habitat loss and fragmentation, water pollution, disease, and invasive species (Speybroeck et al. 2016). This is particularly the case in the heavily impacted regions of Western Europe, where some of its main enemies are other *Pelophylax* frogs of exogenous origin (summarized in Dufresnes and Dubey 2020). In France, Belgium, Switzerland, and western Germany, pool frogs suffer from competition and sexual parasitism through hybridogenesis by Balkan and Anatolian lineages of the marsh frog (*P.* cf. *ridibundus*), imported for the frog leg industry in the middle of the twentieth century (Holsbeek et al. 2008; Dufresnes et al. 2018). In parallel, molecular data revealed that the genetic integrity of western European *P. lessonae* has been compromised by introgressive hybridization by its sister taxon, the Italian pool frog (*P. bergeri*), as a result of putatively ancient translocations from the Apennine Peninsula (Dufresnes et al. 2017; Dufresnes and Dubey 2020). Nowadays, most populations from eastern France and northern Switzerland bear extensive traces of admixture at nuclear and mitochondrial markers (Dufresnes et al. 2017; Dufresnes and Dubey 2020). The conservation situation of *P. lessonae* is thus critical, and its fate in Western Europe depends on the protection of the remaining populations that has not been reached by the *P. bergeri* gene pool.

Specifically, our recent studies emphasized that the Joux Valley, a 30-km-long highland limestone depression isolated within the Jura Mountains in northwestern Switzerland, was the last stronghold of genuine *P. lessonae* north of the Alps (Dufresnes et al. 2017; Dufresnes and Dubey 2020). Given that only a single site has been confirmed (Pontet), it has become of national importance for the species, which is otherwise restricted to the south-Alpine canton of Ticino. Yet, little is known about the amphibian cohorts of the valley (which features > 20 potential amphibian breeding sites), especially whether additional *P. lessonae* populations exist. Details about ecological preferences, such as the terrestrial and aquatic micro-habitats used, phenology, and migration pathways, are also lacking. This is first because the Joux Valley, located ~ 1000 m a.s.l., is an unconventionally high environment for this lowland species – the altitudinal limit for *Pelophylax* in Switzerland is ~1300 m a.s.l. Second, the experience of local wildlife naturalists and managers actually involves *P. lessonae* × *P. bergeri* hybrids, which inhabit the entire Swiss plateau and surroundings (Dufresnes et al. 2017; Dufresnes and Dubey 2020). As suspected in similar cases of trans-Alpine introductions (Dubey et al. 2019a), the ecological niche of *P. lessonae* could be narrower than of the Mediterranean *P. bergeri*, in turn explaining the invasive success of the latter. More generally, the life history of *P. lessonae* is poorly known, as it is often considered together with other syntopic water frogs; additional data would thus be welcome.

Fig. 1 Distributions and amphibian diversity of the aquatic sites ▶ inventoried in the Joux Valley (western Switzerland) by traditional monitoring (top) and eDNA (bottom). The presence of fishes is indicated by black fish symbols. The color codes of each amphibian species are provided in the middle frame. The pontet site (PON) is highlighted in bold. Distribution of *P. lessonae* (green) and *P. bergeri* (black) are indicated on the bottom-right map, with north-Alpine regions of Western Europe consisting of a mixture of both (dashed area), except the Joux Valley (green star). Photo credits: CD and SD

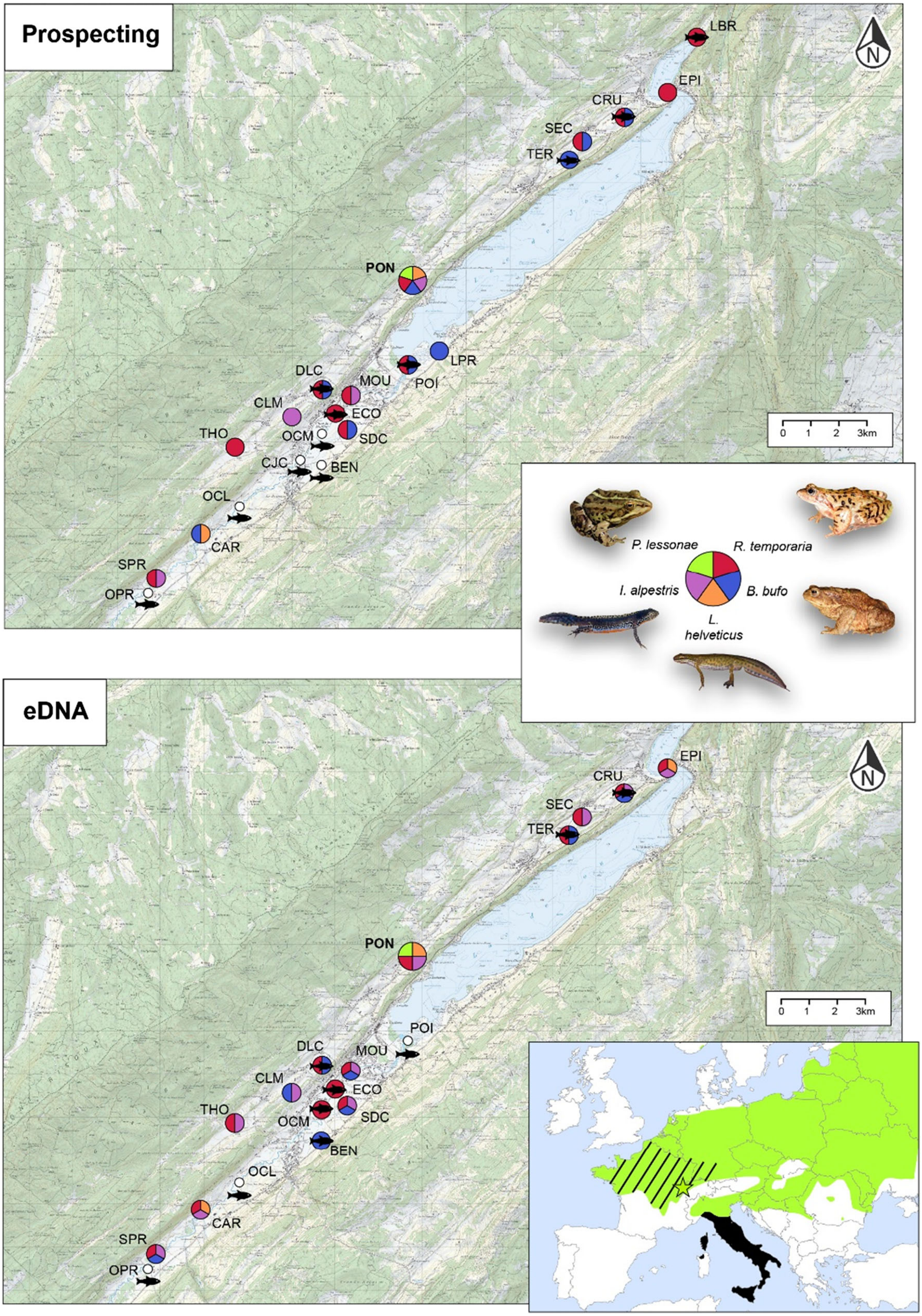
After having identified the Joux Valley as a prime candidate for immediate protection of *P. lessonae* in Western Europe, here we report on the subsequent steps necessary to plan a sustainable management. First, we aimed at mapping the occurrence of the species at the local scale, combining traditional and molecular amphibian monitoring using environmental DNA (eDNA) metabarcoding. Second, we investigated some key aspects of the ecology and life history of pool frogs at the confirmed population of Pontet, namely, the location of their overwintering sites, migration pathways, breeding phenology, and population size. To this end, we applied various surveillance tools throughout the season and experimented the first use of drone aerial photography for amphibian census. Third, we measured relevant abiotic properties of all sites potentially available for amphibians, in order to explore what characteristics may favor the persistence of *P. lessonae*, in the context of future revitalization/translocation efforts.

Methods

Amphibian monitoring in the Joux Valley

All potential amphibian breeding sites of the valley ($n = 21$, Fig. 1) were visited three times during nighttime at regular intervals of about 2 weeks in June–July 2019, which corresponds to the peak of *P. lessonae*'s activity in the area. A few sites are subdivided in several water bodies (CRU, SEC, PON, CLM, CAR), which were all individually prospected. Each visit included 5–10 min of listening for calls, a visual search with a torch, and dip-netting areas of the pond suitable for tadpoles. All amphibian detections were reported, and the presence of fishes (which can predate tadpoles) was also visually assessed. The sites were also scouted at least once by daytime, to properly infer the type of habitat.

In parallel, most sites ($n = 18$) were screened for eDNA in 2019, using the metabarcoding technology of SPYGEN (Le Bourget-du-Lac, France). For two sites (PON and CAR), separate water bodies were analyzed. The Pontet pond (PONp) was analyzed three times (twice in 2019 and once during a preliminary survey in 2018). The approach, developed by Valentini et al. (2016), was identical to our recent eDNA survey in Basel area (Dufresnes et al. 2019), where the full



protocol is exhaustively detailed. Briefly, this consists in the collection of 2 L of water per site and in its filtering on the field, followed by DNA extraction (Pont et al. 2018) and amplification with 12 replicated PCRs per sample using tagged *batra* primers (Valentini et al. 2016). Library preparation and sequencing were performed at Fasteris (Geneva, Switzerland). Three libraries were prepared using the MetaFast protocol (Fasteris) and a paired-end sequencing (2 × 125 bp) was carried out with an Illumina MiSeq with the MiSeq Kit v3 (Illumina), following the manufacturer's instructions. Sequence reads were analyzed using the programs implemented in the OBITools package (Boyer et al. 2016), as described (Dufresnes et al. 2019). Five negative extraction controls and three PCR negatives controls, each with 12 replicates as well, were sequenced in parallel. Filtering parameters included fragment size longer than 20 bp, occurrence higher than 10 reads, and sequence similarity to reference databases above 98%. Sequences identified as "internal" by the *obiclean* software (probably corresponding to PCR/sequencing errors), and below 0.001 of occurrence frequency per taxon and per library (probably corresponding to tag-jumps; Schnell et al. 2015) were discarded. The *batra* primers also amplified fish taxa, allowing to ascertain their presence/absence, although species-level resolution could not be considered since these primers are not reliably species-specific for this vertebrate class.

Species-level identification was possible for all amphibians except for the genus *Pelophylax*, where only the following sets of taxa present in Western Europe can be disentangled from eDNA: (i) *P. ridibundus*/*P. kurtmuelleri*/ *P. bedriagae*; (ii) *P. lessonae*/*P. bergeri*/*P. esculentus*, and (iii) *P. perezi*/ *P. grafi*. This is however not an issue, since only the native *P. lessonae* had previously been identified from genetic analyses in the study area (Dufresnes et al. 2017; Dufresnes and Dubey 2020).

Survey of *P. lessonae* at Pontet

Pontet is located 3 km from the village of Le Chenit on the road leading to Le Pont, in the upland corridor that extends along the northern shore of Lake Joux. The site itself is peatland (high marsh), mostly degraded in a low marsh with an ovale pond of 250m^2 (PONp), resulting from peat exploitation in the first half of the twentieth century. It is heavily vegetated by *Potamogeton natans* at its deepest part (~ 1.5 m), on the northeastern edge. Except for a low marsh on the southern and northern sides, the pond is now immediately enclosed by a forest patch of about 2.4 ha of deciduous trees and spruces. A narrow canal runs along the northern edge of the forest patch (PONc), mainly fed by runoff waters. Farmlands used for pastoral activities surround the area, and mixed forests dominated by spruces extend further south (bordering the lake shore) and north (Jura Mountains). Figure 2 provides an aerial view of the area.

To identify wintering quarters and migrations pathways, drift fences were set up along six sectors (A–F) around Pontet (Fig. 2). Fences were opened 28 times between April 3 and May 29, 2019. All amphibian specimens caught were identified before release.

To inform on the phenology and population size of *P. lessonae* in Pontet, the main pond was monitored by regular summer visits in 2019, during which population density, breeding activity and offspring development were assessed. Specifically, nocturnal searches were conducted in June–July. One issue with traditional census of *Pelophylax*, however, is that frogs tend to dive prematurely when disturbed, thus going unnoticed. To overcome this issue, we took high-resolution photographs of the pond on June 21, at the peak of the calling activity, using a drone (DJI Mavic Air Fly More Combo) flown up to 6 m above pond level. We could then accurately count the individuals present in the water. Finally, from end of July to end of August, three trapping sessions were organized to capture tadpoles/metamorphs, both to confirm the breeding success and assess the progress of the larval cycle. For each session, a total of 20 funnel traps were spread all around the pond (at the water surface) in the late afternoon (16–19 h), and controlled the following morning (9–12 h). The captured animals were identified and released. *Pelophylax* individuals were counted, and the approximate developmental stage of tadpoles was inferred following Gosner (1960).

Properties of the amphibian sites in the Joux Valley

In June–July 2019, all water body candidates for amphibians were characterized for three sets of variables. First, their general morphometry was assessed by estimating size (from aerial photos) and relative maximum water depth (in three classes as A<50 cm<B< 150 cm<C). Second, we measured the following biochemical properties of the water using a multi-purpose field probe (PCE-PHD-1-KIT1, OCE Instruments): pH, temperature, salinity, conductivity, and the concentration of dissolved oxygen (O_2). Because these may be dependent on climatic conditions, we made replicated measurements by independent visits, when possible (1–6, on average 2.6 ± 1.1 visits per site). In complement, we titrated the carbonate hardness (KH) with the sera aqua-test box (sera ©). Third, using that same kit, we evaluated water quality by measuring concentrations of ammonium/ammonia (NH_4/NH_3), nitrogen dioxide (NO_2), nitrate (NO_3), and phosphate (PO_4).

All statistical analyses were conducted in R 3.5.0 (R Core Team 2018). We predicted the presence of each amphibian taxon using multivariate analyses of variance (MANOVA, R package *stats*), combining the morphometric, biochemical (averaged over replicates), and water quality variables, with the occurrence of fish and alpine newts (the main predators of amphibian tadpoles) assessed by the field and eDNA monitoring (see above). We also performed a principal component analysis (PCA) to visualize whether Pontet differs markedly from other sites, and/or which ones are the most similar to it (R package *ade4*).

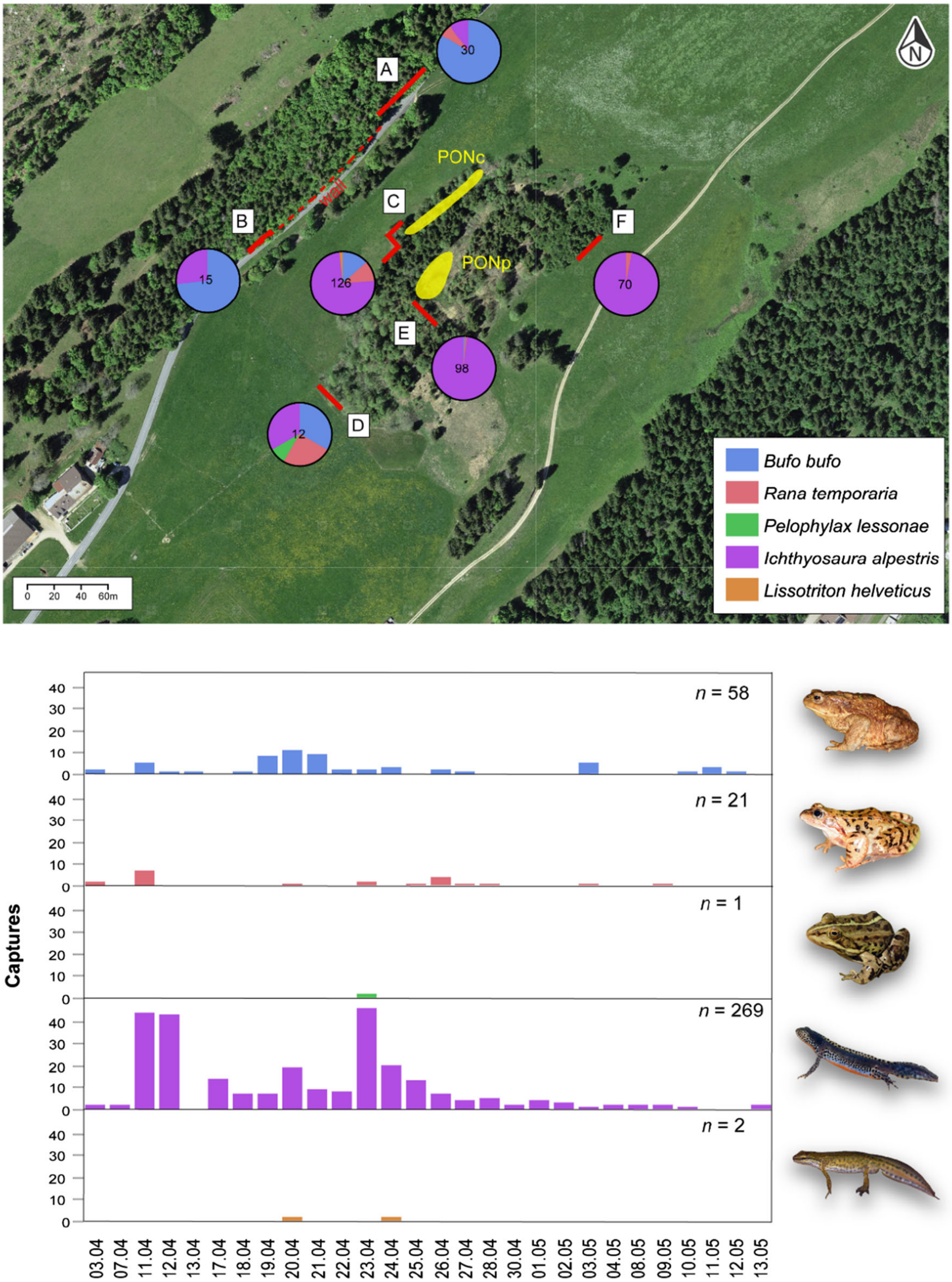


Fig. 2 Aerial view of the Pontet site and surroundings, with location of drift fences (plain red lines), and the proportion of each amphibian captured in spring 2019 (top), as detailed for each day of the survey (bottom). Locations of the pond (PONp) and the forest canal (PONc) are indicated. Photo credits: CD and SD

Results

Amphibian monitoring

No additional population of *P. lessonae* was discovered in the entire Joux Valley, neither by field nor eDNA monitoring (Fig. 1, Table S1). Instead, both approaches confirmed that the species is restricted to the single site of Pontet, in the vegetated pond (PONp) specifically. Four other amphibians were reported and mapped in the region: the common toad (*Bufo bufo*), the common frog (*Rana temporaria*), the alpine newt (*Ichthyosaura alpestris*), and the palmate newt (*Lissotriton helveticus*). The first three were widespread and abundant, while *L. helveticus* was only found at three localities (Fig. 1, Table S1). Fishes were present in about half (46%) of the water bodies investigated (Fig. 1). Details on sequence reads for each species are available in Table S1.

The results obtained from the field and eDNA surveys matched in 78% of cases (Fig. S1). Species were visually observed but not detected by eDNA in 6% of cases (most of them involving *Bufo bufo*—seen on the ground, not in the water). The opposite situation (eDNA detection but no sighting) was more frequent, i.e., 16% of cases (especially for the aquatic *Ichthyosaura alpestris*—putatively hiding on the pond floor).

Survey of *P. lessonae* at Pontet

A single pool frog was captured by the drift fences on April 23, 2019, at the southwestern wood edge (sector D), less than a hundred meters from the pond (Fig. 3). The other species known from Joux were all captured, especially during two migration peaks in early and mid-April, respectively. Spatial and temporal aspects of the amphibian migration in Pontet are displayed in Fig. 3.

Thirteen visits were carried out in Pontet during June–August 2019 (Table 1). Adults were sighted at most occasions, concentrated on the vegetated area on the northeastern side of the pond. Using high-resolution aerial photography (drone), we counted 30 adults on June 21, 2019 (Fig. 3). Similar numbers were roughly estimated by nocturnal census during the first three weeks of June, and amplexus were spotted in mid-June (Table 1). Choruses were the strongest at that time, but males could be heard as late as in mid-August. Large tadpoles (> 5 cm, with developed toes, Gosner stages 36–38) were caught in late-July and early-August. In late-August, however, all were metamorphing (bearing all four limbs, Gosner stages 42–46), while tens of metamorphs were simultaneously spotted on the water vegetation. In addition, tens of newt larvae (*I. alpestris*) and insect larvae (notably *Dytiscus* and *Notonecta*) were trapped during each session.

Properties of the amphibian sites in the Joux Valley

All sites were free of nitrogen pollutants, except two: CARg, located in a gravel pit, and POI, a calm section of the Orbe River. For these, replicate analyses performed at a few days interval yielded identical results. Among the factors bearing the most meaningful variation, pond sizes ranged from 30m^2 (SECo, a water hole) to 2.9 ha (TER, a fishing lake), pH ranged from 4.5 (THO, a bog) to 9.2 (CARg, a quarry pond), KH ranged from 0 (THO) to 12 (PONc, the forest canal of Pontet fed by runoff waters), water temperature ranged from 12.2 (OPR, a fast-flowing river site) to 24.1 °C (CLMf, an exposed flooded field), and dissolved O_2 ranged from 3–4 (most vegetated sites) to 8–9 mg/L (most riverine sites). Moreover, five of the surveyed water bodies were already dried by mid-July. All site measurements are available in Table S1.

The fact that *P. lessonae* occurred at a single locality limited our ability to flag relevant parameters conditioning its persistence, and no variable was significant in the

Fig. 3 Aerial photography using drone technology, illustrating its application for frog census. A total of 30 individuals could be counted on June 21, 2019, from the photographs

Table 1 Monitoring of *P. lessonae* at Pontet in summer 2019, combining nocturnal surveys, drone census ([d]), and captures

Date	Time	Calls	Observations	Captures (traps)
03.06.2019	17:30	+	scattered adults and subadults	
04.06.2019	23:30	+	~ 20–30 adults	
13.06.2019	22:00	+++	~ 20–30 adults (including 2 amplexus)	
19.06.2019	01:30	–	> 10 adults	
21.06.2019	13:00	+	30 adults (including 1 amplexus)[d]	
24.06.2019	15:00	+	3 adults	
10.07.2019	22:15	–	4 adults	
29.07.2019	15:00	–	3 adults	
30.07.2019	10:00	–	5 adults	2 adults, 19 tadpoles
06.08.2019	18:00	–	–	
07.08.2019	10:00	+	–	17 tadpoles
30.08.2019	16:00	–	–	
31.08.2019	10:00	–	> 30 metamorphs	2 tadpoles, 4 metamorphs

MANOVA (Table S3). The PCA did not suggest any unique characteristics of PONp, which received average values for many variables, and thus resembles several other sites, especially vegetated ones (Fig. 4). Among the main features of PONp, we can list the absence of fish and pollutants, a neutral pH (~ 7.1), water temperature about 20 °C (in June–July), water hardness (KH) of ~ 8, and low oxygen concentration (3 mg/L).

A few significant variables predicted the occurrence of the other amphibian species (Table S2, Fig. S2). Specifically, *B. bufo* was preferentially found in deep ponds. Both newts preferred sites free of fish, and *I. alpestris* was further associated with slightly basic and high-oxygenated waters. No variable significantly predicted the presence of *R. temporaria*.

Discussion

Following up on our recent genetic analyses that led us to consider the Joux Valley as the last stronghold of *P. lessonae* in north-Alpine Western Europe (Dufresnes et al. 2017; Dufresnes et al. 2019), here we refine this statement to the single site of Pontet. According to our complementary monitoring approaches, the species is absent in the rest of the valley. It could have hardly gone unnoticed with the

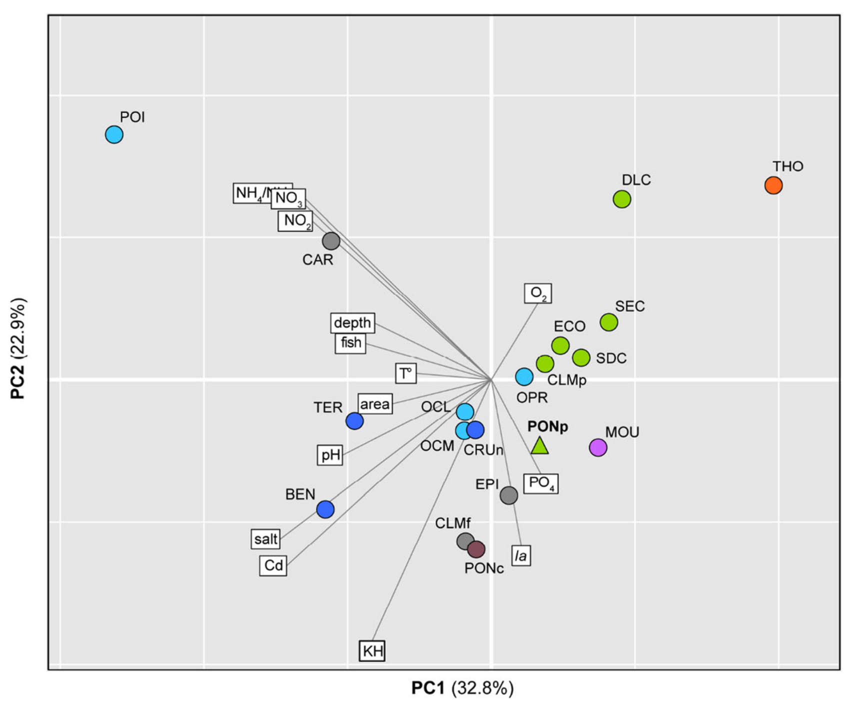


Fig. 4 Principal component analysis (PCA) on 14 characteristics measured in water bodies of the Joux Valley. Their contributions to the first axes displayed are indicated by vectors as follows: pond depth and area, presence of fish and alpine newt (*Ia*), temperature (T°), pH, salinity (salt), conductivity (Cd), carbonate hardness (KH), concentration of dissolved oxygen (O_2), ammonium/ammonia (NH_4/NH_3), nitrogen dioxide (NO_2), nitrate (NO_3), and phosphate (PO_4). The nature of sites is indicated by colors as follows: green, vegetated pond; light blue, river; blue, fish pond; purple, decorative pond; gray, pioneer pond; brown, forest canal; orange, peatland. The Pontet pond (PONp) is distinguished by a triangle

protocols applied here. eDNA metabarcoding is very reliable for amphibian monitoring (up to 0.97 of detection probability; Valentini et al. 2016), as reflected by our low rate of false negatives (6%). Moreover, eDNA was shown to reveal the presence of our target species even outside the breeding season, when traditional surveys cannot (Eiler et al. 2018). Pool frogs are probably absent from the French parts of the valley as well (southwest of our study area): Nocturnal searches at several potential sites in June 2019 were unsuccessful, despite optimal monitoring conditions (CD pers. comm.).

The fact that only a single population of *P. lessonae* persists may be the result of decades of habitat transformation in the valley, i.e., leading to the loss of high marsh and associated water bodies (Grünig 1994). Nowadays, half of the available sites are infested by fishes, and several others dry out in July already, before pool frogs could complete their larval cycle in the area (late-August–September). Several ponds share similarities with Pontet and would be theoretically suitable, but these were restored or created just a few years ago (e.g., CLM, SDC, SEC). In any case, *P. lessonae* will be unable to recolonize the valley from Pontet in the present context. First, the fragmented landscape is improper to its dispersal. Pool frogs are habitat specialists and require continuous humid corridors for movements, such as forested swamps or streams (Covaciu-Marcov et al. 2007; Widenfalk et al. 2018), a matrix absent between the closest suitable ponds (> 4 km distant from Pontet). Second, and although movements over several kilometers have been reported (Smith and Green 2005, and references therein), *P. lessonae* tends to show little mobility, even in favorable habitats. Using telemetry, Widenfalk et al. (2018) recorded almost no movement in a humid forest metapopulation from Sweden, where frogs would hibernate just within 250 m from their breeding sites, buried on the ground and leaf litter. Their observations are consistent with ours: in Pontet, *P. lessonae* probably spends the winter in the woods immediately surrounding the pond. By a capture-mark-recapture approach, Holenweg Peter (2001) found that only 14% of pool frogs had changed ponds at least once over a two-year survey, within a small pond network ($< 2\text{km}^2$) near Zurich, Switzerland (corresponding to *P. lessonae* × *P. bergeri* hybrids). Spatial movements seem to be age- and sex-biased in *Pelophylax* (Sjögren-Gulve 1998a, b; Holenweg Peter 2001), but juvenile dispersal has not been investigated across wide areas (Smith and Green 2005).

Therefore, the resilience of *P. lessonae* in the Joux Valley will require significant improvement of the local connectivity and/or, more realistically, translocation efforts. European pool frogs have been successfully reintroduced in the UK from Scandinavian populations during 2005–2008, after it had gone extinct in the 1990s (Buckley and Foster 2005; Foster et al. 2018). Lessons could be drawn from this experience, but here the small population size at Pontet may hinder its potential as a source population: We never counted more than 30 breeding individuals simultaneously, and always caught less than 20 tadpoles per trapping session. Translocating from elsewhere would be inadequate, given that the Joux population appears genetically differentiated from other European *P. lessonae* populations (Dufresnes et al. 2017; Dubey et al. 2019b). The immediate priority is thus to ensure its sustainability and ideally its growth, which would require specific protection of the entire site (ponds and adjacent woods), and the creation of new suitable habitats nearby (e.g., additional ponds). In a later step, a translocation program to other suitable ponds (e.g., CLM, SDC, SEC) should account for the risk of harvesting such small population (Germano and Bishop 2009). For instance, head starting, i.e., the rearing of eggs, larvae, and juveniles in captivity (Smith and Sutherland 2014), is being implemented to support the reintroduced populations of *P. lessonae* in England (Baker 2018), which could be appropriate here.

On a methodological note, our study emphasizes the use of next-generation tools to monitor endangered amphibians. In addition to limit disturbances, the eDNA approach appears more accurate and cheaper than traditional methods (Valentini et al. 2016) and is thus increasingly implemented for the surveillance of rare species (Rees et al. 2014; Adams et al. 2019). Second, we experimented the use of high-definition aerial photography mounted on drones as a census tool. This has the advantage of leaving the frogs undisturbed, which would otherwise dive or flee. Drones, also referred to as UAV (unmanned aerial vehicles), have been applied to wildlife monitoring programs for some birds or large mammals (Ivošević et al. 2015; Linchant et al. 2015; Šimek et al. 2017), but to our knowledge, our study is the first to deploy this technology for amphibian surveillance. Although the application remains restricted to the specific cases of large conspicuous anurans inhabiting open areas, the scope could be extended to more discrete nocturnal species, through the use of infra-red cameras (Ivošević et al. 2015).

Finally, we stress the fact that the conservation situation of *P. lessonae* is remarkably under-evaluated in Western Europe. Because assessors often lack expertise to provide species-level identifications on the field, pool frogs usually appear as data deficient in many regional lists (e.g., Bourgogne, Varanguin 2014; Rhône-Alpes, De Thiersant and Deliry 2008; Auvergne, Observatoire des amphibians d'Auvergne 2017) or as near threatened in the national lists of France (IUCN France and MNHN 2015) and Switzerland (Schmidt and Zumbach 2005), where it is treated together with the edible frog (*P.* cf. *esculentus*). Although syntopic, these species can have distinct micro-habitat preferences (e.g., Plénet et al. 2000; Pagano et al. 2001) and may experience contrasted population dynamics in the face of threats. For instance, hybridization between pool frogs and invasive marsh frogs (*P.* cf. *ridibundus*) entirely wastes the reproductive efforts of the latter but promotes edible frogs (Pagano et al. 2003). In addition, the discovery of the cryptic invasion by *P. bergeri* is too recent (2017) to have been taken into account by national evaluations. Hence, given that very few “pure” populations may actually remain (Dufresnes et al. 2017), the situation of *P. lessonae* in Western Europe is critical, and our characterization of the last stronghold of the species in Joux will be crucial for its persistence.

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Compliance with ethical standards

Conflict of interest We declare the following conflict of interest: T.D. cofounded SPYGEN, the company that developed the eDNA metabarcoding approach used in this study.

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