First limnological characterization of Lakes Leqinat and Drelaj in Bjeshkët e Nemuna National Park, Kosovo

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Abstract

The Balkan Peninsula is a biodiversity hotspot and hosts numerous mountain lakes, which offer a refuge for a multitude of species. However, previous pristine habitats have been deeply affected by anthropogenic change, such as non-native fish introductions, which calls for multi-species considerations in the last remaining unaltered habitats. We carried out abiotic measurements and biodiversity assessments in two neighbouring alpine lakes, Lakes Leqinat and Drelaj in the Bjeshkët e Nemuna National Park in Kosovo, in August 2018. Lake Leginat is a permanent, stratified water body and exhibits weak oxygen depletion below 3 m. Phytoplankton was dominated by chrysophycean, cryptophycean and chlorophycean algae. Zooplankton consisted of five rotifer species and Daphnia longispina. A mark-recapture experiment yielded a population of alpine newts (Ichthyosaura alpestris) of nearly 4000 adult individuals. In contrast, cold water from the surrounding karst seeps into Lake Drelaj, which is a well-oxygenated temporary lake. Hence phytoplankton and zooplankton biomasses were considerably lower than in Lake Leginat. Phytoplankton was dominated by cryptophycean, chlorophycean, and bacillariophycean algae. Zooplankton consisted of the diaptomid copepod Mixodiaptomus tatricus, the cladoceran Daphnia rosea, and the anostracan Chirocephalus diaphanus. Conservation efforts should ensure that Lake Leqinat remains unstocked as introduced fish would probably destroy the natural community.

Introduction

The Balkan Peninsula is as an important biodiversity hotspot (Griffiths et al. 2004). Mountain lakes harbour unique invertebrate communities often containing large zooplankton species like diaptomid copepods or anostracans (Belmonte et al. 2018; Mancinelli et al. 2019). Under natural, fish-free conditions, amphibians are the native top predators of these aquatic ecosystems (Lejeune et al. 2018; Schabetsberger & Jersabek 1995; Schabetsberger et al. 2006). Among them, newts show an impressive pattern of intraspecific diversity, with several locally described subspecies and phenotypes, especially in Montenegro (see e.g. Radovanović 1951, 1961; Džukić et al. 1990). In neighbouring Kosovo, Bjeshkët e Nemuna National Park was established in 2012 in the Albanian Alps (the Prokletije, or Accursed Mountains). It encompasses 630 km² of mountainous terrain with dense deciduous and coniferous forests and alpine landscapes. So far, only two limnological studies have been conducted in the park, providing information on algae, zooplankton and macrozoobenthos within the Gjeravica Lakes (Živić et al. 1997; Urošević 1997).

The biggest threat to organisms in closed, permanent alpine lakes is stocking with alien fish species. Introduced fish can drive sensitive prey species to ex-

Profile

Protected area Bjeshkët e Nemuna National Park Mountain range Bjeshkët e Nemuna / Prokletije Country Kosovo

tinction (Knapp et al. 2001; Schindler & Parker 2002; Denoël et al. 2005, 2009; Tiberti et al. 2013; Ventura et al. 2017 and references therein). Large (>1 mm) and often pigmented alpine zooplankton organisms can coexist with natural amphibian predators (Schabetsberger & Jersabek 1995), but they disappear after stocking as a result of size-selective predation by alien fish (Brancelj 1999; Schabetsberger et al. 2009). Most native amphibian species also disappear after fish introduction, as their larvae and sometimes even the adults are particularly vulnerable (Pilliod & Peterson 2001; Knapp et al. 2001, 2005; Denoël et al. 2005, 2009; Miró et al. 2018). Although terrestrial landscapes often remain wild or traditionally managed, large declines of newts have been reported due to fish stocking, the primary local driver of their extirpation in the Balkans. This region is a hotspot for paedomorphic populations. Larval structures, such as gills and gill slits, are retained in adult animals. Many paedomorphic populations have become extinct in the Balkans, including all larger populations from Montenegro (Denoël et al. 2005, 2019). The loss of this intraspecific heterochrony represents a massive loss to diversity and there is an urgent need for better conservation of the remaining populations. Lakes in national parks containing newts are becoming rare and are therefore important targets for monitoring.



Figure 1 – Bathymetric map of Lake Leqinat © Google Earth Pro, CNES, Airbus. Insert on top left shows the geographical position of Kosovo in Europe (red) © David Liuzzo (Wikimedia Commons, lizenzed under CreativeCommons-Lizenz by-sa-2.0-de, http://creativecommons.org/licenses/by-sa/2.0/de/legalcode) and an aerial photograph of Lakes Leqinat (right) and Drelaj (left) looking South into the Albanian Alps. © Dini Begolli.

The aim of this preliminary, descriptive study was to support conservation efforts by characterizing the abiotic conditions and the biodiversity of phyto- and zooplankton as well as selected benthic organisms (see Darwall et al. 2018) of Lakes Leqinat and Drelaj in the Bjeshkët e Nemuna National Park. Given the important regulatory role of alpine newts (*Ichthyosaura alpestris*, Laurenti 1761), which are the top predators, and their decline in the region, their population size within Lake Leqinat was assessed. Based on the results, the probability of the survival of alien fish is discussed.

Materials and Methods

Study sites

The two study lakes in western Kosovo are pristine, fishless, high-elevation water bodies just below the timberline that had never been investigated before and could serve as refuges for endemic species. Lake Leqinat is permanent (42° 40.110' N, 20° 5.455' E; 1860 m a.s.l.; Figure 1), whereas Lake Drelaj (42° 40.041' N, 20° 5.956' E; 1801 m a.s.l., Figures 2A and 2B), 0.7 km east of Leqinat, is temporary and dries out in summer



Figure 2 – Lake Drelaj: A on 5 August 2018; B on 27 September 2018. © Linda Grapci-Kotori

or autumn. Both lakes are free of fish and inhabited by alpine newts.

Sampling

Lake Leginat

Lake Leginat was sampled from 1 to 4 August 2018. It was sounded along 6 transect lines (108 soundings in total) across the lake to generate a bathymetric map. Surface area and volume were estimated using Fields R package version 10.0 (Nychka et al. 2017). Profiles of temperature, pH, conductivity and oxygen were measured at 1-m intervals at the deepest point of the lake (4.5 m). Water samples were taken with a 1.5-l Schindler trap. Temperature ($\pm 0.1^{\circ}$ C) was measured using a thermometer mounted inside the trap. Dissolved oxygen, oxygen saturation, pH and conductivity were measured with a HACH HQ30d portable multi-meter. Integrated plankton samples were collected by towing a 30-µm plankton net vertically from the bottom to the surface. Quantitative phytoplankton and zooplankton samples were collected with the Schindler trap at depths of 1, 2 and 3 m. For phytoplankton, samples of 100 ml of unfiltered water were preserved with Lugol's solution (Schwoerbel 1993) and kept in brown glass bottles. Zooplankton samples were filtered through a 30-µm mesh sieve and preserved in 4% formaldehyde. Macrophytes were collected in the littoral area. Samples for diatoms were taken from stones, sand and mud with a spoon or a pipette. Stones were scraped using a toothbrush and the surface biofilm was removed by gentle washing. Parts of submerged macrophytes and mosses were collected by hand and put into plastic vials. All samples were preserved using 4% formaldehyde. Rotifers and nematodes were sampled by snorkeling and towing a small plankton net (20 cm diameter, 30-µm mesh size) through the dense benthic algal mats. Adult trichopterans were caught with entomological nets in the vicinity of the lake. A capture-mark-recapture experiment (Chapman estimator, Krebs 1989) was conducted to estimate the total population size of alpine newts (Ichthyosaura alpestris) from Lake Leginat. The newts were caught by sight with dip nets around the shoreline and by snorkeling in deeper parts of the lake. They were anaesthetized in MS222 (~50 mg $l^{\mbox{-}1})$ and marked by clipping one toe for release and recapture. This method does not affect newt survival (Arntzen et al. 1999) and toes regenerate fast (Gutleb 1991). Snout-vent length (i.e. from the tip of the snout to the end of the cloaca) and total length (i.e. from the tip of the snout to the end of the tail) of 30 males and females each were measured using ruler calipers. Adulthood and sex were determined on the basis of the shape of the cloaca (Denoël 2017).

Lake Drelaj

Lake Drelaj was sampled on 5 August 2018. The surface area was calculated from Google Earth Pro. Temperature, pH, conductivity and oxygen levels were

Table 1 – Phytoplankton species in Lakes Leginat and Drelaj.

Table T – Phyloplankion species in Lakes Leginal and	i Dreidj
Lake Leqinat	
Cyanobacteria	
Aphanocapsa incerta (Lemmermann) G. Cronberg & Kom	árek
Aphanocapsa sp.	
Gloeobacter violaceus Rippka, J.B. Waterbury & Cohen-Ba	zire
Microcystis sp.	
Planktothrix sp.	
Radiocystis geminata Skuja	
Chlorophyceae	
Botryococcus braunii Kützing	
Desmodesmus brasiliensis (Bohlin) E. Hegewald	
Didymocystis sp.	
Elakatothrix genevensis (Reverdin) Hindák	
Neglectella solitaria (Wittrock) Stenclová & Kastovsky	
Oocystis sp.	
Planktosphaeria gelatinosa G.M. Smith	
Scenedesmus sp.	
Tetraëdron minimum (A. Braun) Hansgirg	
Comparison of baileyi Walle	
Cosmarium cf. baileyi Wolle Cosmarium cf. laeve Rabenhorst	
Cosmarium spp.	
Gonatozygon brebissonii De Bary	
Staurastrum sp.	
Staurodesmus sp.	
Zygnema sp.	
Chrysophyceae	
Mallomonas sp.	
Pseudopedinella sp.	
Uroglena sp.	
Dinophyta	
Ceratium hirudinella (O.F. Müller) Dujardin	
Glenodinium sp.	
Gymnodinium sp.	
Cryptophyceae	
Cryptomonas cf. erosa Ehrenberg	
Cryptomonas marssonii Skuja	
Bacillariophyceae	
Achnanthes sp.	
Cymbella sp.	
Melosira varians C. Agardh	
Nitzschia acicularis (Kützing) W. Smith	
Stauroneis sp.	
Laka Daala:	
Lake Drelaj	
Chlorophyceae	
Pseudopediastrum boryanum (Turpin) E. Hegewald	
Scenedesmus sp.	
Conjugatophyceae	
Gonatozygon brebissonii De Bary	
Cryptophyceae	
Cryptomonas erosa Ehrenberg + sp.	
Cryptomonas cf. marssonii Skuja	
Bacillariophyceae	
Bacillariophyceae Amphora ovalis (Kützing) Kützing	
Amphora ovalis (Kützing) Kützing	
Amphora ovalis (Kützing) Kützing Cyclotella sp.	
Amphora ovalis (Kützing) Kützing Cyclotella sp. Cymbella spp.	
Amphora ovalis (Kützing) Kützing Cyclotella sp. Cymbella spp. Cymbopleura inaequalis (Ehrenberg) Krammer	

measured at the surface and above the sediment (3 m depth). One phyto- and one zooplankton sample were taken at 3 m depth. The same methods as described above for Lake Leqinat were applied for phyto-, zooplankton, diatoms and trichopterans. Both lakes were visited again for visual inspection on 26–27 September 2019 with students from Salzburg and Prishtina Universities.

Analyses

Algae were counted under an inverted microscope (Telaval 3, Jena; magnification 40-1000x), applying the method of Utermöhl (1958). Biovolumes were calculated by fitting geometric forms to cell dimensions (Deisinger 1984). The organic content of the samples for diatoms was removed by acid digestion, with the addition of 2 ml of a supersaturated solution of K₂MnO₄ and 4 ml of HCl to a small (ca. 2 ml) subsample. The acid was then removed through a series of water washes. Permanent slides of the cleaned material were mounted with Naphrax®. Slide observations were performed using a Nikon E-80i light microscope, and photomicrographs were taken with a Nikon Coolpix 600 digital camera (40-1000x). Trichopterans were identified under a dissecting microscope (Ibrahimi et al. 2019).

Zooplankton samples were stained with Rose Bengal. Subsamples were counted under the inverted microscope (magnification 40–100x). Biovolumes and biomasses were calculated by approximating natural shapes with geometric formulae for rotifers (Ruttner-Kolisko 1977), and from length–weight regressions for crustaceans (Downing & Rigler 1984). Periphytic nematode samples were mounted on slides in glycerin after a slow, two-step dehydration process (Decraemer et al. 2019).

Results

Lake Leqinat

Lake Leqinat reaches a maximum depth of just 4.5 m, in the southern part of the basin (Figure 1). It has an area of 16 900 m² and a volume of 23 000 m³. Depth profiles of abiotic parameters showed a weakly stratified, oxygen-rich upper layer down to 3 m depth. High pH-values (>9) indicate biogenic decalcification processes. Water of lower oxygen content and higher conductivity was found in the deepest layer (Figure 3).

The phytoplankton community (Table 1) was dominated by the chrysophycean genus *Uroglena* sp., reaching highest densities at around 2 m depth and accounting for 80.5% of the total biovolume (Figure 4A). The cryptophycean species *Cryptomonas marssonii* (9.5%) also reached its highest densities within this intermediate depth layer, while the dominant chlorophycean *Oocystis solitaria* was almost homogeneously distributed throughout the water column, contributing 5.0 to 43.3% of the total biovolume. A total of 391 benthic and periphytic diatom species were recorded in both

Table 2 - Rotifers, nematodes and crustaceans in Lakes Le-
qinat and Drelaj. Euplanktonic species are marked with an
asterisk.

Lake Leqinat
Rotifera
Anuraeopsis fissa (Gosse)*
Ascomorpha saltans indica Koste*
Cephalodella apocolea Myers
Cephalodella edax Hollowday*
Cephalodella forficula (Ehrenberg)
Cephalodella sp.
Cephalodella ventripes (Dixon-Nuttall)
Collotheca sp.
Colurella obtusa (Gosse)
Eothinia elongata (Ehrenberg)
Euchlanis dilatata Ehrenberg
Lecane closterocerca (Schmarda)
Lecane flexilis (Gosse)
Lecane luna (Müller)
Lecane lunaris (Ehrenberg)
Lepadella patella (Müller)
Monommata sp.
Mytilina mucronata (Müller)
Pleurotrocha petromyzon Ehrenberg
Polyarthra Iuminosa Kutikova*
Polyarthra sp.*
Proales fallaciosa Wulfert
Trichocerca bidens (Lucks)
Trichocerca rattus (Müller)
Trichocerca cf. relicta Donner
Nematoda
Eumonhystera cf. barbata Andrassy
Eumonhystera cf. pseudopulbosa (Daday) Andrassy
Mesodorylaimus spec. 1
Tridontulus spec. 1
Plectus spec. 1
Plectus spec. 2
Rhabdolaimus cf. terrestris DeMan
Cladocera
Daphnia longispina (Müller)
Copepoda
Cylopidae Gen. sp.
Lake Drelaj
Rotifera
Bdelloidea Gen spp.
Cladocera
Daphnia rosea Sars
Copepoda
Mixodiaptomus tatricus (Wierzejski)
Anostraca
Chirocephalus diaphanus Prévost

lakes (no separate counts available; see Appendix A, Supplementary Data). A dense mat of *Chara contraria* A. Braun ex Kützing covered large areas of Lake Leqinat down to the deepest part. In shallow regions, *Potamogeton alpinus* Balb. was present. At the shoreline, *Carex vesicaria* L. dominated.

Zooplankton diversity was low, with only five euplanktonic rotifers and a single cladoceran species

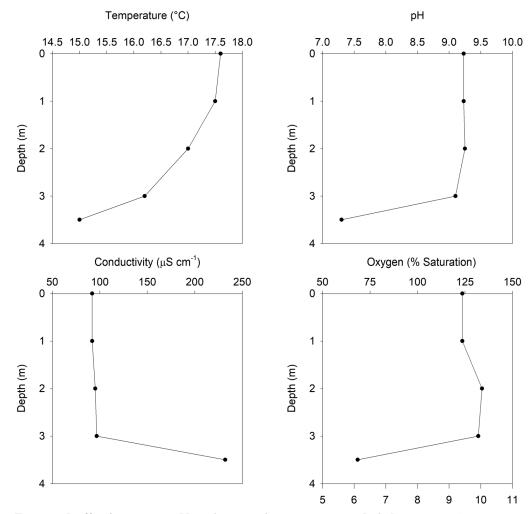


Figure 3 – Profiles of temperature, pH, conductivity and oxygen saturation in Lake Leginat on 2 August 2018.

recorded in the samples; copepods were encountered only as immature developmental stages of Cyclopoida. Densities of *Daphnia longispina* increased with depth. The zooplankton community was dominated by the rotifers *Anuraeopsis fissa* (20.3% of the biomass) and *Polyarthra luminosa* (42.4%), with peak densities at 1 m depth (Figure 5A). A more diverse rotifer fauna was recorded in the benthic *Chara* mats (Table 2). In the sediment and periphytic samples, 7 nematode taxa were observed (Table 2). Six adult trichopteran species were found at Lake Leqinat (*Limnephilus centralis*, *L. flavospinosus*, *L. flavicornis*, *L. stigma*, *Agrypnia varia*, *Oligotricha striata*).

The alpine newt was the only amphibian recorded. Only the metamorphic phenotype was found among the adults. A total of 108 females and 131 males were marked and released. After one day, 136 females and 145 males were recaptured. The capture-mark-recapture experiment yielded a total population size of 1 492 female (683–2 302; 95% confidence interval) and 2 408 male (925–3 891) alpine newts in Lake Leqinat. The total population was estimated to be 3 980 adults (2 262–5 699). Snout-vent length and total length were 49.15 mm (41.9–58.8; median, minimum-maximum) and 85.1 mm (77.1–94.1) for females, and 42.2 mm (38.1–45.4) and 73.2 mm (67.1–78.2) for males.

Lake Drelaj

Lake Drelaj (Figure 2) is 3 m deep but had dried out between September and October. Its maximum surface area is around 3100 m². Cold water from the surrounding karst seeps into the well-oxygenated lake, which in August fed a brook (~ $5 \ l \ s^{-1}$) that emerged from the rocks about 30 m below the lake. On 5 August 2018, at the water surface and at 3 m depth, the temperatures were 9.3 and 5.5°C, Oxygen 9.48 mg l⁻¹ (105% saturation) and 9.27 mg l⁻¹ (98%), pH 8.29 and 8.17, and conductivity 192 µS cm⁻¹. The diatom Cyclotella delicatula dominated the open water community (84.5% of the biomass; Figure 4B). Overall phytoplankton biomass in the cold, clear lake was approximately one tenth of that in Lake Leginat (Figures 4A and 4B). The zooplankton community consisted of three crustacean species: Daphnia rosea, Mixodiaptomus tatricus and Chirocephalus diaphanus. No rotifer species were recorded, other than a few tychoplanktonic bdelloid species. Zooplankton biomass was 6 times less than in Lake Leqinat (Figures 5A and 5B).

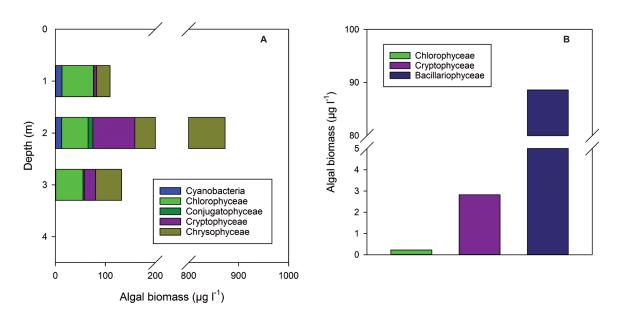


Figure 4 – Phytoplankton. A: Depth profiles of phytoplankton biovolume in Lake Leqinat on 2 August 2018. B: Biovolume of planktonic algae at 3 m depth in Lake Drelaj on 5 August 2018.

The anostracan species *C. diaphanus* reached very high densities in the puddles that remained in September, where adult metamorphosed and larval alpine newts were also found. Two adult trichopteran species (*Allogamus uncatus, Limnephilus bipunctatus*) were recorded in the close vicinity of the lake.

Discussion

Abiotic conditions, phyto- and zooplankton communities

The two alpine lakes differ markedly in their hydrology. Whereas Lake Leqinat is permanent, Lake Drelaj can dry out in summer / autumn. The latter has a visible outflow and comparatively faster water renewal, indicated by colder water temperatures, higher conductivity and lower pH. Lake Leqinat exhibited signs of biogenic decalcification in the upper water column and weak oxygen depletion / ion enrichment in the deepest part of the lake, probably due to summer stagnation, while Lake Drelaj was well oxygenated throughout the water column. Hence phytoplankton and zooplankton biomasses were considerably lower in Lake Drelaj than in Lake Leqinat. Accordingly, species assemblages in the open water also differed markedly. In Lake Leqinat, chrysophycean and chlorophycean species dominated. Zooplankton diversity was low, with only five euplanktonic rotifers, and Daphnia longispina as the sole microcrustacean encountered. Although biomasses were low, species compositions of both phyto- (Oocystis solitaria, Uroglena sp.) and zooplankton (Anuraeopis fissa) reflect a meso- to eutrophic character of the lake and are reminiscent of lowland ponds rather than of alpine lakes. In Lake Drelaj, an impoverished phytoplankton community with few small-sized (nanoplanktonic) species was observed together with three crustacean species in low abundances, presumably as a consequence of the lake's hydrological regime. Euplanktonic rotifers were entirely missing.

No diaptomid copepod was found in Leginat, which may be due to the high feeding pressure of the large alpine newt population (see Dodson 1970; Wissinger et al. 1999). On the other hand, Drelaj contains a considerably smaller newt population (see below) associated with Mixodiaptomus tatricus, a species typical of temporary high-altitude water bodies in the Alps and the Balkans (Jersabek et al. 2001; Mancinelli et al. 2019). We are aware that Daphnia rosea may be a morphotype and belongs to the D. longispina complex (Petrusek et al. 2008; Błędzki & Rybak 2016), but we acknowledge morphological differences and distinct ecological divergences between the two taxa, as known from numerous studies in the Austrian Alps (Gaviria-Melo et al. 2005), by assigning the taxon from Drelaj to D. rosea. Chirocephalus diaphanus is one of the most widespread and tolerant anostracan species in Europe (Brtek & Thiéry 1995; Demeter & Mori 2004). Its presence confirms regular drying of the lake, as the diapausing eggs usually need such drying up for successful development (Brendonck 1996; Zarattini et al. 2002).

Benthic communities

Diatom assemblage is very rich, indicating the high ecological status of the lakes. The large number of species results from the presence of different microhabitats such as macrophytes, stones and pebbles, and fine and coarse sediment, but it probably also reflects low human impact on the lakes. Several rare and endangered diatom species of the flora of Central and Eastern Europe (Lange-Bertalot & Steindorf 1996) have been recorded. The recently discovered *Neidiopsis borealis* (Vidakovic et al. 2019) was also found. Appre-

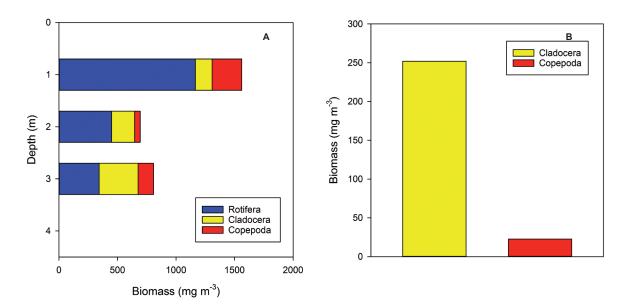


Figure 5 – Zooplankton. A: Depth profiles of zooplankton biomass in Lake Leqinat on 2 August 2018. B: Zooplankton biomass at 3 m depth in Lake Drelaj on 5 August 2018.

ciable macrophyte vegetation was detected only in the permanent Lake Leginat. However, the three species registered (Chara contraria, Potamogeton alpinus and Carex vesicaria) typically co-occur in oligo- to mesotrophic alpine lakes. Carex vesicaria also grows around eutrophic lakes. Nematode taxa represent common sediment and periphytic taxa. The most abundant, Monhystera and Plectida, are deposit feeders essential for ecological functioning at the base of any freshwater food web. Tridontus species are still rarely recorded, because they are often misidentified as Eumonhystera dispar. Three of the trichopteran species are new records for Kosovo and are currently known only from Lake Leqinat: Limnephilus flavospinosus, Limnephilus flavicornis and Oligotricha striata, the last one being known in the Balkan Peninsula from only a few localities (Ibrahimi et al. 2019).

A large alpine newt population inhabits Lake Leqinat. Approximately 4000 metamorphosed adults were found, corresponding to a density of 0.24 individuals per square metre within the lake. Higher densities have been reported for similar-sized lakes in northern Greece (Denoël & Schabetsberger 2003; > 30 000 individuals), but these included a large proportion of paedomorphic individuals. Hridsko Jezero from the Montenegrin part of the mountain range also used to host a population of paedomorphs (Denoël et al. 2001), but now only metamorphosed individuals occur (Denoël et al. 2019). In Leqinat, the sex ratio was slightly biased towards males (0.62). In other populations of alpine newts from the Balkans, sex ratios were found to be either male- or female-biased (Kalezić et al. 1989; Denoël 2003). This may be explained by different sex-specific payoffs across varied environmental conditions. We were unable to conduct a mark-recapture experiment in Drelaj, but the densities were lower, probably due to the particularly unfavourable cold breeding conditions (5°C at the bottom

in summertime). Both locations appear to be new records for the occurrence of the alpine newt (Džukić et al. 2015).

Conservation aspects

Although the lakes so far are pristine, some threats do exist. While in temporary Lake Drelaj, fish introduction poses no threat to the alpine newts, alien fish could potentially survive in Lake Leginat. Since we have no information about the winter oxygen conditions under ice, we cannot speculate about the survival of salmonids, but it is very likely that minnows (Phoxinus sp.) or other species that tolerate low oxygen levels would live through the winter, destroy the alpine newt population, and strongly alter the community of prey organisms (Schabetsberger et al. 2006; Miró et al. 2018). Indeed, many major populations of alpine newts that inhabited mountain lakes in the Balkans, even within National Parks, have now vanished because of fish introductions. Such introductions were particularly detrimental in Montenegro, including in Hridsko Jezero, where fortunately fish have recently disappeared (Denoël et al. 2019). Although we are not aware of any immediate stocking campaigns, hydropower development in the Rugova Valley is an issue and may be associated with stocking campaigns in the region (personal observation). Additionally, a large area of woodland near Lake Leginat has been burnt deliberately. This calls for an enforcement of the protection of the lakes by rangers, and a visitor centre or at least thematic display panels near the lakes to raise awareness of conservation issues.

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