



Colouration in male blue-throated keeled lizards (*Algyroides nigropunctatus*): Evidence for ultraviolet reflectance of throat and lateral patches

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The blue-throated keeled lizard, *Algyroides nigropunctatus*, is distributed along the Adriatic coast from Italy to Greece and is sexually dichromatic. Males display a striking blue on their throat, an orange ventrum, and a dark brown dorsal colouration, but their colouration has never been objectively assessed. Here, we describe the colouration of 13 male blue-throated keeled lizards from Cres Island (Croatia) using spectrophotometry and ultraviolet (UV) photography, and show that the blue throat and the blue spots located on the flanks reflect in the UV part of the spectrum. We discuss the potential role of UV-blue colouration in social signalling.

Key words: Chromatic signal, Ultraviolet, Spectrophotometry, Lizards, Squamates

The lizard genus *Algyroides* comprises four species with disjoint distributions in southern Europe (Arnold & Ovenden, 2010). Compared to other lacertid species, relatively little is known about the behaviour, ecology, or evolution of these lizards, although morphological and molecular analyses have confirmed the monophyly of the genus (Harris et al., 1999). *Algyroides nigropunctatus* Duméril & Bibron (1839), commonly known as the blue-throated keeled lizard or the Dalmatian *Algyroides*, has the largest distribution range of the genus, and is found along the coast of the Balkan peninsula from Italy to Greece (Böhme, 1981). *Algyroides nigropunctatus* inhabits open and semi-open habitats (e.g. open woodland, bushy areas, stonewalls), usually favouring shady areas (Haxhui, 1991; Bressi, 2004; Arnold & Ovenden, 2010). This species exhibits a marked sexual dimorphism in size and shape with males having relatively larger heads than females, which allows the packing of jaw musculature and a compressed braincase (Ljubisavljevic et al., 2011). They are also sexually dichromatic with adult males displaying an orange ventral colouration and a striking blue colouration on their throat (Arnold & Ovenden, 2010; Carlino & Pauwels, 2016), while females are ventrally white to yellow and have no or less bright blue colouration on their throat (Arnold & Ovenden,

2010). Dorsally, males and females are dark brown with black spots present on some of their dorsal scales, to which the species owes its name. Geographical variation in colour pattern has been described, particularly in some insular populations. In the Ionian islands of Lefkada, Kephalliana, and Itaka (Greece) male *A. n. kephallithaticus* are ventrally yellow and their throat shifts from blue to green after the mating season (Arnold & Ovenden, 2010). In other Greek islands like Corfu and Erikoussa, males may exhibit a flashy orange colouration on their throat (unpublished information). However, to the best of our knowledge, no study to date has examined the colour pattern of *A. nigropunctatus* using objective (i.e. independent of the human visual system) methods. Here, we use standard reflectance spectrophotometry along with UV and human-visible photography to provide the first objective description of the colour pattern of males of this species.

In April 2016, we captured 13 adult males (SVL > 59 mm) of *A. nigropunctatus* by noosing in Cres Island (Croatia) in their typical habitat (e.g. shady areas, stone walls with vegetation, open woodland). Upon capture, we took ultraviolet (UV) pictures of every individual using a digital camera (Olympus PEN Mini) converted for UV photography by replacing the standard internal hot mirror filter with a Spectrosil 2000 fused silica filter, which transmits light wavelengths down to 170 nm. The camera was fitted with a UV-transmitting macro lens (Noflexar Novoflex 1:3,5/35mm) and a Baader U-filter with peak transmission between 320 and 380 nm. UV photographs were taken outdoors in the shade using natural illumination. For comparison, we also took pictures identical to the UV ones but in the human-visible range using standard digital cameras.

Lizards were transported in cloth bags to a darkened room where we obtained reflectance spectra of the ventrum, throat, and dorsum using a JAZ spectrophotometer with a R200-7-VIS-NIR reading-illumination probe and a PX-2 xenon strobe light source (Ocean Optics Inc.) for full spectral illumination. We averaged reflectance readings over 5 nm using a kernel

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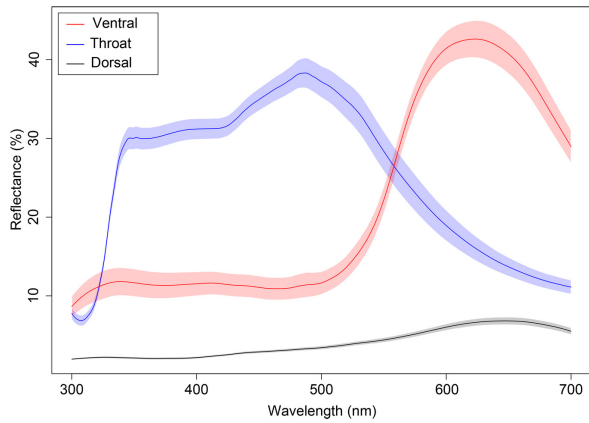


Figure 1. Non-normalised reflectance spectra of the throat (blue), the ventrum (red) and the dorsum (black) of 13 adult males of *A. nigropunctatus*. Reflectance is expressed as mean \pm SEM (coloured area surrounding the line).

smoothing function. We set integration time to 30 ms, scans to average to 10, and boxcar width to 10. For data acquisition, we hand-held the probe over the centre of the throat, ventrum and dorsum of the animals, perpendicular to the surface (i.e. illumination and readings angles were both 90°). An entomological pin attached to the side of the probe allowed us to maintain a constant distance of 5 mm between the tip of the probe and the target surface (see Badiane et al., 2017 for more details). Colour spots measuring less than 2 mm in diameter were ignored as they are beyond the resolution of our spectrophotometer set-up (Badiane et al., 2017). Reflectance spectra were analysed in R using the PAVO package (Maia et al., 2013). All animals were released at their capture sites after a maximum of 24 hours post-capture.

The throat of male *A. nigropunctatus* has a spectrum with a reflectance peak in the blue part of the spectrum

(485 ± 10 nm; mean \pm SEM), a relatively flat plateau extending into the UV range, and then a sharp drop-off at approximately 340 nm (Fig. 1). As expected, spectra of the orange ventrum show a pronounced reflectance peak in the orange-red (621 ± 3 nm). In contrast to the throat and ventrum, reflectance spectra of the dorsum are characteristically flat with little reflectance across the spectrum and show no obvious reflectance peaks (Fig.1). UV photographs confirm that the throat colouration reflects in the UV range and is therefore best characterised as UV-blue (Fig. 2A). The UV-blue colouration of the throat covers the whole throat, sometimes extending over the chest and the supra-labial scales. The ventral orange colouration covers the ventrum and the limbs (ventrally only) and merges with the dorsal colouration on the flanks and neck. In addition, some of the captured males displayed regularly-spaced blue spots (up to 9 on each side) on some of their outer ventral scales (OVS) (Fig. 2B). Unfortunately, these spots were too small to obtain reliable reflectance spectra. However, the UV photographs revealed that these spots are also UV-reflecting (Fig. 2C).

Many lizard species, including lacertids, are capable of seeing in the ultraviolet range of the spectrum (Fleishman et al., 2011; Pérez i de Lanuza & Font, 2013; Martin et al., 2015a) and display UV colours that often appear as blue to the human eye (Whiting et al., 2006; Pérez i de Lanuza et al., 2014). Our results show that the throat of adult males of *A. nigropunctatus* is highly reflective in the UV range of the spectrum. Although this is the first report of UV colouration in *A. nigropunctatus* (see Arribas, 2002), many other lacertids display UV colour patches that purportedly function as social signals, including *Gallotia galloti* and *G. atlantica* (Font & Molina-Borja, 2004; Molina-Borja et al., 2006), *Timon lepidus* (Font et al., 2009), *Lacerta agilis* (Pérez i de Lanuza & Font, 2007), *L. viridis* (Bajer et al., 2010), *Zootoca vivipara* (Martin et al., 2015b), and several species of *Podarcis* (e.g. Marshall & Stevens 2014; Pérez i de Lanuza et al., 2014; our own unpublished data). It is possible that the UV-blue colouration also plays a

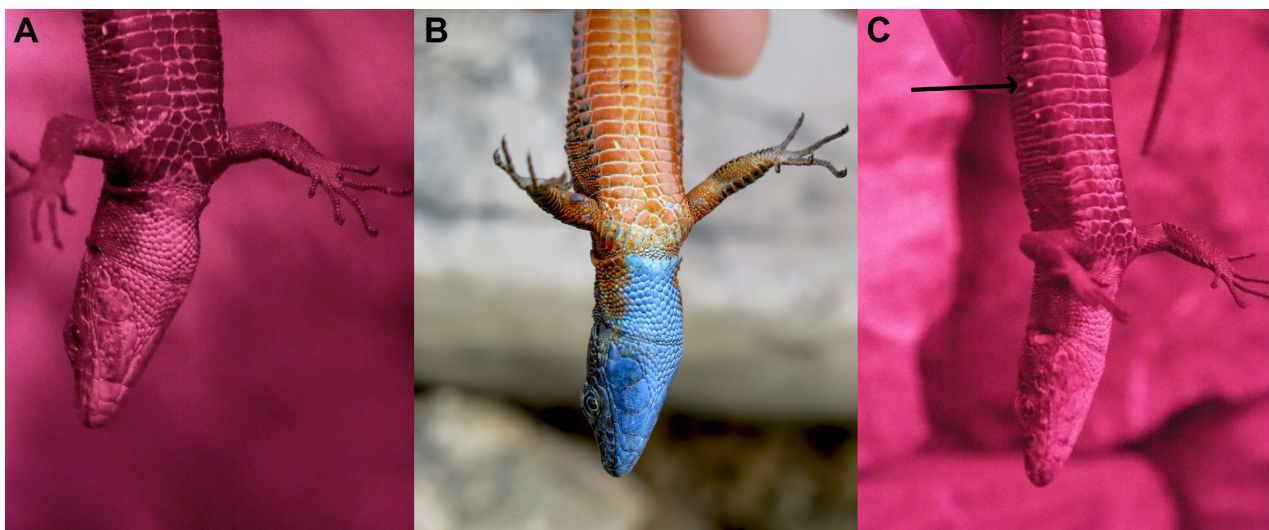


Figure 2. Photographs of a representative male of *A. nigropunctatus* from Cres Island in the UV (A, C, ventrally and laterally respectively) and in the human-visible range (B). The photographs in A and C were taken with a modified digital camera through a UV-transmitting filter that blocks most of the light wavelengths outside the 320-380 nm range. UV-reflecting skin patches in A and C are visible due to their lighter, whitish colouration (UV reflecting spots are indicated by an arrow in C).

role as a social signal in *A. nigropunctatus*, either as an ornament (female choice) or as an armament (male-male competition) because the ventrum and throat of lizards, and particularly of lacertids, are often the target of sexual selection and are used to convey information to conspecifics by means of chromatic signals (Leal & Fleishman, 2004; Bajer et al., 2010; Martin et al., 2015b). For example, males of *Lacerta schreiberi* also exhibit an iridescent UV-blue colouration on their throat (Pérez i de Lanuza & Font, 2014) with a spectral shape similar to the throat of *A. nigropunctatus*, which may function as an indicator of individual quality (Martín & López, 2009). Although we do not have spectral data for the UV-blue OVS, based on their similarity to UV-blue OVS from other lacertids (e.g. *Podarcis*, Pérez i de Lanuza et al., 2013) it is likely that their reflectance peak is also located in the UV range. Interestingly, this is the first lacertid species in which both a UV-blue throat and UV-blue OVS are described as most species studied to date have only one type of UV-blue patch, or they have none. Unfortunately, almost nothing is known about the behaviour of *A. nigropunctatus* and so discussion about the putative functions of these UV-blue patches is speculative.

The body colouration of male *A. nigropunctatus* conforms to the general pattern found in lacertids, with a relatively cryptic dorsum, possibly selected for background-matching, and conspicuous colour patches on the less visible lateral and ventral body surfaces. In many species, the latter are used for signalling and are made more or less visible through a variety of stereotyped movements and/or postural adjustments. The allocation of functionally different colour patches to different body regions (i.e. signal partitioning) allows balancing effective communication with the risks of detection by unintended receivers (Endler, 1992; Marshall & Stevens, 2014). Furthermore, the colours of the throat and ventrum in *A. nigropunctatus* are complementary, each one reflecting in the region of the spectrum where the other does not (see Fig. 1), suggesting that their presence on adjacent patches has been selected to maximise signal conspicuousness (Pérez i de Lanuza & Font, 2016).

Recent technical and methodological advances have expanded the breath of taxa for which spectral data are available (Kemp et al., 2015). However, our knowledge of animal colouration is largely shaped by studies of a handful of model species and very little is known about the colouration of most species alive today. Research on relatively understudied species, such as *A. nigropunctatus*, is a useful complement to studies with model species and essential to uncover general principles of lizard colouration.

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Effect of fish stocking on alpine populations of European common frog (*Rana temporaria*) in the Pyrénées National Park

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The introduction of non-native species is one of the factors driving the global decline of amphibians. We examined the effect of fish stocking in naturally fishless mountain lakes and ponds on the local populations of the European common frog (*Rana temporaria*). We surveyed 215 mountain lakes and ponds and noted the presence or absence of frogs and signs of frog reproduction (i.e. tadpoles, eggs). We compared these data with fish stocking data from the regional park management (all surveyed lakes and ponds). Our results show a strong negative effect of fish stocking on the presence of *R. temporaria*, and an even stronger effect on its breeding presence, but we found a small number of lakes and ponds where coexistence occurred. In addition, the preferential stocking of large, deep lakes and ponds left smaller ponds as the only remaining habitats, a number of which are likely to become temporary due to increased summer temperatures. We recommend a series of measures to conciliate fish stocking for recreational fishing whilst conserving *R. temporaria* populations, which might be extensible to other high mountain environments.

Key words: Fish stocking, *Rana temporaria*, *Oncorhynchus mykiss*, amphibian conservation, Pyrénées National Park

Amphibians have become a cause of great concern in recent years due to their sharply declining populations worldwide and their high extinction rates (McCallum, 2007). Although the exact numbers are in dispute (Stuart et al., 2004; Pimenta et al., 2005; Stuart et al., 2005), there is no doubt that a very large proportion of amphibian species are under severe threat. Some of the main factors that drive the decline of amphibians include climate change (Corn, 2005; Ryan et al., 2014), habitat fragmentation and loss (Cushman, 2006), emerging amphibian diseases (Daszak et al., 1999; North et al., 2015; Yasumiba et al., 2016) and the introduction of non-native species (Adams, 1999).

For many years the last factor has been the main subject of countless studies (reviewed by Kats &

Ferrer, 2003). Specifically, the effects of non-native fish introduction as a potential new predatory pressure on frog populations have received much-needed attention (Knapp & Matthews, 2000; Bosch et al., 2006; Welsh et al., 2006). Introduction of salmonids in lakes and ponds with no native fish fauna is one of the more common ecosystem modifications of mountain lakes (Radomski & Goeman, 1995; Lodge et al., 1998; Rahel, 2000).

Although the introduction of some fish species seems to have low or imperceptible effects on the native communities (Moyle & Light, 1996), other species have been known to produce local extinction of native fauna and substantial changes in ecosystem structure (Anderson, 1980; Herbold & Moyle, 1986; Hrabik et al., 1998). Due to its predatory nature, the introduction of trout in some lakes in the USA has caused a remarkable impact on faunistic groups like Amphibia, zooplankton and benthic macroinvertebrates, leading in some cases to their complete disappearance (Anderson, 1975, 1980; Bradford et al., 1998; Carlisle & Hawkins, 1998; Knapp & Matthews, 2000), as well as the alteration of the food web and algae production (Leavitt et al., 1994).

Fish species tend to become dominant in the lakes' food chain, because they live for many years and their biomass is usually constant from one year to the next. In the case of cold water fish like trout, warm summers make it possible for the fish born that year to reach a sufficient size to resist the winter conditions, and a lack of predators ensures the success of many consecutive cohorts (Wetzel, 1981).

Amphibians living in mountain lakes and ponds are very sensitive to the introduction of salmonids in their habitats, and their populations show significant decreases in response to fish stocking (Knapp & Matthews, 2000) as well as signs of recovery when the practices are stopped (Knapp et al., 2007). The French National Park of the Pyrénées has for some time stocked lakes and ponds with trout for recreational fishing, but the impact of these practices in the native populations of *Rana temporaria* has not been properly evaluated. Our hypothesis is that trout stocking diminishes frog numbers in the lakes and

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ponds where they are introduced. The present study evaluates the impact of rainbow trout (*Oncorhynchus mykiss*) stocking in the populations of *R. temporaria* inhabiting mountain lakes and ponds within the confines of the French National Park of the Pyrénées (Fig. 1A).

During July of 2001 and 2002 we surveyed 215 lakes and ponds in the core of the French National Park of the Pyrénées within an area of ca. 450 square km located from 0°36'54"W to 0° 9'3"E and 42°42'50"N to 42°52'9"N, at a altitude between 1580 and 2747 m a.s.l. (Fig. 1C). Most of the sampled lakes and ponds (Online Appendix 1) are inaccessible by car, so the survey was conducted in two 20-day treks, one each year in July and August (Fig.1B). In summer, frog adults, tadpoles and egg clutches are mainly located in shallow water near the shore (Bradford, 1989). This allowed us to use visual encounter surveys (Crump & Scott, 1994) along the lakes and ponds' entire shoreline to evaluate the presence or absence of adult *R. temporaria* as well as any evidence of ongoing reproduction (by presence of eggs or tadpoles).

We characterised the lakes and ponds by a number of variables: location (longitude and latitude), altitude (m a.s.l.), total surface (m²), pH, conductivity (µS/m), geomorphology (drainage basin) as a vector for isolation and presence of rock, and vegetation in the riparian area. We measured water pH and conductivity on site at the surface and bottom of the lake from samples taken with a Teflon bottom water sampler. We used Hach HQ40d portable pH and conductivity probes (IntelliCAL PHC201, accuracy ±0.002 pH and IntelliCAL CDC401, accuracy ±0.5% from 1 µS/cm–200 mS/cm). We analysed the geomorphology to study the isolation of the lakes and ponds. Other studies from the same sampling surveys have analysed in depth the ecology of these lakes (see Zaharescu et al., 2016a; Zaharescu et al., 2016b; Zaharescu et al., 2017).

As part of the official management policy of the Pyrénées National Park, several different lakes and ponds were stocked for recreational fishing. The fish stocking was carried out by helicopter so that when positioned over the selected lakes, a Park employee (using a climbing harness) descends close to the lake surface holding a bucket of water containing the rainbow trout fry, which are carefully deposited in the water. The park administration provided us with the fish stocking data from 1998-1999, which included 68 of the total surveyed lakes and ponds. Due to the strict control over private stocking in the park, these data were considered reliable. In addition, we found no visual evidence of trout or trout larvae on any of the non-stocked lakes and ponds during our surveys.

Data from the lakes and ponds were analysed by pooling all the lakes and ponds together. Each of the lakes and ponds was characterised according to its combination of presence or absence of rainbow trout fry, frog, and evidence of frog breeding. We then built dispersion diagrams using IBM SPSS Statistics 19.0 (SPSS, Inc., Chicago, IL) statistical package for Windows, in order to examine the effect that the presence of rainbow trout fry had in the frog breeding presence. It is worth noting that evidence of frog breeding presence was considered

as evidence of frog presence, but presence of adult frogs was not considered evidence of frog breeding presence.

We surveyed a total of 215 ponds and lakes that we grouped into four lakes and ponds types: 63 were fish stocked (type A), 42 were non-fish stocked and we did not find frog breeding presence (type B), 5 were fish stocked and we found frog breeding presence (type C), and 105 were non-fish stocked and we did not find either frog presence nor frog breeding presence (type D). We conducted Kruskal-Wallis and Jonckheere-Terpstra non-parametric tests on pond and lake types A, B and C as described above, with type D omitted as it had no biological meaning for this study.

A Kruskal-Wallis H and Jonckheere-Terpstra tests showed statistically significant differences in lake and pond type between some of the considered variables: Total surface (m²) ($\chi^2(2) = 55.976$, $p = 0$; J-T = -6.636, $p = 0$), Altitude m a.s.l. ($\chi^2(2) = 7.345$, $p = 0.025$; J-T = -2.292, $p = 0.022$), Latitude UTM ($\chi^2(2) = 13.19$, $p = 0.001$; J-T = -3.529, $p = 0$), and Longitude UTM ($\chi^2(2) = 0.879$, $p = 0.644$; -T = 0.817, $p = 0.414$). From a geological point of view, the Pyrénées mountain chain runs from east to west, and thus the latitude (N-S) is obviously linked to altitude and geology. Therefore, it does not have any biological meaning in our study. The pH and Conductivity values among the studied lakes were similar and did not show any significant differences. The total surface is the variable that best discriminates the grouping variables studied (see Online Appendix 3).

In order to see the effect of the four studied variables in the presence of frog breeding and frog adults we ran Kruskal-Wallis H and Jonckheere-Terpstra tests, which did not show any significant differences between the lakes and ponds with frog breeding presence and frog adults (Total surface (m²): $\chi^2(2) = 2.052$, $p = 0.359$; J-T = 0.109, $p = 0.913$; Altitude m a.s.l.: $\chi^2(2) = 0.602$, $p = 0.74$; J-T = 0.246, $p = 0.806$; Latitude UTM: $\chi^2(2) = 6.632$, $p = 0.036$; J-T = -0.772, $p = 0.44$; Longitude UTM: $\chi^2(2) = 8.622$, $p = 0.013$; J-T = 1.511, $p = 0.131$) (see Online Appendix 4).

To determine if trout stocking effected frog presence in our study area, the degree of isolation between each water body was estimated from the average distance of the focal water body to the nearest three water bodies (in metres) (Cogalniceanu, 2012). Our studied lakes and ponds were gathered in two groups, lakes and ponds in the west with a lower fish stocking rate (7%) and the lakes and ponds in the east with a higher fish stocking rate (34%). The average distance between water bodies groups was 274.5 m (± 175 m) and 342.9 m (± 236 m) respectively, which supported our hypothesis.

We characterised each lake and pond as a function of the percentage of rock and vegetation present in their riparian area and the mean of the total surface in order to find any differences between lake and pond types [ie. fish stocked (type A), non-fish stocked and frog breeding present (type B), fish stocked and frog breeding present (type C)]. In total 63 lakes and ponds were characterised as type A, 42 as type B and 5 as type C. Of those characterised as type A (Online Appendix 2), the mean percentage of rock and vegetation present in the

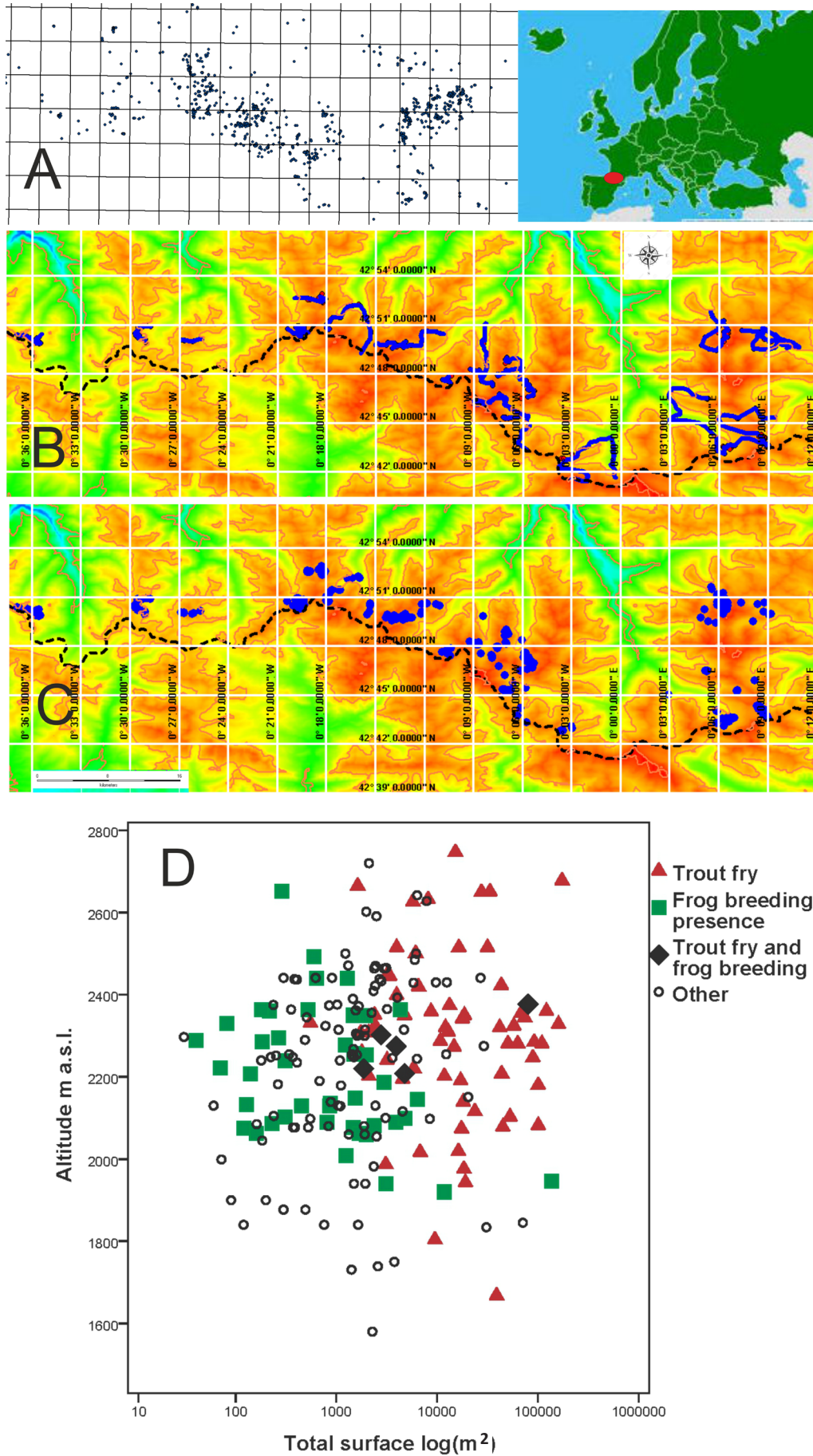


Figure 1. A. Map of the lakes from all the sectors in the French National Park of the Pyrénées. B. Treks surveys carried out during 2001 and 2002 in the core of the Park. C. Sampled lakes. D. Dispersion diagram showing altitude (m a.s.l.) and Total surface log (m²).

riparian area were 73% and 20% respectively, and the mean total surface area was 31680 m². In type B lakes and ponds (Online Appendix 2), the mean percentage of rock and vegetation present in the riparian area were 43% and 44% respectively, and the mean total surface area was 4785 m². Finally, in type C lakes and ponds (Online Appendix 2) the mean percentage of rock and vegetation present in the riparian area were 36% and 42%, and the mean total surface area was 18641 m². We found that the percentage of rock and vegetation present in the riparian area of lakes type B and C were very similar. More detailed descriptive statistics of these and non-significant variables studied can be found in Online Appendix 3.

Of the 215 lakes and ponds surveyed after fish stocking, *R. temporaria* was present in 97 of them, which represented 45.1% of the total. We also found the presence of *R. temporaria* reproduction in 49 of the lakes and ponds surveyed (22.8%). Fish stocking had occurred in 68 lakes and ponds (31.6%). The altitude range where we conducted our research of *R. temporaria* was between 1580 and 2747 m a.s.l., and we found *R. temporaria* from 1739 m, however we did not find *R. temporaria* or any other amphibian at elevations higher than 2652 m a.s.l. Lakes and ponds with bigger surface areas were found to be stocked more frequently, which is consistent with the fact that stocking was done via helicopter and only the largest lakes were accessible (Fig. 1D). When studying the four types of lake and pond distributions we found that the spatial distribution was homogeneous among six major drainage basins studied (Online Appendix 3, Fig. A), distribution across latitudes and altitudes was uniform due to the north-south drainage basin orientation, the distribution across longitudes and the total surface areas was uniform (Online Appendix 3, Fig. B), and the distribution across altitudes and longitudes was uniform (Online Appendix 3, Fig. C).

Our results showed an adverse impact of rainbow trout fish stocking on the presence of *R. temporaria*, a result consistent with other studies of fish stocking impact on amphibian populations (Bradford, 1989; Braña et al., 1996; Bradford et al., 1998; Tyler et al., 1998; Knapp & Mathews, 2000; Knapp et al., 2001; Martinez-Solano et al., 2003; Pope et al., 2008; Pilliod & Peterson, 2001; Knapp, 2005; Orizaola & Braña, 2006; Dan et al., 2012; Băncilă et al., 2017).

This impact can be seen when evaluating frog presence using individual lakes and ponds. Frogs and trout showed very limited overlap (Fig. 1D and Online Appendix 2C) and as a general rule frog presence declined wherever trout presence increased. Trout stocking within the park was biased towards larger lakes and ponds, which were both more popular among trekkers and easier to stock using helicopters. As a consequence, frogs were more frequently found in smaller, harder to reach ponds (Fig. 1D and Online Appendix 2B) which would become preferred breeding sites (Tiberti & Hardenberg, 2012). Many of these smaller ponds tend to be temporary, which forces new cohorts to adapt to a shorter growing period (Newman, 1989). Populations that become specialised in rapid development and an early metamorphosis

at low metamorphic size are known to have a lower degree of phenotypic plasticity (Lind & Johansson, 2007; Miramontes et al., 2018) and lose fitness as a result of their smaller size at metamorphosis (Altwegg & Reyer, 2003). In addition, smaller ponds are likely to see an increase in their temporality with climate change as temperatures in the Central Pyrénées rise (Catalan et al., 2002; El Kenawy et al., 2011; Pérez-Zanón et al., 2016), which results in increasing evaporation in summer and a decrease in the availability of snowmelt water (Lopez-Moreno et al., 2008, 2009). This increase in the temporality of lakes due to climate change has been reported before and has been shown to have deleterious effects on amphibian populations (McMenamin et al., 2008). Such changes can also cause an increase in habitat fragmentation in an alpine habitat that is naturally fragmented, which could also be detrimental to *R. temporaria* populations (Johansson et al., 2005, 2007).

Although it has been shown previously in other protected areas that the only management practice that has a positive effect on the protection and recovery of mountain lakes and ponds and its fauna is the prohibition of fishing (Miró & Ventura, 2013) and other studies have proven that the introduced fish removal has a positive effect in the recovery of the frog populations (Vredenburg, 2004; Knapp, 2007) and other amphibians (Funk & Dunlap, 1999), we recommend considering the lake and pond characteristics before designing the fish stocking strategy (pictures available in Online Appendix 2). Due to recent efforts to study coexistence of predators and preys (Hartman et al., 2014; Kenison et al., 2016; Winandy et al., 2017) and in order to minimise the fish stocking impact, we propose to group lakes and ponds in different classes ordered from minimum to maximum fish stocking impact.

The lakes and ponds susceptible to fish stocking with minimum impact on our studied species are lakes that are used as water reservoirs like Migouelou 2278 m a.s.l.; lakes where the effluent is subterranean and the affluent present a sharp slope making impracticable the displacement of fish (e.g. Remoulis 2017 and 2019 m a.s.l., Badete 2344 m a.s.l., col d'Aratille 2501 m a.s.l., Pouey Laun 2346 m a.s.l. and Nère 2241 m a.s.l.); and lastly lakes with small ponds nearby and interconnected with the main lake and have subterranean effluents (e.g. Batcrabere 2116 and 2180 m a.s.l.).

A second group are the big lakes that are easily accessible (e.g. Suyen 1536 m a.s.l. and Tech 1207 m a.s.l.) and can be fish stocked, as these are appropriate for fish reproduction. These may also be ideal candidates to make neighbouring artificial ponds in order to recuperate amphibian and general fauna, which could be extended to other lakes.

A third group are lakes with a series of nearby and intercommunicated ponds with subterranean effluents (e.g. Houns de Heche 2113 m a.s.l. and lac du Pic Arrouy 2376 m a.s.l.). In this case, to minimise the effect of fish stocking we propose to isolate the lake from the ponds in order to avoid the fish displacement to small neighbouring ponds.

A fourth group would be lakes where the fish could displace through their effluents (e.g. Fache 2332 and 2427 m a.s.l., d'Aratille 2247 m a.s.l.) and also affluent (e.g. Lassiedouat 2200 m a.s.l.). In this case the impact might be higher, thus more effort should be done to avoid and prevent the displacement of the fish through the drainage basin.

A fifth group with the potential to have an extensive impact of fish stocking are small lakes (e.g. Laquets de Micoulaou 2302 and 2333 m a.s.l., Lascarat de Migouelou 2429 m a.s.l., Laquets de Lassiedouat 2220 and 2268 m a.s.l. and Araille 2450 m a.s.l.) where fish would rapidly consume available nutrients; and lakes with flooded lands nearby with a big number of amphibian species (e.g. Plaa de Prat 1656 m a.s.l.). Also, lakes that are physically suitable for amphibians should be conserved to host the most numerous populations of *R. temporaria* in the Park, as in the case of Lake Touest 1955 m a.s.l., or small lakes and ponds along Badete 2344 m a.s.l. and Lacs d'Aratille 2247 and 2315 m a.s.l.

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Supplementary material:

- Appendix 1: pictures of 100 sampled lakes and ponds are available in a pdf file.
- Appendix 2: pictures of the three lakes and ponds types in function of its percentage of rock and vegetation in their riparian area.
- Appendix 3: dispersion diagrams showing different lake types' distribution (longitude (UTM) and latitude (UTM) (Fig. A); longitude (UTM) and total surface log (m²) (Fig. B); longitude (UTM) and altitude (m a.s.l.) (Fig.C).
- Appendix 4: descriptive statistics of the studied variables in the three types of ponds and lakes (A, B and C), one-way ANOVA and non parametric Kruskal-Wallis and Jonckheere-Terpstra tests.
- Appendix 5: non-parametric Kruskal-Wallis and Jonckheere-Terpstra tests to analyse the effect of the total surface area, longitude, latitude and altitude on the distribution of frog breeding and frog adults' presence in the study area.

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