

Assessing the response of amphibians to wildfire according to forest type and bioregion affinity of species

Brahim Chergui¹, César Ayres², Xavier Santos^{3,*}

¹ LESCB URL-CNRST N°18, FS, Abdelmalek Essaadi University, Tetouan, Morocco.

² AHE-Galicia, Barcelona 86 6C, 36211 Vigo, Spain.

³ CIBIO/InBIO (Centro de Investigação em Biodiversidade e Recursos Genéticos da Universidade do Porto), R. Padre Armando Quintas, 4485-661, Vairão, Portugal.

*Correspondence: xsantossantiro@gmail.com

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Climate and socioeconomic factors are modifying fire regimes. In this scenario, some taxa, such as amphibians, may be increasingly vulnerable. We examined the response of amphibian species to fire severity after a 1600-ha fire in a fire-active region located in the north-western Iberian Peninsula. This area is a biogeographical crossroad where Atlantic and Mediterranean amphibian species can coexist in the same ponds. We sampled 33 water points in native (mainly oak) and exotic (eucalyptus) forests. Water points were sampled in two different periods: just after the fire to report direct mortality, and two years after the fire to evidence amphibian resilience. We examined the influence of forest type and fire severity on the number of amphibian species detected per point (species richness). Species richness per point varied depending on both forest type (i.e. higher richness in the native forest) and fire severity. Both Atlantic and Mediterranean groups showed higher species richness at native than at exotic points. The occurrence of Atlantic species did not change with fire whereas the number of Mediterranean species increased at sites affected by high-severity fires. This study identified the negative effect of eucalyptus plantations on amphibians and showed that the response of this taxon to fire is partially shaped by species-specific bioregion affinity

Key words: amphibians; eucalyptus; Galicia; oak forest; refuge; wildfire.

Fire is a key disturbance shaping species composition from ecosystems located in fire-prone regions such as the Mediterranean Basin (BOND *et al.*, 2005). In recent decades, socioeconomic factors (rural abandonment and fuel accumulation) together with climatic factors (rising temperatures and increasingly irregular rainfall cycles) are altering fire regimes, augmenting the frequency, intensity, and extent of fires (MOREIRA *et al.*, 2001; PAUSAS & FERNÁNDEZ-MUÑOZ, 2012). After a fire, the sim-

plification of the burnt habitat gives, at the short term, ecological opportunities to species that prefer open landscapes (BROTONS *et al.*, 2008), whereas long-unburnt habitats are preferred by species adapted to more closed-in mature landscapes (SANTOS *et al.*, 2015; FERREIRA *et al.*, 2016; CHERGUI *et al.*, 2019). Despite these general patterns, the response of animal communities to fire is insufficiently known (PAUSAS & PARR, 2018) and, for many groups, including severely threatened ones as is the case of amphibi-

ans, scant information is available on the consequences posed by fire (Muñoz *et al.*, 2019; GOMES DOS ANJOS *et al.*, 2021). The scarcity of fire-ecology studies targeting amphibian responses may result from an underestimation of the importance of wildfire on the conservation of amphibian populations (PILLIOD *et al.*, 2003).

The literature reviewing the impact of fire on amphibians indicates highly variable responses at the individual, population, and community levels (RUSSELL *et al.*, 1999; BURY *et al.*, 2002). A recent comprehensive study including 68 published works revealed a lack of general responses among 191 amphibian species distributed around the world (GOMES DOS ANJOS *et al.*, 2021), as some species responded positively and others negatively to fire. Although there is little evidence of amphibian direct mortality by flames (JOLLY *et al.*, 2022), negative responses can be caused by post-fire chemical changes in aquatic habitats (SMITH *et al.*, 2011; JAGER *et al.*, 2021), by vegetation changes along migration routes (PILLIOD *et al.*, 2003; HOSSACK & PILLIOD, 2011), and especially by structural changes in terrestrial vegetation around water points (Muñoz *et al.*, 2019). However, amphibians can often survive to fire by burying themselves, moving to aquatic refuges, using the burrows of other animals (MEANS & CAMPBELL, 1981), and resisting heat through skin secretions (RUSSELL *et al.*, 1999; VAN MANTGEM *et al.*, 2015). The quality and extent of breeding habitats before the fire also modulate the response of amphibian populations to this disturbance (ROCHESTER *et al.*, 2010; WESTGATE *et al.*, 2018).

Amphibians are considered an indica-

tor of ecosystem health because they are highly sensitive to disturbance and to habitat change (DE MAYNADIER & HUNTER, 1995; WELSH & DROEGE, 2001; HOSSACK *et al.*, 2006). There is a widespread decline of amphibian species due to multiple factors such as habitat degradation, overexploitation, diseases and climate change (STUART *et al.*, 2004). However, potential threats like wildfires have been scarcely studied, even in fire-prone environments (BURY *et al.*, 2002; PILLIOD *et al.*, 2003; BURY, 2004; MUÑOZ *et al.*, 2019). The present study is a new contribution about the impact of fire on amphibian species. Specifically, we aim to examine the short-term response of amphibians to a large fire occurred in Galicia (north-western Iberian Peninsula). The flames burned patches of cultivated areas, native forest, and eucalyptus plantations. In this region, the amphibian community consists of a mixture of Mediterranean and Atlantic species according to their distribution ranges in one or other bioregions (SILLERO *et al.*, 2009). In this context, the specific objectives were: 1) To identify differences in amphibian species richness between native and eucalyptus forests. This objective has been examined in unburnt water points located outside the burnt perimeter. 2) To identify amphibian mortality after fire by sampling burnt water points immediately after the fire. 3) To estimate the impact of fire severity (i.e. the proportion of burnt vegetation) on amphibian species. Given the dependence of amphibian species to the pond-surrounding vegetation for foraging and refuge, we expect higher impact on more severely affected water points. 4) To compare the impact of fire severity in water

points surrounded by native or eucalyptus forests. Native forest is structurally more complex than monospecific eucalyptus plantations. Accordingly, we expect that amphibian species living in water points located within native forest will be more resilient to fire than those living in non-native plantations (see a similar dynamic for reptile populations in *CHERGUI et al.*, 2019). 5) To compare the impact of fire between Mediterranean and Atlantic species. Fire is considered a landscape modeller of the Mediterranean Basin (*KEELEY et al.*, 2012) and amphibian species living in this

biome are considered to be adapted to this disturbance (*MUÑOZ et al.*, 2019). For this reason, we expect that Mediterranean species will respond to fire better than Atlantic ones.

MATERIALS AND METHODS

Study area and amphibian sampling design

The study area was located in Vigo (Pontevedra province), in the region of Galicia (north-western Spain; Fig. 1). On 15 October 2017, several fires in Galicia burned more than 20 000 ha in the Pontevedra province, devastating 1600 ha in the

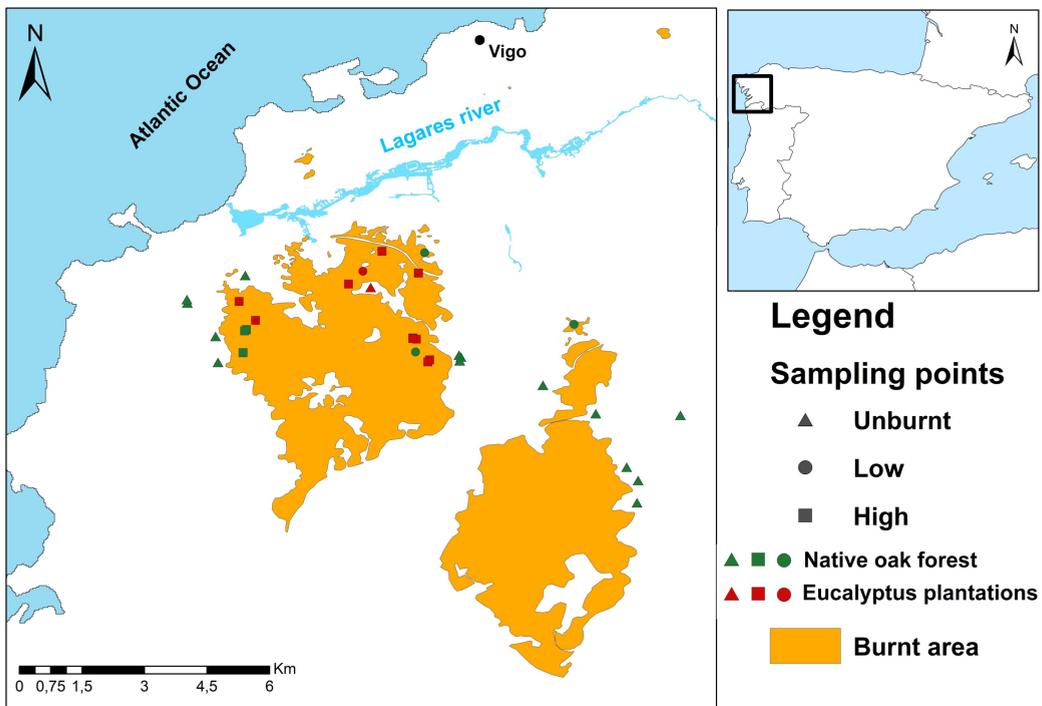


Figure 1: Location of the burnt area on 15 October 2017 in Galicia (north-western Iberia) and position of the sampled water bodies. Points were selected according to the fire severity: ‘high-severity’ when almost all the vegetation surrounding the water point was burnt; ‘low-severity’ when less than 50% of the surrounding vegetation was burnt; and ‘unburnt’ when points were outside the fire perimeter or in unburnt patches inside the burnt area.

metropolitan area of Vigo (CHAS-AMIL *et al.*, 2020), where we later conducted the present study. The landscape is a mixture of patches of small cultivated areas, scrubland dominated by *Ulex* and *Erica*, stands of native forest (mainly *Quercus robur* L. oaks, and also willows and alders in riverine habitats), and plantations of non-native trees (hereafter exotic forest), mainly *Eucalyptus* sp. and also *Acacia* sp. Despite Galicia is a region intensively affected by recurrent fires (DE DIEGO *et al.*, 2019), most of our study area had remained unburnt for at least the last 40 years.

A total of 33 sampling points including ponds, stretches of streams, and water sources, were surveyed to evaluate the impact of fire on the amphibian fauna. Sampling points covered burnt and unburnt areas of native forest (burnt = 6 and unburnt = 14 points) and eucalyptus plantations (burnt = 11 and unburnt = 2 points) (Figs. 1, S1). Points were selected according to the proportion of trees and shrub with burnt canopies (i.e. fire severity) around each water point, assigning the following categories: ‘high-severity’ when almost all the surrounding vegetation to the water point was burnt; ‘low-severity’ when less than 50% of the surrounding vegetation was burnt, which usually corresponded to the edge of the fire perimeter; and ‘unburnt’, when water points were outside the fire perimeter or in unburnt patches inside the fire perimeter.

The methods used to sample amphibians in the study area were visual surveys along streams searching for individuals under refuges, along the shores and in the water. Surveys were conducted during the daytime to facilitate the detection of lar-

vae. Dip-netting was used in ponds and artificial reservoirs to quantify the presence of adults and larvae. Each water point was surveyed approximately during 30 minutes. Water points were sampled twice each year (2017 and 2019, total four samplings) to reduce the probability of failing to detect species that were present in the study area. In 2017, the first sampling was conducted in November, i.e. just after the fire to find dead or dying amphibians in burnt areas, and the second sampling was in December 2017, two months after the blaze. During autumn 2019, the same sampling points were visited twice again.

We have no information about the amphibian community composition in burnt points before the fire. For this reason, the impact of fire was measured by comparing species richness between unburnt and burnt water points, with that difference being considered as an inverse surrogate of amphibian resilience (i.e. the lower the difference, the higher the resilience). Unburnt water points were selected as those composed by similar structural non-native / native vegetation compared to the burnt ones.

Data analysis

The response variable was species richness, as the number of amphibian species found at a given sampling point each sampling year (2017 and 2019). We hypothesised that Mediterranean species would be more adapted to fire (KEELEY *et al.*, 2012) and their resistance to fire would be higher than in Atlantic species. Therefore, the Mediterranean and Atlantic species richness per sampling point were also used separately as response variables in our

analyses. Amphibian species were classified as Mediterranean or Atlantic according to the classification of SILLERO *et al.* (2009), based on the overall distribution of each species within the Mediterranean or Atlantic bioregions. We found a total of three Atlantic species (the palmate newt *Lissotriton helveticus*, the golden-striped salamander *Chioglossa lusitanica*, and the Iberian stream frog *Rana iberica*) and six Mediterranean species (Bosca’s newt *Lissotriton boscai*, the marbled newt *Triturus marmoratus*, the fire salamander *Salamandra salamandra*, the common midwife toad *Alytes obstetricans*, Perez’s frog *Pelophylax perezi*, and the Iberian painted frog *Discoglossus galganoi*). According to SILLERO *et al.* (2009), *S. salamandra* has very similar percentages of assignment to both biogeographical regions, making it difficult to decide in which region this species should be included. In Galicia, it is ubiquitous and we have classified it as Mediterranean due to its high ecological tolerance.

We examined the effect of forest type (native and exotic) and fire severity (high-severity, low-severity, unburnt) on total species richness as well as on Mediterranean and Atlantic species richness separately by Generalized Linear Mixed Models (GLMMs). Forest type, fire severity, and their interaction were considered as categorical fix factors, and ‘year’ as a random effect. We modelled the response variables with a Poisson distribution due to the type of used data (discrete values). Unfortunately, the availability of water points in the study area did not allow for a balancing the number of sampling points between native and exotic points, or among fire severity classes. Analyses were performed using the ‘lmer’ function in the lme4 package (BATES *et al.*, 2015). The significance of fix factors was tested with Wald’s χ^2 tests of the fitted final model using the function ANOVA from the package car (FOX & WEISBERG, 2019).

Table 1: Results of the Generalised linear mixed models to explore the influence of forest type (native forest or exotic eucalyptus), fire severity (unburnt, low severity or high severity), and their interaction on amphibian species richness, as well as the on the richness of Atlantic and Mediterranean species separately .

Response variable	Effect	χ^2	df	P
Species richness	Forest type	11.9954	1	0.0005
	Fire severity	9.1497	2	0.01
	Forest type * fire severity	4.0403	2	0.13
Atlantic species	Forest type	5.2170	1	0.02
	Fire severity	0.6480	2	0.7
	Forest type * fire severity	4.7911	2	0.09
Mediterranean species	Forest type	5.0413	1	0.02
	Fire severity	10.6035	2	0.005
	Forest type * fire severity	0.9949	2	0.6

RESULTS

We recorded a total of 99 occurrences of amphibian species at the 33 surveyed points. On average, we found 1.5 amphibian species per point (range 0-8 species; standard deviation = 1.82). These 99 records corresponded to nine species, i.e. six Mediterranean and three Atlantic species (Table S1). The most frequently recorded species were *S. salamandra* (N = 13 points), *P. perezi* (N = 11 points), *R. iberica* (N = 10 points), and *C. lusitanica* (N = 9 points) (Table S1). The first sampling year, we found 10 individuals from five species dead in or closed to the water at four burnt points, i.e. 24% of the sampled burnt points (Fig. S2). These animals were not found burnt, suggesting that they could have been overheated due to a sudden increase of water temperature.

The GLMMs indicated that the total amphibian species richness was significantly higher in water points located in native forest than points located in exotic points (Table 1, Fig. 2a). This result was also significant when Mediterranean and Atlantic species were analysed separately (Fig. 2c,e). The responses of amphibian species to fire varied according to fire severity, and the number of species was higher at water points severely affected by fire (Fig. 2b). This result was related to the different responses of Atlantic and Mediterranean species. Thus, Atlantic species richness did not vary according to fire severity (Fig. 2d), whereas Mediterranean species richness increased at points affected by high-severity fire (Fig. 2f). None of the interactions between forest type and fire severity resulted significant, suggest-

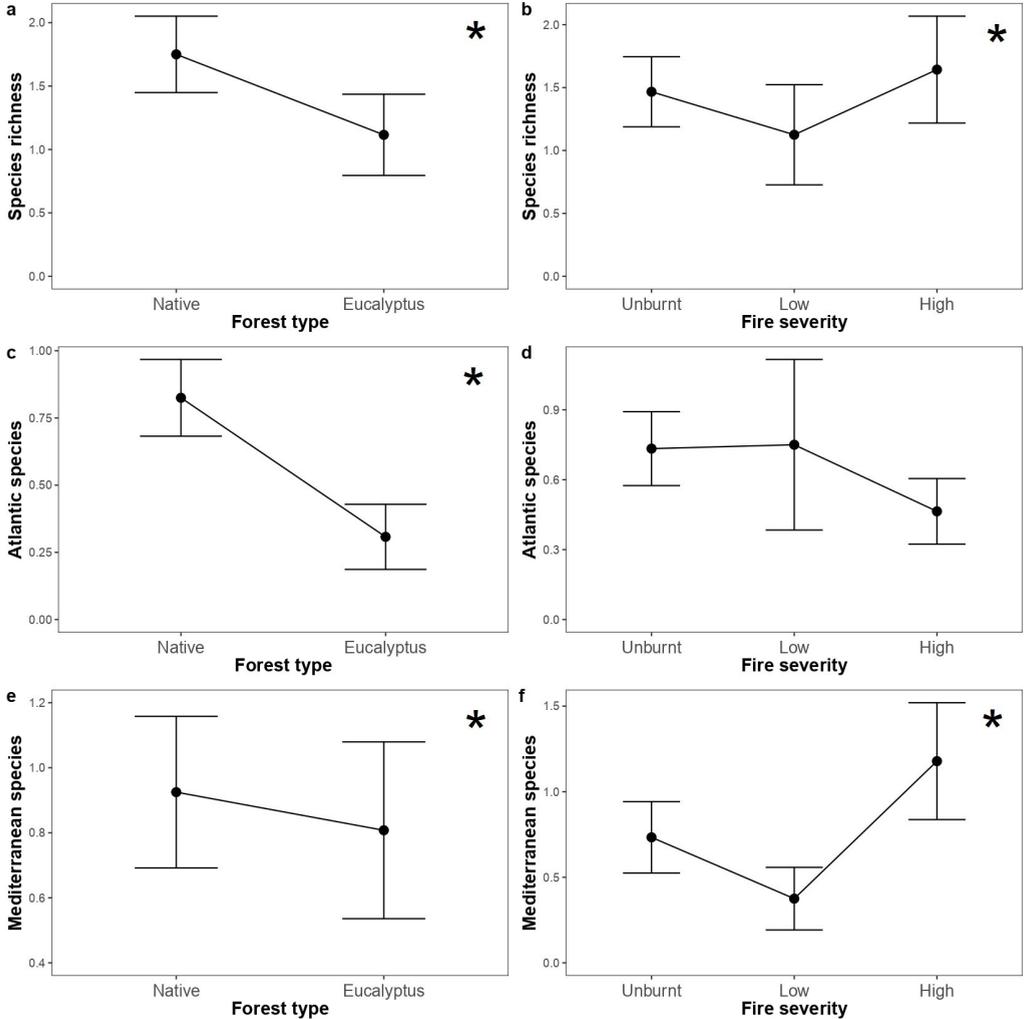
ing that the impact of fire on amphibian species richness resulted similar regardless the water point affected was located on native or exotic forests (Table 1).

DISCUSSION

This study assesses the implications of fire severity and habitat change (from native forest to exotic plantations) on the occurrence of amphibian species. We acknowledge that species occurrence is a coarse measure of environmental impact on a taxon, but the presence of species at breeding water points gives a preliminary evaluation of impact on amphibian species (HOSSACK & CORN, 2007). Amphibians are specially stressed during periods of extended drought (WESTERLING *et al.*, 2006; MORGAN *et al.*, 2008), when large wildfires tend to occur. Thus, amphibians can be challenged both by wildfire and adverse environmental conditions (PECHMANN *et al.*, 1991). We found three main results: 1) amphibian species richness declined in water points located in exotic forests; 2) direct amphibian mortality occurred after fire; and 3) the response of amphibian species to fire severity was complex and related to species-specific bioregion affinity (i.e. Mediterranean or Atlantic species).

Our observations of direct amphibian mortality due to the fire indicate a high impact as at least 24% of sampled points had recently death animals. The proportion is probably higher since we have not observed burnt animals located into the vegetation. A recent review of animal mortality during fire stated that there are no studies reporting direct impact in amphibians (JOLLY *et al.*, 2022). Our novel observation was probably caused by the small size

Figure 2: Generalized Linear Mixed Model plots of the number of amphibian species found at sampled water points, modelled for forest type, fire severity and their interaction. Plots show mean values (symbols) \pm one standard error (whiskers) of total amphibian species richness according to forest type (a) and fire severity (b), and the same for Atlantic species richness (c and d) and Mediterranean species richness (e and f). Asterisks indicate significant differences among groups.



of some water points, whose water temperature would have raised up to lethal levels because of the high fire intensity, and we were able to detect those dead animals because of the first sampling visit that was done few days after the fire.

The effect of habitat type

Our results provide empirical evidence that amphibian species richness declined from native forests to exotic plantations. In natural habitats, amphibian species rich-

ness is positively correlated with landscape heterogeneity (ATAURI & DE LUCIO, 2001) and land-cover composition (IGLESIAS-CARRASCO *et al.*, 2016a,b), as observed in native forests. By contrast, the homogeneous canopy in eucalyptus plantations alters the soil composition and water quality (MONTEIRO *et al.*, 2021). In general, eucalyptus plantations have negative ecological effects such as soil degradation, declining groundwater level, and general biodiversity decline (GODED *et al.*, 2019), including diminished amphibian diversity (VALLAN, 2002; ARNTZEN, 2015). Eucalyptus trees produce leachates that alter water composition where amphibians breed by lowering oxygen level and pH (ABELHO & GRAÇA, 1996; CANHOTO & LARANJEIRA, 2007). Ultimately, these physico-chemical changes can affect amphibian behaviour and development (BURRACO *et al.*, 2018; IGLESIAS-CARRASCO *et al.*, 2022). Moreover, these changes can also influence the structure of macroinvertebrate communities; in fact, eucalyptus plantations are characterized by lower macro-arthropod abundance than native forests (ZAHN *et al.*, 2009). Invertebrates constitute the primary food resource for amphibians, and therefore the density of understory vegetation boosts the number of invertebrates, which in turn can promote the diversity of amphibians (BROWNE *et al.*, 2009). Moreover, anuran larvae are detritivore feeders, which make them extremely sensitive to the quality of plant remains (MAERZ *et al.*, 2010). Overall, these results demonstrate that eucalyptus plantations exert strong adverse effects on amphibian species, similar to those identified for certain aquatic invertebrate groups (GORMAN *et al.*, 2009, 2013).

The effect of fire

Overall, we did not find a negative impact of fire on amphibian species richness. Although we found direct mortality caused by the fire, species richness did not decline after low or high fire severity at the sampled water points. We found that some burnt water points had a rich amphibian community and others did not have any amphibian; for this reason, standard errors were high in plots of burnt water points (see Fig. 2). We speculate that the impact of fire on amphibians can be spatially heterogeneous, depending on local environmental conditions, fire intensity and water point characteristics, but unfortunately these factors were not measured. This spatial heterogeneity at the local scale matches the diversified results obtained at a global scale by GOMES DOS ANJOS *et al.* (2021), and highlight the difficulty in uncovering general responses of animal groups to fire.

At the short-term, fire simplifies the complexity of forest ecosystems by a reduction in several structural variables such as canopy and litter cover (CHERGUI *et al.*, 2018). A reduction in forest canopy exposes the lowest habitat layers to intense radiation and wind (SEMLITSCH *et al.*, 2009), generating warmer surfaces (ZHENG *et al.*, 2000). Given the importance of vegetation structure to many amphibian species (HALVERSON *et al.*, 2003; GRUNDEL *et al.*, 2015), intense fires are expected to negatively affect many amphibians due to changes in micro-environmental conditions (RUSSELL *et al.*, 1999; BURY *et al.*, 2002). However, we did not find negative responses in our study area, and interestingly the response of amphibians to fire

differed according to the bioregion affinity of each species: whereas the presence of Atlantic species in burnt water points did not vary, Mediterranean species increased their presence in severely burnt water points. Contrasting environmental needs of Mediterranean vs. Atlantic amphibian species can explain why these two biogeographical groups of organisms responded differently to fire (see FERREIRA *et al.*, 2016 for a similar result in reptiles). The positive response to fire by Mediterranean amphibians might be because these species benefit from the land-cover openness created by fire (KEELEY *et al.*, 2012; SANTOS *et al.*, 2019). Many of those species have evolved and persisted in fire-prone regions, apparently due to adaptations to fire disturbances (PILLIOD *et al.*, 2003).

Atlantic amphibian species select forested and mild-temperature areas over cleared ones with high temperatures (KATI *et al.*, 2007; SILLERO *et al.*, 2009). In regions affected by high fire activity such as our study area, these species tend to occur in moist environments, which are restricted to areas with very long fire return intervals (PILLIOD *et al.*, 2003). Atlantic species in Galicia appeared to be resilient to fire, probably due to the resprouting capacity of dominant tree species such as oaks and the long-unburnt intervals on some native forests (more than 40 years for the area sampled in the present study). These species are expected to persist in Galician native forests if fire frequency and intensity do not increase. If fire frequency increases, a progressive substitution of Atlantic towards Mediterranean amphibian communities would arise as already observed for reptile communities in high recurrent fire

areas (SANTOS & CHEYLAN, 2013). The positive response of Mediterranean amphibian species to high-severity fire suggests their capacity to colonize burnt areas as it has been documented in southern France (SANTOS *et al.*, 2019).

Concluding remarks

This study documents the impact of exotic forest and fire severity on amphibian species. Whereas eucalyptus plantations had a negative impact on the whole amphibian community, the fire apparently did not impact amphibian presence in burnt water points, and positive responses were observed for Mediterranean amphibians. Eucalyptus plantations represent 22% of the forest cover in Galicia and have increased from a baseline of 6% since the start of the 21st century. Currently, more than 420 000 ha of Galicia are occupied by eucalyptus, representing an increase of 65% in the last two decades (IFN, 2011). These plantations provide economic benefits despite being less suitable for the conservation of biodiversity than are native environments (RAMÍREZ & SIMONETTI, 2011; CODED *et al.*, 2019). Landscape managers and local actors should reconsider the null, even negative, value of exotic plantations for the conservation of biodiversity and apply new models of management (CIDRÁS *et al.*, 2018).

Despite the apparently null impact of fire for amphibian species, climate and socioeconomic factors are modifying fire regimes in many regions towards bigger, more intense and frequent fires (PAUSAS, 2022). This shift in fire regimes can modify the landscape structure and forest composition, which indirectly can affect amphibian

an community composition. Conservationists have to be alert with the potential effect of this processes on threatened species like some of the amphibian species living in the study area, which could decline due to landscape modification.

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