

1 **Title: Amphibian diversity and abundance in ponds is lower in exotic plantations than**
2 **native forests**

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12

13 **Abstract**

14 What effect do tree plantations have on the diversity of native organisms? Some studies show that plantations
15 reduce the diversity and abundance of certain taxa, while other studies suggested that plantations help to
16 conserve biodiversity. Pine and eucalyptus plantations are among the most widespread exotic plantations
17 worldwide, and they have negative effects on many taxa. But how do they affect amphibian diversity and
18 abundance? We barely know. We therefore tallied up the number of amphibian taxa and their abundance from
19 18 ponds in patches of native oak forests, pine or eucalypt plantations. We also quantified water quality by
20 measuring its physicochemistry and identifying the macroinvertebrates present in each pond. There were
21 significantly fewer amphibian species in tree plantations than in native forest. Compared to native forest, the
22 total density of amphibians was also significantly lower in eucalypt, but not pine, plantations. Species varied in
23 the effects of plantations on their presence and abundance. We suggest that the decline in the presence and
24 abundance of amphibians in plantations is linked to the physicochemical of pond water, combined with the
25 relatively low presence of invertebrate. It seems likely that earlier desiccation, greater toxicity, and poor quality
26 detritus in ponds in plantation are key drivers of species decline. The effects of these drivers are expected to
27 worsen as climate change continues.

28

29 **Keywords:** water toxicity, pond desiccation, amphibian conservation, oak forest, eucalypt plantations, pine
30 plantations.

31 **Introduction**

32

33 All over the world vast areas of native forest have been converted into exotic tree plantations. In total, 7% of the
34 global forested area is occupied by plantations [1], which are mainly used to produce paper, timber or charcoal
35 [2]. Plantations have a higher density, but lower diversity, of trees than natural forests [3]. Many studies have
36 shown that plantations reduce the number of species of important animal groups, such as arthropods and birds
37 [4,5]. But in extreme cases where natural forests become scarce, plantations can paradoxically contribute to the
38 conservation of diversity [6–8]. Eucalypt (mostly *Eucalyptus globulus* Labill.) and pine plantations (*Pinus sp.*)
39 are the two most common types of exotic plantations worldwide. We now have information about how pines
40 and eucalypts affect many taxa, including birds [9], mites [10], fish [11], lizards [12] and even native vegetation
41 [13]. In contrast, how plantations affect amphibians is practically unknown (but see [14,15]). Here we address
42 this gap in knowledge.

43

44 As with many non-native plants, eucalypt plantations release toxic substances into the soil [16] and waterways
45 [17], reduce water yields and change soil characteristics [18]. Widely reported declines in stream assemblages,
46 such as stream macroinvertebrates and fungi, in waterways in the catchments of eucalypt and pine plantations
47 [19–21] have been attributed to the toxicity and low quality of their leaf litter. We expect these effects to be
48 stronger in stationary (lentic) water systems, as water quality decline is exacerbated due to the steady
49 accumulation of leaf litter and the lack of water renewal [22].

50

51 Globally, amphibians are among the most threatened animal groups. This is largely due to habitat loss [23],
52 which is particularly devastating as amphibians often have low mobility and high philopatry [24]. Their highly
53 permeable skin also makes them sensitive to toxic substances [25]. Consequently, small changes in the chemical
54 characteristics of their terrestrial or aquatic habitats (depending on the life history phase) can have major effects
55 on amphibian survival and breeding success. For example, the introduction of some exotic plants alters native
56 forest amphibian communities due to seemingly small modifications, such as a changes in the temperature in
57 their preferred microhabitats [26]. Small, ephemeral ponds are common in forests and usually support a high
58 diversity and abundance of amphibians. Many amphibians breed in such ponds, stay there until they complete
59 larval development, and then emerge to forage and hibernation/estivation in nearby terrestrial habitat [27].

60 Lower species diversity and abundance in plantations could be due to negative effects of leachates from their

61 leaf litter on water quality and therefore larval survival and/or adult breeding success; or to plantations lowering
62 the survival of juveniles and adults while on land.

63

64 We investigated how *Eucalypt* and *Pine* plantations affect the diversity and relative density of amphibian adults
65 and larvae. Most European amphibians have terrestrial adults that move to nearby ponds to breed. We therefore
66 focused our sampling efforts in the vicinity of, and in, ponds. We first characterized the amphibian assemblages
67 in ponds under native forests and *Eucalypt* and *Pine* plantations. We then measured physical, chemical and
68 biological properties of these ponds to determine which environmental characteristics might affect amphibian
69 diversity and abundance. We tested whether:

70

- 71 1) replacement of native forest by pine and eucalypt exotic plantations reduces the diversity and density of
72 amphibian species;
- 73 2) the assemblage of macroinvertebrates in ponds predicts amphibian diversity and density, because these
74 assemblages are related to long-term water quality and are themselves a food resource for amphibians;
- 75 3) water chemistry, wetland vegetation and the size/depth of ponds predict amphibian diversity and density.

76

77 **Material and methods**

78 *Study site*

79 We collected data at 18 ponds in Atlantic watersheds of the Basque Country: six under native
80 deciduous forest patches (*Quercus robur* L.), six under eucalypt plantations (*Eucalyptus globulus*) and another
81 six under pine plantations (*Pinus radiata* D.Don). The ponds were totally surrounded for at least 400 meters by
82 the corresponding habitat type. The climate of the study area is mesotemperate oceanic, with an average annual
83 temperature of 11.6-13.1°C and an average annual precipitation of 1200 to >2000 mm [28].

84

85 *Sampling of amphibians and macroinvertebrates*

86 We sampled each pond twice, in mid-March and in late May 2015, to increase the likelihood of finding
87 both winter and spring/summer breeding amphibians. Each sampling bout was less than a week to reduce
88 confounding effects of weather or time in the life cycle on habitat differences. All ponds were dipnetted by MI-
89 C for invertebrates and amphibians (larvae and adults) in a standardized way (effort: 1 minute m⁻²; net size: 1
90 mm mesh, 26 x 21 cm frame). We identified all amphibian species *in situ*, recorded the number of individuals

91 and determined their sex and life cycle stages (larvae, metamorphic, juvenile or adult). We then released them
92 back into the pond. We stored macroinvertebrates in 70% ethanol and transported them to the laboratory for
93 identification to the family level following Tachet et al., 2010. For our statistical analyses we used two
94 biological index based on the sensitivity of invertebrate families to water contamination: the IBMWP (Alba-
95 Tercedor et al., 2002) and the Iberian Average Score Per Taxon (IASPT) [31], calculated as the division of the
96 IBMWP and the number of different taxa.

97

98 *Environmental characteristics*

99 At each pond we measured 8 variables that seem to be important drivers for amphibian and
100 macroinvertebrate diversity in aquatic habitats [32–36]. We measured the water temperature, pH, and
101 conductivity with field WTW multi-parametric sensors. To measure light penetration in water, we used a LI-
102 COR Li-250A light meter placed at the center of the pond and then expressed the pond's turbidity as the light
103 extinction coefficient (K). We also estimated the pond's area and average depth (mean of five measures along
104 the length of the pond). For this, using the longest axis of the pond as reference (a), we defined a number of
105 equidistant and perpendicular transects (b_1 to b_n) (total transects depended on the irregularity of the pond: range
106 2-8). The area of the pond was estimated as a *average (b_1 to b_n). Depth was recorded every 15 cm along those
107 transects and we used the mean using all the depths computed. Finally, we estimated the percentage of the pond
108 covered by submerged and emergent aquatic vegetation.

109

110 *Statistical analyses*

111 We used linear models to test for differences in the measured variables among the three habitat types.
112 When necessary, data were log-transformed to meet model requirements. Multiple regressions by forward
113 selections were performed to predict amphibian richness and total amphibian density [37]. For that, we
114 performed linear models between the dependent variable and each independent variable alone and we retained
115 those independent variables that fit best (lowest AIC). We then added new independent variables that reduced
116 the most the previous AIC values. We stopped adding new variables when reductions of the AIC were smaller
117 than a value of 2 [37].

118

119

120

121 Results

122 *Biodiversity and abundance of amphibians*

123

124 We found 5494 individuals (larvae and adults) from 7 species in ponds under native forests, 885
125 individuals of 2 species under pine plantations and 168 individuals of 4 species under eucalypt plantations (Fig
126 1a). Only *Lissotriton helveticus* (Razoumowsky, 1789) was detected in all the sampled ponds (Table 1). By
127 contrast, *Alytes obstetricans* (Laurenti, 1768), *Rana dalmatina* Fitzinger, 1838 and *Triturus marmoratus*
128 (Latreille, 1800), appeared only in native forests, while *Rana temporaria* Linnaeus, 1758, *Pelophylax perezi*
129 (López Seoane, 1885) and *Salamandra salamandra* (Linnaeus, 1758) appeared in both native forests and
130 eucalypt plantations (Table 1). We found both adults and larvae of *L. helveticus*; only larvae of *A. obstetricans*,
131 *R. dalmatina*, *Rana temporaria*, *S. salamandra*; and only adults of *P. perezi* and *T. marmoratus* (Table 1).
132 The number of species differed among the three habitat types ($F_{2,15} = 9.95$, $p = 0.002$, Fig. 1a). There were
133 significantly more species in ponds in native forests (median: 4) than in either pine (1.5) or eucalypt plantations
134 (2) (Fig 1a), but no difference between the two plantation types (Tukey HSD test: $p = 0.70$). There were also
135 differences in total density between native forests and plantations ($F_{2,15} = 4.29$, $p = 0.034$, Fig 1b). Amphibian
136 density was highest in ponds in native forests (mean \pm SE: 72.53 ± 32.9 no. m^{-2}), intermediate in pine
137 plantations (17.60 ± 7.52 no. m^{-2}) and lowest in eucalypt plantations (8.15 ± 5.53 no. m^{-2}), but the only
138 significantly pairwise different was between native forests and eucalypt plantations (Tukey HSD: $p = 0.026$).
139 Looking at each species separately, only the densities of *A. obstetricans* and *R. temporaria* varied significantly
140 with habitat type. *A. obstetricans* only inhabited native forests (1.77 ± 0.91 no. m^{-2}) (Table 1). *R. temporaria*
141 occurred in all three habitats but at significantly different densities. The density was highest in oak forest (62.99
142 ± 33.26 no. m^{-2}), intermediate in pine plantations (10.37 ± 6.34 no. m^{-2}) and lowest in eucalypt plantations (0.11
143 ± 0.11 no. m^{-2}), although the only significant pairwise difference was between oak forests and eucalypt
144 plantations (Tukey HSD: $p = 0.014$).

145

146 *Environmental characteristics of ponds and relationship with amphibians*

147

148 Ponds in native forests were significantly deeper (23.55 ± 6.85 cm) than those in pine (7.79 ± 0.83 cm)
149 and eucalypt plantations (8.44 ± 2.22 cm) (Table 2). Similarly, pond surface area was bigger in native forests
150 (22 ± 5.90 m^2) than in pine (7.16 ± 0.20 m^2) and eucalypt (5.95 ± 1.59 m^2) plantations (Table 2). Although

151 submerged, emerged and terrestrial vegetation cover varied widely among habitats (range of average values:
152 0.8-23.3, 1.7-10.0 and 29.2-75.0, respectively), there were no significant differences (Table 2). Variation in
153 water physicochemistry (pH, temperature, conductivity) was smaller and it did not differ significantly among
154 habitat types (Table 2). For macroinvertebrates, only the IBMWP, the index of sensitivity of invertebrate
155 families to water contamination, was significant different about habitat types: highest in native forests ($56.67 \pm$
156 9.85), intermediate in pine plantations (31.83 ± 6.26) and lowest in eucalypt plantations (23.33 ± 6.77) (Tukey
157 HSD: $p = 0.022$) (Table 2).

158 Finally, the best model to explain the species richness of amphibians mainly included habitat type
159 (93.0% of the variance explained; $p < 0.001$), but also conductivity (4.5%; $p < 0.001$) and submerged vegetation
160 cover (0.7%; $p = 0.0499$), with less than 2% left unexplained. In contrast, amphibian abundance was explained
161 by habitat type (36.4% of the variance explained; $p = 0.002$), size of the pond (23.4%, $p = 0.003$), terrestrial
162 vegetation cover (11.6%, $p = 0.024$) and by the emerged vegetation cover (7.7%; $p = 0.058$; it was retained by
163 the AIC criterion, despite not being significant in the ANOVA) (Table 3), with 21% left unexplained.

164

165 **Discussion**

166 Species richness of amphibians was lower in ponds in both pine and eucalypt plantations than in native forests,
167 while amphibian density was also significantly lower in eucalypt, but not pine, plantations than in native forests.
168 Far more of the variance among ponds in species richness and density was explained by the habitat type (93 and
169 36%, respectively) than the specific properties of the ponds that we measured (e.g. pond size, depth and aspects
170 of water quality). This suggests that variables that we did not measure that are themselves affected by the
171 habitat type are responsible for the observed differences in amphibian diversity (e.g. toxin levels). We conclude
172 that the replacement of natural forests by plantations is harmful for amphibians, albeit less so when the
173 plantation is pine rather than eucalyptus. Our results also suggest that even the small number of forest patches
174 sampled, the effect of the type of habitat is strong enough to be detected. The heterogeneity of our samplings
175 was controlled, in part, by choosing small and temporary ponds in all the habitats. So that, small sample sizes
176 combined with a low heterogeneity among ponds, seem to be enough to determine habitat modification effects
177 on amphibian diversity.

178

179

180

181 *Plantation effects on water and resource quality*

182

183 We characterized the physicochemistry of the ponds to look at how plantations affect water quality, but we did
184 not detect any differences among the three habitat types. At first glance these findings are unexpected as
185 eucalypt leachates can cause a marked decrease in oxygen level and pH, and an increase in conductivity in only
186 a few days [22]. However, leaves from a range of tree species from alder, to eucalypt and pine can create
187 leachates with very similar characteristics for oxygen level, pH, and conductivity [38]. In contrast, the biotic
188 index for macroinvertebrates (IBMWP), was significantly higher in ponds in native forests than in eucalypt
189 plantations. This index is designed to assess water quality [39] which suggests that eucalypt plantations have
190 more toxic pond water. This conclusion is in line with the much higher concentration of phenolic compounds in
191 eucalypt leachates than in those from other species such as alder or pine [38]. Finally, water conductivity also
192 predicted amphibian richness which suggests that the accumulation of chemicals plays a role in amphibian
193 diversity.

194

195 Anuran larvae are detritivore feeders, which makes them very sensitive to the quality of plant remains [40].
196 Detritus is known to be of lower quality in ponds in plantations than in native deciduous forest [21,41]. This
197 could explain the reduced abundance of *R. temporaria* tadpoles in plantations compared to oak forests. If
198 tadpole survival is lower in plantations due to toxicity of their food resources, the number of individuals that
199 reach adulthood will be lower, reducing the population each generation. Moreover, poor environmental
200 conditions and a low quality diet early in development can reduce adult size, lower energy reserves, decrease
201 competitive ability and, ultimately, reduce fitness [42]. This could lower the ability of some adults to breed, and
202 exacerbate the effect of a smaller adult population size.

203

204 *Plantation effects on habitat quality*

205

206 Ponds in oak natural forests were significantly bigger and deeper than those in plantations, and the IBMWP is
207 known to be lower in ponds that suffer periodic desiccation [43]. The periodic drying out of smaller ponds could
208 further explain the observed differences in both the diversity and abundance of amphibians among the three
209 habitat types. Interestingly, *R. dalmatina* and *T. marmoratus* have a long larval period of about 3 months that
210 extends until summer [44]; and for *A. obstreticans* the tadpoles overwinter in the water, extending the larval

211 cycle to a year [44]. These three species were absent in plantations, possibly because they cannot complete
212 metamorphosis there before the ponds dry out. Both the accumulation of toxic compounds of leachates and the
213 desiccation of the ponds in summer is expected to increase with on-going climate change.

214

215 The rapid drying out of smaller ponds might also explain the apparent habitat differences in aquatic vegetation:
216 coverage is almost absent in ponds in pine plantations. The amount of aquatic vegetation in ponds might explain
217 some of the variation in the density and abundance of amphibians. Amphibian species richness and individual
218 density has been observed to increase with structural complexity of the habitat [45]. Terrestrial vegetation can
219 provide refuge and feeding opportunities for anuran adults. Submerged vegetation provides refuge, food [46]
220 and protection against UV-B radiation. In newts, aquatic vegetation is likely to be related to the oviposition
221 behavior of wrapping each egg individually in leaves to protect them from UV-B radiation and predators [47].

222

223 *Conclusions*

224

225 In sum, the land use seems to be a critical factor behind the differences we found in amphibian diversity and
226 density among ponds. However, some related variables, such as water quality, macroinvertebrate availability (an
227 index of food resources), and the early desiccation of the ponds in tree plantations also play an important role
228 explaining the differences found among habitats. These effects seemed to be more detrimental for some species
229 (*A. obstetricans*, *R. temporaria*, *R. dalmatina* and *T. marmoratus*) than for others (*L. helveticus* and *P. perezi*).
230 Discovering which are the key drivers modulating the populations of amphibians under different land use
231 regimes would allow to preserve the natural biodiversity of this threatened animal group.

232

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243

244 **References**

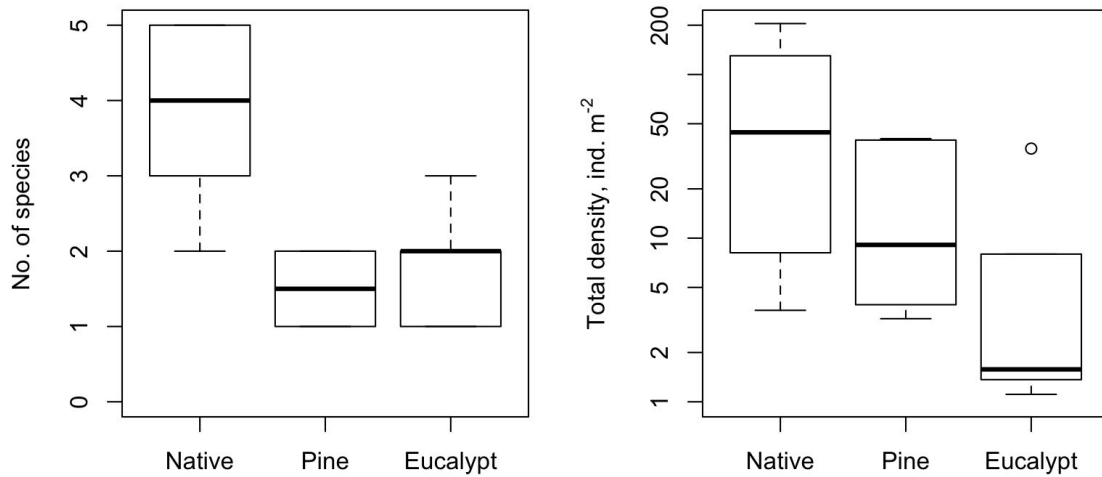
- 245 1. MacDicken K. Global Forest Resources Assessment 2015: What, why and how? For Ecol Manage.
246 2015;352: 3–8. doi: 10.1016/j.foreco.2015.02.006
- 247 2. Buongiorno J, Zhu S, Zhang D, Turner J, Tomberlin D. Overview of the Global Forest Products. In:
248 Elsevier Science, editor. The Global Forest Products Model (GFPM): Structure, Estimation,
249 Applications. USA; 2003. pp. 252–262.
- 250 3. Lindenmayer D, Hobbs R. Fauna conservation in Australian plantation forests—a review. Biol Conserv.
251 2004;119: 151–168. doi: 10.1016/j.biocon.2003.10.028
- 252 4. Fork S, Woolfolk A, Akhavan A, Van Dyke E, Murphy S, Candiloro B, et al. Biodiversity effects and
253 rates of spread of nonnative eucalypt woodlands in central California. Ecol Appl. 2015;25: 2306–2319.
254 doi:10.1890/14-1943.1
- 255 5. Zurita G, Rey N, Varela D, Villagra M. Conversion of the Atlantic Forest into native and exotic tree
256 plantations: Effects on bird communities from the local and regional perspectives. For Ecol Manage.
257 2006;235: 164–173. doi:10.1016/j.foreco.2006.08.009
- 258 6. Brockerhoff E, Jactel H, Parrotta J, Quine C. Plantation forests and biodiversity: oxymoron or
259 opportunity? Biodivers Conserv. 2008;17: 925–951. doi:10.1007/978-90-481-2807-5_1
- 260 7. Heer K, Helbig-Bonitz M. Effects of land use on bat diversity in a complex plantation–forest landscape
261 in northeastern Brazil. J Mammal. 2015;96: 720–731. doi:http: 10.1093/jmammal/gyv068
- 262 8. Mortelliti A, Lindenmayer D. Effects of landscape transformation on bird colonization and extinction
263 patterns in a large-scale, long-term natural experiment. Conserv Biol. 2015;29: 1314–1326. doi:
264 10.1111/cobi.12523
- 265 9. Galván I, Benayas J. Bird species in Mediterranean pine plantations exhibit different characteristics to
266 those in natural reforested woodlands. Oecologia. 2011;166: 305–316. doi:10.1007/s00442-010-1849-0
- 267 10. Rieff G, Natal-da-Luz T, Sousa J. Collembolans and mites communities as a tool for assessing soil
268 quality: effect of eucalyptus plantations on soil mesofauna biodiversity. Curr Sci . 2016;110: 713–719.

- 269 11. Oliveira J, Fernandes F, Ferreira M. Effects of forest management on physical habitats and fish
270 assemblages in Iberian eucalypt streams. For Ecol Manage. 2016;363: 1–10.
271 doi:10.1016/j.foreco.2015.12.011
- 272 12. Mortelliti A, Michael D, Lindenmayer D. Contrasting effects of pine plantations on two skinks: results
273 from a large-scale “natural experiment” in Australia. Anim Conserv. 2015;18: 433–441.
274 doi:10.1111/acv.12190
- 275 13. Tererai F, Gaertner M, Jacobs S. Eucalyptus invasions in riparian forests: effects on native vegetation
276 community diversity, stand structure and composition. For Ecol Manage. 2013;297: 84–93.
277 doi:10.1016/j.foreco.2013.02.016
- 278 14. Arntzen J. Drastic population size change in two populations of the golden-striped salamander over a
279 forty-year period—Are Eucalypt plantations to blame? Diversity. 2015;7: 270–294.
280 doi:10.3390/d7030270
- 281 15. Cruz J, Sarmento P, Carretero M, White P. Exotic fish in exotic plantations: A multi-scale approach to
282 understand amphibian occurrence in the Mediterranean region. PLoS One. 2015;10.
- 283 16. Souto X, Gonzales L, Reigosa M. Comparative analysis of allelopathic effects produced by four forestry
284 species during decomposition process in their soils in Galicia (NW Spain). J Chem Ecol. 1994;20:
285 3005–3015. doi:10.1007/BF02098405
- 286 17. Pozo J, Basaguren A, Elozegui A, Molinero J, Fabre E. Afforestation with *Eucalyptus globulus* and leaf
287 litter decomposition in streams of northern Spain. Hydrobiologia. 1998;101: 373–374.
288 doi:10.1023/A:1017038701380
- 289 18. Florence R. Cultural problems of Eucalyptus as exotics. Commonw For Rev. 1986;65: 141–163.
- 290 19. Ferreira V, Larrañaga A, Gulis V, Basaguren A. The effects of eucalypt plantations on plant litter
291 decomposition and macroinvertebrate communities in Iberian streams. For Ecol Manage. 2015;355:
292 129–138. doi: 10.1016/j.foreco.2014.09.013
- 293 20. Ferreira V, Elozegui A, Gulis V, Pozo J. Eucalyptus plantations affect fungal communities associated
294 with leaf-litter decomposition in Iberian streams. Arch für Hydrobiol. 2006;166: 467–490.
295 doi:10.1127/0003-9136/2006/0166-0467
- 296 21. Martínez A, Larrañaga A, Miguélez A. Land use change affects macroinvertebrate community size
297 spectrum in streams: the case of *Pinus radiata* plantations. Freshw Ecol. 2016;61: 69–79.
298 doi:10.1111/fwb.12680

- 299 22. Canhoto C, Laranjeira C. Leachates of *Eucalyptus globulus* in intermittent streams affect water
300 parameters and invertebrates. *Int Rev Hydrobiol.* 2007;92: 173–182. doi:10.1002/iroh.200510956
- 301 23. Houlihan J, Findlay C, Schmidt B, Meyer A. Quantitative evidence for global amphibian population
302 declines. *Nature.* 2000;404: 752–755. doi:10.1038/35008052
- 303 24. Cushman S. Effects of habitat loss and fragmentation on amphibians: a review and prospectus. *Biol*
304 *Conserv.* 2006;128: 231–240. doi:10.1016/j.biocon.2005.09.031
- 305 25. Rowe CL, Hopkins WA, Bridges C. Physiological ecology of amphibians in relation to susceptibility to
306 natural and anthropogenic factors. In: Linder G, Krest S, Sparling D, editors. *Amphibian Decline: An*
307 *Integrated Analysis of Multiple Stressor Effects.* Pensacola: SETAC press; 2003. pp. 9–57.
- 308 26. Watling J, Hickman C, Orrock J. Invasive shrub alters native forest amphibian communities. *Biol*
309 *Conserv.* 2011;144: 2597–2601. doi: 10.1016/j.biocon.2011.07.005
- 310 27. Semlitsch R, Bodie J. Biological criteria for buffer zones around wetlands and riparian habitats for
311 amphibians and reptiles. *Conserv Biol.* 2003;17: 1219–1228. doi:10.1046/j.1523-1739.2003.02177.x
- 312 28. Euskalmet. Climatología del País Vasco. 2011. [http://www.euskalmet.euskadi.eus/s07-](http://www.euskalmet.euskadi.eus/s07-5853x/es/contenidos/informacion/car_latitud/es_7257/es_latitud.html)
313 [5853x/es/contenidos/informacion/car_latitud/es_7257/es_latitud.html](http://www.euskalmet.euskadi.eus/s07-5853x/es/contenidos/informacion/car_latitud/es_7257/es_latitud.html)
- 314 29. Tachet H, Richoux P, Bournaud M, Usseglio-Polatera P. *Invertébrés d'eau douce : Systématique,*
315 *biologie, écologie.* CNRS, editor. Paris; 2010.
- 316 30. Alba-Tercedor J, Jáimez-Cuéllar P, Álvarez M, Avilés J, Bonada i Caparrós N, Casas J, et al.
317 *Caracterización del estado ecológico de ríos mediterráneos ibéricos mediante el índice IBMWP (antes*
318 *BMWP').* *Limnetica.* 2002;21: 175–185. doi:<http://hdl.handle.net/2445/32903>
- 319 31. Alba-Tercedor J, Sánchez-Ortega A. Un método rápido y simple para evaluar la calidad biológica de las
320 aguas corrientes basado en el de Hellawell (1978). *Limnetica.* 1988;4: 41–56.
- 321 32. Freda J. The influence of acidic pond water on amphibians: a review. *Water Air Soil Pollut.* 1986;30:
322 439–450. doi:10.1007/BF00305213
- 323 33. Oertli B, Joye D, Castella E, Juge R, Cambin D. Does size matter? The relationship between pond area
324 and biodiversity. *Biol Conserv.* 2002;104: 59–70. doi: 10.1016/S0006-3207(01)00154-9
- 325 34. Orizaola G, Braña F. Oviposition behaviour and vulnerability of eggs to predation in four newt species
326 (genus *Triturus*). *Herpetol J.* 2003;13: 121–124.
- 327 35. Brodman R, Ogger J, Bogard T, Long A. Multivariate analyses of the influences of water chemistry and
328 habitat parameters on the abundances of pond-breeding amphibians. *J Freshw Ecol.* 2003;18: 425–436.

- 329 doi:10.1080/02705060.2003.9663978
- 330 36. Raffel T, Rohr J, Kiesecker J. Negative effects of changing temperature on amphibian immunity under
331 field conditions. *Funct Ecol.* 2006;20: 819–828. doi:10.1111/j.1365-2435.2006.01159.x
- 332 37. Lengendre P, Lengendre L. *Numerical Ecology*. 2nd ed. Elsevier Science, editor. Amsterdam; 1998.
- 333 38. Friberg N, Winterbourn M. Interactions between riparian leaves and algal/microbial activity in streams.
334 *Hydrobiologia.* 1996;341: 51–56. doi:10.1007/BF00012302
- 335 39. Jáimez-Cuéllar P, Vivas S, Bonada N, Robles S. Protocolo GUADALMED (prece). *Limnetica.* 2002;21:
336 187–204.
- 337 40. Maerz J, Cohen J, Blossey B. Does detritus quality predict the effect of native and non-native plants on
338 the performance of larval amphibians? *Freshw Biol.* 2010;55: 1694–1704. doi:10.1111/j.1365-
339 2427.2010.02404.x
- 340 41. Molinero J, Pozo J. Impact of a eucalyptus (*Eucalyptus globulus* Labill.) plantation on the nutrient
341 content and dynamics of coarse particulate organic matter (CPOM) in a small stream. *Hydrobiologia.*
342 2004;528: 143–165. doi:10.1007/s10750-004-2338-4
- 343 42. Taborsky B. The influence of juvenile and adult environments on life-history trajectories. *Proc R Soc B.*
344 2006;273: 741–750. doi:10.1098/rspb.2005.3347
- 345 43. Attrill M, Rundle S, Thomas R. The influence of drought-induced low freshwater flow on an upper-
346 estuarine macroinvertebrate community. *Water Res.* 1996;30: 261–268. doi:10.1016/0043-
347 1354(95)00186-7
- 348 44. García-París M, Montori-Faura A, Herrero-Solans P. *Amphibia: Lissamphibia*. Consejo Superior de
349 Investigaciones Científicas, editor. Fauna Ibérica. 2004. p. 640.
- 350 45. Vallan D. Effects of anthropogenic environmental changes on amphibian diversity in the rain forests of
351 eastern Madagascar. *J Trop Ecol.* 2002;18: 725–742. doi:10.1017/S026646740200247X
- 352 46. Waringer-Löschenkohl A. An experimental study of microhabitat selection and microhabitat shifts in
353 European tadpoles. *Amphibia-Reptilia.* 1988;9: 219–236. doi:10.1163/156853888X00314
- 354 47. Alarcos G, Ortiz M, Lizana M, Aragón A. La colonización de medios acuáticos por anfibios como
355 herramienta para su conservación: el ejemplo de Arribes del Duero. *Munibe.* 2003;16: 114–127.
356
357
358

359 Figure 1: (a) Number of species and (b) total density of individuals per pond. ANOVA results for Richness: $F_{2,15}$
360 = 9.95, $p = 0.002$; Tukey HSD comparison: Native > Eucalypt = Pine, and for Total density: $F_{2,15} = 4.29$, $p =$
361 0.034; Tukey HSD: Native > Eucalyptus (the other pairwise tests are non-significant). Note the logarithmic y-
362 axis for total density.
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366 Table 1: Density (mean \pm SE, no. m⁻²) and frequency (out of 6 ponds) for each amphibian species separated in adults and larvae. Comparisons among the three habitat types
 367 after Tukey HSD tests are shown. In bold are species that differ in density among habitats. (N = 6 ponds per habitat type).

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Species	Stage	Native forest			Pine plantations			Eucalypt plantations			Tukey HSD
		Density		Frequency	Density		Frequency	Density		Frequency	
		Mean	SE		Mean	SE		Mean	SE		
<i>L. helveticus</i>	Adults	2.16	0.45	6	3.95	0.76	6	4.66	2.52	6	Pin = Euc = Nat
	Larvae	4.96	2.57	4	3.27	2.02	3	2.69	2.69	1	Nat = Pin = Euc
<i>T. marmoratus</i>	Adults	0.04	0.04	1							Nat = Pin = Euc
<i>S. salamandra</i>	Larvae	0.14	0.12	3				0.44	0.43	2	Euc = Nat = Pin
<i>A. obstetricans</i>	Larvae	1.77	0.91	4							Nat > Pin = Euc
<i>R. temporaria</i>	Larvae	62.99	33.2	5	10.37	6.34	3	0.11	0.11	1	Nat > Euc
<i>P. perezi</i>	Adults	0.44	0.24	3				0.24	0.16	2	Nat = Euc = Pin
<i>R. dalmatina</i>	Larvae	0.02	0.02	1							Nat = Euc = Pin

369 Table 2: Mean and SE of descriptors of the ponds sampled. Comparisons after Tukey HSD tests are shown for each variable. In bold are the variables with significant
 370 differences among habitat types.

Descriptor	Native forest		Pine plantations		Eucalypt plantations		Tukey HSD
	Mean	SE	Mean	SE	Mean	SE	
Mean water depth (cm)	23.55	6.85	7.79	0.83	8.44	2.22	Nat > Euc = Pin
Area (m²)	22.69	5.90	7.16	0.20	5.95	1.59	Nat > Pin = Euc
Submerged vegetation (%)	23.33	11.38	0.83	0.83	20.00	10.88	Nat = Euc = Pin
Emerged vegetation (%)	1.67	1.05	1.67	1.05	10.00	4.28	Euc = Nat = Pin
Terrestrial vegetation (%)	73.33	14.70	29.17	12.81	75.00	11.55	Euc = Nat = Pin
pH	7.34	0.15	6.74	0.45	7.35	0.26	Euc = Nat = Pin
Temperature (°C)	11.67	2.61	8.18	1.28	10.18	0.55	Nat = Euc = Pin
Conductivity (µS cm ⁻¹)	136.52	41.15	91.53	21.25	147.17	42.43	Euc = Nat = Pin
Turbidity (K)	6.40	1.01	6.08	1.23	8.69	1.33	Euc = Nat = Pin
Invertebrate taxa (no. families)	12.00	1.86	7.33	1.12	4.83	0.87	Nat = Pin = Euc
Invertebrate predator taxa (%)	48.88	3.75	59.72	10.19	52.50	12.44	Pin = Euc = Nat
IBMWP	56.67	9.85	31.83	6.26	23.33	6.77	Nat > Euc
IASPT	4.51	0.36	4.18	0.29	4.56	0.59	Nat = Euc = Pin

372 Table 3: Model supported by AIC for Mean density and Total richness of amphibians in the ponds.

Response variable	Variable	Transformation	D	SS	MS	F	P	Variance	AIC	Comparison/Slope
Total amphibian richness	Land use		2	30.5	1.53	32.13	<0.001	93.0	22.05	Nat > Euc = Pin
	Conductivity ($\mu\text{S cm}^{-1}$)	LN	1	1.46	1.46	30.76	<0.001	4.5	5.91	-
	Submerged vegetation (%)	LN	1	0.22	0.22	4.67	0.0499	0.7	2.38	+
	Residuals		1	0.62	0.05			1.9		
Mean amphibian density	Land use		2	16.28	8.14	10.41	0.002	36.4	67.33	Nat > Euc
	Size (m^2)	LN	1	10.45	10.45	13.36	0.003	23.4	61.09	-
	Terrestrial vegetation (%)	LN	1	5.19	5.19	6.63	0.024	11.6	56.98	+
	Emerged vegetation (%)*	Non-transf.	1	3.44	3.44	4.39	0.058	7.7	53.36	-
	Residuals		1	9.39	0.78			21.0		

373 *Although non-significant in the ANOVA, AIC supported the inclusion of this variable in the model.

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