Article

Environmental Factors Affecting Amphibian Communities in River Basins of the Southern Apennines

Alessandro Nessi [1], Sara Cioccarelli [2],*, Paolo Tremolada [1], Pasquale Gariano [3,4], Maria Grandinetti [4], Alessandro Balestrieri [1] and Raoul Manenti [1]

[1] Department of Environmental Science and Policy, University of Milan, I-20133 Milan, Italy; raoul.manenti@unimi.it (R.M.)
[2] Department of Biology, University of Pisa, I-56126 Pisa, Italy
[3] TEA Engineering S.r.l., I-56121 Pisa, Italy
[4] CESRAM—Centro Studi e Ricerca Ambiente Marino, I-88065 Guardavalle, Italy
* Correspondence: sara.cioccarelli@phd.unipi.it

Abstract: The study of the environmental features affecting amphibian communities is a priority task for addressing effective conservation initiatives. In the southern edge of the Apennines (Sila Massif, Calabria Region, Italy), we surveyed the distribution of amphibians in lotic freshwater habitats (eight rivers, 17 sampling stations, 87 transects) and recorded nine environmental variables, including the occurrence of potential predators (fish and Eurasian otter *Lutra lutra*), potentially affecting the distribution and breeding success of amphibian species. A total of seven amphibian species was recorded (75.9% of transects). Fish occurred in all rivers while the otter was found in four rivers (Amato, Lese, Neto, and Savuto). Illuminance and bank heterogeneity were the main factors affecting amphibian reproduction, while neither otter nor fish presence showed significant effects on the amphibian community. Overall, habitat complexity and coevolution history seemed to shape the distribution of amphibians and their breeding sites, while the ongoing recolonization of the study area by the otter is expected to have a negligible impact on the richness of the amphibian community.

Keywords: anurans; freshwater habitat; stream features; breeding sites; Eurasian otter

1. Introduction

Riverine ecosystems make up only 0.006% of the world's freshwaters [1], but host a rich array of biodiversity, which is partially still unknown [2]. In the last centuries, human activities have so deeply altered river systems as to make it hard even to realize the extent to which regulated rivers differ from pristine conditions [3]. Running waters are intensively used for water supply, irrigation, hydroelectric energy and waste-water discharge; consequently, freshwater communities are among the most endangered on Earth [4]. Among the areas hosting a huge number of endemic freshwater species, the Mediterranean basin is one of the most vulnerable [5–7] especially for amphibians, reptiles and invertebrates [8,9]. At a global scale, amphibians are the most threatened vertebrates; it has been estimated that currently about 40% of species are at risk of extinction [10,11], the most dramatic example of ongoing decline in vertebrates [12]. The main causes of amphibian decline are habitat degradation and fragmentation, pollution, introductions of invasive species, climate change, UV radiation, and emerging diseases (i.e.,: *Batrachochytrium dendrobatidis*) [13–17]. In the Mediterranean basin, and in particular, in the Italian Apennines, habitat loss and climate change have been imputed as the major causes of the recent population decline [18,19].

Environmental conditions, such as stream features and landscape composition, affect the distribution of amphibians [20–22] and abiotic parameters—e.g.,: sun exposure, water depth and slope—can affect breeding success [23]. Reproductive success, and the diversity of amphibian communities, are also affected by predator fish, such as salmonids [23,24]. In streams where the risk of desiccation is low, fish predators constitute the main cause of

amphibian mortality and predation is considered the major selective force constraining the distribution of several species to temporary ponds [25–27]. While the role played by both native and alien fish has been widely studied (e.g.,: [27–29]), the effect of native mammal predators in structuring amphibian communities has not yet been fully investigated. Mustelids, especially polecat (*Mustela putorius*), Eurasian otter (*Lutra lutra*) and European badger (*Meles meles*) often prey on adult amphibians, mainly frogs and toads [30–32]. Despite being mainly piscivorous [33,34], in Mediterranean habitats, the Eurasian otter often relies on amphibians as a major alternative-to-fish resource [35–38]. In Italy, as well as throughout most of Europe, this semi-aquatic mustelid suffered a drastic decline in the last two decades of the 20th century [39], but is currently spreading, especially in the southern and central sectors of the peninsula [40], where amphibian availability has been suggested to enhance otter recovery [38]. In turn, the extent to which expanding otter populations may affect amphibian conservation, particularly small populations of endemic species, is still unknown, as it is the impact of fish on their breeding success in second-order streams and rivers of the Mediterranean biogeographical region.

Investigating the combined effects of abiotic conditions and predation pressure on habitat and breeding site selection by amphibian species is pivotal for their conservation because it may highlight the environmental determinants of amphibian occurrence and provide useful indications for conservation-aimed management.

With this main aim, we sampled both amphibians and predators in large streams and rivers of the Sila Massif (South Italy), where otter expansion has been periodically monitored since its rediscovery in 2003 [41]. Specifically, we hypothesized: (i) amphibian distribution to be affected by environmental features depending on each species' habitat preferences, (ii) fish predation to constrain amphibian presence to small lateral streams and isolated pools, and (iii) otter recolonization to limit amphibian abundance.

2. Materials and Methods

2.1. Field Sampling

Surveys were carried out in 17 sampling sites, on eight rivers (Table S1) distributed at different altitudes above sea level (85–1355 m) flowing from the Sila Massif (South Italy, Table S2): the rivers Corace, Alli, Simeri, and Tacina, flowing south into the Squillace Gulf of the Ionian Sea; Savuto and Amato, which flow into the Tyrrhenian Sea; and, on the east-facing slope of the massif, the River Neto with its main tributary, the River Lese (Figure 1). In all sampling sites, surveys for amphibians, fish and otter were performed in parallel in April 2017.
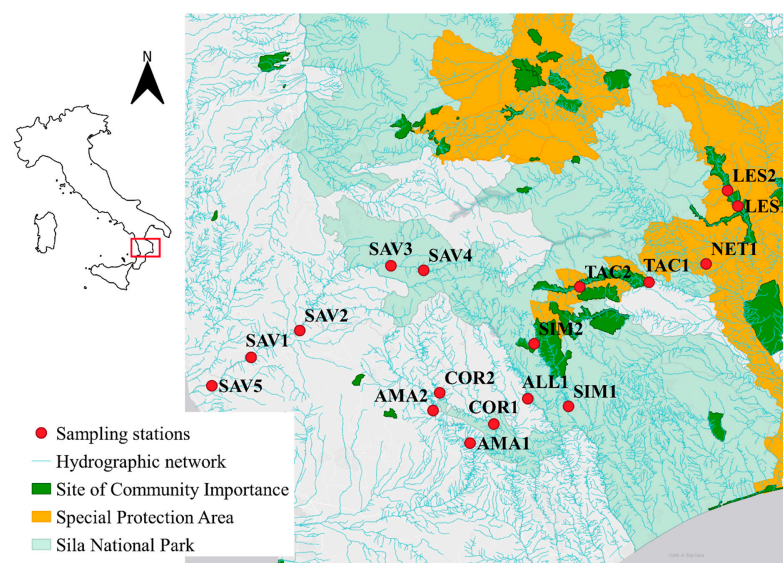
Figure 1. Sampling stations in the study area (Calabria region, Southern Italy).

2.2. Amphibian Monitoring

The monitoring of amphibian populations was performed by calling and visual encounter surveys [42], recording the occurrence of both egg-clutches or tadpoles (breeding) and adults. In the 17 sampling sites, we surveyed a total of 87 transects (Savuto: 24, Amato: 10, Corace: 13, Alli: 3, Simeri: 16, Tacina: 10, Neto: 3, Lese: 8), of which 35 on main river courses, 19 on lateral branches and 33 in isolated pools and ponds (see Tables S3–S17 for details). Transects were split according to fish zonation (cyprinids vs. salmonids), roughly coinciding with the altitudinal ranges 0–400 m (N = 32) and 400–1400 m (N = 55), respectively [38].

Each transect was inspected twice, at one-month intervals, by the same surveyors. Each sampling station consisted of a linear transect (mean length $\pm$ SE = 135.5 $\pm$ 6.1 m), centred on the river stretch where electrofishing was carried out.

2.3. Environmental Features of the Breeding Sites

In each transect, we recorded eight variables describing stream morphology, quality, and ecosystem functioning, which were expected to affect reproduction in amphibians [23,43]. Wet riverbed heterogeneity was visually assessed based on the percent cover of sediments (including organic matter [44]) and classified as follows: 1, absence of diversification, one single lithal element covering almost entirely the site; 2, poorly diversified, two substrates with transect coverage >90%; 3, quite diversified, at least three lithal elements, each of which covering 20–40% of the transect; 4, highly diversified, four or more elements. Bank heterogeneity was assessed using the same procedure. Daytime illuminance (*lux*) was measured using a PCE EM882 luxmeter (accuracy = $\pm$0.01 lux) by performing at least 10 measures of illuminance in the points of the transect receiving more light. Stream dimensions (width and length) and maximum depth were measured by a meter tape and graduated rod, respectively. Percent tree (>3 m) and shrub cover was assessed by eye in a 1 m wide buffer along the whole transect. Transects were also classified according to the watercourse type as follows: 1, main watercourse; 2, pools and ponds; and 3, small lateral branches.

2.4. Otter and Fish Monitoring

Both otters and fish were sampled at the same time as amphibians (spring 2017). Otter occurrence was assessed using the 'Standard Method' suggested by the IUCN/SSC Otter Specialist Group [45], by recording along each transect indisputable signs of presence (spraints and jellies). Briefly, each sampling site was surveyed by walking along both sides of the river and skirting small islands to search for characteristic otter sprainting sites (e.g.,: large rocks, bridges, pool banks, confluences [30,46].

Fish presence was assessed by electrofishing, a technique which provides a reliable estimation of the relative abundance of fish and is more cost-effective than removal sampling [47,48]. On main watercourses, the length of each surveyed stretch was at minimum 10 times the river width (mean $\pm$ SE = 78.2 $\pm$ 3.3 m [49]). Each stretch was sampled twice, as the number of fish caught in the second passage was always less than 25% of the first one [50].

2.5. Statistical Analysis

A site is certainly "occupied" if a species is detected at least once, but non-detections do not necessarily imply that the species is absent [51]. For each amphibian species, we calculate the per sampling detection probability by means of Presence 5.5. The per sampling detection probability was based on detection histories (presence/absence) assuming a constant detection probability across sampling occasions (visits). We then assessed the reliability of the survey as the probability of detecting a species through two visits, following Gomez-Rodriguez et al. [52].

As a first step, the correlation between environmental features was tested using the 'corrplot.mixed' function of R package 'corrplot' [53].

To assess the effect of stream features on the richness of amphibian communities, we applied constrained redundancy analysis (RDA) to fish and otter occurrence and non-correlated (Pearson coefficient < |0.36|) environmental variables. Stream dimensions and maximum depth were correlated as well as wet riverbed and bank heterogeneity, thence the environmental variables used were: bank heterogeneity, illuminance, maximum depth, forest and shrub cover, and watercourses type. The variable "fish occurrence" distinguished sites in which during our samplings we observed at least an individual of one species of fish from those in which no fish were recorded. RDA is a canonical analysis, merging the features of regression and ordination techniques, that allows estimating how much of the variation of the structure of one dataset (i.e., community composition; endogenous dataset) is explained by independent variables (i.e., habitat features; exogenous dataset) [54].

Two RDA analyses were performed, one for adults and the other for "reproduction" (presence of egg clutches or larvae), including only the four species—*Bufo bufo*, *Pelophylax* kl. *esculentus*, *Rana dalmatina*, and *Rana italica*—for which the likelihood of non-detection was <0.05% (see results).

Variance partitioning was used to calculate the independent and joint effects of stream features, while the significance of explained variance was calculated by 10,000 ANOVA-like permutation tests [54]. RDA analyses were performed using 'rda' and 'permutation' functions of R package 'vegan'. To highlight the biotic and abiotic factors affecting amphibian reproduction, for each species we ran a generalized linear mixed effects model (GLMM), that allows accounting for the non-independence of observations by including random factors [55]. Binomial GLMMs were performed using the R package 'glmmTMB' [56], including all possible combinations of environmental variables. The breeding occurrence/absence of each species was the dependent variable, while the river identity was included as a random factor, as multiple sites occurred within the same river. For each model, we calculated the AIC corrected for small sample size (AICc) and Δ-AICc, which is the difference in AICc between a candidate and the model with the lowest AICc (i.e., the best model); for this analysis we used the dredge function of R package 'MuMIn' [57]. Finally, to assess the significance of the variables included in the best model, a likelihood ratio test was carried out, using the drop1 function of R package 'lme4' [58]. All statistical analyses were performed in R 4.0.3 [59].

3. Results

Amphibians occurred in 66 transects (75.9%), spread in all surveyed rivers and sampling areas (Tables S4–S18) except for one station on the River Alli (Table S10). Five species of anurans, Italian stream frog (*Rana italica*), agile frog (*Rana dalmatina*), edible frog (*Pelophylax* kl. *esculentus*), Italian tree frog (*Hyla intermedia*) and common toad (*Bufo bufo*), and two species of urodeles, fire salamander (*Salamandra salamandra*) and spectacled salamander (*Salamandrina terdigitata*) were recorded. Anurans were spread in all habitats (main watercourse, lateral branches and pools), except for Italian tree frogs, which were recorded only in pools (Table 1); urodeles were more selective, with fire salamander larvae occurring only in pools and spectacled salamander adults on the main watercourse. In two transects we found a maximum of three species simultaneously, *R. italica*, *B. bufo* and, respectively, *S. terdigitata* or *R. dalmatina* (see Tables S4–S18 for details). Breeding was recorded for all species (Table 1; see also Tables S4–S18 for details).

Detectability, in general, was high (>0.9) and for the species which showed occurrence >7% the likelihood of failing to detect any species was <0.05%.

Otter presence (Table S19) was detected on four rivers (Amato, Lese, Neto, and Savuto, 50%), for a total of 9 sampling sites (56%; SAV1-4, LES1-2; AMA1-2 and NET1, Figure 1) and 40 amphibian transects (46%).

Table 1. Presence of the species and the evidence of reproduction (eggs or tadpoles) in percentage in respect to all transects and the relative occurrence of each species in the main course of the river, in small lateral branches and in isolated pools and ponds.

Species	Positive Transects (%)	Breeding (%)	Distribution per Habitat (%)		
			Main Courses	Lateral Branches	Pools and Ponds
Rana italica	41.4	26.4	38.8	30.6	30.6
Rana dalmatina	8.0	8.0	14.2	42.9	42.9
P. kl. *esculentus*	12.6	1.2	9.1	27.3	63.6
Hyla intermedia	3.5	2.3	0.0	0.0	100.0
Bufo bufo	32.2	29.9	39.3	21.4	39.3
Salamandra salamandra	1.2	1.2	0.0	0.0	100.0
Salamandrina terdigitata	2.3	2.3	100.0	0.0	0.0

Electrofishing revealed fish occurrence in all sampled rivers and all sampling areas (Table S19), but only in 55 of the transects surveyed for amphibians (63.2%). Seven species were detected: *Salmo* sp., *Squalius squalus* (chub), *Rutilus rubilio* (roach), *Cobitis taenia*, *Anguilla anguilla*, and the allochthonous *Carassius carassius* and *Gambusia holbrooki*. Particularly, salmonids (trout) were detected in 40 transects and cyprinids in 14. Salmonids showed an anomalous colour pattern, suggesting hybridisation between the native Mediterranean trout of the southern Apennines *Salmo ghigi* and the allochthonous Atlantic trout *Salmo trutta*. Salmonids were found on the rivers Alli, Amato, Corace, Savuto, Simeri, and Tacina; while cyprinids, mainly chub and roach, predominated in all rivers below 400 m a.s.l.

The first RDA analysis, addressing the effects of the considered environmental features on the occurrence of adult amphibians of the different species explained 13.7% of the total variance (F = 1.55; *p* = 0.06). The second RDA analysis, investigating the relationships between the environmental features considered and the occurrence of breeding of the different species of amphibians explained a bit more of the total variance (15%), but was significant (F = 1.76; *p* = 0.02). It showed (Figure 2) that *R. italica* occurrence was related to sites with high level of illuminance and bank heterogeneity, while *B. bufo* and *R. dalmatina* preferred high illuminance but poorly diversified banks. No relationship emerged for *P.* kl. *esculentus*. Both otter and fish presence had a negligible effect on the amphibian community.
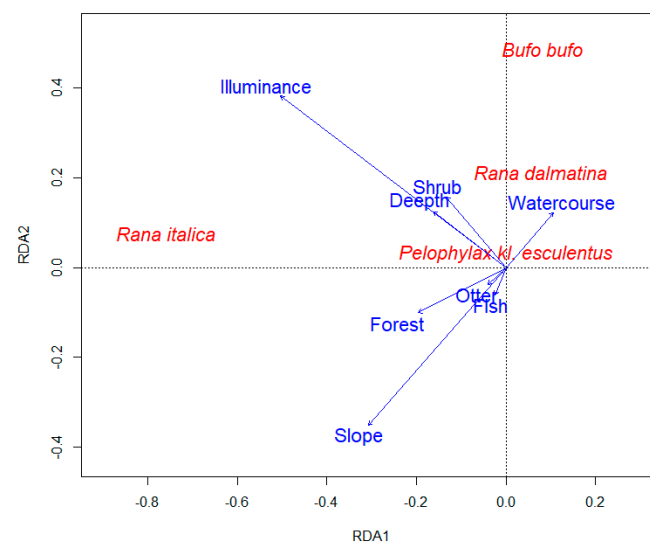
Figure 2. Results of constrained redundancy analysis (RDA) showing the relation between habitat features and the distribution of breeding amphibians. Constraining variables are represented by blue arrows. Otter = otter presence.

GLMMs showed that *R. italica* breeding individuals selected sites with high illuminance and bank heterogeneity, while shrub cover had a slightly negative effect on the choice for breeding sites (Table 2). *R. dalmatina* tadpoles and eggs were found in deep, lateral branches and lentic water bodies (Table 3). Breeding by *B. bufo* was not significantly affected by any of the recorded variables (Table 4). It was not possible to test *P.* kl. *esculentus*, because only one transect was positive for reproduction. In the best models, neither otter nor fish presence were included as significant parameters. The random effect of river identity showed variance <0.01 in all models.

Table 2. Results of binomial GLMMs performed using *Rana italica* breeding as the dependent variable and log-transformed abiotic stream features as independent variables. In bold significant relationships.

Rana italica	Estimate $\pm$ (S.E.)	LRT	Pr (>Chi)
Intercept	$-19.14 \pm (5.66)$		
Illuminance	$4.38 \pm (1.38)$	18.78	**<0.001**
Shrub cover	$-4.75 \pm (2.62)$	3.12	0.077
Slope heterogeneity	$4.05 \pm (2.17)$	3.96	**0.047**

Table 3. Results of binomial GLMMs performed using *Rana dalmatina* breeding as the dependent variable and log-transformed abiotic stream features as independent variables. In bold significant relationships.

Rana dalmatina	Estimate $\pm$ (S.E.)	LRT	Pr (>Chi)
Intercept	$-4.60 \pm (2.06)$		
Water depth	$2.33 \pm (1.17)$	5.40	**0.020**
Forest cover	$4.23 \pm (3.14)$	3.72	0.054
Slope heterogeneity	$-4.04 \pm (2.49)$	2.66	0.103
Watercourse type	$1.19 \pm (0.62)$	4.25	**0.039**

Table 4. Results of the binomial GLMMs performed using *Bufo bufo* breeding as the dependent variable and log-transformed slope heterogeneity as independent variables. In bold significant relationships.

Bufo bufo	Estimate $\pm$ (S.E.)	LRT	Pr (>Chi)
Intercept	$-0.14 \pm (0.60)$		
Slope heterogeneity	$-2.19 \pm (1.40)$	2.50	0.114

4. Discussion

In our study area, the distribution of breeding sites of most amphibians was affected by river features; among the variables considered, illuminance played the main role for most species. The explanation of this pattern can be twofold. First, mean water temperature is expected to be lower in watercourses than in lentic water bodies occurring at the same latitude [60]; therefore, species breeding in rivers may select those parts of the river which are likely to be warmer and enhance tadpole thermoregulatory behavior [61]. Moreover, in Mediterranean areas tadpoles must metamorphose before their habitat dries [62] and many amphibians lay eggs in exposed-to-sunlight and shallow areas to lower hatching time and fasten developmental rates [63]. Particularly, *R. italica* tadpoles are very dark, probably to enhance thermoregulation [64]. Although adults are mainly nocturnal [65,66], both juveniles and adults of several anurans have been observed basking in sunlight, indicating that even nocturnal species rely on exposure to UV-B radiation to synthesize vitamin D3 [67,68]. In most vertebrates, vitamin D3 plays a critical role in regulating calcium metabolism, muscle contraction, organ development and functioning of both the immune and nervous systems [69]. In tadpoles, basking enhances growth and improves anti-predator behaviour [70,71].

Slope and substrate heterogeneity are usually important for breeding as they provide suitable sites for attaching egg-clutches and they can also act as shelters for both breeding individuals and larvae [72]. Nonetheless, *B. bufo* and *R. dalmatina*, differently from *R. italica*, preferred rocky and poorly diversified slopes, which, in turn, may allow easy access to water and female detection by males in the breeding period.

Unexpectedly, in the study area neither fish nor otter occurrence affected breeding. As most anurans provide no parental care and larvae are relatively confined to deposition sites until metamorphosis, the choice of threat-free sites should be of pivotal importance for breeding pairs [73,74]. Fish can have strong top-down impacts on amphibian communities, limiting egg deposition to waters where fish are absent [75,76]. Nevertheless, at the site level structurally complex habitats may lower predation pressure, making tadpoles less vulnerable to fish attacks in vegetated pools [77]. The choice for heterogeneous and illuminated sites may partially depend on the preference for macrophyte-rich pools offering a diversity of microhabitats less exposed to predation risk. Otherwise, amphibians may mediate between abiotic and direct risks; in the Mediterranean region small pools, which usually do not host large fish populations, suffer a high risk of desiccation [78] and amphibian species may prefer to face an unknown degree of predation pressure rather than losing all egg-clutches in dried pools.

Few data about the effect of native mustelids on the use of breeding sites by amphibians are available, while introduced mammal predators may affect amphibian breeding and survival [79,80]. In France, selective predation by polecats on agile frogs was reported to affect their sex ratio and promote monogamy [81]. In island ecosystems of the Baltic Sea, predation by American mink (*Mustela vison*) has been shown to have drastic detrimental effects on anuran population density [82].

While the impact of novel predators on native prey is explained by the lack of long-term coexistence and effective antipredatory responses [83], native predators usually do not have large detrimental effects on their native prey's populations [84].

Amphibian adults are an important secondary food resource for otters throughout southern Europe [34,85–87], including the rivers basin where we performed our monitoring [38]. Otters mainly prey upon amphibians in late winter and spring [37], when adults gather near rivers, small streams and ponds for breeding. Predation is male-biased because males stay near the breeding sites for mating longer than females and calling behaviour exposes them to higher predation risk [37]. This bias lowers the impact of predation on amphibian reproduction success because the males that survive are enough to guarantee female reproduction. Although otters have been reported to rely on a wide diversity of amphibian species [38], in the study area they mostly preyed on the most widespread species, namely Italian agile frogs and bufonids, confirming their generalist feeding behavior. While osteological analyses showed that also the other monitored anurans were preyed on by otters, the two urodeles (fire- and spectacled salamander) were not recorded in otter diet.

5. Conclusions

Our study provided some insight into the ecological factors driving the breeding of a diverse amphibian community of southern Italy. In the monitored sites, amphibian community was mainly affected by abiotic river features, while predation by either expanding otters or fish did not seem to have a detrimental effect on the amphibian community.

The otter occurred on about half of the surveyed rivers allowing a balanced comparison between sites with and without its presence. Considering that the otter is currently expanding in Southern Italy, as in many other European regions, this result seems to indicate that the ongoing recovery of this native predator should not threaten the amphibian populations of conservation interest. Predation by otters on amphibians is usually inversely related to fish availability [38]. Hence, the reinforcement and conservation of fish communities, may further reduce the impact of amphibian predation by otters. Moreover, preying on the most abundant species and specifically on males, otters can be expected to have a negligible impact on the structure of amphibian communities.

As regards fish, in the model we did not distinguish species and age classes, although both can indisputably affect diet composition and feeding habits and then the impact of fish occurrence on amphibians. Hence, predator-prey interactions need further, more detailed studies.

References

1. Shiklomanov, L.A. World freshwater resources. In *Water in Crisis: A Guide to World's Freshwater Resources*; Gleick, P.H., Ed.; Oxford University Press: New York, NY, USA, 1993; pp. 13–24.
2. Dudgeon, D.; Arthington, A.H.; Gessner, M.O.; Kawabata, Z.; Knowler, D.J.; Lévêque, C.; Naiman, R.J.; Prieur-Richard, A.-H.; Soto, D.; Stiassny, M.L.J.; et al. Freshwater biodiversity: Importance, threats, status and conservation challenges. *Biol. Rev. Camb. Philos. Soc.* **2006**, *81*, 163–182. [CrossRef] [PubMed]
3. Ward, J.V.; Tockner, K. Biodiversity: Towards a unifying theme for river ecology. *Freshw. Biol.* **2001**, *46*, 807–819. [CrossRef]
4. Malmqvist, B.; Rundle, S. Threats to the running water ecosystems of the world. *Environ. Conserv.* **2002**, *29*, 134–153. [CrossRef]
5. Myers, N.; Mittermeier, R.A.; Mittermeier, C.G.; da Fonseca, G.A.B.; Kent, J. Biodiversity hotspots for conservation priorities. *Nature* **2000**, *403*, 853–858. [CrossRef] [PubMed]
6. Olson, D.M.; Dinerstein, E.; Powell, G.V.N.; Wikramanayake, E.D. Conservation biology for the biodiversity in crisis. *Conserv. Biol.* **2002**, *16*, 1–3. [CrossRef] [PubMed]
7. Abell, R.; Thieme, M.L.; Revenga, C.; Bryer, M.; Kottelat, M.; Bogutskaya, N.; Coad, B.; Mandrak, N.; Balderas, S.C.; Bussing, W.; et al. Freshwater ecoregions of the world: A new map of biogeographic units for freshwater biodiversity conservation. *BioScience* **2008**, *58*, 403. [CrossRef]
8. Lanza, B. *Anfibi e Rettili (Amphibia-Reptilia)*; CNR: Roma, Italy, 1987; Volume 27.

9. Manenti, R.; Barzaghi, B.; Nessi, A.; Cioccarelli, S.; Villa, M.; Ficetola, G.F. Not Only Environmental Conditions but Also Human Awareness Matters: A Successful Post-Crayfish Plague Reintroduction of the White-Clawed Crayfish (*Austropotamobius pallipes*) in Northern Italy. *Front. Ecol. Evol.* **2021**, *9*, 64. [CrossRef]
10. Bishop, P.J.; Angulo, A.; Lewis, J.P.; Moore, R.D.; Rabb, G.B.; Moreno, J.G. The amphibian extinction crisis—What will it take to put the action into the amphibian conservation action plan? *SAPIENS* **2012**, *5*, 97–111.
11. Ficetola, G.F.; Rondinini, C.; Bonardi, A.; Baisero, D.; Padoa-Schioppa, E. Habitat availability for amphibians and extinction threat: A global analysis. *Divers. Distrib.* **2015**, *21*, 302–311. [CrossRef]
12. Wake, D.B.; Vredenburg, V.T. Are we in the midst of the sixth mass extinction? A view from the world of amphibians. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 11466–11473. [CrossRef]
13. Reading, C.J. Linking global warming to amphibian declines through its effects on female body condition and survivorship. *Oecologia* **2007**, *151*, 125–131. [CrossRef]
14. Wake, D.B. Climate change implicated in amphibian and lizard declines. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 8201–8202. [CrossRef]
15. Lips, K.R.; Diffendorfer, J.; Mendelson, J.R.; Sears, M.W. Riding the wave: Reconciling the roles of disease and climate change in amphibian declines. *PLoS Biol.* **2008**, *6*, e72. [CrossRef] [PubMed]
16. Rohr, J.R.; Raffel, T.R.; Romansic, J.M.; McCallum, H.; Hudson, P.J. Evaluating the links between climate, disease spread, and amphibian declines. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 17436–17441. [CrossRef]
17. Ribeiro, M.C.; Metzger, J.P.; Martensen, A.C.; Ponzoni, F.J.; Hirota, M.M. The Brazilian Atlantic forest: How much is left, and how is the remaining forest distributed? Implications for conservation. *Biol. Conserv.* **2009**, *142*, 1141–1153. [CrossRef]
18. D'Amen, M.; Bombi, P. Global warming and biodiversity: Evidence for climate-linked amphibian declines in Italy. *Biol. Conserv.* **2009**, *142*, 3060–3067. [CrossRef]
19. D'Amen, M.; Pietrangeli, B.; Bologna, M.A. Human provoked amphibian decline in central Italy and the efficacy of protected areas. *Wildl. Res.* **2010**, *37*, 547–557. [CrossRef]
20. Hartel, T.; Schweiger, O.; Ollerer, K.; Cogalniceanu, D.; Arntzen, J.W. Amphibian distribution in a traditionally managed rural landscape of eastern Europe: Probing the effect of landscape composition. *Biol. Conserv.* **2010**, *143*, 1118–1124. [CrossRef]
21. Ficetola, G.F.; Marziali, L.; Rossaro, B.; De Bernardi, F.; Padoa-Schioppa, E. Landscape–stream interactions and habitat conservation for amphibians. *Ecol. Appl.* **2011**, *21*, 1272–1282. [CrossRef]
22. Manenti, R.; De Bernardi, F.; Ficetola, G.F. Pastures vs forests: Do traditional pastoral activities negatively affect biodiversity? The case of amphibian communities. *NW J. Zool.* **2013**, *9*, 284–292.
23. Manenti, R.; Pennati, R. Environmental factors associated with amphibian breeding in streams and springs: Effects of habitat and fish occurrence. *Amphibia-Reptilia* **2016**, *37*, 237–242. [CrossRef]
24. Tiberti, R.; von Hardenberg, A. Impact of introduced fish on common frog (*Rana temporaria*) close to its altitudinal limit in alpine lakes. *Amphibia-Reptilia* **2012**, *33*, 303–307. [CrossRef]
25. Kats, L.B.; Petranka, J.W.; Sih, A. Antipredator defenses and the persistence of amphibian larvae with fishes. *Ecology* **1988**, *69*, 1865–1870. [CrossRef]
26. Holomuzki, J.R. Oviposition sites and fish-deterrent mechanisms of two stream anurans. *Copeia* **1995**, *1995*, 607–613. [CrossRef]
27. Hecnar, S.J.; M'Closkey, R.T. The effects of predatory fish on amphibian species richness and distribution. *Biol. Conserv.* **1997**, *79*, 123–131. [CrossRef]
28. Nunes, A.L.; Fill, J.M.; Davies, S.J.; Louw, M.; Rebelo, A.D.; Thorp, C.J.; Vimercati, G.; Measey, J. A global meta-analysis of the ecological impacts of alien species on native amphibians. *Proc. R. Soc. B* **2019**, *286*, 20182528. [CrossRef]
29. Fayzulin, A.I. Impact Assessment of the Amur Sleeper Perccottus glenii Dybowski, 1877 on Amphibians in Samara Oblast. In *IOP Conference Series: Earth and Environmental Science*; IOP Publishing: Bristol, UK, 2021; Volume 818.
30. Prigioni, C. *La lontra. Una Vita Silenziosa Negli Ambienti Acquatici*; Edagricole: Bologna, Italy, 1997.
31. Roper, T.J. *Badger*; Harper Collins Publishers: London, UK, 2010.
32. Birks, J. *Polecats*; Whittet Books Ltd.: Stansted, UK, 2015.
33. Jedrzejewska, B.; Sidorovich, V.E.; Pikulik, M.M.; Jedrzejewsky, W. Feeding habits of the otter and the American mink in Bialowieza Primeval Forest (Poland) compared to other Eurasian populations. *Ecography* **2001**, *24*, 165–180. [CrossRef]
34. Remonti, L.; Prigioni, C.; Balestrieri, A.; Sgrosso, S.; Priore, G. Trophic flexibility of the otter (*Lutra lutra*) in southern Italy. *Mamm. Biol.* **2008**, *73*, 293–302. [CrossRef]
35. Almeida, D.; Copp, G.H.; Masson, L.; Miranda, R.; Murai, M.; Sayer, C.D. Changes in the diet of a recovering Eurasian otter population between the 1970s and 2010. *Aquat. Conserv.* **2012**, *22*, 26–35. [CrossRef]
36. Balestrieri, A.; Remonti, L.; Vezza, P.; Prigioni, C.; Copp, G.H. Do non-native fish as prey favour the conservation of the threatened indigenous Eurasian otter? *Freshwater Biol.* **2013**, *58*, 995–1007. [CrossRef]
37. Smiroldo, G.; Villa, A.; Tremolada, P.; Gariano, P.; Balestrieri, A.; Delfino, M. Amphibians in Eurasian otter *Lutra lutra* diet: Osteological identification unveils hidden prey richness and male-biased predation on anurans. *Mammal. Rev.* **2019**, *49*, 240–255. [CrossRef]
38. Smiroldo, G.; Gariano, P.; Balestrieri, A.; Manenti, R.; Pini, E.; Tremolada, P. Predation on amphibians may enhance Eurasian otter recovery in southern Italy. *Zool. Sci.* **2019**, *36*, 273–283. [CrossRef]

39. Balestrieri, A.; Remonti, L.; Prigioni, C. Toward Extinction and Back: Decline and Recovery of Otter Populations in Italy. In *Problematic Wildlife—A Cross-Disciplinary Approach*; Angelici, F.M., Ed.; Springer: Cham, Switzerland, 2016. [CrossRef]
40. Giovacchini, S.; Antonucci, A.; Bartolomei, R.; Bandini, M.; Caldarella, M.; De Castro, G.; De Riso, L.; Di Marzio, M.; Fabrizio, M.; Fulco, E.; et al. Conservation status of Eurasian otter *Lutra lutra* in Italy. In Proceedings of the Eurasian Otter Workshop, Online, 26–28 February 2021.
41. Gariano, P.; Balestrieri, A. "Otter, come out!": Taking away the stone on the southernmost Italian *Lutra lutra* population. *Ecoscience* **2018**, *25*, 287–294. [CrossRef]
42. Crump, M.L.; Scott, N.J., Jr. Visual Encounter Surveys. In *Measuring and Monitoring Biological Diversity*; Smithsonian Institution Press: Washington, DC, USA, 1994.
43. Browne, R.K.; Zippel, K. Reproduction and Larval Rearing of Amphibians. *ILAR J.* **2007**, *48*, 214–234. [CrossRef]
44. Vezza, P.; Zanin, A.; Parasiewicz, P. *Manuale Tecnico- Operativo Per la Modellazione e la Valutazione Dell'integrità Dell'habitat Fluviale*; ISPRA–Manuali e Linee Guida 154/2017; Maggio: Roma, Italy, 2017.
45. Reuther, C.; Dolch, D.; Green, R.; Jahrl, J.; Jefferies, D.; Krekemeyer, A.; Kucerova, M.; Madsen, A.B.; Romanowski, J.; Roche, K.; et al. Surveying and Monitoring Distribution and Population Trends of the Eurasian Otter (*Lutra lutra*). *Habitat* **2000**, *12*, 152.
46. Macdonald, S.M.; Mason, C.F. The otter *Lutra lutra* in Southern Italy. *Biol. Conserv.* **1983**, *25*, 95–101. [CrossRef]
47. Fièvet, E.; Bonnet-Arnaud, P.; Mallet, J.P. Efficiency and sampling bias of electrofishing for freshwater shrimp and fish in two Caribbean streams, Guadeloupe Island. *Fish. Res.* **1999**, *44*, 149–166. [CrossRef]
48. Ross, R.M.; Lellis, W.A.; Bennett, R.M.; Johnson, C.S. Landscape determinants of nonindigenous fish invasions. *Biol. Invasions* **2001**, *3*, 347–361. [CrossRef]
49. Verneaux, J. Les poisons et la qualité des cour d'eau. *Ann. Sci. l'Université Fr.-Comté* **1981**, *2*, 33–41.
50. Peterson, J.T.; Thurow, R.F.; Guzevic, J.W. An evaluation of multipass electrofishing for estimating the abundance of stream-dwelling salmonids. *Trans. Am. Fish. Soc.* **1994**, *133*, 462–475. [CrossRef]
51. MacKenzie, D.I. Modeling the probability of resource use: The effect of, and dealing with, detecting a species imperfectly. *J. Wildl. Manag.* **2006**, *70*, 367–374. [CrossRef]
52. Gomez-Rodriguez, C.; Bustamante, J.; Diaz-Paniagua, C.; Guisan, A. Integrating detection probabilities in species distribution models of amphibians breeding in Mediterranean temporary ponds. *Diver. Distrib.* **2012**, *18*, 260–272. [CrossRef]
53. Wei, T.; Simko, V. R Package "Corrplot": Visualization of a Correlation Matrix (Version 0.84). 2017. Available online: https://github.com/taiyun/corrplot (accessed on 2 May 2023).
54. Borcard, D.; Gillet, F.; Legendre, P. *Numerical Ecology with R*; Springer: New York, NY, USA, 2011.
55. Pinheiro, P.; Bates, D. *Mixed-Effect Models in S and S-PLUS*; Springer: New York, NY, USA, 2000.
56. Brooks, M.E.; Kristensen, K.; van Benthem, K.J.; Magnusson, A.; Berg, C.W.; Nielsen, A.; Skaug, M.; Maechler, H.J.; Bolker, B.M. glmmTMB Balances Speed and Flexibility Among Packages for Zero-inflated Generalized Linear Mixed Modeling. *R J.* **2017**, *9*, 378–400. [CrossRef]
57. Barton, K. Mu-MIn: Multi-Model Inference. R Package Version 0.12.2/r18. 2009. Available online: http://R-Forge.R-project.org/projects/mumin/ (accessed on 2 May 2023).
58. Bates, D.; Mächler, M.; Bolker, B.; Walker, S. Fitting Linear Mixed-Effects Models Using lme4. *J. Stat. Softw.* **2015**, *67*, 1–48. [CrossRef]
59. R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2019; Available online: http://www.r-project.org/index.html (accessed on 2 May 2023).
60. Seyedhashemi, H.; Moatar, F.; Vidal, J.P.; Diamond, J.S.; Beaufort, A.; Chandesris, A.; Valette, L. Thermal signatures identify the influence of dams and ponds on stream temperature at the regional scale. *Sci. Total Environ.* **2021**, *766*, 142667. [CrossRef]
61. Catenazzi, A.; Kupferberg, S.J. Variation in thermal niche of a declining river-breeding frog: From counter-gradient responses to population distribution patterns. *Freshw. Biol.* **2017**, *62*, 1255–1265. [CrossRef]
62. Blaustein, A.R.; Wildy, E.L.; Belden, L.K.; Hatch, A. The influence of abiotic and biotic factors on amphibians in ephemeral ponds with special reference to long-toed salamanders (*Ambystoma macrodactylum*). *Isr. J. Zool.* **2001**, *47*, 333–345. [CrossRef]
63. Stebbins, R.C.; Cohen, N.W. *A Natural History of Amphibians*; Princeton University Press: Princeton, NJ, USA, 1995.
64. Ambrogio, A.; Mezzadri, S. *Girini d'Italia-Tadpoles of Italy*; Gravia Edizioni: Piacenza, Italy, 2014.
65. Baran, I.; Atatür, M.K. *Turkish Herpetofauna*; The Republic of Turkey, Ministry of Environment Publications: Ankara, Turkey, 1997.
66. Vignoli, L.; D'Amen, M.; Della Rocca, F.; Bologna, M.A.; Luiselli, L. Contrasted influences of moon phases on the reproduction and movement patterns of four amphibian species inhabiting different habitats in central Italy. *Amphibia–Reptilia* **2014**, *35*, 247–254. [CrossRef]
67. Tattersall, G.J.; Eterovick, P.C.; De Andrade, D.V. Tribute to R.G. Boutilier: Skin colour and body temperature changes in basking Bokermannohyla alvarengai (Bokermann 1956). *J. Exp. Biol.* **2006**, *209*, 1185–1196. [CrossRef]
68. Michaels, C.J.; Preziosi, R. Basking behaviour and ultraviolet B radiation exposure in a wild population of *Pelophylax lessonae* in Northern Italy. *Herpetol. Bull.* **2013**, *124*, 1–8.
69. Wright, K.M.; Whitaker, B.R. *Amphibian Medicine and Captive Husbandry*; Krieger Publishing Company: Malabar, FL, USA, 2001.
70. Seymour, R.S. Behavioral thermoregulation by juvenile Green Toads, *Bufo debilis*. *Copeia* **1972**, *1972*, 572–575. [CrossRef]

71. Lillywhite, H.B.; Licht, P.; Chelgren, P. The role of behavioral thermoregulation in the growth energetics of the toad, *Bufo boreas*. *Ecology* **1973**, *54*, 375–383. [CrossRef]
72. Wells, K. *The Ecology & Behavior of Amphibians*; The University of Chicago Press: Chicago, IL, USA; London, UK, 2007.
73. Woodward, B.D. Predator–prey interactions and breeding-pond use of temporary-pond species in a desert anuran community. *Ecology* **1983**, *64*, 1549–1555. [CrossRef]
74. Binckley, C.A.; Resetarits, W.J. Reproductive decisions under threat of predation: Squirrel treefrog (*Hyla squirella*) responses to banded sunfish (*Enneacanthus obesus*). *Oecologia* **2002**, *130*, 157–161. [CrossRef]
75. Holbrook, J.D.; Dorn, N.J. Fish reduce anuran abundance and decrease herpetofaunal species richness in wetlands. *Freshw. Biol.* **2016**, *61*, 100–109. [CrossRef]
76. Buxton, V.L.; Sperry, J.H. Reproductive Decisions in Anurans: A Review of How Predation and Competition Affects the Deposition of Eggs and Tadpoles. *BioScience* **2017**, *67*, 26–38. [CrossRef]
77. Kloskowski, J.; Nieoczym, M.; Stryjecki, R. Between-habitat distributions of pond tadpoles and their insect predators in response to fish presence. *Hydrobiologia* **2020**, *847*, 1343–1356. [CrossRef]
78. Gasith, A.; Resh, V.H. Streams in Mediterranean climate regions: Abiotic influences and biotic responses to predictable seasonal events. *Ann. Rev. Ecol. System.* **1999**, *30*, 51–81. [CrossRef]
79. Innes, J.; Kelly, D.; Overton, J.C.; Gillies, C. Predation and other factors currently limiting New Zealand forest birds. *N. Z. J. Ecol.* **2010**, *34*, 86–114.
80. Goldson, S.L.; Bourdôt, G.W.; Brockerhoff, E.G.; Byrom, A.E.; Clout, M.N.; McGlone, M.S.; Nelson, W.A.; Popay, A.J.; Suckling, D.M.; Templeton, M.D. New Zealand pest management: Current and future challenges. *J. R. Soc. N. Z.* **2015**, *45*, 31–58. [CrossRef]
81. Lodé, T.; Holveck, M.J.; Lesbarrères, D.; Pagano, A. Sex-biased predation by polecats influences the mating system of frogs. *Proc. R. Soc. B Biol. Sci.* **2004**, *271*, S399–S401. [CrossRef] [PubMed]
82. Ahola, M.; Nordstrom, M.; Banks, P.B.; Laanetu, N.; Korpimaki, E. Alien mink predation induces prolonged declines in archipelago amphibians. *Proc. R. Soc. B Biol. Sci.* **2006**, *273*, 1261–1265. [CrossRef] [PubMed]
83. Lima, S.L.; Dill, L.M. Behavioral decisions made under the risk of predation: A review and prospectus. *Can. J. Zool.* **1990**, *68*, 619–640. [CrossRef]
84. Salo, P.; Korpimaki, E.; Banks, P.B.; Nordstrom, M.; Dickman, C.R. Alien predators are more dangerous than native predators to prey populations. *Proc. R. Soc. B Biol. Sci.* **2007**, *274*, 1237–1243. [CrossRef]
85. Ruiz-Olmo, J.; López-Martín, J.M.; Palazón, S. The influence of fish abundance on the otter (*Lutra lutra*) populations in Iberian Mediterranean habitats. *J. Zool.* **2001**, *254*, 325e336. [CrossRef]
86. Remonti, L.; Balestrieri, A.; Prigioni, C. Altitudinal gradient of Eurasian otter (*Lutra lutra*) food niche in Mediterranean habitats. *Can. J. Zool.* **2009**, *87*, 285–291. [CrossRef]
87. Smiroldo, G.; Balestrieri, A.; Remonti, L.; Prigioni, C. Seasonal and habitat-related variaton of otter *Lutra lutra* diet in a Mediterranean river catchment (Italy). *Folia Zool.* **2009**, *58*, 87–97.