diversity

MDPI

Article

High Evolutionary Potential Maintained in Common Frog (*Rana temporaria*) Populations Inhabiting Urban Drainage Ponds

Robert Jehle [1,*], Jeanette Hall [2,3], Samantha A. Hook [1], Sarenta King [1], Kirsty McArthur [4,5], Alexandre Miró [3], Marcia Rae [2] and David O'Brien [2,3,*]

[1] School of Science, Engineering and Environment, University of Salford, Salford M5 4WT, UK
[2] NatureScot, Great Glen House, Leachkin Road, Inverness IV3 8NW, UK
[3] Highland Amphibian and Reptile Project, c/o Woodlands, Brae of Kinkell, Dingwall IV7 8HZ, UK
[4] School of Applied Sciences, Edinburgh Napier University, Edinburgh EH11 4BN, UK
[5] Centre for Conservation and Restoration Science, Edinburgh Napier University, Edinburgh EH11 4BN, UK
* Correspondence: r.jehle@salford.ac.uk (R.J.); david.obrien@nature.scot (D.O.)

Abstract: Urbanisation leading to habitat change and fragmentation is a recognised global threat to biodiversity. However, it may also offer opportunities for some species. Genetic diversity, one of the three components of biodiversity, is often overlooked in conservation planning and policy. In the present study, we used a panel of seven microsatellite markers to compare the genetic structure of 34 common frog (*Rana temporaria*) populations residing in urban and suburban drainage ponds in Inverness (Scotland) with populations from rural surroundings. As a main finding, the levels of genetic variation were indiscernible between (sub)urban and rural populations. Significant isolation-by-distance was observed only for rural populations, with measures of pairwise genetic differentiation (F_{st}) that were, on average, lower than those in urban and suburban areas. The mean numbers of alleles remained stable between two temporal sets of samples collected at intervals broadly representing one *R. temporaria* generation, but with a tendency of decreasing allelic richness, irrespectively of the site characteristics. Taking these results together, our study revealed that the elevated levels of differentiation between *R. temporaria* populations inhabiting (sub)urban drainage ponds did not lead to increased levels of genetic erosion. Our findings support the importance of well-designed blue–green infrastructure in urban landscapes for the retention of within-species genetic diversity and can help to inform future biodiversity management policies.

Keywords: amphibians; SuDSs; microsatellites; genetic connectivity; urban ecology; land use policy

Citation: Jehle, R.; Hall, J.; Hook, S.A.; King, S.; McArthur, K.; Miró, A.; Rae, M.; O'Brien, D. High Evolutionary Potential Maintained in Common Frog (*Rana temporaria*) Populations Inhabiting Urban Drainage Ponds. *Diversity* **2023**, *15*, 738. https://doi.org/10.3390/d15060738

Academic Editors: Oldřich Kopecký and Michael Wink

Received: 14 April 2023
Revised: 26 May 2023
Accepted: 29 May 2023
Published: 2 June 2023

Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Urbanisation is increasing at a global level and influencing the ecology and evolution of both plants and animals at a local and global scale [1–3]. Blue–green infrastructure is defined as a 'strategically planned network of natural and semi-natural areas with other environmental features designed and managed to deliver a wide range of ecosystem service' [4]. In urban settings, such as cities or towns, this includes waterbodies such as stormwater ponds and other sustainable drainage systems (SuDSs), which can act as islands of vital resources for local wildlife to persist amidst otherwise unsuitable terrain [5–7]. The extent to which populations residing in such environments are able to retain their evolutionary potential and functional diversity under increased levels of fragmentation, however, depends on the requirements of the given species and is a growing area of research [8–11], as well as a policy concern [12].

Suitably managed waterbodies in urban environments harbour relatively high levels of biodiversity compared to other urban habitats that are concentrated in particularly

confined spaces [13–16]. Amphibians are a useful model taxon for investigating the value of such waterbodies within wider blue–green infrastructure. They use urban ponds for reproduction and depend on surrounding terrestrial habitats for foraging, hibernation, aestivation, and dispersal [17–22]. The occurrence and abundance of amphibians are generally shaped by small-scale landscape features, which makes them particularly suited for studying the consequences of habitat fragmentation for the structure of wildlife populations [23,24]. Generalisations of the particular effects of urbanisation on amphibians have, however, been proven difficult to make, suggesting that the diversity of their life histories requires a case-by-case consideration of how specific species respond to environmental settings in given cityscapes [18,25]. This particularly applies to the level of genetic erosion imposed on amphibian populations through the urbanisation of connected habitats that were previously more natural (see [26] for a meta-analysis).

The common frog (*Rana temporaria*) occurs across large parts of the Western Palearctic and is among the most populous European anurans north of the Mediterranean basin [27,28]. Reproduction takes place in spring when adults congregate in ponds at broadly even sex ratios to form local populations. Females deposit spawn clumps containing numbers of eggs on the order of 1–2000, from which tadpoles hatch; these largely metamorphose in the same season [29]. Individuals reach maturity after 2–5 years, attaining a longevity of 6–13 years depending on the latitude and altitude [30]. Facilitated by a wide ecological niche that allows its persistence in human-made habitats and an overall high standing amount of genetic variation [31,32], *R. temporaria* was among the first amphibian species that served to document the genetic consequences of human-induced habitat fragmentation [33,34]. Subsequent studies on *R. temporaria* focused on the interplay between temporal and spatial forces for shaping genetic variation at the wider landscape level [35–37] and on how specific landscape features impede or promote connectivity under a pervasive anthropogenic influence [38,39]. *Rana temporaria* populations have further been shown to suffer from negative fitness consequences of both in- and outbreeding depression when subjected to isolation [34,40,41].

In Scotland, *R. temporaria* occurs in all terrestrial EUNIS habitat categories [42], from sea level to over 1100 m above sea level [43,44]. The city of Inverness in the Scottish Highlands is characterised by a recent rapid expansion that, over the last decades, has resulted in the construction of an array of SuDSs to facilitate water runoff and flood prevention through surface blue–green infrastructure rather than below-ground engineering approaches [7,45]. Previous studies have demonstrated that the local SuDS ponds are inhabited by amphibian communities that are generally indiscernible from those of rural surrounding areas, despite marked variations in the ecological quality of the existing SuDSs depending on their level of maintenance and design [7,46,47]. The aim of the present paper is to expand on these studies by documenting the genetic structure of *R. temporaria* populations in and around Inverness and by documenting whether SuDSs harbour populations that are affected by genetic erosion. More specifically, we used a panel of seven microsatellite markers to genetically compare *R. temporaria* populations inhabiting urban and suburban SuDSs, as well as populations from surrounding rural areas, based on two sets of temporal samples. We further investigated whether the ecological quality of SuDSs is linked to the standing amount of genetic variation of inhabiting *R. temporaria* populations.

2. Methods

2.1. Field Sampling

A total of 34 populations were sampled in and around Inverness in April 2015, April 2019, or both years (Table 1). The SuDS ponds that we considered (n = 22) represented a subsample of the sites described in [47], and we used the Global Human Settlement Layer GHSL R2022A system [48] to divide them into urban (n = 14; corresponding to the Dense Urban Cluster GHSL category) and suburban (n = 8; Suburban GHSL category) depending

on their surrounding areas. The rural ponds (n = 12; combined GHSL categories of Low-Density Rural and Very-Low-Density Rural) used in the study were situated up to a distance of about 20 km from Inverness and were characterised in more detail in [49]. The ecological quality of the SuDS ponds was assessed by recording the presence or absence of 13 freshwater invertebrate groups with different pollution or eutrophication tolerance ranges according to the OPAL protocol, as described in [45,50]. Genetic sampling used a single embryo (egg) collected from separate clumps where possible, and it was preserved in absolute ethanol until DNA extraction. As females produce a single spawn clump each year, samples from separate clumps were, therefore, no more closely related than at the level of a half-sibling. No precise information on adult population sizes was available.

Table 1. Details of sampling sites and descriptive population genetic parameters across 34 *Rana temporaria* populations in and around Inverness (Scotland). n: genetic sample size, with numbers of samples from two different sampling years in brackets; H_o: observed mean heterozygosity; H_e: expected mean heterozygosity, where * denotes deviations from population-wide Hardy–Weinberg equilibria at a Bonferroni-corrected p-value of 0.0015; AL: mean number of alleles per locus; PA: number of private alleles.

Sampling Site	National Grid Reference	n (2015/2019)	H_o	H_e	AL	PA
Urban:						
AA	NH 67100 42136	13 (0/13)	0.68	0.77	5.57	0
BA	NH 69000 44010	10 (0/10)	0.95	0.81	5.86	0
BB	NH 69017 44011	16 (5/11)	0.77	0.75	6.57	1
BD	NH 71372 45055	15 (7/8)	0.78	0.74	6.29	0
IP	NH 68782 43278	14 (0/14)	0.56	0.57	4.29	0
SD	NH 64398 44565	11 (0/11)	0.63	0.64	4.33	0
SP	NH 67295 42107	27 (18/9)	0.77	0.81	9.86	0
TA	NH 71708 44979	10 (10/0)	0.82	0.78	6.14	0
WC	NH71519 45111	17 (8/9)	0.67	0.70	6.43	0
WF	NH 71820 44732	9 (9/0)	0.91	0.78	5.57	1
WH	NH 71904 44662	22 (10/12)	0.79	0.81	10.14	2
WHR	NH 66834 42423	22 (10/12)	0.78	0.81	8.43	0
WP	NH 71646 45212	8 (8/0)	0.84	0.75	7.00	0
WO	NH 68917 44160	14 (14/0)	0.94	0.81 *	7.29	0
Suburban:						
BAA	NH 70069 42351	10 (10/0)	0.69	0.76	5.57	1
DV	NH 67281 41847	10 (10/0)	0.91	0.81	6.57	0
FA	NH 67159 41965	10 (10/0)	0.89	0.81	6.57	0
GR	NH 69399 42517	10 (0/10)	0.71	0.68	4.29	1
HFR	NH 66477 41715	10 (10/0)	0.84	0.82	7.00	1
HH	NH 69068 45577	22 (9/13)	0.75	0.80 *	9.57	2
IC	NH 69221 45070	10 (10/0)	0.93	0.83	7.14	0
MN	NH 66968 41605	21 (10/11)	0.82	0.81 *	6.71	1
Rural:						
AWH	NH 59220 43830	21 (9/12)	0.78	0.77	6.14	0
BW	NH 47930 57240	9 (9/0)	0.74	0.68	5.40	0
DC	NH 63190 42000	11 (11/0)	0.77	0.73	4.57	1
HP	NH 59120 53680	21 (11/10)	0.79	0.76	7.57	2
KM	NH 60120 44180	27 (14/13)	0.80	0.77	10.14	1
LL	NH 53590 49720	9 (9/0)	0.90	0.79	5.43	0
NSE	NH 64320 42650	15 (5/10)	0.78	0.80	7.43	3
NSW	NH 64270 42540	21 (10/11)	0.76	0.74	7.29	0
PH	NH 86420 50310	20 (8/12)	0.74	0.75	6.86	0
RO	NH 6376044270	23 (8/15)	0.70	0.71	8.43	1
SN	NH 63894 44129	9 (0/9)	0.75	0.66	4.43	0
TH	NH 57620 54430	24 (11/13)	0.84	0.77	9.57	0

2.2. Laboratory Work

Samples were genotyped at the seven previously characterised *R. temporaria* microsatellite loci of *BGF*048, *BGF*053, *BGF*106, *BGF142*, *BGF*157, *BGF*250, and *BGF*258 (see [51]); the specific loci were chosen due to their high numbers of alleles and the tri-/tetranucleotide nature of repeat motifs for straightforward scoring. PCRs contained approximately 10 ng of DNA, 5 pmol (5 mmol/L) of each primer, 0.15 mmol/L of each dNTP, 1.5 mmol/L $MgCl_2$, and 0.5–1.0 U Taq polymerase (Advanced Biotechnologies, Columbia, MD, USA) in the manufacturer's buffer for a total volume of 10 μL. The PCR profiles were 94 °C for 2 min, followed by 35 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s. Primers were labelled with fluorochromes (FAM, HEX, or AT-550) and separated via capillary electrophoresis by using an ABI 3130 Genetic Analyser (Applied Biosystems, Waltham, MA, USA) either in-house or commercially through Macrogen. Fragments were sized by using Peak Scanner Software v1.0 (Applied Biosystems).

2.3. Data Analysis

In the first set of analyses, we addressed the question of whether the combined samples from the two sampling years of 2015 and 2019 could be pooled for joint spatial analyses by using the Bayesian clustering approach implemented in the Structure 2.3.4 software (originally described in [52]). The approach assigned individual genotypes to a predefined number of clusters (K) in a given sample (X) in order to achieve Hardy–Weinberg and linkage equilibrium. We estimated the *ln* posterior probabilities for $K = 1$ (implying one gene pool) or $K = 2$ (implying that the sampling years of 2015 and 2019 represented different gene pools) for the 13 populations for which at least eight individuals were sampled in both study years (Table 1, $n = 4$, 2, and 7 for urban, suburban, and rural populations, respectively), followed by calculating $P(K|X)$ by using Bayes' rule. Results were obtained from 10^6 runs after 10^5 burn-ins, without allowing for admixture and using the correlated frequency model as implemented in the software (see also [53,54] for the use of this approach).

After showing that the site-specific samples were better characterised by assuming a single gene pool (see below), we computed the observed (H_o) and expected (H_e) heterozygosities, departures from Hardy–Weinberg equilibria at each locus with Bonferroni corrections to give table-wide significance levels of $P = 0.05$, and measures of F_{is} for each population by using Genepop on the Web by employing the implemented Markov Chain method (10^6 runs) to obtain unbiased estimates of Fisher's exact tests [55]. FStat [56] was used to obtain estimates of allelic richness based on the minimum population sample size. The spatial structure of the populations was further investigated by using the algorithm implemented in BAPS 6.0 [57] to distinguish an enforced substructure (in our case, this was defined on the basis of ponds) from potentially more meaningful partitions reflected in the dataset (for details, see [58]). Bayesian posterior distributions were derived from an MCMC algorithm (we considered 500,000 runs after 100,000 burn-ins), and we set a lower probability bound of 0.05 for partitions to be considered in the final model. Patterns of pairwise spatial genetic differentiation between ponds were further assessed with F_{st} values that were also derived in Genepop by regressing $F_{st}/(1 - F_{st})$ against the geographic distance to test for scenarios of isolation-by-distance by using Mantel tests as implemented in the IBD software ([59]; see also [60] for the general framework). For the 13 populations for which at least eight individuals were sampled in both study years (Table 1, $n = 4$, 2, and 7 for urban, suburban, and rural populations, respectively), we also investigated whether the two temporal samples differed in their overall levels of genetic variation. Based on published information on *R. temporaria* [59], the four-year interval between sampling years broadly represented one generational turnover.

3. Results

The study considered a total of 521 genotypes across the 34 studied populations (n = 208, 103, and 210 for urban, suburban, and rural populations, respectively; Table 1). These genotypes encompassed 273 and 248 samples collected in 2015 and 2019, respectively, resulting in an average of 14.2 total samples per population (range: 8–27). The overall PCR success rate across all loci was 83.0%, with a minimum per-population sample size per locus of n = 3 for the calculation of allelic richness values.

According to the algorithm implemented in the Structure software, the posterior probabilities that samples from a given pond represented a single genetic cluster (K = 1) ranged from 0.77 (rural population *PH*) to 1.00 (rural population *AWH* and suburban population *MN*) with a median of 0.97, confirming that the two sampling years could be merged for joint spatial analyses. The mean number of alleles per locus ranged between 4.29 (rural population *IP*) and 10.14 (urban population *WH*), with an average of 6.70, 6.68, and 6.94 for urban, suburban, and rural populations, respectively, and no significant differences between the groups (one-way ANOVA, d.f. = 2, F = 2.031, p = 0.15; Table 1). The corresponding mean values of allelic richness ranged from 3.53 (rural) over 3.67 (urban) to 3.89 (suburban)—again, without significant differences between the groups (d.f. = 2, F = 0.08, p = 0.92; Figure 1). In total, 31 out of the 34 populations (91.2%) were in Hardy–Weinberg equilibrium at a Bonferroni-adjusted p-value of 0.0015, with heterozygosities exceeding the expected values in 22/34 (64.7%) of cases (Table 1). High heterozygosities were also reflected in slightly negative mean F_{is} values (urban: −0.04; suburban: −0.03; rural: −0.05; Figure 1). For the combined urban and suburban populations, there was no significant correlation between the OPAL scores and the average number of alleles (Spearman rank correlation: r_s = −0.19, p = 0.53) or allelic richness (r_s = −0.16, p = 0.60).

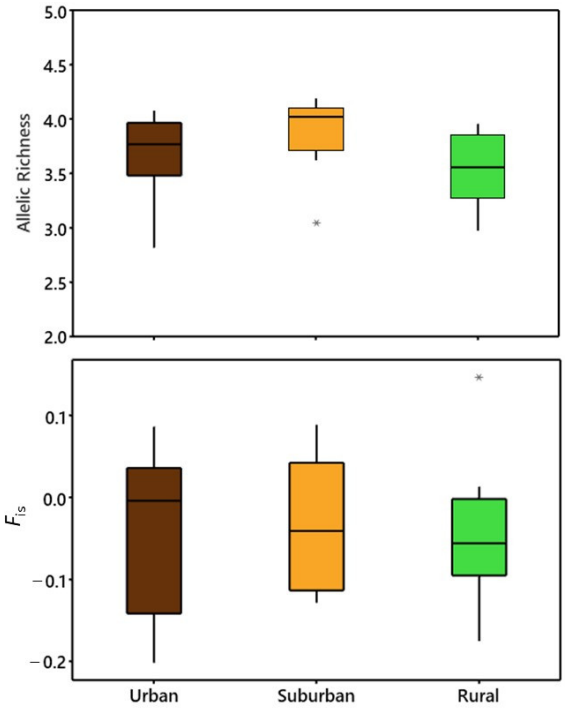

Figure 1. Comparison of allelic richness and F_{is} values for a total of 34 *Rana temporaria* populations inhabiting urban, suburban, and rural ponds in and around Inverness (Scotland). Asterisks denote outlier values.

Across all considered ponds, private alleles were the least common in urban populations (n = 4), followed by suburban (n = 6) and rural populations (n = 8). The average pairwise F_{st} values were larger between urban (0.07) and suburban (0.05) populations than between rural (0.04) populations, suggesting an overall stronger partition of genetic

variation in built-over areas despite the greater overall geographic proximity. This was further reflected by isolation-by-distance scenarios, which were absent in the suburban (Z = 5.27, p = 0.54) and urban populations (Z = 24.40, p = 0.60) and highly significant for rural populations (Z = 12.55, p > 0.01, Figure 2). The algorithm implemented in the BAPS software reduced the 34 ponds to five genetic clusters (Figure 3). One cluster consisted of the single spatially isolated urban SuDS pond *IP*, and another cluster consisted of the spatially adjacent but differentially classified ponds *SN* (rural) and *SD* (urban). The remaining three clusters comprised six, seven, and 18 populations each (Figure 3).

The comparison of the levels of genetic variation between the two sampling years of 2015 and 2019 revealed no marked differences among rural, urban, and suburban sites, but with the majority of populations across all sites being characterised by a slight decrease in allelic richness (Figure 4).

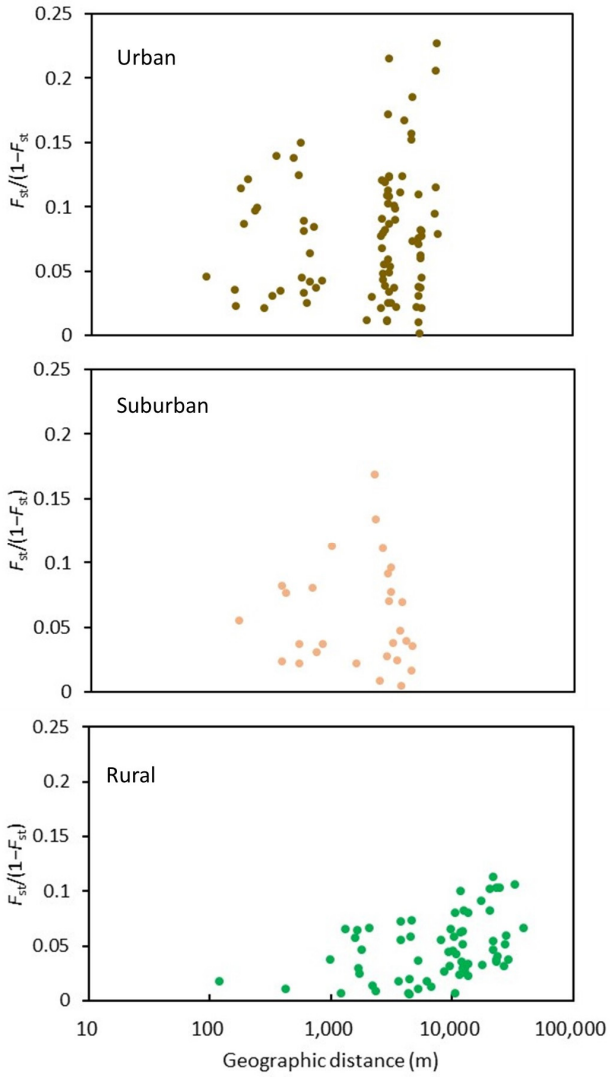

Figure 2. Relationship between log-transformed geographic and genetic distances across urban, suburban, and rural *Rana temporaria* populations in and around Inverness. Mantel tests revealed a significant correlation for rural populations only (for details, see the text).

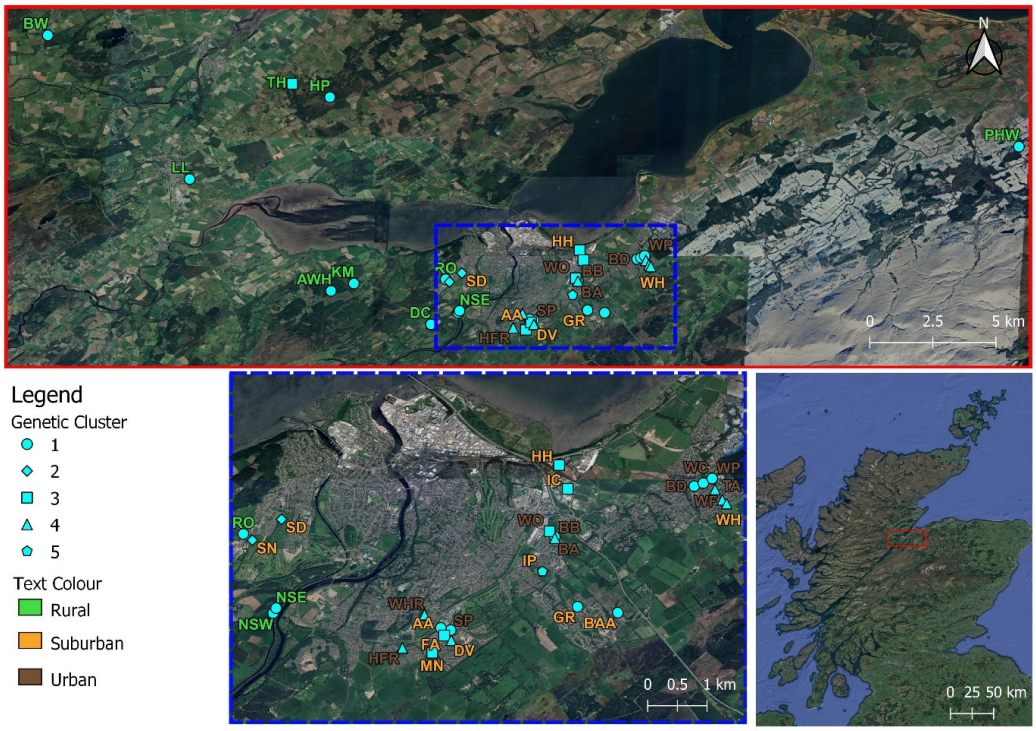

Figure 3. Map of the study area showing all 34 *Rana temporaria* study ponds, their classification according to the Global Human Settlement Layer system (rural, suburban, or urban), and their partitioning into five genetic clusters that were identified by using the BAPS software. The sampling sites were identical to those shown in Table 1. For more details, see the text. The red square in the right figure represents the top figure.

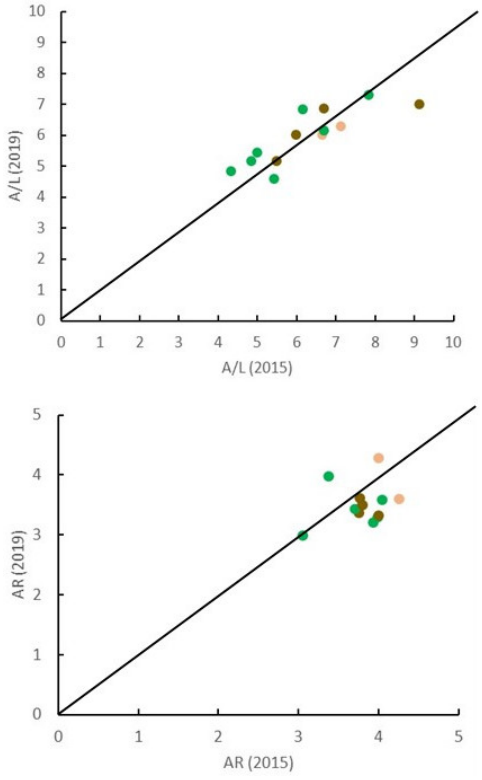

Figure 4. Comparisons of the mean numbers of alleles per locus (A/L) and allelic richness (AR) between the two sampling years (2015 and 2019) for *Rana temporaria* populations in and around Inverness. Values above/below the diagonal represent increases/decreases over time. Green symbols: rural populations; orange symbols: suburban populations; brown symbols: urban populations.

4. Discussion

The present study builds upon existing work that demonstrated that SuDS ponds in Inverness (Scotland) provide suitable breeding habitats for the locally occurring amphibian assemblage, which comprises five species in total [7,46,47]. We used *R. temporaria* as a model species to investigate whether the evolutionary potential of resident populations as measured by the standing amount of genetic variation in seven microsatellite loci was compromised by habitat modification and (sub)urban surroundings. We found that suburban and urban SuDS ponds, despite their overall increasing local levels of genetic differentiation, were not characterised by higher levels of genetic erosion compared to rural sites. Our case study adds to the growing evidence that generalisations on the effects of human-induced habitat fragmentation on amphibians are difficult to infer (e.g., [26]). It also reinforces that urban habitats can provide an important contribution to the preservation of within-species genetic diversity at the landscape scale for species that can persist in human-modified areas. Our microsatellite-based inferences are, however, largely unable to reveal whether urban populations differ from their rural counterparts in more detailed demographic and ecological traits.

Given that our study site was situated in the northwestern periphery of the *R. temporaria* range, the overall high levels of allelic diversity revealed by our study are noteworthy. They exceed those found in another microsatellite-based study conducted in Scotland across favourable habitats in the absence of interpopulation barriers [61]. Although it is impossible to discard local, population-specific reasons for this observation, our choice of loci from a pool of 145 *R. temporaria* candidate markers offered by [51] was based on the most polymorphic loci available and may, in part, explain this difference. We also encountered heterozygosities that, for the majority of the populations, were above their expected values under Hardy–Weinberg equilibrium. While heterozygote advantage can be invoked through mate choice (for an example on the congeneric *R. arvalis*, see [62]) and likely particularly applies under harsh conditions (for an example on *R. temporaria*, see [63]), we preferred to attribute this observation to our sampling regime, which avoided the random collection of full sibs. The high levels of heterozygosities observed also further supported that the pooling of individuals from two sampling years for spatial inferences was justified, as an unconsidered substructure would reduce H_o to below H_e [64].

A main finding arising from the present study is that urban and suburban SuDS ponds are indiscernible from rural ponds with respect to the levels of genetic variation, with no evidence of inbreeding in any of the study populations. Our genetic data suggest that gene flow might be responsible for counteracting the negative consequences of drift, and this is in line with the findings from the clustering approach, which revealed a wide partitioning of 34 ponds into five genetic groups, whereby SuDS ponds were not separated from their rural counterparts (see also, e.g., [65], who found a similar pattern for a urodele species). Our findings, however, contrast with those of a previous study of urban *R. temporaria* populations [34], which revealed reduced levels of genetic variation combined with inbreeding depression in urban populations. The city of Inverness has undergone particularly rapid growth since the late 20th century, resulting in an expansion of the urban area that has led to the rather recent creation and modification of the SuDS ponds under study [7,46]. This suggests two possible explanations: Either there has not yet been sufficient time for genetic erosion to occur, or the green infrastructure associated with SuDSs provides suitable breeding habitats and functional connectivity of terrestrial habitats that are favourable for the conservation of genetic variation in this species. The demographic consequences of habitat modification and population isolation might, therefore, still accumulate over time (but see, e.g., [66] for the maintenance of genetic variation under long-term isolation in another northern European amphibian). However, since several of the ponds sampled have existed for over 20 years (approximately five generations for *Rana temporaria*), signs of genetic erosion would be expected if isolation effects were strong.

Facilitated by a high plasticity in breeding behaviour, diet, and larval development (e.g., [67–69]), *R. temporaria* possesses a wide ecological niche that is known to include

human-influenced habitats (e.g., [36,44]). It is, therefore, not overly surprising that we found no clear links between pond habitat quality as measured with OPAL and genetic variation. SuDS ponds in Inverness have been found to have higher OPAL scores compared to those in Scotland and Britain as a whole, confirming their high value as breeding habitats for *R. temporaria* [7,47]. Environmental conditions are, however, known to influence vital demographic parameters, such as individual longevity and growth, in *R. temporaria* populations [30,70]. From the view of conservation, it would, therefore, be beneficial to compare levels of recruitment, generational turnover, and habitat-dependent life-history parameters, such as diet, between SuDS ponds and rural sites in future studies.

The distribution of genetic variation in given landscapes arises from a combination of spatial and temporal processes. Ponds in rural areas were characterised by a scenario of isolation-by-distance that represented levels of connectivity that were proportional to geographic proximity. This matched previous studies that noted low levels of artificial barriers to amphibian movement, such as major roads and human settlements, in the study area [49,71]. Isolation-by-distance was, however, absent in urban and suburban ponds, whose genetic makeups appeared to be dominated by drift or gene flow that was uncoupled from geographic distance. While landscape genetic analyses are beyond the scope of the present work, it is noteworthy that we revealed overall higher levels of pairwise genetic differentiation (F_{st}) between SuDS ponds compared to rural sites, despite the higher overall number of population–private alleles in the latter. Aside from modified or reduced patterns of gene flow, the observed breakdown of isolation-by-distance in urban and suburban areas might, therefore, also be linked to the spatial scale of investigation (see also, e.g., [72]). Rural populations covered a wider area than their urban and suburban counterparts, which might have led to a more pronounced genetic signal of spatial differentiation.

In our temporal comparison of samples collected within a 4-year interval, we revealed no tendency for changes in mean numbers of alleles per locus, however, alongside a tendency for a reduction in allelic richness across (sub)urban and rural sites. This seems likely to be linked to the rather limited per-population, per-locus sample size, rather than to a scenario of genetic drift, which would result in a higher probability of rare alleles becoming lost than common alleles. Significantly for our study, this supports the evidence that accelerated genetic erosion is not taking place in our urban populations. Our inferences nevertheless reinforce the general importance of temporal genetic monitoring of populations (see, e.g., [73]). However, we refrained from, for example, using the temporal samples to compare effective genetic population sizes among urban, suburban, and rural populations due to the large confidence intervals expected under the given sampling regime and the general caution that is recommended for such an approach due to amphibians' life history [74]. Given the increased availability of amphibian genomes and transcriptomes (for *R. temporaria,* see [75,76]), future research could focus on how habitats such as SuDS ponds shape the distribution of adaptive genetic variation in response to mounting evidence of selection for distinctive phenotypic traits, such as reduced mobility, larger body size, and fewer offspring in dense urban settings ([11]; see also [70] for evidence of local phenotypic adaptation in *R. temporaria* populations).

What do our results tell us about the maintenance of within-species genetic variation of amphibians across human-modified landscapes? When suitably managed, SuDS ponds appear to provide nature-friendly neighbourhoods that enable the retention of evolutionary potential for *R. temporaria* populations. This is an encouraging finding, given that the density of such sites in built-over areas can be higher than, for example, on agricultural land, which has suffered a marked loss of amphibian breeding ponds in recent decades (e.g., [77]). From a policy perspective, attempts to maximise biodiversity conservation opportunities require an understanding of how different habitats contribute to the evolutionary potential of a given species [73]. Our findings reinforce that well-designed and managed urban ecosystems can harbour an integral share of the overall genetic diversity for species that are able to use them for reproduction. While this was not the case in our

study area, urban environments can also act as filters that reduce the overall number of species [18,19,78]. Our findings support the emphasis on increasing the quality and connectivity of blue–green infrastructure both locally—for example, in the developing Scottish Biodiversity Strategy [79]—and globally through Target 12 of the Convention on Biological Diversity [12]. They also add an often-missing genetic dimension to our understanding of the importance of connectivity [71] and contribute to the development of an evidence-based approach to conservation, which is particularly important in times of resource constraints [80]. Whilst the lifecycles of other urban amphibians may be different from that of *R. temporaria*, this set of SuDSs was studied over 12 years, and thus, our work also highlights the importance of long-term studies for informing conservation interventions ([7,47]; see also, e.g., [81]).

Author Contributions: D.O., R.J., and J.H. conceived the study; M.R. and A.M. conducted the fieldwork; S.A.H. and S.K. conducted the laboratory work; R.J. analysed the data with input from D.O., J.H., and K.M.; R.J., J.H., and D.O. drafted the manuscript with input from K.M. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by the Highland Council (fieldwork) and NatureScot (field and laboratory work, project number SERA47).

Institutional Review Board Statement: Sampling was approved by Nature Scot and the University of Salford Ethics Committee.

Data Availability Statement: The datasets generated and/or analysed during the current study are available from the corresponding authors upon request.

Acknowledgements: We are grateful to the landowners for allowing access to their properties and for their enthusiasm for the research.

Conflicts of interest: The authors declare no conflict of interest.

References

1. Douglas, I.; James, P. *Urban Ecology: An Introduction*; Routledge: London, UK, 2014.
2. Johnson, M.T.J.; Munshi-South, J. Evolution of life in urban environments. *Science* **2017**, 358, eaam8327.
3. Schilthuizen, M. *Darwin Comes to Town: How the Urban Jungle Drives Evolution*; Picador: New York, NY, USA, 2019
4. JNCC. Available online: https://jncc.gov.uk/our-work/blue-green-infrastructure/ (accessed on 11 April 2023).
5. Niemelä, J. *Urban Ecology: Patterns, Processes and Applications*; Oxford University Press: Oxford, UK, 2011.
6. Gaston, K.J. *Urban Ecology*; Cambridge University Press: Cambridge, UK, 2013.
7. O'Brien, C.D. Sustainable drainage system (SuDS) ponds in Inverness, UK and the favourable conservation status of amphibians. *Urban Ecosyst*. **2015**, *18*, 321–331.
8. Alberti, M.; Correa, C.; Marzluff, J.M.; Zhou, Y. Global urban signatures of phenotypic change in animal and plant populations. *Proc. Natl. Acad. Sci. USA* **2017**, *114*, 8951–8956.
9. McDonald, R.I.; Mansur, A.V.; Ascensão, F.; Colbert, M.; Crossman, K.; Elmqvist, T.; Gonzalez, A.; Güneralp, B.; Haase, D.; Hamann, M.; et al. Research gaps in knowledge of the impact of urban growth on biodiversity. *Nat. Sustain.* **2020**, *3*, 16–24.
10. Caspi, T.; Johnson, J.R.; Lambert, M.R.; Schell, C.J.; Sih, A. Behavioral plasticity can facilitate evolution in urban environments. *Trends Ecol. Evol.* **2022**, *37*, 1092–1103.
11. Hahs, A.K.; Fournier, B.; Aronson, M.F.J.; Nilon, C.H.; Herrera-Montes, A.; Salisbury, A.; Threlfall, C.G.; Rega-Brodsky, C.C.; Lepczyk, C.A.; Carpenter, E.S.; et al. The global effects of urbanisation on functional diversity across multiple taxa. *Nature Comm*. **2023**, in press.
12. Convention on Biological Diversity. Available online: https://www.cbd.int/ (accessed on 11 April 2023).
13. Hassall, C. The ecology and biodiversity of urban ponds. *WIREs Water* **2014**, *1*, 187–206.
14. Hill, M.J.; Biggs, J.; Thornhill, I.; Briers, R.A.; Gledhill, D.G.; White, J.C.; Wood, P.J.; Hassall, C. Urban ponds as an aquatic biodiversity resource in modified landscapes. *Glob. Chang. Biol.* **2017**, *23*, 986–999.
15. Oertli, B.; Parris, K.M. Review: Toward management of urban ponds for freshwater biodiversity. *Ecosphere* **2019**, *10*, e02810.
16. Hyseni, C.; Heino, J.; Binic, L.M.; Bjelked, U.; Johansson, F. The importance of blue and green landscape connectivity for biodiversity in urban ponds. *Basic Appl. Ecol.* **2021**, *57*, 129–145.
17. Parris, K.M. Urban amphibian assemblages as metacommunities. *J. Anim. Ecol.* **2006**, *75*, 757–764.
18. Hamer, A.J.; McDonnell, M.J. Amphibian ecology and conservation in the urbanising world: A review. *Biol. Cons.* **2008**, *141*, 2432–2449.
19. Hamer, A.J.; Parris, K.M. Local and landscape determinants of amphibian communities in urban ponds. *Ecol. Appl.* **2011**, *21*, 378–390.

20. Hutto, D., Jr.; Barrett, K. Do urban open spaces provide refugia for frogs in urban environments? *PLoS ONE* **2021**, *16*, e0244932.
21. Lee, T.S.; Randall, L.A.; Kahal, N.L.; Kinas, H.L.; Carney, V.A.; Rudd, H.; Baker, T.M.; Sanderson, K.; Creed, I.F.; Moehrenschlager, A.; et al. A framework to identify priority wetland habitats and movement corridors for urban amphibian conservation. *Ecol. Solut. Evid.* **2022**, *3*, e12139.
22. Sauer, E.L.; Cruz, J.; Crone, E.; Lewis, C.; Plumier, E.; Cwynar, B.; Drake, D.; Herrick, B.M.; Preston, D.L. Multiscale drivers of amphibian community occupancy in urban ponds. *Urban Ecosyst.* **2022**, *25*, 1469–1479.
23. Cushman, S.A. Effects of habitat loss and fragmentation on amphibians: A review and prospectus. *Biol. Cons*. **2006**, *128*, 231–240.
24. Schmidt, C.; Munshi-South, J.; Dray, S.; Garroway, C.J. Determinants of genetic diversity and species richness of North American amphibians. *J. Biogeogr.* **2022**, *49*, 2005–2015.
25. Landler, L.; Burgstaller, S.; Schweiger, S. Land-use preferences of the European green toad (*Bufotes viridis*) in the city of Vienna (Austria): The importance of open land in urban environments. *Front. Zool.* **2023**, *20*, 3.
26. Schmidt, C.; Garroway, C.J. The population genetics of urban and rural amphibians in North America. *Mol. Ecol.* **2021**, *30*, 3918–3929.
27. Teacher, A.G.F.; Garner, T.W.J.; Nichols, R.A. European phylogeography of the common frog (*Rana temporaria*): Routes of postglacial colonization into the British Isles, and evidence for an Irish glacial refugium. *Heredity* **2009**, *102*, 490–496.
28. Vences, M.; Hauswaldt, J.S.; Steinfartz, S.; Rupp, O.; Goesmann, A.; Künzel, S.; Orozco-terWengel, P.; Vieites, D.R.; Nieto-Roman, S.; Haas, S.; et al. Radically different phylogeographies and patterns of genetic variation in two European brown frogs, genus *Rana*. *Mol. Phylogenet. Evol.* **2013**, *68*, 657–670.
29. Cummins, C.P. Temporal and spatial variation in egg size and fecundity in *Rana temporaria*. *J. Anim. Ecol.* **1996**, *55*, 303–316.
30. Miaud, C.; Guyétant, R.; Elmberg, J. Variations in life-history traits in the common frog *Rana temporaria* (Amphibia: Anura): A literature review and new data from the French Alps. *J. Zool.* **1999**, *249*, 61–73.
31. Brede, E.G.; Beebee, T.J.C. Consistently different levels of genetic variation across the European ranges of two anurans, *Bufo bufo* and *Rana temporaria*. *Herpetol. J.* **2006**, *16*, 265–271.
32. Brede, E.G.; Beebee, T.J.C. Large variations in the ratio of effective breeding and census population sizes between two species of pond-breeding anurans. *Biol. J. Linn. Soc.* **2006**, *89*, 365–372.
33. Reh, W.; Seitz, A. The influence of land use on the genetic structure of populations of the common frog *Rana temporaria. Biol. Cons.* **1990**, *54*, 239–249.
34. Hitchings, S.P.; Beebee, T.J.C. Genetic substructuring as a result of barriers to gene flow in urban *Rana temporaria* (common frog) populations: Implications for biodiversity conservation. *Heredity* **1997**, *79*, 117–127.
35. Johansson, M.; Primmer, C.R.; Merilä, J. History vs. current demography: Explaining the genetic population structure of the common frog (*Rana temporaria*). *Mol. Ecol.* **2006**, *15*, 975–983.
36. Johansson, M.; Primmer, C.R.; Sahlsten, J.; Merilä, J. The influence of landscape structure on occurrence, abundance and genetic diversity of the common frog, *Rana temporaria*. *Glob. Chang. Biol.* **2005**, *11*, 1664–1679.
37. Lenhardt, P.P.; Brühl, C.A.; Leeb, C.; Theissinger, K. Amphibian population genetics in agricultural landscapes: Does viniculture drive the population structuring of the European common frog (*Rana temporaria*)? *PeerJ* **2017**, *5*, e3520.
38. Safner, T.; Miaud, C.; Gaggiotti, O.; Decout, S.; Rioux, D.; Zundel, S.; Manel, S. Combining demography and genetic analysis to assess the population structure of an amphibian in a human-dominated landscape. *Conserv. Genet*. **2011**, *12*, 161–173.
39. Saarikivi, J.; Knopp, T.; Granroth, A.; Merilä, J. The role of golf courses in maintaining genetic connectivity between common frog (*Rana temporaria*) populations in an urban setting. *Conserv. Genet*. **2013**, *14*, 1057–1064.
40. Sagvik, J.; Uller, T.; Olsson, M. Outbreeding depression in the common frog, *Rana temporaria*. *Conserv. Genet.* **2005**, *6*, 205–211.
41. Johansson, M.; Primmer, C.R.; Merilä, J. Does habitat fragmentation reduce fitness and adaptability? A case study of the common frog (*Rana temporaria*). *Mol. Ecol.* **2007**, *16*, 2693–2700.
42. Davies, C.E.; Moss, D.; O'Hill, M. *EUNIS Habitat Classification. Revision 2004*; European Environment Agency, European Topic Centre on Nature Protection and Biodiversity: Copenhagen, Denmark, 2004.
43. O'Brien, C.D.; O'Brien, D.M.; Hall, J. *Rana temporaria* (European common frog), British altitudinal range extension. *Herp. Bull.* **2014**, *129*, 26.
44. McInerny, C.; Minting, P. *The Amphibians & Reptiles of Scotland*; The Glasgow Natural History Society: Glasgow, Scotland, 2016.
45. Woods-Ballard, B.; Wilson, S.; Udale-Clarke, H.; Illman, S.; Scott, T.; Ashley, R.; Kellagher, R. *The SUDS Manual*; CIRIA report C753; CIRIA: London, UK, 2015.
46. Miró, A.; Hall, J.; Rae, M.; O'Brien, D. Links between ecological and human wealth in drainage ponds in a fast-expanding city, and proposals for design and management. *Landsc. Urban Plan.* **2018**, *180*, 93–102.
47. Rae, M.; Miró, A.; Hall, J.; O'Brien, K.; O'Brien, D. Evaluating the validity of a simple citizen science index for assessing the ecological status of urban drainage ponds. *Ecol. Indic.* **2019**, *98*, 1–8.
48. Pesaresi, M.; Politis, P. *GHS Built-Up Surface Grid, Derived from Sentinel2 Composite and Landsat, Multitemporal (1975–2030)*; European Commission Joint Research Centre (JRC): Seville, Spain, 2022.
49. O'Brien, C.D.; Hall, J.E.; Miró, A.; O'Brien, K.; Jehle, R. A co-development approach to conservation leads to informed habitat design and rapid establishment of amphibian communities. *Ecol. Solut. Evid.* **2021**, *2*, e12038.
50. Davies, L.; Bell, J.N.B.; Bone, J.; Head, M.; Hill, L.; Howard, C.; Hobbs, S.J.; Jones, D.T.; Power, S.A.; Rose, N.; et al. Open Air Laboratories (OPAL): A community-driven research programme. *Environ. Pollut*. **2011**, *159*, 2203–2210.

51. Matsuba, C.; Merilä, J. Isolation and characterization of 145 polymorphic microsatellite loci for the common frog (*Rana temporaria*). *Mol. Ecol. Res*. **2009**, *9*, 555–562.
52. Pritchard, J.K.; Stephens, M.; Donnelly, P. Inference of population structure using multilocus genotype data. *Genetics* **2000**, *155*, 945–959.
53. Jehle, R.; Burke, T.; Arntzen, J.W. Delineating fine-scale genetic units in amphibians: Probing the primacy of ponds. *Conserv. Genet*. **2005**, *6*, 227–234.
54. Jehle, R.; Wilson, G.A.; Arntzen, J.W.; Burke, T. Contemporary gene flow and the spatio-temporal genetic structure of subdivided newt populations (*Triturus cristatus*, *T. marmoratus*). *J. Evol. Biol*. **2005**, *18*, 619–628.
55. Rousset, F. Genepop'007: A complete reimplementation of the Genepop software for Windows and Linux. *Mol. Ecol. Res*. **2008**, *8*, 103–106.
56. Goudet, J. FSTAT (Version 1.2): A computer program to calculate F-statistics. *J. Hered.* **1995**, *86*, 485–486.
57. Cheng, L.; Connor, T.R.; Sirén, J.; Aanensen, D.M.; Corander, J. Hierarchical and spatially explicit clustering of DNA sequences with BAPS software. *Mol. Biol. Evol*. **2013**, *30*, 1224–1228.
58. Corander, J.; Waldmann, P.; Sillanpää, M.J. Bayesian analysis of genetic differentiation between populations. *Genetics* **2003**, *163*, 367–374.
59. Bohonak, A.J. IBD (Isolation by Distance): A program for analysis of isolation by distance. *J. Hered.* **2002**, *93*, 153–154.
60. Rousset, F. Genetic differentiation and estimation of gene flow from *F*-statistics under isolation by distance. *Genetics* **1997**, *145*, 1219–1228.
61. Muir, A.P.; Thomas, R.; Biek, R.; Mable, B.K. Using genetic variation to infer associations with climate in the common frog, *Rana temporaria*. *Mol. Ecol*. **2013**, *22*, 3737–3751.
62. Rausch, A.; Sztatecsny, M.; Jehle, R.; Ringler, E.; Hödl, W. Male body size and parental relatedness but not nuptial colouration influence paternity success during scramble competition in *Rana arvalis*. *Behaviour* **2014**, *151*, 1869–1884.
63. Lesbarrères, D.; Primmer, C.R.; Laurila, A.; Merilä, J. Environmental and population dependency of genetic variability-fitness correlations in *Rana temporaria*. *Mol. Ecol.* **2005**, *14*, 311–323.
64. Wahlund, S. Zusammensetzung von Populationen und Korrelationerscheinungen vom Standpunkt der Vererbungslehre aus betrachtet. *Hereditas* **1928**, *11*, 65–106.
65. Yannic, G.; Helfer, V.; Sermier, R.; Schmidt, B.R.; Fumagalli, L. Fine scale genetic structure in fire salamanders (*Salamandra salamandra*) along a rural-to-urban gradient. *Conserv. Genet.* **2021**, *22*, 275–292.
66. Roth, S.; Jehle, R. High genetic diversity of common toad (*Bufo bufo*) populations under strong natural fragmentation on a northern archipelago. *Ecol. Evol*. **2016**, *6*, 1626–1636.
67. Houston, W.W.K. The food of the common frog, *Rana temporaria*, on high moorland in northern England. *J. Zool.* **1973**, *171*, 153–165.
68. Loman, J. Breeding phenology in *Rana temporaria*. Local variation is due to pond temperature and population size. *Ecol. Evol*. **2016**, *6*, 6202–6209.
69. Lind, M.I.; Persbo, F.; Johansson, F. Pool desiccation and developmental thresholds in the common frog, *Rana temporaria*. *Proc. Roy. Soc. B*. **2008**, *275*, 1073–1080.
70. Richter-Boix, A.; Teplitsky, C.; Rogell, B.; Laurila, A. Local selection modifies phenotypic divergence among *Rana temporaria* populations in the presence of gene flow. *Mol. Ecol.* **2010**, *19*, 716–731.
71. O'Brien, D.; Hall, J.E.; Miró, A.; O'Brien, K.; Falaschi, M.; Jehle, R. Reversing a downward trend in threatened peripheral amphibian (*Triturus cristatus*) populations through interventions combining species, habitat and genetic information. *J. Nat. Conserv*. **2021**, *64*, 126007.
72. Castric, V.; Bernatchez, L. The rise and fall of isolation by distance in the anadromous brook charr (*Salvelinus fontinalis* Mitchill). *Genetics* **2003**, *163*, 983–996.
73. Hoban, S.; Bruford, M.W.; Funk, W.C.; Galbusera, P.; Griffith, M.P.; Grueber, C.E.; Heuertz, M.; Hunter, M.E.; Hvilsom, C.; Stroil, B.K.; et al. Global commitments to conserving and monitoring genetic diversity are now necessary and feasible. *BioScience* **2021**, *71*, 964–976.
74. Kimble, S.J.A.; Unger, S.D.; Williams, R.N. Genetically derived effective population size estimates of herpetofaunal species should be used with caution. *J. Wildl. Manag.* **2023**, *87*, e22340.
75. Price, S.J.; Garner, T.W.J.; Balloux, F.; Ruis, C.; Paszkiewicz, K.H.; Moore, K.; Griffiths, A.G.F. A de novo assembly of the Common Frog (*Rana temporaria*) transcriptome and comparison of transcription following exposure to *Ranavirus* and *Batrachochytrium dendrobatidis*. *PLoS ONE* **2015**, *10*, e0130500.
76. Streicher, J.W.; Wellcome Sanger Institute Tree of Life programme. The genome sequence of the common frog, *Rana temporaria* Linnaeus 1758. *Wellcome Open Res.* **2021**, *6*, 286.
77. Arntzen, J.W.; Abrahams, C.; Meilink, W.R.M.; Iosif, R.; Zuiderwijk, A. Amphibian decline, pond loss and reduced population connectivity under agricultural intensification over a 38 year period. *Biodiv. Conserv.* **2017**, *26*, 1411–1430.
78. Zhang, W.; Li, B.; Shu, X.; Pei, E.; Yuan, X.; Sun, Y.; Wang, T.; Wang, Z. Responses of anuran communities to rapid urban growth in Shanghai, China. *Urban For. Urban Green.* **2016**, *20*, 365–374.
79. Scottish Biodiversity Strategy to 2045: Tackling the Nature Emergency in Scotland. Available online: https://www.gov.scot/publications/scottish-biodiversity-strategy-2045-tackling-nature-emergency-scotland/ (accessed on 11 April 2023).

80. Downey, H.; Bretagnolle, V.; Brick, C.; Bulman, C.R.; Cooke, S.J.; Dean, M.; Edmonds, B.; Frick, W.F.; Friedman, K.; McNicol, C.; et al. Principles for the production of evidence-based guidance for conservation actions. *Conserv. Sci. Pract*. **2022**, *4*, e12663.
81. Moor, H.; Bergamini, A.; Vorburger, C.; Holdegger, R.; Bühler, C.; Egger, S.; Schmidt, B.R. Bending the curve: Simple but massive conservation action leads to landscape-scale recovery of amphibians. *Proc. Natl. Acad. Sci. USA* **2023**, *119*, 2123070119.

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.