

# Environmental DNA metabarcoding elucidates patterns of fish colonisation and co-occurrences with amphibians in temperate wetlands created for biodiversity

Ineta Kačergytė<sup>1</sup>  | Erik Petersson<sup>2</sup> | Debora Arlt<sup>1,3</sup> | Micaela Hellström<sup>4,5</sup> | Jonas Knape<sup>1</sup> | Johan Spens<sup>4,6</sup> | Michał Żmihorski<sup>7</sup> | Tomas Pärt<sup>1</sup>

<sup>1</sup>Department of Ecology, Swedish University of Agricultural Sciences, Uppsala, Sweden

<sup>2</sup>Department of Aquatic Resources, Swedish University of Agricultural Sciences, Drottningholm, Sweden

<sup>3</sup>SLU Swedish Species Information Centre, Swedish University of Agricultural Sciences, Uppsala, Sweden

<sup>4</sup>AquaBiota Water Research, Stockholm, Sweden

<sup>5</sup>MIX Research Sweden, Uppsala, Sweden

<sup>6</sup>Limnordic AB, Örnköldsvik, Sweden

<sup>7</sup>Mammal Research Institute, Polish Academy of Sciences, Białowieża, Poland

## Correspondence

Ineta Kačergytė, Department of Ecology, Swedish University of Agricultural Sciences, Box 7044, Uppsala 750 07, Sweden.  
Email: ineta.kacergyte@slu.se

## Funding information

Belmont Forum and BiodivERsA; Stiftelsen Oscar och Lili Lamms Minne, Grant/Award Number: 2016-0022; Svenska Forskningsrådet Formas; Swedish EPA, Grant/Award Number: [13/361]

## Abstract

1. Wetlands are biodiversity hotspots that provide several essential ecosystem services. On a global scale, wetlands have greatly declined due to human activities. To counteract wetland loss, created wetlands are used as a conservation tool to facilitate biodiversity and provide habitats mainly for birds and amphibians. Fishes are likely to colonise the created wetlands and potentially affect the diversity and occurrence of amphibians. Still, species occurrence data for fish in created wetlands are largely lacking.
2. Using eDNA metabarcoding, we investigated occurrence and co-occurrence patterns of fish and amphibian communities in 52 wetlands (some of which are ponds) created to benefit mainly bird and amphibian communities in south-central Sweden.
3. Altogether, 17 fish and five amphibian species were detected in the created wetlands out of the 32 fish and six amphibian species found in the regional species pool. Amphibians were less common in wetlands physically connected to other wetlands. Connected wetlands were more fish-rich than isolated ones, suggesting potential fish avoidance. Additionally, the amphibian community occupied a narrower environmental niche compared to the fish community. Nevertheless, we observed only five statistically significant negative fish–amphibian species co-occurrences (out of 14 species considered).
4. Even though our results suggest amphibian avoidance/exclusion from the created biodiversity wetlands, they also show that fish and amphibians frequently co-exist. Increased habitat heterogeneity in terms of water vegetation, size, shape, and structure of the wetland could be possible factors enabling the co-existence of these two taxa.
5. With this study, we contribute to the general knowledge of fish occurrence patterns in created biodiversity wetlands. By comparing the frequencies of fish occurrence in natural and created wetlands, we have shown that there was some

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2021 The Authors. *Freshwater Biology* published by John Wiley & Sons Ltd.

mismatch in what is common in natural compared to created wetlands. This mismatch probably comes from species-specific habitat requirements, stocking, and differences in detectability when using eDNA metabarcoding (small species detected) versus conventional multi-mesh gill-net methods (small species missed). Therefore, our results obtained using eDNA metabarcoding can complement the pre-existing knowledge of amphibian and fish associations and increase our understanding of how to create wetlands to facilitate biodiversity of several taxa.

#### KEYWORDS

connectivity, constructed ponds, species interactions, stocking, water community

## 1 | INTRODUCTION

Wetlands are rich ecosystems that provide multiple ecosystem services making them irreplaceable for biodiversity and humans (Kusler, 2012). Nevertheless, natural wetland habitat has been reduced by around 70% worldwide, mainly due to agricultural drainage and other human activities (Davidson, 2014). To counteract this, there is an increase in the creation of new wetlands (Dixon et al., 2016). Many of these wetlands are created to improve wetland biodiversity as compensation for its previous decline due to loss and deterioration of natural biodiversity-rich wetlands; for example, 400 wetlands were created for amphibians in Estonia (Magnus & Rannap, 2019; SEPA, 2019). Wetland protection has worldwide become a high priority, supported by international agreements such as the Ramsar Convention and the International Convention of Biological Diversity (Bobbink et al., 2007).

Amphibians and birds are common focal taxa for the conservation of wetland biodiversity, and the colonisation of created wetlands by these taxa is relatively well-documented in the literature (e.g. Baker & Halliday, 1999; Kačergytė et al., 2021; Porej & Hetherington, 2005; Sebastián-González & Green, 2014; Semlitsch et al., 2015; Shulse et al., 2012). Amphibians in particular seem to benefit from the creation of small wetlands (Magnus & Rannap, 2019), as their occurrence and establishment may depend on hydrology (preferred shallow, standing water), eutrophication (avoid hyper-eutrophication), presence of aquatic vegetation, distance to other wetlands and absence of predatory fish (reviewed by Brown et al., 2012). However, created wetlands are often connected with surrounding waterways for water level regulation, enabling fish colonisation. The colonisation of fish in created wetlands is rather common but generally not systematically recorded (Kristensen et al., 2020; Langston & Kent, 1997; Zimmer et al., 2001), and therefore fish community composition in created wetlands is often unknown. The construction features of created wetlands (e.g. dams vs. connected wetlands) vary, and some can affect the degree of accessibility for colonisations (Beatty et al., 2009; Talley, 2000), where the degree of isolation may be a strong factor diversifying fish species composition (Tonn et al., 1990). Natural colonisation by fish also depends on the movement rates (e.g. how active the fish is) and their regional abundance (Albanese et al., 2009).

Additionally, fish occurrence can be related to human activities due to both legal and illegal stocking (Spens & Ball, 2008; Talley, 2000). The colonisation and presence of fish in created wetlands are likely to affect other taxa either by being a food source (e.g. for birds and newts) or by competition and predation (Bouffard & Hanson, 1997; Elmerg et al., 2010; Kloskowski et al., 2010; Semlitsch et al., 2015). Indirect effects may also manifest through altered water quality, loss of macrophyte diversity and cover via foraging activity, and management practices associated with angling activity (e.g. Lemmens et al., 2013; O'Toole et al., 2009; Schilling et al., 2009).

Fish are reported to have negative impacts on the growth and prevalence of amphibian populations (Pope, 2008; Semlitsch et al., 2015; Shulse et al., 2012) as well as their behaviour (Winandy et al., 2015). Amphibian population declines are also related to habitat loss or deterioration, over-exploitation, climate change and diseases (Cohen et al., 2019; Meurling et al., 2020; Stuart et al., 2004) and therefore are of conservation interest when creating new aquatic habitats. Negative effects of fish on amphibians, especially focusing on prey-predator occurrences, are well documented in natural wetlands (Hartel et al., 2007) and created ponds (Semlitsch, 1987). However, created wetlands constructed for improving wetland biodiversity and including potential amphibian-fish interactions on the competition of resources are poorly investigated. Thus, there is a risk that if fish colonise all created wetlands for biodiversity in a relatively short time, amphibian diversity in this habitat can be reduced due to predation, but also competition and avoidance.

Using the non-invasive technique of environmental DNA (eDNA) metabarcoding as the inventory tool, we surveyed amphibian and fish occurrences in 52 created wetlands in Sweden. Aquatic eDNA is defined as genetic material that has been shed by an organism (extra-organismal DNA) into the surrounding water and can be captured directly from water samples as cellular or subcellular material (Moushomi et al., 2019; Turner et al., 2014; Wilcox et al., 2015). Only a few studies have used eDNA data to test patterns of diversity in wetlands, let alone created ones (see Harper et al., 2020). The advancement of molecular methods makes it possible to identify species in a water column from DNA, enabling a completely new approach for species inventories. Environmental DNA barcoding and metabarcoding have in numerous investigations proven to be

successful at detecting cryptic fish species (Hänfling et al., 2016; McElroy et al., 2020; Pochardt et al., 2020; Sigsgaard et al., 2015) and amphibians (Ficetola et al., 2019). By using eDNA, it is also possible to identify those species where morphological identification is hard or impossible (e.g. larvae, Fujii et al., 2019). In studies comparing eDNA sampling and traditional monitoring (gill-net, traps, electro-fishing, etc.), the overlap, or repeatability, between taxa detected varies depending on how thoroughly the traditional inventories are performed. However, eDNA analysis is generally comparable to, complementary to or more effective than traditional methods for amphibian and fish detection (Ficetola et al., 2019; Fujii et al., 2019; Hänfling et al., 2016; Lawson Handley et al., 2019; Pont et al., 2019). Therefore, eDNA monitoring alone could be sufficient for evaluating fish and amphibian (Bálint et al., 2018; Harper et al., 2020) communities in created wetlands. Additionally, eDNA metabarcoding as an inventory method is non-invasive and reduces the risk of spreading pathogens, whereas traditional surveys might harm or even kill the target species (Snyder, 2003).

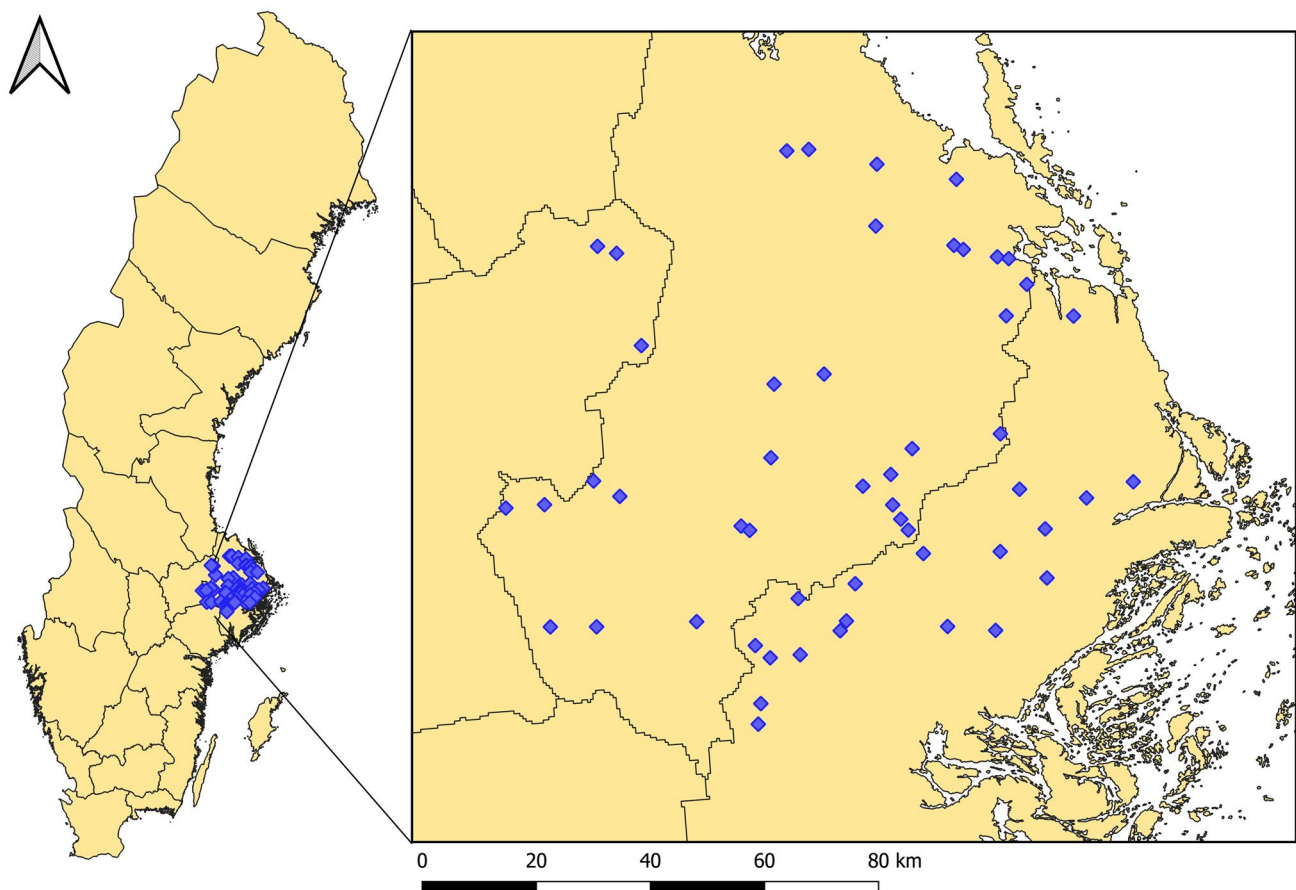
We investigated colonisation patterns and co-occurrences of amphibians and fish in relation to local wetland characteristics, the composition of the surrounding landscape and the species regional pool. For species richness and occurrences of more common species, we predicted: (1) fish to be more likely to occur in wetlands connected with surrounding waterways, while amphibian occurrence

would be less dependent on the connectedness of the wetlands (due to their terrestrial dispersal ability); (2) amphibians to be more likely to occur in small wetlands because fish are less likely to occur and prevail in very small wetlands, whereas fish richness should be higher in larger wetlands (area-richness relationship); and (3) amphibians to be less likely to occur in wetlands with a high species richness of fish due to described predatory interactions. To further explore the observed patterns of fish and amphibian occurrence, we also investigated specific pairwise species co-occurrences to better elucidate possible species interactions that might cause amphibian avoidance or exclusion. Last, we compared fish occurrences in created wetlands to pre-existing data on fish occurrences in natural lakes and fish stocking in the same geographical region to better understand which fish species are likely to colonise created wetlands.

## 2 | METHODS

### 2.1 | Study sites and environmental data

The sampling of fish and amphibian eDNA took place in June and July of 2018 in 52 created wetlands in the Uppland region (Figure 1), Sweden. These wetlands are generally shallow and were created to promote biodiversity on the whole, but primarily



**FIGURE 1** Locations of 52 surveyed created wetlands (59°35'3.9"N 16°46'14.3"E; 60°19'37.0"N 18°25'46.4"E; Sweden)

for birds and, to some extent, amphibian diversity rather than fish (Dietrichson, 2017). They were created at sites where no wetland existed at the time of the creation, although some were created at sites where there had historically been a wetland more than 50 years ago. The latter wetlands had been drained, and the land was used for other purposes, usually pastures or arable land. The wetlands in this study were created between 1990 and 2013 (variable 1) and were surrounded by agricultural, forest or urban landscapes (var. 2). Wetland size (var. 3) varied between 0.53 and 20 ha, and wetlands were covered by different percentages of aquatic vegetation (from completely free of to completely covered; var. 4). Many of the wetlands were physically connected (var. 5) via ditches or stream networks to rivers or lakes. The surrounding area around the wetlands is often seasonally flooded, creating temporary pools (var. 6). These selected six environmental variables (local wetland and landscape characteristics) might explain variation in occurrences of fish and amphibians in created wetlands (see Table 1 for details and explanations). All measurements were obtained by digitising hand-drawn maps in ArcGIS software 10.5. Data on the year of wetland creation were obtained by interviewing locals and assessed from aerial photographs repeated since 1990 (Lantmäteriet, 2020). Landscape composition was determined using the Swedish Terrain Map (GSD Geografiska Sverigedata).

Additionally, we extracted data about fish stocking in the Uppland region (Figure 2, Figure S1) based on the permit information given for landowners, which included localities in lakes and rivers, but none of these permits were given for the created wetlands we investigated. Moreover, we obtained data on fish occurrences in 97 natural lakes using fish monitoring surveys (e.g. gill netting) during

the last 3 decades, covering the overall study area of created wetlands (see Table S1). Even though the natural systems differ from the created wetlands in size and depth, littoral zone, etc., a comparison between natural and created wetland communities would indicate whether some species might prefer or avoid created wetlands as habitat.

## 2.2 | Environmental DNA collection and extraction

Prior to the fieldwork, all collection vessels (jars and buckets) were sterilised (using 6%–10% sodium hypochlorite solution) and cleaned. The filtering equipment, including filters and syringes, were ordered in sterile single-use packages. Footwear was cleaned in bleach (10% sodium hypochlorite) before visiting a location, and the collector avoided entering the water. Five litres of water were collected from each wetland from at least ten subsamples, which were spatially and evenly distributed around the pond following Harper et al., (2019). The water collected from the subsamples was mixed in a clean container and double filtered through enclosed double filters made of 5- $\mu$ m glass fibre and 0.8- $\mu$ m polyethersulfone membranes (NatureMetrics Ltd) until the water volume reached 3,300 ml or the filters clogged. The median volume of water filtered was 1,500 ml, with water volumes ranging from 210 ml to 3,300 ml (Table S2). Negative field filter controls were taken each sampling day to test for potential field contamination by using bottled water that was filtered on-site and treated like the field samples. All samples were fixated in 96% molecular grade ethanol following Spens et al., (2017) and sent to laboratory

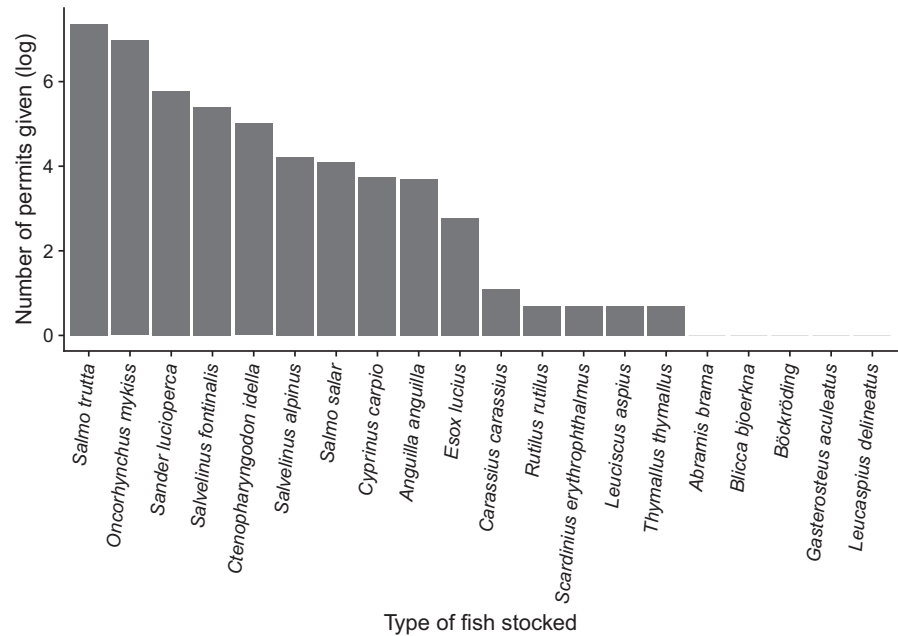
**TABLE 1** Environmental variables included in the analysis for fish and amphibian species richness and occurrence

Environmental characteristic	Description (unit)	Explanations	Range (Mean $\pm$ SD)
Size	The total size of the created wetland water surface (ha)	Larger wetlands may have more species of fish, while amphibians will be more likely to occur in smaller wetlands	0.53–19.98, (4.57 $\pm$ 3.99)
Water vegetation	Cover of wetland area by emergent water vegetation (e.g. <i>Typha</i> , <i>Phragmites</i> , <i>Iris</i> ) and floating water vegetation (e.g. <i>Nymphaea</i> , <i>Lemnoideae</i> , algae; %)	Habitat and possible refuges for amphibians, structural complexity, reflects water productivity, general depth of a wetland	0–98.2, (50.1 $\pm$ 30.4)
Connectivity	Whether the wetland has ditches/streams that connect it to other water bodies (except for dams which are assumed to be isolated in terms of natural fish colonisation probability)	Connectivity makes wetlands colonisable by fish	28 connected, 24 isolated
Flooded area <sup>a</sup>	The proportion of area around the wetland shore that is flooded (50 meters around wetland shore) (%)	Protection from predators and more suitable habitat for amphibian eggs and larvae	0–55.6, (12 $\pm$ 12)
Year of creation <sup>b</sup>	The year when the wetland was created. When this information was not available, the age was estimated using aerial photographs.	Time allows more species to colonise and establish	1990–2013, (2001 $\pm$ 5.6)
Landscape forest	Coverage of forest within 1 km buffer starting from the wetland shore (%)	May affect wetland acidity and productivity	0.5–100, (66 $\pm$ 24.6)

<sup>a</sup>Not applied for fish.

<sup>b</sup>not applied for amphibians as they are able to colonise wetlands during the first years of creation, and all wetlands in this study were at least 5 years old at the time of the survey (Lesbarrères et al., 2010; Petranka et al., 2003).

**FIGURE 2** The frequency of stocking permits given for each species in the Upland region (see Figure S1). In Sweden, the stocking permit is required just for connected wetlands, but none of our inventoried connected wetlands had received such permits



(MoRe Research AB), designated for eDNA purposes only, for extraction following a modified protocol for enclosed filters in Spens et al., (2017). The modification included pooling the lysate after the overnight lysis of the filter capsule DNA and ethanol pellet DNA into one sample in order to gain a higher DNA yield. The extracted DNA samples were sent to a commercial laboratory, NatureMetrics Ltd, for downstream analytical applications, including polymerase chain reaction (PCR), high-throughput sequencing, and bioinformatics (see Appendix S1 for more details). We used MiFish 12S primers (Miya et al., 2015, 2020) and additionally adjusted MiFish primers to match amphibians on the 12S region (for details, see Appendix S1.1). For each step in the pipeline ranging from field collections to PCR, controls were introduced and were treated like the original samples. The control samples used were as follows: negative filter controls in the field, negative extraction controls (one for every 20 samples), negative PCR controls and positive PCR controls (mock community of tropical fishes). All the controls were amplified and sequenced. A detailed description of the 173 negative and 12 positive controls is given in Appendix S1.1.

### 2.3 | Statistical analyses

We used generalised linear models to examine the variation in fish and amphibian species richness (Poisson distribution), and additionally, variation in occurrence (binomial error distribution) for some of the more common fish (e.g. species occurring in >10 sites) and all amphibian species at our study sites, using five out of total six environmental variables relevant for either fish or amphibians (Table 1). A species was assumed to occur at a site when we detected eDNA sequences from it and was considered absent if eDNA of that species was not detected (the minimum number of reads per species was 55 for fish and 33 for amphibians). In the models analysing amphibian

species richness, we additionally added fish species richness as an explanatory variable.

We used the *co-occur* package (Griffith et al., 2016) to infer associations between fish and amphibian species based on presence-absence data. The *co-occur* package investigates observed co-occurrences in relation to a null hypothesis of random species distributions without accounting for environmental data. We excluded those species that occurred in three or fewer sites (nine fish species excluded, analysed 14 remaining species, including all amphibians) for these analyses. Additionally, we performed non-metric multi-dimensional scaling (NMDS) for analyses of community structure following environmental gradients using two dimensions, which can facilitate the interpretation of species distributions. The presence/absence species data were analysed using the Bray-Curtis distance measure and the metaMDS function in the *vegan* package (Oksanen et al., 2019). For NMDS only, we excluded the occurrence of rainbow trout *Oncorhynchus mykiss* as it is non-native and appeared singularly in one wetland, thus acting as rare species in the ordination analyses and appearing outside the species ordination cloud. All analyses were done in R software version 3.6.0 (R Core Team, 2019).

## 3 | RESULTS

The in-silico testing of the MiFish fish 12S primers could immediately distinguish 17 of the 18 fish species included in the analyses in this study. The MiFish primers adapted to amphibians detected five unambiguous amphibian species (see Appendix S2 for more details and Table S3 for the operational taxonomic unit sequences). The eDNA metabarcoding results showed that none of the negative control samples contained any fish or amphibian DNA that were the target species for the metabarcoding pipeline. The DNA was expectedly very inhibited and purified to remove PCR inhibitors using a

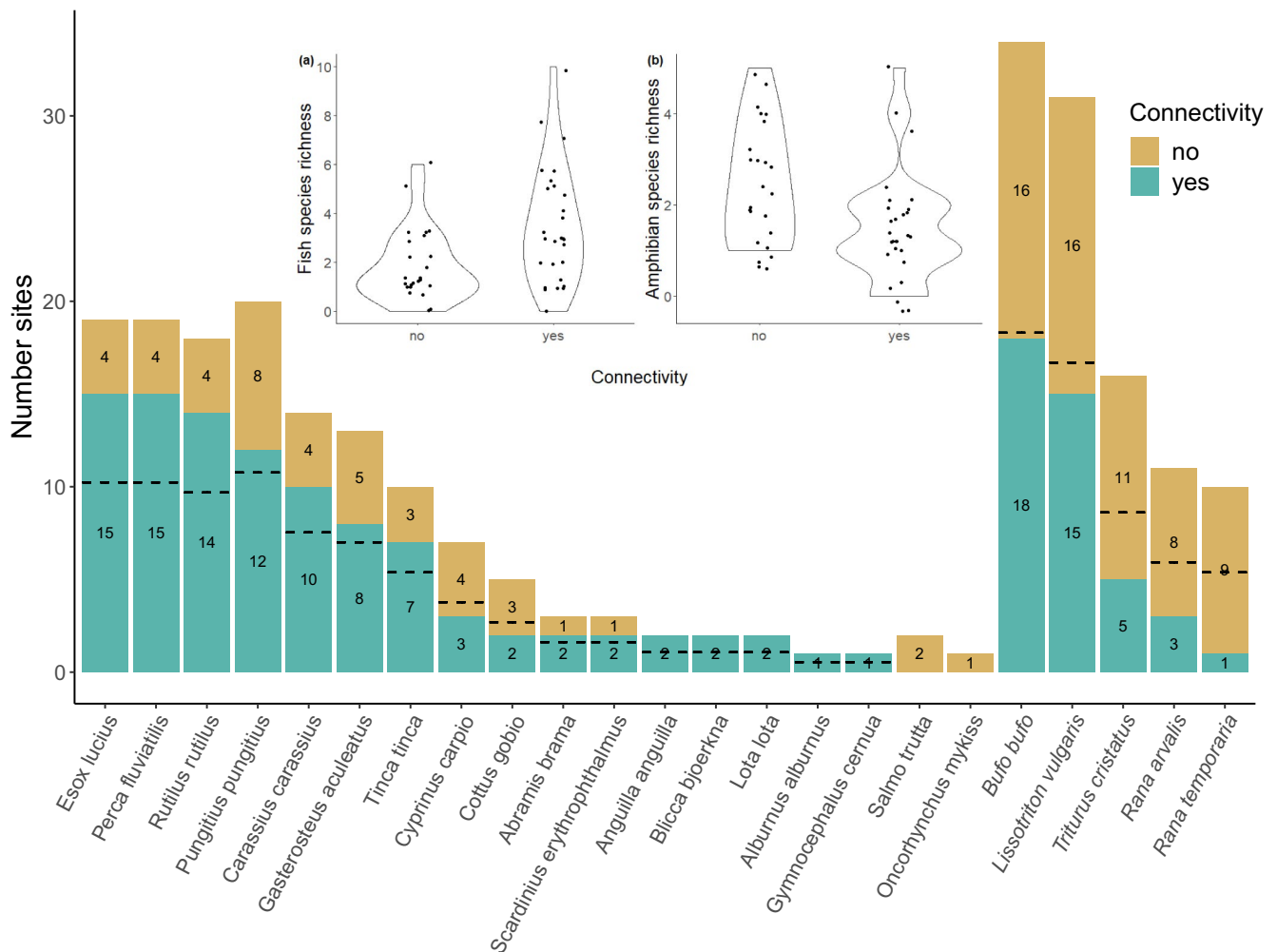
commercial purification kit. The DNA concentrations were measured after cleaning and sufficient for analyses. The results from field and laboratory quality controls, amount of water collected per sample and detailed sequencing results are outlined in Supplementary information, Appendix S2.

In total, we detected 18 fish species in the 52 investigated wetlands (Figure 3, Figure S2, Table S4). The most common fish species was nine-spined stickleback *Pungitius pungitius* (found in 20 wetlands), followed by Eurasian perch *Perca fluviatilis*, northern pike *Esox lucius* (each at 19 wetlands), Eurasian roach *Rutilus rutilus* (18), Crucian carp *Carassius carassius* (14), and three-spined stickleback *Gasterosteus aculeatus* (13). Three species were detected only once. Out of 32 fish species in the region, 15 were not detected in the created wetlands (Table S5).

We detected no fish species at three of the wetlands. At the other 49 wetlands (Figure S2), we found that the number of fish species ranged from one (18 wetlands; commonly *P. pungitius*) to 10 (one wetland), with a median of two. The frequency distribution of

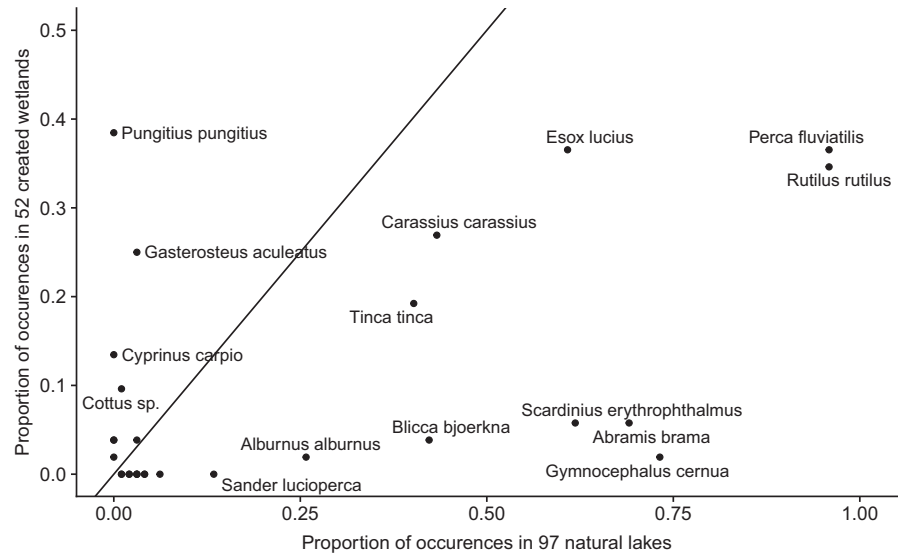
fish species richness was therefore skewed towards most wetlands having few species. The occurrence of some fish species in created wetlands reflected the occurrences of fish species at the regional level as judged from the long-term data from fish surveys in natural lakes (Figure 4, Table S1). However, many species of fish occurred infrequently in both created and natural wetlands, while others showed a mismatch between the frequency of occurrences between created and natural systems. Even though species such as *R. rutilus* and *P. fluviatilis* were common in both inventories, species such as common bream *Abramis brama* or ruffe *Gymnocephalus cernua* that are frequent in natural systems were rare in the created wetlands, while species such as *P. pungitius* and *G. aculeatus* were commonly found in created but not in natural wetlands.

We detected five amphibian species; the most frequently found was the common toad *Bufo bufo* (34 wetlands, Figure 3, Figure S2), followed by the smooth newt *Lissotriton vulgaris* (31) and great crested newt *Triturus cristatus* (16), while moor frog *Rana arvalis* (11) and common frog *Rana temporaria* (10) were least common.



**FIGURE 3** Fish and amphibian species occurrences in connected (turquoise) and isolated (dark yellow) wetlands. The black dashed lines indicate the number of wetlands a species would occur in connected wetlands if the occurrence had been random. The violin plots in the top illustrate (a) fish species richness and (b) amphibian species richness in isolated and connected wetlands. Of the 52 wetlands, 28 were connected and 24 isolated

**FIGURE 4** The comparison of species occurrence frequencies between the 52 created wetlands using eDNA data from the year 2018 (this study) and 97 natural lakes, surveyed by gill mesh nets within the Upland region. Black line represents identity line (i.e. 1:1). Species occurring in low frequencies (<4 sites) are not labelled but include *Abramis ballerus*, *Anguilla anguilla*, *Aspius aspius*, *Cobitis taenia*, *Coregonus albula*, *Coregonus lavaretus*, *Cyprinidae* sp. (only for 2 natural lakes), *Leuciscus idus*, *Leuciscus leuciscus*, *Lota lota*, *Oncorhynchus mykiss*, *Osmerus eperlanus*, *Salmo trutta*, and *Squalius cephalus*



**TABLE 2** Model estimates for each included environmental variable in relation to fish and amphibian species richness [95% confidence interval]

	Fish	Amphibians	Amphibians
Wetland size <sup>a</sup>	-0.01 [-0.19, 0.17]	-0.00 [-0.21, 0.20]	-0.00 [-0.20, 0.20]
Water vegetation	0.11 [-0.07, 0.29]	0.19 [-0.00, 0.39]	0.21 <sup>*</sup> [0.01, 0.40]
Flooded area		0.04 [-0.16, 0.25]	0.03 [-0.18, 0.24]
Connectivity	0.67 <sup>***</sup> [0.30, 1.04]	-0.54 <sup>**</sup> [-0.94, -0.13]	-0.47 <sup>*</sup> [-0.92, -0.02]
Year of creation	0.02 [-0.16, 0.20]		
Landscape forest	-0.19 <sup>*</sup> [-0.34, -0.04]	0.10 [-0.12, 0.32]	0.08 [-0.14, 0.31]
Fish richness			-0.09 [-0.33, 0.16]
AIC	207.83	172.71	174.21
N	52	52	52
Pseudo $r^2$	0.36	0.20	0.21

All variables were mean centred and scaled.

Abbreviation: AIC, Akaike information criterion

<sup>a</sup>Log-transformed.

\* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$

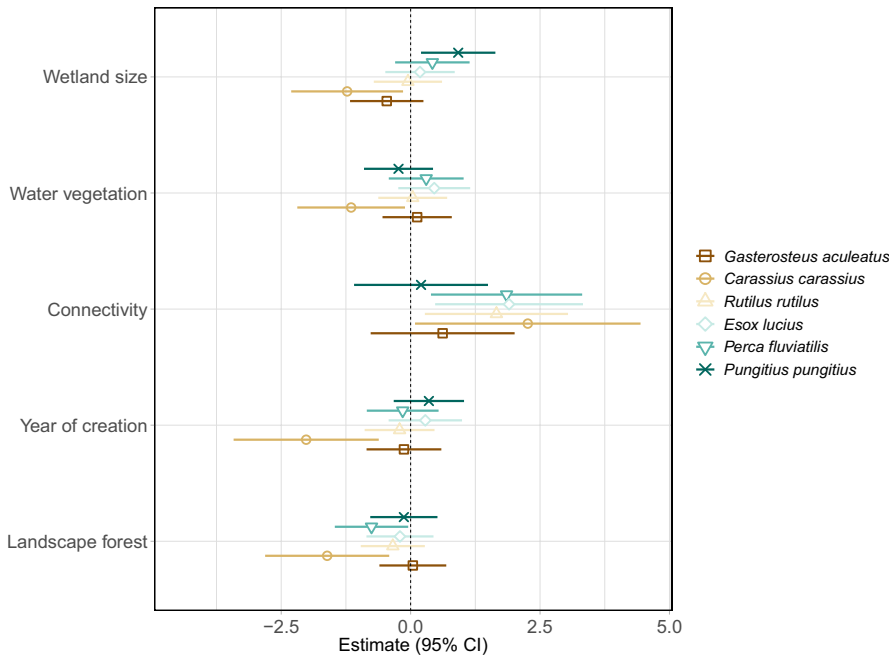
The only species occurring in the region that was not found in our eDNA samples is pool frog *Pelophylax lessonae*, which only breeds at the north-eastern coastal part where we did not sample (Figure 1). The frequency distribution of amphibian species richness was also skewed; most commonly, wetlands were inhabited by just one species (18 wetlands), but three wetlands had all five species (Figure S2). Five wetlands were amphibian-free.

### 3.1 | Fish occurrence patterns

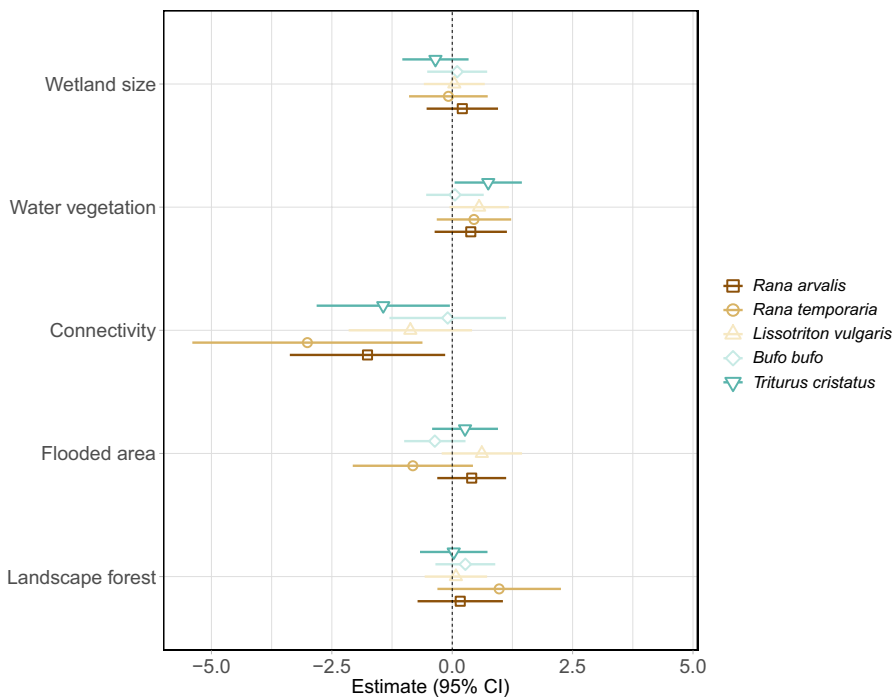
Fish species richness in created wetlands was related to wetland connectivity and the proportion of forest within one kilometre. On average, fish species richness was predicted to be twice as high (confidence interval [CI] = [1.4; 2.8]) in connected (predicted number of species [CI] = 3.43 [2.73; 4.13], when keeping other variables in their means) as compared to isolated wetlands (1.76 [1.19; 2.33], Table 2,

Figure 3a). Fish species richness was 53.7% [32.9; 88.5] lower in forest-dominated landscapes (90% forest cover, Table 2, Figure S3d) than in unforested ones (10% forest cover).

Similarly to the results for fish species richness, when investigating fish species individually, most species tended to occur more frequently in connected wetlands (e.g. *E. lucius*, *P. fluviatilis*, *R. rutilus*, *C. carassius*; Figures 3 and 5), and the occurrence of the most common species *P. fluviatilis* and *C. carassius* was related to landscape type with these species occurring less frequently in more forested wetland locations (Figure 5, Figure S4h,t). Furthermore, the occurrence of two species was related to wetland size; *P. pungitius* occurred more frequently in large wetlands (Figure 5, Figure S4a), while *C. carassius* only occurred in small ones (Figure 5, Figure S4q). *Carassius carassius* occurred more often in less vegetated wetlands (Figure 5, Figure S4r) and more often in older wetlands (Figure 5, Figure S4s), although the estimates for this species are very uncertain (large CI) and thus have to be viewed with caution.



**FIGURE 5** Model estimates for each environmental variable (centred and scaled) included in relation to the occurrence of the six most common fish species. Error bars represent 95% confidence intervals (CI). Wetland size was log-transformed



**FIGURE 6** Model estimates for each considered environmental variable (centred and scaled) in relation to the occurrence of each amphibian species. Error bars represent 95% confidence intervals (CI). Wetland size was log-transformed

For effect plots of all species and environmental variables, see Figures S3-S4.

### 3.2 | Amphibian occurrence patterns

Contrary to the pattern found in fish, model estimates indicated amphibian species richness was 41.5% lower (effect size 0.6 [0.39; 0.88] CI) in connected wetlands (predicted number of species [CI] = 1.45 [1; 1.90], when keeping the other variables at their means) than in isolated ones (2.47 [1.77; 3.18], Figure 3b, Table 2). Amphibian species richness was positively associated with the

proportion of water vegetation (Table 2, Figure S3f), where species richness was 59.9% [35.3; 100.2] higher in wetlands with high cover of water vegetation (90% cover of the wetland) than in those with low water vegetation (10% cover). When looking at species individually, the negative relationship to wetland connectivity (less frequent in connected wetlands) was evident for occurrences of *T. cristatus*, *R. arvalis*, and *R. temporaria*, but not for *B. bufo* and *L. vulgaris* (Figures 3 and 6). The positive relationship to the proportion of water vegetation was especially clear for the occurrence of *T. cristatus* (Figure 6, Figure S5b). Other environmental variables did not show clear relationships with amphibian occurrence (Figure 6, Figures S3 and S5).



Additionally, observed fish species richness did not clearly explain variation in amphibian species richness when added to the full model, i.e. model Akaike information criterion value increased when fish richness was included (Table 2, Figure S6). As toads are poisonous and might not be preyed upon by fish, we also analysed amphibian richness omitting *B. bufo*; however, such analyses did not change our conclusions concerning the lack of fish–amphibian association (fish richness estimate  $-0.1$  [ $-0.4, 0.21$ ]).

### 3.3 | Fish and amphibian co-occurrence

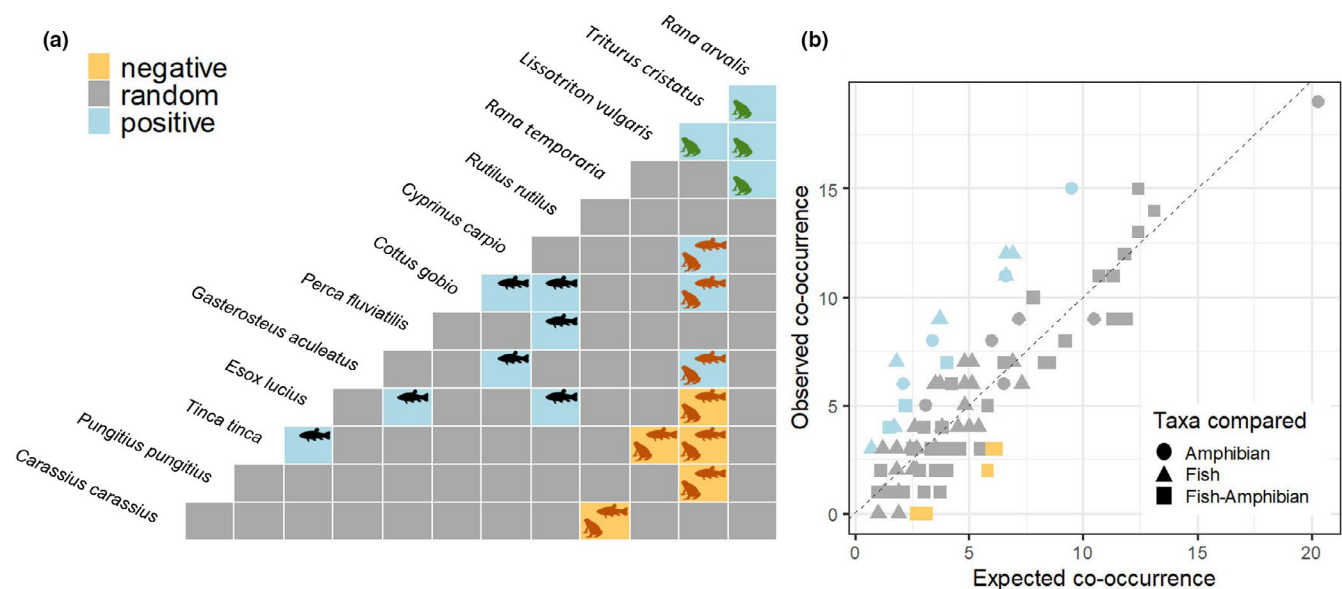
According to the probabilistic species co-occurrence analyses (Figure 7), which identified 91 species pairs, 14 (15.4%) co-occurrences were significantly positive, five (5.5%) significantly negative, and 72 were not statistically clear (79.1%). All negative co-occurrences were between fish and amphibians, while positive pairwise occurrences were within the fish community (7), within the amphibian community (4), or between amphibians and fish (3). *Triturus cristatus* occurred more often with European bullhead *Cottus gobio* (co-occurred 4 times, expected to co-occur 1.5 times under the null hypothesis of random associations), common carp *Cyprinus carpio* (co-occurred 5, expected 2.2), and *G. aculeatus* (co-occurred 7, expected 4). By contrast, *T. cristatus* occurred less frequently with *E. lucius* (co-occurred 2, expected 5.8), tench *Tinca tinca* (co-occurred 0, expected 3.1) and *P. pungitius* (co-occurred 3, expected 6.2). *L. vulgaris* presence was less likely in the presence of *T. tinca* (co-occurred 3, expected 6). *R. temporaria* presence was also less likely in the presence of *C. carassius* (co-occurred 0, expected 2.7). *R. arvalis*

and *B. bufo* did not show higher than expected co-occurrence pattern with fish (see also Figure S7). When it came to within taxa co-occurrences, i.e. either between fish species or between amphibian species, none of the co-occurrences were significantly negative.

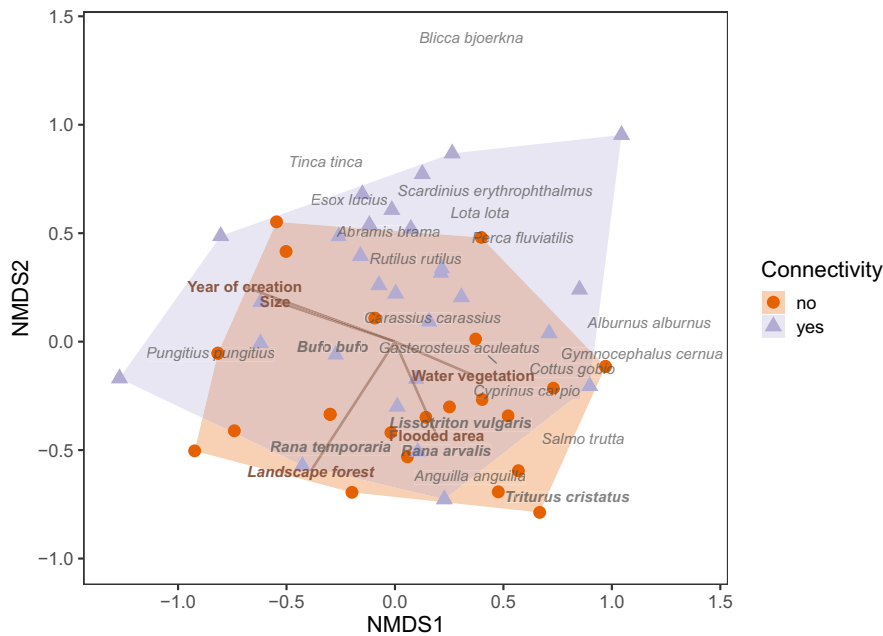
NMDS analyses showed a significant distinction between community composition in connected and isolated wetlands (Figure 8, Figure S8,  $r^2 = 0.09$ ,  $p = 0.006$ , centroid for isolated:  $-0.0218$  [NMDS1],  $-0.2286$  [NMDS2], connected  $0.0187$  [NMDS1],  $0.1959$  [NMDS2]), although it was a weak predictor (stress = 0.2). Other environmental variables did not show a correlation with the community assemblage gradient. Amphibians were concentrated in the central bottom part of the diagram (except for *B. bufo*), while species in the fish community were scattered around in the ordination space with more concentration to the top part of the diagram. Thus, amphibians showed a higher degree of niche similarity as compared to fish.

## 4 | DISCUSSION

We found fish species richness to be higher in connected wetlands, while the opposite was true for amphibian species richness. Additionally, amphibian occurrences showed a more aggregated pattern in the ordination analyses compared to the scattered pattern of fish occurrences. These different patterns in environmental space use might indicate negative associations between fish and amphibians, as we also observed some negative pairwise fish–amphibian co-occurrences. Still, out of the 52 surveyed wetlands, fish and amphibians did co-occur at 44 sites. However,



**FIGURE 7** (a) Probabilistic species co-occurrence matrix and (b) observed-expected plot. Colours represent negative (yellow), positive (blue), and random (grey) species associations. Yellow and light blue colours indicate significant, above 0.95, probabilities. For illustration purposes, associations within amphibians (green, circle) and fish (black, triangle), and between amphibians and fish (brown, square) are marked with appropriate colours and shapes. We excluded species occurring in three or fewer wetlands (*Alburnus alburnus*, *Abramis brama*, *Anguilla anguilla*, *Blicca bjoerkna*, *Gymnocephalus cernua*, *Lota lota*, *Oncorhynchus mykiss*, *Salmo trutta*, *Scardinius erythrophthalmus*), while species with only non-significant associations are not illustrated (*Bufo bufo*, panel a)



**FIGURE 8** The non-metric multidimensional scaling (NMDS) plot of all wetlands (stress 0.2) shows the clustering of communities within connected and isolated wetlands. The arrows show the five fitted environmental gradients; however, none of them were significant. Triangles and circles represent connected and isolated wetlands; shaded areas are the convex hulls of the triangles and points, respectively. Rainbow trout (*Oncorhynchus mykiss*) was excluded because it is non-native and occurred in one wetland only

fish–amphibian co-occurrence when *B. bufo* is excluded, because it is poisonous, dropped to 31 sites (see below). Therefore, we cannot rule out that fish–amphibian interactions may be of importance when amphibians colonise wetlands created for biodiversity. However, the high frequency of fish–amphibian co-occurrences suggests that any pattern of amphibian avoidance or exclusion by fish might be counterbalanced by other characteristics of the wetland, enabling the co-occurrence of these two species groups.

Although we found all amphibian species expected to be in the surveyed created wetlands, we found 17 (out of which two are non-native) fish species expected to also occur in the region, which is 53% of the total species pool. Of these fish species, some were more common while others rarer in created compared to natural wetlands. Such differences between fish species communities in created and natural wetlands may come from specific species adaptations and niche requirements but also from the different inventory methods used. As eDNA metabarcoding may detect species that would be missed by conventional methods to survey fish, our results contribute to the general knowledge of amphibian–fish species co-occurrence patterns, especially so for the associations with small-bodied fish as these may go undetected when using conventional survey methods (see below).

#### 4.1 | Fish occurrence patterns

Connectivity, that is, the physical connection to other waters, was an important predictor associated with fish species richness and occurrence, as more species were found in connected compared to isolated wetlands. This does not come as a surprise, as physical connectivity makes colonisation easier for fish (Hammer, 1992; Streever & Crisman, 1993). Additionally, methane (indicating

anoxia) is significantly correlated with variation in fish community composition in isolated Swedish wetlands (Öhman et al., 2006) as it reduces long-term fish survival. Still, isolated wetlands were able to support up to six fish species with 1.8 species on average. One of the most obvious explanations for the occurrences of some fish species in created wetlands is stocking (cf. Søndergaard et al., 2018), and in Sweden, fish stocking permits are not required for isolated water bodies (SFS, 2020). Therefore, it is likely that some of these occurrences were due to stocking, especially when it comes to non-native species and in isolated wetlands. However, fish occurrence in isolated wetlands could also be related to the creation process, when fish are trapped at the initial filling of a wetland (cf. Snodgrass et al., 1996) or through irrigation (Langston & Kent, 1997) or natural causes, such as terrestrial dispersal (European eel *Anguilla anguilla*, Bergmann, 1978) or flooding events (e.g. *A. brama*, Grift et al., 2001).

When considering the fish species pool in natural lakes of this region, many species were rare in both created and natural wetlands. A few species—*P. fluviatilis*, *E. lucius*, *R. rutilus*, and *C. carassius*—were relatively common in natural lakes and created wetlands. Yet other species common in natural lakes were almost absent in created wetlands. Created wetlands usually are shallow and lack a large pelagic zone, and, therefore, some fish species adapted to large, open or deep waters will not thrive in the created wetlands (e.g. bleak *Alburnus alburnus*). By contrast, few fish species occurred more often in created than in natural wetlands, such as *P. pungitius*, *G. aculeatus*, *C. carpio*, and *C. gobio*. *P. pungitius* and *G. aculeatus* are among the smallest fish species in this region and thus are often missed in the surveys using multi-mesh gill-nets (also used for the natural lake surveys). The catchability of different fish species differs; however, eDNA from these small species was obviously detected in the created wetlands. Additionally, the conditions in created wetlands might be beneficial for species that have a high tolerance to hypoxic conditions (such as

*C. carassius* and *C. carpio*, e.g. Nilsson & Renshaw, 2004; VanRaaij et al., 1996).

Regardless of the source for fish colonisation, some species will initially flourish but might disappear after some time (cf. Degani et al., 1998); therefore, other habitat characteristics not related to colonisation will partly determine whether fish will establish and thus be possible to detect. However, wetland size was not distinctly related to species richness in contrast to what we expected (i.e. due to species–area relationship). This suggests that size does not limit the fish species community or that created wetlands are too small for seeing the species–area relationship, as created wetlands had half of the regional species pool. We also did not find support for our expectation of older wetlands to have more species. This could be because the youngest wetland in our study was at least 5 years old, where most of the species may already have colonised the created wetlands at the time of our surveys (cf. fast colonisers of created wetlands, Kristensen et al., 2020). Additionally, confidence intervals are wide and cover a range of non-trivial effect sizes, and we cannot exclude the possibility that we simply were not able to detect the effect.

The fish community was associated with the landscape context as wetlands with a high proportion of forest in the surroundings tended to have fewer species and occurrences of two species (*C. carassius* and *P. fluviatilis*). During spring, organic acids are usually washed out into the wetlands (minimum pH; cf. Laudon et al., 2000); therefore, wetlands in the forested landscape may be rather acidic compared to those located in agricultural land, as the latter land-use increases soil pH and thus surrounding water bodies (Renberg et al., 1993). Some fish tolerate acidity, but even acid-tolerant *P. fluviatilis* and *C. carassius* occurred less often in wetlands located in a forested landscape. Alternatively, wetlands in open areas (e.g. non-forested) in the flat Upland region may be more prone to flooding than those in forested areas, thus wetlands in non-forested landscapes may be more easily colonised by fish.

## 4.2 | Amphibian occurrence patterns

Avoidance of, or exclusion by, fish is one explanation to why species richness and occurrence of *T. cristatus*, *R. temporaria*, and *R. arvalis*, were higher in isolated wetlands compared to fish-rich connected ones. As illustrated by the NMDS analysis, amphibian species, except *B. bufo*, display a narrower environmental niche space than the fish community. Predatory fish can completely deplete the amphibian larvae from the wetland (Heyer et al., 1975). Therefore, predatory fish are usually negatively related to the amphibian presence (Porej & Hetherington, 2005), but this is not always the case, especially when the fish present are native (Pearl et al., 2005). *Bufo bufo* is an exception from this pattern as this species is toxic and is not usually preyed upon by fish (Manteifel & Reshetnikov, 2002) and can even prefer ponds with fish (Beebee, 1979). Although unlikely, we cannot rule out alternative explanations for the amphibian preference of isolated wetlands, for instance, inflow could contribute to

the influx of pollutants from agricultural fields (Harper et al., 2020), for which amphibians would be sensitive.

In general, out of the nine fish species analysed, only four species displayed a clear negative association with amphibians, of which *E. lucius* could be directly linked to predation, while the other three fish species are probably linked to the competition for food (cf. Reshetnikov, 2003). Similarly to other research (Harper et al., 2020; Magnus & Rannap, 2019), *T. cristatus* was most sensitive to the presence of fish (i.e. three negative associations with fish). However, amphibians often co-occurred with fish, and there were also several positive associations between amphibians and fish, although only with *T. cristatus* (three positive associations). This latter result contrasts the claims that *T. cristatus* altogether avoid fish (Magnus & Rannap, 2019; Skei et al., 2006). Some of the fish species positively co-occurring with amphibians could reduce amphibian larva predation by consuming their predators, including predatory insects (Brown et al., 2012), such as *Aeshna* dragonfly larva (Laurila et al., 2008) or other Odonata species (Johansson & Brodin, 2003). The general amphibian co-occurrence with fish could also reflect variation in habitat quality and productivity. For example, a higher cover of water vegetation is generally important for amphibians as it provides breeding habitat and protection from predators (Shulze et al., 2012). Water vegetation cover was positively related to amphibian species richness and at least to the occurrence of *T. cristatus*, which is in line with previous studies (Brown et al., 2012; Hecnar & M'Closkey, 1997; Shulze et al., 2012). However, some species' relatively low occurrence frequencies reduce the power to detect interspecific associations, which is why an absence of a clear co-occurrence pattern within and between fish and amphibians investigated should be taken with quite some care.

Several studies suggest that many amphibian species prefer small wetlands because fish are more likely to be absent because of unfavourable conditions for fish survival (Harper et al., 2020; Semlitsch et al., 2015). However, we did not find any clear relationship between wetland size and richness or occurrence of amphibians. All our sampled wetlands, however, were larger than 0.5 ha, while the suggested optimal size for many amphibian species may be as small as 0.1 ha (Semlitsch et al., 2015; but see Bancila et al., 2017). Therefore, similarly to studies involving larger ponds (Landi et al., 2014; Porej & Hetherington, 2005), we may have failed to detect such a preference for the smallest wetlands as these were not available in our study area. Furthermore, as most wetlands had fish, probably because they were large enough not to dry out entirely or freeze, we may lack relevant variation in wetland size to detect the amphibian preference for smaller, fish-free wetlands. We also predicted flooded areas creating temporary pools to positively relate to amphibian species as it could provide shelter from predators (Porej & Hetherington, 2005; Tramer, 1977). We found no such clear relationship, probably due to increased connectivity for small-bodied fish (Lyon et al., 2010).

Finally, in contrast to fish, amphibians displayed no clear relationship to the landscape setting of created wetlands, despite the suggestion that forest cover is a positive predictor for amphibian

occurrence (Pearl et al., 2005), either due to dispersal facilitation (Brown et al., 2012) or improved water quality (Simon et al., 2009). The lack of correlation here could be because the Uppland region is rather forested and does not lack moist areas for dispersal and all five species detected in the created wetlands were widespread in this region (Swedish Species Information Centre, SLU, 2020).

### 4.3 | Study limitations when using eDNA metabarcoding

As with any other field survey methods, despite numerous investigations demonstrating that eDNA metabarcoding as a tool to detect amphibian and fish species is an efficient and precise survey method (Lopes et al., 2021, Miya et al., 2020), the use of eDNA analyses has its limitations. First, wetlands are usually rich in humic acids, which easily interfere with molecular analyses (Matheson et al., 2010). The samples in this survey were inhibited, and the inhibition removal process may have reduced the available DNA for sequencing, thus reducing overall detection probability (but see Appendix S2 for comparison with other studies). Second, in this survey we used Miya et al., (2015) MiFish primers for fish and adapted them towards amphibians' 12S region on the reverse primer to avoid amphibian presence being masked by fish. Even though this reduces the risk, there is still a possibility that amphibian species with low eDNA abundance in these ponds may be underrepresented, and other mitochondrial DNA regions might be more suitable (e.g. 16S region). The common and widespread species *R. arvalis* and *R. temporaria* occurred perhaps less frequently than expected, while *T. cristatus* was more frequent in our study sites. Thirdly, falsely detected occurrences (e.g. *Clupea harengus* and *Gadus morhua*) are usually a consequence of contamination from sewage, recreation, excrements from fish-eating birds (Guilfoyle & Schultz, 2017) and by water inflow from adjacent wetlands harbouring fish (Hänfling et al., 2016; Harper et al., 2020), which could have influenced our interpretation of fish presences in connected wetlands or wetlands close to recreational places, although fast degradation of eDNA reduces such risk. Last, we do not have data on fish age that in turn would inform us about fish cohort structure, which could affect predation risk patterns (Kloskowski, 2009).

Nevertheless, eDNA metabarcoding is a powerful tool to detect common species, and, particularly efficient in detecting species that are elusive, static, or rare (Bálint et al., 2018; Hänfling et al., 2016), hard to detect or identify (e.g. early developmental stages, Fujii et al., 2019; Lopes et al., 2017), or, in general, species with low catchability with standard methods, such as multi-mesh gill-nets (Sutela et al., 2008).

## 5 | CONCLUSIONS

When creating wetlands to facilitate biodiversity, fish is not usually a target taxon. Still, fish come almost always with the water, either

by natural colonisation through watercourses (i.e. connectivity) or by stocking. As partly suggested by our study, fish presence may impact the wetland use by communities of other wetland taxa, such as amphibians, based on the observed opposing preferences of connected and isolated wetlands. However, our study also suggests that fish and amphibians can co-exist. The creation of larger wetlands and increased habitat heterogeneity in terms of water vegetation, and shape and structure of the wetland could be possible factors enabling the co-existence of these two taxa, but this requires further studies.

Environmental DNA metabarcoding is a relatively cheap and time-efficient method to conduct surveys of fish and amphibian species occurrences in wetlands. Furthermore, the non-invasive eDNA surveys would also include small-bodied fish that otherwise might be missed by conventional fish surveys using multi-mesh gill-nets. The knowledge about fish community assemblages in created biodiversity wetlands using eDNA metabarcoding, thus provides new and detailed insight into fish occurrence patterns and possible associations with amphibians and other taxa to improve the future creation of wetlands for biodiversity.

### ACKNOWLEDGMENTS

Thanks to AquaBiota Water Research and Hannah Phillips for help with sample collection, MoRe Research for the eDNA extractions and NatureMetrics Ltd for eDNA metabarcoding and bioinformatics. We especially want to thank Anders Kinnerbäck for sharing the fish inventory data of the 97 lakes and Daniel Brelín and Niklas Sjöberg from Uppsala and Stockholm county administrative boards for providing the fish stocking data. We also want to thank Patrik Bohman for the early project discussion and Simon Kärverno and Johan Elmberg for suggestions regarding the manuscript. The research was financially supported by the 2017–2018 Belmont Forum and BiodivERSA joint call for research proposals, under the BiodivScen ERA–Net COFUND program Formas [2018-02440], Research Council FORMAS [215-2014-1425], Swedish EPA [13/361] and Oscar and Lili Lamm's foundation [2016-0022], all to TP.

### DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available from Swedish National Data Service: <https://doi.org/10.5878/gzq7-8q71>

### ORCID

Ineta Kačergytė  <https://orcid.org/0000-0003-4756-8253>

### REFERENCES

- Albanese, B., Angermeier, P. L., & Peterson, J. T. (2009). Does mobility explain variation in colonisation and population recovery among stream fishes? *Freshwater Biology*, 54(7), 1444–1460. <https://doi.org/10.1111/j.1365-2427.2009.02194.x>

- Baker, J. M. R., & Halliday, T. R. (1999). Amphibian colonisation of new ponds in an agricultural landscape. *Herpetological Journal*, 9(2), 55–63.
- Bálint, M., Nowak, C., Márton, O., Pauls, S. U., Wittwer, C., Aramayo, J. L., ... Jansen, M. (2018). Accuracy, limitations and cost efficiency of eDNA-based community survey in tropical frogs. *Molecular Ecology Resources*, 18(6), 1415–1426. <https://doi.org/10.1111/1755-0998.12934>
- Bancila, R. I., Coghincianu, D., Ozgul, A., & Schmidt, B. R. (2017). The effect of aquatic and terrestrial habitat characteristics on occurrence and breeding probability in a montane amphibian: Insights from a spatially explicit multistate occupancy model. *Population Ecology*, 59(1), 71–78. <https://doi.org/10.1007/s10144-017-0575-4>
- Beatty, R. J., Rahel, F. J., & Hubert, W. A. (2009). Complex influences of low-head dams and artificial wetlands on fishes in a Colorado River tributary system. *Fisheries Management and Ecology*, 16(6), 457–467. <https://doi.org/10.1111/j.1365-2400.2009.00695.x>
- Beebee, T. J. C. (1979). Habitats of the British amphibians (2): Suburban parks and gardens. *Biological Conservation*, 15(4), 241–257. [https://doi.org/10.1016/0006-3207\(79\)90046-6](https://doi.org/10.1016/0006-3207(79)90046-6)
- Bergmann, A. (1978). *Aale gehen an Land: E. Studie zur Biologie d. Aals (1. Aufl)*. Ifland.
- Bobbink, R., Beltman, B., Verhoeven, J. T. A., & Whigham, D. F. (2007). *Wetlands: Functioning, Biodiversity Conservation, and Restoration*, Vol. 191. Springer Science & Business Media.
- Bouffard, S. H., & Hanson, M. A. (1997). Fish in waterfowl marshes: Waterfowl Managers' perspective. *Wildlife Society Bulletin*, 146–157.
- Brown, D. J., Street, G. M., Nairn, R. W., & Forstner, M. R. J. (2012). A place to call home: Amphibian use of created and restored wetlands. *International Journal of Ecology*, 2012, 1–11. <https://doi.org/10.1155/2012/989872>
- Cohen, J. M., Civitello, D. J., Venesky, M. D., McMahon, T. A., & Rohr, J. R. (2019). An interaction between climate change and infectious disease drove widespread amphibian declines. *Global Change Biology*, 25(3), 927–937. <https://doi.org/10.1111/gcb.14489>
- Davidson, N. C. (2014). How much wetland has the world lost? Long-term and recent trends in global wetland area. *Marine and Freshwater Research*, 65(10), 934–941. <https://doi.org/10.1071/MF14173>
- Degani, G., Yehuda, Y., Jackson, J., & Gophen, M. (1998). Temporal variation in fish community structure in a newly created wetland lake (Lake Agmon) in Israel. *Wetlands Ecology and Management*, 6(2), 151–157. <https://doi.org/10.1023/A:1008463918715>
- Dietrichson, W. (2017). *Häckande fåglar i anlagda vatten i Uppland Inventering 2012–2014 (2017:02)*. Länsstyrelsen i Uppsala län.
- Dixon, M. J. R., Loh, J., Davidson, N. C., Beltrame, C., Freeman, R., & Walpole, M. (2016). Tracking global change in ecosystem area: The Wetland Extent Trends index. *Biological Conservation*, 193, 27–35. <https://doi.org/10.1016/j.biocon.2015.10.023>
- Elmberg, J., Dessborn, L., & Englund, G. (2010). Presence of fish affects lake use and breeding success in ducks. *Hydrobiologia*, 641(1), 215–223. <https://doi.org/10.1007/s10750-009-0085-2>
- Ficetola, G. F., Manenti, R., & Taberlet, P. (2019). Environmental DNA and metabarcoding for the study of amphibians and reptiles: Species distribution, the microbiome, and much more. *Amphibia-Reptilia*, 40(2), 129–148. <https://doi.org/10.1163/15685381-20191194>
- Fujii, K., Doi, H., Matsuoka, S., Nagano, M., Sato, H., & Yamanaka, H. (2019). Environmental DNA metabarcoding for fish community analysis in backwater lakes: A comparison of capture methods. *PLoS One*, 14(1), e0210357. <https://doi.org/10.1371/journal.pone.0210357>
- Griffith, D. M., Veech, J. A., & Marsh, C. J. (2016). *co-occur: Probabilistic species co-occurrence analysis in R (Version 1.3)*. <https://CRAN.R-project.org/package=cooccur>
- Grift, R. E., Buljse, A. D., Breteler, J. G. P. K., van Densen, W. L. T., Machiels, M., ... Backx, J. J. M. (2001). Migration of bream between the main channel and floodplain lakes along the lower River Rhine during the connection phase. *Journal of Fish Biology*, 59(4), 1033–1055. <https://doi.org/10.1111/j.1095-8649.2001.tb00170.x>
- Guilfoyle, M. P., & Schultz, M. T. (2017). The contribution of double-crested cormorants (*Phalacrocorax auritus*) to silver carp (*Hypophthalmichthys molitrix*) DNA loads in the Chicago Area Waterway System. *Journal of Great Lakes Research*, 43(6), 1181–1185. <https://doi.org/10.1016/j.jglr.2017.09.008>
- Hammer, D. A. (1992). Designing constructed wetlands systems to treat agricultural nonpoint source pollution. *Ecological Engineering*, 1(1), 49–82. [https://doi.org/10.1016/0925-8574\(92\)90025-W](https://doi.org/10.1016/0925-8574(92)90025-W)
- Hänfling, B., Lawson Handley, L., Read, D. S., Hahn, C., Li, J., Nichols, P., ... Winfield, I. J. (2016). Environmental DNA metabarcoding of lake fish communities reflects long-term data from established survey methods. *Molecular Ecology*, 25(13), 3101–3119. <https://doi.org/10.1111/mec.13660>
- Harper, L. R., Buxton, A. S., Rees, H. C., Bruce, K., Brys, R., Halfmaerten, D., ... Hänfling, B. (2019). Prospects and challenges of environmental DNA (eDNA) monitoring in freshwater ponds. *Hydrobiologia*, 826(1), 25–41. <https://doi.org/10.1007/s10750-018-3750-5>
- Harper, L. R., Lawson Handley, L., Hahn, C., Boonham, N., Rees, H. C., Lewis, E., ... Hänfling, B. (2020). Generating and testing ecological hypotheses at the pondscape with environmental DNA metabarcoding: A case study on a threatened amphibian. *Environmental DNA*, 2(2), 184–199. <https://doi.org/10.1002/edn3.57>
- Hartel, T., Nemes, S., Coghincianu, D., Öllerer, K., Schweiger, O., Moga, C.-I., & Demeter, L. (2007). The effect of fish and aquatic habitat complexity on amphibians. *Hydrobiologia*, 583(1), 173. <https://doi.org/10.1007/s10750-006-0490-8>
- Hecnar, S. J., & M'Closkey, R. T. (1997). The effects of predatory fish on amphibian species richness and distribution. *Biological Conservation*, 79(2), 123–131. [https://doi.org/10.1016/S0006-3207\(96\)00113-9](https://doi.org/10.1016/S0006-3207(96)00113-9)
- Heyer, W. R., McDiarmid, R. W., & Weigmann, D. L. (1975). Tadpoles, predation and pond habitats in the tropics. *Biotropica*, 7(2), 100–111. JSTOR. <https://doi.org/10.2307/2989753>
- Johansson, F., & Brodin, T. (2003). Effects of fish predators and abiotic factors on dragonfly community structure. *Journal of Freshwater Ecology*, 18(3), 415–423. <https://doi.org/10.1080/02705060.2003.9663977>
- Kačergytė, I., Arlt, D., Berg, Å., Žmihorski, M., Knape, J., Rosin, Z. M., & Pärt, T. (2021). Evaluating created wetlands for bird diversity and reproductive success. *Biological Conservation*, 257, 109084. <https://doi.org/10.1016/j.biocon.2021.109084>
- Kloskowski, J. (2009). Size-structured effects of common carp on reproduction of pond-breeding amphibians. *Hydrobiologia*, 635(1), 205–213. <https://doi.org/10.1007/s10750-009-9912-8>
- Kloskowski, J., Nieoczym, M., Polak, M., & Pitucha, P. (2010). Habitat selection by breeding waterbirds at ponds with size-structured fish populations. *Naturwissenschaften*, 97(7), 673–682. <https://doi.org/10.1007/s00114-010-0684-9>
- Kristensen, E., Sand-Jensen, K., Kristensen, J. S. B., Pedersen, M. E., Baastrup-Spohr, L., & Kragh, T. (2020). Early fish colonisation and community development in a shallow re-established lake. *Ecological Engineering*, 155, 105956. <https://doi.org/10.1016/j.ecoleng.2020.105956>
- Kusler, J. A. (2012). *Wetland creation and restoration: The status of the science*. Island Press.
- Landi, M., Piazzini, S., & Saveri, C. (2014). The response of amphibian communities to fish and habitat features in Mediterranean permanent ponds. *Biologia*, 69(6), 806–810. <https://doi.org/10.2478/s11756-014-0377-5>
- Langston, M. A., & Kent, D. M. (1997). Fish recruitment to a constructed wetland. *Journal of Freshwater Ecology*, 12(1), 123–129. <https://doi.org/10.1080/02705060.1997.9663515>
- Lantmäteriet (2020). Retrieved from <https://geolex.etjanster.lantmateriet.se/>. 10 September 2020.

- Laudon, H., Westling, O., & Bishop, K. (2000). Cause of pH decline in stream water during spring melt runoff in northern Sweden. *Canadian Journal of Fisheries and Aquatic Sciences*, 57(9), 1888–1900. <https://doi.org/10.1139/f00-131>
- Laurila, A., Lindgren, B., & Laugen, A. T. (2008). Antipredator defenses along a latitudinal gradient in *Rana temporaria*. *Ecology*, 89(5), 1399–1413. <https://doi.org/10.1890/07-1521.1>
- Lawson Lawson Handley, L., Read, D. S., Winfield, I. J., Kimbell, H., Johnson, H., Li, J., ...Hänfing, B. (2019). Temporal and spatial variation in distribution of fish environmental DNA in England's largest lake. *Environmental DNA*, 1(1), 26–39. <https://doi.org/10.1002/edn3.5>
- Lemmens, P., Mergeay, J., De Bie, T., Van Wichelen, J., De Meester, L., & Declercq, S. A. J. (2013). How to maximally support local and regional biodiversity in applied conservation? Insights from pond management. *PLoS One*, 8(8), e72538. <https://doi.org/10.1371/journal.pone.0072538>
- Lesbarrères, D., Fowler, M. S., Pagano, A., & Lodé, T. (2010). Recovery of anuran community diversity following habitat replacement. *Journal of Applied Ecology*, 47(1), 148–156. <https://doi.org/10.1111/j.1365-2664.2009.01748.x>
- Lopes, C. M., Sasso, T., Valentini, A., Dejean, T., Martins, M., Zamudio, K. R., & Haddad, C. F. B. (2017). eDNA metabarcoding: A promising method for anuran surveys in highly diverse tropical forests. *Molecular Ecology Resources*, 17(5), 904–914. <https://doi.org/10.1111/1755-0998.12643>
- Lopes C. M., Baêta D., Valentini A., Lyra M. L., Sabbag A. F., Gasparini J. L., Dejean T., Haddad C. F. B., Zamudio K. R. (2021). Lost and found: Frogs in a biodiversity hotspot rediscovered with environmental DNA. *Molecular Ecology*, 30 (13), 3289–3298. <http://dx.doi.org/10.1111/mec.15594>.
- Lyon, J., Stuart, I., Ramsey, D., & O'Mahony, J. (2010). The effect of water level on lateral movements of fish between river and off-channel habitats and implications for management. *Marine and Freshwater Research*, 61(3), 271. <https://doi.org/10.1071/MF08246>
- Magnus, R., & Rannap, R. (2019). Pond construction for threatened amphibians is an important conservation tool, even in landscapes with extant natural water bodies. *Wetlands Ecology and Management*, 27(2), 323–341. <https://doi.org/10.1007/s11273-019-09662-7>
- Manteifel, Y. B., & Reshetnikov, A. N. (2002). Avoidance of noxious tadpole prey by fish and invertebrate predators: Adaptivity of a chemical defence may depend on predator feeding habits. *Fundamental and Applied Limnology*, 153(4), 657–668. <https://doi.org/10.1127/archivhydrobiol/153/2002/657>
- Matheson, C. D., Gurney, C., Esau, N., & Lehto, R. (2010). Assessing PCR inhibition from humic substances. *The Open Enzyme Inhibition Journal*, 3(1), 38–45. <https://doi.org/10.2174/1874940201003010038>
- McElroy, M. E., Dressler, T. L., Titcomb, G. C., Wilson, E. A., Deiner, K., Dudley, T. L., ... Jerde, C. L. (2020). Calibrating environmental DNA metabarcoding to conventional surveys for measuring fish species richness. *Frontiers in Ecology and Evolution*, 8, 276. <https://doi.org/10.3389/fevo.2020.00276>
- Meurling, S., Kärveemo, S., Chondrelli, N., Cortazar Chinarro, M., Åhlen, D., Brookes, L., ... Laurila, A. (2020). Occurrence of *Batrachochytrium dendrobatidis* in Sweden: Higher infection prevalence in southern species. *Diseases of Aquatic Organisms*, 140, 209–218. <https://doi.org/10.3354/dao03502>
- Miya, M., Gotoh, R. O., & Sado, T. (2020). MiFish metabarcoding: A high-throughput approach for simultaneous detection of multiple fish species from environmental DNA and other samples. *Fisheries Science*, 86, 939–970. <https://doi.org/10.1007/s12562-020-01461-x>
- Miya, M., Sato, Y., Fukunaga, T., Sado, T., Poulsen, J. Y., Sato, K., ... Iwasaki, W. (2015). MiFish, a set of universal PCR primers for metabarcoding environmental DNA from fishes: Detection of more than 230 subtropical marine species. *Royal Society Open Science*, 2(7), 150088. <https://doi.org/10.1098/rsos.150088>
- Moushomi, R., Wilgar, G., Carvalho, G., Creer, S., & Seymour, M. (2019). Environmental DNA size sorting and degradation experiment indicates the state of *Daphnia magna* mitochondrial and nuclear eDNA is subcellular. *Scientific Reports*, 9(1), 12500. <https://doi.org/10.1038/s41598-019-48984-7>
- Nilsson, G. E., & Renshaw, G. M. C. (2004). Hypoxic survival strategies in two fishes: Extreme anoxia tolerance in the North European crucian carp and natural hypoxic preconditioning in a coral-reef shark. *Journal of Experimental Biology*, 207(18), 3131–3139. <https://doi.org/10.1242/jeb.00979>
- Öhman, J., Buffam, I., Englund, G., Blom, A., Lindgren, E., & Laudon, H. (2006). Associations between water chemistry and fish community composition: A comparison between isolated and connected lakes in northern Sweden. *Freshwater Biology*, 51(3), 510–522. <https://doi.org/10.1111/j.1365-2427.2006.01514.x>
- Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Wagner, H. et al (2019). *Vegan: Community Ecology Package (Version 2.5-6)*. <https://CRAN.R-project.org/package=vegan>
- O'Toole, A. C., Hanson, K. C., & Cooke, S. J. (2009). The effect of shoreline recreational angling activities on aquatic and riparian habitat within an urban environment: Implications for conservation and management. *Environmental Management*, 44(2), 324–334. <https://doi.org/10.1007/s00267-009-9299-3>
- Pearl, C. A., Adams, M. J., Leuthold, N., & Bury, R. B. (2005). Amphibian occurrence and aquatic invaders in a changing landscape: Implications for wetland mitigation in the Willamette Valley, Oregon, USA. *Wetlands*, 25(1), 76–88. [https://doi.org/10.1672/0277-5212\(2005\)025\[0076:AOAAI\]2.0.CO;2](https://doi.org/10.1672/0277-5212(2005)025[0076:AOAAI]2.0.CO;2)
- Petranka, J. W., Kennedy, C. A., & Murray, S. S. (2003). Response of amphibians to restoration of a southern Appalachian wetland: A long-term analysis of community dynamics. *Wetlands*, 23(4), 1030–1042. [https://doi.org/10.1672/0277-5212\(2003\)023\[1030:ROATR O\]2.0.CO;2](https://doi.org/10.1672/0277-5212(2003)023[1030:ROATR O]2.0.CO;2)
- Pochardt, M., Allen, J. M., Hart, T., Miller, S. D. L., Yu, D. W., & Levi, T. (2020). Environmental DNA facilitates accurate, inexpensive, and multiyear population estimates of millions of anadromous fish. *Molecular Ecology Resources*, 20(2), 457–467. <https://doi.org/10.1111/1755-0998.13123>
- Pont, D., Valentini, A., Rocle, M., Maire, A., Delaigue, O., Jean, P., & Dejean, T. (2019). The future of fish-based ecological assessment of European rivers: From traditional EU Water Framework Directive compliant methods to eDNA metabarcoding-based approaches. *Journal of Fish Biology*, 98(2), 354–366. <https://doi.org/10.1111/jfb.14176>
- Pope, K. L. (2008). Assessing changes in amphibian population dynamics following experimental manipulations of introduced fish. *Conservation Biology*, 22(6), 1572–1581. <https://doi.org/10.1111/j.1523-1739.2008.00998.x>
- Porej, D., & Hetherington, T. E. (2005). Designing wetlands for amphibians: The importance of predatory fish and shallow Littoral Zones in structuring of amphibian communities. *Wetlands Ecology and Management*, 13(4), 445–455. <https://doi.org/10.1007/s11273-004-0522-y>
- R Core Team (2019). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. <https://www.R-project.org/>
- Renberg, I., Korsman, T., & Anderson, N. J. (1993). A temporal perspective of lake acidification in Sweden. *Ambio*, 22(5), 264–271.
- Reshetnikov, A. N. (2003). The introduced fish, rotan (*Perccottus glenii*), depresses populations of aquatic animals (macroinvertebrates, amphibians, and a fish). *Hydrobiologia*, 510(1), 83–90. <https://doi.org/10.1023/B:HYDR.0000008634.92659.b4>

- Schilling, E. G., Loftin, C. S., & Huryn, A. D. (2009). Effects of introduced fish on macroinvertebrate communities in historically fishless headwater and kettle lakes. *Biological Conservation*, 142(12), 3030–3038. <https://doi.org/10.1016/j.biocon.2009.08.003>
- Sebastián-González, E., & Green, A. J. (2014). Habitat use by waterbirds in relation to pond size, water depth, and isolation: Lessons from a restoration in southern Spain. *Restoration Ecology*, 22(3), 311–318. <https://doi.org/10.1111/rec.12078>
- Semlitsch, R. D. (1987). Interactions between fish and salamander larvae: Costs of predator avoidance or competition? *Oecologia*, 72(4), 481–486. <https://doi.org/10.1007/BF00378972>
- Semlitsch, R. D., Peterman, W. E., Anderson, T. L., Drake, D. L., & Ousterhout, B. H. (2015). Intermediate pond sizes contain the highest density, richness, and diversity of pond-breeding amphibians. *PLoS One*, 10(4), e0123055. <https://doi.org/10.1371/journal.pone.0123055>
- SEPA (2019). *Fördjupad utvärdering av miljömålen 2019*. Myllrande våtmarker. (Report, 6873), 142.
- SFS (2020). *Förordning (1994:1716) om fisket, vattenbruket och fiskerinäringen Svensk författningssamling 1994:1994:1716 t.o.m. SFS 2020:502—Riksdagen*. Retrieved from [https://www.riksdagen.se/sv/dokument-lagar/dokument/svensk-forfattningssamling/forordning-19941716-om-fisket-vattenbruket\\_sfs-1994-1716](https://www.riksdagen.se/sv/dokument-lagar/dokument/svensk-forfattningssamling/forordning-19941716-om-fisket-vattenbruket_sfs-1994-1716) 12 September 2020
- Shulse, C. D., Semlitsch, R. D., Trauth, K. M., & Gardner, J. E. (2012). Testing wetland features to increase amphibian reproductive success and species richness for mitigation and restoration. *Ecological Applications*, 22(5), 1675–1688. <https://doi.org/10.1890/11-0212.1>
- Sigsgaard, E. E., Carl, H., Møller, P. R., & Thomsen, P. F. (2015). Monitoring the near-extinct European weather loach in Denmark based on environmental DNA from water samples. *Biological Conservation*, 183, 46–52. <https://doi.org/10.1016/j.biocon.2014.11.023>
- Simon, J. A., Snodgrass, J. W., Casey, R. E., & Sparling, D. W. (2009). Spatial correlates of amphibian use of constructed wetlands in an urban landscape. *Landscape Ecology*, 24(3), 361–373. <https://doi.org/10.1007/s10980-008-9311-y>
- Skei, J., Ringsby, T., Rønning, L., & Dolmen, D. (2006). Habitat use during the aquatic phase of the newts *Triturus vulgaris* (L.) and *T. cristatus* (Laurenti) in central Norway: Proposition for a conservation and monitoring area. *Amphibia-Reptilia*, 27(3), 309–324. <https://doi.org/10.1163/156853806778189972>
- Snodgrass, J. W., Bryan, A. L., Lide, R. F., & Smith, G. M. (1996). Factors affecting the occurrence and structure of fish assemblages in isolated wetlands of the upper coastal plain, U.S.A. *Canadian Journal of Fisheries and Aquatic Sciences*, 53, 12. <https://doi.org/10.1139/f95-200>
- Snyder, D. E. (2003). Invited overview: Conclusions from a review of electrofishing and its harmful effects on fish. *Reviews in Fish Biology and Fisheries*, 13(4), 445–453. <https://doi.org/10.1007/s11160-004-1095-9>
- Søndergaard, M., Lauridsen, T. L., Johansson, L. S., & Jeppesen, E. (2018). Gravel pit lakes in Denmark: Chemical and biological state. *Science of the Total Environment*, 612, 9–17. <https://doi.org/10.1016/j.scitotenv.2017.08.163>
- Spens, J. S., & Ball, J. P. B. P. (2008). Salmonid or nonsalmonid lakes: Predicting the fate of northern boreal fish communities with hierarchical filters relating to a keystone piscivore. *Canadian Journal of Fisheries and Aquatic Science*, 65(9), 1945–1955. <https://doi.org/10.1139/F08-103>
- Spens, J., Evans, A. R., Halfmaerten, D., Knudsen, S. W., Sengupta, M. E., Mak, S. S. T., ... Hellström, M. (2017). Comparison of capture and storage methods for aqueous microbial eDNA using an optimised extraction protocol: Advantage of enclosed filter. *Methods in Ecology and Evolution*, 8(5), 635–645. <https://doi.org/10.1111/2041-210X.12683>
- Streever, W. J., & Crisman, T. L. (1993). A comparison of fish populations from natural and constructed freshwater marshes in Central Florida. *Journal of Freshwater Ecology*, 8(2), 149–153. <https://doi.org/10.1080/02705060.1993.9664845>
- Stuart, S. N., Chanson, J. S., Cox, N. A., Young, B. E., Rodrigues, A. S. L., Fischman, D. L., & Waller, R. W. (2004). Status and trends of amphibian declines and extinctions worldwide. *Science*, 306(5702), 1783–1786. <https://doi.org/10.1126/science.1103538>
- Sutela, T., Rask, M., Vehanen, T., & Westermark, A. (2008). Comparison of electrofishing and NORDIC gill-nets for sampling littoral fish in boreal lakes. *Lakes & Reservoirs: Science, Policy and Management for Sustainable Use*, 13(3), 215–220. <https://doi.org/10.1111/j.1440-1770.2008.00372.x>
- Swedish Species information Cente, S.L.U. (2020). Retrieved from <https://artfakta.se/>. 16 November 2020.
- Talley, D. M. (2000). Ichthyofaunal utilisation of newly-created versus natural salt marsh creeks in Mission Bay, CA. *Wetlands Ecology and Management*, 8(2), 117–132. <https://doi.org/10.1023/A:1008436301041>
- Tonn, W. M., Magnuson, J. J., Rask, M., & Toivonen, J. (1990). Intercontinental comparison of small-lake fish assemblages: The balance between local and regional processes. *The American Naturalist*, 136(3), 345–375. <https://doi.org/10.1086/285102>
- Tramer, E. J. (1977). Catastrophic mortality of stream fishes trapped in shrinking pools. *American Midland Naturalist*, 97(2), 469. <https://doi.org/10.2307/2425110>
- Turner, C. R., Barnes, M. A., Xu, C. C. Y., Jones, S. E., Jerde, C. L., & Lodge, D. M. (2014). Particle size distribution and optimal capture of aqueous microbial eDNA. *Methods in Ecology and Evolution*, 5(7), 676–684. <https://doi.org/10.1111/2041-210X.12206>
- VanRaaij, M. T. M., VandenThillart, G., Vianen, G. J., Pit, D. S. S., Balm, P. H. M., & Steffens, B. (1996). Substrate mobilisation and hormonal changes in rainbow trout (*Oncorhynchus mykiss*, L) and common carp (*Cyprinus carpio*, L) during deep hypoxia and subsequent recovery. *Journal of Comparative Physiology B-Biochemical Systems and Environmental Physiology*, 166(7), 443–452.
- Wilcox, T. M., McKelvey, K. S., Young, M. K., Lowe, W. H., & Schwartz, M. K. (2015). Environmental DNA particle size distribution from Brook Trout (*Salvelinus fontinalis*). *Conservation Genetics Resources*, 7(3), 639–641. <https://doi.org/10.1007/s12686-015-0465-z>
- Winandy, L., Darnet, E., & Denoel, M. (2015). Amphibians forgo aquatic life in response to alien fish introduction. *Animal Behaviour*, 109, 209–216. <https://doi.org/10.1016/j.anbehav.2015.08.018>
- Zimmer, K. D., Hanson, M. A., & Butler, M. G. (2001). Effects of Fathead Minnow colonization and removal on a Prairie wetland ecosystem. *Ecosystems*, 4(4), 346–357. <https://doi.org/10.1007/s10021-001-0016-1>

## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

**How to cite this article:** Kačergytė, I., Petersson, E., Arlt, D., Hellström, M., Knape, J., Spens, J., Żmihorski, M. & Pärt, T. (2021). Environmental DNA metabarcoding elucidates patterns of fish colonisation and co-occurrences with amphibians in temperate wetlands created for biodiversity. *Freshw Biol.* 66, 1915–1929. <https://doi.org/10.1111/fwb.13800>