Combining environmental DNA and species distribution modeling to evaluate reintroduction success of a freshwater fish

Maria Riaz [iD],[1,2,3,9] Mathias Kuemmerlen,[4,8] Claudia Wittwer,[1,3] Berardino Cocchiararo,[1,3] Imran Khaliq,[5] Markus Pfenninger,[3,6,7] and Carsten Nowak[1,3]

[1]*Conservation Genetics Group, Senckenberg Research Institute and Natural History Museum Frankfurt, 63571, Gelnhausen, Germany*
[2]*Faculty of Biological Sciences, Institute for Ecology, Evolution and Diversity, Goethe University, Max-von-Laue-Straße 9, 60438 Frankfurt am Main, Germany*
[3]*LOEWE Centre for Translational Biodiversity Genomics (LOEWE-TBG), Senckenberganlage 25, 60325, Frankfurt am Main, Germany*
[4]*Department of Systems Analysis, Integrated Assessment and Modelling, Eawag: Swiss Federal Institute of Aquatic Science and Technology, Ueberlandstrasse 133, 8600, Duebendorf, Switzerland*
[5]*Department of Zoology, Ghazi University, Dera Ghazi Khan, Pakistan*
[6]*Molecular Ecology Group, Senckenberg Biodiversity and Climate Research Centre (BiK-F), Senckenberganlage 25, 60325, Frankfurt am Main, Germany*
[7]*Institute for Molecular and Organismic Evolution, Johannes Gutenberg University, Johann-Joachim-Becher-Weg 7, 55128, Mainz, Germany*

Abstract. Active species reintroduction is an important conservation tool when aiming for the restoration of biological communities and ecosystems. The effective monitoring of reintroduction success is a crucial factor in this process. Here, we used a combination of environmental DNA (eDNA) techniques and species distribution models (SDMs) to evaluate the success of recent reintroductions of the freshwater fish *Alburnoides bipunctatus* in central Germany. We built SDMs without and with eDNA presence data to locate further suitable reintroduction sites and potentially overlooked populations of the species. We successfully detected eDNA of *A. bipunctatus* at all reintroduction sites, as well as several adjacent sites mostly in downstream direction, which supports the success of reintroduction efforts. eDNA-based species detection considerably improved SDMs for *A. bipunctatus*, which allowed to identify species presence in previously unknown localities. Our results confirm the usefulness of eDNA techniques as standard tool to monitor reintroduced fish populations. We propose that combining eDNA with SDMs is a highly effective approach for long-term monitoring of reintroduction success in aquatic species.

Key words: Alburnoides bipunctatus; environmental DNA; monitoring; noninvasive species; reintroduction; species distribution models; Water Framework Directive.

Introduction

In recent decades, habitat pollution has led to massive biodiversity declines in European freshwater ecosystems (European Commission 2007). Large-scale efforts to improve water quality as well as river morphology have been conducted to prepare aquatic habitats as demanded by the European Water Framework Directive (WFD). In addition, numerous species reintroduction projects have been conducted to re-establish formerly present species communities and to improve the ecological status of river ecosystems (Jourdan et al. 2018). Important

contributing factors are the highly impaired longitudinal connectivity in most river networks (Fullerton et al. 2010) and the fact that numerous fish species have poor long-distance dispersal capacity. In response, several projects have aimed at restoring fish communities and bring back emblematic, previously widespread species. Prominent examples for recent reintroduction programs involve the release of salmon (*Salmo salar*), Allis shad (*Alosa alosa*), and Atlantic sturgeon (*Acipenser sturio*) in the river Rhine and other major European stream systems (Monnerjahn 2011, Kirschbaum et al. 2011, Scharbert et al. 2011).

Despite the tremendous effort conducted in many of these programs, a considerable proportion of aquatic reintroduction projects fail. While the reasons for this are intensively debated (Sarrazin and Barbault 1996, Jourdan et al. 2018) such as effective monitoring during and after the reintroductions (Griffith et al. 1989,

Wolf et al. 1996, Kleiman and Mallinson 1998) and a science-based, more careful selection of reintroduction sites (Woodroffe and Ginsberg 1999, IUCN 2013). Accordingly, the choice of the optimal release site and the implementation of scientific monitoring are crucial factors for reintroduction success.

The availability of suitable habitat is a key requirement for the successful establishment of reintroduced species (IUCN 1998). Identifying and selecting suitable habitats prior to reintroduction of a species can favor long-term establishment of the species. Species distribution models (SDMs) are frequently applied to identify and assess habitat suitability for species reestablishment in a certain area (Williams et al. 2009). Furthermore, SDMs have been successfully applied to various tasks in freshwater conservation and management (Domisch et al. 2015a). The approach relies on statistically associating species occurrence with the prevailing environmental data to evaluate the habitat suitability. These models have been successfully applied to discover previously unknown populations through the identification of their specific habitat requirements (Guisan et al. 2006). Combined with field detection such as electrofishing, SDM-supported surveys can outperform traditional large-scale surveys in their efficiency (Costa et al. 2010, Guisan et al. 2013).

A next crucial step in species reintroduction programs is an effective long-term monitoring. A reliable and effective monitoring method can provide the required information to evaluate the status as well as spatial and temporal dynamics of the reintroduced population. Obtained data informs about the potential need for adopting further reintroduction action and provides important information for future reintroduction plans (Woodroffe and Ginsberg 1999). Unfortunately, in several cases, monitoring after introducing a species to its native habitat has been either neglected or was done by invasive methods and documented only in grey literature (Sarrazin and Barbault 1996). Particularly for rare species, invasive survey methods may be harmful, (Snyder 2004), dependent on species characteristics, weather conditions, river size, and bed structure (Thomsen and Willerslev 2015, Jerde et al. 2016) and may thus lead to incomplete or misleading information when surveying reintroduction success.

Noninvasive species detection based on DNA shed by species to its environment (eDNA) has been proven particularly suitable to monitor even rare species in a variety of aquatic environments (Jerde et al. 2011, Thomsen et al. 2012). In particular for taxa that are difficult to monitor, eDNA can often provide information on species' distribution faster and with less effort compared to traditional surveys based on catch or observational data (Dejean et al. 2012, Biggs et al. 2015). The use of eDNA has an enormous potential to quickly provide data on the distribution of species to complement the occurrence data used as input in SDMs and improving the underlying knowledge leading to more statistically sound distribution predictions. Interestingly, this approach has only rarely been applied so far (Schmidt et al. 2013, Hunter et al. 2015, Muha et al. 2017, Eiler et al. 2018, Doi et al. 2019) mainly to address imperfect detection. To our knowledge, there is no study in which SDM and eDNA support each other in an iterative manner.

Here, we present a new approach combining eDNA and SDM to evaluate the reintroduction success of a regionally endangered freshwater fish, *A. bipunctatus*, which inhabits fast-flowing and well-oxygenated waters of streams and rivers. In central Europe, this historically abundant species has undergone massive decline in the second half of the past century due to water pollution and river regulations (Rothe 2002). In the central German state of Hessen, for instance, only a few remaining populations have been documented in the north, mainly in the river Eder and in its tributaries (Bobbe 2016). With the overall improvement of water quality in Germany during the past decades and the conduction of ongoing morphological river restoration measures, a successful re-establishment of the species seems plausible. Thus an active reintroduction program of *A. bipunctatus* (Bobbe 2011) has been planned and carried out since 2009 in several stream systems of Hessen, aiming at reestablishing the species in all suitable water bodies in the region. *A. bipunctatus* is sensitive towards changes in water quality and river morphology (Bobbe 2015). Therefore, the establishment and spread of reintroduced populations of the species may negatively be influenced by entry of organic materials and fluctuating negative oxygen contents. Moreover, the presence of physical barriers also hinders the spread of the reintroduced populations to nearby suitable habitats (Bobbe and Korte 2017). While single individuals have been captured in several reintroduction regions up to a maximum of 8 km away from its release point, the overall success of this action has not been systematically evaluated so far. Also, it is unknown to what degree undetected relict populations of this small, difficult-to-monitor species have persisted. In order to maintain a viable population, the species is restocked every year in some of the studied rivers where it was considered unestablished.

We therefore aimed to answer the following questions: (1) Was the reintroduction successful; i.e., can we detect *A. bipunctatus* in the vicinity of the reintroduction sites several years after its release? (2) Do overlooked populations exist that need to be considered in future reintroduction planning? (3) Is the iterative combination of eDNA and SDM a suitable tool to guide the choice of suitable reintroduction sites?

METHODS

Study sites and region

The federal state of Hessen is located in the western central part of Germany, with an approximate area of 21.100 km^2. The main river systems in the north are the Fulda and the Eder, which drain into the Weser

watershed. The Main and the Lahn rivers both drain into the Rhine river in the south and in the west of the state, respectively. In this study, we sampled 115 localities from 33 streams and rivers covering several major river systems of Hessen between April 2017 and February 2018. We categorized the target rivers into four categories: (1) rivers where actual reintroduction took place (hereafter RS, reintroduction sites); (2) rivers predicted by SDMs as suitable habitats (MS for model sites without and eMS for model sites with eDNA-based monitoring results); (3) rivers considered unsuitable, neither comprising reintroduction sites nor predicted by SDM1 (AS, additional sites); and (4) two rivers as negative control sites (NS) as such (Fig. 1).

To evaluate the success of recent reintroductions of *A. bipunctatus*, we sampled all known RS including exact reintroduction localities in Hessen (rivers Kinzig, Nidda, Sinn, Mümling, Lahn, Ulster, and Döllbach), as well as

several upstream and downstream sites from the actual reintroduction localities in 5-km intervals (except for the long stretched river Lahn, where intervals of approximately 10 km were chosen; Fig. 1). To test SDMs generated with and without eDNA data and check for potentially overlooked populations, we included additional 190 samples from 52 MS and eMS predicted by the SDMs (see *eDNA sampling*) as well as 102 samples from 24 AS (see Fig. 1). In total, 392 samples were taken in the course of this study.

eDNA sampling

We collected eDNA samples using glass fiber filters (GFFs) of 2-μm pore size and 47 mm diameter (Millipore Merck, KGaA, Darmstadt, Germany) by filtering a range of water volumes (1–10 L) through a peristaltic pump (Masterflex; Cole-Palmer Instrument Company, East
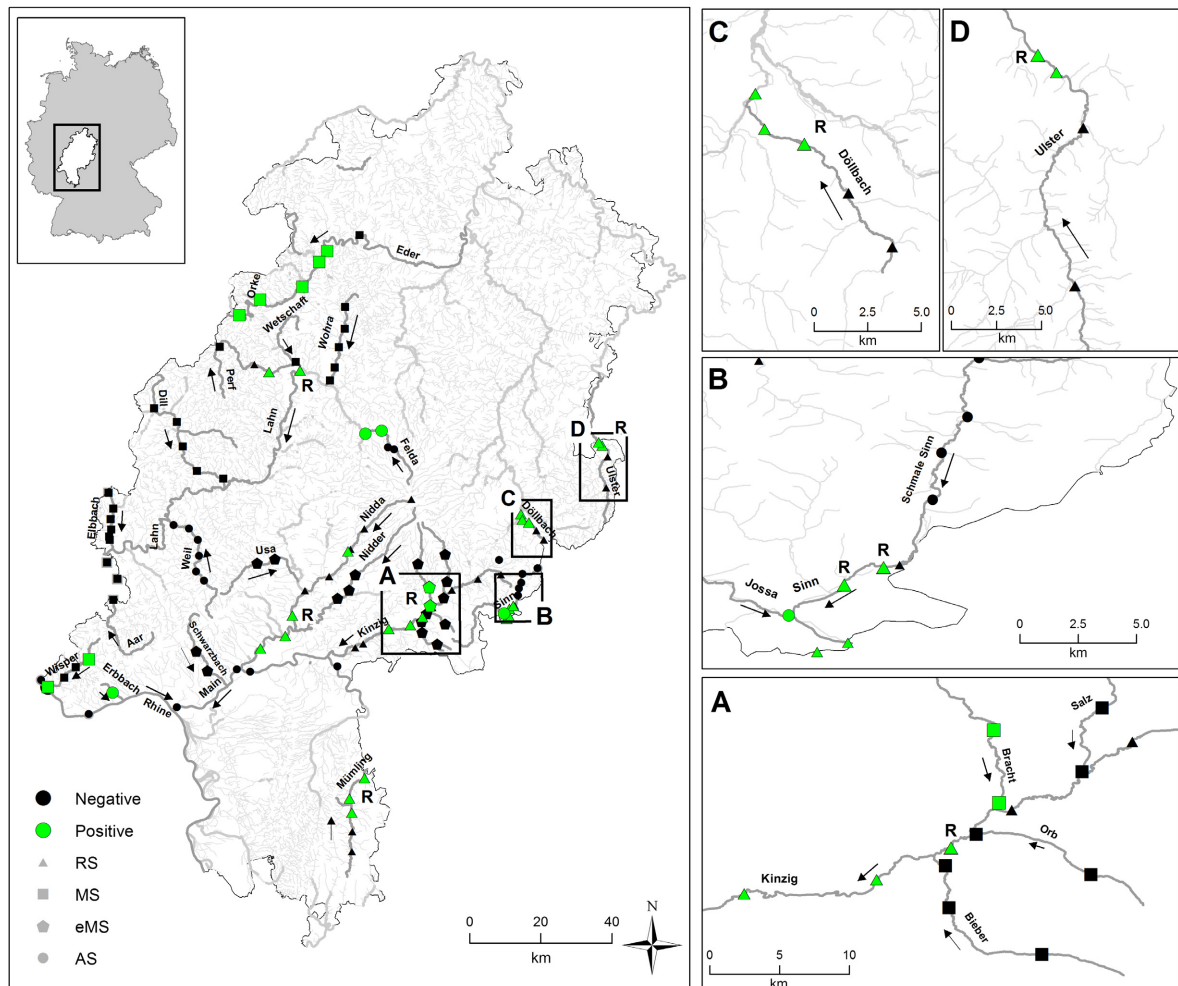

FIG. 1. Sampling locations of the 31 freshwater systems located in central German region of Hessen targeted in this study. Panels A–D are zoomed-in views from the large panel. Black points represent negative sites and green points represent positive sites. Samples were taken from (RS) reintroduction river sites with exact reintroduction localities (R), (MS) model sites without, (eMS) model sites with eDNA-based data, and (AS) additional sites. For exact positions of all 113 localities, see Appendix S1: Table S1.

Bunker Ct Vernon Hills, IL, USA) following (Wittwer et al. 2018a, b). The volume of filtered water depended upon turbidity of the sampled river, weather conditions and also the particle size variations at each site. We transferred each GFF to 50-mL screw-cap tubes with sterile forceps. Samples were transported to the laboratory within few hours in ice coolers and then stored at −20°C until extraction. For each sampling event, equipment and field blanks were taken to monitor the risk of contamination as mentioned in other studies (Piaggio et al. 2014).

Before each sampling event, we decontaminated all equipment using 0.25% peracetic acid or 10% bleach solution, rinsing with 96% ethanol and then exposed to UV light. To further minimize the risk of contamination, we frequently changed gloves and flushed the filtration apparatus with river water at each sampling site (Fukumoto et al. 2015).

Species distribution modeling

Besides eDNA detection of RS, we aimed to check for potentially undetected occurrences of *A. bipunctatus*. For this, SDMs were built using the biomod2 package (Thuiller et al. 2009) for R (R Development Core Team 2014). SDMs were calibrated with environmental data representative of freshwater ecosystems, as well as with occurrence data of the target species. Based on this information, the model is trained to determine habitats considered to be suitable and to predict it across the landscape. Models used occurrence data for *A. bipunctatus* for Germany (*n* = 61; recorded between 2006 and 2015) from the Global Biodiversity Information Facility (GBIF) database. As environmental predictors, we used 10 variables from a freshwater-specific, near-global, environmental variable data set, available for stream networks at a 1-km resolution spatial (Domisch et al. 2015b; *available online*).[10] The considered predictor variables included ecologically relevant environmental factors for freshwater biota, which have been proven to improve model predictions in freshwater ecosystems (Kuemmerlen et al. 2014, Domisch et al. 2015b). Three predictors described atmospheric temperature patterns as surrogates for water temperatures, monthly minima, monthly maxima, and their seasonality (Austin 2007); three more predictors described precipitation patterns during driest and wettest months as well as their seasonality; two predictors described the relative proportion of land used in the upper subcatchment, urban and cultivated; one predictor described topographical inclination (i.e. slope) as a surrogate for flow velocity. The model was calibrated for the entire river network of Germany (85,682 grid cells) but projected only for the river network of the study area, the state of Hessen (4,893 grid cells). The algorithms generalized linear models (GLM), generalized boosted models (GBM), classification tree analysis (CTA), artificial neural networks (ANN),

random forests (RF), and multinomial logistic regression (MAXENT.Tsuruoka) were run for five different pseudo-absence sets (10% of calibration grid cells, *n* = 8,568) and 10 repetitions, for a total of 600 individual models that were aggregated into an ensemble model, using a performance weighted mean. The performance metric of choice was the true skill statistic (TSS; Allouche et al. 2006). As an internal validation procedure, for each individual model, the occurrence data is split into training (70%) and testing (30%) data sets, in order to assess the sensitivity (i.e. true positive rate) and specificity (i.e. true negative rate), from which the TSS is calculated. Finally, binary predictions were projected on the stream network map of Hessen to inform eDNA sampling efforts.

From this initial prediction of habitat suitability (SDM1), 10 MS with large reaches of predicted occurrence were selected for eDNA sampling (see *eDNA sampling*); Elbbach, Aar, Wetschaft, Perf, Dill (Lahn tributries), Lahn, Wisper (Rhine tributaries), Wohra (Weser tributary), Orke (Eder tributary), and Eder (Fulda tributary; Fig. 1). After this sampling, a second model (SDM2) was set up using the presence data generated through the eDNA analysis and using the same parameters as described above. We sampled only a small number of rivers from the predictions of SDM2 to check its efficacy. We sampled (details in eDNA sampling section) seven eMS including Bieber, Orb, Salz, and Bracht (Kinzig tributaries), Nidder, and Usa (Nidda tributary) and Schwarzbach (Main tributary). We also sampled one negative site each from two NS, namely the rivers Nüst and Bieber, where the complete absence of *A. bipunctatus* has recently been shown (Bobbe 2015).

Laboratory procedure and data analysis

We performed all DNA extractions and PCR reactions under UV hoods in separate laboratories assigned for these purposes. We used tissue extracts of fin-clips of two *A. bipunctatus* reference specimens from Riaz et al. (2018) and quantified DNA concentrations with the NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA). We extracted DNA from all water samples by using a modified phenol-chloroform-alcohol method according to Wittwer et al. 2018a, b, .

We used a highly sensitive and specific TaqMan MGB qPCR Assay developed for *A. bipunctatus* by Riaz et al. 2018 and performed qPCR on QuantStudio 3 (Thermo Fisher Scientific) using TaqMan Environmental Master Mixture 2.0 following the thermal conditions of Riaz et al. 2018. All qPCR runs included eight levels of standard concentrations as positive control, no template control (PCR water), either an equipment/field control or an extraction blank in triplicate for ensuring the reliability of the results. We analyzed the qPCR runs by Quantstudio software v1.1 (Appendix S1: Table S1) and

---

[10] http://www.earthenv.org/streams

calculated relative DNA amounts based on the standard curve and obtained cycle threshold (C_t) values.

A site was scored positive if there was amplification of at least two replicates (biological, different water samples of one site; technical, PCR replicate of the same sample) of the same site with DNA quantity equal to or over the limit of detection (LOD = 40.310 C_t, 0.05 pg/μL).

Inhibition test

Although TaqMan® Environmental Master Mix 2.0 is generally considered effective to cope with inhibition effect (Jane et al. 2015), we tested for potential inhibitory effects. For this, a selection of 15 light and dark-colored extracts from all RS and few additional MS, eMS and AS were selected. Extracts were diluted 5, 10, and 100 fold (McKee et al. 2015) and run in triplicates for qPCR.

Results

eDNA-based detection

All 392 samples from 115 sites containing all four river categories were analysed for the presence of eDNA from *A. bipunctatus* by qPCR. We did not detect *A. bipunctatus* from the two NS considered as negative for the species in this study. No cross-contamination was found during field and laboratory handling for all equipment controls, field controls, extraction and PCR blanks. As we found negligible inhibition effects only from a few samples we used concentrated extracts for further analysis in this study.

Species presence was detected at 34 sites (120 samples, 31%). Out of these 34 positive sites, 21 belong to RS (62%) including all eight reintroduction sites (Fig. 1). We detected presence of *A. bipunctatus* at all actual reintroduction localities of the rivers Kinzig, Mümling, Lahn, Döllbach, Ulster, Nidda, and Sinn and also at several downstream sites (Fig. 3). Additionally, a few sites upstream sites gave positive qPCR signals indicating upstream dispersal (Kinzig and Nidda; Figs. 1, 4, Appendix S1: Table S1).

Species distribution modeling

A. bipunctatus was predicted to occur in <10% of the stream network of Hessen (453 grid cells) by the initial model (SDM1, Fig. 2a), which showed moderate performance (TSS = 0.879). eDNA analysis could not confirm *A. bipunctatus* in seven of the MS: Elbbach, Aar, Wetschaft, Perf, Dill, and Wohra. However, we got strong qPCR signals from four MS rivers: the Wisper (Rhine tributary), the Eder, its tributary the Orke, and also from the Lahn (Fig. 3, Appendix S1: Table S1), confirming SDM distribution predictions. Consideration of species detection by eDNA resulted in improved model performance (TSS = 0.961), as well as a considerably larger area of predicted occurrence for *A. bipunctatus*, (1,617 grid cells) (SDM2, Fig. 2b). In one out of seven additional rivers predicted by SDM2 (Bracht) presence of *A. bipunctatus* could be confirmed by eDNA.

Three out of six AS rivers Felda, Erbach, and Jossa were found to be suitable habitats for *A. bipunctatus* through eDNA tests (Fig. 3, Appendix S1: Table S1). Moreover, for the two largest rivers in our study region, Main and Rhine, we obtained weak qPCR signals below the LOD and were thus classified as negative detections based on our established scoring criteria for this study.

Discussion

Evaluation of reintroduction success with eDNA

Our results show a clear pattern, confirming the absence of *A. bipunctatus* in most tested river systems in Hessen. Positive detections at all reintroduction sites (Fig. 3, Appendix S1: Table S2) provide strong evidence for the overall success of the reintroduction efforts and are in line with electrofishing data, which found *A. bipunctatus* individuals following the releases (Bobbe 2016, Bobbe and Korte 2017). We are aware of the fact that a positive detection at a site does not provide clear evidence for successful reproduction and there is yearly restocking conducted to support the reintroduced populations. Thus, detections may derive from surviving or recently restocked animals. However, the fact that we obtained generally strong signals, even in regions considerably distant from the reintroduction sites, as well as in cases where the reintroduced population had not been confirmed by electrofishing (river Mümling; [Bobbe and Korte 2017]) makes this scenario rather unlikely and documents the suitability of eDNA to detect reintroduced populations.

Besides detecting the species at all the reintroduction localities, we found *A. bipunctatus* DNA downstream of the actual reintroduction localities. While these detections may relate to the downstream transport of eDNA fragments, certain factors such as temperature, physical decay, biological degradation, substrate type, flow rate, and increased dilution by influx of water affect downstream eDNA detection probability (Dejean et al. 2011, Barnes et al. 2014, Jane et al. 2015, Jerde et al. 2016, Shogren et al. 2017). For example, in an experimental study, (Shogren et al. 2017) detected eDNA at a maximum distance of up to 21.7 m downstream from the source depending upon the substrate bed. While in this study, we detected eDNA of *A. bipunctatus* as far as 14.5 km downstream of the reintroduction sites. Assuming no dispersal, this would correspond to a minimum travelling time of 27 h for eDNA fragments at an average flow rate of 0.54 km/h (data obtained for Mümling from Hessisches Landesamt für Naturschutz, Umwelt und Geologie) during the month of May 2017 (Fig. 4,
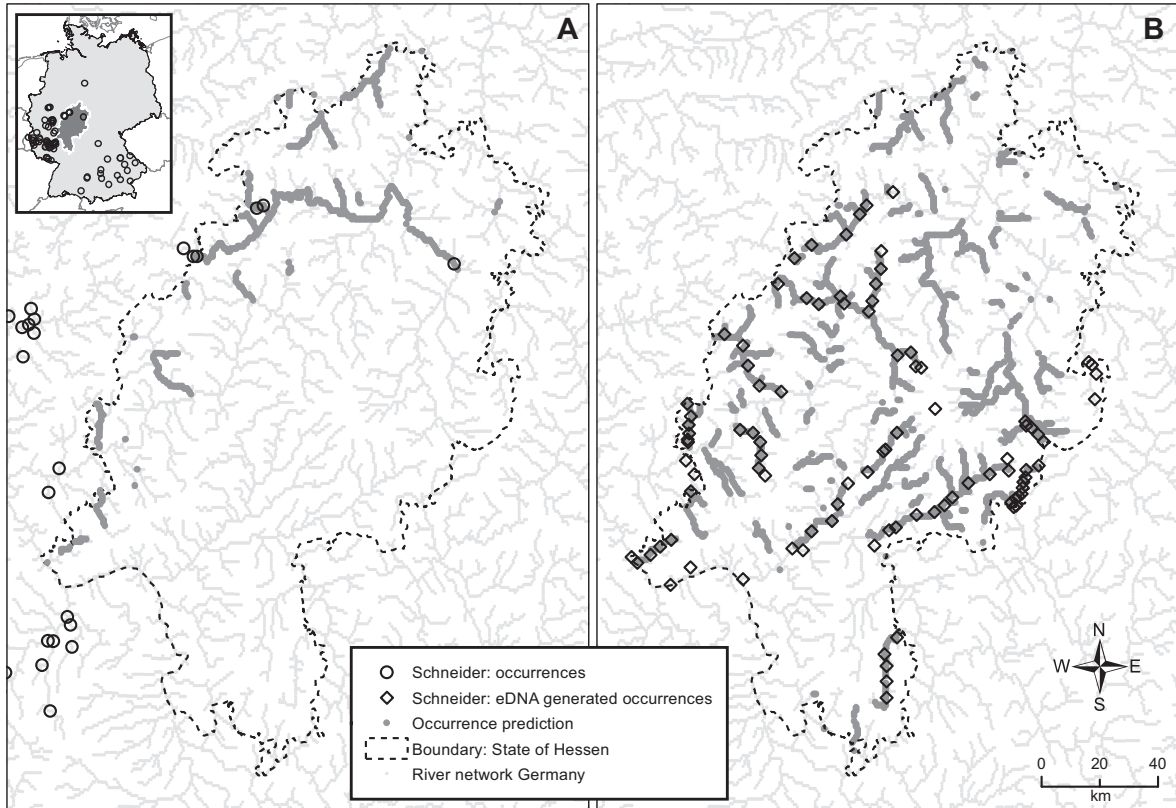
Fig. 2. Predicted suitable habitats for *Alburnoides bipunctatus* projected for the German state of Hessen. (A) Model predicted sites (MS) of SDM1 based on available occurrence data for *A. bipunctatus* for Germany (*n* = 61; from 2006 to 2015) from the Global Biodiversity Information Facility (GBIF). (B) eDNA-model sites (eMS) of SDM2 generated from adding eDNA-based detection data obtained in this study.

Appendix S1: Table S1), Moreover, the presence of seven barriers between the reintroduction site and the last site where the species was detected with eDNA has likely contributed to eDNA retention. Considering the travelling time, physical barriers, and degrading factors affecting persistence of eDNA from other studies (for example, Barnes et al. 2014), we expect the relatively strong eDNA signals (eDNA quantity > LOQ, Appendix S1: Table S1) downstream of the reintroduction site to be derived solely from flowing eDNA traces attributable to the occurrence of fish at or very near the sampled sites. In contrast to two reintroduction rivers, such as Nidda and Kinzig, signal intensity did not decrease in the course of the stream while moving downstream the reintroduction sites. At least for the Mümling site, it may thus be evident that *A. bipunctatus* could have spread from the reintroduction point and there is strong indication of the occurrence of some downstream native populations. As there is no eDNA sampling before the reintroduction process started at any of the studied rivers, disentangling the effects of dispersal from the potential presence of overlooked native populations remains inconclusive. Our results, however, highlights the broad applicability of eDNA to be used to detect the

presence of any native population before initiating the process of reintroduction to make it more targeted and fruitful.

Besides this evidence for downstream dispersal, in some rivers, the patchy upstream detections of *A. bipunctatus* from the reintroduction sites and also detection in some tributaries may again be attributed to previously undiscovered isolated populations of the species. However, as the species was generally considered to be extinct in reintroduction rivers (Bobbe 2011), it is plausible to conclude an up-stream range extension of the reintroduced populations in at least some of the cases. Further evidence of the gradual dispersal of *A. bipunctatus* comes from the fact that three new populations were discovered at non-predicted rivers (Fig. 3, Appendix S1: Table S1). Two of these rivers, the Jossa and the Felda, are tributaries of the reintroduction rivers Sinn and Ohm-Lahn, respectively, while the river Erbach is the Rhine tributary that is reported to contain a relict population (Bobbe 2015). We cannot prove here if these populations behave somewhat different or if it may be due to model inaccuracy. Also, these findings necessitate frequent monitoring following reintroduction of a species not only at or around the reintroduction sites but also in
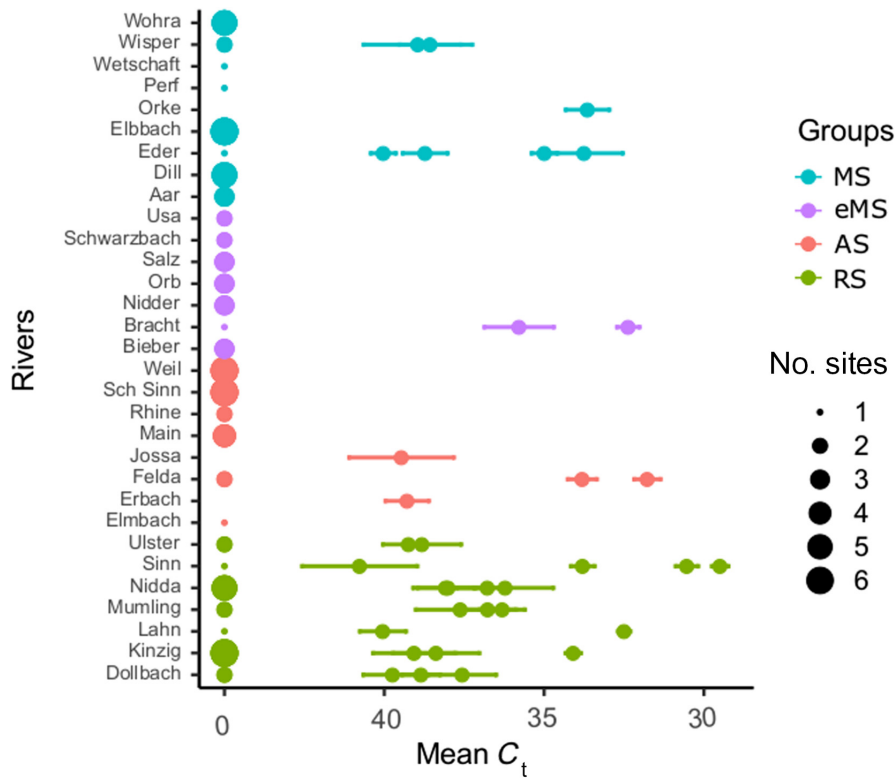
Fig. 3. eDNA-based detections of *A. bipunctatus* in 31 Hessian streams. An illustration of the mean cycle threshold (C_t) values with standard errors from three qPCR replicates of water samples obtained from 113 sampling sites. The *x*-axis indicates the mean C_t values and *y*-axis indicates the sampling rivers. Along the *x*-axis, a C_t value of 0 means absence of DNA in our results, with values in descending order, where higher C_t value means lower DNA concentration and lower C_t value means higher DNA concentration. In the key, "No. sites" represents the number of sites for the samples that showed zero values only, and the size of the circle indicates the number of sites. The mean C_t values other than zero are shown with standard deviation. See Fig. 1 for sampling site categories ("Groups").
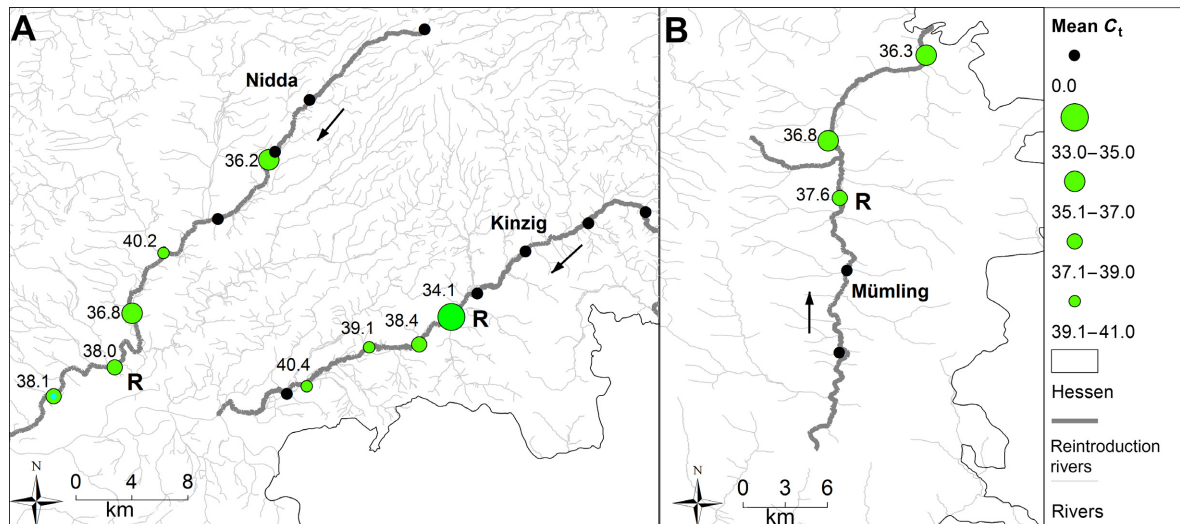

Fig. 4. Mean cycle threshold (C_t) values of eDNA- based *A. bipunctatus* detection from sites downstream of three reintroduction sites: (A) Nidda and Kinzig rivers and (B) river Mümling.

the whole stretch of the rivers and their tributaries to access the species' full range.

We obtained few examples of an isolated, upstream, dispersal or occurrence of the species. For instance, in the Nidda, some detections were made approximately 4 and 21 km upstream from the RS. While in the Wisper (MS), *A. bipunctatus* was also detected to occur at the river mouth and also at a far upstream site. Interestingly, no detection was made between the previous downstream detection site and the upstream detection site. A false positive detection due to contamination during field or/and laboratory handling is highly unlikely, as all controls including field, equipment, extraction, and PCR controls showed no amplification. We also replicated all laboratory procedures and could confirm the positive detection. While eDNA of a species may be transported to the upstream sites by predators such as fish or birds, for instance in their guts and excreted there (Merkes et al. 2014), our findings provide strong evidence for within-catchment dispersal of the species from the actual reintroduction site.

In some cases, such as the large rivers Rhine and Main, weak eDNA signals were obtained in some samples. The intensity of weak eDNA signals is comparable to other downstream detections below the reintroduction sites except the Mümling (Appendix S1: Table S1). It appears likely that the high dilution factor and faster degradation of eDNA due to the high water temperatures of these large rivers may lead to weak eDNA signals. Similar false negative results have been reported in several eDNA studies from sites where species presence was evident (Díaz-Ferguson and Moyer 2014, Port et al. 2016). Thus, additional sampling efforts as well as experimental studies might be required to test for species' occurrence and population density in large water bodies using eDNA.

Our results document the suitability of the eDNA approach for monitoring and range estimation of reintroduced fish species. In this study, we did not explicitly aim for a comparison between traditional monitoring based on electrofishing and eDNA. However, we propose eDNA as a reliable alternative or ideally a valuable complementation to this approach. For instance, electrofishing can be harmful to fishes if improperly applied. Electrofishing is reported to cause spinal damages or hemorrhages as a result of the possible epileptic seizures in over 50% of the fishes examined internally (Snyder 2004). This physical stress and sometimes death of some of the individuals should be worth concerning especially when handling rare and endangered species or vulnerable populations that have recently been reintroduced. In this context, eDNA can be regarded as a nondestructive, noninvasive, effective, detection approach with no obvious threats to animal health.

Combining eDNA with SDM

To delineate the potential range of *A. bipunctatus* and guide the sampling effort in this study, we used SDM based on publicly available data. The initial model (SDM1) was limited in its predictions, with only four rivers, including one reintroduction river, being identified as suitable habitat for *A. bipunctatus*. However, due to few occurrence data points and the fact that the species did not yet recolonize its potential range lead to moderate performing model, as indicated by TSS. Moreover, the model output can be the result of several additional factors: uncertainty related to the occurrence data (e.g. unknown sampling techniques, non-systematic sampling) and possibly an incomplete description of the suitable habitat for *A. bipunctatus* (more environmental predictors at a finer spatial scale). Moreover, while SDMs were calibrated with improved occurrence data in a second model and that they could, in general terms, be calibrated using additional, environmental data at high spatial resolutions; inaccuracies are an intrinsic part of a predictive approach. Implementing posterior field validation can help detect such inaccuracies and potentially improve subsequent SDMs (Barry and Elith 2006, Pearson et al. 2006).

By means of harnessing the efficiency of eDNA techniques to sample rare species in almost any kind of habitat, even those that are otherwise hard to access by traditional methods (Yoccoz 2012, Muha et al. 2017), the second SDM could be calibrated with additional presence data from the eDNA monitoring/survey, yielding a very good performing model as indicated by TSS. While the resulting area of predicted habitat suitability was larger than in the first SDM, a significant portion of the occurrence prediction could not be confirmed with the eDNA method. This result was expected, as the species is known to have disappeared from most former habitats due to massive water pollution and morphological river degradation during past decades. As reintroductions only occurred recently, it seems plausible to assume that the species has only been able to occupy nearby habitats in close proximity to the reintroduction sites. This scenario is confirmed by the eDNA data. Moreover, some degree of dispersal limitations due to vertical barriers or stretches of unsuitable habitats might further hamper the spread of the species in the future. Nevertheless, this procedure resulted in the detection of this rare species at a previously unknown site (river Bracht) and can be considered as a significant knowledge improvement for the distribution of *A. bipunctatus*. In the course of this study, our finding was also confirmed by the electrofishing survey report (Bobbe and Korte 2017). Moreover, predicting a species' distribution is particularly challenging in our case study, because of the local extirpation attributable to a legacy effect of former pollution, combined with the dispersal limitation suggested by our results.

Applications of coupling eDNA and SDM

Combining eDNA and SDMs can serve as a useful active management tool for species reintroduction with the following algorithm: (1) Build an SDM with best

available occupancy information for identification of most suitable habitat. (2) Release the species in those habitats and reevaluate with eDNA to improve the SDMs predictions. (3) Consider additional releases in case new suitable areas are predicted due to model improvement. This process may lead to the identification of most suitable habitat for species that can enhance the chances of establishment of species enormously. Moreover, this study can be considered as an initial exemplary work that can guide conservation managers formerly using electrofishing method or mark recapturing at potential sites to monitor population trends. In addition this combined technique could be utilized for the early identification of invasive species to prevent and manage its spread.

CONCLUSION

Our results provide evidence that (1) *A. bipunctatus* is currently well established in extensive river segments with suitable habitat, (2) some dispersal has occurred after reintroduction efforts, suggesting a slow dispersal and local colonization, (3) diverse dispersal limitations still hamper range expansion, largely preventing the colonization of the majority of potentially suitable habitat; and d) coupling of eDNA and SDM can be a powerful active noninvasive, reliable management tool.

A. bipunctatus, once gone extinct from its native haunts due to decreased water quality and straightening of the water bodies that probably lead to habitat degradation, has regained a lot of its former range in many of the studied rivers. This may forebode a sustainable long-term species establishment. Beyond the focal species here, we recommend additional species reintroductions at potentially suitable sites, involving continuous monitoring using eDNA and SDM to attain a good ecological status of freshwater bodies as given by biotic indices and demanded by WFD.

Our study highlights the potential of eDNA as a robust monitoring technique to evaluate the reintroduction of a rare aquatic species. In particular to our model species *A. bipunctatus*, our findings strongly encourage to conduct additional reintroduction projects in a broader geographical context to reach favorable conservation status of the species and fulfill the criteria of a "good ecological status" under the demands of the European WFD for riverine ecosystems in Hessen and other Central European regions. Our results also underpin the usefulness to combine eDNA with SDM in an iterative approach in the context of reintroduction evaluation and to guide future reintroduction action. We recommend that more studies verify implementation of the combined approach of eDNA and SDM to enhance the success of species reintroductions.

LITERATURE CITED

Allouche, O., A. Tsoar, and R. Kadmon. 2006. Assessing the accuracy of species distribution models: Prevalence, kappa and the true skill statistic (TSS). Journal of Applied Ecology 43:1223–1232.

Austin, M. 2007. Species distribution models and ecological theory: A critical assessment and some possible new approaches. Ecological Modelling 200:1–19.

Barnes, M. A., C. R. Turner, C. L. Jerde, M. A. Renshaw, W. L. Chadderton, and D. M. Lodge. 2014. Environmental conditions influence eDNA persistence in aquatic systems. Environmental Science and Technology 48:1819–1823.

Barry, S., and J. Elith. 2006. Error and uncertainty in habitat models. Journal of Applied Ecology 43:413–423.

Biggs, J., et al. 2015. Using eDNA to develop a national citizen science-based monitoring programme for the great crested newt (*Triturus cristatus*). Biological Conservation 183:19–28.

Bobbe, T. 2011. Erste Erfahrungen bei der Wiederansiedlung des Schneiders in Südhessen. http://flussgebiete.hessen.de/fileadmin/dokumente/5_service/bobbe_wiederansiedlung_schneider_20110405.pdf

Bobbe, T. 2015. Wiederansiedlung des Schneiders *Alburnoides bipunctatus* (BLOCH 1782) in Südhessen 2009 – 2015. https://rp-darmstadt.hessen.de/sites/rp-darmstadt.hessen.de/files/content-downloads/Projektbericht%202015%20Kinzig%2C%20M%C3%BCmling%2C%20Nidda%2C%20Sinn.pdf

Bobbe, T. 2016. Wiederansiedlung des Schneiders *Alburnoides bipunctatus* (BLOCH 1782) in Südhessen 2009 – 2015. https://rp-darmstadt.hessen.de/sites/rp-darmstadt.hessen.de/files/Projektbericht%202016%20Kinzig%2C%20M%C3%BCmling%2C%20Nidda%2C%20Sinn.pdf

Bobbe, T., and E. Korte. 2017. Die Wiedereinbürgerung des Schneiders (*Alburnoides bipunctatus* in der Nidda). https://www.gfg-fortbildung.de/web/images/stories/gfg_pdfs_ver/Hessen/NiddaNidder/2018-Fruehling/18_ninima_v4b.pdf

Costa, G. C., C. Nogueira, R. B. Machado, and G. R. Colli. 2010. Sampling bias and the use of ecological niche modeling

in conservation planning: A field evaluation in a biodiversity hotspot. Biodiversity and Conservation 19:883–899.

Dejean, T., A. Valentini, A. Duparc, S. Pellier-Cuit, F. Pompanon, P. Taberlet, and C. Miaud. 2011. Persistence of environmental DNA in freshwater ecosystems. PLoS ONE 6: 8–11.

Dejean, T., A. Valentini, C. Miquel, P. Taberlet, E. Bellemain, and C. Miaud. 2012. Improved detection of an alien invasive species through environmental DNA barcoding: The example of the American bullfrog *Lithobates catesbeianus*. Journal of Applied Ecology 49:953–959.

Díaz-Ferguson, E. E., and G. R. Moyer. 2014. History, applications, methodological issues and perspectives for the use of environmental DNA (eDNA) in marine and freshwater environments. Revista de Biología Tropical 62:1273–1284.

Doi, H., K. Fukaya, S. ichiro Oka, K. Sato, M. Kondoh, and M. Miya. 2019. Evaluation of detection probabilities at the water-filtering and initial PCR steps in environmental DNA metabarcoding using a multispecies site occupancy model. Scientific Reports 9:1–8.

Domisch, S., G. Amatulli, and W. Jetz. 2015. Near-global freshwater-specific environmental variables for biodiversity analyses in 1 km resolution. Scientific Data 2:150073.

Domisch, S., S. C. Jähnig, J. P. Simaika, M. Kuemmerlen, and S. Stoll. 2015. Application of species distribution models in stream ecosystems: the challenges of spatial and temporal scale, environmental predictors and species occurrence data. Fundamental and Applied Limnology/Archiv für Hydrobiologie 18:45–61.

Eiler, A., A. Löfgren, O. Hjerne, S. Nordén, and P. Saetre. 2018. Environmental DNA (eDNA) detects the pool frog (*Pelophylax lessonae*) at times when traditional monitoring methods are insensitive. Scientific Reports 8:1–9.

European Commission. 2007. Towards sustainable water management in the European Union. First stage in the implementation of the Water Framework Directive 2000/60/EC. Commission staff working document accompanying document to the communication from the commission to the European par. Page COM 128 final. European Commission. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:52007DC0128&from=EN

Fukumoto, S., A. Ushimaru, and T. Minamoto. 2015. A basin-scale application of environmental DNA assessment for rare endemic species and closely related exotic species in rivers: a case study of giant salamanders in Japan. Journal of Applied Ecology 52:358–365.

Fullerton, A. H., K. M. Burnett, E. A. Steel, R. L. Flitcroft, G. R. Pess, B. E. Feist, C. E. Torgersen, D. J. Miller, and B. L. Sanderson. 2010. Hydrological connectivity for riverine fish: measurement challenges and research opportunities. Freshwater Biology 55:2215–2237.

Griffith, B., J. M. Scott, J. W. Carpenter, and C. Reed. 1989. Translocation as a species conservation tool: status and strategy. Science 245:477–480.

Guisan, A., O. Broennimann, R. Engler, M. Vust, N. G. Yoccoz, A. Lehmann, and N. E. Zimmermann. 2006. Using niche-based models to improve the sampling of rare species. Conservation Biology 20:501–511.

Guisan, A., et al. 2013. Predicting species distributions for conservation decisions. Ecology Letters 16:1424–1435.

Hunter, M. E., S. J. Oyler-McCance, R. M. Dorazio, J. A. Fike, B. J. Smith, C. T. Hunter, R. N. Reed, and K. M. Hart. 2015. Environmental DNA (eDNA) sampling improves occurrence and detection estimates of invasive Burmese pythons. PLoS ONE 10:1–17.

IUCN. 1998. IUCN guidelines for re-introductions. Information Press, Oxford, UK.

IUCN/SSC. 2013. IUCN guidelines for reintroductions and other conservation translocations. IUCN Species Survival Commission, Gland, Switzerland.

Jane, S. F., T. M. Wilcox, K. S. Mckelvey, M. K. Young, M. K. Schwartz, W. H. Lowe, B. H. Letcher, and A. R. Whiteley. 2015. Distance, flow and PCR inhibition: EDNA dynamics in two headwater streams. Molecular Ecology Resources 15:216–227.

Jerde, C. L., A. R. Mahon, W. L. Chadderton, and D. M. Lodge. 2011. "Sight-unseen" detection of rare aquatic species using environmental DNA. Conservation Letters 4:150–157.

Jerde, C. L., B. P. Olds, A. J. Shogren, E. A. Andruszkiewicz, A. R. Mahon, D. Bolster, and J. L. Tank. 2016. Influence of stream bottom substrate on retention and transport of vertebrate environmental DNA. Environmental Science and Technology 50:8770–8779.

Jourdan, J., et al. 2018. Reintroduction of freshwater macroinvertebrates: challenges and opportunities. Biological Reviews 94:368–387.

Kirschbaum, F., P. Williot, F. Fredrich, R. Tiedemann, and J. Gessner. 2011. Restoration of the European Sturgeon Acipenser sturio in Germany. In: P. Williot, E. Rochard, N. Desse-Berset, and F. Kirschbaum, editors. Biology and Conservation of the European Sturgeon Acipenser sturio L. 1758. Springer, Berlin, Heidelberg.

Kleiman, D. G., and J. J. C. Mallinson. 1998. Recovery and management committees for Lion Tamarins: partnership in conservation planning and implementation. Conservation Biology 12:27–38.

Kuemmerlen, M., B. Schmalz, B. Guse, Q. Cai, N. Fohrer, and S. C. Jähnig. 2014. Integrating catchment properties in small scale species distribution models of stream macroinvertebrates. Ecological Modelling 277:77–86.

McKee, A. M., S. F. Spear, and T. W. Pierson. 2015. The effect of dilution and the use of a post-extraction nucleic acid purification column on the accuracy, precision, and inhibition of environmental DNA samples. Biological Conservation 183:70–76.

Merkes, C. M., S. G. McCalla, N. R. Jensen, M. P. Gaikowski, and J. J. Amberg. 2014. Persistence of DNA in carcasses, slime and avian feces may affect interpretation of environmental DNA data. PLoS ONE 9:e113346.

Monnerjahn, U. 2011. Atlantic Salmon (*Salmo salar* L.) re-introduction in Germany: a status report on national programmes and activities. Journal of Applied Ichthyology 27:33–40.

Muha, T. P., M. Rodríguez-Rey, M. Rolla, and E. Tricarico. 2017. Using environmental DNA to improve species distribution models for freshwater invaders. Frontiers in Ecology and Evolution 5:158.

Pearson, R. G., W. Thuiller, M. B. Araújo, E. Martinez-Meyer, L. Brotons, C. McClean, L. Miles, P. Segurado, T. P. Dawson, and D. C. Lees. 2006. Model-based uncertainty in species range prediction. Journal of Biogeography 33:1704–1711.

Piaggio, A. J., R. M. Engeman, M. W. Hopken, J. S. Humphrey, K. L. Keacher, W. E. Bruce, and M. L. Avery. 2014. Detecting an elusive invasive species: a diagnostic PCR to detect Burmese python in Florida waters and an assessment of persistence of environmental DNA. Molecular Ecology Resources 14:374–380.

Port, J. A., J. L. O'Donnell, O. C. Romero-Maraccini, P. R. Leary, S. Y. Litvin, K. J. Nickols, K. M. Yamahara, and R. P. Kelly. 2016. Assessing vertebrate biodiversity in a kelp forest ecosystem using environmental DNA. Molecular Ecology 25:527–541.

R Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/

Riaz, M., C. Wittwer, C. Nowak, and B. Cocchiararo. 2018. An environmental DNA assay for the detection of the regionally endangered freshwater fish *Alburnoides bipunctatus* in Germany. Conservation Genetics Resources 1–3.

Rothe, U. 2002. Kurzmitteilung—short communication. Mitteilungen aus dem Museum für Naturkunde in Berlin, Zoologische Reihe 78:183–185.

Sarrazin, F., and R. Barbault. 1996. Reintroduction: challenges and lessons for basic ecology. Trends in Ecology and Evolution 5347:474–478.

Scharbert, A., S. Staas, E. Korte, J. Schneider, F. Rochol, P. Beeck, and H. Klinger. 2011. Technical publication on Allis shad stocking and monitoring measures within the Framework of the LIFE Allis shad project. https://www.lanuv.nrw.de/alosa-alosa/

Schmidt, B. R., M. Kery, S. Ursenbacher, O. J. Hyman, and J. P. Collins. 2013. Site occupancy models in the analysis of environmental DNA presence/absence surveys: a case study of an emerging amphibian pathogen. Methods in Ecology and Evolution 4:646–653.

Shogren, A. J., J. L. Tank, E. Andruszkiewicz, B. Olds, A. R. Mahon, C. L. Jerde, and D. Bolster. 2017. Controls on eDNA movement in streams: transport, retention, and resuspension /704/158/2464 /704/242 /45/77 article. Scientific Reports 7:1–11.

Snyder, D. E. 2004. Invited overview: conclusions from a review of electrofishing and its harmful effects on fish. Reviews in Fish Biology and Fisheries 13:445–453.

Thomsen, P. F., and E. Willerslev. 2015. Environmental DNA - An emerging tool in conservation for monitoring past and present biodiversity. Biological Conservation 183:4–18.

Thomsen, P. F., J. Kielgast, L. L. Iversen, C. Wiuf, M. Rasmussen, M. T. P. Gilbert, L. Orlando, and E. Willerslev. 2012. Monitoring endangered freshwater biodiversity using environmental DNA. Molecular Ecology 21:2565–2573.

Thuiller, W., B. Lafourcade, R. Engler, and M. B. Araújo. 2009. BIOMOD—a platform for ensemble forecasting of species distributions. Ecography 32:369–373.

Williams, J. N., C. Seo, J. Thorne, J. K. Nelson, S. Erwin, J. M. O'Brien, and M. W. Schwartz. 2009. Using species distribution models to predict new occurrences for rare plants. Diversity and Distributions 15:565–576.

Wittwer, C., C. Nowak, D. A. Strand, T. Vrålstad, M. Thines, and S. Stoll. 2018a. Comparison of two water sampling approaches for eDNA-based crayfish plague detection. Limnologica 70:1–9.

Wittwer, C., S. Stoll, D. Strand, T. Vrålstad, C. Nowak, and M. Thines. 2018b. eDNA-based crayfish plague monitoring is superior to conventional trap-based assessments in year-round detection probability. Hydrobiologia 807:87–97.

Wolf, C. M., B. Griffith, C. Reed, and S. A. Temple. 1996. Avian and mammalian translocations: update and reanalysis of 1987 survey data. Conservation Biology 10:1142–1154.

Woodroffe, R., and J. R. Ginsberg. 1999. Conserving the African wild dog *Lycaon pictus*. II. Is there a role for reintroduction? Oryx 33:143–151.

Yoccoz, N. G. 2012. The future of environmental DNA in ecology. Molecular Ecology 21:2031–2038.

Supporting Information

Additional supporting information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/eap.2034/full