Using environmental DNA to monitor the reintroduction success of the Rhine sculpin (*Cottus rhenanus*) in a restored stream

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Abstract

Freshwaters face some of the highest rates of species loss, caused by strong human impact. To decrease this strong impact, ecological restorations are increasingly applied to restore and maintain the natural ecological status of freshwaters. Their ecological status can be determined by assessing the presence of indicator species (e.g. certain fish species), which is called biomonitoring. However, traditional biomonitoring of fish, such as electrofishing, is often challenging and invasive. To augment traditional biomonitoring of fish, the analysis of environmental DNA (eDNA) has recently been proposed as an alternative, sensitive approach. The present study employed this modern approach to monitor the Rhine sculpin (Cottus rhenanus), a fish species that has been reintroduced into a recently restored stream within the Emscher catchment in Germany, in order to validate the success of the applied restorations and to monitor the species' dispersal.

We monitored the dispersal of the Rhine sculpin using replicated 12S end-point PCR eDNA surveillance at a fine spatial and temporal scale. In that way, we investigated if eDNA analysis can be applied for freshwater assessments. We also performed traditional electrofishing in one instance to validate our eDNA-based approach.

We could track the dispersal of the Rhine sculpin and showed a higher dispersal potential of the species than we assumed. We validated the species' dispersal across a potential dispersal barrier via eDNA detection and showed a steep increase of positive detections once the reintroduced population had established. In contrast to that, false negative eDNA results occurred at early reintroduction stages. Our results show that eDNA detection can be used to confirm and monitor reintroductions and to contribute to the assessment and modelling of ecological status of streams.

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Introduction

Freshwaters are heavily impacted due to habitat fragmentation caused by wetland drainage, river straightening and dam building; in combination with poor water quality caused by agricultural and industrial pollution (Jensen et al., 2006). This strong human impact can cause a decrease of the ecological status of freshwaters. Monitoring their ecological status is consequently an important task to assess if actions are needed for restoring and maintaining these ecosystems. Their ecological status can be determined by assessing indicator species, which include algae, benthic macroinvertebrates and fish (Bellinger and Sigee, 2015; Karr, 1981; Resh and Unzicker, 1975).

The Rhine sculpin (*Cottus rhenanus*; Freyhof et al., 2005) and the European bullhead (*Cottus gobio*; Linnaeus, 1758) are two closely related freshwater sculpin species (previously treated as one species) that prefer similar habitats. They require well-oxygenated streams (Colleye et al., 2013) with gravelly to stony stream beds (Wittkugel, 2005), show a stationary behavior with limited home ranges (Ovidio et al., 2009), and are presumed to be incapable of crossing barriers higher than 18-20 cm (based on a study on the European bullhead; Utzinger et al., 1998). These requirements make them good indicators for structure and passability of flowing waters due to their limited movement behavior. The Rhine sculpin used to be resident in the Emscher catchment (North Rhine-Westphalia, Germany), but became locally extinct during the 19th century when the catchment was used as an open sewer system for wastewater disposal (Brink-Kloke et al., 2006). The species survived in only one tributary stream that was less anthropogenically impacted during that period (Donoso-Büchner, 2009). Today, one of the largest European infrastructure projects supports comprehensive river restructuring of the Emscher catchment, with 4.5 billion Euro invested in the project as of 2015 (Böhmer, 2015).The project aims to restore the river and all its tributaries to a near natural ecological status (Schnelle and Wilts, 2016). These efforts make it possible to reintroduce individuals from the isolated Rhine sculpin population into the restored streams, which additionally confirms the restoration success.

Monitoring the reintroduction success of the Rhine sculpin is however logistically challenging. Electrofishing, where fish are temporarily stunned using an electronic device,

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is the established method for collecting individuals. This traditional approach is invasive for ecosystems and not always feasible (Bohlin et al., 1989; Platts et al., 1983). The application of environmental DNA (eDNA) analysis is a promising, and non-invasive alternative for the detection and biomonitoring of species in aquatic environments (Thomsen and Willerslev, 2015). While eDNA studies face several limitations because of eDNA degradation, PCR inhibition, and uncertainties about eDNA production and transportation (Goldberg et al., 2015), it was nevertheless shown that eDNA analysis has potential to effectively infer the richness of fish in streams, lakes and the ocean (Dejean et al., 2012; Hänfling et al., 2016; Jerde et al., 2011; Miya et al., 2015; Takahara et al., 2013; Yamamoto et al., 2016). A recent large-scale comparison has shown reliability and higher success rates when inferring the presence of fish species in rivers via eDNA analysis as compared to electrofishing (Pont et al., 2018).

The potential of eDNA analysis for monitoring fish dispersal has been shown for invasive species (Adrian-Kalchhauser and Burkhardt-Holm, 2016; Laramie et al., 2015; Takahara et al., 2013). However, to our knowledge it has not been applied to investigate the dispersal of reintroduced fish species yet. Therefore, the aim of this study was to use eDNA analysis for monitoring the reintroduction success and dispersal of the Rhine sculpin in a restored stream of the Emscher catchment. In the context of this study, the recently restored stream Borbecker Mühlenbach within the Emscher catchment was considered suitable for re-establishing the Rhine sculpin post restoration. Successful re-establishment of the target species in restored streams indicates that a good ecological status has been achieved, supporting successful restoration. We monitored the fish's dispersal at a fine spatial and temporal scale to test three specific hypotheses: (1) The reintroduced Rhine sculpin individuals will disperse faster upstream than expected from the species' typical stationary behavior (maximum of 149 m in 27 days in an established population = 5.51 m/day; Ovidio et al., 2009) because of the high density of individuals at the reintroduction sites; (2) the reintroduced individuals are not able to cross a potential dispersal barrier and will not be detected upstream of that barrier; and (3) individuals will establish along the entire stream section until the dispersal barrier because of the habitat's expected suitability for the species. To validate our eDNA results with traditional methods,

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we carried out electrofishing in one instance towards the end of our eDNA sampling period.

Material and Methods

Field site

The Borbecker Mühlenbach is a small urban stream categorized as German stream type 6 (fine-grained, carbonic mountain streams; Sommerhäuser and Pottgiesser, 2005; Fig. 1 A) that has its source in Essen, North Rhine-Westphalia, Germany. Like most streams within the Emscher catchment, the Borbecker Mühlenbach was used as an open sewer system for wastewater disposal from the 19th century on. It was recently restored (mainly carried out in 2011, finished in 2014) and today consists of both piped underground sections and restored above ground sections. This study focuses on a 1050 m long above ground section of the Borbecker Mühlenbach that represents a young freshwater ecosystem due to its recent restoration. A loose stone dam is located within the studied stream section, which was deemed to represent a potential dispersal barrier for the Rhine sculpin as it is approximately 40 cm high and blocks the entire width of the stream (Fig. 1 B). Further upstream of the stone dam, the stream Kesselbach enters the Borbecker Mühlenbach. After the studied stream section had been deemed suitable for reintroducing the Rhine sculpin post restoration, 118 Rhine sculpin individuals taken from the river Boye (Bottrop, Germany) were released into the stream at sampling sites 3-5 (Fig. 1 C) on the 23rd of August 2017. To prevent individuals from drifting downstream into underground pipes, a net was installed in front of the pipes.

Sampling

eDNA sampling

Samples were taken 8 days before reintroduction of the Rhine sculpin (negative control samples), and 2, 5 10, 44, 71, 104, 439 and 471 days after the reintroduction. 7 sites were

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sampled for the negative control samples, 14 sites for the first two sampling days, and 15 sites on the following sampling days (Fig. 1 C; sampling site 1a was added 50 m upstream of site 1 after two sampling days). The second last sampling day included both eDNA collection and electrofishing. Due to unexpected findings, we took samples at the last sampling day from site 10 on (sampling sites 11a and 11b were added 50 m and 100 m upstream of site 11). Sampling dates and distances between sampling sites are shown in Tab. 1; capital letters A – H indicate the respective sampling days.

For every eDNA sample, 1 L of water was collected in bottles (sterilized in 4% chlorine bleach overnight after each use) and filtered through sterile 0.45 µm cellulose nitrate filters (Thermo Scientific™ Nalgene™ filtration units, Thermo Fisher Scientific, Waltham, USA) using a vacuum pump. The filtering took place in the field to prevent cross-contamination by other laboratory samples. A separate filter was used for every 500 ml (2 filters per sample). Additionally, an extra filter was exposed to air and then included to the other samples at each sampling day. This was done to check for cross-contamination by air on site (blank sample). The filters were preserved in 96% ethanol and stored at -20°C until extraction.

Electrofishing

On the 5th of November 2018, electrofishing was carried out after taking water samples for eDNA analyses. One person experienced in electrofishing was using a portable backpack device (EFGI 650, Bretschneider, Chemnitz, Germany) and a round anode net with a diameter of 50 cm and a mesh size of 6 mm. The voltage was set to 115 V, the current to 3 A and the pulse frequency to 60 Hz. Another person was walking behind the first, additionally looking for stunned fish. Every sampling site was electrofished along a stretch of 20 m in upstream direction from the point of eDNA sampling, for approximately 15 min. Sites 11a and 11b were not electrofished because they were added after the electrofishing survey, and site 14 was not accessible for electrofishing due to the presence of dense vegetation. We are aware that a stretch length of 20 m does not meet requirements of official electrofishing standards (required is a stretch of 40 times the stream width at minimum; Barbour et al., 1999; Dußling, 2014). However, our goal was not to conduct a traditional survey on the entire fish inventory but to verify the presence

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of the Rhine sculpin at the respective sites. To not overfish the small stream for that purpose, we decided to deviate from the standard approach and to reduce the electrofished stream stretches to 20 m.

Laboratory work

All lab work was carried out in an eDNA laboratory with 45 mins of UV-light sterilization between separate work cycles. DNA was extracted using a modified salt extraction protocol (original protocol by Sunnucks and Hales (1996); for modified protocol see Weiss and Leese (2016)). For the purification, either the NucleoSpin Gel and PCR Clean-up kit (MACHEREY-NAGEL GmbH & Co. KG, Düren, Germany) or the MinElute Reaction Cleanup Kit (QIAGEN Inc., Germantown, Maryland, USA) were used, following the manufacturer's instructions. The final DNA extracts were eluted into 20 µL of water. The success of all extractions and purifications was verified using agarose gel electrophoresis.

Primers targeting the hypervariable mitochondrial 12S rRNA gene of the Rhine sculpin were designed using a reference database containing 272 sequences of the 12S rRNA gene from 57 fish species (Hänfling et al., 2016). These included all native fish species resident in the Borbecker Mühlenbach and Kesselbach. The complete mitochondrial genome of the Rhine sculpin was downloaded from NCBI GenBank (MF326941/NC_036147, both identical), which was representative for the study population in the 12S rRNA gene sequence, and added to the downloaded 12S rRNA dataset (for final mafft (Katoh and Standley, 2013) alignment of 12S rRNA gene sequences see Appendix A).

The presence of Rhine sculpin DNA within the eDNA extracts was tested with nested end-point PCRs using the QIAGEN Multiplex PCR Plus Kit (QIAGEN Inc., Hilden, Germany). For the first PCR step, 2 µL of the extracted eDNA, 0.25 µL of 100 µM universal fish 12S rRNA primers 12S_30F and 12S_1380R (Hänfling et al., 2016), 25 µL of the Multiplex MasterMix and 22.5 µL water were used per reaction. PCR conditions consisted of an initial incubation at 95°C for 15 minutes followed by 30 cycles of 95°C for 30 seconds, 50°C for 30 seconds, and 72°C for 50 seconds, and a final elongation at 72°C for 10 minutes. For the second PCR step, 1 µL template of each PCR product from step 1, 0.25

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193 µL of 100 µM newly designed primers C_12S_377F (5'–AGGCCCAAGTTGACAAACAC–
194 3') and C_12S_731R (5'–GGCGGGTAAAACAAGGAAGG–3'), 12.5 µL of the Multiplex
195 MasterMix and 11.25 µL water were used per reaction. PCR conditions consisted of an
196 initial incubation at 95°C for 15 minutes followed by 30 cycles of 95°C for 20 seconds,
197 63°C for 30 seconds, and 72°C for 1 minute, and a final incubation at 72°C for 5 minutes.
198 The newly designed primers amplify a 344 bp long region of the Rhine sculpin's 12S rRNA,
199 which is located within the 12S rRNA section targeted by the universal fish 12S rRNA
200 primers used in step 1. The designed primer pair is universal for 20 out of 24 species of
201 the Cottus complex with available 12S sequences on GenBank including Baikal sculpins,
202 according to PrimerBlast (NCBI, https://www.ncbi.nlm.nih.gov/tools/primer-blast/). Due to
203 the presence of only the Rhine sculpin in the investigated stream, misamplification of other
204 Cottus species caused by the universality of the primer pair was excluded. We included
205 negative PCR controls into each PCR to check for a clean PCR setup.

206 Half of the extracted DNA of every sample (10 µL) was used as template for the first PCR
207 step to minimize stochasticity effects, leading to five PCR replicates per sample containing
208 2 µL of the extracted DNA each. The presence of the Rhine sculpin was confirmed in a
209 sample if at least one of the five reactions of the second step showed a visible band at
210 expected amplicon length using agarose gel electrophoresis (with 1% agarose gels run
211 for 15 minutes at 80 V). In case the Rhine sculpin was not detected in the first half of the
212 eluate, the remaining eluate was used as an input for further PCRs. If this approach also
213 did not show any signal, then the Rhine sculpin was considered to be absent in a sample.

214 Samples from each sampling day were tested up to site 12. If the species was detected
215 behind the barrier (sites 11 and 12), then sites 13 and 14 were also tested.

216 To confirm the specificity of the primer pair, PCR amplicons of eleven positive replicates
217 from different samples were Sanger sequenced (Eurofins Genomics, Ebersberg,
218 Germany) that all confirmed the identity of the Rhine sculpin.

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Results

No DNA was found in the blank controls, excluding cross-contamination during filtering on site and confirming clean DNA extraction and purification. Moreover, no DNA was found in any negative PCR control of the nested end-point PCRs, confirming clean PCR setup.

The Rhine sculpin was not detected in any negative control sample, confirming its absence in the stream section prior to the reintroduction (Tab. 1). Within the first three months, the Rhine sculpin was detected at least once at every sampling site downstream of the barrier (site 1-10) and not detected upstream of the barrier (site 11 and 12). Samples 9 B and 10 C were positive, confirming the fish's dispersal of 200 m upstream within the first five days and 250 m within the first ten days, respectively. However, the species was not detected in several samples despite the presence of the species further upstream on the same day. In general, positive detections strongly varied among technical replicates, from five detections in five replicates to one detection in ten replicates. Positive detections in all technical replicates of one sample were rare within the first three months, indicating low DNA template concentration in the stream.

After one year, the species was detected at every sampling site downstream of the barrier, with positive detections in all technical replicates of each sample (apart from sample 4 G, of which only four of five technical replicates were positive). In addition, the Rhine sculpin was detected at sites 11 G and 13 G upstream of the barrier.

In contrast to that, we detected Rhine sculpin individuals at every site downstream of the barrier (apart from site 1) by conducting electrofishing at the same day, but no single individual upstream of the barrier. We detected a total number of 113 individuals, including juveniles, with 2-20 individuals found per site (Tab. 1).

After one more month, the species' presence behind the barrier was again confirmed by eDNA detections at sites 11 H, 11a H and 11b H (Tab. 1).

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Discussion

Dispersal of the Rhine sculpin

Based on end-point PCR eDNA detections, we showed that individuals dispersed at least 200 m within the first five days (positive sample 9 B), on average at least 40 m/day, which greatly exceeds daily moving distances of the Rhine sculpin in an established population observed by Ovidio et al. (2009; maximum of 5.51 m/day). This verifies our first hypothesis that the reintroduced individuals disperse faster from their reintroduction sites than expected from their known stationary behavior. An explanation for this pattern is that the initially high density of individuals at each reintroduction site led to high intraspecific competition for habitats. A density-dependent behavioral mechanism to compensate for this high density is the dispersal of smaller individuals to sub-optimal habitats (Davey et al., 2005). Note that maximum moving distances of up to 395 m for the Rhine sculpin during one year and over 250 m for the closely related European bullhead during several months have been observed (Knaepkens et al., 2005, 2006; Ovidio et al., 2009). Furthermore, seasonal migration has been shown for the European bullhead (Crisp et al., 1984). However, none of these studies investigated the fish's movement at a temporal scale comparable to the present study.

The Rhine sculpin was not detected in eDNA samples upstream of site 10 within the first three months and furthermore not detected with electrofishing after one year, indicating a dispersal limitation by the barrier before site 11. However, eDNA detections of the species upstream of the barrier after one year (detections at sites 11 G and 13 G) and moreover after one additional month (detections at sites 11 H, 11a H and 11b H) indicate that at minimum one Rhine sculpin individual was present upstream of the barrier. This result was unexpected (see second hypothesis) and not supported by our electrofishing survey. We based our second hypothesis on the finding that the European bullhead (which used to be a cryptic species including the Rhine sculpin) is unable to cross solid stream barriers of 18-20 cm height (Utzinger et al., 1998). However, the barrier in the present study consists of loose stones and is occasionally flooded during heavy rainfall events. It is possible that juveniles were able to swim through gaps in the lose stone dam or that individuals were able to cross the barrier after heavy rainfalls. Another explanation is

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predator-induced translocation of individuals across the barrier (for example by gray herons) or defecation of predators that ate Rhine sculpins into the stream section upstream of the barrier. Human-induced translocation of individuals across the barrier (for example by hand-netting) can also not be excluded, as the stream is urban and frequently visited by pedestrians. Nevertheless, we consider predator- or human-induced translocation as unlikely. Consequently, we conclude that our results indicate a higher dispersal potential of the species than previously assumed, because the loose stone dam was deemed to represent a substantial dispersal barrier for small, benthic fish.

The inconsistency between eDNA-based and traditional monitoring results for sites upstream of the barrier was unexpected. An explanation for the failure in detecting the species using electrofishing is that individuals were located upstream of the electrofishing sites and hence could not be detected via electrofishing. In that case, they were still releasing eDNA that was transported downstream and collected, making the detection of the individuals possible. However, a more comprehensive electrofishing survey would be needed to confirm this explanation.

The third hypothesis that individuals establish along the entire stream section downstream of the barrier could be confirmed by monitoring results of both surveys. After one year, the Rhine sculpin was detected at every sampling site downstream of the barrier using eDNA and at every sampling site downstream of the barrier except for site 1 using electrofishing. This verified our expectations that the section consists of habitats suitable for the Rhine sculpin. Additional sampling following the Rhine sculpins' next reproduction period is needed to investigate the fish's further dispersal upstream.

The finding of juveniles during the electrofishing survey confirmed reproduction of the species, and the recapture of 113 individuals while 118 individuals were released in total implies an increase in population size. This is a likely source for the highly increased number of positive eDNA-based detections after one year, although the adsorption-release dynamics of eDNA might also have facilitated the detection, i.e. more eDNA being released in contrast to the initial phase (Shogren et al., 2017; Spear et al., 2015).

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Our results confirm successful reintroduction, dispersal, and establishment of the species within the stream Borbecker Mühlenbach. Additionally, the species' establishment indicates good water quality, habitat structure and passability of the stream and consequently indicates a successful stream restoration.

Applicability of eDNA for biomonitoring of fish

Our electrofishing observations suggest that the Rhine sculpin was not able to cross a minor barrier in the stream, which is congruent with previous observations for the closely related European bullhead (Utzinger et al., 1998). However, results of our eDNA analysis revealed that the species was present upstream of the barrier, indicating that the mobility of the Rhine sculpin and its capacity to cross instream obstacles is better than previously assumed. Greater electrofishing efforts, which are typically performed in standardized surveys, might have led to detection of the Rhine sculpin upstream of the barrier. Nevertheless, our observed trend is consistent with previous studies showing that overlooking individuals is the rule rather than the exception with electrofishing (Jerde et al., 2011; Pont et al., 2018). Although analyzing eDNA cannot be used for estimating individual numbers or age structure, its application in biomonitoring presents a promising, non-invasive, and sensitive tool.

Nevertheless, the applicability of eDNA analysis as a monitoring tool is still limited, as many characteristics of eDNA are poorly understood yet. It is known that eDNA release can be affected by various conditions of an individual, for example size, health, sex, fecundity or diet (Goldberg et al., 2015; Klymus et al., 2015); the extent and variation between species and even individuals remain, however, unclear. Moreover, eDNA durability in aquatic environments is strongly influenced by degradation through temperature, pH value, conductivity, UV light and microbes (Barnes et al., 2014), reducing eDNA concentration and hence causing additional bias. Furthermore, eDNA movement processes such as downstream transport, soil retention and soil resuspension constantly affect eDNA concentration (Shogren et al., 2017). Apart from these ecological issues, methodological difficulties can lead to failure in detecting eDNA, caused by stochastic effects and polymerase chain reaction (PCR) inhibition (Taberlet et al., 1996; Thomsen

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and Willerslev, 2015). Together, these may lead to inexplicable patterns of eDNA detection in some studies (observed by Jane et al. (2015) and Laramie et al. (2015)).

eDNA can be transported downstream over long distances in flowing waters (Deiner and Altermatt, 2014; Deutschmann et al., 2019; Jane et al., 2015), which implies that Rhine sculpin eDNA was transported downstream from actual residence sites in our study. This effect was observed for sample 1 G, where the species was detected even though electrofishing failed to detect individuals up to 20 m upstream of the site. Consequently, every detection of the Rhine sculpin downstream of the uppermost detection site could represent false positives rather than true local detection. Visual observation of individuals by traditional surveys is still necessary for the validation of local presence at sites downstream of actual residence sites. However, when validating the general presence of a fish species in flowing waters, this effect might be beneficial, as there is no need to sample the entire stream or river.

In this study the Rhine sculpin was not detected at several sites despite the presence of the species further upstream on the same day. These occurrences represent false negatives, whose presence is consistent with comparable studies (Foote et al., 2012; Jane et al., 2015; Laramie et al., 2015) and can be explained by dilution of DNA by high flows, increasing distance from the DNA source or PCR inhibition (Jane et al., 2015); eDNA dynamics in the system (e.g. retention to stream bottom; Shogren et al., 2017); and the less sensitive end-point PCR approach we used (Turner et al., 2014). Inhibition is especially problematic in eDNA studies, as it can mask even high eDNA copy numbers (Jane et al., 2015) and hence can lead to false negatives although the target organism is present (Goldberg et al., 2015; Thomsen and Willerslev, 2015). Addressing PCR inhibition is therefore one of the major challenges in detecting target species, especially in biomonitoring.

Conclusion

Our study shows that eDNA can provide detailed insights into reintroduction success and fish dispersal. eDNA analysis allowed for monitoring the dispersal of the Rhine sculpin in a small German stream at a fine temporal and spatial scale. The method revealed a

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363 substantially higher realized dispersal potential than previously assumed and verified a
364 successful stream restoration. We conclude that eDNA analysis is thus applicable to
365 investigate the ecological status and fragmentation of streams by proving the presence of
366 an indicator species, making it a useful tool for biomonitoring. Consequently, eDNA
367 analysis holds potential for freshwater assessments and is an effective, non-invasive
368 approach that can be used to augment traditional methods, although several aspects need
369 to be further understood to correctly interpret false negative results.

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513 ## Figures

514

515 Fig. 1: (A) Borbecker Mühlenbach, (B) Barrier before site 11, which was assumed to be
516 a dispersal barrier for the Rhine sculpin, (C) Detailed map of the sampling sites at the
517 stream. For coordinates of and distances between sampling sites see Supplementary
518 Data S2.

519

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Tables

Tab. 1: Results of the Rhine sculpin detection by nested end-point PCRs. Positive samples are coloured in dark green, negative samples in orange. Each sample shows the number of positive technical replicates. White text indicates Sanger sequencing for one of the replicates. Electrofishing results are coloured in light blue and show the number of captured individuals per 20 m section.

Date	Day	Sampling event	Sampling site and distance to site 1 in [m]																					
			1	1a	2	3	4	5	6	7	8	9	10			11	11a	11b		12		13	14
			0	50	100	200	250	300	350	400	450	500	550	600		650	700	750	800	850	1000	1050	
2017-08-15	-8	Neg	0/10			0/10				0/10				0/10	Barrier				0/10		0/10	0/10		
2017-08-23	0					Reintroduction																		
2017-08-25	2	A	1/5		0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10			0/10				0/10				
2017-08-28	5	B	5/5		1/5	1/5	0/10	2/5	0/10	1/10	0/10	1/5	0/10			0/10				0/10				
2017-09-02	10	C	1/5	4/5	3/5	4/5	3/5	1/5	1/5	0/10	1/5	0/10	1/10			0/10				0/10				
2017-10-06	44	D	2/5	4/5	4/7	5/7	0/10	0/10	0/10	2/7	0/10	0/10	0/10			0/10				0/10				
2017-11-02	71	E	2/5	1/5	5/5	3/5	1/5	1/5	1/5	1/5	0/10	1/5	0/10			0/10				0/10				
2017-12-05	104	F	3/5	3/5	4/5	3/5	1/5	0/10	0/10	1/10	0/10	0/10	0/10			0/10				0/10				
2018-11-05	439	G	5/5	5/5	5/5	5/5	4/5	5/5	5/5	5/5	5/5	5/5	5/5			2/10				0/10		1/10	0/10	
		E-fishing	0	18	20	5	15	2	10	13	4	18	8			0				0		0	NA	
2018-12-07	471	H											5/5			5/10	1/10	1/10		0/10		0/10	0/10	

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