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Research Article

The development of an eDNA based detection method for the invasive shrimp *Dikerogammarus haemobaphes*

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Abstract

Dikerogammarus haemobaphes is a freshwater gammarid crustacean native to the Ponto-Caspian region. However, the species is rapidly spreading throughout Western Europe and is classed as a highly invasive species. Here we present a novel eDNA assay aimed at detecting *D. haemobaphes* and demonstrate its suitability with validation steps conducted *in-silico* (computer simulations), *ex-situ* (test of specificity using closely related species) and *in-situ* (within the field). A survey of freshwater systems in the West-Midlands, United Kingdom, highlighted that *D. haemobaphes* was present in 26 out of the 39 sites assessed. We conclude that eDNA detection for *D. haemobaphes* is a promising tool for assessing and mapping the presence/distribution of this invasive amphipod.

Key words: invasive species, early detection, freshwater systems, environmental DNA, Limit of Detection, Limit of Quantification, qPCR

Introduction

Amphipods are a very successful group of invertebrates and many species can impact on the benthic communities and ecosystems of fresh and brackish water systems (van der Velde et al. 2009). Upwards of 1870 species (and sub-species) have been described to date – all of which have been shown to inhabit fresh or inland waters around the world (Väinölä et al. 2008). In some instances, abundances of certain species have been recorded to exceed 5,000 individuals per square metre (Kotta et al. 2013). Their rapid proliferation rates translate to a high potential to function as effective invaders and disrupt natural communities of ecosystems where they are non-native (van der Velde et al. 2009). One example of just such an invader is *Dikerogammarus haemobaphes* (Eichwald, 1841), also referred to as the "demon shrimp". *D. haemobaphes* originates from the Ponto-Caspian region, however the species has been documented to progressively move across much of Central and Western Europe over recent years (Bacela et al. 2009). In the United Kingdom, it was first recorded in 2012 in the river Severn (Constable and Birkby 2016; Aldridge 2018), and has since spread

rapidly through many canal and river networks across the country (Constable and Birkby 2016). The invasion of this species can lead to significant threats to native species, such as *Gammarus pulex* (Linnaeus, 1758), by direct predation, intensifying resource competition and functioning as a vector for new diseases and parasites (Constable and Birkby 2016).

The far-reaching ecological effects of this invasive species (Gallardo and Aldridge 2013) highlight the importance to develop an effective early detection and monitoring system to improve the conservation plans for endangered and functionally important native species. However, existing methods used for detecting amphipods (i.e. kick-sampling, capture-recapture)—especially for those occurring at low population densities—are labour-intensive, often ineffective (Forsström and Vasemägi 2016), time consuming, expensive, ecologically invasive (as it may cause injuries to targeted and non-targeted organisms) (Eiler et al. 2018) and require (in many cases) a high level of taxonomic expertise (Ushio et al. 2017). New detection techniques facilitating area-wide surveys are therefore urgently required.

All aquatic species leave traces of their DNA within their environment. These DNA fragments may originate from eggs, mucus, faeces or shedding of the epidermis (Thomsen and Willerslev 2015) and are referred to as environmental DNA (eDNA) (Ficetola et al. 2008). By sampling an aquatic system and amplifying existing eDNA, it is now possible to determine the presence of a given species via means of targeted barcoded qPCR or metagenomics (Dejean et al. 2011; Thomsen and Willerslev 2015). eDNA methodology has been used successfully over the last 10 years for various target organisms (Hunter et al. 2016; Mauvisseau et al. 2018; Parrondo et al. 2018). eDNA based methods represent a non-invasive tool for assessing species distribution i.e. such methods do not require catching, disturbing or even killing the target organisms. Further, the use of eDNA has been shown to be a highly repeatable and relatively cost effective method as it requires a lower sampling effort than more traditional survey methods (Smart et al. 2016; Evans et al. 2017; Mauvisseau et al. 2018; Parrondo et al. 2018; Bálint et al. 2018).

The aim of our study therefore was to further advance eDNA approaches for surveying aquatic amphipods and develop a targeted barcoded eDNA method for *D. haemobaphes*. We developed and validated a new assay and tested its specificity *in-vitro* and *in-silico* on numerous closely related species and on species, which are likely to share the same habitat as the target organism. The reliability of our assay, i.e. the limit of detection (LOD) and quantification (LOQ), was assessed following the method outlined in (Tréguier et al. 2014). To ensure assay design was optimum, the Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) guidelines were utilised (Bustin et al. 2009) (See Appendix 1). Further, we tested the new assay on 39 sites across the West-Midlands, United

Table 1. Table depicting eDNA detection results, the number of positive qPCR replicates, the mean Cycle threshold (Ct) of positive replicates, the collection date, the type of site and the GPS coordinates of each sampled locations.

Locations	eDNA detection	Number of positive replicates	Mean Ct	Collection date	Type of site	Latitude	Longitude
1	Yes	1/6	36,13276291	01/12/2016	Canal	52,7212	−1,7995
2	No	–	–	01/12/2016	Canal	52,7219	−1,7879
3	Yes	1/6	36,32998657	29/11/2016	Canal	52,6121	−1,7025
4	Yes	4/6	36,40121746	29/09/2016	Canal	52,7597	−2,0982
5	Yes	1/6	35,16476822	29/09/2016	Pond	52,7568	−2,0961
6	Yes	1/6	37,29187012	27/10/2016	Canal	52,6616	−1,9336
7	No	–	–	27/10/2016	Canal	52,6594	−1,9301
8	No	–	–	27/10/2016	Canal	52,6641	−1,9397
9	No	–	–	27/10/2016	Canal	52,6566	−1,9263
10	Yes	1/6	36,51880264	27/10/2016	Canal	52,6379	−1,9706
11	Yes	2/6	36,52320671	23/11/2016	Canal	52,5453	−2,0090
12	Yes	3/6	36,98597972	16/11/2016	Canal	52,5240	−2,0484
13	Yes	1/6	35,91026306	16/11/2016	Canal	52,5154	−2,0494
14	Yes	3/6	36,56238302	16/11/2016	Canal	52,5161	−2,0240
15	No	–	–	13/11/2016	Canal	52,5006	−2,1004
16	Inconclusive	3/6	38,43717448	13/11/2016	Canal	52,4872	−2,1160
17	Yes	6/6	37,4743983	13/11/2016	Canal	52,4750	−2,1268
18	Yes	1/6	36,24039078	13/11/2016	Canal	52,4846	−2,0925
19	Yes	3/6	36,27352524	11/11/2016	Canal	52,4891	−2,0733
20	Yes	3/6	36,4181811	13/11/2016	Canal	52,4822	−2,0606
21	Inconclusive	2/6	40,08729935	11/11/2016	Canal	52,4730	−2,0558
22	No	–	–	11/11/2016	Canal	52,4704	−2,0522
23	No	–	–	11/11/2016	Canal	52,4595	−2,0397
24	No	–	–	12/12/2016	Canal	52,4890	−1,4584
25	No	–	–	12/12/2016	Canal	52,5910	−1,3951
26	No	–	–	12/12/2016	Canal	52,6577	−1,4455
27	Yes	2/6	36,7260437	12/12/2016	Canal	52,6800	−1,4947
28	Yes	1/6	37,28199768	01/12/2016	Canal	52,7262	−1,7862
29	Yes	1/6	37,62047958	29/09/2016	Canal	52,7542	−2,0964
30	Yes	3/6	36,47432709	29/09/2016	Canal	52,7378	−2,0942
31	No	–	–	11/10/2016	Pond	52,5274	−2,1582
32	No	–	–	06/10/2016	Reservoir	52,4949	−2,1279
33	Yes	1/6	37,29760361	27/09/2016	Canal	52,4770	−2,1548

Kingdom, in order to validate the assay in the field and assess the distribution of *D. haemobaphes* (Table 1 and Appendix 2). Based on our results, we present the efficiency of our developed assay and propose recommendations for field sampling protocols.

Materials and methods

Primers and probe design

Species specific primers, targeting the Cytochrome C Oxidase subunit 1 (i.e. COI) mitochondrial gene of *D. haemobaphes* were designed using the Geneious Pro R10 Software https://www.geneious.com (Kearse et al. 2012). A probe (6-FAM – TTCTTAATATGCGCGCCCCAGGC – BHQ-1) was designed to complement both the forward primer (EY-COI-DhF 5' – GGAGCTTCCTCTATTCTTGGCGCAATT – 3'), and the reverse primer (EY-COI-DhR 5' – GGCCGTGATAAAGACAGACCAGACAAA – 3') in order to increase specificity of the reaction. This resulted in a 117 bp fragment of DNA from the COI region when amplified. Sequences from 23

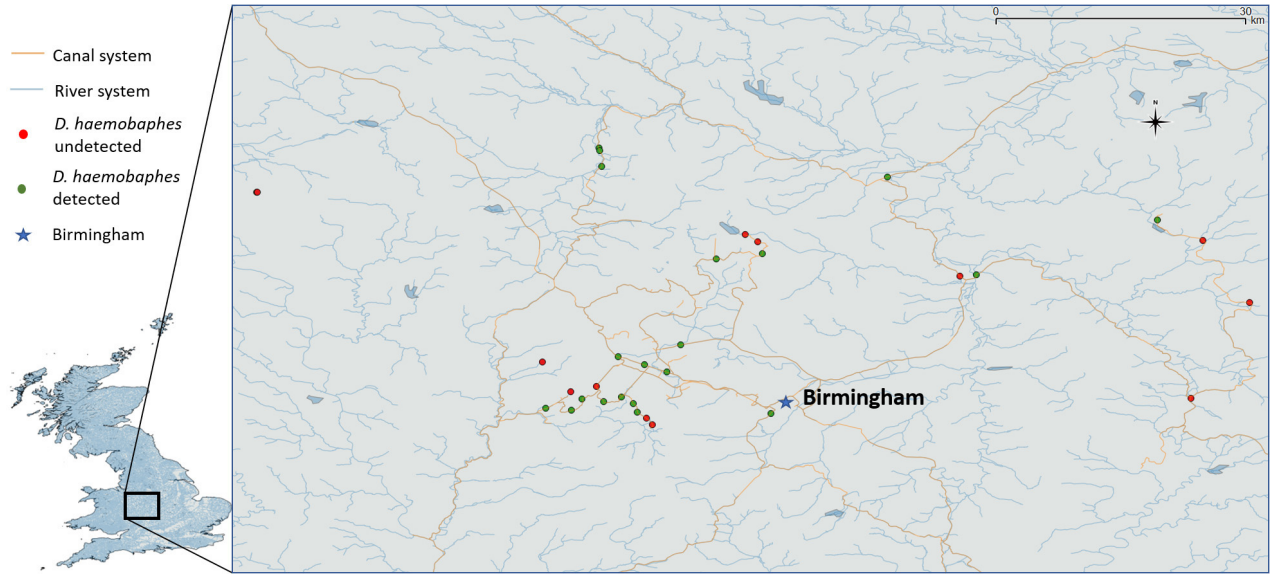

Figure 1. Map of the canal and rivers system showing the sampling locations (n = 33) screened for the presence of *D. haemobaphes* in the United Kingdom. DNA of the targeted species was detected in the green locations. Red locations showed the places found to be negative to eDNA detection.

species (which are either taxonomically similar to *D. haemobaphes*, or likely to be present within the same habitats) were utilised during the development and assessment of the assay sensitivity (Table S1). The assay was then tested against extracted DNA from the following species to further ensure specificity; *G. pulex,* the killer shrimp *Dikerogammarus villosus* (Sowinsky, 1894), *Gammarus fossarum* (Koch, 1836), *Sigara fossarum* (Leach, 1817), *Cloeon dipterum* (Linnaeus, 1761), the spinycheek crayfish *Faxonius limosus* (Rafinesque, 1817), the signal crayfish *Pacifastacus leniusculus* (Dana, 1952), the noble crayfish *Astacus astacus* (Linnaeus, 1758), the narrow-clawed *Pontastacus leptodactylus* (Eschscholtz, 1823), the Louisiana crayfish *Procambarus clarkii* (Girard, 1852) and the white-clawed crayfish *Austropotamobius pallipes* (Lereboullet, 1858). DNA was also extracted from four individual *D. haemobaphes*.

eDNA samples

33 locations spanning canals, rivers and reservoirs were sampled across the West Midlands in the United Kingdom (Figure 1). Sampling was conducted between the 29th September 2016 and the 12th December 2016 (Table 1). At each location, a 1 L water sample was collected with a sterile polypropylene ladle (see Figure 2). To acquire the 1 L water sample, 25 "sub-samples" of 40 ml of water were collected and placed into a sterile plastic bag (Whirl-Pak® 1242 ml Stand-Up Bag Merck®, Darmstadt, Germany) for homogenisation. Out of the 1 L sample, we transferred 6 × 15 mL into 6 sterile falcon tubes (Falcon™ 50 mL Conical Centrifuge Tube, Fisher Scientific, Ottawa, Canada) containing 1.5 mL 3M sodium acetate and 33.5 mL of absolute ethanol using sterile disposable plastic pipettes. All samples were then stored at −20 °C before further analysis. Furthermore, at 6 additional locations, kick-sampling was

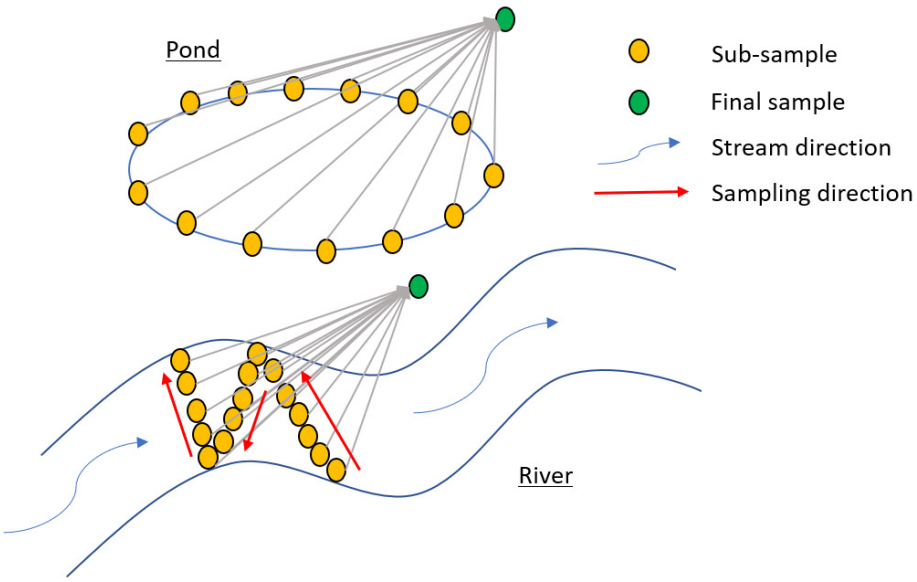

Figure 2. Representation of the eDNA sampling protocol in respective freshwater systems. For each location, 25 sub-samples of 40 mL were taken to obtain a representative 1 L final sample of the location. In small river system or canal, sub-samples are taken from across the river/canal and sampled from downstream to upstream. This ensures disturbed sediment washes downstream from the collection point at any given time.

undertaken (in addition to the eDNA sampling protocol mentioned above – Appendix 2). However, results from these 6 locations were only used for confirming the specificity of the eDNA assay to traditional sampling methods and not utilised in the spatial analysis which was conducted on the 33 original sites (highlighted above).

DNA extraction

Laboratory equipment was disinfected using 10% bleach solution and ethanol and decontaminated under UV lights (Mauvisseau et al. 2017; Parrondo et al. 2018). DNA extraction was performed in a clean, PCR free room. The Qiagen DNeasy® Blood and Tissue Extraction Kit was used for eDNA extraction following manufacturers' guidelines. eDNA from the water samples were extracted following the methods outlined in (Tréguier et al. 2014). DNA pellets from all 6 falcon tubes were pooled together and hence a total water volume of 90 mL per location was analysed. The final DNA elution volume was 100 µL.

PCR and qPCR amplification

PCR amplifications were performed on a Gen Amp PCR System 9700 (Applied Biosystem). A 25 µL reaction was run for each sample, consisting of 12.5 µL of PCRBIO Ultra Mix Red (PCRBIOSYSTEMS), 1 µL of each primer (10 µM), 9.5 µL of ddH_2O and 1 µL of template DNA. qPCR amplifications were performed on an ABI StepOnePlus™ Real-Time PCR (Applied Biosystems) in the same final volume of 25 µL. In contrast to standard PCR, the mixture for qPCR consisted of; 12.5 µL of TaqMan™

Environmental Master Mix 2.0 (ThermoFisher Scientific, UK), 1 µL of each primer (10 µM), 1 µL of probe (2.5 µM), 6.5 µL of ddH_2O and 3 µL of extracted DNA. Both PCR and qPCR were performed under the following protocol. Initial denaturation at 50 °C for 2 min and 95 °C for 10 min, followed by 45 cycles of 95 °C for 15 s and 64 °C for 1 min.

A standard curve was established by analysing a 1:10 dilution series of DNA extracted from *D. haemobaphes* (164.1 ng/µL, Nanodrop 2000 Spectrophotometer, ThermoFisher Scientific) following the MIQE Guidelines (Bustin et al. 2009). Dilutions ranged from 10^{-1} to 10^{-9} with 10 technical replicates used for each of the dilution steps. This meant we could work out the Limit of Detection (LOD). We defined the LOD as the last dilution in the standard curve at which eDNA is detected with a threshold cycle (Ct) below 45. The Limit of Quantification (LOQ) was also assessed. LOQ was defined as the last dilution in the standard curve at which eDNA is detected and quantified in at least 90% of the qPCR replicates with a threshold cycle below 45 (Bustin et al. 2009; Hunter et al. 2016). All eDNA samples were then analysed with six technical replicates (Cowart et al. 2018) on a qPCR plate including six negative controls and a positive control dilution series from 10^{-1} to 10^{-5} in duplicate.

Statistical analysis

A standard dilution was undertook for the assay in order to determine the LOD and the LOQ (Bustin et al. 2009; Tréguier et al. 2014; Hunter et al. 2016). Then, the likelihood of obtaining similar eDNA detection results in locations geographically close to each other was also explored utilising the GPS coordinates from the original 33 sampled locations. For this, a matrix distance was calculated containing pairwise distances (in meters) between all data points. Further, we established a second matrix, containing the information on the constancy of eDNA detection between two locations. eDNA based detection was considered as consistent if the target species was either present or absent in both locations. We then assessed (utilising a logit-regression analysis) whether the probability of obtaining consistent results decreased with the increase in distance between sampling locations. Regression analyses were performed with logged and non-logged data and the most parsimonious model was chosen based on the Akaike Information Criterion (AIC). Residuals of the regression were checked for autocorrelation, Cook's distance and systematic trends of residuals. All statistical analyses were performed with R version 3.4.1 (R Core Team 2018).

Results

The assay designed in this study was found to be species-specific to *D. haemobaphes*. Both PCR and qPCR did not result in any positive detection of non-target species in all *in-vitro* tests (i.e. none of the PCR and

qPCR controls showed any amplification during this study). The LOD and the LOQ of the assay determined by an analysis of the standard curve (Slope = −3.577, Y inter = 18.037, R^2 = 0.937, Eff% = 90.341) was assessed and revealed a LOD of 1.641 pg DNA μl^{-1} at 38.236 ± 0.915Ct and a LOQ of 16.41 pg DNA μl^{-1} at 34.90 ± 0.690 Ct.

We confirmed the efficiency of our developed assay using both kick-sampling and eDNA analysis (Appendix 2). We were able to detect *D. haemobaphes* using both methods in 4 locations and had an inconclusive result in another location due to a Ct value over the LOD. One location was positive only with eDNA methodology. The blank control (see Appendix 2) and all negative control technical replicates showed no amplification with PCR or qPCR during the whole experiment.

eDNA of *D. haemobaphes* was detected in 21 of the 33 tested locations (Table 1). Mean Ct values from the positive field samples ranged from 35.2 to 40.1. Notably, two sites showed a high mean Ct value, which ranged well above the established LOD. However, both of these sites contained technical replicates with Ct values below the LOD. As the mean Ct values from all positive locations were above the established LOQ, only the presence and absence data were utilised for further assessments.

The analysis of matrices containing the physical distance of sampling locations and the consistency of eDNA measurements revealed that an increase of the distance between two locations increased the probability to attain different eDNA detection results ($p < 0.023$). Interestingly sampling locations within close proximity to each other showed a relatively low eDNA based detection consistency of 68% (Figure 4). We evaluated two versions of the regression model containing either logged or non-logged physical distance values. While both versions resulted in a significant influence of physical distances, the linear model (non-logged data) was more parsimonious (lower AIC). The relationship showed a large scattering around predictions indicating that there is likely to be a number of factors not included in the analysis influencing the consistency of *D. haemobaphes* detection.

Discussion

In this study, we introduce a novel eDNA assay, which can be used to assess the presence of *D. haemobaphes*. The approach was shown to be highly sensitive and no false positives were identified, either via our *ex-situ* or *in-situ* validation tests. Further, we were able to demonstrate that the assay can successfully detect *D. haemobaphes* in various habitats including lotic and lentic systems such as ponds, canals and faster flowing rivers. Design of a novel eDNA based method mean that, in contrast to traditional tools such as kick-sampling for example, the environment does not need to be disturbed when any survey is undertaken. Further, the use of eDNA eliminates

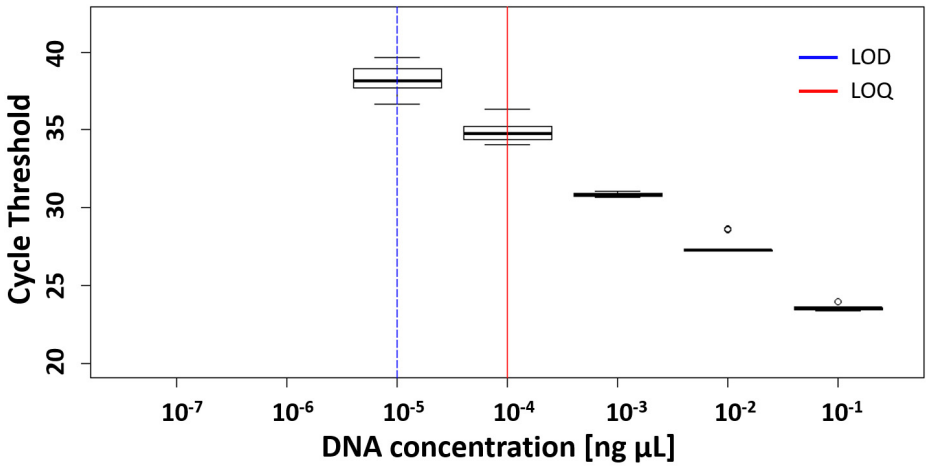

Figure 3. Standard curve used for determining the Limit of Quantification (LOQ) and Limit of Detection (LOD) relating Ct (threshold cycle) of the qPCR targeting the COI region of *D. haemobaphes* to DNA dilution steps. The standard curve was obtained using a dilution series with 10 replicates per concentration, with a DNA sample starting of 164.1 ng DNA μL^{-1}. The Ct represents the number of qPCR amplification cycles required for a positive detection. The LOD was found to be 1.641 pg DNA μl^{-1} at 38.2 ± 0.9 Ct (with 10/10 replicates showing an amplification) and the LOQ was found to be 16.41 pg DNA μl^{-1} at 34.9 ± 0.7 Ct (with 8/10 replicates showing an amplification).

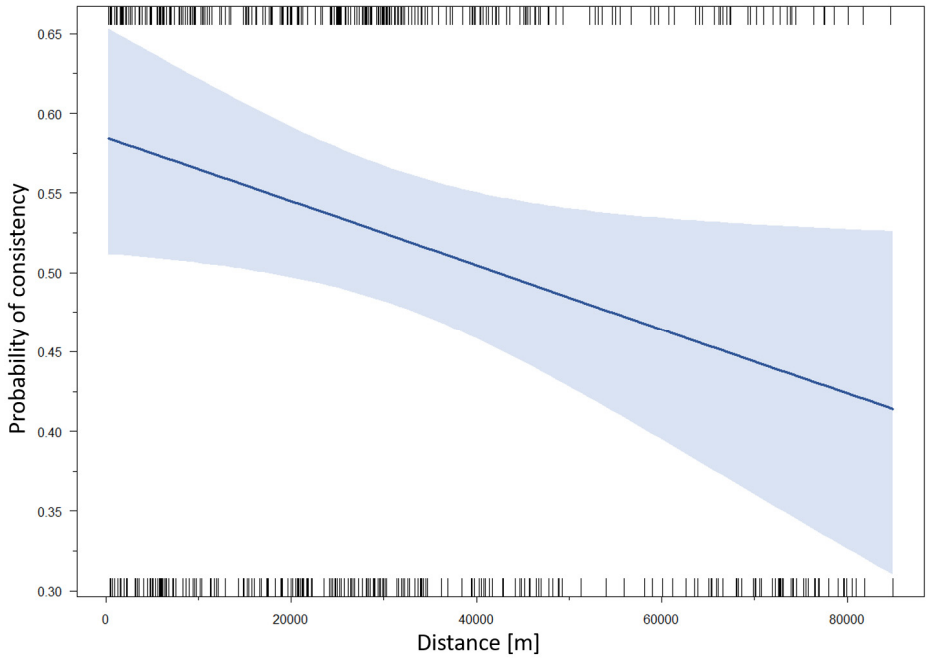

Figure 4. Relationship between the probability of obtaining the same eDNA detection result in two different sampling locations (i.e. probability of consistency) and the distance between sites. Distances between sites are stated in meters. The blue line and the blue shaded area reflect the regression equation and its confidence interval, respectively. Black ticks on the upper and lower edge of the graph represent data points.

the need for high level taxonomic expertise. However, despite the specificity of the approach and the reliability of detection, our field assessment highlights several possible ways we can improve the sampling protocols.

First, the amount of eDNA detected was generally low and ranged above the established LOQ in all sites (Table 1, Figure 3). This indicates that our field sampling protocol for *D. haemobaphes* was in contrast to protocols

developed for other aquatic invertebrates (Yusishen et al. 2018) and therefore not suitable for the accurate quantification of DNA traces, as a proxy for population densities. There are a number of possible explanations for this. For example, population densities may be low for this species and/or *D. haemobaphes* may only shed a reduced amount of DNA into its environment (Buxton et al. 2017). A corroborating reason, however, may be the choice of sampling method utilised in this study (Piggott 2016). Here we opted to use the established ethanol precipitation method introduced by (Tréguier et al. 2014), which is commercially available for the detection of the endangered Great Crested Newt *Triturus cristatus* (Laurenti, 1768) (Harper et al. 2018). This method results in the extraction of eDNA from only 90 mL of water. In contrast, the use of filters is becoming a more widespread and practiced method for eDNA surveys and more often results in the filtration of upwards of 250 mL of water (Rees et al. 2014). Indeed, other studies focusing on macroinvertebrates have utilised filtration successfully to detect eDNA, but as of yet there is no consensus on the optimal filter type (Mächler et al. 2014; Niemiller et al. 2017). Further studies will therefore benefit from a detailed assessment of sampling design when utilising eDNA of any given species. Moreover, our analysis revealed that an increase of the distance between two locations increased the probability of obtaining different eDNA results between two locations. This is an expected result because samples taken from the same canal-section or from the same river reach are more likely to have a similar habitat suitability than sections far apart from each other. However, sampling locations within close proximity to each other showed a relatively low eDNA detection consistency of 68%. One factor contributing to this finding might be a large habitat heterogeneity. Indeed, tributaries to channels can be in close proximity but may very well have a different species composition than the main channel of the river potentially explaining different result in sampling locations separated by only a few hundred meters. Anthropogenic interventions (such as dams) and-or variation in habitat quality (such as levels of pollution), can also affect the presence and/or dispersion of any given species (invasive or not) and would therefore be picked up as variation in the consistency in the eDNA assays of close environmental replicates.

However, we want to acknowledge that the sampling protocol utilised here may also have caused relatively large inconsistency in eDNA results of adjacent sampling sites. For example, sampling a small amount of water (90 mL) could increase the stochasticity of eDNA detection (Foote et al. 2012; Wilcox et al. 2016). Protocols of eDNA capture and extraction often varies between studies and for different target species (Deiner et al. 2015). Therefore, the sampling methodology could influence the reliability in the eDNA detection of any given organism and/or any given eDNA assay. The

filtration of large amounts of water is one possible approach to reduce the variability of eDNA detection in samples from the same location (Adrian-Kalchhauser and Burkhardt-Holm 2016) and this has been recommended by several studies for detecting eukaryotes in freshwater ecosystems (Deiner et al. 2015; Hinlo et al. 2017). Alternatively, using multiple field replicates for each sampling locations could also allow for the reduction in the variability of eDNA detection and improve the detectability of the target species even at low abundance. A detailed assessment of the effect of sampling method choice/water volume utilised on the consistency of eDNA detection would be an interesting next step to further improve the efficiency and reliability of eDNA based surveys.

In conclusion, our study illustrates a novel and reliable method to assess the presence of *D. haemobaphes* populations. As this proposed assay is non-invasive and can be utilized in a citizen science type program, it can be easily brought into existing biodiversity management plans – especially those tackling the spread of invasive species.

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Author contributions

Q.M., E.Y. and M.S., designed the experiment and methodology; C.T. collected field samples; Q.M., E.Y. and C.T., performed extraction and qPCR; Q.M. and A.B. analysed the data. The manuscript was written by Q.M and M.S and reviewed by all authors.

Competing interest

The authors declare no competing interests.

References

Adrian-Kalchhauser I, Burkhardt-Holm P (2016) An eDNA Assay to Monitor a Globally Invasive Fish Species from Flowing Freshwater. *PLoS ONE* 11: e0147558, https://doi.org/10.1371/journal.pone.0147558

Aldridge DC (2018) GB Non-native Species Rapid Risk Assessment for *Dikerogammarus haemobaphes* (Eichwald, 1841). http://www.nonnativespecies.org/downloadDocument. cfm?id=870

Bacela K, Konopacka A, Grabowski M (2009) Reproductive biology of *Dikerogammarus haemobaphes*: an invasive gammarid (Crustacea: Amphipoda) colonizing running waters in Central Europe. *Biological Invasions* 11: 2055–2066, https://doi.org/10.1007/s10530-009-9496-2

Bálint M, Nowak C, Márton O, Pauls SU, Wittwer C, Aramayo JL, Schulze A, Chambert T, Cocchiararo B, Jansen M (2018) Accuracy, limitations and cost efficiency of eDNA-based community survey in tropical frogs. *Molecular Ecology Resources* 18: 1415–1426, https://doi.org/10.1101/176065

Bustin SA, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M, Mueller R, Nolan T, Pfaffl MW, Shipley GL, Vandesompele J, Wittwer CT (2009) The MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments. *Clinical Chemistry* 55: 611–622, https://doi.org/10.1373/clinchem.2008.112797

Buxton AS, Groombridge JJ, Zakaria NB, Griffiths RA (2017) Seasonal variation in environmental DNA in relation to population size and environmental factors. *Scientific Reports* 7: 46294, https://doi.org/10.1038/srep46294

Constable D, Birkby NJ (2016) The impact of the invasive amphipod *Dikerogammarus haemobaphes* on leaf litter processing in UK rivers. *Aquatic Ecology* 50: 273–281, https://doi.org/10.1007/s10452-016-9574-3

Cowart DA, Breedveld KGH, Ellis MJ, Hull JM, Larson ER (2018) Environmental DNA (eDNA) applications for the conservation of imperiled crayfish (Decapoda: Astacidea) through monitoring of invasive species barriers and relocated populations. *Journal of Crustacean Biology* 38: 257–266, https://doi.org/10.1093/jcbiol/ruy007

Deiner K, Walser JC, Mächler E, Altermatt F (2015) Choice of capture and extraction methods affect detection of freshwater biodiversity from environmental DNA. *Biological Conservation* 183: 53–63, https://doi.org/10.1016/j.biocon.2014.11.018

Dejean T, Valentini A, Duparc A, Pellier-Cuit S, Pompanon F, Taberlet P, Miaud C (2011) Persistence of Environmental DNA in Freshwater Ecosystems. *PLoS ONE* 6: e23398, https://doi.org/10.1371/journal.pone.0023398

Eiler A, Löfgren A, Hjerne O, Nordén S, Saetre P (2018) Environmental DNA (eDNA) detects the pool frog (*Pelophylax lessonae*) at times when traditional monitoring methods are insensitive. *Scientific Reports* 8: 5452, https://doi.org/10.1038/s41598-018-23740-5

Evans NT, Shirey PD, Wieringa JG, Mahon AR, Lamberti GA (2017) Comparative Cost and Effort of Fish Distribution Detection via Environmental DNA Analysis and Electrofishing. *Fisheries* 42: 90–99, https://doi.org/10.1080/03632415.2017.1276329

Ficetola GF, Miaud C, Pompanon F, Taberlet P (2008) Species detection using environmental DNA from water samples. *Biology Letters* 4: 423–425, https://doi.org/10.1098/rsbl.2008.0118

Foote AD, Thomsen PF, Sveegaard S, Wahlberg M, Kielgast J, Kyhn LA, Salling AB, Galatius A, Orlando L, Gilbert MTP (2012) Investigating the Potential Use of Environmental DNA (eDNA) for Genetic Monitoring of Marine Mammals. *PLoS ONE* 7: e41781, https://doi.org/10.1371/journal.pone.0041781

Forsström T, Vasemägi A (2016) Can environmental DNA (eDNA) be used for detection and monitoring of introduced crab species in the Baltic Sea? *Marine Pollution Bulletin* 109: 350–355, https://doi.org/10.1016/j.marpolbul.2016.05.054

Gallardo B, Aldridge DC (2013) Priority setting for invasive species management: risk assessment of Ponto-Caspian invasive species into Great Britain. *Ecological Applications* 23: 352–364, https://doi.org/10.1890/12-1018.1

Harper LR, Handley LL, Hahn C, Boonham N, Rees HC, Gough KC, Lewis E, Adams IP, Brotherton P, Phillips S, Hänfling B (2018) Needle in a haystack? A comparison of eDNA metabarcoding and targeted qPCR for detection of great crested newt (*Triturus cristatus*). *Ecology and Evolution* 8: 6330– 6341, https://doi.org/10.1002/ece3.4013

Hinlo R, Gleeson D, Lintermans M, Furlan E (2017) Methods to maximise recovery of environmental DNA from water samples. *PLoS ONE* 12: e0179251, https://doi.org/10.1371/journal.pone.0179251

Hunter ME, Dorazio RM, Butterfield JSS, Meigs-Friend G, Nico LG, Ferrante JA (2016) Detection limits of quantitative and digital PCR assays and their influence in presence-absence surveys of environmental DNA. *Molecular Ecology Resources* 17: 221–229, https://doi.org/10.1111/1755-0998.12619

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A (2012) Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28: 1647–1649, https://doi.org/10.1093/bioinformatics/bts199

Kotta J, Pärnoja M, Katajisto T, Lehtiniemi M, Malavin S, Reisalu G, Panov V (2013) Is a rapid expansion of the invasive amphipod *Gammarus tigrinus* Sexton, 1939 associated with its niche selection: a case study in the Gulf of Finland, the Baltic Sea. *Aquatic Invasions* 8: 319–332, https://doi.org/10.3391/ai.2013.8.3.08

Mächler E, Deiner K, Steinmann P, Altermatt F (2014) Utility of environmental DNA for monitoring rare and indicator macroinvertebrate species. *Freshwater Science* 33: 1174–1183, https://doi.org/10.1086/678128

Mauvisseau Q, Parrondo M, Fernández MP, García L, Martínez JL, García-Vázquez E, Borrell YJ (2017) On the way for detecting and quantifying elusive species in the sea: The *Octopus vulgaris* case study. *Fisheries Research* 191: 41–48, https://doi.org/10.1016/j.fishres.2017.02.023

Mauvisseau Q, Coignet A, Delaunay C, Pinet F, Bouchon D, Souty-Grosset C (2018) Environmental DNA as an efficient tool for detecting invasive crayfishes in freshwater ponds. *Hydrobiologia* 805: 163–175, https://doi.org/10.1007/s10750-017-3288-y

Niemiller ML, Porter ML, Keany J, Gilbert H, Fong DW, Culver DC, Hobson CS, Kendall KD, Davis MA, Taylor SJ (2017) Evaluation of eDNA for groundwater invertebrate detection and monitoring: a case study with endangered *Stygobromus* (Amphipoda: Crangonyctidae). *Conservation Genetics Resources* 10: 247–257, https://doi.org/10.1007/s12686-017-0785-2

Parrondo M, Clusa L, Mauvisseau Q, Borrell YJ (2018) Citizen warnings and post checkout molecular confirmations using eDNA as a combined strategy for updating invasive species distributions. *Journal for Nature Conservation* 43: 95–103, https://doi.org/10.1016/j.jnc.2018.02.006

Piggott MP (2016) Evaluating the effects of laboratory protocols on eDNA detection probability for an endangered freshwater fish. *Ecology and Evolution* 6: 2739–2750, https://doi.org/10.1002/ece3.2083

R Core Team (2018) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/

Rees HC, Maddison BC, Middleditch DJ, Patmore JRM, Gough KC (2014) REVIEW: The detection of aquatic animal species using environmental DNA - a review of eDNA as a survey tool in ecology. *Journal of Applied Ecology* 51: 1450–1459, https://doi.org/10.1111/1365-2664.12306

Smart AS, Weeks AR, Rooyen AR van, Moore A, McCarthy MA, Tingley R (2016) Assessing the cost-efficiency of environmental DNA sampling. *Methods in Ecology and Evolution* 7: 1291–1298, https://doi.org/10.1111/2041-210x.12598

Thomsen PF, Willerslev E (2015) Environmental DNA – An emerging tool in conservation for monitoring past and present biodiversity. *Biological Conservation* 183: 4–18, https://doi.org/10.1016/j.biocon.2014.11.019

Tréguier A, Paillisson JM, Dejean T, Valentini A, Schlaepfer MA, Roussel JM (2014) Environmental DNA surveillance for invertebrate species: advantages and technical limitations to detect invasive crayfish *Procambarus clarkii* in freshwater ponds. *Journal of Applied Ecology* 51: 871–879, https://doi.org/10.1111/1365-2664.12262

Ushio M, Murakami H, Masuda R, Sado T, Miya M, Sakurai S, Yamanaka H, Minamoto T, Kondoh M (2017) Quantitative monitoring of multispecies fish environmental DNA using high-throughput sequencing. *bioRxiv* 113472, https://doi.org/10.1101/113472

Väinölä R, Witt JDS, Grabowski M, Bradbury JH, Jazdzewski K, Sket B (2008) Global diversity of amphipods (Amphipoda; Crustacea) in freshwater. *Hydrobiologia* 595: 241–255, https://doi.org/10.1007/s10750-007-9020-6

Velde G van der, Leuven RSEW, Platvoet D, Bacela K, Huijbregts MAJ, Hendriks HWM, Kruijt D (2009) Environmental and morphological factors influencing predatory behaviour by invasive non-indigenous gammaridean species. *Biological Invasions* 11: 2043–2054, https://doi.org/10.1007/s10530-009-9500-x

Wilcox TM, McKelvey KS, Young MK, Sepulveda AJ, Shepard BB, Jane SF, Whiteley AR, Lowe WH, Schwartz MK (2016) Understanding environmental DNA detection probabilities: A case study using a stream-dwelling char *Salvelinus fontinalis*. *Biological Conservation* 194: 209–216, https://doi.org/10.1016/j.biocon.2015.12.023

Yusishen ME, Eichorn FC, Anderson WG, Docker MF (2018) Development of quantitative PCR assays for the detection and quantification of lake sturgeon (*Acipenser fulvescens*) environmental DNA. *Conservation Genetics Resources* 1–3, https://doi.org/10.1007/s12686-018-1054-8

Supplementary material

The following supplementary material is available for this article:

Appendix 1. MIQE Guidelines, *Dikerogammarus haemobaphes*.
Appendix 2. Additional kick-sampling and eDNA survey.
Table S1. Mismatches between primers/probe and COI targeting sequences of *D. haemobaphes* and other co-occurring species.