Effect of artificial barriers on the distribution of the invasive signal crayfish and Chinese mitten crab

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The role of river obstacles in preventing or facilitating the dispersal and establishment of aquatic invasive species is controversial. Novel detection tools like environmental DNA (eDNA) can be used for monitoring aquatic invasive species (AIS) such as the American signal crayfish (Pacificastacus leniusculus) and the Chinese mitten crab (Eriocheir sinensis), providing information on the effect of barriers on their distribution. We analysed eDNA from both water and surface sediment in three river catchments (Medway, Dee and Stour; Great Britain), with differing levels of connectivity, to determine spatial distribution of the two species, and assessed the effect of barriers on their eDNA detection. Positive eDNA detections were obtained within confirmed sites for both species in all catchments, with evidence of species overlap in the River Medway. Upstream barriers in the Medway positively influenced detection success of mitten crab lower in the catchment while detection success of signal crayfish was higher in the highly fragmented catchment (River Medway). This information on the role of river barriers on AIS distribution and eDNA detection is important for management strategies and for predicting both future dispersal and likelihood of new colonisations in previously uninvaded fragmented catchments.

The introduction of aquatic invasive species (AIS) within the last century has been largely influenced by the expansion of aquaculture and the lack of adequate ballast water treatment. The successful dispersal and establishment of AIS often results in negative consequences for native biota, through competition for resources, introduction of novel pathogens and through habitat transformation and/or degradation. Thus, understanding the factors affecting the spread of invasive species is critical, and the influence of anthropogenic activities and man-made structures needs to be considered in addition to the ecological capabilities of the species.

Fragmentation of aquatic systems as a consequence of the presence of man-made structures such as roads, locks and culverts, or through natural barriers such as waterfalls has direct impacts on both native and non-native biota. Habitat alteration and associated stressors acting on native species in fragmented ecosystems are likely to facilitate the establishment of AIS compared to fully connected habitats. The installation of barriers can cause fundamental changes in the lotic ecosystems, including reduction in flow variability and fine sediment accumulation upstream of the barrier, which often can remove native species at a local scale through either stress or dispersal to a more favourable environment, thus opening a niche for invading species. Dams create novel impoundments, where AIS can be up to 300 times more likely to occur than in natural lakes. Additionally, impoundments are considered to act as a ‘bridge’ habitat in some cases, increasing the risk of invasion of natural lentic systems by residing AIS within close proximity. River barriers may act as an efficient barrier for solely aquatic invaders, however the influence on AIS which are not limited to movement through water is rarely considered.

The North American signal crayfish (Pacificastacus leniusculus) and the Chinese mitten crab (Eriocheir sinensis) represent two of the most successful AIS in the world but the factors determining their dispersal success are largely unknown. Human-mediated dispersal has contributed to the expansion of the invasive signal crayfish, which was intentionally imported and farmed in Great Britain from 1970 to 1990. Signal crayfish is a voracious invader, introduced through a combination of purposeful stocking implants and escape events and has caused a 90% decline in native white-clawed crayfish (Austropotamobius pallipes) through competition and transmission of crayfish plague (Aphanomyces astaci). The Chinese mitten crab, which is becoming increasingly abundant across Great Britain, is an additional host of the crayfish plague pathogen. Ballast water and mariculture are the major pathways by which AIS are introduced to new areas, however eDNA can be used to detect the presence of AIS in areas where invasive species are not currently present.

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main vectors of introduction for mitten crabs and, similar to signal crayfish, mitten crabs are notorious for their destructive nature towards native biodiversity and banks and levees of lakes and rivers21–23.

Overland dispersal in invasive crayfish species has been reported numerous times12–27 in a number of species (e.g. red swamp crayfish (Procambarus clarkii); signal crayfish), including mitten crabs28. If faced with unfavourable conditions and/or barriers, crayfish and mitten crabs are known to exit the water to find more suitable habitats and to overcome barriers12,24–27. Although man-made cross-channel barriers and natural barriers (rapos and waterfalls) could in some cases restrict the upstream dispersal of crayfish15,26,29,30, signal crayfish have been reported to disperse downstream more often than upstream to colonise new locations31–33, and therefore it is unclear whether river barriers inhibit the natural movement and dispersal of this species31,33. In contrast, barriers such as dams are likely to impede the migration of mitten crabs, which tends to occur upstream, limiting the dispersal of the species34.

In the field, it is difficult to assess the relative effects of barriers on presence and dispersal of mitten crabs using traditional surveys such as direct observation and trapping35,36. Conventional traps are size-biased towards smaller individuals, often failing to trap mitten crabs with carapaces >19 mm35,37. Fyke nets have proven to be effective at catching mitten crabs when they are in large numbers37, however they pose problems with by-catch of native fish and mammals37. Thus, the inefficiency of conventional methods can result in false negatives when assessing the upstream migration of mitten crabs in relation to barriers. Trapping also has variable efficiencies in detecting signal crayfish36, but the development of novel molecular techniques (environmental DNA) has enabled fine-scale detection across a variety of waterbodies39–43.

The environmental DNA (eDNA) approach has been increasingly used for detection and, potentially, quantification of AIS, with eDNA successfully detected both in aqueous samples and in aquatic sediment and some studies suggest that DNA concentration is higher in surface water than in sediments15,24–26,44. In addition, eDNA has the potential to aid understanding of how river barriers can limit the upstream progression of a range of aquatic species, including invasive species33, and has been used to identify the successful upstream migration of migratory fishes over barriers31,50. One of the limitations of eDNA sampling in flowing systems is that the source of the extracellular DNA cannot be easily determined51,52. Invertebrate eDNA has previously been successfully detected up to 1.5 km downstream from the DNA source41 but the persistence of eDNA in riverine systems from source to sample site depends on numerous factors, including flow rate51,53,54. During periods of low flow, DNA is more likely to sink into the substratum and bind to the sediment, reducing the distance travelled downstream55,56 and potentially increasing the longevity of the DNA47–49.

Using eDNA methods, multiple species can be detected at once, either by using universal primers39 or by undertaking the multiplex approach39,58, by which rivers can be surveyed for presence/absence of target species simultaneously at various locations of the catchment. High resolution melt (HRM) profiling combined with eDNA quantitative PCR (qPCR) is an emerging analysis technique which allows the detection of single-base variations in DNA sequences by differences in double stranded DNA product melt temperature39,59. The PCR product melt temperature (tm) depends on the sequence composition, fragment length and the choice of qPCR MasterMix used in the PCR reaction39,59. The main advantage of using HRM analysis over conventional probe-based qPCR assays for eDNA presence/absence, is the ease of distinguishing non-target amplifications from true melt peaks of target species, which limits the rate of type I errors60,61. In addition, adopting the HRM probe-based qPCR analyses for eDNA presence/absence, is the ease of distinguishing non-target amplifications from true melt peaks of target species, which limits the rate of type I errors60,61. In addition, adopting the HRM approach allows the use of multiple primer pairs within the same qPCR reaction to detect more than one species simultaneously. This multiplexing approach has been previously implemented to detect a combination of signal crayfish, white-clawed crayfish and crayfish plague oomycete A. astaci, from eDNA samples39.

In this study, we assessed the presence of both signal crayfish and mitten crab within three catchments in Great Britain with different degrees of fragmentation, using different eDNA sample types (water and sediment). We aimed to investigate the potential of eDNA in identifying the effects of barrier presence on limiting the dispersal of these species, upstream in the case of mitten crabs (through determining the upstream limit of eDNA detection) and downstream in the case of the crayfish.

Materials and Methods
Sample sites and eDNA collection. Samples were collected in July/October 2016 from three river systems: the River Medway (14 barriers; Figs 1 and 2; Table S1), the River Dee (four barriers; Figs 3 and 4; Table S1) and the River Stour (no barriers; Figs 1 and 2), with the assistance of North Wales Wildlife Trust (Dee). The River Medway spans 113 km from West Sussex to Sheerness62, the River Dee is considerably more connected than the River Stour (no barriers; Figs 1 and 2), with the assistance of North Wales Wildlife Trust (Dee). The River

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our own studies using 15 mL water volume for detecting signal crayfish\textsuperscript{39}, as well as other work on crayfish\textsuperscript{42,59} and several aquatic species\textsuperscript{42,66,68}. Negative controls consisting of ultrapure water in place of DNA were taken both before sampling and at the end of each sampling effort per site to test for any DNA carryover between sites potentially resulting in false positives. In addition to water samples, two 5 g sediment samples were collected at each site where possible for all river systems. Due to lack of sediment cohesion at a majority of sites, a sterile 15 mL Falcon tube was used to collect 5 mL from the top 2 cm of sediment\textsuperscript{49}. We collected eDNA water samples prior to collecting sediment samples, to ensure DNA being collected was derived from the water and not from re-suspension of fragments from the sediment during collection\textsuperscript{49}. Sediment was stored on ice and then kept frozen at \(-80{\textdegree}C\) until DNA extraction. Environmental conditions including temperature, flow rate, shade cover, bank consistency (concrete vs. mud/clay) and also bank angle (rounded up to nearest 5°) in relation to river/pond water were recorded for each site (Table 1).

**DNA extraction and qPCR optimisation.** Previously designed primers for crayfish\textsuperscript{39} (ApalPlen16SF: 5\textsuperscript{\prime} - AGTTCCTTTAGGAAAGTGCTG-3\textsuperscript{\prime} and ApalPlen16SR: 5\textsuperscript{\prime} - CTCTTTAACATCGAGGTGTC-3\textsuperscript{\prime}) were used to amplify a 83 bp product of both target species. Primers were assessed \textit{in vitro} for mitten crab using positive control tissue (leg muscle) from eight mitten crab individuals from three populations (Maidstone, Kent; Chester, Cheshire; Leeds, Yorkshire). Mitten crab DNA was extracted using Qiagen\textsuperscript{80} DNeasy Blood and Tissue Kit (Qiagen, UK), eluted in 200 \(\mu\)l, and amplified in end-point PCR with the above primers using the following
protocol: 95 °C for 3 min, followed by 40 cycles of 95 °C for 30 s, 61 °C for 30 s and 72 °C for 45 s with a final elongation step of 72 °C for 10 min. All amplified PCR products were checked for the correct amplicon sizes using a 2% agarose gel electrophoresis. To confirm the species identity, PCR products were analysed using Sanger Sequencing on an ABI Prism 277 DNA sequencer. Resulting sequences were aligned using BioEdit v. 5.0.9 (using the ClustalW program) and inputted to BLAST69.

Mitten crab qPCR-HRM optimisation. Optimisation of the primers above has previously been undertaken for signal crayfish39. Here, specific in vitro testing of RT-qPCR-HRM analysis was performed for mitten crab DNA only using SsoFast EvaGreen® qPCR Supermix (BioRad, UK). The cycling protocol was carried out using a Bio-Rad CFX96 Touch Real-Time PCR Detection System (Bio-Rad, UK) and began with 15 min of denaturation at 98 °C, followed by 40 cycles of 95 °C for 15 s and 61.5 °C for 30 s. After the 40 cycles, a HRM step was applied to the RT-qPCR reactions, which consisted of applying a temperature gradient ranging from 65 °C to 95 °C in 0.1 °C/10 s increments, to melt the amplified qPCR product for assessment of consistency of amplicon tm. Resulting efficiency value for mitten crab DNA at pre-determined annealing temperature (61.5 °C) was 105.8%, \( R^2 = 0.997 \) (previously determined efficiency of 100.2%, \( R^2 = 0.986 \) for signal crayfish, and 107.9%39). Limit of detection (LOD) and limit of quantification (LOQ) were determined for mitten crab by running a dilution series ranging from 5 ng/µl to \( 5 \times 10^{-7} \) ng/µl, using a mitten crab DNA pool. HRM analysis for mitten crab DNA was conducted on seven individuals to account for any degree of intraspecific variation in qPCR product tm. Overall, melt curves generated from species-specific product tms (signal crayfish: 73.8 °C ± 0.2; mitten crab: 73.2 °C ± 0.2) were analysed to assess the presence/absence of all species.
Table 1. Site information for eDNA sample collection in the River Medway (M), River Stour (S) and River Dee (D) including site name, GPS coordinates of site, water temperature (°C), flow rate (m/s), shade level (0–3), sediment collection status (yes/no), bank consistency (concrete vs. mud/clay) and bank angle in relation to waterbody (left and right in relation to downstream direction of water flow).

| Site            | Site Name         | Site Type | River System | GPS          | Temp. (°C) | Flow Rate (m/s) | Shade (0–3) | Sediment collected? | Bank consistency | Bank angle left (°) | Bank angle right (°) |
|-----------------|-------------------|-----------|--------------|--------------|------------|-----------------|--------------|----------------------|-------------------|---------------------|---------------------|
| M1              | Tombridge Castle  | River     | Medway       | TQ 59089 46489 | 17         | 0.2             | 0            | No                   | Concrete          | 90                  | 90                  |
| M2              | Tudeley Brook     | Stream    | Medway       | TQ 67472 48254 | 15         | 0.3             | 0            | No                   | Mud/clay          | 75                  | 55                  |
| M3              | Pottenden Lake    | Pond      | Medway       | TQ 60810 51347 | 17         | N/A             | 2            | Yes                  | Mud/clay          | 30                  | N/A                 |
| M4              | Canon Landing     | River     | Medway       | TQ 68987 49924 | 18         | 0.2             | 1            | No                   | Concrete          | 90                  | 90                  |
| M5              | Horse Farm        | River     | Medway       | TQ 72866 48687 | 16         | 0.25            | 1            | No                   | Mud/clay          | 70                  | 80                  |
| M6              | Summerhill Road   | Stream    | Medway       | TQ 77297 46511 | 14         | 0.1             | 3            | Yes                  | Mud/clay          | 85                  | 85                  |
| M7              | Green Lane        | Stream    | Medway       | TQ 72843 45680 | 13         | 0.2             | 1            | Yes                  | Mud/clay          | 65                  | 70                  |
| M8              | Teston Bridge     | River     | Medway       | TQ 70880 53290 | 15         | 0.2             | 1            | Yes                  | Mud/clay          | 90                  | 90                  |
| M9              | Farleigh Station  | River     | Medway       | TQ 73478 53564 | 16         | 0.4             | 0            | No                   | Concrete          | 90                  | 90                  |
| M10             | Asda              | River     | Medway       | TQ 75665 55630 | 17         | 0.3             | 0            | No                   | Concrete          | 90                  | 90                  |
| M11             | Leybourne Lakes   | Lake      | Medway       | TQ 70192 59812 | 19         | N/A             | 2            | Yes                  | Mud/clay          | 30                  | N/A                 |
| S1              | Gore Street       | Stream    | Stour        | TR 26937 63415 | 17         | 0.25            | 2            | No                   | Mud/clay          | 55                  | 50                  |
| S2              | Grove Ferry Road  | River     | Stour        | TR 23499 63189 | 18         | 0.1             | 1            | No                   | Mud/clay          | 65                  | 70                  |
| S3              | Fordwich          | River     | Stour        | TR 17922 59782 | 17         | 0.5             | 1            | Yes                  | Concrete          | 90                  | 90                  |
| S4              | NRW Bala          | River     | Dee          | SH 93341 35505 | 15         | N/A             | 0            | Yes                  | Mud/clay          | 25                  | 55                  |
| S2              | Dilan             | River     | Dee          | SJ 02021 37388 | 15         | N/A             | 1            | Yes                  | Mud/clay          | 50                  | 45                  |
| S3              | Carrog            | River     | Dee          | SJ 02080 37443 | 16         | N/A             | 0            | Yes                  | Mud/clay          | 20                  | 60                  |
| S4              | Llangollen Serpents | River  | Dee          | SJ 20848 43565 | 15         | N/A             | 3            | Yes                  | Mud/clay          | 85                  | 50                  |
| S5              | Halton Woods      | River     | Dee          | SJ 29494 40857 | 14         | N/A             | 2            | Yes                  | Mud/clay          | 50                  | 45                  |
| S6              | Eyton Hall        | River     | Dee          | SJ 36286 44256 | 13         | N/A             | 1            | Yes                  | Mud/clay          | 55                  | 60                  |
| S7              | Sutton Green      | River     | Dee          | SJ 41383 47928 | 13         | N/A             | 0            | Yes                  | Mud/clay          | 50                  | 25                  |
| S8              | Caldecott         | River     | Dee          | SJ 42500 51100 | 13         | N/A             | 3            | Yes                  | Mud/clay          | 55                  | 70                  |
| S9              | Holt              | River     | Dee          | SJ 40307 56900 | 14         | N/A             | 3            | Yes                  | Mud/clay          | 60                  | 65                  |
| S10             | Eccleston         | River     | Dee          | SJ 41592 62289 | 13         | N/A             | 2            | Yes                  | Mud/clay          | 70                  | 65                  |
| S11             | Chester Meadows   | River     | Dee          | SJ 41701 66539 | 14         | N/A             | 0            | Yes                  | Mud/clay          | 65                  | 90                  |

For assessing the ability to detect both target invasive species in the same reaction, different volume ratios were combined for the two target species (crayfish DNA and mitten crab from 1:9 μl through to 9:1 μl signal crayfish: mitten crab DNA at 5 ng/μl) and amplified in triplicate.

Analysis of eDNA field samples. DNA extraction was performed using Qiagen® DNeasy Powerlyzer PowerSoil Kit (Qiagen, UK), for both field eDNA water samples (n = 177; Table 2) and sediment eDNA samples (n = 39; Table 2), including negative controls, following the manufacturer's instructions, apart from a reduction in the elution volume from 60 μl to 50 μl, to maximise DNA yield. We opted for Qiagen® DNeasy PowerSoil Kit for all samples based on the effectiveness of the kit to reduce inhibitors and produce high DNA yields[10,11]. Sediment samples were extracted in triplicate, resulting in a total of 117 sediment extractions. DNA extractions were undertaken in a dedicated eDNA area within an extraction cabinet, fully equipped with flow-through air system and UV light and to minimise contamination; additionally, dedicated eDNA laboratory coat and nitrile gloves were worn during the process.

Amplifications were undertaken in triplicate using the protocol previously described, with the final optimised qPCR reactions carried out in a final volume of 10 μl, containing 2 μl of SsoFast™ EvaGreen® (Bio-Rad, UK), 0.25 μl of each primer (10 μM), 1 μl of template DNA at 5 ng/μl and 3.5 μl of ultrapure water. Melt curves generated from species-specific product tms (signal crayfish: 73.8 °C ± 0.2 °C; mitten crab: 73.2 °C ± 0.2) were analysed to assess the presence/absence of target species in field samples. Samples which had at least two out of three PCR replicates with corresponding target tms for either or both species, with a melt rate above 200 -d(RFU)/dT were considered positive. In addition, qPCR reactions were carried out at sites positive for either signal crayfish or mitten crab DNA at 5 ng/μl amplification negative controls consisting of HPLC water and extraction of both target species positive control DNA once all the eDNA samples were loaded and sealed to prevent false positive signals in the eDNA samples. Amplification negative controls consisting of HPLC water and extraction negative controls were also added in the same well location on each plate test for contamination in eDNA samples.

To confirm mitten crab presence in field samples, a subset of four positive amplifications were cloned and sequenced. Out of 21 successfully transformed clones (seven per sample), between five and seven sequences
matched 100% with mitten crab on BLAST\(^9\), non-specific amplification was observed in remaining clones. In addition, all positive control clones (seven) for mitten crab matched 100% on BLAST.

**Statistical analysis.** We employed a generalized linear modelling approach in R v.3.4.3\(^72\) to model detection success (i.e. the proportion of samples that tested positive for signal crayfish and mitten crabs at each site) for both water and sediment eDNA samples as a function of the number of river barriers both upstream and downstream of each positive site and river identity (n: 2 rivers; Medway and Dee). This approach aimed to test whether barriers limit the upstream or downstream migration of each species, by assessing whether an increasing number of barriers makes it more difficult to detect the species eDNA upstream or downstream. River identity also served as fragmentation status (Medway: highly fragmented; Dee: partially fragmented). We considered that either species was present at a site if two of the three PCR replicates per sample (6 samples per site) tested positive for target species. A quasibinomial log-link was used to correct for overdispersion.

### Results

#### Mitten crab detection limits.

Results from a 10-fold dilution series revealed that for mitten crab the limit of detection (LOD) was 0.005 ng/µl for the qPCR assay, which is the same LOD as the predetermined value for both signal crayfish and white-clawed crayfish\(^9\). No overlap in qPCR product tm was observed between the two species (Fig. S1; Table S3) and it was possible to detect presence of either species in a single reaction based on the diagnostic melt curve shape produced when combining varying ratios of pooled DNA for both species. Results from mixed proportions of signal crayfish and mitten crab displayed only signal crayfish melt curves from 9:1 to 7:3 µl signal crayfish: mitten crab ratios whereas from 6:4 to 1:9 µl ratios, the melt curves were diagnostic for just mitten crabs (Fig. S1; Table S3).

#### Detection success and spatial distribution.

Signal crayfish DNA was successfully detected in six out of the 11 sites sampled in the River Medway, whereas mitten crab DNA was only detected in three sites (Table 3; Figs S2 and S3; Tables S4 and S5). For all three catchments, both signal crayfish and mitten crab were detected within sections of the catchment where there has been visual confirmation of both species\(^20\), which confirms the utility of the essay in the field. As expected, positive sites for signal crayfish were located in the upper reaches of the river area sampled. Signal crayfish DNA was detected further downstream than previously reported in the Dee and Medway catchments\(^65\), however it was not possible to determine whether this represents downstream dispersal or downstream transport of eDNA from an upstream source\(^51,53\). Similarly, mitten crab DNA was detected further upstream than previously reported from visual surveys in the Medway and the Stour (Fig. S4; Tables S6 and S7), however, in the River Dee, mitten crab DNA was not detected at the uppermost extent of their known range\(^20\). Three sampling sites in the River Medway overlapped for both target species (M7, M10, M11) and similarly signal crayfish and mitten crab were detected at two of the same sites in the River Dee (D7 and D8; Figs S3 and S6; Tables S8 and S9). The River Stour also had an overlap in detection of both species in site ST3. Results from amplifying positive signal crayfish and mitten crab samples with HOT FIREPol® EvaGreen® qPCR multiplex indicated that there was no A. astaci present in any of the three catchments.

#### Detection success in relation to barriers.

In comparison to the River Dee, the seven positive sites for signal crayfish in the Medway catchment were distributed at regular intervals down the catchment, whereas positive sites in the Dee for this species were located at very start of sample area (D1) and then clustered further downstream of three weirs (D7–D9). Despite presence of six locks within close proximity to one another in the upper Medway catchment (Fig. 1; Table S1), and three weirs situated within 15 km in the River Dee (Fig. 2; Table S1), signal crayfish DNA was detected above these barriers at M1 and D1 respectively. In contrast, despite previous records of mitten crab around Teston lock at M8, there was no DNA detected in either sediment or water samples from this site. However, there were positive detections of this target species directly upstream of Allington Lock at site M10, where mitten crab was previously known to accumulate at lock gates as has been observed in other impounded catchments\(^7,24\). Mitten crab DNA in the River Dee was detected at three sites upstream of Chester Weir, a barrier which is known to be passable for this species, however was not detected any further upstream than site D7.

Water and sediment samples did not perform to the same extent, with water samples producing a greater proportion of positive detections (Figs S2–S6; Tables S4–S9). For signal crayfish, there was no effect of barriers upstream or downstream (deviance = 43.31, df = 19, P = 0.091; Table S10) or on positive detection of the

### Table 2.

| River System | Location         | Number of Known River Obstructions | Month/Year | Number of sites sampled | Total number of eDNA water samples collected* | Total number of eDNA sediment samples collected |
|--------------|------------------|-----------------------------------|------------|-------------------------|-----------------------------------------------|-------------------------------------------------|
| Medway       | SE England       | 15                                | July/2016  | 11                      | 78                                            | 18                                              |
| Dee          | N Wales          | 4                                 | September/2016 | 11                  | 78                                            | 18                                              |
| Stour        | SE England       | 0                                 | July/2016  | 3                       | 21                                            | 3                                               |
| **TOTAL**    |                  |                                   |            | **25**                  | **177**                                       | **39**                                          |
species in water samples or sediment samples (deviance = 56.05, df = 13, P = 0.794; Table S10). There was however an effect of river identity on positive detections of signal crayfish in water samples (deviance = 43.31, df = 19, P < 0.05), with a significantly higher detection success in the River Medway (highly fragmented) compared to the River Dee (partially fragmented; Table S10). For mitten crab, the number of barriers upstream of sampling site had a positive effect on detection success in water samples (deviance = 10.14, df = 19, P < 0.05) but not sediment samples (deviance = 17.16, df = 13, P = 0.997; Tables S11 and S12).

Table 3. Number of water and sediment samples collected, positive sites for both water and sediment samples for each species (signal crayfish (SC) and mitten crab (MC)) and total number of positive sites for each sample type.

| Catchment | Total Water Sample Sites | Total Sediment Sample Sites | No. Positive SC sites (Water) | No. Positive SC sites (Sediment) | No. Positive MC sites (Water) | No. Positive MC sites (Sediment) |
|-----------|--------------------------|----------------------------|-------------------------------|---------------------------------|------------------------------|---------------------------------|
| Medway    | 11                       | 5                          | 4                             | 3                               | 3                            | 1                               |
| Dee       | 11                       | 11                         | 1                             | 2                               | 1                            | 2                               |
| Stour     | 3                        | 3                          | 1                             | 1                               | 1                            | 1                               |
| Total     | 25                       | 17                         | 6                             | 6                               | 5                            | 4                               |

Discussion
Here, we have identified a negative effect of barrier presence on the upstream distribution of mitten crab and apparent lack of effect of barriers in the downstream presence of signal crayfish, by comparing water eDNA detection in river catchments with differing levels of fragmentation. This effect was not observed in sediment in any of both species, which could reflect the temporal differences in detection between water and sediment47,49.

Species presence compared to previous records. In all three catchments, both signal crayfish and mitten crab eDNA was detected in close proximity to locations of previous records. The exception to this being lack of mitten crab DNA near the estuary in the River Stour (ST1) and positive detections of signal crayfish in the lentic system at M11. Due to the one-way flow of eDNA in lotic systems, upstream detections of target species DNA suggest that individuals have progressed upstream; this also applies to sediment samples53,75. Signal crayfish are known to migrate both up and downstream anything from 1 to 4 km upstream and 1.5 to 6 km downstream per year48, therefore the high proportion of positive sites in the Medway catchment could suggest that this species has expanded beyond its previously considered range75. Similarly, positive detections of signal crayfish immediately downstream of Lake Bala in the River Dee, indicates upstream range expansion in this catchment49. Due to the catadromous nature of mitten crabs, juvenile crabs are known to migrate up to 750 km upstream to mature73,74, therefore the uppermost extent of DNA detection in a river is likely to be within close proximity to the true upstream extent of species occupation75,77–79. Similar to signal crayfish, within the Medway and Dee we detected mitten crab further upstream than previously reported, again suggesting upstream range expansion of the species75.

In both the Rivers Medway and Dee, signal crayfish and mitten crab were detected in the same sampling site, both in sediment and water samples, in locations which match the recorded downstream extent of signal crayfish and upstream extent of mitten crab30,76; this could indicate that both species are occupying the same stretches of the Medway and Dee around these sites, which has already been observed in other catchments in the UK80. Overlapping zones between signal crayfish and mitten crab are expected to result in negative impacts on local biota in comparison to single-species zones due to a combination of niche partitioning and predatory overlap80.

Effect of sample type on species presence. Water and aquatic sediments are known sources of eDNA, and both sample types have been directly used in a range of non-invasive surveys and monitoring techniques44,49,79,81. The observed higher within-sample detection rate in sediment samples correlates with results from additional studies on DNA detection from sediment40,82–85, and could be a result of both the ecology of both target species and increased temporal longevity of DNA in sediment, resulting in higher detectability across sample replicates47,49,86–88. We found that a greater number of sampling sites were positive for target species in water samples in comparison with sediment samples. This is unexpected because both target species are benthic by nature, it was expected that eDNA would be more likely to be detected in the sediments than in surface waters, as aqueous DNA from crabs and crayfish is most likely to originate from faeces which sink rapidly into the substratum87–89, due to lack of mucus exuded46,73,86,88. The conditions of the aquatic sediment can enable DNA to remain detectable for a longer period of time (at least 132 days for fish vs 25 days in water samples49), in comparison to the varying temporal persistence of DNA in sediment89, it is difficult to determine the time of DNA deposition, which can be a problem for assessing current presence/absence, as deposits from past occupancy can result in false positives for target species77–79. There is little information regarding the longevity of invertebrate eDNA in sediments, however due to the cross-over in detection success between sediment and water samples, it is likely that detections in sediment for signal crayfish and mitten crabs represent more current-occupancy than past-occupancy49,87.

Here, we have validated the use of HRM for the analysis of field eDNA samples (as opposed to use for optimisation only46,86), which is still incipient, both for water and sediment, although this method has been extensively used as a highly discriminative method for identifying species46,92,93. We have observed that the use of the same primers can present problems when the amount of eDNA from one of the species is considerably larger than for the other. To overcome this problem, we used a large number of replicates per sampling location and sequenced
the resulting PCR in cases when a single species was detected. When collecting a large number of replicates is not possible, optimisation of specific primers may be the best alternative approach.

**Barrier influence on presence and DNA detectability.** The presence of the locks and flood gates in the River Medway appeared to have an influence over the DNA detection of mitten crab in this river. Detection likelihood of mitten crab increased with the number of barriers upstream of the sampling site, which indicates that barriers in the Medway are restricting the upstream movement of this species. During their upstream migration, mitten crabs have been known to aggregate at barriers, especially when banks are too steep to navigate around barrier on land and the presence of large structures such as dams and flood gates are known to considerably slow down its upstream migration. This congregation of individuals is likely to result in a stronger eDNA signal further downstream, because density is known to be the major contributing factor to successful DNA detection in numerous aquatic species. The most upstream record of mitten crabs was in the River Beult, a tributary of the River Medway, which branches off from the main river ~5 km before a series of six consecutive locks. Our detections of mitten crab DNA from water samples taken in this tributary, suggests this species is present here as opposed to the main river as no detections were found any further upstream of the River Medway. In contrast with the mitten crab, we found that the barriers did not affect the presence of the signal crayfish, probably due to the fact signal crayfish mainly disperse in a downstream direction. In the River Stour, mitten crab appears to have expanded its range as this species was detected alongside signal crayfish in the most upstream site sampled. Mitten crabs had previously only been reported in the mouth of the estuary in this river system, and successful upstream range expansion could be as a result of the high levels of connectivity in the Stour.

Significantly higher detection success for signal crayfish in the River Medway compared to the River Dee could be the result of varying hydrological conditions, crayfish abundance or the difference in seasonality between the sampling period for each river. Some studies have reported reduction of DNA detection for signal crayfish in the winter months (November – February) due to the winter torpor signal crayfish undergo as part of their annual life cycle. Temperature is considered to be the main driver for reduction in crayfish activity, which can directly correspond to the amount of eDNA being released into the local environment. However, our previous work on signal crayfish eDNA during October resulted in the species being detected in all reported locations, suggesting a substantial level of detection during the autumn. Additionally, temperatures in the River Medway and River Dee were not very different between July (average 16.1 °C across all sampling sites) and October (average 14.1 °C across all sampling sites; Table 1), and therefore we expected the levels of crayfish activity, and eDNA shedding rates, to be comparable. It is thus unlikely that the difference in detection rate of signal crayfish DNA is as a direct result of the seasonality in this case.

The detectability of eDNA in a flowing river depends on both biotic and abiotic factors such as distance from source, water velocity, and temperature. The presence of a series of locks along a section of river, as seen in the River Medway, could have the potential to create ‘mini-lentic systems’ upstream of each obstacle, and therefore eDNA is more likely to settle and bind to sediment, as opposed to being carried downstream. Further research into the fate of DNA in fragmented river systems should be investigated to address this concept.

Overall, assessing the influence of barriers on invasive species presence and distribution is important for informing management strategies. Long-term persistence of mitten crabs depends on the ability of juveniles to migrate upstream and colonise suitable freshwater habitats, therefore river obstacles can have a great influence over colonisation success. Additionally, being able to detect sites of predicted range overlap between signal crayfish and mitten crabs using eDNA is important for informing management strategies of critical areas for invasive species control, particularly for species which experience complex trophic interactions and are potentially synergistic. Our work suggests that sampling in the proximity of obstacles can increase species detectability through eDNA, particularly for species like the mitten crab which tend to concentrate downstream of the barriers. We also found that water samples can outperform sediment samples for DNA detection of benthic species, highlighting the ability to detect sufficient quantities of DNA in flowing systems to determine current distribution.

**Data Availability**

All data generated or analysed during this study are included in this published article (and its Supplementary Information files).

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**Author Contributions**

C.V.R. and S.C. designed the study, C.V.R. analysed the data, C.G.D.L. performed statistical analyses, C.V.R., S.C. and C.G.D.L. wrote the paper.

**Additional Information**

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