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## Data in Brief

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### Data Article

# Data on optimisation of a multiplex HRM-qPCR assay for native and invasive crayfish as well as the crayfish plague in four river catchments

Chloe Victoria Robinson, Tamsyn M. Uren Webster,  
Sofia Consuegra\*

Swansea University, Singleton Park, Swansea SA2 8PP, Wales, UK

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#### ABSTRACT

The data presented here corresponds to the research paper “Simultaneous detection of invasive signal crayfish, endangered white-clawed crayfish and the crayfish plague using environmental DNA”. A crayfish-specific assay was designed and optimised using three real-time PCR supermixes (SYBR™ Green, SsoFast™ EvaGreen® and HOT FIREPol® EvaGreen®). Diagnostic high resolution melt (HRM) data from direct application of assay on both *ex-situ* eDNA water samples and field samples from four catchments (two in Wales, two in England) is presented in this article, displaying positive HRM profiles for invasive signal crayfish (*Pacifastacus leniusculus*), native white-clawed crayfish (*Austropotamobius pallipes*) and crayfish plague causal agent (*Aphanomyces astaci*).

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### Specifications Table

|                            |   |
|----------------------------|---|
| Subject area               | Biology   |
| More specific subject area | Detection of invasive, native crayfish and crayfish plague in environmental DNA water samples using HRM-qPCR analysis |

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\* Corresponding author.

E-mail address: [s.consuegra@swansea.ac.uk](mailto:s.consuegra@swansea.ac.uk) (S. Consuegra).

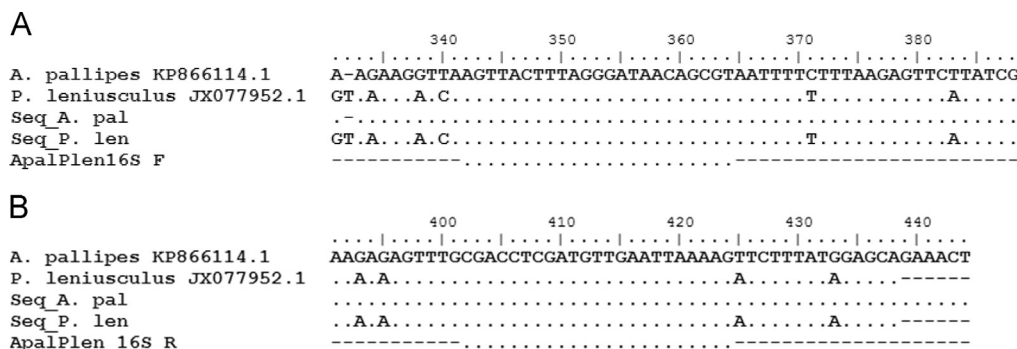
<https://doi.org/10.1016/j.dib.2018.05.134>

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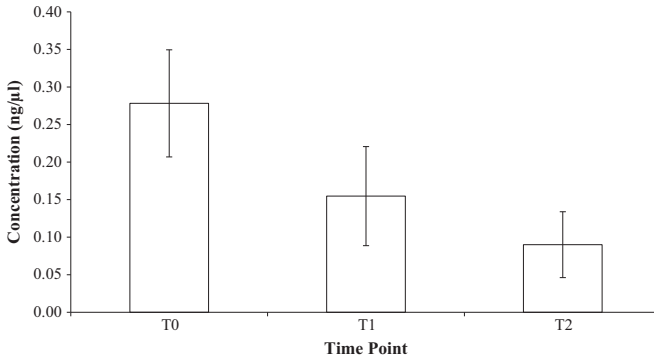
|                          |  |
|--------------------------|--|
| Type of data             | Sequence alignment, tables and figures   |
| How data was acquired    | Sequence alignment was achieved using GenBank and BioEdit (ver. 7.2.5)<br>DNA concentrations determined using Qubit™ 4 Fluorometer (Thermo-Fisher Scientific, UK)<br>qPCR data achieved using CFX96 Touch™ Real-Time PCR Detection System (C1000 Touch™ chassis, Bio-Rad, UK)  |
| Data format              | Raw  |
| Experimental factors     | DNA extracted from water and tissue samples using Qiagen DNeasy® Blood and Tissue extraction kit (QIAGEN, UK)  |
| Experimental features    | Assessment of presence/absence of signal crayfish, white-clawed crayfish and crayfish plague DNA in water samples from four river catchments   |
| Data source location     | Signal crayfish tank water samples from Cardiff University<br>Native crayfish tank water samples from Cynrig Hatchery<br>Water samples from the River Wye catchment (Builth Wells to Bough-rood, UK)<br>Water samples from the River Itchen catchment (Bishop's Sutton to Easton, UK)<br>Water samples from the River Taff catchment (Nant-ddu to Treharris, UK)<br>Water samples from the River Medway catchment (Tonbridge to Leybourne, UK) |
| Data accessibility       | Data in full is provided with this article   |
| Related research article | Robinson, C.V., Uren Webster, T.M., Cable, J., James, J., Consuegra, S. Simultaneous detection of invasive signal crayfish, endangered white-clawed crayfish and the crayfish plague using environmental DNA. <i>Biological Conservation</i> 222, 241–252. [1]   |

## Value of the data

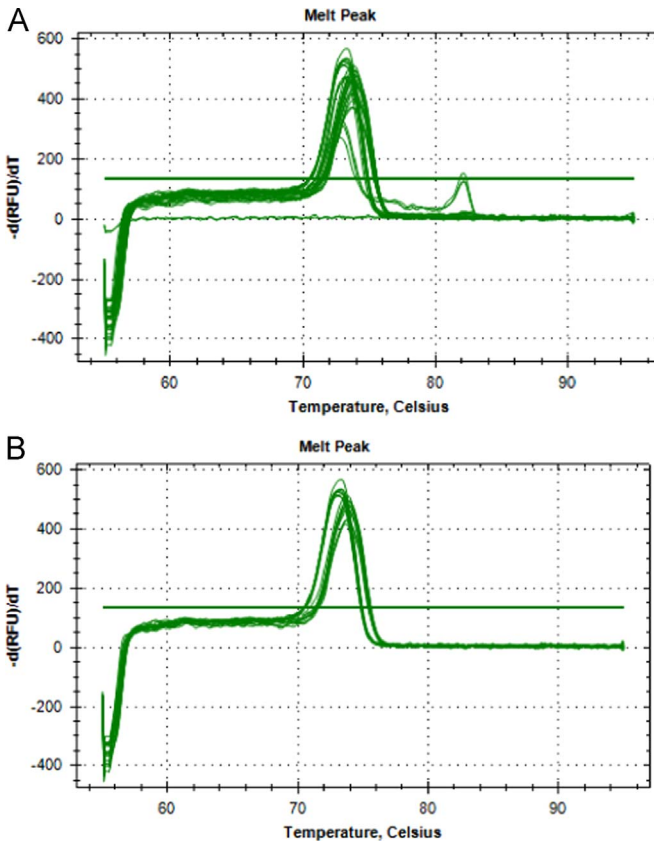
- The data shows that melting curve differences between native and invasive crayfish can be used for management purposes by screening eDNA water samples.
- The protocol successfully amplifies invasive and native crayfish and can detect their infection status.
- The comparison of HRM-qPCR outputs using SYBR™ Green and SsoFast™ Evagreen® suggested that the second qPCR mastermix provided greater sensitivity and reproducibility.
- Temporal concentration measurements indicated that eDNA degraded  $3 \times$  in 48 h under controlled conditions.



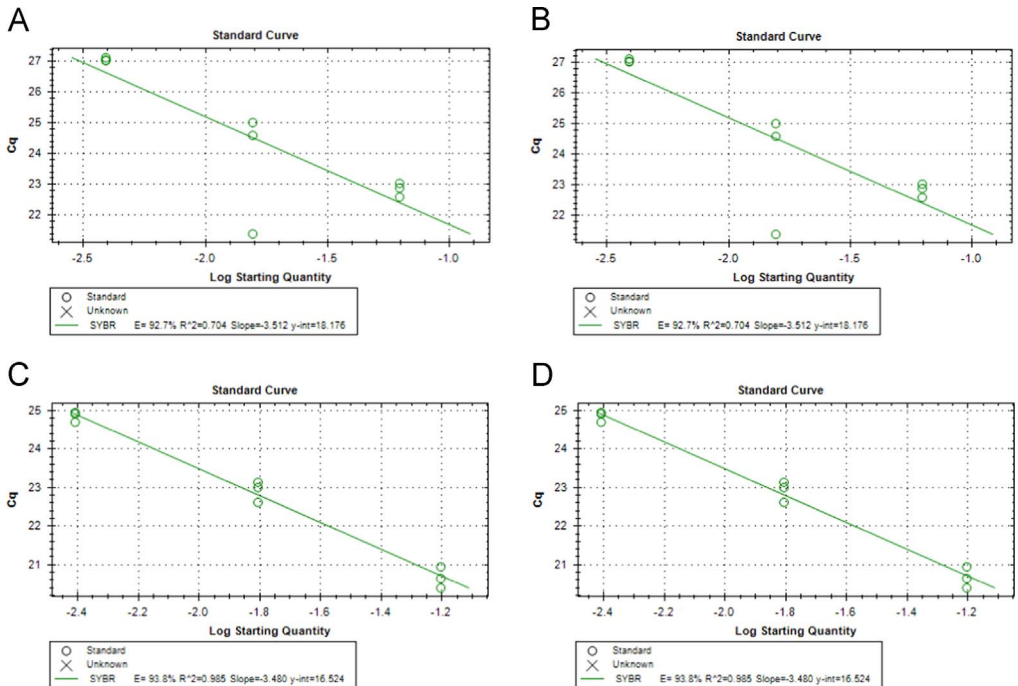
**Fig. 1.** Alignment of DNA fragments from eDNA samples for both *Pacifastacus leniusculus* and *Austropotamobius pallipes* and ApalPlen16S forward (a) and reverse (b) primers against reference sequences. Seq\_A.pal = positive in-situ *Austropotamobius pallipes* eDNA fragment; Seq\_P. len = positive in-situ *Pacifastacus leniusculus* eDNA fragment.



**Fig. 2.** Average eDNA concentration across all *ex-situ* samples for the three time points (T0 = time of crayfish removal; T1 = 24 h post crayfish removal; T2 = 48 h post crayfish removal).



**Fig. 3.** Melt peaks from SYBR™ Green dilution series (A) and from SsoFast™ EvaGreen® dilution series (B) for *Pacifastacus leniusculus* and *Austroptamobius pallipes* DNA pools.



**Fig. 4.** Efficiency outputs from SYBR™ Green dilution series for *Pacifastacus leniusculus* (A) and *Austroptamobius pallipes* (B); efficiency outputs from SsoFast™ EvaGreen® dilution series for (B) for *Pacifastacus leniusculus* (C) and *Austroptamobius pallipes* (D).

## 1. Data

Data presented in Section 1.1 includes a sequence alignment of *Pacifastacus leniusculus* and *Austroptamobius pallipes* 16s mtDNA 83 bp product with binding sites respective forward (ApalPlen16S\_F) and reverse (ApalPlen16S\_R) primers and nucleotide base differences between the two species (Fig. 1).

In Section 1.2, data is presented on the average eDNA concentrations of tank water samples collected from tanks containing *P. leniusculus* at three time points (Fig. 2).

The data presented in Section 1.3 consists of the SYBR™ Green Supermix and SsoFast™ EvaGreen® Supermix qPCR optimization results of both *P. leniusculus* and *A. pallipes* DNA, including the qPCR melt curve graphs (Fig. 3), standard curves with efficiency values (Fig. 4) and raw melt data (Table 1). In addition, Subsection 1.3 includes qPCR melt curve graphs (Fig. 5) and raw melt data (Table 2) for amplifications of mixed proportions of both *P. leniusculus* and *A. pallipes* DNA in the same reaction tube and *ex-situ* *P. leniusculus* tank eDNA amplifications (Fig. 5; Table 3). Data on the qPCR melt curve graphs and raw melt data for HOT FIREPol® EvaGreen® qPCR optimisation with *P. leniusculus* and the crayfish plague causal agent (*Aphanomyces astaci*) DNA are presented in Subsection 1.3 in Fig. 6 and Table 4.

In Section 1.4, data represents SsoFast™ EvaGreen® qPCR product melt curve graphs (Fig. 7) and raw melt output (Table 5) from positive eDNA water sample amplifications collected in the Bachowey and Duhonw rivers around crayfish traps containing *P. leniusculus*.

Section 1.5 contains both qPCR melt curve graphs and raw melt information from positive amplifications from the Sgithwen and Bachowey catchments using both SsoFast™ EvaGreen® and HOT FIREPol® EvaGreen® mastermixes (Fig. 8, Table 6).

**Table 1**

Melt peak data from SYBR™ Green and SsoFast™ EvaGreen® dilution series for *Pacificastacus leniusculus* (s\_pool) and *Austroptotamobius pallipes* (n\_pool).

| Mastermix          | Sample ID | Concentration (ng/μl) | Melt Temperature (°C) |
|--------------------|-----------|-----------------------|-----------------------|
| SYBR™ Green        | s_pool    | 5                     | 72.50                 |
| SYBR™ Green        | s_pool    | 5                     | 72.80                 |
| SYBR™ Green        | s_pool    | 5                     | 72.80                 |
| SYBR™ Green        | n_pool    | 5                     | 73.70                 |
| SYBR™ Green        | n_pool    | 5                     | 73.70                 |
| SYBR™ Green        | n_pool    | 5                     | 73.70                 |
| SYBR™ Green        | s_pool    | 0.5                   | 73.10                 |
| SYBR™ Green        | s_pool    | 0.5                   | 73.40                 |
| SYBR™ Green        | s_pool    | 0.5                   | 73.30                 |
| SYBR™ Green        | n_pool    | 0.5                   | 73.70                 |
| SYBR™ Green        | n_pool    | 0.5                   | 73.70                 |
| SYBR™ Green        | n_pool    | 0.5                   | 73.70                 |
| SYBR™ Green        | n_pool    | 0.5                   | 73.70                 |
| SYBR™ Green        | s_pool    | 0.05                  | 73.40                 |
| SYBR™ Green        | s_pool    | 0.05                  | 73.40                 |
| SYBR™ Green        | s_pool    | 0.05                  | 73.30                 |
| SYBR™ Green        | n_pool    | 0.05                  | 73.80                 |
| SYBR™ Green        | n_pool    | 0.05                  | 73.70                 |
| SYBR™ Green        | n_pool    | 0.05                  | 73.60                 |
| SYBR™ Green        | s_pool    | 0.005                 | 73.70                 |
| SYBR™ Green        | s_pool    | 0.005                 | 73.30                 |
| SYBR™ Green        | s_pool    | 0.005                 | 73.20                 |
| SYBR™ Green        | n_pool    | 0.005                 | 73.70                 |
| SYBR™ Green        | n_pool    | 0.005                 | 73.70                 |
| SYBR™ Green        | s_pool    | 0.0005                | 72.90                 |
| SYBR™ Green        | s_pool    | 0.0005                | 73.00                 |
| SYBR™ Green        | s_pool    | 0.0005                | 73.00                 |
| SYBR™ Green        | n_pool    | 0.0005                | 73.80                 |
| SYBR™ Green        | n_pool    | 0.0005                | 73.70                 |
| SYBR™ Green        | n_pool    | 0.0005                | 73.70                 |
| SYBR™ Green        | MB        | N/A                   | None                  |
| SYBR™ Green        | MB        | N/A                   | None                  |
| SYBR™ Green        | MB        | N/A                   | None                  |
| SsoFast™ EvaGreen® | s_pool    | 5                     | 82.10                 |
| SsoFast™ EvaGreen® | s_pool    | 5                     | 72.50                 |
| SsoFast™ EvaGreen® | s_pool    | 5                     | 72.80                 |
| SsoFast™ EvaGreen® | s_pool    | 5                     | 72.80                 |
| SsoFast™ EvaGreen® | n_pool    | 5                     | 73.70                 |
| SsoFast™ EvaGreen® | n_pool    | 5                     | 73.70                 |
| SsoFast™ EvaGreen® | n_pool    | 5                     | 73.70                 |
| SsoFast™ EvaGreen® | s_pool    | 0.5                   | 73.10                 |
| SsoFast™ EvaGreen® | s_pool    | 0.5                   | 73.40                 |
| SsoFast™ EvaGreen® | s_pool    | 0.5                   | 73.30                 |
| SsoFast™ EvaGreen® | n_pool    | 0.5                   | 73.70                 |
| SsoFast™ EvaGreen® | n_pool    | 0.5                   | 73.70                 |
| SsoFast™ EvaGreen® | n_pool    | 0.5                   | 73.70                 |
| SsoFast™ EvaGreen® | s_pool    | 0.05                  | 73.70                 |
| SsoFast™ EvaGreen® | s_pool    | 0.05                  | 73.70                 |
| SsoFast™ EvaGreen® | s_pool    | 0.05                  | 73.70                 |
| SsoFast™ EvaGreen® | n_pool    | 0.05                  | 73.80                 |
| SsoFast™ EvaGreen® | n_pool    | 0.05                  | 73.70                 |
| SsoFast™ EvaGreen® | n_pool    | 0.05                  | 73.70                 |
| SsoFast™ EvaGreen® | s_pool    | 0.005                 | 73.70                 |
| SsoFast™ EvaGreen® | s_pool    | 0.005                 | 73.70                 |
| SsoFast™ EvaGreen® | s_pool    | 0.005                 | 73.60                 |
| SsoFast™ EvaGreen® | n_pool    | 0.005                 | 73.70                 |
| SsoFast™ EvaGreen® | n_pool    | 0.005                 | 73.70                 |
| SsoFast™ EvaGreen® | n_pool    | 0.005                 | 73.70                 |
| SsoFast™ EvaGreen® | n_pool    | 0.005                 | 73.80                 |
| SsoFast™ EvaGreen® | n_pool    | 0.005                 | 73.70                 |
| SsoFast™ EvaGreen® | s_pool    | 0.0005                | 72.90                 |

Table 1 (continued)

| Mastermix                      | Sample ID | Concentration (ng/ $\mu$ l) | Melt Temperature ( $^{\circ}$ C) |
|--------------------------------|-----------|-----------------------------|----------------------------------|
| SsoFast™ EvaGreen <sup>®</sup> | s_pool    | 0.0005                      | 73.00                            |
| SsoFast™ EvaGreen <sup>®</sup> | s_pool    | 0.0005                      | 73.00                            |
| SsoFast™ EvaGreen <sup>®</sup> | n_pool    | 0.0005                      | 73.80                            |
| SsoFast™ EvaGreen <sup>®</sup> | n_pool    | 0.0005                      | 73.70                            |
| SsoFast™ EvaGreen <sup>®</sup> | n_pool    | 0.0005                      | 73.70                            |
| SsoFast™ EvaGreen <sup>®</sup> | MB        | N/A                         | None                             |
| SsoFast™ EvaGreen <sup>®</sup> | MB        | N/A                         | None                             |
| SsoFast™ EvaGreen <sup>®</sup> | MB        | N/A                         | None                             |
| SsoFast™ EvaGreen <sup>®</sup> | MB        | N/A                         | None                             |
| SsoFast™ EvaGreen <sup>®</sup> | MB        | N/A                         | None                             |
| SsoFast™ EvaGreen <sup>®</sup> | MB        | N/A                         | None                             |

Sample ID: s\_pool Signal crayfish DNA pool, n\_pool Native crayfish DNA pool, MB Amplification negative controls.

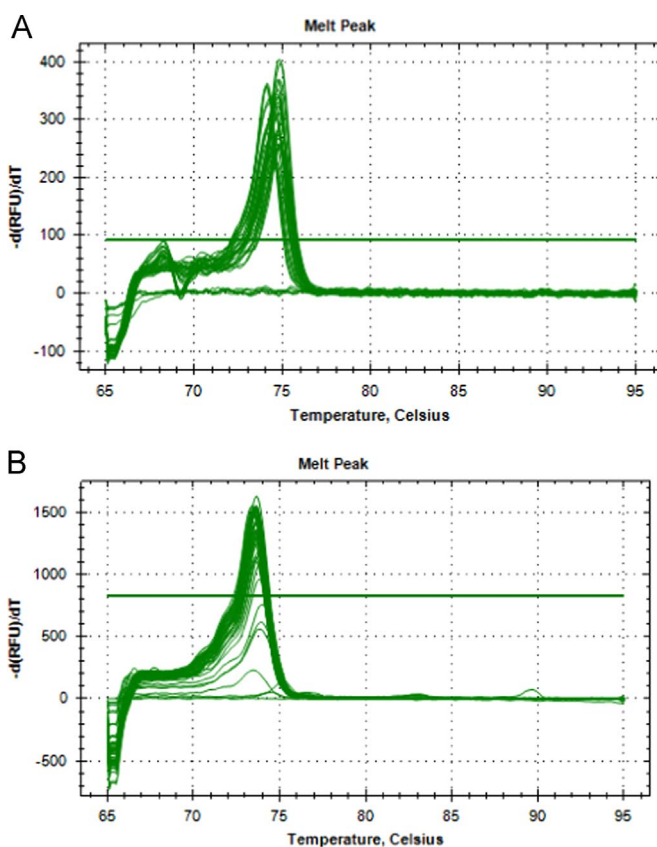


Fig. 5. Melt peaks from SsoFast™ EvaGreen<sup>®</sup> mixed DNA ratios of crayfish species (*Pacifastacus leniusculus* and *Austroptamobius pallipes*) qPCR amplifications (A). 10:90 *Pacifastacus leniusculus*: *Austroptamobius pallipes* to 90:10 *Pacifastacus leniusculus*: *Austroptamobius pallipes*. Melt peaks from SsoFast™ EvaGreen<sup>®</sup> ex-situ *Pacifastacus leniusculus* eDNA qPCR amplifications (B).

**Table 2**

Melt curve data from SsoFast™ EvaGreen® mixed DNA ratios of crayfish species (*Pacifastacus leniusculus* and *Austropotamobius pallipes*) qPCR amplifications.

| Mastermix          | Sample ID | Melt Temperature (°C) |
|--------------------|-----------|-----------------------|
| SsoFast™ EvaGreen® | 10:90S:N  | 74.20                 |
| SsoFast™ EvaGreen® | 10:90S:N  | 74.10                 |
| SsoFast™ EvaGreen® | 10:90S:N  | 74.20                 |
| SsoFast™ EvaGreen® | 90:10S:N  | 74.80                 |
| SsoFast™ EvaGreen® | 90:10S:N  | 74.90                 |
| SsoFast™ EvaGreen® | 90:10S:N  | 74.90                 |
| SsoFast™ EvaGreen® | 20:80S:N  | 74.30                 |
| SsoFast™ EvaGreen® | 20:80S:N  | 74.30                 |
| SsoFast™ EvaGreen® | 20:80S:N  | 74.30                 |
| SsoFast™ EvaGreen® | 30:70S:N  | 74.30                 |
| SsoFast™ EvaGreen® | 30:70S:N  | 74.40                 |
| SsoFast™ EvaGreen® | 30:70S:N  | 68.30                 |
| SsoFast™ EvaGreen® | 30:70S:N  | 74.40                 |
| SsoFast™ EvaGreen® | 40:60S:N  | 74.70                 |
| SsoFast™ EvaGreen® | 40:60S:N  | 74.70                 |
| SsoFast™ EvaGreen® | 40:60S:N  | 74.60                 |
| SsoFast™ EvaGreen® | 50:50S:N  | 74.70                 |
| SsoFast™ EvaGreen® | 50:50S:N  | 74.70                 |
| SsoFast™ EvaGreen® | 50:50S:N  | 74.70                 |
| SsoFast™ EvaGreen® | 50:50S:N  | 74.70                 |
| SsoFast™ EvaGreen® | 60:40S:N  | 74.70                 |
| SsoFast™ EvaGreen® | 60:40S:N  | 74.70                 |
| SsoFast™ EvaGreen® | 60:40S:N  | 74.70                 |
| SsoFast™ EvaGreen® | 70:30S:N  | 74.80                 |
| SsoFast™ EvaGreen® | 70:30S:N  | 74.80                 |
| SsoFast™ EvaGreen® | 70:30S:N  | 74.70                 |
| SsoFast™ EvaGreen® | 80:20S:N  | 74.80                 |
| SsoFast™ EvaGreen® | 80:20S:N  | 74.70                 |
| SsoFast™ EvaGreen® | 80:20S:N  | 74.70                 |
| SsoFast™ EvaGreen® | PC_SC     | 74.00                 |
| SsoFast™ EvaGreen® | PC_SC     | 74.10                 |
| SsoFast™ EvaGreen® | PC_SC     | 74.10                 |
| SsoFast™ EvaGreen® | PC_NC     | 74.80                 |
| SsoFast™ EvaGreen® | PC_NC     | 74.80                 |
| SsoFast™ EvaGreen® | PC_NC     | 74.90                 |
| SsoFast™ EvaGreen® | MB        | None                  |
| SsoFast™ EvaGreen® | MB        | None                  |
| SsoFast™ EvaGreen® | MB        | None                  |
| SsoFast™ EvaGreen® | MB        | None                  |
| SsoFast™ EvaGreen® | MB        | None                  |
| SsoFast™ EvaGreen® | MB        | None                  |

Sample ID: #:# Ratio of DNA mix, S Signal crayfish, N Native crayfish, PC\_SC Signal crayfish positive DNA control, PC\_NC Native crayfish positive DNA control, MB Amplification negative control.

Data displayed in Section 1.6 includes the SsoFast™ EvaGreen® qPCR product melt curve graphs and raw melt data from positive detections of both *P. leniusculus* and *A. pallipes* at the same site in the River Medway and Itchen (Fig. 9, Table 7). To conclude, Table 8 provides raw melt data on the absence of *A. astaci* DNA at sites in the River Medway and Itchen where both *P. leniusculus* and *A. pallipes* DNA were detected.

### 1.1. Sequence alignment of 16s mtDNA qPCR product for target crayfish species

See Fig. 1.



**Table 3**Melt peak data from SsoFast™ EvaGreen® *ex-situ* *Pacifastacus leniusculus* eDNA qPCR amplifications.

| Mastermix          | Sample ID | Melt Temperature (°C) |
|--------------------|-----------|-----------------------|
| SsoFast™ EvaGreen® | 1_T1      | None                  |
| SsoFast™ EvaGreen® | 1_T1      | 73.70                 |
| SsoFast™ EvaGreen® | 1_T1      | 73.70                 |
| SsoFast™ EvaGreen® | 7_T0      | 73.70                 |
| SsoFast™ EvaGreen® | 7_T0      | 73.70                 |
| SsoFast™ EvaGreen® | 7_T0      | 73.70                 |
| SsoFast™ EvaGreen® | 1_T0      | None                  |
| SsoFast™ EvaGreen® | 1_T0      | 73.70                 |
| SsoFast™ EvaGreen® | 1_T0      | 73.70                 |
| SsoFast™ EvaGreen® | 8_T1      | 73.70                 |
| SsoFast™ EvaGreen® | 8_T1      | 73.70                 |
| SsoFast™ EvaGreen® | 8_T1      | 73.70                 |
| SsoFast™ EvaGreen® | 1_T2      | 73.90                 |
| SsoFast™ EvaGreen® | 1_T2      | 73.70                 |
| SsoFast™ EvaGreen® | 8_T0      | 73.70                 |
| SsoFast™ EvaGreen® | 8_T0      | 73.70                 |
| SsoFast™ EvaGreen® | 8_T0      | 73.70                 |
| SsoFast™ EvaGreen® | 3_T1      | None                  |
| SsoFast™ EvaGreen® | 3_T1      | 73.70                 |
| SsoFast™ EvaGreen® | 3_T1      | 73.70                 |
| SsoFast™ EvaGreen® | 8_T2      | 73.70                 |
| SsoFast™ EvaGreen® | 8_T2      | 73.70                 |
| SsoFast™ EvaGreen® | 8_T2      | 73.70                 |
| SsoFast™ EvaGreen® | 3_T0      | None                  |
| SsoFast™ EvaGreen® | 3_T0      | 73.80                 |
| SsoFast™ EvaGreen® | 3_T0      | 73.80                 |
| SsoFast™ EvaGreen® | 9_T1      | 73.70                 |
| SsoFast™ EvaGreen® | 9_T1      | 73.70                 |
| SsoFast™ EvaGreen® | 9_T1      | 73.70                 |
| SsoFast™ EvaGreen® | 6_T1      | 73.80                 |
| SsoFast™ EvaGreen® | 6_T1      | 73.70                 |
| SsoFast™ EvaGreen® | 6_T1      | 73.70                 |
| SsoFast™ EvaGreen® | 9_T0      | 73.60                 |
| SsoFast™ EvaGreen® | 9_T0      | 73.70                 |
| SsoFast™ EvaGreen® | 6_T0      | 73.80                 |
| SsoFast™ EvaGreen® | 6_T0      | 73.70                 |
| SsoFast™ EvaGreen® | 6_T0      | 73.70                 |
| SsoFast™ EvaGreen® | 7_T1      | None                  |
| SsoFast™ EvaGreen® | 7_T1      | 73.60                 |
| SsoFast™ EvaGreen® | 7_T1      | 73.70                 |
| SsoFast™ EvaGreen® | PC_SC     | 73.70                 |
| SsoFast™ EvaGreen® | PC_SC     | 73.60                 |
| SsoFast™ EvaGreen® | PC_SC     | 73.70                 |
| SsoFast™ EvaGreen® | MB        | None                  |
| SsoFast™ EvaGreen® | MB        | None                  |
| SsoFast™ EvaGreen® | MB        | None                  |

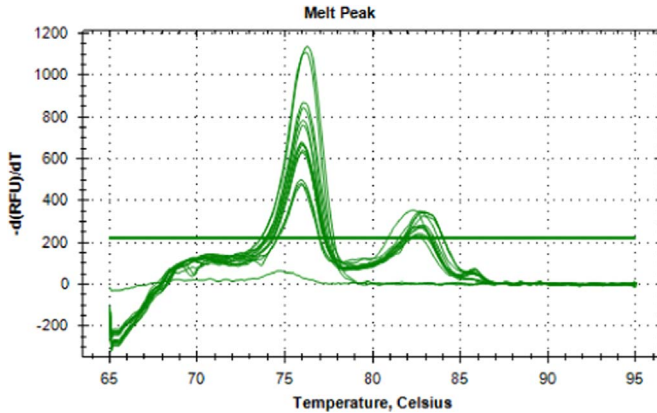
Sample ID: # Tank, T0 Time zero, T1 Time 1 (24 hrs after removal), T2 Time 2 (48 hours after removal), PC\_SC Signal crayfish positive DNA control, MB Amplification negative control.

### 1.2. eDNA yield data from *ex-situ* samples

See Fig. 2.

### 1.3. Data on qPCR optimisation for SYBR™ Green, SsoFast™ EvaGreen® and HOT FIREPol® EvaGreen® mastermixes

See Fig. 3.



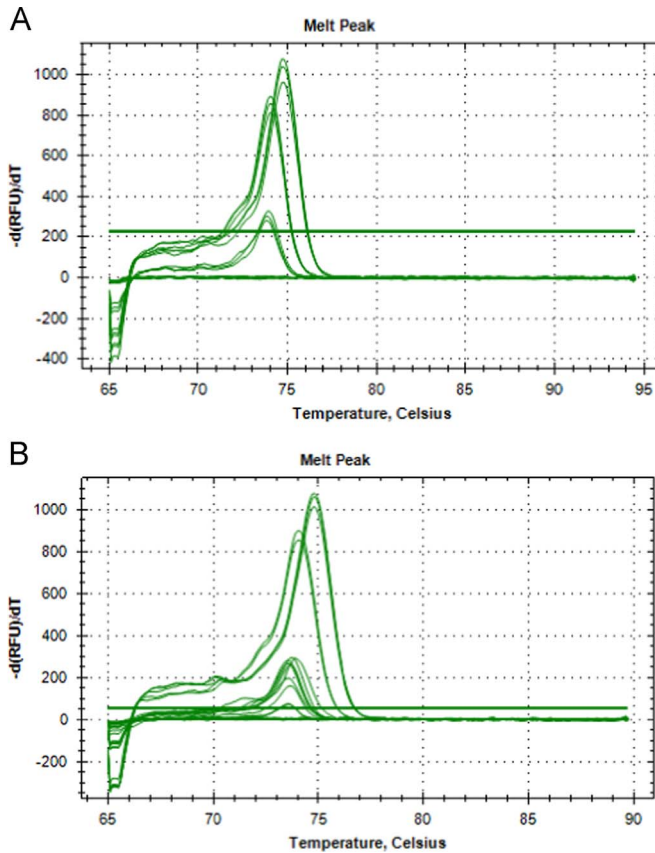
**Fig. 6.** Melt peaks for HOT FIREPol<sup>®</sup> EvaGreen<sup>™</sup> qPCR multiplex optimised reactions using an *Aphanomyces astaci*-infected *Pacifastacus leniusculus* DNA pool.

**Table 4**

Melt peak data for HOT FIREPol<sup>®</sup> EvaGreen<sup>™</sup> qPCR multiplex optimised reactions. 164 – 287 = *Aphanomyces astaci*-infected *Pacifastacus leniusculus* individuals.

| Mastermix                                      | Sample ID | Melt Temperature (°C) |
|--|-----------|-----------------------|
| HOT FIREPol <sup>®</sup> EvaGreen <sup>™</sup> | 287       | 82.90                 |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>™</sup> | 287       | 75.80                 |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>™</sup> | 287       | 82.90                 |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>™</sup> | 287       | 75.80                 |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>™</sup> | 287       | 82.90                 |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>™</sup> | 287       | 75.80                 |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>™</sup> | 281       | 82.80                 |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>™</sup> | 281       | 75.90                 |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>™</sup> | 281       | 82.70                 |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>™</sup> | 281       | 75.90                 |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>™</sup> | 281       | 82.80                 |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>™</sup> | 281       | 75.90                 |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>™</sup> | 164       | 82.70                 |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>™</sup> | 164       | 75.90                 |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>™</sup> | 164       | 82.30                 |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>™</sup> | 164       | 75.90                 |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>™</sup> | 164       | 82.80                 |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>™</sup> | 164       | 75.80                 |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>™</sup> | 278       | 82.30                 |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>™</sup> | 278       | 75.90                 |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>™</sup> | 278       | 82.80                 |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>™</sup> | 278       | 75.80                 |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>™</sup> | 278       | 82.80                 |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>™</sup> | 278       | 75.80                 |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>™</sup> | 278       | 82.80                 |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>™</sup> | 278       | 75.80                 |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>™</sup> | 278       | 82.80                 |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>™</sup> | 278       | 75.80                 |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>™</sup> | MB        | None                  |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>™</sup> | MB        | None                  |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>™</sup> | MB        | None                  |

Sample ID: # Infected crayfish individual.



**Fig. 7.** Melt peaks from SsoFast™ EvaGreen® eDNA qPCR amplifications for the trap water samples from the Bachowey (A) and Duhonw catchments (B).

1.4. Positive trap water sample amplifications for *Pacifastacus leniusculus* in the Wye catchment

See Fig. 7.

1.5. Data from positive field eDNA amplifications for *Pacifastacus leniusculus*, *Austropotamobius pallipes* and *Aphanomyces astaci* in the Wye catchment

See Fig. 8.

1.6. Data from field eDNA samples positive for *Pacifastacus leniusculus* and *Austropotamobius pallipes* in the same site in the River Medway and Itchen

See Fig. 9.

**Table 5**

Melt peak data from SsoFast™ EvaGreen® eDNA qPCR amplifications for the trap water samples from the Bachowey and Duhonw catchments.

| Mastermix          | Catchment | Sample ID | Melt Temperature (°C) |
|--------------------|-----------|-----------|-----------------------|
| SsoFast™ EvaGreen® | Bachowey  | L3B       | 73.80                 |
| SsoFast™ EvaGreen® | Bachowey  | L3B       | 73.90                 |
| SsoFast™ EvaGreen® | Bachowey  | L3B       | 73.90                 |
| SsoFast™ EvaGreen® | Bachowey  | L4A       | 73.90                 |
| SsoFast™ EvaGreen® | Bachowey  | L4A       | 73.70                 |
| SsoFast™ EvaGreen® | Bachowey  | L4A       | 73.70                 |
| SsoFast™ EvaGreen® | Bachowey  | L4B       | 73.80                 |
| SsoFast™ EvaGreen® | Bachowey  | L4B       | 73.70                 |
| SsoFast™ EvaGreen® | Bachowey  | L4B       | 73.70                 |
| SsoFast™ EvaGreen® | Duhonw    | L5B       | 73.70                 |
| SsoFast™ EvaGreen® | Duhonw    | L5B       | 73.70                 |
| SsoFast™ EvaGreen® | Duhonw    | L5C       | None                  |
| SsoFast™ EvaGreen® | Duhonw    | L5C       | None                  |
| SsoFast™ EvaGreen® | Duhonw    | L5C       | None                  |
| SsoFast™ EvaGreen® | N/A       | PC_SC     | 74.10                 |
| SsoFast™ EvaGreen® | N/A       | PC_SC     | 74.00                 |
| SsoFast™ EvaGreen® | N/A       | PC_SC     | 74.00                 |
| SsoFast™ EvaGreen® | N/A       | PC_NC     | 74.80                 |
| SsoFast™ EvaGreen® | N/A       | PC_NC     | 74.80                 |
| SsoFast™ EvaGreen® | N/A       | PC_NC     | 74.80                 |
| SsoFast™ EvaGreen® | N/A       | MB        | None                  |
| SsoFast™ EvaGreen® | N/A       | MB        | None                  |
| SsoFast™ EvaGreen® | N/A       | MB        | None                  |
| SsoFast™ EvaGreen® | N/A       | MB        | None                  |
| SsoFast™ EvaGreen® | N/A       | MB        | None                  |
| SsoFast™ EvaGreen® | N/A       | MB        | None                  |

Sample ID: L# Location number with subsample letter, PC\_SC Signal crayfish positive DNA control, PC\_NC Native crayfish positive DNA control, MB Amplification negative control.

## 2. Experimental design, materials and methods

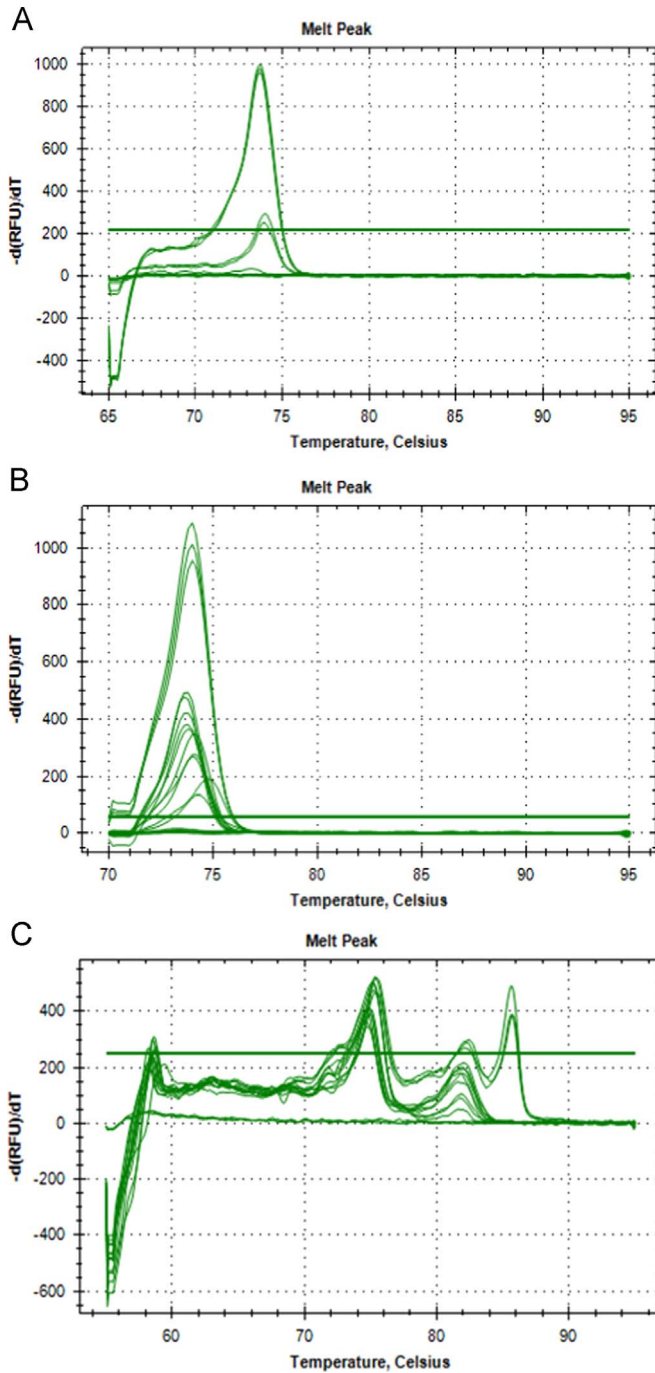
Methodologies that produced the data presented in this article are fully detailed in [1]. Below, the qPCR protocol for both SsoFast™ EvaGreen® and HOT FIREPol® EvaGreen® are described to complement data provided here.

### 2.1. Sample collection

Water samples were collected at six locations in the River Wye catchment, seven sites in the River Taff catchment, both in Wales, and at 29 sites in the Itchen and Medway rivers, Southern England as detailed in [1]. An ex-situ experiment was also conducted with *P. leniusculus* in three 2 L isolated tanks from where water samples were collected 24 and 48 h after removal of the crayfish [1].

### 2.2. qPCR analysis protocol

DNA from the ex-situ eDNA and tissue samples for *P. leniusculus* and *A. pallipes* were extracted using Qiagen® DNeasy Blood and Tissue Kit (Qiagen, UK). Crayfish specific primers were designed using Primer3, then tested using Beacon Primer Designer (ver. 2.1, PREMIER Biosoft), and finally checked for cross-amplification using NCBI Primer-BLAST [2] and fresh tissue samples as described in [1].



**Fig. 8.** Melt peaks from SsoFast™ EvaGreen® eDNA qPCR amplifications for the Bachowey catchment 2015 samples (A), Sgithwen and Bachowey catchment 2016 samples (B) and HOT FIREPol® EvaGreen® eDNA qPCR amplifications from Bachowey (C).

**Table 6**

Melt peak data from SsoFast™ EvaGreen® eDNA qPCR amplifications for the Bachowey catchment 2015 samples and Sgithwen and Bachowey catchment 2016 samples.

| Mastermix              | Catchment | Year | Sample ID | Melt Temperature (°C) |
|------------------------|-----------|------|-----------|-----------------------|
| SsoFast™ EvaGreen®     | Sgithwen  | 2017 | 2B        | 74.80                 |
| SsoFast™ EvaGreen®     | Sgithwen  | 2017 | 2B        | 74.80                 |
| SsoFast™ EvaGreen®     | Bachowey  | 2016 | 3         | 73.70                 |
| SsoFast™ EvaGreen®     | Bachowey  | 2016 | 3         | 73.70                 |
| SsoFast™ EvaGreen®     | Bachowey  | 2016 | 3         | 73.70                 |
| SsoFast™ EvaGreen®     | Bachowey  | 2017 | 4G        | 74.00                 |
| SsoFast™ EvaGreen®     | Bachowey  | 2017 | 4G        | 74.20                 |
| SsoFast™ EvaGreen®     | Bachowey  | 2017 | 4G        | 74.20                 |
| SsoFast™ EvaGreen®     | Bachowey  | 2017 | 4F        | 73.80                 |
| SsoFast™ EvaGreen®     | Bachowey  | 2017 | 4F        | 73.80                 |
| SsoFast™ EvaGreen®     | Bachowey  | 2017 | 4I        | 73.80                 |
| SsoFast™ EvaGreen®     | Bachowey  | 2017 | 4I        | 73.80                 |
| SsoFast™ EvaGreen®     | Bachowey  | 2017 | 4I        | 73.70                 |
| SsoFast™ EvaGreen®     | N/A       | N/A  | PC_SC     | 74.00                 |
| SsoFast™ EvaGreen®     | N/A       | N/A  | PC_SC     | 74.00                 |
| SsoFast™ EvaGreen®     | N/A       | N/A  | PC_SC     | 73.90                 |
| SsoFast™ EvaGreen®     | N/A       | N/A  | PC_NC     | 74.80                 |
| SsoFast™ EvaGreen®     | N/A       | N/A  | PC_NC     | 74.80                 |
| SsoFast™ EvaGreen®     | N/A       | N/A  | PC_NC     | 74.80                 |
| SsoFast™ EvaGreen®     | N/A       | N/A  | PC_NC     | 74.80                 |
| SsoFast™ EvaGreen®     | N/A       | N/A  | MB        | None                  |
| SsoFast™ EvaGreen®     | N/A       | N/A  | MB        | None                  |
| SsoFast™ EvaGreen®     | N/A       | N/A  | MB        | None                  |
| SsoFast™ EvaGreen®     | N/A       | N/A  | EB1       | None                  |
| SsoFast™ EvaGreen®     | N/A       | N/A  | EB1       | None                  |
| SsoFast™ EvaGreen®     | N/A       | N/A  | EB1       | None                  |
| SsoFast™ EvaGreen®     | N/A       | N/A  | EB2       | None                  |
| SsoFast™ EvaGreen®     | N/A       | N/A  | EB2       | None                  |
| SsoFast™ EvaGreen®     | N/A       | N/A  | EB2       | None                  |
| SsoFast™ EvaGreen®     | N/A       | N/A  | EB3       | None                  |
| SsoFast™ EvaGreen®     | N/A       | N/A  | EB3       | None                  |
| SsoFast™ EvaGreen®     | N/A       | N/A  | EB3       | None                  |
| HOT FIREPol® EvaGreen® | Bachowey  | 2016 | 10        | 75.10                 |
| HOT FIREPol® EvaGreen® | Bachowey  | 2016 | 10        | 83.90                 |
| HOT FIREPol® EvaGreen® | Bachowey  | 2016 | 10        | 75.20                 |
| HOT FIREPol® EvaGreen® | Bachowey  | 2016 | 10        | 83.00                 |
| HOT FIREPol® EvaGreen® | Bachowey  | 2016 | 10        | 75.00                 |
| HOT FIREPol® EvaGreen® | Bachowey  | 2016 | 10        | 82.90                 |
| HOT FIREPol® EvaGreen® | Bachowey  | 2016 | 11        | 75.20                 |
| HOT FIREPol® EvaGreen® | Bachowey  | 2016 | 11        | 82.90                 |
| HOT FIREPol® EvaGreen® | Bachowey  | 2016 | 11        | 75.20                 |
| HOT FIREPol® EvaGreen® | Bachowey  | 2016 | 11        | 82.90                 |
| HOT FIREPol® EvaGreen® | Bachowey  | 2016 | 11        | 75.20                 |
| HOT FIREPol® EvaGreen® | Bachowey  | 2016 | 11        | 82.90                 |
| HOT FIREPol® EvaGreen® | Bachowey  | 2016 | 14        | 75.20                 |
| HOT FIREPol® EvaGreen® | Bachowey  | 2016 | 14        | 83.00                 |
| HOT FIREPol® EvaGreen® | Bachowey  | 2016 | 14        | 75.20                 |
| HOT FIREPol® EvaGreen® | Bachowey  | 2016 | 14        | 83.00                 |
| HOT FIREPol® EvaGreen® | Bachowey  | 2016 | 14        | 75.10                 |
| HOT FIREPol® EvaGreen® | Bachowey  | 2016 | 14        | 82.90                 |
| HOT FIREPol® EvaGreen® | Bachowey  | 2016 | 14        | 75.10                 |
| HOT FIREPol® EvaGreen® | Bachowey  | 2016 | 14        | 82.90                 |
| HOT FIREPol® EvaGreen® | Bachowey  | 2016 | 14        | 75.60                 |
| HOT FIREPol® EvaGreen® | Bachowey  | 2016 | 14        | 83.00                 |
| HOT FIREPol® EvaGreen® | N/A       | N/A  | PC POOL   | 75.70                 |
| HOT FIREPol® EvaGreen® | N/A       | N/A  | PC POOL   | 82.90                 |
| HOT FIREPol® EvaGreen® | N/A       | N/A  | PC POOL   | 75.70                 |
| HOT FIREPol® EvaGreen® | N/A       | N/A  | PC POOL   | 83.00                 |
| HOT FIREPol® EvaGreen® | N/A       | N/A  | PC POOL   | 75.70                 |
| HOT FIREPol® EvaGreen® | N/A       | N/A  | PC POOL   | 82.90                 |

**Table 6** (continued)

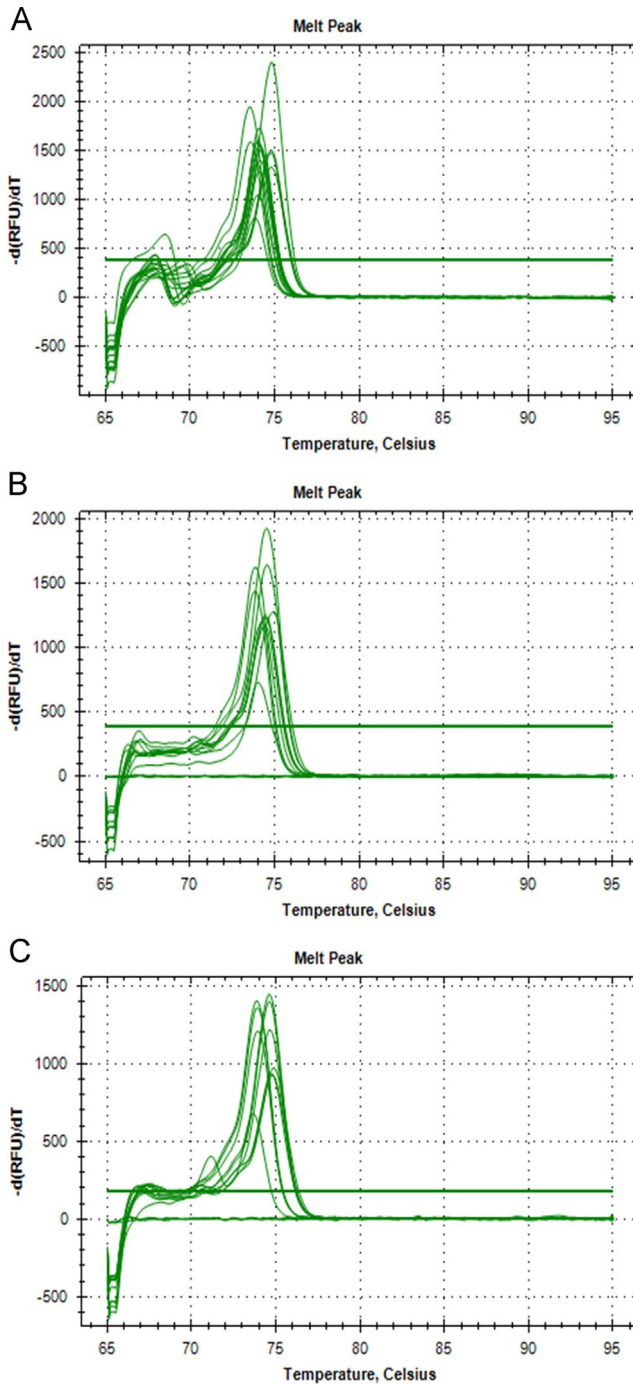
| Mastermix                                      | Catchment | Year | Sample ID | Melt Temperature (°C) |
|--|-----------|------|-----------|-----------------------|
| HOT FIREPol <sup>®</sup> EvaGreen <sup>®</sup> | N/A       | N/A  | MB        | None                  |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>®</sup> | N/A       | N/A  | MB        | None                  |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>®</sup> | N/A       | N/A  | MB        | None                  |

Sample ID: # Wye catchment sample with corresponding subsample letter, PC\_SC Signal crayfish positive DNA control, PC\_NC Native crayfish positive DNA control, MB Amplification negative control, EB# Extraction negative control.

Water samples were amplified in triplicate using optimised SsoFast™ EvaGreen<sup>®</sup> supermix assay to assess presence of *P. leniusculus* and *A. pallipes* through diagnostic melt peak temperature of resulting qPCR products. Reactions were undertaken in 10 µl volumes using a CFX96 Real-Time PCR detection system (Bio-Rad, UK) consisting of 5 µl SsoFast™ EvaGreen<sup>®</sup> supermix, 0.25 µl each forward and reverse primer (ApalPlen16S), 3.5 µl ultrapure water and 1 µl DNA. PCR protocol began with 15 min of denaturation at 95 °C, followed by 40 cycles of 95 °C for 10 s and 61.5 °C for 30 s. A melt gradient step was applied to the end of RT-qPCR reactions, ranging from 55 °C to 95 °C in 0.1 °C increments. Once qPCR products were analysed for presence/absence of *P. leniusculus* and *A. pallipes*, qPCR amplifications were repeated for positive sites using 2 × HOT FIREPol<sup>®</sup> EvaGreen<sup>®</sup> multiplex mix with 0.4 µl of primer mix (5 µM), 6.6 µl of ultrapure water and 1 µl template DNA. Cycling conditions were as follows: activation at 95 °C for 12 min, 40 cycles of 95 °C for 15 s, 61.5 °C for 20 s and 72 °C for 20 s. After the PCR reaction, a melt gradient was applied, which ran from 65 °C to 95 °C by raising 1 °C for 10 s each step. Resulting melt peaks from the multiplex qPCR were then assessed to determine presence/absence of *A. astaci* in *P. leniusculus*/*A. pallipes* positive sites.

The results of the ex situ study indicated that DNA concentration decreased slightly but remained fairly constant across the three time points and was still detectable (melt peak above threshold) at the end of the third time point. DNA quantity was fairly uniform across all tanks, which is to be expected as there was equal biomass of crayfish in each tank, which is known to correlate with the amount of eDNA detected in other aquatic species [3,4].

Our approach is still subject to factors affecting the sensitivity of the eDNA analyses, such as number and type of samples collected, volume of water sampled, types of waterbody sampled and differences in laboratory techniques [5–7]. Larger water volumes can increase detectability of eDNA, but there is a trade-off between volume and number of samples, and we have shown that our method can detect infected crayfish even in small volume samples, while allowing to maximize coverage [8–10].



**Fig. 9.** Melt peaks from SsoFast™ EvaGreen® eDNA qPCR amplifications for the 2016 Medway catchment site 5 (A), 2017 Medway catchment site 5 (B) and Itchen catchment at site 8 (C).



**Table 7**

Melt peak data from SsoFast™ EvaGreen® eDNA qPCR amplifications for the 2016 Medway catchment site 5, 2017 Medway catchment site 5 and Itchen catchment at site 8.

| Mastermix          | Catchment | Year | Sample ID | Melt Temperature (°C) |
|--------------------|-----------|------|-----------|-----------------------|
| SsoFast™ EvaGreen® | Medway    | 2016 | M5A       | 68.00                 |
| SsoFast™ EvaGreen® | Medway    | 2016 | M5A       | 74.10                 |
| SsoFast™ EvaGreen® | Medway    | 2016 | M5A       | 74.10                 |
| SsoFast™ EvaGreen® | Medway    | 2016 | M5A       | None                  |
| SsoFast™ EvaGreen® | Medway    | 2016 | M5B       | 67.90                 |
| SsoFast™ EvaGreen® | Medway    | 2016 | M5B       | 74.00                 |
| SsoFast™ EvaGreen® | Medway    | 2016 | M5B       | 68.00                 |
| SsoFast™ EvaGreen® | Medway    | 2016 | M5B       | 74.00                 |
| SsoFast™ EvaGreen® | Medway    | 2016 | M5B       | 68.50                 |
| SsoFast™ EvaGreen® | Medway    | 2016 | M5B       | 74.80                 |
| SsoFast™ EvaGreen® | Medway    | 2016 | M5C       | 74.10                 |
| SsoFast™ EvaGreen® | Medway    | 2016 | M5C       | 74.10                 |
| SsoFast™ EvaGreen® | Medway    | 2016 | M5C       | 74.80                 |
| SsoFast™ EvaGreen® | Medway    | 2016 | M5D       | 74.10                 |
| SsoFast™ EvaGreen® | Medway    | 2016 | M5D       | 74.00                 |
| SsoFast™ EvaGreen® | Medway    | 2016 | M5D       | 74.80                 |
| SsoFast™ EvaGreen® | Medway    | 2016 | M5E       | 67.90                 |
| SsoFast™ EvaGreen® | Medway    | 2016 | M5E       | 74.00                 |
| SsoFast™ EvaGreen® | Medway    | 2016 | M5E       | 73.80                 |
| SsoFast™ EvaGreen® | Medway    | 2016 | M5E       | 74.80                 |
| SsoFast™ EvaGreen® | N/A       | N/A  | PC_SC     | 73.70                 |
| SsoFast™ EvaGreen® | N/A       | N/A  | PC_SC     | 73.70                 |
| SsoFast™ EvaGreen® | N/A       | N/A  | PC_NC     | 74.80                 |
| SsoFast™ EvaGreen® | N/A       | N/A  | PC_NC     | 74.80                 |
| SsoFast™ EvaGreen® | N/A       | N/A  | MB        | None                  |
| SsoFast™ EvaGreen® | N/A       | N/A  | MB        | None                  |
| SsoFast™ EvaGreen® | N/A       | N/A  | MB        | None                  |
| SsoFast™ EvaGreen® | Medway    | 2017 | M5B       | 74.00                 |
| SsoFast™ EvaGreen® | Medway    | 2017 | M5B       | None                  |
| SsoFast™ EvaGreen® | Medway    | 2017 | M5B       | 74.00                 |
| SsoFast™ EvaGreen® | Medway    | 2017 | M5C       | 74.60                 |
| SsoFast™ EvaGreen® | Medway    | 2017 | M5C       | 74.60                 |
| SsoFast™ EvaGreen® | Medway    | 2017 | M5C       | 74.60                 |
| SsoFast™ EvaGreen® | N/A       | N/A  | PC_SC     | 73.90                 |
| SsoFast™ EvaGreen® | N/A       | N/A  | PC_SC     | 73.80                 |
| SsoFast™ EvaGreen® | N/A       | N/A  | PC_SC     | 73.90                 |
| SsoFast™ EvaGreen® | N/A       | N/A  | PC_NC     | 74.60                 |
| SsoFast™ EvaGreen® | N/A       | N/A  | PC_NC     | 74.60                 |
| SsoFast™ EvaGreen® | N/A       | N/A  | PC_NC     | 74.90                 |
| SsoFast™ EvaGreen® | N/A       | N/A  | MB        | None                  |
| SsoFast™ EvaGreen® | N/A       | N/A  | MB        | None                  |
| SsoFast™ EvaGreen® | N/A       | N/A  | MB        | None                  |
| SsoFast™ EvaGreen® | Itchen    | 2017 | I8E       | 74.00                 |
| SsoFast™ EvaGreen® | Itchen    | 2017 | I8E       | 74.00                 |
| SsoFast™ EvaGreen® | Itchen    | 2017 | I8F       | 74.00                 |
| SsoFast™ EvaGreen® | Itchen    | 2017 | I8F       | 74.00                 |
| SsoFast™ EvaGreen® | Itchen    | 2017 | I8A       | 73.70                 |
| SsoFast™ EvaGreen® | Itchen    | 2017 | I8A       | 73.70                 |
| SsoFast™ EvaGreen® | Itchen    | 2017 | I8C       | 74.80                 |
| SsoFast™ EvaGreen® | Itchen    | 2017 | I8C       | 74.90                 |
| SsoFast™ EvaGreen® | Itchen    | 2017 | I8C       | 74.70                 |
| SsoFast™ EvaGreen® | N/A       | N/A  | PC_SC     | 73.90                 |
| SsoFast™ EvaGreen® | N/A       | N/A  | PC_SC     | 73.90                 |
| SsoFast™ EvaGreen® | N/A       | N/A  | PC_SC     | 73.90                 |
| SsoFast™ EvaGreen® | N/A       | N/A  | PC_NC     | 74.60                 |
| SsoFast™ EvaGreen® | N/A       | N/A  | PC_NC     | 74.60                 |
| SsoFast™ EvaGreen® | N/A       | N/A  | PC_NC     | 74.60                 |
| SsoFast™ EvaGreen® | N/A       | N/A  | MB        | None                  |
| SsoFast™ EvaGreen® | N/A       | N/A  | MB        | None                  |

**Table 7** (continued)

| Mastermix                      | Catchment | Year | Sample ID | Melt Temperature (°C) |
|--------------------------------|-----------|------|-----------|-----------------------|
| SsoFast™ EvaGreen <sup>®</sup> | N/A       | N/A  | MB        | None                  |

Sample ID: M# Medway catchment sample with corresponding subsample letter, I# Itchen catchment sample with corresponding subsample letter, PC\_SC Signal crayfish positive DNA control, PC\_NC Native crayfish positive DNA control, MB Amplification negative control.

**Table 8**

Melt peak data from HOT FIREPol<sup>®</sup> EvaGreen<sup>®</sup> eDNA qPCR amplifications from the Medway and Itchen catchments, at sites where both *Pacifastacus leniusculus* and *Austropotamobius pallipes* DNA was detected in the same site.

| Mastermix                                      | Catchment | Year | Sample ID | Melt Temperature (°C) |
|--|-----------|------|-----------|-----------------------|
| HOT FIREPol <sup>®</sup> EvaGreen <sup>®</sup> | Medway    | 2016 | M5A       | 76.90                 |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>®</sup> | Medway    | 2016 | M5A       | 76.00                 |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>®</sup> | Medway    | 2016 | M5A       | 76.00                 |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>®</sup> | Medway    | 2016 | M5B       | 76.00                 |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>®</sup> | Medway    | 2016 | M5B       | 76.00                 |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>®</sup> | Medway    | 2016 | M5B       | 75.90                 |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>®</sup> | Medway    | 2016 | M5C       | None                  |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>®</sup> | Medway    | 2016 | M5C       | 75.90                 |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>®</sup> | Medway    | 2016 | M5C       | 75.90                 |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>®</sup> | Medway    | 2016 | M5D       | 75.90                 |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>®</sup> | Medway    | 2016 | M5D       | None                  |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>®</sup> | Medway    | 2016 | M5D       | 75.90                 |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>®</sup> | Medway    | 2016 | M5E       | 75.80                 |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>®</sup> | Medway    | 2016 | M5E       | None                  |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>®</sup> | Medway    | 2016 | M5E       | None                  |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>®</sup> | Medway    | 2016 | M5F       | 75.90                 |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>®</sup> | Medway    | 2016 | M5F       | 75.90                 |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>®</sup> | Medway    | 2016 | M5F       | 75.80                 |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>®</sup> | Medway    | 2017 | M5C       | 75.90                 |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>®</sup> | Medway    | 2017 | M5C       | 75.90                 |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>®</sup> | Medway    | 2017 | M5C       | 76.90                 |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>®</sup> | Itchen    | 2017 | I8C       | 76.90                 |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>®</sup> | Itchen    | 2017 | I8C       | 76.90                 |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>®</sup> | Itchen    | 2017 | I8C       | None                  |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>®</sup> | Itchen    | 2017 | I8F       | 75.90                 |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>®</sup> | Itchen    | 2017 | I8F       | None                  |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>®</sup> | Itchen    | 2017 | I8F       | None                  |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>®</sup> | N/A       | N/A  | PC_SC     | 75.90                 |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>®</sup> | N/A       | N/A  | PC_SC     | 76.00                 |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>®</sup> | N/A       | N/A  | PC_SC     | 75.90                 |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>®</sup> | N/A       | N/A  | PC_AA     | 82.90                 |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>®</sup> | N/A       | N/A  | PC_AA     | 82.90                 |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>®</sup> | N/A       | N/A  | PC_AA     | 82.90                 |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>®</sup> | N/A       | N/A  | PC_AA     | 82.90                 |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>®</sup> | N/A       | N/A  | MB        | None                  |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>®</sup> | N/A       | N/A  | MB        | None                  |
| HOT FIREPol <sup>®</sup> EvaGreen <sup>®</sup> | N/A       | N/A  | MB        | None                  |

Sample ID: M# Medway catchment sample with corresponding subsample letter, I# Itchen catchment sample with corresponding subsample letter, PC\_SC Signal crayfish positive DNA control, PC\_NC Native crayfish positive DNA control, PC\_AA Crayfish plague positive DNA control, MB Amplification negative control.

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### Transparency document. Supporting information

Transparency data associated with this article can be found in the online version at <https://doi.org/10.1016/j.dib.2018.05.134>.

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