The prevalence of *Aphanomyces astaci* in invasive signal crayfish from the UK and implications for native crayfish conservation

J. JAMES[1]*†, S. NUTBEAM-TUFFS[2,3]†, J. CABLE[1], A. MRUGAŁA[4],
N. VIÑUELA-RODRIGUEZ[4], A. PETRUSEK[4] and B. OIDTMANN[3]

[1] *School of Biosciences, Cardiff University, Cardiff CF10 3AX, UK*
[2] *The Roslin Institute, University of Edinburgh, Easter Bush, Midlothian EH25 9RG, UK*
[3] *Centre for Environment, Fisheries and Aquaculture Science (Cefas), The Nothe, Weymouth, Dorset DT4 8UB, UK*
[4] *Department of Ecology, Faculty of Science, Charles University, Viničná 7, CZ-12844 Prague 2, Czech Republic*

SUMMARY

The crayfish plague agent, *Aphanomyces astaci*, has spread throughout Europe, causing a significant decline in native European crayfish. The introduction and dissemination of this pathogen is attributed to the spread of invasive North American crayfish, which can act as carriers for *A. astaci*. As native European crayfish often succumb to infection with *A. astaci*, determining the prevalence of this pathogen in non-native crayfish is vital to prioritize native crayfish populations for managed translocation. In the current study, 23 populations of invasive signal crayfish (*Pacifastacus leniusculus*) from the UK were tested for *A. astaci* presence using quantitative PCR. Altogether, 13 out of 23 (56·5%) populations were found to be infected, and pathogen prevalence within infected sites varied from 3 to 80%. Microsatellite pathogen genotyping revealed that at least one UK signal crayfish population was infected with the *A. astaci* genotype group B, known to include virulent strains. Based on recent crayfish distribution records and the average rate of signal crayfish population dispersal, we identified one native white-clawed crayfish (*Austropotamobius pallipes*) population predicted to come into contact with infected signal crayfish within 5 years. This population should be considered as a priority for translocation.

Key words: *Pacifastacus leniusculus*, crayfish plague, white-clawed crayfish, *Austropotamobius pallipes*, Ark Sites.

INTRODUCTION

Crayfish plague, caused by the oomycete *Aphanomyces astaci*, is arguably one of the most deadly invasive parasites of freshwater ecosystems worldwide (Lowe *et al*. 2004; DAISIE, 2009). The pathogen is thought to have been first introduced into Europe (Italy) in 1859, and has subsequently spread throughout most of the continent (reviewed by Alderman, 1996; Holdich, 2003). In the second half of the 20th century the spread of *A. astaci* throughout Europe was facilitated by the movement of non-native North American (henceforth referred to as American) crayfish (reviewed by Alderman, 1996; Holdich, 2003). Whilst American crayfish are often asymptomatic carriers of the pathogen, in native European crayfish infection is usually fatal (Unestam and Weiss, 1970; Diéguez-Uribeondo *et al*. 1997; Bohman *et al*. 2006; Kozubíková *et al*. 2008; Oidtmann, 2012). Therefore, preventing the spread of this pathogen in regions with populations of highly susceptible hosts is a conservation priority.

One of the main American crayfish species responsible for spreading *A. astaci* in Europe, the signal crayfish (*Pacifastacus leniusculus*), was first introduced into the UK from Sweden during the 1970s for aquaculture (e.g. Holdich and Reeve, 1991; Alderman, 1996; Holdich *et al*. 1999, 2014). This corresponded with mass declines in Britain's historically abundant native white-clawed crayfish (*Austropotamobius pallipes*) (see Holdich and Reeve, 1991; Holdich and Sibley, 2009; Holdich *et al*. 2009, 2014; James *et al*. 2014), to such an extent that since 2010 they have been categorized as endangered (IUCN, 2015). Whilst it was widely considered that reductions in native crayfish were, at least partially, due to the transmission of *A. astaci* from signal crayfish, screening and detection of this pathogen in the UK did not occur until the early 1980s (Alderman, 1996). One of the first suspected outbreaks of plague in the UK was recorded from the River Lee, Thames catchment, England in 1981 (Alderman, 1996). The pathogen has since been reported in native crayfish from several other sites in England as well as Wales and Ireland (Alderman *et al*. 1984, 1990; Holdich and Reeve, 1991; Alderman, 1996; Lilley *et al*. 1997; Holdich, 2003). However, these reports have been based on pathogen morphology and disease symptoms in native European crayfish. Given that there are no morphological features that distinguish *A. astaci* from non-pathogenic *Aphanomyces* species (Royo

* Corresponding author: School of Biosciences, Cardiff University, Cardiff, CF10 3AX, UK. E-mail: jamesj12@cardiff.ac.uk
† Both authors contributed equally to this study.

et al. 2004; Oidtmann, 2012), molecular confirmation is essential (Oidtmann et al. 2006; Vrålstad et al. 2009). The only reports in the scientific literature of A. astaci detection in the UK using molecular methods are from another introduced crayfish species, Orconectes cf. virilis (see Tilmans et al. 2014), which is restricted to a single catchment (James et al. 2016).

Gaining a comprehensive understanding of A. astaci distribution in the UK is essential for native crayfish conservation. It is generally considered that the only way of ensuring the sustainability of white-clawed crayfish in the UK is through the establishment of isolated 'Ark Sites' free from non-native crayfish and at low risk of their invasion (Peay, 2009). Resources for implementing such conservation measures are, however, limited and so the selection of native crayfish populations for translocation needs to be a well-informed process. Native crayfish populations in close vicinity to A. astaci-infected invasive crayfish populations are at higher risk of extirpation than those neighbouring uninfected ones (Söderbäck, 1994; Westman et al. 2002; Schulz et al. 2006; Dunn et al. 2009; Schrimpf et al. 2013). Co-existence of native crayfish with invasive crayfish for several years has been observed in the absence of A. astaci (see Söderbäck, 1994; Westman et al. 2002; Schulz et al. 2006; Dunn et al. 2009; Schrimpf et al. 2013). Therefore, it is of greater urgency to translocate native crayfish populations at high risk of A. astaci transmission, than those in close proximity to uninfected invasive crayfish.

Here, we used quantitative PCR (qPCR) to assess the prevalence and intensity of infection with A. astaci in 23 populations of invasive signal crayfish in England and Wales. Using these data in combination with long-term white-clawed crayfish distribution records (James et al. 2014) we identified native crayfish populations at high risk of infection with A. astaci (determined by their proximity to an A. astaci-infected signal crayfish population). Given that A. astaci genotypes differ in virulence (Makkonen et al. 2012; Becking et al. 2015), when possible, we also genotyped the strain of A. astaci.

METHODS

For this study, invasive signal crayfish (P. leniusculus) from the UK were screened for the presence of A. astaci using similar molecular methods in two separate laboratories: the Centre for Environment, Fisheries and Aquaculture Science, Weymouth, UK (Cefas); and Charles University, Prague, Czech Republic (CUNI). At all sites, signal crayfish were captured from rivers and ponds using baited traps. Upon collection, animals were transported to the laboratory and humanely euthanized by exposure to chloroform vapour or freezing at −80 °C, before being stored individually in falcon tubes containing 95% molecular grade ethanol. Samples collected between September 2009 and July 2010 from 17 sites were processed at Cefas (n = 8–30 animals per site), whereas those harvested during May–September 2014 were analysed at CUNI (n = 20–30 animals per site, Table 1).

From each crayfish, a section of tail fan and soft abdominal cuticle were harvested for A. astaci screening. For animals processed in CUNI soft cuticle from two limb joints and any sections of melanized cuticle were also collected and pooled (Svoboda et al. 2014). At Cefas tissue samples from the tail fan and soft abdominal cuticle were analysed separately (mean: 60 and 78 mg of tissue per host sample for the tail fan and soft abdominal cuticle, respectively). For these samples, tissue disruption was conducted in fast prep tubes containing lysis matrix A (MP Biomedicals, Cambridge, UK) and DNA subsequently extracted using the Qiamp DNeasy Biorobot investigator kit (Qiagen, Hilden, Germany), according to the manufacturer's guidelines. At CUNI, for each animal, all collected tissue samples were amassed (40–50 mg per host sample) and ground together in liquid nitrogen. DNA was then extracted using DNeasy tissue kit (Qiagen, Hilden, Germany) in accordance with manufacturer's instructions.

All samples were tested for A. astaci presence with the TaqMan MGB qPCR as described in Vrålstad et al. (2009) with the following slight alterations at Cefas and CUNI, respectively: an elongation of the decontamination step from 2 to 5 min (Tuffs and Oidtmann, 2011), an increase in the annealing temperature from 58 to 60 °C and decreased synthesis time from 60 to 30 s (Svoboda et al. 2014). At Cefas and CUNI qPCRs were run on a Step one Plus real-time cycler (Applied Biosystems) and an iQ5 BioRad thermal cycler, respectively. Negative controls were used in every step of the procedure; these remained negative in all cases. The amount of A. astaci DNA in each sample was estimated based on the calibration curve of a set of standards. At Cefas the quantity of pathogen DNA in these standards ranged from 1 ng to 10 fg, in a 10-fold dilution series. At CUNI four standards were included containing 3×4^{10}, 3×4^{8}, 3×4^{4} and 3×4^{2} PCR forming units (PFU) of pathogen DNA. At Cefas each sample was run in triplicate and an average taken when calculating pathogen DNA concentrations. At CUNI each isolate was run twice, undiluted and a 10-fold diluted replicate to test for inhibition that may affect the efficacy of pathogen detection (Vrålstad et al. 2009; Strand et al. 2011). Based on the strength of the PCR signal, we assigned the relative level of A. astaci infection to semi-quantitative agent levels (A0–A7; according to Vrålstad et al. 2009; Kozubíková et al. 2011). Samples designated as A2 or higher were considered positive for A.

Table 1. Prevalence (95% CI) and infection intensity of *Aphanomyces astaci* in 23 populations of invasive signal crayfish (*Pacifastacus leniusculus*) from the UK, where infection intensities are reported as semi-quantitative agent levels (Vrålstad *et al*. 2009): uninfected (A0–A1) and infected (A2–A5).

Population (location)	Catchment	Lat/long (approx.)	Prevalence % (95% CI)	No. animals tested (*n*)	Agent level (range)
Wales					
*Sirhowy River (Caerphilly)	Usk	51°39′28″N, 003°11′23″W	0 (0–17)	30	A0
*Dderw farm pond (Powys)	N/A	52°01′45″N, 003°15′28″W	0 (0–17)	30	A0–A1
*Bachowey River 1 (Powys)	Wye	52°06′12″N, 003°12′58″W	50 (27–73)	20	A0–A3
Bachowey River 2 (Powys)	Wye	52°06′34″N, 003°13′51″W	23 (10–42)	30	A0–A3
*Gavenny River (Monmouthshire)	Usk	51°50′28″N, 003°00′11″W	47 (28–66)	30	A0–A3
*Mochdre Brook (Powys)	Severn	52°30′14″N, 003°20′53″W	75 (51–91)	20	A0–A4
England					
Broadmead Brook (Wiltshire)	Bristol Avon	51°29′24″N, 002°15′28″W	0 (0–17)	30	A0
St Catherine's Brook (South Gloucestershire)	Bristol Avon	51°25′59″N, 002°18′33″W	0 (0–17)	30	A0
Sutton Bingham Reservoir (Somerset)	N/A	50°54′01″N, 002°38′03″W	0 (0–17)	30	A0
River Wharfe 1 (North Yorkshire)	Yorkshire Ouse	54°06′09″N, 002°02′07″W	0 (0–17)	30	A0
River Riddle (Cumbria)	Derwent	54°15′03″N, 002°37′34″W	0 (0–17)	30	A0
Fenny Beck (West Yorkshire)	Yorkshire Ouse	53°39′00″N, 001°44′18″W	0 (0–17)	29	A0
Great Ouse (Suffolk)	Great Ouse	52°20′10″N, 000°31′50″E	0 (0–17)	30	A0
*River Lugg (Herefordshire)	Wye	52°10′01″N, 002°42′01″W	0 (0–17)	30	A0–A1
Tetbury Avon (Wiltshire)	Bristol Avon	51°35′30″N, 002°06′45″W	3 (0–17)	30	A0–A3
River Hamps (Staffordshire)	Trent	53°04′53″N, 001°54′27″W	20 (3–56)	10	A0–A3
River Wharfe 2 (North Yorkshire)	Yorkshire Ouse	54°03′56″N, 002°00′05″W	38 (9–76)	8	A0–A3
River Evenlode (Oxfordshire)	Thames	51°48′08″N, 001°21′53″W	28 (12–46)	29	A0–A3
River Thame (Aylesbury)	Thames	51°45′14″N, 001°01′14″W	80 (58–90)	30	A0–A3
River Wid (Norfolk)	Thames	52°33′42″N, 000°27′23″E	19 (6–38)	27	A0–A3
River Ash (Hertfordshire)	Thames	51°48′34″N, 000°00′22″E	7 (1–22)	30	A0–A3
River Lea (Hertfordshire)	Thames	51°47′60″N, 000°04′32″W	17 (6–35)	30	A0–A4
Bently Brook (Derbyshire)	Trent	53°01′54″N, 001°44′15″W	10 (2–27)	30	A0–A5

*Animals were processed in Charles University (Prague).

astaci presence. These data were used to determine the prevalence of *A. astaci* in the studied populations, and its 95% confidence intervals. Confidence intervals were calculated using the function 'epi. conf' included in the library 'epiR' (Stevenson *et al*. 2013) for the statistical package R version 3·2 (R Core Team, 2013).

Pathogen genotyping was only conducted for *A. astaci*-infected crayfish that were tested at CUNI (samples had been processed at Cefas before microsatellite genotyping became available for *A. astaci*). As most of these crayfish harboured relatively low infection intensities (A2–A3), pathogen genotyping was only possible for crayfish from one population, the Mochdre Brook (Wales). From this population, pathogen DNA from one crayfish (harbouring an A3 agent level infection) was analysed using nine *A. astaci*-specific microsatellite markers (Grandjean *et al*. 2014). Genotyping was attempted for another crayfish from this population but, presumably due to the relatively small amount of pathogen DNA present, this was un-successful. Prior to genotyping the sample was concentrated using a Concentrator Plus 5305 (Eppendorf) to increase pathogen DNA concentration. The results were compared with the *A. astaci* reference strains described in Grandjean *et al*. (2014).

We assessed native white-clawed crayfish populations at potential risk from the 13 signal crayfish populations where we detected *A. astaci* using recent (2009 onwards) native crayfish distribution records (Craybase database, James *et al*. 2014). In this regard, we are aware that it is not possible to declare those signal crayfish populations where the pathogen was not detected as uninfected. As such it should be noted that in the context of native crayfish risk assessment the purpose of this study is only to show where *A. astaci* definitely is present (or has been recently) and highlight surrounding native crayfish populations potentially at risk of disease. For these purposes, sites where *A. astaci* was detected were mapped and any native crayfish populations, not already exposed to signal crayfish, within a 7·5, 10, 12 or 15 km aerial radius were recorded. Locations harbouring native crayfish were searched for within the signal crayfish records contained in Craybase (7166 in total, James *et al*. 2014), and only those not already invaded were considered for risk assessment purposes. Buffer zones (i.e. 7·5, 10, 12 and 15 km) were selected on the basis that the average rate of signal crayfish population expansion along a river in the UK has been estimated as 1·5 km year^{-1} (Bubb *et al*. 2004; although it should be acknowledged that the rate of signal crayfish dispersal is faster in other European countries, e.g. Hudina *et al*. 2009; Weinländer and Füreder, 2009). Therefore, we presume that populations within 7·5 km of each other are predicted to come into contact within 5 years, providing that
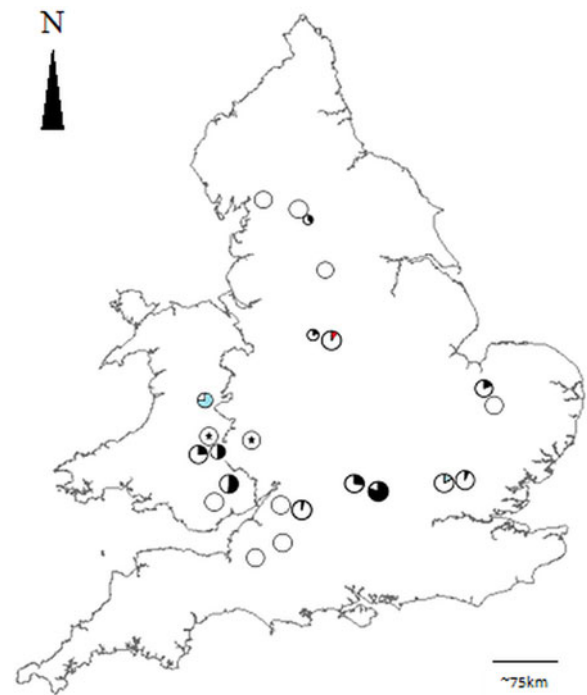

Fig. 1. Location of the invasive signal crayfish populations tested for *Aphanomyces astaci* in the current study using qPCR. For each population, the percentage of crayfish tested that were infected with *A. astaci* (i.e. the pathogen prevalence) is shown using a pie chart, with the shaded portion of each chart representing infected individuals, and the diameter of the circle the sample size ($n = 8$–30). Black shading indicates that the highest infection intensity (reported as semi-quantitative agent levels, see Vrålstad *et al*. 2009) detected was A3, blue A4 and red A5. White circles show populations where the pathogen was not detected at any level (A0). Circles containing black stars represent those populations where trace levels of the pathogen (A1) were amplified. As an infection intensity of A1 is considered below the limit of detection for the method used (Vrålstad *et al*. 2009), these populations are classed as uninfected; although the possibility of them harbouring *A. astaci* at a low prevalence remains.

they inhabit connected waterbodies. These analyses were performed using ArcGIS version 10·3 mapping software.

RESULTS

Aphanomyces astaci was detected in 56·5% (13 out of 23) signal crayfish populations from Wales and England (Table 1, Fig. 1). Among the infected populations, prevalence ranged from 3 to 80% at generally low infection intensities (agent levels A2–A3) with the exception of Mochdre Brook in Wales, and Bently Brook and River Lee in England (Table 1, Fig. 1). At two sites, trace amounts of pathogen DNA (below the limit of detection for the methods used i.e. agent levels A1) were detected in tested isolates from single crayfish specimens (Table 1, Fig. 1). The agent level A1

Table 2. Comparison of allele sizes of nine micro-satellite loci from the reference strains of the *Aphanomyces astaci* genotype group B (Grandjean *et al*. 2014) and an *A. astaci*-positive signal crayfish (*Pacifastacus leniusculus*) from a UK population (Mochdre Brook).

Locus	Reference sequence (VI 03555)	UK population
Aast 2	142	142
Aast 4	87	87
Aast 6	148	148
Aast 7	215	215
Aast 9	164/182	164
Aast 10	132	132
Aast 12	226/240	226/240
Aast 13	202	202
Aast 14	248	248

should not be considered as a reliable detection of *A. astaci* (Vrålstad *et al*. 2009) but such observations should raise concern about its potential presence in these populations. A multilocus microsatellite geno-type of *A. astaci* was only obtained from the Mochdre Brook signal crayfish population. This was identical to the reference axenic culture of the genotype group B strain at eight loci, but was homo-zygous rather than heterozygous at the Aast9 locus (Table 2).

As we were only able to test a fraction of the signal crayfish populations in the UK (see James *et al*. 2014 for detailed distribution information) for *A. astaci*, comprehensively assessing the risk this pathogen poses to native crayfish in the UK was beyond the scope of the current study. Nevertheless, we located ten native crayfish populations (confirmed extant at some time point between 2009 and 2014) within 15 km of an *A. astaci*-infected signal crayfish population (Table 3). Of these, the popula-tion in River Cilcenni, South Wales, was closest (within 7·5 km) to infected signal crayfish (Table 3). These infected crayfish from the Bachowey River were also within 15 km of an add-itional six extant native crayfish populations (Table 3). Due to the low spatial resolution of the river network data available it was, however, often not possible to determine if the waterbodies har-bouring these native and invasive crayfish popula-tions were connected.

DISCUSSION

Using molecular diagnostics, we provide the first comprehensive study of *A. astaci* prevalence in inva-sive signal crayfish populations from England and Wales. Whilst this affirms the perceived role of *A. astaci* causing native crayfish declines (Holdich, 2003), not all signal crayfish populations tested

appeared to be infected. In fact, *A. astaci* was only detected in just over half (57%) of the tested UK signal crayfish populations, and within these popula-tions the prevalence varied between 3 and 80%. While we cannot definitively declare those popula-tions where we did not detect *A. astaci* as uninfected, our data show that, among signal crayfish popula-tions, pathogen prevalence varies widely. Our findings contradict the traditional assumption that all American crayfish are carriers of *A. astaci* (see Cerenius *et al*. 2003), but are in agreement with other DNA-based studies focusing on distribution and prevalence of this pathogen. Recently, popula-tions of American crayfish, in which *A. astaci* had not been detected, were reported in other European countries (Kozubíková *et al*. 2009; Skov *et al*. 2011; Filipová *et al*. 2013; Schrimpf *et al*. 2013; Tilmans *et al*. 2014). The situation in the UK seems to almost mirror that reported from France, with 53% (24 out of 45) of signal crayfish populations being infected with *A. astaci* and the pathogen prevalence ranging from 8 to 80% (Filipová *et al*. 2013).

In the current study, microsatellite genotyping revealed the presence of an *A. astaci*-positive DNA isolate congruent with the reference genotype group B strain (Grandjean *et al*. 2014) at eight of the nine loci tested. Such intra-genotype group vari-ation has been reported previously (Grandjean *et al*. 2014; Mrugała *et al*. 2016), therefore, it is likely that the DNA isolate from the UK belongs to genotype group B. This is perhaps unsurprising given that, within Europe, group B strains of *A. astaci* were first isolated from invasive signal crayfish in Sweden (Huang *et al*. 1994), which is considered as the country of origin for most signal crayfish intro-duced into the UK during the 1970s and 1980s (Holdich *et al*. 1999). Isolation of this highly viru-lent strain of *A. astaci* (see Makkonen *et al*. 2012) may explain the mass mortalities of native white-clawed crayfish in the UK following the introduc-tion of signal crayfish (e.g. James *et al*. 2014). Although chronic *A. astaci* infections have been observed in other white-clawed crayfish and other native European crayfish (e.g. Jussila *et al*. 2011; Pârvulescu *et al*. 2012; Kokko *et al*. 2012; Schrimpf *et al*. 2012; Kušar *et al*. 2013; Maguire *et al*. 2016), these may be caused by the less virulent strains from the 'old' genotype group, A (Makkonen *et al*. 2012). The ability to identify *A. astaci* strains and their virulence would help inform risk assess-ment for native crayfish populations in the future, although better characterization of all *A. astaci* gen-otypes is required before this can be exploited fully.

Given that the long-term conservation of native crayfish in the UK is generally considered to be dependent upon the translocation of animals into 'Ark Sites' (Peay, 2009), and that resources for implementing such measures are limited; targeting

Table 3. *Location and year of the most recent record of native white-clawed crayfish (*Austropotamobius pallipes*) populations (data from CrayBase: James et al. 2014) in close vicinity to an* Aphanomyces astaci-*infected invasive signal crayfish (*Pacifastacus leniusculus*) population.*

White-clawed crayfish			Signal crayfish	
Population	Location (country)	Most recent record	Population(s)	Proximity (km)
Cilcenni	Wales	2009	Bachowey River 1 and 2	7·5
Scithwen	Wales	2014	Bachowey River 1 and 2	10·5
Clettwr	Wales	2014	Bachowey River 1 and 2	10·5
Rhiwiau Brook	Wales	2009	Bachowey River 1 and 2	12
Llynfi Dulas	Wales	2014	Bachowey River 1 and 2	15
River Ennig	Wales	2011	Bachowey River 1 and 2	15
Cwm Sheppard Brook	Wales	2010	Bachowey River 1 and 2	15
Nant Onnau Fach	Wales	2010	Gavenny River	10·5
Lurscombe	England	2009	Tetbury Avon	15
Winterburn beck	England	2010	River Wharfe 2	10·5

removal of native crayfish populations at the greatest risk of extirpation is critical. Native European crayfish can co-exist with American crayfish for extended periods of up to 30 years in the absence of *A. astaci* (see Westman *et al.* 2002; Schulz *et al.* 2006; Dunn *et al.* 2009; Skov *et al.* 2011; Schrimpf *et al.* 2013), but are often rapidly extirpated if this pathogen is present (e.g. Holdich and Reeve, 1991; Vennerström *et al.* 1998; Bohman *et al.* 2006; Kozubíková *et al.* 2008). Therefore, native white-clawed crayfish populations in close vicinity to *A. astaci*-infected signal crayfish are predicted to be at greater risk of local extinction than those neighbouring uninfected signal crayfish. Considering that only a portion of the signal crayfish populations existing in the UK were screened in the current study, and of these only around half were infected with *A. astaci*, increased testing for this pathogen is needed to comprehensively assess native crayfish populations at greatest risk of disease transmission. Nevertheless, for the 13 signal crayfish populations where we detected *A. astaci* we identified one white-clawed crayfish population recorded since 2009, located within 7·5 km. This native crayfish population inhabits the Cilcenni within the Wye catchment, South Wales, and was most recently detected in 2009. Given its proximity to infected signal crayfish, we recommend that translocating a subset of individuals from this population into an 'Ark Site' is considered as a priority, although we acknowledge that increased screening of signal crayfish for *A. astaci* may reveal other native crayfish populations at greater risk of extirpation. Determining the exact order of translocation priority for the ten native crayfish populations within 15 km of an *A. astaci*-infected signal crayfish population is beyond the scope of the current study. For extant populations, factors that should be considered when assessing translocation priority include: proximity to infected crayfish, connectivity of water bodies housing native and infected invasive crayfish (particularly considering the ability of the pathogen not only to be transmitted via spores in the water, but potentially also with fish; Oidtmann *et al.* 2002; Svoboda *et al.* 2017), prevalence of *A. astaci* in the nearest infected crayfish population, density of crayfish present in the native crayfish and neighbouring infected signal crayfish population, and whether any barriers in the environment exist that may prevent animals from either population dispersing. Additionally, as native crayfish populations can be rapidly extirpated by crayfish plague, surveying to confirm the persistence of populations under consideration for translocation should always be a prerequisite.

Within the UK, this is currently the only comprehensive study that uses molecular methods to confirm the presence, and determine the prevalence of, *A. astaci* in invasive signal crayfish populations. The current study also provides the first record of *A. astaci* genotype group B (known to contain virulent strains) from signal crayfish in the UK. The presence and prevalence of *A. astaci*, however, varied between populations. Although we cannot definitively declare those signal crayfish populations where we did not detect *A. astaci* as uninfected, our findings show that pathogen prevalence can vary from very low to very high. Therefore, from a conservation perspective, the risk posed to native crayfish from different invasive crayfish populations may be asymmetric. As such, considering *A. astaci* prevalence data will improve risk assessments for native crayfish populations. Based on our findings we recommend increased *A. astaci* screening, using appropriate pathogen-specific molecular methods, of non-native crayfish populations in the UK, to fully assess the risks to native crayfish and target populations for translocation. As part of this, those populations where we detected trace levels (i.e. below the limit of detection) of *A. astaci* should be re-tested to ascertain whether they are harbouring a low prevalence infection.

REFERENCES

Alderman, D. J. (1996). Geographical spread of bacterial and fungal dis-
eases of crustaceans. *Scientific and Technical Review of the Office
International des Epizooties* **15**, 603–632.
Alderman, D. J., Polglase, J. L., Frayling, M. and Hogger, J. (1984).
Crayfish plague in Britain. *Journal of Fish Diseases* **7**, 401–405.
Alderman, D. J., Holdich, D. and Reeve, I. (1990). Signal crayfish as
vectors in crayfish plague in Britain. *Aquaculture* **86**, 3–6.
**Becking, T., Mrugała, A., Delaunay, C., Svoboda, J., Raimond, M.,
Viljamaa-Dirks, S., Petrusek, A., Grandjean, F. and Braquart-
Varnier, C.** (2015). Effect of experimental exposure to differently virulent
Aphanomyces astaci strains on the immune response of the noble crayfish
Astacus astacus. *Journal of Invertebrate Pathology* **132**, 115–124.
Bohman, P., Nordwall, F. and Edsman, L. (2006). The effect of the
large-scale introduction of signal crayfish on the spread of crayfish plague in
Sweden. *Bulletin Français de la Pêche et de la Pisciculture* **380–381**, 1291–1302.
Bubb, D. H., Thom, T. J. and Lucas, M. C. (2004). Movement and dis-
persal of the invasive signal crayfish *Pacifastacus leniusculus* in upland
rivers. *Freshwater Biology* **49**, 357–368.
**Cerenius, L., Bangyeekhun, E., Keyser, P., Söderhäll, I. and
Söderhäll, K.** (2003). Host prophenoloxidase expression in freshwater
crayfish is linked to increased resistance to the crayfish plague fungus,
Aphanomyces astaci. Cellular Microbiology **5**, 353–357.
DAISIE, 2009. *Handbook of Alien Species in Europe*. Springer, Dordrecht.
Diéguez-Uribeondo, J., Temiño, C. and Múzquiz, J. L. (1997). The
crayfish plague fungus (*Aphanomyces astaci*) in Spain. *Bulletin Français
de la Pêche et de la Pisciculture* **347**, 753–763.
Dunn, J. C., McClymont, H. E., Christmas, M. and Dunn, A. M.
(2009). Competition and parasitism in the native white clawed crayfish
Austropotamobius pallipes and the invasive Signal Crayfish *Pacifastacus
leniusculus* in the UK. *Biological Invasions* **11**, 315–324.
**Filipová, L., Petrusek, A., Matasová, K., Delaunay, C. and
Grandjean, F.** (2013). Prevalence of the crayfish plague pathogen
Aphanomyces astaci in population of the signal crayfish *Pacifastacus lenius-
culus* in France: evaluating the threat to native crayfish. *PLoS ONE* **8**,
e70157.
**Grandjean, F., Vrålstad, T., Diéguez-Uribeondo, J., Jelić, M.,
Mangombi, J., Delaunay, C., Filipová, L., Rezinciuc, S.,
Kozubíková-Balcarová, E., Guyonnet, D., Viljamaa-Dirks, S. and
Petrusek, A.** *et al*. (2014). Microsatellite markers for direct genotyping
of the crayfish plague pathogen *Aphanomyces astaci* (Oomycetes) from
infected host tissues. *Veterinary Microbiology* **170**, 317–324.
Holdich, D. M. (2003). Crayfish in Europe – an overview of the taxonomy,
legislation, distribution, and crayfish plague outbreaks. In *Management
and Conservation of Crayfish*, Conference Proceedings, Nottingham, UK,
November 7th 2002 (ed. Holdich, D. M. and Sibley, P. J.), pp. 15–34.
Environment Agency, Rio House, Bristol, UK.
Holdich, D. M. and Reeve, I. D. (1991). Distribution of freshwater
crayfish in the British Isles, with particular reference to crayfish plague,
alien introduction and water quality. *Aquatic Conservation Marine and
Freshwater Ecosystems* **1**, 139–158.
Holdich, D. M. and Sibley, P. J. (2009). ICS and NICS in Britain in the
2000s. In *Crayfish Conservation in the British Isles* (ed. Brickland, J.,
Holdich, D. M. and Imhoff, E. M.), March 25, 2009, Leeds, UK, pp. 13–33.
Holdich, D. M., Rogers, W. D. and Reynolds, J. D. (1999). Native and
alien crayfish in the British Isles. In *Crayfish in Europe as Alien Species:*

How to Make the Best of a Bad Situation? (ed. Gherardi, F. and
Holdich, D. M.), Crustacean Issues 11, pp. 221–235. A. A. Balkema,
Rotterdam.
Holdich, D. M., Palmer, M. and Sibley, P. J. (2009). The indigenous
status of *Austropotamobius pallipes* (Lereboullet) in Britain. In *Crayfish
Conservation in the British Isles* (ed. Brickland, J., Holdich, D. M. and
Imhoff, E. M.), March 25, 2009, Leeds, UK, pp. 1–12.
Holdich, D. M., James, J., Jackson, C. and Peay, S. (2014). The North
American signal crayfish, with particular reference to its success as an inva-
sive species in Great Britain. *Ethology Ecology and Evolution* **26**, 232–262.
Huang, T., Cerenius, L. and Söderhall, K. (1994). Analysis of genetic
diversity in the crayfish plague fungus, *Aphanomyces astaci*, by random
amplification of polymorphic DNA. *Aquaculture* **126**, 1–9.
Hudina, S., Faller, M., Lucic, A., Klobucar, G. and Maguire, I.
(2009). Distribution and dispersal of two invasive crayfish species in the
Drava River basin, Croatia. *Knowledge and Management of Aquatic
Ecosystems* **394–395**, 9.
IUCN (2015). The IUCN red list of threatened species [online]. www.
iucnredlist.org [accessed on 28.10.15].
James, J., Slater, F. and Cable, J. (2014). A.L.I.E.N. databases: address-
ing the lack in establishment of non-natives databases. *Crustaceana* **87**,
1192–1199.
**James, J., Thomas, J. R., Ellis, A., Young, K. A., England, J. and
Cable, J.** (2016). Over-invasion in a freshwater ecosystem: newly intro-
duced virile crayfish (*Orconectes virilis*) outcompete established invasive
signal crayfish (*Pacifastacus leniusculus*). *Marine and Freshwater
Behaviour and Physiology* **49**, 9–18.
Jussila, J., Makkonen, J., Vainikka, A., Kortet, R. and Kokko, H.
(2011). Latent crayfish plague (*Aphanomyces astaci*) infection in a robust
wild noble crayfish (*Astacus astacus*) population. *Aquaculture* **321**, 17–20.
**Kokko, H., Koistinen, L., Harlioğlu, M. M., Makkonen, J., Aydin, H.
and Jussila, J.** (2012). Recovering Turkish narrow clawed crayfish
(*Astacus leptodactylus*) populations carry *Aphanomyces astaci*. *Knowledge
and Management of Aquatic Ecosystems* **404**, 1–7.
**Kozubíková, E., Petrusek, A., Ďuriš, Z., Martín, M. P., Diéguez-
Uribeondo, J. and Oidtmann, B.** (2008). The old menace is back:
recent crayfish plague outbreaks in the Czech Republic. *Aquaculture* **274**,
208–217.
**Kozubíková, E., Filipová, L., Kozák, P., Ďuriš, Z., Diéguez-
Uribeondo, J., Oidtmann, B. and Petrusek, A.** (2009). Prevalence of
the crayfish plague pathogen Aphanomyces astaci in invasive American
crayfishes in the Czech Republic. *Conservation Biology* **22**, 1204–1213.
Kozubíková, E., Vrålstad, T., Filipová, L. and Petrusek, A. (2011). Re-
examination of the prevalence of *Aphanomyces astaci* in North American
crayfish populations in Central Europe by TaqMan MGB real-time
PCR. *Diseases of Aquatic Organisms* **97**, 113–125.
Kušar, D., Vrezec, A., Ocepek, M. and Jenčič, V. (2013). *Aphanomyces
astaci* in wild crayfish populations in Slovenia: first report of persistent
infection in a stone crayfish (*Austropotamobius torrentium*) population.
Diseases of Aquatic Organisms **103**, 157–169.
Lilley, J. H., Cerenius, L. and Söderhäll, K. (1997). RAPD evidence
for the origin of crayfish plague outbreaks in Britain. *Aquaculture* **157**,
181–185.
Lowe, S., Browne, M., Boudjelas, S. and De Poorter, M. (2004). 100 of
the World's Worst Invasive Alien Species. A selection from the Global
Invasive Species Database. The Invasive Species Specialist Group
(ISSG), a specialist group of the Species Survival Commission (SSC) of
the IUCN, Gland, Switzerland.
**Maguire, I., Jelić, M., Klobučar, G., Delpy, M., Delaunay, C. and
Grandjean, F.** (2016). Prevalence of the pathogen *Aphanomyces astaci* in
freshwater crayfish populations in Croatia. *Diseases of Aquatic Organisms*
118, 45–53.
Makkonen, J., Jussila, J., Kortet, R., Vainikka, A. and Kokko, H.
(2012). Differing virulence of *Aphanomyces astaci* isolates and elevated
resistance of noble crayfish *Astacus astacus* against crayfish plague.
Diseases of Aquatic Organisms **102**, 129–136.
Mrugała, A., Kawai, T., Kozubíková-Balcarová, E. and Petrusek, A.
(2016). *Aphanomyces astaci* presence in Japan: a threat to the endemic and
endangered crayfish species *Cambaroides japonicus*? *Aquatic Conservation:
Marine and Freshwater Ecosystems*, doi: 10.1002/aqc.2674.
Oidtmann, B. (2012). Crayfish plague (*Aphanomyces astaci*). In *Manual of
Diagnostic Tests for Aquatic Animals 2012*. Chapter 2·2·1, pp. 101–118.
World Organisation of Animal Health, Office international des epizooties,
Chapter 2·2·1, pp. 101–118. Paris.
Oidtmann, B., Heitz, E., Rogers, D. and Hoffman, R. W. (2002).
Transmission of crayfish plague. *Diseases of Aquatic Organisms* **52**, 159–167.
**Oidtmann, B., Geiger, S., Steinbauer, P., Culas, A. and
Hoffmann, R. W.** (2006). Detection of *Aphanomyces astaci* in North

American crayfish by polymerase chain reaction. *Diseases of Aquatic Organisms* **72**, 53–64.

Pârvulescu, L., Schrimpf, A., Kozubíková, E., Cabanillas Resino, S., Vrålstad, T., Petrusek, A. and Schulz, R. (2012). Invasive crayfish and crayfish plague on the move: first detection of the plague agent *Aphanomyces astaci* in the Romanian Danube. *Diseases of Aquatic Organisms* **98**, 85–94.

Peay, S. (2009). Selection criteria for "ark sites" for white-clawed crayfish. In *Crayfish Conservation in the British Isles* (ed. Brickland, J., Holdich, D. M. and Imhoff, E. M.), March 25, 2009, Leeds, UK, pp. 63–70.

R Core Team (2013). *R: A Language and Environment for Statistical Computing.* R Foundation for Statistical Computing, Vienna, Austria.

Royo, F., Andersson, G., Bangyeekhun, E., Múzquiz, J. L., Söderhäll, K. and Cerenius, L. (2004). Physiological and genetic characterisation of some new *Aphanomyces* strains isolated from freshwater crayfish. *Veterinary Microbiology* **104**, 103–112.

Schrimpf, A., Pârvulescu, L., Copilaş Ciocianu, D., Petrusek, A. and Schulz, R. (2012). Crayfish plague in the Danube Delta – a potential threat to biodiversity in southeastern Europe. *Aquatic Invasions* **7**, 503–510.

Schrimpf, A., Maiwald, T., Vrålstad, T., Schulz, H. K., Śmietana, P. and Schulz, R. (2013). Absence of the crayfish plague pathogen (*Aphanomyces astaci*) facilitates coexistence of European and American crayfish in central Europe. *Freshwater Biology* **58**, 1116–1125.

Schulz, H. K., Smietána, P., Maiwald, T., Oidtmann, B. and Schulz, R. (2006). Case studies on the co-occurrence of *Astacus astacus* (L.) and *Orconectes limosus* (Raf.): snapshots of a slow displacement. *Freshwater Crayfish* **15**, 212–219.

Skov, C., Aarestup, K., Sivebaek, F., Pedersen, S., Vrålstad, T. and Berg, S. (2011). Non-indigenous signal crayfish *Pacifastacus leniusculus* are now common in Danish streams: preliminary status for national distribution and protective actions. *Biological Invasions* **13**, 1269–1274.

Söderbäck, B. (1994). Interactions among juveniles of two freshwater crayfish species and a predatory fish. *Oecologia* **100**, 229–235.

Stevenson, M., Nunes, T., Sanchez, J., Thornton, R., Reiczigel, J., Robison-Cox, J., Sebastiani, P., Solymos, P., Yoshida, K., Jones, G., Pirikahu, S., Firestone, S. and Kyle, R. (2013) epiR: An R package for the analysis of epidemiological data. R package version 0.9. Online. Available at: https://cran.r-project.org/web/packages/epiR/index.html.

Strand, D. A., Holst-Jensen, A., Viljugrein, H., Edvardsen, B., Klaveness, D., Jussila, J. and Vrålstad, T. (2011). Detection and quantification of the crayfish plague agent in natural waters: direct monitoring approach for aquatic environments. *Diseases of Aquatic Organisms* **95**, 9–17.

Svoboda, J., Strand, D. A., Vrålstad, T., Grandjean, F., Edsman, L., Kozák, P., Kouba, A., Fristad, R. F., Koca, S. B. and Petrusek, A. (2014). The crayfish plague pathogen can infect freshwater-inhabiting crabs. *Freshwater Biology* **59**, 918–929.

Svoboda, J., Mrugała, A., Kozubíková-Balcarová, E. and Petrusek, A. (2017). Hosts and transmission of the crayfish plague pathogen *Aphanomyces astaci*: a review. *Journal of Fish Diseases* **40**, 127–140.

Tilmans, M., Mrugala, A., Svoboda, J., Engelsma, M. Y., Petie, M., Soes, D. M., Nutbeam-Tuffs, S., Oidtmann, B., Rossink, I. and Petrusek, A. (2014). Survey of the crayfish plague pathogen presence in the Netherlands reveals a new *Aphanomyces astaci* carrier. *Journal of Invertebrate Pathology* **120**, 74–79.

Tuffs, S. and Oidtmann, B. (2011). A comparative study of molecular diagnostic methods designed to detect the crayfish plague pathogen, *Aphanomyces astaci*. *Veterinary Microbiology* **153**, 343–353.

Unestam, T. and Weiss, D. W. (1970). The host-parasite relationship between freshwater crayfish and the crayfish disease fungus *Aphanomyces astaci*: responses to infection by a susceptible and a resistant species. *Journal of General Microbiology* **60**, 77–90.

Vennerström, P., Söderhäll, K. and Cerenius, L. (1998). The origin of two crayfish plague (*Aphanomyces astaci*) epizootics in Finland on noble crayfish, *Astacus astacus*. *Annales Zoologici Fennici* **35**, 43–46.

Vrålstad, T., Knutsen, A. K., Tengs, T. and Holst-Jensen, A. (2009). A quantitative TaqMan® MGB real-time polymerase chain reaction based assay for detection of the causative agent of crayfish plague *Aphanomyces astaci*. *Veterinary Microbiology* **137**, 146–155.

Weinländer, M. and Füreder, L. (2009). The continuing spread of *Pacifastacus leniusculus* in Carinthia (Austria): potential reasons, invasiveness and ecological effects. In *Proceedings of the Regional European Crayfish Workshop: Future of Native Crayfish in Europe*, Písek, p. 53.

Westman, K., Savolainen, R. and Julkunen, M. (2002). Replacement of the native crayfish *Astacus astacus* by the introduced species *Pacifastacus leniusculus* in a small, enclosed Finnish lake: a 30-year study. *Ecography* **25**, 53–73.