

1 **Competition and parasitism in the native White Clawed Crayfish *Austropotamobius***  
2 ***pallipes* and the invasive Signal Crayfish *Pacifastacus leniusculus* in the UK**

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## 1 **Abstract**

2 Many crayfish species have been introduced to novel habitats worldwide, often threatening  
3 extinction of native species. Here we investigate competitive interactions and parasite infections  
4 in the native *Austropotamobius pallipes* and the invasive *Pacifastacus leniusculus* from single  
5 and mixed species populations in the UK. We found *A. pallipes* individuals to be significantly  
6 smaller in mixed compared to single species populations; conversely *P. leniusculus* individuals  
7 were larger in mixed than in single species populations. Our data provide no support for  
8 reproductive interference as a mechanism of competitive displacement and instead suggest  
9 competitive exclusion of *A. pallipes* from refuges by *P. leniusculus* leading to differential  
10 predation. We screened fifty-two *P. leniusculus* and twelve *A. pallipes* for microsporidian  
11 infection using PCR. We present the first molecular confirmation of *Thelohania contejeani* in the  
12 native *A. pallipes*; in addition, we provide the first evidence for *T. contejeani* in the invasive *P.*  
13 *leniusculus*. Three novel parasite sequences were also isolated from *P. leniusculus* with an  
14 overall prevalence of microsporidian infection of 38 % within this species; we discuss the identity  
15 of and the similarity between these three novel sequences. We also screened a subset of fifteen  
16 *P. leniusculus* and three *A. pallipes* for *Aphanomyces astaci*, the causative agent of crayfish  
17 plague and for the protistan crayfish parasite *Psorospermium haeckeli*. We found no evidence for  
18 infection by either agent in any of the crayfish screened. The high prevalence of microsporidian  
19 parasites and occurrence of shared *T. contejeani* infection lead us to propose that future studies  
20 should consider the impact of these parasites on native and invasive host fitness and their  
21 potential effects upon the dynamics of native-invader systems.

22

23 *Keywords:* *Austropotamobius pallipes*; competitive exclusion; differential predation;  
24 invasion; microsporidia; *Pacifastacus leniusculus*; parasites

## 1 **Introduction**

2 Parasites can play important roles in biological invasions: invading species may bring with  
3 them parasites or diseases which may detrimentally affect native species (Ohtaka et al. 2005;  
4 Rushton et al. 2000), or may themselves acquire parasites from their new environment (Bauer  
5 et al. 2000; Krakau et al. 2006). Alternatively invading species may lose their parasites,  
6 potentially giving them an advantage over native species (Torchin et al. 2003; Torchin et al.  
7 2001). Parasites have been shown to be important mediators of interspecific interactions  
8 (Hatcher et al. 2006): they may confer a competitive advantage to the host species (Yan et al.  
9 1998), alter dominance relationships and predation hierarchies (MacNeil et al. 2003a), and  
10 may promote species exclusion or coexistence (MacNeil et al. 2003b; Prenter et al. 2004).  
11 By mediating native-invader interactions, parasites can play a key role in the outcome of a  
12 biological invasion (MacNeil et al. 2003a; MacNeil et al. 2003b; Prenter et al. 2004). For  
13 example, in Northern Ireland, the acanthocephalan parasite *Echinorynchus truttae* reduces the  
14 predatory impact of the invasive amphipod *Gammarus pulex* on the native *G. duebeni celticus*  
15 (MacNeil et al. 2003b).

16

17 The North American Signal Crayfish, *Pacifastacus leniusculus* (Dana), has become  
18 established throughout Britain as a result of escapes from farms (Holdich et al. 2004). The  
19 species is highly invasive and commonly leads to the displacement of Britain's only native  
20 crayfish *Austropotamobius pallipes* (Lereboullet) (Bubb et al. 2006; Kemp et al. 2003) As a  
21 result, populations of *A. pallipes* are now concentrated in central and northern England  
22 (Souty-Grosset et al. 2006) where they are of global importance, representing the densest  
23 concentrations of the species within Europe (Holdich 2003). The mechanism by which *A.*  
24 *pallipes* is displaced varies between populations. In some cases, the native species is  
25 displaced through competitive interactions, (Bubb et al. 2006); however the exact mechanism

1 by which this occurs is unclear. In many water courses in the south of England, extinction of  
2 *A. pallipes* has resulted from crayfish plague (Kemp et al. 2003). The invasive crayfish, *P.*  
3 *leniusculus*, commonly acts as a reservoir for *Aphanomyces astaci* (the causative agent of  
4 crayfish plague), which is fatal to the native species (Holdich 2003).

5  
6 Also of interest are two further parasites. The microsporidian parasite *Thelohania*  
7 *contejeani* (Henneguy), infects *Austropotamobius pallipes* causing porcelain disease and is  
8 the most widely recorded parasitic infection of this species (Alderman and Polglase 1988).  
9 Whilst the pathology of *T. contejeani* is not as severe as that of crayfish plague it can be a  
10 serious threat within crayfish aquaculture (Edgerton et al. 2002) and may cause changes in the  
11 ecology of its host through changes in diet (Chartier and Chaisemartin 1983); however the  
12 consequences of infection by many pathogen groups in European freshwater crayfish are  
13 largely poorly understood (Edgerton et al. 2004). Microsporidia are widespread in crustacean  
14 hosts (Edgerton et al. 2002; Terry et al. 2004) and can cause significant mortality (Alderman  
15 and Polglase 1988). A second parasite, the protist *Psorospermium haeckeli* (Haeckel) infects  
16 crayfish and has recently been isolated from *A. pallipes* (Rogers et al. 2003) and *Pacifastacus*  
17 *leniusculus* (Dieguez-Uribeondo et al. 1993). The influence of these parasites upon  
18 native/invasive interactions in crayfish is unknown.

19  
20 In the UK, Yorkshire is a stronghold for *A. pallipes*: although *P. leniusculus* is present  
21 within the county in substantial numbers, it has not yet displaced many native populations and  
22 mixed populations do exist (Peay and Rogers 1999). Here we investigate possible  
23 competitive interactions by comparing the sizes of native and invading individuals in single  
24 species versus mixed species populations. Secondly we use PCR screening and sequence  
25 analysis to compare parasite diversity in the native and invasive crayfish, focusing on

1 microsporidian parasites.

2

### 3 **Materials and Methods**

#### 4 *Animal collection and measurement*

5 A total of seven *A. pallipes* populations, four *P. leniusculus* populations and three mixed  
6 species sites were surveyed between June and August 2005 (Table 1). Sites in the Wharfe  
7 catchment were similar to each other and were typified by boulders and smaller stones  
8 overlying gravel. Sites in the Dearne catchment (including Cawthorne Dike) were also  
9 similar to each other and were typified by boulders and small stones overlying deep silt. Sites  
10 were surveyed for crayfish using a standardised manual survey of selected refuges within a  
11 site (Peay 2003). Selection of similar sized refuges at each site ensures no size bias during  
12 collection (Peay 2003). For each crayfish individual captured we recorded the species, size  
13 (carapace length) and sex. In addition any signs of disease, breeding or moult were recorded:  
14 microsporidian infections when at high burden typically cause opacity of muscle tissues as a  
15 result of spore replication and muscle pathology (Alderman and Polglase 1988);  
16 *Aphanomyces astaci* can be identified by the appearance of brown melanisations on the  
17 exoskeleton of the infected animal (Alderman and Polglase 1988). Following assessment,  
18 crayfish were set aside to prevent duplication of records, until the population assessment of  
19 the site had been completed. All *Austropotamobius pallipes* were then released; *P. leniusculus*  
20 were stored at -20 °C.

21

#### 22 *Statistical analysis*

23 Statistical analyses were conducted using R version 4.2.1. ([www.R-project.org](http://www.R-project.org)). Linear mixed  
24 effects models (LMM) were fitted to the size distribution data for each species separately  
25 using Maximum Likelihood fits. Size was used as the dependent variable with population

1 (single vs. mixed species) and sex as fixed factors; site identity was included in the model as a  
2 random factor to control for any inter-site differences in size composition.

3

4 **Table 1.** Field sites sampled during the study. All populations were surveyed for size  
5 distribution; <sup>b</sup> denotes populations from which *P. leniusculus* or dead *A. pallipes* were  
6 obtained for parasite screening

| Site Name                  | Watercourse    | Site Grid Reference | Population composition |
|----------------------------|----------------|---------------------|------------------------|
| Cawthorne South            | Cawthorne Dike | SE299087            | <i>P. leniusculus</i>  |
| Road Bridge <sup>b</sup>   | Cawthorne Dike | SE295088            | Mixed                  |
| Haigh <sup>b</sup>         | River Dearne   | SE300116            | <i>P. leniusculus</i>  |
| Burnsall <sup>b</sup>      | River Wharfe   | SE025622            | <i>P. leniusculus</i>  |
| Lobwood                    | River Wharfe   | SE077518            | Mixed                  |
| Addingham                  | River Wharfe   | SE082500            | Mixed                  |
| Footbridge                 | River Wharfe   | SE122484            | <i>A. pallipes</i>     |
| Riverside Gardens          | River Wharfe   | SE113480            | <i>A. pallipes</i>     |
| Denton Stones <sup>b</sup> | River Wharfe   | SE132482            | <i>A. pallipes</i>     |
| Fenay <sup>b</sup>         | Fenay Beck     | SE179160            | <i>P. leniusculus</i>  |
| Adel Dam                   | Adel Beck      | SE275407            | <i>A. pallipes</i>     |
| Meanwood <sup>b</sup>      | Meanwood Beck  | SE281385            | <i>A. pallipes</i>     |
| Grange Park                | Wyke Beck      | SE341363            | <i>A. pallipes</i>     |
| Gipton                     | Wyke Beck      | SE342353            | <i>A. pallipes</i>     |

7

8 In order to determine whether parasite prevalence differed between sexes or sizes of *P.*  
9 *leniusculus*, a Generalized Linear Model (GLM) with binomial error distributions was fitted

1 to the data. Microsporidian presence or absence was used as the dependent variable with size  
2 and sex as fixed factors.

3

4 Non-significant fixed factors were removed from the maximal models in a stepwise  
5 fashion until only factors significant at the 5 % level remained.

6

### 7 *Screening for microsporidian parasites*

8 Fifty-two *P. leniusculus* from the field collection (Table 1) were screened for microsporidia  
9 (Table 2). As *A. pallipes* is classified as vulnerable (IUCN 2004) and protected under  
10 Schedule 5 of the Wildlife and Countryside Act (1981), we did not screen live animals  
11 collected from the field; however twelve dead *A. pallipes* obtained from sites detailed in  
12 Table 1 were screened for microsporidia. Sampling was carried out towards the end of the  
13 breeding season when most young have hatched and dispersed (Holdich 2003). However, one  
14 female *P. leniusculus* still had two eggs attached; as many microsporidia are vertically  
15 transmitted (Dunn and Smith 2001) we also screened these to test for the presence of  
16 vertically transmitted parasites.

17

18 Crayfish tissue (approximately 0.25g) was dissected from tail muscle between the 3<sup>rd</sup>  
19 and 4<sup>th</sup> pleonites, being careful to avoid sampling gut tissue. Eggs from the single gravid  
20 female sampled were collected and homogenised. DNA was extracted using a chloroform  
21 extraction described by Doyle and Doyle (1987) with modifications described in McClymont  
22 et al. (2005).

23

24

25

1 Table 2. Results of PCR screen for microsporidian infection in *P. leniusculus*. Summary of  
 2 PCR results for *P. leniusculus*; for site grid references refer to Table 1.

| Site                  | Number of individuals screened | Number of infected individuals | Observed Prevalence |
|-----------------------|--------------------------------|--------------------------------|---------------------|
| Cawthorne Road Bridge | 16                             | 7                              | 0.44                |
| Burnsall              | 13                             | 5                              | 0.38                |
| Haigh                 | 4                              | 3                              | 0.75                |
| Fenay                 | 19                             | 5                              | 0.26                |
| Total                 | 52                             | 20                             | 0.38                |

3  
 4 PCR of the host cytochrome C oxidase 1 (CO1) gene was used to confirm the quality of  
 5 the DNA extraction before PCR for microsporidian SSU rDNA was carried out. Primers used  
 6 for detection of host DNA were LCO1490 and HCO2198, which amplify a fragment of the  
 7 CO1 gene (Folmer et al. 1994). The CO1 PCR protocol was as described in McClymont et al.  
 8 (2005). Positive controls containing DNA extracted from microsporidium infected crayfish  
 9 muscle stored in ethanol and negative controls containing deionised water in place of DNA  
 10 were included for each reaction; the total reaction volume was 25 µl.

11  
 12 Three primer sets were used for detection of microsporidian SSU rDNA. V1f  
 13 (Vossbrinck and Woese 1986) and 1492r (Weiss et al. 1994) are specific for *T. contejeani*  
 14 (Lom et al. 2001), whilst both V1f and 530r (Baker et al. 1995), and 18sf (Baker et al. 1995)  
 15 and 964r (McClymont et al. 2005) are general microsporidian primers. The PCR reaction  
 16 mixture and protocols are as described by McClymont et al. (2005); annealing temperatures  
 17 and PCR product lengths are shown in Table 3.



1 Positive controls containing DNA extracted from microsporidium infected crayfish muscle  
 2 stored in ethanol and negative controls containing deionised water in place of DNA were  
 3 included for each reaction; the total reaction volume was 25 µl for initial parasite detection.  
 4 PCR protocols were all carried out on a Hybaid Omn-E Thermal Cycler (Hybaid Ltd,  
 5 Waltham, Massachusetts, USA).

6  
 7 Table 3. PCR annealing temperatures and approximate expected product length for primers  
 8 used in parasite detection and for sequencing

| Primers     | Annealing temperature/°C | Product length/bp |
|-------------|--------------------------|-------------------|
| V1f-1492r   | 50                       | 1500              |
| 18sf-964r   | 50                       | 900               |
| V1f-530r    | 60                       | 600               |
| 350f-964r   | 60                       | 800               |
| 18sf-350r   | 50                       | 600               |
| 18sf-530r   | 50                       | 700               |
| HA3bf-HG4r  | 60                       | 1500              |
| HG4f-HG4r   | 50                       | 1200              |
| HG4f-1492r  | 50                       | 600               |
| Thelof-580r | 50                       | 1400              |
| BACF-1492r  | 50                       | 800               |

9  
 10 *Sequencing and phylogenetic analysis of microsporidia*  
 11 Different primer sets gave positive bands in different individuals suggesting the presence of  
 12 more than one microsporidian parasite within *P. leniusculus*. Therefore additional primers  
 13 were used in order to obtain longer sequences: these were 580r (Vossbrinck et al. 1993),

1 Ha3Bf (Gatehouse and Malone 1998), HG4r (Gatehouse and Malone 1998), 350f (Weiss and  
2 Vossbrinck 1998), HG4f (Gatehouse and Malone 1998), 1342r (McClymont et al. 2005) and  
3 350r (5'-CCAAGGACGGC-AGCAGGCGCGAAA-3'), together with new primers Thelof  
4 (5'-TCGTAGTTCCG-CGCAGTAAACTA-3') and BACF (5'-  
5 ATATAGGAACAGATGATGGC-3'). Annealing temperatures for all primer combinations  
6 are given in Table 3. Where PCR products were to be sequenced the amounts of reagents in  
7 the reaction mixture were doubled to give a total reaction volume of 50 µl.

8

9 50 µl of each PCR product were electrophoresed through a 2 % agarose TAE gel in  
10 standard TAE buffer, stained with ethidium bromide and visualised by UV light to ensure  
11 successful amplification of the PCR product. PCR products were excised from the gel and  
12 purified using a QIAQuick Gel Purification Kit (Qiagen, Crawley, UK) and were sequenced  
13 on an ABI 3130xl capillary sequencer at the University of Leeds.

14

15 The closest matching sequence to each sequence generated within this study was  
16 determined using the NCBI-BLAST database (Altschul et al. 1997) and a percentage  
17 sequence similarity calculated using the pairwise alignment function in BioEdit (Hall 2005).

18

### 19 *Screening for Aphanomyces astaci and Psorospermium haeckeli*

20 In addition, a subset of fifteen *Pacifastacus leniusculus* and three *Austropotamobius pallipes*  
21 from the field collection were screened for the presence of *Aphanomyces astaci* and of  
22 *Psorospermium haeckeli*

23

24 Tissue was dissected from the eye to screen for the presence of *A. astaci* as in the early  
25 stages of the infection mycelium are known to be present within the cornea (Vogt 1999).

1 DNA extraction was performed and confirmed as described previously. Primers 525 and 640  
2 were used to screen for *A. astaci*, with an expected product length of 115 bp (Oidtmann et al.  
3 2004). The reaction mixture comprised 0.625 U of GoTaq Taq polymerase and 5 µl 5 x  
4 GoTaq buffer (giving a final concentration of 1.5 mM MgCl<sub>2</sub> per reaction) (Promega,  
5 Southampton, UK), 0.04 mM dNTPs, 10 pmol of each primer, 1 µl DNA and deionised water  
6 in a total reaction volume of 25 µl. No positive control material was available; a negative  
7 control containing deionised water in place of DNA was included for each PCR reaction. The  
8 PCR protocol is as described in Oidtmann et al. (2004).

9  
10 To screen for *Psorospermium haeckeli*, tissue was dissected from the subepidermal  
11 connective tissue as high parasite burdens have been reported from this tissue type (Henttonen  
12 1996). DNA extraction was performed and confirmed as described previously. Primers Pso-  
13 1 (Bangyeekhun et al. 2001) and ITS-4 (White et al. 1990) were used to screen for *P. haeckeli*  
14 with expected product lengths of 1300 or 1500 bp (Bangyeekhun et al. 2001). The reaction  
15 mixture comprised 1.25 U of GoTaq Taq polymerase, 5 µl 5 x GoTaq buffer (Promega,  
16 Southampton, UK), 2 mM MgCl<sub>2</sub>, 0.08 mM dNTPs, 20 pmol of each primer, 1 µl of DNA  
17 and deionised water in a total reaction volume of 25 µl. No positive control was available; a  
18 negative control containing deionised water in place of DNA was included for each PCR.  
19 The PCR protocol is as described in Bangyeekhun et al. (2001).

## 21 **Results**

### 22 *Sizes of animals in single and mixed populations*

#### 23 *Austropotamobius pallipes*

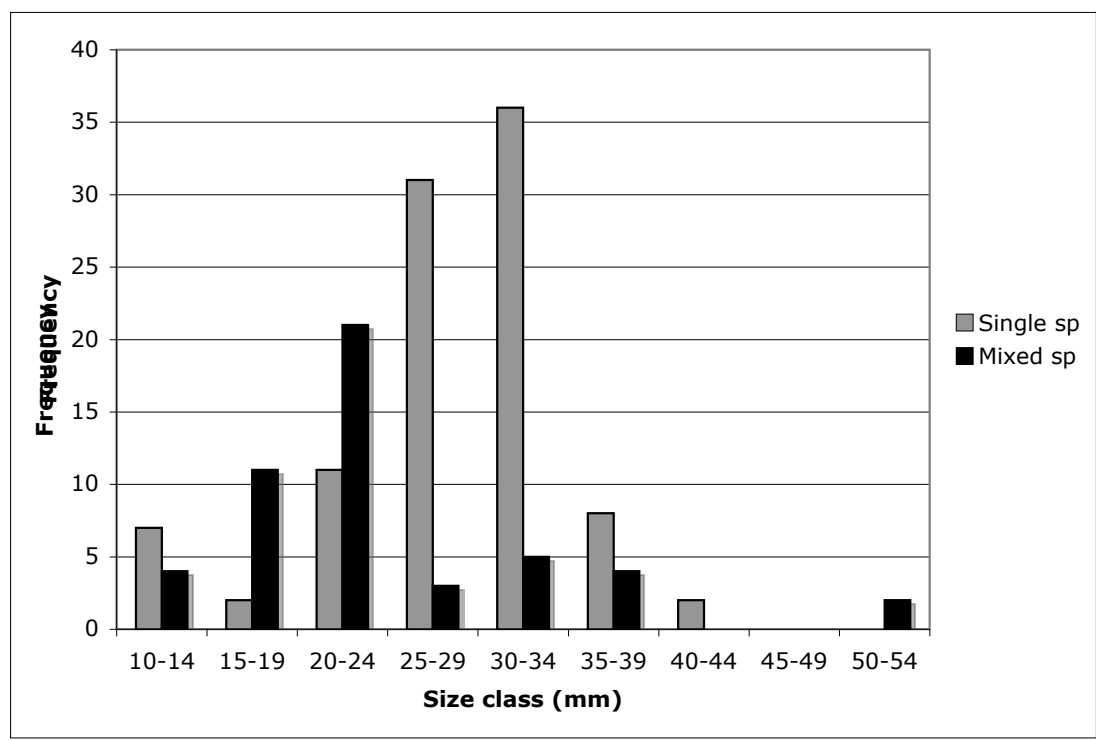
24 Following stepwise deletion of non-significant fixed effects from the Maximal model,  
25 population composition (single vs. mixed species) was the only significant term remaining in

1 the Minimum Adequate Model (LMM,  $F_{1,73}$ ,  $p=0.025$ ) indicating a significant difference in  
2 size composition of single and mixed species populations. The mean size of *A. pallipes* was  
3 28.5 mm in single species populations and 22.5 mm in mixed populations (Fig. 1).

4

5 Fig 1. Size distributions of *Austropotamobius pallipes* in single species and mixed species  
6 populations. *A. pallipes* individuals in single species populations were significantly larger  
7 than those in mixed species populations (LMM,  $F_{1,73}$ ,  $p=0.025$ )

8



9

10

11 *Pacifastacus leniusculus*

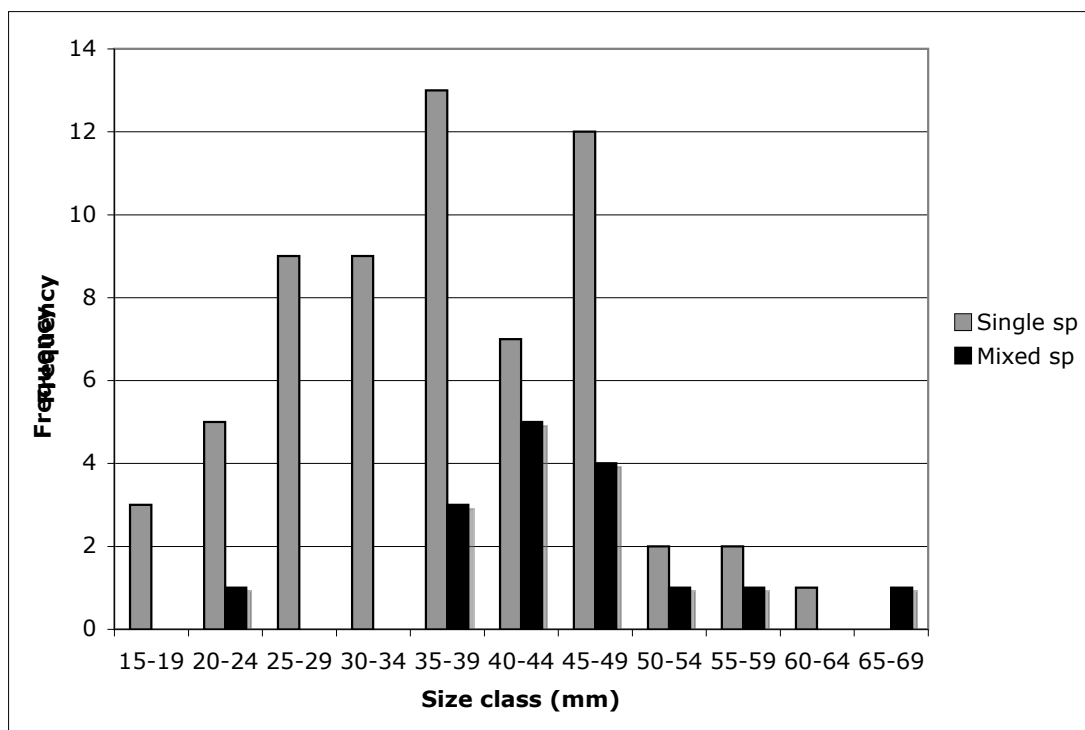
12 Following stepwise deletion of non-significant fixed effects, population composition (single  
13 vs. mixed species) was the only significant term remaining in the Minimum Adequate Model  
14 (LMM,  $F_{1,72}$ ,  $p=0.028$ ). *P. leniusculus* individuals in mixed populations were significantly  
15 larger than their counterparts in single species populations with a mean size of 36.3 mm in

1 single species populations and 46.0 mm in mixed species populations (Fig. 2). Very few  
2 juveniles were observed in the mixed species sites.

3

4 Fig 2. Size distributions of *Pacifastacus leniusculus* in single species and mixed species  
5 populations. *P. leniusculus* individuals in single species populations were significantly  
6 smaller than individuals in mixed species populations (LMM,  $F_{1,72}$ ,  $p=0.028$ )

7



8

### 9 *Microsporidian parasites*

10 All twelve *A. pallipes* individuals tested showed clinical signs of microsporidian infection  
11 through an opacity of the abdominal musculature; these all tested positive for microsporidian  
12 infection through PCR screening. As we were only able to screen dead individuals from the  
13 field, we were unable to estimate the prevalence of microsporidian infection for this species.

14

15 The prevalence of microsporidian infection in *P. leniusculus* ranged from 0.26 to 0.75,

1 with an overall prevalence across all populations of 0.38 (Table 2). Six of the twenty infected  
2 individuals showed clinical signs of infection through an opacity of the abdominal  
3 musculature; one of these was dead when collected. There was no significant difference  
4 between the frequency of infection of males versus females (GLM,  $p_{47}=0.181$ ) and there was  
5 no significant difference in sizes of infected versus uninfected individuals (GLM,  $p_{48}=0.831$ ).

6

### 7 *Parasite sequences*

8 We obtained multiple sequences from 4 distinct microsporidian parasite species (Table 4).  
9 Three of these parasites, *Bacillidium* sp. PLFB32, *Microsporidium* sp. PLWB7A and  
10 *Vittaforma* sp. PLDH3, had not previously been reported from crayfish hosts and represent  
11 novel microsporidian sequences; the fourth, *Thelohania contejeani*, despite having been  
12 previously recorded in crayfish, had not been sequenced from either of the two study species.

13

14 Forty-four sequences from 29 individuals were 98 % -100 % identical to *T. contejeani*  
15 isolated from the crayfish *Astacus fluviatilis* in France (Lom et al. 2001). These sequences  
16 were obtained from 17 *P. leniusculus* and 12 *Austropotamobius pallipes*. We detected two  
17 strains of *T. contejeani* within each crayfish species, corresponding to strains TcC2 and TcC3  
18 described by Lom et al. (2001). We found strain TcC2 in 7 individuals: 3 *A. pallipes* and 4 *P.*  
19 *leniusculus*. We sequenced strain TcC3 from 18 individuals: 8 *A. pallipes* and 10 *P.*  
20 *leniusculus*. Four samples were not sequenced across the variable region and so could belong  
21 to either strain. In three cases we sequenced both strains from the same host, twice in *A.*  
22 *pallipes* and once in *P. leniusculus*.

- 1 Table 4. Summary of microsporidian parasite diversity in *A. pallipes* and microsporidian diversity and prevalence *P. leniusculus*. It should be
- 2 noted that only dead individuals of *A. pallipes* were screened for parasites, and so prevalence cannot be estimated for this species.

| <b>Parasite</b>   | <b>% similarity</b>  | <b><i>A. pallipes</i></b> | <b><i>P. leniusculus</i></b> | <b>Genbank Accession numbers</b>                       |
|---|--|---------------------------|------------------------------|--|
| <i>Thelohania contejeani</i><br>isolates Tcc2PL, Tcc3PL,<br>Tcc2AP and Tcc3AP | 98-100% similarity to <i>Thelohania</i><br><i>contejeani</i> (AF492593 and AF492594)   | 12/12                     | 17/52                        | AM261747, AM261750,<br>AM261751, AM261752,<br>AM261753 |
| <i>Vittaforma</i> sp. isolate PLDH3   | 95% similarity to <i>Microsporidium</i> sp.<br>CRANFA (AJ966723)<br><br>93% similarity to <i>Vittaforma</i> -like<br>parasite (AY375044) | Absent                    | 1/52                         | AM261754   |
| <i>Bacillidium</i> sp. isolate<br>PLFB32                                      | 97% similarity to <i>Bacillidium</i><br><i>vesiculoformis</i> (AJ581995)   | Absent                    | 1/52                         | AM261748   |
| <i>Microsporidium</i> sp. isolate<br>PLWB7A                                   | 75% similarity to <i>Bacillidium</i><br><i>vesiculoformis</i> (AJ581995)   | Absent                    | 1/52                         | AM261749   |

1 Two sequences isolated from one *P. leniusculus* had 97 % sequence similarity  
2 to *Bacillidium vesiculoformis*, a species that has to date only been described from the  
3 oligochaete worm *Nais simplex* in Scotland. One sequence isolated from a *P.*  
4 *leniusculus* egg had 75 % sequence similarity to *B. vesiculoformis*; the parent crayfish  
5 tested negative for microsporidian infection.

6  
7 Two sequences isolated from a single *P. leniusculus* host had 95 % sequence  
8 similarity to *Microsporidium sp.* CRANFA isolated from the amphipod crustacean  
9 *Crangonyx floridanus* in Florida (Galbreath 2005), and 93 % sequence similarity to a  
10 *Vittaforma*-like parasite isolated from a human host (Sulaiman et al. 2003).

11  
12 We found no clinical/visible signs of *Aphanomyces astaci* infection in any of  
13 the individuals sampled. No evidence was found for infection by either *A. astaci* or  
14 *Psorospermium haeckeli* in any of subset the individuals screened for these parasites  
15 by PCR.

16  
17 **Discussion**

18 *Competitive interactions*

19 In mixed populations the size distributions of both species differ from those in single  
20 species populations. *Austropotamobius pallipes* tend to be smaller in mixed  
21 populations (Fig. 1) whereas *Pacifastacus leniusculus* tend to be larger (Fig. 2).  
22 Displacement mechanisms proposed in other native-invader crayfish systems include  
23 reproductive interference (Westman et al. 2002); competitive exclusion from refuges  
24 resulting in differential predation (Vorburger and Ribic 1999); and differential  
25 susceptibility to diseases (Alderman and Polglase 1988).



1

2           In Finland, where *P. leniusculus* displaces the native *Astacus astacus*, it is  
3 thought that reproductive interference by dominant *P. leniusculus* males results in the  
4 majority of *A. astacus* females producing only sterile eggs (Westman et al. 2002).  
5 Our data provide no support for this mechanism of displacement in our study system  
6 as smaller *Austropotamobius pallipes* were more common in mixed populations (Fig.  
7 1); this is in direct contrast to the pattern of fewer small *A. pallipes* in mixed  
8 populations that would be predicted by reproductive interference (Westman et al.  
9 2002).

10

11           Our data show large *A. pallipes* to be under-represented in mixed populations  
12 (Fig. 1), which may reflect competitive exclusion by the larger (Lowery 1988) and  
13 more dominant (Vorburger and Ribi 1999) invader from limited refuges (Bubb et al.  
14 2006), since small *P. leniusculus* and large *A. pallipes* overlap in size (Fig. 1, 2). *P.*  
15 *leniusculus* has been shown to oust other crayfish species from refuges (Söderbäck  
16 1995) which would leave larger *A. pallipes* more vulnerable to predation (Söderbäck  
17 1994, after Söderbäck 1992) and result in the reduction of large *A. pallipes* in the  
18 mixed populations seen within our study.

19

20           The absence of juvenile *P. leniusculus* from mixed populations (Fig. 2) is  
21 interesting, and implies that *A. pallipes* may in fact be influencing the population  
22 structure of the invading species. The moulting of juvenile *P. leniusculus* is  
23 synchronized, resulting in reduced intraspecific cannibalism (referenced in  
24 Ahvenharju et al. 2005). However, interspecific predation by the native *A. pallipes*  
25 (Gil-Sánchez and Alba-Tercedor 2006) as well as other predators such as fish

1 (Söderbäck 1992) may underpin the observed reduction in juvenile *P. leniusculus* in  
2 mixed populations.

3 *Parasitism in native and invasive crayfish*

4 Four species of microsporidia were detected in the invasive crayfish *P. leniusculus*.  
5 In contrast, only one microsporidian parasite was detected from *A. pallipes* although  
6 the sample size was small. The overall prevalence of microsporidian infection in *P.*  
7 *leniusculus* was 38 % (Table 2). This prevalence is higher than previous reports of  
8 visible microsporidiosis in *A. pallipes* in Britain (9 %, (Brown and Bowler 1977); 26  
9 % (Rogers et al. 2003); 30 % (Evans and Edgerton 2002)), France (0-8%, Chartier  
10 and Chaisemartin 1983) and Spain (1%, Dieguez-Uribeondo et al. 1993), probably  
11 reflecting a higher detection efficiency by PCR.

12  
13 The *T. contejeani* sequences we obtained were identical to those previously  
14 isolated from *Astacus fluviatilis* (Genbank accession numbers AF492593 and  
15 AF492594, Lom et al. 2001). This is, to our knowledge, the first molecular  
16 confirmation of *T. contejeani* infecting *P. leniusculus*, as well as the first report of the  
17 parasite in an invasive species in Europe. Whilst *T. contejeani* has previously been  
18 reported from *Austropotamobius pallipes* in the UK (Brown and Bowler 1977;  
19 Edgerton et al. 2002; Rogers et al. 2003), these reports were based on light  
20 microscopy and lack the ultrastructural or molecular information to confirm species  
21 identity (Dunn and Smith 2001). This is the first molecular confirmation of the  
22 presence of *T. contejeani* infecting *A. pallipes*.

23  
24 The presence of *T. contejeani* in the invasive *P. leniusculus* leads to the question  
25 of how the parasite has come to infect this species. Firstly, *P. leniusculus* may have

1 brought the parasite with it from its native range. *T. contejeani* has been reported  
2 from a number of crayfish hosts (Graham and France 1986; Quilter 1976), and there is  
3 a single report of *T. contejeani* from *P. leniusculus* in its native range in California  
4 (McGriff and Modin 1983); but identification is based on spore size, and molecular or  
5 ultrastructural confirmation is lacking. The pattern of infection in the current study  
6 leads us to suggest that it is more likely that *P. leniusculus* in the UK has acquired *T.*  
7 *contejeani* from the native host. *T. contejeani* was detected in *A. pallipes* only sites,  
8 in mixed sites and in sites where only *P. leniusculus* occurred. Furthermore, identical  
9 sequences were found in the native and invading species (Table 4). These data fit a  
10 pattern of transmission from the native *A. pallipes* to the invading species in mixed  
11 sites. Detailed studies of the fitness effects of *T. contejeani* and its mode of  
12 transmission within and between crayfish species are required.

13  
14 In addition, our *T. contejeani* sequences had 98-100 % sequence similarity to  
15 the unclassified microsporidium, *Microsporidium* sp. JES2002H, which was detected  
16 in three species of amphipod in France (Terry et al. 2004). We suggest that  
17 *Microsporidium* sp. JES2002H and *T. contejeani* may be the same species, although  
18 confirmation awaits ultrastructural analysis of *Microsporidium* sp. JES2002H.

19  
20 In one *P. leniusculus* we found a microsporidium sequence with 97 % sequence  
21 similarity to *Bacillidium vesiculoformis*, a parasite previously described from the  
22 oligochaete worm *Nais simplex* in Scotland, UK (Morris et al. 2005). The sequence  
23 similarity indicates that the parasite is likely to be in the same genus as *B.*  
24 *vesiculoformis*; however further molecular and morphological analysis would be  
25 required to confirm this. This is the first record of a *Bacillidium* spp. in crayfish and

1 supports Morris et al's (2005) suggestion that *B. vesiculoformis* is a generalist  
2 parasite.

3

4 The *Vittaforma*-like parasite sequenced from *P. leniusculus* had closest  
5 sequence similarity (95 %) to an unidentified *Microsporidium* sp. CRANFA  
6 sequenced from *Crangonyx floridanus* in Florida (Galbreath 2005), which suggests  
7 that this may be a parasite originating in the native range of *P. leniusculus*.

8

9 We also sequenced a novel parasite from a *P. leniusculus* egg. This parasite was  
10 unlike other microsporidia and had the closest sequence similarity (75 %) to *B.*  
11 *vesiculoformis*. The presence of the parasite in the egg suggests vertical transmission  
12 (Terry et al. 2004), widespread amongst microsporidian parasites (Dunn and Smith  
13 2001). Muscle tissue from the mother tested negative for microsporidian infection,  
14 but ovarian tissue was not screened in this study.

15

16 We found no evidence of *Aphanomyces astaci* or *Psorospermium haeckeli*  
17 within our study populations (although our results should be treated with caution  
18 owing to the absence of positive control material). The absence of crayfish plague  
19 may explain the persistence of mixed species populations in Yorkshire and highlights  
20 the need for vigilance in preventing plague from spreading into these rivers.

21

22 In summary, our size distribution data are in accord with a pattern of  
23 competitive exclusion of *Austropotamobius pallipes* from refuges leading to  
24 differential predation. We provide the first molecular evidence for the presence of the  
25 microsporidian parasite *T. contejeani* in both *A. pallipes* and *Pacifastacus leniusculus*.

1 We also detected three novel microsporidian sequences in *P. leniusculus*. This raises  
2 the question of the effects of these parasites on host fitness as well as their potential  
3 influence on native - invader interactions.

4

5

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