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

Jenny C. Dunn[1]*, H. Elizabeth McClymont[1], Martin Christmas[2] & Alison M. Dunn[1].

[1]*Institute of Integrative and Comparative Biology, L. C. Miall Building, University of Leeds, Clarendon Way, Leeds, LS2 9JT, UK.*

[2]*Environment Agency, Phoenix House, Global Avenue, Leeds, LS11 8PG, UK.*

* Author and address for correspondence:
Institute of Integrative and Comparative Biology,
L. C. Miall Building,
University of Leeds,
Clarendon Way,
Leeds,
LS2 9JT

Tel: +44 (0) 113 3433078
E-mail: J.C.Dunn01@leeds.ac.uk

Abstract

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

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

Introduction

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

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

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

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

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

microsporidian parasites.

Materials and Methods

Animal collection and measurement

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

Statistical analysis

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

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

Table 1. Field sites sampled during the study. All populations were surveyed for size distribution; [b] denotes populations from which *P. leniusculus* or dead *A. pallipes* were obtained for parasite screening

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

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

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

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

Screening for microsporidian parasites

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

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

Table 2. Results of PCR screen for microsporidian infection in *P. leniusculus.* Summary of 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

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

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

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

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

Primers	Annealing temperature/^{o}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

Sequencing and phylogenetic analysis of microsporidia

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

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

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

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

Screening for Aphanomyces astaci and Psorospermium haeckeli

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

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

DNA extraction was performed and confirmed as described previously. Primers 525 and 640 were used to screen for *A. astaci*, with an expected product length of 115 bp (Oidtmann et al. 2004). The reaction mixture comprised 0.625 U of GoTaq Taq polymerase and 5μl 5 x GoTaq buffer (giving a final concentration of 1.5 mM $MgCl_2$ per reaction) (Promega, Southampton, UK), 0.04 mM dNTPs, 10 pmol of each primer, 1 μl DNA and deionised water in a total reaction volume of 25 μl. No positive control material was available; a negative control containing deionised water in place of DNA was included for each PCR reaction. The PCR protocol is as described in Oidtmann et al. (2004).

To screen for *Psorospermium haeckeli*, tissue was dissected from the subepidermal connective tissue as high parasite burdens have been reported from this tissue type (Henttonen 1996). DNA extraction was performed and confirmed as described previously. Primers Pso-1 (Bangyeekhun et al. 2001) and ITS-4 (White et al. 1990) were used to screen for *P. haeckeli* with expected product lengths of 1300 or 1500 bp (Bangyeekhun et al. 2001). The reaction mixture comprised 1.25 U of GoTaq Taq polymerase, 5 μl 5 x GoTaq buffer (Promega, Southampton, UK), 2 mM $MgCl_2$, 0.08 mM dNTPs, 20 pmol of each primer, 1 μl of DNA and deionised water in a total reaction volume of 25 μl. No positive control was available; a negative control containing deionised water in place of DNA was included for each PCR. The PCR protocol is as described in Bangyeekhun et al. (2001).

Results

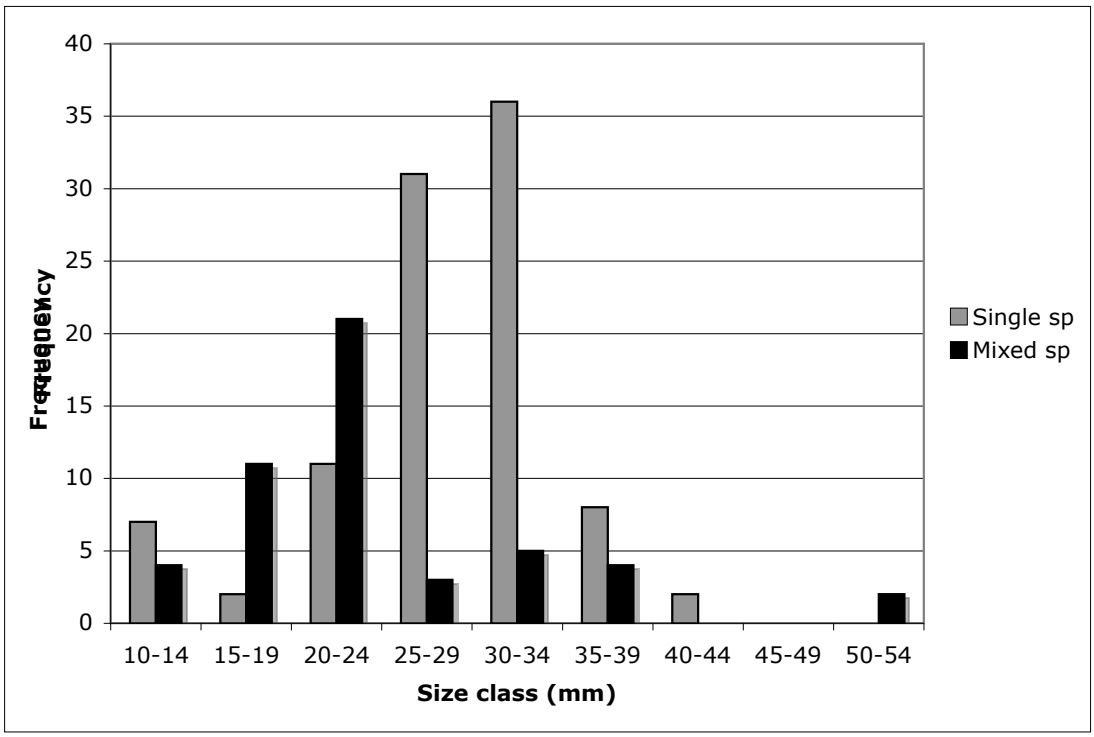
Sizes of animals in single and mixed populations

Austropotamobius pallipes

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

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

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

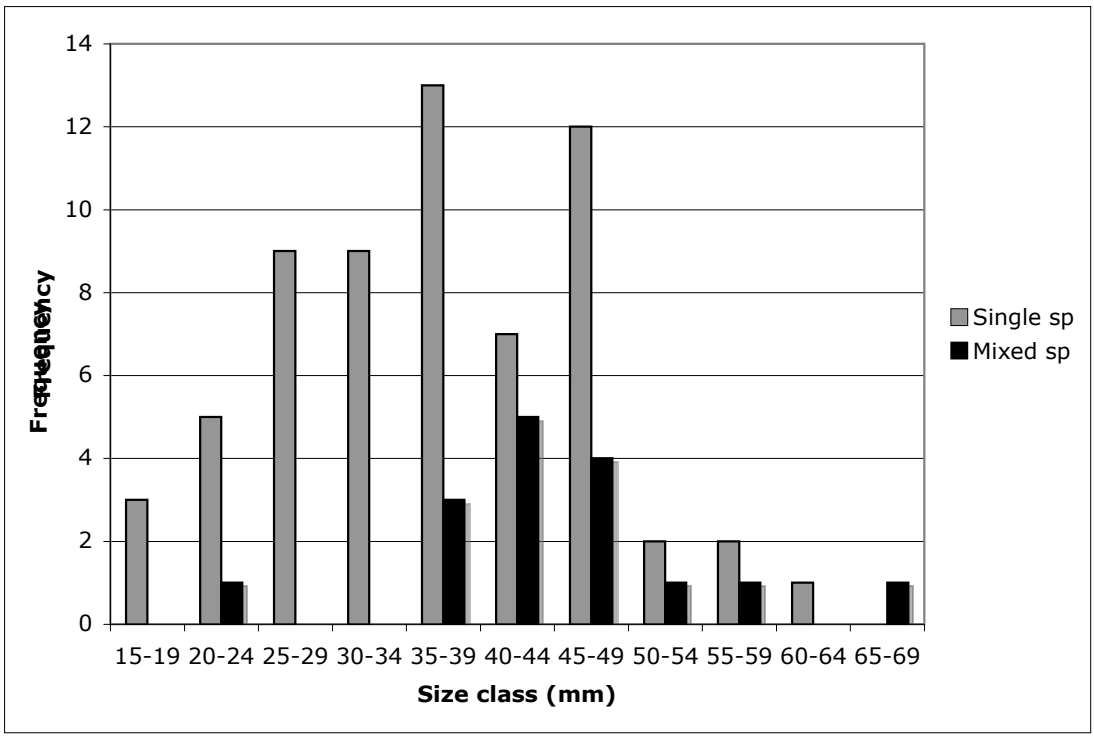


Pacifastacus leniusculus

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

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

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



Microsporidian parasites

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

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

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

Parasite sequences

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

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

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

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

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

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

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

Discussion

Competitive interactions

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

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

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

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

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

Parasitism in native and invasive crayfish

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

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

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

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

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

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

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

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

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

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

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

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

Acknowledgements

Stephanie Peay provided advice and help with fieldwork. Qiu Yang provided advice and assistance for the molecular work. Elizabeth Moodie provided positive control material in the form of microsporidian infected crayfish muscle. Fieldwork was carried out under licence from English Nature.

References

Ahvenharju T, Savolainen R, Tulonen J et al. (2005) Effects of size grading on growth, survival and cheliped injuries of signal crayfish (*Pacifastacus leniusculus* Dana) summerlings (age 0+). Aquac Res 36: 857-867

Alderman DJ and Polglase JL (1988) Pathogens, Parasites and Commensals. In: Holdich DM and Lowery RS (eds) Freshwater Crayfish: Biology, Management and Exploitation. Croom Helm, London

Altschul SF, Madden TL, Schäffer AA et al. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25: 3389 - 3402

Baker MD, Vossbrinck CR, Didier ES et al. (1995) Small Subunit Ribosomal DNA Phylogeny of Various Microsporidia with Emphasis on AIDS Related Forms. J Eukaryot Microbiol 42: 564 - 570

Bangyeekhun E, Ryynänen HJ, Henttonen P et al. (2001) Sequence analysis of the ribosomal internal transcribed spacer DNA of the crayfish parasite *Psorospermium haeckeli*. Dis Aquat Organ 46: 217 - 222

Bauer A, Trouve S, Gregoire A et al. (2000) Differential influence of *Pomphorhynchus laevis* (Acanthocephala) on the behaviour of native and invader gammarid species. Int J Parasitol 30: 1453 - 1457

Brown DJ and Bowler K (1977) A population study of the British freshwater crayfish *Austropotamobius pallipes* (Lereboullet). Freshwater Crayfish 3: 33 - 49

Bubb DH, Thom TJ and Lucas MC (2006) Movement, dispersal and refuge use of co-occurring introduced and native crayfish. Freshwater Biol 51: 1359 - 1368

Chartier L and Chaisemartin C (1983) Effect of *Thelohania* infection on populations of *Austropotamobius pallipes* in granitic and calcareous habitats. Comptes Rendus des Seances de L'Academie des Sciences, Paris 297: 441 - 443

Dieguez-Uribeondo J, Pinedo-Ruiz J, Cerenius L et al. (1993) Presence of *Psorospermium haeckeli* (Hilgendorf) in a *Pacifastacus leniusculus* (Dana) population of Spain. Freshwater Crayfish 9: 286 - 288

Doyle JJ and Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull 19: 11 - 15

Dunn AM and Smith JE (2001) Microsporidian life cycles and diversity: the relationship between virulence and transmission. Microbes Infect 3: 381 - 388

Edgerton BF, Evans LH, Stephens FJ et al. (2002) Synopsis of freshwater crayfish diseases and commensal organisms. Aquaculture 206: 57 - 135

Edgerton BF, Henttonen P, Jussila J et al. (2004) Understanding the Causes of Disease in European Freshwater Crayfish. Conserv Biol 18: 1466 - 1474

Evans LH and Edgerton BF (2002) Pathogens, parasites and commensals. In: Holdich DM (ed) Biology of freshwater crayfish. Blackwell Science, Oxford, United Kingdom

Folmer O, Black M, Hoeh W et al. (1994) DNA primers for amplification of mitochondrial cytochrome C oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotechnol 3: 294 - 299

Galbreath JGS (2005) The impact of intercontinental invasion on host genetic and microsporidian parasite diversity in the freshwater amphipod *Crangonyx pseudogracilis*. Dissertation, University of Leeds

Gatehouse HS and Malone LA (1998) The ribosomal RNA gene of *Nosema apis* (Microspora): DNA sequence for small and large subunit rRNA genes and evidence of a large tandem repeat size. J Invertebr Pathol 71: 97 - 105

Gil-Sánchez JM and Alba-Tercedor J (2006) The Decline of the Endangered Populations of the Native Freshwater Crayfish (*Austropotamobius pallipes*) in Southern Spain: It is Possible to Avoid Extinction? Hydrobiologia 559: 113 - 122

Graham I and France R (1986) Attempts to transmit experimentally the microsporidian *Thelohania contejeani* in freshwater crayfish (*Orconectes virilis*). Crustaceana 51: 208 - 211

Hall T (2005) BioEdit: Biological sequence alignment editor for Win95/98/NT/2K/XP. Accessed 07/06/2005. http://www.mbio.ncsu.edu/BioEdit/bioedit.html

Hatcher MJ, Dick JTA and Dunn AM (2006) How parasites affect interactions between competitors and predators. Ecol Lett 9: 1253 - 1271

Henttonen P (1996) The Parasite *Psorospermium* in Freshwater Crayfish. Dissertation, University of Kuopio

Holdich DM (2003) Ecology of the White-Clawed Crayfish. Conserving Natura 2000 Rivers Ecology Series No. 1. English Nature, Peterborough

Holdich DM, Sibley P and Peay S (2004) The White-Clawed Crayfish - a decade on. British Wildlife 15: 153 - 164

IUCN (2004) IUCN Red List of Threatened Species. Accessed 07/04/2005. www.redlist.org

Kemp E, Birkinshaw N, Peay S et al. (2003) Reintroducing the White-Clawed Crayfish *Austropotamobius pallipes*. Conserving Natura 2000 Rivers Conservation Techniques Series No. 1. English Nature, Peterborough

Krakau M, Thieltges DW and Reise K (2006) Native Parasites Adopt Introduced Bivalves of the North Sea. Biol Invasions 8: 919 - 925

Lom J, Nilsen F and Dykova (2001) *Thelohania contejeani* Henneguy, 1892: dimorphic life cycle and taxonomic affinities, as indicated by ultrastructural and molecular study. Parasitol Res 87: 860 - 872

Lowery RS (1988) Growth, moulting and reproduction. In: Holdich DM and Lowery RS (eds) Freshwater Crayfish: Biology, Management and Exploitation. Croom Helm, London

MacNeil C, Dick JTA, Hatcher MJ et al. (2003a) Parasite-mediated predation between native and invasive amphipods. P Roy Soc B-Biol Sci 270: 1309 - 1314

MacNeil C, Fielding NJ, Dick JTA et al. (2003b) An acanthocephalan parasite mediates intraguild predation between invasive and native freshwater amphipods (Crustacea). Freshwater Biol 48: 2085 - 2093

McClymont HE, Dunn AM, Terry RS et al. (2005) Molecular data suggest that microsporidian parasites in freshwater snails are diverse. Int J Parasitol 35: 1071 - 1078

McGriff D and Modin J (1983) *Thelohania contejeani* parasitism of the crayfish, *Pacifastacus leniusculus*, in California. Calif Fish Game 69: 178 - 183

Morris DJ, Terry RS, Ferguson KB et al. (2005) Ultrastructural and molecular characterization of *Bacillidium vesiculoformis* n. sp. (Microspora: Mrazekiidae) in the freshwater oligochaete *Nais simplex* (Oligochaeta: Naididae). Parasitology 130: 31 - 40

Ohtaka A, Gelder SR, Kawai T et al. (2005) New records and distributions of two North American branchiobdellidan species (Annelida: Clitellata) from introduced signal crayfish, *Pacifastacus leniusculus*, in Japan. Biol Invasions 7: 149 - 156

Oidtmann B, Schaefers N, Cerenius L et al. (2004) Detection of genomic DNA of the crayfish plague fungus *Aphanomyces astaci* (oomycete) in clinical samples by PCR. Vet Microbiol 100: 269 - 282

Peay S (2003) Monitoring the White-clawed Crayfish *Austropotamobius p. pallipes*. Conserving Natura 2000 Rivers Monitoring Series No. 1. English Nature, Peterborough

Peay S and Rogers D (1999) The peristaltic spread of signal crayfish in the River Wharfe, Yorkshire, England. Freshwater Crayfish 12: 665 - 677

Prenter J, MacNeil C, Dick JTA et al. (2004) Roles of parasites in animal invasions. Trends Ecol Evol 19: 385

Quilter CG (1976) Microsporidian parasite *Thelohania contejeani* Henneguy from New Zealand freshwater crayfish. New Zeal J Mar Fresh 10: 225 - 231

Rogers D, Hoffman R and Oidtmann B (2003) Diseases in selected *Austropotamobius pallipes* populations in England. In: Management and Conservation of Crayfish. Proceedings of a conference held on 7th November 2002. Environment Agency, Bristol

Rushton SP, Lurz PWW, Gurnell J et al. (2000) Modelling the spatial dynamics of parapoxvirus disease in red and grey squirrels: a possible cause of decline in the red squirrel in the UK? J Appl Ecol 37: 997 - 1012

Söderbäck B (1992) Predator avoidance and vulnerability of two co-occurring crayfish species, *Astacus astacus* (L.) and *Pacifastacus leniusculus* (Dana). Ann Zool Fenn 29: 253 - 259

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

Söderbäck B (1995) Replacement of the native crayfish *Astacus astacus* by the introduced species *Pacifastacus leniusculus* in a Swedish lake: possible causes and mechanisms. Freshwater Biol 33: 291 - 304

Souty-Grosset C, Holdich D, Noël P et al. (eds) (2006) Atlas of crayfish in Europe. Muséum National d'Histoire Naturelle, Paris

Sulaiman IM, Matos O, Lobo ML et al. (2003) Identification of a New Microsporidian Parasite Related to *Vittaforma corneae* in HIV-Positive and HIV-Negative Patients from Portugal. J Eukaryot Microbiol 50: 586 - 590

Terry RS, Smith JE, Sharpe RG et al. (2004) Widespread vertical transmission and associated host sex-ratio distortion within the eukaryotic phylum Microspora. P Roy Soc B-Biol Sci 271: 1783 - 1789

Torchin ME, Lafferty KD, Dobson AP et al. (2003) Introduced species and their missing parasites. Nature 421: 628 - 629

Torchin ME, Lafferty KD and Kuris AM (2001) Release from parasites as natural enemies: increased performance of a globally introduced marine crab. Biol Invasions 3: 333 - 345

Vogt G (1999) Diseases of European freshwater crayfish, with particular emphasis on interspecific transmission of pathogens. In: Gherardi F and Holdich DM (eds) Crayfish in Europe as Alien Species: How to make the best of a bad situation? pp 87-103. AA Balkema, Rotterdam, The Netherlands

Vorburger C and Ribi G (1999) Aggression and competition for shelter between a native and an introduced crayfish in Europe. Freshwater Biol 42: 111 - 119

Vossbrinck CR, Baker MD, Didier ES et al. (1993) Ribosomal DNA Sequences of *Encephalitozoon hellem* and *Encephalitozoon cuniculi*: Species Identification and Phylogenetic Construction. J Eukaryot Microbiol 40: 354 - 362

Vossbrinck CR and Woese CR (1986) Eukaryotic ribosomes that lack a 5.8S RNA. Nature 320: 287 - 288

Weiss LM and Vossbrinck CR (1998) Microsporidiosis: molecular and diagnostic aspects. In: Tzipori S (ed) Advances in Parasitology. Academic Press, San Diego

Weiss LM, Zhu X, Cali A et al. (1994) Utility of microsporidian rRNA in diagnosis and phylogeny: a review. Folia Parasit 41: 81 - 90

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

White TJ, Bruns T, Lee S et al. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ et al (eds) PCR protocols: a guide to methods and applications. Academic Press, San Diego

Yan G, Stevens L, Goodnight CJ et al. (1998) Effects of a tapeworm parasite on the competition of Tribolium beetles. Ecology 79: 1093 - 1103