Horizontal transmission of *Thelohania contejeani* in the endangered white-clawed (*Austropotamobius pallipes*) and the invasive signal crayfish (*Pacifastacus leniusculus*)

EMILY M. IMHOFF[1], ROBERT J. G. MORTIMER[2], MARTIN CHRISTMAS[3] *and* ALISON M. DUNN[1]*

[1] *Faculty of Biological Sciences, University of Leeds, Leeds LS2 9JT, UK*
[2] *School of Earth and the Environment, University of Leeds, Leeds LS2 9JT, UK*
[3] *Environment Agency, Phoenix House, Global Avenue, Millshaw LS11 8PG, UK*

(*Received 26 November 2011; revised 9 April 2012; accepted 9 April 2012; first published online 20 July 2012*)

SUMMARY

The microsporidian parasite *Thelohania contejeani* causes porcelain disease and has been implicated in mass mortalities in populations of the endangered European crayfish *Austropotamobius pallipes*. However, the route of parasite transmission is not known. This paper investigates the horizontal transmission of *T. contejeani* between *A. pallipes* hosts as well as its transmissibility to the invasive signal crayfish (*Pacifastacus leniusculus*). Field collected juvenile *A. pallipes* and *P. leniusculus* were assigned to 1 of 3 experimental treatments; fed heavily infected *A. pallipes* tissue, exposed to water from tanks housing heavily parasitized *A. pallipes*, and a control group to provide an estimate of the baseline infection levels in the field. After 26 weeks, abdominal muscle samples were screened by PCR for *T. contejeani*. Infection was significantly higher in the treatment groups (83% in the cannibalism treatment, 42% in the water exposure treatment) than in the control group (4%), providing evidence for horizontal transmission of the parasite between *A. pallipes* hosts. Cannibalism and scavenging are common amongst crayfish, providing transmission opportunities in the field. The study also provides the first direct evidence for transmission of the parasite from an indigenous European crayfish species to the invasive signal crayfish, with 50% of *P. leniusculus* in each treatment, and 8% of control animals infected. We discuss the possibility that high density populations of the invasive signal crayfish may serve either as reservoirs or sinks for the parasite.

Key words: *Thelohania contejeani*, microsporidia, invasive species, biological invasion, *Austropotamobius pallipes*, *Pacifastacus leniusculus*.

INTRODUCTION

The microsporidian *Thelohania contejeani* commonly causes porcelain disease in populations of indigenous endangered white-clawed (*Austropotamobius pallipes*) and vulnerable noble (*Astacus astacus*) crayfish throughout Europe (e.g. Mazylis, 1978; Diéguez-Uribeondo *et al*. 1997). Porcelain disease is a chronic infection that results in the deaths of infected crayfish, and has been implicated in past crayfish mass mortalities (Henneguy and Thélohan, 1892; Duffield, 1933; Pixell Goodrich, 1956). Commonly, prevalence in white-clawed crayfish populations range from 0·2 to 10% (Cossins and Bowler, 1974; O'Keefe and Reynolds, 1983; Mori and Salvidio, 2000; Hutchings, 2009), but higher rates can occur (e.g. 30%, Schäperclaus, 1954; 18%, Pixell Goodrich, 1956; 30%, O'Keefe and Reynolds, 1983; 18–50%, Imhoff *et al*. 2009; >80%, J. Brickland, personal communication).

The primary site of infection is the muscle cells, where sporogony occurs, although the mature spores may be found in other body tissues extracellularly (Cossins and Bowler, 1974; Oidtmann *et al*. 1996). Infected muscle becomes filled with spores and takes on an opaque white colouration which is visible externally and gives rise to the common name of microsporidiosis in crayfish – porcelain disease. Lom *et al*. (2001) found that *T. contejeani* exhibits 2 different routes of sporogony simultaneously within the same host tissue. The first route occurs inside a sporophorous vesicle and results in the formation of 8 uninucleated (haploid) spores with 9–10 turns of the polar filament. The second route occurs in 'vacuole-like compartments' and results in the formation of single diplokaryotic spores with 5–7 turns of the polar filament (Lom *et al*. 2001). Molecular and ultra-structural data indicate that *T. contejeani* is closely related to *T. montirivilorum* and *T. parastaci* which occur in Australian crayfish species and which have similar developmental patterns in the muscle tissue (Moodie *et al*. 2003*a*, *b*).

The route of transmission of *T. contejeani* between hosts is not well understood. A proposed life cycle of the congeneric parasite *T. parastaci* was provided by Moodie *et al*. (2003*b*), who proposed that diplokaryotic spores may be involved in vertical transmission and that uninucleate spores may be involved in

* Corresponding author: Institute of Integrative and Comparative Biology, University of Leeds, Leeds LS2 9JT, UK. Tel: +44 (0)113 3432856. Fax: +44 (0) 113 3432835. E-mail: a.dunn@leeds.ac.uk

doi:10.1017/S0031182012000777

horizontal transmission. Observations of *T. contejeani* in haemolymph (Cossins and Bowler, 1974; Oidtmann *et al*. 1996) suggest that, following intrusion of the parasite into the crayfish, the parasite is transported via the haemolymph to the muscle cells (Moodie *et al*. 2003*b*). However, the method of transmission to new hosts remains poorly understood. A number of transmission routes have been suggested, including direct cannibalism of infected crayfish tissue (Voronin, 1971), uptake of spores released from dead and decaying infected crayfish (Cossins and Bowler, 1974), the use by the parasite of an intermediate host (Graham and France, 1986; Lom *et al*. 2001) and vertical (transovarial) transmission from mother to eggs (Vey and Vago, 1973; Cossins and Bowler, 1974; Moodie *et al*. 2003*b*).

The white-clawed crayfish is indigenous to Europe and was recently classified as Endangered on the International Union for Conservation of Nature Red List (IUCN, 2010). A greater understanding of the transmission of *T. contejeani* may help instruct conservation efforts, because fatal porcelain disease caused by *T. contejeani* is common in populations of these crayfish and can occur at high rates (Imhoff *et al*. 2009). Furthermore, the parasite has recently been found in populations of the invasive, non-indigenous signal crayfish (*Pacifastacus leniusculus*) at high prevalence (25–75%), and sequence comparison suggests that the invader has acquired the parasite from its new range (Dunn *et al*. 2009).

This study investigates horizontal transmission between white-clawed crayfish by experimentally exposing crayfish to spores through feeding of infected tissue and through contaminated water. To determine whether signal crayfish are able to acquire the parasite from the indigenous white-clawed crayfish, signal crayfish were also exposed to infection.

MATERIALS AND METHODS

Experimental animals

A total of 98 juvenile white-clawed crayfish were collected from the River Fury in Northern Ireland and 98 juvenile signal crayfish were collected from the River Wharfe in England. White-clawed crayfish that were used to initiate the infection were visibly infected with *T. contejeani* and were collected from Wyke Beck, England. Signal crayfish were held at the University of Leeds under license from CEFAS. White-clawed crayfish ranged from 7 to 18·2 mm in carapace length (mean = 13·8 mm) at the beginning of the experiment, and signal crayfish from 8·4 to 27·2 mm (mean = 20 mm). The crayfish were housed in plastic tubs measuring 58 cm by 74 cm, divided into 35 individual 10 cm by 10 cm units. Dechlorinated tap water was provided, with constant aeration, at 6 cm deep and was changed every 2 weeks. The crayfish unit divider had a mesh bottom to allow water circulation. Crayfish were fed crustacean food pellets (Hikari Crab Cuisine) and detrital leaves throughout the experiment.

Horizontal transmission treatments

To test for direct horizontal transmission between *A. pallipes* hosts, crayfish were exposed to *T. contejeani* spores through feeding of infected tissue and through contaminated water. To determine whether signal crayfish are able to acquire the parasite from the indigenous white-clawed crayfish, signal crayfish were also exposed to infection. As limited juvenile white-clawed crayfish were available, transmission from signal to white-clawed crayfish was not tested. Each crayfish was randomly assigned to 1 of 3 experimental groups. To test for transmission via cannibalism/predation or scavenging, crayfish were fed fresh abdominal muscle tissue from heavily infected adult white-clawed crayfish. Feeding of *T. contejeani*-infected tissue occurred every 2 weeks, with 6 tissue feedings in total. At each feeding, each individual crayfish was provided with approximately 100 mg of tissue. All crayfish readily consumed the infected tissue. To test for transmission via spores released from infected crayfish into the water, crayfish were exposed to water from an aquarium housing multiple infected adult white-clawed crayfish in various stages of infection. Three hundred ml of this water was poured directly into the appropriate experimental tanks following the water change performed every 2 weeks. The control group did not receive tissue or exposure to contaminated water, but were otherwise treated the same as the other groups. Thus, the control group provided an estimate of the base rate of infection for the populations (juvenile crayfish were not screened for parasitism at the onset of the experiment owing to the likelihood that tissue sampling would lead to mortality in these small individuals (Imhoff *et al*. 2010)). All groups were monitored for 26 weeks and overall growth and survival data were recorded. To determine overall growth rate, the carapace length of each crayfish was measured at the beginning and end of the experimental period (or following death), growth rate was estimated as the total change in length divided by the number of weeks the crayfish was housed. At the end of the experiment all remaining crayfish were frozen individually and 24 crayfish were randomly selected (using www.random.org) from each treatment to be screened for *T. contejeani* by PCR.

Parasite screening

To screen the animals for infection with *T. contejeani*, tissue samples (7–15 mg) were removed from each crayfish's abdominal muscle taking care to avoid the gut and cuticle. Individuals were also examined for visible signs of infection. DNA was extracted from

https://doi.org/10.1017/S0031182012000777 Published online by Cambridge University Press

Table 1. PCR protocols showing primers and expected fragment sizes, reagent quantities/concentrations, and PCR cycles for each reaction

(Protocol for inner nest is that of El-Matbouli *et al*. 2006.)

Primers		Outer nest V1f/1492r	Inner nest F3/B3
Expected fragment size		1360 bp	215 bp
Reagents	ddH2O (μl)	10·9	14
	5x Taq buffer (μl)	5	2
	$MgCl_2$ (mM)	1·5	1·37
	dNTPs (μM)	0·2	0·2
	F primer (μM)	0·4	0·4
	R primer (μM)	0·4	0·4
	Promega GoTaq (u)	0·625	0·5
	Template (μl)	6	1
Total volume (μl)		25	20
PCR	Initial denature	95 °C 5 min	95 °C 5 min
	Cycles	95 °C 1 min 50 °C 1 min 10 s (40) 72 °C 1 min 30 s	95 °C 45 s 66 °C 45 s (38) 72 °C 45 s
	Final extension	72 °C 10 min	72 °C 5 min

each sample using a phenol:chloroform method based on that of Kocher *et al*. (1989). A nested PCR to amplify a portion of microsporidian small subunit ribosomal DNA was carried out using general microsporidian primers (all primers presented in 5′–3′ orientation) V1f (CACCAGGTTGATTCTGCCTGAC) and 1492r (GGTTACCTTGTTACGACTT) for the outer nest (Weiss *et al*. 1994), and *T. contejeani*-specific primers F3 (AGCTAGTATGTAGGGTAAGGGC) and B3 (ACTCTTGGAGCTGGAATTACCG) for the inner nest (El-Matbouli *et al*. 2006). See Table 1 for PCR protocols. Multiple negative and positive controls were included in each reaction. To confirm successful extraction of DNA, PCRs using host primers HCO2198 (TAAACTTCAGGGTGACCAAAAAATCA) and LCO1490 (GGTCAACAAATCATAAAGATATTGG) were also carried out (Folmer *et al*. 1994). All PCR products were visualized by electrophoresis in 2% agarose gels stained with ethidium bromide. All samples were screened twice, and were considered positive when both screenings yielded a readily discernible band of the expected number of base pairs on the gel. Two white-clawed and 2 signal crayfish samples were sequenced to further confirm the presence of *T. contejeani*.

Data analysis

Statistical analyses were performed using the programs Excel (Microsoft, 2007) and R 2.10.1 (www.r-project.org). To determine predictors of survival and infection, generalised linear models (GLM, logistic regression type) were constructed and tested. Explanatory variables tested for survival included species and treatment group, and variables for infection included sex, growth rate, and treatment group. To examine any differences in growth among the experimental groups and between uninfected and infected (those which screened positive by PCR) crayfish, ANOVA tests were performed after examining the data to ensure the appropriate assumptions were met. Significance was accepted at probabilities of 0·05 or less in all original test analyses. Where *post-hoc* multiple comparisons were required, alpha was adjusted using the Bonferroni method.

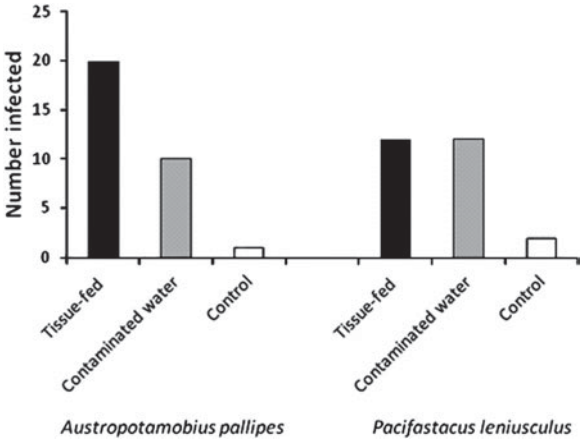

Fig. 1. Horizontal transmission of *Thelohania contejeani* to *Austropotamobius pallipes* and *Pacifastacus leniusculus*. The graph shows the numbers of crayfish in each treatment group that tested positive for *T. contejeani* by PCR; (24 individuals per treatment group were screened for infection. Treatments; Tissue-fed; animals were fed infected muscle tissue from parasitized *A. pallipes*, Contaminated water; animals were exposed to water from tanks housing infected *A. pallipes*.

RESULTS

Parasite screening

For both white-clawed and signal crayfish, the frequency of infection was higher in crayfish exposed to infected tissue or to contaminated water than in the control treatments (Fig. 1), providing evidence for

https://doi.org/10.1017/S0031182012000777 Published online by Cambridge University Press

Table 2. Results of comparisons of infection status between treatment groups for white-clawed and signal crayfish samples

(Alpha level of significance = 0·0167 after Bonferroni correction needed for multiplicity of tests.)

Comparison	Deviance	D.F.	P
White-clawed crayfish			
Tissue and water	6·09	1,37	0·0136
Water and control	9·94	1,41	0·0016
Tissue and control	30·37	1,42	$3{\cdot}56\times10^{-8}$
Signal crayfish			
Tissue and water	0·0009	1,40	0·9764
Water and control	10·377	1,40	0·0013
Tissue and control	11·103	1,44	0·0009

horizontal transmission of *T. contejeani*. None of the signal or white-clawed crayfish which tested positive or negative for *T. contejeani* infection by PCR showed visible signs of infection. Of the control animals, 1 white-clawed crayfish and 2 signal crayfish tested positive for *T. contejeani*, indicating low parasite prevalence in the field.

Among white-clawed crayfish, each treatment group had significantly different rates of infection. The tissue-fed group had a higher rate of infection than the group exposed to contaminated water, which in turn had a higher rate of infection than the control group (Fig. 1, Table 2). There was no difference in the sex ratios assigned to the different treatments, ($\chi^2 = 0{\cdot}99$, D.F. = 2, $P = 0{\cdot}605$). However, females were more likely to become infected than males (deviance = 7·61, D.F. = 1, $P = 0{\cdot}0058$).

Among signal crayfish, treatment group was the only significant predictor of infection status (deviance = 13·89, D.F. = 2, $P = 0{\cdot}00096$). Infection was significantly higher in animals exposed to infected tissue or to contaminated water than in animals from the control group. However, there was no difference in the infection rate in animals exposed to infected tissue or to contaminated water (Fig. 1, Table 2). Sex was not a significant predictor of infection status (deviance = 1·387, D.F. = 1, $P = 0{\cdot}239$).

Growth and survival

Growth rates did not differ among the treatment groups for either crayfish species (Table 3). White-clawed crayfish had a mean growth rate (± S.E.) of 0·102 ± 0·005 mm/week and growth rate did not differ among the three treatments ($F_{2,83} = 0{\cdot}774$, $P = 0{\cdot}46$). Signal crayfish had a mean growth rate of 0·095 ± 0·006 mm/week and growth rate did not differ significantly among treatment groups ($F_{2,85} = 0{\cdot}415$, $P = 0{\cdot}66$). As not all crayfish became infected following exposure, growth rate was also compared between crayfish that were found to be infected and uninfected at the end of the experiment. There was no difference in growth rate between white-clawed crayfish which screened positive or negative for parasite presence at the end of the experiment (0·102 ± 0·008 mm/week and 0·105 ± 0·008 mm/week; $F_{1,64} = 0{\cdot}066$, $P = 0{\cdot}80$). Likewise there was no significant difference between signal crayfish which screened positive or negative (0·108 ± 0·010 mm/week and 0·104 ± 0·007 mm/week, $F_{1,63} = 0{\cdot}016$, $P = 0{\cdot}90$).

Survival was greater than 78% in all treatments (Table 3) and did not differ between the treatments (deviance = 2·01, D.F. = 2, $P = 0{\cdot}4$) or between white-clawed and signal crayfish (deviance = 0·18, D.F. = 1, $P = 0{\cdot}7$).

DISCUSSION

Thelohania contejeani was transmitted experimentally between white-clawed crayfish via ingestion of infected tissue as well as through exposure to contaminated water, with direct ingestion of infected tissue leading to a higher rate of transmission (83% *vs* 42%). This study presents an improvement in the understanding of the transmission route of *T. contejeani* in freshwater crayfish. There will be frequent opportunities for direct horizontal transmission of *T. contejeani* in the wild. Cannibalism is common in crayfish, with larger adults and juveniles preying on smaller individuals (Abrahamsson, 1966; Mason, 1977), providing opportunities for ingestion of infected tissue. Crayfish use refuges in close proximity to each other as well as coming into contact when foraging and mating (Peay, 2002), providing opportunities for spore transmission via the water. This study also provides the first evidence for direct transmission of *T. contejeani* from infected white-clawed crayfish to the invasive signal crayfish, both by consumption of infected tissue and through exposure to contaminated water. Transmission success to the signal crayfish was high (50%) in both treatments.

Previous attempts to transmit *T. contejeani* to healthy crayfish have been of mixed success. Voronin (1971) and Mazylis (1978) may have induced infection in the noble crayfish *Astacus astacus* by injection of spores into the stomach and by feeding infected tissue to apparently healthy crayfish. However, others have been critical of these results, suggesting that the infections which arose were likely present in the crayfish prior to experimental treatment (Graham and France, 1986). Later experiments were unable to replicate the findings of Voronin and Mazylis. Fischer (1992) and Hoffman *et al*. (1999) attempted transmission in noble crayfish via cannibalism and injection of spores into the stomach, as well as via a snail intermediate host, but no transmission of the parasite was observed. Similarly, Graham and France (1986) found no evidence of direct or indirect transmission of *T. contejeani* to the North American crayfish species, *Orconectes virilis*.

https://doi.org/10.1017/S0031182012000777 Published online by Cambridge University Press

Table 3. Survival and growth rates of crayfish from the 3 experimental groups; tissue; fed with tissue from *Thelohania contejeani*-infected *Austropotamobius pallipes*, water; exposed to contaminated water from tanks that housed infected *A. pallipes*, control; animals were not fed infected tissue or exposed to contaminated water

Species	Group	Number	Survival (%)	Mean growth rate (mm/week)
A. pallipes	Tissue	33	88%	0·09 (±0·007 S.E.)
A. pallipes	Water	33	79%	0·11 (±0·012)
A. pallipes	Control	32	91%	0·10 (±0·008)
P. leniusculus	Tissue	31	90%	0·10 (±0·009)
P. leniusculus	Water	33	85%	0·09 (±0·011)
P. leniusculus	Control	34	88%	0·09 (±0·010)

These earlier studies relied on visual examination and light microscopy for parasite detection, and may have missed infections at low burden and low pathogenicity. The use of PCR in the current study increases the sensitivity of detection (Imhoff *et al*. 2010), as well as providing an estimate for the base rate of infection in the field population. None of the crayfish tested showed visible signs of infection, suggesting that parasite burden is low during the initial months of infection. This is supported by our observation that growth and survival did not differ between infected and uninfected crayfish of either species over the 6-month study period.

There is considerable current interest in the creation of 'ark sites' as refuges for the endangered white-clawed crayfish in the United Kingdom (Kemp *et al*. 2003; Horton, 2009). Crayfish populations are moved from a location where they are threatened by non-indigenous species or habitat degradation to a water body deemed safe and appropriate for the population's survival (the 'ark site'). Our findings lead us to recommend that infected individuals are removed from the population before translocation, in agreement with Diéguez-Uribeondo *et al*. (1997). Mazylis (1978) recommended quarantining a population for 6 months prior to introduction to an ark site, to allow visual identification and removal of infected individuals, but for many ark site translocations this is not feasible due to the number of crayfish involved. Non-lethal molecular screening of individuals provides a more sensitive method of parasite detection (Imhoff *et al*. 2010), although it may be beyond the scope of conservationists. In such a case, critical visual inspection and subsequent removal of infected crayfish must suffice. There has also been recent interest in using captive-reared white-clawed crayfish to supplement wild populations (Nightingale, 2009). Again we recommend the removal of any infected crayfish from the breeding programme, as our findings indicate that they can transmit the parasite to healthy juveniles via shared water supply.

Parasites can play a key role in mediating interspecific interactions including competition and thus can influence the success and outcome of biological invasions (Prenter *et al*. 2004; Tompkins *et al*. 2011; Dunn *et al*. 2012). Invaders may introduce parasites to a biological community, or may acquire parasites in their new range. The current study provides evidence for efficient, direct horizontal transmission of the endemic parasite *T. contejeani* to the invasive crayfish *P. leniusculus* through ingestion of infected *A. pallipes* tissue and through contaminated water. *A. pallipes* and *P. leniusculus* frequently occur in sympatry at the edges of the invasion zone and intraguild predation is common among crayfish, hence opportunities for transmission will be common in the field, and previous studies have reported the infection in wild *P. leniusculus* populations (Dunn *et al*. 2009). The outcome of this shared infection will depend on the relative competence of the native and invading host species (Dunn, 2009; Hatcher *et al*. 2012). One the one hand, if the invasive species is a less competent host, it may act as a sink for the parasite, leading to a reduction in prevalence in native hosts. For example, Solter and Maddox (1998) investigated transmission of endemic N. American microsporidia to the invasive gypsy moth *Lymantria dipar*. Although gypsy moth larvae that were experimentally exposed to microsporidia spores developed infections, subsequent transmission to new hosts was very low. If the signal crayfish acts as a sink for *T. contejeani* then the tendency of signal crayfish to form dense populations may lead to a reduction in the impact of *T. contejeani* on the native white-clawed crayfish when the species occur in sympatry. Conversely, an invasive host species may act as a reservoir for transmission of the infection into native hosts, leading to an increase in parasite prevalence. The process of spillover (where the reservoir host is the original host species) or spillback (where the reservoir is a more recently acquired host, Poulin *et al*. 2011; Hatcher *et al*. 2012) can also mediate invasion success (Dunn, 2009; Tompkins *et al*. 2011). For example, the invasion of the signal crayfish and resulting extirpation of the native European crayfish has been facilitated in many populations by outbreaks of crayfish plague, caused

https://doi.org/10.1017/S0031182012000777 Published online by Cambridge University Press

by the fungus *Aphanomyces astaci*. The disease was co-introduced with North American crayfish species, in which it causes little pathogenicity, but spillover to native crayfish causes high mortality (Holdich and Reeve, 1991; Alderman, 1996). Although transmission from the invasive to the native species remains to be tested, it is possible that *P. leniusculus* may act as a reservoir for spillback of the native parasite *T. contejeani* to *A. pallipes*, potentially facilitating extirpation of the native species. The potential for spillback from the invasive signal crayfish to the endangered native species is of concern as signal crayfish reach higher population densities than do white-clawed crayfish (Arrignon and Roché, 1983; Holdich and Domaniewski, 1995; Guan and Wiles, 1996). Current policies to restrict the movement of signal crayfish and promote conservation of the white-clawed crayfish in isolated habitats (Holdich *et al*. 2004; Peay, 2009) may therefore be important to control transmission of *T. contejeani* as well as plague.

ACKNOWLEDGEMENTS

The authors thank N. Wilson and J. Dick of Queens University, Belfast for assistance in collecting and caring for the Irish white-clawed crayfish. A. Dubuffet and N. Haddaway of University of Leeds assisted with molecular work and care of signal crayfish. H. Soliman provided advice regarding the use of *T. contejeani*-specific primers and J. E. Smith on PCR. J. Brickland provided data on a population of crayfish with high parasite prevalence.

FINANCIAL SUPPORT

E. M. Imhoff acknowledges support from the Marie Curie Early Stage Training and the Environment Agency; A. M. Dunn acknowledges support from NERC NE/D011000/1.

REFERENCES

Abrahamsson, S. A. A. (1966). Dynamics of an isolated population of the crayfish *Astacus astacus* Linné. *Oikos* **17**, 96–107.

Alderman, D. J. (1996). Geographical spread of bacterial and fungal diseases of crustaceans. *Revue Scientifique et Technique de l'Office International des Epizooties*. **15**, 603–632.

Arrignon, J. C. V. and Roché, B. (1983). Population of the crayfish *Austropotamobius pallipes pallipes* Lereboullet in a brook of Corsica, France. *Freshwater Crayfish* **5**, 229–238.

Cossins, A. R. and Bowler, K. (1974). An histological and ultrastructural study of *Thelohania contejeani* Henneguy, 1892 (Nosematidae), Microsporidian parasite of the crayfish *Austropotamobius pallipes* Lereboullet. *Parasitology* **68**, 81–91.

Diéguez-Uribeondo, J., Pinedo-Ruíz, J. and Muzquiz, J. L. (1997). *Thelohania contejeani* in the province of Alava, Spain. *Bulletin Francais de la Pêche et de la Pisciculture* **347**, 749–752.

Duffield, J. E. (1933). Fluctuations in numbers among freshwater crayfish, *Potamobius pallipes* Lereboullet. *Journal of Animal Ecology* **2**, 184–196.

Dunn, A. M. (2009). Parasites and biological invasions. *Advances in Parasitology* **68**, 161–184.

Dunn, A. M., Torchin, M. E., Hatcher, M. J., Kotanen, P. M., Blumenthal, D. M., Byers, J. E., Coon, C. A. C, Frankel, V. M., Holt, R. D., Hufbauer, R. A., Kanarek, A. R., Shierenbeck, K. A., Wolfe, L. M. and Perkins, S. E. (2012). Indirect effects of parasites on invasions. *Functional Ecology* (in the Press).

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.

El-Matbouli, M. and Soliman, H. (2006). Molecular diagnostic methods for detection of *Thelohania contejeani* (Microsporidia), the causative agent of porcelain disease in crayfish. *Diseases of Aquatic Organisms* **69**, 205–211.

Fischer, H. (1992). The porcelain disease of the freshwater crayfish *Astacus astacus* L., caused by *Thelohania contejeani*. DVM Thesis, Tierärztliche Fakultät, Universität München, München, Germany.

Folmer, O., Black, M., Hoeh, W., Lutz, R. and Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates *Molecular Marine Biology and Biotechnology* **3**, 294–299.

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

Guan, R. and Wiles, P. R. (1996). Growth, density and biomass of crayfish, *Pacifastacus leniusculus*, in a British lowland river. *Aquatic Living Resources* **9**, 265–272.

Hatcher, M. J. and Dunn, A. M. (2011). *Parasites in Ecological Communities*. Cambridge University Press, Cambridge, UK.

Hatcher, M. J., Dick, J. and Dunn, A. M. (2012). Disease emergence and invasions. *Functional Ecology* (in the Press)

Henneguy, G. and Thélohan, P. (1892). Myxosporidies parasites des muscles chez quelques crustacés décapodes. *Annuales Micrographie* **4**, 617–641.

Hoffman, R. W., El-Matbouli, M., Oidtmann, B. and Fischer, H. (1999). Light and electron microscopic studies on porcelain disease (*Thelohania contejeani*) in noble crayfish (*Astacus astacus*). *Freshwater Crayfish* **12**, 933–934.

Holdich, D. M. and Domaniewski, J. C. J. (1995). Studies on a mixed population of the crayfish *Austropotamobius pallipes* and *Pacifastacus leniusculus* in England. *Freshwater Crayfish* **10**, 37–45.

Holdich, D. M. and Reeve, I. D. (1991). The distribution of freshwater crayfish in the British Isles with particular reference to crayfish plague, alien introductions and water quality. *Aquatic Conservation* **1**, 139–158.

Holdich, D., Sibley, P. and Peay, S. (2004). The white-clawed crayfish – a decade on. *British Wildlife* **15**, 153–164.

Horton, M. P. (2009). Establishing the island of Ireland's first ark site for the white-clawed crayfish *Austropotamobius pallipes* in the Ballinderry river system, Co. Tyrone. In *Crayfish Conservation in the British Isles* (ed. Brickland, J., Holdich, D. M. and Imhoff, E. M.), *Proceedings of a conference held on 25 March 2009, Leeds, UK*, pp. 87–94.

Hutchings, A. (2009). Monitoring *Austopotamobius pallipes* (Lereboullet) in a chalk stream in southern England. In *Crayfish Conservation in the British Isles* (ed. Brickland, J., Holdich, D. M. and Imhoff, E. M.), *Proceedings of a conference held on 25 March 2009, Leeds, UK*, pp. 99–119.

Imhoff, E., Mortimer, R. J. G., Christmas, M. and Dunn, A. M. (2011). Invasion progress of the signal (*Pacifastacus leniusculus* (Dana)) crayfish and displacement of the native white-clawed crayfish (*Austropotamobius pallipes* (Lereboullet)) in the River Wharfe, UK. *Freshwater Crayfish* **18**, 45–53.

Imhoff, E., Mortimer, R. J. G., Christmas, M. and Dunn, A. M. (2010). Non-lethal tissue sampling allows molecular screening for microsporidian parasites in signal (*Pacifastacus leniusculus* Dana) and vulnerable white-clawed crayfish (*Austropotamobius pallipes* Lereboullet). *Freshwater Crayfish* **17**, 145–150.

Imhoff, E., Mortimer, R. J. G., Christmas, M. and Dunn, A. M. (2009). Porcelain disease in white-clawed and signal crayfish in the UK. In *Crayfish Conservation in the British Isles* (ed. Brickland, J., Holdich, D. M. and Imhoff, E. M.), *Proceedings of a conference held on 25 March 2009, Leeds, UK*, pp. 49–56.

IUCN, 2010. IUCN Red List of Threatened Species. Version 2010.4. www.iucnredlist.org. Accessed 14 February 2011.

Kemp, E., Birkinshaw, N., Peay, S. and Hiley, P. D. (2003). Reintroducing the white-clawed crayfish *Austropotamobius pallipes*. *Conserving Natura 2000 Rivers Conservation Techniques Series No. 1*. English Nature, Peterborough, UK.

Kocher, T. D., Thomas, W. K., Meyer, A., Edwards, S. V., Paabo, S., Villablanca, F. X. and Wilson, A. C. (1989). Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proceedings of the National Academy of Sciences, USA* **86**, 6196–6200.

Lom, J., Nilson, F. and Dykova, I. (2001). *Thelohania contejeani* Henneguy, 1892: dimorphic life cycle and taxonomic affinities, as

indicated by ultrastructural and molecular study. *Parasitology Research* **87**, 860–872.

Mason, J. C. (1977). Reproductive efficiency of *Pacifastacus leniusculus* in culture. *Freshwater Crayfish* **3**, 101–117.

Mazylis, A. (1978). *Astacus astacus* L. infected with *Thelohania contejeani* Henneguy. *Freshwater Crayfish* **4**, 472–473.

Moodie, E. G., Le Jambre, L. F. and Katz, M. E. (2003*a*). *Thelohania montirivulorum* sp. nov. (Microspora: Thelohaniidae), a parasite of the Australian freshwater crayfish, *Cherax destructor* (Decapoda: Parastacidae): fine ultrastructure, molecular characteristics and phylogenetic relationships. *Parasitology Research* **91**, 215–228.

Moodie, E. G., Le Jambre, L. F. and Katz, M. E. (2003*b*). *Thelohania parastaci* sp. nov. (Microspora: Thelohaniidae), a parasite of the Australian freshwater crayfish, *Cherax destructor* (Decapoda: Parastacidae). *Parasitology Research* **91**, 151–165.

Mori, M. and Salvidio, S. (2000). The occurrence of *Thelohania contejeani* Henneguy, a microsporidian parasite of the crayfish *Austropotamobius pallipes* (Lereboullet), in Liguria Region (NW Italy). *Journal of Limnology* **59**, 167–169.

Nightingale, J. (2009). Conservation of the white-clawed crayfish in South-west England. In *Crayfish Conservation in the British Isles* (ed. Brickland, J., Holdich, D. M. and Imhoff, E. M.), *Proceedings of a conference held on 25 March 2009, Leeds, UK*, pp. 85–86.

Oidtmann, B., El-Matbouli, M., Fischer, H., Hoffmann, R., Klärding, K., Schmid, I. and Schmidt, R. (1996). Light microscopy of *Astacus astacus* L. under normal and selected pathological conditions, with special emphasis to porcelain disease and crayfish plague. *Freshwater Crayfish* **11**, 465–480.

O'Keefe, C. and Reynolds, J. D. (1983). The occurrence of crayfish diseases and their significance in Ireland. *Freshwater Crayfish* **5**, 299–306.

Peay, S. (2002). *Monitoring the White-clawed Crayfish*. English Nature, Peterborough, UK.

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.), *Proceedings of a conference held on 25 March 2009, Leeds, UK*, pp. 49–56.

Pixell Goodrich, H. (1956). Crayfish epidemics. *Parasitology* **46**, 480–483.

Poulin, R., Paterson, R. A., Townsend, C. R., Tompkins, D. M. and Kelly, D. W. (2011). Biological invasions and the dynamics of endemic diseases in freshwater ecosystems. *Freshwater Biology* **56**, 676–688.

Prenter, J., MacNeil, C., Dick, J. T. A. and Dunn, A. M. (2004). Roles of parasites in animal invasions. *Trends in Ecology and Evolution* **19**, 385–390.

Schäperclaus, W. (1954). *Fischkrankheiten*. Akademie-Verlag, Berlin, Germany.

Solter, L. F. and Maddox, J. V. 1998. Physiological host specificity of microsporidia as an indicator of ecological host specificity. *Journal of Invertebrate Pathology* **71**, 207–216.

Tompkins, D. M., Dunn, A. M., Smith, M. J. and Telfer, S. (2011). Wildlife diseases, from individuals to ecosystems. *Journal of Animal Ecology* **80**, 19–38.

Vey, A. and Vago, C. (1973). Protozoan and fungal diseases of *Austropotamobius pallipes* Lereboullet in France. *Freshwater Crayfish* **1**, 165–180.

Voronin, V. N. (1971). New data on microsporidiosis of the crayfish, *Astacus astacus* (L. 1758). *Parazitologya* **5**, 186–191.

Weiss, L. M., Zhu, X., Cali, A., Tanowitz, H. B. and Wittner, M. (1994). Utility of microsporidian ribosomal-RNA in diagnosis and phylogeny – a review. *Folia Parasitologia* **41**, 81–90.